Population genomics, systematics, and phylogenomics of the tapeworm order Trypanorhyncha

By
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Kaylee S. Herzog
Ph.D., University of Kansas, 2022
M.A., University of Kansas, 2016
B.Sc., State University of New York (SUNY) College at Oneonta, 2014

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Chairperson Dr. Kirsten Jensen

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Dr. Paulyn Cartwright

_____

Dr. Mark T. Holder

_____

Dr. W. Leo Smith

_____

Dr. Eduardo Rosa-Molinar

Date Defended: April 29, 2022

The dissertation committee for Kaylee S. Herzog certifies that this is the approved version of the following dissertation:

Population genomics, systematics, and phylogenomics of the tapeworm order Trypanorhyncha

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Chairperson Dr. Kirsten Jensen

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Abstract

The order Trypanorhyncha is a ubiquitous and speciose group of commercially relevant marine tapeworms. As adults, trypanorhynchs inhabit the guts of sharks and rays (i.e., elasmobranchs). They sport attachment organs with highly characteristic hooked tentacles and are further unique among the nine orders of shark and ray-hosted tapeworms in demonstrating variable degrees of specificity for their elasmobranch hosts. Adult trypanorhynchs from elasmobranchs, and their larval counterparts from bony fishes, molluscs, and crustaceans, have been collected for centuries. Despite this attention, however, the order remains notorious as "the most chaotic and confused of tapeworm groups" (Wardle & McLeod, 1952; *The Zoology of Tapeworms* pg. 287). Drivers of chaos include the misinterpretation of distinguishing morphological features, species descriptions based only on larval specimens, and an evolutionary hypothesis for the order that is at odds with a classification based on morphology. This dissertation leverages a variety of data types and methodological approaches to present a refined understanding of diversity, morphology, population genomics, and evolutionary history for the trypanorhynch tapeworms. In Chapter 1, the trypanobatoid family Rhinoptericolidae Carvajal & Campbell, 1975 is revised based on global collections and an integrative taxonomic approach. Membership in the family is increased through the description of new species and the transfer of previously described species. The importance of scanning electron microscopy for accurate interpretation of tentacular armature is demonstrated, and the first comprehensive evaluation of intraspecific and intrageneric divergence in the tapeworm barcoding gene for trypanorhynch tapeworms is presented. Finally, a phylogenetic analysis based on sequence data is the first to recover a monophyletic Rhinoptericolidae, and a novel schematic to aid comprehension of line drawings and scanning electron micrographs of the tentacular armature is introduced. Chapter 2 represents the first investigation of population genomics for trypanorhynch tapeworms, and for elasmobranch tapeworms more broadly. A restriction-site associated DNA (RAD) sequencing approach is used to characterize population structure and genetic diversity for two species of trypanorhynchs that demonstrate relaxed host specificity. These are *Rhinoptericola*

megacantha Carvajal & Campbell, 1975 (suborder Trypanobatoida) and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 (suborder Trypanoselachoida). For each species, worms were sampled from multiple species of their known elasmobranch hosts from across their known geographic distributions. Sampling also emphasized sequencing worms from multiple conspecific host individuals within and between geographic regions, and sequencing multiple worms from the same host individual, wherever possible. For *R. megacantha*, population structure coincides with geographic region rather than definitive host species. For *C. gracilis*, limited population structure is found. Both species exhibit high levels of homozygosity and elevated F_{IS} values. Conspecific tapeworms collected from the same host individual are as, or more, genetically divergent from one another as from conspecifics collected from different host individuals. Additionally, examination of material from tens of carcharhiniform sharks suggests that adults of *C. gracilis* may be restricted to parasitizing species in the family Carcharhinidae Jordan & Evermann, 1896. Chapter 3 presents the first phylogenomic hypothesis for the order Trypanorhyncha. Multispecies coalescent and multigene concatenation-based tree building approaches are employed to analyze data for hundreds of orthologous loci from more than 200 trypanorhynchs representing all major lineages within the order. These analyses expand on prior sequencing efforts for the order, both in terms of the number of loci sequenced and the proportion of trypanorhynch diversity represented. Support for the two existing suborders is recovered, but need for major changes to inclusion in, and organization of, superfamilies, families, and genera is evident—particularly for the suborder Trypanobatoida. Support is recovered for multiple independent losses of the rhyncheal system, and new taxa hosted by species of elasmobranchs previously unexamined for trypanorhynch tapeworms are identified as targets for future descriptive work.

Author's Disclaimer

All taxonomic actions in this work are hereby disclaimed for nomenclatural purposes, as recommended in Article 8 of the International Code of Zoological Nomenclature (Ride et al. 1999).

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Introduction

The tapeworm order Trypanorhyncha

An introduction to tapeworms in the order Trypanorhyncha

It is estimated that approximately 20,000 species of tapeworms inhabit the guts of the world's vertebrates (Caira et al., 2017). This diversity is presently organized into 19 tapeworm orders, each with its own unique suite of morphologies and vertebrate host associations. Remarkably, nearly half of the 19 orders of tapeworms are comprised of species that exclusively parasitize sharks and rays (elasmobranchs) as adults (Caira & Jensen, 2014; Caira et al., 2014). Of these, the order Trypanorhyncha is the most speciose, with over 330 valid species in more than 80 genera described to date (Beveridge et al., 2017; Caira et al., 2021). Given the great number of species of elasmobranchs for which trypanorhynch faunas have yet to be explored, however, these counts underestimate global diversity in the order (Beveridge et al., 2017). At the subordinal level, the Trypanorhyncha is divided into the Trypanobatoida and the Trypanoselachoida, members of which parasitize primarily rays and primarily sharks, respectively.

As a group, trypanorhynchs have been described and reported from a broad diversity of shark and ray hosts, representing species in every elasmobranch order (Palm, 2004; Caira et al., 2021). Some species of trypanorhynchs have even been reported to parasitize chimaeras, or rat fishes—the closest extant relatives of elasmobranchs (e.g., Beveridge, 1990). It is worth mentioning, however, that to date, not all groups of elasmobranchs have been surveyed evenly for trypanorhynchs. For example, the trypanorhynch faunas of deep-sea sharks remain notoriously understudied (Beveridge et al., 2017), and speciose ray groups such as the family Dasyatidae Jordan, 1888 (stingrays) and the order Rajiformes (skates) have been undersampled relative to their diversity.

In concert with their broad definitive host associations, trypanorhynchs collectively enjoy a circumglobal distribution (Palm, 2004). Though a handful of species are known from freshwater stingrays or riverine bull sharks (e.g., Watson & Thorson, 1976; Campbell et al., 1999), the bulk of trypanorhynch diversity is marine (Palm, 2004; Caira et al., 2021). Like other elasmobranch tapeworms, trypanorhynchs are found in the intestines of their shark and ray hosts

(Caira & Jensen, 2014). Unlike their relatives in other orders, however, trypanorhynchs can also be found as adults in the stomachs of sharks (e.g., Palm, 1999; Palm & Beveridge, 2002) and in the stomachs, cloacae, and even gall bladders and nephridial systems of devil rays (family Mobulidae Gill, 1893) (Campbell & Beveridge, 2006; Palm et al., 2019).

Morphologically, trypanorhynchs are united by their possession of a complex attachment organ, or scolex, that is highly characteristic. Trypanorhynch scoleces are comprised of two or four weakly muscular lappets, or bothria, and four hooked tentacles. Each tentacle can be independently everted and retracted using an intricate interplay of muscular and hydraulic components. This system, including the four tentacles, is known as the rhyncheal system. As the rhyncheal system is a feature entirely unique to the trypanorhynchs, its presence allows one to immediately identify a tapeworm as belonging to the order Trypanorhyncha. The rhyncheal system is thus the prominent morphological synapomorphy for the order, though this feature has been lost secondarily in its entirety in the four species of *Aporhynchus* Nybelin, 1819 and the two species of *Nakaycestus* Caira, Kuchta & Desjardins, 2010 (see Beveridge, 1990; Caira et al., 2010; Noever et al., 2010).

For the majority of species that do possess a rhyncheal system, the shape, size, and arrangement of hooks along a tentacle are diagnostic at the species level. These features of the tentacular armature do not tend to vary within a species, but do vary between species, and they have thus traditionally formed the basis of trypanorhynch taxonomy and classification (Campbell & Beveridge, 1994). Recent authors, however, have shed light on the potential systematic utility of additional features of the scolex and the larval tapeworm body (e.g., Palm, 1997). Variation in tentacular armatures across the order is immense, and armatures of individual species are often highly complex, as reflected in the now-expansive vocabulary contrived for their description (summarized by Palm, 2004). For example, the metabasal armature of *Halysiorhynchus macrocephalus* (Shipley & Hornell, 1906) Pintner, 1913—which parasitizes a variety of ray hosts in the Indo-Pacific—is described as poeciloacanthous multiatypical, with solid, heteromorphic hooks arranged in ascending half-spiral rows of eight hooks each (Beveridge &

Campbell, 1992). Rows begin with hooks 1(1') on the internal tentacle surface and terminate with hooks 8(8') on the external tentacle surface. Hooks 7(7') and 8(8') are described as found in satellite positions relative to hooks 1(1')–6(6') and flank a double-winged chainette element on the external surface. Additionally, *H. macrocephalus* possesses a characteristic basal armature (i.e., a unique hook pattern in the proximal portions of each tentacle that gives way to the metabasal hook pattern described above). The characteristic basal armature of *H. macrocephalus* is described as heteroacanthous typical, consisting of approximately three ascending half-spiral rows of eight homeomorphic hooks each without a chainette element (Beveridge & Campbell, 1992). As illustrated by the example of *H. macrocephalus*, the complex vocabulary used to describe trypanorhynch armatures is in most cases necessary to accurately document existing morphological diversity; however, this vocabulary can certainly make trypanorhynch identification and description challenging for non-experts.

In addition to being known for their distinctive tentacles, the trypanorhynchs are notorious among elasmobranch tapeworms for their variable degrees of host specificity. Most tapeworms of elasmobranchs are highly host specific and parasitize only a single species of shark or ray as adults (Caira & Jensen, 2014), but trypanorhynchs are more catholic. Host specificity in the order ranges from species that exhibit the one-to-one associations common in other elasmobranch tapeworms (e.g., Campbell & Beveridge, 2006) to species that parasitize tens of elasmobranchs, including both sharks and rays, representing multiple host families and orders (e.g., Beveridge, 1990; Schaeffner & Beveridge, 2012, 2014). Degree of host specificity is even known to vary between congeners. For example, *Grillotia australis* Beveridge & Campbell, 2001 is known to parasitize only the Australian angelshark, *Squatina australis* Regan, 1906, whereas *Grillotia erinaceus* (van Beneden, 1858) Guiart, 1927 has been reported from 24 species of sharks and rays spanning ten genera, seven families, and four orders (Beveridge & Campbell, 2001; Menoret & Ivanov, 2012).

As trypanorhynchs are obligate internal parasites, a species' geographic distribution is intimately tied to its degree of host specificity. Ranges for individual species are thus known

to vary within the order as widely as do host associations. For example, the grey smooth-hound, *Mustelus californicus* Gill, 1864, is found only in the Gulf of California and along the Pacific coast of Baja and southern California (Ebert et al., 2021). Correspondingly, *Dollfusiella macrotrachelus* (Heinz & Dailey, 1974) Beveridge, Neifar & Euzet, 2004, which is specific to the grey smooth-hound, is known only from off southern California (Heinz & Dailey, 1974). Contrastingly, *Tentacularia coryphaenae* Bosc, 1797 is known from eleven species in two families of carcharhiniform sharks, including blue sharks, which have been known to migrate across nearly 10,000 km of open ocean (Palm, 2004; Ebert et al., 2021). Predictably, *T. coryphaenae* has been reported from numerous tropical and temperate localities around the world (Palm, 2004).

Though elasmobranchs serve as the definitive hosts for trypanorhynchs, they represent only a subset of host species known for members of the order. Like all tapeworms, trypanorhynchs have complex, multi-host life-cycles and are trophically transmitted. This means they parasitize several intermediate hosts as larvae prior to a final elasmobranch host in which they mature and reproduce, and they move between hosts when their current host is consumed by a suitable next host. Life-cycles and intermediate host use remain largely a mystery for most species of elasmobranch tapeworms (Jensen & Bullard, 2010), but not so for trypanorhynchs: Thousands of reports of trypanorhynch larvae exist from an impressive diversity of intermediate hosts. These include molluscs, holothurians, cnidarians, salps, and, most notably, crustaceans and bony fishes (e.g., Shipley, 1903; Dollfus, 1923; Bates, 1990; Palm, 2004). Trypanorhynchs are even occasionally reported as larvae from sharks and rays, implicating elasmobranchs as not only definitive hosts, but also potential intermediate hosts in the life-cycles of some species. For example, species of *Hepaoxylon* Bosc, 1811—which as adults parasitize large pelagic sharks such as the great white shark and the blue shark—have been reported as larvae from a variety of species of smaller sharks, including sleeper sharks, lantern sharks, catsharks, and the frilled shark (Dollfus, 1942; Palm, 2004). Trypanorhynch larvae have been known to cause inflammatory responses and mechanical damage in their intermediate hosts (e.g., Brill et al., 1987; Palm et

al., 1994). As many of these intermediate host species are targets of global marine fisheries, trypanorhynch larvae are often considered commercially significant pathogens (Palm, 2004).

Such immense knowledge of intermediate host use for the order is possible because a rhyncheal apparatus is present in both larval and adult trypanorhynchs. This is highly unusual, as the majority of elasmobranch tapeworms do not have larval stages that exhibit adult morphologies (Jensen & Bullard, 2010). For trypanorhynchs, this means that, if tentacles are sufficiently everted, larvae can be confidentially identified to species based on morphology alone. This, in combination with the ubiquity of trypanorhynch larvae in marine ecosystems, has led to their being routinely encountered and reported on from around the world. In fact, the literature contains more reports of larval trypanorhynchs from intermediate hosts than reports of adults from elasmobranchs. Curiously, this has also enabled researchers to describe new species of trypanorhynchs based solely on larval specimens (e.g., Escalante & Carvajal, 1984; Palm, 2010; Beveridge & Campbell, 2013). At present, 73 species of trypanorhynchs (representing approximately 20% of valid species) are described based exclusively on larval specimens collected from intermediate hosts (Caira et al., 2021).

Despite unparalleled knowledge of intermediate host associations, no complete trypanorhynch life-cycle is known from nature. Indeed, there is only a single species of trypanorhynch for which a life-cycle has even been experimentally completed (Sakanari & Moser, 1989). Based on that study, however, and on ample data on intermediate host use and marine food webs, life-cycles that include 2–4 intermediate hosts have been proposed for a number of species of trypanorhynchs. Overall, two generalized life-cycle patterns have been hypothesized: In both types, copepods serve as first intermediate hosts, but the two types differ in whether an invertebrate or a teleost serves as the obligate second intermediate host. For some species, the use of teleosts as third, and even fourth, intermediate hosts has also been suggested (Palm, 2004). Ample knowledge of host use by trypanorhynch larvae has additionally allowed for the first studies of specificity at the level of intermediate hosts in elasmobranch tapeworms (Palm & Caira, 2008).

Challenges

As a result of their ubiquity, morphological distinctiveness, and notable host associations, trypanorhynchs have been collected and reported on for centuries. Despite this attention, significant gaps still exist in our knowledge of evolutionary history, host use and specificity, and genetic diversity in the order. The current hypotheses for the evolutionary history of the Trypanorhyncha are based on ~3,500 base pairs of DNA sequence data and limited taxon sampling (i.e., ~64% of genera and ~26% of species) (Palm et al., 2009; Olson et al., 2010; Beveridge et al., 2017). These hypotheses suggests rampant non-monophyly at every level of trypanorhynch higher classification and elusive morphological synapomorphies for groups with seemingly strong molecular support. For example, the superfamily Eutetrarhynchoidea Guiart, 1927 is non-monophyletic with respect to the superfamily Tentacularioidea Poche, 1926, and houses the speciose and non-monophyletic genera *Dollfusiella* Campbell & Beveridge, 1994 and *Prochristianella* Dollfus, 1946. Diversity within the Eutetrarhynchoidea is organized into four "novel clades" of species whose monophyly is well supported in phylogenetic analyses but for which morphological synapomorphies that unite their members cannot be readily identified based on the information in existing species descriptions. (For an in-depth discussion of non-monophyly within the current system of trypanorhynch classification, see the introduction of Chapter 3, herein.) A novel, stable system of higher classification for the order as informed by a well-resolved and comprehensive phylogenomic evolutionary hypothesis is sorely needed. Such a system would allow for the resolution of existing non-monophyly as well as streamline the determination of higher-level associations for new species awaiting description. Furthermore, an understanding of the evolutionary history for the order would allow for, for example, tests of whether the patterns of trypanorhynch evolution mirror those of shark and rays, or whether degree of host specificity is correlated with species-level diversity within a clade.

In addition to their unresolved evolutionary history, the trypanorhynchs are also plagued by significant taxonomic disorder at the level of individual species. The order is, in fact, infamous as "the most chaotic and confused of tapeworm groups" (Wardle & McLeod, 1952;

pg. 287). Drivers of chaos include missing type material, incomplete or insufficiently detailed species descriptions, conflicting interpretations of key morphological features, and frequent misidentification of type and voucher specimens (Campbell & Beveridge, 1994; Palm, 2004). This confusion not only makes it difficult to identify existing species and describe new taxa, but also muddies our understanding of host associations in the order. Descriptions of species based only on larval specimens are a prime example. Because 73 species of trypanorhynchs have been described based solely on larvae, any knowledge of definitive elasmobranch host associations and strobila and proglottid anatomy are missing for a fifth of trypanorhynch diversity. This dearth of critical information has reinforced emphasis on features of the scolex in species delimitation. Because of the often-impenetrable terminology used to describe trypanorhynch armatures, emphasis on scolex features in descriptions and keys can make accurate identification of species prohibitively challenging to non-experts. This is well-exemplified by the numerous keys to species that are relegated to using only minutely variable scolex features to distinguish species (e.g., Palm, 2004; Schaeffner & Beveridge, 2013a, 2013b; Schaeffner, 2016). Additionally, though a rhyncheal system is present in larval trypanorhynchs, it may not always be fully developed, and so measurements of scolex features for species described from larvae may not be representative or comparable to measurements for species described based on adult specimens. A lack of information on strobila and proglottid anatomy for all species additionally hampers our understanding of trypanorhynch evolution, as it makes it impossible to identify such features as morphological synapomorphies for groups. For example, the proposed synapomorphies for the superfamily Tentacularioidea are a ventro-submarginal genital pore and a uterus that develops laterally from the distal end of the uterine duct (both feature of mature proglottids) (Beveridge et al., 2017). The possession of these features, however, cannot be verified for the ~40% of tentacularioids known only from larval specimens, and so keys to species cannot include these features. Moving forward, the field of trypanorhynch taxonomy would benefit from a focus on descriptions of new species based on material from adult specimens. The field would additionally benefit from a focus on making connections between species described based on larvae and

their adult counterparts from elasmobranchs. Such connections would not only allow for the redescription of these species, but also point to potential trypanorhynch life-cycles and illuminate trophic interactions between marine species.

Rigorous investigations of genetic diversity are also lacking for the Trypanorhyncha. Though variability in degree of host specificity and geographic range are hallmarks for the order, few studies have attempted to assess the impact these traits may have on intraspecific genetic diversity or population structure in trypanorhynchs. The handful of studies that have attempted such investigations (Palm et al., 2007; Haseli et al., 2017; Salmani & Haseli, 2017) are limited by single-locus sequence data, unverified host identifications, and insufficient intraspecific sampling. (For an in-depth analysis of these studies, see the discussion section of Chapter 1, herein.) More broadly, data for the tapeworm barcoding gene, 28S rRNA, have only been generated for ~30% of species of trypanorhynchs, and rarely from multiple specimens for the same species representing different hosts or localities. Publicly available genome-scale datasets and genomic resources for trypanorhynchs are non-existent. Association of trypanorhynchs with multiple species of hosts across variably broad geographic ranges makes them particularly suited for studies of how these biological factors might interact within a complex life-cycle to structure parasite populations and genomic diversity, and are worth investigating with modern methods and data types.

Aims of this dissertation

This dissertation leverages global collections and a variety of methodological approaches to address population genomic and phylogenomic questions in the Trypanorhyncha, as well as to systematically revise targeted groups within in order.

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Chapter 1

A synergistic, global approach to revising the trypanorhynch tapeworm family Rhinoptericolidae (Trypanobatoida)*

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A synergistic, global approach to revising the trypanorhynch tapeworm family Rhinoptericolidae (Trypanobatoida)

Kaylee S. Herzog and Kirsten Jensen

Department of Ecology & Evolutionary Biology and the Biodiversity Institute, University of Kansas, Lawrence, KS, United States

ABSTRACT

Since 2010, the trypanorhynch tapeworm family Rhinoptericolidae *Carvajal & Campbell, 1975* has housed just two distinctive, monotypic genera (*Rhinoptericola Carvajal & Campbell, 1975* and *Nataliella Palm, 2010*). However, global collections of tapeworms from sharks and rays over the last more than three decades brought to light the need for major revision of the family by suggesting a much greater species-level diversity for the nominal genus *Rhinoptericola*. Through synonymy and the description of new species, the number of species in the genus is increased from one to eight. A phylogenetic analysis of the D1–D3 gene region of 28S rRNA (28S), including seven of the now nine species of rhinoptericolids, and a broad sampling of the other Trypanobatoida is the first to recover a monophyletic Rhinoptericolidae. In addition to systematic revision, this study allowed for the first evaluation of the degree of intraspecific *vs* interspecific variation in 28S for adult trypanorhynchs across the various hosts and geographic localities from which they have been reported, suggesting a relatively consistent boundary for *Rhinoptericola*. It is further suggested that detailed scanning electron microscopy (SEM) images of both the basal and metabasal armatures greatly aid in the interpretation of hook arrangement and shape. A schematic to streamline determination of the tentacular surface presented in scanning electron micrographs and line drawings of trypanorhynchs is presented for species with both two and four bothria.
In combination, these methodological refinements can now be used as a model to resolve issues of classification and non-monophyly within both major lineages of the Trypanorhyncha. As a result of the taxonomic work, *Rhinoptericola megacantha Carvajal & Campbell, 1975* (previously only known from the American cownose ray from the Chesapeake Bay and the Ticon cownose ray from the Gulf of Mexico, Venezuela, and Brazil) is now known from an additional species of cownose ray and a species of stingray, and is revealed to have a transatlantic distribution. Data from SEM suggest a simpler interpretation of hook arrangement in the metabasal armature for *Rhinoptercola* and—in combination with 28S sequence data—support *Shirleyrhynchus Beveridge & Campbell, 1988* (a former rhinoptericolid) as its junior synonym. The three species formerly assigned to *Shirleyrhynchus* are thus transferred to *Rhinoptericola*. Data from light microscopy on whole-mounted specimens and histological sections, SEM, and 28S showed the eutetrarhynchid *Prochristianella jensenae Schaeffner & Beveridge, 2012b* to be morphologically consistent with species of *Rhinoptericola* and it is thus transferred to the genus. The type series of *P. jensenae* was determined to be mixed, representing two distinct species which are here

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Corresponding author
Kaylee S. Herzog,
ksherzog@ku.edu

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redescribed and described as new, respectively. Two additional novel species of *Rhinoptericola* are described from cownose rays from off Mozambique and the Gulf of California.

Subjects Biodiversity, Marine Biology, Parasitology, Taxonomy, Zoology
Keywords *Rhinoptericola*, *Shirleyrhynchus*, Scanning electron microscopy, 28S rRNA, Tentacular armature, Elasmobranchs, Synonymy, *Prochristianella jensenae*, Phylogeny, Species boundaries

INTRODUCTION

The monotypic family Rhinoptericolidae *Carvajal & Campbell, 1975* was erected to accommodate the genus *Rhinoptericola Carvajal & Campbell, 1975* and its type species, *Rhinoptericola megacantha Carvajal & Campbell, 1975*. Since then, the family has been synonymized, resurrected, moved between three superfamilies, and has variously included members of several unusual trypanorhynch genera. In light of the significant changes proposed in this study to the species diversity, degree of host specificity, interrelationships, and the interpretation of the tentacular armature of the family as a whole or its members, a summary of its convoluted history is warranted.

Carvajal & Campbell (1975) described *R. megacantha* based on worms from a single adult American cownose ray, *Rhinoptera bonasus* (Mitchill, 1815), collected from the Chesapeake Bay, Virginia, USA, as possessing a heteroacanthous atypical metabasal armature (*i.e.*, an armature with hooks arranged in paired principal rows with one or more intercalary hook[s] between those rows). The authors distinguished the new species from the other heteroacanthous atypical trypanorhynchs known at the time (species in the families Otobothriidae Dollfus, 1942 and Mustelicolidae Dollfus, 1969) based on its unique morphology: possession of four bothria and a uterus bifurcated at the posterior end, and lack of bothrial pits. They thus justified the creation of a new family. In addition to describing *R. megacantha* as an atypical heteroacanth, the authors (mistakenly) reported that the species lacks prebulbar organs and made no mention of gland cells in the bulbs.

Nearly two decades later, *Campbell & Beveridge (1994)* formally allied the Rhinoptericolidae with the other families of heteroacanthous atypical trypanorhynchs, placing them together in the superfamily Otobothrioidea Dollfus, 1942. Shortly thereafter, *Palm (1995, 1997)* published a revised classification for the trypanorhynchs which emphasized morphological features other than tentacular armature. In the classification of *Palm (1997)*, *Rhinoptericola* was moved to the family Pterobothriidae Pintner, 1931 within the superfamily Tentacularioidea Poche, 1926 based on its reported lack of bothrial pits and prebulbar organs, and its possession of four bothria and a heteroacanthous atypical metabasal armature, thus making Rhinoptericolidae a junior synonym of Pterobothriidae.

In the first cladistic analysis for the trypanorhynchs, based on 44 morphological characters coded for 49 genera, *Beveridge, Campbell & Palm (1999)* recovered *Rhinoptericola* in a clade with members of the families Shirleyrhynchidae *Campbell & Beveridge, 1994* and Mixodigmatidae Dailey & Vogelbein, 1982, a group the authors

referred to as "Clade 5". As they did not recover *Rhinoptericola* allied with the otobothriids or pterobothriids, *Beveridge, Campbell & Palm (1999)* rejected the classifications of *Campbell & Beveridge (1994)* and *Palm (1997)* and resurrected the Rhinoptericolidae from synonymy. They also noted that the families in their Clade 5 share morphological features with the family Eutetrarhynchidae Guiart, 1927, members of which form a sister group to Clade 5 in their analysis. Though this comparison was made, the authors maintained in their discussion that *R. megacantha* lacked prebulbar organs (a feature shared by all eutetrarhynchids). Superfamilial placements were not discussed for any taxa in this analysis.

In his formative opus on the order Trypanorhyncha, *Palm (2004)* made Shirleyrhynchidae a junior synonym of Rhinoptericolidae, reclassifying both shirleyrhynchid genera (*i.e.*, *Shirleyrhynchus Beveridge & Campbell, 1988* and *Cetorhinicola Beveridge & Campbell, 1988*) as rhinoptericolids. He also moved the Rhinoptericolidae—at that time containing, for the first time since its creation, three genera—to the superfamily Eutetrarhynchoidea Guiart, 1927. In his revised familial diagnosis, *Palm (2004)* specified a heteroacanthous typical metabasal armature for the rhinoptericolids. Both *Shirleyrhynchus* and *Cetorhinicola* were originally described as typical heteroacanths (see *Beveridge & Campbell, 1988*), but unlike the former shirleyrhynchids, *Rhinoptericola* was described as possessing intercalary hooks (*Carvajal & Campbell, 1975*). *Palm (2004)* did not mention this significant change for *Rhinoptericola* in his discussion of the newly circumscribed Rhinoptericolidae, except to say that the possession of a heteroacanthous typical armature was a feature that unified the three genera. Furthermore, he did not mention the presence or absence of prebulbar organs in *Rhinoptericola* even though he had, for the first time, classified the Rhinoptericolidae as belonging to a superfamily for which morphological synapomorphies include the presence of prebulbar organs.

To piece together the complete picture of the redefinition of the Rhinoptericolidae by *Palm (2004)*, one must read his discussion sections for *Rhinoptericola* and *R. megacantha*. It is in these sections where Palm reported that a reexamination of type material of *R. megacantha* revealed the lack of intercalary hooks and the presence of prebulbar organs, thus justifying his earlier taxonomic and systematic changes at the family level. He did not, however, provide any description, photograph, or illustration to demonstrate how the hooks of *R. megacantha* which were originally described by *Carvajal & Campbell (1975)* as intercalary hooks could be reinterpreted as belonging to principal rows, or to demonstrate the presence of prebulbar organs in this species.

Palm et al. (2009) produced the first phylogenetic hypothesis for the order Trypanorhyncha based on molecular sequence data (18S rRNA and partial 28S rRNA). They included one specimen each of *R. megacantha* and *Nataliella marcelli Palm, 2010* (as "Unidentified gen. nov. sp. nov. [Hp 47, pl]"), as well as a specimen identified therein as *Shirleyrhynchus aetobatidis* (*Shipley & Hornell, 1906*) *Beveridge & Campbell, 1998*. In that analysis, *R. megacantha* was recovered as the sister taxon to a clade containing *N. marcelli* + the Tentaculariidae Poche, 1926, while the specimen identified as *S. aetobatidis* was recovered deeply embedded within a clade of eutetrarhychid taxa, thus

rendering the Rhinoptericolidae of *Palm (2004)* paraphyletic. *Olson et al. (2010)* later published an alternative hypothesis, also based on 18S rRNA and partial 28S rRNA, but their analysis included only *R. megacantha* (recovered as sister to the tentacullariids) and the specimen identified as *S. aetobatidis* (similarly recovered embedded among eutetrarhynchids). In both analyses, a monophyletic Tentacullariidae were recovered embedded within the eutetrarhynchoids, resulting in a paraphyletic Eutetrarhynchoidea.

The next significant contribution to the taxonomic history of the Rhinoptericolidae was made by *Palm (2010)*, wherein he resurrected the Shirleyrhynchidae to once again comprise the genera *Shirleyrhynchus* and *Cetorhinicola*, and formally described *N. marcelli* as a new genus and species belonging to the Rhinoptericolidae (now containing only *Rhinoptericola* and *Nataliella* *Palm, 2010*). The inclusion of *N. marcelli* in the Rhinoptericolidae necessitated revision of the familial diagnosis to accommodate its homeoacanthous metabasal armature. It is in this revised familial diagnosis that, for the first time, the family Rhinoptericolidae was explicitly defined by its members possessing the unique combination of four bothria, prebulbar organs, and a heteroacanthous typical (or homeoacanthous) metabasal armature, but lacking gland cells in the bulbs (*Palm, 2010*).

The removal of *Shirleyrhynchus* and *Cetorhinicola* from the Rhinoptericolidae was not explicitly justified by *Palm (2010)*. *Schaeffner (2016)* speculated that the decision was perhaps based on an interpretation of the results of the molecular phylogenetic analyses of *Palm et al. (2009)* and *Olson et al. (2010)*, in which the specimen identified as *S. aetobatidis* was recovered as deeply embedded among eutetrarhynchids. *Schaeffner (2016)* reexamined the hologenophore of this specimen and reidentified it as the eutetrarhynchid *Parachristianella indonesiensis* *Palm, 2004*. Thus, if *Palm (2010)* resurrected the Shirleyrhynchidae based on the results of these analyses, he was perhaps unknowingly misled by this misidentification.

Despite elucidating this specimen identification error and making extensive taxonomic revisions within the genus *Shirleyrhynchus*, *Schaeffner (2016)* refrained from making any change at the family level. In the most recent review of the order by *Beveridge et al. (2017)*, the authors confirmed (*Rhinoptericola* + (*Nataliella* + Tentacullariidae)) of *Palm et al. (2009)* as the accepted relationship between those taxa and commented on the paraphyletic nature of the Rhinoptericolidae, but similarly refrained from making taxonomic or systematic changes. Thus, the classification of *Palm (2010)* (*i.e.*, a Rhinoptericolidae inclusive of *Rhinoptericola* and *Nataliella*, and a Shirleyrhynchidae inclusive of *Shirleyrhynchus* and *Cetorhinicola*) had been accepted for the last decade prior to this study. Both *Rhinoptericola* and *Nataliella* have remained monotypic since their descriptions.

Findings from recent global elasmobranch collections once more call into question the identity of the Rhinoptericolidae, necessitating its revision. The status of the family also has implications for resolving the non-monophyly of other groups within the Trypanobatoida (see *Beveridge et al., 2017*). The goal of this study was to use the Rhinoptericolidae as a model for applying a novel, multi-pronged approach for stabilizing the taxonomy and classification of trypanorhynch tapeworms. The contributions of this

study to trypanorhynch systematics include assessment of the validity of the Rhinoptericolidae, expansion of its membership *via* synonymy and the description of new species, redescriptions of its valid members, and expansion of the geographic range and known host species for the type species of *Rhinoptericola*, *R. megacantha*. The broader conceptual contributions of this work include a comprehensive assessment of generic and specific boundaries for species of trypanorhynchs based on sequence data, reinterpretations of tentacular armature facilitated by scanning electron microscopy (SEM) data, and the introduction of a visual tool to effectively communicate the tentacle surfaces depicted in line drawings and scanning electron micrographs (SEMs).

MATERIALS AND METHODS

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:CE2287DE-C097-4EA5-84D4-7DC7E8F3BE7A. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

Specimen collection

In total, representatives of six species of *Rhinoptericola* were recovered from 67 batoid host individuals representing three families, seven genera, and 14 species. Host taxonomy follows *Last et al. (2016)*. Disk width, sex, collection date, and collection locality are provided for each host individual in Table 1; the unique host code is also provided and can be used in the Global Cestode Database (www.elasmobranchs.tapewormdb.uconn.edu) (*Caira, Jensen & Barbeau, 2021*) to access additional specimen information. Host identifications follow *Naylor et al. (2012)* and *Fernando et al. (2019)* (see Table 1).

The body cavity of each batoid was opened with a mid-ventral longitudinal incision, and the spiral intestine was removed and opened with a longitudinal incision. Spiral intestines were fixed in one of three ways: (1) the entire spiral intestine and its contents were fixed in 95% ethanol, (2) a subset of worms was removed from the spiral intestine and fixed in 95% ethanol, and the spiral intestine and its remaining contents were fixed in 10% seawater-buffered formalin, or (3) the entire spiral intestine and its contents were fixed in 10% seawater-buffered formalin. Spiral intestines fixed in 95% ethanol were permanently stored in 95% ethanol at −20 °C at the University of Kansas (KU) or the University of Connecticut (UConn) while those fixed in formalin were later transferred to 70% ethanol at KU or UConn for permanent storage.

Collections were conducted under the following permits (by country): *Queensland, Australia:* General Fisheries Permit No. PRM04598E issued to Lyle & Cadel Squire for

Table 1 Size, sex, and collection data for the batoid specimens found to host species of *Rhinoptericola Carvajal & Campbell, 1975* as part of this study.

Host family: Host species	Host code	Disk width (cm)	Sex	Collection date	Collection locality	Species hosted
Aetobatidae: *Aetobatus ocellatus*	CM03-29	73	?	Jun. 7, 2003	Weipa (12°35′11″S, 141°42′34″E), Queensland, Australia, Gulf of Carpentaria	Rj
Aetobatidae: *Aetobatus ocellatus*	CM03-44	80	female	Jun. 10, 2003	Weipa (12°35′11″S, 141°42′34″E), Queensland, Australia, Gulf of Carpentaria	Rj
Dasyatidae: *Hemitrygon bennetti*	VN-42*	38	male	Mar. 12, 2010	Cat Ba (20°43′31.1″N, 107°02′54.9″E), Haiphong Province, Viet Nam, Gulf of Tonkin, South China Sea	Rb
Dasyatidae: *Himantura tutul*	KA-71	73.5	female	Nov. 29, 2006	Pagatan market (03°36′36.00″S, 115°54′59.40″E), South Kalimantan, Indonesia, Java Sea	Rb
Dasyatidae: *Hypanus say*	CH-22	41	female	Jun. 18, 2013	Charleston (32°47′18.08″N, 79°53′18.77″W), South Carolina, USA, Charleston Harbor, Atlantic Ocean	Rme
Dasyatidae: *Maculabatis gerrardi*	KA-75	54	male	Nov. 29, 2006	Pagatan market (03°36′36.00″S, 115°54′59.40″E), South Kalimantan, Indonesia, Java Sea	Rb
Dasyatidae: *Maculabatis gerrardi*	KA-82	48	female	Nov. 30, 2006	Gusungnge near Pagatan (03°36′46.10″S, 115°55′05.10″E), South Kalimantan, Indonesia, Java Sea	Rb
Dasyatidae: *Pastinachus ater*	KA-32*	87	male	Nov. 23, 2006	Sei Kerbau (00°31′44.50″S, 117°09′32.90″E), East Kalimantan, Indonesia, Makassar Strait	Rs
Dasyatidae: *Pastinachus ater*	KA-47*	86	female	Nov. 26, 2006	Muara Pasir (01°45′58.92″S, 116°23′36.09″E), East Kalimantan, Indonesia, Makassar Strait	Rb
Dasyatidae: *Pastinachus ater*	NT-105*	123	female	Nov. 19, 1999	East of Wessel Islands (11°17′44″S, 136°59′48″E), Northern Territory, Australia, Arafura Sea	Rb
Dasyatidae: *Pastinachus solocirostris*	BO-164	44	female	May 14, 2003	Sematan (01°48′15.45″N, 109°46′47.17″E), Sarawak, Malaysia, South China Sea	Rs
Dasyatidae: *Pastinachus solocirostris*	BO-165	39	male	May 14, 2003	Sematan (01°48′15.45″N, 109°46′47.17″E), Sarawak, Malaysia, South China Sea	Rs
Dasyatidae: *Pastinachus solocirostris*	BO-177	45	female	May 15, 2003	Sematan (01°48′15.45″N, 109°46′47.17″E), Sarawak, Malaysia, South China Sea	Rs
Dasyatidae: *Pastinachus solocirostris*	BO-267	39.5	female	May 20, 2003	Mukah (02°53′52.16″N, 112°05′44.12″E), Sarawak, Malaysia, South China Sea	Rs
Dasyatidae: *Pastinachus solocirostris*	KA-44	69	female	Nov. 26, 2006	Muara Pasir (01°45′58.92″S, 116°23′36.09″E), East Kalimantan, Indonesia, Makassar Strait	Rb
Rhinopteridae: *Rhinoptera bonasus*	CH-3	88	female	Jun. 27, 2012	Awendaw (33°02′07.78″N, 79°32′47.24″W), South Carolina, USA, Bulls Bay, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera bonasus*	CH-17	82.5	male	Jun. 17, 2013	Charleston (32°45′2.53″N, 79°53′48.28″W), South Carolina, USA, Charleston Harbor, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera bonasus*	CH-18	91	female	Jun. 17, 2013	Charleston (32°45′2.53″N, 79°53′48.28″W), South Carolina, USA, Charleston Harbor, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera bonasus*	CH-19	92	female	Jun. 17, 2013	Charleston (32°44′51.30″N, 79°53′44.07″W), South Carolina, USA, Charleston Harbor, Atlantic Ocean	Rme

Table 1 (continued)

Host family: Host species	Host code	Disk width (cm)	Sex	Collection date	Collection locality	Species hosted
Rhinopteridae: *Rhinoptera bonasus*	CH-29	87	female	Jun. 19, 2013	Awendaw (33°02′07.78″N, 79°32′47.24″W), South Carolina, USA, Bulls Bay, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera bonasus*	CH-30	93	female	Jun. 19, 2013	Awendaw (33°02′07.78″N, 79°32′47.24″W), South Carolina, USA, Bulls Bay, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera bonasus*	CH-32	66	male	Jun. 20, 2013	Charleston (32°45′2.53″N, 79°53′48.28″W), South Carolina, USA, Charleston Harbor, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera bonasus*	CH-40	92	male	Jun. 15, 2015	Charleston, South Carolina, USA, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera bonasus*	CH-43	94	female	Jun. 15, 2015	Charleston, South Carolina, USA, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera bonasus*	CH-44	88.7	male	Jun. 15, 2015	Charleston, South Carolina, USA, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	BE-10	89	male	May 18, 2012	Gales Point Manatee (17°13′1.0″N, 88°19′01.4″W), Belize, Inner Channel, Caribbean Sea	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	BE-11	88	female	May 18, 2012	Gales Point Manatee (17°13′1.0″N, 88°19′01.4″W), Belize, Inner Channel, Caribbean Sea	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	BE-15	87.5	female	May 19, 2012	Gales Point Manatee (17°13′1.0″N, 88°19′01.4″W), Belize, Inner Channel, Caribbean Sea	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	CH-15	58	male	Jun. 17, 2013	Awendaw (33°0′34.27″N, 79°29′8.82″W), South Carolina, USA, Bulls Bay, 5 Fathom Creek, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-49	92	male	Jun. 19, 2005	South side of East Ship Island (30°14′24.54″N, 88°52′25.25″W), Mississippi, USA, Gulf of Mexico	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-156*	?	?	Aug. 2005	Ship Island (30°13′13.53″N, 88°54′52.48″W), Mississippi, USA, Gulf of Mexico	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-298	97	female	Apr. 25, 2006	West tip of Horn Island (30°14′37.70″N, 88°46′37.62″W), Mississippi, USA, Gulf of Mexico	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-299*	?	?	Apr. 21, 2006	Horn Island (30°14′1.44″N, 88°40′5.47″W), Mississippi, USA, Gulf of Mexico	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-300*	?	?	Apr. 21, 2006	Horn Island (30°14′1.44″N, 88°40′5.47″W), Mississippi, USA, Gulf of Mexico	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-301*	?	?	Apr. 21, 2006	Horn Island (30°14′1.44″N, 88°40′5.47″W), Mississippi, USA, Gulf of Mexico	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-305*	81	female	Mar. 28, 2006	Horn Island (30°15′04″N, 88°42′42″W), Mississippi, USA, Gulf of Mexico	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-375	?	?	Aug. 27, 2006	West of south tip of Chandeleur Islands (29°57′9.54″N, 88°50′38.98″W), Louisiana, USA, Gulf of Mexico	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-441	102	female	Oct. 7, 2006	Gulf Coast Research Lab (30°23′33.55″N, 88°47′51.79″W), Ocean Springs, Mississippi, USA, Gulf of Mexico	Rme
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-591*	101.5	male	Jun. 7, 2009	Horn Island (30°14′1.44″N, 88°40′5.47″W), Mississippi, USA, Gulf of Mexico	Rme
Rhinopteridae: *Rhinoptera javanica*	VN-94	144.5	male	Mar. 18, 2010	Long Hai (10°22′60.00″N, 107°13′60.00″E), Ba Ria Province, Viet Nam, South China Sea	Rb

(Continued)

Table 1 (continued)

Host family: Host species	Host code	Disk width (cm)	Sex	Collection date	Collection locality	Species hosted
Rhinopteridae: *Rhinoptera jayakari*	MZ-1	85	female	Jun. 23, 2016	Tofo (23°47′33.02″S, 35°31′16.38″E), Inhambane, Mozambique, Mozambique Channel	Rmo
Rhinopteridae: *Rhinoptera jayakari*	MZ-2	85	female	Jun. 23, 2016	Tofo (23°47′33.02″S, 35°31′16.38″E), Inhambane, Mozambique, Mozambique Channel	Rmo
Rhinopteridae: *Rhinoptera jayakari*	MZ-3	90	female	Jun. 23, 2016	Tofo (23°47′33.02″S, 35°31′16.38″E), Inhambane, Mozambique, Mozambique Channel	Rmo
Rhinopteridae: *Rhinoptera jayakari*	MZ-4	92	female	Jun. 23, 2016	Tofo (23°47′33.02″S, 35°31′16.38″E), Inhambane, Mozambique, Mozambique Channel	Rmo
Rhinopteridae: *Rhinoptera marginata*	SE-78	54.5	female	Jan. 12, 2003	St. Louis (16°1′28″N, 16°30′33″W), Senegal, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera marginata*	SE-84	74	female	Jan. 13, 2003	St. Louis (16°1′28″N, 16°30′33″W), Senegal, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera marginata*	SE-85	56	female	Jan. 13, 2003	St. Louis (16°1′28″N, 16°30′33″W), Senegal, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera marginata*	SE-135	84	female	Jan. 3, 2004	St. Louis (16°1′28″N, 16°30′33″W), Senegal, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera marginata*	SE-137	74	female	Jan. 3, 2004	St. Louis (16°1′28″N, 16°30′33″W), Senegal, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera marginata*	SE-138	84.5	female	Jan. 3, 2004	St. Louis (16°1′28″N, 16°30′33″W), Senegal, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera marginata*	SE-139	86	female	Jan. 3, 2004	St. Louis (16°1′28″N, 16°30′33″W), Senegal, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera marginata*	SE-145	46	female	Jan. 4, 2004	St. Louis (16°1′28″N, 16°30′33″W), Senegal, Atlantic Ocean	Rme
Rhinopteridae: *Rhinoptera neglecta*	AU-85	138	female	Aug. 11, 1997	Dundee Beach (12°45′33″S, 130°21′7″E), Northern Territory, Australia, Fog Bay, Timor Sea	Rb, Rj
Rhinopteridae: *Rhinoptera neglecta*	AU-86	144	female	Aug. 11, 1997	Dundee Beach (12°45′33″S, 130°21′7″E), Northern Territory, Australia, Fog Bay, Timor Sea	Rj
Rhinopteridae: *Rhinoptera neglecta*	AU-87	129	male	Aug. 11, 1997	Dundee Beach (12°45′33″S, 130°21′7″E), Northern Territory, Australia, Fog Bay, Timor Sea	Rb
Rhinopteridae: *Rhinoptera neglecta*	CM03-31	131	male	Jun. 8, 2003	Weipa (12°35′11″S, 141°42′34″E), Queensland, Australia, Gulf of Carpentaria	Rj
Rhinopteridae: *Rhinoptera neglecta*	CM03-43	127	male	Jun. 10, 2003	Weipa (12°35′11″S, 141°42′34″E), Queensland, Australia, Gulf of Carpentaria	Rb, Rj
Rhinopteridae: *Rhinoptera neglecta*	NT-87	99	male	Nov. 16, 1999	East of Wessel Islands (11°17′44″S, 136°59′48″E), Northern Territory, Australia, Arafura Sea	Rb
Rhinopteridae: *Rhinoptera steindachneri*	BJ-1	71.5	male	Jul. 22, 1993	Puertecitos (30°20′58″N, 114°38′22″W), Baja California, Mexico, Gulf of California	Rh
Rhinopteridae: *Rhinoptera steindachneri*	BJ-274	82	male	Aug. 20, 1993	Santa Rosalia (27°19′51″N, 112°15′30″W), Baja California Sur, Mexico, Gulf of California	Rh
Rhinopteridae: *Rhinoptera steindachneri*	BJ-317*	76	male	Aug. 27, 1993	Loreto (25°49′52″N, 111°19′38″W), Baja California Sur, Mexico, Gulf of California	Rh
Rhinopteridae: *Rhinoptera steindachneri*	BJ-355*	74	male	Sept. 1, 1993	Loreto (25°49′52″N, 111°19′38″W), Baja California Sur, Mexico, Gulf of California	Rh

Table 1 (continued)

Host family: Host species	Host code	Disk width (cm)	Sex	Collection date	Collection locality	Species hosted
Rhinopteridae: *Rhinoptera steindachneri*	BJ-595	79.5	female	Jun. 7, 1996	Bahia de Los Angeles (28°59′9″N, 113°32′53″W), Baja California, Mexico, Gulf of California	Rh
Rhinopteridae: *Rhinoptera steindachneri*	BJ-672	78	male	Jun. 9, 1996	Bahia de Los Angeles (28°59′9″N, 113°32′53″W), Baja California, Mexico, Gulf of California	Rh
Rhinopteridae: *Rhinoptera steindachneri*	BJ-684	71	male	Jun. 12, 1996	Santa Rosalia (27°19′51″N, 112°15′30″W), Baja California Sur, Mexico, Gulf of California	Rh
Rhinopteridae: *Rhinoptera steindachneri*	BJ-696*	54	male	Jun. 13, 1996	Santa Rosalia (27°19′51″N, 112°15′30″W), Baja California Sur, Mexico, Gulf of California	Rh
Rhinopteridae: *Rhinoptera steindachneri*	BJ-707	79	female	Jun. 14, 1996	Santa Rosalia (27°19′51″N, 112°15′30″W), Baja California Sur, Mexico, Gulf of California	Rh

Notes:
Asterisks (*) indicate host specimens for which the identification was not verified using NADH2 sequence data.
Rme, *Rhinoptericola megacantha* Carvajal & Campbell, 1975; Rb, *Rhinoptericola butlerae* (Beveridge & Campbell, 1988) n. comb.; Rj, *Rhinoptericola jensenae* (Schaeffner & Beveridge, 2012b) n. comb.; Rs, *Rhinoptericola schaeffneri* n. sp.; Rmo, *Rhinopericola mozombiquensis* n. sp.; Rh, *Rhinoptericola hexacantha* n. sp.

05 May 2004–04 Jul. 2004 by Delegate of the Chief Executive, Queensland Fisheries Service. *Belize:* Permit No. 000016-12 issued to Janine N. Caira, Kirsten Jensen, Fernando P. L. Marques, and Roy Polonio by Fisheries Administrator Beverly Wade of the Belize Fisheries Department (Ministry of Forestry, Fisheries and Sustainable Development), Belize. *Indonesian Borneo (Kalimantan):* Nos. 06252/SU.3/KS/2006 and 3861/SU.3/KS/2007 from LIPI in Jakarta, and 1586/FRP/SM/VII/2008 from RISTEK in Jakarta. *Malaysian Borneo:* UPE:40/200/19SJ.924 and UPE:40/200/19SJ.925 from the Economic Planning Unit in Kuala Lumpur, No. JKM 100-24/13/1/223(59) from the Chief Minister's Department, Kota Kinabalu, Sabah, and SBC-RA-0050-JNC from the Sarawak Biodiversity Center, Sarawak, Kuching. *Mexico:* No. 120496-213-03 issued to Janine N. Caira (University of Connecticut) by the Secretaria de Medio Ambiente Recursos Naturales y Pesca, Mexico. *Mozambique:* Permit No. 13 dated 16 Jun. 2016 by Director General Bartolomen Soto of the Ministério da Terra, Ambiente E Desenvolvimento Rural (Administração Nacional das Áreas de Conservação); specimens export follows International Veterinary Certificate for Exportation of Biological Products No. 21AMOS/DEV/2016 issued 01 Jul. 2016, signed by Maria Emilio Pinto of the Ministério Da Agricultura E Segurança Alimentar (Direcção Nacional De Veterinária), Maputo, Mozambique. *Senegal:* Permit No. 006087 issued by the Ministère de L'Éducation, Dakar, Senegal. *Sri Lanka:* Collections were conducted under a letter of no objection (as species are not protected under national law and are from dead fisheries specimens) with reference number WL/3/2/74/17, dated 4th January 2018, issued by the Department of Wildlife Conservation, Sri Lanka; samples were exported under a letter of no objection with reference number WL/3/2/74/17, dated 14th March 2018, issued by the Department of Wildlife Conservation, Sri Lanka. Collections were conducted under the following protocols approved by the Institutional Animal Care and Use Committee at the University

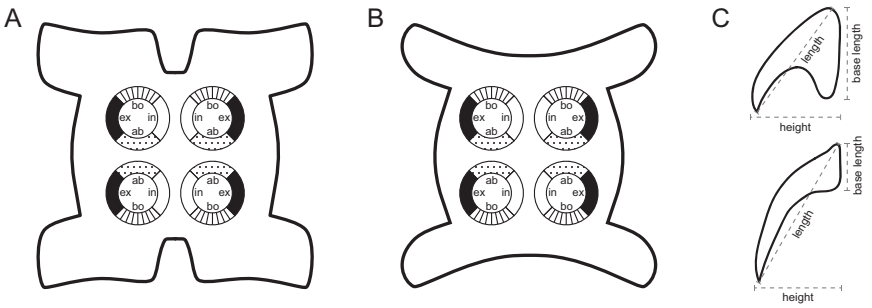

Figure 1 Explanation of the tentacle surface schematics and hook measurement conventions. (A) Key to the patterns used to indicate tentacle surfaces pictured for species with four bothria. (B) Key to the patterns used to indicate tentacle surfaces pictured for species with two bothria. (C) Diagram of the hook measurements made for hooks of differing shapes (modified from *Palm (2004)*).
Full-size DOI: 10.7717/peerj.12865/fig-1

of Connecticut (in chronological order): C010 0202, C010 0102, A04-177, A04-176, A08-044, A11-030, A14-030, and A17-039.

Specimen preparation and examination

Specimen preparation as whole mounts or vouchers for examination with light microscopy, as whole or partial specimens for examination with scanning electron microscopy (SEM), and for histological sectioning of specimens embedded in glycol methacrylate follows (*Herzog & Jensen (2017)* and *Herzog & Jensen (2018)*. Generation of line drawings and photomicrographs of histological sections follows *Herzog & Jensen (2018)*. Measurements were taken using INFINITY ANALYZE v.7.0.26.20 image analysis software (Teledyne Lumenera, Ottawa, ON, Canada). Measurements are reported in micrometers unless otherwise specified and are presented as ranges followed in parentheses by the mean, standard deviation, number of specimens measured, and total number of measurements taken if more than a single measurement was made per worm. Means were calculated as the sum of all measurements taken, divided by the total number of measurements taken, regardless of the number of measurements made per worm. Measurements of reproductive organs were made in mature terminal proglottids only unless otherwise specified. Only ranges are presented if four or fewer total measurements were taken. For redescriptions where the holotype was remeasured, measurement values for the holotype are given in brackets following each series of measurements.

Scolex length to width ratios were based on scolex total lengths and scolex maximum widths; scolex maximum widths were measured at the pars bothrialis or pars bulbosa, depending on the specimen. Visual representations of the terms used to describe hook measurements and the patterns shown beneath line drawings and scanning electron micrographs to describe tentacle surfaces are given in Fig. 1. Oncotaxy follows *Campbell & Beveridge (1994)*. Microthrix terminology follows *Chervy (2009)*. Shape terminology follows *Clopton (2004)*. Museum abbreviations are as follows: Australian Helminthological

Collection (AHC), South Australian Museum (SAM), Adelaide, South Australia, Australia; Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico; H. W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska, USA; Lawrence R. Penner Parasitology Collection (LRP), Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, USA; Laboratorio de Artrópodos Venenosos (LAV), Museo de Invertebrados G. B. Fairchild (MIUP), Universidad de Panama, Panama City, Panama; Museu de Zoologia Universidade de São Paulo (MZUSP), São Paulo, Brazil; Museum Zoologicum Bogoriense (MZB), Center for Biology, Indonesian Institute of Science, Cibinong, Jakarta-Bogor, Java, Indonesia; Muzium Zoologi (MZUM or MZUM[P]), Universiti Malaya, Kuala Lumpur, Malaysia; Naturhistorisches Museum Wien (VNHM; formerly NMV), Vienna, Austria; Queensland Museum (QM), Invertebrate Collection, Worms & Echinoderms Department, South Brisbane, Australia; Sarawak Biodiversity Center (SBC), Kuching, Sarawak, Malaysia; National Museum of Natural History (USNM; formerly USNPC), Smithsonian Institution, Washington, D. C., USA; Zoological Reference Collection (ZRC), Lee Kong Chian Natural History Museum, National University of Singapore, Singapore, Republic of Singapore.

DNA extraction and sequencing

Sequence data for the D1–D3 gene region of the 28S rRNA gene (hereafter 28S) were generated for 32 specimens representing six species of *Rhinoptericola* preserved in 95% ethanol. Specimens from which sequence data were generated were photographed using a Lumenera INFINITY3-6UR 6.0 megapixel USB 3 microscopy camera (Teledyne Lumenera, Ottawa, ON, Canada) attached to a Leica MZ16 dissecting microscope (Leica Microsystems, Buffalo Grove, IL, USA). Portions of each specimen were used for genomic DNA extraction; partial scoleces, scolecles only, or scoleces and partial strobilae were prepared as whole-mounted hologenophore vouchers *sensu* *Pleijel et al. (2008)* following the methods described above. Host specimen numbers and accession numbers for hologenophores and GenBank sequences for the specimens for which sequence data were generated as part of this study are given in Table 2.

Genomic DNA was extracted from a portion of each specimen using a MasterPure™ Complete DNA and RNA Purification Kit (Epicentre® Biotechnologies, Madison, WI, USA) and the following modified extraction protocol: Tissue was placed in 100 µl Tissue and Cell Lysis Solution in individual standard sterile 1.5 mL microcentrifuge flip-top tubes and incubated at 65 °C for 1 h. Following incubation, 1.5 µL Proteinase K (50 µg/µL) was added to each tube. Tubes were incubated at 55 °C for 1–3 h and vortexed briefly one to three times over the course of the incubation. Tubes were vortexed again and subsequently incubated at 37 °C for 10 min. Tubes were briefly centrifuged, 0.5 µL RNase A was added, and tubes were incubated at 37 °C for an additional 15 min. Following the incubation at 37 °C, tubes were placed on ice for 4 min, then centrifuged. Immediately following addition of 58 µL MPC Protein Precipitation Reagent, tubes were vortexed for 20 s, returned to ice, and subsequently centrifuged at 15,000 rpm for 7 min. After centrifugation, the supernatant was removed and placed in an individual 1.5 mL DNA

Table 2 Specimens of the species of *Rhinoptericola* *Carvajal & Campbell, 1975* from which sequence data for the D1–D3 region of the 28S rRNA gene were generated as part of this study with their host species, hologenophore and GenBank accession numbers, and sequence lengths.

Species	Host species	Host code	Hologenophore accession no. (Lab specimen no. or nos.)	GenBank accession no.	Sequence length (bp)
Rhinoptericola megacantha *Carvajal & Campbell, 1975*					
	Rhinoptera bonasus	CH-17	LRP 10437 (CH-17-1-DNAV)	OL412720	1,413
	Rhinoptera bonasus	CH-18	LRP 10438 (KW393-DNAV)	OL412723	1,413
	Rhinoptera bonasus	CH-29	LRP 10439 (CH-29-1-DNAV)	OL412721	1,413
	Rhinoptera bonasus	CH-3	LRP 10440 (CH-3-1-DNAV)	OL412716	1,413
	Rhinoptera bonasus	CH-30	LRP 10441 (CH-30-1-DNAV)	OL412722	1,413
	Rhinoptera brasiliensis	BE-10	LRP 10432 (KW399)	OL412724	1,415
	Rhinoptera brasiliensis	BE-11	LRP 10433 (BE-11-3-DNAV)	OL412715	1,413
	Rhinoptera brasiliensis	CH-15	LRP 10434 (CH-15-1-DNAV)	OL412717	1,413
	Rhinoptera brasiliensis	CH-15	LRP 10435 (CH-15-4-DNAV)	OL412718	1,413
	Rhinoptera brasiliensis	CH-15	LRP 10436 (CH-15-5-DNAV)	OL412719	1,413
	Rhinoptera brasiliensis	MS05-156	LRP 10442 (MS05-156-1-DNAV)	OL412726	1,413
	Rhinoptera brasiliensis	MS05-156	LRP 10443 (MS05-156-2-DNAV)	OL412727	1,413
	Rhinoptera brasiliensis	MS05-298	LRP 10444 (MS05-298-20-DNAV)	OL412728	1,413
	Rhinoptera brasiliensis	MS05-298	LRP 10445 (MS05-298-22-DNAV)	OL412729	1,413
	Rhinoptera brasiliensis	MS05-298	LRP 10446 (MS05-298-24-DNAV)	OL412730	1,411
	Rhinoptera brasiliensis	MS05-305	LRP 10447 (MS05-305-4-DNAV)	OL412732	1,413
	Rhinoptera brasiliensis	MS05-305	LRP 10448 (MS05-305-3-DNAV)	OL412731	1,413
	Rhinoptera brasiliensis	MS05-375	LRP 10449 (MS05-375-1-DNAV)	OL412733	1,413
	Rhinoptera brasiliensis	MS05-49	LRP 10450 (MS05-49-2-DNAV)	OL412725	1,413
	Rhinoptera marginata	SE-139	LRP 10451 (SE-139-1-DNAV)	OL412735	1,414
	Rhinoptera marginata	SE-84	LRP 10452 (SE-84-1-DNAV)	OL412734	1,413
Rhinoptericola butlerae (*Beveridge & Campbell, 1988*) **n. comb.**					
	Hemitrygon bennetti	VN-42	LRP 10558 (KW382)	OL412711	1,415
	Maculabatis gerrardi	KA-75	LRP 10552 (JW774; KA-75-1-DNAV)	OL412709	1,246
	Rhinoptera neglecta	AU-87	LRP 10550 (AU-87-1-DNAV)	OL412708	1,415
	Rhinoptera neglecta	CM03-43	LRP 10553 (JW775; CM03-43-1-DNAV)	OL412710	1,415
Rhinoptericola jensenae (*Schaeffner & Beveridge, 2012b*) **n. comb.**					
	Rhinoptera neglecta	AU-86	LRP 10570 (AU-86-1-DNAV)	OL412712	1,426
	Rhinoptera neglecta	CM03-31	LRP 10571 (KW766)	OL412714	1,426
	Rhinoptera neglecta	CM03-43	LRP 10572 (CM03-43-2-DNAV)	OL412713	1,426
Rhinoptericola schaeffneri **n. sp.**					
	Pastinachus ater	KA-32	LRP 10601 (KW1316; KA-32-4-DNAV)	OL412737	841
Rhinoptericola mozambiquensis **n. sp.**					
	Rhinoptera jayakari	MZ-4	LRP 10659 (KW217)	OL412738	1,131
	Rhinoptera jayakari	MZ-4	LRP 10660 (MZ-4-1-DNAV)	OL412739	1,414
Rhinoptericola hexacantha **n. sp.**					
	Rhinoptera steindachneri	BJ-684	LRP 10721 (KW1039)	OL412736	1,424

LoBind® microcentrifuge flip-top tube (Eppendorf® North America, Enfield, CT, USA). Following addition of 0.5 µL of molecular biology grade glycogen (20 mg/µL; ThermoFisher Scientific™, Waltham, MA, USA) to the supernatant, tubes were gently inverted 30–40 times each and allowed to incubate at RT for 30 min, followed by incubation at 4 °C overnight. Tubes were subsequently centrifuged at 15,000 rpm for 10 min to produce a pellet of DNA. Pellets were washed twice with the addition of 100 µL molecular grade 75% ethanol followed by centrifugation at 12,000 rpm for 1.5 min. After the final wash, ethanol was removed, and DNA was resuspended in 60 µL of TE Buffer diluted 1:3 with molecular grade water. Tubes were then incubated at 65 °C for 1 h and briefly vortexed twice over the course of this incubation, and subsequently flicked firmly, centrifuged, and incubated at RT for 1–3 h.

Following DNA extraction, 28S was amplified using the protocol of *Herzog & Jensen (2018)*, the forward primer ZX-1 (5′–ACCCGCTGAATTTAAGCATAT–3′) (modified from *Van der Auwera, Chapelle & De Wächter, 1994*) and the reverse primer 1500R (5′–GCTATCCTGAGGGAAACTTCG–3′) (*Olson et al., 2003*; *Tkach et al., 2003*). Polymerase chain reaction (PCR) products were purified and sequenced by GENEWIZ (South Plainfield, NJ, USA) or ACGT, Inc. (Wheeling, IL, USA) using single pass primer extension. The primers ZX-1 and 1500R and, in some cases, the internal sequencing primer 300F (5′–CAAGTACCGTGAGGGAAAGTTG–3′) (*Littlewood, Curini-Galletti & Herniou, 2000*) were used for sequencing.

Phylogenetic methods

Raw reads were assembled using Geneious Prime 2019.1.3 (https://www.geneious.com) following either a *de novo* or reference mapping approach. Assembled sequences were combined into a matrix with 150 28S sequences downloaded from GenBank representing 144 ingroup sequences (72 representatives of the suborder Trypanobatoida and 72 representatives of the suborder Trypanoselachoida) (*Anglade & Randhawa, 2018*; *Caira et al., 2014*; *Dallarés, Carrassón & Schaeffner, 2017*; *De Silva et al., 2021*; *Faria de Menezes et al., 2018*; *Haseli, Bazghalee & Palm, 2017*; *Jun et al., 2018*; *Olson et al., 2010*; *Olson et al., 2001*; *Palm, Waeschenbach & Littlewood, 2007*; *Palm et al., 2009*; *Schaeffner, Gasser & Beveridge, 2011*; *Schaeffner & Marques, 2018*; *Waeschenbach et al., 2007*) and six outgroup taxa (*Bray & Olson, 2004*; *Caira et al., 2020*; *Caira et al., 2014*; *Fyler, Caira & Jensen, 2009*; *Healy et al., 2009*). For ingroup taxa, updated names follow *Beveridge, Koehler & Appy (2021)*, *Haseli, Bazghalee & Palm (2017)*, *Palm (2010)*, and *Schaeffner & Beveridge (2012a)*. Ingroup taxa were selected based on sequence length, broad representation across major clades of trypanorhynchs, and replication of multiple specimens within species (where available) for comparison with species of *Rhinoptericola*. Outgroup taxa were selected based on representation across the acetabulate and non-acetabulate orders of elasmobranch tapeworms (*i.e.*, one species each from the Onchoproteocephalidea, Phyllobothriidea, Lecanicephalidea, Diphyllidea, Litobothriidea, and Rhinebothriidea). Taxon names, higher classifications, and GenBank accession numbers for all ingroup and outgroup sequences downloaded from GenBank and included in the analysis are given in Table S1.

Sequences were trimmed, then aligned using PRANK v.170427 (*Löytynoja & Goldman, 2005*; *Löytynoja, 2014*) using default settings with the exception of the removal of the "+F" flag. A GTR+I+Γ model of sequence evolution was determined to be the best fit for the dataset by jModelTest v.2.1.7 (*Darriba et al., 2012*; *Guindon & Gascuel, 2003*); goodness of fit was evaluated based on corrected Akaike Information Criterion (AICc) values. A maximum likelihood (ML) tree searching analysis and a ML bootstrap analysis with 1,000 bootstrap replicates were conducted using GARLI v.2.01 (*Zwickl, 2006*) on the University of Kansas Center for Research Computing Shared Community Cluster. Default GARLI configurations were used with the following alternations: "streefname=" was set to "random", "attachmentspertaxon=" was set to "364" and "outputphyliptree=" was set to "1". For the ML tree searching analysis "searchreps=" was set to "1000" and for the ML bootstrap analysis "searchreps=" was set to "1" and "bootstrapreps=" was set to "1000". Clades with bootstrap values of 95% or greater were considered to have high nodal support. Bootstrap values were displayed on the best resulting ML topology using SumTrees v.4.5.2 in DendroPy v.4.5.2 (*Sukumaran & Holder, 2010*; Sukumaran, J. and M. T. Holder. SumTrees: Phylogenetic Tree Summarization. 4.5.2. Available at https://github.com/jeetsukumaran/DendroPy).

For assessment of levels of intra- and interspecific divergence within *Rhinoptericola*, the 32 trimmed sequences for specimens of the six species of *Rhinoptericola* generated herein and the single trimmed 28S sequence for *R. megacantha* available in GenBank (DQ642792) were aligned using MUSCLE v.3.8.425 (*Edgar, 2004a*; *Edgar, 2004b*) in Geneious Prime 2019.1.3 with default settings and 1,000 iterations.

RESULTS

All reports of species of *Rhinoptericola* from the literature and this study are summarized in Table 3.

Taxonomic descriptions and redescriptions

Rhinoptericolidae *Carvajal & Campbell, 1975*

Synonym: Shirleyrhynchidae *Campbell & Beveridge, 1994*.

Type genus: *Rhinoptericola Carvajal & Campbell, 1975* (syn. *Shirleyrhynchus Beveridge & Campbell, 1988*).

Other genera: *Nataliella Palm, 2010*.

Diagnosis (modified from *Palm, 2010*)

Scolex craspedote or acraspedote, elongate, slender. Bothria four in number, elliptoid, with free lateral and posterior margins, arranged in dorsal and ventral pairs, not overlapping pars bulbosa; bothrial pits absent. Pintner's cells absent. Rhyncheal apparatus present. Tentacle sheaths sinuous. Prebulbar organs present. Bulbs long; gland cells in bulbs absent; retractor muscles originate at base of bulbs. Pars postbulbosa present or absent. Tentacles long, with slight basal swelling. Characteristic basal armature present; hooks heteromorphous, solid or hollow, arranged in quincunxes or indistinct rows; macrohooks present or absent; billhooks present or absent. Metabasal armature heteroacanthous typical heteromorphous or homeoacanthous homeomorphous; hooks

Table 3 Records of host associations, geographic distributions, and specimens deposited for species of *Rhinoptericola Carvajal & Campbell, 1975*.

Valid name	Host family: Host species	Locality	Name in original report if different from valid name	Specimens deposited	Source of report
***Rhinoptericola megacantha Carvajal & Campbell, 1975* (type species)**					
	Rhinopteridae: *Rhinoptera bonasus*	**Atlantic Ocean: Chesapeake Bay, Virginia, USA**		USNM 73835 (ht), USNM 73836* (pt); HWML 34972 (v)	*Carvajal & Campbell (1975)*; this study
	Dasyatidae: *Hypanus say*	Atlantic Ocean: Charleston, South Carolina, USA		LRP 10453 (hg)	This study
	Rhinopteridae: *Rhinoptera bonasus*	Atlantic Ocean: Charleston, South Carolina, USA		LRP 10454–10535 (v), LRP 10537–10539 (v), LRP 10544–10546 (v), LRP 10437–10441 (hg); USNM 1661577 (v), USNM 1661582–1661583 (v)	This study
	Rhinopteridae: *Rhinoptera bonasus* or *R. brasiliensis* (as *Rhinoptera bonasus*)	Caribbean Sea: Caimare Chico, Zulia, Venezuela, Gulf of Venezuela		HWML 21032 (v)	*Mayes & Brooks (1981)*
	Rhinopteridae: *Rhinoptera brasiliensis*	Atlantic Ocean: Charleston, South Carolina, USA		LRP 10434–10436 (hg); USNM 1661578–1661581 (v)	This study
	Rhinopteridae: *Rhinoptera brasiliensis* (as *Rhinoptera bonasus*)	Gulf of Mexico: Mississippi, USA		BMNH 2008.5.21.1* (hg)	*Palm et al. (2009)*, *Olson et al. (2010)*
	Rhinopteridae: *Rhinoptera brasiliensis*	Gulf of Mexico: Mississippi and Louisiana, USA		LRP 10536 (v), LRP 10540–10542 (v), LRP 10442–10450 (hg); USNM 1661576 (v), USNM 1661584–1661586 (v)	This study
	Rhinopteridae: *Rhinoptera brasiliensis*	Caribbean Sea: Gales Point Manatee, Belize		LRP 10432–10433 (hg)	This study
	Rhinopteridae: *Rhinoptera brasiliensis*	Southern and southeastern Brazil		No material deposited	*Napoleão et al. (2015)*
	Rhinopteridae: *Rhinoptera marginata*	Atlantic Ocean: St. Louis, Senegal		LRP 10543 (v), LRP 10451–10452 (hg); USNM 1661587 (v)	This study
***Rhinoptericola butlerae* (*Beveridge & Campbell, 1988*) n. comb.**					
	Dasyatidae: *Hemitrygon fluviorum* (as *Dasyatis fluviorum*)	**Coral Sea: Queensland, Australia**	*Shirleyrhynchus butlerae*	AHC 44088 (ht), AHC 22773 (pt), AHC 17565* (v); USNM 1375081 (pt); BMNH 1987.5.1.1* (pt),	*Beveridge & Campbell (1988)*
	Dasyatidae: *Hemitrygon bennetti*	South China Sea: Haiphong Province, Cat Ba Island, Viet Nam		LRP 10558 (hg)	This study
	Dasyatidae: *Himantura tutul*	Java Sea: South Kalimantan, Indonesia		LRP 10555–10556 (v); QM G239455 (v)	This study

(Continued)

Table 3 (continued)

Valid name	Host family: Host species	Locality	Name in original report if different from valid name	Specimens deposited	Source of report
	Dasyatidae: *Himantura tutul* (as *Himantura uarnak*)	Java Sea: South Kalimantan, Indonesia	*Shirleyrhynchus aetobatidis*	LRP 10560 (v)	*Schaeffner & Beveridge (2014)*
	Dasyatidae: *Maculabatis gerrardi*	Java Sea: South Kalimantan, Indonesia		LRP 10552 (hg), LRP 10557 (v)	This study
	Dasyatidae: *Maculabatis gerrardi* (as *Himantura gerrardi*)	Java Sea: South Kalimantan, Indonesia	*Shirleyrhynchus aetobatidis*	LRP 10559 (v)	*Schaeffner & Beveridge (2014)*
	Dasyatidae: *Maculabatis gerrardi* (as *Himantura gerrardi*)	Sulu Sea: Sabah, Malaysia	*Shirleyrhynchus aetobatidis*	USNM 1394285* (v)	*Schaeffner & Beveridge (2014)*
	Dasyatidae: *Pastinachus ater* (as *Dasyatis sephen*)	Timor Sea: Northern Territory, Australia	*Shirleyrhynchus butlerae*	AHC 17542* (v)	*Beveridge & Campbell (1988)*
	Dasyatidae: *Pastinachus ater*	Arafura Sea: Northern Territory, Australia		QM G239456 (v)	This study
	Dasyatidae: *Pastinachus ater*	Makassar Strait: East Kalimantan, Indonesia		LRP 10554 (v)	This study
	Dasyatidae: *Pastinachus ater* (as *Pastinachus atrus*)	Makassar Strait: East Kalimantan, Indonesia	*Shirleyrhynchus aetobatidis*	LRP 10562 (v)	*Schaeffner & Beveridge (2014)*
	Dasyatidae: *Pastinachus solocirostris*	Makassar Strait: East Kalimantan, Indonesia		LRP 10548–10549 (v)	This study
	Dasyatidae: *Pastinachus solocirostris*	Makassar Strait: East Kalimantan, Indonesia	*Shirleyrhynchus aetobatidis*	LRP 10561 (v)	*Schaeffner & Beveridge (2014)*
	Hemiscylliidae: *Chiloscyllium punctatum*	South China Sea: Sarawak, Malaysia	*Shirleyrhynchus aetobatidis*	USNM 1394286 (v)	*Schaeffner & Beveridge (2014)*
	Rhinopteridae: *Rhinoptera javanica*	South China Sea: Ba Ria Province, Viet Nam		LRP 10547 (v)	This study
	Rhinopteridae: *Rhinoptera neglecta*	Gulf of Carpentaria: Queensland, Australia		LRP 10553 (hg)	This study
	Rhinopteridae: *Rhinoptera neglecta* (as *Rhinoptera* sp.)	Arafura Sea: Northern Territory, Australia	*Shirleyrhynchus aetobatidis*	AHC 28567* (v)	This study
	Rhinopteridae: *Rhinoptera neglecta*	Arafura Sea: Northern Territory, Australia		LRP 10563–10569 (v)	This study
	Rhinopteridae: *Rhinoptera neglecta*	Timor Sea: Northern Territory, Australia		LRP 10551 (v), LRP 10550 (hg); QM G239454 (v)	This study
Rhinoptericola panamensis (*Schaeffner, 2016*) **n. comb.**					

Table 3 (continued)

Valid name	Host family: Host species	Locality	Name in original report if different from valid name	Specimens deposited	Source of report
	Urotrygonidae: *Urotrygon aspidura*	**Pacific Ocean: Veraguas, Panama**	*Shirleyrhynchus panamensis*	MIUP-LAV-002 (ht); USNM 1298205–1298206 (pt)	*Schaeffner (2016)*
	Potamotrygonidae: *Styracura pacifica* (as *Himantura pacifica*)	Pacific Ocean: Veraguas, Panama	*Shirleyrhynchus panamensis*	MZUSP No 7766* (pt)	*Schaeffner (2016)*
***Rhinoptericola aetobatidis* (*Shipley & Hornell, 1906*) n. comb.**					
	Aetobatidae: *Aetobatus ocellatus* (as *Aetobatus narinari*)	**Laccadive Sea: Dutch Bay Spit, Sri Lanka**	*Tetrarhynchus aetobatidis*	VNHM 2099* (ht, missing)	*Shipley & Hornell (1906)*
	Dasyatidae: *Brevitrygon* sp. 1 or *B. imbricata* (as *Trygon walga*)	Laccadive Sea: Dutch Bay Spit, Sri Lanka	*Tetrarhynchus aetobatidis*	no material deposited	*Shipley & Hornell (1906)*
	Dasyatidae: *Neotrygon indica* or *N. caerulofasciata* (as *Trygon kuhlii*)	Laccadive Sea: Pearl Banks, Sri Lanka	*Tetrarhynchus aetobatidis*	no material deposited	*Southwell (1924)*
***Rhinoptericola jensenae* (*Schaeffner & Beveridge, 2012b*) n. comb.**					
	Dasyatidae: *Pastinachus solocirostris*	**South China Sea: Sarawak, Malaysia**	*Prochristianella jensenae*	ZRC.PLA.0409 (ht), ZRC.PLA.0411 (pt); AHC 35409 (pt), AHC 35412 (pt), AHC 35414 (pt, left-most worm), AHC 35416 (pt); LRP 7844 (pt), LRP 7846–7847 (pt); USNM 1400164 (pt, slides 1 & 3); LRP 10658 (v, worm 2)	*Schaeffner & Beveridge (2012b)*, *Schaeffner & Beveridge (2014)*
	Aetobatidae: *Aetobatus ocellatus*	Gulf of Carpentaria: Queensland, Australia		QM G239457 (v); USNM 1661573–1661574 (v)	This study
	Dasyatidae: *Pastinachus ater* (as *Pastinachus atrus*)	Indian Ocean: Nickol Bay, Australia	*Prochristianella jensenae*	AHC 35450 (pt)	*Schaeffner & Beveridge (2012b)*
	Dasyatidae: *Himantura australis* or *H. leoparda* (as *Himantura uarnak*)	Indian Ocean: Nickol Bay, Australia	*Prochristianella jensenae*	AHC 35449 (pt)	*Schaeffner & Beveridge (2012b)*
	Rhinopteridae: *Rhinoptera neglecta*	Timor Sea: Northern Territory, Australia		LRP 10570 (hg); QM G239458–G239459 (v)	This study
	Rhinopteridae: *Rhinoptera neglecta*	Gulf of Carpentaria: Queensland, Australia	*Prochristianella jensenae*	AHC 35441–35443 (pt), AHC 35445–35448 (pt)	*Schaeffner & Beveridge (2012b)*
	Rhinopteridae: *Rhinoptera neglecta*	Gulf of Carpentaria: Queensland, Australia		AHC 36891–36893 (v); LRP 10573–10600 (v), LRP 10571–10572 (hg); QM G239460–G2394602 (v); USNM 1661575 (v)	This study
***Rhinoptericola schaeffneri* n. sp.**					
	Dasyatidae: *Pastinachus solocirostris*	**South China Sea: Sarawak, Malaysia**		USNM 1400164† (v, slides 2, 4 & 5); MZUM(P) 2021.1 (H) (ht), MZUM(P) 2021.2 (P)–2021.3 (P) (pt); LRP 10602 (pt); SBC-P-00077 (pt); USNM 1661588 (pt), USNM 1661590 (pt)	This study

(Continued)

Table 3 (continued)

Valid name	Host family: Host species	Locality	Name in original report if different from valid name	Specimens deposited	Source of report
	Dasyatidae: *Pastinachus ater*	Makassar Strait: East Kalimantan, Indonesia	*Prochristianella jensenae*	MZB Ca 168–169† (v)	*Schaeffner & Beveridge (2012b)*
	Dasyatidae: *Pastinachus ater*	Makassar Strait: East Kalimantan, Indonesia		LRP 10601 (hg); MZB Ca 211 (pt)	This study
	Dasyatidae: *Pastinachus gracilicaudus*	Sulu Sea: Sabah, Malaysia	*Prochristianella jensenae*	AHC 35422–35425† (v)	*Schaeffner & Beveridge (2012b)*
	Dasyatidae: *Pastinachus solocirostris*	Makassar Strait: East Kalimantan, Indonesia	*Prochristianella jensenae*	MZB Ca 170–172† (v)	*Schaeffner & Beveridge (2012b)*
	Dasyatidae: *Pastinachus solocirostris*	Makassar Strait: East Kalimantan, Indonesia		LRP 10603–10656 (pt); USNM 1661589 (pt), USNM 1661591 (pt)	This study
	Dasyatidae: *Pastinachus solocirostris*	Java Sea: West Kalimantan, Indonesia	*Prochristianella jensenae*	MZB Ca 173† (v), MZB Ca 175† (v)	*Schaeffner & Beveridge (2012b)*
	Dasyatidae: *Pastinachus solocirostris*	South China Sea: Sarawak, Malaysia	*Prochristianella jensenae*	AHC 35408† (v), AHC 35410–35411† (v), AHC 35413† (v), AHC 35414† (v, right-most worm), AHC 35415† (v), AHC 35417–35421† (v), AHC 35426† (v), AHC 35428† (v, middle worm), AHC 35429–35432† (v), AHC 35433† (v, immature worm with tentacles everted), AHC 35434–35440† (v); LRP 7843† (v), LRP 7845† (v), LRP 7848–7849† (v); USNM 1400163† (v, slide 1); ZRC.PLA.0410† (v), ZRC.PLA.0412–0413† (v); LRP 10658 (v, worms 1 and 3), LRP 10657 (v)	*Schaeffner & Beveridge (2014)*
***Rhinoptericola mozambiquensis* n. sp.**					
	Rhinopteridae: *Rhinoptera jayakari*	**Mozambique Channel: Inhambane, Mozambique**		USNM 1661599 (ht), USNM 1661596–1661598 (pt), USNM 1661600–1661610 (pt); LRP 10661–10720 (pt), LRP 10659–10660 (hg)	This study
***Rhinoptericola hexacantha* n. sp.**					
	Rhinopteridae: *Rhinoptera steindachneri*	**Gulf of California: Mexico**		CNHE 11612 (ht), CNHE 11613–11614 (pt); LRP 10722–10772 (pt), LRP 10721 (hg); USNM 1661592–1661595 (pt)	This study
***Rhinoptericola jensenae* or *Rhinoptericola schaeffneri* n. sp.**					
	Dasyatidae: *Pastinachus solocirostris*	South China Sea: Sarawak, Malaysia	*Prochristianella jensenae*	AHC 35414† (pt, middle worm; tentacles not everted far enough to identify), AHC 35427† (pt, tentacles not everted far enough to identify), AHC 35428† (pt, bottom-most worm), AHC 35433† (pt, immature worm with tentacles retracted); USNM 1400163† (pt, slide 2; tentacles not everted far enough to identify)	*Schaeffner & Beveridge (2012b)*

Table 3 (continued)

Valid name	Host family: Host species	Locality	Name in original report if different from valid name	Specimens deposited	Source of report
	Dasyatidae: *Pastinachus solocirostris*	Java Sea: West Kalimantan, Indonesia	*Prochristianella jensenae*	MZB Ca 174*† (pt)	*Schaeffner & Beveridge (2012b)*
	Rhinopteridae: *Rhinoptera neglecta*	Gulf of Carpentaria: Queensland, Australia, Indian Ocean	*Prochristianella jensenae*	AHC 35444† (pt, tentacles not everted far enough to identify)	*Schaeffner & Beveridge (2012b)*

Notes:
Type hosts and localities are given in bold. Asterisks (*) indicate material that was not confirmed as part of this study; daggers (†) indicate type specimens of *Prochristianella jensenae* *Schaeffner & Beveridge, 2012b*.
ht, holotype; pt, paratype(s); hg, hologenophore(s); v, voucher specimen(s).

solid or hollow, arranged in alternating ascending half-spiral rows with hook files 1 and (1′) separated, or arranged in quincunxes. Band of hooks, chainette elements and intercalary hooks absent.

