MOLECULAR PHYLOGENY OF THE SHINING LEAF BEETLES (COLEOPTERA: CHRYSOMELIDAE: CRIOCERINAE)

By

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THESIS ABSTRACT

Shining leaf beetles (Coleoptera, Chrysomelidae, Criocerinae; ~1500 spp) are considered amongst the earliest diverging leaf beetle lineage to attack early angiosperms. Although they are distributed worldwide, little is known about their biology and evolutionary relationships. Schmitt (1988) generated the first morphology-based phylogeny using four genera: ((*Lilioceris* + *Crioceris*) + (*Lema* + *Oulema*)). Teo (1999) is the second phylogenetic hypothesis (unpublished), this morphology-based phylogeny shows ((Pseudocriocerii + Criocerini (Lemini). Vencl *et al* (2004) proposed a phylogenetic hypothesis for the Central American genera ((*Crioceris* + *Metopoceris*) + (*Lema* + (*Neolema* + *Oulema*)). These three studies sampled a subset of the recognized genera and lacked large outgroup representation. Nevertheless, they provide a general understanding about phylogenetic relationships in Criocerinae. In this research I tested: 1) the systematic position of Criocerinae, 2) the monophyly of the subfamily, and 3) the intrageneric relationships by generating a molecular dataset and developing a new phylogentic hypothesis of evolutionary relationships. I sampled 76 species in 7 genera of Criocerinae and 9 outgroups from other chrysomelid subfamilies, to generate a molecular data set of three molecular markers (COI, 18S, and 28S). Phylogenetic analyses using parsimony, maximum likelihood, and posterior probabilities show strong support (> 0.90 posterior probabilities/ 1 – 0.75 bootstrap) for placing Criocerinae within the Sagrinae clade of Chrysomelidae, as either sister group to Donaciinae or Sagrinae. The monophyly of Criocerinae has been supported by several morphological characters—stridulatory apparatus and frontal grooves in adults, and ambulatory warts, dorsal anus, and fecal shield in larvae). Yet, this phylogenetic analyses showed no support for the monophyly of this subfamily.

My phylogenetic analyses do not clarify the pattern of evolution in Criocerinae because systematic relationships within Criocerinae, at tribal or generic levels, were not recovered from our tree topologies (individual genes and combined data analyses). Our most resolved phylogeny was recovered using posterior probabilities and these results were consistent with Teo's (1999) strict consensus topology. Both phylogenies are not fully resolved and show that *Lema* Fabricius and *Lilioceris* Reitter are not monophyletic. Additionally, parametric bootstrapping was performed to test the monophyly of each genus and tribe. The only significant improvement was constraining *Neolema* Monrós as monophyletic (better ML scores, but not MP score).

Using the most resolved topology, I examined the geographic pattern of species distributions. I found that species clusters are more related to their geographical distribution (i.e., the existence of Nearctic-Neotropical and Oriental species clusters). Similarly, I examined host plant record patterns with this tree topology, and observed that most of my sampled criocerinae species are monocot feeders. However, some species in certain genera (e.g., *Lema*, *Lilioceris*, *Neolema*, *Metopoceris* Heinze) are also feeding on eudicot plants.

Future research in Criocerinae needs to focus on developing stronger diagnostic characters for the subfamily since traditional characters supporting it monophyly also occur in other chrysomelid subfamilies (e.g., Sagrinae & Hispinae). Future research should also sample members of Pseudocriocerini and Criocerini, which have been thought to be basal in the evolution of the subfamily. These will ultimately contribute to resolving the evolutionary patterns in Criocerinae.

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INTRODUCTION

Chrysomelidae, commonly known as leaf beetles, is one of the most diverse families of phytophagous beetles with >220 genera and > 35,000 species (Jolivet, 1988; Arnett *et al*., 2002). They are widely distributed with the exception of the Polar Regions (Seeno & Wilcox, 1982). According to Bouchard *et al.*'s (2011), 12 subfamilies are recognized today: Bruchinae Latreille, Cassidinae Gyllenhal, Chrysomelinae Latreille, Criocerinae Latreille, Cryptocephalinae Gyllenhal, Donaciinae Kirby, Eumolpinae Hope, Galerucinae Latreille, Lamprosomatinae Lacordaire, Sagrinae Leach, Spilophoriinae Chapuis, and Synetinae LeConte & Horn. Although there is a general agreement on subfamily distinctions, systematics within subfamilies is ambiguous, especially in the monophyly and rank of genera and tribes (Arnett *et al.*, 2002).

The chrysomelid subfamily Criocerinae Latreille, 1807, commonly known as "shining leaf beetles", is a relatively small group comprising *ca*.1500 described species classified in 3 tribes and 21 genera (Table 1; Seeno & Wilcox, 1982; White, 1993; Schmitt, 1996; Arnett *et al*., 2002). Even though 21 genera are recognized, the majority of species (67%) are currently in one genus, *Lema* Fabricius, 1798 (Figure. 1; White, 1993; Arnett *et al*., 2002).

Criocerine leaf beetle adults are characterized for being small, glabrous, with a shiny appearance. The main diagnostic characters of the subfamily are: 1) The adult head has a frons with distinct diverging grooves; 2) the head and pronotum are narrower than the elytra; 3) the pronotum medially or basally constricted; and 4) stridulatory files developed on the 7^{th} tergite. The larva has the anus dorsally-oriented (Monrós, 1960; Crowson, 1967; Schmitt, 1988, 1996; Arnett *et al*., 2002). Criocerines occur in temperate, subtropical and tropical regions of the world (Schmitt, 1988; Monrós, 1960). They are phytophagous, feeding mainly from the leaf surface of monocots

and eudicots (Vencl & Aiello, 1997; Monrós, 1960), and like most leaf beetles, criocerines exhibit a strong plant association for feeding and mating (Jolivet & Petitpierre, 1981).

Historically, our understanding of Criocerinae is based on the Palearctic (Schmitt, 2010), North American (White, 1993), and South East Asian fauna (Gressitt, 1965; Kimoto & Gressitt, 1979), where more taxonomists are located. Monrós (1960) provided the most detailed subfamily description, highlighting characteristics for generic identification, biology, and biogeography.

Schmitt (1985, 1988) described phylogenetic patterns of the main genera: ((*Lilioceris* + *Crioceris*) + (*Lema* + *Oulema*)), where members of the Criocerini tribe were considered to be less derived than the members of Lemini. Host plant associations where also traced in this phylogeny, where the basal genera *Lilioceris* and *Crioceris* are considered recorded feeding only monocots and *Lema* and *Oulema* are also recorded feeding from eudicots. Teo (1999) developed a morphology based phylogeny using 16 genera, 56 taxa and 67 characters. This work supported the hypothesis that Criocerinae is sister group to Cassidinae, and that Pseudocriocerini and Criocerini are less derived in the evolution of the subfamily: ((Pseudocriocerini + Criocerini (Lemini)). Vencl *et al*. (2004) developed a molecular phylogenetic hypothesis for Criocerinae, sampling 21 Panamanian and Costa Rican species and one mitochondrial marker (COI, 427–641 base pairs long). This work does not conform to current cladistics standards since the taxon sampling omitted outgroups and is geographically skewed to Central America. However, it provides a hypothesis about the internal relationships between the species present in Central America.

Previous work done in Criocerinae has greatly contributed to the understanding of the systematic of the group by providing us this key characters for identification, biological facts as well as hypotheses for evolutionary patterns in Criocerinae. However, in some this cases some of these hypotheses were geographically skewed, presented a poor outgroup representation (one

outgroup or none), and did not include statistical support for branch position. For these reason, a more rigorous phylogeny of Criocerinae is sorely needed to uncover evolutionary relationships within the subfamily and with other chrysomelid subfamilies. This will also clarify the sequence of speciation events, evolution of morphological and behavioral characters, host plant association, chemical defenses, and other characters in this subfamily.

Systematic position of Criocerinae

There are several hypotheses regarding the placement of Criocerinae within Chrysomelidae. Traditionally, Criocerinae systematic position has been related to the subfamilies, Sagrinae, Bruchinae, Donaciinae and Hispinae, based on morphological characters (e.g. prothrorax smaller than elytra, frontal grooves, reduction of wing venation in adults and in larvae the presence of ambularoty warts and fecal shield as few examples; Monrós, 1960; Schmitt, 1988; Reid, 1995)

Chapuis (1874) created the Eupedes for the subfamilies Criocerinae, Donaciinae and Sagrinae based on pronotal and elytral characters ("pronotum narrower than elytra, without lateral margins"; Figure. 2). Monrós (1960) proposed a new classification for the group by creating a new group, the Crioceriformes (Figure. 3) to contain Criocerinae, Donaciinae, Sagrinae and Bruchinae. Subsequent morphological studies have produced substantial evidence to support the monophyly of this "Crioceriformes" clade (Monrós, 1960; Mann & Crowson, 1983, Reid, 1995).

The monophyly for Donaciinae + Criocerinae + Sagrinae group is supported by the presence of frontoclypleal grooves, a median depression on basal and apical ventrites in males, an elytral suture explanate, pronotal margins lost, the sclerite MEG of penis present, the presence of

basal sac sclerites, and a pubescent scutellum. Each of these characters are present in most members of the Donaciinae, Criocerinae and Sagrinae clade with some exceptions (Monrós, 1960; Askevold, 1990; Reid, 1995). Reid (1995) considered Bruchinae as derived from Sagrinae because of the shape and number of stemmata on the larval head, and the structure of the clypeus and ligula in adults.

Mann and Crowson (1981) proposed another hypothesis where Criocerinae and Hispinae form a monophyletic group (Figure. 4), based on the presence of bifid setea in the tarsi in adults, and 3 segmented antennae, i-segmented labial palpi and several ocelli on each side in the larvae. Mann and Crowson hypothesis differs from previous studies where Criocerinae is usually associated with Sagrinae, Bruchinae and Donaciinae. Chen (1985) included Sagrinae, Bruchinae, Donaciinae, and Criocerinae with the Crioceridae, based on the presence of a bifid setae on the 3rd tarsal segment. He provided two alternative subfamilial relationships: 1) Crioceridae, contained within the Chrysomelidae, Eumolpidae and Hispidae clade, more closely related to Chyrsomelidae (Chrysomelinae, Galerucinae, Alticinae and Synetinae; Figure. 5A); and 2) Sister group to Hispidae (Cassidinae & Hispinae, Figure. 5B).

Jolivet (1988) proposed the relationship: Criocerinae + (Chrysomelinae, Galerucinae and Alticinae), based on male genitalia (tegmen; Figure. 6). Reid (1995) supported Mann and Crowson's (1981) hypothesis with further evidence for this monophyletic group: adult with reduced tegmen and reduced wing venation, and larva with 6 stemmata (Mann & Crowson, 1981, 1983; Reid, 1995). Schmitt (1988) generated a hypothesis for generic relationships (*Lilioceris* + *Crioceris* (*Lema* + *Oulema*)), based on morphological characters (e.g., vertex, tarsal claws and aedeagus).

Reid (1995) is the first modern analysis of chrysomelid subfamily relationships. He generated a new matrix of 71 characters for 29 taxa (Figure. 7). His analyses located Criocerinae as sister group to Hispinae, within the same clade as Donaciinae, Sagrinae and Bruchinae (((Criocerinae + Hispinae) + Donaciinae) + (Sagrinae + Bruchinae)), based on the reduction of the tegmen and wing venation in adults, and plesiomorphic number of stemmata, one segmented palpi, and paronychial appendix on tibia in larvae.

Teo (1999) developed a phylogeny for Criocerinae based on 53 species and 67 morphological characters, and one outgroup, Hispinae. A total of nine discrete clades are apparent in this topology corresponding to either Criocerini or Lemini (Figure 37). These clusters have some correlation with their geographical distribution (e.g., African *Crioceris* Geoffroy, African Lemini; South American Lemini). Criocerini species were described as polyphyletic, where *Pseudocrioceris* Pic as the most basal genus of Criocerinae, followed by *Ovamela* Fairmaire and *Manipuria* Jacoby. Lemini species where contained within the same clade, but *Lema* was not monophyletic and present in several clades. This work provided insights about intraspecific relationships, as well characters that should be taken in account for the systematic classification of the group.

Molecular phylogenetic studies of Chrysomelidae only began in the 1990's, bringing a very different set of data to the question of relationships. Hunt *et al*. (2007) proposed the first coarse phylogeny for coleopteran subfamilies using three molecular markers (18S, 16S and Cox I; Figure. 8). Their results suggested that (Bruchinae + (Criocerinae + Donaciinae)) are in the same clade, and more distantly related is Sagrinae in the adjacent clade. Gómez-Zurita *et al*. (2008) developed a new phylogeny for Chrysomelidae using three molecular markers, two nuclear (18S rRNA and 28rRNA) and one mitochondrial marker (16S rRNA; Figure. 9). This phylogeny suggests that

Criocerinae, Sagrinae and Donaciinae are monophyletic groups, but their relationship with Synetinae is uncertain.

In contrast, Marvaldi *et al*. (2008) analyzed a dataset of two nuclear markers (18S rRNA and 28rRNA) for 96 species and found (((Criocerinae + Donaciinae) + Eumolpinae) + Synetinae + Sagrinae; Figure. 10). Gómez-Zurita (2008) used three molecular markers (rrnL, LSU, SSU) for 167 species and found (Donaciinae + Bruchinae + Criocerinae) as monophyletic. Sagrinae was not sampled but placed as sister group of Bruchinae in this phylogenetic hypothesis.

Current molecular phylogenies discard the hypothesis that suggested that Criocerinae and Hispinae were a monophyletic group (Hunt *et al*., 2007; Marvaldi *et al*., 2007; Gómez-Zurita *et al*., 2008). Available phylogenies suggest that Criocerinae belongs to the clade of Donaciinae and Bruchinae. Relationships with Sagrinae and Synetinae within the chrysomelid evolution are still unclear, to be determined with the development of new phylogenies that include a larger set of taxa and greater number of molecular markers. However, we have only begun to explore the full array of evolutionary characters; larval morphology and their interesting behaviors (e.g., fecal shield, gregariousness) have not been integrated into phylogenetic datasets of Criocerinae.

Monophyly of Criocerinae, Latreille, 1807

Criocerine adults are recognized by their small size (3–8 mm), glabrous appearance and brilliant and metallic colors (Figures. 11–22). The pronotum is basally or medially constricted unmarginated pronotum ("hourglass" shape) and lacks a marginal bead (Figures. 23–25; Vencl *et al*., 2004). The adults have a prognathous head and the mouthparts are not ventrally deflexed (Figure. 26A) and possess a mandibular mola (Figure. 26B Reid, 1995). The head is usually wider

than the pronotum (Figure. 26A and Figures 23–25; Arnett *et al*., 2002). The antennae is clavate to slightly filiform, reach beyond the humeri, and the insertions are located near the lower margin of the eyes. The eyes are emarginated (Figure. 26A) and the head presents a postocular constriction (Figure. 26A: Monrós, 1960; Arnett *et al*., 2002). The head also has inter-antennal frontoclypeal grooves (Figure. 26A; Schmitt, 1988; Arnett *et al*., 2002; Vencl *et al*., 2004).

On thorax, the mesothorax exhibits a reduced number of setae in the mesoscutum relative to Sagrinae (Schmitt, 1988). The elytra have ten striae, shallow impressions or lines (Arnett *et al*., 2002; Triplehorn & Johnson, 2005). The first ventrite of the abdomen is as long as the next two combined and sternites III and IV are not fused (Arnett *et al*., 2002; Reid, 1995). Adults possess stridulatory files on tergite VII (Figure. 27); the pygidium is covered by the elytra (Schmitt, 1988; Arnett *et al*., 2002). The tarsi are 5–5–5, but pseudotetramerous (the fourth tarsomere is reduced); the third tarsomere has bifid setae ventrally (Arnett *et al*., 2002). Useful diagnostic characters of the aedeagus include the number and orientation of aedeagal folds; *Lema* has a single medial fold overlaying lateral folds whereas *Neolema* has lateral aedeagal folds meeting, concealing a central fold. In contrast, *Oulema* Gozis has three folds, but the lateral folds do not conceal the central fold (Vencl *et al*., 2004).

The larva has a three-segmented maxillary palpi and labrum with three pairs of setae on the disc (Schmitt, 1988; Reid, 1995). The larval abdomen is globular shaped, with distant ventral ampullae. Egg bursters are located on the first abdominal segment of instar I larvae (Reid, 1995). Criocerine larvae lack a posterior divergence from the midline in the epicranium and lack a bilobed paronychial appendix (Schmitt, 1988; Reid, 1995). Larvae have the anal opening in a dorsal position, similar to larva of Alticini (Galerucinae); this is distinct from the caudoventral opening of larvae of other Chrysomelidae (Figure 28 B). This dorsal position is related to the deposition of

feces on the dorsum of body, is probably part of a defensive mechanism against predators (Arnett *et al*., 2002; Vencl *et al.*, 2004).

Internal relationships of Criocerinae

Criocerinae is currently recognized as comprising three tribes and twenty-one genera (Table 1; Seeno and Wilcox 1982; White, 1993). Tarsal claw morphology is the fundamental characteristic used to separate tribes. Tarsal claws separated at the base are observed in Criocerinini, (Heinze 1962 and Pseudocriocerini Heinze 1962; connate tarsal claws occur in the tribe Lemini, Heinze 1962) (Monrós, 1960; Arnett *et al*., 2002; Figure. 29). Tarsal claws, pronotal morphology, and elytral puncture patterns are the main characteristics used at present in generic determination (Vencl *et al*., 2004). Although twenty-one genera are recognized, most diversity of Criocerinae is in one genus, *Lema* (Figure. 1; Arnett *et al*., 2002).

Biology

Criocerinae is a small lineage within Chrysomelidae, but members exhibit many interesting morphological, life history, and behavioral traits. Hypotheses about host-plant driven diversification in Chrysomelidae (Jolivet & Verma, 2002; Farrell & Sequeira, 2004; Gómez-Zurita et al., 2008) also applies to criocerines. They also exhibit many intricate and unusual behaviors that may have driven their diversification, e.g., life cycle and seasonality traits, defensive behaviors (e.g., presence of fecal shield and cycloalexy in larva, and stridulation, a form of sound production in adults); and also serve as biological controls of plant pest species (Arnett *et al*., 2002).

Seasonality and life cycle

Criocerinae life cycle will vary depending in the geographical region they inhabit. Neotropical criocerines are active all year round and will undergo two or more full cycles per year (White, 1993; Vencl *et al*., 2004). In temperate four-season regions, adults emerge in early spring when mating takes place. In the northern hemisphere, some Criocerinae have only one full cycle per year (e.g., *Lema puncticollis* Curtis, *Oulema melanopus* Linnaeus). Other species (e.g., *Crioceris asparagi* (Linnaeus), *Lilioceris lili*, *Lema trilinea* White) undergo two or more full cycles per year (Vencl *et al*., 1994; White, 1993).

The female will lay between 200–400 eggs, which varies with temperature, over a period of 2-3 months (Green, 1939; Schmitt, 1988, White 1993, Selman, 1994; Hawkeswood, 2009). Criocerine eggs are yellow or brown; they are arranged in small groups and attached to the foliar surface of the host plant (Figure 28 A). The eggs usually hatch in 2–10 days (Hodson, 1929; White, 1993; Selman, 1994; Hawkeswood, 2009). Most females will only oviposit the first year of life, although in some species of Criocerinae oviposition has also been reported during their second year of life as seen in *Lilioceris lili* (Scopoli) (White, 1993).

Once the egg hatches, the newly emerged larva is commonly white, yellow or gray. Criocerines have four instars, the larvae can be solitary (Vasconcellos-Neto & Jolivet, 1994) or gregarious (e.g., *Lema latipennis* Pic and *Lema apicales* Lacordaire (Monrós, 1960; Vasconcellos-Neto & Jolivet, 1994; Vencl & Morton, 1999)). Early instars tend to be gregarious, but later instars disperse and begin to feed individually. Mature larvae migrate to the soil near the host plant for pupation (Monrós, 1960; White, 1993). The larvae make a cocoon from oral discharges that solidify into a white case (White, 1993). Pupation lasts between 8–22 days before the adult emerges.

Food preference and host plant

All criocerines are phytophagous and are considered mono– and olygophagous, like other chrysomelids (Jolivet & Petitpierre, 1981). (Jolivet & Petitpierre, 1981). Because of their presumed basal position in Chrysomelidae evolution, Criocerinae are considered to be amongst the earliest diverging leaf beetle lineage to attack early angiosperms (Vencl *et al*., 2004). Both adults and larvae mainly feed on the leaf surface of monocotylenous and dicotyledonous plants, and some non-angiosperms plants like Cycads (Vencl & Aiello, 1997; Monrós, 1960; Jolivet & Petitpierre, 1981; Vencl *et al*., 2004).

The known Criocerine host plants are summarized in Table 3. Criocerinae are primarily associated with monocot plants (e.g., Liliaceae, Asparagaceae, Dioscoraceae, Commelinaceae), occasionally feeding on eudicot plants (Solanaceae, Fabaceae, Asteraceae), as well as some non-angiosperm plants like *Cycas* and ferns (Sengupta, & Behura, 1957; Schmitt, 1988; White, 1993; Jolivet & Hawkeswood, 1995; Hawkeswood, 2009). Criocerine adults and larvae feed on different parts of the plant, leaving holes in leaves or making furrows in the leaf surface (Monrós, 1960; White, 1993; Vencl *et al*, 2004; Bienkowski, 2010). On monocotyledonous hosts, adults and larvae have been reported to feed between ribs in the leaf epidermis (Monrós, 1960; White, 1993; Vencl *et al*., 2004). In eudicotyledon plants, Criocerinae feed from the surface of the leaf blade, leaving only the thicker leaf ribs (Mónros, 1960; White, 1993; Vencl *et al*., 2004). Larvae tend to chew long strips between leaf veins; some larvae have also been found feeding from roots and stems (e.g., *Oulema pumila* Vencl & Aiello and *Lema quadrivittata* Boheman; Vencl & Aiello, 1997; Monrós, 1960). Adults cause major damage to the leaf surface during the spring (White, 1993, Vencl *et al*., 2004). Criocerine not only feed on leaf surfaces, but adults of some species can eat pollen, flowers and seeds (Monrós, 1960, Jolivet & Hawkeswood, 1995).

