Journal of the Marine Biological Association of the United Kingdom, 2008, 88(8), 1663–1672.
doi:10.1017/S0025315408002257 Printed in the United Kingdom

Phylogenetics of Hydroidolina (Hydrozoa: Cnidaria)

PAULYN CARTWRIGHT[1], NATHANIEL M. EVANS[1], CASEY W. DUNN[2], ANTONIO C. MARQUES[3], MARIA PIA MIGLIETTA[4], PETER SCHUCHERT[5] AND ALLEN G. COLLINS[6]

[1]Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66049, USA, [2]Department of Ecology and Evolutionary Biology, Brown University, Providence RI 02912, USA, [3]Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil, [4]Department of Biology, Pennsylvania State University, University Park, PA 16802, USA, [5]Muséum d'Histoire Naturelle, CH-1211, Genève, Switzerland, [6]National Systematics Laboratory of NOAA Fisheries Service, NMNH, Smithsonian Institution, Washington, DC 20013, USA

Hydroidolina is a group of hydrozoans that includes Anthoathecata, Leptothecata and Siphonophorae. Previous phylogenetic analyses show strong support for Hydroidolina monophyly, but the relationships between and within its subgroups remain uncertain. In an effort to further clarify hydroidolinan relationships, we performed phylogenetic analyses on 97 hydroidolinan taxa, using DNA sequences from partial mitochondrial 16S rDNA, nearly complete nuclear 18S rDNA and nearly complete nuclear 28S rDNA. Our findings are consistent with previous analyses that support monophyly of Siphonophorae and Leptothecata and do not support monophyly of Anthoathecata nor its component subgroups, Filifera and Capitata. Instead, within Anthoathecata, we find support for four separate filiferan clades and two separate capitate clades (Aplanulata and Capitata sensu stricto*). Our data however, lack any substantive support for discerning relationships between these eight distinct hydroidolinan clades.*

Keywords: phylogenetics, Hydroidolina, Hydrozoa, Cnidaria

Submitted 30 November 2007; accepted 12 May 2008; first published online 29 July 2008

INTRODUCTION

Hydroidolina (=Leptolina) is a clade of hydrozoans comprising Leptothecata (=Leptomedusae, Thecata), Anthoathecata (=Anthomedusae, Athecata) and Siphonophorae (Collins, 2002; Marques & Collins, 2004; Collins *et al.*, 2006). Amongst the approximately 3220 valid species of Hydroidolina (Bouillon *et al.*, 2006), there exist vast amounts of diversity in the morphologies of hydroids and medusae as well as in life cycles. Uncovering a robust phylogeny for Hydroidolina would shed insight into the patterns underlying this diversity and provide a framework for generating hypotheses concerning processes responsible for their evolution. In addition, molecular phylogenies of Hydroidolina could help serve as a guide to taxonomic classification, which has been somewhat problematic, in large part due to inconsistencies in classifications of hydroids and medusae (e.g. Bouillon, 1985, 1994).

Hydrozoan phylogenetics has seen much progress in recent years, particularly in revealing major hydrozoan lineages and questioning others. For example, phylogenetic analyses have shown that Hydrozoa comprises two well-supported, reciprocally monophyletic clades, Trachylina and Hydroidolina (Marques & Collins, 2004; Marques, 2001a; Collins, 2002; Collins *et al.*, 2006; Van Iten *et al.*, 2006). Siphonophorae is a clade (Collins, 2002; Dunn *et al.*, 2005), but its phylogenetic position within Hydroidolina is uncertain (Collins, 2002; Collins *et al.*, 2006). Similarly, there is strong support for the monophyly of Leptothecata (Collins *et al.*, 2006; Leclère *et al.*, 2007), but no well-supported hypotheses have emerged regarding its relationship with other hydroidolinans. Molecular phylogenetic studies do not support the monophyly of Anthoathecata and instead suggest that it is a paraphyletic assemblage that has given rise to one or more hydroidolinan groups (Marques & Collins, 2000; Marques, 2001a; Collins, 2002; Collins *et al.*, 2006; Van Iten *et al.*, 2006).

Although these studies illuminated the phyletic status of the three main groups of Hydroidolina, the relationships within and between these groups remain uncertain (Collins *et al.*, 2006). In addition, with exception to studies on Kirchenpaueriidae (Peña Cantero & Marques, 1999), Corynidae (Collins *et al.*, 2005), Siphonophorae (Dunn *et al.*, 2005), Tubulariidae (Marques & Migotto, 2001), Campanulariidae (Govindarajan *et al.*, 2006), Hebellidae–Lafoeidae (Marques *et al.*, 2006) and Plumularioidea (Leclère *et al.*, 2006), relationships within component hydroidolinan groups have not been studied within a detailed phylogenetic framework. In an effort to further clarify relationships within Hydroidolina, we greatly augmented the published molecular dataset of hydroidolinan taxa using three molecular markers, the nuclear large (28S) and small (18S) subunit rDNAs and the mitochondrial large subunit rDNA (16S). We present combined phylogenetic analyses of 97 hydroidolinan taxa (plus 13 trachyline taxa as outgroups) under maximum likelihood (ML) and parsimony (MP) criteria. The augmented dataset reveals new evolutionary

Corresponding author:
P. Cartwright
Email: pcart@ku.edu

patterns in morphology, although a more thorough sampling is needed to further clarify these patterns. These data suggest that a molecular phylogenetic approach is promising for guiding future taxonomic classifications but further study is needed to elucidate phylogenetic patterns of the deeper nodes within Hydroidolina.

MATERIALS AND METHODS

Taxa sampled, DNA isolation, amplification and sequencing

The 110 hydrozoan taxa used in this study are arranged taxonomically in Table 1, including GenBank accession and museum voucher numbers. The sequences in Table 1 comprise both published and new DNA sequences generated for this study. Although most new sequences correspond to museum voucher specimens, some were included that had no associated vouchers, but for which published sequences of other markers were generated from the same DNA pool. For new sequences, genomic DNA was extracted using Qiagen DNeasy kits according to the manufacturer's protocol (QIAGEN Inc., Mississauga, ON) or a standard phenol/chloroform protocol. The latter method involved tissue digestion with proteinase K (20 mg/ml) in a lysis buffer (20 mM Tris–CL pH 8.0, 5 mM EDTA pH 8.0, 400 mM NaCl, 2% SDS), extraction with phenol/chloroform (1:1), precipitation with 2.5 vol. 95% EtOH and elution in TE or H_2O.