Strobila apolytic or euapolytic. Proglottids acraspedote. Testes medullary, arranged in two columns in single layer essentially anterior to ovary. External and internal seminal vesicles absent. Cirrus unarmed. Genital atrium absent. Genital pores separate, unilateral, at or anterior to mid-level of proglottid; male and female genital pores at same level. Vagina medial in proglottid; vaginal sphincter absent; seminal receptacle present. Ovary terminal in proglottid, H-shaped in dorsoventral view, tetralobed in cross-section, with lobulated margins. Vitellarium follicular; follicles circumcortical, extending entire length of proglottid, interrupted dorsally and ventrally by ovary, partially interrupted ventrally by terminal genitalia. Uterus saccate, medial, dorsal to vagina, bifurcated at posterior end, extending from anterior margin of ovary to anterior margin of proglottid. Uterine pore present or absent. Excretory vessels four, arranged in one dorsal and one ventral pair on each lateral margin of proglottid. Eggs single, essentially spherical, non-embryonated; polar filaments absent. Plerocercus larval stage present, or larvae unknown. Parasites of Rhinopteridae Jordan & Evermann, 1896, and Dasyatidae Jordan, 1888 (Myliobatiformes), also in Aetobatidae White & Naylor, 2016, Potamotrygonidae Garman, 1877, and Urotrygonidae McEachran, Dunn & Miyake, 1996 (Myliobatiformes), and Hemiscylliidae Gill, 1862 (Orectolobiformes) as adults; parasites of Acanthuridae Bonaparte, 1832 (Acanthuriformes), Scombridae Rafinesque, 1815 (Scombriformes), and Lutjanidae Gill, 1861 and Priacanthidae Günther, 1859 (Perciformes) as larvae.

Remarks: The original diagnosis of the family Rhinoptericolidae by *Carvajal & Campbell (1975)* was revised thrice prior to this study (*Campbell & Beveridge, 1994*; *Palm, 2004*; *Palm, 2010*). The revised diagnosis herein is modified from the most recent diagnosis by *Palm (2010)*. It incorporates the novel scolex morphologies represented by the new species described in this study, as well as clarifies and expands on the details of rhinoptericolid proglottid anatomy. As all rhinoptericolids described to date possess a characteristic basal

armature, this feature is newly added to the familial diagnosis. The possession of solid or hollow hooks in the metabasal armature is also added to accommodate the morphology of a new species described herein. With respect to proglottid anatomy, the familial diagnosis of *Palm (2010)* was limited to the mention of pore position in the anterior third of the proglottid, and the presence of seminal vesicles. The diagnosis is expanded here significantly to include the description of a number of additional proglottid features. Deviating from *Palm (2010)*, the family is now known to also include species with a genital pore at the mid-level of the proglottid, and external and internal seminal vesicles are considered to be absent in all species with known proglottid anatomies.

Shirleyrhynchidae is considered a junior synonym of Rhinoptericolidae, but *Cetorhinicola acanthocapax* *Beveridge & Campbell, 1988* is not herein transferred to the Rhinoptericolidae. No specimens of *C. acanthocapax* preserved in 95% EtOH were available from which to generate sequence data. In the absence of molecular evidence, morphology alone is used to inform its higher-level associations. Though, like the rhinoptericolids, *C. acanthocapax* possesses prebulbar organs and four bothria, unlike rhinoptericolids, it possesses gland cells in the bulbs, laciniated proglottids, testes arranged in multiple columns, a genital atrium, a vagina strongly recurved anterior to the cirrus sac, and a uterus that is not bifurcated at the posterior end (*Beveridge & Campbell, 1988*; *Beveridge & Duffy, 2005*). These significant differences in morphology are deemed sufficient to warrant the exclusion of *C. acanthocapax* from the Rhinoptericolidae at present. Furthermore, adults of *C. acanthocapax* solely parasitize basking sharks (*Beveridge & Campbell, 1988*; *Beveridge & Duffy, 2005*), while adults of rhinoptericolids are known almost exclusively from myliobatiforms. *Cetorhinicola* now is considered a genus *incertae sedis* within the superfamily Eutetrarhynchoidea.

Rhinoptericola* *Carvajal & Campbell, 1975

Synonym: *Shirleyrhynchus* *Beveridge & Campbell, 1988*.

Type species: *Rhinoptericola megacantha* *Carvajal & Campbell, 1975*.

Other species: *Rhinoptericola aetobatidis* (*Shipley & Hornell, 1906*) n. comb.; *Rhinoptericola butlerae* (*Beveridge & Campbell, 1988*) n. comb.; *Rhinoptericola hexacantha* n. sp.; *Rhinoptericola jensenae* (*Schaeffner & Beveridge, 2012b*) n. comb.; *Rhinoptericola mozambiquensis* n. sp.; *Rhinoptericola panamensis* (*Schaeffner, 2016*) n. comb.; *Rhinoptericola schaeffneri* n. sp.

Diagnosis (modified from *Palm, 2004*)

Scolex acraspedote, elongate, slender. Bothria four in number, elliptoid to deeply ovoid, with free lateral and posterior margins, arranged in dorsal and ventral pairs, not overlapping pars bulbosa; bothrial pits absent. Pintner's cells absent. Rhyncheal apparatus present. Tentacle sheaths sinuous. Prebulbar organs present. Bulbs long; gland cells in bulbs absent; retractor muscles originate at base of bulbs. Pars postbulbosa short or absent. Tentacles long, with slight basal swelling. Characteristic basal armature present; hooks heteromorphous, solid or hollow, arranged in indistinct rows; macrohooks present or absent; billhooks present or absent. Metabasal armature heteroacanthous typical; hooks

heteromorphous, solid or hollow, arranged in alternating ascending half-spiral rows of 6–9 hooks each; hook files 1 and 1′ separated.

Worms apolytic or euapolytic. Proglottids acraspedote. Testes numerous, medullary, arranged in two columns in single layer essentially anterior to ovary. Vas deferens extending from near anterior margin of ovary to anterior margin of cirrus sac, entering cirrus sac at its antero-medial margin; external and internal seminal vesicles absent. Cirrus sac ovoid to elliptoid in shape, bent anteriorly or not, containing coiled cirrus; cirrus unarmed. Genital atrium absent. Genital pores separate, unilateral, at or anterior to mid-level of proglottid; male and female genital pores at same level. Vagina medial in proglottid; vaginal sphincter absent; seminal receptacle present. Ovary terminal in proglottid, H-shaped in dorsoventral view, tetralobed in cross-section, with lobulated margins. Vitellarium follicular; follicles circumcortical, extending entire length of proglottid, interrupted dorsally and ventrally by ovary, partially interrupted ventrally by terminal genitalia. Uterus saccate, medial, dorsal to vagina, bifurcated at posterior end, extending from anterior margin of ovary to anterior margin of proglottid. Uterine pore present or absent. Excretory vessels four, arranged in one dorsal and one ventral pair on each lateral margin of proglottid. Eggs single, essentially spherical, non-embryonated; polar filaments absent. Parasites of rays (Myliobatiformes) and Hemiscylliidae (Orectolobiformes) as adults. Cosmopolitan.

Remarks: Prior to this study, *Campbell & Beveridge (1994)* and *Palm (2004)* amended the original diagnosis of *Rhinoptericola* based on the features of the type and only species, *R. megacantha*. *Palm (2004)* reinterpreted the metabasal armature as heteroacanthous typical (rather than atypical) and determined the presence (rather than absence) of prebulbar organs. These features were confirmed in the present study for all members of *Rhinoptericola*. *Palm (2004)* also interpreted *R. megacantha* to possess five hooks per principal row; however, with the addition of data on new species, species transferred to the genus, and reinterpretation of the hooks of *R. megacantha*, species of *Rhinoptericola* are now collectively considered to possess six or more hooks per principal row. Additional changes include that, with the exception of one euapolytic species, species of *Rhinoptericola* are now considered to be apolytic *sensu* *Caira, Jensen & Healy (1999)*, and that the cirrus was found to be unarmed, rather than armed with spinitriches.

The synonymy of *Shirleyrhynchus* with *Rhinoptericola* is supported by both morphological and molecular data (see results of the phylogenetic analysis). *Beveridge & Campbell (1988)* noted strong morphological similarity between the proglottid anatomy of *Shirleyrhynchus* and *Rhinoptericola*, and distinguished the genera solely based on metabasal armature type: heteroacanthous typical armatures in species of *Shirleyrhynchus* and heteroacanthous atypical armatures in species of *Rhinoptericola*. Now that species of *Rhinoptericola* are interpreted to be typical heteroacanths as well, there is no compelling morphological evidence to justify maintaining *Shirleyrhynchus* as a separate genus.

Nataliella* *Palm, 2010

Synonyms: None.

Type and only species: *Nataliella marcelli* Palm, 2010.
Type specimens: Holotype and two paratypes (MPM 15751 [formerly MPM 23200]) and one paratype (MPM 15752 [formerly MPM 23201]).
Voucher specimens: ZMB 7439 (hologenophore; missing).

Remarks: Palm (2010) assigned the genus *Nataliella*, and its type and only species, *Nataliella marcelli*, to the family Rhinoptericolidae based on the results of a molecular phylogenetic analysis (Palm et al., 2009) and a scolex morphology unique among tapeworms and shared between *N. marcelli* and *R. megacantha* (*i.e.*, elongate scoleces with four bothria and prebulbar organs, but without gland cells in the bulbs). The presence (or absence) of these features was confirmed following examination of detailed photomicrographs of the holotype of *N. marcelli* (MPM 15751 [formerly MPM 23200]). Unlike species of *Rhinoptericola*, however, *N. marcelli* was described as possessing a homeoacanthous metabasal armature (*i.e.*, a metabasal armature with hooks arranged in quincunxes). This differs markedly from paired rows of hooks known for species of *Rhinoptericola*, but observations of photomicrographs of the holotype were insufficient to conclusively assess armature type for *N. marcelli*.

Unfortunately, proglottid anatomy is not known for *N. marcelli* as it was described solely from larval specimens collected from teleosts (families Acanthuridae, Scombridae, Lutjanidae, and Priacanthidae; see Palm, 2010). Despite this lack of information on proglottid anatomy, *Nataliella* is here retained in the Rhinoptericolidae based on shared scolex features. No information is known about definitive host associations for *N. marcelli* but given that the species was described from relatively large bony fishes (between 20 and 79 cm standard length; Froese & Pauly, 2019), the definitive host is likely a shark.

***Rhinoptericola megacantha* Carvajal & Campbell, 1975**
Figures 2–6
Synonyms: None.

Redescription (based on holotype and 26 voucher specimens: five gravid worms, 11 mature worms, one immature worm, cross-sections of one scolex and one partial strobila, lactophenol and glycerin egg preparations from one gravid proglottid, and four scoleces, one detached proglottid, and one partial strobila prepared for SEM):

Worms apolytic (Fig. 2A); mature worms 10.7–38.6 mm (24.2 ± 8.4; 12) [38.6 mm] long, gravid worms 23.7–31.6 mm (n = 4) long, maximum width at level of pars bothrialis, pars bulbosa or terminal proglottid; proglottids 39–74 (56 ± 17.0; 5) [56] in total number in mature and 22–74 (51 ± 15.5; 17) in total number in gravid worms.

Scolex (Figs. 2B, 4A and 4B) acraspedote, elongate, slender, 2,616–5,078 (3,973 ± 659.1; 18) [4,019] long, length:width ratio 2.8–6.4 (4.7 ± 1.3; 13):1 [5.2:1]. Pars bothrialis 369–902 (571 ± 127.3; 15) [581] long by 529–963 (751 ± 119.0; 15) [529] wide, with four bothria (Figs. 2B, 4A, 4B, 5A); bothria elliptoid to deeply ovoid, 320–625 (469 ± 79.2; 17; 40) [427–514] long by 188–332 (248 ± 42.3; 13; 28) wide, with free lateral and posterior margins, arranged in dorsal and ventral pairs, not overlapping pars bulbosa; bothrial pits absent. Pintner's cells absent. Pars vaginalis 1,173–2,609 (1,831 ± 417.8; 18) [1,730] long by

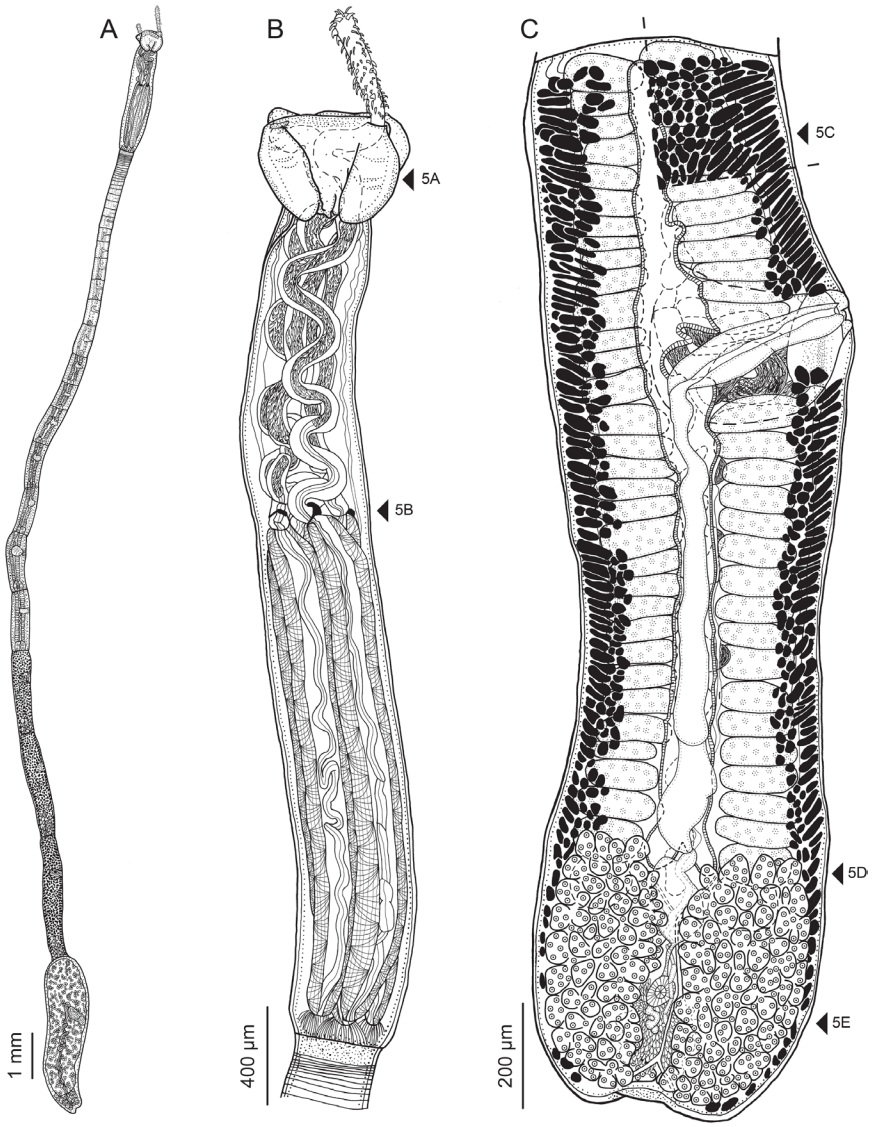

Figure 2 Line drawings of *Rhinoptericola megacantha* *Carvajal & Campbell, 1975*. (A) Whole worm (USNM 1661579; voucher). (B) Scolex (USNM 1661577; voucher). (C) Terminal proglottid (USNM 1661584; voucher); circumcortical vitelline follicles are drawn only on the lateral margins and in the region delimited by dashed lines. Arrowheads indicate the level at which the sections in Fig. 5 were taken.
Full-size DOI: 10.7717/peerj.12865/fig-2

378–793 (586 ± 129.2; 18) [591] wide at midpoint; tentacle sheaths sinuous. Pars bulbosa 1,458–2,410 (2,083 ± 324.9; 18) [2,185] long by 492–741 (593 ± 81.5; 18) [516] wide at midpoint; bulbs very narrowly oblong, thick-walled, muscular, 1,367–2,483 (2,078 ± 321.6;

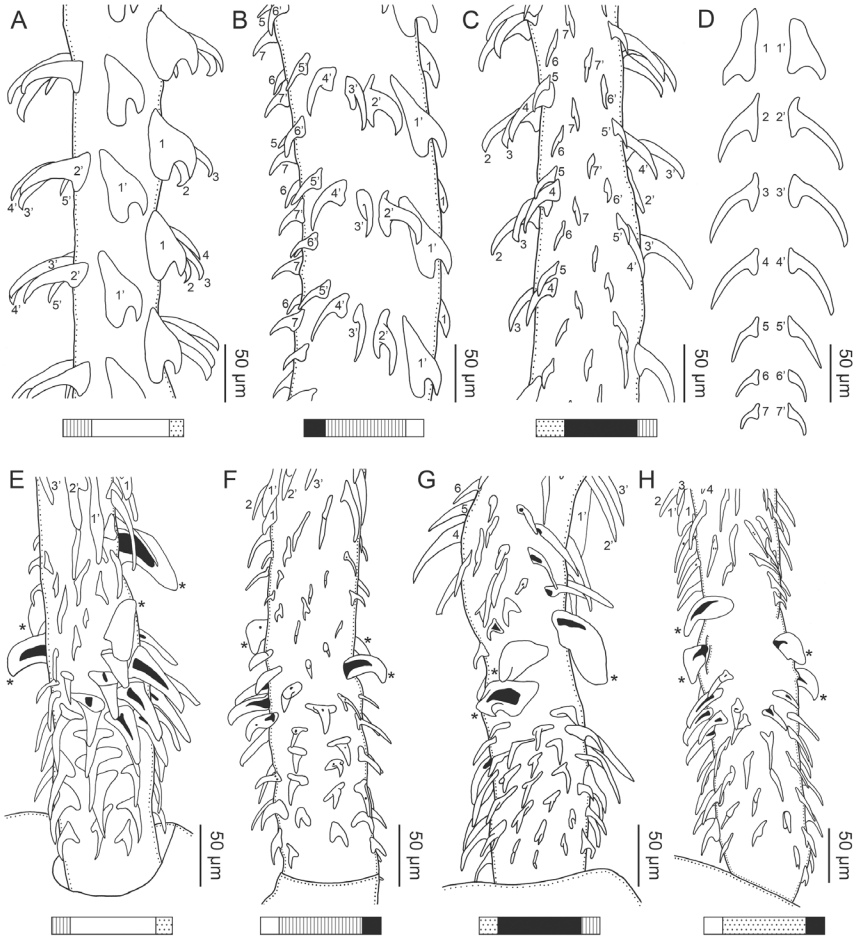

Figure 3 Line drawings of the tentacular armature of *Rhinoptericola megacantha* *Carvajal & Campbell, 1975*. (A) Metabasal armature, internal surface (LRP 10538; voucher). (B) Metabasal armature, bothrial surface (USNM 1661582; voucher). (C) Metabasal armature, external surface (LRP 10538; voucher). (D) Comparison of metabasal hook shapes. (E) Basal armature, internal surface (USNM 73836; holotype). (F) Basal armature, bothrial surface (USNM 1661576; voucher). (G) Basal armature, external surface (USNM 73836; holotype). (H) Basal armature, antibothrial surface (USNM 1661579; voucher). Asterisks (*) in E–H indicate macrohooks. Full-size DOI: 10.7717/peerj.12865/fig-3

18; 53) [2,156–2,176] long by 172–306 (231 ±.34.2; 18; 45) [172–195] wide; bulb length: width ratio 4.8–12.7 (9.1 ± 1.6; 18; 45):1 [11.1–12.7:1]; prebulbar organs present; gland cells inside bulbs absent; retractor muscles in bulbs 24–55 (38 ± 7.0; 18; 52) [29–39] wide, originating at base of bulbs. Pars postbulbosa short, 41–128 (79 ± 27.3; 18) [122] long. Scolex length ratio (pars bothrialis length:pars vaginalis length:pars bulbosa length) 1:2.2–6.2 (3.3 ± 1.1; 15):2.4–5.0 (3.7 ± 0.8; 15) [1:3.0:3.8].

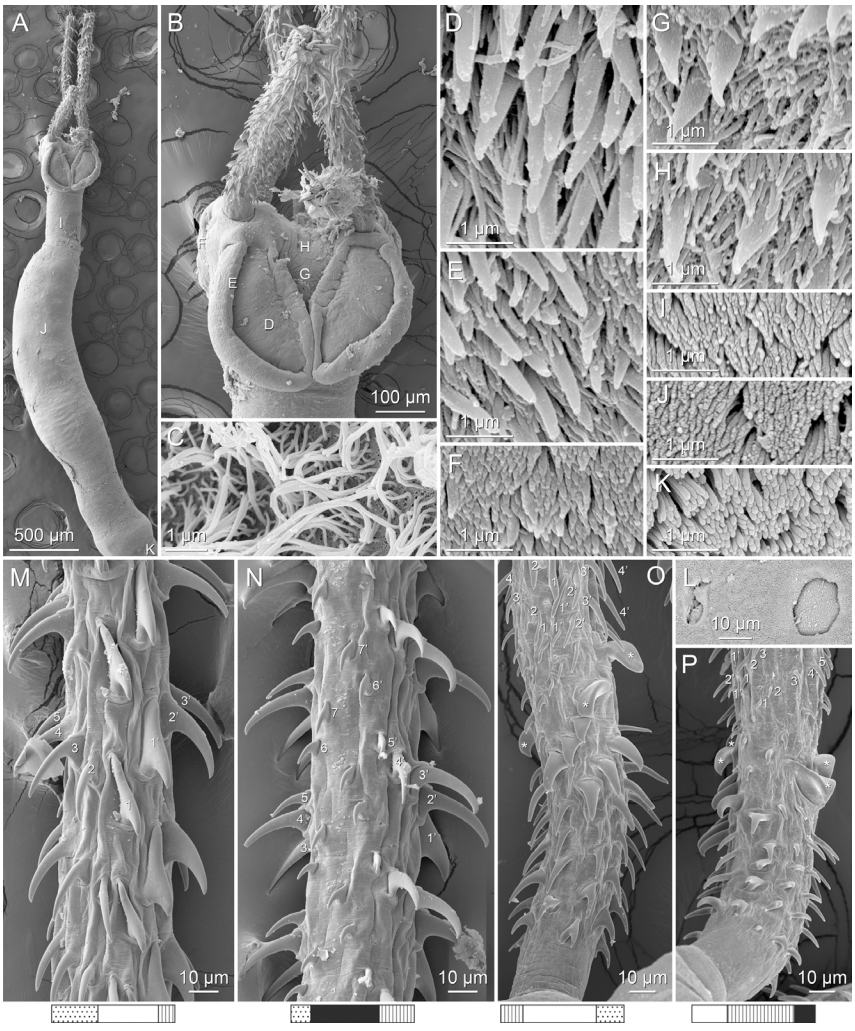

Figure 4 **Scanning electron micrographs of *Rhinoptericola megacantha* *Carvajal & Campbell, 1975*.** (A) Scolex; small letters indicate the location of details shown in (I–K). (B) Bothria and tentacular armature; small letters indicate the location of details shown in (D–H). (C) Surface of everted cirrus. (D) Distal bothrial surface. (E) Proximal bothria surface near the bothrial rim. (F) Bothrial surface away from the bothrial rim. (G) Surface of the scolex proper between the bothria. (H) Surface of the scolex proper at the apex. (I) Surface of the pars vaginalis. (J) Surface of the pars bulbosa. (K) Strobilar surface. (L) Separate male and female genital pores. (M) Metabasal armature, internal surface. (N) Metabasal armature, external surface. (O) Basal armature, internal surface. (P) Basal armature, bothrial surface. Asterisks (*) in O and P indicate macrohooks. Full-size DOI: 10.7717/peerj.12865/fig-4

Tentacles long, with slight basal swelling, rarely retracted into bulbs, at least 2,206 long, 56–109 (84 ± 13.1; 15; 30) [56–72] wide at base, 81–118 (98 ± 9.9; 14; 22) [82–94] wide at basal swelling, 68–106 (89 ± 11.2; 14; 27) [76–84] wide in metabasal region.

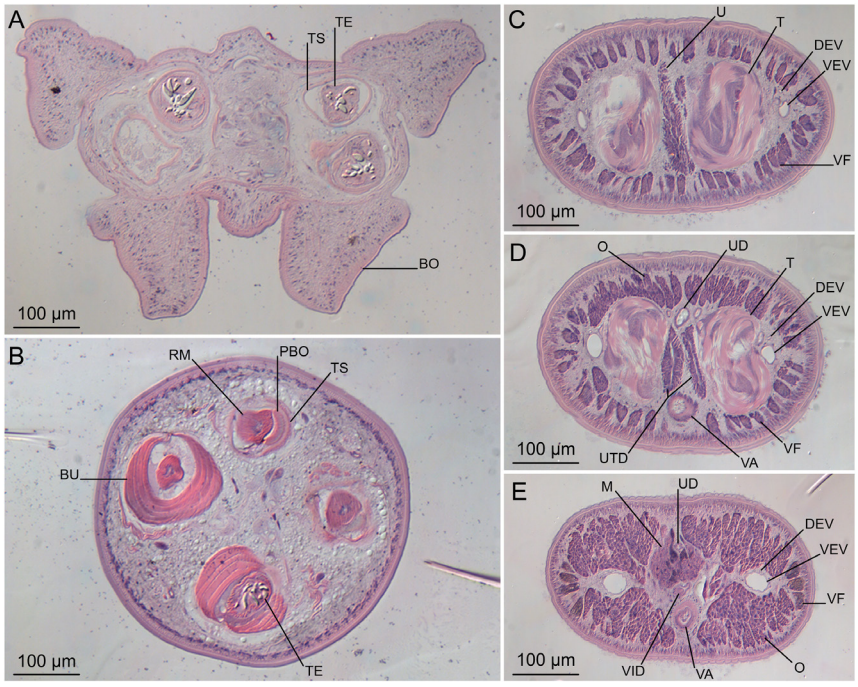

Figure 5 **Light micrographs of cross-sections of *Rhinoptericola megacantha* *Carvajal & Campbell, 1975*.** (A) Scolex at the level of the bothria. (B) Scolex at the level of the prebulbar organs. (C) Mature proglottid anterior to the genital pores. (D) Mature proglottid at the anterior margin of the ovary. (E) Mature proglottid at the level of the Mehlis' gland. Abbreviations: BO, bothrium; BU, bulb; DEV, dorsal excretory vessel; M, Mehlis' gland; O, ovary; PBO, prebulbar organ; RM, retractor muscle; T, testis; TE, tentacle; TS, tentacle sheath; U, uterus; UD, uterine duct; UTD, uterine diverticulum; VA, vagina; VEV, ventral excretory vessel; VID, vitelline duct; VF, vitelline follicle.
Full-size DOI: 10.7717/peerj.12865/fig-5

Characteristic basal armature present (Figs. 3E–3H, 4O and 4P), 237–368 (306 ± 29.4; 13; 23) [237–293] long from base of tentacle to start of metabasal armature, consisting of 60–76 (64 ± 2.8; 9) [66] hooks arranged in 8–11 [11] indistinct rows; hooks in posterior-most rows 1–3 uncinate, solid, with or without slight anterior base extensions; hooks in rows 3–6 falcate to bent spiniform or hastate, solid or hollow; hooks in rows 5–7 triangular and dorsoventrally flattened or falcate with or without recurved tips, solid or hollow; four macrohooks in rows 8–9; macrohook on internal surface, amorphous, blunt, solid; macrohooks on external surface uncinate, dorsoventrally flattened, rebated, with recurved tips, solid or hollow; macrohook on antibothrial surface, plow-shaped, hollow, with region devoid of hooks immediately posterior; macrohooks 30–73 (47 ± 9.7; 14; 36) long, 20–57 (35 ± 8.0; 14; 36) high, base 15–29 (20 ± 4.0; 14; 36) long; hooks in anterior-most rows 10–11 spiniform to falcate or rosethorn-shaped, small, thin, solid or hollow; billhooks absent.

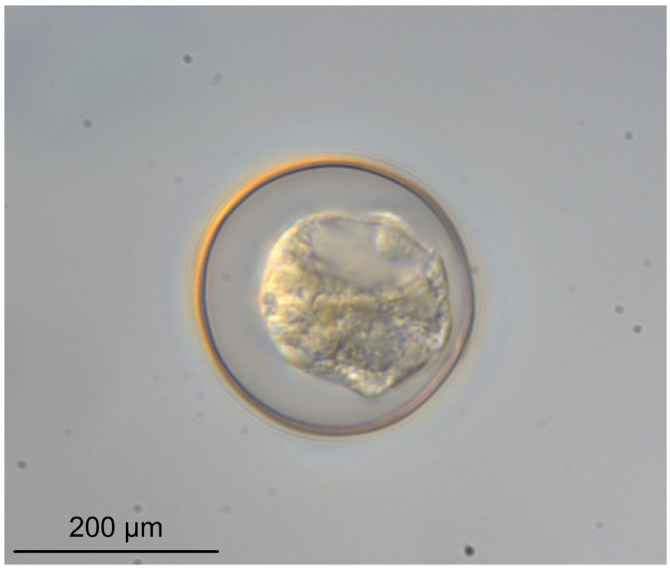

Figure 6 **Light micrograph of an egg of *Rhinoptericola megacantha* Carvajal & Campbell, 1975 (USNM 1661583; voucher).** Full-size DOI: 10.7717/peerj.12865/fig-6

Metabasal armature (Figs. 3A–3D, 4M and 4N) heteroacanthous typical; hooks heteromorphous, solid, arranged in alternating ascending half-spiral rows of seven hooks each; rows originating with hooks 1(1′) on internal surface, terminating with hooks 7(7′) in near single file on external surface; hooks 1(1′)–3(3′) not angled towards gap between hooks 1(1′). Hook files 1 and 1′ slightly separated, 14–27 (21 ± 5.5; 5; 6) apart. Hooks 1(1′) uncinate with prominent anterior base extensions, 45–119 (81 ± 16.2; 15; 38) long, 20–68 (39 ± 11.7; 15; 38) high, base 45–103 (67 ± 15.2; 15; 38) long. Hooks 2(2′) falcate, with slightly recurved tips and slight anterior base extensions, 42–100 (71 ± 11.1; 14; 30) long, 27–72 (45 ± 11.3; 14; 30) high, base 26–83 (41 ± 10.4; 14; 30) long. Hooks 3(3′) falcate, with slightly recurved tips and slight anterior base extensions, 47–100 (73 ± 11.3; 11; 26) long, 28–69 (47 ± 10.5; 11; 26) high, base 21–42 (27 ± 5.4; 11; 26) long. Hooks 4(4′) falcate, with slightly recurved tips and slight anterior base extensions, 53–80 (66 ± 8.6; 10; 19) long, 21–57 (43 ± 9.9; 10; 19) high, base 15–29 (22 ± 3.0; 10; 19) long. Hooks 5(5′) falcate with slight anterior base extensions, 33–67 (48 ± 7.9; 11; 24) long, 15–37 (26 ± 5.1; 11; 24) high, base 13–22 (18 ± 2.5; 11; 24) long. Hooks 6(6′) falcate to uncinate with tips extending beyond hook base, with slight anterior base extensions, 25–48 (36 ± 6.0; 12; 24) long, 12–38 (21 ± 5.6; 12; 24) high, base 10–22 (17 ± 3.3; 12; 24) long. Hooks 7(7′) falcate to uncinate with tips extending beyond hook base, with slight anterior base extensions, 22–45 (35 ± 5.4; 12; 22) long, 14–31 (20 ± 4.3; 12; 22) high, base 12–25 (19 ± 3.6; 12; 22) long.

Distal bothrial surfaces (Fig. 4D) with long narrow gladiate spinitriches and capilliform filitriches. Proximal bothrial surfaces near bothrial rims (Fig. 4E) with long narrow gladiate

spinitriches and capilliform filitriches, away from bothrial rims (Fig. 4F) with short narrow gladiate spinitriches and acicular filitriches. Scolex proper at apex (Fig. 4H) and between bothria (Fig. 4G) with gladiate spinitriches and acicular to capilliform filitriches. Pars vaginalis (Fig. 4I), pars bulbosa (Fig. 4J), and strobila (Fig. 4K) with capilliform filitriches.

Proglottids acraspedote. Neck 57–257 (124 ± 51.3; 16) long. Immature proglottids 17–64 (41 ± 12.8; 17) [44] in number, wider than long, becoming longer than wide with maturity. Mature proglottids 3–21 (9 ± 4.0; 17) [12] in number; terminal mature proglottids in mature worms 1,629–3,170 (2,232 ± 455.0; 12) [3,170] long by 402–945 (598 ± 173.6; 12) [680] wide. Gravid proglottids 1–4 (n = 4) in number; terminal gravid proglottids 2,295–3,260 (n = 4) long by 624–1,209 (n = 4) wide.

Testes 41–67 (57 ± 6.6; 16) [58] in total number, 20–26 (23 ± 1.9; 15) [23] pre-poral, 21–43 (34 ± 5.7; 15) [35] post-poral, 39–137 (77 ± 20.9; 16; 48) [80–137] long by 85–218 (133 ± 34.2; 15; 45) [161–193] wide, in field from anterior margin of proglottid to ovary, slightly overlapping anterior margin of ovary, arranged in two columns (Figs. 2C, 5C and 5D), essentially in single layer (Figs. 5C and 5D). Vas deferens extending from near anterior margin of ovary to anterior margin of cirrus sac, entering cirrus sac at its antero-medial margin, coiled primarily anterior to cirrus sac; external and internal seminal vesicles absent. Cirrus sac ovoid to elliptoid, occasionally bent anteriorly, 241–672 (449 ± 121.7; 14) [672] long by 149–350 (225 ± 51.6; 15) [269] wide, containing coiled cirrus; cirrus unarmed, thin-walled. Genital atrium absent. Genital pores separate (Fig. 4L), at same level, unilateral, 60–79% (71% ± 4.5%; 17) [75%] of proglottid length from posterior margin of proglottid in mature proglottids and 65–74% (n = 4) in gravid proglottids. Vagina thick-walled, weakly sinuous, extending from ootype along midline of proglottid to anterior margin of cirrus sac, then laterally at level of cirrus sac, terminating in female genital pore, greatly expanded when sperm-filled; vaginal sphincter absent; seminal receptacle present. Ovary terminal in proglottid, H-shaped in dorsoventral view, tetralobed in cross-section, 283–662 (476 ± 109.2; 16) [584] long by 243–599 (437 ± 109.5; 14) [508] wide, with lobulated margins; ovarian isthmus near center of ovary. Mehlis' gland near posterior margin of ovary. Vitellarium follicular; follicles circumcortical, 15–79 (27 ± 13.1; 16; 47) [30–37] long by 12–77 (32 ± 13.0; 15; 44) [28–57] wide, extending entire length of proglottid, interrupted dorsally and ventrally by ovary, partially interrupted ventrally by terminal genitalia; post-ovarian vitelline follicles absent. Uterus saccate, medial, dorsal to vagina (Figs. 2C, 5D), bifurcated at posterior end (Figs. 2C, 5D), extending from anterior margin of ovary to anterior margin of proglottid. Uterine duct entering uterus at mid-level. Uterine pore absent. Excretory vessels four, arranged in one dorsal and one ventral pair on each lateral margin of proglottid. Eggs (Fig. 6) single, essentially spherical, 15–23 (17 ± 2.2; 4; 12) in diameter *in situ*, 26–29 (27 ± 1.0; 1; 10) in diameter *ex situ*, non-embryonated; polar filaments absent.

Type host: Rhinoptera bonasus (Mitchill, 1815) (Rhinopteridae: Myliobatiformes).
Additional hosts: *Rhinoptera brasiliensis* Müller, 1836 and *Rhinoptera marginata* (Geoffroy St. Hilaire, 1817) (Rhinopteridae: Myliobatiformes); *Hypanus say* (Lesueur, 1817) (Dasyatidae: Myliobatiformes).

Type locality: **Atlantic Ocean, Virginia, USA:** Chesapeake Bay.

Additional localities: **Atlantic Ocean, Brazil:** Southern and southeastern Brazil. **Atlantic Ocean, Senegal:** St. Louis (16°1′28″N, 16°30′33″W). **Atlantic Ocean, South Carolina, USA:** Awendaw (33°02′07.78″N, 79°32′47.24″W; 33°0′34.27″N, 79°29′8.82″W), Bull's Bay; and Charleston (32°45′2.53″N, 79°53′48.28″W; 32°44′51.30″N, 79°53′44.07″W; 32°47′18.08″N, 79°53′18.77″W), Charleston Harbor. **Caribbean Sea, Belize:** Gales Point Manatee (17°13′1.0″N, 88°19′01.4″W), Inner Channel. **Caribbean Sea, Venezuela:** Caimare Chico, Zulia, Gulf of Venezuela. **Gulf of Mexico, Louisiana, USA:** Chandeleur Islands (29°57′9.54″N, 88°50′38.98″W). **Gulf of Mexico, Mississippi, USA:** East Ship Island (30°14′37.70″N, 88°46′37.62″W); Horn Island (30°14′1.44″N, 88°40′5.47″W; 30°14′24.54″N, 88°52′25.25″W; 30°15′04″N, 88°42′42″W); off the Gulf Coast Research Lab, Ocean Springs (30°23′33.55″N, 88°47′51.79″W); and Ship Island (30°13′13.53″N, 88°54′52.48″W).

Site of infection: Spiral intestine.

Type specimens: Holotype (USNM 1369398 [originally USNPC 73835]) and one paratype (USNM 1369399 [originally USNPC 73836]).

Voucher specimens: HWML 21032 (*Mayes & Brooks, 1981*), HWML 34972; BMNH 2008.5.21 (hologenophore; *Olson et al., 2010*); LRP 10454–10546 (this study), LRP 10432–10453 (hologenophores; this study); USNM 1661576–1661587 (this study).

Museum specimens examined: Holotype (USNM 1369398) and two voucher specimens (HWML 21032 and HWML 34972).

Remarks: As the type species of the genus, this species has received relatively little attention since its detailed description by *Carvajal & Campbell (1975)*. In his treatment of the species based on examination of the holotype and paratype, *Palm (2004)* presented a revised version of the description of *Carvajal & Campbell (1975)* using updated terminology. The two most significant changes *Palm (2004)* made were the reinterpretation of the metabasal armature from heteroacanthous atypical to heteroacanthous typical, and the observation of the presence, rather than absence, of prebulbar organs. The redescription herein is based on the holotype (which was remeasured), and new voucher material. It includes the first detailed scanning electron micrographs of the hooks and microthrix pattern for the species (Fig. 4). The species is redrawn from specimens from the type host, *Rhinoptera bonasus*, and from *Rhinoptera brasiliensis* (Figs. 2, 3). Photomicrographs of cross-sections (Fig. 5) and an egg (Fig. 6) are provided, and the known definitive host associations and geographic range for the species are expanded.

Most significant in this redescription is the reinterpretation of the metabasal armature. *Carvajal & Campbell (1975)* and *Palm (2004)* both interpreted the metabasal armature to comprise five hooks per principal row with an additional row of three small hooks on the external surface (see fig. 4 of *Carvajal & Campbell, 1975*). The metabasal armature is reinterpreted here to simply consist of seven hooks per principal row (see Figs. 3C, 4N);

the rows of three small hooks on the external surface observed by *Carvajal & Campbell (1975)* and *Palm (2004)* are now considered part of the principal rows. Additional changes include recognizing *R. megacantha* to be apolytic rather than euapolytic *sensu Caira, Jensen & Healy (1999)* (see Fig. 2A), to possess a cirrus that is unarmed rather than armed (see Fig. 4C), and to possess genital pores that are unilateral rather than irregularly alternating (see Fig. 2A).

Not unexpectedly, given the greater number of measured specimens on which this redescription is based compared to the original description of *Carvajal & Campbell (1975)* (*i.e.*, 18 *vs* six, respectively), ranges for most measurements were expanded, or differ slightly, from those in the original description (see Table S2). There are, however, two instances where ranges differ largely: *Carvajal & Campbell (1975)* reported a total length of 35–65 mm while the specimens examined in this study (including the holotype) measured 10.7–38.6 mm in total length for mature worms and 23.7–31.6 mm for gravid worms; similarly, *Carvajal & Campbell (1975)* reported terminal proglottids of *R. megacantha* to be 2,200–4,000 µm long (without specifying maturity) while we report total lengths of 1,629–3,170 µm and 2,295–3,260 µm for mature and gravid terminal proglottids, respectively. Interestingly, the holotype—a mature, non-gravid worm—was one of the longest specimens measured in this study, and possessed the longest terminal proglottid. This suggests that the additional five specimens measured by *Carvajal & Campbell (1975)* that were not included here may also be particularly large worms.

Prior to this study, *R. megacantha* had been reported from the American cownose ray, *Rhinoptera bonasus*, from both the Chesapeake Bay, USA (*Carvajal & Campbell, 1975*) and the Gulf of Venezuela, Venezuela (*Mayes & Brooks, 1981*), as well as from the Ticon cownose ray, *Rhinoptera brasiliensis*, from the Gulf of Mexico, USA (as *Rhinoptera bonasus; Call, 2007*) and from the Atlantic coast of Brazil (*Napoleão et al., 2015*). Based on updated geographic distributions for species of *Rhinoptera* van Hasselt, 1824 (see *Last et al., 2016*), the identity for the host of *R. megacantha* from the Gulf of Venezuela is uncertain and could have been either *Rhinoptera bonasus* or *Rhinoptera brasiliensis*. Additional voucher material used for this redescription further expands the hosts and geographic localities from which *R. megacantha* is known to include an additional species of cownose ray, the Lusitanian cownose ray, *Rhinoptera marginata*, from Senegal, as well as the bluntnose stingray, *Hypanus say*, from off South Carolina, USA. Thus, *R. megacantha* is now understood to parasitize three species of cownose rays (Rhinopteridae) and one species of stingray (Dasyatidae) from both sides of the Atlantic Ocean, including the Chesapeake Bay, South Carolina, the Gulf of Mexico, Belize, Venezuela, Brazil, and Senegal.

***Rhinoptericola butlerae* (*Beveridge & Campbell, 1988*) n. comb.**

Figure 7

Synonym: *Shirleyrhynchus butlerae Beveridge & Campbell, 1988*.

Redescription (based on holotype, six paratypes, and 10 voucher specimens: one gravid worm, one mature worm, three immature worms, and four complete scoleces and one partial scolex prepared for SEM):

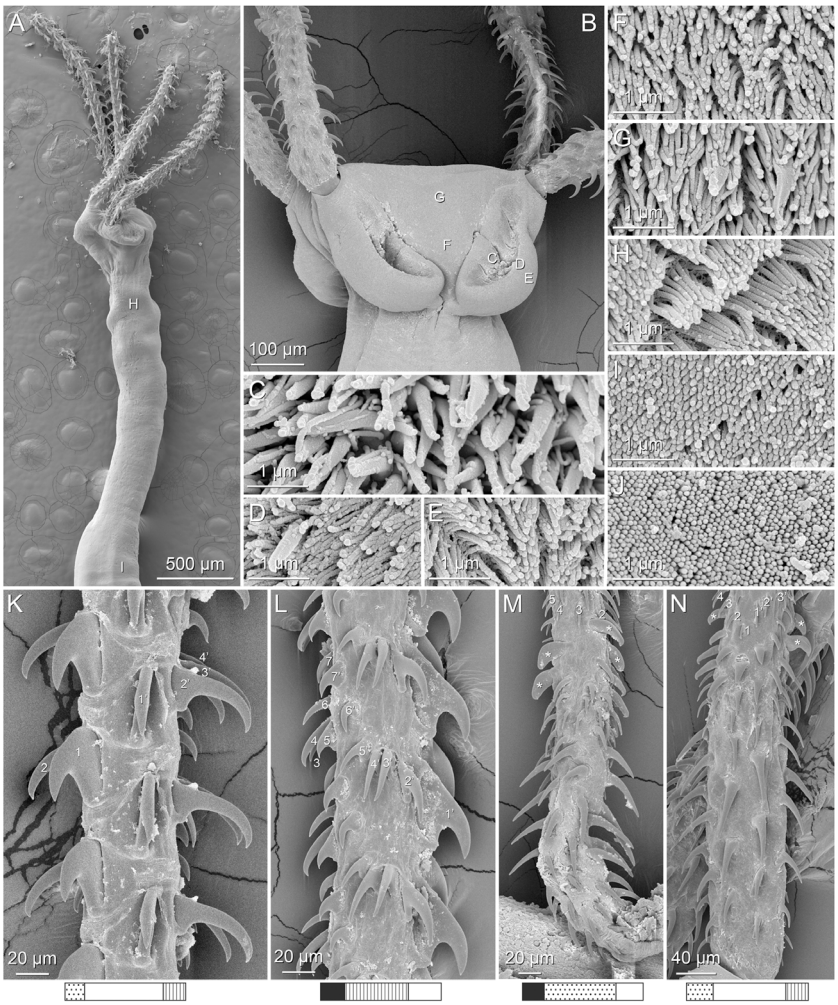

Figure 7 Scanning electron micrographs of *Rhinoptericola butlerae* (*Beveridge & Campbell, 1988*) n. comb. (A) Scolex; small letter indicates the location of details shown in (H–I). (B) Bothria and basal armature; small letters indicate the location of details shown in (C–G). (C) Distal bothrial surface. (D) Proximal bothrial surface near the bothrial rim. (E) Proximal bothria surface away from the bothrial rim. (F) Surface of the scolex proper between the bothria. (G) Surface of the scolex proper at the apex. (H) Surface of the pars vaginalis. (I) Surface of the pars bulbosa. (J) Strobilar surface. (K) Metabasal armature, internal surface. (L) Metabasal armature, bothrial surface. (M) Basal armature, antibothrial surface. (N) Basal armature, internal surface. Asterisks (*) in M and N indicate macrohooks.

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Worms apolytic; mature worms 15.5–18.9 mm (n = 3) [15.5 mm] long, gravid worms 22.7 mm (n = 1) long, maximum width at level of pars bothrialis or pars bulbosa; proglottids 42–51 (n = 3) [42] in total number in mature and 50 (n = 1) in total number in gravid worms.

Scolex (Figs. 7A and 7B) acraspedote, elongate, slender, 4,533–5,899 (5,081 ± 441.1; 11) [4,533] long, length:width ratio 5.0–8.9 (6.4 ± 1.2; 9):1 [5.7:1]. Pars bothrialis 418–714 (599 ± 90.2; 11) [622] long by 664–952 (794 ± 100.3; 9) [790] wide, with four bothria (Figs. 7A and 7B); bothria elliptoid to very deeply ovoid, 373–653 (493 ± 70.2; 11; 28) [492–519] long by 169–273 (223 ± 33.2; 11; 21) [218] wide, with free lateral and posterior margins, arranged in dorsal and ventral pairs, not overlapping pars bulbosa; bothrial pits absent. Pintner's cells absent. Pars vaginalis 2,478–3,420 (2,854 ± 306.0; 11) [2,591] long by 348–785 (562 ± 117.6; 11) [447] wide at midpoint; tentacle sheaths sinuous. Pars bulbosa 1,752–2,476 (2,101 ± 238.0; 11) [1,752] long by 558–1,059 (662 ± 139.2; 11) [558] wide at midpoint; bulbs very narrowly oblong, thick-walled, muscular, 1,641–2,450 (2,047 ± 244.5; 11; 30) [1,641–1,745] long by 186–307 (233 ± 28.7; 11; 29) [203–220] wide; bulb length:width ratio 5.8–11.3 (8.9 ± 1.5; 11; 27):1 [7.9–8.1:1]; prebulbar organs present; gland cells inside bulbs absent; retractor muscles in bulbs 20–56 (38 ± 10.3; 10; 28) [31–53] wide, originating at base of bulbs. Pars postbulbosa short, 76–273 (151 ± 64.7; 11) [136] long. Scolex length ratio (pars bothrialis length:pars vaginalis length:pars bulbosa length) 1:3.7–6.1 (4.8 ± 0.7; 11):2.8–5.1 (3.6 ± 0.7; 11) [1:4.2:2.8].

Tentacles long, with slight basal swelling, rarely retracted into bulbs, at least 2,219 long, 82–159 (101 ± 19.1; 10; 24) [105] wide at base, 83–143 (107 ± 16.2; 9; 22) wide at basal swelling, 77–136 (102 ± 20.2; 9; 23) wide in metabasal region.

Characteristic basal armature present (Figs. 7M and 7N), 354–492 (431 ± 38.8; 9; 18) [492] long from base of tentacle to start of metabasal armature, consisting of 83–99 (91 ± 6.4; 5) [83] hooks arranged in 8–12 [12] indistinct rows; hooks in posterior-most rows 1–3 uncinate, solid, with or without slight anterior base extensions; hooks in rows 3–7 falcate to spiniform or hastate, large, thin, and erect when falcate, solid or hollow; hooks in rows 7–9 triangular and dorsoventrally flattened or falcate with or without recurved tips, solid or hollow; 3–4 macrohooks in rows 9–10; one macrohook on internal surface uncinate, dorsoventrally flattened, rebated to amorphous and blunt, occasionally small enough as to be unrecognizable as macrohook; two macrohooks on external surface, uncinate, dorsoventrally flattened, rebated, with recurved tips, solid or hollow; one anterior-most macrohook on antibothrial surface, plow-shaped to uncinate, dorsoventrally flattened, rebated, hollow, with region devoid of hooks immediately posterior; macrohooks 32–63 (46 ± 8.9; 5; 13) long, 26–56 (39 ± 8.6; 5; 13) high, base 11–28 (18 ± 4.8; 5; 13) long; hooks in anterior-most rows 11–12 spiniform to falcate or rosethorn-shaped, small, thin, solid or hollow; billhooks absent.

Metabasal armature (Figs. 7K and 7L) heteroacanthous typical; hooks heteromorphous, solid, arranged in alternating ascending half-spiral rows of seven hooks each; rows originating with hooks 1(1′) on internal surface, terminating with hooks 7(7′) in near single file on external surface; hooks 1(1′)–3(3′) not angled towards gap between hooks 1(1′). Hook files 1 and (1′) slightly separated, 24–42 (n = 2; 4) apart. Hooks 1(1′) uncinate

with prominent anterior base extensions, 65–126 (86 ± 18.2; 9; 25) long, 38–82 (54 ± 11.9; 9; 25) high, base 53–102 (72 ± 13.1; 9; 25) long. Hooks 2(2′) falcate, with slightly recurved tips and slight anterior base extensions, 66–125 (97 ± 23.5; 5; 12) long, 42–99 (70 ± 20.8; 5; 12) high, base 31–66 (47 ± 11.1; 5; 12) long. Hooks 3(3′) falcate, with slightly recurved tips and slight anterior base extensions, 62–119 (92 ± 22.9; 5; 9) long, 44–108 (68 ± 23.1; 5; 9) high, base 27–42 (34 ± 5.6; 5; 9) long. Hooks 4(4′) falcate, with slightly recurved tips and slight anterior base extensions, 55–99 (71 ± 16.7; 4; 9) long, 29–70 (46 ± 13.5; 4; 9) high, base 19–39 (26 ± 6.7; 4; 9) long. Hooks 5(5′) falcate with slight anterior base extensions, 36–75 (56 ± 13.5; 5; 13) long, 20–59 (37 ± 11.2; 5; 13) high, base 14–26 (20 ± 4.6; 5; 13) long. Hooks 6(6′) falcate to uncinate with tips extending beyond hook base, with slight anterior base extensions, 24–69 (39 ± 12.9; 9; 25) long, 12–38 (23 ± 6.2; 9; 25) high, base 10–31 (20 ± 4.9; 9; 25) long. Hooks 7(7′) falcate to uncinate with tips extending beyond hook base, with slight anterior base extensions, 20–64 (39 ± 12.7; 9; 26) long, 15–37 (24 ± 5.7; 9; 26) high, base 18–31 (26 ± 3.6; 9; 26) long.

Distal bothrial surfaces (Fig. 7C) with long narrow gladiate spinitriches and capilliform filitriches. Proximal bothrial surfaces near bothrial rims (Fig. 7D) with short narrow gladiate spinitriches and capilliform filitriches, away from bothrial rims (Fig. 7E) with acicular filitriches. Scolex proper at apex (Fig. 7G) with gladiate spinitriches and acicular filitriches, and between bothria (Fig. 7F) with acicular to capilliform filitriches. Pars vaginalis (Fig. 7H), pars bulbosa (Fig. 7I), and strobila (Fig. 7J) with capilliform filitriches.

Proglottids acraspedote. Neck 155–164 (n = 2) long. Immature proglottids 35–46 (n = 3) [35] in number, wider than long, becoming longer than wide with maturity. Mature proglottids 5–7 (n = 2) [7] in number; terminal mature proglottids in mature worms 1,085–1,529 (n = 2) [1,085] long by 293–500 (n = 2) [500] wide. Gravid proglottids two (n = 1) in number; terminal gravid proglottids 1,480 by 683 (n = 1) wide; detached gravid proglottids 1,735–2,213 (n = 3) long by 747–766 (n = 3) wide.

Testes 50–60 (54 ± 4.4; 3; 5) [57] in total number, 19–28 (n = 4) [21–28] pre-poral, 29–32 (n = 4) [32] post-poral, 51–60 (55 ± 4.2; 2; 6) [51–60] long by 90–157 (114 ± 23.0; 2; 6) [118–157] wide, in field from anterior margin of proglottid to ovary, slightly overlapping anterior margin of ovary, arranged in two columns, essentially in single layer. Vas deferens extending from near anterior margin of ovary to anterior margin of cirrus sac, entering cirrus sac at its antero-medial margin, coiled primarily anterior to cirrus sac; external and internal seminal vesicles absent. Cirrus sac elliptoid, bent anteriorly, 241 (n = 1) long by 195 (n = 1) wide, containing coiled cirrus; cirrus unarmed, thin-walled. Genital atrium absent. Genital pores separate, at same level, unilateral, 64–72% (n = 3) [64%] of proglottid length from posterior margin in mature proglottids.

Vagina thick-walled, weakly sinuous, extending from ootype along midline of proglottid to anterior margin of cirrus sac, then laterally at level of cirrus sac, terminating in female genital pore, greatly expanded when sperm-filled; vaginal sphincter absent; seminal receptacle present. Ovary terminal in proglottid, H-shaped in dorsoventral view, tetralobed in cross-section, 509 long by 237–383 (n = 2) [383] wide, with lobulated margins; ovarian isthmus near center of ovary. Mehlis' gland near posterior margin of ovary. Vitellarium follicular; follicles circumcortical, 11–21 (16 ± 3.3; 3; 9) [14–16] long by

8–31 (20 ± 8.8; 3; 9) [8–13] wide, extending entire length of proglottid, interrupted dorsally and ventrally by ovary, partially interrupted ventrally by terminal genitalia; post-ovarian vitelline follicles absent. Uterus saccate, medial, dorsal to vagina, bifurcated at posterior end, extending from anterior margin of ovary to anterior margin of proglottid. Uterine duct not observed. Uterine pore present. Excretory vessels four, arranged in one dorsal and one ventral pair on each lateral margin of proglottid. Eggs single, essentially spherical, 19–21 (n = 2) in diameter *in situ*, non-embryonated; polar filaments absent.

Type host: Hemitrygon fluviorum (Ogilby, 1908) (Dasyatidae: Myliobatiformes).

Additional hosts: *Hemitrygon bennetti* (Müller & Henle, 1841), *Himantura tutul* Borsa, Durand, Shen, Alyza, Solihin & Berrebi, 2013, *Maculabatis gerrardi* (Gray, 1851), *Pastinachus ater* (Macleay, 1883), and *Pastinachus solocirostris* Last, Manjaji & Yearsley, 2005 (Dasyatidae: Myliobatiformes); *Rhinoptera javanica* Müller & Henle, 1841 and *Rhinoptera neglecta* Ogilby, 1912 (Rhinopteridae: Myliobatiformes); *Chiloscyllium punctatum* Müller & Henle, 1838 (Hemiscyliidae; Orectolobiformes).

Type locality: **Coral Sea, Australia:** Deception Bay, Queensland.

Additional localities: **Arafura Sea, Australia:** East of Wessel Islands (11°17′44″S, 136°59′48″E), Northern Territory. **Gulf of Carpentaria, Australia:** Weipa (12°35′11″S, 141°42′34″E), Queensland. **Timor Sea, Australia:** Dundee Beach (12°45′33″S, 130°21′7″E), Northern Territory, Fog Bay. **Java Sea, Indonesia:** Gusungnge near Pagatan market (03°36′46.10″S, 115°55′05.10″E), South Kalimantan; and Pagatan market (03°36′36.00″S, 115°54′59.40″E), South Kalimantan. **Makassar Strait, Indonesia:** Muara Pasir (01°45′58.92″S, 116°23′36.09″E), East Kalimantan. **South China Sea, Malaysia:** Mukah (02°53′52.16″N, 112°05′44.12″E), Sarawak. **South China Sea, Viet Nam:** Cat Ba (20°43′31.1″N, 107°02′54.9″E), Haiphong Province, Gulf of Tonkin; and Long Hai (10°22′60.00″N, 107°13′60.00″E), Ba Ria Province.

Site of infection: Spiral intestine.

Type specimens: Holotype (AHC 44088 [originally SAM V4088]), seven paratypes (AHC 22773 [originally SAM S2773]; whole mounts, serial sections and mounted tentacles), one paratype (BMNH 1987.5.1.1), and one paratype (USNM 1375081 [originally USNPC 79701]).

Voucher specimens: LRP 10559–10569 (*Schaeffner & Beveridge, 2014*), LRP 10547–10549, LRP 10551, and LRP 10554–10557 (this study), LRP 10550, LRP 10552, LRP 10553, and LRP 10558 (hologenophores, this study); QM G239454–G239456 (this study).

Museum specimens examined: Holotype (AHC 44088), eight paratypes (AHC 22773-2, AHC 22773-3, AHC 22773-6, AHC 22773-7, AHC 22773-8, AHC 22773-12–14 [sections of one specimen], AHC 22773-15 [sections of one specimen], and USNM 1375081), and one voucher specimen (USNM 1394286 [originally USNPC 99285]).

Table 4 Number of base pair differences (excluding missing data and ambiguous base calls) in the D1–D3 regions of the 28S rRNA gene for species of *Rhinoptericola* *Carvajal & Campbell, 1975* based on a 1,429 bp MUSCLE alignment.

Species	*R. megacantha*	*R. butlerae*	*R. jensenae*	*R. schaeffneri*	*R. mozambiquensis*	*R. hexacantha*
Rhinoptericola megacantha (n = 22)	0–2	20–24	56–59	63–64	63–70	57–59
Rhinoptericola butlerae n. comb. (n = 4)		0–2	54–57	59–60	67–70	58–59
Rhinoptericola jensenae n. comb. (n = 3)			0	36–37	25	53–54
Rhinoptericola schaeffneri n. sp. (n = 1)				–	43	57
Rhinoptericola mozambiquensis n. sp. (n = 2)					2	59–66
Rhinoptericola hexacantha n. sp. (n = 1)						–

Note:
These comparisons include data for a specimen of *Rhinoptericola megacantha* downloaded from GenBank (DQ642792). All but four sequences compared were ≥1,411 bp in total length: a specimen each of *Rhinoptericola megacantha* *Carvajal & Campbell, 1975*, *Rhinoptericola butlerae* (*Beveridge & Campbell, 1988*) n. comb., *Rhinoptericola schaeffneri* n. sp., and *Rhinoptericola mozambiquensis* n. sp. (1,262, 1,246, 841, and 1,131 bp, respectively).

Remarks: Rhinoptericola butlerae bears a strong morphological similarity to *R. megacantha*, but the two are readily distinguished from one another based on features of the basal armature. *Rhinoptericola butlerae* has a greater total number of hooks in the basal armature as compared to *R. megacantha* (*i.e.*, 83–99 *vs* 60–67, respectively). Anterior to the first one to three rows of uncinate, solid hooks in the basal armature, *R. butlerae* possesses several rows of large, thin, erect, widely-spaced hastate hooks; in *R. megacantha*, these hastate hooks are smaller, thicker, less erect, and more densely packed—a difference easily observed in scanning electron micrograph comparisons between the two species (see Fig. 7N for *R. butlerae vs* Fig. 4P for *R. megacantha*). While the ranges for the total lengths of their basal armatures (from base of tentacle to start of metabasal armature) overlap slightly, *R. butlerae* tends to have a longer basal armature as compared to *R. megacantha* (*i.e.*, 354–492 µm *vs* 237–368 µm, respectively). The two species also differ slightly in their scolex microthrix patterns: *R. butlerae* possesses only acicular to capilliform filitriches on the scolex proper between the bothria (see Fig. 7F) while *R. megacantha* possesses both gladiate spinitriches and acicular to capilliform filitriches in the same region (see Fig. 4G). In addition to these morphological differences, the two species differ in 28S sequence data by 20–24 base pairs (bp) (see Table 4).

Though *Beveridge & Campbell (1998)* and *Palm (2004)* each provided updated descriptions for species of *Shirleyrhynchus*, both works were published at a time when *R. butlerae* (as *Shirleyrhynchus butlerae* *Beveridge & Campbell, 1988*) was considered a junior synonym of *Shirleyrhynchus aetobatidis* (now *Rhinoptericola aetobatidis*; see below) and so neither redescription reliably characterizes the morphology of *R. butlerae*, alone. *Schaeffner (2016)* considered *R. butlerae* to be valid (as *S. butlerae*) and provided updated measurements and interpretations for select features of the scolex based on a reexamination of six paratypes. We confirmed the presence of seven, rather than eight, hooks per principal row in the metabasal armature (see Fig. 7L) and the arrangement of the hooks in the basal armature as being in rows, rather than in quincunxes (see Figs. 7M and 7N), as suggested by *Schaeffner (2016)*. However, unlike *Schaeffner (2016)*, who reinterpreted the orientation of principal rows in the metabasal armature as starting on the antibothrial tentacle surface and terminating on the bothrial tentacle surface, we observed

the internal-to-external orientation reported by *Beveridge & Campbell (1988)* (see Figs. 7K and 7L and Fig. S1). Additionally, we suggest that *R. butlerae* possesses three or four—rather than four—macrohooks in the basal armature; for several specimens examined from both type and voucher material, what would positionally be considered the fourth macrohook was indistinguishable in size from the surrounding hooks.

The original line drawings by *Beveridge & Campbell (1988)*—in combination with the reinterpretation of the armature provided by *Schaeffner (2016)*—are sufficiently detailed to obviate the need for new illustrations. Instead, this redescription provides the first SEM data for the species and an updated interpretation of the proglottid anatomy. The following changes from the original description by *Beveridge & Campbell (1988)* are made based on examination of new material and the majority of the type material: cirrus sac unarmed rather than armed, genital pores unilateral rather than irregularly alternating, and the absence rather than presence of a genital atrium. Combining data for the remeasured holotype and six paratypes of *R. butlerae* with measurements from new material changed the ranges for most morphological features only negligibly from those presented in the original description (see Table S2). Notable differences include total number of proglottids in gravid worms (up to 38 *vs* 50 herein) and ovary length (180–260 µm *vs* 509 µm herein).

The known host associations for *R. butlerae* are expanded significantly herein (see Table 3) from having been originally described from *Hemitrygon fluviorum* and *Pastinachus ater* (Dasyatidae) to include *Hemitrygon bennetti*, *Himantura tutul*, *Maculabatis gerrardi*, and *Pastinachus solocirostris* (Dasyatidae), *Rhinoptera javanica* and *Rhinoptera neglecta* (Rhinopteridae), and *Chiloscyllium punctatum* (Hemiscylliidae). The reports from *Hima. tutul*, *M. gerrardi*, *P. solocirostris*, and *C. punctatum* are, however, originally attributable to *Schaeffner & Beveridge (2014)*. In their paper, *Schaeffner & Beveridge (2014)* reported *R. butlerae* from these four host species from Indonesia and Malaysia (*i.e.*, the island of Borneo), but as these reports occurred during a time when the name *Shirleyrhynchus butlerae* was still considered a junior synonym of *Shirleyrhynchus aetobatidis*, they were made using the name *S. aetobatidis*. Examination of voucher specimens associated with these reports (*i.e.*, one each from *Hima. tutul*, *M. gerrardi*, *P. solocirostris*, and *C. punctatum*), augmented by additional new voucher material from all but *C. punctatum*, confirmed them to be *R. butlerae* (see Table 3). The geographic distribution is also expanded herein northward from Australia to Viet Nam.

***Rhinoptericola panamensis* (*Schaeffner, 2016*) n. comb.**
Synonym: *Shirleyrhynchus panamensis Schaeffner, 2016*.

Type host: Urotrygon aspidura (Jordan & Gilbert, 1882) (Urotrygonidae: Myliobatiformes).

Additional hosts: Styracura pacifica (Beebe & Tee-Van, 1941) (Potamotrygonidae: Myliobatiformes).

Type locality: **Pacific Ocean, Panama:** Off Palo Seco (7°34′33.5″N, 81°00′42.8″W), Veraguas, Golfo de Montijo.

Additional localities: **Pacific Ocean, Panama:** Playa de Caleta off Isla Cebaco (7°29′37.9″N, 81°13′21.8″W), Veraguas, Golfo de Montijo.

Site of infection: Spiral intestine.

Type specimens: Holotype (MIUP-LAV-002), two paratypes (USNM 1298205–1298206), and one paratype (MZUSP 7766).

Museum specimens examined: Holotype (MIUP-LAV-002) and two paratypes (USNM 1298205–1298206).

Remarks: Schaeffner (2016) described *Rhinoptericola panamensis* based on four whole-mounted specimens and two specimens prepared for SEM, all of which were immature worms. Examination of the holotype and two paratypes was sufficient to confirm that the scolex morphology of *R. panamensis* aligns with the revised generic diagnosis for *Rhinoptericola* (*i.e.*, four bothria, pre-bulbar organs, no gland cells in the bulbs, a characteristic basal armature, and a heteroacanthous typical heteromorphous metabasal armature with six or more hooks per principal row). Thus, the species is hereby transferred to the genus *Rhinoptericola* despite having no knowledge of its proglottid anatomy. The reexamination of type material, however, also allowed for the reinterpretation of, and collection of additional information on, aspects of the metabasal and basal armatures. We observed the principal rows in the metabasal armature to begin on the internal tentacle surface and terminate on the external tentacle surface, as opposed to the bothrial to antibothrial orientation specified in the original description by Schaeffner (2016) (see Fig. S1). Additionally, as it has become clear that total number of hooks in the basal armature can be an important feature for distinguishing between species of *Rhinoptericola*, it is here noted that the holotype of *R. panamensis* possesses 60 hooks in the basal armature. This easily distinguishes *R. panamensis* from *R. butlerae*, which possesses 83–99 hooks in the basal armature.

Based on quantitative features of the scolex, *R. panamensis* is morphologically indistinguishable from *R. megacantha* (see Fig. S2; as *R. panamensis* was originally described in the genus *Shirleyrhynchus*, the two species were not compared to one another prior to this study). They are similarly identical in terms of qualitative features of the scolex. Both have characteristic basal armatures with four macrohooks and a similar hook shape, number, and arrangement throughout, and metabasal armatures with seven hooks per principal row that begin on the internal tentacle surface and terminate on the external tentacle surface. In terms of scolex microthrix patterns, Schaeffner (2016) described *R. panamensis* as possessing distal bothrial surfaces with gladiate spinitriches and proximal bothrial surfaces with acicular to capilliform filitriches (see figs. 4E and 4F of Schaeffner, 2016), whereas *R. megacantha* is herein redescribed as possessing distal and proximal bothrial surfaces with both gladiate spinitriches and capilliform (or acicular) filitriches (see Figs. 4D and 4E). This is the only morphological difference between the two species and warrants further investigation.

Despite being essentially indistinguishable based on the morphological data at hand, the two species are not synonymized until proglottid anatomy can be assessed for

R. panamensis and material preserved in 95% ethanol for *R. panamensis* is available for DNA sequencing to confirm conspecificity with *R. megacantha*.

***Rhinoptericola aetobatidis* (*Shipley & Hornell, 1906*) n. comb.**
Synonyms: *Tetrarhynchus aetobatidis* *Shipley & Hornell, 1906*; *Tentacularia aetobatidis* (*Shipley & Hornell, 1906*) Southwell, 1929; *Shirleyrhynchus aetobatidis* (*Shipley & Hornell, 1906*) *Beveridge & Campbell, 1988*.

Type host: Aetobatus ocellatus (Kuhl, 1823) (as *Aetobatis* [*sic*] *narinari* [Euphrasen, 1790]) (Aetobatidae: Myliobatiformes).

Additional hosts: Brevitrygon imbricata (Bloch & Schneider, 1801) or *Brevitrygon* sp. 1 *sensu* *Fernando et al. (2019)* (as *Trygon walga* Müller & Henle, 1841; see *Jensen & Guyer, 2021*) and *Neotrygon indica* Pavan-Kumar, Kumar, Pitale, Shen & Borsa, 2018 or *Neotrygon caeruleopunctata* Last, White & Séret, 2016 (as *Trygon kuhlii* [*sic*] Müller & Henle, 1841) (Dasyatidae: Myliobatiformes).

Type locality: **Laccadive Sea, Sri Lanka:** Dutch Bay Spit, Gulf Mannar.

Site of infection: Spiral intestine.

Type specimens: Holotype (VNHM 2099 [originally NMV 2099]; missing).

Remarks: Rhinoptericola aetobatidis has a complex taxonomic history that was well summarized by *Schaeffner (2016)*. He also provided updated illustrations, scolex measurements, and morphological interpretations based on reexamination of the holotype. For this study, the holotype (VNHM 2099) of the species was requested from the Natural History Museum in Vienna for examination, but unfortunately was reported missing (P. Frade, 2020, pers. comm.). The decision here to transfer *R. aetobatidis* to *Rhinoptericola* was thus based on the report of its scolex morphology as given by *Schaeffner (2016)* (*i.e.*, four bothria, the presence of prebulbar organs but lack of gland cells in the bulbs, a characteristic basal armature, and a heteroacanthous typical heteromorphous metabasal armature). These features are consistent with, and unique to, members of the genus *Rhinoptericola*. Because the holotype of *R. aetobatidis* was an immature specimen, the proglottid anatomy of *R. aetobatidis* remains unknown.

Based on the illustrations and interpretations of the armature of the holotype of *R. aetobatidis* by *Schaeffner (2016)*, the species is distinguished easily from *R. megacantha*, *R. butlerae*, and *R. panamensis* by its possession of two (*vs* more than two) macrohooks in the basal armature, and an orientation of metabasal hook rows from external to internal (*vs* from internal to external) tentacle surfaces. Eight specimens of the type host (*Aetobatus ocellatus*) collected in 2018 from the type locality (off Sri Lanka) were examined as part of this study, but unfortunately, no specimens of *R. aetobatidis* were found in those host specimens, nor in specimens of *A. ocellatus* examined from Australia, Indonesia, and the Solomon Islands.

Consideration of older and more recent host reports for *R. aetobatidis*, beyond those from its type host, revealed both to be in need of revision. *Shipley & Hornell (1906)*

reported *Trygon walga* and *Southwell (1924)* reported *T. kuhlii* as hosts of *R. aetiobatidis*, both from Sri Lanka. In light of information presented by *Fernando et al. (2019)* and *Last et al. (2016)* (see also *Jensen & Guyer, 2021*) on the elasmobranchs of Sri Lanka, the identities of these host species are doubtful. Based on their distributions, *Brevitrygon imbricata* or *Brevitrygon* sp. 1 *sensu Fernando et al. (2019)* are the most likely candidates for the host species reported as *T. walga*, and *Neotrygon indica* or *N. caeruleopunctata* could either be the host species reported as *T. kuhlii*. Given the potential for *R. aetobatidis* to parasitize species in multiple genera of batoids in Sri Lanka, we examined three specimens of *N. indica* and one specimen each of *Narcine* cf. *lingula sensu Fernando et al. (2019)*, *Pastinachus ater*, and *Himantura tutul* collected from Sri Lanka in 2018 in search of specimens of *R. aetobatidis*, but none were found. Thus, the host records of *R. aetobatidis* fom Sri Lanka remain uncertain. More recently, *Schaeffner & Beveridge (2014)* reported *Shirleyrhynchus aetobatidis* from the dasyatids *Himantura tutul*, *Maculabatis gerrardi*, and *Pastinachus ater* (as *Himantura uarnak* [Gmelin, 1789], *Himantura gerrardi* [Gray, 1851], and *Pastinachus atrus* [MacLeay, 1883], respectively), and from *P. solocirostris* and the hemiscylliid *Chiloscyllium punctatum*, during a time when *S. aetobatidis* was the valid name with *S. butlerae* its junior synonym (see Remarks section for *R. butlerae*). Voucher specimens of *Schaeffner & Beveridge (2014)* from each of these host species were examined and have been found to be consistent with *R. butlerae* (see Table 3). Finally, as mentioned in the Introduction, the specimen of *R. aetobatidis* (as *Shirleyrhynchus aetobatidis*; LRP 4275) from *Himantura australis* (as *Himantura* cf. *uarnak*) included by *Palm et al. (2009)* and *Olson et al. (2010)* in their phylogenetic analysis was subsequently determined to be misidentified and is actually a specimen of the eutetrarhynchid *Parachristianella indonesiensis* (see *Schaeffner, 2016*).

***Rhinoptericola jensenae* (*Schaeffner & Beveridge, 2012b*) n. comb.**
Figures 8–10

Synonym: *Prochristianella jensenae Schaeffner & Beveridge, 2012b*, in part.

Redescription (based on four paratypes and 17 voucher specimens: three gravid worms, five mature worms, four immature worms, one incomplete worm, cross-sections of one terminal proglottid, and three scoleces prepared for SEM):

Worms apolytic (Fig. 8A); mature worms 3.4–6.3 mm (4.6 ± 1.2; 7) long, gravid worms 4.3–5.1 mm (n = 3) long, maximum width at level of pars bothrialis, pars bulbosa, or gravid proglottid; proglottids 6–11 (9 ± 1.6; 7) in total number in mature and 10–12 (n = 3) in total number in gravid worms.

Scolex (Figs. 8B, 10A and 10B) acraspedote, elongate, slender, 1,133–1,962 (1,603 ± 224.0; 17) long, length:width ratio 3.3–7.8 (5.3 ± 1.3; 15):1. Pars bothrialis 185–329 (274 ± 39.2; 17) long by 214–357 (286 ± 36.8; 17) wide, with four bothria (Figs. 8B, 10B); bothria elliptoid to narrowly elliptoid, 179–282 (231 ± 28.2; 17; 48) long by 68–135 (99 ± 24.3; 6; 18) wide, with free lateral and posterior margins, arranged in dorsal and ventral pairs, not overlapping pars bulbosa; bothrial pits absent. Pintner's cells absent. Pars vaginalis 701–1,336 (1,085 ± 185.4; 16) long by 109–191 (155 ± 23.8; 17) wide at

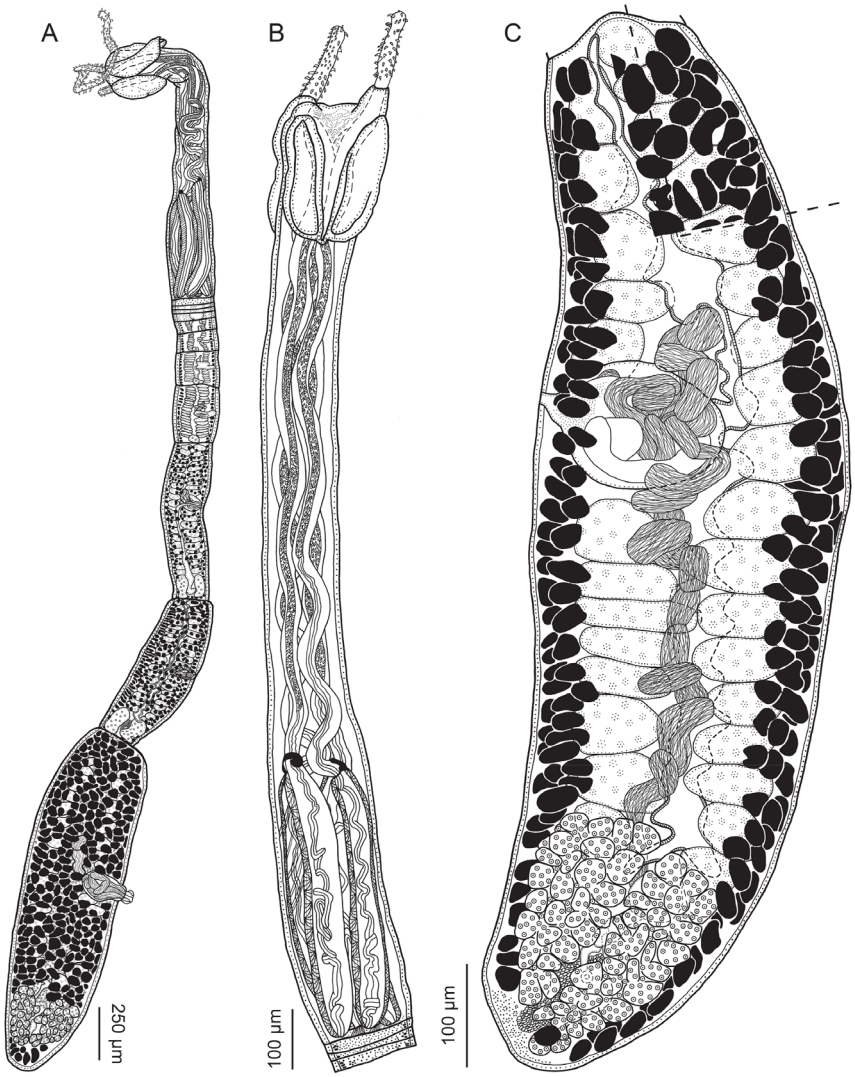

Figure 8 Line drawings of *Rhinoptericola jensenae* (*Schaeffner & Beveridge, 2012b*) n. comb. (A) Whole worm (QM G239457; voucher). (B) Scolex (QM G239461; voucher). (C) Terminal proglottid (QM G239460; voucher); circumcortical vitelline follicles are drawn only on the lateral margins and in the region delimited by dashed lines. Full-size DOI: 10.7717/peerj.12865/fig-8

midpoint; tentacle sheaths sinuous. Pars bulbosa 383–626 (524 ± 75.4; 16) long by 152–252 (208 ± 26.0; 16) wide at midpoint; bulbs very narrowly oblong, thick-walled, muscular, 342–624 (495 ± 81.9; 17; 47) long by 53–92 (72 ± 9.4; 17; 50) wide; bulb length:width ratio 1:4.7–11.3 (7.0 ± 1.5; 17; 46):1; prebulbar organs present; gland cells inside bulbs

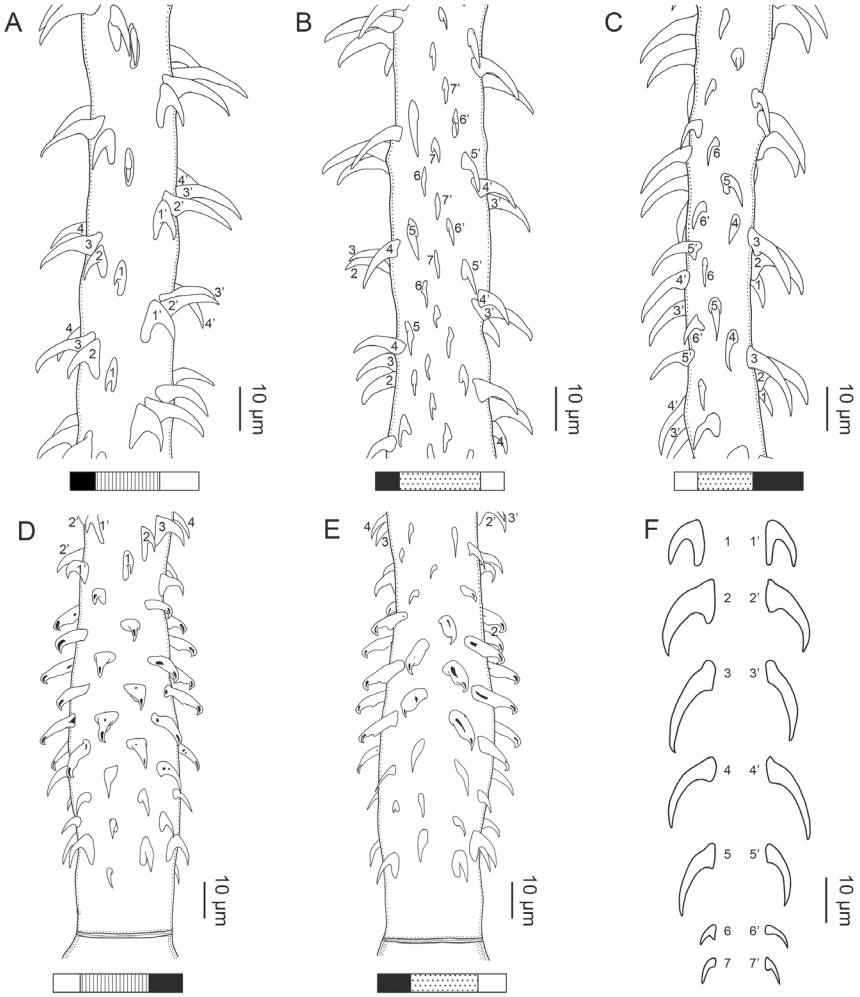

Figure 9 Line drawings of the tentacular armature of *Rhinoptericola jensenae* (*Schaeffner & Beveridge, 2012b*) n. comb. (A) Metabasal armature, bothrial surface (USNM 1661573; voucher). (B) Metabasal armature, antibothrial surface (USNM 1661573; voucher). (C) Metabasal armature, distal antibothrial surface, showing a reduction to six hooks per principal row (LRP 10574; voucher). (D) Basal armature, bothrial surface (QM G239461; voucher). (E) Basal armature, antibothrial surface (QM G239461; voucher). (F) Comparison of metabasal hook shapes.