Determinant factors for food selection in chrysomelids are color vision, gradients of concentration of chemical substances in plants, olfactive organs in antennae, chemosensory cells located in the labrum and palps that help the beetle in their food selection as well as host plant evolutionary patterns (Jolivet & Petitpierre, 1981; Jolivet & Verma, 2002; Farrell & Sequeira, 2004; Gómez-Zurita *et al*., 2008). This group may have evolved characteristics and behaviors associated with living on open surfaces. Living within exposed foliage may have led to the evolution of high sensory capabilities, such as an increased eye emargination in *Oulema*, which appear to have resulted in the antennal more widely separated (Crowson, 1981).

Behavior and defense mechanism

Criocerinae larvae and adults have developed several particular defensive behaviors against natural enemies (Monrós, 1960; White, 1993; Schmitt, 1994; Vencl *et al*., 2004). Immature stages have a greater rate of mortality than adults due to a greater exposure to natural enemies as they feed on the leaf surface (Vencl *et al*., 2004, 2009). Immature stages have a greater rate of mortality than adults due to a greater exposure to natural enemies as they feed on the leaf surface (Vencl *et al*., 2004; Vencl *et al*., 2009). Protective behaviors against natural enemies that have evolved in Criocerinae, include aggressive displays, biting, dorsal secretions, dorsal shield, case bearing, toxicity, regurgitation of liquids, exsanguination, and reflex immobilization in the larvae. Adults present aposematic coloration, secretion of allomones, stridulation, homochromy and mimicry (Monrós, 1960, White, 1993; Schmitt, 1988; 1994; Pasteels *et al*., 1994, Vasconcellos-Neto & Jolivet, 1994; Vencl *et al*., 2004,). Some of these traits have been reported in several Chrysomelidae lineages (Seeno & Wilcox, 1982, Vasconcellos-Neto & Jolivet, 1994; Chaboo, 2007; Santiago-Blay *et al.*, 2012).

Criocerine larvae have a distinctive fecal shield (Monrós, 1960), that is composed of a combination of feces, water and sometimes exuviae and plant derived metabolites (Morton & Vencl, 1998, Vencl *et* al., 1999). It is hypothesized that the fecal shield act acts as camouflage, insulation, or a defense against predators by serving as a physical barrier like the fecal shields of some other chrysomelids (Olmstead, 1994; Morton & Vencl, 1998). Some evidence suggests that the composition of the shield increases its effectives. Criocerinae host plants, especially Commelinaceae and Solanaceae, are known to contain secondary compounds such as terpenoids, phenolics and alkaloids (Whitman *et al*., 1990; Morton & Vencl, 1998).

A few criocerine species have larva that form circled-wagon formations termed 'cycloalexy' (Santiago-Blay *et al.*, 2012). Cycloalexy is a circular or quasicircular aggregation of insects (adults and immature stages), and has been reported in six orders of insects. This behavior has been mainly considered defensive, but it also appears to be related to thermoregulation, feeding and reproduction. Cycloalexy comprises a coordinated movement (for example threating postures, regurgitation and biting movements), to minimize the impact of predation and parasitism of potential predators or parasitoids (Santiago-Blay *et al.*, 2012). In Chrysomelidae, cycloalexy has been reported in Criocerinae, Chrysomelinae, Cassidinae and Galerucinae larvae (Vasconcellos-Neto & Jolivet, 1994; Santiago-Blay *et al.*, 2012). To date, only four species of *Lema* and *Lilioceris* have been reported as exhibiting cycloalexy—*Lema reticulosa* Clark, *Lema apicalis* Lacordaire, *Lilioceris nigropectoralis* (Pic), and *Lilioceris formosa* Heinze (Santiago-Blay *et al.*, 2012).

Adult criocerine beetles are known to release allomones (amino acid derivatives) from pronotal and elytral glands when they are disturbed. These allomones are probably used for defensive purposes, and can present interspecific variations (Pasteels *et al*., 1994). Another mechanism of defense observed in adult Criocerinae is stridulation (Schmitt & Traue, 1990;

Schmitt, 1994). Adults stridulate by rubbing two surfaces, a file and scrapper, together. This behavior has been found in seven subfamilies of Chrysomelidae (three types of stridulatory devices; Seeno & Wilcox, 1982). Criocerinae produce sounds by the friction of stridulatory files located in the seventh abdominal tergite and a row of chitinized teeth on the hind margin of the elytra (Schmitt & Traue, 1990). This behavior has been attributed to disturbing predators or parasitoids' in *Stethopachys formosa* Baly stridulation may be a form of communication between conspecifics (Schmitt, 1994).

Adults show other additional defenses. Mimetic complexes (e.g., *Lema* (Criocerinae) and *Diabotrica* (Galerucinae; Gahan; 1891; Balsbaugh, 1988), dodging predators or feigning death (e.g., *Crioceris asparagi),* flight or stridulate when captured (e.g., *Crioceris duodecimpunctata* (Rettenmeyer, 1970) have all documented.

Natural enemies and predators

Chrysomelid eggs and larvae have many predators and parasitoids. Chrysomelidae are predated and parasitized by members of the insect orders Hemiptera, Dermaptera, Odonata, Neuroptera, Coleoptera, Lepidoptera, Mecoptera, Diptera and Hymenoptera (Selman, 1994). Hymenopterans are the principal parasitoids of shining leaf beetle (Schmitt, 1988; Selman, 1994; Vencl *et al*., 2004); members of the Myrmaridae and Eulophidae are parasites of Criocerinae eggs (Selman, 1994). Some parasitoids are specific in host selection, distinguishing between close related species; e.g., eulophid adults can distinguish the eggs of *Crioceris asparragi* and *Crioceris duodecimpunctata* and parasitize only the eggs of *C. asparragi* (Selman, 1994).Other orders reported to predate criocerine are: Heteroptera (e.g., Nabidae, Reduviidae and Pentatomidae),

Neuroptera (e.g., Chrysopidae), and Coleoptera (e.g., Carabidae, Coccinellidae, Melyridae and Staphylinidae, Table 4; Cox, 1996), Diptera (e.g., Tachinidae), Odonata (e.g., Chrysopidae), and as well as birds (Passeriformes; Table 4; Schmitt, 1988).

Our general understanding of Criocerinae is mostly based on pest species (e.g. *Oulema melanopus*; *Crioceris asparagi*). Still, their biology and evolutionary relationships are unknown (Schmitt, 1988; Vencl *et al*., 2004). Morphology is only one source of evolutionary evidence to define evolutionary relationships. For this reason, the development of a molecular phylogenetic hypothesis would contribute to a greater understanding of the group.

The overarching goal of the present study is to develop a new phylogenetic hypothesis of Criocerinae, based on a more densely sampled data matrix and more molecular markers. This study seeks five objectives: 1) test the monophyly of Criocerinae, 2) identify its sister group, 3) better resolve internal relationships of the main genera, 4) identify species clusters within *Lema*, and 5) identify the pattern of host plant selection in the subfamily.

MATERIALS AND METHODS

Ingroup and outgroup taxon sampling

Following Seeno and Wilcox (1982), I constructed the taxon sampling to include the most representative genera of Criocerinae (Table 2); and taxa representing the main biogeographical regions. I assembled a data matrix for 76 criocerine species in 7 genera (Figure. 1A; Table 2). Nine outgroups were sampled, guided by the molecular and morphological phylogenies of Reid (1995), Farrell (1998); Hunt *et al*. (2007), Marvaldi *et al.* (2008), and Gómez-Zurita (2008; Table 2).

Data collection

This research is based on a cryo-collection assembled by a criocerine specialist, Dr. Frederic Vencl, Stony Brook University (Table 2). The collection was previously identified by comparison with type specimens of the following institutions: Museum of Comparative Zoology, Harvard University, Cambridge, United States (MCZH); American Museum of Natural History, New York, United States (AMNH**)**; National Museum of Natural History, Smithsonian Institution, Washington D.C., United States (USNM); British Museum Natural History, London, England, United Kingdome (BMNH), BBM Bishop Museum Collection, Honolulu, Hawaii, United States; Museum der Naturkunde für Humbolt Universität zu Berlin (MNHB), Berlin, Germany; and Institut Royal des Sciences Naturelles (IRSN), Brussels, Belgium. The cryo-collection and extracts will be deposited in the United States National Museum (USNM).

Cryotissues representing 23 species were loaned by Dr. Michael Whiting and Dr. Shawn Clark, Department of Zoology and Entomology, Brigham Young University, Provo, Utah, USA (BYU). Dr. Yoko Matsumura, Institut für Spezielle Zoologie und Evolutionsbiologie, Friedrich-Schiller-Universität Jena, Jena, Germany (UOJ), contributed five criocerine species. I collected specimens of *Crioceris asparagi* Linnaeus in Lawrence, Kansas.

I identified specimen provided by BYU and UOJ, using Monrós (1960) for genera and I used several keys for species: Warchalowski (2010) for Palearctic species, Kimoto & Gressitt (1979), Mohamedsaid (2004) and Warchalowski (2010) for oriental species; and Vencl (2004) for Neotropical species. Dr. Frederic Vencl confirmed species identification and I also confirmed species with imaged types in the MCZ type Database, Harvard University, Cambridge MA (http://insects.oeb.harvard.edu/mcz/).

DNA extractions

DNA was isolated and purified using DNeasy® extraction kits (Qiagen) following the manufacturer's protocol for animal tissue. Which follows a process of lysis, ethanol precipitation, mini column purification and elution of the DNA in AE Buffer.

PCR amplification

Primer selection was guided by recent molecular work performed in the Chrysomelidae and other related taxa (Table 5). Preliminary work to identify the most suitable markers was done using 12S, 18S, 28S, wingless and COI. Only three molecular markers 28S, 18S and COI yielded good quality PCR product. A modified protocol of Giribet (1999) was used to standardize 28S,

with 28Sa and 28Sb as primers (Table 5). Amplification cycles were performed in a Biorad MyCycler (see protocols in Table 6). COI was amplified using a modified protocol of Kubisz *et al* (2012, Table 6), using the primers TL2-N-3014 and C1-J-2183 (Table 5). For 18S, specific primers were designed using available sequences of 18S from Genbank through Geneious v.5.4 (Table 6). Amplification protocols were modified from Marvaldi (2008; Table 6). PCR products were visualized in 2% agarose gels and unincorporated primers and DNTPs were removed from PCR product using ExoSAP-IT (GE USB Corporation) using a 2:5 proportion.

Sequencing

Cycle sequencing reactions were performed in the KU-NHM Molecular lab using a Big Dye Terminator 3.1 chemistry (Applied Biosciences) and the corresponding primers, with a sequencing profile (96°C/3 min; 35 cycles of 96°C/15 s; 50°C/15 s; 60°C/3 min). Reactions were purified using Performa ® DTR Ultra 96-Well Plate Kit and run in an Applied Biosystems 3730xl DNA Analyzer. Data was compared to generate a consensus sequence for each taxon using Geneious v.5.4.7. and queried using the Basic Local Alignment Search tool to confirm the nature of the samples. Additional sequence data were downloaded from GenBank (NCBI; Table 2 &Appendix 1.1).

Sequence alignment

DNA sequences were edited and preliminarily aligned using Geneious v.5.4.7. The alignment of consensus sequences was done using MAFFT v.7.036 (Katon *et al*., 2002) using a G-INS-I model; the correct translation of amino acids was checked using Geneious v.5.4.7. for protein

coding genes. The individual alignments for each gene were then concatenated in Mesquite 2.75 (Maddison & Maddison, 2011). This aligned matrix (three genes) was subjected to phylogenetic analyses.

Phylogenetic Analysis

Phylogenetic analysis was performed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses (BA) for individual genes, as well as combined data sets. Parsimony analysis were done using PAUP* v.4.0b1 (Swofford, 2002), using heuristic searches (10,000 stepwise random additions), as well as TBR branch swapping. Clade support was estimated using bootstrap pseudoreplicates (Felsenstein, 1985) using PAUP and Garli. ML analysis were performed using Garli (Genetic Algorithm for Rapid Likelihood Inference) v.2.0.1. (Zwickl, 2006) using GTR + I + G for the combined sequences and the default settings through 5,000,000 generations. Bayesian analyses were done using Mr. Bayes 3.2 (Ronquist and Huelsenbeck, 2003) as well as BEAST 1.4.7 (Drummond *et al*., 2012). Estimated parameters for Bayesian analyses as calculated by JModelTest 2.1.4. (Table 7; Darriba *et al*., 2012). The outcome of each analyses was visualized in Mesquite were consensus trees were calculated using Majority-rule criterion.

Resulting topologies for each gene were compared to detect areas of incongruence that are strongly supported by non-parametric bootstrap values and/ or posterior probabilities. Bootstrap values $\geq$ 70 % are considered to indicate strong support (Hillis & Bull, 1993). Clades with posterior probabilities $\geq$ 0.95 are considered strongly supported, special caution was put into nodes of high posterior probability values and low bootstrap to avoid over-estimate of confidence (Alfaro *et al*., 2003).

Parametric bootstrapping and hypothesis testing

Parametric bootstrapping was performed to test the monophyly of Criocerinae, its two tribes, individual genera, and geographic clusters of species. A total of twenty-one hypotheses were tested using constraints in the evolution of the group using PAUP* v.4.0b1 (Table 9–11), were parsimony and likelihood scores were calculated. Additionally, LScores (e.g., base frequencies, rates of the GTR matrix, proportion of invariable sites, and shape) were calculated for each constraint and tested using Seq gen v1.3.3. (Rambaut & Grass, 1997) to simulate data scores. The original data were compared with the constrained hypothesis scores.

RESULTS

Characterization of the molecular data

For these sampled taxa I obtained a total of ~5059 bp from three molecular markers: mitochondrial COI (~1695 bp), and nuclear 28S rDNA (~1399 bp) and 18S rDNA (~1965 bp; Appendix 1.1). The alignment included 2332 constant sites, 1386 variable but parsimony-uninformative sites, and 1343 parsimony-informative sites. As expected, the mitochondrial gene COI exhibited more variability than the nuclear genes, 18S and 28S (Table 8).

Phylogenetic analyses

Phylogenetic analysis for individual genes as well for a combined data matrix showed consistency across tree topologies under different models of evolution (MP, ML and posterior probability, Figures. 30–36). All these analyses produced consistent unresolved trees. These analyses did not recover monophyly of Criocerinae, tribes, or genera. However, the placement of Criocerinae as a basal subfamily within Chrysomelidae was confirmed. ML and posterior probability analyses show that *Crioceris* species tend to associated with either Sagrinae or Donaciinae species. Tree topologies show limited evidence for certain species clusters, which are more mostly related to geographical distribution of species and host plant association.

Individual gene analyses

At the individual gene analyses, the parsimony 50% majority rule consensus tree for COI showed the best resolution among individual gene tree topologies (Figure 30A). This parsimony tree has good bootstrap support (1.0) for placing Criocerinae within Chrysomelidae. Only five clades with good bootstrap support are apparent, each one containing small clusters of species of *Lema*, *Oulema*, *Neolema*, *Lilioceris,* and *Crioceris*. The ML and posterior probability trees for COI were also poorly resolved, only supporting the placement of Criocerinae within Chrysomelidae and some species clusters. Most of the COI topologies (Figure 30A, B, C) show polytomies, evidencing uncertainty in the pattern of evolution in Criocerinae. However, species clusters found in the COI ML topology were observed in all COI tree topologies (Figure 30A, B and C).

The analysis of the two nuclear genes, 28S and 18s, revealed more variability within 28S (Table 8). The analyses of parsimony for 28S gave more resolution than ML or posterior probabilities (Figure 31). All 28S topologies support the placement of Criocerinae within Chrysomelidae, suggesting that Criocerinae and Sagrinae are sister taxa when Donaciinae is defined as outgroup in the ML and MP tree topologies. The most derived subfamilies of Chrysomelidae are located within the Chrysomelid clade without any resolution of subfamilial relationships in all tree topologies for 28S (31 A, B and C). Some species clusters appeared consistently across tree topologies.

From all the molecular markers tested, 18S had the most conserved sequences across sampled data (Table 8). Posterior probabilities gave a more resolved tree than ML or MP (Figure 32 C). As seen in 28S parsimony topologies, 18S showed a good support for placing Criocerinae within the Chrysomelidae clade and sister group of Sagrinae. Subfamilial relationships with other chrysomelid subfamilies are still uncertain. However, in this topology we can see six discrete

clades, which contain in some cases clusters of species of the same genera and geography. We also encountered some of the same species clusters recorded in 28S gene tree; i.e., *Stethopachys javeti* Baly and *Lema papuana* Lacordaire as sister species representing the Australian fauna, or a small clade containing most *Lilioceris* species (*L. merdigera*, *L. unicolor*, *L. nigripes* Fabricius and *L. quadripustulata* Fabricius).

Combined analysis of molecular markers

In a combined analysis of the molecular markers, COI, 28S, 18S, criocerine tree topologies did not show evidence for the monophyly of the subfamily when using MP, ML and posterior probabilities (Figures 33–36). Most resolution was seen on the 50% majority rule consensus tree using a posterior probability criterion (Figure 35). This topology shows twelve clusters of species. Some of these clusters are related to genera, but mostly to their geographical distribution (Figure 37). Although this phylogeny was better resolved, branch support was poor for most clades. Better posterior probabilities values were generated using Mr. Bayes 3.2 for this phylogenetic hypothesis (Figure 36). Clades with posterior probability higher than ≥ 0.8 were considered well supported and values between 0.6 – 0.7 had some level of support.

Other evolutionary approaches used in this research such as ML and MP did not show great resolution for intraspecific relationships (Figure 33–34). Still, these two phylogenies supports Gómez-Zurita's (2008) hypothesis of Criocerinae as positioned within the basal clade of Chrysomelidae, sister group to Donaciinae and Sagrinae (bootstrap support 1.0), while most derived chrysomelids (Galerucinae, Chrysomelinae, Cassidinae, Cryptocephalinae) were located within Criocerinae in a separate clade. Both ML and MP trees show similar unresolved tree

topologies, with more resolution in the ML tree. Also, a few clusters of species are found in both phylogenies for *Lema*, *Neolema*, *Oulema, Lilioceris* and *Crioceris* species.

The combined data analyses showed more resolution than individual gene analyses, but did not resolved intraspecific and interspecific relationships of Criocerinae. In spite of this, I was able to test different subfamilial hypotheses (Monrós, 1960; Mann & Crowson, 1981; Jolivet, 1988; Reid, 1995; Gómez-Zurita, 2008) by using an extended set of outgroups. As a result, all the gene trees found show posterior probability and bootstrap support for Criocerinae as a basal subfamily. Most derived subfamilies (Chrysomelinae, Bruchinae, Eumolpinae, Galerucinae and Hispinae) are located in a separate clade within the Criocerinae clade.

The pattern of evolution in Criocerinae is unclear. Phylogenies developed in this research showed some evidence for placing Criocerine within the Chrysomelidae clade (≥ 0.8–1.0 posterior probabilities, 1.0 bootstrap; Figures 33–36). Relationships within Criocerinae at tribal or generic level where not recovered from these topologies. Some of the species clusters observed in these tree topologies where shared, especially between ML and posterior probabilities. The resulting 50% majority rule consensus phylogram from BEAST 1.4.7 (Figure 36) was able to recover most of the species clusters from MP, ML and Mr. Bayes 3.2 posterior topology.

Parametric bootstrapping and tree searching

Parametric bootstrapping was done to test twenty-one subfamilial, tribal, generic and geographical constraints (Table 9–11). Additional tree searching was performed including or excluding taxa, which did not improve the topology or scores of the trees analyzed. From the proposed hypothesis constraints, constraint nine was the only hypothesis that improved Lk (Table 10). This constrained *Neolema* as monophyletic: ((*Neolema*) Outgroups, *Lema*, *Oulema*,

Sthethopachys, *Lilioceris*, *Crioceris*, *Metopoceris*). Although this hypothesis only improved the likelihood scores, it still shows some evidence of monophyly for these Neotropical and Nearctic species. The remaining hypotheses (Table 12–14) did not improve the likelihood or parsimony score through parametric bootstrapping.

Geography

By associating the geographical to our most resolved topology (Figure 36), I found some species clusters are correlated to their distribution, were Neotropical, Neotropical-Nearctic, Oriental, Oriental-African and Palearctic clusters of species are apparent in our tree topology (Figure 37).

Host plant association

The addition of known host plant records (collecting information/literature; Appendix 1.2) to this phylogeny, showed that most Criocerinae sampled feed from monocot plants (Figure 37), especially from Commelinaceae and Dioscoreaceae. Some members of *Lema*, *Neolema* and *Metopoceris* secondarily feed on Solanaceae (Eudicotyledon). Additionally, two species of *Lilioceris* were recorded feeding from Cycadales and Magnoliids, which are considered more basal in the evolution of plants (II, 2003). Although my phylogeny does not contain all generic and species representation for Criocerinae, it provides a general view of host plant selection in Criocerinae and some species clustering with biogeographical significance.

DISCUSSION

This study aimed to assemble a better-sampled and more rigorous phylogeny for Criocerinae with better tests of subfamily monophyly and position within Chrysomelidae, and internal relationships in Criocerinae. My dataset includes an increased geographical representation and more outgroups than considered in previous studies. I discuss and compare my results with other phylogenetic studies that focused on Criocerinae (Schmitt, 1988; Reid, 1995; Teo, 1999 and Vencl *et al*., 2004), and other patterns observed in this phylogenetic hypothesis associated to host plant records and geographic distribution of the sampled taxa.

