Observations on the natural history, systematics and phylogeny of the Criocerinae of Costa Rica and Panama

Fredric V. Vencl,[1,2] André Levy,[1] R. Geeta,[1] Gwen Keller[2] and Donald M. Windsor[2]
[1]*Department of Ecology and Evolution, The State University of New York at Stony Brook, Stony Brook, NY, USA;* [2]*The Smithsonian Tropical Research Institute, UNIT 0948, APO AA 34002, Panama*

Introduction

Although not among the largest, the Criocerinae, or shining leaf beetles, with nearly 1400 described species, form an importantly basal subfamily within the remarkably successful radiation of the Chrysomelidae. As their vernacular name indicates, shining leaf beetles are glabrous, with a brilliant, metallic sheen. Even though many species are quite colorful, shining leaf beetles have not been the favorites of collectors. Most species are small (3-8 mm), and all are quite rapid fliers, making their capture a challenge. With a nearly world wide distribution, shinning leaf beetles feed on the members of at least fifteen, both mono- and dicotyledonous plant families (Jolivet & Hawkeswood, 1995). Despite a trend toward eclecticism in host associations at higher taxon levels, there is also a strong pattern of dietary conservatism at the species level. Although most of their host plants are monocots, there are scattered reports of criocerines feeding on cycads and on ferns (Hawkeswood, 1992). Most species appear to be monophagous or narrowly oligophagous (Jolivet, 1988). However, conclusions on diet ranges must be provisional, because few systematic host-choice experiments have been attempted across a wide range of species. Furthermore, only 20% of host plants are presently known, and many of these records are themselves dubious (Jolivet & Hawkeswood, 1995). Thus, a final assessment of any broad phylogenetic patterns in host associations within the Criocerinae must await further documentation from more systematic fieldwork. However, we can say with confidence that the vast majority of species are found outside the forest canopy, on secondary-growth plants, inhabiting forest tree-fall gaps, edges, and other similarly disturbed areas. All life stages are intimately associated with the host plant. Although rare in the rest of the Chrysomelidae, leaf surface grazing of both adults and larvae is the chief trophic habit within the Criocerinae. A bizarre synapomorphic character, the peculiar dorsal orientation of the larval anus, which promotes the formation of a fecal shield, supports the close relationship among the subfamily's members. The significance of the combination of leaf grazing, shielded larvae, and the overall eclecticism of dietary associations within the subfamily will be examined below.

Plant feeding, especially leaf grazing, represents an enormous ecological hurdle (Strong *et al.*, 1984). Although a minority of insect lineages has successfully managed to colonize the plant resource spectrum, these few lineages contain most species (Mitter & Farrell, 1991). For example, beetles are the most diverse group of organisms on the planet, with over one-half of them being phytophagous. However, the reasons for their numerical dominance remain obscure, but somehow seem to be positively correlated with plant feeding (Farrell, 1998).

The Criocerinae were among the first leaf beetle lineages to attack angiospermous plants, and also among the very first to enter the leaf surface adaptive zone, a trophic niche fraught with numerous biotic and abiotic perils. Predation, desiccation, plant chemical, and physical defenses are perhaps among the most formidable hazards. Because the Criocerinae are considered to be very basal within the Chrysomelidae radiation (Monrós, 1960; Jolivet, 1988; Farrell, 1998), they make appropriate models to investigate the reasons for the marked success of the Chrysomelidae in particular, and of the phytophagous Coleoptera in general.

Address for correspondence: Fredric V. Vencl, Department of Ecology and Evolution, The State University of New York at Stony Brook, Stony Brook, NY 11794-5245, USA. *Email:* fvencl@life.bio.sunysb.edu

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Of Criocerinae natural history, it can well be said that our ignorance greatly exceeds our knowledge. With the exception of a few agricultural pests, such as the cereal, lily, three-lined potato, and asparagus beetles, virtually nothing is known about the natural history of the vast majority of species, especially the denizens of the tropics, which lack economic importance (but see Aiello & Vencl, 2004). Against this paucity, basic biological observations of the kind we present here, not only serve to increase our understanding, but also form a necessary foundation for advanced research. The qualitative observations we present here will be essential to investigators starting on experimental studies of this poorly known, but rich tropical fauna.