Full-size DOI: 10.7717/peerj.12865/fig-9

absent; retractor muscles in bulbs 9–24 (14 ± 3.5; 17; 51) wide, originating at base of bulbs. Pars postbulbosa short or absent, 6–42 (19 ± 11.5; 13) long when present. Scolex length ratio (pars bothrialis length:pars vaginalis length:pars bulbosa length) 1:2.5–5.6 (3.9 ± 0.7; 16):1.3–3.0 (1.9 ± 0.4; 16).

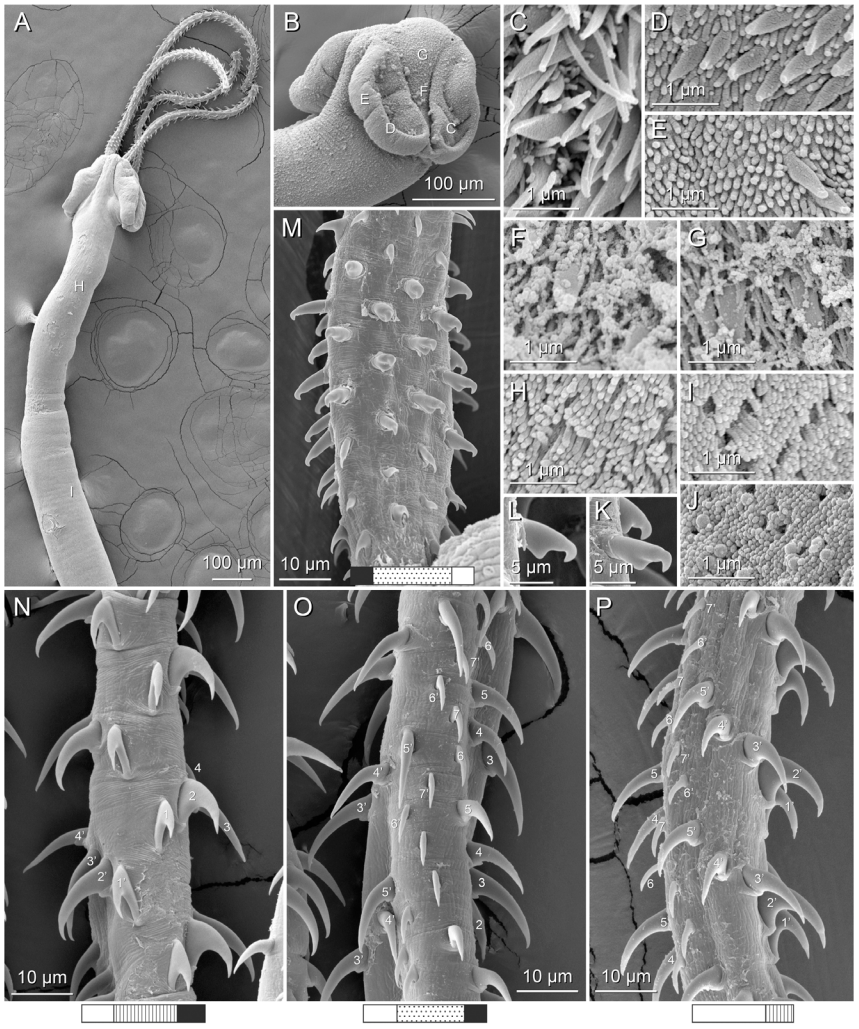

Figure 10 Scanning electron micrographs of *Rhinoptericola jensenae* (*Schaeffner & Beveridge, 2012b*) n. comb. (A) Scolex; small letters indicate the location of details shown in (H–I). (B) Bothria; small letters indicate the location of details shown in (C–G). (C) Distal bothrial surface. (D) Proximal bothrial surface near the bothrial rim. (E) Proximal bothria surface away from the bothrial rim. (F) Surface of the scolex proper between the bothria. (G) Surface of the scolex proper at the apex. (H) Surface of the pars vaginalis. (I) Surface of the pars bulbosa. (J) Strobilar surface. (K) and (L) Falcate, erect, dorsoventrally flattened billhooks with short forward protrusions on their lower surface and mucronate tips (*i.e.*, "can opener-shaped" billhooks) on the antibothrial surface of the basal armature. (M) Basal armature, antibothrial surface. (N) Metabasal armature, bothrial surface. (O) Metabasal armature, antibothrial surface. (P) Metabasal armature, internal surface. Full-size DOI: 10.7717/peerj.12865/fig-10

Tentacles long, with slight basal swelling, not seen retracted into bulbs, at least 1,003 long, 22–35 (30 ± 3.8; 11; 20) wide at base, 26–38 (32 ± 3.4; 10; 17) wide at basal swelling, 12–26 (20 ± 3.6; 8; 15) wide in metabasal region.

Characteristic basal armature present (Figs. 9D, 9E, 10M), 78–97 (87 ± 6.9; 7; 11) long from base of tentacle to start of metabasal armature, consisting of 6–8 indistinct rows of hooks; hooks in posterior-most rows 1–3 uncinate with or without tips extending beyond hook base and with or without slight anterior base extensions to falcate, solid; billhooks in rows 4–8; billhooks falcate, erect, dorsoventrally flattened, solid or hollow, with and without short forward protrusions on lower surface, with recurved mucronate tips; mucronate tips solid or hollow; macrohooks absent.

Metabasal armature (Figs. 9A–9C, 10N–10P) heteroacanthous typical; hooks heteromorphous, solid, arranged in alternating ascending half-spiral rows of seven hooks each (Fig. 9B), reduced to six hooks per row more distally (Fig. 9C); rows originating with hooks 1(1′) on bothrial surface, terminating with hooks 7(7′) or 6(6′) in near single file on antibothrial surface; hooks 1(1′)–3(3′) not angled towards gap between hooks 1(1′). Hook files 1 and (1′) slightly separated, 4–9 (7 ± 1.4; 5; 10) apart. Hooks 1(1′) uncinate, with or without tips extending beyond hook base, 10–13 (12 ± 1.0; 4; 11) long, 6–9 (8 ± 1.1; 4; 11) high, base 8–11 (10 ± 0.8; 4; 11) long. Hooks 2(2′) falcate, with slightly recurved tips and slight anterior base extensions, 14–21 (17 ± 2.2; 5; 14) long, 7–13 (11 ± 1.6; 5; 14) high, base 6–10 (8 ± 1.3; 5; 14) long. Hooks 3(3′) falcate, with slightly recurved tips and slight anterior base extensions, 10–22 (18 ± 3.5; 5; 15) long, 8–16 (11 ± 2.1; 5; 15) high, base 5–7 (6 ± 0.7; 5; 15) long. Hooks 4(4′) falcate, with or without slightly recurved tips, with or without slightly slight anterior base extensions, 10–18 (15 ± 2.6; 4; 11) long, 6–14 (9 ± 2.3; 4; 11) high, base 4–6 (5 ± 0.6; 4; 11) long. Hooks 5(5′) falcate, with or without slightly recurved tips, with or without slightly slight anterior base extensions, 8–17 (12 ± 3.0; 5; 12) long, 4–11 (7 ± 2.7; 5; 12) high, base 4–5 (4 ± 0.5; 5; 12) long. Hooks 6(6′) falcate, with slightly recurved tips and slight anterior base extensions, 6–7 (7 ± 0.5; 3; 6) long, 3–5 (4 ± 0.8; 3; 6) high, base 2–4 (3 ± 0.8; 3; 6) long. Hooks 7(7′) falcate, with slightly recurved tips and slight anterior base extensions, 5–6 (n = 3; 4) long, 3 (n = 3; 4) high, base 3–4 (n = 3; 4) long.

Distal bothrial surfaces (Fig. 10C) with gladiate spinitriches and acicular and capilliform filitriches. Proximal bothrial surfaces near bothrial rims (Fig. 10D) with small gladiate spinitriches and acicular filitriches, away from bothrial rims (Fig. 10E) with few small gladiate spinitriches and acicular filitriches. Scolex proper at apex (Fig. 10G) and between bothria (Fig. 10F) with gladiate spinitriches and capilliform filitriches. Pars vaginalis (Fig. 10H), pars bulbosa (Fig. 10I), and strobila (Fig. 10J) with capilliform filitriches.

Proglottids acraspedote. Neck absent. Immature proglottids 5–10 (8 ± 1.4; 10) in number, wider than long, becoming longer than wide with maturity. Mature proglottids 1–2 (2 ± 0.5; 10) in number; terminal mature proglottids in mature worms 897–1,844 (1,305 ± 371.2; 7) long by 237–461 (306 ± 74.7; 7) wide. Gravid proglottids one (n = 3) in number; terminal gravid proglottids 1,065–1,527 (n = 3) long by 462–530 (n = 3) wide.

Testes 31–38 (36 ± 2.3; 8) in total number, 13–21 (17 ± 2.4; 8) pre-poral, 17–20 (18 ± 1.1; 8) post-poral, 31–111 (55 ± 20.0; 9; 27) long by 53–114 (80 ± 17.6; 7; 21) wide, in

field from anterior margin of proglottid to ovary, slightly overlapping anterior margin of ovary, arranged in two columns (Fig. 8C), essentially in single layer. Vas deferens extending from mid-level of ovary to level anterior to cirrus sac, entering cirrus sac at its antero-medial margin, coiled primarily at level of and anterior to cirrus sac; external and internal seminal vesicles absent. Cirrus sac ovoid to elliptoid, 143–198 (169 ± 24.4; 8) long by 86–159 (123 ± 30.3; 10) wide, containing coiled cirrus; cirrus unarmed, thin-walled. Genital atrium absent. Genital pores separate, at same level, unilateral, 56–70% (62% ± 4.8%; 10) of proglottid length from posterior margin of proglottid in mature proglottids, 54–61% (n = 3) in gravid proglottids. Vagina thick-walled, weakly sinuous, extending from ootype along midline of proglottid to anterior margin of cirrus sac, then laterally at level of cirrus sac, terminating in female genital pore, greatly expanded when sperm-filled; vaginal sphincter absent; seminal receptacle present. Ovary terminal in proglottid, H-shaped in dorsoventral view, tetralobed in cross-section, 108–447 (257 ± 120.9; 10) long by 119–243 (186 ± 40.0; 8) wide, with lobulated margins; ovarian isthmus near center of ovary. Mehlis' gland near posterior margin of ovary. Vitellarium follicular; follicles circumcortical, 10–51 (24 ± 10.7; 10; 30) long by 22–39 (30 ± 5.0; 8; 24) wide, extending entire length of proglottid, interrupted dorsally and ventrally by ovary, partially interrupted ventrally by terminal genitalia; post-ovarian vitelline follicles present. Uterus saccate, medial, dorsal to vagina, bifurcated at posterior end, extending from anterior margin of ovary to anterior margin of proglottid. Uterine duct not observed. Uterine pore absent. Excretory vessels four, arranged in one dorsal and one ventral pair on each lateral margin of proglottid. Eggs single, essentially spherical, 14–21 (n = 3) in diameter *in situ*, non-embryonated; polar filaments absent.

Type host: Pastinachus solocirostris Last, Manjaji & Yearsley, 2005 (Dasyatidae: Myliobatiformes).

Additional hosts: *Rhinoptera neglecta* Ogilby, 1912 (Rhinopteridae: Myliobatiformes); *Aetobatus ocellatus* (Kuhl, 1823) (Aetobatidae: Myliobatiformes); *Pastinachus ater* (Macleay, 1883) and *Himantura australis* (Ramsay & Ogilby, 1886) or *Himantura leoparda* Manjaji-Matsumoto & Last, 2008 (as *H. uarnak*) (Dasyatidae: Myliobatiformes).

Type locality: **South China Sea, Malaysia:** Sematan (01°48′15.45″N, 109°46′47.17″E), Sarawak.

Additional localities: **Gulf of Carpentaria, Australia:** Weipa (12°35′11″S, 141°42′34″E), Queensland. **Indian Ocean, Australia:** Nickol Bay (20°42′0″S, 116°51′0″E), Western Australia. **Timor Sea, Australia:** Dundee Beach (12°45'33"S, 130°21'7"E), Northern Territory, Fog Bay.

Site of infection: Spiral intestine.

Type specimens (verified): Holotype (ZRC.PLA.0409 [originally MZUM(P) 2012.04]), one paratype (ZRC.PLA.0411 [originally MZUM(P) 2012.06]), three paratypes (LRP 7844, LRP 7846–7847), 13 paratypes (AHC 35409, AHC 35412, AHC 35414 [mixed slide, see

Table 3], AHC 35416, AHC 35441–35443, AHC 35445–35450), and one paratype (USNM 1400164 slides 1 and 3 [originally USNPC 105182]).

Type specimens (unverified): Five paratypes (AHC 35414 [mixed slide, see Table 3], AHC 35427, AHC 35428 [mixed slide, see Table 3], AHC 35433 [mixed slide, see Table 3], AHC 35444), one paratype (MZB Ca 174), and one paratype (USNM 1400163 slide 2 [originally USNPC 105181], see Table 3).

Voucher specimens: LRP 10658 (*Schaeffner & Beveridge, 2014* [mixed slide, see Table 3]), LRP 10573–10600 (this study), LRP 10570–10572 (hologenophores; this study); AHC 36891–36893 (this study); QM G239457–G239462 (this study); USNM 1661573–1661575 (this study).

Museum specimens examined: All verified and unverified type specimens excepting one paratype (MZB Ca 174).

Remarks: This species was originally described as the only member of the genus *Prochristianella* Dollfus, 1946 to lack gland cells in the bulbs. The authors noted the morphological similarity to species of *Rhinoptericola* and *Shirleyrhynchus* in this regard but refrained from assigning the species to either genus because it possessed two, rather than four, bothria (*Schaeffner & Beveridge, 2012b*). Following the examination of type and new material it is now clear that the species possesses four bothria. In fact, the line drawing of the scolex and the scanning electron micrograph of the bothria in *Schaeffner & Beveridge (2012b*; figs. 4B and 6B, respectively) both seem to show four bothria.
The possession of four bothria and pre-bulbar organs but a lack of gland cells in the bulbs immediately disqualifies this species from inclusion in *Prochristianella* and those features, as well as its tentacular armature, support the transfer of the species to *Rhinoptericola*. The inclusion of *Rhinoptericola jensenae* in the genus is further supported by its proglottid anatomy. Like the other species of *Rhinoptericola* with known proglottid anatomies, it possesses testes in two columns that overlap the ovary, separate male and female genital pores, a seminal receptacle, circumcortical vitelline follicles, and a uterus bifurcated at the posterior end (see Figs. 8A and 8C). Sequence data also support its inclusion in the genus (see results of phylogenetic analysis).

Unexpectedly, examination of the holotype and 63 of 64 paratypes of *R. jensenae* revealed that the type series is mixed and includes specimens with two distinct tentacular armatures. The holotype (ZRC.PLA.0409 [originally MZUM 2012.04]) and a subset of the paratypes possess a metabasal armature arranged in rows of seven hooks with rows of six hooks more distally on the tentacle, while the remaining paratypes possess a metabasal armature arranged in rows of nine hooks with rows of eight, and then seven, hooks more distally on the tentacle (see Table 3). These latter paratypes with the alternate morphology are described below as the new species *Rhinoptericola schaeffneri* n. sp. While most of the 63 paratypes examined were easily assigned to either *R. jensenae* or *R. schaeffneri* n. sp., six paratypes were problematic (*i.e.*, AHC 35414, AHC 35427–35428,

AHC 35433, AHC 35444, and USNM 1400163 slide 2). These paratypes either had multiple worms of different species mounted on the same slide (referred to as "mixed slides" above), worms with tentacles fully retracted or insufficiently everted to allow for identification to the level of species, or a combination thereof. Notes on these problematic specimens are given in Table 3. Identification as *R. jensenae* or *R. schaeffneri* n. sp. was not possible for the one unexamined paratype (*i.e.*, MZB Ca 174); verification for this specimen is needed.

Rhinoptericola jensenae sensu stricto, as redescribed above, is easily distinguished from its congeners based on differences in overall size and features of the basal armature. *Rhinoptericola jensenae* differs from *R. megacantha* and *R. butlerae* in being smaller in total length (<6.5 mm *vs* >10 mm in *R. megacantha* and *R. butlerae*) and possessing fewer proglottids (<13 *vs* >22 in *R. megacantha* and *R. butlerae*). From *R. panamensis* and *R. aetobatidis*—for which features of the strobila are unknown—*R. jensenae* is readily differentiated based on its possession of a shorter scolex (<2 mm *vs* >2.6 mm in *R. panamensis* and *R. aetobatidis*) and shorter bulbs (<0.63 mm *vs* >1.3 mm in *R. panamensis* and *R. aetobatidis*). *Rhinoptericola jensenae* also lacks, rather than possesses, macrohooks in its characteristic basal armature, further distinguishing it from all four of its larger congeners.

The host species, host associations, and geographic localities reported above in the taxonomic summary for *R. jensenae* are based on new material and the type specimens examined that are morphologically consistent with the redescription. The revised type series comprises specimens from *Rhinoptera neglecta* (Rhinopteridae) from Australia and from three species of dasyatids: *Pastinachus solocirostris* from Malaysia, *P. ater* from Australia, and a species reported by *Schaeffner & Beveridge (2012b)* as *Himantura uarnak* from Australia. According to *Last et al. (2016)*, the only members of the *H. uarnak* complex found in Western Australia, and thus the only members that are candidate hosts for *R. jensenae*, are *Himantura australis* and *H. leoparda*; verification is required. Based on new material, *Aetobatus ocellatus* is reported as a host for the first time. *Rhinoptericola jensenae* is thus restricted to the Indo-Pacific region, parasitizing batoids from Australia and Malaysia (see Table 3). Interestingly, the type specimens of *R. jensenae* deposited by *Schaeffner & Beveridge (2012b)* remain the only reports of this species from Malaysia. Examination of seven specimens of the type host *P. solocirostris*, two specimens of *P. ater*, and two specimens of *Pastinachus gracilicaudus* Last & Manjaji-Matsumoto, 2010 in search of *R. jensenae* yielded no additional material. Instead, specimens of *P. solocirostris* and *P. ater* were found to be parasitized by specimens of the new species, *Rhinoptericola schaeffneri* n. sp., described below. In fact, all new material of *R. jensenae* used in this study came from Australia (see Table 3).

***Rhinoptericola schaeffneri* n. sp.**

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Figures 11–14, 15A and 15B

Synonym: *Prochristianella jensenae Schaeffner & Beveridge, 2012b*, in part.

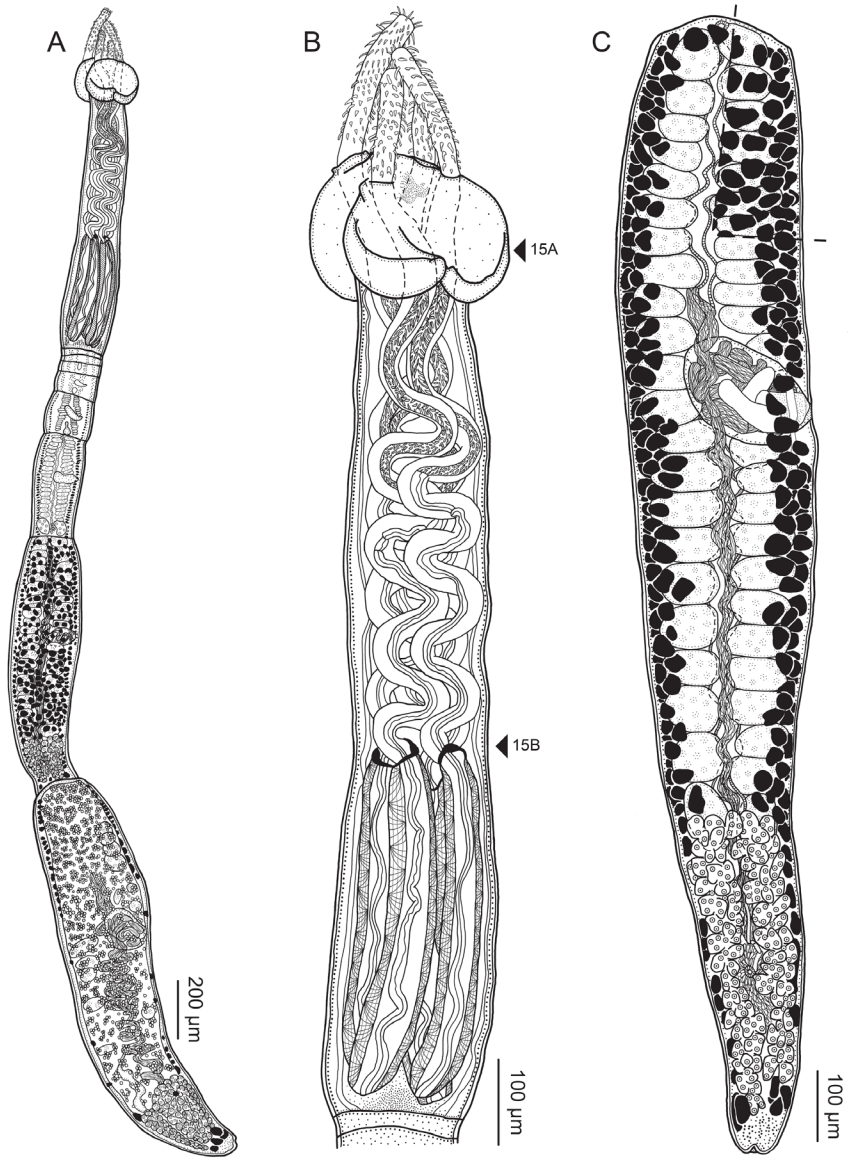

Figure 11 Line drawings of *Rhinoptericola schaeffneri* n. sp. (A) Whole worm (MZUM[P] 2021.1 [H]; holotype). (B) Scolex (MZUM[P] 2021.1 [H]; holotype); arrowheads indicate the level at which the sections in Fig. 15 were taken. (C) Terminal proglottid (USNM 1661588; paratype); circumcortical vitelline follicles are drawn only on the lateral margins and in the region delimited by dashed lines.
Full-size DOI: 10.7717/peerj.12865/fig-11

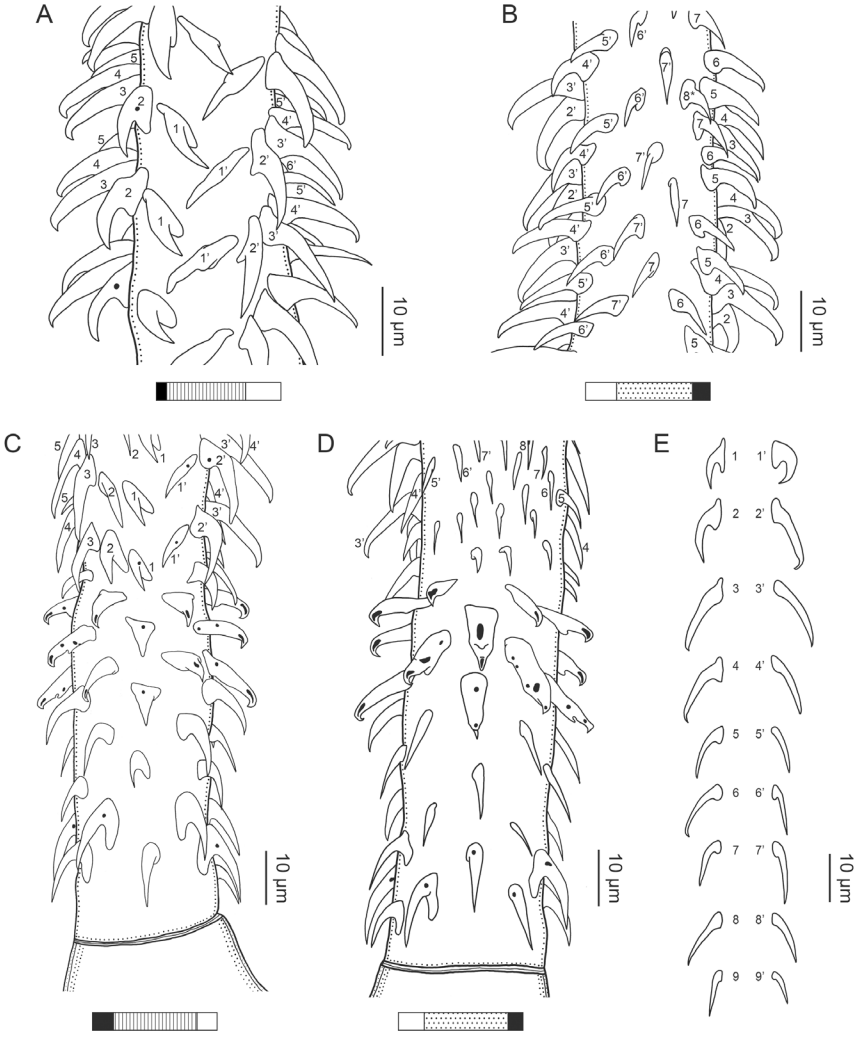

Figure 12 Line drawings of the tentacular armature of *Rhinoptericola schaeffneri* n. sp. (A) Metabasal armature, bothrial surface (USNM 1661589; paratype). (B) Metabasal armature, antibothrial surface (USNM 1661589; paratype), also showing an errant eighth hook shared between the principal rows, denoted with an asterisk (*). (C) Basal armature, bothrial surface (LRP 10602; paratype). (D) Basal armature, antibothrial surface (LRP 10602; paratype). (E) Comparison of metabasal hook shapes.
Full-size DOI: 10.7717/peerj.12865/fig-12

Description (based on one gravid worm, five mature worms, one incomplete worm, five scoleces, cross-sections of one scolex and four scoleces prepared for SEM, and two voucher specimens [AHC 35423 and AHC 35424]):

Worms apolytic (Fig. 11A); mature worms 3.4–6.8 mm (4.4 ± 1.1; 7) long, gravid worms 2.6 mm (n = 1) long, maximum width at level of pars bothrialis, pars bulbosa, or terminal

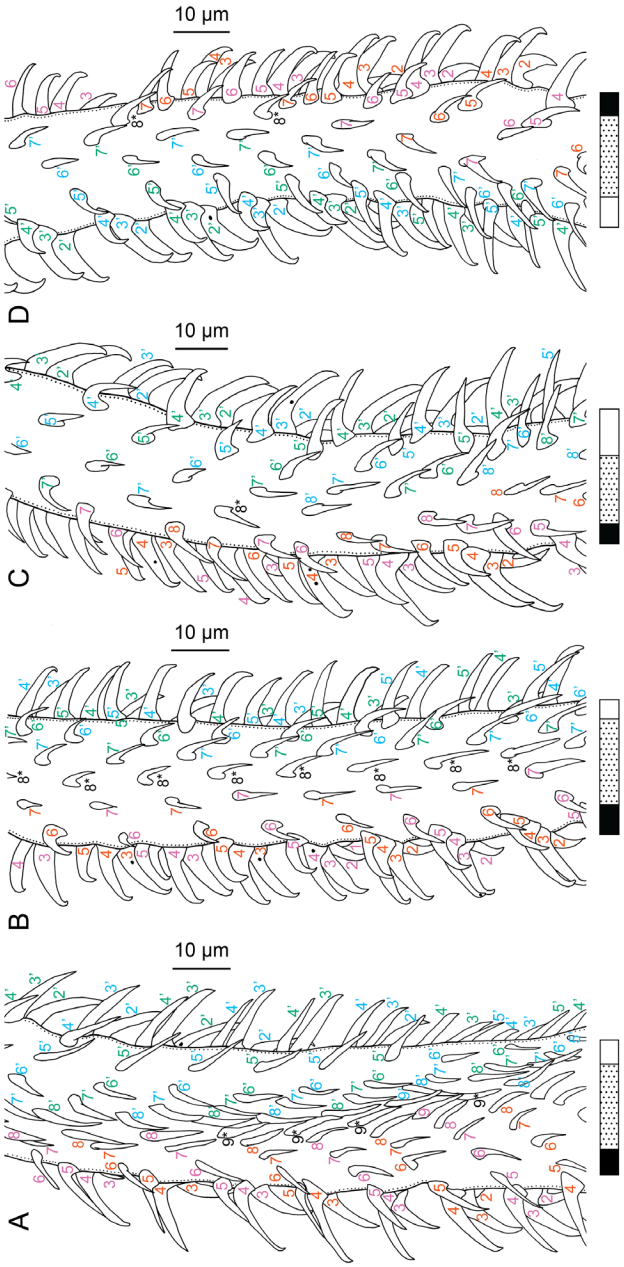

Figure 13 Line drawings of the tentacular armature on the antibothrial surface of *Rhinoptericola schaeffneri* n. sp. showing variation in hook number for principal rows along the tentacle. (A) Meta-basal armature immediately anterior to the basal armature; nine hooks transitioning to eight hooks per principal row (AHC 35424; voucher [paratype of *Prochristianella jensenae* Schaeffner & Beveridge, 2012b]).

Figure 13 (continued)
(B) Metabasal armature ~320 µm anterior to the basal armature; paired principal rows sharing an eighth hook (LRP 10603; paratype). (C) Metabasal armature ~205 µm anterior to the basal armature; eight hooks transitioning to seven hooks per principal row (LRP 10604; paratype). (D) Metabasal armature ~305 µm anterior to the basal armature; seven hooks with an occasional eighth hook per principal row (USNM 1661589; paratype). Hooks are colored by principal row. For hooks 8(8′) and 9(9′), hooks missing their complementary hook are denoted in black font with an asterisk (*).
Full-size DOI: 10.7717/peerj.12865/fig-13

proglottid; proglottids 6–10 (7 ± 1.6; 6) in total number in mature and 7 (n = 1) in total number in gravid worms.

Scolex (Figs. 11B, 14A and 14B) acraspedote, elongate, slender, 938–1,619 (1,216 ± 189.3; 13) long, length:width ratio 2.9–6.7 (4.9 ± 1.1; 13):1. Pars bothrialis 171–327 (227 ± 45.0; 13) long by 204–302 (235 ± 28.9; 13) wide, with four bothria (Figs. 11B, 14A and 14B, 15A); bothria elliptoid, 135–246 (188 ± 34.3; 13; 35) long by 61–100 (87 ± 10.4; 8; 15) wide, with free lateral and posterior margins, arranged in dorsal and ventral pairs, not overlapping pars bulbosa; bothrial pits absent. Pintner's cells absent. Pars vaginalis 536–1,022 (728 ± 165.4; 13) long by 116–208 (174 ± 24.8; 13) wide at midpoint; tentacle sheaths sinuous. Pars bulbosa 168–298 (203 ± 35.7; 13) long by 11–92 (31 ± 21.1; 13) wide at midpoint; bulbs very narrowly oblong, thick-walled, muscular, 360–573 (449 ± 54.8; 13; 38) long by 51–98 (67 ±11.0; 13; 37) wide; bulb length:width ratio 1:5.2–8.2 (6.8 ± 0.9; 13; 37):1; prebulbar organs present; gland cells inside bulbs absent; retractor muscles in bulbs 8–36 (15 ± 6.6; 13; 37) wide, originating at base of bulbs. Pars postbulbosa short, 11–92 (31 ± 21.1; 13) long. Scolex length ratio (pars bothrialis length:pars vaginalis length:pars bulbosa length) 1:2.4–4.6 (3.3 ± 0.7; 12):1.2–2.8 (2.2 ± 0.4; 12).

Tentacles long, with slight basal swelling, not seen retracted into bulbs, at least 535 long, 21–34 (27 ± 3.6; 10; 21) wide at base, 23–39 (29 ± 4.5; 10; 16) wide at basal swelling, 21–29 (24 ± 2.7; 10; 15) wide in metabasal region.

Characteristic basal armature present (Figs. 12C and 12D), 49–78 (64 ± 7.7; 10; 19) long from base of tentacle to start of metabasal armature, consisting of 5–6 indistinct rows of hooks; hooks in posterior-most row uncinate, with tips extending beyond hook base, solid; hooks in rows 2–3 on bothrial and internal surfaces uncinate, with tips extending beyond hook base, solid or hollow, and on antibothrial and external surfaces spiniform, solid; hooks in rows 4–6 on bothrial surface triangular with recurved tips, dorsoventrally flattened, solid or hollow, and on antibothrial, internal, and external surfaces billhooks; billhooks falcate, erect, dorsoventrally flattened, solid or hollow, with (Fig. 14N) and without (Fig. 14O) short forward protrusions on lower surface, with recurved mucronate tips; mucronate tips solid or hollow; macrohooks absent.

Metabasal armature (Figs. 12A, 12B, 12E, 13A–13D, 14K and 14L) heteroacanthous typical, heteromorphous; metabasal hooks solid or hollow, arranged in alternating ascending half-spiral rows of nine hooks immediately anterior to basal armature (Fig. 13A), reduced to eight (Figs. 13B, 13C, 14L) and then seven (Figs. 13C and 13D) hooks per row more distally; rows originating with hooks 1(1′) on bothrial surface,

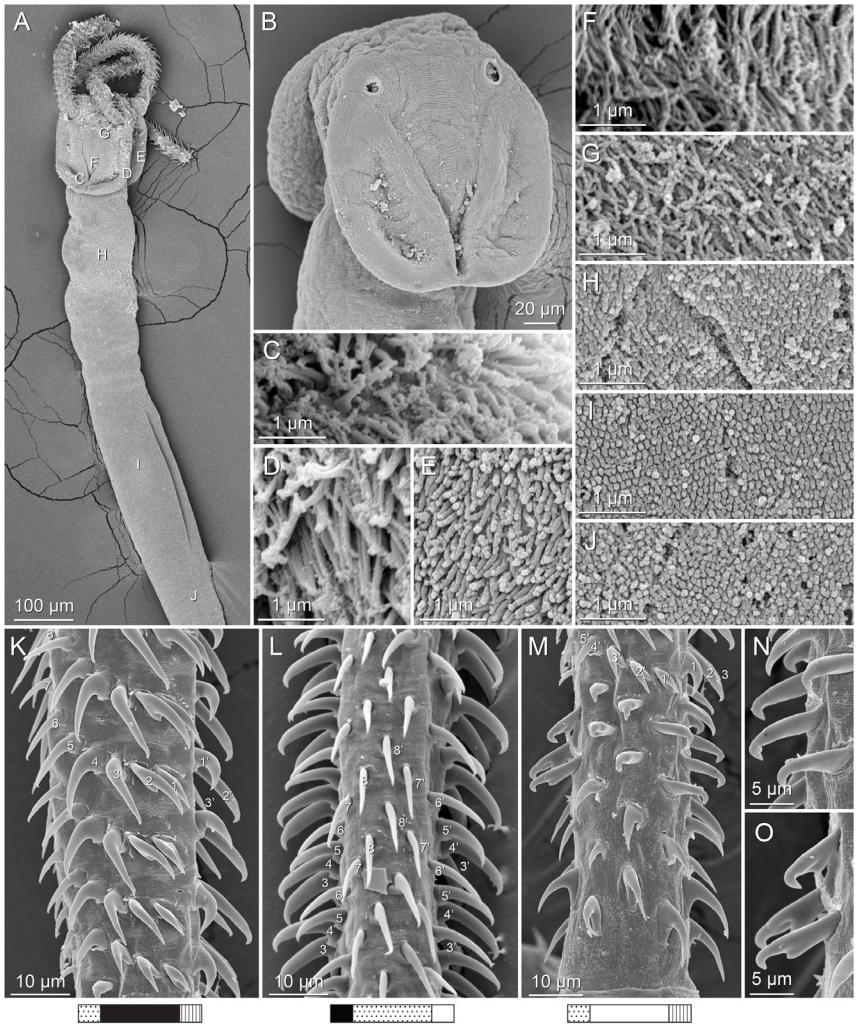

Figure 14 Scanning electron micrographs of *Rhinoptericola schaeffneri* n. sp. (A) Scolex; small letters indicate the location of details shown in (C–J). (B) Bothria. (C) Distal bothrial surface. (D) Proximal bothrial surface near the bothrial rim. (E) Proximal bothrial surface away from the bothrial rim. (F) Surface of the scolex proper between the bothria. (G) Surface of the scolex proper at the apex. (H) Surface of the pars vaginalis. (I) Surface of the pars bulbosa. (J) Strobilar surface. (K) Metabasal armature, external surface. (L) Metabasal armature, antibothrial surface. (M) Basal armature, internal surface. (N) Falcate, erect, dorsoventrally flattened billhooks with mucronate tips on the bothrial and internal surfaces of the basal armature. (O) Falcate, erect, dorsoventrally flattened billhooks with short forward protrusions on their lower surface and mucronate tips (*i.e.*, "can opener-shaped" billhooks) on the antibothrial and external surfaces of the basal armature.

Full-size DOI: 10.7717/peerj.12865/fig-14

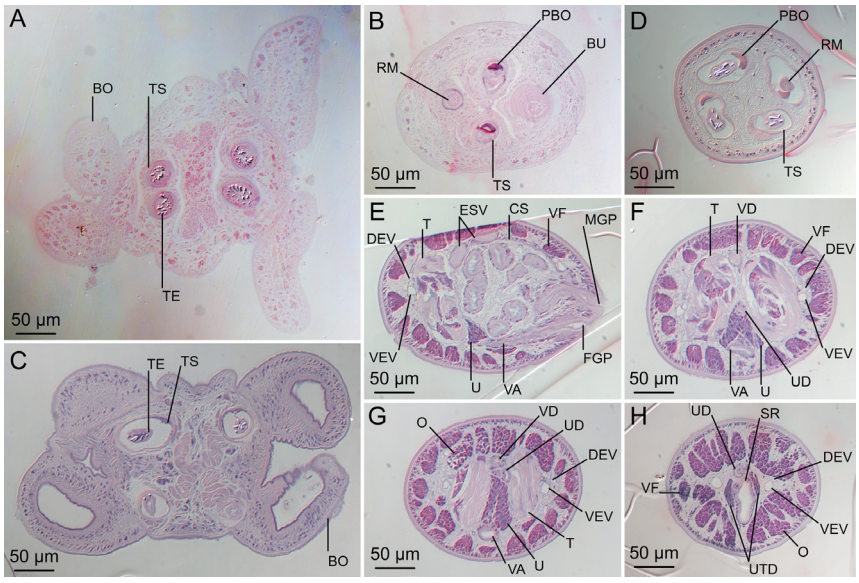

Figure 15 Light micrographs of cross-sections of *Rhinoptericola schaeffneri* n. sp. (A–B) and *Rhinoptericola mozambiquensis* n. sp. (C–H). (A) Scolex at the level of the bothria. (B) Scolex at the level of the prebulbar organs. (C) Scolex at the level of the bothria. (D) Scolex at the level of the prebulbar organs. (E) Mature proglottid at the level of the genital pores. (F) Mature proglottid between ovary and genital pores. (G) Mature proglottid at the anterior margin of the ovary. (H) Mature proglottid anterior to the ootype region. Abbreviations: BO, bothrium; BU, bulb; CS, cirrus sac; ESV, external seminal vesicle; DEV, dorsal excretory vessel; FGP, female genital pore; MGP, male genital pore; O, ovary; PBO, prebulbar organ; RM, retractor muscle; SR, seminal receptacle; T, testis; TE, tentacle; TS, tentacle sheath; U, uterus; UD, uterine duct; UTD, uterine diverticulum; VA, vagina; VEV, ventral excretory vessel; VD, vas deferens; VF, vitelline follicle. Full-size DOI: 10.7717/peerj.12865/fig-15

terminating with hooks 9(9′), 8(8′) or 7(7′) in near single file on antibothrial surface; hooks 1(1′)–3(3′) conspicuously angled towards gap between hooks 1(1′). Hook files 1 and (1′) slightly separated, 5–9 (6 ± 1.2; 8; 12) apart at base. Hooks 1(1′) occasionally with overlapping tips, uncinate, with tips extending beyond hook base, with or without anterior base extensions, 7–13 (11 ± 2.0; 7; 12) long, 4–8 (6 ± 1.1; 7; 12) high, base 4–10 (7 ± 2.2; 7; 12) long. Hooks 2(2′) uncinate with tips extending beyond hook base to falcate with slightly recurved tips and anterior base extensions, 11–19 (16 ± 2.5; 8; 13) long, 5–12 (8 ± 2.3; 8; 13) high, base 5–9 (6 ± 1.4; 8; 13) long. Hooks 3(3′) falcate, with recurved tips and anterior base extensions, 15–25 (18 ± 3.4; 8; 11) long, 5–14 (9 ± 2.4; 8; 11) high, base 4–7 (5 ± 1.0; 8; 11) long. Hooks 4(4′) falcate, with slightly recurved tips and anterior base extensions, 13–19 (16 ± 1.9; 7; 10) long, 4–12 (8 ± 2.4; 7; 10) high, base 2–6 (5 ± 1.2; 7; 10) long. Hooks 5(5′) hastate to falcate with slightly recurved tips, 11–16 (14 ± 1.9; 8; 11) long, 5–11 (8 ± 2.0; 8; 11) high, base 2–5 (4 ± 1.0; 8; 11) long. Hooks 6(6′) uncinate, with tips extending beyond hook base, 3–13 (10 ± 3.0; 8; 11) long, 4–10 (6 ± 2.3; 8; 11) high, base 3–12 (4 ± 2.6; 8; 11) long. Hooks 7(7′) uncinate with tips extending beyond hook

base to falcate, 9–13 (11 ± 1.5; 5; 6) long, 6–10 (7 ± 1.6; 5; 6) high, base 3–5 (4 ± 0.6; 5; 6) long. Hooks 8(8′) uncinate with tips extending beyond hook base to falcate, 13–18 (n = 2; 3) long, 5–9 (n = 2; 3) high, base 4 (n = 2; 3) long. Hooks 9(9′) falcate, 7–11 (9 ± 1.2; 6; 8) long, 2–4 (3 ± 0.7; 4; 5) high, base 3–4 (3 ± 0.5; 6; 8) long.

Distal bothrial surfaces (Fig. 14C) with small gladiate spinitriches and acicular and capilliform filitriches. Proximal bothrial surfaces near bothrial rims (Fig. 14D) with gladiate spinitriches and capilliform filitriches, away from bothrial rims (Fig. 14E) with acicular filitriches. Scolex proper near and at apex (Fig. 14G) with acicular filitriches and between bothria (Fig. 14F) with capilliform filitriches. Pars vaginalis (Fig. 14H), pars bulbosa (Fig. 14I), and strobila (Fig. 14J) with acicular filitriches.

Proglottids acraspedote. Neck absent. Immature proglottids 4–8 (5 ± 1.3; 7) in number, wider than long, becoming longer than wide with maturity. Mature proglottids 1–2 (2 ± 0.5; 7) in number; terminal mature proglottids in mature worms 1,325–1,658 (1,465 ± 117.0; 7) long by 174–365 (262 ± 59.6; 8) wide. Gravid proglottids 1 (n = 1) in number; terminal gravid proglottids 1,605 (n = 1) long by 369 (n = 1) wide.

Testes 36–49 (43 ± 4.1; 8) in total number, 20–24 (23 ± 1.8; 8) pre-poral, 13–25 (20 ± 4.2; 8) post-poral, 33–88 (60 ± 15.8; 8; 23) long by 64–122 (83 ± 15.2; 6; 16) wide, in field from anterior margin of proglottid to ovary, slightly overlapping anterior margin of ovary, arranged in two columns (Fig. 11C), essentially in single layer. Vas deferens extending from mid-level of ovary to level slightly anterior to cirrus sac, entering cirrus sac at its antero-medial margin, coiled primarily anterior to cirrus sac; external and internal seminal vesicles absent. Cirrus sac ovoid to elliptoid, occasionally bent anteriorly, 128–190 (164 ± 22.6; 6) long by 84–170 (133 ± 27.2; 8) wide, containing coiled cirrus; cirrus unarmed, thin-walled. Genital atrium absent. Genital pores separate, at same level, unilateral, 48–65% (57% ± 7.0%; 8) of proglottid length from posterior margin of proglottid in mature proglottids and 66% (n = 1) in gravid proglottids. Vagina thick-walled, weakly sinuous, extending from ootype along midline of proglottid to anterior margin of cirrus sac, then laterally at level of cirrus sac, terminating in female genital pore, greatly expanded when sperm-filled; vaginal sphincter absent; seminal receptacle present. Ovary terminal in proglottid, H-shaped in dorsoventral view, tetralobed in cross-section, 128–190 (164 ± 22.6; 6) long by 84–170 (133 ± 27.2; 8) wide, with lobulated margins; ovarian isthmus near center of ovary. Mehlis' gland near posterior margin of ovary. Vitellarium follicular; follicles circumcortical, 17–54 (25 ± 8.9; 7; 21) long by 24–46 (31 ± 7.4; 5; 15) wide, extending entire length of proglottid, interrupted dorsally and ventrally by ovary, partially interrupted ventrally by terminal genitalia; post-ovarian vitelline follicles present. Uterus saccate, medial, dorsal to vagina, bifurcated at posterior end, extending from anterior margin of ovary to anterior margin of proglottid. Uterine duct not observed. Uterine pore absent. Excretory vessels four, arranged in one dorsal and one ventral pair on each lateral margin of proglottid. Eggs single, essentially spherical, 13–16 (n = 3) in diameter *in situ*, non-embryonated; polar filaments absent.

Type host: Pastinachus solocirostris Last, Manjaji & Yearsley, 2005 (Dasyatidae: Myliobatiformes).

Additional hosts: *Pastinachus ater* (Macleay, 1883) and *Pastinachus gracilicaudus* Last & Manjaji-Matsumoto, 2010 (Dasyatidae: Myliobatiformes).

Type locality: **South China Sea, Malaysia:** Sematan (01°48′15.45″N, 109°46′47.17″E), Sarawak.

Additional localities: **Makassar Strait, Indonesia:** Muara Pasir (01°45′58.92″S, 116°23′36.09″E), East Kalimantan; and Sei Kerbau (00°31′44.50″S, 117°09′32.90″E), East Kalimantan. **South China Sea, Malaysia:** Mukah (02°53′52.16″N, 112°05′44.12″E), Sarawak. **Sulu Sea, Malaysia:** Kampung Tetabuan (06°01′10.32″N, 117°42′14.76″E), Sabah.

Site of infection: Spiral intestine.

Type specimens: Holotype (MZUM[P] 2021.1 [H]), two paratypes (MZUM[P] 2021.2 [P]–2021.3 [P]), five paratypes (LRP 10602–10656), one paratype (SBC-P-00077), one paratype (MZB Ca 211), and four paratypes (USNM 1661588–1661591).

Voucher specimens: AHC 35408, AHC 35410–11, AHC 35413, AHC 35415, AHC 35417–26, AHC 35428 (mixed slide, see Table 3), AHC 35429–32, AHC 35433 (mixed slide, see Table 3), AHC 35434–40; MZB Ca 168–75; LRP 7843, LRP 7845, LRP 7848–9; USNM 1400163 slide 1 (originally USNPC 105181), USNM 1400164 slides 2, 4, and 5 (originally USNPC 105182); and ZRC.PLA.0410 (originally MZUM[P] 2012.05), ZRC. PLA.0412–3 (originally MZUM[P] 2012.07–8) (all originally deposited as paratypes of *Prochristianella jensenae*; *Schaeffner & Beveridge, 2012b*); LRP 10657 (*Schaeffner & Beveridge, 2014*), LRP 10658 (*Schaeffner & Beveridge, 2014* [mixed slide, see Table 3]); LRP 10601 (hologenophore, this study).

Museum specimens examined: All voucher specimens.

Etymology: This species is named for Dr. Bjoern C. Schaeffner for his contributions to trypanobatoid taxonomy.

Remarks: Rhinoptericola schaeffneri n. sp. is erected for new material and the paratypes of *Prochristianella jensenae* that were found to not be conspecific with *R. jensenae* as redescribed above. *Rhinoptericola schaeffneri* n. sp. can be distinguished from all species of *Rhinoptericola*—including *R. jensenae*—by its unique metabasal armature: *R. schaeffneri* n. sp. possesses nine hooks per row immediately anterior to the basal armature (see Fig. 13A), diminishing to eight, and then seven, hooks per row more distally on the tentacle (see Figs. 13B–13D), while its congeners possess either seven hooks per principal row (*e.g.*, see Fig. 3C), or seven hooks per principal row proximally, diminishing to six hooks per principal row more distally on the tentacle (*e.g.*, see Figs. 9B and 9C). *Rhinoptericola schaeffneri* n. sp. is similarly unique in terms of the shape and size of its metabasal hooks along a row: in *R. schaeffneri* n. sp., hooks 1(1′)–3(3′) are consistently angled towards the space between hook files 1 and (1′), and hooks gradually diminish in size along a row (see Figs. 12, 13, 14K and 14L). In the other five species of

Rhinoptericola, hooks 1(1′)–3(3′) are not angled towards the space between hook files 1 and (1′), and there is both a stark physical separation and change in hook size between hooks 5(5′) and 6(6′) (*e.g.*, see Figs. 10O and 10P for *R. jensenae*).

Rhinoptericola schaeffneri n. sp. can be distinguished further from *R. megacantha* and *R. butlerae* based on its shorter total length (<7 mm *vs* >10 mm in *R. megacantha* and *R. butlerae*) and fewer number of proglottids (<11 *vs* >22 in *R. megacantha* and *R. butlerae*), and from *R. panamensis* and *R. aetobatidis* based on its shorter scolex (<1.7 mm *vs* >3.8 mm in *R. panamensis* and *R. aetobatidis*) and shorter bulbs (<0.6 mm *vs* >1.9 mm in *R. panamensis* and *R. aetobatidis*). This new species is similar in size to *R. jensenae* but the two can be further distinguished based on metabasal hook shape: in *Rhinoptericola schaeffneri* n. sp., metabasal hooks 5(5′)–7(7′) are thinner and more elongate than those in *R. jensenae* (see Figs. 14L *vs* 10O). *Rhinoptericola schaeffneri* n. sp. is only known from species of cowtail rays (genus *Pastinachus* Forsskål, 1775) and only from the waters off Malaysia and Indonesia.

It should be noted that three of the paratypes of *Prochristianella jensenae* (*i.e.*, AHC 35414, AHC 35428, and AHC 35433) and one voucher specimen (*i.e.*, LRP 10658) consist of slides with specimens confirmed as *R. schaeffneri* mounted alongside worms of other species (including, for AHC 35414 and LRP 10658, specimens of *R. jensenae*). Notes on these specimens are given in Table 3.

***Rhinoptericola mozambiquensis* n. sp.**

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Figures 15C–15H, 16–18

Description (based on five gravid worms, 16 mature worms, one immature worm, cross-sections of one scolex and one partial strobila, and three scoleces and one partial strobila prepared for SEM):

Worms apolytic (Fig. 16A); mature worms 2.6–4.8 mm (3.7 ± 0.6; 16) long, gravid worms 1.6–5.9 mm (4.2 ± 1.7; 5) long, maximum width at level of pars bothrialis or terminal proglottid; proglottids 5–10 (7 ± 1.3; 16) in total number in mature and 6–10 (9 ± 1.6; 5) in total number in gravid worms.

Scolex (Figs. 16A, 16B, 18A) acraspedote, elongate, slender, 1,122–1,862 (1,389 ± 206.6; 22) long, length:width ratio 2.4–5.6 (3.5 ± 0.9; 19):1. Pars bothrialis 192–380 (251 ± 51.8; 18) long by 215–357 (277 ± 46.6; 19) wide, with four bothria (Figs. 15D, 16A, 16B, 18A); bothria narrowly elliptoid to very deeply ovoid, 133–273 (194 ± 33.2; 20; 54) long by 55–164 (110 ± 23.2; 16; 42) wide, with free lateral and posterior margins, arranged in dorsal and ventral pairs, not overlapping pars bulbosa; bothrial pits absent. Pintner's cells absent. Pars vaginalis 724–1,371 (932 ± 179.9; 22) long by 120–225 (162 ± 25.3; 22) wide at midpoint; tentacle sheaths sinuous. Pars bulbosa 379–577 (461 ± 54.9; 22) long by 160–237 (191 ± 19.1; 22) wide at midpoint; bulbs very narrowly oblong, thick-walled, muscular, 343–565 (452 ± 50.5; 21; 66) long by 50–95 (68 ± 10.1; 22; 66) wide; bulb length: width ratio 4.4–11.0 (6.7 ± 1.2; 22; 64):1; prebulbar organs present; gland cells inside bulbs absent; retractor muscles in bulbs 10–23 (15 ± 2.8; 22; 66) wide, originating at base of

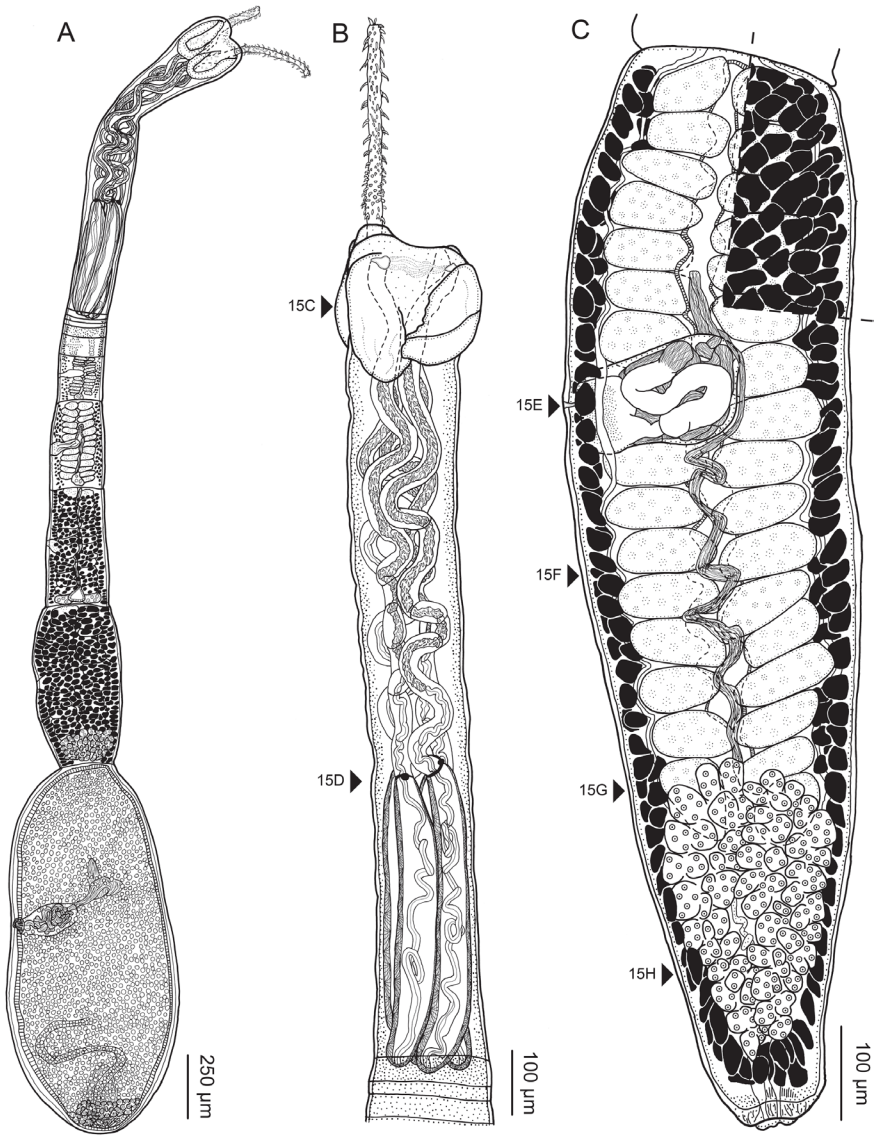

Figure 16 Line drawings of *Rhinoptericola mozambiquensis* n. sp. (A) Whole worm (USNM 1661599; holotype). (B) Scolex (USNM 1661596; paratype). (C) Terminal proglottid (USNM 1661598; paratype); circumcortical vitelline follicles are drawn only on the lateral margins and in the region delimited by dashed lines. Arrowheads indicate the level at which the sections in Fig. 15 were taken.
Full-size DOI: 10.7717/peerj.12865/fig-16

bulbs. Pars postbulbosa short or absent, 10–18 (n = 3) long when present. Scolex length ratio (pars bothrialis length:pars vaginalis length:pars bulbosa length) 1:2.9–4.6 (3.9 ± 0.5; 18):1.2–2.4 (1.9 ± 0.3; 18).

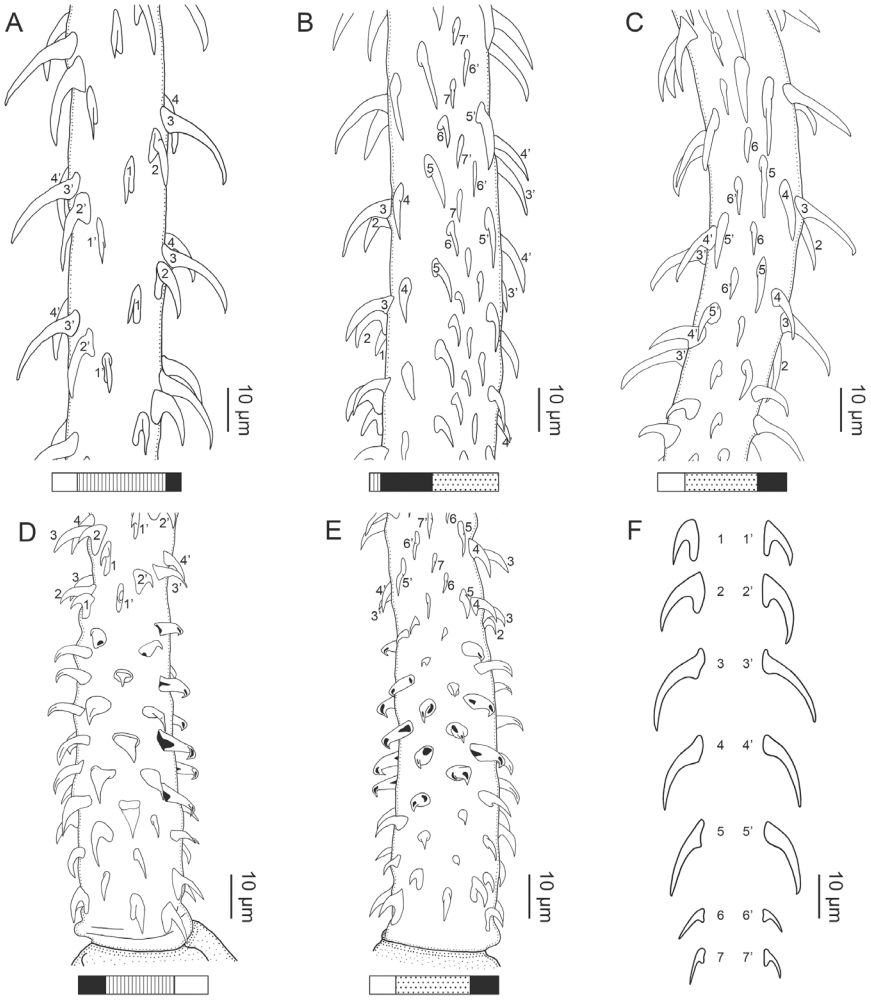

Figure 17 Line drawings of the tentacular armature of *Rhinoptericola mozambiquensis* n. sp. (A) Metabasal armature, bothrial surface (USNM 1661596; paratype). (B) Metabasal armature, external and antibothrial surfaces (USNM 1661597; paratype). (C) Metabasal armature, antibothrial surface more distal on the tentacle showing a reduction to six hooks per principal row (LRP 10661; paratype). (D) Basal armature, bothrial surface (LRP 10663; paratype). (E) Basal armature, antibothrial surface (LRP 10663; paratype). (F) Comparison of metabasal hook shapes.

Full-size DOI: 10.7717/peerj.12865/fig-17

Tentacles long, with slight basal swelling, occasionally retracted into bulbs, at least 1,007 long, 19–48 (29 ± 5.2; 21; 43) wide at base, 21–38 (31 ± 3.8; 19; 34) wide at basal swelling, 15–34 (21 ± 3.9; 19; 36) wide in metabasal region.

Characteristic basal armature present (Figs. 17D, 17E, 18P), 71–133 (91 ± 12.3; 17; 26) long from base of tentacle to start of metabasal armature, consisting of 6–7 indistinct rows

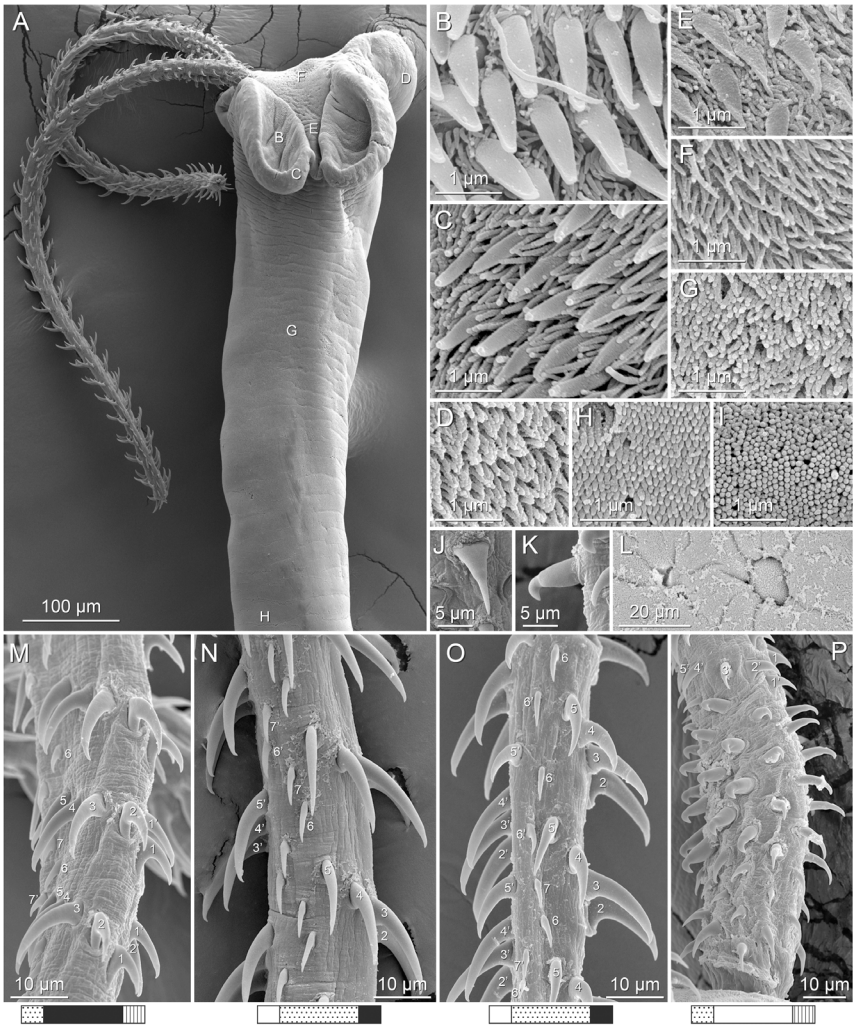

Figure 18 Scanning electron micrographs of *Rhinoptericola mozambiquensis* n. sp. (A) Scolex; small letters indicate the location of details shown in (B–H). (B) Distal bothrial surface. (C) Proximal bothria surface near the bothrial rim. (D) Proximal bothria surface away from the bothrial rim. (E) Surface of the scolex proper between the bothria. (F) Surface of the scolex proper at the apex. (G) Surface of the pars vaginalis. (H) Surface of the pars bulbosa. (I) Strobilar surface. (J) Triangular dorsoventrally flattened hook with the tip extending well beyond the hook base on the bothrial surface of the basal armature. (K) Falcate, erect, dorsoventrally flattened billhook with a mucronate tip on the internal and external surfaces of the basal armature. (L) Separate male and female genital pores. (M) Metabasal armature, external surface. (N) Metabasal armature, antibothrial surface. (O) Metabasal armature, distal antibothrial surface showing the transition from seven to six hooks per principal row. (P) Basal armature, internal surface.
Full-size DOI: 10.7717/peerj.12865/fig-18

of hooks; hooks in posterior-most rows 1–3 uncinate with or without tips extending beyond hook base and with or without slight anterior base extensions to falcate, solid; hooks in rows 4–7 on bothrial surface triangular, dorsoventrally flattened, with tips extending well beyond hook base, solid, and on antibothrial, internal, and external surfaces billhooks; billhooks falcate, erect, dorsoventrally flattened, solid or hollow, with recurved mucronate tips; mucronate tips solid or hollow; macrohooks absent.

Metabasal armature (Figs. 17A–17C, 17F, 18M–18O) heteroacanthous typical; hooks heteromorphous, solid, arranged in alternating ascending half-spiral rows of seven hooks each, reducing to six hooks each more distally (Figs. 17C, 18O); rows originating with hooks 1(1′) on bothrial surface, terminating with hooks 7(7′) or 6(6′) in near single file on antibothrial surface; hooks 1(1′)–3(3′) not angled towards space between hook files 1 and (1′). Hook files 1 and (1′) slightly separated, 3–9 (5 ± 1.3; 14; 23) apart. Hooks 1(1′) uncinate, with or without tips extending beyond hook base, 8–15 (13 ± 1.8; 15; 29) long, 6–15 (8 ± 1.9; 15; 29) high, base 6–11 (9 ± 1.2; 15; 29) long. Hooks 2(2′) falcate, with slightly recurved tips and slight anterior base extensions, 14–21 (18 ± 1.8; 17; 30) long, 8–15 (12 ± 2.0; 17; 30) high, base 6–11 (8 ± 1.2; 17; 30) long. Hooks 3(3′) falcate, with slightly recurved tips and slight anterior base extensions, 14–24 (20 ± 2.4; 18; 33) long, 9–18 (13 ± 2.3; 18; 33) high, base 5–9 (6 ± 1.1; 18; 33) long. Hooks 4(4′) falcate, with or without slightly recurved tips, with or without slightly slight anterior base extensions, 14–21 (17 ± 2.0; 17; 23) long, 4–16 (10 ± 2.5; 17; 23) high, base 4–6 (6 ± 0.6; 17; 23) long. Hooks 5(5′) falcate, with or without slightly recurved tips, with or without slight anterior base extensions, 13–19 (17 ± 1.7; 13; 15) long, 5–15 (10 ± 2.5; 13; 15) high, base 5–7 (6 ± 0.7; 13; 15) long. Hooks 6(6′) falcate, with slightly recurved tips and slight anterior base extensions, 6–8 (7 ± 0.8; 10; 13) long, 3–5 (4 ± 0.8; 10; 13) high, base 3–5 (3 ± 0.7; 10; 13) long. Hooks 7(7′) falcate, with slightly recurved tips and slight anterior base extensions, 6–8 (7 ± 0.8; 9; 11) long, 2–5 (4 ± 0.8; 9; 11) high, base 3–5 (4 ± 0.8; 9; 11) long.

Distal bothrial surfaces (Fig. 18B) with large gladiate spinitriches and acicular to capilliform filitriches. Proximal bothrial surfaces near bothrial rims (Fig. 18C) with small gladiate spinitriches and capilliform filitriches, away from bothrial rims (Fig. 18D) with capilliform filitriches only. Scolex proper near and at apex (Fig. 18F) with acicular to capilliform filitriches and between bothria (Fig. 18E) with small gladiate spinitriches and acicular to capilliform filitriches. Pars vaginalis (Fig. 18G), pars bulbosa (Fig. 18H), and strobila (Fig. 18I) with capilliform filitriches.

Proglottids acraspedote. Neck absent. Immature proglottids 4–9 (6 ± 1.4; 21) in number, wider than long, becoming longer than wide with maturity. Mature proglottids 0–2 (1 ± 0.5; 21) in number; terminal mature proglottids in mature worms 708–1,562 (1,101 ± 229.5; 16) long by 232–419 (306 ± 53.0; 16) wide. Gravid proglottids one (n = 5) in number; terminal gravid proglottids 1,407–1,970 (1,735 ± 261.1; 5) long by 427–690 (n = 4) wide.

Testes 23–35 (29 ± 2.8; 16) in total number, 11–18 (15 ± 1.7; 16) pre-poral, 12–18 (14 ± 1.7; 16) post-poral, 38–114 (64 ± 17.9; 18; 45) long by 46–118 (88 ± 14.4; 15; 36) wide, in field from anterior margin of proglottid to ovary, slightly overlapping anterior

margin of ovary, arranged in two columns (Fig. 16C), essentially in single layer (Fig. 15F). Vas deferens extending from near mid-level of ovary to slightly anterior to anterior margin of cirrus sac, entering cirrus sac at its antero-medial margin; external and internal seminal vesicles absent. Cirrus sac ovoid to elliptoid, 164–206 (183 ± 10.7; 12) long by 84–178 (133 ± 23.9; 16) wide, containing coiled cirrus; cirrus unarmed, thin-walled. Genital atrium absent. Genital pores separate (Figs. 15E, 18L), at same level, unilateral, 56–70% (64% ± 3.8%; 16) of proglottid length from posterior margin of proglottid in mature proglottids and 58–65% (n = 3) in gravid proglottids. Vagina thick-walled, weakly sinuous, extending from ootype along midline of proglottid to anterior margin of cirrus sac, then laterally at level of cirrus sac, terminating in female genital pore, greatly expanded when sperm-filled; vaginal sphincter absent; seminal receptacle present. Ovary terminal in proglottid, H-shaped in dorsoventral view, tetralobed in cross-section (Fig. 15H), 187–427 (277 ± 62.3; 15) long by 156–266 (193 ± 36.3; 11) wide, with lobulated margins; ovarian isthmus near center of ovary. Mehlis' gland near posterior margin of ovary. Vitellarium follicular; follicles circumcortical, 16–48 (30 ± 7.8; 20; 50) long by 18–64 (34 ± 8.4; 17; 42) wide, extending entire length of proglottid, interrupted dorsally and ventrally by ovary and partially interrupted ventrally by terminal genitalia; post-ovarian vitelline follicles present. Uterus saccate, medial, dorsal to vagina, bifurcated at posterior end (Fig. 15H), extending from anterior margin of ovary to anterior margin of proglottid. Uterine duct not observed. Uterine pore absent. Excretory vessels four, arranged in one dorsal and one ventral pair on each lateral margin of proglottid. Eggs single, essentially spherical, 7–18 (12 ± 4.6; 2; 6) in diameter *in situ*, non-embryonated; polar filaments absent.

Type host: Rhinoptera jayakari Boulenger, 1895 (Rhinopteridae: Myliobatiformes).

Type locality: **Mozambique Channel, Mozambique:** Tofo (23°47′33.02″S, 35°31′16.38″E), Inhambane.

Site of infection: Spiral intestine.

Type specimens: Holotype (USNM 1661599), 14 paratypes (USNM 1661596–1661598, USNM 1661600–1661610), and 11 paratypes (LRP 10661–10720).

Voucher specimens: LRP 10659–10660 (hologenophores, this study).

Etymology: This species is named for its country of origin, Mozambique.

Remarks: Rhinoptericola mozambiquensis n. sp. is distinguished from *R. megacantha* and *R. butlerae* based on its shorter total length (<6 mm *vs* >10 mm in *R. megacantha* and *R. butlerae*) and fewer proglottids (<11 *vs* >22 in *R. megacantha* and *R. butlerae*). It is distinguished from *R. panamensis* and *R. aetobatidis* based on its shorter scolex (<1.9 mm *vs* >3.8 mm in *R. panamensis* and *R. aetobatidis*) and shorter bulbs (<0.6 mm *vs* >1.9 mm in *R. panamensis* and *R. aetobatidis*). This new species can also be distinguished from its four larger congeners by its lack of macrohooks in the basal armature; *R. megacantha*, *R. butlerae*, *R. panamensis*, and *R. aetobatidis* all possess two to four macrohooks in the

basal armature. Though similar in overall size, *R. mozambiquensis* n. sp. has a unique metabasal armature as compared to *R. schaeffneri*. It possesses seven hooks per principal row diminishing to six hooks per principal row more distally, while *R. schaeffneri* possesses nine hooks per principal row immediately anterior to the basal armature, diminishing to eight, and then seven, hooks per row more distally.

This new species is most morphologically similar to *R. jensenae*. Both species possess a metabasal armature with seven hooks per principal row diminishing to six hooks per row more distally, a basal armature of similar length that lacks macrohooks, and similar total lengths, scolex lengths, numbers of proglottids, and numbers of testes. *Rhinoptericola mozambiquensis* n. sp. is distinguished from *R. jensenae*, however, based on the shape of hooks in the anterior portion of the basal armature. In this tentacle region, both species possess billhooks that are falcate, erect, and dorsoventrally flattened with recurved mucronate tips; however, in *R. jensenae*, a subset of these billhooks have short forward protrusions on their lower surface (*i.e.*, are "can opener-shaped"; see Figs. 9D, 9E, 10K–10M)—a feature conspicuously absent in *R. mozambiquensis* n. sp. (see Figs. 17D, 17E, 18K, 18P). Additionally, *R. mozambiquensis* n. sp. possesses triangular, solid, dorsoventrally flattened hooks with tips extending well beyond the hook base on the bothrial surface of its basal armature (see Figs. 17D, 18J, 18P) which are absent in *R. jensenae* (see Fig. 9D). Molecular data similarly support the two as separate species (see results of phylogenetic analysis). Unlike its congeners, *R. mozambiquensis* n. sp. is described from only a single species of host and has a geographic distribution restricted to the waters off Mozambique.

***Rhinoptericola hexacantha* n. sp.**

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Figures 19–21

Description (based on eight mature worms, two immature worms, one detached mature proglottid, cross-sections of one scolex, and two scoleces prepared for SEM):

Worms euapolytic (Fig. 19A); 2.5–6.4 mm (3.7 ± 1.4; 8) long, maximum width at level of pars bothrialis; proglottids 4–10 (7 ± 1.9; 9) in total number.

Scolex (Figs. 19B, 21A and 21B) acraspedote, elongate, slender, 686–1,368 (973 ± 238.6; 10) long, length:width ratio 1.6–3.2:1 (n = 4). Pars bothrialis 153–291 (223 ± 47.6; 7) long by 227–362 (289 ± 48.1; 7) wide, with four bothria (Figs. 19A, 19B, 21A, 21B); bothria elliptoid to deeply ovoid, 123–219 (177 ± 26.0; 9; 21) long by 75–124 (96 ± 14.2; 7; 15) wide, with free lateral and posterior margins, arranged in dorsal and ventral pairs, not overlapping pars bulbosa; bothrial pits absent. Pintner's cells absent. Pars vaginalis 383–1,045 (551 ± 202.9; 10) long by 139–240 (205 ± 37.9; 10) wide at midpoint; tentacle sheaths sinuous. Pars bulbosa 326–699 (472 ± 117.4; 10) long by 144–259 (203 ± 32.8; 10) wide at midpoint; bulbs very narrowly oblong, thick-walled, muscular, 285–610 (457 ± 84.5; 10; 28) long by 50–102 (74 ± 12.5; 10; 27) wide; bulb length:width ratio 3.8–12.1 (6.4 ± 2.1; 10; 25):1; prebulbar organs present; gland cells inside bulbs

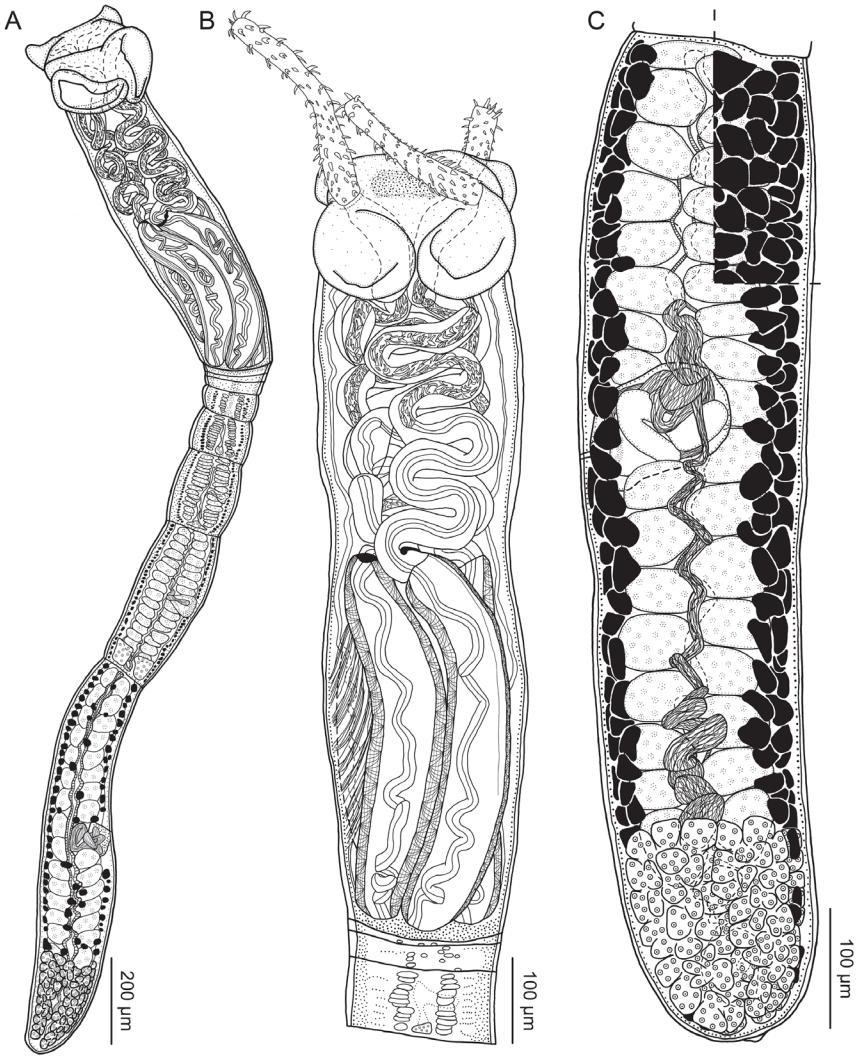

Figure 19 Line drawings of *Rhinoptericola hexacantha* n. sp. (A) Whole worm (USNM 1661594; paratype); specimen is not mature enough to exhibit circumcortical vitelline follicles in the terminal proglottid. (B) Scolex (CNHE 11612; holotype). (C) Terminal proglottid (USNM 1661593; paratype); circumcortical vitelline follicles are drawn only on the lateral margins and in the region delimited by dashed lines. Full-size DOI: 10.7717/peerj.12865/fig-19

absent; retractor muscles in bulbs 8–31 (14 ± 5.6; 10; 29) wide, originating at base of bulbs. Pars postbulbosa short or absent, 7–8 (n = 2) long when present. Scolex length ratio (pars bothrialis length:pars vaginalis length:pars bulbosa length) 1:2.0–4.3 (2.7 ± 0.8; 7):1.7–2.9 (2.2 ± 0.5; 7).

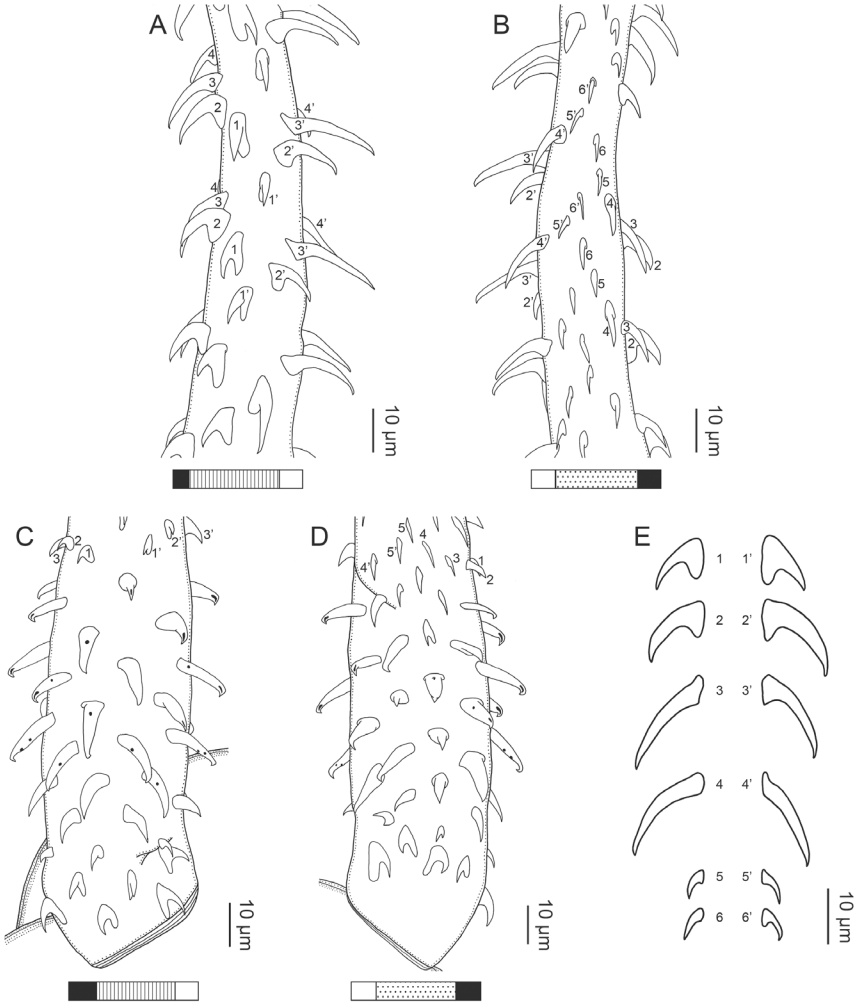

Figure 20 Line drawings of the tentacular armature of *Rhinoptericola hexacantha* n. sp. (A) Metabasal armature, bothrial surface (LRP 10723; paratype). (B) Metabasal armature, antibothrial surface (LRP 10723; paratype). (C) Basal armature, bothrial surface (CNHE 11612; holotype). (D) Basal armature, antibothrial surface (CNHE 11612; holotype). (E) Comparison of metabasal hook shapes.
Full-size DOI: 10.7717/peerj.12865/fig-20

Tentacles long, with slight basal swelling, occasionally retracted into bulbs, at least 360 long, 25–32 (29 ± 2.5; 3; 6) wide at base, 26–37 (33 ± 4.5; 6; 3) wide at basal swelling, 16–24 (20 ± 3.0; 3; 6) wide in metabasal region.