An approximately 600 bp fragment of 16S was amplified using a modified forward primer (F1Mod: TCGACTGTTTA CCAAAAACATA) and reverse primer (R2: ACGGAATGA ACTCAAATCATGTAAG) from Cunningham & Buss (1993). Amplifications of 16S were conducted with the following thermal profile: 5 minutes (min.) at 94°C; 5 cycles of 50 seconds(s) at 94°C, 50 s at 45°C and 1 min. at 72°C; 30 cycles of 5 s at 94°C, 50 s at 50°C and 1 min. at 72°C; 10 min. at 72°C. An approximately 1.8 kb portion of the gene coding for 18S was amplified with universal eukaryotic primers as described by Medlin *et al.* (1988). Nearly complete, an approximately 3 kb portion of the gene coding for 28S was amplified and sequenced according to Evans *et al.* (2008).

All gene fragments were purified and sequenced by Cogenics, Inc. (Houston, TX) and assembled and edited using Sequencher v4.5 (Gene Code Co., 2005). Sequences for each marker were aligned using the program MUSCLE (Edgar, 2004). Regions containing alignment ambiguities were removed using Gblocks v0.91b (Castresana, 2000) with default parameters except the minimum length block was set to 5 and half the taxa were allowed to be gaps for any given position (Table 2). The three datasets were concatenated into one combined dataset.

Phylogenetic analysis

Phylogenetic analyses were performed on individual markers and on the combined dataset using both maximum likelihood (ML) and parsimony (MP) criteria. ML searches were performed using GARLI v0.951.OsX-GUI (Zwickl, 2006) under an assumed GTR + I + G model with rates estimated from the data. The assumed model of nucleotide substitution was selected by using the Akaike information criterion (AIC) as implemented in ModelTest (Posada & Crandall, 2000). For the combined dataset the ML analysis was repeated 10 times from random starting trees using default termination conditions. Each run gave identical topologies and similar likelihood scores. 100 bootstrap replications were run in GARLI v0.951.0sX-GUI (Zwickl, 2006) under the same parameters.

MP analyses were performed using PAUP* 4.0.0b10 (Swofford, 1998). Heuristic analyses were run using 500 random addition sequences and TBR branch swapping. 100 bootstrap replications were run using 10 random addition sequences per replicate and TBR branch swapping. Most parsimonious trees were summarized as a strict consensus.

The concatenated, Gblocked DNA alignment and corresponding trees can be found in TreeBASE (http://www.treebase.org/treebase/index.html, accession No. S2066).

RESULTS AND DISCUSSION

After excluding the ambiguously aligned regions, the combined dataset of nearly complete 28S rDNA, nearly complete 18S rDNA and partial 16S rDNA contained 5046 characters, 1699 of which are parsimony informative. Information about individual markers is shown in Table 2. The markers were analysed separately under a ML optimality criterion and in a combined dataset under ML and MP optimality criteria. There is incongruence in topologies between the individual markers and very little support for most of the nodes in the 16S and 18S datasets (not shown). By contrast, the 28S topology is almost identical to the combined dataset (not shown) but the combined dataset shows a higher frequency of well-supported nodes (bootstrap values >50%), than the 28S topology (not shown). Given that the 28S and combined dataset are congruent but the combined dataset gives better overall support values, we concluded that the combined dataset provides the most robust hypotheses. Thus, all subsequent discussions are confined to the analyses of the combined dataset (Figures 1 & 2).

MP and ML analyses both support the monophyly of Hydroidolina (Figures 1 & 2). Trachyline relationships are treated in detail in this volume (see Collins *et al.*) and are therefore not discussed here. The hydroidolinan taxa included in these analyses sort out into eight different monophyletic clades (Figures 1 & 2; Table 1). The composition of taxa in these clades is identical in the ML and MP analyses (Figures 1 & 2). Both optimality criteria support the monophyly of Leptothecata and Siphonophorae. In the ML and MP topologies, 'Anthoathecata' is a polyphyletic assemblage with leptothecates and siphonophores derived within anthoathecate lineages. Although it should be noted that all of the nodes separating the different anthoathecate lineages are weakly supported, consistent with previous phylogenetic analyses (Collins, 2002; Marques & Collins, 2004; Collins *et al.*, 2005, 2006; Dunn *et al.*, 2005; Van Iten *et al.*, 2006; Leclère *et al.*, 2007). The separate anthoathecate clades that emerge from both the ML and MP analyses are Aplanulata (Collins *et al.*, 2005), Capitata *sensu stricto* and four filiferan clades (Figures 1 & 2; Table 1). The composition and relationships within these major clades are discussed below.

Relationships among these major clades of Hydroidolina are uncertain. There is very little bootstrap support (<50%) in the deeper nodes under both optimality criteria and there is incongruence in the ML and MP topologies between the

Table 1. Taxon and sequence list. A complete list of sequences used in the analyses with GenBank accession numbers and museum voucher numbers. Those in bold represent new sequences generated for this study.