In support of research into larval shield chemical ecology and diet evolution, the results of which are reported elsewhere (Vencl & Morton, 1999), the present study began as a survey of host associations and biogeography of the shining leaf beetles of Costa Rica and Panama (hereinafter referred to as CRP). We present the most contemporary list of known and estimated species, together with their host associations, for the CRP region. Many criocerine species and host plants are recorded for the first time here. Although the present work is not intended to be a taxonomic revision, we have nevertheless found it necessary to change the status of several species. These changes reconcile some names with a recent, comprehensive revision, and serve to clarify the systematics of the Neotropical Criocerinae and of their North American counterparts. We present a preliminary phylogenetic hypothesis, based on molecular evidence, which reveals a clear historical signature upon host plant colonization events in the New World. Finally, after summarizing host data and phylogenetic relationships, we propose a chemoecological hypothesis that attempts to explain the apparently contrary patterns of host shifts among unrelated plant families with the abiding pattern of dietary specialization evidenced within individual species. We suggest that both top-down, ecological, and bottom-up, plant driven factors have acted upon the constitution of larval chemical defenses, and thus, have guided the observed directionality of host shifts within the subfamily.

At the end of this paper, we provide two appendices: a species key and a diagnostic description for each species. Used in combination, we hope that they will provide sufficient basic natural historical and taxonomic information to facilitate specific identifications, and thereby provide a more solid foundation for the formulation of more robust hypotheses and more detailed experiments. Eventually, such advanced studies will elucidate the very unusual history of phytophagy within the Criocerinae, and perhaps also predict the uncertain future of the group vis-à-vis their fire-scored and threatened habitats. We further hope that this study will promote more detailed studies of the selective processes that drive diversification, and diet evolution within leaf beetles and, in the limit, lead to the clearer recognition of the imprint of history upon the contemporary structure of Neotropical plant-herbivorous insect communities.

Neotropical habitat conversion proceeds unabated, and at an alarming rate, despite much publicity to the contrary. Consequently, the chance to gain insights into the Criocerinae, and many other insect groups inhabiting similarly traumatized habitats may soon be lost. Such groups, including the Criocerinae, likely harbor insights into the fascinating, and potentially pharmaceutically important, nexus between plant chemistry and beetle defenses. These opportunities may soon be irrevocably lost. As weighty as this sad caveat is, we hope the present study begins to promptly redress our ignorance, and to foster more intensive investigations, hopefully before time runs out on shining leaf beetles, their habitats, and their associated flora.

Scope of the study and methods

The checklist for the Criocerinae of the World by Monrós (1960), in addition to Blackwelder's (1946) checklist of Central and South America, together, form the basis of the list presented herein. We incorporate the synonomies reported in both checklists in order to arrive at a conservative estimate of the CRP Criocerinae fauna. We then add our field observations, and reconcile the differences.

The New World members of the Criocerinae number over 460 described species, fully accounting for 33% of the subfamily's worldwide fauna. Based on the Monrós' (1960) checklist, a third of these, some 150 species, are thought to occur in Central America. Several species are included in the present survey, which have disjunct distributions, that is, they occur on either side of the CRP region, but have yet to be found within it. Costa Rica and Panama together represent a land area less than one-twentieth of one percent of the Earth's unfrozen surface, yet comprise 4% of the total Criocerinae estimated to exist worldwide, and well over three times as many as recorded from the much larger North American region.

Although we have collected in all parts of the study area, our efforts have been greatest in Sarapiqui, Palo Verde, Cauhita, and the Maseta Central of Costa Rica, as well as in Central and Western Panama. Sweeping usually yields familiar species that reside along roadsides, trails, and forest edges. The under-representation of most species in collections is due not only to their rarity and small size, but also chiefly to the exceptional ability of shining leaf beetles to avoid capture, especially during beating and sweeping activities. Because of their tendency to take immediate upward flight when disturbed, most criocerine species are relatively difficult to collect, especially in very warm conditions. They often escape before their presence has even been detected. However,

since their escape flight is brief and vertical, shining leaf beetles can often be aerially netted, or swept after they alight nearby, a meter or so above their host plants.

We generally avoided sweeping, until close examination of adjacent vegetation was completed. Feeding observations consisted of carefully looking at leaf abaxial surfaces, while moving slowly through the foliage. We collected year round. The most productive time of the year for collecting was at the beginning and end of the rainy season, from May-June and from November-December, respectively.

According to Monrós' (1960) list, the Criocerinae fauna of CRP can be conservatively estimated at between 50 to 60 species. With increased collecting effort, the area's fauna will undoubtedly exceed the current estimate, but will likely remain within the same order of magnitude. We do not expect a significant increase in the number of species to be discovered, even if the forest canopy is canvassed thoroughly. Further collecting in the Cordillera Talamanca and Darién regions, at mid-elevations, should be a priority, and will surely yield additional records and new species. As for the many forms that apparently belong to large species-complexes on the same hosts, a few new species may be differentiated by means of more detailed taxonomic scrutiny, and with the application of species-diagnostic techniques such as allozymes or microsatellite DNA (*e.g.*, Futuyma & McCafferty, 1990; Molbo *et al.*, 2002).