Characteristic basal armature present (Figs. 20C, 20D, 21N), 84–95 (89 ± 4.9; 3; 5) long from base of tentacle to start of metabasal armature, consisting of 5–7 indistinct rows of

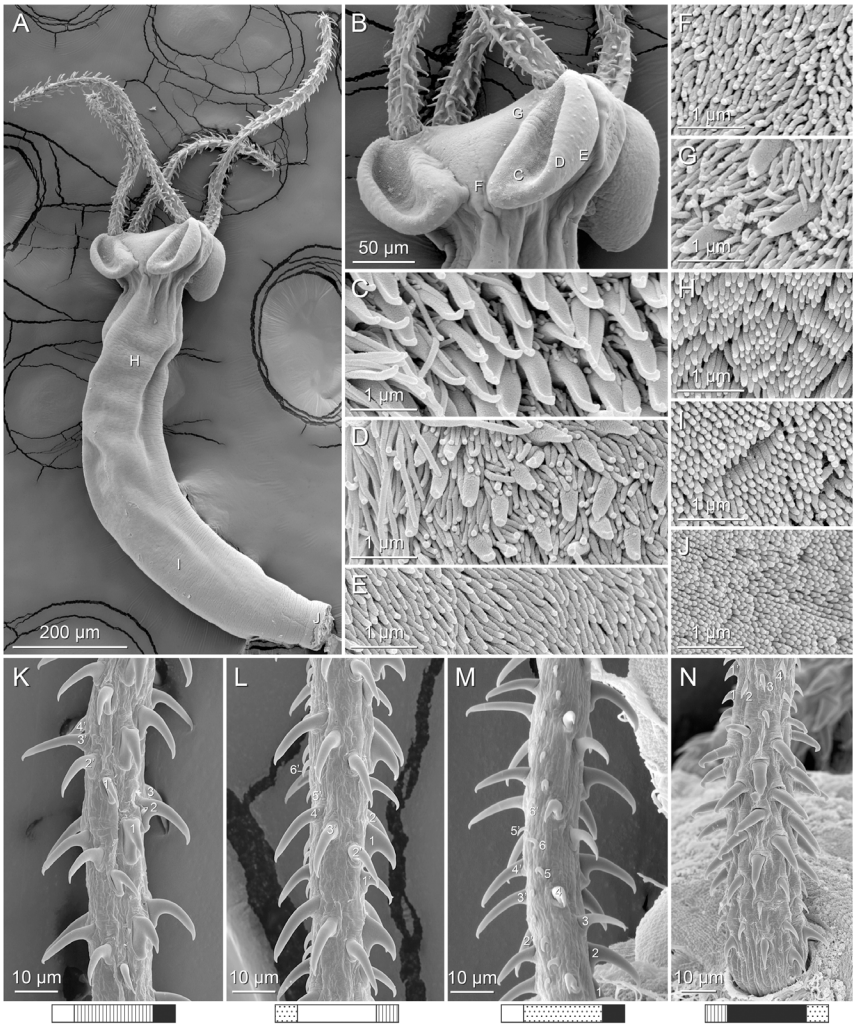

Figure 21 Scanning electron micrographs of *Rhinoptericola hexacantha* n. sp. (A) Scolex; small letters indicate the location of details shown in (H–J). (B) Bothria and basal armature; small letters indicate the location of details shown in (C–G). (C) Distal bothrial surface. (D) Proximal bothrial surface near the bothrial rim. (E) Proximal bothria surface away from the bothrial rim. (F) Surface of the scolex proper between the bothria. (G) Surface of the scolex proper at the apex. (H) Surface of the pars vaginalis. (I) Surface of the pars bulbosa. (J) Strobilar surface. (K) Metabasal armature, bothrial surface. (L) Metabasal armature, internal surface. (M) Metabasal armature, antibothrial surface. (N) Basal armature, external surface. Full-size DOI: 10.7717/peerj.12865/fig-21

hooks; hooks in posterior-most 2–3 rows uncinate, solid; anterior 4–5 rows with spiniform hooks and billhooks; billhooks falcate, erect, dorsoventrally flattened, solid or hollow, with recurved mucronate tips; mucronate tips solid or hollow; macrohooks absent.

Metabasal armature (Figs. 20A, 20B, 20E, 21K–21M) heteroacanthous typical; hooks heteromorphous, solid, arranged in alternating ascending half-spiral rows of six hooks each; rows originating with hooks 1(1′) on bothrial surface, terminating with hooks 6(6′) in near single file on antibothrial surface; hooks 1(1′)–3(3′) not angled towards the space between hooks files 1 and (1′). Hook files 1 and (1′) slightly separated, 3–6 (n = 3) apart. Hooks 1(1′) uncinate, slightly recurved, 10–13 (n = 3) long, 6–9 (n = 3) high, base 6–11 (n = 3) long. Hooks 2(2′) falcate, with recurved tips, 12–19 (n = 3) long, 8–12 (n = 3) high, base 5–8 (n = 3) long. Hooks 3(3′) falcate, with recurved tips and anterior base extensions, 13–22 (n = 3) long, 9–14 (n = 3) high, base 4–7 (n = 3) long. Hooks 4(4′) falcate, with recurved tips and anterior base extensions, 11–20 (n = 3) long, 6–11 (n = 3) high, base 4–5 (n = 3) long. Hooks 5(5′) falcate to uncinate, 6–7 (n = 3) long, 3–4 (n = 3) high, base 2–4 (n = 3) long. Hooks 6(6′) falcate to uncinate, 5–7 (n = 3) long, 3–4 (n = 3) high, base 2–3 (n = 3) long.

Distal bothrial surfaces (Fig. 21C) with large gladiate spinitriches and acicular to capilliform filitriches. Proximal bothrial surfaces near bothrial rims (Fig. 21D) with small gladiate spinitriches and acicular to capilliform filitriches, away from bothrial rims (Fig. 21E) with acicular to capilliform filitriches only. Scolex proper near and at apex (Fig. 21G) with small gladiate spinitriches and capilliform filitriches, between bothria with acicular to capilliform filitriches only (Fig. 21F). Pars vaginalis (Fig. 21H), pars bulbosa (Fig. 21I), and strobila (Fig. 21J) with capilliform filitriches.

Proglottids acraspedote. Neck absent. Immature proglottids 3–8 (5 ± 1.6; 9) in number, wider than long, becoming longer than wide with maturity. Mature proglottids 1–2 (1 ± 0.5; 8) in number; terminal mature proglottids 820–1,649 (1,153 ± 260.7; 7) long by 195–288 (227 ± 38.3; 7) wide, free mature proglottids 1,303 (n = 1) long by 328 (n = 1) wide. Gravid proglottids not observed.

Testes 30–35 (32 ± 2.1; 7) in total number, 16–19 (17 ± 1.1; 7) pre-poral, 12–17 (15 ± 1.8; 7) post-poral, 37–93 (58 ± 14.7; 7; 21) long by 53–98 (72 ± 10.9; 7; 18) wide, in field from anterior margin of proglottid to ovary, slightly overlapping anterior margin of ovary, arranged in two columns (Fig. 19C), essentially in single layer. Vas deferens extending from near mid-level of ovary to slightly anterior of anterior margin of cirrus sac, entering cirrus sac at its antero-medial margin, coiled primarily anterior to cirrus sac; external and internal seminal vesicles absent. Cirrus sac ovoid to elliptoid, 106–181 (129 ± 27.7; 6) long by 82–154 (121 ± 26.8; 7) wide, containing coiled cirrus; cirrus unarmed, thin-walled, 185 long (n = 1) by 72 wide (n = 1) at base, 26 wide (n = 1) at tip when everted. Genital atrium absent. Genital pores separate, at same level, unilateral, 55–64% (60% ± 3.0%; 8) of proglottid length from posterior margin of proglottid. Vagina thick-walled, weakly sinuous, extending from ootype along midline of proglottid to anterior margin of cirrus sac, then laterally at level of cirrus sac, terminating in female genital pore, greatly expanded when sperm-filled; vaginal sphincter absent; seminal receptacle present. Ovary terminal in proglottid, H-shaped in dorsoventral view, tetralobed in cross-section, 180–379 (242 ± 69.1; 7) long by 114–241 (160 ± 53.8; 7) wide, with lobulated margins; ovarian isthmus near center of ovary. Mehlis' gland near posterior margin of ovary. Vitellarium follicular; follicles circumcortical, 16–38 (29 ± 5.4; 8; 24) long

by 21–47 (32 ± 7.0; 7; 21) wide, extending entire length of proglottid, interrupted dorsally and ventrally by ovary, partially interrupted ventrally by terminal genitalia; post-ovarian vitelline follicles present. Uterus saccate, medial, dorsal to vagina, bifurcated at posterior end, extending from anterior margin of ovary to anterior margin of proglottid. Uterine duct not observed. Uterine pore absent. Excretory vessels four, arranged in one dorsal and one ventral pair on each lateral margin of proglottid. Eggs not observed.

Type host: Rhinoptera steindachneri Evermann & Jenkins, 1891 (Rhinopteridae: Myliobatiformes).

Type locality: **Gulf of California, Mexico:** Loreto (25°49′52″N, 111°19′38″W), Baja California Sur.

Additional localities: **Gulf of California, Mexico:** Bahia de Los Angeles (28°59′9″N, 113°32′53″W), Baja California; Puertecitos (30°20′58″N, 114°38′22″W), Baja California; and Santa Rosalia (27°19′51″N, 112°15′30″W), Baja California Sur.

Site of infection: Spiral intestine.

Type specimens: Holotype (CNHE 11612), three paratypes (CNHE 11613–11614), five paratypes (LRP 10722–10772), and four paratypes (USNM 1661592–1661595).

Voucher specimen: LRP 10721 (hologenophore, this study).

Etymology: This species is named for its possession of six hooks per principal row in the metabasal armature throughout the tentacle length, a unique feature among species of *Rhinoptericola*.

Remarks: Rhinoptericola hexacantha n. sp. differs from all known species of *Rhinoptericola* in consistently having six hooks per principal row in the metabasal armature *vs* having seven hooks per row proximally and six hooks per row more distally (*R. jensenae* and *R. mozambiquensis*) or seven or more hooks per row (*R. megacantha*, *R. butlerae*, *R. panamensis*, *R. aetobatidis*, and *R. schaeffneri*). It is further distinguished from the species of *Rhinoptericola* for which features of the strobila are known (*i.e.*, *R. megacantha*, *R. butlerae*, *R. jensenae*, *R. schaeffneri*, and *R. mozambiquensis*) in being euapolytic rather than apolytic. *Rhinoptericola hexacantha* n. sp. is shorter in total length than *R. megacantha* and *R. butlerae* (<6.5 mm *vs* >10 mm in *R. megacantha* and *R. butlerae*) and has a shorter scolex than *R. panamensis* and *R. aetobatidis* (<1.4 mm *vs* >3.8 mm in *R. panamensis* and *R. aetobatidis*). It also lacks macrohooks in the basal armature, further distinguishing it from its larger congeners (*i.e.*, *R. megacantha*, *R. butlerae*, *R. panamensis*, and *R. aetobatidis*) which possess basal armatures with two to four macrohooks.

Rhinoptericola hexacantha n. sp. is further distinguished from *R. jensenae*, *R. schaeffneri*, and *R. mozambiquensis* by the shape of hooks in the anterior portion of the basal armature. In this region of the tentacle, *R. hexacantha* n. sp. possesses only billhooks that are falcate, erect, and dorsoventrally flattened with recurved mucronate tips, while *R. jensenae*, *R. schaeffneri*, and *R. mozambiquensis* each possess billhooks of this

shape in addition to either billhooks with short forward protrusions on their lower surface (*i.e.*, "can opener-shaped" billhooks in *R. jensenae* and *R. schaeffneri*), or triangular, solid, dorsoventrally flattened hooks with tips extending well beyond the hook base (in *R. mozambiquensis*). *Rhinoptericola hexacantha* n. sp. has a geographic distribution restricted to the Gulf of California, and like *R. mozambiquensis*, is known from only one species of cownose ray host (in this case, *Rhinoptera steindachneri*).

Phylogenetic analysis

For 29 of the 32 specimens sequenced, 1,411–1,426 bp of 28S were generated; for GenBank nos. OL412709 (*R. butlerae*), OL412737 (*R. schaeffneri*), and OL412738 (*R. mozambiquensis*), 1,246 bp, 841 bp, and 1,131 bp were generated, respectively (see Table 2). The initial matrix of 182 sequences, including sequences from six outgroup specimens, was trimmed to a maximum length of 1,510 bp. PRANK produced an alignment of 1,836 positions, including 946 invariable sites and 890 variable sites, which was used for tree searching and bootstrapping analyses. The resulting most likely topology with nodal support provided as bootstrap values (BS) is shown in Fig. 22 with the monophyletic Trypanoselachoida collapsed. For the MUSCLE alignment of 1,429 bp of 28S data for only the 32 specimens of *Rhinoptericola* sequenced herein and the single 28S sequence for *R. megacantha* available in GenBank (DQ642792), intraspecific divergence ranged from 0–2 bp and interspecific divergence ranged from 20–70 bp (excluding ambiguous base calls, see Table 4).

The genus *Rhinoptericola* was recovered as a monophyletic group (BS 100) sister to *Nataliella marcelli*; thus, a monophyletic Rhinoptericolidae were recovered, though the two genera are united with relatively low nodal support (BS 81). For the four species of *Rhinoptericola* for which replicate individuals could be sequenced, all replicates were recovered as reciprocally monophyletic groups with high nodal support (BS 96 or 100). *Rhinoptericola megacantha* and *R. butlerae* (both relatively large species with total lengths >10 mm) were recovered as a monophyletic group (BS 100) sister to the small species *R. hexacantha* (total length <6.5 mm; BS 92); the remaining three small species (*i.e.*, *R. jensenae*, *R. schaeffneri*, and *R. mozambiquensis*; total lengths <6.8 mm) formed a monophyletic group (BS 88).

DISCUSSION

Current status of the Rhinoptericolidae

The Rhinoptericolidae now includes the monotypic *Nataliella marcelli* and eight species of *Rhinoptericola* comprising *R. megacantha*, four species transferred to *Rhinoptericola*, and three new species. Rhinoptericolids are the only trypanorhynchs known to possess a scolex with four bothria and pre-bulbar organs, but to lack gland cells in the bulbs, and are thus united as a family by this unique combination of morphological features. *Nataliella marcelli* is unique among rhinoptericolids in possessing metabasal hooks arranged in quincunxes (*i.e.*, a homeoacanthous metabasal armature), while all species of *Rhinoptericola* are now known to possess hooks arranged in paired rows (*i.e.*, heteroacanthous typical armatures). *Rhinoptericola* is the third genus of

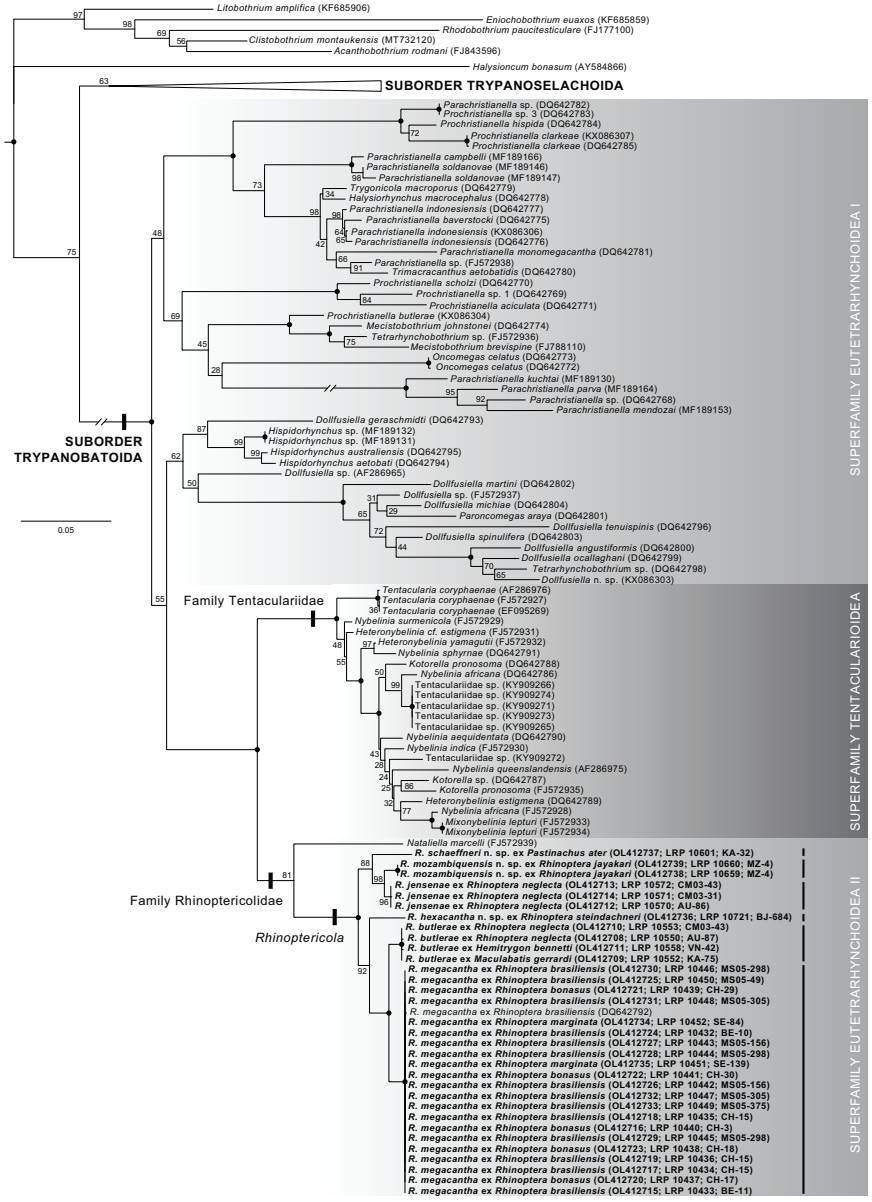

Figure 22 Phylogeny of the Trypanorhyncha resulting from a maximum likelihood analysis of the D1–D3 region of the 28S rRNA gene showing the placement of rhinopterícolid taxa. Taxon labels are presented as the species name and host species, followed in parentheses by the GenBank and hologenophore accession numbers, and the host code, or, for sequences downloaded from GenBank, the GenBank accession number only. Taxon labels in bold represent the sequences generated as part of this study. The clade of Trypanoselachoida is collapsed. Nodal support is given as bootstrap (BS) values generated from 1,000 BS replicates; nodes with BS values equal to 100 are represented by solid black circles. Branch length scale bar at left indicates nucleotide substitutions per site. Full-size DOI: 10.7717/peerj.12865/fig-22

trypanorhynchs known to possess species with dorsoventrally flattened billhooks with mucronate tips (*i.e.*, those found in the in the basal armatures of *R. hexacantha*, *R. jensenae*, *R. mozambiquensis*, and *R. schaeffneri*). Hooks of this type have only been reported previously for species of *Hemionchos* Campbell & Beveridge, 2006 and *Mobulocestus* Campbell & Beveridge, 2006—both unusual genera parasitizing devil rays (Campbell & Beveridge, 2006). Though proglottid anatomy remains unknown for *N. marcelli*, *R. aetobatidis*, and *R. panamensis*, the six species of *Rhinoptericola* for which proglottid anatomies are known share a combination of features unique among trypanorhynchs: they possess circumcortical vitelline follicles that are interrupted dorsally and ventrally by the ovary, testes in two columns that overlap the anterior region of the ovary, a uterus that is bifurcated at the posterior end, a seminal receptacle, an unarmed cirrus sac, and separate male and female genital pores, and they lack external and internal seminal vesicles. To our knowledge, *Rhinoptericola* is the first genus of trypanorhynchs in which orientation of the metabasal armature (*e.g.*, internal to external, external to internal, or bothrial to antibothrial) is known to vary between species.

In terms of species differences, within *Rhinoptericola*, much like in other trypanorhynch genera, species differ, for example, in total length, scolex size, total number of proglottids, and total number of testes. Interestingly, there appear to be larger bodied species with macrohooks in the basal armature (*R. megacantha*, *R. butlerae*, *R. aetobatidis*, and *R. panamensis*) and smaller bodied species with billhooks in the basal armature (*R. jensenae*, *R. schaeffneri*, *R. hexacantha*, and *R. mozambiquensis*); however, these groups of species are not reciprocally monophyletic (see Fig. 22). In addition, species of *Rhinoptericola* vary in their tentacular armature. Though all possess characteristic basal, and heteroacanthous typical metabasal, armatures, they vary in the shape and total number of hooks in the basal armature, the presence or absence and number of macrohooks in the basal armature, the number of hooks per principal row in the metabasal armature (and whether this number is variable along the tentacle), and the shape of their metabasal hooks. The following key to species of rhinoptericolids will aid future work on this group:

A key to species of the family Rhinoptericolidae

1. Metabasal hooks arranged in quincunxes ..... *Nataliella marcelli*
- Metabasal hooks arranged in paired principal rows 2
2. Scolex total length >2.6 mm; macrohooks present and billhooks absent in basal armature 3
- Scolex total length <2.6 mm; macrohooks absent and billhooks present in basal armature 6
3. Characteristic basal armature with two macrohooks only... *Rhinoptericola aetobatidis*
- Characteristic basal armature with more than two macrohooks 4
4. Characteristic basal armature with >80 hooks ..... *Rhinoptericola butlerae*
- Characteristic basal armature with <70 hooks 5

5. Distal and proximal bothrial surfaces with both gladiate spinitriches and capilliform or acicular filitriches..... *Rhinoptericola megacantha*
- Distal bothrial surfaces with gladiate spinitriches and proximal bothrial surfaces with acicular to capilliform filitriches ..... *Rhinoptericola panamensis*
6. Metabasal armature with nine hooks per principal row immediately anterior to the basal armature, reducing to eight, and then seven, hooks more distally on the tentacle..... *Rhinoptericola schaeffneri*
- Fewer than eight hooks per principal throughout metabasal armature 7
7. Six hooks per principal row throughout metabasal armature; basal armature with billhooks without short forward protrusions on their lower surface, only (Fig. 21N) ..... *Rhinoptericola hexacantha*
- Seven hooks per principal row reducing to six hooks per principal row more distally on the tentacle; basal armature with billhooks without short forward protrusions on their lower surface, and, in addition, either billhooks with short forward protrusions on their lower surface or triangular hooks with tips extending well beyond the hook base (Figs. 10K, 10M, 18J, 18K, 18P)..... 8
8. Basal armature with triangular, solid, dorsoventrally flattened hooks with tips extending well beyond the hook base (Figs. 18J, 18K, 18P)..... *Rhinoptericola mozambiquensis*
- Basal armature with billhooks with short forward protrusions on their lower surface (*i.e.*, "can opener-shaped" billhooks) (Figs. 10K–10M) ..... *Rhinoptericola jensenae*

Excluding *N. marcelli* (which was described from larval worms from intermediate hosts, only), rhinoptericolids have now been reported from a diverse array of definitive elasmobranch hosts from various geographic localities (see Table 3). They are known to parasitize species from five batoid families in addition to hemiscylliid sharks and have been reported from various localities in the eastern and western Atlantic Ocean, the Gulf of Mexico, the Gulf of California, and off Malaysia, Indonesia, Viet Nam, Australia, Sri Lanka, and Mozambique.

Though a fair number of reports from dasyatid stingrays exist, cownose rays (in the genus *Rhinoptera*) and cowtail rays (in the genus *Pastinachus*) appear to most commonly serve as hosts for species of *Rhinoptericola*. Thus, species in these genera that have yet to be examined represent the most likely targets for additional rhinoptericolid diversity. These include the one remaining species of *Rhinoptera* (the African cownose ray *Rhinoptera peli* Bleeker, 1863 inhabiting the eastern Central Atlantic) and the two remaining species of *Pastinachus* (the cowtail ray *Pastinachus sephen* (Forsskål, 1775) known to occur in the northern Indian Ocean, and the starrynose cowtail ray *Pastinachus stellurostris* Last, Fahmi & Naylor, 2010 known from the Indo-Malay Archipelago) (*Last et al., 2016*). For *N. marcelli*, which is known from relatively large bony fishes from off the coast of Hawaii, large sharks found in Hawaiian waters (*e.g.*, carcharhinids and lamniforms) seem the most likely targets for adult worms.

Rhinoptericolid monophyly, interrelationships, and intraspecific *versus* interspecific sequence divergence

This study is the first to recover a monophyletic Rhinoptericolidae based on sequence data—albeit with relatively lackluster nodal support for the sister relationship between *Rhinoptericola* and *Nataliella* (BS 81; see Fig. 22). Unfortunately, the identification of the specimen of *N. marcelli* from which the 28S sequence data were generated (GenBank no. FJ572939; *Palm et al., 2009*) could not be verified. Requests to the Berlin Natural History Museum to examine the hologenophore (ZMB 7439) revealed the specimen to be missing (B. Neuhaus, 2019, pers. comm.).

All six species of *Rhinoptericola* sequenced represent evolutionarily distinct lineages within a monophyletic genus in this analysis (see Fig. 22), but relationships between species are subject to change with the addition of data for more genes. The strongly supported sister relationship between the Tentacullariidae and the Rhinoptericolidae still renders a Eutetrarhynchoidea inclusive of the Rhinoptericolidae paraphyletic (see Fig. 22). However, the goal of this single-locus analysis was to support species boundaries rather than to infer higher-level relationships within the order; thus, we do not advocate extrapolating this result based on a single gene to support reorganization at the level of superfamily.

For the four species of *Rhinoptericola* sequenced herein for which intraspecific replication was possible, 28S proved useful for confirming conspecificity for specimens with uniform morphologies from different hosts and geographic localities (see Table 2). For *R. megacantha* for example, sequences from 22 specimens collected from the American cownose ray (*Rhinoptera bonasus*) from off the eastern USA, the Lusitanian cownose ray (*Rhinoptera marginata*) from Senegal, and the Ticon cownose ray (*Rhinoptera brasiliensis*) from Belize and the Gulf of Mexico showed remarkably little sequence divergence. For *R. butlerae*, the four specimens sequenced demonstrated this same low level of divergence despite having been collected from two individual Australian cownose rays (*Rhinoptera neglecta*) from Australia and from the whitespotted whipray (*M. gerrardi*) and the Bennett's stingray (*Hemi. bennetti*) from Indonesia. The two individuals of *R. mozambiquensis* sequenced from the shorttail cownose ray (*Rhinoptera jayakari*) from Mozambique differed from one another by only 2 bp, and the four individuals of *R. jensenae* from Australian cownose rays (*Rhinoptera neglecta*) from two localities in northern Australia were identical in sequence. It was unfortunately not possible to sequence multiple individuals for *R. schaeffneri* and *R. hexacantha*, but as both species demonstrate relatively restricted geographic distributions and host associations (*i.e.*, the roughnose, narrow, and broad cowtail rays [*P. solocirostris*, *P. gracilicaudus*, and *P. ater*, respectively] from Indonesia and Malaysia, and the Pacific cownose ray [*R. steindachneri*] from the Gulf of California, respectively) it seems unlikely that additional replicates would deviate from the pattern of intraspecific divergence observed in other species of *Rhinoptericola*. For pairs of morphologically similar species (*i.e.*, *R. megacantha* and *R. butlerae*, and *R. jensenae* and *R. mozambiquensis)* differences in 28S, in combination

with differing host associations and geographic ranges, all support the species boundaries based on morphology.

The inclusion of sequence data for six of eight species of *Rhinoptericola* combined with the inclusion of replicate specimens for four of those species in the phylogenetic analysis allowed for assessment of intra- and interspecific sequence variation for 28S in the genus. Prior to this study, exploration of intraspecific sequence divergence for trypanorhynchs was limited to three investigations. For the eutetrarhynchid *Prochristianella clarkeae* Beveridge, 1990, Salmani & Haseli (2017) and Haseli, Bazghalee & Palm (2017) reported 0% divergence in 657 bp of 28S for four specimens (three specimens from the cowtail stingray *Pastinachus sephen* in the Persian Gulf and one specimen from the eyebrow wedgefish, *Rhynchobatus palpebratus* Compagno & Last, 2008 [as *Rhynchobatus* cf. *australiae*] from Australia) and 0.07% divergence in 1,367 bp of 28S for one of the specimens from the Persian Gulf and the one from Australia. Haseli, Bazghalee & Palm (2017) provided intraspecific comparisons for the eutetrarhynchids *Parachristianella indonesiensis* and *Parachristianella monomegacantha* Kruse, 1959. For *Para. indonesiensis*, they reported divergences of 0.47% and 0.71% in 1,266 bp of 28S for three specimens (one specimen each from *Past. sephen* from the Persian Gulf, the Australian whipray, *Himantura australis* [as *Hima.* cf. *uarnak*] from Malaysia, and *Rhyn. palpebratus* from Australia). For *Para. monomegacantha*, they reported a divergence of 1.66% in 664 bp of 28S for two specimens (one specimen each from *Past. sephen* from the Persian Gulf and *Pateobatis* cf. *jenkinsii* [as *Himantura draco* Compagno & Heemstra, 1984] from Australia). Palm, Waeschenbach & Littlewood (2007) found 848 bp of 28S to be identical for six specimens of the tentacullariid *Tentacularia coryphaenae* Bosc, 1802; five of the specimens were larvae collected from bony fishes from Indonesia and one specimen was an adult collected from the blue shark, *Prionace glauca* (Linnaeus, 1758), from off the coast of Montauk, NY, USA.

In addition to intraspecific sequence divergence, Haseli, Bazghalee & Palm (2017) evaluated levels of interspecific divergence between *Prochristianella butlerae* Beveridge, 1990, and four described and four undescribed species of *Prochristianella*, estimating anywhere from 12.80–25.10% divergence in 28S depending on sequence fragment length and the species to which *Proc. butlerae* was compared. It is worth noting that these comparisons represent only minimally the various hosts and geographies from which the species sequenced have been reported. For example, while Salmani & Haseli (2017) and Haseli, Bazghalee & Palm (2017) included four individuals of *Proc. clarkeae* from two host species representing two batoid orders, the species is known from 39 species of batoids in 20 genera and four orders from four countries (Beveridge, 1990; Schaeffner & Beveridge, 2012b; Schaeffner & Beveridge, 2014). Additionally, in none of these three studies were the identities of the hosts specimens verified *via* DNA barcoding.

The dense sampling in the present study allowed us to assess levels of intra- and interspecific sequence divergence in 28S for adult trypanorhynchs across the various hosts and geographic localities from which they are known. The boundary between intra- and interspecific sequence divergences within *Rhinoptericola* was clear, with specimens within a species varying by 0–0.14% (0–2 bp) and specimens between species varying by

1.4–4.9% (20–70 bp) (see Table 4). With the exception of the comparison by *Haseli, Bazghalee & Palm (2017)* for *Para. monomegacantha* (which we assume represents an interspecific comparison based on the results of their phylogenetic analysis; see fig. 1), these estimates are consistent with the results of the previous studies. It appears that, based on data for replicate species and specimens of *Prochristianella* (Eutetrarhynchidae) and *Rhinoptericola* (Rhinoptericolidae), as well as replicate specimens for the tentaculariid *T. coryphaenae*, levels greater than ~1% divergence in 28S represent an interspecific boundary, while levels less than ~1% divergence represent intraspecific variation. However, this working hypothesis should be scrutinized by data for additional genera in the Trypanobatoida and the Trypanoselachoida.

Relaxed host specificity in combination with varying geographic ranges and complex morphologies can make species identification and delimitation comparatively challenging in trypanorhynch tapeworms, but this study clearly demonstrates the great potential of 28S to aid in the process. Unfortunately, to date, 28S data are only available in GenBank for fewer than 30% of the 329 valid species of trypanorhynchs (*Caira, Jensen & Barbeau, 2021*). Furthermore, 28S data representing multiple specimens of the same species sequenced from multiple host individuals are available for fewer than 10% of all species, and the replicates which are available rarely come from multiple species of elasmobranchs. The results of this study suggest that, if at all possible, deposition of 28S data should become regular practice when describing or redescribing species of trypanorhynchs.

Recommendations for future taxonomic work on trypanorhynch tapeworms

Examination of scoleces with SEM allowed for an updated understanding of armature patterns in species of *Rhinoptericola* that helped unite the genus morphologically, but also proved particularly useful for comparing hook pattern and shape between congeners. For example, SEMs clearly show that hooks are arranged in paired rows of seven hooks each in *R. megacantha* (see Fig. 4N), which allowed for revised diagnoses for the species and the genus, and in turn led to the synonymy with *Shirleyrhynchus*. Scanning electron micrographs of the tentacular armature are now available for seven of eight species of *Rhinoptericola* (*i.e.*, are presented for the six species described or redescribed herein and for *R. panamensis* [see *Schaeffner, 2016*], but are lacking for *R. aetobatidis*). These data clearly demonstrate that hooks are arranged in paired rows in all species of *Rhinoptericola* imaged, and further confirm their possession of four (rather than two) bothria.

In addition to elucidating features that unite the genus, SEMs were also useful for distinguishing between congeners. For example, SEMs clearly illustrate the differences in hook size and shape in the basal armature of *R. megacantha vs R. butlerae* (see Figs. 4O and 4P *vs* 7M and 7N). These differences, in addition to differences in the total number of hooks in the basal armature and a difference in microthrix pattern, are the basis for their morphological distinction. Similarly, for *R. jensenae* and *R. mozambiquensis*, SEMs clearly illustrate the differences in hook shape in the basal armature that are important for distinguishing between the two species (see "can opener-shaped" billhooks in Figs. 10K and 10L for *R. jensenae vs* triangular hooks and billhooks without short forward

protrusions on their lower surfaces in Figs. 18J and 18K for *R. mozambiquensis*). Ultimately, supplementing more traditional line drawings with SEMs proved crucial for consistent, accurate interpretation of scolex morphology. Though increasingly common, SEM is not yet standard practice in descriptions of new species of trypanorhynchs. The results of this study suggest that, as for 28S data, detailed SEMs of bothria and both basal and metabasal armature should become essential parts of all descriptions and redescriptions of trypanorhynchs, if at all possible.

Three species of *Rhinoptericola* were discovered to possess a reduction in hook number per principal row along the tentacle. *Rhinoptericola jensenae* and *R. mozambiquensis* possess principal rows with relatively straightforward transitions from seven hooks to six hooks more distally on the tentacle (see Fig. 9C for *R. jensenae* and Figs. 17C and 18O for *R. mozambiquensis*). For *R. schaeffneri*, both new material and paratypes of *Proc. jensenae* deposited by *Schaeffner & Beveridge (2012b)* demonstrated zones of transition that are comparatively more complicated. Generally, this species possesses principal rows with nine hooks immediately anterior to the basal armature, reducing to eight and then seven hooks more distally on the tentacle (see Figs. 13, 14K and 14L), but specimens with tentacle regions with unpaired hooks shared between two rows (Figs. 13A–13C) or the errant reappearance of hooks 8(8′) following a reduction to seven hooks per row (Figs. 12B, 13D) are not uncommon. While a reduction in hook number along the tentacle has been described for other trypanorhynchs (*e.g.*, *Eutetrarhynchus ruficollis* [Eysenhardt, 1829] Pintner, 1913, *Prochristianella cairae* *Schaeffner & Beveridge, 2012b*, *Prochristianella scholzi* *Schaeffner & Beveridge, 2012b*) (see *Schaeffner & Beveridge, 2012b*; *Beveridge, Koehler & Appy, 2021*), *R. schaeffneri* is, to our knowledge, the first species for which such complex zones of transition have been thoroughly documented and illustrated. These data underscore, that, moving forward, careful examination of specimens with tentacles in various degrees of eversion is advisable so as not to overlook potentially similar patterns in other species.

Introduced as part of this study is a graphical representation of tentacle surfaces for two- and four-bothriate trypanorhynchs (Figs. 1A and 1B). Herein, bars which illustrate the tentacle surface pictured are provided beneath line drawings and SEMs of tentacular armature. Information on the surfaces pictured in these images has been traditionally difficult to convey. For example, when looking at a scanning electron micrograph or line drawing centered on the bothrial surface for either a two- or four-bothriate trypanorhynch, a portion of the flanking tentacle surfaces are inherently also pictured as a result of the cylindrical nature of tentacles. For the bothrial surface, these flanking surfaces can be either the external surface to the left and internal surface to the right, or internal surface to the left and external surface to the right, depending on the position of the imaged or drawn tentacle relative to the other three tentacles (see Fig. 1). To date, this information has rarely been specified in figure captions or otherwise made clear with supplemental figures, except perhaps in cases where these distinctions have proven to be especially complex or of particular systematic importance (*e.g.*, figs. 2, 3, and 6 of *Schaeffner, 2016*). The importance

of tentacle surface designations is summarized by *Palm (2004)*, but despite seemingly well-established generalizations, authors often disagree on the assignment of hooks 1(1′) to a particular surface. As an example, herein *R. butlerae* and *R. panamensis* are both reported to possess principal rows that begin on the internal tentacle surface, but *Schaeffner (2016)* reported *R. butlerae* to possess principal rows beginning on the antibothrial surface (at odds with both the original description and the redescription herein; see Fig. S1) and reported *R. panamensis* to possess principal rows beginning on the bothrial surface (also at odds with the reassessment herein; see Fig. S1). Given the importance of tentacular armature (and its orientation) in trypanorhynch identification and higher classification, and the obvious challenges with its interpretation, a simplified method for clarifying authors' evaluations seems warranted. The practice of including bars similar to those pictured herein beneath line drawings and SEMs of trypanorhynch tentacles with patterns corresponding to those in Fig. 1 provides such a method.

CONCLUSIONS

In terms of broader contributions to the field of trypanorhynch taxonomy and systematics, this study: (1) increases the number of species of *Rhinoptericola* from one to eight and the number of species of rhinoptericolids from two to nine, and greatly expands known host associations and geographic distributions for species of *Rhinoptericola*; (2) corrects and simplifies the interpretation of hook arrangement in species of *Rhinoptericola*; (3) represents the first comprehensive assessment of the degree of intra- *vs* interspecific variation in 28S for elasmobranch tapeworms demonstrating relaxed host specificity; (4) demonstrates the importance of integrating scolex and proglottid anatomy and morphology (as seen with light microscopy) with both data on tentacular armature and hook shape (as seen with SEM) and 28S data for trypanorhynch species delimitation; and (5) provides a novel schematic to streamline communication of the tentacular surface presented in SEMs and line drawings and make clear the authors' interpretations of these important images. This methodological framework can be readily applied to the study of other groups of trypanorhynchs in need of revision towards a stable classification for the group, and ultimately, elucidation of its evolutionary history.

The following taxonomic actions were taken herein: (1) *Shirleyrhynchus* became a junior synonym of *Rhinoptericola* and all three species in the genus *Shirleyrhynchus* were transferred to the genus *Rhinoptericola* creating the new combinations *Rhinoptericola aetobatidis*, *Rhinoptericola butlerae*, and *Rhinoptericola panamensis*; (2) the family name Shirleyrhynchidae became a junior synonym of the family name Rhinoptericolidae; (3) *Cetorhinicola acanthocapax*, formerly of the Shirleyrhynchidae, is now considered a taxon incertae sedis within the superfamily Eutetrarhynchoidea; (4) the species *Prochristianella jensenae* was transferred to the genus *Rhinoptericola*, creating the new combination *Rhinoptericola jensenae*; (5) the type series of *Proc. jensenae* was split into two species: *R. jensenae* was redescribed based on the holotype, a subset of paratypes, and new material, and the new species *Rhinoptericola schaeffneri* was described based

on the subset of paratypes of *Proc. jensenae* not considered conspecific with *R. jensenae* and new material; and (6) the new species *Rhinoptericola mozambiquensis* and *Rhinoptericola hexacantha* were described based on new material.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Kaylee S. Herzog conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
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The following information was supplied regarding the deposition of DNA sequences:

The 32 sequences of rhinoptericolids are available at GenBank: OL412708 to OL412739.

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All measurement data on which descriptions and redescriptions are based are available in the Supplemental File.

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Rhinoptericola schaeffneri n. sp. LSID: urn:lsid:zoobank.org:act:EC3B77B4-BD65-4425-8EE9-DC9763B891DD

Rhinoptericola mozambiquensis n. sp. LSID: urn:lsid:zoobank.org:act:958674CF-3029-4E37-A709-289E0354E2DF

Rhinoptericola hexacantha n. sp. LSID: urn:lsid:zoobank.org:act:0D1C299F-11FF-415D-B2BA-88BC60FD5E1E

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Supplemental Table 1. Higher classification, taxon name, GenBank accession number, and sequence length prior to trimming for all ingroup and outgroup sequences downloaded from GenBank and included in the maximum likelihood analysis of the D1–D3 region of the 28S rRNA gene. Asterisks (*) indicate a change in taxon name from the GenBank entry following Beveridge, Koehler & Appy (2021), Haseli, Bazghalee & Palm (2017), Palm (2010), or Schaeffner & Beveridge (2012a).

Higher classification	Taxon name	GenBank accession no.	Sequence length prior to trimming (bp)
Diphyllidea (Outgroup)	*Halysioncum bonasum*	AY584866	1,242
Lecanicephalidea (Outgroup)	*Eniochobothrium euaxos*	KF685859	1,516
Litobothriidea (Outgroup)	*Litobothrium amplifica*	KF685906	1,388
Onchoproteocephalidea (Outgroup)	*Acanthobothrium rodmani*	FJ843596	1,304
Phyllobothriidea (Outgroup)	*Clistobothrium montaukensis*	MT732120	1,175
Rhinebothriidea (Outgroup)	*Rhodobothrium paucitesticulare*	FJ177100	1,368
Trypanobatoida	*Dollfusiella angustiformis**	DQ642800	1,370
Trypanobatoida	*Dollfusiella geraschmidti*	DQ642793	1,285
Trypanobatoida	*Dollfusiella martini*	DQ642802	1,274
Trypanobatoida	*Dollfusiella michiae*	DQ642804	1,379
Trypanobatoida	*Dollfusiella* n. sp.*	KX086303	1,506
Trypanobatoida	*Dollfusiella ocallaghani*	DQ642799	1,377
Trypanobatoida	*Dollfusiella* sp.	AF286965	1,342
Trypanobatoida	*Dollfusiella* sp.	FJ572937	1,512
Trypanobatoida	*Dollfusiella spinulifera*	DQ642803	1,473
Trypanobatoida	*Dollfusiella tenuispinis*	DQ642796	1,196
Trypanobatoida	*Halysiorhynchus macrocephalus*	DQ642778	1,215
Trypanobatoida	*Heteronybelinia* cf. *estigmena*	FJ572931	1,530
Trypanobatoida	*Heteronybelinia estigmena*	DQ642789	1,176
Trypanobatoida	*Heteronybelinia yamagutii*	FJ572932	1,530
Trypanobatoida	*Hispidorhynchus aetobati**	DQ642794	1,204
Trypanobatoida	*Hispidorhynchus australiensis**	DQ642795	1,220
Trypanobatoida	*Hispidorhynchus* sp.	MF189131	1,300
Trypanobatoida	*Hispidorhynchus* sp.	MF189132	1,300
Trypanobatoida	*Kotorella pronosoma*	DQ642788	1,428
Trypanobatoida	*Kotorella pronosoma*	FJ572935	1,529
Trypanobatoida	*Kotorella* sp.	DQ642787	1,160
Trypanobatoida	*Mecistobothrium brevispine*	FJ788110	1,315
Trypanobatoida	*Mecistobothrium johnstonei*	DQ642774	1,419
Trypanobatoida	*Mixonybelinia lepturi*	FJ572933	1,531
Trypanobatoida	*Mixonybelinia lepturi*	FJ572934	1,531
Trypanobatoida	*Nataliella marcelli**	FJ572939	1,519
Trypanobatoida	*Nybelinia aequidentata*	DQ642790	1,306
Trypanobatoida	*Nybelinia africana*	DQ642786	1,172
Trypanobatoida	*Nybelinia africana*	FJ572928	1,531
Trypanobatoida	*Nybelinia indica*	FJ572930	1,532
Trypanobatoida	*Nybelinia queenslandensis*	AF286975	4,190
Trypanobatoida	*Nybelinia sphyrnae*	DQ642791	1,174
Trypanobatoida	*Nybelinia surmenicola*	FJ572929	1,530
Trypanobatoida	*Oncomegas celatus**	DQ642772	1,401
Trypanobatoida	*Oncomegas celatus**	DQ642773	1,106
Trypanobatoida	*Parachristianella baverstocki*	DQ642775	1,385
Trypanobatoida	*Parachristianella campbelli*	MF189166	1,016
Trypanobatoida	*Parachristianella indonesiensis**	DQ642776	1,266
Trypanobatoida	*Parachristianella indonesiensis*	DQ642777	1,384

Trypanobatoida	*Parachristianella indonesiensis*	KX086306	1,509
Trypanobatoida	*Parachristianella kuchtai*	MF189130	1,299
Trypanobatoida	*Parachristianella mendozai*	MF189153	1,298
Trypanobatoida	*Parachristianella monomegacantha*	DQ642781	1,378
Trypanobatoida	*Parachristianella parva*	MF189164	1,296
Trypanobatoida	*Parachristianella soldanovae*	MF189146	1,271
Trypanobatoida	*Parachristianella soldanovae*	MF189147	1,270
Trypanobatoida	*Parachristianella* sp.	DQ642768	1,134
Trypanobatoida	*Parachristianella* sp.	DQ642782	1,266
Trypanobatoida	*Parachristianella* sp.	FJ572938	1,522
Trypanobatoida	*Paroncomegas araya*	DQ642801	1,336
Trypanobatoida	*Prochristianella aciculata**	DQ642771	1,182
Trypanobatoida	*Prochristianella butlerae*	KX086304	1,494
Trypanobatoida	*Prochristianella clarkeae*	DQ642785	1,372
Trypanobatoida	*Prochristianella clarkeae*	KX086307	1,452
Trypanobatoida	*Prochristianella hispida**	DQ642784	1,061
Trypanobatoida	*Prochristianella scholzi**	DQ642770	1,072
Trypanobatoida	*Prochristianella* sp. 1	DQ642769	1,394
Trypanobatoida	*Prochristianella* sp. 3	DQ642783	1,214
Trypanobatoida	*Rhinoptericola megacantha*	DQ642792	1,262
Trypanobatoida	*Tentacularia coryphaenae*	AF286976	2,126
Trypanobatoida	*Tentacularia coryphaenae*	EF095269	1,530
Trypanobatoida	*Tentacularia coryphaenae*	FJ572927	1,530
Trypanobatoida	Tentaculariidae sp.	KY909265	1,265
Trypanobatoida	Tentaculariidae sp.	KY909266	1,265
Trypanobatoida	Tentaculariidae sp.	KY909271	1,255
Trypanobatoida	Tentaculariidae sp.	KY909272	1,253
Trypanobatoida	Tentaculariidae sp.	KY909273	1,255
Trypanobatoida	Tentaculariidae sp.	KY909274	1,835
Trypanobatoida	*Tetrarhynchobothrium* sp.	DQ642798	1,130
Trypanobatoida	*Tetrarhynchobothrium* sp.	FJ572936	1,558
Trypanobatoida	*Trimacracanthus aetobatidis*	DQ642780	1,268
Trypanobatoida	*Trygonicola macroporus*	DQ642779	1,111
Trypanoselachoida	*Ancipirhynchus afossalis*	JF907576	1,296
Trypanoselachoida	*Aporhynchus menezesi*	KF685908	1,537
Trypanoselachoida	*Aporhynchus norvegicus*	FJ572947	1,537
Trypanoselachoida	*Bathygrillotia rowei**	DQ642765	1,256
Trypanoselachoida	*Callitetrarhynchus gracilis*	AF286970	1,319
Trypanoselachoida	*Callitetrarhynchus gracilis*	DQ642758	1,198
Trypanoselachoida	*Callitetrarhynchus gracilis*	FJ572957	1,534
Trypanoselachoida	*Callitetrarhynchus gracilis*	MG694210	1,451
Trypanoselachoida	*Callitetrarhynchus speciosus*	DQ642759	1,399
Trypanoselachoida	*Chimaerarhynchus rougetae*	DQ642744	1,412
Trypanoselachoida	*Dasyrhynchus giganteus*	FJ788109	1,293
Trypanoselachoida	*Dasyrhynchus variouncinatus*	FJ572950	1,532
Trypanoselachoida	*Dasyrhynchus variouncinatus*	FJ572951	1,532
Trypanoselachoida	*Diesingium lomentaceum*	DQ642760	1,389
Trypanoselachoida	*Floriceps minacanthus*	AF286971	1,284
Trypanoselachoida	*Floriceps saccatus*	DQ642757	1,307
Trypanoselachoida	*Floriceps saccatus*	FJ572958	1,536
Trypanoselachoida	*Fossobothrium perplexum*	DQ642752	1,396
Trypanoselachoida	*Gilquinia robertsoni*	FJ572944	1,538
Trypanoselachoida	*Gilquinia squali*	AF286966	1,341
Trypanoselachoida	*Gilquinia squali*	FJ572945	1,538

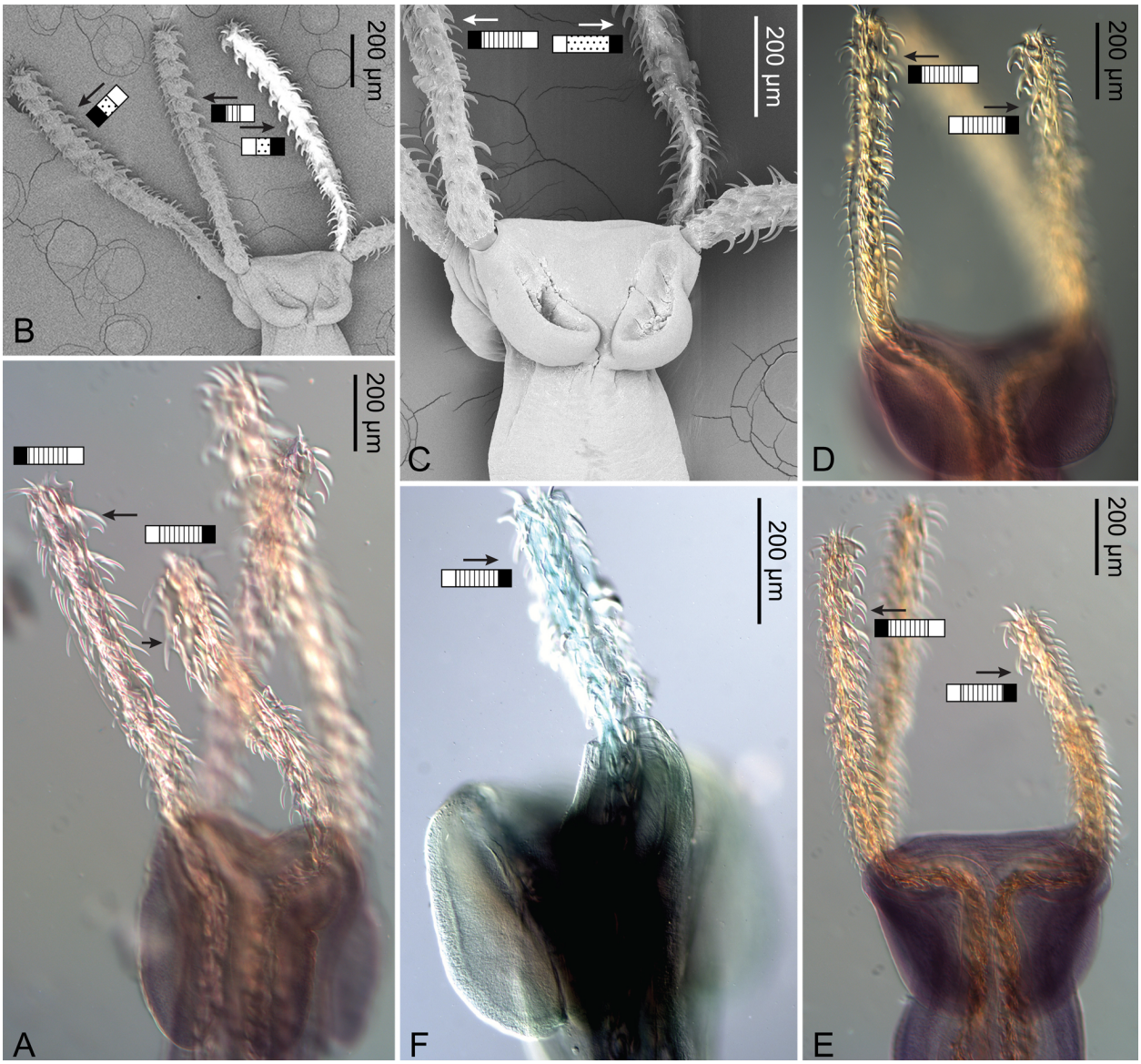
Trypanoselachoida	*Gilquinia squali*	FJ572946	1,538
Trypanoselachoida	*Grillotia erinaceus*	AF286967	4,258
Trypanoselachoida	*Grillotia pristiophori*	DQ642763	1,382
Trypanoselachoida	*Grillotia yuniariae*	FJ572952	1,531
Trypanoselachoida	*Grillotiella exilis*	FJ572953	1,533
Trypanoselachoida	*Gymnorhynchus isuri*	DQ642747	1,409
Trypanoselachoida	*Hepatoxylon* sp.	AF286969	1,325
Trypanoselachoida	*Hepatoxylon trichiuri*	FJ572943	1,543
Trypanoselachoida	*Heterosphyriocephalus oheolumiae*	FJ572941	1,553
Trypanoselachoida	*Heterosphyriocephalus oheolumiae*	FJ572942	1,553
Trypanoselachoida	*Heterosphyriocephalus tergestinus*	KX570645	1,269
Trypanoselachoida	*Heterosphyriocephalus tergestinus*	KX570646	1,300
Trypanoselachoida	*Heterosphyriocephalus tergestinus*	KX570647	1,272
Trypanoselachoida	*Hornelliella annandalei*	DQ642762	1,302
Trypanoselachoida	*Hornelliella annandalei*	FJ572956	1,555
Trypanoselachoida	*Iobothrium elegans*	DQ642754	1,392
Trypanoselachoida	*Lacistorhynchus dollfusi*	DQ642761	1,316
Trypanoselachoida	*Lacistorhynchus tenuis*	FJ572955	1,535
Trypanoselachoida	*Molicola* sp.	FJ572949	1,542
Trypanoselachoida	*Molicola* sp.	KX712337	1,298
Trypanoselachoida	*Molicola* sp.	KX712338	1,299
Trypanoselachoida	*Molicola* sp.	KX712339	1,317
Trypanoselachoida	*Molicola* sp.	KX712340	1,509
Trypanoselachoida	*Molicola* sp.	KX712341	1,511
Trypanoselachoida	*Molicola uncinatus**	DQ642746	1,298
Trypanoselachoida	*Otobothrium carcharidis*	DQ642749	1,247
Trypanoselachoida	*Otobothrium cysticum*	FJ572962	1,531
Trypanoselachoida	*Otobothrium penetrans*	FJ572961	1,536
Trypanoselachoida	*Otobothrium propecysticum*	DQ642751	1,393
Trypanoselachoida	*Otobothrium* sp.	DQ642750	1,244
Trypanoselachoida	*Paragrillotia similis*	FJ572954	1,390
Trypanoselachoida	*Paragrillotia similis*	KF685909	1,399
Trypanoselachoida	*Parotobothrium balli*	DQ642756	1,396
Trypanoselachoida	*Parotobothrium balli*	FJ572959	1,533
Trypanoselachoida	*Pintneriella musculicola*	FJ572948	1,544
Trypanoselachoida	*Poecilancistrium caryophyllum*	FJ788108	1,283
Trypanoselachoida	*Proemotobothrium linstowi*	DQ642755	1,401
Trypanoselachoida	*Proemotobothrium* sp.	DQ642753	1,263
Trypanoselachoida	*Protogrillotia* sp.*	DQ642767	1,397
Trypanoselachoida	*Pseudogilquinia microbothria*	DQ642766	1,114
Trypanoselachoida	*Pseudogilquinia pillersi*	AF286964	1,340
Trypanoselachoida	*Pseudolacistorhynchus heroniensis*	AF286968	1,326
Trypanoselachoida	*Pseudotobothrium arii*	DQ642748	1,401
Trypanoselachoida	*Pseudotobothrium dipsacum*	AF286972	2,093
Trypanoselachoida	*Pterobothrium lintoni*	AF286973	1,182
Trypanoselachoida	*Pterobothrium platycephalum*	DQ642764	1,275
Trypanoselachoida	*Sagittirhynchus aculeatus*	DQ642745	1,161
Trypanoselachoida	*Sphyriocephalus* sp.	AF286974	1,430
Trypanoselachoida	*Sphyriocephalus viridis*	FJ572940	1,608
Trypanoselachoida	*Symbothriorhynchus tigaminacantha*	FJ572960	1,534
Trypanoselachoida	*Vittirhynchus squali*	DQ642743	1,404

Supplemental Table 2. Differences between the measurement ranges presented in the original descriptions *vs* in this study for *Rhinoptericola megacantha* Carvajal & Campbell, 1975 and *Rhinoptericola butlerae* (Beveridge & Campbell, 1988) n. comb. Measurements highlighted in light gray represent any expansion/contraction from the range given in the original description; measurements highlighted in dark gray represent a notable change from the original description. Measurements are given in µm unless otherwise indicated.

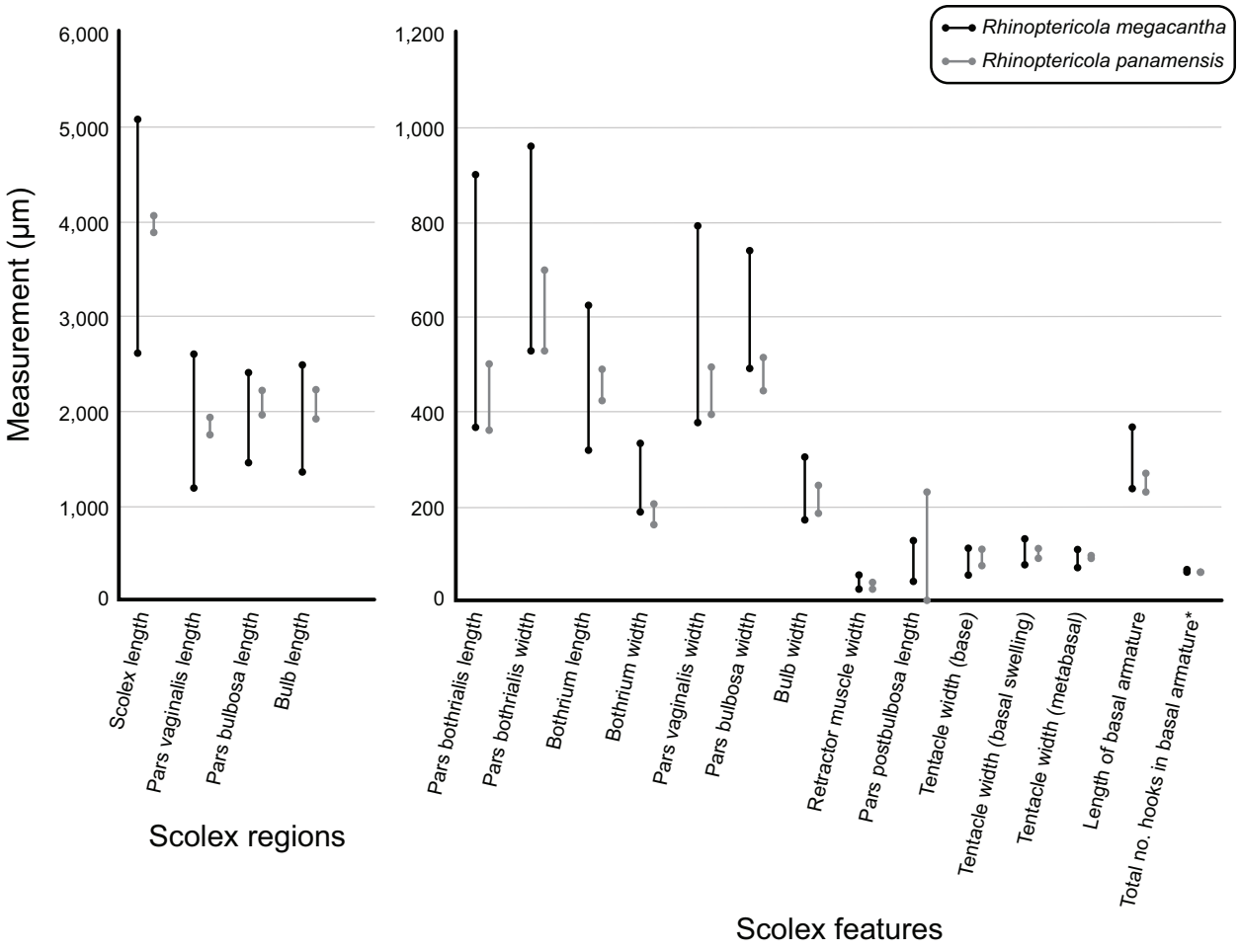
Measurement	*Rhinoptericola megacantha*		*Rhinoptericola butlerae*	
	Original description*	This study	Original description†	This study
Total length (mature)	35–65 mm	10.7–38.6 mm	22 mm	15.5–18.9 mm
Total length (gravid)		23.7–31.6 mm		22.7 mm
Maximum width	700–970	657–1,209		664–1,059
Total no. proglottids (mature)	55–77	39–74	38	42–51
Total no. proglottids (gravid)		22–74		50
Scolex length		2,616–5,078	4,370–5,200	4,533–5,899
Scolex length:width ratio		1:2.8–1:6.4		1:5.0–1:8.9
Pars bothrialis length	550–650	369–902	450–630	418–714
Pars bothrialis width		529–963		664–952
Bothrium length	450–552	320–625		373–653
Bothrium width	300–368	188–332		169–273
Pars vaginalis length	1,600–2,100	1,173–2,609	2,420–3,050	2,478–3,420
Pars vaginalis width		378–793		348–785
Pars bulbosa length	2,100–2,600	1,458–2,410		1,752–2,476
Pars bulbosa width		492–741		558–1,059
Bulb length		1,367–2,483	1,620–2,030	1,641–2,450
Bulb width	150–230	172–306	120–230	186–307
Bulb length:width ratio		1:4.8–1:12.7		1:5.8–1:11.3
Retractor muscle width in bulbs		24–55		20–56
Pars postbulbosa length	70–100	41–128	110–270	76–273
Maximum tentacle length recorded	2,300	2,206		2,219
Tentacle width (base)		56–109		82–159
Tentacle width (basal swelling)	90	81–118	80–110	83–143
Tentacle width (metabasal)	80	68–106	30–50	77–136
Length of basal armature	280	237–368		354–492
No. rows of hooks in basal armature		8–11	10–11	8–12
Total no. hooks in basal armature		60–67		83–99
Hooks per row in metabasal armature	5	7	8	7‡
Length of non-proglottized region		57–257		155–164
No. immature proglottids		17–64		35–46
No. mature proglottids		3–21		5–7
No. gravid proglottids		0–4		0–2
Terminal mature proglottid length	2,200–4,000	1,629–3,170	810–1,400	1,085–1,529
Terminal gravid proglottid length		2,295–3,260	1,130–2,050	1,480
Terminal mature proglottid width	850	402–945	500–700	293–500
Terminal gravid proglottid width		624–1,209		683
Free gravid proglottid length				1,735–2,213
Free gravid proglottid width				747–766
Total no. testes	53–63	41–67	32–45	50–60
No. testes pre-poral		20–26		19–28
No. testes post-poral		21–43		29–32
Testis length		39–137		51–60
Testis width		85–218		90–157
Cirrus sac length		241–672	300	241
Cirrus sac width		149–350	150	195
Genital pore psn. (% from post.; mature)	near ant. 1/3	60–79%	60–65%	64–74%

Genital pore psn. (% from post.; gravid)	near ant. 1/3	65–74%		
Ovary length	500	283–662	180–260	509
Ovary width	250	243–599		237–383
Vitelline follicle length		15–79		11–21
Vitelline follicle width		12–77		8–31
Egg diameter	26	15–23		19–21

* Based on based on six specimens.
† Based on ten specimens.
‡ This number of hooks was previously reported by Schaeffner (2016).



Supplemental Figure 1. Anterior portion of scoleces of *Rhinoptericola buterlae* (Beveridge & Campbell, 1988) n. comb. (A–E) and *Rhinoptericola panamensis* (Schaeffner, 2016) n. comb. (F) illustrating the internal to external orientation of the tentacular armature.(A) Voucher specimen; QM G239458. (B) and (C) Voucher specimen (see Fig. 7B). (D) Voucher specimen; not deposited. (E) Voucher specimen; QM G239455. (F) Paratype; USNM 1298205. Arrows indicate hooks 1(1') and keys to tenacle surfaces pictured follow Fig. 1.



Supplemental Figure 2. Graphs illustrating the overlapping measurement ranges between *Rhinoptericola megacantha* Carvajal & Campbell, 1975 and *Rhinoptericola panamensis* (Schaeffner, 2016) n. comb. for regions and features of the scolex. Asterisk (*) indicates a scolex feature that is a count rather than a measurement.

Chapter 2

First insights into population structure and genetic diversity in relation to host specificity in trypanorhynch tapeworms (Cestoda: Trypanorhyncha) using a multiplexed shotgun genotyping approach

ABSTRACT

Little is known about the population genetics of tapeworms in the order Trypanorhyncha. To date, most studies on trypanorhynchs that incorporate sequence data have focused on species identifications or interrelationships between species. This study presents the first population genomic data for trypanorhynch tapeworms, and for elasmobranch tapeworms more broadly. Multiplexed shotgun genotyping (MSG) datasets were generated to characterize component population structure and infrapopulation diversity for a species in each of the two trypanorhynch suborders: *Rhinoptericola megacantha* Carvajal & Campbell, 1975 in the Trypanobatoida, and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 in the Trypanoselachoida. The two species demonstrate different degrees of relaxed host specificity, allowing for identification of potential correlations between host specificity and genetic structure. For both species, one to five replicate tapeworm specimens were sequenced from the same shark or ray host individual, from multiple host individuals representing the same host species within and across geographic regions, and from multiple host species. For *R. megacantha*, population structure coincided with geographic region rather than definitive host species. For *C. gracilis*, limited population structure was found, suggesting a potential link between degree of host specificity and genetic structure in trypanorhynch tapeworms. It was determined that conspecific trypanorhynchs collected from the same host individual can be as, or more, genetically divergent from one another than from conspecifics collected from different host individuals. For both species, high levels of homozygosity and elevated F_{IS} values were documented. In addition, only minor intraspecific variation was identified in partial 28S rRNA data generated for both species, supporting the ability of the tapeworm barcoding gene to confirm conspecificity even for tapeworms that are not strictly host specific. Finally, based on examination of material from over 150 carcharhiniform sharks, it is suggested that adults of *C. gracilis* may be restricted to parasitizing requiem sharks (family Carcharhinidae Jordan & Evermann, 1896).

INTRODUCTION

The genomic revolution has made what was once reserved for the likes of fruit flies and monkeyflowers accessible to an astonishing diversity of non-model species. It is now easier and more cost-effective than ever before to build genome-scale datasets suitable for addressing population-level questions for almost any organism of interest, and researchers in non-model systems are rising to meet the challenge. An unprecedented number of population genomic studies centered on non-model species have been published in recent decades (see Ellegren, 2014; da Fonseca et al., 2016), and the number of such studies and breadth of taxonomic diversity they encompass only continue to expand.

Even as population genomic workflows become increasingly accessible, however, parasitic species still lag their free-living counterparts on the trend. Though studies of population genomics are relatively common for parasites important to human health and animal husbandry (e.g., Redman et al., 2015; Neafsey et al., 2021; Shortt et al., 2021), genomic techniques have rarely been applied to investigate population-level questions for parasites that lack anthropogenic import. This is particularly true for tapeworms. To date, population genomics in tapeworms has been restricted to a handful of species that infect humans and livestock (e.g., Addy et al., 2017), and a species each infecting freshwater teleosts, clawed frogs, and birds (Brabec et al., 2016; Fraija-Fernández et al., 2021). Collectively, these studies include species from only three of the 19 currently recognized orders of tapeworms (Caira et al., 2017), thus representing only a fraction of diversity in the group.

Perhaps one of the most intriguing groups of tapeworms from a perspective of population-level structure and diversity is the order Trypanorhyncha. The Trypanorhyncha is one of eight orders of tapeworms whose members exclusively parasitize rays and sharks (i.e., elasmobranchs) as adults. Presently, over 330 species of trypanorhynchs are recognized (Caira et al., 2021) and are divided between two suborders who members parasitize primarily either rays (Trypanobatoida) or sharks (Trypanoselachoida) as adults (Olson et al., 2010; Beveridge et al., 2017).

Trypanorhynchs have long been thought to be the only elasmobranch tapeworms for which strict host specificity is the exception rather than the rule (Caira & Jensen, 2014). The reality is, however, that adult trypanorhynchs exhibit a range of degrees of specificity for their definitive elasmobranch hosts. For example, each species of *Hemionchos* Campbell & Beveridge, 2006 is highly host specific and is known to parasitize only a single species of devil ray (family Mobulidae Gill, 1893) (Campbell & Beveridge, 2006), but individual species of *Prochristianella* Dollfus, 1946 have been reported from 39 species of rays representing four orders (Beveridge, 1990; Schaeffner & Beveridge, 2012, 2014).

Much like their relatives in other orders of tapeworms that parasitize elasmobranchs, trypanorhynchs also vary in the breadth of their geographic ranges. For trypanorhynchs, variation in geographic range occurs somewhat in concert with varying degrees of host specificity. For example, the host-specific *Prochristianella caribbensis* (Kovacs & Schmidt, 1980) Beveridge, 1990 is known only from the yellow stingray, *Urobatis jamaicensis* (Cuvier, 1816), from Jamaica, while *Proemotobothrium linstowi* (Southwell, 1912) Beveridge & Campbell, 2001 has been reported from the ocellated eagle ray, *Aetobatus ocellatus* (Kuhl, 1823) (as *Aetobatis narinari* [Euphrasen, 1790]), the bottlenose wedgefish, *Rhynchobatus australiae* Whitley, 1939, the whitespotted wedgefish, *Rhynchobatus djiddensis* (Forsskål, 1775), and a species of requiem shark (family Carcharhinidae Jordan & Evermann, 1896), collectively from Australia, India, Sri Lanka, and Mozambique (Beveridge & Campbell, 2001; Palm, 2004; Herzog & Jensen, unpublished data). Such variation allows for many unique combinations of host use and geographic range in this group, highlighting the trypanorhynchs as ideal candidates for studies of how these life history traits may influence population-level structure and genetic diversity.

The first insights into the population genomics of trypanorhynch tapeworms are presented here. Two focal species were selected for this investigation, representing each of the two trypanorhynch suborders: *Rhinoptericola megacantha* Carvajal & Campbell, 1975 (family Rhinoptericolidae Carvajal & Campbell, 1975) in the suborder Trypanobatoida, and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pinter, 1931 (family Lacistorhynchidae Guiart,

1927) in the suborder Trypanoselachoida. Both species demonstrate relaxed host specificity, or are euryxenous sensu Caira et al. (2003), but they vary in their degree of euryxeny and in the geographic ranges they span. Adults of *R. megacantha* are known from three species of cownose rays (family Rhinopteridae Jordan & Evermann, 1896) and a species of stingray (family Dasyatidae Jordan, 1888), and they are restricted to the Atlantic Ocean (Herzog & Jensen, 2022). *Callitetrarhynchus gracilis* is comparably less host specific and more broadly distributed. Prior to this study, adults of *C. gracilis* had been reported from 20 species of carcharhiniform sharks representing eight genera and three families (i.e., the Carcharhinidae, Sphyrnidae Gill, 1872, and Triakidae Gray, 1851), and from a species each of ginglymostomatid wobbegong and dasyatid stingray (Nakajima & Egusa, 1972a–c, 1973; Heinz & Dailey, 1974; Watson & Thorson, 1976; São Clemente & Gomes, 1989; Palm, 1995; Beveridge & Campbell, 1998; Palm & Overstreet, 2000; Olson et al., 2001; Palm, 2004; Owens, 2008; Haseli et al., 2010; Olson et al., 2010; Méndez & González, 2013; Schaeffner & Beveridge, 2014; Mhaisen et al., 2018). These reports include localities across the world's tropical and temperate oceans, and even include a report from the bull shark, *Carcharhinus leucas* (Müller & Henle, 1839), from freshwater river systems in Costa Rica (Watson & Thorson, 1976).

The goal of this investigation was to leverage next generation sequencing methods and previous global collections of trypanorhynchs from elasmobranchs with an emphasis on broad and deep sampling to address the following questions: (1) Is population-level genetic diversity in trypanorhynch tapeworms that demonstrate relaxed host specificity structured by definitive host species or geography? (2) Does degree of host specify correlate with observed patterns of population structure? (3) Are conspecific trypanorhynchs more genetically similar within a host individual than between host individuals?

MATERIALS AND METHODS

Sampling strategy and specimen collection

As a result of global collections of tapeworms made over recent decades, trypanorhynchs

from hundreds of elasmobranchs appropriately preserved for DNA sequencing were already in-hand. To assess whether conspecific tapeworms collected from a single individual ray or shark (i.e., an infrapopulation) are more genetically similar to one another than those collected from different host individuals, multiple conspecific trypanorhynchs from a single host individual were sampled, for up to five tapeworms per host. To assess the structure of trypanorhynch populations across their known host species and geographic ranges (i.e., the structure of a component population), conspecific trypanorhynchs from multiple host individuals of the same host species, both within and across geographic regions, were sequenced. This process was replicated for as many host species and geographic localities as was possible (see Table 1, Fig. 1).

Elasmobranch intestines or intestinal contents fixed in 95% ethanol were examined for tapeworms. Prior to examination, spiral intestines had been removed from the body cavity and opened with a longitudinal incision. Intestines and their contents were either fixed entirely in 95% ethanol, or intestines were fixed in 10% seawater-buffered formalin and a portion of their contents (including tapeworms) were fixed in 95% ethanol. All ethanol-fixed material was transferred to the University of Kansas (KU) or the University of Connecticut (UConn) and stored in a freezer prior to examination for tapeworms.

For *R. megacantha*, sampling was informed by the comprehensive summary of its known host species and geographic localities compiled by Herzog and Jensen (2022). Specimens of *R. megacantha* were recovered from 19 cownose rays collectively representing one genus and three species in the family Rhinopteridae: ***Rhinoptera bonasus*** (Mitchill, 1815) (n=5), ***Rhinoptera brasiliensis*** Müller, 1836 (n=9), and ***Rhinoptera marginata*** Saint-Hilaire, 1817 (n=5). More than one tapeworm was recovered from eight cownose ray host individuals (see Table 1). All reports to date of adults of *R. megacantha* from elasmobranchs, and the combinations of host species and geographic locality represented by specimens included in this study, are summarized in Table 2.

For *C. gracilis*, sampling was informed by reports from the literature of adults from elasmobranchs, summarized herein in Table 2. Based on these reports, species of carcharhiniform sharks in the families Carcharhinidae (requiem sharks), Galeocerdidae Herman, 2010 (tiger

sharks), Sphyrnidae (hammerhead and bonnethead sharks), and Triakidae (houndsharks) were targeted, as they collectively represent the greatest number of reports of adults of *C. gracilis*. For carcharhinids, material from 120 specimens of 30 species from 17 geographic localities was examined: ***Carcharhinus acronotus*** (Poey, 1860) (n=1; Gulf of Mexico), ***Carcharhinus amblyrhynchoides*** (n=3; Borneo, India), ***Carcharhinus amboinensis*** **1** sensu Naylor et al. (2012) (n=1; Australia), ***Carcharhinus brachyurus*** (Günther, 1870) (n=2; Korea), ***Carcharhinus brevipinna*** (Müller & Henle, 1839) (n=10; Gulf of Mexico, Senegal, Borneo), ***Carcharhinus*** **cf.** ***cautus*** (n=1; Solomon Islands), ***Carcharhinus coatesi*** (Whitley, 1939) (n=2; Australia), ***Carcharhinus falciformis*** (Müller & Henle, 1839) (n=2; Florida), ***Carcharhinus isodon*** (Müller & Henle, 1839) (n=6; South Carolina, Gulf of Mexico), ***Carcharhinus leucas*** (n=1; Senegal), ***Carcharhinus*** **cf.** ***leucas*** sensu Naylor et al. (2012) (n=1; Borneo), ***Carcharhinus limbatus*** (Müller & Henle, 1839) (n=12; South Carolina, Florida, Gulf of Mexico), ***Carcharhinus*** **cf.** ***limbatus*** sensu Naylor et al. (2012) (n=4; Australia, Borneo, Sri Lanka), ***Carcharhinus obscurus*** (Lesueur, 1818) (n=2; Florida, Senegal), ***Carcharhinus sealei*** (n=2; Borneo), ***Carcharhinus sorrah*** (Müller & Henle, 1839) (n=4; Borneo, Thailand, Taiwan, Vietnam), ***Carcharhinus*** **cf.** ***sorrah*** sensu Naylor et al. (2012) (n=1; Australia), ***Carcharhinus tilstoni*** (Whitley, 1950) (n=1; Australia), ***Lamiopsis tephrodes*** Fowler, 1905 (n=4; Borneo**),** ***Loxodon*** **cf.** ***macrorhinus*** sensu Naylor et al. (2012) (n=3; Mozambique), ***Negaprion acutidens*** (Rüppell, 1837) (n=8; Australia), ***Prionace glauca*** (n=23; Montauk, NY), ***Rhizoprionodon*** **cf.** ***acutus*** **1** sensu Naylor et al. (2012) (n=3; Senegal), ***Rhizoprionodon*** **cf.** ***acutus*** **3** sensu Naylor et al. (2012) (n=1; Borneo), ***Rhizoprionodon longurio*** (Jordan & Gilbert, 1882) (n=1; Gulf of California), ***Rhizoprionodon oligolinx*** Springer, 1964 (n=2; Borneo, Sri Lanka), ***Rhizoprionodon terraenovae*** (Richardson, 1836) (n=10; South Carolina, Florida, Gulf of Mexico, Atlantic Ocean), ***Scoliodon*** **cf.** ***lauticaudus*** (n=1; India), ***Scoliodon macrorhynchos*** (Bleeker, 1852) (n=6; Taiwan, Vietnam), and ***Triaenodon obesus*** (Rüppell, 1837) (n=2; Solomon Islands).

For galeocerdid sharks, a single specimen for ***Galeocerdo*** **cf.** ***cuvier*** sensu Naylor et al. (2012) from the Gulf of Mexico was examined.

For sphyrnid sharks, material from 29 specimens of seven species from 10 geographic localities was examined: ***Sphyrna lewini* 1** sensu Naylor et al. (2012) (n=7; Florida, Gulf of Mexico), ***Sphyrna lewini* 2** sensu Naylor et al. (2012) (n=3; Gulf of California, Taiwan), ***Sphyrna* cf. *lewini*** (n=1; Borneo), ***Sphyrna mokarran* 1** sensu Naylor et al. (2012) (n=3; Florida, Gulf of Mexico), ***Sphyrna mokarran* 2** sensu Naylor et al. (2012) (n=3; Australia), ***Sphyrna tiburo*** (n=5; South Carolina, Gulf of Mexico), and ***Sphyrna zygaena*** (Linnaeus, 1758) (n=7; Gulf of California, Ecuador, Senegal, Taiwan, Japan).

For triakid sharks, material from nine specimens of three species from five geographic localities was examined: ***Galeorhinus galeus*** (Linnaeus, 1758) (n=1; New Zealand), ***Mustelus canis*** (n=7; Long Island Sound, Rhode Island, Atlantic Ocean), and ***Mustelus* cf. *antarcticus*** (n=1; Solomon Islands).

In addition to carcharhiniforms, *C. gracilis* has been reported from the tawny nurse shark *Nebrius ferrugineus* (Lesson, 1831) and the estuary stingray, *Hemitrygon fluviorum* (Ogilby, 1908) (see Table 2). Four specimens of ***Nebrius ferrugineus*** from Australia were examined. No material from ***Hemitrygon fluviorum*** was available for examination.

Ultimately, specimens of *C. gracilis* were recovered from 17 requiem sharks collectively representing five species and two genera in the family Carcharhinidae: ***Carcharhinus brevipinna*** (n=4), ***Carcharhinus isodon*** (n=1), ***Carcharhinus limbatus*** (n=6), ***Carcharhinus* cf. *limbatus*** (n=1), and ***Rhizoprionodon terraenovae*** (n=5). More than one tapeworm was recovered from 12 requiem shark host individuals (see Table 1). The combinations of host species and geographic locality represented by specimens included in this study are summarized in Table 2.

For each host individual from which tapeworms included in this study were recovered, Table 3 lists disk width (rays) or total length (sharks), sex, collection date, collection locality, the number of tapeworms recovered, and the number of tapeworms recovered that were ultimately included in final datasets. A unique field identification code is also provided for each host individual in Table 3. This code can be entered online in the Global Cestode Database (www.elasmobranchs.tapewormdb.uconn.edu) (Caira et al., 2021) to access additional

information about each host. For most hosts individuals, identifications were confirmed by Naylor et al. (2012) using sequence data for the NADH2 gene; for others, NADH2 sequence data were generated from liver tissue preserved in 95% ethanol and sequenced at UConn following the methods outlined in Fernando et al. (2019) (Caira et al., unpublished data; see Table 3).

Maps of sampling localities with the number of individuals of each host species sampled from each locality, the number of tapeworms recovered from each host individual, and the number of tapeworms recovered that were ultimately included in finalized datasets are provided in Figure 1. To visualize sampling localities, geographic coordinates were plotted to maps in *R* v. 4.0.3 (R Core Team [2020]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.Rproject.org/) via *RStudio* v. 1.3.1093 (RStudio Team [2020]. RStudio: Integrated Development for R. RStudio. PBC, Boston, MA, USA. http://www.rstudio.com/) using the packages *maps* v. 3.4.0 (Becker et al., 2021), *mapdata* v. 2.3.0 (Becker et al., 2018), *maptools* v. 1.12 (Bivand & Lewin-Koh, 2022), and *scales* v. 1.1.1 (Wickham & Seidel, 2020).

Ecological terminology used to define levels of tapeworm populations follows Bush et al. (1997). Ray taxonomy follows Last et al. (2016). Shark taxonomy follows Naylor et al. (2012) and Ebert et al. (2021).

Collections in from Australia were made under the auspices of Richard Mounsey and Julie Lloyd, formerly of Darwin Fisheries. Material from Belize was collected under permit no. 00001612 issued to Janine N. Caira, Kirsten Jensen, Fernando P.L. Marques, and Roy Polonio by Fisheries Administrator Beverly Wade of the Belize Fisheries Department (Ministry of Forestry, Fisheries and Sustainable Development), Belize. Material from Florida was collected under the auspices of the University of Southern Mississippi Gulf Coast Research Laboratory and/or the National Marine Fisheries Service, Southeast Fisheries Science Center, Panama City, Florida, USA. Collections in the Gulf of Mexico were made under the auspices of the University of Southern Mississippi Gulf Coast Research Laboratory. Material from the northern Atlantic Ocean was collected under the auspices of the National Oceanic and Atmospheric Administration

and the National Marine Fisheries Service. Material from Senegal was collected under permit no. 006087 issued by the Ministère de L'Éducation, Dakar, Senegal. Collections in South Carolina were made under the auspices of the South Carolina Department of Natural Resources (Bryan Frazier and Ashley Shaw) and the College of Charleston (Isaure de Buron).

Specimen vouchering and DNA extraction

Prior to DNA extraction, each tapeworm was photographed using a Lumenera INFINITY3-6UR 6.0 megapixel USB 3 microscopy camera (Teledyne Lumenera, Ottawa, ON, Canada) attached to a Leica MZ16 dissecting microscope (Leica Microsystems, Buffalo Grove, IL, USA). After photo voucher acquisition, microscissors were used to remove a piece of the strobila and/or scolex of each specimen for DNA extraction. To ensure accurate genotyping, DNA was intentionally not extracted from gravid proglottids (i.e., proglottids containing eggs). Methods for DNA extraction follow Herzog and Jensen (2022). Whole-mounted hologenophores sensu Pleijel et al. (2008) were then generated from the remaining portions each specimen not utilized for DNA extraction. Methods for hologenophore preparation follow Herzog and Jensen (2018). Hologenophores consisted of either a scolex and partial strobila, or a complete or partial scolex only. A subset of hologenophores is deposited at the Lawrence R. Penner Parasitology Collection (LRP) Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, USA.