Data performance

The shining leaf beetles (Criocerinae, Chrysomelidae) have proven to be difficult to classify within Chrysomelidae. I found that Criocerinae is not monophyletic, and it has a complex pattern of evolution across its members. Phylogenetic relationships found in this study show a strong support (> 0.90 posterior probabilities/ 1 – 0.75 bootstrap), for placing Criocerinae within Chrysomelidae, as members of the Sagrinae clade as shown in previous phylogenetic studies (Figures 9–10; Hunt *et* al., 2007; Marvaldi *et al*., 2008; Gómez-Zurita *et al*., 2008). However, the pattern of speciation events within Criocerinae is still unclear. My phylogenetic analyses and combined data analysis found a lack of resolution for individual genes (Figure 30-36). Most resolution was seen with a combined data set, were the most resolved phylogenetic tree was the posterior probability tree using BEAST 1.4.7 (Figure 35).

Outgroup selection

Overall, our analyses show consistency across tree topologies, positioning *Crioceris* species within the Sagrinae clade, either as a sister group to Donaciinae or Sagrinae. The synamomorphies that support these relationships [Donaciinae: prosternal process Chaupis (1874) and, lack of pronotal margin Askevold (1990); Sagrinae: presence of frontal grooves (Schmitt, 1985, 1988)], are difficult to determine, and very much focused comparative morphological study across subfamilies.

The remaining outgroups used for the analyses are located within a larger unresolved clade, grouped in two main clades, one representing Eumolpinae clade (Hispinae, Eumolpinae and Cryptocephalinae) and the other representing the Chrysomelinae clade (Chrysomelinae and Galerucinae) in the MP and ML trees (Figure 33–34). These results are consistent with previous phylogenetic studies (Hunt *et al*., 2007; Gómez-Zurita *et al*., 2008 and Marvaldi *et al*., 2008), where Criocerinae is sister group to Donaciinae or Bruchinae, and within the Sagrinae clade (Figures 8–10).

In the posterior probabilities analyses, two outcomes are observed. The output from Mr. Bayes was consistent with ML and MP tree topologies, with the exception of the position of *Diabotrica duodecimpuncta* (Mannerheim; Galerucinae, Chrysomelidae). This outgroup was placed as a basal subfamily in this tree with low support (0.57 posterior probability, Figure 36). A second outcome was generated using BEAST 1.4.7. This topology shows partially recovered relationship for outgroups, with a clear Eumolpinae clade, and another clade containing several members of different clades including Bruchinae, Galerucinae, and *Lilioceris subcostata* (Pic) with low support (0.53 posterior probabilities). This relationship is not maintained in any other tree topology developed in this study.

The placement of Criocerinae in Chrysomelidae evolution has been controversial and several hypothesis have been developed around Donaciinae, Bruchinae, Cassidinae and Sagrinae (Chaupius, 1874; Monrós, 1960; Mann & Crowson, 1981; Chen 1985; Jolivet, 1988; Reid, 1995; Teo, 1999; Hunt *et al*., 2007; Gómez-Zurita *et al*., 2008, Marvaldi *et al*., 2008; Figures 2–10). Previous morphological and molecular phylogenies for Criocerinae did not include outgroups or had selected only one subfamily as outgroup. With the addition of a larger number of outgroups we were able to support the placing of Criocerinae as a basal subfamily in the evolution of chrysomelids. My analyses discard Hispinae as sister group of Criocerinae. Even though these two subfamilies share common features (reduced tegmen and wing venation in adults and; larvae with reduced number of stemmata, paronychial appendix on tibiae, one segmented palpi, and the production of a fecal shield as a defensive mechanism; Reid, 1995; Vencl & Morton, 1998), they are clearly not related. This suggests that some morphological features and behaviors evolved several times in Chrysomelidae. For example, some defensive behaviors such as defense rings (cycloalexy) are present in Chrysomelinae, Cassidinae, Criocerinae, and Galerucinae (Vasconcellos-Neto & Jolivet, 1994; Santiago-Blay *et al.*, 2012), and the production of fecal defense structure present in larvae of Criocerinae, Cassidinae, Camptosomata (Cryptocephalinae + Lamprosomatinae), and Galerucinae larvae (Chaboo, 2011).

Even though my phylogenetic results do not suggest relationships with Bruchinae, there are strong morphological and molecular evidences for placing Bruchinae in the Sagrinae clade (Donaciinae + Sagrinae + Criocerinae + Bruchinae; Mónros, 1960; Mann & Crowson, 1981; Hunt *et al.*, 2007; Gómez-Zurita *et al*., 2008). An available sequence loci for *Callosobruchus maculatus* (Bruchinae, Chrysomelidae) from GenBank could not be aligned with my preliminary data set; for these reasons I did not include more Bruchinae for these analysis.

Monophyly and internal relationships

Our resulting tree topologies show Criocerinae is not a monophyletic group (Figures 30–36), showing unresolved tree topologies and some species clusters. The monophyly of the subfamily is supported by the presence of the stridulatory apparatus in the seventh tergite, and frontal grooves in adults, ambulatory warts in the larval abdomen, as well as the dorsal anus and fecal shield in larvae (Schmitt, 1988). Diagnostic characteristics found in Criocerinae are also found in other Chrysomelidae subfamilies like Sagrinae and Hispinae (Schmitt; 1988; Reid, 1995). These characteristics have been valuable in identifying members of the subfamily, but a more detailed evaluation is needed to consider them diagnostic characteristics.

Status of Criocerinae tribes

Traditionally, tribes have been diagnosed by tarsal morphology: Simple and free pretarsal claws are present in Criocerinini and Pseudocriocerinae, and connate in Lemiini (Monrós, 1960, Arnett *et al.,* 2002; Vencl & Leschen, 2014). Genera are diagnosed mainly by morphological characteristics of the adult tarsal claws, pronotal morphology, and elytral puncture patterns (Table 15; Vencl *et al*., 2004). Our analyses were not able to recover intraspecific and tribal relationships previously proposed. Because some diagnostic characters are considered to be ambiguous or present early in the evolution of Chrysomelidae (Schmitt, 1988; Vencl & Leschen, 2014). My results suggests that more detailed evaluation of diagnostic generic characters are still needed. Still, some evidence of monophyly for *Neolema* was observed through parametric bootstrapping (Table 11).

Some species clusters are clearly observed across tree topologies (Figure 30–36), particularly in the gene trees for 28S and 18S. For example, these trees show clades containing

only *Lilioceris*, *Neolema,* and *Oulema* species. Several clades containing *Lema* species have moderate to high support (≥0.60 bootstrap / posterior probability). This suggests that *Lema* is probably not monophyletic. *Stethopachys* Baly on the other hand, is found to be strongly supported as a sister group of *Lema* (0.81 posterior probability, Figure 31C) in the 28S gene tree, sister species to *Lema* sp 1. (Papua New Guinea). Therefore, *Stethopachys* rests within Lemini.

In a combined analysis, these clusters of species were also conserved across topologies. However, most relationships are still unresolved, and clear patterns of speciation cannot be discussed. *Crioceris* appears to be more basal in the evolution of the group by clustering with Donaciinae and Sagrinae, and not with *Lilioceris* as Schmitt (1985) suggested. We observed some level of concordance with Teo's (1999; Figure. 37) strict consensus phylogeny, with all three tribes represented. Both Teo's phylogenies and mine have some unresolved clades. In Teo's (1999) phylogeny, most Lemini where contained within the same clade, and Criocerini was described as polyphyletic, which is consistent with my results. Both phylogenies show that *Lema* and *Lilioceris* are not monophyletic genera.

Effect of geography on phylogeny

Geography of sampled species seemed to impact Teo's (1999) phylogeny and my phylogenetic hypothesis, where Oriental and New World clusters are observed. Still, the separation between tribes and genus was not observed as in Teo's (1999) morphology-based phylogeny. Although Teo's (1999) study is valuable for understanding of morphological character evolution, her analysis did not include more outgroups and bootstrap support for her resolved relationships.

Vencl *et al*. (2004) found that *Crioceris* and *Metopoceris* were basally positioned in their sequence-based phylogenetic hypothesis of Criocerinae They also found two discrete clades

containing Lemiini species. The first clade includes only *Lema* species, while a second clade includes mainly *Neolema* and *Oulema* species. These results are partially supported by our molecular phylogeny where *Crioceris* is the most basal genera, while *Metopoceris* rests within the main unresolved clade. Vencl et al.'s (2004)'s phylogenetic hypothesis gave us an insight about internal relationships of the subfamily for Panamanian and Costa Rican species (5 genera, 21 species), but these patterns could not be generalized for all criocerine species since this work lacks other geographic and outgroup representation. Still, some of their species relationships are also supported in our phylogeny; for example, *Neolema sallaei* as sister to *Neolema plumbea*, and *Lema bouchardi* as sister to *Lema obliterata*.

Analyses done in Criocerinae suggests that this phylogenetic problem would likely benefit from better taxon sampling, especially adding members of Pseudocriocerini and Criocerini, like *Pseudocrioceris*, *Ovamela* and *Sigrisma*, which are considered basal in the evolution of the group and share several characters with other Chrysomelid subfamilies that are considered to be basal in the evolution of the Chrysomelidae (Reid, 1995; Teo, 1999). The addition of rare and underrepresented genera will give us a better understanding about the speciation patterns of Criocerinae at a molecular level, and should be considered for future work performed in the subfamily.

Host plants

The pattern of host plant selection in Criocerinae has been explained under a co-diversification hypothesis between plants and insects (Crowson, 1981; Schmitt, 1988) where independent mechanisms of diversification for each Chrysomelidae subfamily (coevolution or co-radiation), as is thought determined host-plant association (Crowson, 1981; Schmitt, 1988;

Gómez-Zurita, 2007). We were able to trace host plant association based on host records of the species in the cryotissue collection and on published host plant records (Figure 39).

The current records of criocerine host plants altogether indicate that most Criocerinae are feeding from monocots plants (Jolivet, 1988; Schmitt, 1988). I found that most of my sampled taxa are feeding from Commelinales and Discoreales. These results support Gómez-Zurita's (2008) hypothesis, which discard a co-radiation hypothesis (from gymnosperm to angiosperm). The phylogenetic hypotheses generated herein suggests that Criocerinae possibly evolved from a monocot-eating ancestor, and some of its members have been able to change their host plant; either feeding from non-angiosperms like Cycadophyta in the case of *Lilioceris*, or from eudicots as seen in *Lema*, *Neolema*, and *Metopoceris*.

Criocerinae are considered mono- and olygophagous insects (Jolivet & Petitpierre, 1981). *Lilioceris* and *Crioceris* are associated mainly with monocots, but occasionally feeding from eudicots (e.g., *Lilioceris lilii* feed *on Lilium* and *Solanum*; Schmitt, 1988). *Lema* and *Neolema* are feed on monocots and eudicots (Jolivet, 1988; Schmitt, 1988, White, 1993). The correlation between our phylogeny and host plant records show similar patterns: C*rioceris*, the most basal genera, feeds from monocotyledonous plants (Asparagales; Weise, 1893; Schmitt, 1988; White, 1993), and most of the remaining genera from resolved clades are reported to feed from other groups of plants. For example, all documented *Oulema* species feed on members of the order Poales, especially Poaceae (Schmitt, 1988; White, 1993; Jolivet & Hawkeswood, 1994). *Oulema melanopus* Linnaeus, an agricultural pest of cereal crops in United States and Europe (White, 1993). *Sthethopachys* feed from Orchidaceae (Jolivet & Hawkeswood, 2009) and *Metopoceris* feed from Solanaceae (Vencl *et al*., 2004). Some derived genera like *Lema* select several families of monot and eudicot plants: Commelinaceae, Fabaceae, Zingiberaceae, Poaceae, Agavaceae, Solanaceae, Saxifragales, Pedaliaceae, Asteraceae, Brassicaceae, Hippocastaceae, Araceae,

Zingiberaceae, Malvaceae, Rubiaceae, Rutaceae, Dioscoraceae, Rosaceae, Iridaceae, Pandanaceae, Orchidaceae, Polygonaceae, Convulvulaceae, Cucurbitaceae, Arecaceae and Musaceae; (Schmitt, 1988; White, 1993). While *Neolema* is found associated with Monocots: Commelinaceae, Araceae, Poaceae, Maranthaceae and Eudicots: Asteraceae, Rosaceae, Fabaceae and Solanaceae (White, 1993; Vencl *et al*., 2004).

Lilioceris species were found feeding from monocots, except for *Lilioceris nigripes* and *Lilioceris quadripustulata*, which were observed to feed on Stangeriaceae (Cycadophyta) and Annonaceae (Magnolids). Previous host plant records mention *Lilioceris* species nourishing from Cycadophyta members, which are considered more basal in the evolution of plants. For example *Lilioceris clarkii* Baly is associated with the genus *Cycas* (Crowson, 1981), *Lilioceris nigripes* with *Cycas*, *Bowenia* and *Macrozamia*, and all members of the Cycadophyta and other *Lilioceris* species (*L. fuscomaulata* Clark & *L. chamelus* Duvivier) were observed in Smilacaceae (monocot; Hawkeswood, 2009).

The evolution towards the selection of certain groups of host plants are probably related to the presence of secondary compounds, which have been shown to be used in defensive mechanisms during larval stages (Morton & Vencl, 1997; Vencl & Morton, 1999; Vencl *et al*., 2014). Commelinaceae and Solanaceae, which are common host plants of Criocerinae, are known to contain secondary compounds such as terpenoids, phenolics and alkaloids, which are incorporated into fecal shields (e.g. *Neolema sexpunctata* (Olivier) and *Lema trilinea*) and can act as deterrents against predators (Whitman *et al*., 1990; Morton & Vencl, 1998; Morton & Aiello, 1998). This is reflected in our phylogeny where some small clades maintain the same family of host plants, or even members of the same order. For example C*rioceris* feed from Asparagaceae and Amarylidaceae, members of the order Asparagales.

Information about host plants and general biological aspects in Criocerinae is limited, especially for rare genera. Host plants are documented for most of the taxa analyzed herein. Resolved clades with a particular order of plants that include this gap, could help predict possible host plant order or family. Extensive field and associated data will help incorporate more biological facts, and a more complete view about the evolution of host plant selection in the group.

Even though Gómez-Zurita (2007) rejects a co-radiation hypothesis and provides a new calibrated tree for Chrysomelidae, host plant association is a still an important factor of the evolution of any phytophagous insect, due to their strong association during their biological cycle. The addition of more biological facts and new fossil records can give us a more detailed insight on the pattern of evolution of the group and the factors that derived the diversification in Criocerinae.

Future challenges in Criocerinae

Our phylogenetic results with the lack of unambiguous support for monophyly of criocerine tribes and some genera casts doubt on past explanations of evolutionary pattern. Our contribution offers a pool of molecular data towards resolving these complex issues of phylogeny, taxonomy, and explanation of evolutionary patterns. Our data contributes to resolving the phylogenetic backbone of Criocerinae despite the lack of sampling of all tribes and genera.

Parametric bootstrapping was performed to discard the existence of discrete groups within Criocerinae (genera, tribe and also geographic association of species). The only significant improvement in scores was seen when constraining *Neolema* as monophyletic which improve ML scores (Table 11), which suggest that the genus could be a monophyletic but did not improve MP scores.

Future studies that build on ours will benefit by sampling a wider distribution, including underrepresented genera from specific geographic areas, like African, Oriental and Pacific regions. Sampling more characters (morphological / behavior / molecular), and by developing more rigorous models of sequence evolution will help produce more congruent and well resolved phylogenies (Nabhan & Sakar, 2012).

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TABLE AND FIGURES

TABLE 1. Criocerinae classification according to Seeno and Wilcox (1982) and White (1993) which includes *Neolema* as a new genus for the subfamily. Sampled genera are in bold-faced text.

Tribe	**Genus**	**Author/year**	**Distribution**
Pseudocriocerini Heinze 1962	*Pseudocrioceris*	Pic 1916	Java, Madagascar
Criocerini Latreille 1807	*Ovamela*	Fairmaire 1887	Madagascar
	Metopoceris	Heinze 1931	Central America
	Lilioceris	Reitter 1912	World wide
	Mecoprosopus	Chujo 1951	China
	Crioceris	Muller 1764	World wide
	Elisabethana	Heinze 1928	Africa
	Sigrisma	Fairmaire 1888	Africa
	Manipuria	Jacoby 1908	India
Lemiini Heinze 1962	*Trichonotolema*	Heinze 1927	Africa
	Atactolema	Heinze 1927	Africa
	Lema	Fabricius 1798	World wide
	Neolema	Monrós, 1951	North and South America
	Mimolema	Pic 1921	World wide
	Oulema	Gozis 1886	Europe, Asia and Africa
	Onholema	Heinze 1943	Asia
	Incisolema	Pic 1916	Africa
	Plectonycha	Lacardaire 1845	South America
	Stethopachys	Baly 1861	Asia, Australia
	Lagriolema	Gressitt 1965	New Guinea
	Papulema	Gressitt 1965	New Guinea

TABLE 2. Criocerinae species and outgroups used in the phylogenetic analysis.

Voucher #	Genus	Species	Author	Locality information	Latitude	Longitude
SMCR001	*Lema*	*approximata*	Jacoby 1888	Panama, Colon, Santa Rita	9.3	-80
SMCR002	*Lema*	*balyana*	Jacoby, 1908	Malaysia, Pahang, Bukit Fraser	3.7	102
DQ001931	*Lema*	*biannularis*	Clark, 1866	Panama / Costa Rica region	—	—
SMCR003	*Lema*	*bicincta*	Lacordaire, 1845	Costa Rica, Cartago, Turrialba	9.9	-84
DQ001935	*Lema*	*bitaeniata*	Chevrolat, 1833	Panama / Costa Rica region	—	—
DQ001936	*Lema*	*bouchardi*	Baly, 1879	Panama / Costa Rica region	—	—
SMCR004	*Lema*	*cingulata*	Clark, 1866	Costa Rica, San Jose, San Antonio	9.2	-84
SMCR005	*Lema*	*cyanea*	Fabricius, 1798	Malaysia, Kelatan, Gua Bama	4.3	102
SMCR006	*Lema*	curaca	Monrós, 1958	Peru, Madre de Dios, Posadas Amazonas, Rio Tambopata	-13	-70
SMCR007	*Lema*	*daturaphila*	Kogan & Goeden, 1970	Japan, okinawa, Yonaguni island, Sonai	24	123
SMCR008	*Lema*	*delauneyi*	Baly, 1889	Malaysia, Kelatan, Kuala Lipis	4.3	102
SMCR009	*Lema*	*feae*	Jacoby, 1892	Malaysia, Kelatan, Kuala Lipis	4.3	102
SMCR010	*Lema*	*fleutiauxi*	Baly, 1889	Malaysia, Kelatan, Kenerong river, 5 km S Gugung stong	5.3	102
SMCR011	*Lema*	*foveipennis*	Jacoby, 1888	Panama, Panama, Cerro Campana	8.7	-80
DQ001929	*Lema*	*fulvipes*	Jacoby, 1866	Panama / Costa Rica region	—	—
DQ155990	*Lema*	*gallaeciana*	(Heyden, 1870)	Great Britain	—	—
SMCR012	*Lema*	*hamata*	Lacordaire, 1845	Costa Rica, San Jose, El Valle	7.9	-81
DQ001939	*Lema*	*immaculipennis*	Clark, 1866	Panama	—	—
SMCR013	*Lema*	*insularis*	Jacoby, 1888	Ecuador, Loja, Parque Nacional Podocarpus	8.8	-80
SMCR014	*Lema*	*nigromaculata*	Jacoby, 1880	Panama, Panama, Cerro Campana	8.7	-80
DQ001940	*Lema*	*obliterata*	Jacoby, 1888	Panama	—	—
SMCR015	*Lema*	*obscura*	Fabricius, 1801	Panama, Colon, Barro Colorado, Pipeline rd	9.1	-80
SMCR016	*Lema*	*perplexa*	Baly, 1889	Thailand, Chang Mai, Wachirithan Falls	19	98.9

SMCR017	*Lema*	*phungi*	Pic, 1924	Malaysia, Kelatan, Gua Musang	102	4.89
SMCR018	*Lema*	*praeclara*	Clark, 1865	Costa Rica, San Jose, San Antonio	9.2	-84
SMCR019	*Lema*	*rangoonensis*	Jacoby, 1892	Malaysia, Kelatan, Kuala Lipis	4.3	102
SMCR020	*Lema*	*regularis*	Jacoby, 1888	Panama, Panama, Cerro Campana	8.7	-80
SMCR022	*Lema*	*ropunctata*	Gebler, 1830	Japan, Teramaci Guhuku	35	136
SMCR023	*Lema*	*rufotestaceae*	Clark, 1866	Malaysia, Kelatan, N of Kuala Lipis	4.3	102
SMCR024	*Lema*	*saigonensis*	Pic, 1923	Malaysia, Kelatan, Kuala Lipis	4.3	102
SMCR025	*Lema*	*stolida*	Lacordaire, 1845	Panama, Darien, Cana	8.1	-82
SMCR026	*Lema*	*trabeata*	Lacordaire, 1845	Panama, Colon, Barro Colorado, Pipeline rd	9.1	-80
SMCR027	*Lema*	*transversofasciata*	Jacoby, 1880	Costa Rica, San Jose, Escazu 2 km	9.9	-84
DQ001944	*Lema*	*trivittata*	Say, 1824	United States, New York, Long Island, Shelter Island	41	-72
SMCR028	*Lema*	*vridana*	Jacoby, 1880	Ecuador, Zamora-Chinchipe, Reserva Biologica San Francisco	-4	-79
SMCR029	*Lema*	*viridicolor*	Pic, 1947	Thailand, Chang Mai, Doi Sutep	19	99.1
SMCR030	*Lema*	sp1. Ghana		Ghana, Greater Accra, Snai Hills Research	5.9	0.06
SMCR031	*Lema*	*externevittata*	Pic, 1943	India, Maharashtra, Mulshi, Tamhini village	18	73.4
SMCR032	*Lema*	*constrictofaciata*	Jacoby, 1908	India, Maharashtra, Mulshi	18	73.5
SMCR033	*Lema*	*nr. hopei*	Jacoby, 1908	India, Kerala, Malapuura, Kuttippuram	11	76
SMCR034	*Lema*	*lacordairei*		India, Kerala, Wayanad, Kalpetta, 15 km E	12	76.1
SMCR035	*Lema*	sp1. Namibia		Namibia, Waterberg, Onojoka Spring	-20	17.4
SMCR036	*Lema*	sp1. Papua New Guinea		Papua New Guinea , Chimbu, Wara Sera Research	-7	145
SMCR037	*Lema*	sp 2. Papua New Guinea		Papua New Guinea , Chimbu, Wara Sera Research	-7	145
SMCR038	*Lema*	sp 1. Peru		Peru, Madre de Dios, Posadas Amazonas, Rio Tambopata	-13	-70
SMCR039	*Lema*	sp 1. Malaysia		Malaysia, Perak, Gual Tempurong, IpodGepong	4.5	101