The authors have observed and collected Criocerinae throughout CPR for over fifteen years. Direct field observations of herbivory provided the bulk of the host affiliation data. To wit, a plant was considered a host by direct observation of adult feeding, or by larval feeding and subsequent rearing to adulthood. Host plants were identified by botanists at STRI, or by consultation with Croat (1978) or Gentry (1993). A voucher collection of adults and food plants is maintained at the Smithsonian Tropical Research Institute in Panama, and at the senior author's home institution.

Beetle identifications were made by consultation with Jacoby (1880; 1888), Lacordaire (1845), and other primary 19th century sources. In addition, over 1700 museum specimens were examined during the study. Field-collected material was compared to type material in order to verify identifications whenever possible. The museums visited by the senior author included the American Museum of Natural History, the National Museum of Natural History, the Museum of Comparative Zoology (MCZ), the Carnegie Museum, the Museum of the Catholic University, Quito, Ecuador, and Smithsonian Tropical Research Institution collection, in Panama. The Bowditch collection in the MCZ proved invaluable for confirming many identifications. In addition to field effort, Costa Rican distributional data were also obtained from collections at InBio, San José, and from Catie in Turrialba.

Male genitalia were dissected out with a pin, placed in ethyl acetate, washed in warm KOH, and mounted on points with glue. Genitalia were drawn in dorsal view using a camera lucida and #2 lead pencil.

Specimens for the molecular study were collected and preserved whole in 70% ethanol. Genomic DNA was isolated from whole specimens using the CTAB method (Winnepenninckx *et al.*, 1993). A 635-bp fragment of cytochrome oxidase I (COI) was amplified by Polymerase Chain Reaction (PCR) using primers C1-J-1718 (GGAGGATTTGGAAATTGATTAGTTCC) and C1-N-2248 (AACGAATCCTAGGAGTCCAAT). For a subset of species, a section nested within this fragment was amplified using alternative primers C1-J-1672 (GGAGCACCTGATATAGCTTTCCC), C1-N-2161(AATCCCCG-TAAAATTAAATAAACTTC), C1-N-1556 (TATTAGGAGCCCCTGCTGATATG). Forty amplification cycles were performed on a PTC-200 (94∞C for 60 seconds, 48∞C for 60 seconds, and 72∞C for 60 seconds). PCR products were cleaned using GENECLEAN II Kit. Both strands were sequenced with the amplification primers on an automated sequencer at Davis Sequencing, LLC. Sense and anti-sense sequence data for each specimen were compiled and edited using the Chromas program (v 1.45). Clustal X (v. 1.81) was used to align all sequences. An average of 562 bp sequence data per species was obtained, corresponding to positions 1671-2255 in the mitochondrial genome of *Crioceris duodecimpunctata* (GENBANK accession no. AF467886), which was used as the outgroup. Maximum-parsimony (MP) and maximum likelihood (ML) analyses were performed using PAUP* (v. 4.0b10). In MP analyses, characters were weighted equally; in ML analyses the HKY85 model was used for nucleotide transitions. Trees were obtained using the heuristic search function (starting trees obtained by random additions (100), TBR branch swapping, holding one tree at every step). Support for MP tree was assessed by analysis of 1000 bootstrap replicates. Host plant usage was coded as a four-state character (commelinoid, solenoid, dioscoreoid, asperigoid). These character states were optimized on the MP and ML trees by MP using McClade (v. 4.0).

A brief taxonomic history

In the earlier taxonomic works, it was frequently necessary to describe a species from a single specimen, or a very short, cobbled together series of specimens; otherwise, most species would have remained unnamed. The 19th century describer often had no choice, since, as noted above, most shinning leaf beetles were relatively hard to collect in quantity. Thus, the taxonomist had little to work with in the way of robust series. Many species were patchily distributed, often in remote habitats, and at low densities. As a consequence, the range of phenotypic