Taxonomic hierarchy		**28S**	**18S**	**16S**	**Voucher**
Anthoathecata					
Capitata					
Cladocorynidae	*Cladocoryne floccosa*	EU272551	EU272608	AY512535	
Corynidae	*Dipurena ophiogaster*	EU272560	EU272615	**EU305473**	KUNHM 2803
Corynidae	*Sarsia nipponica*	**EU305530**	**EU448096**	**EU448100**	KUNHM 2627
Moerisiidae	*Moerisia* sp.	AY920801	AF358083	AY512534	
Pennariidae	*Pennaria disticha*	EU272581	AY920762	AM088481	
Polyorchidae	*Scrippsia pacifica*	AY920804	AF358091	AY512551	
Porpitidae	*Porpita* sp.	AY920803	AF358086	AY512529	
Porpitidae	*Velella* sp./*V. velella*	EU272597	AF358087	**EU305487**	
Solanderiidae	*Solanderia ericopsis*	EU272593	EU272636	AY78788	MHNG INVE 29593
Solanderiidae	*Solanderia secunda*	**EU305533**	**EU305502**	**EU305484**	KUNHM 2611
Zancleidae	*Zanclea prolifera*	EU272598	EU272639	**EU305488**	KUNHM 2793
Aplanulata					
Candelabridae	*Candelabrum cocksii*	AY920796	AY920758	AY512520	MHNG INVE 29591
Corymorphidae	*Corymorpha pendula*	**EU305510**	**EU305494**	**EU448098**	KUNHM 2962
Corymorphidae	*Euphysora bigelowi*	EU272563	EU272618	**EU448099**	KUNHM 2829
Hydridae	*Hydra circumcincta*	AY026371	AF358080	AY512521	
Tubulariidae	*Ectopleura dumortieri*	EU272561	EU272616	**EU305474**	
Tubulariidae	*Ralpharia gorgoniae*	EU272590	EU272633	**EU305482**	KUNHM 2778
Tubulariidae	*Zyzzyzus calderi*	EU272599	EU272640	**EU305489**	KUNHM 2777
Filifera I					
Eudendriidae	*Eudendrium californicum*	**EU305513**	**EU305492**	**EU305475**	KUNHM 2850
Eudendriidae	*Eudendrium capillare*	**EU305514**		**EU305476**	KUNHM 2625
Eudendriidae	*Eudendrium racemosum*	EU272562	EU272617	AY787896	
Filifera II					
incertae sedis	*Brinckmannia hexactinellidophila*	EU272550	EU272607	AM183123	MHNG INVE 38148
Laingiidae	*Fabienna sphaerica*	AY920797	AY920767	AM183133	MHNG INVE 33453
Proboscidactylidae	*Proboscidactyla flavicirrata*	**EU305527**	**EU305500**	**EU305480**	USNM 1074994
Proboscidactylidae	*Proboscidactyla ornata*	EU272587	EU272631	**EU305481**	KUNHM 2767
Ptilocodiidae	*Hydrichthella epigorgia*	EU272569	EU272622	**EU305478**	KUNHM 2665
Filifera III					
Hydractiniidae	*Clava multicornis*	EU272552	EU272609	**EU305471**	
Hydractiniidae	*Clavactinia gallensis*	EU272553	EU272610	**EU448101**	MHNG INVE 33470
Hydractiniidae	*Hydractinia* sp.	**EU305518**	**EU305495**	**EU305477**	KUNHM 2876
Hydractiniidae	*Hydractinia symbiolongicarpus*	EU272568	EU272621		
Hydractiniidae	*Podocoryne carnea*	AY920802	AF358092	AY512513	
Stylasteridae	*Adelopora crassilabrum*	EU272541	EU272642		USNM 1027760
Stylasteridae	*Conopora anthohelia*	**EU305509**			
Stylasteridae	*Crypthelia cryptotrema*	EU272558	EU272641		USNM 1027758
Stylasteridae	*Lepidopora microstylus*	EU272572	EU272644		USNM 1027724
Stylasteridae	*Pseudocrypthelia pachypoma*	EU272589	EU272643		USNM 1027728
Filifera IV					
Bougainvilliidae	*Dicoryne conybearei*	EU272559	EU272614	AM183141	MHNG INVE 32949
Bougainvilliidae	*Bimeria vestita*	EU272548	EU272605	AM183130	
Bougainvilliidae	*Bougainvillia carolinensis*	EU272549	EU272606		
Bougainvilliidae	*Bougainvillia fulva*	**EU305507**	**EU305490**	**EU305470**	KUNHM 2816
Bougainvilliidae	*Garveia annulata/Garveia* sp.	EU272564	AY920766		KUNHM 2860
Bougainvilliidae	*Koellikerina fasciculata*	EU272571	EU272623	AM183129	
Bougainvilliidae	*Pachycordyle pusilla*	EU272579	EU272627	AM183132	MHNG INVE 32953
Bougainvilliidae	*Pruvotella (Garvia) grisea*	EU272588	EU272632	AM183131	MHNG INVE 34436
Oceaniidae	*Cordylophora caspia*	EU272556	EU272612	**EU305472**	
Oceaniidae	*Corydendrium* sp.	EU272557	EU272613		KUNHM 2764
Oceaniidae	*Rhizogeton nudus*	EU272592	EU272635	AY787883	MHNG INVE 35757
Oceaniidae	*Turritopsis dohrnii*	EU272596	EU272638	AY787889	MHNG INVE 29753
Oceaniidae	*Turritopsis nutricula*	**EU305538**	**EU305504**	**EU305486**	KUNHM 2817
Pandeidae	*Hydrichthys boycei*	EU272570	**EU305496**	**EU448102**	MHNG INVE 37417
Pandeidae	*Leuckartiara octona*	EU272573	EU272624	AM411421	
Pandeidae	*Neoturris breviconis*	**EU305524**	**EU448097**	**EU448103**	KUNHM 002961
Pandeidae	*Pandea* sp.	EU272580	AY920765		
Rathkeidae	*Lizzia blondina*	EU272574	EU272625	AM411417	
Rathkeidae	*Rathkea octopunctata*	EU272591	EU272634	**EU305483**	KUMIP 314321
Leptothecata					
Conica					
Aequoreidae	*Aequorea aequorea*	**EU305505**	AF358076	AY512518	
Aequoreidae	*Aequorea floridana*	**EU305506**			USNHM PENDING