SMCR040	*Lema*	*femorata*	Guérin-Méneville, 1829	Malaysia, Sabalu, Inobong, Crocker Range National Park	5.6	116
SMCR041	*Neolema*	*Dorsalis*	(Olivier, 1791)	Brazil, Piaui, Toca du Pinga	-7	-41
SMCR042	*Neolema*	*ephippium*	(Lacordaire, 1845)	United States, Florida, Osceola	28	-81
SMCR043	*Neolema*	*eremita*	(Jacoby, 1888)	Panama, Chiriqui, Continental divide trail	8.4	-82
SMCR044	*Neolema*	*gundlachiana*	Suffrian, 1874	United States, Florida, Key Biscane	26	-80
SMCR045	*Neolema*	*hexastigma*	(Lacordaire, 1845)	Panama, Darien, Cana	8.1	-82
SMCR046	*Neolema*	*plumbea*	(Chevrolat, 1835)	United States, Maryland, Annapolis, Arundal	39	-77
SMCR047	*Nolema*	*relucens*	Jacoby, 1888	Panama, Colon, Barro Colorado, Pipeline rd	9.1	-80
SMCR048	*Neolema*	*sallaei*	(Jacoby, 1835)	Panama, Bocas del Toro, Weckso	9.3	-82
SMCR049	*Neolema*	*sexpunctata*	(Olivier, 1808)	United States, Maryland, Annapolis, Arundal	39	-77
SMCR050	*Neolema*	spp.		Panama, Panama, Old Gamboa rd	9.1	-80
SMCR051	*Oulema*	*atrosuturalis*	Pic, 1923	Japan, okinawa, Yonaguni island, Sonai	24	123
DQ001947	*Oulema*	*elongata*	White, 1993	Panama / Costa Rica region	—	—
SMCR052	*Oulema*	*melanopus*	Linnaeus, 1758	United States, Utah, Spanish Fork River Hwy 147	40	-112
SMCR053	*Oulema*	*oryzae*	(Kuwayama, 1929)	Japan, Fukushima, Naganuma	37	140
BMNH:832838	*Oulema*	*rufocyanea*	Suffrian, 1847	Great Britain	—	—
SMCR054	*Oulema*	sp 1. India		India, Maharashtra, Kondye 1.5 km SE	17	73.6
BMNH#704404	*Stethopachys*	*javeti*	Baly, 1861	New Caledonia, South Province, Mt. Humbolt	-22	166
SMCR055	*Crioceris*	*asparagi*	(Linnaeus, 1758)	United States, Kansas, Douglas, Lawrence	39	-95
FJ000446	*Crioceris*	*duodecimpunctata*	(Linnaeus, 1758)	Holartic	—	—
JF775781	*Crioceris*	*quinquepunctata*	(Scopoli, 1763)	Czech Republic	—	—
SMCR056	*Lilioceris*	*merdigera*	(Linnaeus, 1758)	Germany, Oberammergau, Lain Valley	—	—
SMCR057	*Lilioceris*	*nigripes*	(Fabricius, 1775)	Australia, Queensland, Beatrice river	-18	146
SMCR058	*Lilioceris*	*nigropectoralis*	(Pic, 1928)	Malaysia, Selangor, For. Inst. Malaysia	101	3.35

SMCR059	*Lilioceris*	*quadripustulata*		Malayasia, Selangor, FRIM, Kepong	3.2	102
SMCR060	*Lilioceris*	*subcostata*	(Pic, 1921)	Thailand, Chang Mai, Doi Anklang	20	99
SMCR061	*Lilioceris*	*unicolor*	(Fabricius, 1787)	India, Maharashtra, Mulshi	18	73.5
BMNH#704401	*Lilioceris*	*spp.*		Malaysia, Sabah, Batu, ca. 25 km SE Sapulut	5.3	116
SMCR062	*Lilioceris*	*sp1*. India		India, Maharashtra, Mulshi	18	73.5
DQ001949	*Metopoceris*	*spp.*		Panama / Costa Rica region	—	—
SMCR063	**Altica*	*viridicyanea*	(Baly, 1874)	China	32	106
SMCR064	**Bruchidius*	sp.	Zschach, 1788	Spain, Avila, Aviente	40.78	-4.8
BMNH#704481	**Charidotella*	*sexpunctata*	(Fabricius, 1781)	Canada, Ontario, Lanark, Almonte	45	-76
SMCR065	**Cryptocephalus*	*iridipennis*	Chapuis, 1876	Australia, Queensland, Brisbane, Mt. Coo-tha	-27	153
SMCR066	**Diabotrica*	*undecimpuctata*	Linnaeus	United States, Kansas, Douglas, Lawrence	39	-95
SMCR067	**Donacia*	*vulgaris*		Japan, Tokyo, Akiruno	35.73	139
BMNH#704384	**Paropsis*	*maculata*	(Marsham, 1808)	Australia, Queensland, Brisbane, Mt. Coo-tha	-27	153
SMCR068	**Plateumaris*	*flavipes*	(Kirby, 1837)	Canada, Manitoba	55	-97
SMCR069	**Sagra*	*femorata*	(Drury, 1773)	Indonesia	-6	107

*Outgroup

TABLE 3. Host plant records for criocerine beetles at family level.

Genus	Rank	Family
Lilioceris	Cycadopsida	Cycadaceae, Zamiaceae
	Monocot	Liliaceae, Dioscoraceae, Smilacaceae, Xanthorrhoeaceae, Asparagaceae, Amaryllidaceae, Nolinoideae, Pandanaceae, Smilacaceae
	Eudicot	Cucurbitaceae, Salicaceae, Solanaceae
Crioceris	Monocot	Asparagaceae, Liliaceae
	Magnoliids	Lauraceae
Lema	Pterydophyta	Fern
	Monocot	Commelinaceae, Poaceae, Musaceae, Dioscoreaceae, Iridaceae, Zingiberaceae, Costaceae, Pandanaceae, Arecaceae, Asparagaceae, Cyperaceae, Liliaceae, Orchidaceae, Iridaceae
	Eudicot	Solanaceae, Asteraceae, Pedaliaceae, Brassicaceae, Fabaceae, Amaranthaceae, Convulvulaceae, Polygonaceae, Rubiaceae, Sapindaceae, Fagaceae, Malvaceae, Cucurbitaceae, Apiaceae, Rosaceae, Saxifragales
Neolema	Magnoliids	Lauraceae
	Monocot	Commelinaceae, Cyperaceae, Poaceae, Araceae
	Eudicot	Asteraceae, Rosaceae, Fabaceae, Malvaceae, Cucurbitaceae, Brassicaceae, Polygonaceae
Oulema	Pteridophyta	Pteridaceae

	Magnoliids	Piperaceae
	Monocot	Poaceae, Commelinaceae, Polygonaceae, Cyperaceae, Araceae, Asparagaceae, Dioscoraceae,
	Eudicot	Asteraceae, Solanaceae, Brassicaceae, Convolvulaceae, Rutaceae, Fabaceae, Rosaceae
Elisabethana	Monocot	Asparagaceae
Sigrisma	Monocot	Asparagaceae
Plectonycha	Monocot	Poaceae, Basellaceae,
Stethopachys	Monocot	Orchidaceae

TABLE 4. Parasitoids and predators of Criocerinae

Criocerinae genus	**Order**	**Family**
Lilioceris sens. lat.	Heteroptera	Nabidae
Lilioceris merdigera	Hymenoptera	Ichneumonidae
	Diptera	Tachinidae
Crioceris sens. lat.	Heteroptera	Pentatomidae, Reduvidae
	Neuroptera	Chrysopidae
	Coleoptera	Coccinellidae
Crioceris asparagi	Hymenoptera	Eulophidae, Vespidae, Sphecidae
	Diptera	Tachinidae
	Coleoptera	Coccinellidae
	Neuroptera	Chrysopidae
	Odonata	Coenagrionidae
	Hemiptera	Reduviidae, Nabidae, Pentatomidae
Lema sens. lat.	Heteroptera	Nabidae, Pentatomidae
	Neuroptera	Chrysopidae
	Coleoptera	Meliridae, Coccinellidae
Lema bilineata	Hymenoptera	Formicidae
	Heteroptera	Reduviidae
	Araneae	-
Lema cyanella	Hymenoptera	Eulophidae
		Ichneumonidae
Oulema sens. lat.	Coleoptera	Carabidae, Coccinellidae, Staphylinidae, Elateridae
Oulema galleciana	Hymenoptera	Ichneumonidae, Pteromalidae
Oulema haffmannseggi	Hymenoptera	Chalcididae
Oulema melanopus	Coleoptera	Coccinellidae
	Hemiptera	Pentatomidae, Nabidae
	Neoptera	Chrysopidae
	Acari	-
	Hymenoptera	Mymaridae
		Ichneumonidae
		Eulophidae
Oulema oryzae	Coleoptera	Coccinellidae, Staphyllinidae
	Hymenoptera	Mymaridae, Ichneumonidae, Pteromalidae

TABLE 5. Molecular markers used for this research.

Molecular Marker	Primer	Sequence	Author
Nuclear	28Sa	GACCCGTCTTGAAGCACG	Whiting *et al*., 1997
	28Sb	CCCACAGCGCCAGTTCTGCTTACC	Whiting *et al*., 1997
Nuclear	18S (f)	CCGGCACGGGGAGGTAGTGA	This study
	18S (r)	TCGGAGGAACGTCGGCGGAT	This study
Mitochondrial	CIJ-2183 (COI)	CAACATTTATTTTGATTTTTTGG	Simons *et al*., 1994
	TL2-N 3014 (COI)	TCCAATGCACTAATCTGCCATATTA	Simons *et al*., 1994

TABLE 6. Thermocycling conditions used to amplify mitochondrial and nuclear genes using the polymerase chain reaction (PCR). Protocols for 28S were modified from Giribet *et al.* (1999), for 18S from Marvaldi *et al* (2008), and modified for COI from Kubisz *et al*. (2012).

Gene	Protocol
COI	1 cycle: 2 min 95°C 38 cycle: 30 s 95°C, 30 s 50°C, 1 min 72°C 1 cycle: 7 min 72°C
18S	1 cycle: 2 min 95°C 38 cycle: 30 s 95°C, 30 s 58°C, 1 min 72°C 1 cycle: 10 min 72°C
28S	1 cycle: 2 min 95°C 35 cycle: 30 s 95°C, 30 s 50°C, 1 min 72°C 1 cycle: 10 min 72°C

TABLE 7. Estimated parameters for Bayesian analysis using JModel test 2.1.4 (K= Optimized free parameters, I= Proportion of invariable sites, Γ= Gamma distributed rates among sites)

Genes	Best-fit Model	K	-ln likelihood	I	Γ
COI	JC+I+G	184	22355.4374	0.1090	0.5300
28S	GTR+G	185	11145.8432	0.0000	0.8670
18S	JC+I+G	188	5346.8967	0.0000	0.0200

TABLE 8. Proportion of Parsimony informative (PI) and Invariable characters.

Gen	No. of PI	Proportion of PI	No. of Invariable sites	Proportion of invariable sites
COI	863	0.509144543	589	0.347492625
28S	326	0.233023588	593	0.423874196
18S	152	0.07735369	1150	0.58524173

TABLE 9. Hypothesis testing with subfamilial and tribal constraints using parametric bootstrapping.

Constraint 1	(Outgroups) (Criocerinae)
Constraint 2	(Outgroups) (Sagrinae, Donaciinae (Criocerinae)
Constraint 3	(Outgroups) (Sagrinae, Donaciinae ((*Lema*) (*Neolema*) (*Oulema*)(*Stethopachys*) (*Metopoceris*) (*Lilioceris*) (*Crioceris*))
Constraint 4	(Outgroups) (Sagrinae, Donaciinae) (Criocerini) (Lemini))
Constraint 5	(Outgroups) (Sagrinae, Donaciinae (((*Lema*) (*Neolema*) (*Oulema*)(*Stethopachys*)) ((*Metopoceris*) (*Lilioceris*) (*Crioceris*)))
Constraint 6	(Outgroups) (Sagrinae, Donaciinae (Criocerini) (Lemini))
Constraint 7	(Outgroups, Sagrinae, Donaciinae (Criocerini) (Lemini))

TABLE 10. Hypothesis testing – generic constraints using parametric bootstrapping

Constraint 8	((*Lema*) Outgroups, *Neolema, Oulema, Sthethopachys, Lilioceris, Crioceris, Metopoceris*)
Constraint 9	((*Neolema*) Outgroups, *Lema, Oulema, Sthethopachys, Lilioceris, Crioceris, Metopoceris*)
Constraint 10	((*Oulema*) Outgroups, *Lema, Neolema, Sthethopachys, Lilioceris, Crioceris, Metopoceris*)
Constraint 11	((*Sthethopachys*) Outgroups, *Lema, Neolema, Oulema, Lilioceris, Crioceris, Metopoceris*)
Constraint 12	((*Lilioceris*) Outgroups, *Lema, Neolema, Oulema, Sthethopachys, Crioceris, Metopoceris*)
Constraint 13	((Crioceris) Outgroups, *Lema, Neolema, Oulema, Sthethopachys, Lilioceris, Metopoceris*)
Constraint 14	((Metopoceris) Outgroups, *Lema, Neolema, Oulema, Sthethopachys, Lilioceris, Crioceris*)

TABLE 11. Hypothesis testing – geographical constraints using parametric bootstrapping

Constraint 15	(Outgroups (Donacinae, Sagrinae ((Oriental Lemini) (African Lemini) (Neotropical Lemini) (Paleartic Lemini) (Oceanic Lemini)) ((Neotropical/Neartic Criocerini)
Constraint 16	(Outgroups (Donacinae, Sagrinae (Oriental Lemini) (African Lemini) (Neotropical Lemini) (Paleartic Lemini) (Oceanic Lemini) ((Neotropical/Neartic Crocerini) (Neotropical/Neartic Crocerini) (Paleartic Crocerini) (Oriental Crocerini)))
Constraint 17	(Outgroups, Donacinae, Sagrinae (Oriental Lemini) (African Lemini) (Neotropical Lemini)(Paleartic Lemini) (Oceanic Lemini) ((Neotropical/Neartic Criocerini) (Neotropical/Neartic Criocerini) (Paleartic Criocerini) (Oriental Criocerini)))
Constraint 18	(Outgroups, Donacinae, Sagrinae ((Oriental Lemini) (African Lemini) (Neotropical Lemini)(Paleartic Lemini) (Oceanic Lemini) ((Neotropical/Neartic Criocerini) (Neotropical/Neartic Criocerini) (Paleartic Criocerini) (Oriental Criocerini)))
Constraint 19	(Outgroups (Donacinae, Sagrinae ((Oriental Criocerinae) (Neotropical/ Neartic Criocerinae) (African Criocerinae) (Paleartic Criocerinae) (Oceanic Criocerinae)))
Constraint 20	(Outgroups (Donacinae, Sagrinae (Oriental Criocerinae) (Neotropical/ Neartic Criocerinae) (African Criocerinae) (Paleartic Criocerinae) (Oceanic Criocerinae))))
Constraint 21	(Outgroups, Donacinae, Sagrinae ((Oriental Criocerinae) (Neotropical/ Neartic Criocerinae) (African Criocerinae) (Paleartic Criocerinae) (Oceanic Criocerinae)))

TABLE 12. Likelihood scores for subfamilial and tribal constraints.

		Constraints							
		Original Data	C1	C2	C3	C4	C5	C6	C7
-In L		36668.07	38264.15	37490.71	40223.46	38720.09	40287.74	38728.96	39223.5
	A	0.257641	0.261316	0.265637	0.288994	0.271072	0.288583	0.271101	0.271033
Base	C	0.220986	0.211038	0.215892	0.201048	0.210763	0.200647	0.210884	0.207218
Freq	T	0.250111	0.241266	0.245119	0.226098	0.238679	0.2261	0.238449	0.228926
	G	0.271604	0.28638	0.273351	0.283861	0.279486	0.284671	0.279566	0.292823
Rate Matrix	AC	0.98719	1.11047	0.96247	0.91322	1.02774	0.89685	1.02557	0.9384
	AG	4.3814	4.80362	4.39471	4.38031	4.7721	4.2859	4.77656	4.56931
	AT	4.25301	4.16999	4.1318	4.38031	4.17511	3.34875	4.16638	3.59603
	CG	0.91143	1.09555	0.9595	1.17035	1.13725	1.14929	1.14069	1.10893
	CT	5.76453	6.2809	5.90742	5.96195	6.2891	5.91494	6.28157	5.76294
	GT	1	1	1	1	1	1	1	1
Shape		0.319827	0.345323	0.315468	0.295217	0.315938	0.294443	0.314943	0.321059
P_inv		0.110922	0.283257	0.13297	0.232236	0.217119	0.232722	0.216738	0.292744
Parsimony Score		6515	6515	6515	6515	6515	6515	6515	6515

TABLE 13. Likelihood scores for generic constraints

		Constraints							
		Original Data	C8	C9	C10	C10	C11	C12	C13
-In L		36668.07	38019.24	36633.29	37881.97	N/A	36964.79	37744.46	N/A
	A	0.257641	0.264825	0.25744	0.260867	N/A	0.254302	0.263197	N/A
Base	C	0.220986	0.214498	0.220586	0.216649	N/A	0.219869	0.216319	N/A
Freq	T	0.250111	0.240936	0.251324	0.243981	N/A	0.252879	0.243087	N/A
	G	0.271604	0.279741	0.27065	0.278503	N/A	0.27295	0.277397	N/A
Rate Matrix	AC	0.98719	0.9089	0.97147	0.96366	N/A	1.05273	0.94315	N/A
	AG	4.3814	4.236	4.32487	4.41392	N/A	4.51869	4.302	N/A
	AT	4.25301	3.9631	4.26988	4.10293	N/A	4.45314	3.99967	N/A
	CG	0.91143	0.99055	0.93697	1.01553	N/A	1.00207	0.98628	N/A
	CT	5.76453	5.56308	5.80182	5.62403	N/A	6.14391	5.62831	N/A
	GT	1	1	1	1	N/A	1	1	N/A
Shape		0.319827	0.331285	0.314225	0.339638	N/A	0.355643	0.33257	N/A
P_inv		0.110922	0.15741	0.100696	0.201668	N/A	0.220586	0.199518	N/A
Parsimony Score		6515	6939	6517	6823	N/A	6692	6763	N/A

*N/A – PAUP cannot calculate scores for constraints containing only one taxa

TABLE 14. Likelihood scores for geographical constraints.