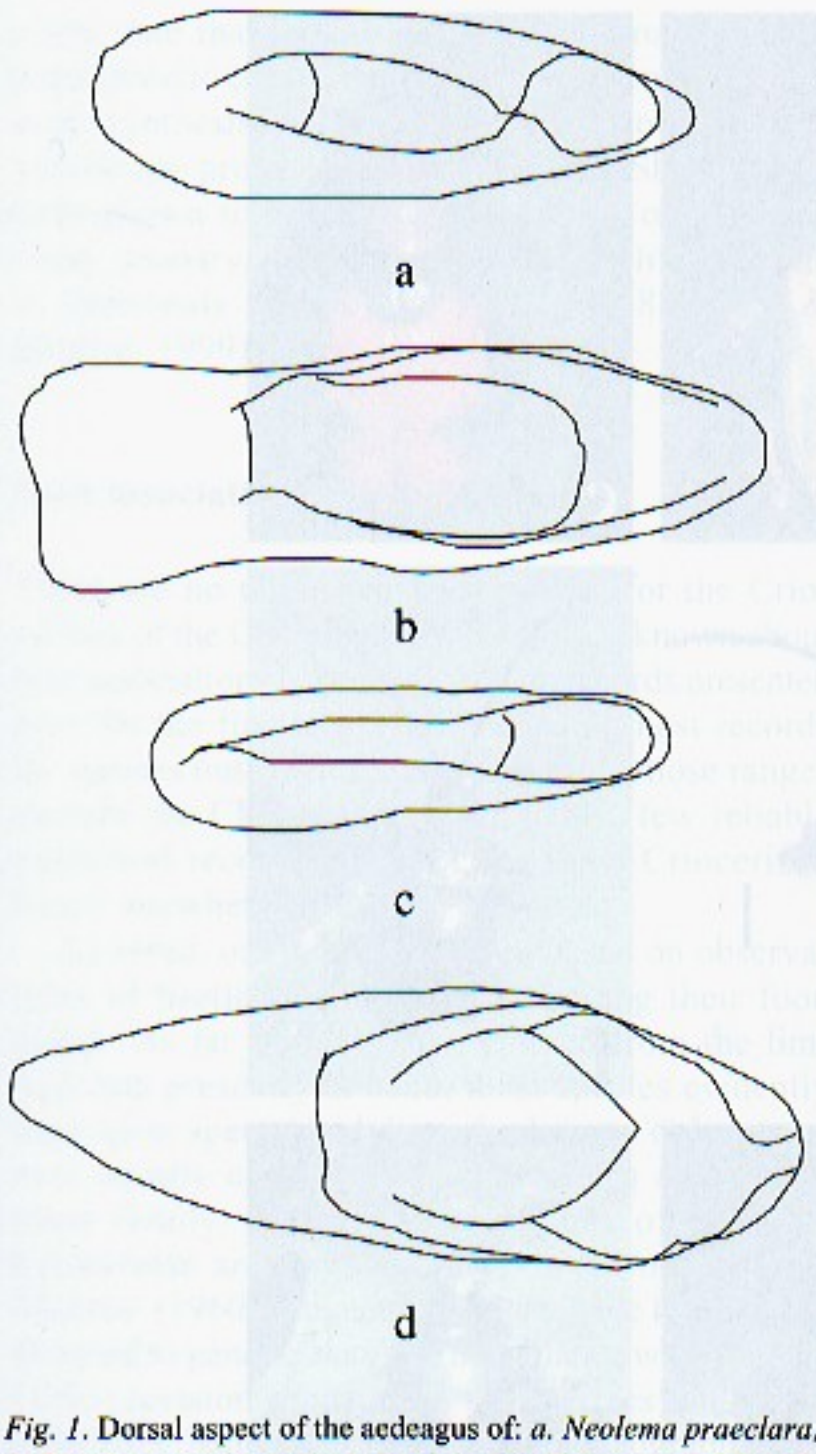

Fig. 1. Dorsal aspect of the aedeagus of: *a. Neolema praeclara; b; Lema immaculipennis; c. Neolema bispilota;* and *d. Metopoceris gemmans.*

Neolema – most species of *Neolema* are distinguished by having the ninth elytral stria with a gap of five to twelve punctures. The head is constricted rather deeply behind the eyes. The antennae are greater than the body length. The pronotal constriction varies from deep to moderately deep, and is located medially to submedially. Some members of *Neolema* are distinguished by having the disc of a solid orange to red color, decorated with black spots. Many others have a solid blue-colored disc. Members of *Neolema* have the bases of their tarsal claws touching (connate). The lateral folds of the aedeagus meet to conceal the central fold (Fig. 1a).