Continued

Table 1. Continued

Taxonomic hierarchy		28S	18S	16S	Voucher
Aequoreidae	*Aequorea victoria*	AY920799	AF358077	**EU305469**	KUNHM 2867
Aequoreidae	*Rhacostoma atlantica*	**EU305528**	**EU305501**		
Aglaopheniidae	*Aglaophenia tubiformis*	EU272543	EU272601	AY787914	MHNG INVE 29967
Blackfordiidae	*Blackfordia virginica*	AY920800	AF358078	AY512516	
Eirenidae	*Eutima sapinhoa*	**EU305515**	**EU305493**		
Haleciidae	*Halecium muricatum*	EU272565	EU272619	AY787915	MHNG INVE 29028
Halopterididae	*Halopteris minuta*	EU272567	EU272620	AY787912	MHNG INVE 25073
Halopterididae	*Monostaechas quadridens*	**EU305521**	**EU305497**	DQ855941	
Hebellidae	*Anthohebella parasitica*	EU272545	EU272603	AY787918	MHNG INVE 29762
Lafoeidae	*Lafoea dumosa*	**EU305520**		AY787917	MHNG INVE 29952
Laodiceidae	*Melicertissa* sp.	AY920798	AF358075	AY512515	
Malagazziidae	*Octophialucium indicum*	EU272577	EU272626	AY787897	MHNG INVE 29970
Melicertidae	*Melicertum octocostatum*	EU272575	AY920757	**EU305479**	USNM 1073342
Mitrocomidae	*Tiaropsidium kelseyi*	**EU305537**	AF358079	**EU305485**	
Plumulariidae	*Nemertesia antennina*	**EU305523**	**EU305498**	AY787910	MHNG INVE 29954
Plumulariidae	*Plumularia hyalina*	**EU305525**	**EU305499**	AY787913	MHNG INVE 25333
Sertulariidae	*Abietinaria filicula*	EU272540	EU272600	AY787899	MHNG INVE 29947
Sertulariidae	*Diphasia fallax*	**EU305511**	**EU305491**	AY787901	MHNG INVE 29950
Sertulariidae	*Hydrallmania falcata*	**EU305519**		AY787900	MHNG INVE 29948
Sertulariidae	*Sertularia cupressina*	**EU305531**		AY787905	MHNG INVE 29949
Sertulariidae	*Sertularia perpusilla*	**EU305532**		AY787894	MHNG INVE 29765
Sertulariidae	*Thuiaria thuja*	**EU305536**	**EU305503**	AY787908	MHNG INVE 29951
Proboscidoidea					
Campanulariidae	*Clytia noliformis*	EU272554	EU272611	DQ064792	
Siphonophorae					
Calycophorae					
Clausophyidae	*Kephyes ovata*	**EU305508**	AY937336	AY935294	YPM 35349
Diphyidae	*Sulculeolaria quadrivalvis*	EU272594	AY937353	AY935311	YPM 35357
Hippopodiidae	*Hippopodius hippopus*	**EU305517**	AY937341	AY935314	YPM 35045
Prayidae	*Nectadamas diomedeae*	**EU305522**	AY937348	AY935306	YPM 35352
Prayidae	*Nectopyramis* sp./*N. natans*	AY026377	AF358068	AY935307	
Prayidae	*Praya dubia*	**EU305526**	AY937326	AY935285	YPM 35346
Prayidae	*Rosacea flaccida*	**EU305529**	AY937328		YPM 35041
Physonectae					
Agalmatidae	*Agalma elegans*	EU272542	AY937313	AY935271	YPM 35029
Agalmatidae	*Cordagalma cordiforme*	EU272555	AY937317	AY935275	YPM 35032
Agalmatidae	*Halistemma rubrum*	EU272566	AY937358	AY935316	YPM 35359
Agalmatidae	*Nanomia bijuga*	EU272576	AY937338	AY935296	YPM 35043
Agalmatidae	*Stephanomia amphytridis*	**EU305535**	AY937322	AY935280	YPM 35076
Apolemiidae	*Apolemia* sp.	EU272546	AY937331	AY935290	YPM 35090
Erennidae	*Erenna* sp.	**EU305512**	AY937361	AY935319	YPM 35362
Forskaliidae	*Forskalia edwardsi*	**EU305516**	AY937320	AY935278	YPM 35036
Physophoridae	*Physophora hydrostatica*	EU272582	AY937342	AY935300	YPM 35046
Rhodaliidae	*Stephalia dilata*	**EU305534**	AY937357	AY935315	YPM 35358
Cystonectae					
Physaliidae	*Physalia physalis*	**EU448095**	AY358065	AY935284	YPM 35345
Trachylina					
Aeginidae	*Aegina citrea*	AY920789	AF358058	EU293997	
Cuninidae	*Solmissus marshalli*	AY920790	AF358060		
Cuninidae	*Solmundella bitentaculata*	EU247797	EU247812		MHNG 31746
Cuninidae	*Solmundella bitentaculata*			EU293998	USNM 1107456
Halicreatidae	*Haliscera conica*	EU247797	AF358064	EU293981	
Oliandiasidae	*Limnocnida tanganyicae*	AY920795	AY920755	EU293972	USNM 1075114
Oliandiasidae	*Aglauropsis aeora*	AY920793	AY920754	EU293973	USNM 1073327
Oliandiasidae	*Astrohydra japonica*	AY920794		EU293975	
Oliandiasidae	*Olindias sambaquiensis*	EU247809	EU247814		
Rhopalonematidae	*Aglantha digitale*	AY920791	EU247821	EU293985	USNM 1073329
Rhopalonematidae	*Aglaura hemistoma*	EU247803	EU247818		MHNG 31745
Rhopalonematidae	*Aglaura hemistoma*			EU293984	KUMIP 314322
Rhopalonematidae	*Pantachogon haeckeli*	AY920792	AF358062		
Rhopalonematidae	*Pantachogon haeckeli*			EU293988	USNM 111078
Rhopalonematidae	*Rhopalonema velatum*	EU247804	EU247819	EU293992	
Tetraplatiidae	*Tetraplatia volitans*	DQ002502	DQ002501	EU293999	KUMIP 314322

KUMIP, University of Kansas Museum of Invertebrate Paleontology; KUNHM, University of Kansas Natural History Museum; MHNG, Muséum d'Histoire Naturelle de Genève; YPM, Yale–Peabody Museum; USNM, US National Museum of Natural History.