		Constraints							
		Original Data	C 14	C15	C16	C16	C17	C18	C19
-In L		36668.07	40912.04	40927.18	40250.82	40823.41	40500.52	39827.08	40500.84
	A	0.257641	0.287738	0.277727	0.266131	0.271318	0.269505	0.268855	0.269401
Base	C	0.220986	0.220918	0.204309	0.210466	0.208932	0.204127	0.211873	0.204075
Freq	T	0.250111	0.221006	0.228496	0.230683	0.230436	0.228664	0.231718	0.22933
	G	0.271604	0.291255	0.289468	0.29272	0.289315	0.297703	0.287553	0.297591
Rate Matrix	AC	0.98719	0.90972	0.93292	0.97316	0.9503	1.06778	0.95537	1.07173
	AG	4.3814	4.38525	4.77751	4.73553	4.95951	4.90588	4.67898	4.91278
	AT	4.25301	3.27815	3.86144	3.79517	4.03129	3.78449	3.89163	3.79837
	CG	0.91143	1.14035	1.14331	1.09007	1.11347	1.16331	1.04455	1.16583
	CT	5.76453	5.72076	6.20398	5.64803	5.98959	6.02895	5.74041	6.0528
	GT	1	1	1	1	1	1	1	1
Shape		0.319827	0.318944	0.603892	0.332193	0.605716	0.311354	0.322356	0.311369
P_inv		0.110922	0.108869	0.438638	0.293751	0.445446	0.30277	0.266123	0.302155
Parsimony score		6515	6515	6515	6515	6515	6515	6515	6015

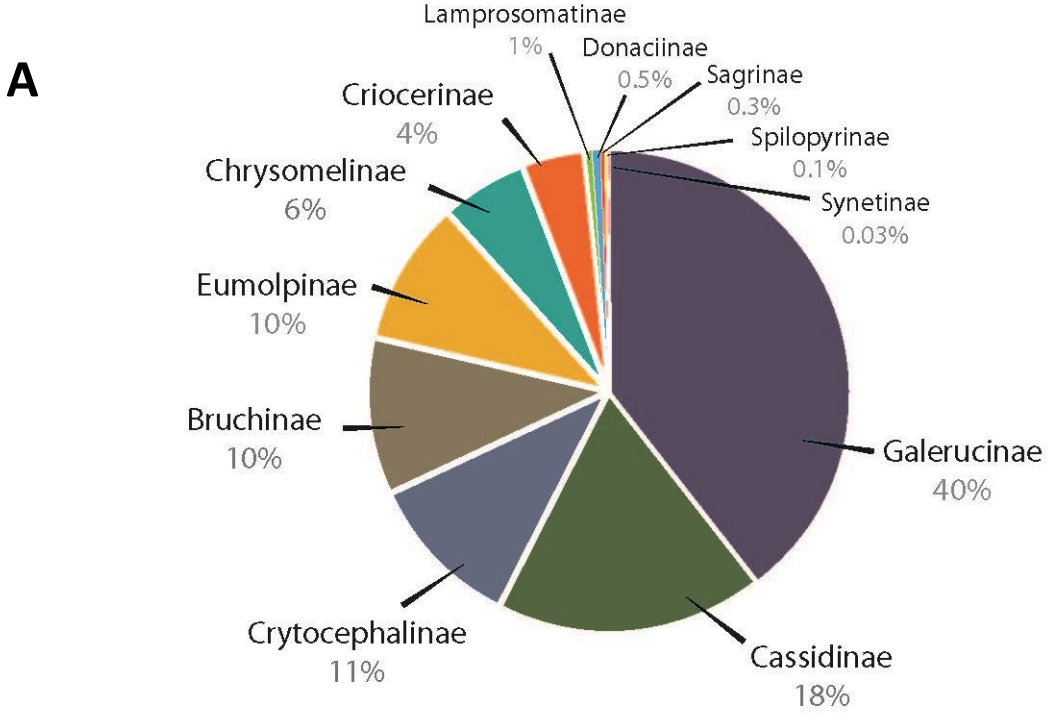
TABLE 15. Diagnostic characters for sampled criocerine genera

Genera	Characters
Lilioceris Reitter 1912	Free tarsal claws.[1] Divided vertex[2] Transverse depression behind the eyes.[1] Constriction near the middle portion of the pronotum.[1,3] Hypognathus head.[3] Fronto clypeal grooves between the antennae.[3] Antennae with variable shapes as long as half of the body size.[3] Elytral broader than pronotum, with punctures variable in pattern.[3] Robust legs.[3] Present in the Old World (Africa, China and South East Asia), also present in North America as pest species of Liliaceae.[4]
Crioceris Geoffroy 1762	Free tarsal claws and pronotum constricted near basis [1] Lacks transverse depression behind eyes.[1] Short antennae (less than half of the body length), cylindrical and robust, to some extent thickened in the apex.[3] Pronotum narrower than head, with a slight fronto clypeal grooves.[3] Aedeagus with notched apex.[2] Present in the Old World; invasive species in the United States, Canada and Mexico; pest of *Asparagus officinallis.*[2,4]
Metopoceris Heinze 1931	Free tarsal claws.[5] Size ranges between 10–16 mm, coloration characterized for having brilliant metallic sheen.[5] Pronotum with an hourglass shape and unmarginated.[5] Antennae and femora are short and robust.[5] Present in only in Central America.[6]
Lema Fabricius, 1798	Connate tarsal claws.[4] Constriction in the head behind eyes.[5] Antennae tubercles are widely spaced, shorter than half of the body length with a broader apical segment.[5] Pronotum constricted at medial line.[5] Elytra with color patterns, longitudinal or transversal stripes, sometimes spots. In elytra, the 9th stria has complete punctuation.[5] Aedeagus in dorsal side has a single, medial fold overlaying the lateral folds.[5] Great variation in phenotype.[3] Worldwide distribution with exception of the poles.[3,4]
Neolema Monrós 1951	Connate tarsal claws.[4] Ninth stria with a gap of five to twelve punctures.[5] Head constricted behind the eyes and antennae greater than body length. [5]

	Pronotum constriction varies from medial to sub-medial and can be deep to moderately deep. [5] Lateral folds of the aedeagus meet to conceal a central. [5] Mostly Neotropicalbut a with few Nearctic species.[4]
Oulema Des Gozis 1886	Connate tarsal claws.[4] Lack of elytral patterns.[3,5] Disc is composed of a solid color, often glabrous blue or black.[3,5] Head not constricted behind eyes. [3,5] Presence of frontal tubercles and a deep furrow in the vertex. [3,5] Antennae length is greater or equal to the length of the body. [3,5] Pronotum is cylindrical or weakly constricted sub medially or near the base[3,5] Punctures of the ninth stria are complete.[3,5] Aedeagus composed of three folds.[5] Present in Palearctic, Africa, Nearctic, Neotropical and Oriental regions.[4,6]
Stethopachys Baly, 1861	Connate tarsal claws.[4] Elongated body.[3,7] Filiform antennae, with four additional antennal segments. [3,7] Medially constricted pronotum, meso and meta sternum slightly projected. [3,7] Elytra with 10 stria [3,7] Hind femur weakly swollen.[7] Present only in New Caledonia, Queensland and Papua New Guinea.[7]

(References: [1]Warchalowski, 2010; [2]Schmitt, 1988; [3]Monrós, 1960; [4]Arnett *et al*., 2002; [5]Vencl *et* al., 2005; [6]Seeno and Wilcox, 1982; [7]Gressitt, 1965).

Figure 1. Chrysomelid diversity. A. Chrysomelid subfamilial status of classification after Bouchard *et al* (2011) representing 12 subfamilies (modified from Chaboo, 2007). B. Criocerinae diversity represented at genus level. The most representative genus in this subfamily is *Lema* with 67% of the total number, followed by *Lilioceris*, *Neolema* and *Oulema.* The remaining 17 genus described for the subfamily only represent 7 % of the described species for Criocerinae.



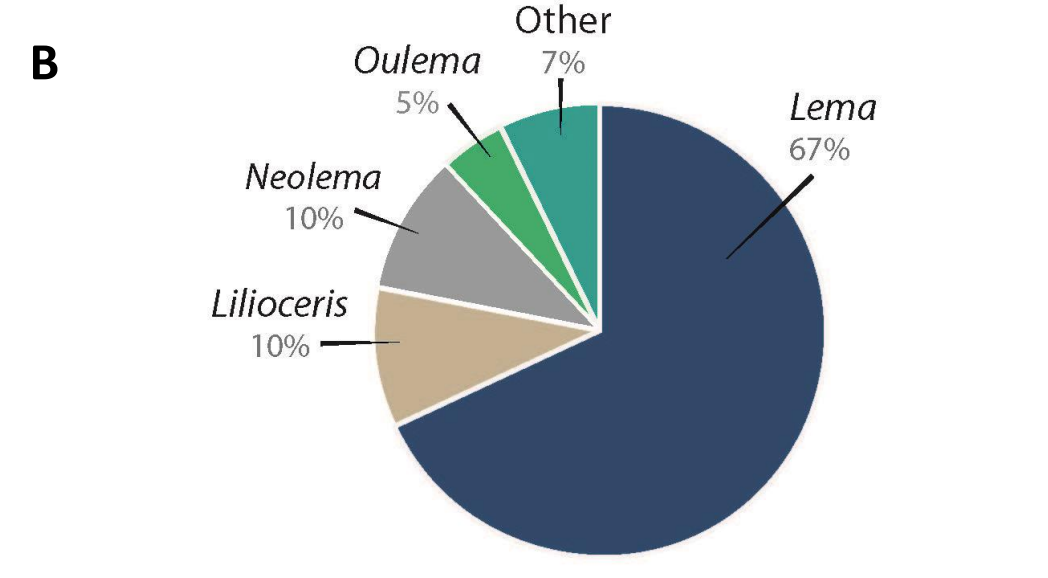
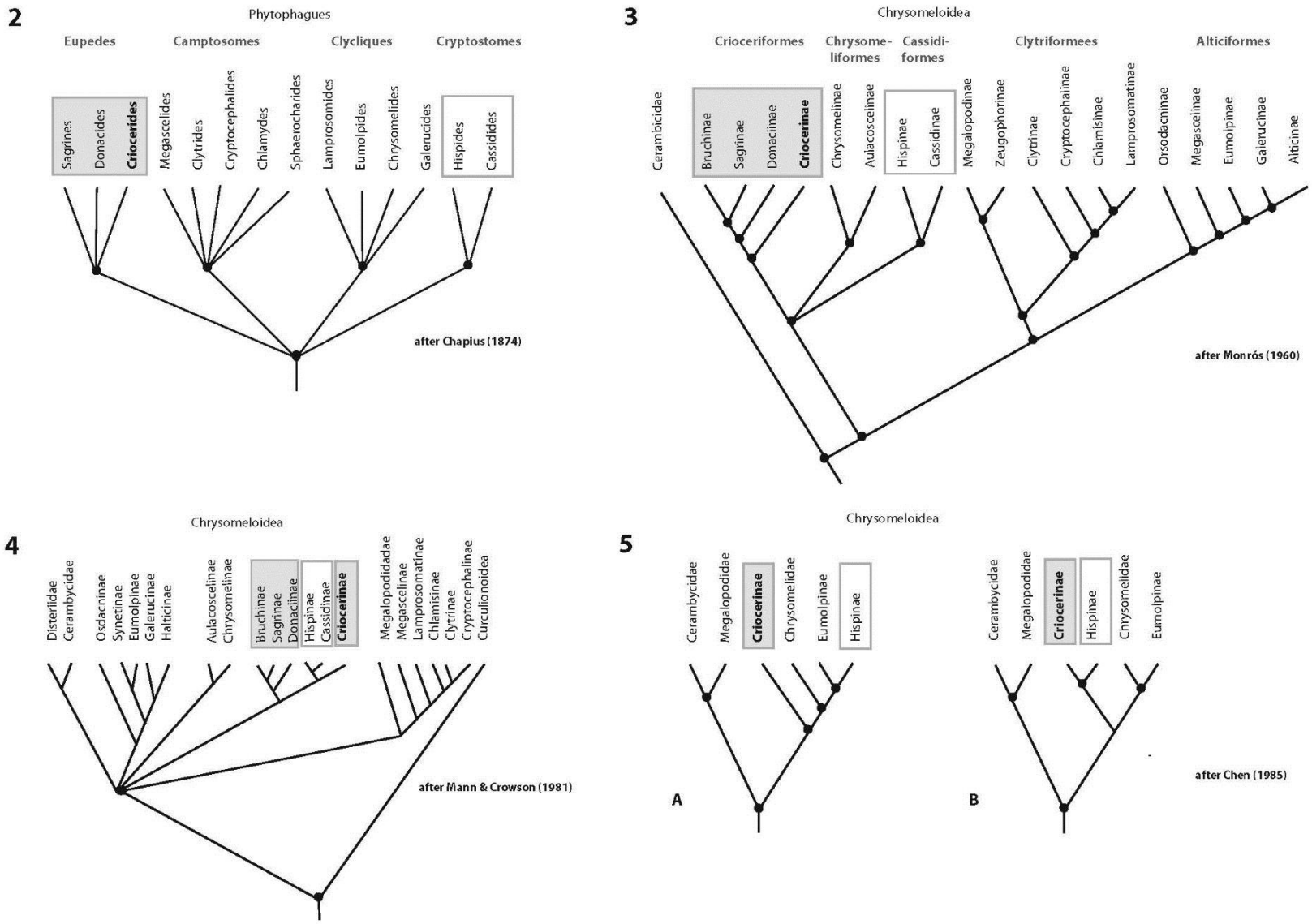


Figure 2–5. Hypotheses of phylogenetic relationships in Chrysomeloidea: 2. Chaupius (1874); 3. Monrós (1960); 4. Mann & Crowson (1981); and 5. Chen (1985).



Figureure 6–7. Hypothesis of phylogenetic relationships in Chrysomeloidea. 6. Jolivet (1988); 7. Reid (1995).

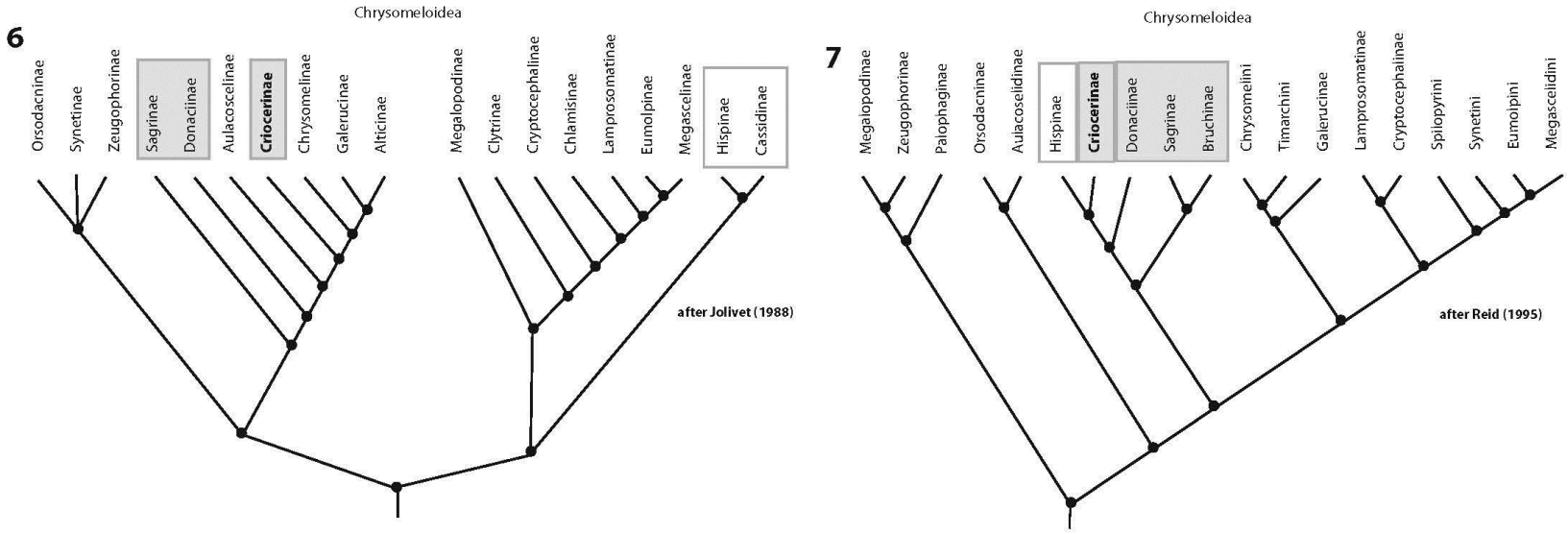


Figure 8–10. Diagrams representing hypotheses of phylogenetic relationships in Chrysomeloidea using a molecular approach. 8. Hunt *et al* (2007); 9. Gómez-Zurta *et al* (2008) and 10. Marvaldi *et al* (2008).

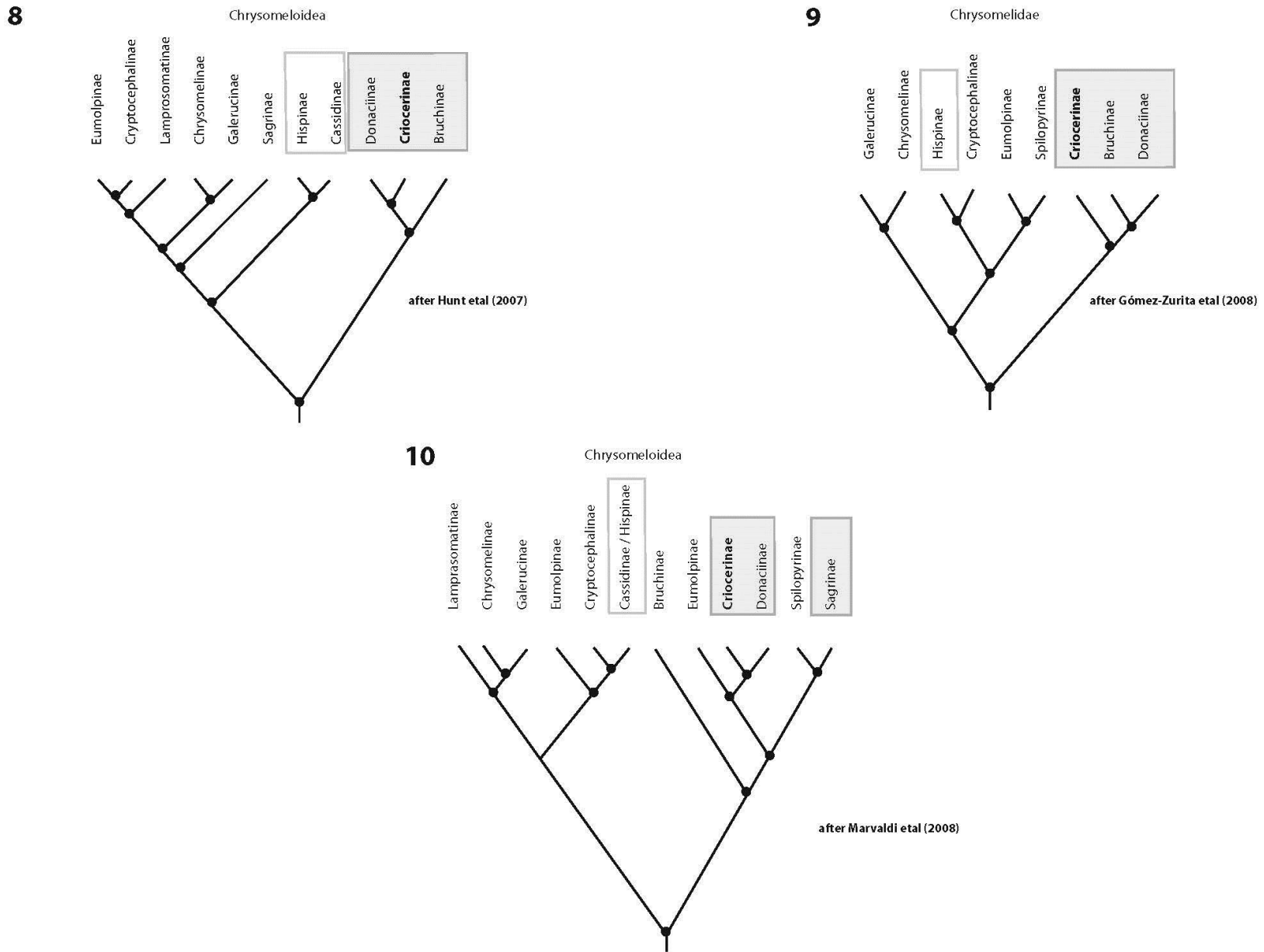


Figure 11–22. Criocerinae diversity representation: 11. *Crioceris asparagi*; 12. *Crioceris duodecimopunctata*; 13. *Lilioceris quadripustulata*; 14. *Lilioceris unicolor*; 15. *Lema viridana*; 16. *Lema insularis*; 17. *Neolema dorsalis*; 18. *Lema externivittata*; 19. *Lema femorata*; 20. *Lema cordairei*; 21. *Stethopachys fasciata*; 22. *Oulema melanopus*.

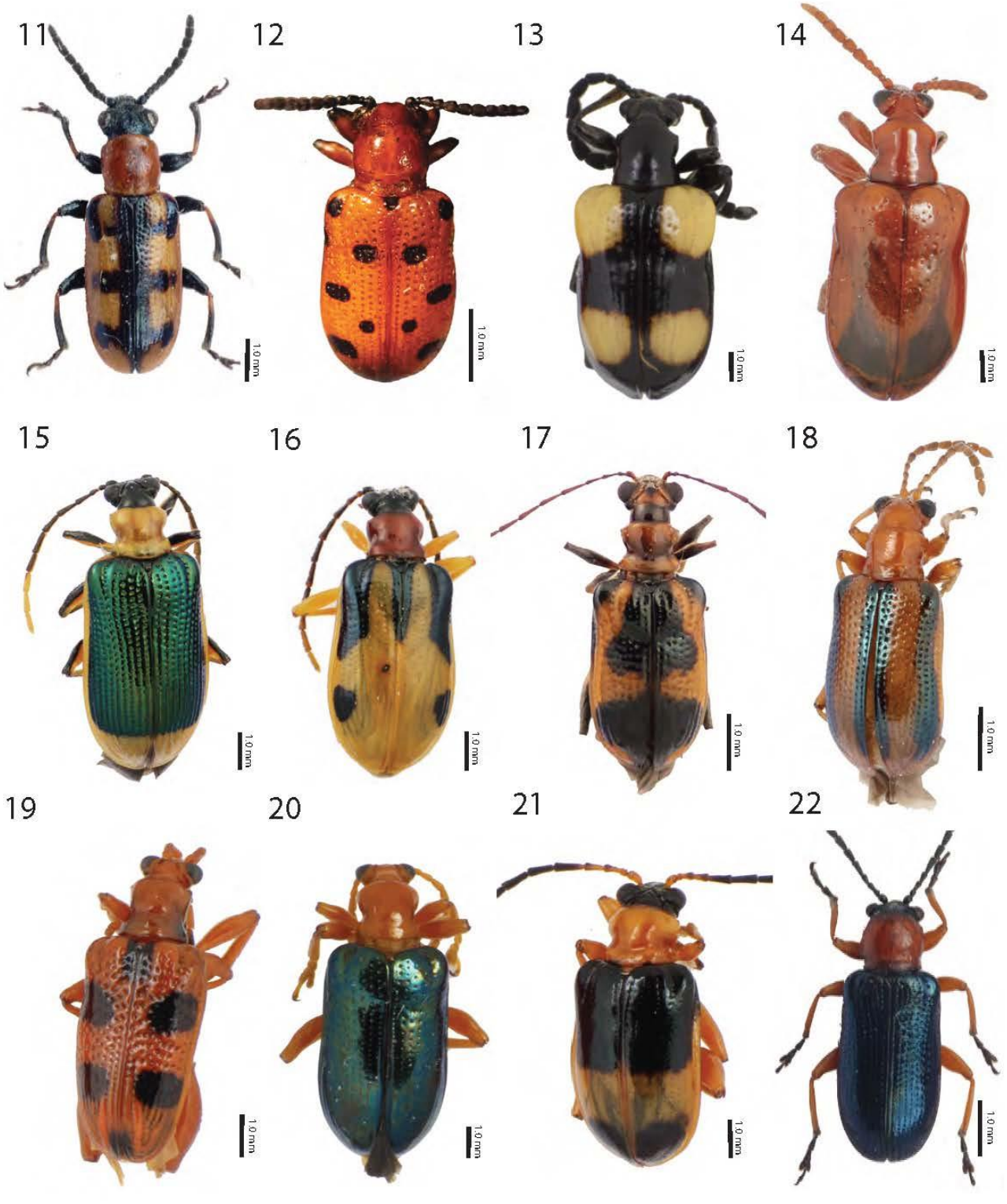


Figure 23–25. Pronota of criocerine adults, dorsal view. 23. *Lema insularis* Jacoby 1888. 24. *Oulema sp*. 25. *Crioceris asparagi* (Linnaeus, 1758).