Sanger sequencing and distance-based analysis of partial 28S rRNA data

To confirm species identifications based on morphology, sequence data for the D1–D3 gene region of the 28S rRNA gene (hereafter 28S) were generated for a subset of specimens (i.e., all 39 specimens of *R. megacantha* and 39 of 47 specimens of *C. gracilis*; see Table 1). Methods for amplification of 28S and Sanger sequencing follow Herzog and Jensen (2022). A subset of the 28S sequences generated for *R. megacantha* are deposited in GenBank (see Table 1).

Following Sanger sequencing, raw reads for 28S were assembled in *Geneious Prime*

v. 2019.1.3 (https://www.geneious.com) de novo or guided by mapping to a reference sequence. Assembled sequences were first trimmed in *Geneious Prime*, then aligned with an outgroup sequence using *MUSCLE* v. 3.8.425 (Edgar, 2004a, b) with default settings and 1,000 iterations. For *R. megacantha*, *Rhinoptericola butlerae* (Beveridge & Campbell, 1988) Herzog & Jensen, 2022, was chosen as an outgroup (GenBank no. OL412708) (Herzog & Jensen, 2022), and for *C. gracilis*, *Callitetrarhynchus speciosus* (Linton, 1897) Carvajal & Rego, 1985 (GenBank no. DQ642759) (Olson et al., 2010) was chosen as an outgroup. Outgroup sequences were selected based on sequence length and completeness, and phylogenetic position relative to the species of interest. Dendrograms were generated from 28S alignments using the *Geneious Tree Builder* specifying a Jukes-Cantor genetic distance model and a neighbor-joining tree building method.

Restriction enzyme selection, library preparation, and next generation sequencing

Restriction enzyme selection for the generation of multiplexed shotgun genotyping (MSG) datasets was informed by virtual simulation of enzyme digestion using custom python scripts (courtesy of J.K. Kelly, KU) in *Python* v. 2.7.16 (Rossum & Drake, 2009). The cut site sequences for the restriction enzymes AseI, Bfal, CviQI, MseI, and NdeI (New England BioLabs, Ipswich, MA, USA), which are all molecularly compatible with the MSG library preparation protocol, were tested using draft genomes for *Rhinoptericola megacantha* (~351 Mb) and *Pseudolacistorhynchus heroniensis* (Sakanari, 1989) Palm, 2004 (~1 Gb) (Caira, Jockusch, Ralicki, Wegryzn, and Jensen, unpublished data). As a genome for *C. gracilis* is not available, the draft genome of *P. heroniensis*—a member of the same family, Lacistorhynchidae—was used. Results of virtual digestions indicated MseI as the most suitable enzyme based on cut site frequency in repetitive versus non-repetitive genomic regions and the size distribution of fragments generated. Physical test digestions using MseI were then performed at the University of Kansas Genome Sequencing Core (KU GSC) using extracted genomic DNA for one specimen of each species of interest (i.e., CH156 for *R. megacantha* and MS05214 for *C. gracilis*; see Table 1). Digested DNA was cleaned up and concentrated using a bead clean up protocol with

2x AMPure XP beads (Beckman Coulter Life Sciences, Indianapolis, IN, USA), quality checked using an Ivitrogen Qubit assay (ThermoFisher Scientific, Waltham, MA, USA), and run on a TapeStation 2200 agarose gel (Agilent, Santa Clara, CA, USE) to visualize the proportion of DNA within a given size range.

Following virtual and physical test digestions, extracted genomic DNA for all specimens was used to generate two MSG libraries using MseI and the protocol of Andolfatto et al. (2011) with the following modifications: (1) Unique in-line barcodes were ligated to digested DNA prior to pooling to allow for bioinformatic identification of each specimen after sequencing; and (2) following bead purification, pooled libraries were run on a Blue Pippin 2% agarose gel cassette (Sage Science, Beverly, MA, USA) to elute DNA fragments within a 300–400 bp range. Each of the two multiplexed libraries was sequenced on a single flow cell of an Illumina NextSeq 550 High Output Next Generation Sequencer (Illumina, San Diego, CA, USA) for 75 bp single end reads. All specimens were included in the first library. The second library consisted of a subset of specimens for which insufficient read counts were generated following the first round of library preparation and sequencing (see Table 1). Library preparation and sequencing was completed by the KU Genome Sequencing Core.

Single nucleotide polymorphism (SNP) dataset generation

To separately generate single nucleotide polymorphism (SNP) datasets from raw next generation sequence data for each of the two species of interest, *Stacks* v. 2.53 (Catchen et al., 2013; Rochette et al., 2019) was used. First, specimens were demultiplexed and low-quality reads and adaptor contamination were removed using the *process_radtags* module with the *-r*, *-c*, and *-q* flags specified. Further filtering was performed using *Trimmomatic* v. 0.39 (Bolger et al., 2014) to remove remaining low quality reads and adaptor contamination, and to enforce a consistent read length of 70 bp. Read quality was visualized using *FastQC* v. 0.11.7 (Andrews, 2010) and *MultiQC* v. 1.7 (Ewels et al., 2016). Throughout the process of SNP dataset generation, file conversion was accomplished using *PGDSpider* v. 2.1.1.5 (Lischer &

Excoffier, 2012) unless otherwise stated.

SNP dataset generation: *Rhinoptericola megacantha*

Availability of the above-mentioned reference genome for *R. megacantha* allowed for use of a reference-guided alignment approach in *Stacks*. First, an index database was built from the reference genome using *Bowtie 2* v. 2.3.5.1 (Langmead & Salzberg, 2012). For each of the 39 specimens, demultiplexed and quality filtered reads were aligned to the index using *Bowtie 2* with the *--no-unal* and *--sensitive* flags. The program *SAMtools* v. 1.9 (Li et al., 2009) was used to convert the generated sequence alignment map (SAM) files to binary alignment map (BAM) files and to subsequently sort BAM files. The *gstacks* module of *Stacks* was used to generate a bylocus dataset, and to genotype specimens at each SNP for each locus using default settings. The *populations* module of *Stacks* was used to export two unfiltered SNP datasets as variant call format (VCF) files using the *--vcf* flag: one dataset containing all 39 specimens (hereafter the "complete" dataset) and one dataset excluding the six specimens collected from Senegal (hereafter the "no-Senegal" dataset) (see Table 1). For both datasets, all specimens were specified as belonging to a single population in the *populations* module.

Filtering of the complete and no-Senegal SNP datasets was performed iteratively based on various metrics of data quality and completeness in *R* v. 4.0.3 via *RStudio* v. 1.3.1093 using the packages *SNPfiltR* v 0.1.1 (DeRaad, 2022) and *VCFR* v. 1.12.0 (Knaus & Grünwald, 2017). For both datasets, minimum and maximum read depths of 6 and 100, respectively, and a minimum genotype quality score of 30, were enforced. All loci were constrained to be biallelic, and heterozygous genotypes falling outside of an allele balance range of 0.25–0.75 were excluded. Ten specimens with ≥90% missing data (of 39 specimens) were removed from the complete dataset, and seven specimens with ≥90% missing data (of 33 specimens) were removed from the no-Senegal dataset (see Table 1). For both datasets, a SNP completeness cutoff of 80% was specified to ensure no retained specimen had >50% missing data. Linkage disequilibrium between loci was minimized by enforcing a minimum distance of 10,000 bp between SNPs

following estimates of linkage disequilibrium decay by Branca et al. (2011). Filtered versions of the complete dataset and the no-Senegal dataset were then exported. An additional filtering step to remove singletons by enforcing a minimum minor allele count of 3 was then employed following Linck and Battey (2019) for both datasets. These minimum minor allele count-filtered versions of the complete and no-Senegal datasets were then exported.

SNP dataset generation: *Callitetrarhynchus gracilis*

As a reference genome is not available for *C. gracilis*, the *Stacks* de novo approach was used for this species. After demultiplexing and initial quality filtering, parameter testing was performed in *Stacks* using the *denovomap.pl* wrapper. Fifteen of the 47 specimens of *C. gracilis* sequenced were chosen for parameter testing based on possession of high read counts and representation across the range of geographic localities and host species sampled. Following Paris et al. (2017), values for the number of raw reads required to form a stack in the *ustacks* module (*-m*), the number of mismatches allowed between stacks to merge them into a putative locus in the *ustacks* module (*-M*) and the number of mismatches allowed between putative loci during catalog construction in the *cstacks* module (*-n*) were varied. In addition to default settings (i.e., *-m 3*, *-M 2*, *-n 1*), values of *-m* (*2*, *4*, *6*, *8*, *10*) (*-M 2*, *-n 1*) and *-n -M* (*2*, *4*, *6*, *8*) (*-m 3*) were tested, with the *minsamplesperpop 0.80* flag employed and all specimens specified as belonging to a single population. Following parameter testing, *-m 4*, *-M 4*, *-n 4* was determined to be the most optimal combination of parameter settings.

Following Cerca et al. (2021), specimens with high proportions of missing data (i.e., "bad apples") were then identified. Briefly, the *populations* module of *Stacks* was used generate exploratory SNP datasets, both with the *-r 0.4* flag enforced and without an *-r* flag specified, for each of the following combinations of specimens: (1) all 47 specimens, (2) the six specimens collected from South Carolina, (3) the seven specimens collected from Florida and the northern Atlantic Ocean, (4) the 29 specimens collected from the Gulf of Mexico, and (5) the four specimens collected from Senegal (see Table 1). For each iteration of *populations*,

each combination of specimens was specified as comprising a single population. Proportions of missing data and mean depth of coverage for each specimen in each of the ten resulting exploratory datasets were assessed separately using the --*missingindv* and --*depth flags* in *VCFtools* v. 0.1.16 (Danecek et al., 2011). Eleven specimens were designated as "bad apples" based on high proportions of missing data and low mean depths of coverage across analyses, and were excluded from future datasets (see Table 1).

Demultiplexed and initially quality filtered reads for the 36 specimens of *C. gracilis* retained (i.e., the "good apples") were assembled into stacks using the *ustacks* module with the *-m 4*, *-M 4*, and --*deleverage* flags specified. The *cstacks* module was then used to create a catalog of consensus loci with the *n 4* flag and default settings. The *sstacks*, *tsv2bam*, and *gstacks* modules were used with default settings to match individual stacks to the catalog, transpose data from orientation by specimen to orientation by locus, and genotype specimens at each SNP for each locus. The *populations* module was used to export two unfiltered SNP datasets as VCF files using the --*vcf* flag: one "complete" dataset containing all 36 specimens, and one "no-Senegal" dataset excluding the three specimens collected from Senegal (see Table 1). For both datasets, all specimens were specified as belonging to a single population in the *populations* module.

As for *R. megacantha* (see above), additional filtering was performed iteratively in *R* v. 4.0.3 via *RStudio* v. 1.3.1093 using the packages *SNPfiltR* and *VCFR* v. 1.12.0. For both datasets, minimum and maximum read depths of 5 and 100, respectively, and a minimum genotype quality score of 30, were enforced. All loci were constrained to be biallelic, and heterozygous genotypes falling outside of an allele balance range of 0.25–0.75 were excluded. Four additional specimens with ≥96% missing data (of 36 retained specimens) were removed from the complete dataset and two additional specimens with ≥96% missing data (of 33 retained specimens) were removed from the no-Senegal dataset (see Table 1). For both datasets, a SNP completeness cutoff of 65% was specified to ensure no retained specimen had >50% missing data, and only a single SNP per locus was retained. Filtered versions of the complete dataset and the no-Senegal dataset were then exported. As with *R. megacantha*, additional versions filtered

by enforcing a minimum minor allele count of 3 were then generated and exported for both the complete dataset and the no-Senegal dataset.

Population genomic methods

Population genetic structure was assessed using discriminant analysis of principal components (*DAPC*) (Jombart et al., 2010) implemented in *R* v. 4.0.3 via *RStudio* v. 1.3.1093 using the package *adegenet* (Jombart, 2008; Jombart & Ahmed, 2011), and with *STRUCTURE* v. 2.3.4 (Pritchard et al., 2000; Falush et al., 2003) implemented via the wrapper program *structure_threader* v. 1.3.10 (Pina-Martins et al., 2017). For *DAPC* analyses, which have been shown to be robust to the inclusion of singletons (Linck & Battey, 2019), datasets not filtered for minimum minor allele count were used. For *STRUCTURE*, which has been shown to be sensitive to the inclusion of singletons (Linck & Battey, 2019), datasets filtered for a minimum minor allele count of 3 were used. The following k-values were tested in *STRUCTURE*: 1–6 (*C. gracilis* complete dataset), 1–5 (*C. gracilis* no-Senegal and *R. megacantha* complete datasets), and 1–4 (*R. megacantha* no-Senegal dataset). For each k-value for each dataset, ten independent *STRUCTURE* runs were completed, each with 1,000,000 generations with the first 50,000 generations discarded as burn-in. For *DAPC* analyses, the most likely number of populations was determined using the Bayesian information criterion (BIC) and the Akaike information criteria (AIC) and the number of retained principal components was determined through ascore optimization. For *STRUCTURE*, the most likely number genetic bins (i.e., k-value) was determined using likelihood scores and the Evanno ΔK method (Evanno et al., 2005) implemented in *structureHarvester* v. A.2 (Earl & VonHoldt, 2012).

Phylogenetic analysis of SNP datasets was completed using *RAxML* v. 8.2.11 (Stamatakis, 2014). The complete dataset not filtered for minimum minor allele count was used for both species. For each dataset, loci were first concatenated for each specimen, and invariant sites were removed using the *Python 3* script *raxml_ascbias* (ascbias.py; https://github.com/btmartin721/raxml_ascbias#raxml_ascbias) in *Python* v. 3.9.7 (Rossum &

Drake, 2009). For both analyses, a GTR+Γ model of nucleotide substitution was specified with the *-m ASC_GTRGAMMA* flag, and standard ascertainment bias correction was specified with the *--asccorr=lewis* flag to account for omission of invariant sites. To yield better likelihood scores, use of the median (rather than the mean) for the discrete Γ model of rate heterogeneity was specified with the *-u* flag. As outgroups were not included in either *RAxML* analysis, the resulting most likely topologies were rooted to maximize subtree balance. Nodal support was assessed with 1,000 rapid bootstrap replicates via the *-f a* and *-# 1000* flags. Bootstrap values (BS) were displayed on the most likely tree topology using *SumTrees* v. 4.5.2 (Sukumaran, J. and M. T. Holder. SumTrees: Phylogenetic Tree Summarization. 4.5.2. Available at https://github.com/jeetsukumaran/DendroPy) implemented in *DendroPy* v. 4.5.2 (Sukumaran & Holder, 2010).

The *populations* module of *Stacks* was used to generate population-level summary statistics and corrected AMOVA F_{ST} values separately for both species using the complete datasets not filtered for minimum minor allele count. Because whether genetic diversity is structured by geography or definitive host species was a primary question of this study, two iterations of *populations* were run for both species specifying different subpopulation groupings. The two iterations run for *R. megacantha* were (1) an iteration specifying the specimens from Senegal, the specimens from Belize, and the specimens from South Carolina and the Gulf of Mexico as three separate populations, and (2) an iteration specifying the specimens from *Rhinoptera brasiliensis, Rhinoptera bonasus*, and *Rhinoptera marginata* as three separate populations. The two iterations run for *C. gracilis* were (1) an iteration specifying six separate populations for the specimens from Senegal, Australia, South Carolina, Florida, the Gulf of Mexico, and the northern Atlantic Ocean, and (2) an iteration specifying five separate populations for the specimens hosted by *Carcharhinus brevipinna, Carcharhinus limbatus*, *Carcharhinus* cf. *limbatus, Carcharhinus isodon*, and *Rhizoprionodon terraenovae*.

To visualize levels of genetic divergence between conspecific specimens within and between infrapopulations, pairwise distances were calculated and plotted separately for both

species using the package *adegenet* in *R* v. 4.0.3 via *RStudio* v. 1.3.1093. For each species, distances were calculated separately for both the complete and no-Senegal datasets not filtered for minimum minor allele count. Comparisons graphed include distances between members of the same infrapopulation for all infrapopulations where SNP data for more than a one specimen was available, and distances between members of each unique pair of infrapopulations.

RESULTS

Sampling across known host species and geographic localities

The 39 specimens of *R. megacantha* included in analyses were collected from South Carolina (*Rhinoptera bonasus* and *Rhinoptera brasiliensis*), the Gulf of Mexico (*Rhinoptera brasiliensis*), Belize (*Rhinoptera brasiliensis*), and Senegal (*Rhinoptera marginata*) (see Table 1, Fig. 1). Specimens from the only other host species from which *R. megacantha* has been reported (i.e., *Hypanus say* [Lesueur, 1817]), and from three additional geographic localities from which it is known (i.e., the Chesapeake Bay, the Gulf of Venezuela, and Brazil) were unavailable (see Table 2). These 39 specimens do not represent novel reports of host species or geographic localities for *R. megacantha* (see Table 2).

The 47 specimens of *C. gracilis* included in analyses herein were collected from specimens of *Carcharhinus brevipinna* (Senegal, Gulf of Mexico), *Carcharhinus isodon* (Gulf of Mexico), *Carcharhinus limbatus* (South Carolina, Florida, Gulf of Mexico), *Carcharhinus* cf. *limbatus* (Australia), and *Rhizoprionodon terraenovae* (South Carolina, Florida, Gulf of Mexico, the northern Atlantic Ocean) (see Table 1, Fig. 1). *Carcharhinus brevipinna*, *Carcharhinus isodon*, and *Carcharhinus* cf. *limbatus* represent novel host reports for *C. gracilis*, and *Carcharhinus limbatus* from South Carolina and Florida, and *Rhizoprionodon terraenovae* from South Carolina and Florida represent novel combinations of host species and locality for the species (see Table 2). No specimens of *C. gracilis* were recovered from any of the galeocerdid, sphyrnid, triakid, or orectolobiform sharks examined. Species of hosts from which *C. gracilis* has been reported that were examined, but from which *C. gracilis* was not recovered, are indicated in

bold font in Table 2.

The following species of carcharhiniform sharks not previously known to host *C. gracilis*, but for which material was available, were examined, but found to not be infected with *C. gracilis*: 17 species of carcharhinids (including species of *Scoliodon* Müller & Henle, 1838, *Loxodon* Müller & Henle, 1838, and *Triaenodon* Müller & Henle, 1837); the single species of galeocerdid; four species of *Sphyrna* Rafinesque, 1810; and three species of triakids (including a species of *Galeorhinus* Blainville, 1816).

28S Sanger data and conspecificity

For *R. megacantha*, 28S data were successfully generated for all 39 specimens included in this study, representing all three species of cownose rays and all four geographic localities, and five unique combinations of host species and locality (Table 1, Fig. 1A). The 39 (of 47) specimens of *C. gracilis* for which 28S data were successfully generated collectively represent all five species of requiem sharks and all five geographic sampling localities, and 11 unique combinations of host species and locality (Table 1, Fig. 1B, C). The lengths of the final trimmed 28S *MUSCLE* alignments were 1,428 bp for *R. megacantha* (39 ingroup sequences and one outgroup sequence) and 1,440 bp for *C. gracilis* (39 ingroup sequences and one outgroup sequence). For *R. megacantha*, individual specimens differed from one another by 0–3 bp (0–0.21%), excluding ambiguous base calls. For *C. gracilis*, individual specimens differed from one another by 0–5 bp (0–0.35%), excluding ambiguous base calls.

The results of neighbor-joining analyses for both species are presented in Figure 2. No grouping of specimens by host individual, host species, or geographic sampling locality was evident for *R. megacantha* (Fig. 2A) or *C. gracilis* (Fig. 2B). Thirteen sequences for specimens of *R. megacantha* contained disproportionally high percentages of missing data (i.e., ≥200 bp) because of stretches of ambiguous base calls at the ends or in the middle of the sequence (Table 1). These specimens frequently grouped together in the neighbor-joining analysis (Fig. 2A). Three sequences for specimens of *C. gracilis* also contained disproportionally high percentages

of missing data (Table 1); two of these specimens were recovered on short branches basal to the remaining ingroup specimens (Fig. 2B).

SNP dataset generation

In total, eight final filtered SNP datasets were generated for population genomic analyses (four datasets for each of the two species). For each species, these include: (1) a complete dataset; (2) a complete dataset additionally filtered for minimum minor allele count; (3) a no-Senegal dataset; and (4) a no-Senegal dataset additionally filtered for minimum minor allele count. The number of specimens, number of loci, and programs used to analyze each of the eight final filtered SNP datasets are presented in Table 4. After filtering, 29 (of 39) and 26 (of 34) specimens were retained in the complete and no-Senegal datasets, respectively, for *R. megacantha* (Table 4). The specimens retained in the complete dataset represented 14 of 19 cownose ray host individuals sampled, and the specimens retained in the no-Senegal dataset represented 11 of 14 cownose ray host individuals sampled. Multiple specimens from a single host individual were retained for seven cownose rays for both the complete and no-Senegal datasets (Table 1, Fig. 1A). After filtering, 32 (of 47) and 21 (of 43) specimens were retained in the complete and no-Senegal datasets, respectively, for *C. gracilis* (Table 4). The specimens retained in the complete dataset represented 15 of 17 requiem shark host individuals sampled, and the specimens retained in the no-Senegal dataset represented 14 (of 15) requiem shark host individuals sampled. Multiple specimens from a single host individual were retained for seven requiem sharks for both the complete and no-Senegal datasets (Table 1, Fig. 1B, C).

Component population structure: *DAPC* and *STRUCTURE*

Results from *DAPC* analyses for both species are presented in Figure 3. For analysis of the complete dataset for *R. megacantha* with *DAPC*, both AIC and BIC preferred a k-value of 2, separating specimens collected from the eastern Atlantic (i.e., from Senegal; hosted by *Rhinoptera marginata*) from those collected from the western Atlantic (hosted by *Rhinoptera*

bonasus and *Rhinoptera brasiliensis*) (Fig. 3A). A k-value of 2 was also preferred by both AIC and BIC for the no-Senegal dataset for *R. megacantha*, separating specimens from Belize (hosted by *Rhinoptera brasiliensis*) from those collected from South Carolina and the Gulf of Mexico (hosted by *Rhinoptera bonasus* and *Rhinoptera brasiliensis*) (Fig. 3B).

For analysis of the complete dataset for *C. gracilis*, BIC preferred a k-value of 2 (Fig. 3C) while AIC preferred a k-value of 3 (Fig. 3D). At k=2, one cluster comprised a group of six specimens collected from Florida, the Gulf of Mexico, and Australia (hosted by *Carcharhinus brevipinna, Carcharhinus limbatus, Carcharhinus* cf. *limbatus*, and *Rhizoprionodon terraenovae*) while the second cluster comprised the remaining 26 specimens collected from South Carolina, Florida, the Gulf of Mexico, Senegal, and the northern Atlantic Ocean (hosted by *Carcharhinus brevipinna, Carcharhinus isodon, Carcharhinus limbatus*, and *Rhizoprionodon terraenovae*) (Fig. 3C). At k=3, the clustering pattern was identical to that recovered at k=2, with the exception of the single specimen from Senegal (hosted by *Carcharhinus brevipinna*) being recovered in its own cluster (Fig. 3D). For the no-Senegal dataset for *C. gracilis*, a k-value of 2 was preferred, recovering the same two multi-specimen clusters described above for the complete dataset at k=3 (Fig. 3E).

Results from *STRUCTURE* analyses for *R. megacantha* are presented in Figure 4. For analysis of the complete dataset, the Evanno ΔK method preferred a k-value of 2, separating specimens collected from the eastern Atlantic (i.e., from Senegal; hosted by *Rhinoptera marginata*) from those collected from the western Atlantic (hosted by *Rhinoptera bonasus* or *Rhinoptera brasiliensis*) (Fig. 4A), thus mirroring the results from the *DAPC* analysis. A k-value of 5 had the greatest likelihood value for this dataset. Obvious patterns of clustering by host individual, host species, or geographic sampling locality were not revealed by k-values of 3–4 for the specimens collected from the western Atlantic, but at k=5, the three specimens from Belize are distinguishable from nearly all specimens from the more northern western Atlantic collecting localities. All k-values tested for the complete dataset indicated little genomic variation among specimens from the western Atlantic (Fig. 4A). For k-values of 4 and 5, nine of

ten *STRUCTURE* replicates binned genetic diversity into only three and four groups, respectively (Fig. 4A). Results from the single iteration for each of the k=4 and k=5 runs that returned genetic diversity binned in as many groups as specified by the k-value are presented in Supplemental Figure 1. For the single replicate for k=5 that binned genetic diversity into five groups, the three specimens collected from Belize are distinguishable from the specimens collected from South Carolina and the Gulf of Mexico (Supplemental Fig. 1).

For analysis of the no-Senegal dataset for *R. megacantha* with *STRUCTURE*, the Evanno ΔK method preferred a k-value of 2, separating specimens from Belize (hosted by *Rhinoptera brasiliensis*) from those collected from South Carolina and the Gulf of Mexico (hosted by *Rhinoptera bonasus* and *Rhinoptera brasiliensis*) (Fig. 4B), again mirroring the results of the *DAPC* analysis. The highest k-value tested (in this case, k=4) had the highest likelihood value. No obvious patterns of clustering by host individual, host species, or geographic sampling locality were evident for the specimens collected from South Carolina and the Gulf of Mexico for k-values of 3 and 4 for the no-Senegal dataset (Fig. 4B).

Results from *STRUCTURE* analyses for *C. gracilis* are presented in Figure 5. For analysis of the complete dataset, the Evanno ΔK method preferred a k-value of 2, while a k-value of 6 had the greatest likelihood value (Fig. 5A). For k=2, the individual from Australia hosted by *Carcharhinus* cf. *limbatus*, the individual from Florida hosted by *Rhizoprionodon terraenovae*, and four individuals from the Gulf of Mexico hosted by *Carcharhinus* cf. *limbatus, Carcharhinus brevipinna*, and *Rhizoprionodon terraenovae* were recovered as sharing similar genetic backgrounds (Fig. 5A; primarily yellow bars for k=2), echoing the results of the *DAPC* analyses (Fig. 3C–E; dark blue cluster). These individuals were consistently recovered as sharing similar genetic backgrounds for k-values 3–5 (Fig. 5A; primarily purple bars for k=3–5), with the specimen from Australia hosted by *Carcharhinus* cf. *limbatus* and the specimen from *Carcharhinus brevipinna* from the Gulf of Mexico identified as most similar to one another at k=6 (Fig. 5A; primarily grey bars for k=6). Additionally, for k-values 4–6, the specimen from Senegal hosted by *Carcharhinus brevipinna* was consistently recovered as distinct from

specimens from the western Atlantic and Australia. No obvious clustering by host individual or host species was evident for any k-values tested for the complete dataset for *C. gracilis*, but for k-values 4–6, a general pattern of genetic similarity shared between specimens from the Gulf of Mexico, Florida, and the northern Atlantic Ocean—to the exclusion of specimens South Carolina, and the group of six specimens discussed above—was evident (Fig. 5A).

For analysis of the no-Senegal dataset for *C. gracilis* with *STRUCTURE*, the Evanno ΔK method preferred a k-value of 2, and the greatest k-value tested (in this case, k=5) had the greatest likelihood value (Fig. 5B). Only a single specimen was excluded to create the no-Senegal dataset for *C. gracilis*, and overall, comparable patterns of genetic similarity for the remaining specimens at k=2–5, as described above for the complete dataset, were recovered (Fig. 5B).

Phylogenetic Analysis: *RAxML*

Following concatenation and removal of invariant sites from complete SNP datasets for both species, final alignment lengths were 1,843 sites for *R. megacantha* and 1,501 sites for *C. gracilis*. The most likely topologies as inferred by *RAxML* are presented in Figure 6 for *R. megacantha* and Figure 7 for *C. gracilis*. For both analyses, most shallow nodes did not receive strong BS support. For *R. megacantha*, the most likely topology showed three well-supported clades with BS ≥99: a clade containing the three specimens from Senegal (hosted by *Rhinoptera marginata*; BS=100), sister to a clade containing all 26 specimens from western Atlantic (hosted by *Rhinoptera bonasus* and *Rhinoptera brasiliensis*; BS=100). Relationships among the specimens from the western Atlantic were not strongly supported, excepting the three specimens from Belize (hosted by *Rhinoptera brasiliensis*), which formed a well-supported clade (BS=99) within the larger group (Fig. 6). For *C. gracilis*, beyond the fact that two of the three specimens sequenced from the host individual CH-50 (*Carcharhinus limbatus* from South Carolina) were recovered as sister to one another with strong support (BS=100), no pattern of grouping by host individual, host species, or geographic sampling locality was evident (Fig. 7). The single

specimen from Senegal hosted by *Carcharhinus brevipinna* was recovered as subtended on a relatively long branch (Fig. 7).

Population-level summary statistics and pairwise distances

Relevant population-level summary statistics (e.g., number of polymorphic sites, number of private alleles, observed homozygosity, F_{IS}, π) for both species for the four iterations of *populations* described above are presented in Table 5, and corrected AMOVA F_{ST} values for both species are presented in Figure 8; F_{ST} values for comparisons involving a subpopulation containing a single specimen are not reported. For *R. megacantha*, the three specimens from Senegal (hosted by *Rhinoptera marginata*) had a high proportion of private alleles (i.e., ~47%) despite comprising a relatively small proportion of the total dataset (i.e., three of 29 specimens) (Table 5). Additionally, F_{ST} values were elevated (i.e., ~0.38–0.61) between specimens of *R. megacantha* from Senegal (hosted by *Rhinoptera marginata*) and other subpopulations in both the by-geography (Fig. 8A) and by-host species (Fig. 8B) comparisons. For *C. gracilis*, F_{ST} values ranged from ~0.04–0.14 in the by-geography comparisons (Fig. 8C) and from ~0.05–0.11 in the by-host species comparisons (Fig. 8D). Observed homozygosity was elevated (i.e., >0.91) for all subpopulations tested for both species, and values of F_{IS} ranged from ~0.01–0.2 for *R. megacantha* and from ~0.03–0.23 for *C. gracilis* for all subpopulations containing more than a single specimen (Table 5).

Results of pairwise distance calculations within and between infrapopulations for both species are presented in Figure 9 (for the no-Senegal datasets) and Supplemental Figure 2 (for the complete datasets). For both species, only a single specimen from each host collected from Senegal was included in SNP datasets (i.e., one specimen of *R. megacantha* from each of three specimens of *Rhinoptera marginata* from Senegal, and one specimen of *C. gracilis* from one individual of *Carcharhinus brevipinna* from Senegal; see Fig. 1). Inclusion of specimens from Senegal—which are genetically divergent from their conspecifics (Figs. 6, 7)—in plots of pairwise distances made plots challenging to interpret, and data for only a single tapeworm

per host individual did not allow for comparisons of genetic distances within versus between infrapopulations for specimens from Senegal. Thus, results from comparisons of genetic distances between conspecifics within and between infrapopulations are here based on plots generated from no-Senegal datasets for both species (Fig. 9).

For both *R. megacantha* (Fig. 9A) and *C. gracilis* (Fig. 9B), the lowest pairwise distances were recovered between specimens from different infrapopulations (i.e., from different host individuals). Based on range and variability, plots of pairwise distances within an infrapopulation were indistinguishable from those representing comparisons between two infrapopulations. For both species, pairwise distances between conspecific specimens within an infrapopulation were as high, or higher, than those between specimens from different infrapopulations (Fig. 9).

DISCUSSION

Sampling across known host species and geographic localities

For both species of interest, efforts were made to sequence tapeworms from all possible species of elasmobranchs and geographic localities from which they have been reported. For *R. megacantha*, these efforts were generally successful (see Table 2). The report of *R. megacantha* from *H. say* comes from a single specimen discovered after the sequence data for this study had been generated, making it unfortunately impossible to include here. Additionally, no specimens from the Chesapeake Bay (the type locality for this species; Carvajal & Campbell, 1975), the Gulf of Venezuela, or Brazil preserved for DNA sequencing were available. Given the recent comprehensive study by Herzog and Jensen (2022) on *R. megacantha*, sampling for this study revealed no new host associations or locality records.

In contrast to *R. megacantha, C. gracilis* had been reported from a diversity of elasmobranch hosts from around the world prior to this study (see Table 2). These data suggested *C. gracilis* to be less host specific and more widely distributed as compared to *R. megacantha* (a driving factor in its selection as a focal species for this study). Including specimens of *C. gracilis* hosted by representatives of each of the families of its known hosts from as many geographic

regions as possible was thus a major goal during the initial design of this study. Unfortunately, these efforts were largely unsuccessful.

Despite examining material from 120 carcharhinid sharks representing 30 species—including the majority of host species from which *C. gracilis* has been reported (see Table 2)—*C. gracilis* was only recovered from four species of *Carcharhinus* Blainville, 1816 and one species of *Rhizoprionodon* Whitley, 1929, including novel reports from *Carcharhinus brevipinna*, *Carcharhinus isodon*, and *Carcharhinus* cf. *limbatus*. In addition, the geographic range for the species is extended slightly northward to include South Carolina and Florida (see Table 2). In light of previous reports, it was surprising that no specimens of *C. gracilis* were recovered from sharks other than carcharhinids.

There are several potential explanations for this result. Firstly, it is plausible that as a species, *C. gracilis* exhibits low prevalence, sensu Bush et al. (1997), in its definitive hosts. Unfortunately, detailed prevalence data for adults of *C. gracilis* (and for most species of trypanorhynchs) are lacking. Data for some well-studied species, however, suggest that trypanorhynchs can vary greatly in their prevalence in definitive shark hosts. For example, after examining 49 specimens of the dusky smooth-hound, *Mustelus canis* (Triakidae), Cislo and Caira (1993) reported a prevalence of 6% and 74%, respectively for the two species of trypanorhynchs recovered. Alarcos et al. (2006) reported a prevalence of 35% for the single species of trypanorhynch recovered from 20 specimens of the narrownose smooth-hound, *Mustelus schmitti* Springer, 1939 (Triakidae), and Preti et al. (2020) reported a prevalence of 11% and 42%, respectively, for the two species of trypanorhynchs recovered from 19 specimens of the common thresher shark, *Alopias vulpinus* (Bonnaterre, 1788) (Lamniformes: Alopiidae, Bonnaterre, 1788). For 217 specimens of the spiny dogfish, *Squalus acanthias*, Linnaeus, 1758 (Squaliformes: Squalidae Bonaparte, 1834), Pickering and Caira (2014) reported a prevalence of 27–37% for the single species of trypanorhynch recovered, depending on the season during which sampling occurred. Of the 43 species of sharks examined herein for *C. gracilis*, material from more than five host individuals was available for only 11 species. Thus, if *C. gracilis*

exhibits low prevalence in its definitive hosts, it is possible that an insufficient number of specimens of all host species were examined to recover it.

Misidentification of specimens by the authors of previous reports may provide an additional explanation for why specimens of *C. gracilis* were not recovered from a greater number of the host species examined. Misidentification of trypanorhynchs is, unfortunately, quite common. In fact, during his reexamination of trypanorhynch type and voucher material, Palm (2004; pg. 347) noted that "many" specimens identified as *C. gracilis* in museums around the world represented misidentifications, and presented a much reduced list of hosts for the species.

Examination of material from a relatively large number of sphyrnid hosts led to routine recovery of specimens of *Mecistobothrium penaeus* (Feigenbaum, 1975) Palm, 2004 (i.e., *Mecistobothrium* 2 *penaeus*; see Chapter 3). This species of eutetrarhynchid trypanorhynch superficially resembles *C. gracilis.* Both species are relatively small tapeworms with two bothria, short muscular bulbs, highly sinuous tentacle sheaths and metabasal tentacular armatures comprised of hooks that are relatively uniform in shape and size. They further lack bothrial pits and a characteristic basal armature. *Mecistobothrium penaeus* and *C. gracilis* are most readily distinguished from one another by the presence of pre-bulbar organs and gland cells in the bulbs in *M. penaeus* (both features that are absent in species of *C. gracilis*); however, it is plausible that previous authors may have misidentified specimens of *M. penaeus* from sphyrnid hosts as *C. gracilis*. This argument is strengthened when considering that the two reports of *C. gracilis* from sphyrnids come from two species of hammerhead sharks from Japan and the Gulf of Mexico, respectively (see Table 2). Examination herein of material from eight hammerheads from the Gulf of Mexico did not result in recovery of any specimens of *C. gracilis* despite recovery of *C. gracilis* herein from each of four species of carcharhinid sharks from the Gulf of Mexico (Table 1, Fig. 1B), suggesting that adults of *C. gracilis* are indeed found at this locality, but likely not in sphyrnids.

A plausible candidate for misidentification was also identified from the triakid hosts examined. Specimens of the genus *Lacistorhynchus* Pintner, 1913 were recovered from triakids.

Lacistorhynchus and *Callitetrarhynchus* Pintner, 1931 belong to the same trypanorhynch family (Lacistorhynchidae) and share a suite of morphological similarities. Species in both genera have two bothria, short muscular bulbs, highly sinuous tentacle sheaths and metabasal tentacular armatures comprised of hollow hooks that are relatively uniform in shape and size, with intercalary hooks and a chainette element, and lack bothrial pits, pre-bulbar organs, and gland cells in the bulbs. Notably, species of *Lacistorhynchus* possess a characteristic basal armature which is lacking in species of *Callitetrarhynchus*, but it is plausible that this subtle feature was overlooked by previous researchers, subsequently leading to misidentifications of species of *Lacistorhynchus* as *C. gracilis.*

In addition to sharks, *C. gracilis* had been reported as an adult from a single species of ray, the estuary stingray, *Hemitrygon fluviorum*. Unfortunately, no new material from estuary stingrays was available for examination. However, photos of the single museum specimen on which this report is based were examined. The specimen is deposited in the South Australian Museum's Australian Helminthological Collection (AHC 24942) and comes from an estuary stingray from Queensland, Australia (Palm, 2004). The tentacles of this specimen are not everted, making its confident identification to the level of species nearly impossible. However, other features of the scolex that can be observed (i.e., the shape and extent of the muscular bulbs, the sinuousness of the tentacle sheaths, and the size and shape of the bothria) do not align with the morphological diagnosis of *C. gracilis*. Thus, it seems likely this host report, too, may be the result of a misidentification. Ultimately, additional material from estuary stingrays (ideally specimens with tentacles everted and/or preserved in 95% ethanol to allow for sequencing of 28S) needs to be examined to support or invalidate this report.

In combination, these results suggest: (1) *C. gracilis* may be found at low prevalence in its definitive hosts; (2) *C. gracilis* may parasitize a broad diversity of host species but may only be found in a particular set of its known host species within a given geographic region; and/or (3) the suite of definitive host species *C. gracilis* parasitizes may be restricted to species in the carcharhiniform family Carcharhinidae (requiem sharks). A comprehensive exploration of

this hypothesis was outside of the scope of the present study, but based on these results, future investigations of definitive host use by *C. gracilis* are warranted.

28S Sanger data

Data for the D1–D3 gene regions of 28S rRNA confirmed conspecificity for specimens of *R. megacantha* and *C. gracilis,* respectively. Within a species, specimens were found to differ from one another by fewer than 5 bp (i.e., <0.35%; see Fig. 2). This degree of divergence in partial 28S for conspecific specimens falls comfortably within the bounds of what is presently known for trypanorhynchs. Herzog and Jensen (2022) previously reported species of *Rhinoptericola* Carvajal & Campbell, 1975 (including *R. megacantha*) to differ from one another by 0–2 bp in partial 28S. These authors also summarized the results of previous studies in which multiple specimens of the same species of trypanorhynchs were sequenced for 28S and reported a consistent boundary of <1% intraspecific sequence divergence across the species studied. It is worth noting that Herzog and Jensen (2022), and the studies summarized therein, focused on species in the suborder Trypanobatoida; thus, the data generated herein for *C. gracilis* represent the first assessment of the degree of intraspecific variation in partial 28S for a trypanoselachoid. As a near-identical level of intraspecific divergence was discovered for *C. gracilis* as compared to species of trypanobatoids, these data tentatively suggest that a consistent degree of intraspecific divergence in 28S can be expected for species across the two suborders of trypanorhynch tapeworms.

All 39 specimens of *R. megacantha* and the 39 (of 47) specimens of *C. gracilis* for which 28S data were successfully generated comprise eight and ten within-host individual comparisons, respectively (see Table 1, Fig. 1). In some instances, specimens from the same host individual grouped together based on 28S. For example, for *R. megacantha*, MS053051 and MS053052 (from *Rhinoptera brasiliensis* from the Gulf of Mexico) were recovered as sister to one another (Fig. 2A), and for *C. gracilis*, MS05-403-2, MS05-403-3, and MS05-403-4 (from *Carcharhinus limbatus* from the Gulf of Mexico) and MS05-488-1, MS05-488-2, and MS05-488-3 (also

from *Carcharhinus limbatus* from the Gulf of Mexico) shared identical 28S sequences (Fig. 2B). Overwhelmingly, however, specimens did not group together based on host individual, host species, or geographic sampling locality for either species based on 28S (Fig. 2). This result was wholly expected, given that only ~1,400 bp of 28S were analyzed, and conspecific individuals were exceedingly genetically similar (i.e., <0.35% divergence). Thus, 28S appears a reliable indicator of conspecificity for multiple species of euryxenous and broadly distributed trypanorhynchs.

Several specimens included in 28S analyses had ~15% missing data for the region of 28S sequenced due to short sequences and/or short internal sections of unreliable, low-quality base calls masked with ambiguities. It is of course possible that these missing data skewed the assessment of levels of intraspecific divergence (particularly for *R. megacantha*, which had the higher proportion of such specimens; see Fig. 2A). However, this seems unlikely given that 26 and 36 complete 28S sequences were included in the analyses for *R. megacantha* and *C. gracilis*, respectively, collectively representing all host species and localities sampled for each species (see Table 1; Fig. 1). Overall, results from analysis of 28S data support the respective conspecificity of specimens of *R. megacantha* and *C. gracilis* sampled from across a range of their known host species and geographic localities.

High rates of homozygosity and *STRUCTURE*

High levels of homozygosity (i.e., >91%) were inferred for both *R. megacantha* and *C. gracilis* (see Table 5). It is therefore important to take into consideration how high levels of homozygosity can potentially bias the results of *STRUCTURE* analyses. A hallmark of unaccounted for subpopulation structure is fewer heterozygotes in a population than would be expected under Hardy-Weinberg equilibrium (HWE) (Wahlund, 1928). *STRUCTURE* operates by binning genetic data into n=k clusters such that deviations from HWE within each bin are minimized. *STRUCTURE* assumes random mating and therefore assumes that lower-than-expected heterozygosity is necessarily the result of unaccounted for substructure.

Thus, for hermaphroditic species like tapeworms, in which reduced heterozygosity could be expected as a result of high rates of selfing, there is the risk that *STRUCTURE* will infer subpopulation structure that is not biologically real (Falush et al., 2003; Gao et al., 2007). It is therefore possible that the results from *STRUCTURE* analyses presented herein were biased by the reduced levels of heterozygosity inferred for both focal species. However, for both species, the results from *STRUCTURE* align with the results inferred from *DAPC* and *RAxML* analyses, population-level summary statistics, and plots of pairwise distances in all cases. Additionally, the Evanno ΔK method preferred a k-value of 2 for all *STRUCTURE* analyses (Figs. 4, 5) (i.e., preferred the lowest degree of substructure possible among individuals). Thus, though results from *STRUCTURE* may have been biased by high levels of homozygosity, the overall conclusions drawn about population structure for both species are based on concordant results from a variety of analyses, all of which support the same results inferred by *STRUCTURE.*

SNP data: *Rhinoptericola megacantha*

Component population-level structure in *Rhinoptericola megacantha*: Definitive host species versus geography

For *R. megacantha*, *DAPC*, *STRUCTURE*, and *RAxML* analyses, population-level summary statistics, and plots of pairwise distances all clearly distinguished specimens collected from the eastern Atlantic (i.e., Senegal) from those collected from the western Atlantic (i.e., South Carolina, the Gulf of Mexico, and Belize) (Table 5, Figs. 3A, 4A, 6, 8A; Supplemental Fig. 2A). Moreover, specimens of *R. megacantha* from Senegal were collected from a different species of host as compared to their western conspecifics: Specimens from Senegal come from *Rhinoptera marginata* while specimens from the western Atlantic come from either *Rhinoptera bonasus* or *Rhinoptera brasiliensis*. According to distributions maps for these three species of cownose rays, *Rhinoptera marginata* is restricted to inshore waters from Portugal to Congo (including the Mediterranean Sea) while *Rhinoptera bonasus* and *Rhinoptera brasiliensis* are found only off the eastern coasts of North, Central, and South America (Last et al., 2016). None

of these three species has been documented from both sides of the Atlantic Ocean.

Given that specimens of *R. megacantha* from Senegal and the western Atlantic are separated by ~6,500–7,500 km of open ocean and are found in allopatric species of cownose ray hosts, it is unsurprising that all analyses based on SNP data suggest that they represent distinct subpopulations. Since the species of cownose ray hosts from which specimens were recovered differ between the two regional localities, however, whether these subpopulation-level differences are driven by definitive host species or geography cannot be reliably disentangled. Nevertheless, specimens sampled from within the western Atlantic do have the potential to address this question.

Within the western Atlantic, analyses based on SNP data suggest geography to play a more important role than definitive host species in structuring genetic diversity in *R. megacantha*. If host species played the more important role, one would expect to find that specimens collected from *Rhinoptera brasiliensis* (regardless of their collection locality) are more genetically similar to one another than they are to specimens collected from *Rhinoptera bonasus*. In fact, results herein suggest the opposite: All SNP-based analyses suggest that the specimens collected from *Rhinoptera brasiliensis* from Belize comprise a subpopulation that is genetically distinct from specimens collected from South Carolina and the Gulf of Mexico hosted by either *Rhinoptera brasiliensis* or *Rhinoptera bonasus* (Figs. 3B, 4B, 6, 8B).

Small sample sizes are an obvious potential source of error, and it is worth noting that sample sizes for *R. megacantha* were less than optimal in some cases. After filtering, only three (of six) specimens of *R. megacantha* hosted by two (of four) individuals of *Rhinoptera bonasus* were included in SNP datasets, versus 20 specimens hosted by seven individuals of *Rhinoptera brasiliensis*. However, only three specimens each were sampled from both Senegal (three host individuals) and Belize (two host individuals), and analyses still recovered distinct genomic signatures for these sets of specimens. Thus, despite limits in sampling, geography—rather than definitive host species—appears to be the primary driver of population structure in *R. megacantha*.

Few studies have probed the population genetics of parasites, and even fewer have focused on tapeworms specifically, making it challenging to place the results of this study into perspective. There is, however, some evidence from previous investigations (based on data from gel electrophoresis, single loci, or microsatellite markers) to support a predominant role of geography in genetic structure for taxa that are not strictly host specific. For example, for the dog tapeworm *Echinococcus granulosus* Batsch, 1786, which parasitizes canids as adults and is known for both its sylvatic and domestic life-cycles, researchers found that genetic diversity of hydatid cysts in sheep (domestic) and marsupial (sylvatic) intermediate hosts across mainland Australia and Tasmania was structured by geography rather than host species, and that 90% of the genetic variation found across mainland populations could be recovered from a single host species and sampling locality (Lymbery et al., 1990, 1997). For the diphyllobothriidean tapeworm *Ligula intestinalis* (Linnaeus, 1758) Gmelin, 1790, which sequentially parasitizes copepods, bony fishes, and birds, researchers analyzed data from 15 microsatellite markers for adult and larval specimens collected from bony fishes (Cyprinidae Linnaeus, 1758) and birds from Canada, Europe, Africa, Russia, China, and Australia, and found genetic differentiation between specimens collected from different geographic regions (Štefka et al., 2009). (In contrast to the findings, herein however, these authors reported at least some degree of structure correlated with host species for populations in Europe and Ethiopia, where relatively broad spectra of host species could be sampled.) For the fox tapeworm *Echinococcus multilocularis* Leuckart, 1863, researchers characterized mitochondrial haplotypes for 76 larval and adult worms collected from voles, red foxes, dogs, and humans from the USA, Europe, China, and Japan, and found distinct clustering of haplotypes by broad geographic region (Nakao et al., 2009). The findings herein (i.e., the importance of geography over definitive host species in structuring genomic diversity in *R. megacantha*) thus align with previous genetic research on tapeworms. A comprehensive explanation for these findings, however, may prove challenging. Given that this study is the first of its kind for elasmobranch tapeworms, potential explanations for the observed patterns are worth exploring despite sampling limitations and the lack of

knowledge about the life history of *R. megacantha*.

As stated above, the genetic difference observed between subpopulations on either side of the Atlantic Ocean was expected given the vast distance between these two regions, and, concurrently, the use of different allopatric species of definitive hosts across these two regions. Potentially more challenging to explain is the genetic differentiation between specimens from the Caribbean (i.e., Belize) versus more northern collecting localities despite apparent panmixia between subpopulations in the eastern Atlantic and the Gulf of Mexico (Figs. 3A, B, 4B). This pattern is intriguing in light of numerous studies for a wide range of marine taxa that document phylogeographic breaks between populations in the Gulf of Mexico and the Atlantic (Avise, 1992; Neigel, 2009). This population structure is further perplexing given that the three localities in question are all connected by the strong oceanic Loop Current (Geyer et al., 2020) which has the potential to disperse eggs of *R. megacantha* between these localities (i.e., the only free-living stage of the life-cycle) and maintain genetic homogeneity between all three subpopulations. Additional factors potentially contributing to the patterns of genetic structure observed for *R. megacantha* may thus include definitive host biology, intermediate host use and biology, and chemical oceanography, and the true explanation likely lies in a combination of these, and perhaps additional factors.

The most obvious explanation for observed patterns would be that individual cownose rays routinely move between the Gulf of Mexico and the east coast of the USA, but not between these localities and the Caribbean. Available data on the movement of cownose rays, however, suggest that this is unlikely—at least for *Rhinoptera bonasus*. Seasonal migratory movements of individuals of *Rhinoptera bonasus* from summer to fall assessed with satellite tags suggest that rays migrate south along the Atlantic Coast of the USA during the year but end their migration to overwinter in waters off the east coast of Florida, and that individual rays do not round the cape of Florida or enter the Gulf of Mexico (Omori & Fisher, 2017; Ogburn et al., 2018). Since genetic data similarly suggest that *Rhinoptera bonasus* does not occur in the Gulf of Mexico (Naylor et al., 2012), it seems unlikely that movements of this host species are responsible for

connecting subpopulations of *R. megacantha.* In contrast, *Rhinoptera brasiliensis* has only been recognized to co-occur with *Rhinoptera bonasus* relatively recently, and so little is known about the movements of *Rhinoptera brasiliensis* in the northern Atlantic region. While individuals of *Rhinoptera brasiliensis* have been reported from off the Atlantic Coast of the USA, including from South Carolina (i.e., the single specimen from South Carolina included in this study), North Carolina (Naylor et al., 2012), and potentially even as far north as New Jersey (Stoeckle et al., 2020), data on the movement of individuals between the Gulf of Mexico and the Atlantic, or between the Caribbean and the Gulf of Mexico, are lacking.

If movement of individual cownose rays between the Caribbean, the Gulf of Mexico, and/or the Atlantic Coast of the USA ultimately does not occur, an alternative hypothesis for subpopulation connectivity and substructure in *R. megacantha* would involve intermediate host use and biology. Like all tapeworms, *R. megacantha* has a complex, multi-host life-cycle and is trophically transmitted, meaning its larvae parasitize several intermediate hosts prior to being consumed by the final ray host in which they mature and reproduce. Unfortunately, the complete natural life-cycle of *R. megacantha* (or of any species of elasmobranch tapeworm) has yet to be elucidated, and nothing concrete is known about intermediate host use for this species. Data on partial life-cycles and intermediate host use in other species of trypanorhynchs suggest that members of the order almost universally utilize copepods as first intermediate hosts, followed by an invertebrate or vertebrate second intermediate host, and in some cases a vertebrate third intermediate host (Palm, 2004). It thus seems likely that *R. megacantha* would utilize copepods as first intermediate hosts, and at least a second intermediate host that is a prey item of cownose rays, to complete its life-cycle. That specimens of *R. megacantha* are routinely encountered in individuals of both *Rhinoptera bonasus* and *Rhinoptera brasiliensis* further suggests that it uses one or more intermediate hosts that are commonly—rather than accidentally—consumed by cownose rays.

The diets of cownose rays in North America have been relatively well-studied, in part, because of the implication of these rays as nuisance predators of commercially important

shellfish stocks (Merriner & Smith, 1979; Smith & Merriner, 1985). In general, *Rhinoptera bonasus* and *Rhinoptera brasiliensis* appear to be opportunistic generalists with diets dependent on the local abundance of benthic invertebrate prey. For example, in the Chesapeake Bay, *Rhinoptera bonasus* has been documented to consume mostly bivalve mollusks—especially soft-shell clams, razor clams, and Baltic macoma—and crustaceans, with notable variability between feeding sites (Smith, 1980; Smith & Merriner, 1985; Fisher, 2010). Rays in the Gulf of Mexico (presumably *Rhinoptera brasiliensis*; Naylor et al., 2012) appear to preferentially feed on bivalves in estuaries, but feed mainly crustaceans and polychaetes in open waters (Collins et al., 2007; Ajemian & Powers, 2012). The feeding habits of cownose rays in the Caribbean have not been documented. Available data thus suggest that the second intermediate host (or hosts) of *R. megacantha* are most likely benthic invertebrates such as bivalves and/or crustaceans.

If the observed pattern of population structure in *R. megacantha* is attributable to copepod or macroinvertebrate intermediate host movement, rather than cownose ray movement, it would be expected, based on these results, that: (1) *Rhinoptericola megacantha* utilizes different intermediate hosts in the Caribbean versus the northern Atlantic and Gulf of Mexico; and (2) the intermediate host (or hosts) utilized in the northern Atlantic and the Gulf of Mexico is/are sufficiently vagile to allow for connectivity between the two regions. Some data are available to support these possibilities. In their formative work establishing marine ecoregions for the world's costal and shelf areas, Spalding et al. (2007) classify both the northern Gulf of Mexico and the southern and mid-eastern coastal USA as belonging to the same province (i.e., the Warm Temperate Northwest Atlantic) while the Caribbean constitutes a separate province (i.e., the Tropical Northwestern Atlantic). According to these authors, provinces represent areas with distinct biotas and some level of endemism, and are bounded by discrete abiotic features. Similar communities of invertebrates shared between the Gulf of Mexico and Atlantic Coast of the USA, but differences in communities between these regions and Belize, are thus expected. Similarly, survey work on benthic macroinvertebrates and copepods supports Gulf-to-Atlantic distributions for some taxa. Engle and Summers (2000) found that 17–31% of species of benthic

macroinvertebrates found in the Gulf of Mexico could also be found in South Florida and along the Atlantic Coast from Florida to Virginia, and multiple species of copepods are known to possess Gulf-to-Atlantic distributions (e.g., Schizas et al., 1999; Drumm & Kreiser, 2012). However, movement of individual invertebrates between the Gulf and the Atlantic is more challenging to support. As is nicely summarized by, for example, Drumm and Kreiser (2012), phylogeographic studies for a wide variety of marine species with Gulf-to-Atlantic distributions have documented distinct genetic differences between populations in the two regions, suggesting little connectivity even for vagile species. Avise (1992) famously hypothesized that such concordant phylogeographic patterns across taxa are likely due to the extent of the Florida peninsula into subtropical waters, creating a biogeographic barrier to dispersal. Given that the Loop Current, which connects the Gulf of Mexico to the Atlantic Gulf Stream, also connects the Caribbean Sea to the Gulf of Mexico (Geyer et al., 2020), hypotheses of eggs of *R. megacantha* being carried from the Gulf to the Atlantic via currents seem an inadequate explanation for the patterns of subpopulation structure observed herein. It is of course still possible that, despite the trends for taxa studied to date, there are indeed invertebrate intermediate hosts of *R. megacantha* that facilitate population connectivity between the Gulf and the Atlantic, but more information on intermediate host use in *R. megacantha* is sorely needed before such hypotheses can be entertained.

Infrapopulation structure in *Rhinoptericola megacantha*: Genetic diversity within versus between host individuals

In addition to evaluating the relative importance of definitive host species versus geography in structuring genetic diversity in *R. megacantha*, a goal of this study was to determine the genetic similarity of specimens recovered from a single host individual (i.e., diversity at the infrapopulation level). For the seven instances where SNP data for more than a one specimen of *R. megacantha* from a single host individual could be analyzed, comparisons of pairwise distances clearly demonstrate that as much, or more, genetic diversity can be found

within an infrapopulation as between infrapopulations (Fig. 9A). *STRUCTURE* results similarly suggest that infrapopulations comprise specimens that are not one another's closest relatives, but rather encompass a mix of genetic backgrounds (Fig. 4B). Furthermore, specimens collected from the same host individual were almost never recovered as one another's closest relatives in the *RAxML* analysis (Fig. 6), further supporting this assessment. It is worth noting, however, that nodal support was lacking for nearly all relationships in the resulting most likely *RAxML* topology except for strong support for specimens from Belize and Senegal as distinct clades. This lack of nodal support is perhaps not surprising, however, given the overall high levels of genetic similarity across specimens suggested by *STRUCTURE* and *DAPC* analyses (Figs. 3A–B, 4). Overall, these results suggest that infrapopulations of *R. megacantha* are not made up exclusively of individuals that are one another's closest relatives, and can in fact contain as much genetic diversity as exists between infrapopulations.

From the limited data available, it seems infrapopulations comprised of genetically dissimilar individuals (i.e., mixed infections) are not uncommon for tapeworms. For *Echinococcus granulosus*, Lymbery and Thompson (1989) documented genetic differences between protoscoleces recovered from different hydatid cysts from the same intermediate host individual, and Nakao et al. (2003) found evidence from microsatellite markers to suggest mixed infections within a single fox definitive hosts for the fox tapeworm *Echinococcus multilocularis*. Additionally, for the pork tapeworm, *Taenia solium* Linnaeus, 1758, researchers sampling naturally infected pigs in Peru reported instances of multiple tapeworm genotypes within a single pig (Pajuelo et al., 2017). Other researchers have provided theory and evidence to support why genetically heterogenous infrapopulations at the level of the definitive host should, in fact, be expected for parasites with complex life-cycles. Rauch et al. (2005) characterized the population genetics of the eye-fluke *Diplostomum pseudospathaceum* Niewiadomska, 1984 (Class Trematoda: Order Diplostomida), a member of yet another class of parasitic flatworms also known for their complex, multi-host life histories. This species uses freshwater snails as first intermediate hosts and bony fishes as second intermediate hosts before maturing to adulthood

and sexually reproducing in the guts of gulls. Using microsatellite markers, Rauch and coauthors determined that in natural populations, the majority of *D. pseudospathaceum* released from a single snail first intermediate host were genetic clones, but that fish second intermediate hosts were typically infected with a diverse array of eye-fluke genotypes. They therefore argued for the maintenance of more than one intermediate host in a parasite's life-cycle as a strategy for increasing genetic diversity at the level of the definitive host, thus reducing the risk of inbreeding during a reproductive event. Though this experiment was done in a fluke system rather than a tapeworm system, enough parallels exist between the life histories of members of the two classes to draw a similar conclusion for *R. megacantha*. It is thus hypothesized that the presence of genetically diverse tapeworm infrapopulations in a single cownose ray is likely the result of the gradual accumulation of dissimilar tapeworm genotypes within a single host individual at each subsequent step in the life-cycle of *R. megacantha.*

Population-level summary statistics: *Rhinoptericola megacantha*

The large proportion of specimens (i.e., 23 of 29) that were collected from *Rhinoptera brasiliensis*—and specifically the 20 specimens collected from *Rhinoptera brasiliensis* from the Gulf of Mexico—encompassed the greatest proportion of genetic diversity sampled for *R. megacantha*, as reflected in high proportions of polymorphic sites and private alleles reported for the subpopulations that include these specimens (Table 5). Filtering led to the inclusion of only three specimens each in the Belize and Senegal subpopulations (in the by-geography comparison), and in the *Rhinoptera bonasus*-hosted and *Rhinoptera marginata*-hosted subpopulations (in the by-host species comparison). Thus, population summary statistics—and particularly F_{ST} values—for these each of these subpopulations should be evaluated with caution. That being said, the F_{ST} values reported herein are corrected AMOVA F_{ST} values, which, unlike binomial F_{ST} values, are not biased by differences in sample sizes between subpopulations. Furthermore, more recent evaluations of F_{ST} calculations highlight the importance of number of loci versus number of individuals: Simulations show that F_{ST} values are not artificially inflated by

small sample sizes if the number of biallelic markers sampled is sufficiently great (i.e., multiple thousands of loci, as sampled herein) (Willing et al., 2012). Potential caveats aside, summary statistics and F_{ST} values for possible subpopulations of *R. megacantha* seem to support the same patterns as *DAPC*, *STRUCTURE*, and *RAxML* analyses: distinct subpopulations on either side of the Atlantic Ocean and more apparent differentiation between Belize and the Gulf of Mexico + South Carolina than exists between specimens collected from *Rhinoptera bonasus* versus *Rhinoptera brasiliensis* (Table 5, Fig. 8A–B).

Though the goal of this study was not to investigate the mating systems of trypanorhynch tapeworms, it is interesting to note that consistently high levels of homozygosity (i.e., >0.91) and elevated F_{IS} values were recovered across subpopulations of *R. megacantha* (Table 5). The few studies available for comparison corroborate these findings as typical for tapeworms, and suggest they indicate some degree of selfing and/or inbreeding. For example, hydatid cysts of the dog tapeworm *Echinococcus granulosus* sampled from sheep and marsupial intermediate hosts across Australia and Tasmania were fully homozygous at all loci surveyed (Lymbery et al., 1990, 1997). Similarly, Nakao et al. (2003) found levels of homozygosity to range from ~90–93% at two microsatellite markers sequenced for over 100 adults of *Echinococcus multilocularis* sampled from foxes, and suggested these levels indicate a mix of both selfing and outcrossing. For the diphyllobothriidean tapeworm *Schistocephalus solidus* (Müller, 1776) Steenstrup, 1857, investigators reported relatively high levels of homozygosity (i.e., 77–80%) and a positive inbreeding coefficient of 0.3 for worms sampled from intermediate hosts (i.e., bony fishes), which they suggested is indicative of selfing (Binz et al., 2000). That tapeworms regularly engage in selfing and/or inbreeding is supported not only by their hermaphroditism, but also by empirical data. Investigators who conducted what is perhaps the most comprehensive assessment of inbreeding and mating system structure for a species of tapeworm to date found that for *Oochoristica javaensis* Kennedy, Killick & Beverley-Burton, 1982—which parasitizes gekkonid lizards as adults—selfing, kin-mating, and outcrossing all contribute to reproduction (Detwiler et al., 2017; Detwiler & Criscione, 2017).

SNP data: *Callitetrarhynchus gracilis*

Component population-level structure in *Callitetrarhynchus gracilis*: Definitive host species versus geography

For *C. gracilis*, results from *DAPC*, *STRUCTURE*, and *RAxML* analyses indicated no apparent genomic structure aligned with definitive host species or geographic locality beyond a distinction between the single specimen collected from Senegal and its remaining 31 conspecifics collected from Australia or the western Atlantic in some analyses. This distinction was evident in pairwise distance analyses (Supplemental Fig. 2B), in *DAPC* when using AIC instead of BIC to inform clustering patterns (Fig. 3D), and at higher k-values (i.e., k=4–6) in *STRUCTURE* analyses (Fig. 5A). For *DAPC*, however, BIC preferred a k-value of 2, grouping the specimen from Senegal with 25 of its conspecifics. For *STRUCTURE*, the k-value preferred by the Evanno ΔK method for the complete dataset for *C. gracilis* was k=2, and at this k-value, the specimen from Senegal is similarly indistinguishable from its conspecifics. Additionally, comparisons of pairwise distances within versus between infrapopulations of *C. gracilis* that include the specimen from Senegal clearly showed that, while this specimen is genetically dissimilar from its conspecifics, the degree of dissimilarly is not nearly as extreme as observed for the three specimens of *R. megacantha* collected from Senegal (see Supplemental Fig. 2B versus A).

Regrettably, no specimens of *C. gracilis* from Belize were available to assess whether the same pattern of genomic differentiation between the Caribbean and the Gulf of Mexico and western Atlantic observed for *R. megacantha* also holds for *C. gracilis*. It is worth noting, however, that the single specimen of *C. gracilis* collected from Australia was indistinguishable from those collected from the western Atlantic based on both 28S data and SNP data. Of course, sampling only a single individual from the Indian Ocean is less than ideal, and this small sample size may have introduced biases, but in all, these results suggest little genetic differentiation for *C. gracilis* across its range.

Little genomic differentiation across a broad range of host species and geographic regions makes sense in light of the circumglobal distributions and apparently high vagilities of

both the definitive and intermediate hosts of *C. gracilis.* As discussed above, the diversity of definitive host species parasitized by *C. gracilis* may indeed be narrower than current estimates based on reports in the literature suggest (see above, and Table 2). However, even considering only the five species of requiem sharks parasitized by the specimens of *C. gracilis* sequenced here (an overly conservative estimate of its actual range of suitable definitive hosts), *C. gracilis* parasitizes a suite of sharks that, as species, have broad geographic distributions. *Carcharhinus brevipinna* is found circumglobally in warm-temperate to tropical waters; *Carcharhinus limbatus* and *Carcharhinus* cf. *limbatus* are found throughout tropical and subtropical seas in the western Atlantic, and in the eastern Atlantic and Indo-Pacific, respectively; and *Carcharhinus isodon* and *Rhizoprionodon terraenovae* are both known from the Caribbean, Gulf of Mexico, and eastern coast of the USA, with some reports from eastern coastal South American (Gallo et al., 2010; Naylor et al., 2012; Ebert et al., 2021). These five species of requiem sharks alone qualify *C. gracilis* as circumglobally distributed in temperate and tropical oceans.

A broad geographic distribution for a species is, however, not necessarily indicative of the movement of individuals. Some data on the movements of individual sharks are available for four of the five species of sharks represented in this study. Kohler and Turner (2018) summarized the results of over five decades of shark mark and recapture data from the National Marine Fisheries Service in collaboration with recreational and commercial anglers. This titanic report includes movement data for individuals of *Carcharhinus brevipinna* (n=1,750), *Carcharhinus isodon* (n=2,865), *Carcharhinus limbatus* (n=10,551), and *Rhizoprionodon terraenovae* (n=5,055). Individuals of all four species were tagged and/or recaptured off North Carolina and the Gulf of Mexico, and individuals of all but *Carcharhinus isodon* were tagged and/or recaptured in the Caribbean Sea. No individuals of any of the four species were tagged or recaptured in the South Atlantic or in the eastern North Atlantic Ocean. These data further documented individuals of *Carcharhinus limbatus* and *Carcharhinus isodon* that moved into the Caribbean Sea, individuals of *Carcharhinus brevipinna* and *Carcharhinus isodon* that moved into the Gulf of Mexico, and individuals of *Carcharhinus isodon* that moved out of the Gulf

of Mexico (Kohler & Turner, 2018). In total, these data suggest that four of the five species of carcharhinid hosts of *C. gracilis* included in this study are quite vagile across their range (at least, within the waters off the Eastern Seaboard). The limited genetic divergence and structure recovered herein for *C. gracilis* thus may very well be attributable to movements of its definitive shark hosts.

Of course, species of carcharhinids other than the five species confirmed herein may—and likely do—host *C. gracilis*. If so, the movements of these species within their respective geographic ranges would also be important to consider. Estimates suggest that approximately half of carcharhinids are migratory or likely to be migratory (Species Survival Commission, 2007); however, studies of costal species of carcharhinids reveal that gene flow tends to be limited across large stretches of ocean (Dudgeon et al., 2012 and citations therein). It is worth mentioning, however, that the most wide-ranging species of shark known—the blue shark, *Prionace glauca*—is a carcharhinid. Individual blue sharks have been documented to make trans-oceanic migrations (Kohler & Turner, 2018; Ebert et al., 2021) and blue sharks have been reported as a definitive host of *C. gracilis* (Table 2). (Examination of material from 23 blue sharks collected off Montauk, NY, USA herein revealed no specimens of *C. gracilis*, however; see above.) Ultimately, a more refined picture of definitive host use by *C. gracilis* based on material that is vouchered and identified correctly, and ideally tied to 28S sequence data, is needed before the biology of additional species of definitive hosts can be confidently linked to patterns of population-level structure in *C. gracilis*.

As was the case for *R. megacantha*, it is important to mention the complex, multi-host life-cycle of *C. gracilis*, for the distributions and movements of intermediate hosts may be just as important, if not more important, than those of definitive hosts when considering tapeworm population structure. Unlike *R. megacantha*, for which no data on intermediate host use are available, reports of larvae of *C. gracilis* from intermediate hosts abound in the literature. Over 140 species of teleosts representing tens of families worldwide have been reported as intermediate hosts of *C. gracilis* (see Palm, 2004). Of course, as observed by

Palm (2004) and corroborated herein, it seems likely that a number of these reports represent misidentifications; however, even if *C. gracilis* parasitizes only a fraction of the teleosts from which it has been reported, its intermediate host use would still be impressively broad. Based on his own collections and observations of material deposited in museums, Palm (1997, 2004) hypothesized a four-host life-cycle for *C. gracilis*, with copepods servings as first intermediate hosts, clupeids (herrings) serving as second intermediate hosts, scombrids (mackerels and tunas) and epinephelids (groupers) serving as third intermediate hosts, and carcharhinids (requiem sharks) serving as definitive hosts. Clupeids and epinephelids are found worldwide in tropical to temperate coastal waters, and scombrids are globally distributed in the marine tropics and subtropics (Hastings et al., 2015; Nelson et al., 2016). Scombrids in particular are known for their expansive ranges and migratory behavior. For example, the Atlantic bluefin tuna, *Thunnus thynnus* (Linnaeus, 1758)—which has been reported as an intermediate host of *C. gracilis* (Palm, 2004)—is known to migrate great distances, and exhibits little genetic structure between the Atlantic and Mediterranean (Nelson et al., 2016). A hypothesized life-cycle for *C. gracilis* that involves large, vagile, wide-ranging species like scombrids and carcharhinids is supported by the pattern of limited genetic structure recovered for the species herein.

Though in general, SNP-based analyses support a model of little population-level structure in *C. gracilis*, the *DAPC* analysis using AIC over BIC to inform clustering patterns (Fig. 3D), the higher k-values tested in *STRUCTURE* analyses (Fig. 5A), and the comparisons of pairwise distances between infrapopulations (Supplemental Fig. 2B) all revealed genetic differentiation between the single specimen collected from Senegal and its conspecifics. Puzzlingly, these analyses indicated little differentiation between specimens collected from the western Atlantic and Australia. It is worth noting that while four specimens of *C. gracilis* from Senegal were sequenced (i.e., two specimens each from two individuals of *Carcharhinus brevipinna*), it was only possible to include a single specimen in the finalized complete datasets following filtering. Given the high proportion of specimens from Senegal that were pruned from final datasets due to low-quality data, it is of course possible that the genetic differentiation

observed for the single specimen retained results from low-quality input DNA for this specimen. However, relatively strict thresholds for minimum read depth, minimum genotype quality score, and SNP completeness were employed during filtering (see above) to ensure that only high-quality data were ultimately analyzed. Regardless, sample size within Senegal for *C. gracilis* is regrettably small, which alone may have biased the results.

Unlike for *R. megacantha*, which utilizes a different species of definitive host in Senegal as compared to the western Atlantic, the single specimen of *C. gracilis* from Senegal included in SNP-based analyses comes from a host species represented by individuals collected from both the western and eastern Atlantic: In addition to the specimen of *C. gracilis* from *Carcharhinus brevipinna* from Senegal, it was possible to include five specimens of *C. gracilis* from two individuals of *Carcharhinus brevipinna* from the Gulf of Mexico (Table 1, Fig. 1). Given this replication within a host species and across geographic regions, any observed differences—if not attributable to low-quality data or small sample size—are more likely driven by oceanographic or biogeographic barriers as opposed to differences in definitive host species.

It is challenging to explain what could cause a specimen from the eastern Atlantic Ocean to be identified as genetically distinct from specimens collected from the western Atlantic and Indian Oceans when specimens from the latter subpopulations appear largely undifferentiated; presumably, any factors that promote genetic continuity between populations in the western North Atlantic and the Indo-Pacific (e.g., currents transporting tapeworm eggs, definitive and/or intermediate host movements, etc.) would similarly allow for continuity with populations off Senegal. It is possible that the Mid-Atlantic Ridge, which separates the western and eastern halves of the Atlantic Ocean, presents a barrier to dispersal for even the most vagile hosts of *C. gracilis*. There is some evidence to support such a conclusion. Approximately 30% of species of Atlantic reef teleosts found in the Tropical Eastern Atlantic (the region that includes coastal Senegal) are endemic to the region, and only 20% of all species of reef teleosts found in the region are known to possess trans-Atlantic distributions (Floeter et al., 2008). Therefore, it is possible that the intermediate teleost hosts used by *C. gracilis* in the Tropical Eastern Atlantic are

isolated within the region. If that isolation cannot be overcome by the movements of intermediate invertebrate or definitive shark hosts, this could produce a genetically distinct subpopulation of *C. gracilis*. Ultimately, however, a clearer picture of intermediate and definitive host use by *C. gracilis*, and more comprehensive sampling of specimens across the circumglobal range of this species, are needed to better understand this result.

For other species of tapeworms with wide geographic ranges and relaxed host specificity, patterns of limited genetic structure have similarly been documented. For example, for the taeniid tapeworm *Echinococcus ortleppi* López-Neyra & Soler Planas, 1943, Addy et al. (2017) found that parsimony haplotype networks indicated no structuring by host, country, or geographic region for worms sampled from cattle, goats, camels, sheep, pig, and oryx from sub-Saharan Africa, Europe and South America. Additionally, for the highly invasive Asian fish tapeworm *Schyzocotyle acheilognathi* (Yamaguti, 1934) Brabec, Waeschenbach, Scholz, Littlewood & Kuchta, 2015, Brabec et al. (2016) sequenced complete mitochondrial genomes and nuclear rRNA operons for a worm each from the USA, Mexico, China, South Africa, Japan, the Czech Republic, Ethiopia, and Turkey (representing various species of teleost hosts) and found few genetic differences between specimens. Finally, Fraija-Fernández et al. (2021) sequenced the mitogenomes and ribosomal operon for *Ligula* cf. *intestinalis* collected from two subspecies of geographically isolated ringed seal (Finland versus Russia) and found them to be nearly identical. Thus, in the context of the results for other species of tapeworms with comparable life-histories, the results for *C. gracilis* seem to follow previously documented patterns.

Infrapopulation structure in *Callitetrarhynchus gracilis*: Genetic diversity within versus between host individuals

For the seven instances where SNP data for more than a one specimen of *C. gracilis* from a single host individual could be analyzed, comparisons of pairwise distances within and between infrapopulations, and results from *DAPC* and *STRUCTURE* suggest that specimens

within an infrapopulation are not necessarily more genetically similar to one another than are specimens between infrapopulations (Figs. 3C–E, 5, 9B; Supplemental Fig. 2B). Additionally, specimens collected from the same host individual were almost never recovered as one another's closest relatives in the *RAxML* analysis (Fig. 7), further supporting this assessment. It is important to note, however, that almost no deeper-level relationships recovered in the most likely *RAxML* topology received strong nodal support. This likely due to limited genetic variation between specimens overall, as suggested by the low k-values that were preferred in both *DAPC and STRUCTURE* analyses (Figs. 3C–E, 5).

These results for infrapopulation-level genetic diversity for *C. gracilis* essentially mirror those recovered for *R. megacantha*. Possible explanations, including context from other tapeworm systems, are discussed in detail for *R. megacantha*; those considerations are similarly applicable for *C. gracilis* (see above). Intriguingly, a slightly wider range of pairwise distances within and between infrapopulations was recovered for the no-Senegal dataset for *C. gracilis* as compared to *R. megacantha* (Fig. 9; Supplemental Fig. 2). This is potentially due to the slightly greater number of specimens included in no-Senegal SNP datasets for *C. gracilis* as compared to *R. megacantha* (i.e., 29 versus 26, respectively; see Table 4) and the greater number of host individuals from which infrapopulations could be compared (i.e., 13 sharks rays versus 11 rays in no-Senegal datasets; see Table 1; Fig. 1). It may alternatively indicate that this subpopulation of *C. gracilis* truly boasts more standing genetic variation than does that of *R. megacantha*.

Population-level summary statistics: *Callitetrarhynchus gracilis*

The large proportion of specimens collected from the Gulf of Mexico (i.e., 21 of 32) and those from *Carcharhinus limbatus* (i.e., 16 of 32) encompassed the greatest proportion of genetic diversity sampled for *C. gracilis* in the by-geography and by-host species comparisons, respectively: High proportions of polymorphic sites and private alleles were recovered for both of these purported subpopulations (Table 5). Sampling limitations or data filtering (or a combination thereof) led to low samples sizes for South Carolina, the northern Atlantic

Ocean, Senegal, and Australia subpopulations in the by-geography comparisons, and for the *Carcharhinus* cf. *limbatus*-hosted and *Carcharhinus isodon*-hosted subpopulations in the by-host species comparisons. As discussed for *R. megacantha*, population summary statistics—and particularly F_{ST} values—for these subpopulations should thus be evaluated cautiously (see above). Potential caveats in the interpretations of these results aside, in general, summary statistics and F_{ST} values for possible subpopulations of *C. gracilis* support the same patterns as *DAPC*, *STRUCTURE*, and *RAxML* analyses: limited subpopulation-level structure by definitive host species or geographic region (Table 5; Fig. 8C, D).

Additionally, consistently high levels of homozygosity (i.e., >0.94) and elevated F_{IS} values were found for all subpopulations of *C. gracilis* (Table 5), mirroring the results for *R. megacantha*. Context from other tapeworm systems on expected levels of homozygosity and potential levels of selfing and/or inbreeding is provided and discussed for *R. megacantha*, and those same considerations are relevant for *C. gracilis* (see above).

Definitive host specificity and population-level genetic structure

As part of the design of this study, species of trypanorhynchs with variable degrees of host specificity were specifically targeted to allow for examination of the impact of specificity on population-level genetic structure. Considering the differing degrees of host specificity for *C. gracilis* and *R. megacantha*, as well as the differing vagilities and distributions of their definitive and intermediate hosts (documented and hypothesized; see above), it is predictable that less genetic structure was recovered for *C. gracilis* overall (Figs. 3–5, 8; Supplemental Fig. 2). For obvious reasons, relaxed host specificity and increased host vagility should contribute to reduced genetic structure in parasites, and vice-versa (Nadler, 1995; Huyse et al., 2005). Unfortunately, few studies to date have comprehensively tested this prediction across comparable species, making it challenging to place these results in context.

Perhaps the most comparable data come from lice and their avian hosts. In this system, body lice in the genus *Physconelloides* Ewing, 1927 are known to be more host specific than

wing lice in the genus *Columbicola* Ewing, 1929. When comparing mitochondrial sequences for species in both genera, researchers found that species of *Physconelloides* exhibited a greater degree of genetic structure—among both host species and geographic localities—as compared to less specific species of *Columbicola* (Johnson et al., 2002; Clayton & Johnson, 2003). Any parallels between the results herein and those of Clayton, Johnson, and coauthors must of course be drawn cautiously. Despite their shared parasitic lifestyles, avian louse and marine tapeworms have inarguably different biologies and life histories (e.g., arthropods versus flatworms, terrestrial versus marine host habitats, single-host versus multi-host life-cycles, direct versus trophic transmission, etc.), and those differences are likely to affect patterns of genetic structure. However, the results herein and those from studies of avian lice suggest that host specificity may indeed provide a reliable indicator for expected degree of genetic structure, and further, that this predictability may hold across a broad diversity of host-parasite systems.

CONCLUSIONS AND FUTURE DIRECTIONS

This study is the first to leverage genome-scale data to investigate population structure in elasmobranch tapeworms. The study design utilized a restriction site-associated DNA sequencing approach to characterize component population structure and infrapopulation diversity in the trypanorhynch tapeworms *Rhinoptericola megacantha* (suborder Trypanobatoida) and *Callitetrarhynchus gracilis* (suborder Trypanoselachoida). Efforts were made to sample both deeply within, and broadly across, the range of definitive host species and geographic localities from which each species is known. In summary:

1. Component population-level genetic structure in *R. megacantha* corresponds to geographic region rather than definitive host species, and component populations of *C. gracilis* have relatively little genetic structure, suggesting a positive correlation between degree of host specificity and degree of genetic structure in trypanorhynchs.

2. Conspecific trypanorhynchs collected from the same host individual can be as, or more, genetically divergent from one another as from conspecifics collected from different host individuals, highlighting the potential importance of second and/or third intermediate hosts in maintaining genetic diversity at the infrapopulation level for tapeworms.
3. High levels of homozygosity (>91%) and positive F_{IS} values revealed for both *R. megacantha* and *C. gracilis* suggest individuals of both species commonly engage in inbreeding, including even kin-mating and/or selfing.
4. Limited divergence (<0.35%) in the tapeworm barcoding gene, 28S, was found among conspecific specimens for both *R. megacantha* (39 specimens, three host species, four geographic localities) and *C. gracilis* (38 specimens, five host species, four geographic localities). This result aligns with those of previous investigations of intraspecific variation in 28S for trypanorhynch tapeworms.
5. Despite its reputation as an extremely euryxenous species, adults of *C. gracilis* may be restricted to species of requiem sharks (family Carcharhinidae) as definitive hosts.

As discussed, limited sampling necessitates cautious interpretations of these first insights into the population genomics of trypanorhynch tapeworms. Nevertheless, context from studies of other species of tapeworms and other parasite-host systems showed the results of this study to align with the current understanding of parasite population genetics. Furthermore, the role of intermediate hosts in creating and/or maintaining genetic structure in trypanorhynch populations is not to be underestimated.

Moving forward, comparable studies for additional species of marine tapeworms that demonstrate different, variable combinations of host specificity and geographic distributions (e.g., host specific and geographically restricted, less host specific and globally distributed, etc.) are needed. Ideally, these would include data for adults of other trypanorhynchs, and for species in additional orders of elasmobranch tapeworms, as well as for species of marine tapeworms that parasitize hosts other than elasmobranchs as adults. Locally dense and simultaneously geographically broadly-sampled systems are, however, difficult to come by.

In addition to sampling adult tapeworms from definitive hosts, comprehensive sampling of larval stages from intermediate hosts will be necessary to provide the most complete picture of population structure for any species of marine tapeworm. Sampling of larvae could, for example, inform whether movements of intermediate hosts contribute to genetic continuity between subpopulations of *R. megacantha*, or whether larvae of *C. gracilis* exhibit more local genetic structure than do adults. Trypanorhynchs, owning to their unique attachment organ morphologies, are the only group of elasmobranch tapeworms for which larval stages can be confidently identified to the level of species based on morphology, thus highlighting them as excellent candidates for future investigations focused at the suprapopulation level (i.e., all developmental phases of a species of parasite at one time and place sensu Bush et al., 1997).