Table 2. Summary of genetic markers used in this study.

Marker	Primer source	Length after gblocks (bp) (% retained)	No. of parsimony informative characters (% informative)
28S	Evans *et al.*, 2008	2959 (81%)	969 (33%)
18S	Medina *et al.*, 2001	1648 (82%)	407 (25%)
16S	Cunningham & Buss, 1993	439 (55%)	323 (74%)

major clades. Given the inconclusiveness of these results, any discussion of relationships between major hydroidolinan clades would be premature. By contrast, within each of the major clades, the topologies in the ML and MP analyses are largely congruent and most of the nodes within these clades display high bootstrap support (Figures 1 & 2). Thus we focus our discussion below on the composition and relationships within these clades.

Capitata *sensu stricto*

Capitata is traditionally defined by the presence of capitate tentacles at some stage in its life cycle (Reese, 1957; Petersen, 1990). Recent molecular phylogenetic analyses have questioned the monophyly of Capitata and instead suggest that there are two clades, Aplanulata (*sensu* Collins *et al.*, 2005) and non-Aplanulata capitates (Collins, 2002; Collins *et al.*, 2005, 2006). Our ML and MP analyses provide strong support (bootstrap values = 100 and 96 respectively) for a clade of capitates to the exclusion of aplanulata taxa. We refer to this clade as Capitata *sensu stricto* herein. Within Capitata *sensu stricto* the topologies between the ML and MP analyses are nearly identical (Figures 1 & 2). Both optimality criteria indicate support for the suborder Zancleida including Cladocorynidae, Porpitidae and Zancleidae (*sensu* Peterson, 1990), but also including Solanderiidae. *Moerisia* and *Pennaria* together form a sister taxon to the Zancleida clade (MP; Figure 2) or as successive sister taxa (ML; Figure 1). A Corynidae + Polyorchidae clade is strongly supported under both optimality criteria (Figures 1 & 2). These topologies are largely consistent with that of Collins *et al.* (2005, 2006).

Aplanulata

Aplanulata (Collins *et al.*, 2005) is a clade supported by previous molecular phylogenetic analyses (Collins *et al.*, 2006) and is united by the lack of a ciliated planula stage (Petersen, 1990). Our analyses of Corymorphidae, Hydridae, Candelabridae and Tubulariidae representatives provide strong support for the monophyly of Aplanulata (bootstrap values = 100 for ML and MP) (Figures 1 & 2). Although our sampling is limited, within Aplanulata, there is strong support and nearly complete congruence between ML and MP topologies and these relationships are largely consistent with that recovered from Collins *et al.* (2005) that used partial 16S data. Corymorphidae and Tubulariidae are both monophyletic and there is strong support for a Corymorphidae + Tubulariidae clade (bootstrap values = 100 for ML and MP). The *Hydra* + *Candelabrum* clade is the sister group to the rest of Aplanulata in the MP analysis (Figure 2) but are successive sister taxa in the ML analysis (Figure 1). As discussed in Collins *et al.* (2006), there are other putative Aplanulata families that await future sampling and analyses.

Filifera I: Eudendriidae

Our MP and ML analyses provide strong support for a Eudendriidae clade (bootstrap values = 98 for ML and 85 for MP), apart from other filiferan clades (Figures 1 & 2). Eudendriidae as a clade distinct from other filiferans is supported by many synapomorphies including the absence of desmoneme nematocysts, a styloid-shaped gonophore and a trumpet-shaped hypostome (Marques, 1996). Because of these unique traits, a possible sister-group relationship of the Eudendriidae with other filiferans remains dubious (Marques, 1996, 2001b).

Filifera II: *Fabienna/Proboscidactyla/Brinckmannia/Hydrichthella*

The monophyly of *Fabienna* + *Proboscidactyla* + *Brinkmannia* is well supported (bootstrap values = 94 for ML and 79 for MP) but the node that includes *Hydrichthella* as its sister taxon has relatively low support (bootstrap values = 57 for ML and MP). An association between the Laingiomedusae *Fabienna* and Proboscidactylidae is supported by morphological evidence including a solid ring canal and macrobasic euryteles (Schuchert, 1996). A previous molecular analysis supported this relationship (Collins *et al.*, 2006). In addition, there are a number of morphological features that support the association of *Fabienna/Proboscidactyla* with *Brinckmannia* (Filifera *incertae sedis*) and the ptilocodiid *Hydrichthella*. Schuchert & Reiswig (2006) argued for a close relationship between *Brinckmannia* and Proboscidactylidae based on the shape of their hydranths and 16S sequence similarity. Although the polyp stage in *Fabienna* is unknown, the other taxa share a synapomorphy of hydranths with reduced tentacles: in *Brinckmannia*, hydranths have no tentacles (Schuchert & Reiswig, 2006), *Hydrichthella* has no tentacles on its gastrozooids (dactylozooids have many tentacles) and *Proboscidactyla* has only two tentacles on its hydranths. Interestingly, many of the species in this group are closely associated with another invertebrate as a substrate: *Hydrichthella* is found on an octocoral, *Brinckmannia* within the tissues of a hexactinellid sponge and *Proboscidatyla* on tubes of sabellid polychaetes. In addition, although the ptilocodiid *Hydrichthella* does not have a medusa, the medusae of *Fabienna* are strikingly similar to that of another ptilocodiid species, *Thecocodium quadratum* (Collins *et al.*, 2006).

Filifera III: Hydractiniidae/Stylasteridae

Our ML and MP analyses are congruent in identifying a clade that includes Hydractiniidae and Stylasteridae (bootstrap values = 86 for ML and <50 for MP). There is strong support for monophyly of Stylasteridae (bootstrap values = 100 for ML and 99 for MP) (Figures 1 & 2).