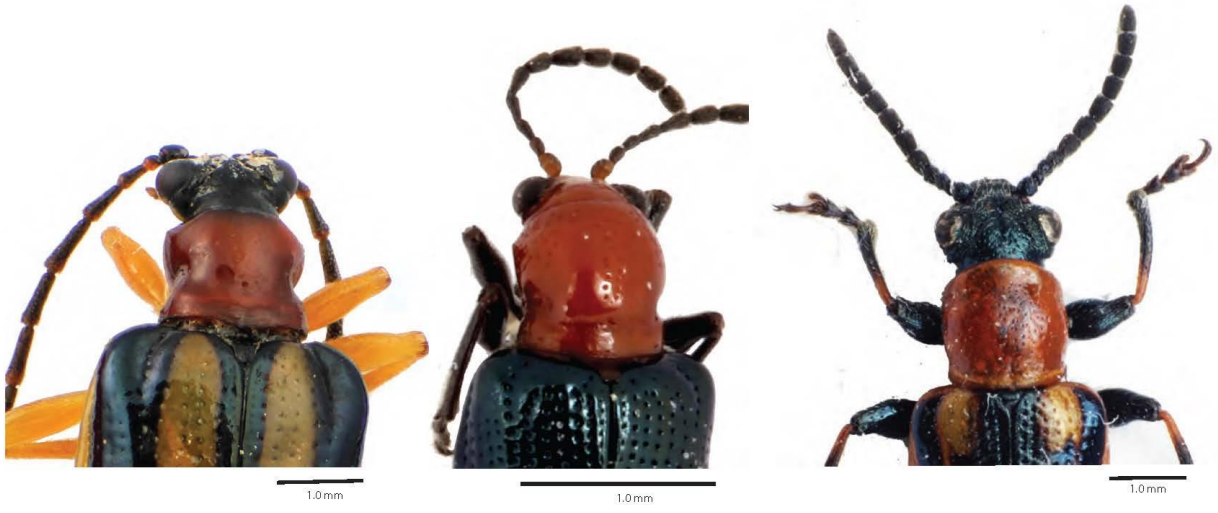


Figure 26. Head of criocerine adult, anterior view. A. *Lilioceris* sp. and B. *Crioceris asparagi* (Linnaeus, 1758) mandible, lateral view of molar area and teeth.

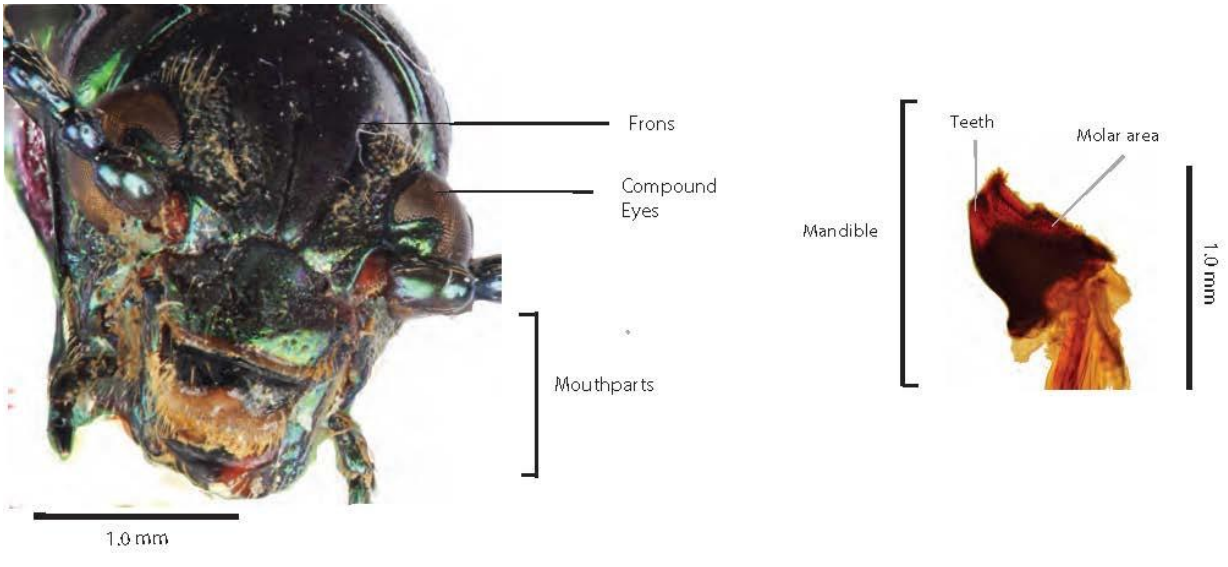


Figure 27. Stridulatory files of *Crioceris asparagi* (Linnaeus, 1758).

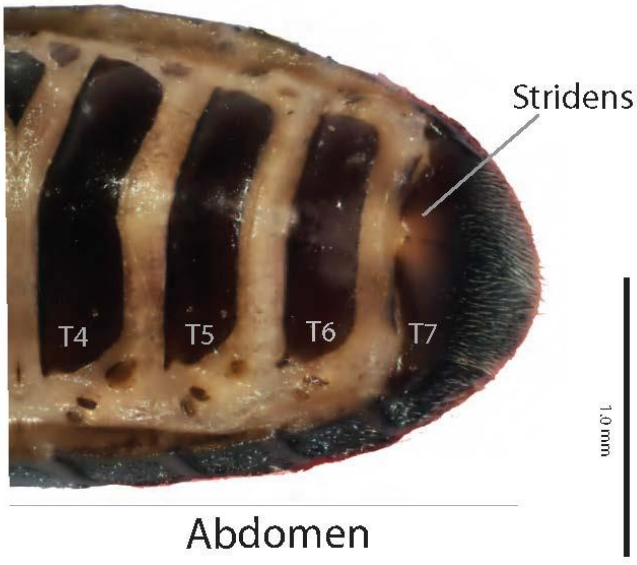


Figure 28. Life cycle of a *Crioceris asparagi*. A. Eggs; B. Larvae, and C. Adult.

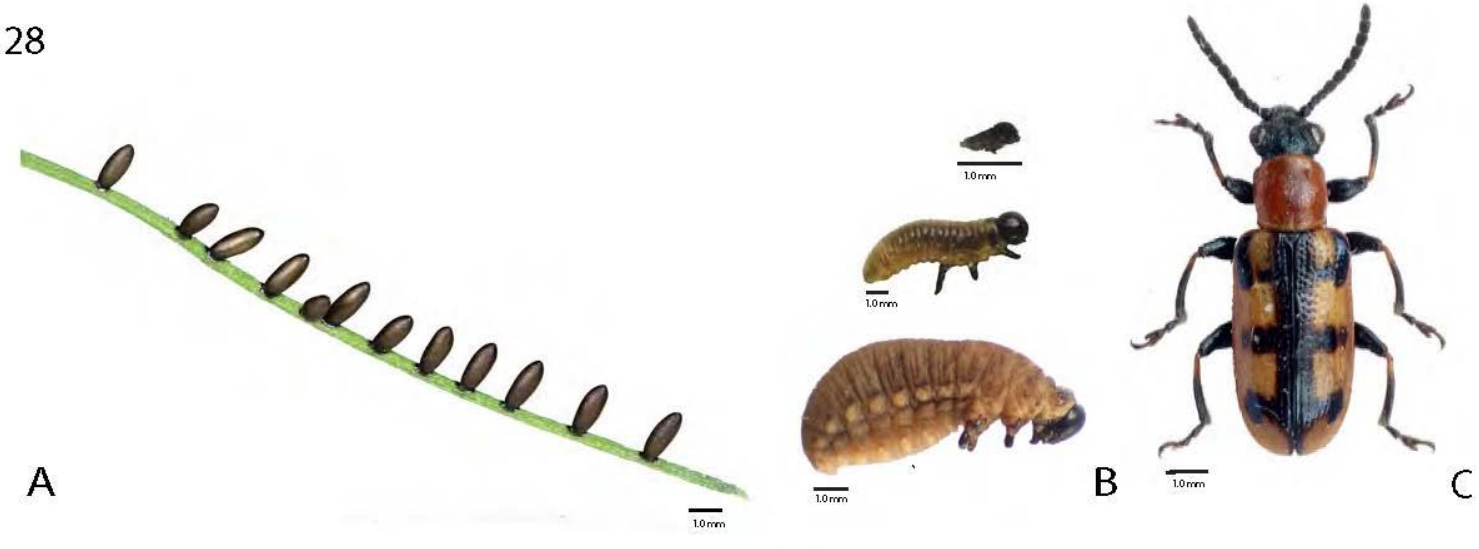


Figure 29. Criocerinae tarsal claws: free claws present in A. *Lilioceris* sp. (Criocerini) and connate claws in B. *Lema trilineata* (Lemiini).

Figure **30.** Gene Tree for COI using three evolutionary models: A) parsimony, B) maximum likelihood, C) posterior probabilities.

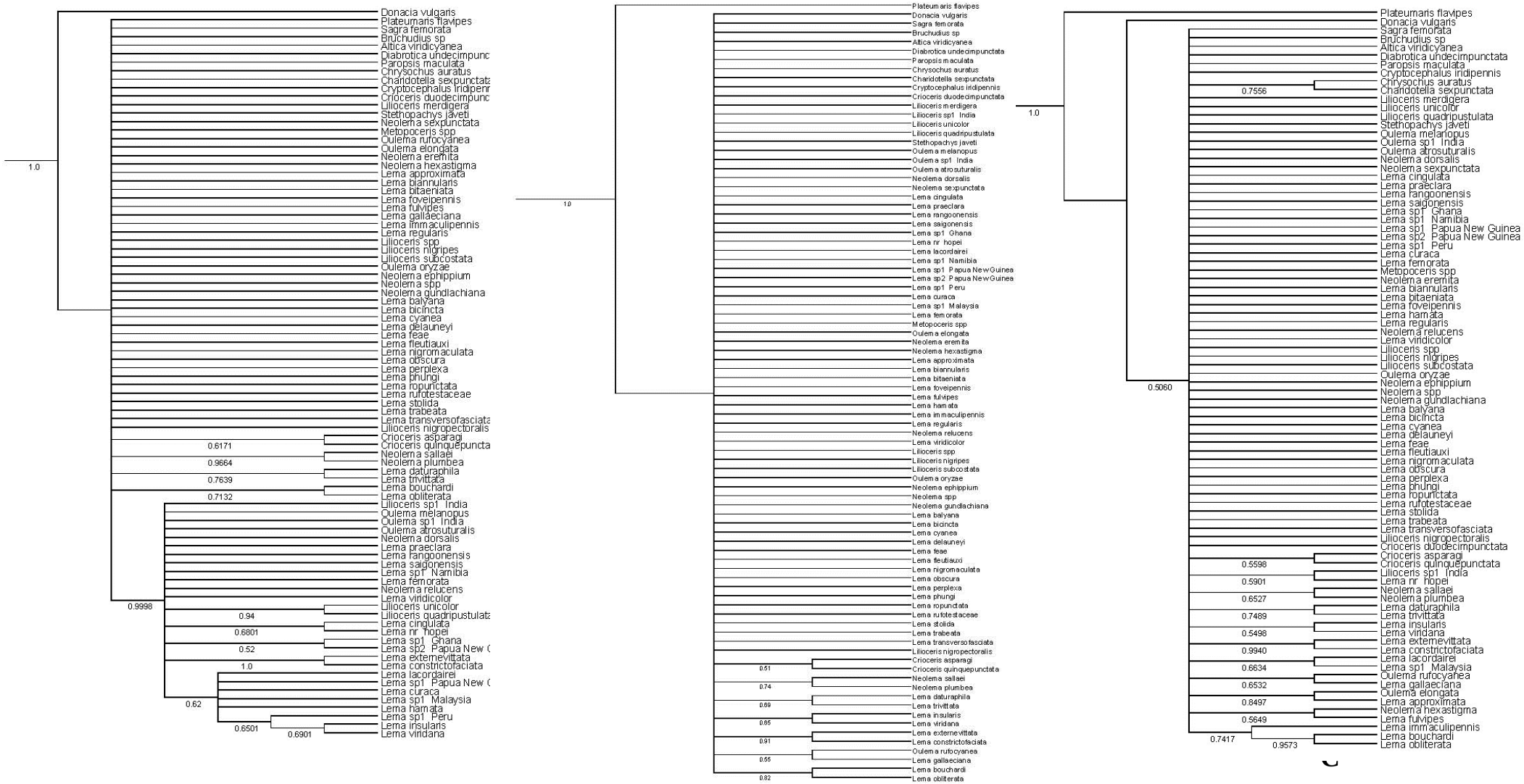


Figure 31. Gene Tree for 28S using three evolutionary models: A) parsimony, B) maximum likelihood, C) posterior probabilities.

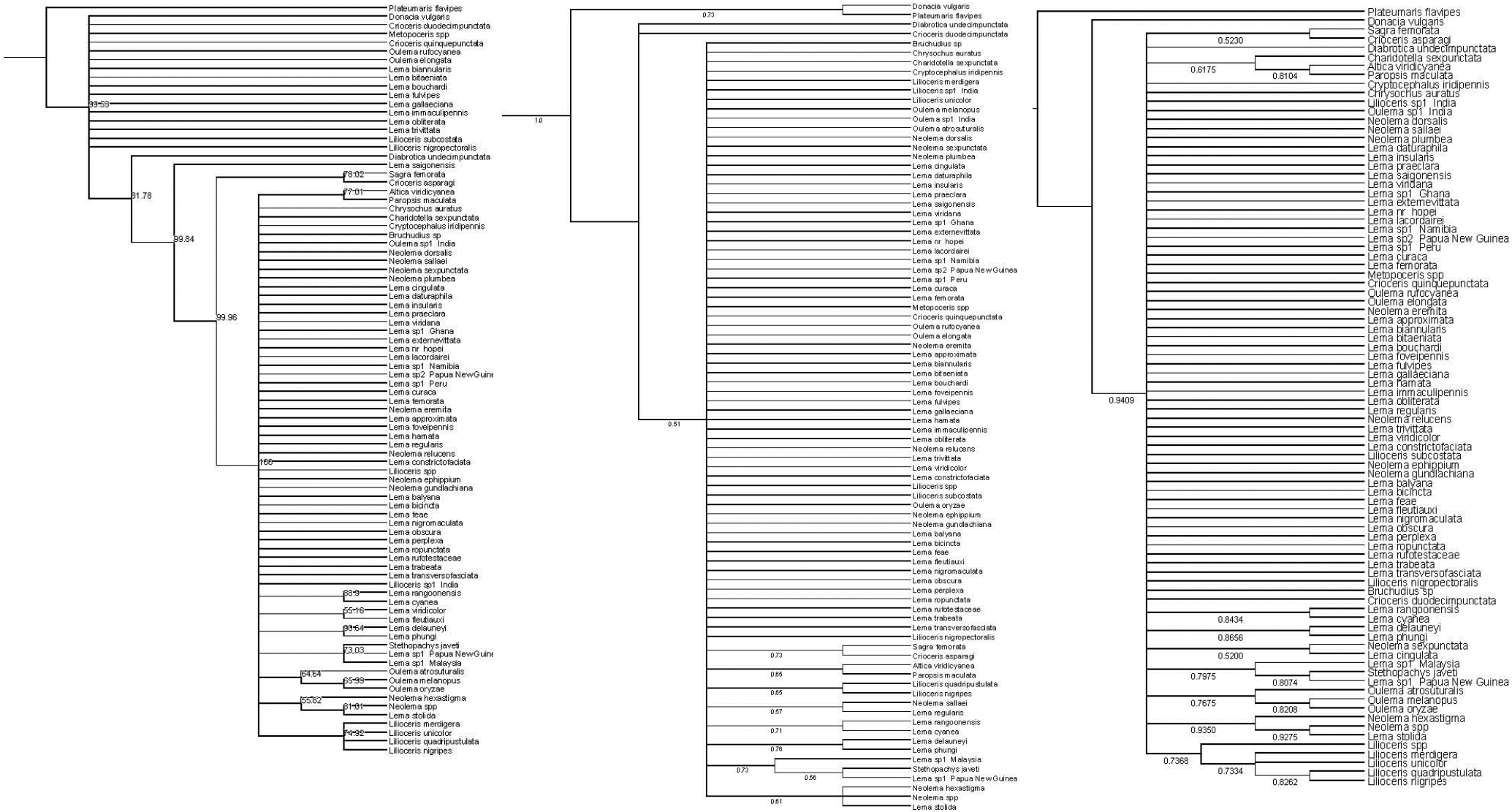


Figure 32. Gene Tree for 18S using three evolutionary models: A) Parsimony, B) Maximum Likelihood, C) Posterior Probabilities.

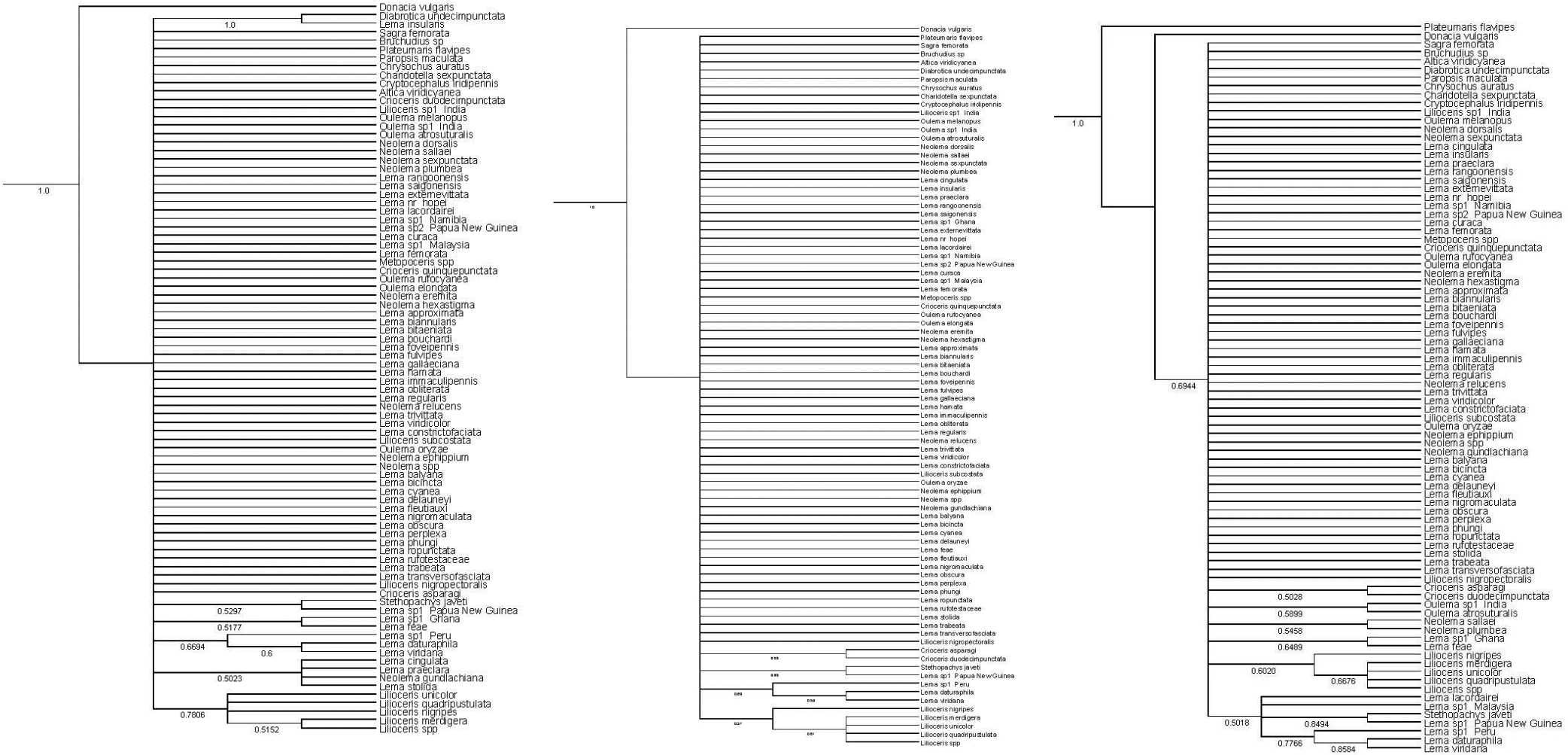


Figure 33. Consensus tree of the 3 molecular markers (COI, 28S and 18S) using MP as criterion and majority rule consensus.