Ultimately, the results of this study provide a foundation on which future investigations of marine tapeworm populations genomics can build. This work also supplements the growing body of literature highlighting the immensely complex interplay between biological, ecological, and oceanographic factors in structuring populations of marine species.

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Table 1. Specimens sequenced for Illumina next generation sequencing (NGS) following multiplexed shotgun genotyping (MSG) library preparation and/or for Sanger sequencing of partial 28S rRNA (D1–D3 gene regions). Single asterisk (*) indicates that specimen underwent two rounds of MSG library preparation and NGS; double asterisks (**) indicate read counts following demultiplexing and initial quality filtering with *Stacks process_radtags* and *Trimmomatic*; dagger (†) indicates that data were generated by colleagues at the University of Connecticut (H. Ralicki, pers. comm.); double dagger (‡) indicates a host name sensu Naylor et al. (2012).

Species	Laboratory specimen no.	No. reads retained**	Reason for removal from final MSG datasets	No. bp 28S	28S GenBank accession no. (Hologenophore accession no.)	Host species	Host code	Sampling locality
Rhinoptericola megacantha								
	KW399	1,120,666		1,413†	OL412724 (LRP 10432)	*Rhinoptera brasiliensis*	BE-10	Belize
	BE-11-1*	707,855	SNPfiltR	1,420		*Rhinoptera brasiliensis*	BE-11	Belize
	BE-11-2*	869,764	SNPfiltR	1,292		*Rhinoptera brasiliensis*	BE-11	Belize
	BE-11-3	9,444,812		1,420	OL412715 (LRP 10433)	*Rhinoptera brasiliensis*	BE-11	Belize
	BE-11-4	1,283,042		1,410		*Rhinoptera brasiliensis*	BE-11	Belize
	CH-3-1*	1,030,419	SNPfiltR	1,413	OL412716 (LRP 10440)	*Rhinoptera bonasus*	CH-3	South Carolina
	CH-15-1*	3,156,060		1,421	OL412717 (LRP 10434)	*Rhinoptera brasiliensis*	CH-15	South Carolina
	CH-15-3	4,191,960		1,420		*Rhinoptera brasiliensis*	CH-15	South Carolina
	CH-15-4	4,672,947		1,380	OL412718 (LRP 10435)	*Rhinoptera brasiliensis*	CH-15	South Carolina
	CH-15-5*	3,611,116		1,422	OL412719 (LRP 10436)	*Rhinoptera brasiliensis*	CH-15	South Carolina
	CH-15-6	1,534,189		1,418		*Rhinoptera brasiliensis*	CH-15	South Carolina
	CH-17-1*	126,479	SNPfiltR	1,417	OL412720 (LRP 10437)	*Rhinoptera bonasus*	CH-17	South Carolina
	CH-29-1	2,349,107		1,420	OL412721 (LRP 10439)	*Rhinoptera bonasus*	CH-29	South Carolina
	CH-29-2	1,435,022		1,425		*Rhinoptera bonasus*	CH-29	South Carolina
	CH-30-1*	154,402	SNPfiltR	1,421	OL412722 (LRP 10441)	*Rhinoptera bonasus*	CH-30	South Carolina
	CH-43-1	1,412,893		1,425		*Rhinoptera bonasus*	CH-43	South Carolina
	MS05-49-1	5,569,289		1,416		*Rhinoptera brasiliensis*	MS05-49	Gulf of Mexico
	MS05-49-2*	1,433,907		1,420	OL412725 (LRP 10450)	*Rhinoptera brasiliensis*	MS05-49	Gulf of Mexico
	MS05-156-1	1,733,092		1,413	OL412726 (LRP 10442)	*Rhinoptera brasiliensis*	MS05-156	Gulf of Mexico
	MS05-156-2*	5,495,368		1,421	OL412727 (LRP 10443)	*Rhinoptera brasiliensis*	MS05-156	Gulf of Mexico
	MS05-156-3	901,702		1,136		*Rhinoptera brasiliensis*	MS05-156	Gulf of Mexico
	MS05-298-20	5,558,729		1,419	OL412728 (LRP 10444)	*Rhinoptera brasiliensis*	MS05-298	Gulf of Mexico
	MS05-298-21	1,219,614		1,425		*Rhinoptera brasiliensis*	MS05-298	Gulf of Mexico
	MS05-298-22*	1,157,913		1,424	OL412729 (LRP 10445)	*Rhinoptera brasiliensis*	MS05-298	Gulf of Mexico
	MS05-298-23*	930,097	SNPfiltR	1,426		*Rhinoptera brasiliensis*	MS05-298	Gulf of Mexico
	MS05-298-24	920,720		1,415	OL412730 (LRP 10446)	*Rhinoptera brasiliensis*	MS05-298	Gulf of Mexico
	MS05-305-1	1,540,560		1,420		*Rhinoptera brasiliensis*	MS05-305	Gulf of Mexico
	MS05-305-2*	115,747	SNPfiltR	1,424		*Rhinoptera brasiliensis*	MS05-305	Gulf of Mexico
	MS05-305-3	1,889,779		1,419	OL412731 (LRP 10448)	*Rhinoptera brasiliensis*	MS05-305	Gulf of Mexico
	MS05-305-4*	8,422,210		1,415	OL412732 (LRP 10447)	*Rhinoptera brasiliensis*	MS05-305	Gulf of Mexico
	MS05-305-5	1,281,232		1,412		*Rhinoptera brasiliensis*	MS05-305	Gulf of Mexico
	MS05-375-1	13,950,789		1,413	OL412733 (LRP 10449)	*Rhinoptera brasiliensis*	MS05-375	Gulf of Mexico
	MS05-591-1*	1,803,326		1,000		*Rhinoptera brasiliensis*	MS05-591	Gulf of Mexico
	SE-78-1*	541,933	SNPfiltR	1,414		*Rhinoptera marginata*	SE-78	Senegal

SE-84-1*	749,650	SNPfiltR	1,413	OL412734 (LRP 10452)	*Rhinoptera marginata*	SE-84	Senegal
JW555	21,645,555		1,413†		*Rhinoptera marginata*	SE-84	Senegal
SE-85-1	1,164,486		1,155		*Rhinoptera marginata*	SE-85	Senegal
SE-138-1*	30,263	SNPfiltR	1,158		*Rhinoptera marginata*	SE-138	Senegal
SE-139-1*	10,527,402		1,413	OL412735 (LRP 10451)	*Rhinoptera marginata*	SE-139	Senegal
Callitetrarhynchus gracilis							
AU-3-1	5,630,185		1,431		*Carcharhinus* cf. *limbatus*‡	AU-3	N. Territory, Australia
CH-8-1*	1,304,745	bad apple	1,433		*Rhizoprionodon terraenovae*	CH-8	South Carolina
CH-8-2*	1,411,674	bad apple	1,433		*Rhizoprionodon terraenovae*	CH-8	South Carolina
CH-8-3*	1,242,686	bad apple	1,443		*Rhizoprionodon terraenovae*	CH-8	South Carolina
CH-50-1	8,070,627		1,433		*Carcharhinus limbatus*	CH-50	South Carolina
CH-50-2*	18,035,193		1,443		*Carcharhinus limbatus*	CH-50	South Carolina
KW840	15,303,104		1,433†		*Carcharhinus limbatus*	CH-50	South Carolina
JW474	25,058,762		1,433†		*Rhizoprionodon terraenovae*	DEL-5	Florida
DEL-9-1*	38,171,518				*Carcharhinus limbatus*	DEL-9	Florida
DEL-9-2	19,922,341				*Carcharhinus limbatus*	DEL-9	Florida
DEL-9-3*	7,735,470				*Carcharhinus limbatus*	DEL-9	Florida
DEL-9-4*	938,324	bad apple	1,433		*Carcharhinus limbatus*	DEL-9	Florida
DEL-9-5*	4,091,167		1,433		*Carcharhinus limbatus*	DEL-9	Florida
MS05-5-1*	2,674,933	SNPfiltR			*Carcharhinus brevipinna*	MS05-5	Gulf of Mexico
MS05-5-2	41,506,914		1,433		*Carcharhinus brevipinna*	MS05-5	Gulf of Mexico
MS05-5-3	3,272,653		1,433		*Carcharhinus brevipinna*	MS05-5	Gulf of Mexico
MS05-5-4	4,454,885		1,433		*Carcharhinus brevipinna*	MS05-5	Gulf of Mexico
MS05-5-5	7,926,344		1,433		*Carcharhinus brevipinna*	MS05-5	Gulf of Mexico
MS05-21-2*	3,522,108		1,433		*Rhizoprionodon terraenovae*	MS05-21	Gulf of Mexico
MS05-21-3*	5,110,408		1,433		*Rhizoprionodon terraenovae*	MS05-21	Gulf of Mexico
MS05-21-4	5,192,806		1,439		*Rhizoprionodon terraenovae*	MS05-21	Gulf of Mexico
MS05-24-1	4,201,041		1,433		*Carcharhinus limbatus*	MS05-24	Gulf of Mexico
MS05-83-1	3,612,175				*Carcharhinus isodon*	MS05-83	Gulf of Mexico
MS05-83-2	8,573,745		1,433		*Carcharhinus isodon*	MS05-83	Gulf of Mexico
MS05-83-3*	7,970,821		1,433		*Carcharhinus isodon*	MS05-83	Gulf of Mexico
MS05-396-1*	36,062,437		1,433		*Rhizoprionodon terraenovae*	MS05-396	Gulf of Mexico
JW477*	4,522,503	bad apple	1,433†		*Rhizoprionodon terraenovae*	MS05-396	Gulf of Mexico
MS05-403-1*	2,867,690	bad apple	1,433		*Carcharhinus limbatus*	MS05-403	Gulf of Mexico
MS05-403-2*	1,242,006	SNPfiltR	1,439		*Carcharhinus limbatus*	MS05-403	Gulf of Mexico
MS05-403-3*	20,996,906		1,443		*Carcharhinus limbatus*	MS05-403	Gulf of Mexico
MS05-403-4*	21,070,495		1,445		*Carcharhinus limbatus*	MS05-403	Gulf of Mexico
MS05-403-5*	14,023,118		1,436		*Carcharhinus limbatus*	MS05-403	Gulf of Mexico
MS05-457-1*	4,667,177		1,433		*Carcharhinus brevipinna*	MS05-457	Gulf of Mexico
MS05-488-1*	2,941,599	bad apple	1,433		*Carcharhinus limbatus*	MS05-488	Gulf of Mexico
MS05-488-2*	4,035,585	bad apple	1,433		*Carcharhinus limbatus*	MS05-488	Gulf of Mexico
MS05-488-3*	10,186,793		1,407		*Carcharhinus limbatus*	MS05-488	Gulf of Mexico
MS05-488-4*	1,229,041	bad apple	1,433		*Carcharhinus limbatus*	MS05-488	Gulf of Mexico
MS05-489-3*	11,808,355		1,433		*Carcharhinus limbatus*	MS05-489	Gulf of Mexico
MS05-489-4	5,020,369		1,443		*Carcharhinus limbatus*	MS05-489	Gulf of Mexico
MS05-489-5*	15,639,315		1,445		*Carcharhinus limbatus*	MS05-489	Gulf of Mexico
MS05-489-6*	20,044,128		1,433		*Carcharhinus limbatus*	MS05-489	Gulf of Mexico
MS05-489-7*	5,327,316	bad apple	1,433		*Carcharhinus limbatus*	MS05-489	Gulf of Mexico

VJ-6-1	5,028,827		1,433	*Rhizoprionodon terraenovae*	VJ-6	N. Atlantic Ocean
SE-61-1	1,698,962	SNPfiltR		*Carcharhinus brevipinna*	SE-61	Senegal
SE-61-2*	7,562,834			*Carcharhinus brevipinna*	SE-61	Senegal
SE-81-1*	12,002,596	bad apple		*Carcharhinus brevipinna*	SE-81	Senegal
SE-81-2-HUP*	18,338	SNPfiltR	775	*Carcharhinus brevipinna*	SE-81	Senegal

Table 2. Definitive host associations of *Rhinoptericola megacantha* Carvajal & Campbell, 1975 and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 with updated host identifications. Bolded host names indicate host species that were examined as part of this study, but from which specimens of *C. gracilis* were not recovered; asterisks (*) indicates a host name sensu Naylor et al. (2012); double asterisks (**) indicate a report from freshwater; dagger (†) indicates a report as *Callitetrarhynchus* cf. *gracilis.*

Host order: Host family	Host species	Locality	Source of report	New report herein	Included herein
Rhinoptericola megacantha					
Myliobatiformes: Rhinopteridae	*Rhinoptera bonasus*	Chesapeake Bay, VA, USA	Carval & Campbell (1975)		
		Charleston, SC, USA	Herzog & Jensen (2022); this study		★
Myliobatiformes: Rhinopteridae	*Rhinoptera bonasus* or *Rhinoptera brasiliensis* (as *Rhinoptera bonasus*)	Gulf of Venezuela	Mayes & Brooks (1981)		
Myliobatiformes: Rhinopteridae	*Rhinoptera brasiliensis*	Charleston, SC, USA	Herzog & Jensen (2022); this study		★
Myliobatiformes: Rhinopteridae	*Rhinoptera brasiliensis* (as *Rhinoptera bonasus* prior to Herzog & Jensen, 2022)	Gulf of Mexico	Palm et al. (2009); Olson et al. (2010); Herzog & Jensen (2022); this study		★
Myliobatiformes: Rhinopteridae	*Rhinoptera brasiliensis*	Belize	Herzog & Jensen (2022); this study		★
		S. and S.E. Brazil	Napoleão et al. (2015)		
Myliobatiformes: Rhinopteridae	*Rhinoptera marginata*	Senegal	Herzog & Jensen (2022); this study		★
Myliobatiformes: Dasyatidae	*Hypanus say*	Charleston, SC, USA	Herzog & Jensen (2022)		
Callitetrarhynchus gracilis					
Carcharhiniformes: Carcharhinidae	***Carcharhinus amblyrhynchoides***	Queensland, Australia	Palm (2004)		
Carcharhiniformes: Carcharhinidae	***Carcharhinus amboinensis***	N. Territory, Australia	Palm (2004); Olson et al. (2010)		
Carcharhiniformes: Carcharhinidae	*Carcharhinus brevipinna*	Gulf of Mexico	This study	★	★
		Senegal	This study	★	★
Carcharhiniformes: Carcharhinidae	*Carcharhinus* cf. *dussumieri**	Iran, Persian Gulf	Haseli et al. (2010)		
Carcharhiniformes: Carcharhinidae	*Carcharhinus fitzroyensis*	N. Territory, Australia	Palm (2004)		
Carcharhiniformes: Carcharhinidae	*Carcharhinus isodon*	Gulf of Mexico	This study	★	★
Carcharhiniformes: Carcharhinidae	***Carcharhinus leucas***	Costa Rica	Watson & Thorson (1976)**		
		Gulf of Mexico	Palm (2004); Méndez & González (2013)		
Carcharhiniformes: Carcharhinidae	*Carcharhinus limbatus*	Charleston, SC, USA	This study	★	★
		Florida, USA	This study	★	★
		Gulf of Mexico	Palm & Overstreet (2000); Owens (2008); this study		★
Carcharhiniformes: Carcharhinidae	*Carcharhinus* cf. *limbatus**	N. Territory, Australia	This study	★	★
Carcharhiniformes: Carcharhinidae	*Carcharhinus melanopterus*	Queensland, Australia	Olson et al. (2001)		
Carcharhiniformes: Carcharhinidae	***Carcharhinus obscurus***	Japan	Nakajima & Egusa (1972a)		
Carcharhiniformes: Carcharhinidae	***Carcharhinus sorrah*** (as *Carcharhinus* cf. *sorrah)*	Iran, Persian Gulf	Haseli et al. (2010)		

Carcharhiniformes: Carcharhinidae	***Lamiopsis tephrodes***	Borneo	Schaeffner & Beveridge (2014)		
Carcharhiniformes: Carcharhinidae	*Negaprion brevirostris*	Japan	Nakajima & Egusa (1972a)		
Carcharhiniformes: Carcharhinidae	***Prionace glauca***	California, USA	Heinz & Dailey (1974)		
Carcharhiniformes: Carcharhinidae	*Rhizoprionodon* cf. *acutus* 2* (as *Rhizoprionodon acutus*)	Japan	Nakajima & Egusa (1972a)		
Carcharhiniformes: Carcharhinidae	***Rhizoprionodon* cf. *acutus* 1*** (as *Rhizoprionodon acutus*)	South Africa	Palm (2004)		
Carcharhiniformes: Carcharhinidae	*Rhizoprionodon acutus*	Iran, Persian Gulf	Haseli et al. (2010)		
Carcharhiniformes: Carcharhinidae	***Rhizoprionodon* cf. *acutus* 3*** (as *Rhizoprionodon acutus* 3)	Borneo	Schaeffner & Beveridge (2014)		
Carcharhiniformes: Carcharhinidae	*Rhizoprionodon terraenovae*	Charleston, SC, USA	This study	★	★
		Florida, USA	This study	★	★
		Gulf of Mexico	Palm (2004); this study		★
		Senegal	Palm (2004)		
Carcharhiniformes: Sphyrnidae	***Sphyrna lewini* 1*** (as *Sphyrna lewini*)	Gulf of Mexico	Palm (1995)		
Carcharhiniformes: Sphyrnidae	***Sphyrna zygaena***	Japan	Nakajima & Egusa (1972b)		
Carcharhiniformes: Triakidae	***Mustelus canis***	Rio Grande do Sul, Brazil	São Clemente & Gomes (1989)		
Carcharhiniformes: Triakidae	*Mustelus moisis*	Iraq, Persian Gulf	Mhaisen et al. (2018)†		
Carcharhiniformes: Triakidae	*Triakis scyllium*	Japan	Nakajima & Egusa (1972c); Nakajima & Egusa (1973)		
Orectolobiformes: Ginglymostomatidae	***Nebrius ferrugineus***	Sri Lanka	Beveridge & Campbell (1998)		
Myliobatiformes: Dasyatidae	*Hemitrygon fluviorum* (as *Dasyatis fluviorum*)	Queensland, Australia	Palm (2004)		

Table 3. Size, sex, and detailed collection locality data for elasmobranch specimens hosting specimens of *Rhinoptericola megacantha* Carvajal & Campbell, 1975 and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 for which sequence data were generated as part of this study. Asterisks (*) indicate an identification not verified with NADH2 sequence data; double asterisks (**) indicate a host name sensu Naylor et al. (2012).

Family: Species	Field code	Disk width/ total length (cm)	Sex	Collection date	Collection locality	Species hosted (No. worms sequenced: No. worms for which 28S was generated: No. worms retained in final SNP datasets)
Rhinopteridae: *Rhinoptera brasiliensis*	BE-10	89 DW	M	May 18, 2012	Gales Point Manatee (17°13'1.0"N, 88°19'01.4"W), Belize, Inner Channel, Atlantic Ocean	Rm (1:1:1)
Rhinopteridae: *Rhinoptera brasiliensis*	BE-11	88 DW	F	May 18, 2012	Gales Point Manatee (17°13'1.0"N, 88°19'01.4"W), Belize, Inner Channel, Atlantic Ocean	Rm (4:4:2)
Rhinopteridae: *Rhinoptera bonasus*	CH-3	88 DW	F	Jun. 27, 2012	Awendaw (33°02'07.78"N, 79°32'47.24"W), South Carolina, USA, Bulls Bay, Atlantic Ocean	Rm (1:1:0)
Rhinopteridae: *Rhinoptera brasiliensis*	CH-15	58 DW	M	Jun. 17, 2013	Awendaw (33°0'34.27"N, 79°29'8.82"W), South Carolina, USA, Bulls Bay, 5 Fathom Creek, Atlantic Ocean	Rm (5:5:5)
Rhinopteridae: *Rhinoptera bonasus*	CH-17	82.5 DW	M	Jun.17, 2013	Charleston (32°45'2.53"N, 79°53'48.28"W), South Carolina, USA, Charleston Harbor, Atlantic Ocean	Rm (1:1:0)
Rhinopteridae: *Rhinoptera bonasus*	CH-29	87 DW	F	Jun. 19, 2013	Awendaw (33°02'07.78"N, 79°32'47.24"W), South Carolina, USA, Bulls Bay, Atlantic Ocean	Rm (2:2:2)
Rhinopteridae: *Rhinoptera bonasus*	CH-30	93 DW	F	Jun. 19, 2013	Awendaw (33°02'07.78"N, 79°32'47.24"W), South Carolina, USA, Bulls Bay, Atlantic Ocean	Rm (1:1:0)
Rhinopteridae: *Rhinoptera bonasus*	CH-43	94 DW	F	Jun. 15, 2015	Charleston, South Carolina, USA, Atlantic Ocean	Rm (1:1:1)
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-49	92 DW	M	Jun. 19, 2005	South side of East Ship Island (30°14'24.54"N, 88°52'25.25"W), Mississippi, USA, Gulf of Mexico	Rm (2:2:2)
Rhinopteridae: *Rhinoptera brasiliensis**	MS05-156	?	?	Aug. 2005	Ship Island (30°13'13.53"N, 88°54'52.48"W), Mississippi, USA, Gulf of Mexico	Rm (3:3:3)
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-298	97 DW	F	Apr. 25, 2006	West tip of Horn Island (30°14'24.54"N, 88°52'25.25"W), Mississippi, USA, Gulf of Mexico	Rm (5:5:4)
Rhinopteridae: *Rhinoptera brasiliensis**	MS05-305	81 DW	F	Mar. 28, 2006	Horn Island (30°15′04″N, 88°42′42″W), Mississippi, USA, Gulf of Mexico	Rm (5:5:4)
Rhinopteridae: *Rhinoptera brasiliensis*	MS05-375	?	?	Aug. 27, 2006	West of south tip of Chandeleur Islands (29°57'9.54"N, 88°50'38.98"W), Louisiana, USA, Gulf of Mexico	Rm (1:1:1)
Rhinopteridae: *Rhinoptera brasiliensis**	MS05-591	101.5 DW	M	Jun. 7, 2009	Horn Island (30°14'1.44"N, 88°40'5.47"W), Mississippi, USA, Gulf of Mexico	Rm (1:1:1)
Rhinopteridae: *Rhinoptera marginata*	SE-78	54.5 DW	F	Jan. 12, 2003	St. Louis (16°1'28"N, 16°30'33"W), Senegal, Atlantic Ocean	Rm (1:1:0)
Rhinopteridae: *Rhinoptera marginata*	SE-84	74 DW	F	Jan. 13, 2003	St. Louis (16°1'28"N, 16°30'33"W), Senegal, Atlantic Ocean	Rm (2:2:1)
Rhinopteridae: *Rhinoptera marginata*	SE-85	56 DW	F	Jan. 13, 2003	St. Louis (16°1'28"N, 16°30'33"W), Senegal, Atlantic Ocean	Rm (1:1:1)
Rhinopteridae: *Rhinoptera marginata*	SE-138	84.5 DW	F	Jan. 3, 2004	St. Louis (16°1'28"N, 16°30'33"W), Senegal, Atlantic Ocean	Rm (1:1:0)
Rhinopteridae: *Rhinoptera marginata*	SE-139	86 DW	F	Jan. 3, 2004	St. Louis (16°1'28"N, 16°30'33"W), Senegal, Atlantic Ocean	Rm (1:1:1)
Carcharhinidae: *Carcharhinus* cf. *limbatus***	AU-3	204 TL	F	Aug. 4, 1997	Darwin (12°20'11"S, 130°54'39"E), Northern Territory, Australia, Buffalo Creek, Timor Sea, Indian Ocean	Cg (1:1:1)
Carcharhinidae: *Rhizoprionodon terraenovae*	CH-8	93.5 TL	M	Jun. 28, 2012	Wadmalaw Point (32°37'45.84"N, 80°16'02.26"W), South Carolina, USA, North Edisto River, Atlantic Ocean	Cg (3:3:0)
Carcharhinidae: *Carcharhinus limbatus*	CH-50	149 TL	F	Jun. 18, 2015	Charleston, South Carolina, USA, Bulls Bay, Atlantic Ocean	Cg (3:3:3)

Carcharhinidae: *Rhizoprionodon terraenovae*	DEL-5	67.5 TL	M	Apr. 18, 2001	Florida (24°47.56'N , 80°39.79'W), USA, Atlantic Ocean	Cg (1:1:1)
Carcharhinidae: *Carcharhinus limbatus*	DEL-9	154.6 TL	M	Apr. 22, 2001	Florida, (28°0.8'N , 80°27'W), USA, Atlantic Ocean	Cg (5:2:4)
Carcharhinidae: *Carcharhinus brevipinna*	MS05-5	103 TL	F	Jun. 15, 2005	South of the Shallow Fields (29°37'22.8"N, 88°30'11"W), Mississippi, USA, Gulf of Mexico	Cg (5:4:4)
Carcharhinidae: *Rhizoprionodon terraenovae*	MS05-21	88 TL	M	Jun. 16, 2005	North of West end of Horn Island, Mississippi, USA, Gulf of Mexico	Cg (3:3:3)
Carcharhinidae: *Carcharhinus limbatus*	MS05-24	80 TL	M	Jun. 16, 2005	North of West end of Horn Island, Mississippi, USA, Gulf of Mexico	Cg (1:1:1)
Carcharhinidae: *Carcharhinus isodon*	MS05-83	82 TL	M	Jun. 21, 2005	Round Island (30°17'42.45"N, 88°35'11.55"W), Mississippi, USA, Gulf of Mexico	Cg (3:2:3)
Carcharhinidae: *Rhizoprionodon terraenovae*	MS05-396	86 TL	M	Oct. 3, 2006	Florida (29°46'3"N, 85°21'4"W), USA, St. Joe's Bay, Gulf of Mexico	Cg (2:2:1)
Carcharhinidae: *Carcharhinus limbatus*	MS05-403	100 TL	M	Oct. 4, 2006	Florida (29°59'37"N, 85°31'48"W), USA, Crooked Island Bay, Gulf of Mexico	Cg (5:5:3)
Carcharhinidae: *Carcharhinus brevipinna**	MS05-457	79 TL	M	Oct. 8, 2006	Southwest end of Horn Island (30°14'9"N, 88°46'2"W), Mississippi, USA, Gulf of Mexico	Cg (1:1:1)
Carcharhinidae: *Carcharhinus limbatus**	MS05-488	78 TL	F	May 22, 2007	Florida (29°40'8.05"N, 85°13'30.17"W), USA, Indian Pass, Gulf of Mexico	Cg (4:4:1)
Carcharhinidae: *Carcharhinus limbatus**	MS05-489	79 TL	M	May 22, 2007	Florida (29°40'8.05"N, 85°13'30.17"W), USA, Indian Pass, Gulf of Mexico	Cg (5:5:4)
Carcharhinidae: *Rhizoprionodon terraenovae**	VJ-6	94.4 TL	F	Jan. 25, 1997	USA, Atlantic Ocean	Cg (1:1:1)
Carcharhinidae: *Carcharhinus brevipinna*	SE-61	71.5 TL	M	Jan. 11, 2003	Ouakam (14°42'54"N, 17°29'28"W), Senegal, Atlantic Ocean	Cg (2:0:1)
Carcharhinidae: *Carcharhinus brevipinna*	SE-81	86 TL	F	Jan. 12, 2003	St. Louis (16°1'28"N, 16°30'33"W), Senegal, Atlantic Ocean	Cg (2:1:0)

Abbreviations: Cg–*Callitetrarhynchus gracilis*; DW–disk width; F–female; M–male; Rm–*Rhinoptericola megacantha*; TL–total length.

Table 4. Number of individuals, number of loci, and population genomic analyses conducted for each of the final filtered single nucleotide polymorphism datasets generated for *Rhinoptericola megacantha* Carvajal & Campbell, 1975 and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931.

Species	Dataset	No. specimens	No. loci	Analyses conducted
Rhinoptericola megacantha				
	complete	29 of 39	2,568	*DAPC*; *RAxML*; summary statistics
	no-Senegal	26 of 34	2,221	*DAPC*; pairwise distances
	MAC complete	29 of 39	1,958	*STRUCTURE*
	MAC no-Senegal	26 of 34	1,408	*STRUCTURE*
Callitetrarhynchus gracilis				
	complete	32 of 47	3,908	*DAPC*; *RAxML*; summary statistics
	no-Senegal	31 of 43	3,273	*DAPC*; pairwise distances
	MAC complete	32 of 47	1,603	*STRUCTURE*
	MAC no-Senegal	31 of 43	1,294	*STRUCTURE*

Abbreviations: MAC–filtered for a minimum minor allele count of 3.

Table 5. Population-level summary statistics for the complete single nucleotide polymorphism datasets generated for *Rhinoptericola megacantha* Carvajal & Campbell, 1975 and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931. For each species, the *populations* module of *Stacks* was run by grouping specimens by either geographic collection locality or host species.

Species	Grouping	Sub-population	n	No. sites analyzed	No. (%) polymorphic sites	No. (%) private alleles	Observed (expected) homozygosity	F_{IS}	π
Rhinoptericola megacantha									
	Geography	SC and GoM	23	2,568	1,297 (50.51%)	944 (36.76%)	0.94971 (0.9251)	0.17954	0.07689
		Belize	3	2,566	322 (12.55%)	76 (2.96%)	0.94557 (0.95067)	0.01236	0.06116
		Senegal	3	2,537	620 (24.44%)	1,181 (46.55%)	0.91493 (0.89404)	0.07882	0.13043
	Host	Rbon	3	2,568	381 (14.84%)	18 (0.70%)	0.94224 (0.94835)	0.01139	0.06412
		Rbra	23	2,568	1,356 (52.80%)	915 (35.65%)	0.95027 (0.92225)	0.19593	0.07981
		Rmar	3	2,537	620 (24.43%)	1,181 (46.55%)	0.91493 (0.89404)	0.07882	0.13043
Callitetrarhynchus gracilis									
	Geography	SC	3	3,866	473 (12.23%)	268 (6.93%)	0.9634 (0.95339)	0.04062	0.06064
		FL	5	3,908	866 (22.16%)	513 (13.13%)	0.95347 (0.93322)	0.06801	0.07786
		GoM	21	3,908	2,648 (67.76%)	2,111 (54.02%)	0.96095 (0.92215)	0.23197	0.08064
		N. Atlantic	1	2,972	111 (3.73%)	102 (3.43%)	0.96265 (0.98133)	———	0.03735
		Senegal	1	2,455	131 (5.33%)	236 (9.61%)	0.94664 (0.97332)	———	0.05336
		Australia	1	2,788	146 (5.24%)	90 (3.22%)	0.94763 (0.97382)	———	0.05237
	Host	Cbre	6	3,906	1,009 (25.83%)	639 (16.36%)	0.95662 (0.92667)	0.09787	0.08403
		Clim	16	3,908	2,260 (57.83%)	1,696 (43.4%)	0.96215 (0.91978)	0.22408	0.08388
		Ccflim	1	2,788	146 (5.24%)	90 (3.23%)	0.94763 (0.97382)	———	0.05237
		Ciso	3	3,854	446 (11.57%)	255 (6.62%)	0.95909 (0.9566)	0.0266	0.05703
		Rter	6	3,907	951 (24.34%)	574 (14.69%)	0.95712 (0.93183)	0.07912	0.07827

Abbreviations: Cbre–*Carcharhinus brevipinna*; Ccflim–*Carcharhinus* cf. *limbatus*; *Ciso*–*Carcharhinus isodon*; Clim–*Carcharhinus limbatus*; FL–Florida; GoM–Gulf of Mexico; Rbon–*Rhinoptera bonasus*; Rbra–*Rhinoptera brasiliensis*; Rmar–*Rhinoptera marginata*; Rter–*Rhizoprionodon terraenovae*; SC–South Carolina.

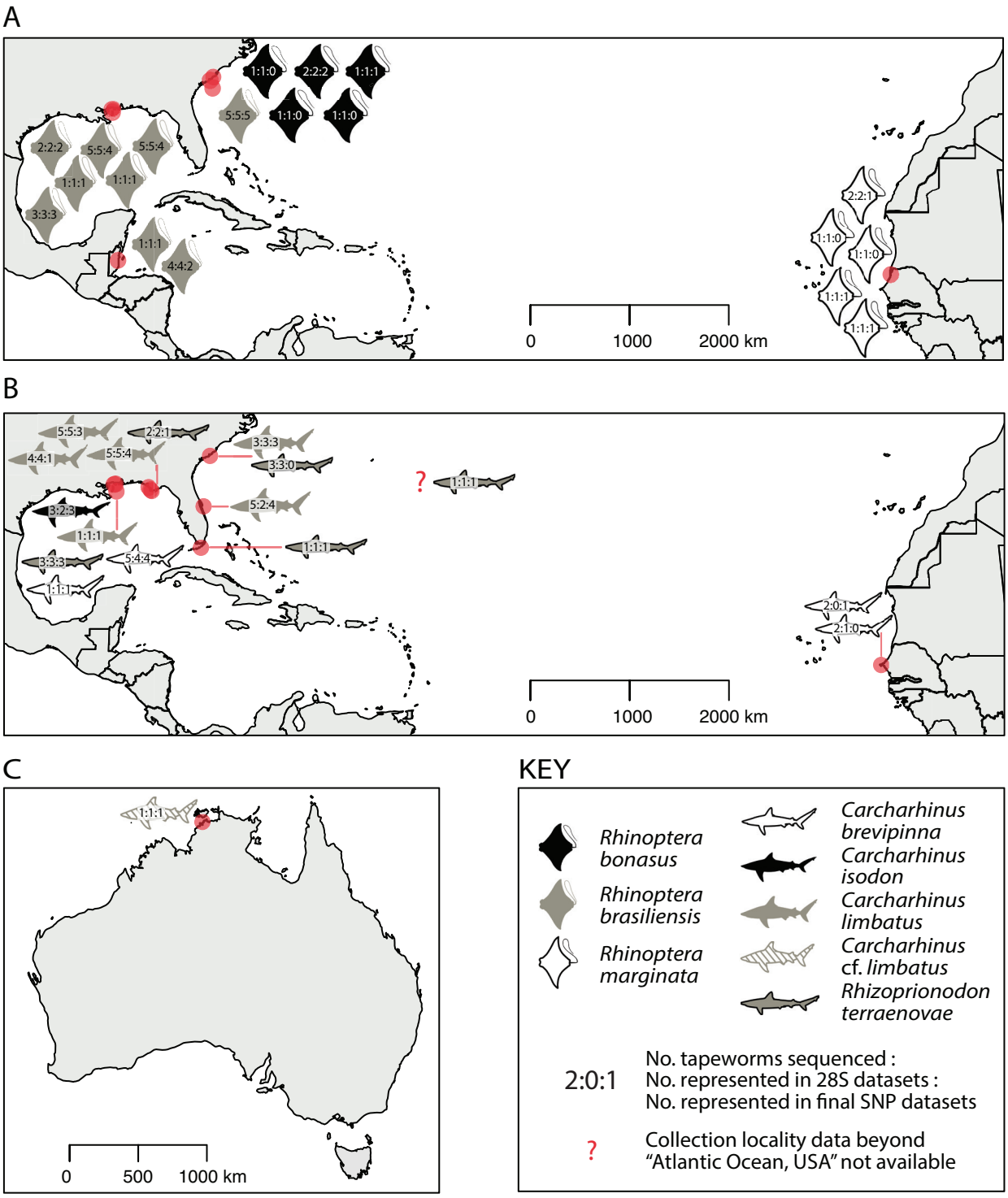


Figure 1. Maps of sampling localities indicating the number of individuals of each host species sampled per locality and the number of tapeworms sequenced per host individual for *Rhinoptericola megacantha* Carvajal & Campbell, 1975 (A) and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 (B–C). Abbreviations: 28S–partial 28S rRNA; SNP–single nucleotide polymorphism.

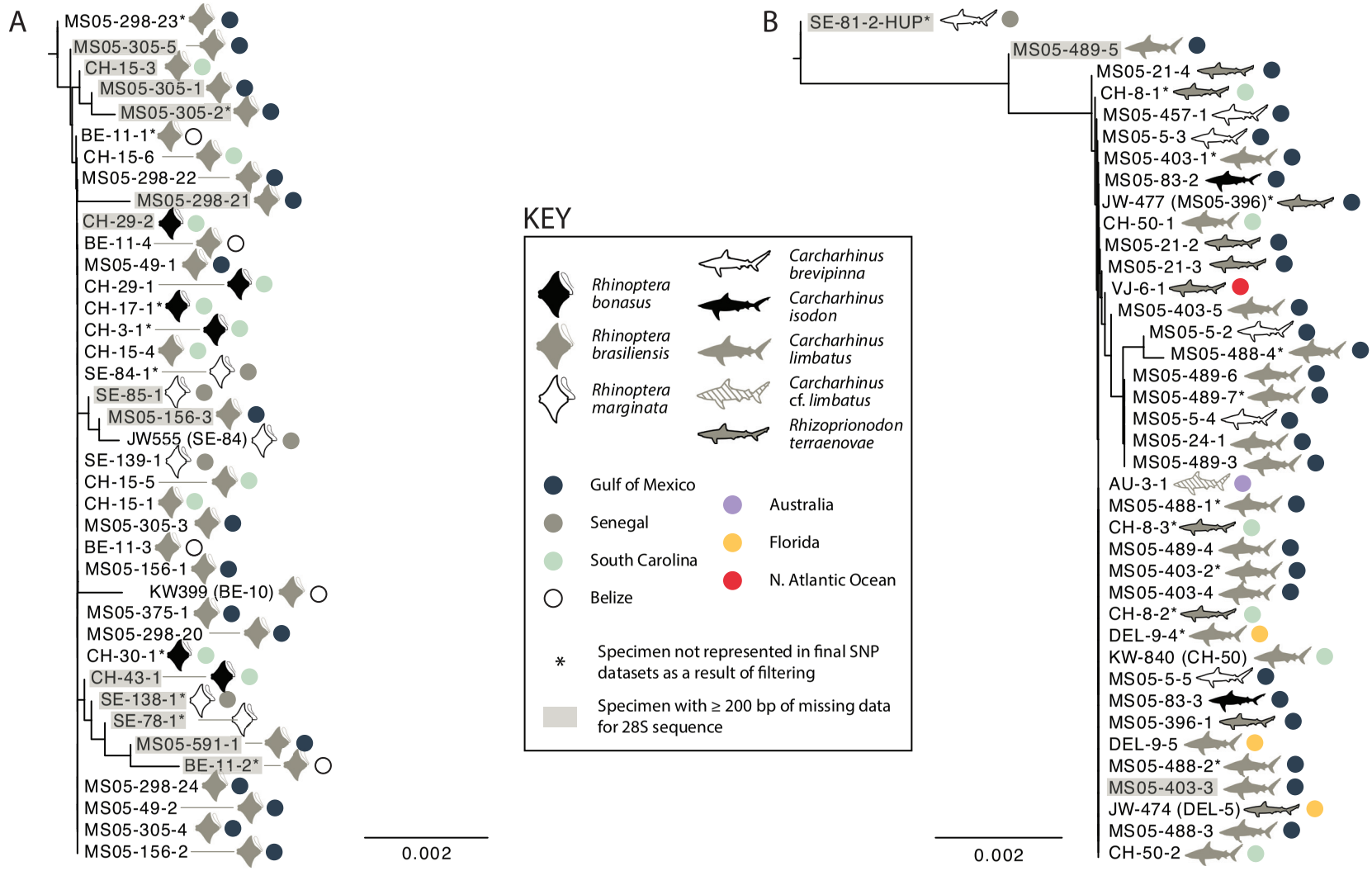


Figure 2. Neighbor-joining trees generated from sequence data for the D1–D3 gene regions of 28S rRNA for *Rhinoptericola megacantha* Carvajal & Campbell, 1975 (A) and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 (B). For both trees, the outgroup taxon was pruned. Taxon labels are unique lab specimen numbers comprising unique host accession numbers and specimen numbers, or unique specimen numbers followed in parentheses by host accession numbers, and are followed by graphic representations of host species and geographic locality. Scale bars indicate pairwise distances.

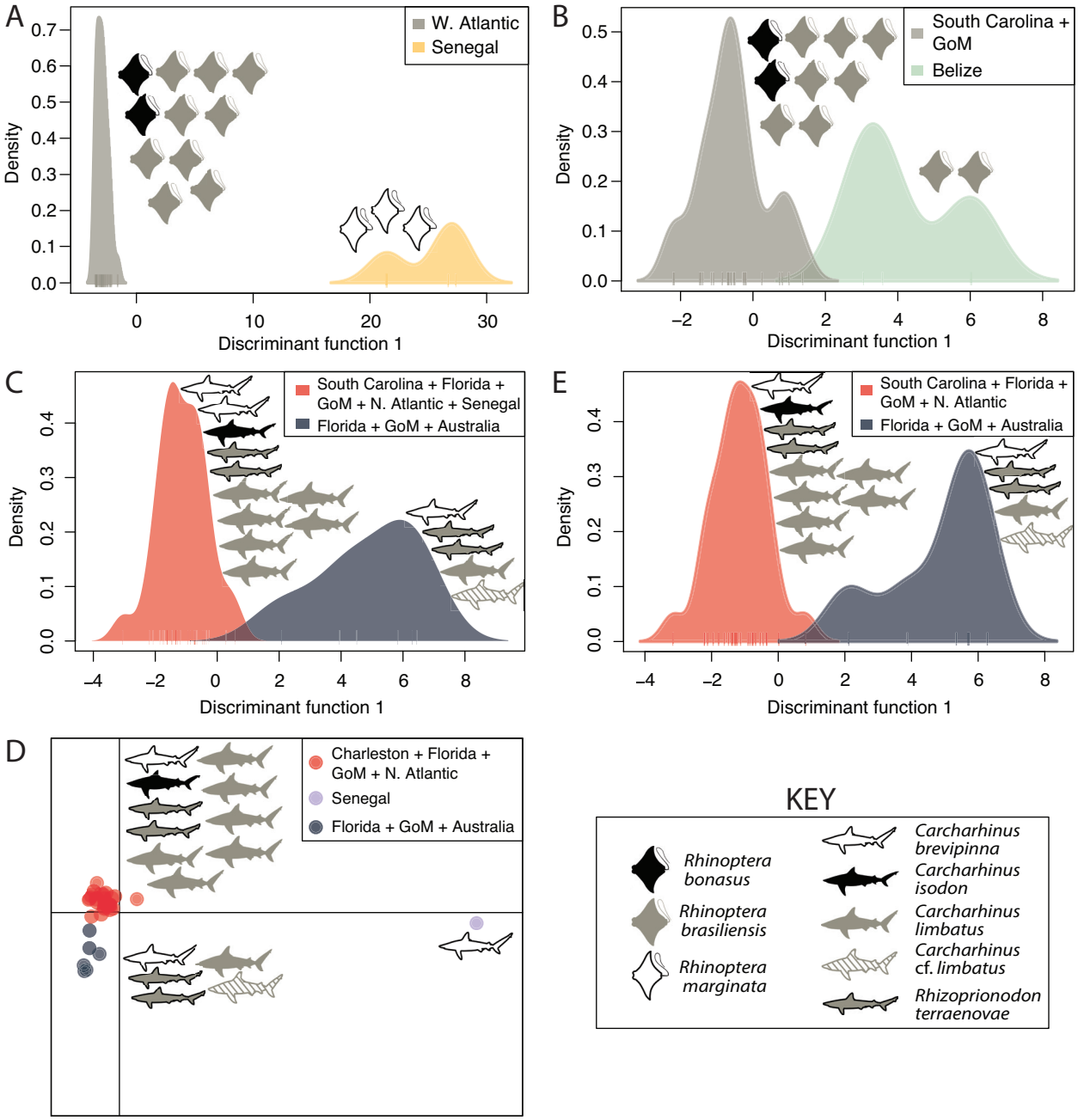


Figure 3. Results of discriminant analysis of principle components (*DAPC*) for *Rhinoptericola megacantha* Carvajal & Campbell, 1975 (A–B) and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 (C–E). Graphic representations of the number of individuals of each host species from which specimens grouped into a cluster were collected are presented alongside each cluster. Plots (A) and (C–D) were generated using complete single nucleotide polymorphism datasets not filtered for minor allele count; plots (B) and (E) were generated using no-Senegal single nucleotide polymorphism datasets not filtered for minor allele count. For both datasets for *R. megacantha* (A–B) and for the no-Senegal dataset for *C. gracilis* (E), AIC and BIC each preferred two clusters. For the complete dataset for *C. gracilis*, BIC preferred two clusters (C) while AIC preferred three clusters (D). Abbreviations: GoM–Gulf of Mexico.

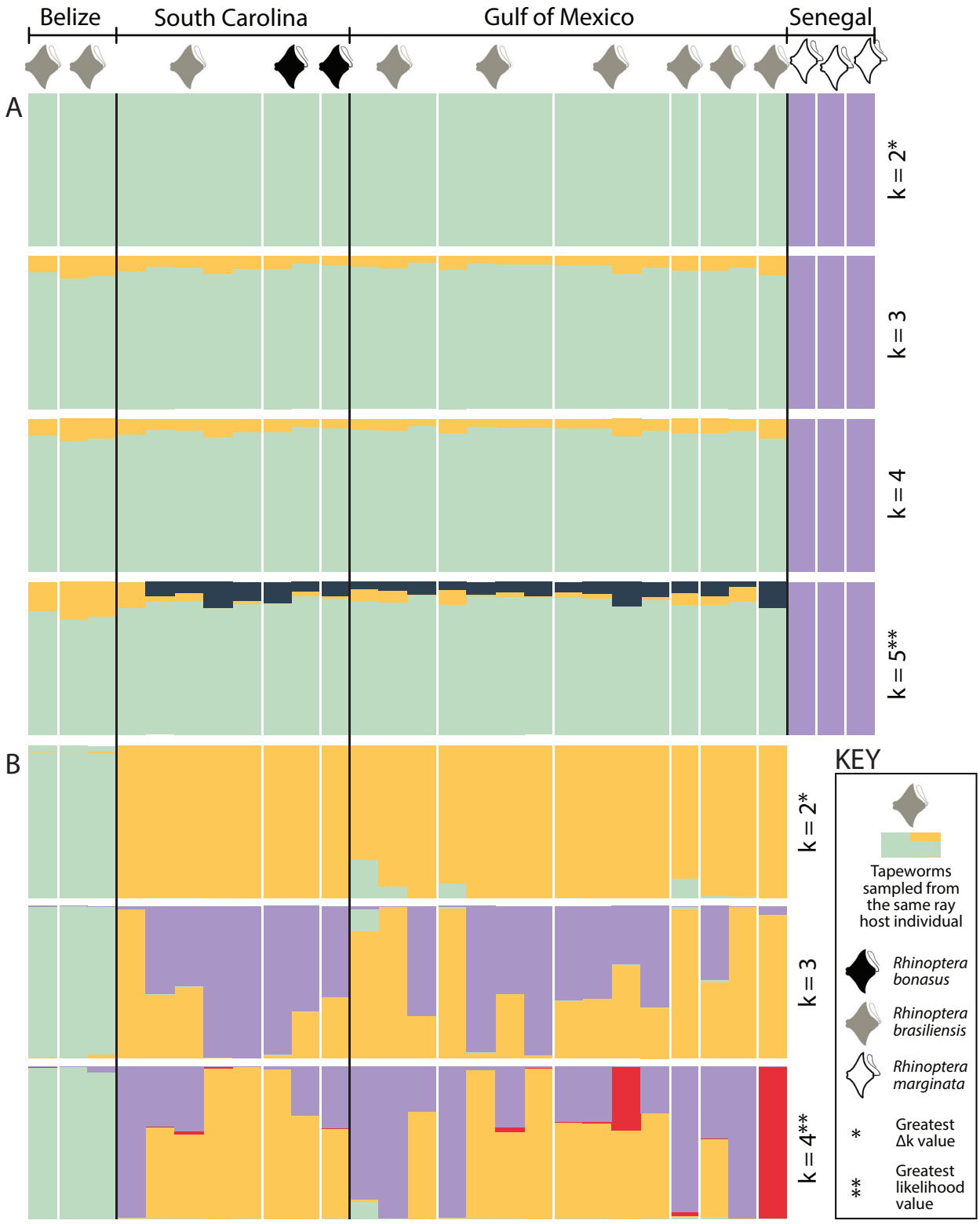


Figure 4. Results of *STRUCTURE* analyses for *Rhinoptericola megacantha* Carvajal & Campbell, 1975 for complete (A) and no-Senegal (B) single nucleotide polymorphism datasets. Black lines separate sampling localities and white lines separate host individuals within a sampling locality.

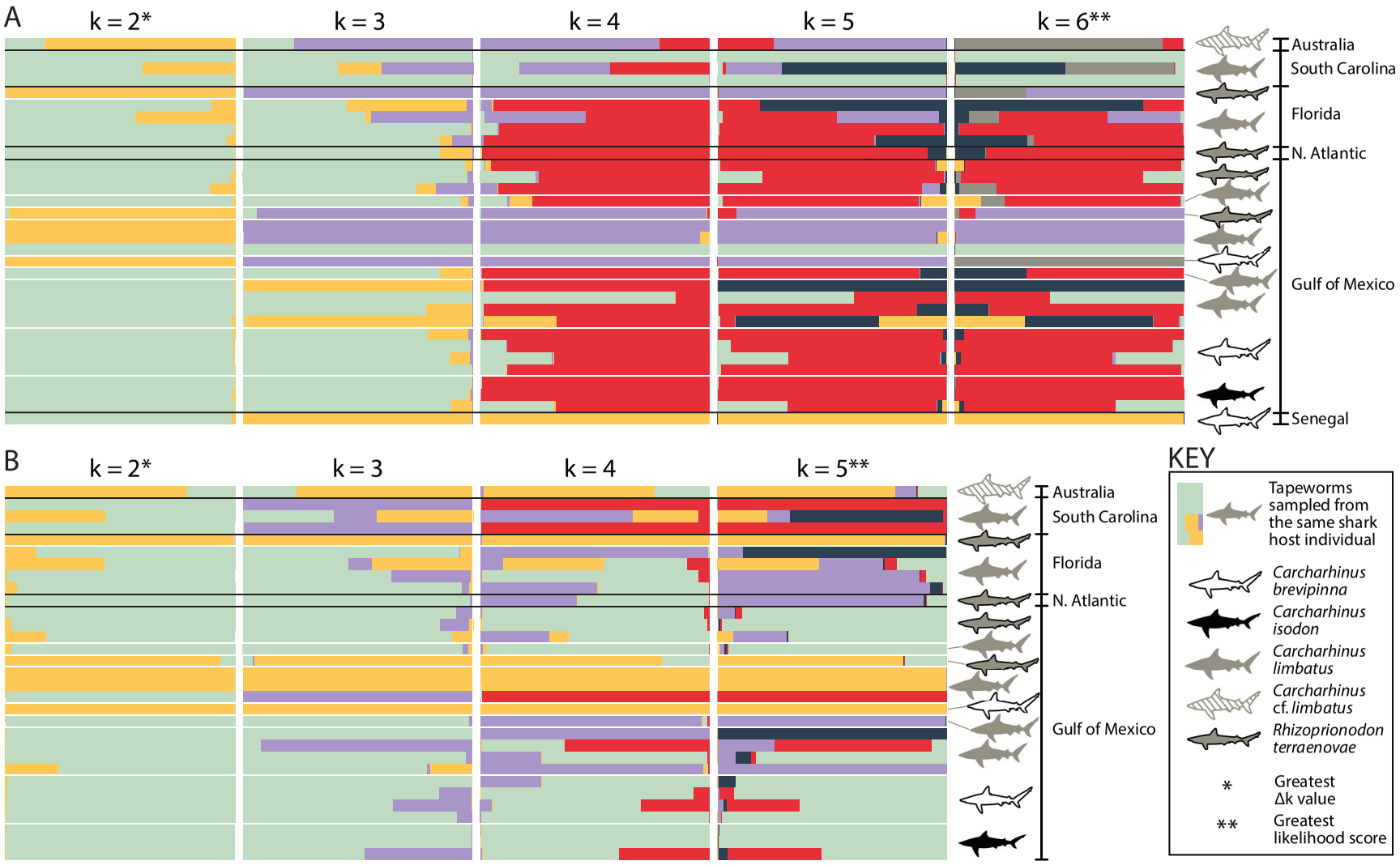


Figure 5. Results of *STRUCTURE* analyses for *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 for complete (A) and no-Senegal (B) single nucleotide polymorphism datasets. Black lines separate sampling localities and white lines separate host individuals within a sampling locality.

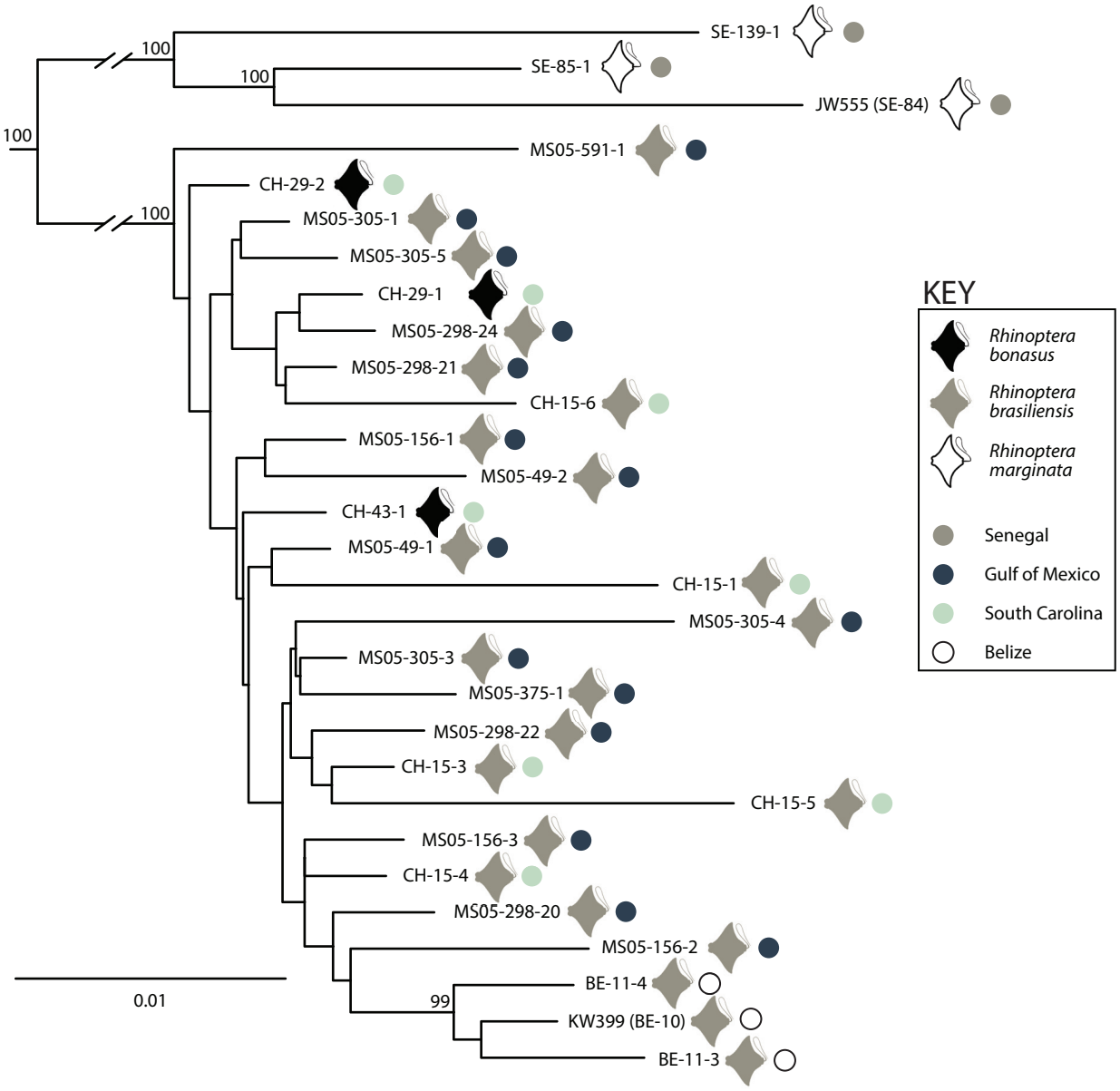


Figure 6. Tree resulting from the *RAxML* maximum likelihood phylogenetic analysis for *Rhinoptericola megacantha* Carvajal & Campbell, 1975. Tree is based on the complete single nucleotide polymorphism dataset and is rooted to maximize subtree balance. Taxon labels are unique lab specimen numbers comprising unique host accession numbers and specimen numbers, or unique specimen numbers followed in parentheses by host accession numbers, and are followed by graphic representations of host species and geographic locality. Nodal support is given as bootstrap (BS) values generated from 1,000 rapid BS replicates; BS values <50 are not shown. Scale bar indicates nucleotide substitutions per site.

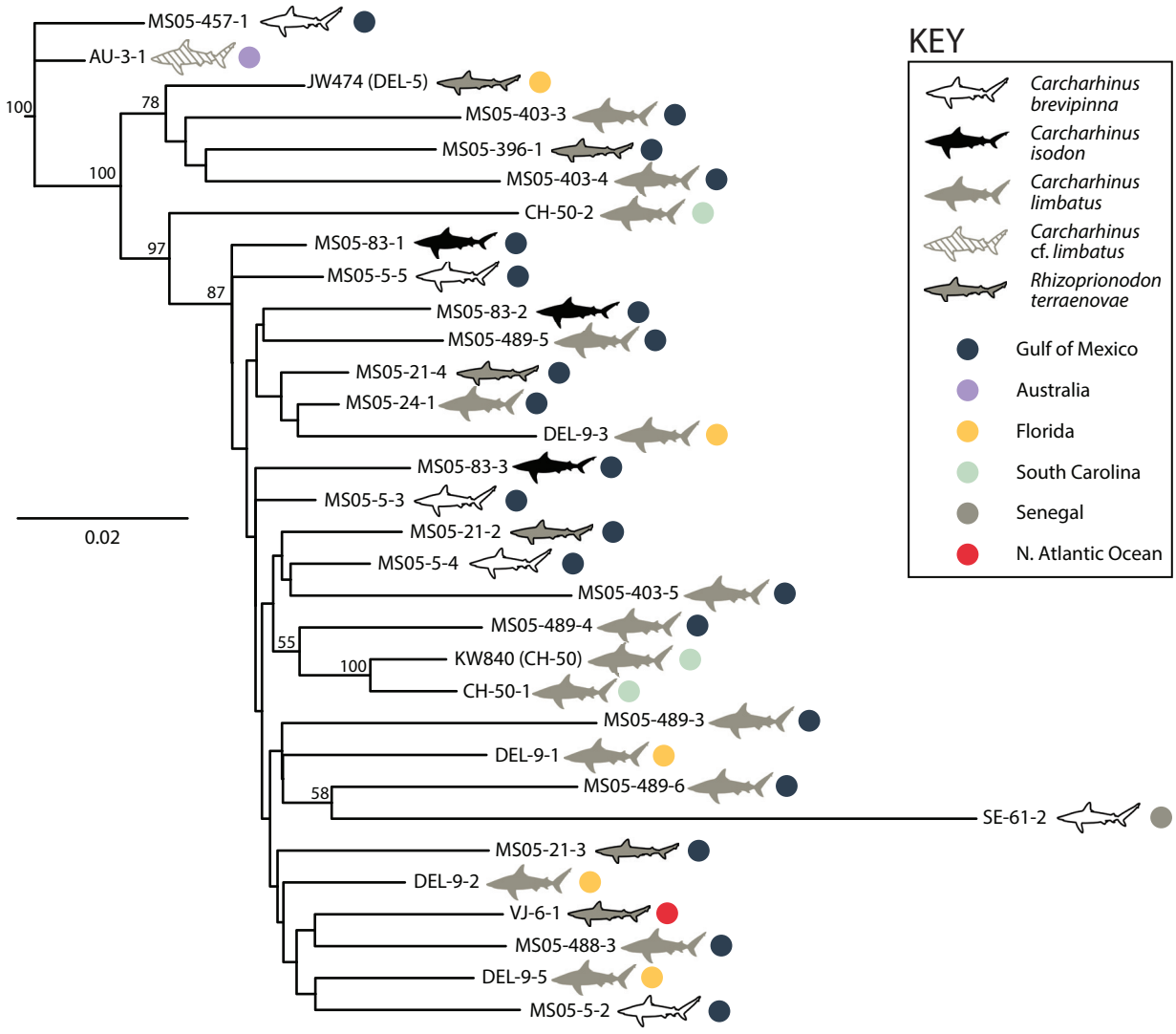


Figure 7. Tree resulting from the *RAxML* maximum likelihood phylogenetic analysis for *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931. Tree is based on the complete single nucleotide polymorphism dataset and is rooted to maximize subtree balance. Taxon labels are unique lab specimen numbers comprising unique host accession numbers and specimen numbers, or unique specimen numbers followed in parentheses by host accession numbers, and are followed by graphic representations of host species and geographic locality. Nodal support is given as bootstrap (BS) values generated from 1,000 rapid BS replicates; BS values <50 are not shown. Scale bar indicates nucleotide substitutions per site.

A

	Belize	South Carolina + Gulf of Mexico	Senegal
Belize (n=3)		0.0543	0.6082
South Carolina + Gulf of Mexico (n=23)			0.3906
Senegal (n=3)			

B

	R. brasiliensis	*R. bonasus*	*R. marginata*
R. brasiliensis (n=23)		0.0245	0.3823
R. bonasus (n=3)			0.5823
R. marginata (n=3)			

C

	Australia	South Carolina	Gulf of Mexico	Florida	Senegal	N. Atlantic
Australia (n=1)		——	——	——	——	——
South Carolina (n=3)			0.1402	0.0441	——	——
Florida (n=5)				0.0382	——	——
Gulf of Mexico (n=21)					——	——
Senegal (n=1)						——
N. Atlantic (n=1)						

D

	C. cf. limbatus	*C. limbatus*	*R. terraenovae*	*C. brevipinna*	*C. isodon*
C. cf. *limbatus* (n=1)		——	——	——	——
C. limbatus (n=16)			0.0446	0.0475	0.0463
R. terraenovae (n=6)				0.0894	0.1176
C. brevipinna (n=6)					0.1138
C. isodon (n=3)					

Figure 8. Corrected AMOVA F_{ST} values calculated using the *populations* module of *Stacks* for *Rhinoptericola megacantha* Carvajal & Campbell, 1975 grouped by geographic sampling locality (A) or host species (B), and for *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 grouped by geographic sampling locality (C) or host species (D). The number of tapeworm specimens in each subpopulation is specified in parentheses; F_{ST} values are not reported for comparisons involving subpopulations with only a single individual.

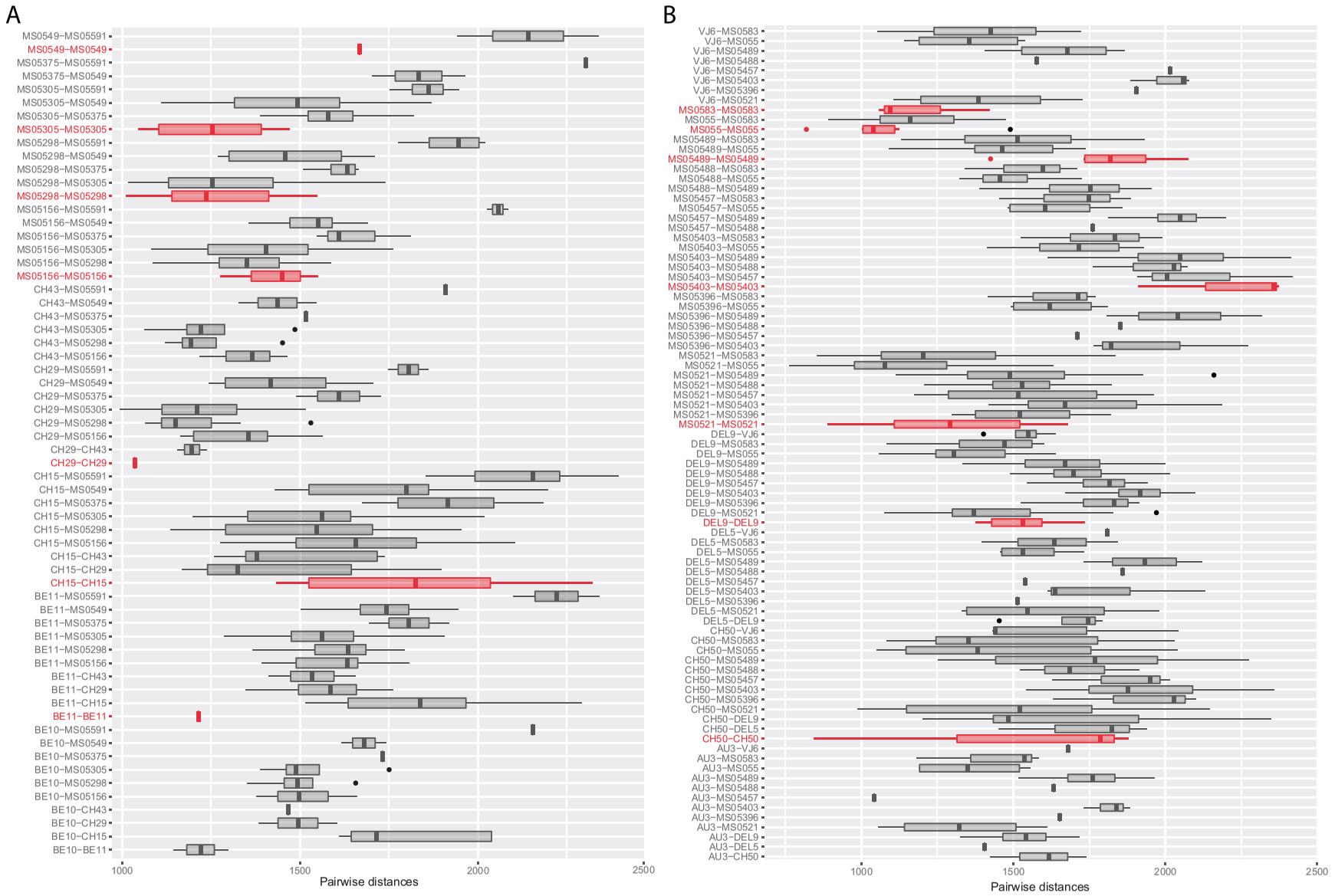
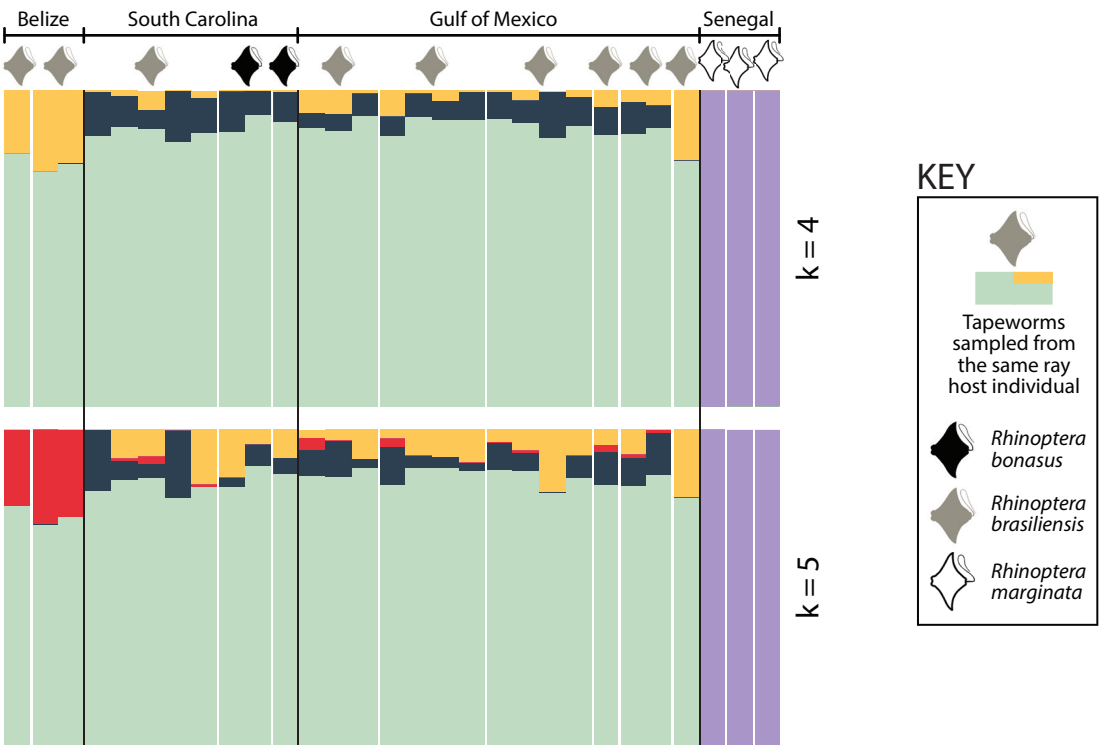
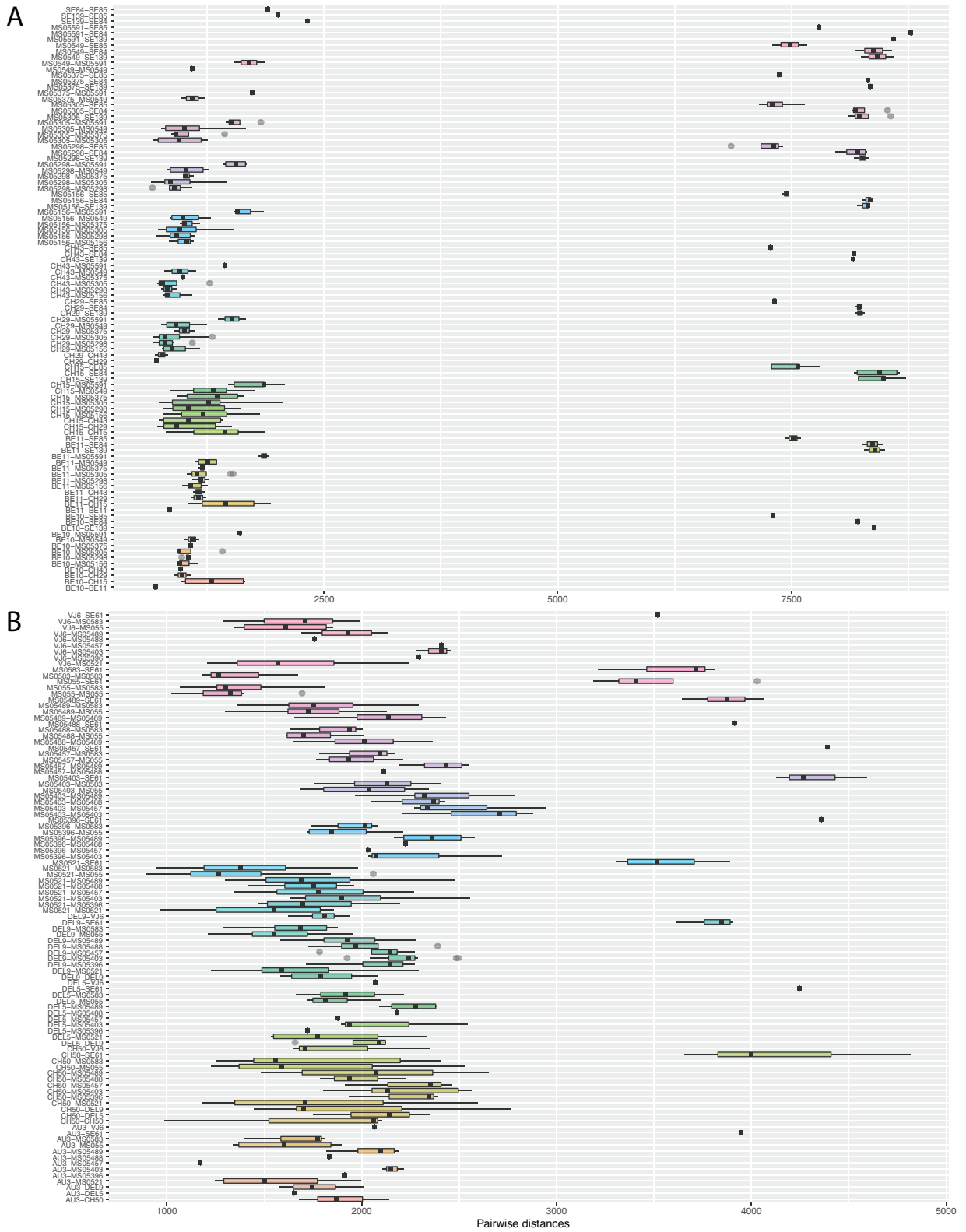


Figure 9. Plots of pairwise distances between tapeworms within an infracommunity and between pairs of infracommunities for *Rhinoptericola megacantha* Carvajal & Campbell, 1975 (A) and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 (B)

◄ **Figure 9 (continued) based on no-Senegal single nulceotide polymorphism datasets.** Comparisons within an infracommunity (i.e., between tapeworms from a single host individual) are highlighted in red.



Supplemental Figure 1. Alternate, less common binning patterns produced by *STRUCTURE* for k-values of 4 and 5 for *Rhinoptericola megacantha* Carvajal & Campbell, 1975 based on the complete single nucleotide polymorphism dataset. Black lines separate sampling localities and white lines separate host individuals within a sampling locality.



Supplemental Figure 2. Plots of pairwise distances between tapeworms within an infracommunity and between pairs of infracommunities for *Rhinoptericola megacantha* Carvajal & Campbell, 1975 (A) and *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 (B) based on complete single nucleotide polymorphism datasets.

Chapter 3

A first phylogenomic hypothesis of evolutionary relationships for the elasmobranch tapeworm order Trypanorhyncha

ABSTRACT

Since its recognition as an order in 1863, numerous studies have focused on clarifying relationships in the elasmobranch tapeworm order Trypanorhyncha. Most attempts to organize trypanorhynch diversity based on morphology emphasized features of the scolex—particularly those of the distinctive and characteristic hooked tentacles and their associated internal muscular and hydraulic network, both of which are unique to members of the order. More recent studies utilized sequence data to produce phylogenetic hypotheses for trypanorhynch interrelationships based on two to four genes each. These hypotheses have revealed troubling amounts of non-monophyly at the level of genus, family, and even superfamily, calling into question previous systems of classification based largely on scolex morphology. This study presents the first phylogenomic hypothesis for interrelationships in the order Trypanorhyncha. Data for approximately 400 gene regions for over 200 vouchered trypanorhynch ingroup specimens are analyzed in multispecies coalescent and concatenated maximum likelihood frameworks. The sampled specimens comprise at least 120 species of trypanorhynchs from 130 species of elasmobranch hosts collected from dozens of sampling localities around the world. All four trypanorhynch superfamilies, and 14 of the 17 recognized major clades, are represented, as are eight genera previously not included in phylogenetic analyses based on sequence data. Topologies produced by the two tree-building methods were to a great extent congruent. They support the monophyly of the two previously established suborders and the two superfamilies in the suborder Trypanoselachoida. They do not, however, support continued recognition the two trypanobatoid superfamilies as they are presently circumscribed. Novel genera and species were identified, as were specimens which represent the first adults reported from elasmobranchs for several species described based on larval specimens. Several genera were indicated to be non-monophyletic, and hypotheses of patterns of trait evolution and loss are proposed for three significant scolex structures. Ultimately, this first phylogenomic hypothesis provides a framework to inform needed revisionary and descriptive work in the Trypanorhyncha, toward the broader goal of aligning the classification of the order with its evolutionary history.

INTRODUCTION

Summary of the study of trypanorhynch interrelationships

The Trypanorhyncha is the most speciose of the orders whose members exclusively parasitize sharks and rays (i.e., elasmobranchs) as adults. The first reports of trypanorhynchs in the literature date back to 1684, when Francesco Redi, Italian biologist and "father of modern parasitology" (Amici, 2001), reported larval trypanorhynchs from the viscera of the herring smelt, *Argentina sphyraena* Linnaeus, 1758 (Redi, 1684; Wardle & McLeod, 1952). Over a century and a half later, the order Trypanorhyncha was formally erected to house tapeworms parasitic in elasmobranchs as adults with an attachment organ with four eversible hooked tentacles (Diesing, 1863). Much has been learned about the biology of trypanorhynch tapeworms in the over 330 years since the first reports of individual trypanorhynchs. In that time, hundreds of new taxa have been described, host associations for the order have been greatly expanded, potential life-cycles for individual species have been proposed, and the understanding of trypanorhynch morphology has been deepened and refined. With few exceptions, trypanorhynchs are united by their possession of an attachment organ with bothria (rather than bothridia) and a rhyncheal system with hooked tentacles, and by their possession of proglottids with a vagina ventral to the uterus (Campbell & Beveridge, 1994). Because of these compelling synapomorphies, the monophyly of the Trypanorhyncha has rarely been challenged. Despite the well-supported monophyly of the order itself, however, a robust hypothesis for evolutionary relationships within the order is lacking. This has in turn hamstrung efforts to establish a stable system of trypanorhynch classification. These knowledge gaps are certainly not born from lack of effort, however. Over the last century, the community of trypanorhynch tapeworm researchers has spent significant time and resources attempting to resolve trypanorhynch interrelationships.

These efforts most notably began in the early twentieth century with Guiart (1927), who established the two suborders Atheca and Thecaphora. Guiart's Atheca housed species with plerocercoid larval types (i.e., those without a blastocyst) while his Thecaphora housed those with plerocerus larval types (i.e., those with a blastocyst) (Guiart, 1927, 1931). Dollfus

(1942) then contributed arguably the first most comprehensive treatment of the order with his *Études critiques sur les tétrarhynques du Muséum de Paris*. At the start of this formative work, Dollfus explicitly warns that it "…is essentially a contribution to the precise knowledge of the external and internal morphological characteristics and the geographical distribution of the trypanorhynchs; it is neither a systematic work, nor a summary of all trypanorhynchs." (Dollfus, 1942, pg. 2; translated). Ironically, *Études* served as the most widely accepted system of trypanorhynch classification for the better part of five decades. Dollfus, it seems, disagreed with the decision of Guiart to recognize suborders based on the presence or absence of a blastocyst in larval worms, but nevertheless worked within that existing system to recognize four groups of families: Homéacanthes (Atheca), and Heteracantha Typica, Heteracantha Atypica, and Pécilacanthes (Thecaphora). These groups were distinguished based on the possession of two versus four bothria, and on features of the tentacular armature. Many of the terms Dollfus created to describe differences in armature types are still used today. Though *Études* does not include an explicit hypothesis of trypanorhynch interrelationships, it does include hypotheses of broad evolutionary trends within the order. For example, Dollfus suggested that solid hooks are plesiomorphic while hollow hooks are apomorphic; that hooks arranged in spirals are plesiomorphic while hooks arranged in paired rows or files are apomorphic; and that two-bothriate taxa evolved independently from four-bothriate ancestors multiple times.

In the decades following the publication of *Études* by Dollfus (1942), several authors contributed their own takes on trypanorhynch classification. For example, in 1952, Wardle & McLeod published *The Zoology of Tapeworms*, in which they recognized three families in the Thecaphora and seven families in the Atheca. Yamaguti (1959) similarly recognized three families of trypanorhynchs with a blastocyst as larvae, but eight (rather than seven) families of trypanorhynchs without a blastocyst. Joyeux and Baer (1961) did not subscribe to suborders in their classification, but they maintained the Homeacanthides, Heteracanthides, and Poecilacanthides sensu Dollfus (1942), recognizing three, five, and four families within them, respectively. In Schmidt's (1986) *Handbook of Tapeworm Identification*, he did recognize

suborders based on the presence versus absence of a blastocyst in larval worms, and also provided detailed keys to 14 families and numerous genera.

Though these various changes to the classification were proposed, the framework laid by Dollfus (1942) remained, to a great extent, unaltered until 1994. In their seminal work, Campbell and Beveridge (1994) abandoned the use of suborders and proposed the informal group names Homeacanthoidea, Heteracanthoidea, Otobothrioidea, and Poecilacanthoidea, which they referred to as superfamilies. Moreover, they redefined membership in each group and family, and erected both new families and new genera. In keeping with the precedent set by Dollfus, the classification of Campbell and Beveridge (1994) emphasized the importance of features of the tentacular armature, and applied pattern isometry to inform differences in these features. It also emphasized additional features of the scolex (such as the presence or absence of bothrial pits) as well as features of the terminal genitalia (such as the presence or absence of hermaphroditic ducts and accessory seminal vesicles). In addition to their revised classification, Campbell and Beveridge (1994) proposed plesiomorphic and apomorphic states for thirteen morphological characters and illustrated potential interrelationships among trypanorhynch families.

Palm (1995, 1997) provided the next revised system of classification for the order, and argued for a reduced emphasis on features of the tentacular armature in trypanorhynch classification. His three superfamilies—the Tentacularioidea Poche, 1926; Otobothrioidea Dollfus, 1942; and Eutetrarhynchoidea Guiart, 1927—were distinguished based on the possession of a scolex with versus without bothrial pits, and with versus without pre-bulbar organs. He further recognized 12 families based on the possession of a scolex with two versus four bothria, and the possession of a larval stage with versus without a blastocyst, and reassigned existing genera within these 12 families. Though the superfamilies of Palm (1995, 1997) were not circumscribed by features of the tentacular armature, Palm did place some emphasis on armature type for classification at lower taxonomic levels (e.g., hooks arranged in spirals or simple rows versus hooks arranged in rows with some degree of modification).

Beveridge et al. (1999) presented the first cladistic analysis for the order Trypanorhyncha.

They coded 44 morphological characters for 49 genera and produced an evolutionary hypothesis via parsimony analysis. Their analysis did not support a monophyletic superfamily Tentacularioidea, nor did it support the possession of bothrial pits as a synapomorphy for any group. Both of these results were at odds with the classification proposed by Palm (1995, 1997). These authors also placed the results of their analysis in the context of associations with elasmobranch hosts. For example, they noted that early-diverging genera tended to share both hooks arranged in spirals and an association with lamniform and/or carcharhiniform shark hosts, while other clades parasitized either primarily sharks or primarily rays, or species in both host groups. Finally, they noted that their character polarization was necessarily subjective because of the unknown position of the Trypanorhyncha relative to other orders of tapeworms, and they cited the challenges of coding characters for genera described insufficiently or based only on larval specimens.

Olson et al. (2001) presented one of the first hypotheses for interrelationships among tapeworm orders based on sequence data. Though their goal was not to untangle trypanorhynch interrelationships, but rather cestode interrelationships more broadly, they did include 13 species of trypanorhynchs representing 12 genera in their analysis of sequence data for the complete small ribosomal subunit (18S rDNA) and partial large ribosomal subunit (28S rDNA). Curiously, a monophyletic Trypanorhyncha was not supported in their analyses, but it is worth noting for the context of future studies based on sequence data that the two reciprocally monophyletic clades they recovered contained taxa hosted by primarily sharks and primarily rays, respectively.