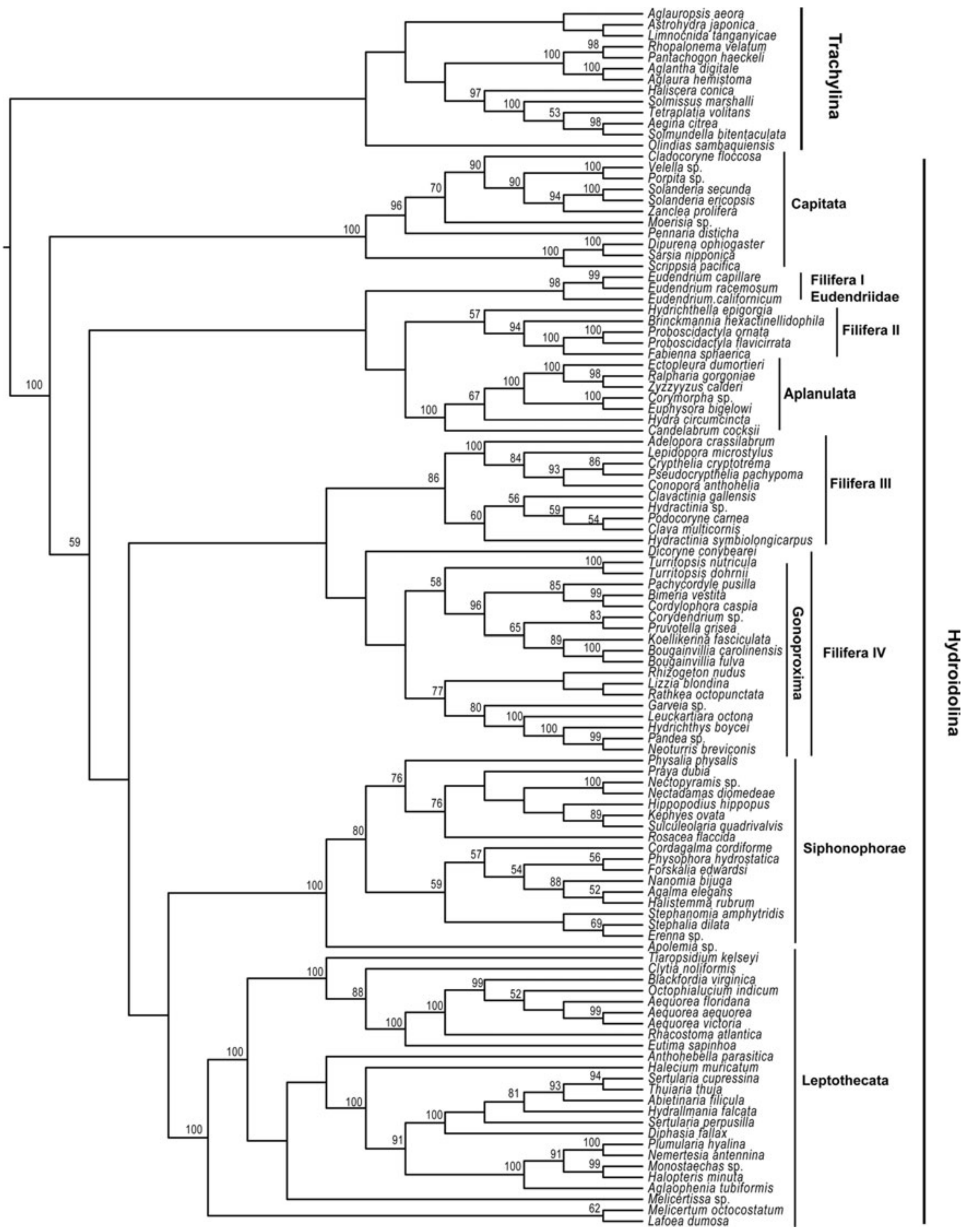

Fig. 1. Phylogenetic hypothesis among 110 hydrozoan taxa, based on a maximum likelihood criterion of a combined dataset of nearly complete 28S, nearly complete 18S and partial 16S rDNA sequences. Bootstrap values greater that 50 are indicated above nodes. The assumed model (GTR + I + G) with six substitutions rates estimated from the data (A-C, 0.8735; A-G, 2.9730; A-T, 1.6586; C-G, 0.8463; C-T, 5.2641; G-T, 1.0000), an assumed proportion of invariant sites (0.5740) and a gamma shaped parameter of (0.6021).

Although the hydractiniids are monophyletic in the ML analysis (bootstrap value = 60) (Figure 1) the MP analysis places them as paraphyletic relative to the stylasterids (Figure 2). Our analyses show *Clava multicornis* as the sister taxon to the hydractiniid *Podocoryne carnea* with strong support in both ML and MP trees (bootstrap values = 94 for ML and 89 for MP). *Clava* has traditionally been placed in the family Clavidae, although Schuchert (2001) argued, based on the similarities of *Clava* to other hydractiniids (Bouillon *et al.*, 1997), that the genus *Clava* should be moved to the hydractiniids and the other Clavidae genera moved to the nominal family Oceaniidae (Schuchert, 2004). Our analysis supports the interpretation that *Clava* is a hydractiniid.

The close relationship between the Hydractiniidae and Stylasteridae families has previously been suggested based