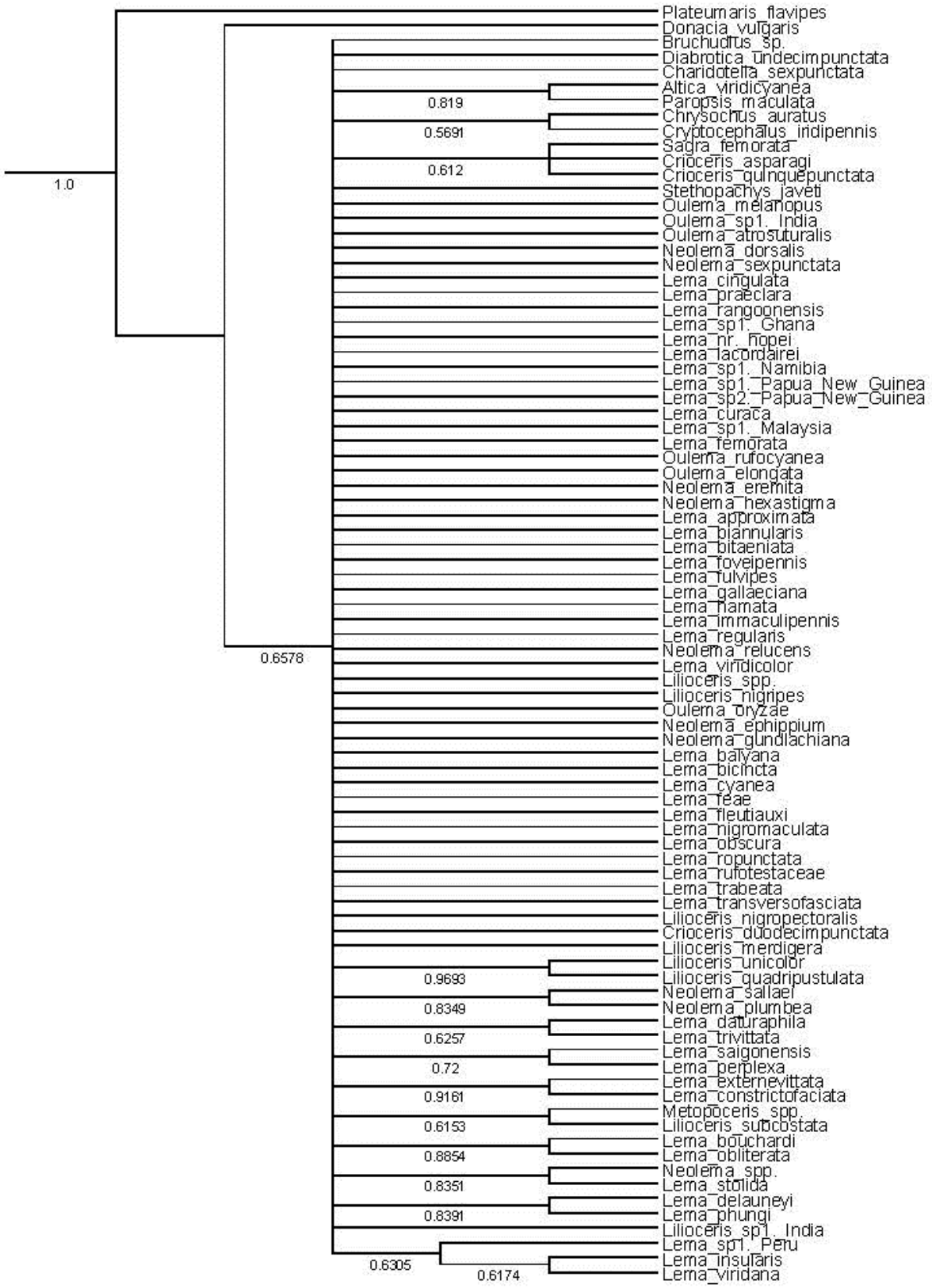


Figure 34. Consensus tree of the 3 molecular markers (COI, 28S and 18S) using ML as criterion and majority rule consensus.

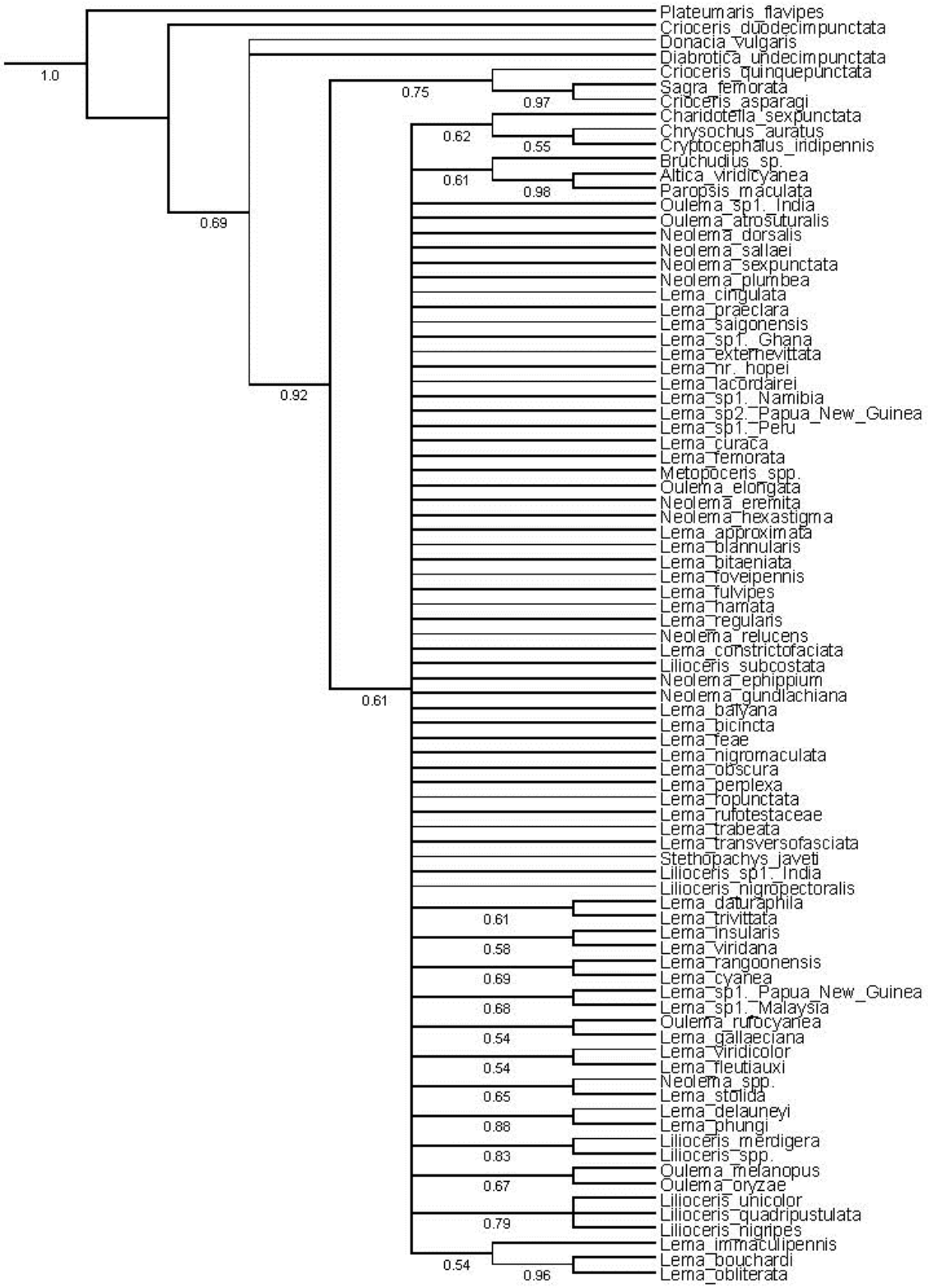


Figure 35. Consensus tree of the 3 molecular markers (COI, 28S and 18S) using posterior probabilities in BEAST.

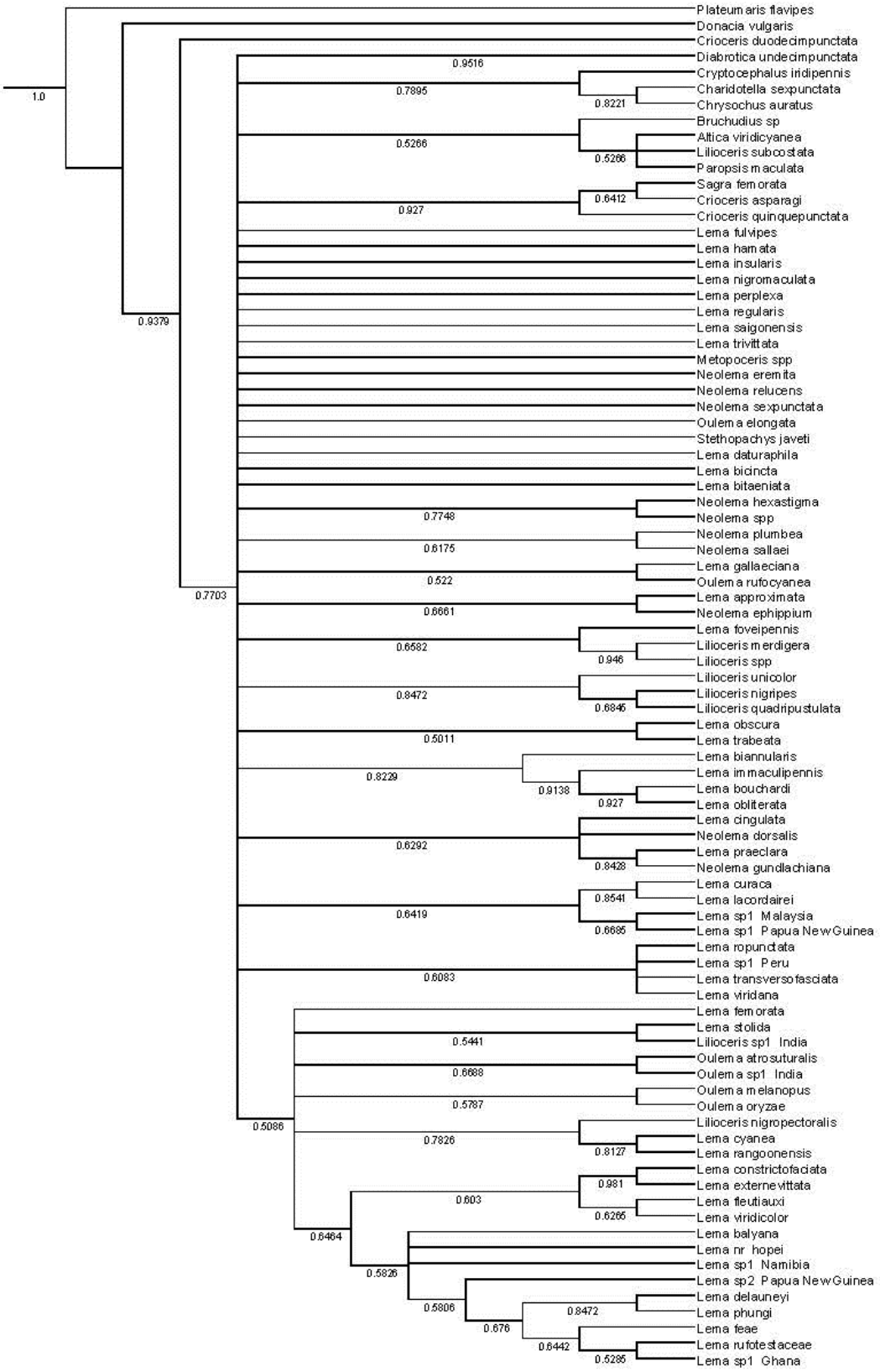


Figure 36. Consensus tree of the 3 molecular markers (COI, 28S and 18S) using posterior probabilities in Mr. Bayes.

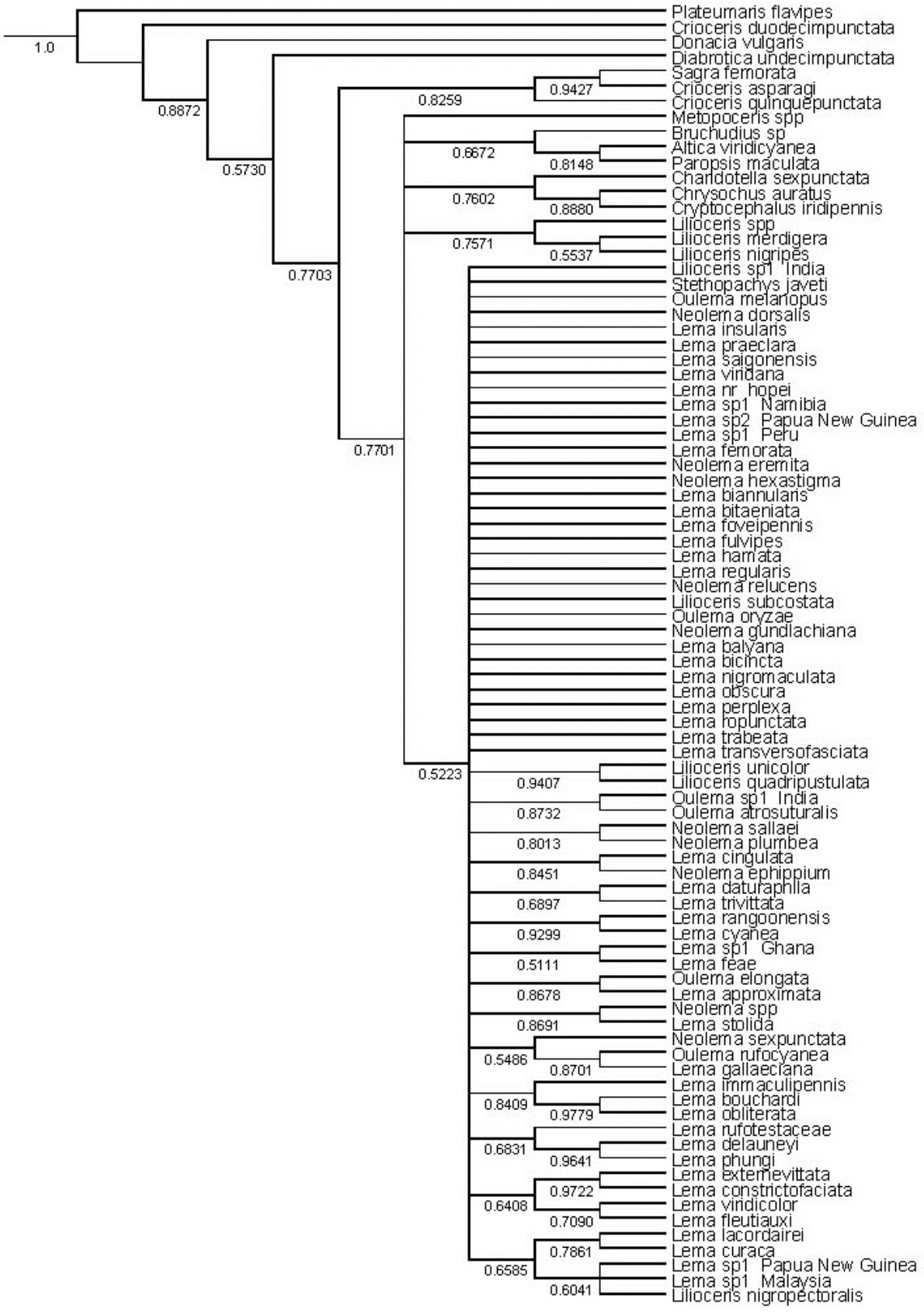


Figure 37. Morphological phylogeny of Criocerinae beetle by Teo (1999) using strict consensus as criterion.

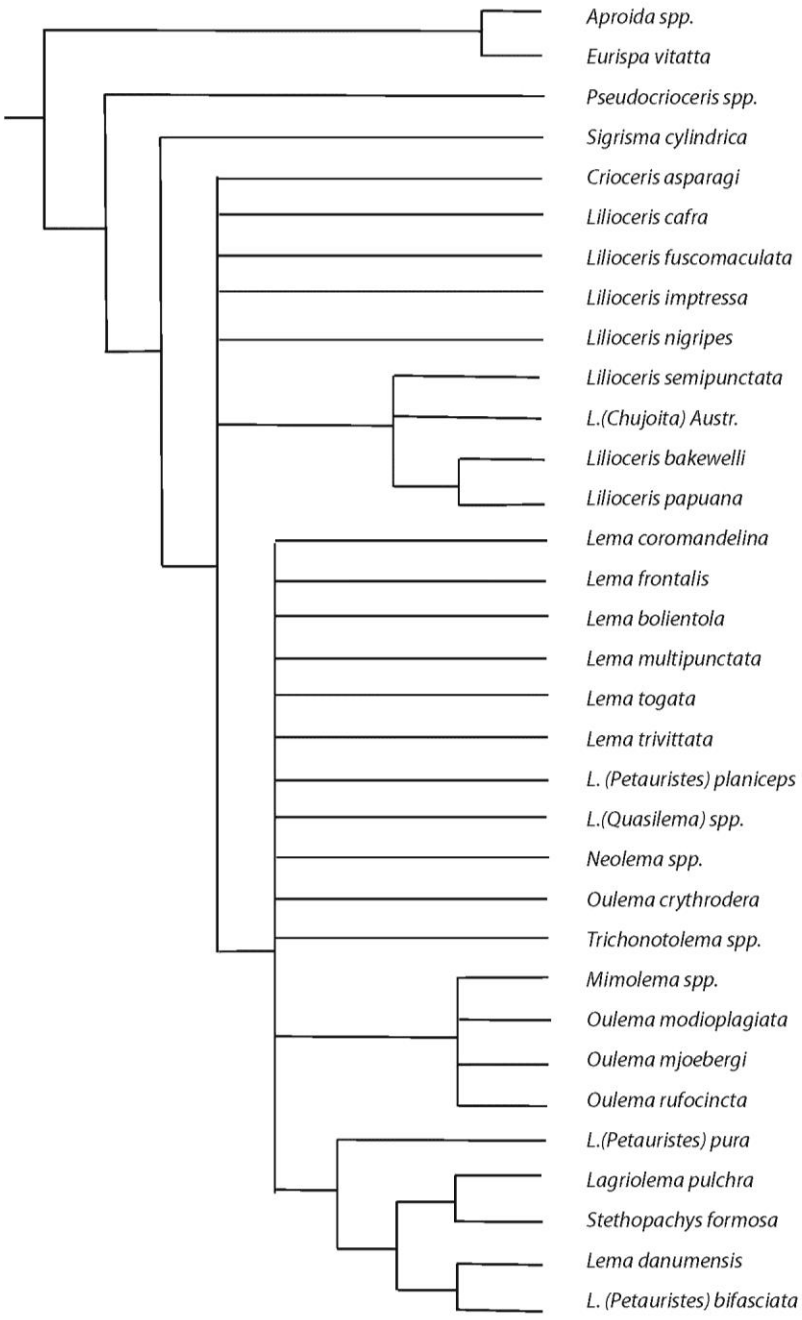


Figure 38. Posterior probability tree topology correlated with geographical regions