Perhaps the most significant modern work on trypanorhynch systematics is the monograph of Palm (2004), titled *The Trypanorhyncha Diesing, 1863*. In addition to presenting a revised system of classification and a cladistic analysis for the order based on morphological characters, Palm provided keys to superfamilies, families, genera, and species, as well as an in-depth treatment of every species of trypanorhynch known at the time. Five superfamilies were recognized in the revised classification of Palm (2004): the Tentacularioidea, Otobothrioidea, and Eutetrarhynchoidea (sensu Palm, 1995, 1997), as well as the Gymnorhynchoidea Dollfus, 1935

and Lacistorhynchoidea Guiart, 1927. Characteristics of the scolex (including presence versus absence of bothrial pits and pre-bulbar organs, position of insertion of retractor muscles within the muscular bulbs, and armature type) all featured prominently in his key to superfamilies. Keys to lower-level taxa emphasized a wide array of additional features of the scolex and proglottids—including number of bothria, possession of solid versus hollow hooks, possession of a characteristic basal armature, and number of testes—as well as features of the larval tapeworm body. The cladistic analysis of Palm (2004) was based on 60 morphological characters coded for 66 trypanorhynch genera. Palm cited what he saw as notable differences between his analysis and that of Beveridge et al. (1999), including the development of novel terminology to describe armature patterns and the first inclusion of data on microthrix patterns. Palm recovered strong support for the Gymnorhynchoidea, Tentacularioidea, and Eutetrarhynchoidea as monophyletic, but recovered otobothrioids embedded within the Lacistorhynchoidea, leaving the later non-monophyletic.

The first true attempts to leverage sequence data to understand evolutionary relationships within the order Trypanorhyncha were those of Palm et al. (2009) and Olson et al. (2010). Both studies included data for complete 18S rDNA and partial 28S rDNA for taxa representing all five superfamilies recognized by Palm (2004). Palm et al. (2009) based their study on data for the 80 species of Olson et al. (2010) and further expanded their sampling to include approximately 20 additional species. In addition to sequence data, Olson et al. (2010) coded 45 morphological characters for the specimens they sequenced, and utilized ancestral character state reconstruction to inform the evolution of elasmobranch host associations. Palm et al. (2009) recovered support for two suborders: (Tentacularioidea + Eutetrarhynchoidea) and (Lacistorhynchoidea + Gymnorhynchoidea + Otobothrioidea), but found a paraphyletic Eutetrarhynchoidea with respect to the Tentacularioidea, and a paraphyletic Lacistorhynchoidea with respect to the Otobothrioidea. Olson et al. (2010) recovered support for the same two suborders as Palm et al. (2009), and the results of their ancestral character state reconstruction strongly supported these two suborders as ancestrally shark-hosted and ancestrally ray-hosted, respectively. They thus

proposed the subordinal names Trypanobatoida for (Tentacularioidea + Eutetrarhynchoidea) (primarily parasites of batoids as adults) and Trypanoselachoida for (Lacistorhynchoidea + Gymnorhynchoidea + Otobothrioidea) (primarily parasites of sharks as adults). Like Palm et al. (2009), Olson et al. (2010) found the Eutetrarhynchoidea to be paraphyletic with respect to the Tentacularioidea, but unlike Palm et al., found support for the Lacistorhynchoidea, Gymnorhynchoidea, and Otobothrioidea each as monophyletic superfamilies. Analyses in both studies recovered non-monophyletic genera in both suborders. Furthermore, both sets of authors commented on the highly homoplasious nature of characteristics of the tentacular armature and noted multiple independent evolutions of four-bothriate taxa. The mapping of coded morphological characters by Olson et al. (2010) also revealed other features previously popular for use in trypanorhynch classification to be homoplasious, including the presence of pre-bulbar organs, the presence of a uterine pore, and the body types of larval worms. Importantly, for both studies, most specimens from which molecular data (and morphological data in the case of Olson et al., 2010) were generated were vouchered as hologenophores sensu Pleijel et al. (2008).

The most recent comprehensive treatment of trypanorhynch classification and interrelationships to date is that of Beveridge et al. (2017). The phylogenetic analysis therein is based on a subset of previously generated sequences for complete 18S rDNA and partial 28S rDNA (largely those of Palm et al., 2009 and Olson et al., 2010), as well as novel sequence data for the mitochondrial genes 16S rDNA and COI. Additionally, vouchers of previously-sequenced specimens were reexamined and, in a few cases, their identification was corrected. These authors made clear that the purpose of their analysis was not to present a novel, comprehensive hypothesis for the evolutionary history of the order, but rather to summarize the most up-to-date understanding of trypanorhynch interrelationships at the time, and to highlight outstanding systematic issues. As the analysis of Beveridge et al. (2017) represents the most recent hypothesis of trypanorhynch evolution, a brief recapitulation of the state of trypanorhynch interrelationships and higher classification as summarized therein is warranted. The following synopsis—with some added context and appraisal—will allow for comparison of how the results

of this study align with or differ from the prior understanding of the evolutionary history of the order.

Current status of subordinal and superfamilial interrelationships

Beveridge et al. (2017) found support for the two suborders of Palm et al. (2009) and Olson et al. (2010), and recognized four superfamilies: the Gymnorhynchoidea and Lacistorhynchoidea (suborder Trypanoselachoida), and the Eutetrarhynchoidea and Tentacularioidea (suborder Trypanobatoida). Both trypanoselachoid superfamilies were found to be reciprocally monophyletic; however, within the Trypanobatoida, Beveridge et al. (2017)—like Palm et al. (2009) and Olson et al. (2010)—found the Eutetrarhynchoidea (represented therein by 16 of 26 valid genera) to be non-monophyletic with respect to a monophyletic Tentacularioidea. Specifically, their analysis supported four clades of "eutetrarhynchoids" ("Novel Clades 1–4") in a polytomy with the "eutetrarhynchoid" family Rhinoptericolidae Carvajal & Campbell, 1975, which itself was non-monophyletic with respect to the clade of tentacularioids. Despite once again recovering a non-monophyletic Eutetrarhynchoidea, the authors justified continual recognition of two separate trypanobatoid superfamilies based on stark morphological differences between their members: "Eutetrarhynchoids" are united by their possession of a pre-bulbar organ and gland cells within the bulbs (though the later are lacking in rhinoptericolids) while tentacularioids possess ventro-submarginal genital pores and a uterus that develops laterally from the distal end of the uterine duct.

Current status of the superfamily Gymnorhynchoidea

Beveridge et al. (2017) recovered strong support for a monophyletic superfamily Gymnorhynchoidea sister to the superfamily Lacistorhynchoidea. Within the Gymnorhynchoidea, they recognized the monophyletic families Gymnorhynchidae Dollfus, 1935 and Sphyriocephalidae Pintner, 1913 (previously considered a tentacularioid taxon by Palm, 2004; see below), as well as a clade containing the families Gilquiniidae Dollfus, 1942

and Aporhynchidae Poche, 1926. They recovered the family Rhopalothylacidae Guiart, 1935 in a polytomy with the Gymnorhynchidae and Sphyriocephalidae. The authors noted that gymnorhynchoids are united by their possession of a modified external seminal vesicle and a uterus deviated towards the genital pore, but it is not clear based on their summary whether all members of each family possess both, or even one, of these features. They also noted that while all gymnorhynchoids possess hollow hooks, they may possess either a heteroacanthous typical, a homeoacanthous, or a poeciloacanthous metabasal armature. With the exception of the sphyriocephalids, which are united in possessing retractor muscles that do not enter the bulbs, morphological synapomorphies uniting each of the gymnorhynchoid families are uncertain. It is worth noting that the Gymnorhynchoidea houses the only trypanorhynchs described to lack a rhyncheal system: species in the genera *Aporhynchus* Nybelin, 1819 and *Nakayacestus* Caira, Kuchta, & Desjardins, 2010 (family Aporhynchidae).

Current status of the superfamily Lacistorhynchoidea

The otobothrioids included in the analysis of Beveridge et al. (2017) were recovered deeply embedded within the superfamily Lacistorhynchoidea. Thus, these authors did not recognize the fifth superfamily of Palm (2004) (i.e., the Otobothrioidea), but rather recognized this group of taxa as the family Otobothriidae Dollfus, 1942 within the superfamily Lacistorhynchoidea. In addition to the Otobothriidae, Beveridge et al. (2017) found support for six clades of lacistorhynchoids: the families Hornelliellidae Yamaguti, 1954, Dasyrhynchidae Dollfus, 1942, and Lacistorhynchidae Guiart, 1927; a clade containing members of the families Grillotiidae Dollfus, 1969 and Pterobothriidae Pintner, 1931; a clade they referred to as Novel Clade 5; and the species *Ancipirhynchus afossalis* Schaeffner, Gasser & Beveridge, 2011 subtended on a long branch. *Ancipirhynchus afossalis* was recovered in a polytomy with the Dasyrhynchidae, Lacistorhynchidae, and Otobothriidae, and that clade of taxa was in turn recovered in a polytomy with the Grillotiidae + Pterobothriidae and a clade containing the Hornelliellidae sister to Novel Clade 5. According to Beveridge et al. (2017), lacistorhynchoids

are united in their possession of a hermaphroditic duct (excepting *A. afossalis*, which appears to lack this feature; Schaeffner et al., 2011).

The family Otobothriidae is defined by the possession of bothrial pits, and though the monophyly of the family was well-supported in their analysis, Beveridge et al. (2017) did not recover the nominal genus *Otobothrium* Linton, 1890 as monophyletic. The Hornelliellidae consists of only *Hornelliella annandalei* Hornell, 1912. The species is unique in its posession of a hermaphroditic vesicle and a pseudo-chainette element formed by the terminal hooks in each row of the metabasal armature. The Dasyrhynchidae was circumscribed to contain four genera, but a morphological synapomorphy uniting dashyrhynchids has yet to be proposed despite support for their monophyly based on molecular data. The three genera Beveridge et al. (2017) validated as members of the Lacistorhynchidae are united in their possession of a metabasal armature with nine hooks per principal row, with hooks 7(7') and 8(8') occupying a "satellite position" relative to hooks 1(1')–6(6'), and hooks 9(9') forming a median chainette. Beveridge et al. (2017) found species of the Grillotiidae and Pterobothriidae non-monophyletic with respect to one another; however, they maintained the use of both family names because the result was not strongly-supported, and because of what they cite as prominent differences in scolex morphology between species in the two families. Grillotiids are united by a suite of features of the scolex and aramture (i.e., two bothria, a heteroacanthous atypical metabasal aramture, and a band of hooks on the external tentacle surface), none of which are unique to the family. Contrastingly, pterobothriids are unique in their possession of four bothria borne on pedicels. The Novel Clade 5 of Beveridge et al. (2017) contains the genera *Paragrillotia* Dollfus, 1969 and *Pseudolacistorhynchus* Palm, 1995, but a compelling synapomorphy for the clade (beyond the association of both genera with orectolobiform sharks as adults) was not proposed. Finally, Beveridge et al. (2017) recovered *A. afossalis* on a long branch in a polytomy with three monophyletic families. Despite possessing a number of morphological features in common with lacistorhynchids, it was described as an otobothriid, but lacks the bothrial pits characteristic of the family (Schaeffner et al., 2011). As both the Lacistorhynchidae and Otobothriidae were

included in the polytomy with this species, its phylogenetic affinities within the superfamily were not addressed and remain uncertain.

Current status of the superfamily Tentacularioidea

The superfamily Tentacularioidea was recovered as monophyletic by Beveridge et al. (2017). The authors noted that members of this superfamily are united by two strong morphological synapomorphies (see above), but admitted that the presence of these features—both of which are observed in mature proglottids—remains to be verified for the substantial proportion of tentacularioids described based only on larval specimens. Within the superfamily, Beveridge et al. (2017) recognized two families: the Tentaculariidae Poche, 1926 and the Paranybeliniidae Schmidt, 1970. (Based on the results of their phylogenetic analysis, they considered the Sphyriocephalidae, which were included in the Tentacularioidea by Palm [2004], to represent a family within the Trypanoselachoida; see above.) Tentaculariids are united in possessing a genital pore in the anterior margin of the proglottid while paranybeliniids are united in their possession of bothria with tegumental grooves (Palm, 2004); however, as the single valid paranybelinid species was described based only on larvae, it is not known whether members of this family also possess a genital pore in the anterior margin of the proglottid. Beveridge et al. (2017) noted that several of the six valid tentacularioid genera appear to be non-monophyletic (a result also recovered by Olson et al., 2010) and cited a need for improved phylogenetic sampling within the group.

Current status of the superfamily "Eutetrarhynchoidea"

Within the Eutetrarhynchoidea, Novel Clade 1 of Beveridge et al. (2017) contained two species of *Prochristianella* Dollfus, 1946 and the genera *Progrillotia* Dollfus, 1946, *Oncomegas* Dollfus, 1929, and *Mecistobothrium* Heinz & Dailey, 1974. The authors cited the possession of two bothria, hollow hooks, and a metabasal armature with a distinct space between hooks 1(1') as a suite of unifying morphological features for the clade, but noted that this combination of

features is not unique to this group of species.

Their Novel Clade 2 contained the remaining two species of *Prochristianella* included in the analysis (rendering the genus non-monophyletic), as well as species in the genera *Parachristianella* Dollfus, 1946, *Trimacracanthus* Beveridge & Campbell, 1987, *Halysiorhynchus* Pintner, 1913, and *Trygonicola* Beveridge & Campbell, 1998. The possession of solid hooks and a metabasal armature with a distinct space between hooks 1(1') was said to unite the members of Novel Clade 2, but, as with Novel Clade 1, this combination of features is not unique to members of the clade. Additionally, species in Novel Clade 2 possess either two or four bothria, and either a heteroacanthous or poeciloacanthous metabasal armature, further complicating the identification of a morphological synapomorphy for the group.

Novel Clade 3 housed all but one species of *Dollfusiella* Campbell & Beveridge, 1994 included in the analysis, as well as species of *Tetrarhynchobothrium* Diesing, 1850, and *Paroncomegas* Campbell, Marques & Ivanov, 1999. In addition to being spread across two of the four novel "eutetrarhynchoid" clades, the species of *Dollfusiella* in Novel Clade 3 were recovered as non-monophyletic therein. Species in Novel Clade 3 are said to possess solid hooks and a metabasal armature without a distinct space between hooks 1(1'), but this combination of features is not unique to the group. Like Novel Clade 2, metabasal armature types vary within Novel Clade 3, with species possessing either a heteroacanthous or homeoacanthous armature.

The fourth and final novel "eutetrarhynchoid" clade of Beveridge et al. (2017)—Novel Clade 4—contained the single remaining species of *Dollfusiella* included in the analysis (i.e., *Dollfusiella geraschmidti* [Dollfus, 1974] Beveridge & Jones, 2000) and the genus *Hispidorhynchus* Schaeffner & Beveridge, 2012. The morphological features uniting the members of Novel Clade 4 are the possession of hollow hooks and a heteroacanthous metabasal armature without a distinct space between hooks 1(1'). Like Novel Clades 1, 2, and 3, however, this combination of features is not unique to the members of this group.

In addition to their four novel clades, Beveridge et al. (2017) recognized a paraphyletic Rhinoptericolidae inclusive of the genera *Rhinoptericola* Carvajal & Campbell, 1975 and

Nataliella Palm, 2010 as belonging to the Eutetrarhychoidea. They cited the possession of solid hooks and four bothria as morphological features uniting rhinoptericolids, but this combination of features is not unique to the family. It is worth noting that in their recent treatment of the family, Herzog and Jensen (2022) recovered a monophyletic Rhinoptericolidae inclusive of an expanded *Rhinoptericola* and the monotypic *Nataliella* in an analysis based on partial 28S rRNA data. They also confirmed the family as sister to a monophyletic Tentacularioidea, thus rendering the superfamily "Eutetrarhynchoidea" non-monophyletic, as was recovered in previous studies. Herzog and Jensen (2022) proposed a suite of unique proglottid features as potential synapomorphies for the family but were unable to verify the presence of these features in *Nataliella marcelli* Palm, 2010 because the species was described based only on larval worms.

In summary, the "Eutetrarhynchoidea" is today recognized as a non-monophyletic assemblage of five clades whose species parasitize mainly rays as adults. The monophyly of each of the five clades is well-supported based on molecular data, but morphological features that may unite their members have yet to be identified. Additionally, the relatively speciose "eutetrarhynchoid" genera *Prochristianella* (20 species) and *Dollfusiella* (31 species) (Caira et al., 2021) are known to be non-monophyletic. The possession of solid versus hollow hooks is supported as a potential feature that distinguishes the two clades of species of *Prochristianella*, but morphologic features that distinguish clades of species of *Dollfusiella* remain elusive.

Aims of the present study

Despite the generation of several phylogenetic hypotheses based on sequence data for the order Trypanorhyncha, to date, no formal attempt has been made—beyond the establishment of suborders—to revise trypanorhynch classification as informed by these hypotheses (Beveridge et al., 2017). This is perhaps largely because the results of phylogenetic analyses (all based on fewer than 5,000 bp of sequence data) have not indicated a clear way forward. Instead, they have revealed, at every taxonomic level, significant conflict between molecular signal and previous classifications based largely on scolex morphology. For example, they support the monophyly of

groups for which morphological synapomorphies are unknown, and have rendered a number of seemingly morphologically cohesive (or at least easily recognizable) genera non-monophyletic. Revising higher classification in the order has become the metaphorical "third rail" of trypanorhynch systematics, and lack of a stable system of classification has fettered the global community of trypanorhynch researchers describing new taxa. Resolution of the non-monophyly found at all levels of trypanorhynch higher classification is sorely needed to bring classification for the order in line with its evolutionary history, and to provide a stable systematic framework within which to describe novel trypanorhynch diversity.

Revising all levels of higher classification across the order Trypanorhyncha to prioritize monophyly and morphological diagnosability is the ultimate goal. If Herzog & Jensen's (2022) recent revision of the family Rhinoptericolidae is any indication, achieving this goal will require comprehensive global sampling (to inform species boundaries and how variable shared morphological features can be within a group), as well as the careful reexamination of many type specimens, and a combination of data from light microscopy, scanning electron microscopy, histological sectioning, and DNA sequencing. The first sequence data for type species will also be needed to inform revisionary work for several non-monophyletic genera. In short, achieving this goal is long-term, and falls beyond the scope of any single study. Rather, the revision of trypanorhynch classification will be best based tackled in stages, on a group-by-group basis. However, our present hypothesis of trypanorhynch evolution (sensu Palm et al., 2009; Olson et al., 2010; Beveridge et al., 2017) is not fully resolved, making it challenging to assess which taxa even constitute well-supported clades in need of systematic revision.

The goal of this study is thus to produce the first phylogenomic hypothesis for the order Trypanorhyncha. Next-generation sequence data for over 400 nuclear gene regions were generated via a target enrichment approach for over 200 vouchered trypanorhynch tapeworm specimens representing all recognized trypanorhynch superfamilies, and from 130 species of elasmobranch hosts. Ortholog data were analyzed using multispecies coalescent and concatenated tree building approaches. These data greatly expand on prior phylogenetic

sampling efforts for the order, both in terms of the number of loci sequenced and the proportion of trypanorhynch diversity represented, particularly within the superfamily Eutetrarhynchoidea. This first phylogenomic dataset for trypanorhynchs informs a more well-resolved evolutionary hypothesis for the Trypanorhyncha. It is further underpinned by morphologically informative voucher specimens to aid in diagnosing clades. Ultimately, it can serve as a framework to guide future targeted taxonomic and systematic revisionary work within the order.

MATERIALS AND METHODS

Trypanorhynch sampling and outgroup selection

In total, 211 trypanorhynch ingroup specimens and eight outgroup specimens representing three additional non-acetabulate tapeworm orders (i.e., six diphyllideans, one bothriocephalidean, and one diphyllobothriidean) were included in this study (see Table 1). Sequence data for all 211 trypanorhynchs and for six of the seven outgroup specimens were generated de novo through collaborative efforts by researchers at the University of Kansas (KU; Kirsten Jensen and Kaylee Herzog) and University of Connecticut (UConn; Janine Caira, Jill Wegrzyn, Elizabeth Jockusch, and Hannah Ralicki), described in detail below. For the single outgroup specimen representing the order Diphyllobothriidea (i.e., *Schistocephalus solidus* [Müller, 1776] Steenstrup, 1857), sequence data were bioinformatically pulled by Hannah Ralicki (UConn) from a publicly available draft genome of *S. solidus* downloaded from WormBase ParaSite (Howe et al., 2017) (BioProject accession no. PRJEB527; Parasite Genomic Group at the Wellcome Trust Sanger Institute and Martin Kalbe [Max-Planck Institute for Evolutionary Biology]).

Vouchering and DNA extraction for tapeworm specimens

Tapeworms selected for this study and fixed in 95% EtOH were first photographed; most were photographed at KU using a Lumenera INFINITY3-6UR 6.0 megapixel USB 3 microscopy camera (Teledyne Lumenera, Ottawa, ON, Canada) attached to a Leica MZ16

dissecting microscope (Leica Microsystems, Buffalo Grove, IL, USA), and some were photographed at UConn. Each tapeworm specimen was assigned a unique identification code at the time of photo voucher generation. Tapeworms were then cut using microscissors to separate portions of the body to be used for DNA extraction from those to be prepared as whole-mounted hologenophores sensu Pleijel et al. (2008) for identification purposes. In most cases, the entire scolex and at least one proglottid were prepared as a hologenophore, but for some smaller specimens (e.g., <2 mm in total length) only a scolex or part of a scolex was prepared as a hologenophore to ensure sufficient input tissue for DNA extraction. Whole-mounted hologenophores were prepared at KU following the methods outlined in Herzog and Jensen (2018). For tens of specimens, DNA extraction was completed at KU following the methods outlined in Herzog and Jensen (2022), and extracted DNA was sent to UConn for sequencing. For the remaining specimens, the portion(s) of the body designated for DNA extraction were placed in individual labeled 1.5 mL flip-top tubes in 95% EtOH and sent to UConn for both DNA extraction and sequencing. Unique identification codes, higher classifications, and species-level identifications for each tapeworm included in this study are provided in Table 1.

Elasmobranch host identification

Tapeworms were recovered from elasmobranch hosts whose identity was verified using NADH2 sequence data. These data were generated by H. Ralicki (UConn) from liver tissue preserved in 95% EtOH following the methods outlined in Fernando et al. (2019). Unique identification codes, higher classifications, and species-level identifications for all host specimens from which tapeworms sequenced as part of this study were collected are given in Table 1. The unique identification code of each host specimen (e.g., NT-96) can be entered online in the Global Cestode Database (tapewormdb.uconn.edu) to access additional information about the host, including size, sex, collection locality, etc. Ray taxonomy follows Naylor et al. (2012) and Last et al. (2016), and shark taxonomy follows Naylor et al. (2012) and Ebert et al. (2021).

Target enrichment and dataset assembly

Target enrichment libraries were prepared for each specimen by H. Ralicki (UConn) using extracted DNA fragmented in 1X TE buffer and a NEBNext® Ultra™ II DNA Library Preparation Kit for Illumina® (New England BioLabs, Ipswich, MA, USA) and modified protocol. Baits used for target enrichment of exonic gene regions were designed by H. Ralicki and E. Jockusch (UConn) using a myBaits Custom Target Capture Kit (Arbor Biosciences) with 120bp RNA probes. Bait design was informed by analysis using *OrthoFinder* (Emms & Kelly, 2015, 2019) of newly-generated draft genomes for 15 species of elasmobranch tapeworms (including two species of trypanorhynchs representing both suborders and one species of diphyllidean; see Table 1). Indexed target enrichment libraries were sequenced on an Illumina® HiSeq4000 for paired-end 150 bp reads at the University of California, Berkeley DNA Sequencing Facility (Berkeley, CA, USA). Following demultiplexing, adaptor trimming, and quality filtering, H. Ralicki utilized the *HybPiper* pipeline (Johnson et al., 2016), which relies on the *SPAdes* assembly algorithm (Bankevich et al., 2012), to generate assemblies for orthologous exonic gene regions for each of the specimens sequenced. Homologous regions were bioinformatically pulled from the genome of *S. solidus* downloaded from WormBase ParaSite (see above) and included in assemblies. *HybPiper* assemblies were saved in a FASTA-formatted file; these data were the starting point for phylogenetic analyses conducted as part of this study, described in detail below.

Multiple sequence alignment: *MACSE*

The *MACSE* v. 2.04 pipeline (i.e., Multiple Alignment of Coding SEquences Accounting for Frameshifts and Stop Codons) (Ranwez et al., 2011) was utilized to generate multiple sequence alignments (MSAs) from each *HybPiper* assembly. The MSA software *MAFFT* v. 7.305 (Katoh & Standley, 2013) was utilized within the *MACSE* pipeline. Two iterations of *MACSE* were run, with the first iteration creating initial nucleotide and amino acid MSAs for each assembly and the second iteration refining the MSAs created in the first iteration. The

--*min_percent_NT_at_ends 1* flag was specified in both iterations to remove gappy alignment extremities, and the --*no_prefiltering* and --*min_seqToKeepSite 4* flags were specified in the second iteration, ensuring final versions of each MSA contained data for at least four sequences at each site. Both the nucleotide and the amino acid MSAs output by *MACSE* were saved as FASTA-formatted files; only nucleotide MSAs were used for downstream phylogenetic analyses. For instances in which an orthologous gene region was represented by more than a single copy, alignments were visualized in *Geneious Prime* v. 2019.1.3 (https://www.geneious.com), and one copy was selected for downstream analysis based on alignment length, taxon inclusion, and mean coverage.

Tree-building: *RAxML-NG*, *TreeShrink*, and *ASTRAL-III*

The multispecies coalescent-based tree-building approach implemented in *ASTRAL-III* (i.e., Accurate Species Tree ALgorithm) requires as input a gene tree for each locus of interest. Thus, a gene tree was generated for each of the final refined single-copy nucleotide MSAs produced by *MACSE* using the maximum likelihood optimality criterion-based software *RAxML-NG* v. 0.9.0 (Kozlov et al., 2019). For *RAxML-NG* analyses, a GTR+FO+G4m model of sequence evolution was specified and the --*brlen scaled* and --*tree rand{4}* flags were employed. The most likely gene trees output by *RAxML-NG* were then concatenated into a single file.

There is little evidence to support the notion that *ASTRAL* analyses are significantly improved by the removal of entire gene trees from datasets; rather, they appear to be more sensitive to the inclusion of gene trees with tips that demonstrate unexpected branching patterns (Mirarab, 2019). Therefore, *TreeShrink* v. 1.3.9 (Mai & Mirarab, 2017, 2018) was used to remove taxa with abnormally long branch lengths from gene trees prior to analysis with *ASTRAL-III*. A species tree was then generated from the set of "shrunken" gene trees using *ASTRAL-III* v. 5.7.3 (Sayyari & Mirarab, 2016; Zhang et al., 2018). The --*branch-annotate 3* flag was specified to *ASTRAL-III* to ensure output of nodal support values as the posterior probability of the main resolution, or "local PP" sensu Sayyari and Mirarab (2016). The *ASTRAL-III* species tree was

post-processed in *R* v. 4.0.3 (R Core Team [2020]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.Rproject.org/) via *RStudio* v. 1.3.1093 (RStudio Team [2020]. RStudio: Integrated Development for R. RStudio. PBC, Boston, MA, USA. http://www.rstudio.com/) using the packages *ape* v. 5.62 (Paradis & Schliep, 2019) and *treeio* v. 1.14.4 (Wang et al., 2020). Post-processing included rerooting the tree at the clade of outgroup taxa, recoding terminal branch lengths from "NA" (as output by *ASTRAL-III*) to "0.1" to improve ease of interpretation, and renaming tip labels according to updated species identifications. Nodes in the species tree output from *R* were then rotated in *FigTree* v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) to enforce a ladderized topology.

Tree-building: *IQ-TREE*

In addition to the multispecies coalescent-based tree-building approach implemented in *ASTRAL-III*, a multi-gene concatenation approach was implemented in *IQ-TREE*. First, the final refined single-copy nucleotide MSAs produced by *MACSE* were concatenated into a single partitioned alignment using the *Perl* script *catfasta2phyml* (catfasta2phyml.pl; https://github.com/nylander/catfasta2phyml) in *Perl* v. 5.18.4 (Wall et al., 1994). This script also generated a partition file. Then, *IQ-TREE* v. 2.1.3 (Nguyen et al., 2015) was run for the partitioned concatenated alignment. The *-m TESTMERGE* flag was specified to *IQ-TREE* to use *ModelFinder* to evaluate the ideal partition-merging scheme and the preferred evolutionary model for each merged partition based on Bayesian information criterion (BIC) scores (Chernomor et al., 2016; Kalyaanamoorthy et al., 2017). Additionally, the *-B 1000* flag was specified to perform 1,000 ultrafast bootstrap replicates (UFBS) (Hoang et al., 2018). The resulting most likely tree with nodal support values represented by ultrafast bootstrap replicates output by *IQ-TREE* was then post-processed using *treeio* v. 1.14.4 in *R* v. 4.0.3 *RStudio* v. 1.3.1093 to rename tip labels according to updated species identifications. Nodes in the species tree output from *R* were then rotated in *FigTree* v. 1.4.4 to enforce a ladderized topology.

RESULTS

As stated in the Introduction, revising higher classification in the order Trypanorhyncha to prioritize monophyly and morphological diagnosability is the ultimate goal. This study is but a first step toward achieving that goal. As such, the Results (and Discussion) section herein will focus on individual groups and clades to: (1) describe their interrelationships and host associations; (2) highlight taxonomic issues; and (3) provide hypotheses for morphological features that may serve useful in diagnosing groups or clades. This study can thus serve as a framework moving forward to guide future descriptive or revisionary work that will necessarily be approached on a group-by-group basis.

Every attempt was made to identify each specimen to the level of species. In the cases where confident species-level identifications were not possible, "cf." was used to indicate tenuous identifications (e.g., *Parachristianella* cf. *baverstocki*) and "sp." was used to indicate uncertain assignment within a genus (e.g., *Parachristianella* sp. 1). Resulting non-monophyly necessitated the adoption of additional naming conventions. For genera recovered as paraphyletic, clades were assigned numbers (e.g., *Dollfusiella* 1, *Dollfusiella* 2, etc.), and paraphyletic groups are indicated with quotation marks (e.g., superfamily "Eutetrarhynchoidea"). In the few cases where identification was more complicated (usually involving novel taxa), specimens were assigned temporary names to indicate morphological similarity to known taxa (e.g., *Prochristianella* 1 n. sp. cf. *Oncomegas*).

Trypanorhynch and elasmobranch host diversity represented

Sequencing efforts for the order Trypanorhyncha at the generic level prior to this study versus herein are summarized in Table 2. The analyses for this study included representatives of 40 of the 82 valid trypanorhynch genera. The first sequence data were generated for representatives of the two trypanoselachoid genera *Nakayacestus* and *Pristiorhynchus* Schaeffner & Beveridge, 2013, and for the following six trypanobatoid genera: *Fellicocestus* Campbell & Beveridge, 2006; *Hemionchos* Campbell & Beveridge, 2006; *Poecilorhynchus* Schaeffner &

Beveridge, 2013; *Pseudochristianella* Campbell & Beveridge, 1990; *Trigonoglobium* Dollfus, 1929, and *Zygorhynchus* Beveridge & Campbell, 1988 (see Table 2).

The 211 trypanorhynch ingroup specimens conservatively represent 123 species (see Table 1). They include 46 trypanoselachoids and 165 trypanobatoids, and represent all four of the superfamilies and 14 of the 17 families and novel clades recognized by Beveridge et al. (2017) (see Table 1), as well as three additional clades of trypanobatoid taxa (see Table 1, Fig. 2). The three clades recognized by Beveridge et al. (2017) for which at least one representative was not included in this analysis are the monotypic gymnorhynchoid family Rhopalothylacidae, the monotypic lacistorhynchoid family Hornelliellidae, and the lacistorhynchoid family Dasyrhynchidae, comprising four genera. In several instances, a species of trypanorhynch is represented in analyses by multiple specimens collected from different species of hosts and/or geographic regions (see Table 1).

The trypanorhynchs sequenced herein were recovered from 169 specimens of 130 species of elasmobranch hosts representing 18 families and four orders of rays, and 16 families and five orders of sharks (see Table 1). Prior to this study, no trypanorhynchs had been sequenced from hosts in nine of the 18 families of rays and six of the 16 families of sharks represented herein (Palm et al., 2009; Olson et al., 2010; Beveridge et al., 2021; Oosthuizen et al., 2021). For the rays, these families are: Gymnuridae Fowler, 1934; Mobulidae Gill, 1893; Myliobatidae Bonaparte, 1838; Narcinidae Gill, 1862; Platyrhinidae Jordan, 1923; Plesiobatidae Nishida, 1990; Rhinobatidae Müller & Henle, 1837; Urotrygonidae McEachran, Dunn & Miyake, 1996; and Zanobatidae Fowler, 1928. For the sharks, these families are: Alopiidae Bonaparte, 1838; Hemigaleidae Compagno, 1984; Hemiscylliidae Gill, 1862; Parascylliidae Gill, 1862; Pentanchidae Smith, 1912; and Squatinidae Bonaparte, 1838 (see Table 1).

In total, the 169 host specimens from which trypanorhynchs were recovered were collected from all the one world's oceans and major water bodies except for the Arctic and Southern Oceans. Collection localities include the following 28 countries and geographic regions: Australia (CM03, JO, NT), Baja (BJ), Belize (BE), Borneo (BO), South Carolina, USA

(CH), Chile (WMO), Costa Rica (CRP), Ecuador (EC), Egypt (EG), Florida, USA (DEL, GC), the Gulf of Mexico, USA (MS05), India (IN), Jamaica (JM), Japan (JN), Kalimantan (KA), Long Island Sound, USA (AI, DR, LIT, RI), Mexico (MX), Mozambique (MZ), Rhode Island, USA (RDM), Senegal (SE), the Solomon Islands (SO), South Africa (AF), Sri Lanka (SL), Taiwan (TW), the Azores, Portugal (AZ), the Falkland Islands (FA), Trinidad & Tobago (TT14), and Vietnam (VN) (see Table 1).

Multiple sequence alignment

The *MACSE* pipeline generated 517 nucleotide MSAs from the input *Hybpiper* assemblies. Of these, 363 MSAs represented orthologous gene regions present in only a single copy and 154 MSAs represented orthologous gene regions represented by more than one copy (i.e., 39 multi-copy orthologs represented by 2–6 copies each). After one copy was selected to represent each of the 39 multi-copy orthogroups, 402 MSAs remained for downstream phylogenetic analysis. These 402 MSAs—representing 448,194 bp in total—ranged in length from 105–3,732 bp (mean=1,115 bp ± 565.48 bp; median=987 bp). Guanine-cytocine content ("GC-content") for each MSA ranged from 41–70% (mean=52% ± 5.67%; median=52%). Each alignment contained 4–216 of the 219 taxa (mean=167 ± 53.96; median=195) and the number of alignments a given taxon was present in ranged from 4–390 (mean=307 ± 66.10; median=311).

Phylogenetic trees and interrelationships: *RAxML-NG*, *TreeShrink*, and *ASTRAL-III*

Gene trees were successfully constructed using *RAxML-NG* for all 402 final *MACSE* nucleotide MSAs. Alignments used to build gene trees were composed of 0–80% gaps (mean=41% ± 17.46%; median=44%) and 15–71% invariant sites (mean=33% ± 6.78%; median=33%). *TreeShrink* removed less than 1% of all tips across all 402 gene trees produced by *RAxML-NG*. Individual taxa were removed from 0–50% (mean=1.26% ± 4.61%; median=0.32%) of the gene trees in which they had been present prior to filtering. Plots illustrating the proportion of gene trees from which individual taxa were removed are provided in Figure 1. The topology

of the species tree produced by *ASTRAL-III* containing all clades of ingroup and outgroup taxa is presented in Figure 2. Detailed subtrees are presented for specific clades in Figure 3 (suborder Trypanoselachoida), Figure 4 (superfamily Tentacularioidea + subset of members of the superfamily "Eutetrarhynchoidea"), and Figure 5 (majority of members of the superfamily "Eutetrarhynchoidea"). The *ASTRAL-III* analysis recovered strong support (i.e., localPP=1) for the reciprocally monophyletic suborders Trypanobatoida and Trypanoselachoida (Figs. 2–4).

Superfamily Gymnorhynchoidea

A monophyletic Gymnorhynchoidea sensu Beveridge et al. (2017) was recovered (Figs. 2, 3), represented by 12 specimens (see Table 1). Within the superfamily, the families Gymnorhynchidae and Sphyriocephalidae were reciprocally monophyletic with strong support, sister to a well-supported clade containing members of the families Gilquiniidae and Aporhynchidae (Fig. 3). Within this later clade, neither the Gilquiniidae nor the Aporhynchidae were recovered as monophyletic, nor were the genera *Aporhynchus* and *Deanicola* Beveridge, 1990; thus, the three aporhynchids included in this analysis which lack a rhyncheal system (i.e., two species of *Aporhynchus* and one species of *Nakayacestus*) were not recovered as one another's closest relatives (Fig. 3).

Superfamily Lacistorhynchoidea

A monophyleyic Lacistorhynchoidea sensu Beveridge et al. (2017) was recovered for the 34 lacistorhynchoids included herein (localPP=1), sister to the superfamily Gymnorhynchoidea (Figs. 2, 3). Within the Lacistorhynchoidea, the family Otobothriidae was recovered as monophyletic with strong support, sister to a clade containing to the remaining lacistorhynchoids (Fig. 3). The genus *Otobothrium* was not recovered as monophyletic. The single specimen of *Symbothriorhynchus* Yamaguti, 1952 was found sister to a species of *Otobothrium* with strong support, and a clade containing the genera *Proemotobothrium* Beveridge & Campbell, 2001, *Pristiorhynchus,* and *Fossobothrium* Beveridge & Campbell, 2005 was found sister to one

of the two subclades of species of *Otobothrium*, albeit with low support (localPP=0.44) (Fig. 3). The remaining lacistorhynchoids were recovered in two sister clades. These comprise a clade containing the genus *Ancipirhynchus* Schaeffner, Gasser & Beveridge, 2011 sister to the monophyletic Lacistorhynchidae, with that clade in turm sister to Novel Clade 5 sensu Beveridge et al. (2017) and the Grillotiidae + Pterobothriidae (Fig. 3). Though Novel Clade 5 is well-supported, the genera *Paragrillotia* and *Pseudolacistorhynchus* therein were non-monophyletic with respect to one another (Fig. 3).

Superfamily Tentacularioidea and allied "eutetrarhynchoid" taxa

The six tentacularioids included herein were recovered as one another's closest relatives, supporting a monophyletic Tentacularioidea sensu Beveridge et al. (2017) (Figs. 2, 4). However, this superfamily was recovered deeply embedded with the superfamily "Eutetrarhynchoidea," rendering the later paraphyletic (Figs. 2, 4, 5). Within the Tentacularioidea, the genus *Nybelinia* Poche, 1926 was non-monophyletic with respect to the genus *Kotorella* Euzet & Radujkovic, 1989 (Fig. 2). The clade of tentacularioids was recovered with strong support as sister to a clade containing the "eutetrarhynchoid" genera *Fellicocestus* (one specimen) and *Hemionchos* (four specimens) (Figs. 2, 4), both of which represent genera sequenced for the first time herein. The clade containing the tentacularioids + *Fellicocestus* and *Hemionchos* was found sister to a monophyletic Rhinoptericolidae (10 specimens) (Figs. 2, 4). The two species of *Rhinoptericola* represented by more than a single specimen were found to be monophyletic, though both clades contained a weakly-supported sister relationship between two of three specimens (Fig. 4). The clade containing the tentacularioids + *Fellicocestus* and *Hemionchos* + the rhinpotericolids was recovered as sister to Novel Clade 4 sensu Beveridge et al. (2017) (represented herein by three specimens of the genus *Hispidorhynchus*) with a localPP=1 (Figs. 2, 4, 5). That clade in turn was found to be sister to a clade containing the remaining eutetrarhynchoids (Figs. 2, 4, 5).

Remaining members of the superfamily "Eutetrarhynchoidea"

Within the largest clade of "eutetrarhynchoid" taxa (142 specimens), support was found for Novel Clades 1, 2, and 3 sensu Beveridge et al. (2017), as well as two additional clades not recognized by Beveridge et al. (2017) (Figs. 2, 5). The newly-sequenced genera *Trigonoglobium* (solid metabasal hooks) and *Poecilorhynchus* (hollow metabasal hooks) were recovered in a clade sister to the remaining "eutetrarhynchoid" taxa (localPP=0.99; Figs. 2, 5). This later clade consisted of three subclades: (1) Novel Clade 1 sensu Beveridge et al. (2017), found to contain taxa with both solid and hollow metabasal hooks; (2) Novel Clade 2 sensu Beveridge et al. (2017), members of which possess solid metabasal hooks (Figs. 2, 5); and (3) a clade of taxa with hollow metabasal hooks consisting of Novel Clade 3 sensu Beveridge et al. (2017) sister to a clade containing some species of *Prochristianella* and *Mecistobothrium*. Novel Clade 2 was recovered as sister to the subclade containing Novel Clade 3 and the additional clade of taxa, with that clade in turn sister to Novel Clade 1; however, the internodes supporting the relationships between these subclades are small and have low local posterior probabilities (i.e., 0.26–0.69; Figs. 2, 5). Thus, these subclades essentially form a polytomy, sister to *Trigonoglobium* + *Poecilorhynchus*.

Within Novel Clade 1, *Oncomegas* Dollfus, 1929 (hollow metabasal hooks) was recovered as monophyletic, sister to a clade containing: (1) a representative of a new genus (solid metabasal hooks); (2) all but one specimen of *Mecistobothrium* Heinz & Dailey, 1974 (i.e., *Mecistobothrium* 1; hollow metabasal hooks); (3) species of *Dollfusiella* hosted by aetobatid eagle rays (i.e., *Dollfusiella* 5; solid metabasal hooks); and (4) a clade of species of *Prochristianella* (i.e., *Prochristianella* 1; solid metabasal hooks) (Fig. 5).

Novel Clade 2 was composed of a well-supported clade of species of *Parachristianella* (localPP=1), within which the reciprocally monophyletic genera *Trygonicola* and *Halysiorhynchus* were recovered as deeply embedded, rendering *Parachristianella* non-monophyletic with respect to these two genera (Fig. 5). Sister to the clade of *Parachristianella, Trygonicola*, and *Halysiorhynchus* was a clade of representatives of the

newly-sequenced genus *Pseudochristianella*, with this largest clade of taxa in turn sister to a clade containing *Prochristianella clarkeae* Campbell & Beveridge, 2002 and *Prochristianella* cf. *clarkeae* (Fig. 5) (i.e., *Prochristianella* 2).

Novel Clade 3 contained the majority of specimens of *Dollfusiella* included herein (i.e., *Dollfusiella* 1, 2, 3, and 4). *Dollfusiella* 1 was non-monophyletic with respect to *Paroncomegas myliobatidis* (Woodland, 1934) Campbell, Marques & Ivanov, 1999 and the genus *Tetrarhynchobothrium* (Fig. 5). The clade of species of *Dollfusiella* 1, *Tetrarhynchobothrium*, and *Paroncomegas* was sister to a clade containing the remaining species of *Dollfusiella* (i.e., *Dollfusiella* 2, 3, and 4) and representatives of the genus *Zygorhynchus*—another genus newly sequenced herein (Fig. 5). Novel Clade 3 was recovered as sister to a clade containing the remaining species of *Prochristianella* (i.e., *Prochristianella* 3) (Fig. 5). *Prochristianella* 3 was non-monophyletic with respect to *Mecistobothrium penaeus* (Feigenbaum, 1975) Schmidt, 1986 (i.e., *Mecistobothrium* 2) (Fig. 5).

Phylogenetic trees and interrelationships: *IQ-TREE*

The concatenated alignment used for the *IQ-TREE* analysis contained 448,194 sites, including 243,455 parsimony-informative sites, 58,607 singleton sites, and 146,132 invariable sites. The model and partition-finder analyses within *IQ-TREE* reduced the number of partitions in the concatenated alignment from 402 (i.e., one partition per exon) to 64. Of the 64 partitions, five contained a single exon, and the remaining 79 partitions contained 2–18 exons (mean=7 ± 4.49; median=6). The following models of sequence evolution were assigned to the 64 partitions: GTR+F+I+G4 (44 partitions), TIM2+F+I+G4 (10 partitions), SYM+I+G4 (5 partitions), TVM+F+I+G4 (3 partitions), TN+F+I+G4 (1 partition), and TIM3e+I+G4 (1 partition).

The resulting most likely species tree produced by *IQ-TREE* is presented in Figure 6. Its topology is essentially identical to that of the species tree produced by *ASTRAL-III*, with four notable differences. Firstly, the two analyses differed significantly in their placements of the clade containing species of *Prochristianella* 3 and *Mecistobothrium* 2 *penaeus*.

ASTRAL-III recovered the clade of these six specimens sister Novel Clade 3 with strong support (localPP=0.99; Fig. 5) whereas *IQ-TREE* recovered this clade of specimens sister to Novel Clade 2 with low support (UFBS=64; Fig. 6). Secondly, the two methods differed in their placement of the three specimens of *Ancipirhynchus* included herein. Whereas *ASTRAL-III* recovered *Ancipirhynchus* sister to the Lacistorhynchidae (localPP=0.99; Fig. 3), *IQ-TREE* recovered the genus sister to a clade containing all other non-otobothriid lacistorhynchoids (UFBS=0.98; Fig. 6). Thirdly, the clade containing *Proemotobothrium*, *Pristiorhynchus*, and *Fossobothrium* was recovered among species of *Otobothrium* and the single species of *Symbothriorhynchus* in the *ASTRAL-III* analysis (localPP=0.44; Fig. 3) but was recovered sister to a clade of all species of *Otobothrium* (inclusive of *Symbothriorhynchus*) in the *IQ-TREE* analysis (UFBS=100; Fig. 6). Fourthly, a specimen of *Parachristianella* (i.e., KW923) was recovered on a short branch amongst its congeners in the *ASTRAL-III* topology (localPP=0.42; Fig. 5) but was recovered on an abnormally long branch sister to all other members of Novel Clade 2 in the *IQ-TREE* topology (UFBS=47; Fig. 6).

The *IQ-TREE* topology recovered the bothriocephalid *Marsipometra hastata* (Linton, 1897) Cooper, 1917 as the sister taxon to the order Trypanorhyncha with strong support (UFBS=100; Fig. 6). The diphyllobothriid *S. solidus* was further recovered by *IQ-TREE* as sister to *Marsipometra* + Trypanorhyncha (UFBS=100), with diphyllideans sister to the remaining ingroup and outgroup specimens (Fig. 6).

DISCUSSION

Sampling efforts and trypanorhynch diversity represented

Except for data for *Schistocephalus solidus* which were downloaded from a publicly available genome, all data analyzed herein were generated de novo, and they comprise the largest phylogenetic dataset for trypanorhynchs to date by a substantial margin. The 211 ingroup specimens included the first representatives sequenced for eight trypanorhynch genera previously unrepresented in phylogenetic analyses. These include a genus each in the "Aporhynchidae"

and Otobothriidea (suborder Trypanoselachoida) and six genera in the "Eutetrarhynchoidea" (suborder Trypanobatoida) (see Table 2). As a result of this expanded sampling, representatives of 64 of the 82 valid trypanorhynch genera (~78%) have now been included in at least one phylogenetic analysis based on sequence data to date (Palm et al., 2009; Olson et al., 2010; Schaeffner et al., 2011; Beveridge et al., 2017, 2021) (see Table 2). Those that remain unrepresented include ten genera of lacistorhynchoids, four genera of tentacularioids, and four genera of "eutetrarhynchoids" (see Table 2).

Twenty-three genera (representing all four superfamilies) were included in previous studies but were not included here (Palm et al., 2009; Olson et al., 2010; Beveridge et al., 2017, 2021; Oosthuizen et al., 2021) (see Table 2). It is of course possible that the lack of representation for these genera has significantly affected the results of the analyses herein. However, representatives of all but three of the 17 major clades or families recognized by Beveridge et al. (2017) were included here, plus representatives of three additional clades (see Fig. 2). Thus, perhaps only those genera belonging to the three major clades or families not represented here have the potential to have significantly impacted the results. These include the four genera in the lacistorhynchoid family Dasyrhynchidae, and *Pintneriella* Yamaguti, 1934 and *Hornelliella* Yamaguti, 1954—the sole genera in the gymnorhynchoid family Rhopalothylacidae and lacistorhynchoid family Hornelliellidae, respectively.

Sampling efforts herein were focused particularly on expanding "eutetrarhynchoid" representation with the goal of clarifying interrelationships within this most confused of superfamilies. These efforts are reflected in the uneven specimen-level representation with respect to superfamilies, as 159 of 211 ingroup specimens (~75%) were "eutetrarhynchoids" (see Table 1). This focus is further reflected in the uneven distribution of the eight newly-sequenced genera included herein, as six of eight were "eutetrarhynchoid" genera (see Table 2).

Interrelationships and host associations at the subordinal level

Strong support was recovered for the suborders Trypanoselachoida and Trypanobatoida,

as erected by Olson et al. (2010). The reciprocal monophyly of these two clades was well supported in both the multispecies coalescent and concatenated maximum likelihood-based analyses (see Figs. 2, 6), and the taxa recovered as belonging to each suborder herein align with the results of previous ordinal-level studies (Palm et al., 2009; Olson et al., 2010; Beveridge et al., 2017). Morphological synapomorphies that may diagnose each suborder beyond broad host associations, however, are still unclear and require more detailed investigation.

The two trypanorhynch suborders were named to reflect the primary definitive host associations of their members (Olson et al., 2010). Exceptions, however, are known for each suborder, and were also recovered in this study. Herein, ten of 46 trypanoselachoids were collected from rays. *Grillotia patagonica* Menoret & Ivanov, 2012 (KW102) was recovered from the graytail skate, *Bathyraja griseocauda* (Norman, 1937); *Fossobothrium perplexum* Beveridge & Campbell, 2005 (KW502) and *Pristiorhynchus palmi* Schaeffner & Beveridge, 2013 (KW307) were recovered from the knifetooth sawfish, *Anoxypristis cuspidata* (Latham, 1794); *Proemotobothrium* sp. (KW668) was recovered from the leopard whipray, *Himantura leoparda* Manjaji-Matsumoto & Last, 2008; and all six specimens of *Pterobothrium* were recovered from butterfly rays (Gymnuridae) or stingrays (Dasyatidae Jordan, 1888) (see Table 1). These findings are not unusual: *Grillotia patagonica* was originally described from an arhynchobatid skate (Menoret & Ivanov, 2012), the knifetooth sawfish is the type host of both *F. perplexum* and *P. palmi* (see Beveridge & Campbell, 2005; Schaeffner & Beveridge, 2013c), and species of *Proemotobothrium* and *Pterobothrium* are known to parasitize rays (e.g., Palm, 2004; Herzog & Jensen, unpublished data). These findings do, however, slightly expand the suite of ray hosts from which trypanoselachoids are known.

Fifteen of the 165 trypanobatoids were recovered from shark hosts. These host records are in line with known host associations for these taxa. The four species of *Nybelinia* included here and collected from sharks corroborates the fact that species of tentaculariids for which definitive host associations are known more commonly parasitize carcharhiniform and lamniform sharks than they do rays (Palm, 2004; Caira et al., 2021). Species of *Zygorhynchus*

have been described from nurse sharks in addition to dasyatids (Palm, 2004), and the single species of *Poecilorhynchus—Poecilorhynchus perplexus* Schaeffner & Beveridge, 2013—was described from the brownbanded bambooshark, *Chiloscyllium punctatum* Müller & Henle, 1838 (Schaeffner & Beveridge, 2013b) (see Table 1). Additionally, though its type host is a ray, *Trigonoglobium spinuliferum* (Southwell, 1911) Dollfus, 1929 had been previously reported from the snaggletooth shark, *Hemipristis elongata* (Klunzinger, 1871) (Beveridge, 1990; see Table 1).

The four remaining trypanobatoid specimens recovered from shark hosts constitute more surprising records. These include: *Mecistobothrium* 2 *penaeus* (JW497) from the bonnethead, *Sphyrna tiburo* (Linnaeus, 1758); a specimen of *Parachristianella* (JW788) from the common smooth-hound, *Mustelus mustelus* Linnaeus, 1758; and two species of *Dollfusiella* 1 from the Taiwan saddled carpetshark, *Cirrhoscyllium formosanum* Teng, 1959, and the smalleye pygmy shark, *Squaliolus aliae* Teng, 1959, respectively (see Table 1). *Mecistobothrium* 2 *penaeus* was described as *Renibulbus penaeus* Feigenbaum, 1975 based on larval worms collected from prawns from coastal Florida, USA. It was transfered to the genus *Mecistobothrium* by Palm (2004) and is here referred to as "*Mecistobothrium* 2 *penaeus"* to reflect the non-monophyly of the genus *Mecistobothrium* (see Figs. 5, 6). This specimen (i.e., JW497) from a bonnethead from the Gulf of Mexico is the first record of an adult worm for the species, and the first report of its definitive elasmobranch host associations. It also represents the first report of a species of *Mecistobothrium* from a shark host, further supporting that this specimen (which did not group with its congeners in this study; see Figs. 5, 6), in fact, represents a distinct genus (see further discussion of the non-monophyly of *Mecistobothrium*, below). The specimen of *Parachristianella* recovered from the common smooth-hound is similarly intriguing. Species of *Parachristianella* are known to demonstrate relaxed host specificity and have been reported from a broad diversity of rays (e.g., Palm, 2004); however, to the best of my knowledge, this specimen (i.e., JW788) represents the first report of a species of *Parachristianella* from a shark. Like *Parachristianella,* species of *Dollfusiella* have been previously reported from species in numerous families of batoids, but they have also been reported from a number of sharks (e.g.,

the nurse shark and tawny nurse shark, the sandbar shark, the blacktip reef shark, the broadnose sevengill shark, and several species of houndsharks; Palm, 2004). *Dollfusiella* 1 sp. 2 (i.e., KW220) from the Taiwan saddled carpetshark appears to constitute the first report of a species of *Dollfusiella* from a parascylliid, and *Dollfusiella* 1 sp. 4 (i.e., JW400) collected from the smalleye pygmy shark appears to represent the first report of a species of *Dollfusiella* from any species of squaliform shark (see Table 1).

Validity and interrelationships of superfamilies

Within the suborder Trypanoselachoida, the superfamilies Gymnorhynchoidea and Lacistorhynchoidea sensu Beveridge et al. (2017) were recovered as monophyletic with strong support in both analyses (Figs. 2, 3, 6). As summarized by Beveridge et al. (2017), the presence of a hermaphroditic duct represents a compelling morphological synapomorphy to unite the lacistorhynchoids, but a morphological feature that unites gymnorhynchoids is lacking at present. Whether all taxa in the superfamily possess a modified external seminal vesicle and/or a uterus deviated towards the genital pore remains to be verified. Answering this question should be prioritized in future studies of members of the group.

Within the suborder Trypanobatoida, the superfamily Tentacularioidea was recovered as monophyletic, but deeply embedded within a paraphyletic superfamily "Eutetrarhynchoidea" in both analyses (Figs. 2, 4, 6). This result was similarly recovered by all three of the previous ordinal-level studies of trypanorhynch interrelationships based on sequence data (Palm et al., 2009; Olson et al., 2010; Beveridge et al., 2017). The inclusion here of the first sequence data for six previously unrepresented "eutetrarhynchoid" genera further supports the non-monophyly of the superfamily, as *Hemionchos* and *Fellicocestus* were recovered as sister to the tentacularioids with strong support (Figs. 2, 4, 6). The results of this study thus support either (1) revising the circumscription of each superfamily, or (2) abandoning the use of trypanobatoid superfamilies entirely.

If the first solution is preferred, the results herein suggest a superfamily inclusive of

the Tentaculariidae, *Hemionchos* and *Fellicocestus,* the Rhinoptericolidae, and Novel Clade 4, and a superfamily consisting of the remaining "eutetrarhynchoids" (see Figs. 2, 4, 6). At present, however, morphological features (or combinations of features) that would diagnose each of these two clades are lacking. A ventro-submarginal genital pore is a potential candidate to unite the former clade, as this feature is currently used to diagnose the Tentacularioidea. Species of *Hemionchos, Fellicocestus, Hispidorhynchus*, and *Rhinoptericola* were not described as possessing ventro-submarginal genital pores (Campbell & Beveridge, 2006; Schaeffner & Beveridge, 2012; Herzog & Jensen, 2022), but it is possible that, without the context of a close relationship to tentacularioids, this feature was overlooked by previous authors.

Alternatively, superfamilies may not prove an accurate reflection of the history of trypanobatoid evolution and should perhaps be done away with in this suborder entirely. Regardless, available data overwhelmingly support abandoning use of the superfamily Tentacularioidea and superfamily "Eutetrarhynchoidea" sensu Beveridge et al. (2017) in favor of generic names or family or clade names, only, until evidence for monophyletic, morphologically diagnosable trypanobatoid superfamilies can be demonstrated.

Superfamily Gymnorhynchoidea

Analyses herein included representatives of four of the five recognized families in the superfamily Gymnorhynchoidea sensu Beveridge et al. (2017). Unfortunately, no specimens of *Pintneriella* (the sole representative of the Rhopalothylacidae) were available for inclusion. The single specimen of *Pintneriella* included in past analyses was collected from the smalltooth sand tiger, *Odontaspis ferox* (Risso, 1810), from Indonesia (Palm et al., 2009). This host species belongs to a family of sharks (i.e., the Odontaspididae Müller & Henle, 1839) from which no trypanorhynchs were available for this study (see Table 1). Nevertheless, the familial interrelationships recovered herein for the clades of included gymnorhynchoids did not differ from those of previous studies (Palm et al., 2009; Olson et al., 2010; Beveridge et al., 2017).

As in previous studies, members of the "Gilquiniidae" and "Aporhynchidae" were here

found to form a well-supported clade (see Fig. 3). Unlike previous studies, however, analyses herein included the first sequence data generated for a species of *Nakayacestus*. With this addition of data for *Nakayacestus*, at least one representative of all 14 valid gymnorhynchoid genera has now been sequenced (see Table 2). Species of *Nakayacestus*—like species of *Aporhynchus*—lack a rhyncheal system, and together, these two genera are known to comprise the "Aporhynchidae" (see Caira et al., 2010). Both the *ASTRAL-III* and *IQ-TREE* analyses recovered the gilquiniid genus *Deanicola* and the aporhynchid genus *Aporhynchus* as non-monophyletic, rendering the two families non-monophyletic relative to one another (see Fig. 3). Interrelationships within this clade were strongly supported in both analyses (i.e., localPP=1 or UFBS=100). The past analyses of Palm et al. (2009) and Olson et al. (2010) recovered a single specimen of *Aporhynchus* sister to all gilquiniid taxa, but Beveridge et al. (2017) appear likely to have recovered a result similar to that of the present study. They represent the gilquiniid genera + *Aporhynchus* as a single collapsed clade referred to as "Gilquiniidae + Aporhynchidae" (see their fig. 3) and state "Genera of the Gilquiniidae included in this clade were *Aporhynchus* (currently placed in a separate family Aporhynchidae)…" (Beveridge et al., 2017; pg. 415). This result is intriguing because it suggests multiple independent losses of the rhyncheal system over the course of trypanorhynch evolution. If this result receives support in the future, it is likely the two families will be synonymized, with Aporhynchidae being the older name. Beveridge et al. (2017) state that members of the Gilquiniidae (presumably sensu stricto) are united in possessing an accessory seminal vesicle. Accessory seminal vesicles have also been described for both valid species of *Aporhynchus* (see Noever et al., 2010) and for both valid species of *Nakayacestus* (see Caira et al., 2010). The presence of an accessory seminal vesicle thus seems a compelling morphological synapomorphy to unite species in both families.

Superfamily Lacistorhynchoidea

A monophyletic superfamily Lacistorhynchoidea was strongly supported by both phylogenetic analyses herein, consisting of the same clades or families as recovered in previous

studies (see Figs. 2, 3, 6) (Palm et al., 2009; Olson et al., 2010; Beveridge et al., 2017) excepting representatives of the Dasyrhynchidae and Hornelliellidae, which were missing herein. Like Olson et al. (2010), but unlike Palm et al. (2009) and Beveridge et al. (2017), strong support was recovered for a monophyletic Otobothriidae sister to the remaining lacistorhynchoids (see Figs. 3, 6), evoking support for the fifth trypanorhynch superfamily (i.e., the Otobothrioidea) sensu Palm (2004). The Otobothrioidea was relegated to family-level status by Beveridge et al. (2017) based on the results of their analysis. Otobothriids are distinguished from all other trypanorhynchs by their possession of bothrial pits. This morphological synapomorphy—in addition to strong evidence for its monophyly—easily supports the clade as unique from other lacistorhynchoids, but whether it should be recognized at the familial or superfamilial level remains to be determined.

Within the clade of otobothriids, *ASTRAL-III* and *IQ-TREE* both recovered three subclades: (1) *Proemotobothrium* sister to *Pristiorhynchus* + *Fossobothrium*; (2) four species of *Otobothrium* (as *Otobothrium* 1 herein); and (3) four species of *Otobothrium* (as *Otobothrium* 2 herein) non-monophyletic with respect to *Symbothriorhynchus uranoscopi* Yamaguti, 1952. The two tree-building methods recovered different interrelationships among these subclades, however. The *ASTRAL-III* topology supports *Proemotobothrium* + (*Pristiorhynchus* + *Fossobothrium*) as embedded among the two clades of species of *Otobothrium* while the *IQ-TREE* topology supports this clade sister to a monophyletic *Otobothrium* (inclusive of *S. uranoscopi*). Relationships among the three subclades were poorly supported in both analyses, however; thus, the analyses herein, unfortunately, do not serve to clarify whether the genus *Otobothrium* is monophyletic. It is worth noting that Palm et al. (2009) and Beveridge et al. (2017) did not recover a monophyletic *Otobothrium* in their analyses (based on five and 12 representatives of the genus, respectively), but Olson et al. (2010) (based on three representatives) did. Though this study included the first sequence data for the sole species of *Pristiorhynchus* (see Tables 1, 2), only five of 12 valid otobothriid genera were represented herein compared to eight of 12 represented in previous analyses (see Table 2; Palm et al., 2009;

Olson et al., 2010; Beveridge et al., 2017). Future studies should focus on the generation of sequence data for the as-of-yet unrepresented otobothriid genera to help clarify interrelationships among this suite of morphologically distinctive taxa, and to inform the monophyly of the genus *Otobothrium*.

Regardless of whether *Otobothrium* is fated to be split or expanded, it seems clear based on the results of the phylogenetic analyses herein that *S. uranoscopi* will likely be synonymized under the generic name *Otobothrium* (Figs. 3, 6). The genus *Symbothriorhynchus* houses two valid species, both of which were described based on larval specimens collected from stargazer fishes (family Uranoscopidae Jordan & Evermann, 1898). The specimen of *S. uranoscopi* included herein collected from a species of great hammerhead shark (i.e., JW499; see Table 1) thus constitutes the first report of an adult of this species, and the first record of its definitive host associations. (Adults of its lone congener *Symbothriorhynchus tigaminacantha* Palm, 2004 have also been reported from hammerheads [Schaffner & Beveridge, 2013b].) The genus *Symbothriorhynchus* was distinguished from other genera of trypanorhynch tapeworms with bothrial pits by having comparatively "very small" (Palm, 2004; pg. 395) bothrial pits and a "characteristic" (Palm, 2004; pg. 395) metabasal armature composed of rows of five principal hooks with a single hook per intercalary row, and a longitudinal band of three or four hooks on the external tentacle surface (Yamaguti, 1952; Palm, 2004). Members of the genus *Otobothrium* are known to possess intercalary hooks and principal rows of 6–7 hooks "often overrunning on the external surface" (Palm, 2004; pg. 405). Examination of the hologenophore of JW499 revealed that what Yamaguti (1952) interpreted as a band of hooks is likely the continuation of the principal and intercalary rows on the external surface (though this observation of course needs to be confirmed in type specimens of both species of *Symbothriorhynchus*, as well as with additional new material prepared for scanning electron microscopy). If this observation is supported, the only difference between *Symbothriorhynchus* and *Otobothrium* would be the size of the bothrial pits, which does not seem a compelling-enough morphological feature to maintain the two as separate genera—especially with strong evidence from sequence data suggesting they

are congeners (see Figs. 3, 6 herein and fig. 1 of Palm et al. [2009]).

In addition to a monophyletic Otobothriidae, the *ASTRAL-III* and *IQ-TREE* analyses indicated support for the Lacistorhynchidae, Grillotiidae, and Pterobothriidae, and Novel Clade 5 sensu Beveridge et al. (2017) (see Figs. 3, 6). The genera within these clades were recovered as monophyletic, except for *Paragrillotia* and *Pseudolacistorhynchus* (Novel Clade 5), which appear to be non-monophyletic with respect to one another. This is the first study to include sequence data for more than a single representative of each genus (Palm et al., 2009; Olson et al., 2010; Beveridge et al., 2017). The genera *Paragrillotia* and *Pseudolacistorhynchus* are remarkably similar in terms of their overall scolex and proglottid morphologies; essentially the only feature differentiating the two genera is that species of *Paragrillotia* are reported to possess a poecilacanthous multiatypical metabasal armature while species of *Pseudolacistorhynchus* are reported to possess a heteroacanthous typical metabasal armature (Palm, 1995; Beveridge & Justine, 2007). Whether they, in fact, represent separate genera is a topic worthy of future investigation. *Paragrillotia* sp. collected from the blacktip shark, *Carcharhinus limbatus* (Müller & Henle, 1839) (i.e., KW843; see Table 1) appears to represent the first report of a member of this clade from a non-orectolobiform definitive host (Beveridge et al., 2017).

In addition to the lacistorhynchoid clades discussed above, both the *ASTRAL-III* and *IQ-TREE* analyses recovered the three specimens of *Ancipirhynchus* included here as a monophyletic group (Figs. 3, 6). They differed, however, in their placement of the genus among the other lacistorhynchoid clades: *ASTRAL-III* recovered it as sister to the Lacistorhynchidae while *IQ-TREE* recovered it as sister to a clade containing all lacistorhynchoids except the otobothriids—both with strong nodal support. Beveridge et al. (2017) similarly recovered their representative of the genus in an uncertain position within the superfamily. The genus houses a single species, *Ancipirhynchus afossalis*, which was described as the only member of the family Otobothriidae to lack bothrial pits (hence its specific epithet; Schaeffner et al., 2011). Though the results of the analyses herein do not appear to resolve the placement of *Ancipirhynchus* within the superfamily Lacistorhynchoidea, it seems clear that it is not a member of the Otobothriidae.

In the description of *A. fossalis*, the authors note "striking" (Schaeffner et al., 2011; pg. 13) morphological similarities between the metabasal armature of the species and that of species of the family Lacistorhynchidae, but they additionally cite several morphological differences between *A. fossalis* and lacistorhynchids (Schaeffner et al., 2011). Determining whether *A. fossalis* should be considered a member of the Lacistorhynchidae, or whether it constitutes a new monotypic lacistorhynchoid family, will require additional investigation.

Superfamily Tentacularioidea and allied "eutetrarhynchoid" taxa

Both phylogenetic analyses conducted herein recovered two well-supported clades of trypanobatoids: one clade contained the tentacullariids, the genera *Hemionchos* and *Fellicocestus*, the rhinoptericolids, and the genus *Hispidorhynchus*, and the second clade contained the remaining taxa circumscribed as eutetrathynchoids (see Figs. 2, 6). Whether these clades could (or should) be considered superfamilies is discussed, above. Sampling of tentacularioids herein was sparse compared to their diversity. Presently, the superfamily consists of 57 species of tentaculariids in seven genera and two species of paranybeliniids in two genera (Beveridge et al., 2017; Palm et al., 2019, 2020), but only six specimens representing two genera and (at most) five species of tentaculariids were included in this study (see Table 1). Beyond the fact that these six specimens were recovered in a well-supported clade in both analyses, the limited sampling herein does not allow for much discussion of interrelationships within the superfamily. It is worth noting that previous studies based on sequence data found most of the tentacularioid genera to be non-monophyletic (Palm et al., 2009; Olson et al., 2010). It is clear that untangling interrelationships among these taxa requires further study.

Hemionchos and *Fellicocestus* are two of the six "eutetrarhynchoid" genera for which representatives were sequenced for the first time herein (see Tables 1, 2). That these genera were recovered in a clade with tentacularioids and rhinoptericolids is not surprising: tentacularioids are the only trypanobatoids that lack both pre-bulbar organs and gland cells in the bulbs (Palm, 2004), and rhinoptericolids and species of *Hemionchos, Fellicocestus*, and *Mobulocestus*

Campbell & Beveridge, 2006 are the only trypanorhynchs that possess pre-bulbar organs but lack gland cells in the bulbs (Campbell & Beveridge, 2006; Herzog & Jensen, 2022). Species of *Hemionchos* are further united with rhinoptericolids in their shared possession of dorsoventrally flattened billhooks with mucronate tips (Campbell & Beveridge, 2006; Herzog & Jensen, 2022). Thus, finding species of *Hemionchos* and *Fellicocestus* allied with tentacularioids and rhinoptericolids is not surprising. Finding species of *Hemionchos* and *Fellicocestus* more closely related to tentacularioids than to species of *Rhinoptericola* (with which they share more scolex features) was, however, unexpected. A morphological feature shared by members of this clade (beyond a shared loss of gland cells in the bulbs) is presently lacking. Examination of hologenophores for specimens of *Hispidorhynchus*—which were recovered as sister to the clade of tentacularioids, *Hemionchos* and *Fellicocestus*, and rhinoptericolids (see Figs. 2, 4, 5, 6)—confirmed that species in this genus indeed possesses both pre-bulbar organs and gland cells in the bulbs, as described by Schaeffner and Beveridge (2012). If this topology is correct, it suggests a loss of gland cells in the bulbs along the internode leading to the clade containing rhinoptericolids, *Hemionchos* and *Fellicocestus*, and tentacularioids, and a loss of pre-bulbar organs in tentacularioids (see Fig. 4).

To date, the sole species of *Fellicocestus* and the three species of *Mobulocestus* are the only elasmobranch tapeworms not known from the stomach or intestine of their definitive hosts. Rather, *Fellicocestus mobulae* Campbell & Beveridge, 2006 inhabits the gall bladder the giant devilray, *Mobula mobular* (Bonnaterre, 1788), and the three species of *Mobulocestus* are known from the nephridial system or cloaca of the smoothtail mobula, *Mobula thurstoni* (Lloyd, 1908) (Campbell & Beveridge, 2006). *Mobulocestus* is one of four "eutetrarhynchoid" genera that have yet to be included in a phylogenetic analysis based on sequence data (see Table 2), but based on the results herein and its lack of gland cells in the bulbs (Campbell & Beveridge, 2006), it is expected to group with *Fellicocestus* and *Hemionchos*. Also missing from this analysis is the second of the two rhinoptericolid genera (i.e., *Nataliella*). Herzog and Jensen (2022) recovered the sole species in the genus, *N. marcelli*, sister to rhinoptericolids based on 28S rRNA sequence

data with lackluster nodal support. Sequence data for that species had been downloaded from GenBank for that analysis (Herzog & Jensen, 2022), and representatives of *N. marcelli* were not available to include in the present study. Results from analysis of a preliminary unpublished 28S dataset inclusive of *N. marcelli*, *Rhinoptericola*, *Fellicocestus*, *Hemionchos*, and tentacularioids, however, suggest that the species groups among these taxa.

That the genus *Hispidorhynchus* was recovered as sister to the aforementioned clade of taxa is puzzling. As mentioned above, the four valid species of *Hispidorhynchus* all possess pre-bulbar organs and gland cells in the bulbs, in stark contrast to the lack of one or both features in members of the aforementioned clade. Species of *Hispidorhynchus* additionally have a metabasal armature morphologically similar to that of species of species of *Dollfusiella*, further differentiating them from their apparent clade mates. Though the sister relationship between *Hispidorhynchus* and the clade of the tentacularioids and their allied "eutetrarhynchoids" was strongly supported in both analyses herein, the internode supporting that relationship was relatively short (see Figs. 4, 6). Additionally, this analysis did not include *Dollfusiella geraschmidti*, which—according to Beveridge at al. (2017)—comprises Novel Clade 4 along with species of *Hispidorhynchus*. The sister relationship recovered between *Hispidorhynchus* and the Tentacularioidea et al. herein should thus be interpreted cautiously until expanded taxon sampling and more detailed morphological study can provide clarification.

Remaining members of the superfamily "Eutetrarhynchoidea"

Both the *ASTRAL-III* and *IQ-TREE* analyses recovered the majority of the "eutetrarhynchoids" included herein in a clade sister to a clade containing Novel Clade 4, the Rhinoptericolidae, the genera *Hemionchos* and *Fellicocestus*, and the Tentacularioidea. This former clade contained most of the specimens sequenced as part of this study (i.e., 141 of 211 ingroup specimens; ~69%). The five subclades recovered within this clade correspond with Novel Clades 1, 2, and 3 sensu Beveridge et al. (2017), and two additional subclades of taxa not reported on in previous studies. The first of these additional subclades was recovered as sister to

all other taxa in the clade, and comprises specimens of *Trigonoglobium spinuliferum* (Southwell, 1911) Dollfus, 1929 and *Poecilorhynchus perplexus* (see Figs. 5, 6). This is the first time either genus has been included in a phylogenetic analysis based on sequence data (see Table 2). *Trigonoglobium* houses two valid species (Dollfus, 1929; Beveridge, 1990) and *Poecilorhynchus* is monotypic (Schaeffner & Beveridge, 2013b). Species of *Trigonoglobium*—with their unique tri-lappeted craspedote proglottids and set of unusual vaginal sphincters (Beveridge, 1990)—are morphologically distinctive, and it is unclear at present what features may unite them with *Poecilorhynchus*. *Poecilorhynchus perplexus* had been previously known only from its type host, the brownbanded bamboo shark, *Chiloscyllium punctatum* Müller & Henle, 1838, and so the specimen herein (i.e., JW159; see Table 1) from the epaulette shark, *Hemiscyllium ocellatum* (Bonnaterre, 1788), represents a novel host report for the species.

The second additional subclade of taxa not reported on in previous studies consisted of one of three lineages of *Prochristianella* recovered herein (i.e., *Prochristianella* 3) and *Mecistobothrium* 2 *penaeus* (see Figs. 5, 6). Whether this subclade was allied with Novel Clade 2 or Novel Clade 3 varied between the two tree-building methods, and so its position within the suborder is uncertain. Palm et al. (2009), Olson et al. (2010), and Beveridge et al. (2017) each recovered a non-monophyletic *Prochristianella*, but only found *Prochristianella*-like specimens in two groups: species with hollow metabasal hooks, which were allied with members of Novel Clade 1, and species with solid metabasal hooks, which were allied with members of Novel Clade 2. Like previous studies, the analyses herein recovered representatives of *Prochristianella butlerae* Beveridge, 1990 (i.e., *Prochristianella* 2; species with solid hooks) allied with the solid-hooked members of Novel Clade 2 (see Figs 5, 6). Unlike previous studies, however, herein, the clade of species of *Prochristianella* with hollow hooks (i.e., *Prochristianella* 3) was recovered as an independent lineage of uncertain and variable position within the suborder, while the clade of species of *Prochristianella* that were allied with members of Novel Clade 1 (i.e., *Prochristianella* 1) were found to have solid metabasal hooks (see Fig. 5).

Which of these three clades has claim to the generic name *Prochristianella* remains to

be determined. The type species of *Prochristianella—Prochristianella papillifer* (Poyarkoff, 1909) Dollfus, 1957—was described based on larval worms collected from mud shrimp from the Bay of Biscay, but adults have since been reported from stingrays in the genus *Dasyatis* Rafinesque, 1810 (see Beveridge et al., 2004). To date, *P. papillifer* has not been included in a phylogenetic analysis based on sequence data, and so its position within the Trypanobatoida is unknown. A specimen of *Prochristianella* from the common stingray (a host of *P. papillifer* as reported by Beveridge et al. 2004) from Egypt has been set aside to be sequenced in the near future (i.e., KW1206; K. Jensen and J. N. Caira, pers. comm.). Hopefully this specimen can be confirmed as *P. papillifer*, and its future inclusion in phylogenetic analyses can inform which lineage of *Prochristianella*-like taxa will retain the generic name. The redescription of *P. papillifer* asserts that the species possess hollow metabasal hooks (Beveridge et al., 2004), suggesting it may ultimately group with the clade of *Prochristianella* 3 and *Mecistobothrium* 2 *penaeus* (see Fig. 5).

The remaining three clades corresponded to Novel Clades 1–3 sensu Beveridge et al. (2017). Novel Clade 1 of Beveridge et al. (2017) housed the genera *Oncomegas, Mecistobothrium,* and *Progrillotia,* and two species of *Prochristianella* with hollow hooks. Novel Clade 1 herein was composed of *Oncomegas*, all specimens of *Mecistobothrium* except the representative of *Mecistobothrium* 2 *penaeus* (see above)*,* the solid-hooked species of *Prochristianella* 1, a single representative of a new genus from the giant electric ray (i.e., KW527; see Table 1), and the clade of *Dollfusiella* 5 cf. *aetobati* collected from aetobatid eagle rays (see Figs. 5, 6). Examination of the hologenophores of specimens of *Dollfusiella* 5 suggests that they represent a new genus. Given the extensive sampling of trypanobatoids herein, it is unsurprising that additional diversity was found in Novel Clade 1. Based on this result, the clade is no longer restricted to taxa with hollow metabasal hooks, but rather houses species with both hook types (see Fig. 4). Unfortunately, a specimen of *Progrillotia* was not available to include as part of this study, so whether this genus is a member of this expanded concept of Novel Clade 1 remains to be confirmed.

Novel Clade 2 of Beveridge et al. (2017) contained the genera *Parachristianella, Halysiorhynchus, Trygonicola,* and *Trimacracanthus*, and two species of *Prochristianella* with solid hooks. Herein, Novel Clade 2 contained all of those taxa (except for *Trimacracanthus,* which was not sequenced in this study), and additionally included the genus *Pseudochristianella*, which was sequenced for the first time herein (see Figs. 5, 6). The specimens of *Prochristianella* 2 and *Pseudochristianella* formed reciprocally monophyletic groups, but the genera *Halysiorhynchus* and *Trygonicola* were recovered deeply embedded within the genus *Parachristianella* (see Figs. 5, 6). *Parachristianella* is a distinctive and morphologically cohesive genus of 15 species (Caira et al., 2021). Its members are small worms (<1 cm) with two stout bothria, a heteroacanthous typical metabasal armature with widely divergent hooks 1(1') and solid hooks that decrease in size regularly along a principal row, and a characteristic basal armature without a basal swelling. Contrastingly, *Halysiorhynchus macrocephalus* Shipley & Hornell, 1906) Pintner, 1913 and *Trygonicola macroporus* (Shipley & Hornell, 1906) Beveridge & Campbell, 1998 are both large worms (>3 cm) with four bothria and poecilacanthous typical metabasal armature with solid hooks and widely divergent hooks 1(1') and a distinctive chainette element (Beveridge & Campbell, 1992, 1998). Whether *Parachristianella* should be expanded to accommodate species in these genera or instead be separated into several monophyletic genera to amend its present paraphyly requires more detailed morphological investigation. Sequence data have yet to be generated for the type species of the genus, *Parachristianella trygonis* Dollfus, 1946, and so its phylogenetic placement relative to other species in the genus remains unknown.

The final clade of "eutetrarhynchoids" corresponds to Novel Clade 3 of Beveridge et al. (2017). Their Novel Clade 3 contained all but a single species of *Dollfusiella* (i.e., *D. geraschmidti*, which was recovered instead in their Novel Clade 4), and the genera *Tetrarhynchobothrium* and *Paroncomegas*. In the present study, Novel Clade 3 housed all species of *Dollfusiella* except the representatives of *Dollfusiella* 5 recovered in Novel Clade 1 (see above), as well as *Tetrarhynchobothrium* and *Paroncomegas*, and the newly sequenced genus *Zygorhynchus* (see Table 2; Figs. 5, 6). *Dollfusiellla geraschmidti* was unfortunately

not represented in this analysis. *Dollfusiella* 2, 3, and 4 were recovered in a subclade with *Zygorhynchus*, and the representatives of *Tetrarhynchobothrium* and *Paroncomegas* placed among the large clade of specimens of *Dollfusiella* 1 (see Figs. 5, 6).

The future of *Dollfusiella* is uncertain. The morphologically distinctive genus currently comprises 31 valid species (Caira et al., 2021). These species are characterized by the possession of two bothria, a heteroacanthous typical metabasal armature of numerous small hollow hooks without a distinctive space between hooks 1(1'), a characteristic basal armature and basal swelling, and circumcortical vitelline follicles. Many species also have large microtriches on their scoleces that are visible with light microscopy (Schaeffner & Beveridge, 2013a). These features, in combination, lend an instantly recognizable *Dollfusiella* gestalt. That the genus *Dollfusiella* was recovered as non-monophyletic herein is not a novel result. Both Palm et al. (2009) and Olson et al. (2010) recovered representatives of *Tetrarhynchobothrium* and *Paroncomegas* clustered among specimens of *Dollfusiella* in their analyses (in addition to recovering *D. geraschmidti* allied with rhinoptericolids and tentaculariids). Members of the genus *Paroncomegas* share all of the distinguishing features (above) of the genus *Dollfusiella* but differ in two ways: (1) their characteristic basal armature includes a short chainette of macrohooks; and (2) their testes are arranged in multiple irregular columns, rather than in two or four regular columns (Campbell et al., 1999). Species of *Tetrarhynchobothrium* are also morphologically similar to species of *Dollfuseilla* but differ in their possession of: (1) a homeoacanthous (rather than heteroacanthous) metabasal armature; (2) testes arranged in multiple irregular columns (rather than in two or four regular columns); and (3) two internal seminal vesicles (rather than one) (Beveridge & Campbell, 1988). That the representatives of these genera have been repeatedly recovered among species of *Dollfusiella* warrants a detailed morphological investigation into generic boundaries to determine the best approach to resolving the non-monophyly of what is herein referred to as *Dollfusiella* 1.

The remaining species of *Dollfusiella* included here were recovered in Novel Clade 1 (i.e., *Dollfusiella* 5; see above) or in a clade with specimens of *Zygorhynchus* sister to rest

of Novel Clade 3 (see Figs. 5, 6). Like *Tetrarhynchobothrium* and *Paroncomegas*, species of *Zygorhynchus* share a number of morphological features in common with species of *Dollfusiella*, but are distinct in lacking a basal swelling and in possessing testes arranged in multiple irregular columns and a heavily muscular vagina with two internal diverticula (Beveridge & Campbell, 1988). Again, detailed morphological investigation will be required to determine generic boundaries in Novel Clade 3 considering these results. It is also worth noting that no phylogenetic analysis to date seems to have included the type species of *Dollfusiella*, *Dollfusiella australis* (Prudhoe, 1969) Campbell & Beveridge, 1994. *Dollfusiella australis* was described from the gummy shark, *Mustelus antarcticus* Günther, 1870, from Tasmania, but it has since also been reported from the whiskery shark, *Furgaleus macki* (Whitley, 1943), and the broadnose sevengill shark, *Notorynchus cepedianus* (Péron, 1807), from South Australia (Beveridge, 1990). (No specimens of *Dollfusiella* from shark hosts were included in this—or any previous—phylogenetic analysis.) Future sampling efforts should prioritize including specimens of *D. australis* in phylogenetic analyses to help inform questions of monophyly for the genus.