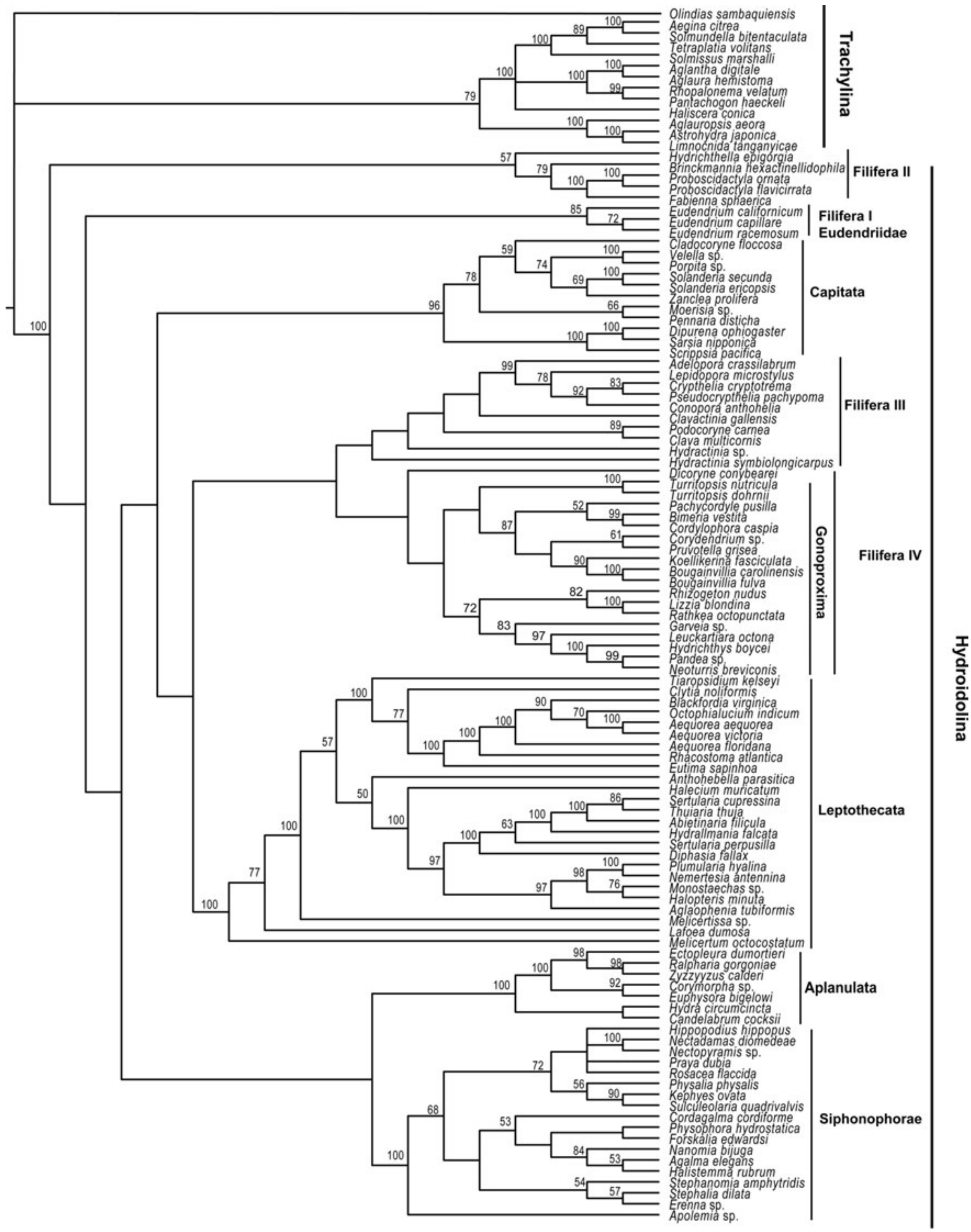

Fig. 2. Phylogenetic hypothesis among 110 hydrozoan taxa, based on parsimony criterion of a combined dataset of nearly complete 28S, nearly complete 18S and partial 16S rDNA sequences. Bootstrap values greater that 50 are indicated above nodes. Topology is a strict consensus of the 10 most parsimonious trees (5046 characters). Length: 13246 steps, CI – 0.26; RI – 0.59.

on a number of synapomorphies including polymorphic polyps and the perisarc or skeleton covered stolons (Bouillon, 1978; Petersen, 1979). Bouillon (1978) placed these families in the superfamily Hydractinoidea, which also includes Ptilocodiidae, Rathkeidae and Rhysiidae. Though we did not sample any members of Rhysiidae, our analyses do not support Hydractinoidea, as sampled Ptilocodiidae and Rathkeidae members are placed outside this clade.

Filifera IV: Gonoproxima + *Dicoryne*—Bougainvilliidae/Oceaniidae/Pandeidae/Rathkeidae

Our ML and MP analyses support the monophyly of a clade that includes representatives of Bougainvilliidae, Oceaniidae, Pandeidae and Rathkeidae. This clade has relatively weak support (bootstrap values <50 for ML and MP) and must be viewed as tentative. Within the clade the topologies are congruent between the ML and MP analyses. Of the four families, Pandeidae and Rathkeidae are monophyletic, whereas Bougainvilliidae and Oceaniidae are polyphyletic. Although association of these four families is somewhat surprising, they all share a striking synapomorphy. The species in all four families bear gonophores on hydrocauli, pedicels, or stolons and not on the hydranth body. The shift of the gonophores from the hydranth body to the region below is an apomorphy (Schuchert, 2001). Backed by this synapomorphy, we name this clade Gonoproxima. The 'bougainvilliid' *Dicoryne*, which is distinct from other bougainviliid species in that it produces gonophores on blastostyles, is placed as the sister taxon to Gonoproxima in both the ML and MP analyses (Figures 1 & 2). Interestingly, many taxa included in this group have perisarc extending over the hydranth body, either as a gelatinous structure or pseudohydrotheca.

Two species within Gonoproxima, *Cordylophora caspia* (sampled here) and *Pachycordyle kubotai* (not sampled) live in fresh water. In our analyses, *Cordylophora* and *Pachycordyle*, which also contains brackish and marine species, are indicated to be close relatives under both optimality criteria, forming a clade with the bougainvilliid *Bimeria*. With denser taxon sampling within Gonoproxima and more targeted phylogenetic analyses, it should be possible to ascertain whether the fresh water habit was evolved one or more times in this clade and potentially whether freshwater species are descended from ancestors that lived in brackish environments.

Siphonophorae

The siphonophores have historically been split into three major groups, Cystonectae, Physonectae and Calycophorae. Collins (2002) placed the cystonect *Physalia* as sister to the other included siphonophores and suggested that Physonectae may be paraphyletic with respect to the Calycophorae. A later study (Dunn *et al.*, 2005), that considered additional taxa and two genes (18S and 16S) found that cystonects form a monophyletic group that is sister to the remaining siphonophores and the paraphyly of Physonectae was recovered with significant support. Dunn *et al.* (2005) erected the name Codonophora to refer to the clade comprising taxa assigned to Physonectae and Calycophorae (i.e. the clade that is sister to Cystonectae).