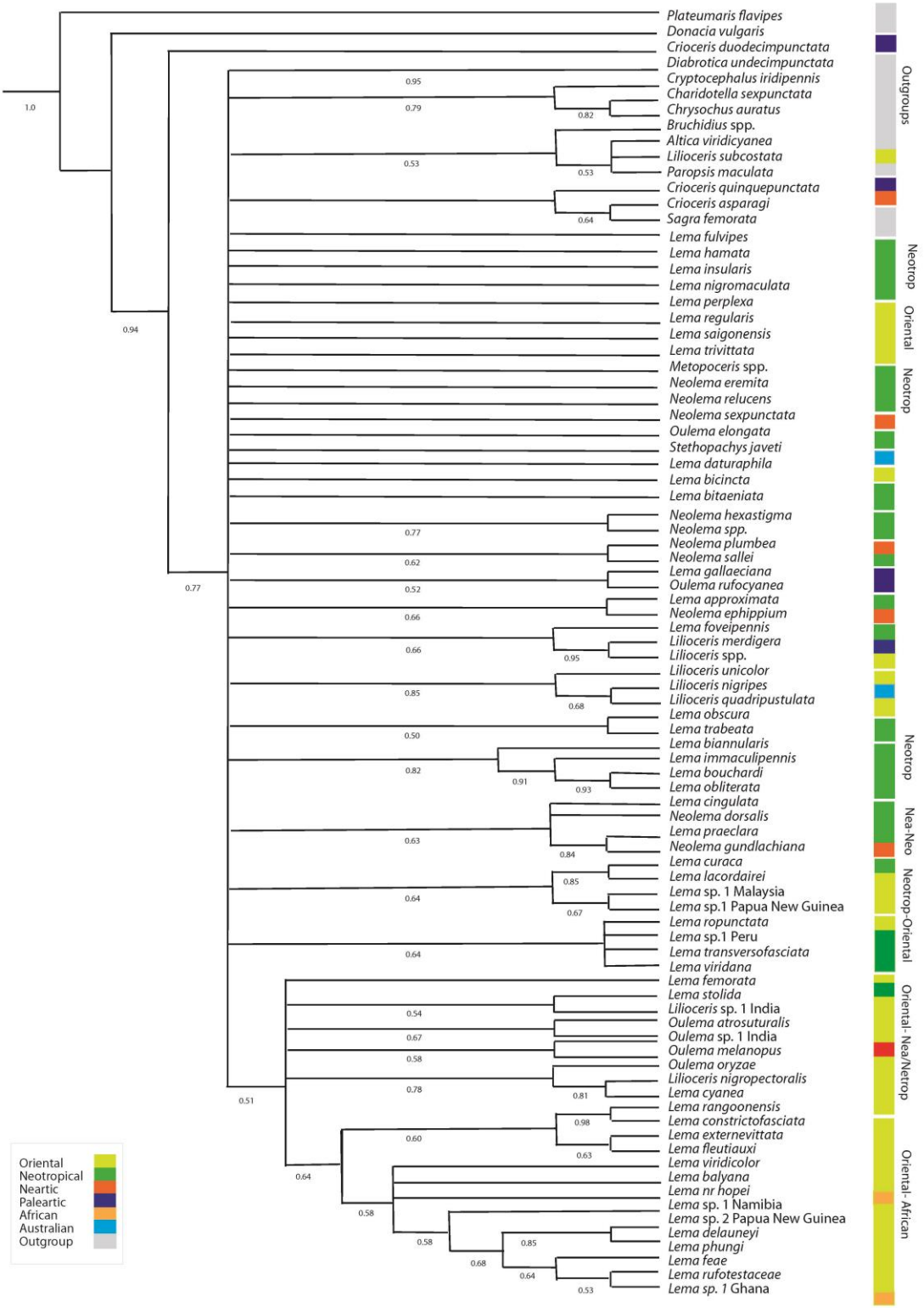
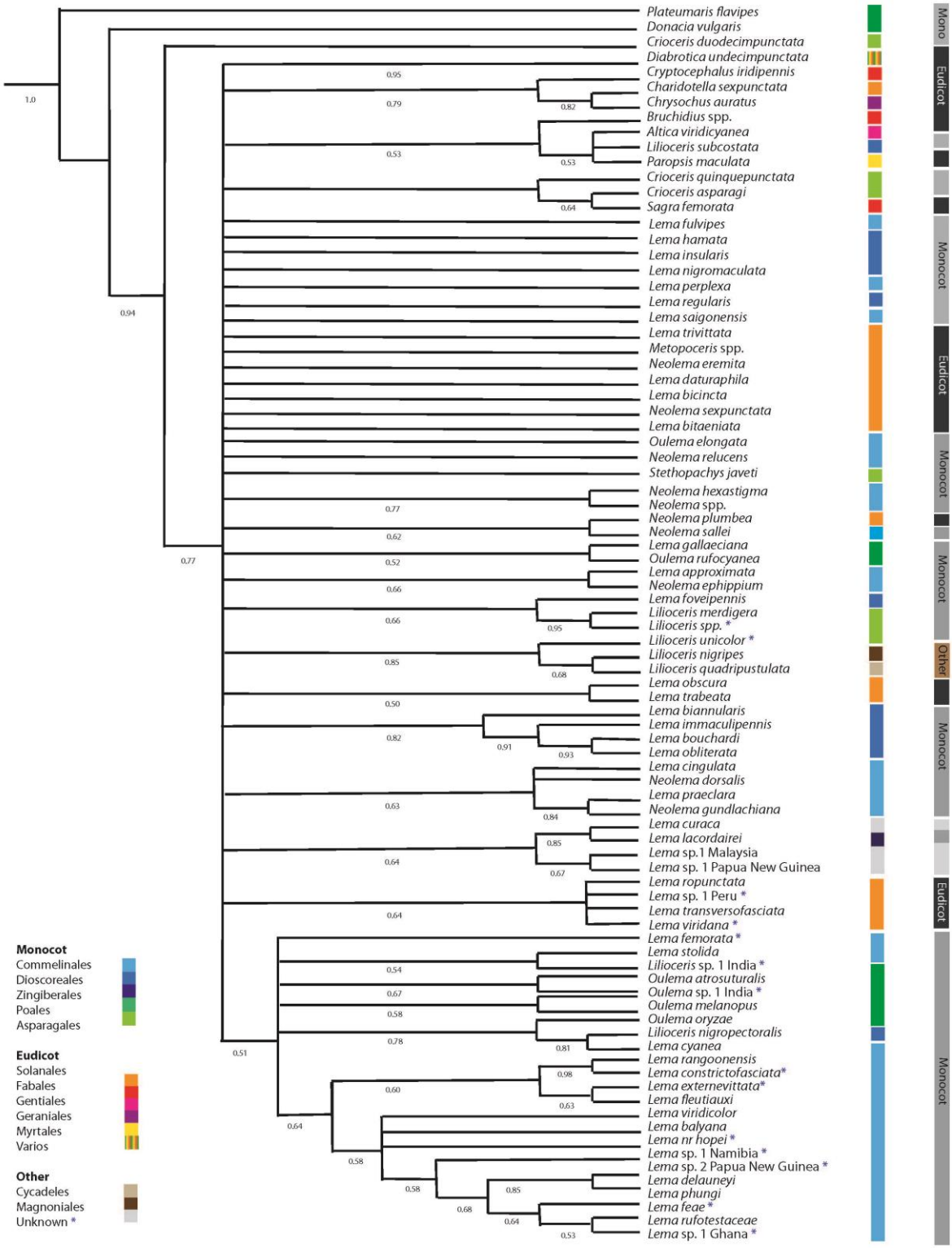


Figure 39. Molecular phylogeny of Criocerinae beetles with host plant association records.



Appendix 1.1 Profile of amplification for each individual gene and reference of sequences acquired in Genebank.

Voucher #	Specimen	COI	28S	18S
SMCR001	*Lema approximata*	This study	This study	—
SMCR002	*Lema balyana*	—	This study	This study
DQ001931	*Lema biannularis*	Vencl et al., 2004	—	—
SMCR003	*Lema bicincta*	—	This study	—
DQ001935	*Lema bitaeniata*	Vencl et al., 2004	—	—
DQ001936	*Lema bouchardi*	Vencl et al., 2004	—	—
SMCR004	*Lema cingulata*	This study	This study	This study
SMCR005	*Lema cyanea*	—	This study	This study
SMCR006	*Lema curaca*	This study	This study	This study
SMCR007	*Lema daturaphila*	This study	This study	This study
SMCR008	*Lema delauneyi*	—	This study	This study
SMCR009	*Lema feae*	—	This study	This study
SMCR010	*Lema fleutiauxi*	—	This study	This study
SMCR011	*Lema foveipennis*	This study	This study	—
DQ001929	*Lema fulvipes*	Vencl et al., 2004	—	—
DQ155990	*Lema gallaeciana*	Hunt et al., 2013	—	—
SMCR012	*Lema hamata*	This study	This study	—
DQ001939	*Lema immaculipennis*	Vencl et al., 2004	—	—
SMCR013	*Lema insularis*	This study	This study	This study
SMCR014	*Lema nigromaculata*	—	This study	—
DQ001940	*Lema obliterata*	Vencl et al., 2004	—	—
SMCR015	*Lema obscura*	—	This study	—
SMCR016	*Lema perplexa*	—	This study	—
SMCR017	*Lema phungi*	—	This study	This study
SMCR018	*Lema praeclara*	This study	This study	This study
SMCR019	*Lema rangoonensis*	This study	This study	This study
SMCR020	*Lema regularis*	This study	This study	—

SMCR022	*Lema ropunctata*	—	This study	—
SMCR023	*Lema rufotestaceae*	—	This study	This study
SMCR024	*Lema saigonensis*	This study	This study	This study
SMCR025	*Lema stolida*	—	This study	This study
SMCR026	*Lema trabeata*	—	This study	—
SMCR027	*Lema transversofasciata*	—	This study	—
DQ001944	*Lema trivittata*	Vencl et al., 2004	—	—
SMCR028	*Lema vridana*	This study	This study	This study
SMCR029	*Lema viridicolor*	This study	This study	—
SMCR030	*Lema* sp1. Ghana	This study	This study	This study
SMCR031	*Lema externevittata*	This study	This study	This study
SMCR032	*Lema constrictofaciata*	This study	This study	—
SMCR033	*Lema* nr. hopei	This study	This study	This study
SMCR034	*Lema lacordairei*	This study	This study	This study
SMCR035	*Lema* sp1. Namibia	This study	This study	This study
SMCR036	*Lema* sp1. Papua New Guinea	This study	This study	This study
SMCR037	*Lema* sp 2. Papua New Guinea	This study	This study	This study
SMCR038	*Lema* sp 1. Peru	This study	This study	This study
SMCR039	*Lema* sp 1. Malaysia	This study	This study	This study
SMCR040	*Lema femorata*	This study	This study	This study
SMCR041	*Neolema dorsalis*	This study	This study	This study
SMCR042	*Neolema ephippium*	—	This study	This study
SMCR043	*Neolema eremita*	This study	This study	—
SMCR044	*Neolema gundlachiana*	—	This study	This study
SMCR045	*Neolema hexastigma*	This study	This study	—
SMCR046	*Neolema plumbea*	This study	This study	This study
SMCR047	*Nolema relucens*	This study	This study	—
SMCR048	*Neolema sallaei*	This study	This study	This study

SMCR049	*Neolema sexpunctata*	This study	This study	This study
SMCR050	*Neolema* spp.	—	This study	This study
SMCR051	*Oulema atrosuturalis*	This study	This study	This study
DQ001947	*Oulema elongata*	Vencl et al., 2004	—	—
SMCR052	*Oulema melanopus*	This study	This study	This study
SMCR053	*Oulema oryzae*	—	This study	—
BMNH:832838	*Oulema rufocyanea*	This study	—	—
SMCR054	*Oulema* sp 1. India	This study	This study	This study
BMNH#704404	*Stethopachys javeti*	Gómez-Zurita & Vogler, 2009	Gómez-Zurita et al., 2007	Gómez-Zurita et al., 2007
SMCR055	*Crioceris asparagi*	Gómez-Zurita & Vogler, 2009	This study	This study
FJ000446	*Crioceris duodecimpunctata*	Kajtoch, 2011	Marvaldi et al., 2009	Marvaldi et al., 2009
JF775781	*Crioceris quinquepunctata*	Kajtoch, 2013	—	—
SMCR056	*Lilioceris merdigera*	Gómez-Zurita & Vogler, 2009	Gómez-Zurita et al., 2007	Farrel et al., 1998
SMCR057	*Lilioceris nigripes*	—	This study	This study
SMCR058	*Lilioceris nigropectoralis*	—	—	This study
SMCR059	*Lilioceris quadripustulata*	This study	This study	This study
SMCR060	*Lilioceris subcostata*	—	This study	—
SMCR061	*Lilioceris unicolor*	This study	This study	This study
BMNH#704401	*Lilioceris* spp.	Gómez-Zurita & Vogler, 2009	Gómez-Zurita et al., 2007	Gómez-Zurita et al., 2007
SMCR062	*Lilioceris* sp1. India	This study	This study	This study
DQ001949	*Metopoceris* spp.	Vencl et al., 2004	—	—
SMCR063	**Altica viridicyanea*	Ge et al., 2011	Ge et al., 2011	Ge et al., 2011
SMCR064	**Bruchidius* spp.	Gómez-Zurita & Vogler, 2009	Gómez-Zurita & Vogler, 2009	Gómez-Zurita *et al*., 2007
BMNH#704481	**Charidotella sexpunctata*	Gómez-Zurita & Vogler, 2009	Gómez-Zurita et al., 2007	Gómez-Zurita et al., 2007

SMCR065	**Cryptocephalus iridipennis*	Vogler, 2009	Gómez-Zurita *et al*., 2007	Gómez-Zurita *et al*., 2007
SMCR066	**Diabotrica undecimpuctata*	Kim et al.,2003	Kim et al.,2003	Szalanski,1997
SMCR067	**Donacia vulgaris*	Hayashi & Sota, 2013	Hayashi & Sota, 2013	Hunt *et al*., 2013
BMNH#704384	**Paropsis maculata*	Gómez-Zurita & Vogler, 2009	Gómez-Zurita et al., 2007	Gómez-Zurita et al., 2007
SMCR068	**Plateumaris flavipes*	Sota et al., 2008	Sota et al., 2008	Marvaldi et al., 2009
SMCR069	**Sagra femorata*	Kergoat et al., 2011	Marvaldi et al., 2009	Marvaldi et al., 2009

***Outgroups**

Appendix 1.2. Host plant associations of Criocerinae and outgroups.

Voucher #	Specimen	Host plant	Family	Eudicot/ Monocot	
SMCR001	*Lema approximata*	*Commelina erecta*	Commelinaceae	Monocot	Vencl pers. ob
SMCR002	*Lema balyana*	*Commelina diffusa*	Commelinaceae	Monocot	Vencl pers. ob
DQ001931	*Lema biannularis*	*Dioscorea spp.*	Dioscoraceae	Monocot	Vencl pers. ob
SMCR003	*Lema bicincta*	*Solanum nigrum*	Solanaceae	Eudicot	Vencl pers. ob
DQ001935	*Lema bitaeniata*	*Witheringia solanacea*	Solanaceae	Eudicot	Vencl pers. ob
DQ001936	*Lema bouchardi*	*Dioscorea spp.*	Dioscoraceae	Monocot	Vencl pers. ob
SMCR004	*Lema cingulata*	*Commelina diffusa*	Commelinaceae	Monocot	Vencl pers. ob
SMCR005	*Lema cyanea*	*Commelina diffusa*	Commelinaceae	Monocot	Vencl pers. ob
SMCR006	*Lema curaca*	—	—	—	—
SMCR007	*Lema daturaphila*	—	Solanaceae	Eudicot	Vencl pers. ob
SMCR008	*Lema delauneyi*	*Dictyospermum ovatum*	Commelinaceae	Monocot	Vencl pers. ob
SMCR009	*Lema feae*	—	—	—	—
SMCR010	*Lema fleutiauxi*	*Commelina peludosa*	Commelinaceae	Monocot	Vencl pers. ob
SMCR011	*Lema foveipennis*	*Calathea spp.*	Maranthaceae	Monocot	Vencl pers. ob
DQ001929	*Lema fulvipes*	*Commelina rufipes.*	Commelinaceae	Monocot	Vencl pers. ob
DQ155990	*Lema gallaeciana*	*Triticum spp.*	Poaceae	Monocot	Vencl pers. ob
SMCR012	*Lema hamata*	*Dioscorea spp.*	Dioscoraceae	Monocot	Vencl pers. ob
DQ001939	*Lema immaculipennis*	*Dioscorea mexicana*	Dioscoraceae	Monocot	Vencl pers. ob
SMCR013	*Lema insularis*	*Dioscorea mexicana*	Dioscoraceae	Monocot	Vencl pers. ob
SMCR014	*Lema nigromaculata*	*Dioscorea spp.*	Dioscoraceae	Monocot	Vencl pers. ob
DQ001940	*Lema obliterata*	*Dioscorea mexicana*	Dioscoraceae	Monocot	Vencl pers. ob
SMCR015	*Lema obscura*	*Withuringia solanacea*	Solanaceae	Eudicot	Vencl pers. ob
SMCR016	*Lema perplexa*	*Commelina peludosa*	Commelinaceae	Monocot	Vencl pers. ob
SMCR017	*Lema phungi*	*Commelina diffusa*	Commelinaceae	Monocot	Vencl pers. ob

SMCR018	*Lema praeclara*	*Tradescantia (Campelia) zonona*	Commelinaceae	Monocot	Vencl pers. ob
SMCR019	*Lema rangoonensis*	*Commelina difussa*	Commelinaceae	Monocot	Vencl pers. ob
SMCR020	*Lema regularis*	*Dioscorea spp.*	Dioscoraceae	Monocot	Vencl pers. ob
SMCR022	*Lema ropunctata*	*Lycium spp.*	Solanaceae	Monocot	Vencl pers. ob
SMCR023	*Lema rufotestaceae*	*Triticum aestivum*	Poaceae	Monocot	Vencl pers. ob
SMCR024	*Lema saigonensis*	*Commelina diffusa*	Commelinaceae	Monocot	Vencl pers. ob
SMCR025	*Lema stolida*	*Tradescantia (Campelia)zanonia*	Commelinaceae	Monocot	Vencl pers. ob
SMCR026	*Lema trabeata*	*Physalis* spp.	Solanaceae	Eudicot	Vencl pers. ob
SMCR027	*Lema transversofasciata*	*Acnistus* spp.	Solanaceae	Eudicot	Vencl pers. ob
DQ001944	*Lema trivittata*	*Datura stromonium*	Solanaceae	Eudicot	Vencl pers. ob
SMCR028	*Lema vridana*	—	—	—	—
SMCR029	*Lema viridicolor*	*Commelina spp.*	Commelinaceae	Monocot	Vencl pers. ob
SMCR030	*Lema* sp1. Ghana	—	—	—	—
SMCR031	*Lema externevittata*	—	—	—	—
SMCR032	*Lema constrictofaciata*	—	—	—	—
SMCR033	*Lema* nr. hopei	—	—	—	—
SMCR034	*Lema lacordairei*	*Curcuma spp.*	Zingiberaceae	Monocot	Ravindran *et al.*, 2007
SMCR035	*Lema* sp1. Namibia	—	—	—	—
SMCR036	*Lema* sp1. Papua New Guinea	—	—	—	—
SMCR037	*Lema* sp 2. Papua New Guinea	—	—	—	—
SMCR038	*Lema* sp 1. Peru	—	—	—	—
SMCR039	*Lema* sp 1. Malaysia	—	—	—	—
SMCR040	*Lema femorata*	—	—	—	—
SMCR041	*Neolema dorsalis*	*Commelina erecta*	Commelinaceae	Monocot	Vencl pers. ob
SMCR042	*Neolema ephippium*	*Tradescantia pendula*	Commelinaceae	Monocot	Vencl pers. ob

SMCR043	*Neolema eremita*	*Withuringia solanacea*	Solanaceae	Eudicot	Vencl pers. ob
SMCR044	*Neolema gundlachiana*	*Commelina diffusa*	Commelinaceae	Monocot	Vencl pers. ob
SMCR045	*Neolema hexastigma*	*Commelina diffusa*	Commelinaceae	Monocot	Vencl pers. ob
SMCR046	*Neolema plumbea*	*Solanum dulcamara*	Solanaceae	Eudicot	Vencl pers. ob
SMCR047	*Nolema relucens*	*Commelina erecta*	Commelinaceae	Monocot	Vencl pers. ob
SMCR048	*Neolema sallaei*	*Tripogandra serrulata*	Commelinaceae	Monocot	Vencl pers. ob
SMCR049	*Neolema sexpunctata*	*Solanum dulcamara*	Solanaceae	Eudicot	Vencl pers. ob
SMCR050	*Neolema* spp.	*Callisia cordifolia*	Commelinaceae	Monocot	Vencl pers. ob
SMCR051	*Oulema atrosuturalis*	*Oryza sativa*	Poaceae	Monocot	Heinrichs *et al.*,1994
DQ001947	*Oulema elongata*	*Commelina erecta* / *Tradescantia* spp.	Commelinaceae	Monocot	White *et al*., 1993
SMCR052	*Oulema melanopus*	*Triticum aestivum* / *Zea maiys*	Poaceae	Monocot	White *et al*., 1993
SMCR053	*Oulema oryzae*	*Oryzae spp.*	Poaceae	Monocot	Vencl pers. ob
BMNH:832838	*Oulema rufocyanea*	*Commelina diffusa*	Commelinaceae	Monocot	Vencl pers. ob
SMCR054	*Oulema* sp 1. India	—	—	—	—
BMNH#704404	*Stethopachys javeti*	—	Orchidaceae	Monocot	Jourdan H. & C. Mille, 2006
SMCR055	*Crioceris asparagi*	*Asparagus officinalis*	Asparagaceae	Monocot	
FJ000446	*Crioceris duodecimpunctata*	*Asparagus officinalis*	Asparagaceae	Monocot	White *et al*., 1993
JF775781	*Crioceris quinquepunctata*	*Allium spp.*	Amarylidaceae	Monocot	Schmit, 1998
SMCR056	*Lilioceris merdigera*	*Allium, Polygonatum* and *Lilium*	Amarylidaceae/Asparagaceae / Liliaceae	Monocot	Schmit, 1998
SMCR057	*Lilioceris nigripes*	—	Stangeriaceae	Cycadales	Vencl pers. ob
SMCR058	*Lilioceris nigropectoralis*	*Dioscorea alata*	Dioscoreae	Monocot	Vencl pers. ob
SMCR059	*Lilioceris quadripustulata*	*Ovaria sp.*	Anonaceae	Magnoniales	Mohamed Said, 2004
SMCR060	*Lilioceris subcostata*	*Dioscorea alata*	Dioscoreae	Monocot	Vencl pers. ob
SMCR061	*Lilioceris unicolor*	—	—	—	—

BMNH#704401	*Lilioceris* spp.	—	—	—	—
SMCR062	*Lilioceris* sp1. India	—	—	—	—
DQ001949	*Metopoceris* spp.	—	Solanaceae	Eudicot	Vencl *et al*., 2004
SMCR063	**Altica viridicyanea*	*Geranium wilfordii*	Geraniaceae	Eudicot	Xue *et al*., 2009
SMCR064	**Bruchidius* spp.	*Cercis spp.*	Fabaceae	Eudicot	Kergoat *et al*., 2007
BMNH#704481	**Charidotella sexpunctata*	*Ipomea* spp., *Convolvulus* spp.	Convolvulaceae	Eudicot	Clark *et al*., 2004
SMCR065	**Cryptocephalus iridipennis*	*Panicum* sp.	Poaceae	Monocot	Hawkeswood, 1988
SMCR066	**Diabotrica undecimpuctata*	Many hostplants	Cucurbitaceae, Malvaceae, Fabaceae	Eudicot	EPPO, 2013
SMCR067	**Donacia vulgaris*	*Typha latifolia L., T. angustifolia L., Sparganium spp*	Typhaceae	Monocot	Borowiec, 1984
BMNH#704384	**Paropsis maculata*	*Eucalyptus*	Myrtaceae	Eudicot	Walker, 2006
SMCR068	**Plateumaris flavipes*	*Carex, Eleocharis, Scirpus*	Cyperaceae	Monocot	Clark *et al*., 2004
SMCR069	**Sagra femorata*	*Pueraria montana*	Fabaceae	Eudicot	Van Driesche *et al*., 2002

*Outgroup