Relationship of the Trypanorhyncha to other non-acetabulate tapeworms

The trypanorhynchs are considered non-acetabulate eucestodes. The outgroup specimens included representatives of three additional orders of non-acetabulate eucestodes: the Bothriocephalidea (parasites of bony fishes and amphibians), Diphyllobothriidea (parasites of birds, mammals, and herptiles), and Diphyllidea (parasites of elasmobranchs) (see Table 1). In total, eight orders of non-acetabulate eucestodes are recognized. The orders not included here are the Caryophyllidea, Spathebothriidea, and Haplobothriidea (parasites of bony fishes), and the Litobothriidea (parasites of lamniform sharks).

Interrelationships among the eucestode orders are uncertain and resolving the backbone of the tapeworm tree of life is a topic of ongoing investigation. The most comprehensive attempt to understand tapeworm interrelationships to date is that of Caira et al. (2014). Therein, the Trypanorhyncha was recovered as sister to a clade containing the Diphyllobothriidea,

the Litobothriidea, and a clade of all orders of acetabulate eucestodes. A prior study which included additional orders of tapeworms, but fewer representatives within each order, variously recovered the Trypanorhyncha sister to the Diphyllidea or sister to a clade containing the Bothriocephalidea, the Litobothriidea, and the acetabulate eucestodes, depending on which combination of four genes was analyzed (Waeschenbach et al., 2012).

Herein, the *IQ-TREE* analysis recovered support for *Marsipometra hastata* (a member of the order Bothriocephalidea hosted by the American paddlefish) as sister to the order Trypanorhyncha with strong support (see Fig. 6). The goal of this study was of course to examine interrelationships within the order Trypanorhyncha—not to investigate relationships between the orders of non-acetabulate eucestodes—yet this result is still worth noting. The currently accepted working hypothesis for tapeworm interrelationships includes the Diphyllidea as the sister group to the Trypanorhyncha (Waeschenbach et al., 2012; Caira et al., 2014). Clearly, additional studies (and particularly those including representatives of acetabulate orders) are needed before the trypanorhynchs can be accurately placed relative to the other orders of tapeworms.

CONCLUSIONS AND FUTURE DIRECTIONS

This study presents the first hypothesis for interrelationships in the elasmobranch tapeworm order Trypanorhyncha based on a phylogenomic dataset. Data for 402 genes generated via target enrichment for 211 vouchered trypanorhynch tapeworm specimens were analyzed using both multispecies coalescent and multi-gene concatenation-based tree-building approaches. Representatives of all four trypanorhynch superfamilies and 14 of 17 named or recognized clades were included, as were representatives of eight genera that had not been included in a phylogenetic analysis based on sequence data prior to this study. The topologies preferred by the two tree-building methods were largely concordant. They supported:

1. Two reciprocally monophyletic trypanorhynch suborders, the Trypanoselachoida and Trypanobatoida, sensu Olson et al. (2010).

2. Two reciprocally monophyletic superfamilies, the Gymnorhynchoidea and the Lacistorhynchoidea sensu Beveridge et al. (2017), within the suborder Trypanoselachoida.
3. A non-monophyletic superfamily "Eutetrarhynchoidea" as it is presently circumscribed.
4. A monophyletic trypanobatoid superfamily Tentacularioidea, but only as a clade deeply embedded within the superfamily "Eutetrarhynchoidea".
5. Numerous short branches subtending major clades of trypanorhynchs, particularly within the superfamily "Eutetrarhynchoidea".
6. The trypanoselachoid genera *Aporhynchus, Deanicola, Otobothrium, Paragrillotia*, and *Pseudolacistorhynchus* as non-monophyletic.
7. The trypanobatoid genera *Dollfusiella*, *Mecistobothrium*, *Parachristianella,* and *Prochristianella* as non-monophyletic.
8. Multiple independent losses of the rhyncheal system among gymnorhynchoid taxa.
9. A single evolutionary loss of pre-bulbar organs among in trypanobatoid taxa.
10. A single evolutionary loss of gland cells in the bulbs in the tentacularioids.
11. The order Bothriocephalidea as potentially the sister taxon to the order Trypanorhyncha.

A combination of data from species tree topologies, host associations, and examination of whole-mounted hologenophore voucher specimens, in the context of the results of previous phylogenetic analyses (Palm et al., 2009; Olson et al., 2010; Beveridge et al., 2017), suggests the following:

1. As in other orders of tapeworms with members with highly variable and homoplasious scolex morphologies (e.g., the Rhinebothriidea [see Reyda et al., 2016] and the Lecanicephalidea [see Jensen et al., 2016]) features of proglottids are more likely to serve as informative morphological synapomorphies for higher-level taxa in the order Trypanorhyncha than are features of scoleces.
2. The possession of hollow versus solid metabasal hooks will likely prove a useful character for distinguishing between trypanorhynch genera, but perhaps will not be a reliable indicator of family-level groupings as previously suggested.

3. Numerous trypanorhynchs were sequenced as part of this study that represent either novel taxa, or the first report of adult specimens from elasmobranch definitive hosts for their representative species, foreshadowing future descriptive and redescriptive work.
4. A number of crucial type species have yet to be represented in phylogenetic analyses; their future inclusion should be prioritized to inform the resolution of non-monophyletic genera.

This study is by no means a resolution of trypanorhynch interrelationships. The phylogenomic hypotheses put forth here (and their underlying voucher specimens) can, however, serve as a much-needed jumping-off point to inform critical revisionary and descriptive work in the order. In addition to this work, feasible next steps should involve expanding the taxon representation in this phylogenomic dataset to include representatives of the remaining outstanding valid trypanorhynch genera and type species.

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Table 1. Unique identification number, higher classification, species identification, and host specimen information for all ingroup and outgroup specimens in the phylogenetic analyses. Assignments to suborders, superfamilies, and families or clades follows Beveridge et al. (2017). Elasmobranch taxonomy follows Last et al. (2016) and Naylor et al. (2012) (batoids) and Ebert et al. (2021) and Naylor et al. (2012) (sharks). Single asterisk (*) indicates that sequence data were downloaded from a publicly available genome rather than generated de novo. Double asterisks (**) indicate a specimen for which a draft genome was generated and used to inform bait design for target enrichment to generate data for this study.

JW/KW	Order	Subord.	Superfam.	Family/Clade	Species	Host order: Host family	Host species	Host no.
KW339	Bothriocephalidea	—	—	—	*Marsipometra hastata*	Acipenseriformes: Polyodontidae	*Polyodon spathula*	OK-6
—	Diphyllobothriidea	—	—	—	*Schistocephalus solidus** (BioProject PRJEB527)	—	—	—
JW425	Diphyllidea	—	—	—	*Ahamulina* sp.	Carcharhiniformes: Pentanchidae	*Apristurus macrostomus*	TW-43
JW494	Diphyllidea	—	—	—	*Coronocestus* sp.	Carcharhiniformes: Proscylliidae	*Eridacnis radcliffei*	TW-65
JW615	Diphyllidea	—	—	—	*Coronocestus* sp.	Carcharhiniformes: Triakidae	*Hemitriakis* sp. A	SO-35
JW240b	Diphyllidea	—	—	—	*Echinobothrium rhynchobati***	Rhinopristiformes: Glaucostegidae	*Glaucostegus* cf. *typus*	BO-120
JW265	Diphyllidea	—	—	—	*Echinobothrium* sp.	Rhinopristiformes: Platyrhinidae	*Platyrhina tangi*	TW-60
KW216	Diphyllidea	—	—	—	*Halysioncum* sp.	Myliobatiformes: Rhinopteridae	*Rhinoptera jayakari*	MZ-4
JW506	Trypanorhyncha	—	—	—	Unidentifiable specimen	—	—	—
KW259	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Dollfusiella* 5 cf. *aetobati* 1	Myliobatiformes: Aetobatidae	*Aetobatus ocellatus*	SO-34
KW408	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Dollfusiella* 5 cf. *aetobati* 2	Myliobatiformes: Aetobatidae	*Aetobatus narinari*	BE-8
KW263	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Dollfusiella* 5 cf. *aetobati* 2	Myliobatiformes: Aetobatidae	*Aetobatus ocellatus*	VN-38
KW570	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Mecistobothrium* 1 sp. 1	Myliobatiformes: Dasyatidae	*Pastinachus gracilicaudus*	KA-162
KW269	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Mecistobothrium* 1 sp. 2	Myliobatiformes: Rhinopteridae	*Rhinoptera bonasus*	CH-40
JW740	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Mecistobothrium* 1 sp. 2	Myliobatiformes: Rhinopteridae	*Rhinoptera brasiliensis*	MX-73
JW745	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Mecistobothrium* 1 sp. 2	Myliobatiformes: Rhinopteridae	*Rhinoptera brasiliensis*	MX-76
KW527	Trypanorhyncha	TB	"EU"	Novel Clade 1	n. gen. cf. *Prochristianella* n. sp.	Torpediniformes: Narcinidae	*Narcine entemedor*	EC-20
KW736	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Oncomegas* sp. 1	Myliobatiformes: Dasyatidae	*Hypanus guttatus*	TT14-11
KW310	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Oncomegas* sp. 2	Myliobatiformes: Dasyatidae	*Megatrygon microps*	NT-108
KW474	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Oncomegas* sp. 2	Myliobatiformes: Dasyatidae	*Pateobatis* cf. *jenkinsii*	NT-71
JW689	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Oncomegas* sp. 2	Myliobatiformes: Dasyatidae	*Urogymnus acanthobothrium*	NT-96
JW201	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Prochristianella* 1 cf. *butlerae*	Myliobatiformes: Myliobatidae	*Aetomylaeus vespertilio*	CM03-61
KW533	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Prochristianella* 1 cf. *fragilis*	Myliobatiformes: Dasyatidae	*Neotrygon australiae*	NT-62
KW563	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Prochristianella* 1 cf. *macracantha*	Myliobatiformes: Dasyatidae	*Neotrygon orientalis*	BO-322
KW856	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Prochristianella* 1 cf. *macracantha*	Myliobatiformes: Dasyatidae	*Pastinachus ater*	SO-8
KW929	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Prochristianella* 1 cf. *macracantha*	Myliobatiformes: Dasyatidae	*Taeniura lymma* 1	KA-420
KW591	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Prochristianella* 1 n. sp.	Myliobatiformes: Dasyatidae	*Pastinachus ater*	KA-32
JW221	Trypanorhyncha	TB	"EU"	Novel Clade 1	*Prochristianella* 1 n. sp. cf. *Oncomegas*	Myliobatiformes: Dasyatidae	*Maculabatis bineeshi*	IN-60
KW858	Trypanorhyncha	TB	"EU"	Novel Clade 2	*Halysiorhynchus* cf. *macrocephalus*	Myliobatiformes: Dasyatidae	*Himantura australis*	SO-13
KW482	Trypanorhyncha	TB	"EU"	Novel Clade 2	*Halysiorhynchus* cf. *macrocephalus*	Myliobatiformes: Dasyatidae	*Pateobatis uarnacoides*	KA-81
KW672	Trypanorhyncha	TB	"EU"	Novel Clade 2	*Halysiorhynchus macrocephalus*	Myliobatiformes: Dasyatidae	*Himantura leoparda*	NT-37
KW600	Trypanorhyncha	TB	"EU"	Novel Clade 2	*Halysiorhynchus macrocephalus*	Myliobatiformes: Dasyatidae	*Pastinachus ater*	NT-44
KW520	Trypanorhyncha	TB	"EU"	Novel Clade 2	*Halysiorhynchus macrocephalus*	Rhinopristiformes: Rhinidae	*Rhina ancylostoma*	NT-91
JW793	Trypanorhyncha	TB	"EU"	Novel Clade 2	*Halysiorhynchus macrocephalus*	Rhinopristiformes: Rhinidae	*Rhynchobatus palpebratus*	NT-66
KW659	Trypanorhyncha	TB	"EU"	Novel Clade 2	*Halysiorhynchus* n. sp.	Myliobatiformes: Dasyatidae	*Maculabatis pastinacoides*	KA-203
JW263	Trypanorhyncha	TB	"EU"	Novel Clade 2	*Parachristianella* cf. *baverstocki*	Myliobatiformes: Dasyatidae	*Hemitrygon bennetti*	TW-46
KW626	Trypanorhyncha	TB	"EU"	Novel Clade 2	*Parachristianella* cf. *baverstocki*	Myliobatiformes: Dasyatidae	*Maculabatis pastinacoides*	KA-203

JW236	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *baverstocki*	Rhinopristiformes: Glaucostegidae	*Glaucostegus* cf. *typus*	BO-120
KW969	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *indonesiensis*	Myliobatiformes: Dasyatidae	*Telatrygon biasa*	KA-182
JW785	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *indonesiensis*	Myliobatiformes: Dasyatidae	*Himantura australis*	BO-82
KW699	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *indonesiensis*	Myliobatiformes: Dasyatidae	*Himantura undulata*	KA-326
KW175	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *indonesiensis*	Rhinopristiformes: Rhinidae	*Rhynchobatus australiae*	MZ-15
KW911	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha* 1	Myliobatiformes: Dasyatidae	*Taeniura lymma* 2	KA-98
JW786	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha* 2	Myliobatiformes: Dasyatidae	*Himantura tutul*	KA-71
KW682	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha* 2	Myliobatiformes: Dasyatidae	*Himantura tutul*	KA-71
KW377	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha* 3	Myliobatiformes: Dasyatidae	*Hemitrygon akajei*	JN-1
KW923	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha* 4	Myliobatiformes: Dasyatidae	*Taeniura lymma* 1	KA-358
JW790	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha/heteromegacantha* 1	Myliobatiformes: Dasyatidae	*Neotrygon australiae*	NT-62
JW784	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha/heteromegacantha* 2	Myliobatiformes: Dasyatidae	*Himantura australis*	BO-82
KW1044	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha/heteromegacantha* 2	Myliobatiformes: Dasyatidae	*Urogymnus acanthobothrium*	NT-96
KW176	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha/heteromegacantha* 2	Rhinopristiformes: Rhinidae	*Rhynchobatus australiae*	MZ-15
JW788	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha/heteromegacantha* 3	Carcharhiniformes: Triakidae	*Mustelus mustelus*	AF-144
JW783	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha/heteromegacantha* 3	Myliobatiformes: Dasyatidae	*Dasyatis chrysonota*	AF-158
KW324	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha/heteromegacantha* 3	Myliobatiformes: Dasyatidae	*Dasyatis chrysonota*	AF-158
KW378	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha/heteromegacantha* 3	Myliobatiformes: Dasyatidae	*Hemitrygon akajei*	JN-1
JW789	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha/heteromegacantha* 3	Myliobatiformes: Myliobatidae	*Myliobatis aquila*	AF-50
JW269	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha/heteromegacantha* 3	Myliobatiformes: Plesiobatidae	*Plesiobatis daviesi*	TW-98
KW279	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *monomegacantha/heteromegacantha* 3	Rhinopristiformes: Rhinobatidae	*Acroteriobatus annulatus*	AF-141
KW776	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *trygonis*	Myliobatiformes: Myliobatidae	*Myliobatis tobijei*	TW-45
KW1033	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *trygonis*	Rajiformes: Rajidae	*Okamejei acutispina*	TW-31
KW761	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *trygonis*	Rajiformes: Rajidae	*Okamejei hollandi*	TW-132
JW728	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *trygonis*	Rajiformes: Rajidae	*Okamejei kenojei*	JN-43
JW268	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* cf. *trygonis*	Rhinopristiformes: Platyrhinidae	*Platyrhina tangi*	TW-63
KW823	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella indonesiensis*	Myliobatiformes: Dasyatidae	*Maculabatis bineeshi*	IN-19
JW791	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella indonesiensis*	Myliobatiformes: Dasyatidae	*Pastinachus solocirostris*	BO-464
KW579	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella indonesiensis*	Myliobatiformes: Dasyatidae	*Pastinachus solocirostris*	BO-464
JW794	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella indonesiensis*	Myliobatiformes: Dasyatidae	*Urogymnus lobistoma*	BO-480
JW254	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella indonesiensis*	Rhinopristiformes: Glaucostegidae	*Glaucostegus obtusus*	IN-8
KW326	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 1	Myliobatiformes: Dasyatidae	*Dasyatis marmorata*	SE-317
JW787	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 1	Myliobatiformes: Dasyatidae	*Hypanus rudis*	SE-222
JW581	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 1	Rhinopristiformes: Rhinobatidae	*Rhinobatos rhinobatos*	SE-240
JW792	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 1	Rhinopristiformes: Rhinobatidae	*Rhinobatos rhinobatos*	SE-62
JW795	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 1	Rhinopristiformes: Zanobatidae	*Zanobatus schoenleinii*	SE-201
KW869	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 2	Myliobatiformes: Dasyatidae	*Himantura uarnak*	EG-1
KW752	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 3	Myliobatiformes: Dasyatidae	*Maculabatis astra*	NT-90
KW669	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 4	Myliobatiformes: Dasyatidae	*Himantura leoparda*	NT-32
KW751	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 4	Myliobatiformes: Dasyatidae	*Maculabatis astra*	NT-90
KW598	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 4	Myliobatiformes: Dasyatidae	*Pastinachus ater*	NT-44
KW465	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 4	Myliobatiformes: Dasyatidae	*Pateobatis fai*	NT-33
KW1037	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 5	Rajiformes: Rajidae	*Rostroraja equatorialis* 2	EC-51
KW414	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Parachristianella* sp. 5	Rhinopristiformes: Trygonorrhinidae	*Zapteryx xyster*	CRP-7
KW327	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Prochristianella* 2 cf. *clarkeae* 1	Myliobatiformes: Dasyatidae	*Dasyatis* sp.	TW-25
KW803	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Prochristianella* 2 cf. *clarkeae* 2	Myliobatiformes: Dasyatidae	*Neotrygon indica*	SL-46
KW690	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Prochristianella* 2 *clarkeae*	Myliobatiformes: Dasyatidae	*Himantura australis*	BO-82
JW229	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Prochristianella* 2 *clarkeae*	Rhinopristiformes: Glaucostegidae	*Glaucostegus thouin*	KA-70
KW419	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Pseudochristianella* sp. 1	Rhinopristiformes: Trygonorrhinidae	*Zapteryx xyster*	CRP-39
KW320	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Pseudochristianella* sp. 2	Myliobatiformes: Dasyatidae	*Dasyatis chrysonota*	AF-90

KW356	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Pseudochristianella* sp. 3	Myliobatiformes: Dasyatidae	*Fontitrygon margarita*	SE-292
KW760	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Pseudochristianella* sp. 3	Myliobatiformes: Dasyatidae	*Fontitrygon margaritella*	SE-214
KW870	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Trygonicola* cf. *macroporus*	Myliobatiformes: Dasyatidae	*Himantura uarnak*	EG-1
KW170	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Trygonicola* cf. *macroporus*	Rhinopristiformes: Rhinidae	*Rhynchobatus australiae*	MZ-15
KW613	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Trygonicola macroporus*	Myliobatiformes: Dasyatidae	*Maculabatis macrura*	KA-86
JW796	Trypanorhyncha	TB	“EU”	Novel Clade 2	*Trygonicola macroporus*	Myliobatiformes: Dasyatidae	*Pastinachus ater*	SL-64
KW892	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 cf. *martini*	Myliobatiformes: Dasyatidae	*Taeniura lymma*	EG-10
KW883	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 cf. *martini*	Myliobatiformes: Dasyatidae	*Taeniura lymma* 3	MZ-28
JW777	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 cf. *ocallaghani*	Myliobatiformes: Dasyatidae	*Maculabatis gerrardi*	BO-466
KW654	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 cf. *spinulifera*	Myliobatiformes: Myliobatidae	*Myliobatis californica*	BJ-586
JW180	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 cf. *tenuispinis*	Myliobatiformes: Urotrygonidae	*Urobatis tumbesensis*	EC-56
JW780	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 1	Myliobatiformes: Dasyatidae	*Pastinachus solocirostris*	BO-164
KW952	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 1	Myliobatiformes: Myliobatidae	*Aetomylaeus maculatus*	BO-179
KW959	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 1	Myliobatiformes: Myliobatidae	*Aetomylaeus maculatus*	BO-179
KW220	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 2	Orectolobiformes: Parascylliidae	*Cirrhoscyllium formosanum*	TW-93
KW777	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 3	Myliobatiformes: Myliobatidae	*Myliobatis tobijei*	TW-45
KW442	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 4	Myliobatiformes: Dasyatidae	*Bathytoshia centroura*	RDM-250
JW400	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 4	Squaliformes: Dalatiidae	*Squaliolus aliae*	TW-117
KW671	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 5	Myliobatiformes: Dasyatidae	*Himantura leoparda*	NT-32
KW749	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 5	Myliobatiformes: Dasyatidae	*Maculabatis astra*	NT-26
JW781	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 5	Myliobatiformes: Dasyatidae	*Pateobatis fai*	NT-33
KW282	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 6	Myliobatiformes: Dasyatidae	*Brevitrygon heterura*	KA-88
KW284	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 7	Myliobatiformes: Dasyatidae	*Brevitrygon heterura*	KA-99
JW212	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 7	Myliobatiformes: Dasyatidae	*Maculabatis bineeshi*	IN-55
JW235	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 7	Rhinopristiformes: Glaucostegidae	*Glaucostegus* cf. *typus*	BO-120
KW340	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 8	Myliobatiformes: Dasyatidae	*Fluvitrygon oxyrhynchus*	KA-186
KW686	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 9	Myliobatiformes: Dasyatidae	*Himantura tutul*	KA-392
JW778	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 9	Myliobatiformes: Dasyatidae	*Maculabatis pastinacoides*	BO-100
KW492	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 9	Myliobatiformes: Dasyatidae	*Pateobatis uarnacoides*	BO-118
KW868	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 10	Myliobatiformes: Dasyatidae	*Himantura uarnak*	EG-1
KW455	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 11	Myliobatiformes: Dasyatidae	*Pateobatis bleekeri*	IN-14
JW219	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 12	Myliobatiformes: Dasyatidae	*Maculabatis bineeshi*	IN-60
JW232	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 13	Rhinopristiformes: Glaucostegidae	*Glaucostegus thouin*	KA-70
KW519	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 14	Rhinopristiformes: Rhinidae	*Rhina ancylostoma*	NT-103
KW212	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 sp. 15	Myliobatiformes: Dasyatidae	*Maculabatis* cf. *gerrardi* 6	MZ-12
JW247	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 *spinulifera*	Rhinopristiformes: Glaucostegidae	*Glaucostegus obtusus*	IN-8
KW364	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 *spinulifera*	Rhinopristiformes: Glaucostegidae	*Glaucostegus typus*	CM03-75
JW776	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 *spinulifera*	Myliobatiformes: Dasyatidae	*Hypanus sabinus*	MS05-253
JW168	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 1 *spinulifera*	Myliobatiformes: Urotrygonidae	*Urobatis jamaicensis*	JM-33
KW275	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 2 sp. 1	Rhinopristiformes: Rhinobatidae	*Acroteriobatus annulatus*	AF-155
JW245	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 2 sp. 1	Rhinopristiformes: Glaucostegidae	*Glaucostegus cemiculus*	SE-117
KW411	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 3 sp.	Rajiformes: Rajidae	*Rostroraja equatorialis* 1	CRP-35
KW653	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 4 sp. 1	Myliobatiformes: Myliobatidae	*Myliobatis californica*	BJ-586
JW779	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Dollfusiella* 4 sp. 2	Myliobatiformes: Myliobatidae	*Myliobatis aquila*	AF-50
KW769	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Paroncomegas myliobatis*	Myliobatiformes: Dasyatidae	*Urogymnus granulatus*	SO-9
KW685	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Tetrarhynchobothrium* sp.	Myliobatiformes: Dasyatidae	*Himantura tutul*	KA-392
KW490	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Tetrarhynchobothrium* sp.	Myliobatiformes: Dasyatidae	*Pateobatis uarnacoides*	KA-386
JW782	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Tetrarhynchobothrium* sp.	Myliobatiformes: Dasyatidae	*Urogymnus lobistoma*	KA-385
JW77	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Zygorhynchus ginglymostomatis*	Orectolobiformes: Ginglymostomatidae	*Ginglymostoma cirratum*	BE-1
JW351	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Zygorhynchus ginglymostomatis*	Orectolobiformes: Ginglymostomatidae	*Ginglymostoma cirratum*	BE-1

JW163	Trypanorhyncha	TB	“EU”	Novel Clade 3	*Zygorhynchus* cf. *ginglymostomatis*	Orectolobiformes: Ginglymostomatidae	*Ginglymostoma unami*	BJ-425
JW73	Trypanorhyncha	TB	“EU”	Novel Clade 3	cf. *Zygorhynchus* sp.	Orectolobiformes: Ginglymostomatidae	*Ginglymostoma cirratum*	GC-1
KW652	Trypanorhyncha	TB	“EU”	Novel Clade 4	*Hispidorhynchus* cf. *paulinae*	Myliobatiformes: Myliobatidae	*Myliobatis californica*	BJ-586
JW744	Trypanorhyncha	TB	“EU”	Novel Clade 4	*Hispidorhynchus* cf. *paulinae*	Myliobatiformes: Rhinopteridae	*Rhinoptera brasiliensis*	MX-76
KW764	Trypanorhyncha	TB	“EU”	Novel Clade 4	*Hispidorhynchus* cf. *paulinae*	Myliobatiformes: Urotrygonidae	*Urobatis maculatus*	BJ-95
KW382	Trypanorhyncha	TB	“EU”	Rhinoptericolidae	*Rhinoptericola butlerae*	Myliobatiformes: Dasyatidae	*Hemitrygon bennetti*	VN-42
JW774	Trypanorhyncha	TB	“EU”	Rhinoptericolidae	*Rhinoptericola butlerae*	Myliobatiformes: Dasyatidae	*Maculabatis gerrardi*	KA-75
JW775	Trypanorhyncha	TB	“EU”	Rhinoptericolidae	*Rhinoptericola butlerae*	Myliobatiformes: Rhinopteridae	*Rhinoptera neglecta*	CM03-43
KW1039	Trypanorhyncha	TB	“EU”	Rhinoptericolidae	*Rhinoptericola hexacantha*	Myliobatiformes: Rhinopteridae	*Rhinoptera steindachneri*	BJ-684
KW766	Trypanorhyncha	TB	“EU”	Rhinoptericolidae	*Rhinoptericola jensenae*	Myliobatiformes: Rhinopteridae	*Rhinoptera neglecta*	CM03-31
KW393	Trypanorhyncha	TB	“EU”	Rhinoptericolidae	*Rhinoptericola megacantha*	Myliobatiformes: Rhinopteridae	*Rhinoptera bonasus*	CH-18
KW399	Trypanorhyncha	TB	“EU”	Rhinoptericolidae	*Rhinoptericola megacantha*	Myliobatiformes: Rhinopteridae	*Rhinoptera brasiliensis*	BE-10
JW555	Trypanorhyncha	TB	“EU”	Rhinoptericolidae	*Rhinoptericola megacantha*	Myliobatiformes: Rhinopteridae	*Rhinoptera marginata*	SE-84
——	Trypanorhyncha	TB	“EU”	Rhinoptericolidae	*Rhinoptericola megacantha***	Myliobatiformes: Rhinopteridae	*Rhinoptera bonasus*	CH-19
KW217	Trypanorhyncha	TB	“EU”	Rhinoptericolidae	*Rhinoptericola mozambiquensis*	Myliobatiformes: Rhinopteridae	*Rhinoptera jayakari*	MZ-4
KW819	Trypanorhyncha	TB	“EU”	Undetermined	*Fellicocestus mobulae*	Myliobatiformes: Mobulidae	*Mobula mobular*	TW-214
KW817	Trypanorhyncha	TB	“EU”	Undetermined	*Hemionchos maior*	Myliobatiformes: Mobulidae	*Mobula mobular*	TW-208
KW818	Trypanorhyncha	TB	“EU”	Undetermined	*Hemionchos mobulae*	Myliobatiformes: Mobulidae	*Mobula mobular*	TW-208
KW644	Trypanorhyncha	TB	“EU”	Undetermined	*Hemionchos mobulae*	Myliobatiformes: Mobulidae	*Mobula munkiana*	BJ-275
KW816	Trypanorhyncha	TB	“EU”	Undetermined	*Hemionchos striatus*	Myliobatiformes: Mobulidae	*Mobula thurstoni*	BJ-429
JW133	Trypanorhyncha	TB	“EU”	Undetermined	*Poecilorhynchus perplexus*	Orectolobiformes: Hemiscylliidae	*Chiloscyllium punctatum*	BO-431
JW127	Trypanorhyncha	TB	“EU”	Undetermined	*Poecilorhynchus perplexus*	Orectolobiformes: Hemiscylliidae	*Chiloscyllium punctatum*	BO-73
JW159	Trypanorhyncha	TB	“EU”	Undetermined	*Poecilorhynchus perplexus*	Orectolobiformes: Hemiscylliidae	*Hemiscyllium ocellatum*	JO-12
JW485	Trypanorhyncha	TB	“EU”	Undetermined	*Trigonoglobium spinuliferum*	Carcharhiniformes: Hemigaleidae	*Hemipristis elongata*	NT-102
KW493	Trypanorhyncha	TB	“EU”	Undetermined	*Prochristianella* 3 cf. *aciculata*	Myliobatiformes: Dasyatidae	*Pateobatis uarnacoides*	BO-118
KW768	Trypanorhyncha	TB	“EU”	Undetermined	*Prochristianella* 3 cf. *aciculata*	Myliobatiformes: Dasyatidae	*Urogymnus granulatus*	SO-17
KW872	Trypanorhyncha	TB	“EU”	Undetermined	*Prochristianella* 3 cf. *kostadinovae*	Myliobatiformes: Dasyatidae	*Himantura uarnak*	EG-2
KW213	Trypanorhyncha	TB	“EU”	Undetermined	*Prochristianella* 3 cf. *kostadinovae*	Myliobatiformes: Dasyatidae	*Maculabatis* cf. *gerrardi* 6	MZ-12
JW185	Trypanorhyncha	TB	“EU”	Undetermined	*Prochristianella* 3 sp.	Myliobatiformes: Dasyatidae	*Taeniurops grabatus*	SE-121
JW497	Trypanorhyncha	TB	“EU”	Undetermined	*Mecistobothrium* 2 *penaeus*	Carcharhiniformes: Sphyrnidae	*Sphyrna tiburo*	MS05-432
KW576	Trypanorhyncha	TB	TE	Tentaculariidae	*Kotorella* sp.	Myliobatiformes: Dasyatidae	*Pastinachus solocirostris*	BO-164
KW916	Trypanorhyncha	TB	TE	Tentaculariidae	*Kotorella* sp.	Myliobatiformes: Dasyatidae	*Taeniura lymma* 1	BO-122
JW502	Trypanorhyncha	TB	TE	Tentaculariidae	*Nybelinia* 1 sp. 1	Carcharhiniformes: Sphyrnidae	*Sphyrna zygaena*	JN-7
JW444	Trypanorhyncha	TB	TE	Tentaculariidae	*Nybelinia* 1 sp. 2	Lamniformes: Lamnidae	*Isurus oxyrinchus*	DR-1
KW287	Trypanorhyncha	TB	TE	Tentaculariidae	*Nybelinia* 2 sp. 1	Carcharhiniformes: Carcharhinidae	*Carcharhinus* cf. *leucas*	BO-97
KW841	Trypanorhyncha	TB	TE	Tentaculariidae	*Nybelinia* 2 sp. 1	Carcharhiniformes: Carcharhinidae	*Carcharhinus limbatus*	CH-50
JW368	Trypanorhyncha	TS	GY	“Aporhynchidae”	*Aporhynchus* 1 cf. *pickeringae*	Squaliformes: Etmopteridae	*Aculeola nigra*	WMO-1
JW372	Trypanorhyncha	TS	GY	“Aporhynchidae”	*Aporhynchus* 2 *menezesi*	Squaliformes: Etmopteridae	*Etmopterus spinax*	AZ-9
JW429	Trypanorhyncha	TS	GY	“Aporhynchidae”	*Nakayacestus tanyderus*	Carcharhiniformes: Pentanchidae	*Galeus sauteri*	TW-49
JW402	Trypanorhyncha	TS	GY	“Gilquiniidae”	*Deanicola* 1 cf. *minor*	Squaliformes: Centrophoridae	*Deania calcea*	AZ-111
JW412	Trypanorhyncha	TS	GY	“Gilquiniidae”	*Deanicola* 2 cf. *protentus*	Squaliformes: Centrophoridae	*Deania quadrispinosum* 2	TW-119
JW382	Trypanorhyncha	TS	GY	“Gilquiniidae”	*Gilquinia* cf. *squali*	Squaliformes: Squalidae	*Squalus acanthias*	RDM-152
JW411	Trypanorhyncha	TS	GY	“Gilquiniidae”	*Gilquinia* cf. *squali*	Squaliformes: Etmopteridae	*Etmopterus princeps*	AZ-104
JW280a	Trypanorhyncha	TS	GY	Gymnorhynchidae	*Gymnorhynchus isuri*	Lamniformes: Lamnidae	*Isurus oxyrinchus*	AI-37
JW452	Trypanorhyncha	TS	GY	Gymnorhynchidae	*Molicola* sp.	Lamniformes: Lamnidae	*Isurus paucus*	TW-217
JW445	Trypanorhyncha	TS	GY	Sphyriocephalidae	*Hepatoxylon trichiuri*	Carcharhiniformes: Carcharhinidae	*Prionace glauca*	AI-38
JW453	Trypanorhyncha	TS	GY	Sphyriocephalidae	*Sphyriocephalus pelorosoma*	Lamniformes: Alopiidae	*Alopias pelagicus*	BJ-715
JW628	Trypanorhyncha	TS	GY	Sphyriocephalidae	*Sphyriocephalus pelorosoma*	Lamniformes: Alopiidae	*Alopias vulpinus*	RI-33
KW1020	Trypanorhyncha	TS	LA	Grillotiidae	*Grillotia patagonica*	Rajiformes: Arhynchobatidae	*Bathyraja griseocauda*	FA-99
JW460	Trypanorhyncha	TS	LA	Grillotiidae	*Grillotia* sp.	Squatiniformes: Squatinidae	*Squatina californica*	BJ-293

KW831	Trypanorhyncha	TS	LA	Lacistorhynchidae	*Callitetrarhynchus gracilis*	Carcharhiniformes: Carcharhinidae	*Carcharhinus brevipinna*	MS05-5
KW840	Trypanorhyncha	TS	LA	Lacistorhynchidae	*Callitetrarhynchus gracilis*	Carcharhiniformes: Carcharhinidae	*Carcharhinus limbatus*	CH-50
JW534	Trypanorhyncha	TS	LA	Lacistorhynchidae	*Diesingium antarcticum*	Carcharhiniformes: Triakidae	*Mustelus* cf. *antarcticus*	SO-40
JW520	Trypanorhyncha	TS	LA	Lacistorhynchidae	*Diesingium lomentaceum*	Carcharhiniformes: Triakidae	*Mustelus mustelus*	SE-244
JW540	Trypanorhyncha	TS	LA	Lacistorhynchidae	*Lacistorhynchus dollfusi*	Carcharhiniformes: Triakidae	*Mustelus canis*	LIT-2
JW193	Trypanorhyncha	TS	LA	Lacistorhynchidae	*Lacistorhynchus tenuis*	Carcharhiniformes: Triakidae	*Hemitriakis japanica*	TW-37
JW532	Trypanorhyncha	TS	LA	Lacistorhynchidae	*Lacistorhynchus tenuis*	Carcharhiniformes: Triakidae	*Mustelus* cf. *antarcticus*	SO-40
JW75	Trypanorhyncha	TS	LA	Novel Clade 5	*Paragrillotia similis*	Orectolobiformes: Ginglymostomatidae	*Ginglymostoma cirratum*	GC-1
KW843	Trypanorhyncha	TS	LA	Novel Clade 5	*Paragrillotia* sp.	Carcharhiniformes: Carcharhinidae	*Carcharhinus limbatus*	CH-50
JW109b	Trypanorhyncha	TS	LA	Novel Clade 5	*Pseudolacistorhynchus* 1 *heroniensis***	Orectolobiformes: Ginglymostomatidae	*Nebrius ferrugineus*	CM03-16
JW85	Trypanorhyncha	TS	LA	Novel Clade 5	*Pseudolacistorhynchus* 2 *nanus*	Orectolobiformes: Stegostomatidae	*Stegostoma fasciatum*	NT-109
KW298	Trypanorhyncha	TS	LA	Undetermined	*Ancipirhynchus afossalis*	Carcharhiniformes: Carcharhinidae	*Carcharhinus coatesi*	NT-101
JW490	Trypanorhyncha	TS	LA	Undetermined	*Ancipirhynchus afossalis*	Carcharhiniformes: Hemigaleidae	*Paragaleus tengi*	KA-23
JW399	Trypanorhyncha	TS	LA	Undetermined	*Ancipirhynchus* sp.	Squaliformes: Squalidae	*Squalus rancureli*	SO-38
KW502	Trypanorhyncha	TS	LA	Otobothriidae	*Fossobothrium perplexum*	Rhinopristiformes: Pristidae	*Anoxypristis cuspidata*	NT-89
KW583	Trypanorhyncha	TS	LA	Otobothriidae	*Otobothrium* 1 cf. *crenacolle*	Carcharhiniformes: Carcharhinidae	*Carcharhinus falciformis*	DEL-11
JW483	Trypanorhyncha	TS	LA	Otobothriidae	*Otobothrium* 1 cf. *parvum*	Carcharhiniformes: Carcharhinidae	*Triaenodon obesus*	SO-45
KW842	Trypanorhyncha	TS	LA	Otobothriidae	*Otobothrium* 1 sp. 1	Carcharhiniformes: Carcharhinidae	*Carcharhinus limbatus*	CH-50
KW835	Trypanorhyncha	TS	LA	Otobothriidae	*Otobothrium* 1 sp. 1	Carcharhiniformes: Carcharhinidae	*Carcharhinus limbatus*	MS05-24
KW297	Trypanorhyncha	TS	LA	Otobothriidae	*Otobothrium* 2 cf. *carcharidis*	Carcharhiniformes: Carcharhinidae	*Carcharhinus coatesi*	NT-14
KW296	Trypanorhyncha	TS	LA	Otobothriidae	*Otobothrium* 2 cf. *carcharidis*	Carcharhiniformes: Carcharhinidae	*Carcharhinus sorrah*	BO-48
JW565	Trypanorhyncha	TS	LA	Otobothriidae	*Otobothrium* 2 cf. *mugilis*	Carcharhiniformes: Carcharhinidae	*Carcharhinus cf. cautus*	SO-12
JW60	Trypanorhyncha	TS	LA	Otobothriidae	*Otobothrium* 2 sp. 2	Carcharhiniformes: Carcharhinidae	*Lamiopsis tephrodes*	BO-74
KW307	Trypanorhyncha	TS	LA	Otobothriidae	*Pristiorhynchus palmi*	Rhinopristiformes: Pristidae	*Anoxypristis cuspidata*	NT-65
KW668	Trypanorhyncha	TS	LA	Otobothriidae	*Proemotobothrium* sp.	Myliobatiformes: Dasyatidae	*Himantura leoparda*	NT-32
JW499	Trypanorhyncha	TS	LA	Otobothriidae	*Symbothriorhynchus uranoscopi*	Carcharhiniformes: Sphyrnidae	*Sphyrna mokarran* 2	CM03-78
KW763	Trypanorhyncha	TS	LA	Pterobothriidae	*Pterobothrium* sp. 1	Myliobatiformes: Gymnuridae	*Gymnura* cf. *poecilura* 1	KA-37
JW716	Trypanorhyncha	TS	LA	Pterobothriidae	*Pterobothrium* sp. 1	Myliobatiformes: Gymnuridae	*Gymnura sereti*	SE-247
KW891	Trypanorhyncha	TS	LA	Pterobothriidae	*Pterobothrium* sp. 2	Myliobatiformes: Dasyatidae	*Taeniura lymma*	EG-10
KW471	Trypanorhyncha	TS	LA	Pterobothriidae	*Pterobothrium* sp. 3	Myliobatiformes: Dasyatidae	*Pateobatis* cf. *jenkinsii*	NT-71
KW466	Trypanorhyncha	TS	LA	Pterobothriidae	*Pterobothrium* sp. 3	Myliobatiformes: Dasyatidae	*Pateobatis fai*	KA-436
KW1043	Trypanorhyncha	TS	LA	Pterobothriidae	*Pterobothrium* sp. 3	Myliobatiformes: Dasyatidae	*Urogymnus acanthobothrium*	NT-96

Abbreviations: “EU”–“Eutetrarhynchoidea”; GY–Gymnorhynchoidea; LA–Lacistorhynchoidea; Superfam.–Superfamily; Subord.–Suborder; TB–Trypanobatoida; TE–Tentacularioidea; TS–Trypanoselachoida.

Table 2. Sequencing efforts for representatives of the 82 valid trypanorhynch genera prior to versus in this study. List of valid genera and their higher classifications follows Beveridge et al. (2017); Haseli and Malekpour Fard (2017); and Palm et al. (2020). Bolded genera have not been included in any phylogenetic analysis based on sequence data to date. Stars in the "Sequenced in present study" column indicate a genus for which the first sequence data from a representative species were generated as part of this study.

Genus	Sequenced prior to present study	Sequenced in present study
Suborder Trypanoselachoida: Superfamily Gymnorhynchoidea		
Aporhynchus	•	•
Nakayacestus		★
Chimaerarhynchus	•	
Deanicola	•	•
Gilquinia	•	•
Sagittirhynchus	•	
Vittirhynchus	•	
Gymnorhynchus	•	•
Molicola	•	•
Plesiorhynchus	•	
Pintneriella	•	
Hepatoxylon	•	•
Heterosphyriocephalus	•	
Sphyriocephalus	•	•
Suborder Trypanoselachoida: Superfamily Lacistorhynchoidea		
Ancipirhynchus	•	•
Dasyrhynchus	•	
Grillotiella	•	
Protogrillotia	•	
Pseudogilquinia	•	
Bathygrillotia	•	
Campbelliella		
Grillotia	•	•
Microbothriorhynchus		
Pseudogrillotia		
Hornelliella	•	
Bombycirhynchus		
Callitetrarhynchus	•	•
Diesingium	•	•
Floriceps	•	
Lacistorhynchus	•	•
Paragrillotia	•	•
Pseudolacistorhynchus	•	•
Diplootobothrium		
Fossobothrium	•	•
Iobothrium	•	
Olgaella		
Otobothrium	•	•
Parotobothrium	•	
Poecilancistrium	•	
Poeciloacanthum		
Pristiorhynchus		★
Proemotobothrium	•	•
Pseudotobothrium	•	
Symbothriorhynchus	•	•
Cavearhynchus		
Pterobothrioides		
Stragulorhynchus		
Pterobothrium	•	•
Suborder Trypanobatoida: Superfamily Tentacularioidea		
Paranybelinia		
Pseudonybelinia		
Heteronybelinia	•	
Kotorella	•	•

Kotorelliella		
Mixonybelinia	•	
Nybelinia	•	•
Reimeriella		
Tentacularia	•	
Suborder Trypanobatoida: Superfamily “Eutetrarhynchoidea”		
Mecistobothrium	•	•
Oncomegas	•	•
Progrillotia	•	
Prochristianella	•	•
Halysiorhynchus	•	•
Parachristianella	•	•
Pseudochristianella		★
Trimacracanthus	•	
Trygonicola	•	•
Dollfusiella	•	•
Paroncomegas	•	•
Tetrarhynchobothrium	•	•
Zygorhynchus		★
Hispidorhynchus	•	•
Nataliella	•	
Rhinoptericola	•	•
Cetorhinicola		
Didymorhynchus		
Eutetrarhynchus	•	
Fellicocestus		★
Hemionchos		★
Mixodigma		
Mobulocestus		
Poecilorhynchus		★
Trigonolobium		★
82 genera	**56 genera**	**40 genera**

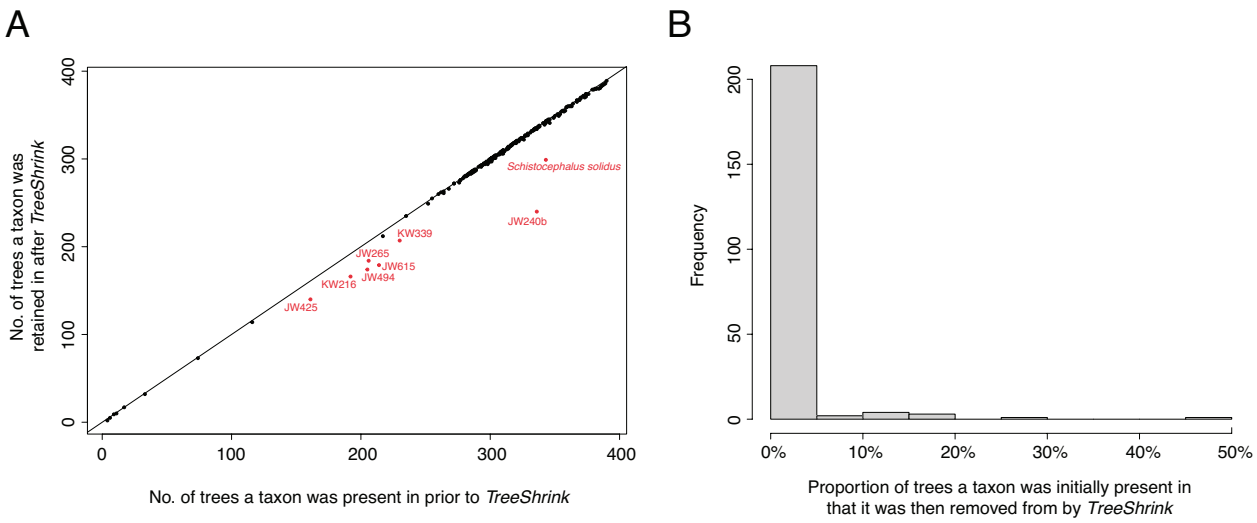


Figure 1. Scatter plot (A) and histogram (B) illustrating the proportion of gene trees from which individual specimens were removed by *TreeShrink*. In (A), specimens removed from a comparatively large proportion of gene trees in which they were present are highlighted in red with their unique specimen identification number or species name; see Table 1 for additional data for these specimens. The line plotted in (A) is y=x.

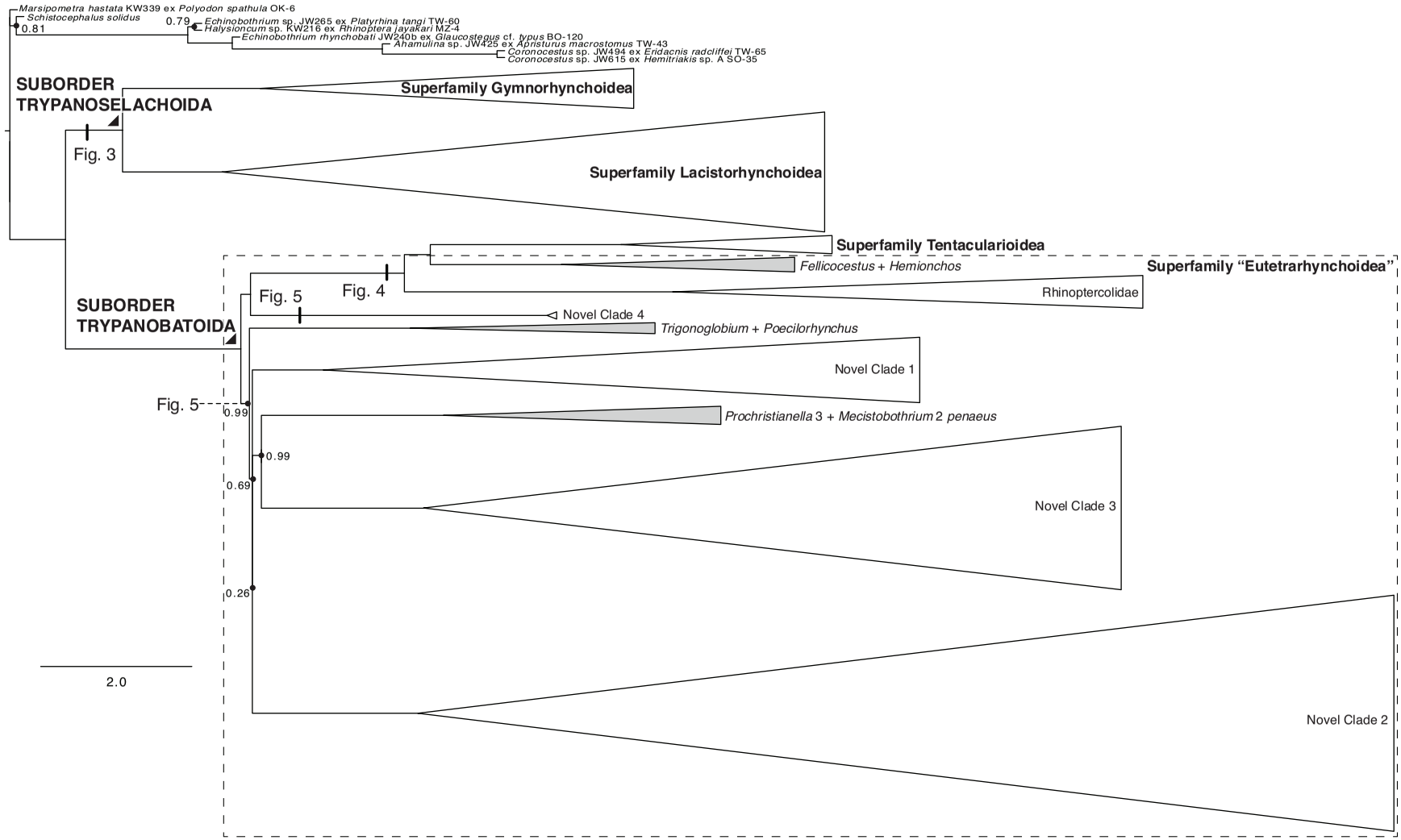


Figure 2. Schematic of species tree topology for the order Trypanorhyncha generated by *ASTRAL-III* based on 211 ingroup and eight outgroup specimens and 402 input maximum likelihood gene trees. Nodal support values are local posterior probabilities (localPP) and are equal to 1 unless otherwise indicated by a solid black circle and a specified localPP value. Scale indicates branch lengths measured in coalescent units (CU). Clade names follow Beveridge et al. (2017); clades not represented in Beveridge et al. (2017) are shaded in grey. Figure numbers associated with nodes correspond to additional figures herein of detailed topologies within a clade.

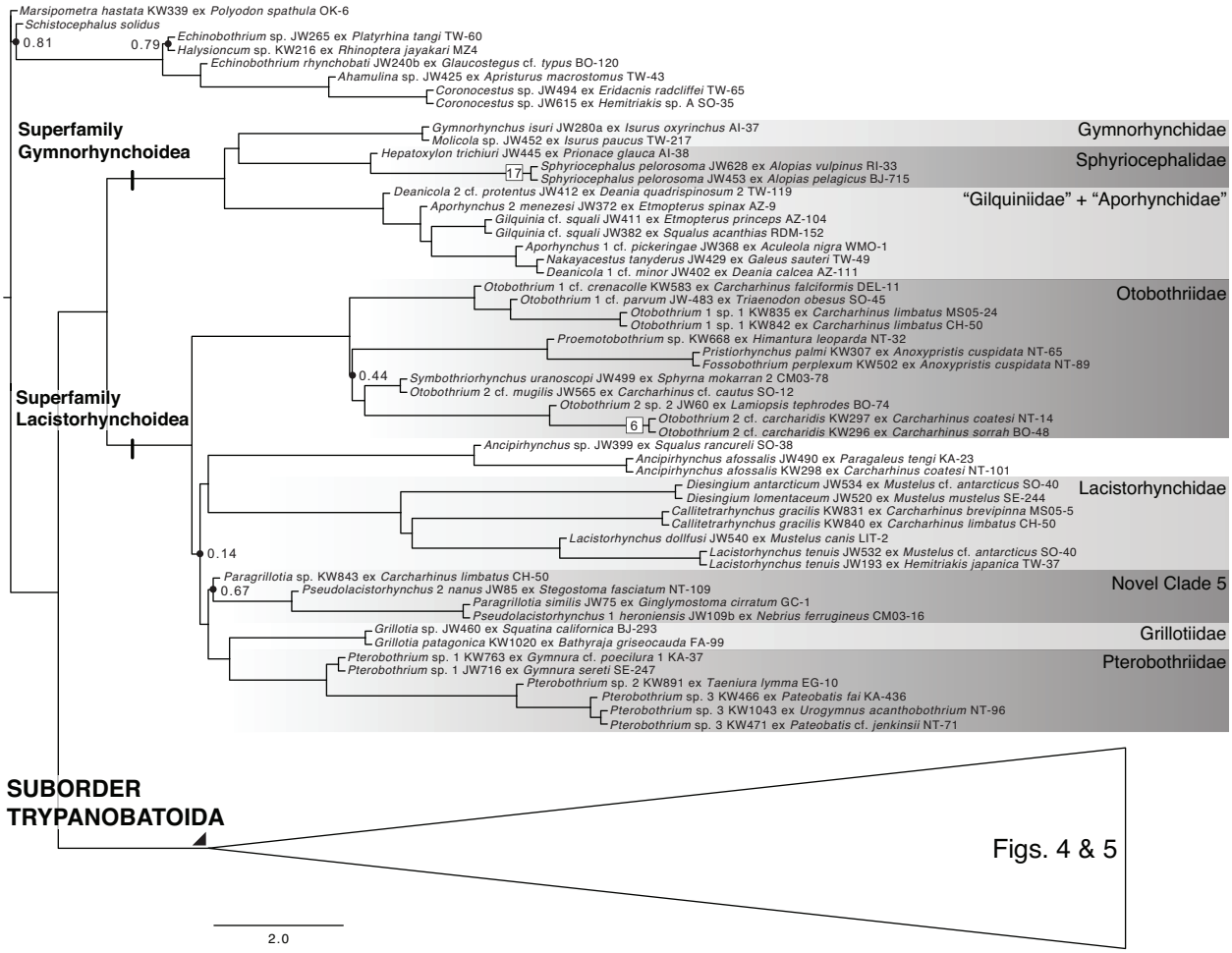


Figure 3. *ASTRAL-III* species tree topology expanded for the suborder Trypanoselachoida. Nodal support values are local posterior probabilities (localPP) and are equal to 1 unless otherwise indicated by a solid black circle and a specified localPP value. White squares indicate nodes supported by fewer than 20 genes, with the number of genes on which they are based specified. Scale indicates branch lengths measured in coalescent units (CU). Clade names follow Beveridge et al. (2017). Figure numbers associated with nodes correspond to additional figures herein of detailed topologies within a clade. Taxon labels consist of species-level specimen identification followed by unique specimen identification number, host species, and unique host specimen number.

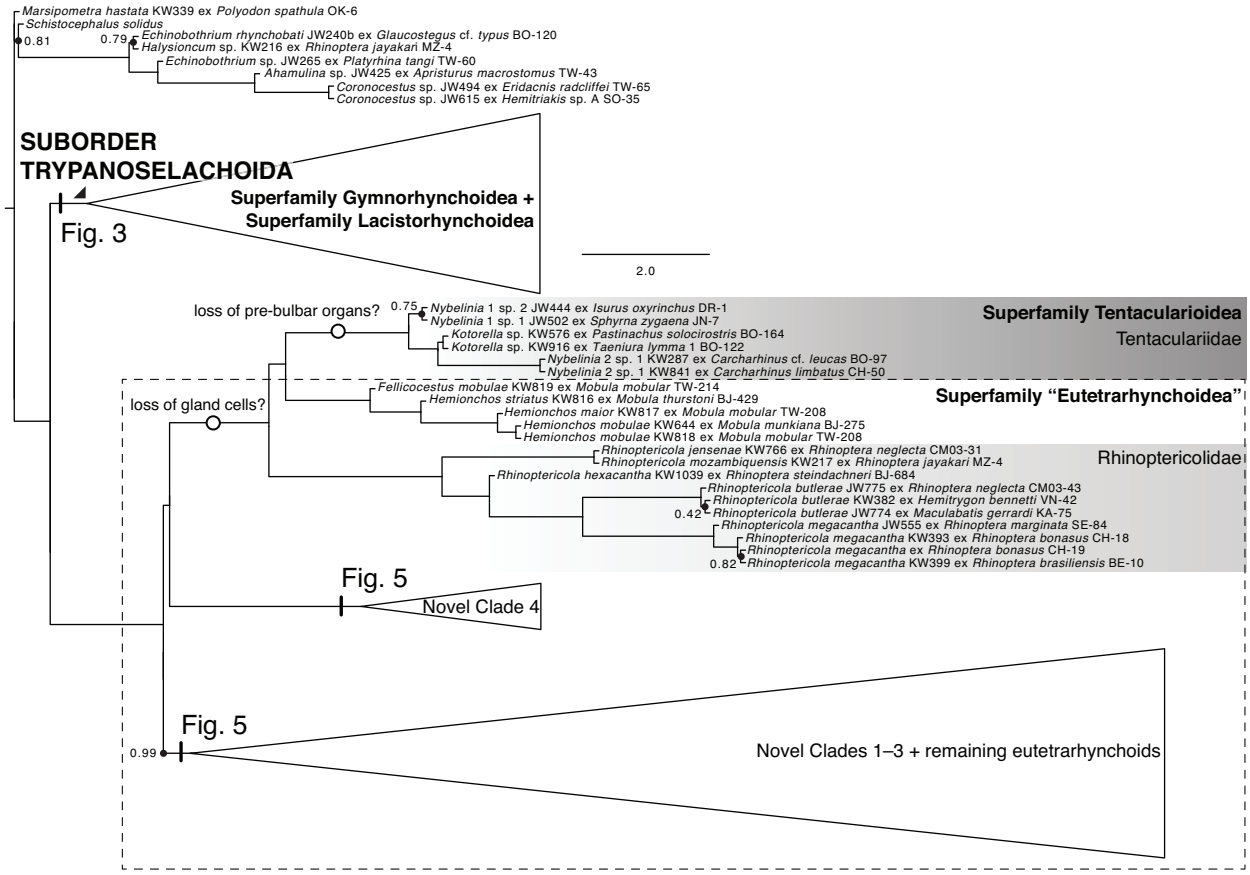
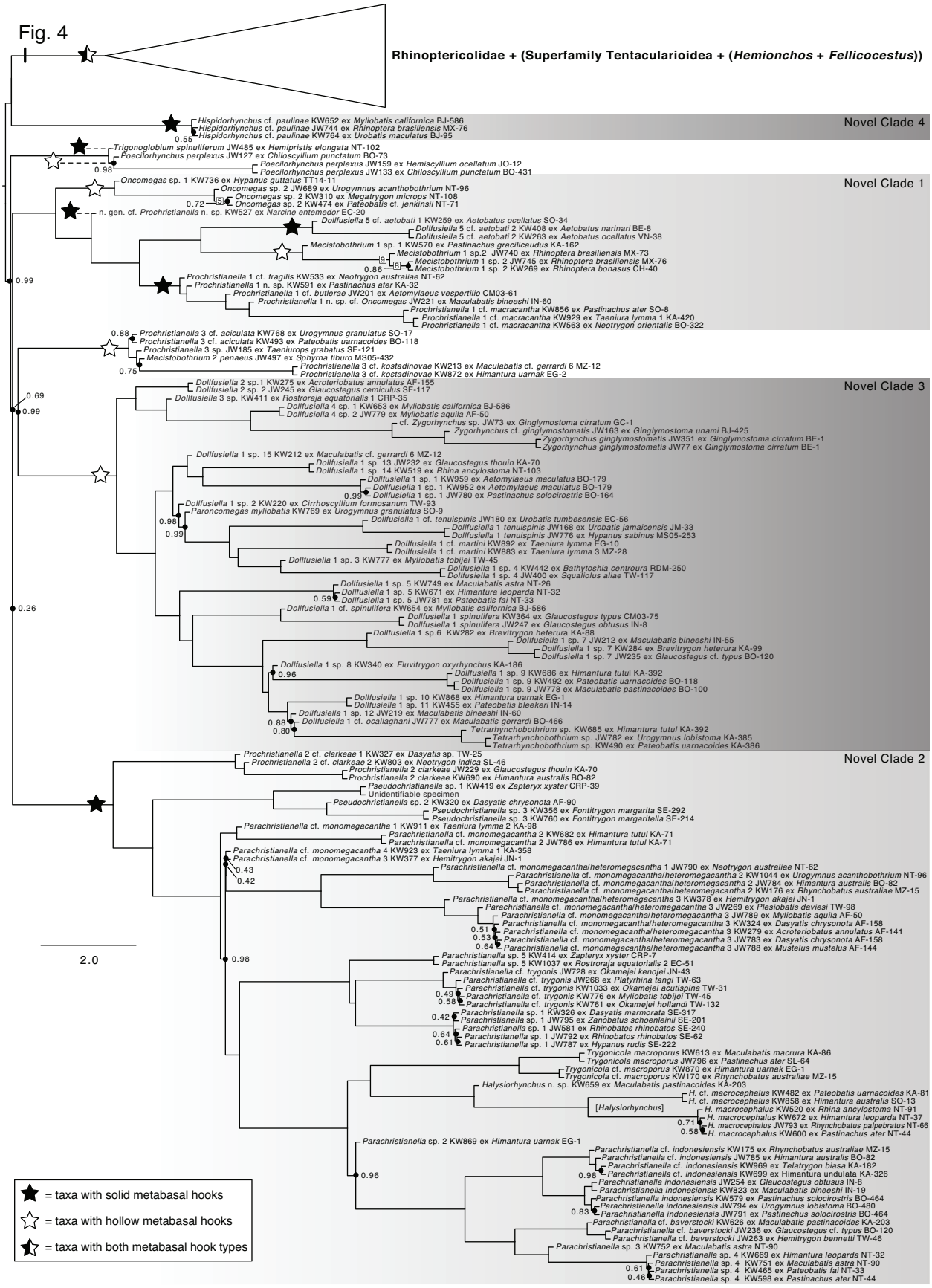
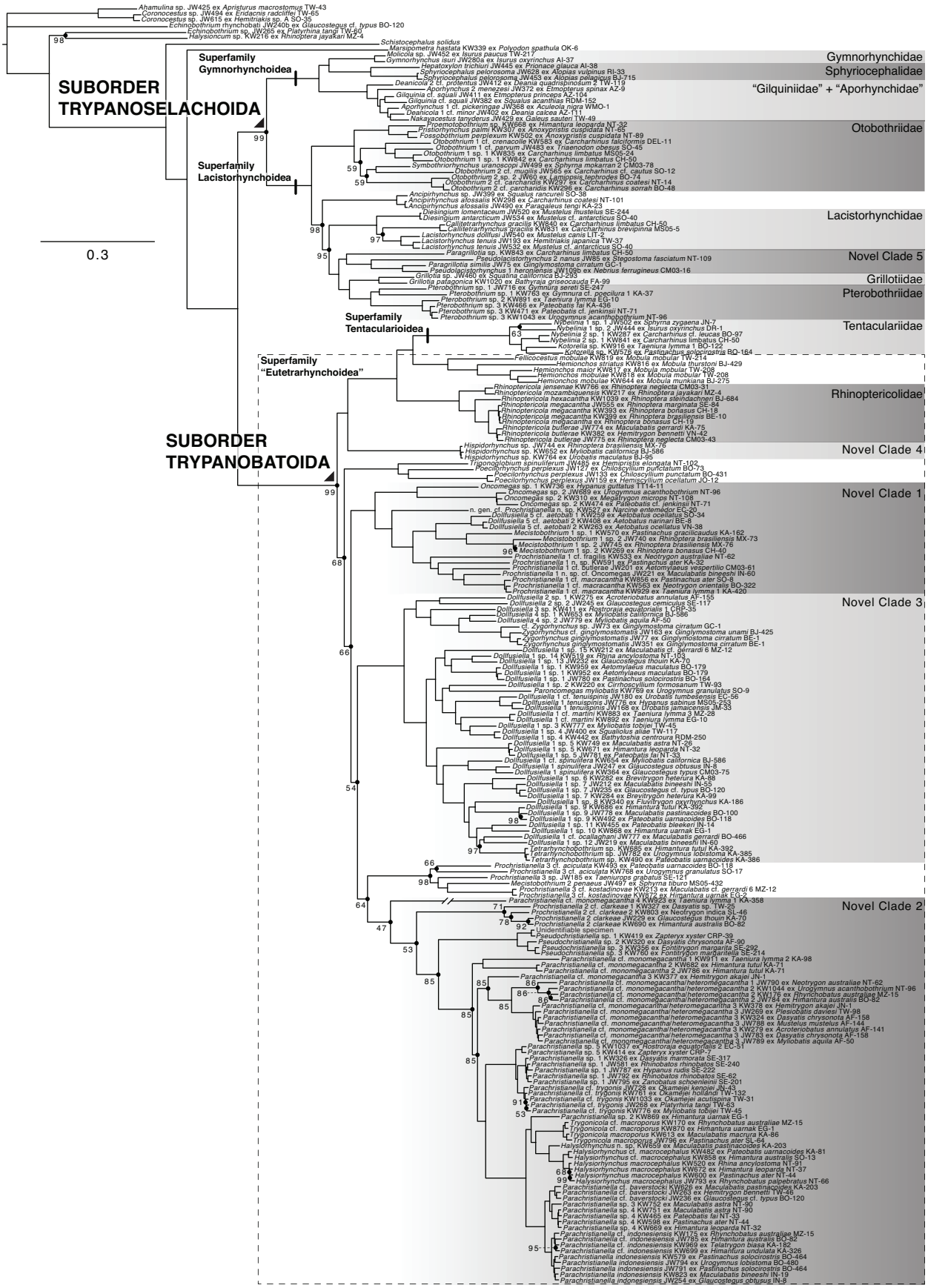


Figure 4. *ASTRAL-III* species tree topology expanded for the superfamily Tentacularioidea and allied "eutetrarhynchoid" taxa. Nodal support values are local posterior probabilities (localPP) and are equal to 1 unless otherwise indicated by a solid black circle and a specified localPP value. Scale indicates branch lengths measured in coalescent units (CU). Clade names follow Beveridge et al. (2017). Open circles on internodes indicate hypotheses for patterns of character evolution. Figure numbers associated with nodes correspond to additional figures herein of detailed topologies within a clade. Taxon labels consist of species-level specimen identification followed by unique specimen identification number, host species, and unique host specimen number.

Fig. 4


Rhinoptericolidae + (Superfamily Tentacularioidea + (*Hemionchos* + *Fellicocestus*))
Novel Clade 4
Novel Clade 1
Novel Clade 3
Novel Clade 2
Hispidorhynchus cf. *paulinae* KW652 ex *Myliobatis californica* BJ-586
Hispidorhynchus cf. *paulinae* JW744 ex *Rhinoptera brasiliensis* MX-76
Hispidorhynchus cf. *paulinae* KW764 ex *Urobatis maculatus* BJ-95
Trigonoglobium spinuliferum JW485 ex *Hemipristis elongata* NT-102
Poecilorhynchus perplexus JW127 ex *Chiloscyllium punctatum* BO-73
Poecilorhynchus perplexus JW159 ex *Hemiscyllium ocellatum* JO-12
Poecilorhynchus perplexus JW133 ex *Chiloscyllium punctatum* BO-431
Oncomegas sp. 1 KW736 ex *Hypanus guttatus* TT14-11
Oncomegas sp. 2 JW689 ex *Urogymnus acanthobothrium* NT-96
Oncomegas sp. 2 KW310 ex *Megatrygon microps* NT-108
Oncomegas sp. 2 KW474 ex *Pateobatis* cf. *jenkinsi* NT-71
n. gen. cf. *Prochristianella* n. sp. KW527 ex *Narcine entemedor* EC-20
Dollfusiella 5 cf. *aetobati* 1 KW259 ex *Aetobatus ocellatus* SO-34
Dollfusiella 5 cf. *aetobati* 2 KW408 ex *Aetobatus narinari* BE-8
Dollfusiella 5 cf. *aetobati* 2 KW263 ex *Aetobatus ocellatus* VN-38
Mecistobothrium 1 sp. 1 KW570 ex *Pastinachus gracilicaudus* KA-162
Mecistobothrium 1 sp.2 JW740 ex *Rhinoptera brasiliensis* MX-73
Mecistobothrium 1 sp. 2 JW745 ex *Rhinoptera brasiliensis* MX-76
Mecistobothrium 1 sp. 2 KW269 ex *Rhinoptera bonasus* CH-40
Prochristianella 1 cf. *fragilis* KW533 ex *Neotrygon australiae* NT-62
Prochristianella 1 n. sp. KW591 ex *Pastinachus ater* KA-32
Prochristianella 1 cf. *butlerae* JW201 ex *Aetomylaeus vespertilio* CM03-61
Prochristianella 1 n. sp. cf. *Oncomegas* JW221 ex *Maculabatis bineeshi* IN-60
Prochristianella 1 cf. *macracantha* KW856 ex *Pastinachus ater* SO-8
Prochristianella 1 cf. *macracantha* KW929 ex *Taeniura lymma* 1 KA-420
Prochristianella 1 cf. *macracantha* KW563 ex *Neotrygon orientalis* BO-322
Prochristianella 3 cf. *aciculata* KW768 ex *Urogymnus granulatus* SO-17
Prochristianella 3 cf. *aciculata* KW493 ex *Pateobatis uarnacoides* BO-118
Prochristianella 3 sp. JW185 ex *Taeniurops grabatus* SE-121
Mecistobothrium 2 *penaeus* JW497 ex *Sphyrna tiburo* MS05-432
Prochristianella 3 cf. *kostadinovae* KW213 ex *Maculabatis* cf. *gerrardi* 6 MZ-12
Prochristianella 3 cf. *kostadinovae* KW872 ex *Himantura uarnak* EG-2
Dollfusiella 2 sp.1 KW275 ex *Acroteriobatus annulatus* AF-155
Dollfusiella 2 sp. 2 JW245 ex *Glaucostegus cemiculus* SE-117
Dollfusiella 3 sp. KW411 ex *Rostroraja equatorialis* 1 CRP-35
Dollfusiella 4 sp. 1 KW653 ex *Myliobatis californica* BJ-586
Dollfusiella 4 sp. 2 JW779 ex *Myliobatis aquila* AF-50
cf. *Zygorhynchus* sp. JW73 ex *Ginglymostoma cirratum* GC-1
Zygorhynchus cf. *ginglymostomatis* JW163 ex *Ginglymostoma unami* BJ-425
Zygorhynchus ginglymostomatis JW351 ex *Ginglymostoma cirratum* BE-1
Zygorhynchus ginglymostomatis JW77 ex *Ginglymostoma cirratum* BE-1
Dollfusiella 1 sp. 15 KW212 ex *Maculabatis* cf. *gerrardi* 6 MZ-12
Dollfusiella 1 sp. 13 JW232 ex *Glaucostegus thouin* KA-70
Dollfusiella 1 sp. 14 KW519 ex *Rhina ancylostoma* NT-103
Dollfusiella 1 sp. 1 KW959 ex *Aetomylaeus maculatus* BO-179
Dollfusiella 1 sp. 1 KW952 ex *Aetomylaeus maculatus* BO-179
Dollfusiella 1 sp. 1 JW780 ex *Pastinachus solocirostris* BO-164
Dollfusiella 1 sp. 2 KW220 ex *Cirrhoscyllium formosanum* TW-93
Paroncomegas myliobatis KW769 ex *Urogymnus granulatus* SO-9
Dollfusiella 1 cf. *tenuispinis* JW180 ex *Urobatis tumbesensis* EC-56
Dollfusiella 1 *tenuispinis* JW168 ex *Urobatis jamaicensis* JM-33
Dollfusiella 1 *tenuispinis* JW776 ex *Hypanus sabinus* MS05-253
Dollfusiella 1 cf. *martini* KW892 ex *Taeniura lymma* EG-10
Dollfusiella 1 cf. *martini* KW883 ex *Taeniura lymma* 3 MZ-28
Dollfusiella 1 sp. 3 KW777 ex *Myliobatis tobijei* TW-45
Dollfusiella 1 sp. 4 KW442 ex *Bathytoshia centroura* RDM-250
Dollfusiella 1 sp. 4 JW400 ex *Squaliolus aliae* TW-117
Dollfusiella 1 sp. 5 KW749 ex *Maculabatis astra* NT-26
Dollfusiella 1 sp. 5 KW671 ex *Himantura leoparda* NT-32
Dollfusiella 1 sp. 5 JW781 ex *Pateobatis fai* NT-33
Dollfusiella 1 cf. *spinulifera* KW654 ex *Myliobatis californica* BJ-586
Dollfusiella 1 *spinulifera* KW364 ex *Glaucostegus typus* CM03-75
Dollfusiella 1 *spinulifera* JW247 ex *Glaucostegus obtusus* IN-8
Dollfusiella 1 sp.6 KW282 ex *Brevitrygon heterura* KA-88
Dollfusiella 1 sp. 7 JW212 ex *Maculabatis bineeshi* IN-55
Dollfusiella 1 sp. 7 KW284 ex *Brevitrygon heterura* KA-99
Dollfusiella 1 sp. 7 JW235 ex *Glaucostegus* cf. *typus* BO-120
Dollfusiella 1 sp. 8 KW340 ex *Fluvitrygon oxyrhynchus* KA-186
Dollfusiella 1 sp. 9 KW686 ex *Himantura tutul* KA-392
Dollfusiella 1 sp. 9 KW492 ex *Pateobatis uarnacoides* BO-118
Dollfusiella 1 sp. 9 JW778 ex *Maculabatis pastinacoides* BO-100
Dollfusiella 1 sp. 10 KW868 ex *Himantura uarnak* EG-1
Dollfusiella 1 sp. 11 KW455 ex *Pateobatis bleekeri* IN-14
Dollfusiella 1 sp. 12 JW219 ex *Maculabatis bineeshi* IN-60
Dollfusiella 1 cf. *ocallaghani* JW777 ex *Maculabatis gerrardi* BO-466
Tetrarhynchobothrium sp. KW685 ex *Himantura tutul* KA-392
Tetrarhynchobothrium sp. JW782 ex *Urogymnus lobistoma* KA-385
Tetrarhynchobothrium sp. KW490 ex *Pateobatis uarnacoides* KA-386
Prochristianella 2 cf. *clarkeae* 1 KW327 ex *Dasyatis* sp. TW-25
Prochristianella 2 cf. *clarkeae* 2 KW803 ex *Neotrygon indica* SL-46
Prochristianella 2 *clarkeae* JW229 ex *Glaucostegus thouin* KA-70
Prochristianella 2 *clarkeae* KW690 ex *Himantura australis* BO-82
Pseudochristianella sp. 1 KW419 ex *Zapteryx xyster* CRP-39
Unidentifiable specimen
Pseudochristianella sp. 2 KW320 ex *Dasyatis chrysonota* AF-90
Pseudochristianella sp. 3 KW356 ex *Fontitrygon margarita* SE-292
Pseudochristianella sp. 3 KW760 ex *Fontitrygon margaritella* SE-214
Parachristianella cf. *monomegacantha* 1 KW911 ex *Taeniura lymma* 2 KA-98
Parachristianella cf. *monomegacantha* 2 KW682 ex *Himantura tutul* KA-71
Parachristianella cf. *monomegacantha* 2 JW786 ex *Himantura tutul* KA-71
Parachristianella cf. *monomegacantha* 4 KW923 ex *Taeniura lymma* 1 KA-358
Parachristianella cf. *monomegacantha* 3 KW377 ex *Hemitrygon akajei* JN-1
Parachristianella cf. *monomegacantha/heteromegacantha* 1 JW790 ex *Neotrygon australiae* NT-62
Parachristianella cf. *monomegacantha/heteromegacantha* 2 KW1044 ex *Urogymnus acanthobothrium* NT-96
Parachristianella cf. *monomegacantha/heteromegacantha* 2 JW784 ex *Himantura australis* BO-82
Parachristianella cf. *monomegacantha/heteromegacantha* 2 KW176 ex *Rhynchobatus australiae* MZ-15
Parachristianella cf. *monomegacantha/heteromegacantha* 3 KW378 ex *Hemitrygon akajei* JN-1
Parachristianella cf. *monomegacantha/heteromegacantha* 3 JW269 ex *Plesiobatis daviesi* TW-98
Parachristianella cf. *monomegacantha/heteromegacantha* 3 JW789 ex *Myliobatis aquila* AF-50
Parachristianella cf. *monomegacantha/heteromegacantha* 3 KW324 ex *Dasyatis chrysonota* AF-158
Parachristianella cf. *monomegacantha/heteromegacantha* 3 KW279 ex *Acroteriobatus annulatus* AF-141
Parachristianella cf. *monomegacantha/heteromegacantha* 3 JW783 ex *Dasyatis chrysonota* AF-158
Parachristianella cf. *monomegacantha/heteromegacantha* 3 JW788 ex *Mustelus mustelus* AF-144
Parachristianella sp. 5 KW414 ex *Zapteryx xyster* CRP-7
Parachristianella sp. 5 KW1037 ex *Rostroraja equatorialis* 2 EC-51
Parachristianella cf. *trygonis* JW728 ex *Okamejei kenojei* JN-43
Parachristianella cf. *trygonis* JW268 ex *Platyrhina tangi* TW-63
Parachristianella cf. *trygonis* KW1033 ex *Okamejei acutispina* TW-31
Parachristianella cf. *trygonis* KW776 ex *Myliobatis tobijei* TW-45
Parachristianella cf. *trygonis* KW761 ex *Okamejei hollandi* TW-132
Parachristianella sp. 1 KW326 ex *Dasyatis marmorata* SE-317
Parachristianella sp. 1 JW795 ex *Zanobatus schoenleinii* SE-201
Parachristianella sp. 1 JW581 ex *Rhinobatos rhinobatos* SE-240
Parachristianella sp. 1 JW792 ex *Rhinobatos rhinobatos* SE-62
Parachristianella sp. 1 JW787 ex *Hypanus rudis* SE-222
Trygonicola macroporus KW613 ex *Maculabatis macrura* KA-86
Trygonicola macroporus JW796 ex *Pastinachus ater* SL-64
Trygonicola cf. *macroporus* KW870 ex *Himantura uarnak* EG-1
Trygonicola cf. *macroporus* KW170 ex *Rhynchobatus australiae* MZ-15
Halysiorhynchus n. sp. KW659 ex *Maculabatis pastinacoides* KA-203
H. cf. *macrocephalus* KW482 ex *Pateobatis uarnacoides* KA-81
H. cf. *macrocephalus* KW858 ex *Himantura australis* SO-13
[*Halysiorhynchus*]
H. macrocephalus KW520 ex *Rhina ancylostoma* NT-91
H. macrocephalus KW672 ex *Himantura leoparda* NT-37
H. macrocephalus JW793 ex *Rhynchobatus palpebratus* NT-66
H. macrocephalus KW600 ex *Pastinachus ater* NT-44
Parachristianella sp. 2 KW869 ex *Himantura uarnak* EG-1
Parachristianella cf. *indonesiensis* KW175 ex *Rhynchobatus australiae* MZ-15
Parachristianella cf. *indonesiensis* JW785 ex *Himantura australis* BO-82
Parachristianella cf. *indonesiensis* KW969 ex *Telatrygon biasa* KA-182
Parachristianella cf. *indonesiensis* KW699 ex Himantura undulata KA-326
Parachristianella indonesiensis JW254 ex *Glaucostegus obtusus* IN-8
Parachristianella indonesiensis KW823 ex *Maculabatis bineeshi* IN-19
Parachristianella indonesiensis KW579 ex *Pastinachus solocirostris* BO-464
Parachristianella indonesiensis JW794 ex *Urogymnus lobistoma* BO-480
Parachristianella indonesiensis JW791 ex *Pastinachus solocirostris* BO-464
Parachristianella cf. *baverstocki* KW626 ex *Maculabatis pastinacoides* KA-203
Parachristianella cf. *baverstocki* JW236 ex *Glaucostegus* cf. *typus* BO-120
Parachristianella cf. *baverstocki* JW263 ex *Hemitrygon bennetti* TW-46
Parachristianella sp. 3 KW752 ex *Maculabatis astra* NT-90
Parachristianella sp. 4 KW669 ex *Himantura leoparda* NT-32
Parachristianella sp. 4 KW751 ex *Maculabatis astra* NT-90
Parachristianella sp. 4 KW465 ex *Pateobatis fai* NT-33
Parachristianella sp. 4 KW598 ex *Pastinachus ater* NT-44
0.55
0.98
0.72
0.99
0.86
0.88
0.75
0.69
0.99
0.26
0.98
0.99
0.99
0.59
0.96
0.88
0.80
0.43
0.42
2.0
0.98
0.49
0.58
0.42
0.64
0.61
0.71
0.58
0.96
0.98
0.83
0.61
0.46
= taxa with solid metabasal hooks
= taxa with hollow metabasal hooks
= taxa with both metabasal hook types

◄ **Figure 5.** ***ASTRAL-III*** **species tree topology expanded for the largest clade of "eutetrarhynchoid" taxa, with important metabasal hook characteristics mapped for individual subclades.** Nodal support values are local posterior probabilities (localPP) and are equal to 1 unless otherwise indicated by a solid black circle and a specified localPP value. White squares indicate nodes supported by fewer than 20 genes, with the number of genes on which they are based specified. Scale indicates branch lengths measured in coalescent units (CU). Clade names follow Beveridge et al. (2017). Figure numbers associated with nodes correspond to additional figures herein of detailed topologies within a clade. Taxon labels consist of species-level specimen identification followed by unique specimen identification number, host species, and unique host specimen number.


SUBORDER TRYPANOSELACHOIDA
SUBORDER TRYPANOBATOIDA
Superfamily Gymnorhynchoidea
Superfamily Lacistorhynchoidea
Superfamily Tentacularioidea
Superfamily "Eutetrarhynchoidea"
Gymnorhynchidae
Sphyriocephalidae
"Gilquiniidae" + "Aporhynchidae"
Otobothriidae
Lacistorhynchidae
Novel Clade 5
Grillotiidae
Pterobothriidae
Tentacularriidae
Rhinoptericolidae
Novel Clade 4
Novel Clade 1
Novel Clade 3
Novel Clade 2
0.3

◄ **Figure 6. Maximum likelihood species tree topology for the order Trypanorhyncha generated by *IQ-TREE* based on 211 ingroup and eight outgroup specimens and 402 genes grouped into 64 partitions.** Nodal support values are based on 1,000 ultrafast bootstrap replicates (UFBS) and are equal to 100 unless indicated by a solid black circle and a specified UFBS value. Scale indicates nucleotide substitutions per site. Clade names follow Beveridge et al. (2017). Taxon labels consist of species-level specimen identification followed by unique specimen identification number, host species, and unique host specimen number.