Our ML and MP analyses are consistent with the findings of Collins (2002) and Dunn *et al.* (2005) in that Siphonophorae is a strongly supported monophyletic group (bootstrap values = 100 for ML and MP). Our ML and MP analysis also recovered Physonectae as paraphyletic and Calycophorae derived within this clade (Figures 1 & 2). We did not however find support for Codonophora (*sensu* Dunn *et al.*, 2005). Our ML analysis placed the cystonect *Physalia* as the sister taxon to Calycophorae (Figure 1), not as the earliest diverging member of siphonophores (Collins 2002; Dunn *et al.*, 2005). The MP analysis also recovered a probable paraphyletic Physonectae, but unlike the ML analyses, *Physalia* was nested within Calycophorae (Figure 2). Under both optimality criteria, the physonect, *Apolemia*, was the earliest diverging siphonophore. Given that we have only one cystonect representative (*Physalia*) and that its placement is dependent on optimality criteria, we view the placement of Cystonectae relative to other siphonophores as equivocal and await further study.

Leptothecata

Our ML and MP analyses found strong support for the Leptothecata clade (bootstrap values = 100 for ML and MP) (Figures 1 & 2). Sampling was concentrated amongst the Conica subgroup, with the inclusion of only one Proboscoida representative, *Clytia noliformis*. This sampling is therefore insufficient to address the question of monophyly of its subgroups, Conica and Proboscoida.

The ML and MP topologies within Leptothecata are nearly congruent except for the placement of *Lafoea* relative to *Melicertum* (discussed below). The traditional taxonomy of Leptothecata, including the relationships of its higher groups, is largely based on similarities in the morphology of the hydrotheca and nematotheca (e.g. Bouillon, 1985, 1994). Many groups found in our analyses corroborate Bouillon's hypotheses, including the monophyly of the Plumularioidea taxa, Plumulariidae and Halopterididae, Sertulariidae and the affinities of these with Haleciidae. Recent molecular and morphological analyses also have corroborated or are consistent with these hypotheses (Leclère *et al.*, 2007).

The affinities of the Hebellidae and Lafoeidae, based on morphological characters, were investigated by Marques *et al.* (2006). Although the authors hypothesized the exclusive monophyly of each family, they considered the possibility that the families are distantly related, a finding consistent with our analyses.

Campanulinida is a group of leptothecates including many diverse families: Aequoreidae, Blackfordiidae, Eirenidae, Laodiceidae, Malagazziidae, Melicertidae and Mitrocomidae. The Campanulinida taxa belonging to Aequoreidae, Blackfordiidae, Eirenidae, Malagazziidae and Mitrocomidae are a strongly supported clade that also includes *Clytia noliformis* (bootstrap values = 100 for ML and MP). The Campanulinida belonging to Melicertidae, *Melicertum octocostatum* is the sister taxon to the rest of the Leptothecata in the MP analysis (Figure 2). This analysis corroborates the hypothesis of an early divergence of *M. octocostatum* (Collins *et al.*, 2006), a species that lacks a theca but has typical leptothecate medusae. The ML analysis places *Melicertum* + *Lafoea dumosa* as the sister taxon to the rest of Leptothecata (Figure 1).

CONCLUSIONS

Anthoathecata represents a diverse order of hydroidolinans that traditionally comprises two suborders, Filifera and Capitata (reviewed in Daly *et al.*, 2007). Although our analyses and previous molecular phylogenetic analyses (Marques & Collins, 2000; Marques, 2001a; Collins, 2002; Collins *et al.*, 2006; Van Iten *et al.*, 2006) do not support the monophyly of Anthoathecata, the dissolution or re-definition of Anthoathecata is premature and should await clarification of relationships between major hydroidolinan clades. Capitata in the traditional sense comprises two clades, the Aplanulata, recognized by the lack of a free-swimming planula (Petersen, 1990) and Capitata *sensu stricto*. Given that there is strong support for these two groups and that there is no support for the monophyly of traditional 'Capitata' in these analyses and in previous phylogenetic analyses (Collins, 2002; Collins *et al.*, 2005, 2006), the validity of Capitata in the traditional sense is questioned. If these clades are indeed separate, then Aplanulata should be referred to as its own order, separate from Capitata *sensu stricto*. Re-defining Capitata however, should await further clarification of Hydroidolina phylogeny. Our analyses do not support the monophyly of Filifera but this too is preliminary as the nodes separating the filiferan subgroups are weakly supported (Figures 1 & 2).

The new augmented dataset used in our analyses provide support for four distinct filiferan clades. Notably, all of these clades possess compelling morphological synapomorphies; Gonoproxima is characterized by gonophores on regions of the colony proximal to the hydranth; Eudendriidae displays distinct polyp and hypostome morphology; the *Fabienna/Proboscidactyla/Brinckmannia/Hydrichthella* clade displays polyps with a reduced number of tentacles and the Hydractiniidae/Sylasteridae clade displays polymorphism. A more comprehensive sampling of hydroidolinan families should provide greater insight into these emerging patterns.

Despite increased sampling, relationships between major hydroidolinan clades remain elusive. The lack of resolution suggests that the initial radiation of Hydroidolina may have been rapid, leaving little clues regarding the sequence of hydroidolinan diversification. New molecular markers, especially if combined with other types of data, may prove helpful in resolving these deep nodes.

ACKNOWLEDGEMENTS

We would like to thank A. Lindner for DNA samples and anonymous referees for helpful comments. This study was supported from grants from NSF AToL EF-0531779 (to P.C. and A.G.C.). A.C.M. has financial support from CNPq (55.7333/2005-9, 490348/2006-8, 305735/2006-3) and FAPESP (2004/09961-4).

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Correspondence should be addressed to:
Paulyn Cartwright
Department of Ecology and Evolutionary Biology
University of Kansas, Lawrence, KS 66049, USA
email: pcart@ku.edu