Oulema – species of *Oulema* can be recognized principally by the lack of any elytral patterning; the disc is always of a solid color, most often a glabrous blue or blue-black. The eyes are more weakly notched than those of *Lema*. The head is not constricted behind the eyes. The presence of frontal tubercles, or productions of the frons, or of a deep furrow in the vertex, is often encountered in the members of *Oulema*. The antennae are greater or equal to the body length. The unmargined pronotum is cylindrical or weakly constricted submedially or near the base. The punctures of the ninth elytral stria are complete. The members of *Oulema* have the bases of their tarsal claws touching (connate). The aedeagus is composed of three folds, of which the lateral two do not meet to conceal the central one, as they do in *Neolema* (Fig. 1c).

Biology

All members of the Criocerinae are phytophagous. Adults and larvae of most species are grazers on leaf surfaces. Adults of some species feed on pollen, flowers, or seeds (*e.g.*, *Neolema dorsalis* and *N. approximata*). The larvae of a few species bore in roots or stems. Only two leaf miners are known in the entire subfamily. These are *Oulema pumila* and *N. quadrivittata* (Vencl & Aiello 1997; Monrós 1960). Early-instar larvae are gregarious, forming phalanxes or cycloalexic, or 'circled-wagon' formations (Vasconcellos-Neto & Jolivet, 1994). These formations presumably serve to amplify the defensive effectiveness of their shields. Shield defenses are discussed elsewhere (Vencl & Morton, 1999). Later instars separate and begin to feed individually. As larvae reach the end of the fourth instar, they migrate to the substrate, where pupation occurs. Metamorphosis occurs in a chamber formed from a frothy, oral discharge that solidifies into a white case.

Patterns of host plant leaf damage indicate adult beetle use. For example, members of *Lema* and *Metopoceris* chew irregular holes within the leaf margins of their dicotyledonous hosts. They consume the entire leaf down to, and including most of the petiole. An entire plant can suffer defoliation. Interestingly, *Dioscorea*-feeders consume their host's leaves like *Lema* species do. On the other hand, *Neolema* and *Oulema* species chew narrow furrows parallel to the veins in the adaxial mesophyll of their monocotyledonous hosts. These differences in chewing patterns seem to reflect differences in host plant leaf venation. Whether these venation patterns affect feeding ability or efficiency is unknown.

The CRP Criocerinae exhibit different degrees of seasonality. A few species, such as *Neolema dorsalis* and *N. sallaei*, are present all year round at various densities, as are their hosts. In contrast, beetle species that feed on host plants that grow seasonally from tubers, such as *Calathea* or *Dioscorea*, disappear during the dry season along with their hosts.

The enemies of shining leaf beetles are chiefly hymenopterans that attack the larval stage. *Azteca* and *Ectatomma* are among the most important leaf patrolling ants that inflict heavy losses on larvae. Asopine pentatomid and reduviid bugs are also important sources of larval mortality. For example, *Montina* (Reduviidae), and the pentatomids, *Podisus*, *Stiretrus* and *Apiomerus*, are ubiquitous larval predators in the CRP region. Predators and parasitoids are reviewed by Schmitt (1988) and Cox (1996). All adults stridulate when handled, and it is likely this

variation was often underestimated. Differences, often minor, in shape, color pattern, and punctation, became the sole basis for distinguishing among many closely allied forms. Since many species are members of mimetic complexes, the picture of variation was further complicated at the local level (Gahan, 1891). Although ecological and behavioral data often prove useful in distinguishing similar species, identifications were not informed by such information until the late 1940s.

The genus *Lema* Fabricius (1798), *sensu lato*, is distributed world wide, and contains the vast majority of criocerines, approaching 1200 described species. The number of species increased significantly with Lacordaire (1845). He used the punctation of the ninth elytral stria as a diagnostic character and, thereupon, formed two divisions within *Lema*, *sensu lato*. His first division had the row of punctures within the ninth stria complete, while his second division had the ninth row's punctures interrupted in the middle for several pits. This character has been retained in all systematic schemes to date.

Des Gozis (1886) assigned *Lema* species with an uninterrupted ninth elytral stria and a cylindrical pronotum to the genus *Oulema*, also with a worldwide distribution. Heinze (1931) placed the large, metallic green colored Central American members of the worldwide genus *Crioceris* into a new genus, *Metopoceris*.

Monrós (1960) listed world species of the Criocerinae and made nomenclatural changes. He expanded upon Lacordaire's system by placing New World species of the First Division, those with continuous punctation of the ninth stria, into two subgenera, *Quasilema* within *Lema,* and *Hapsidolemoides* within *Oulema*. Species with a large gap in the punctation of the ninth elytral stria, members of Lacordaire's Second Division, were placed in a new *Lema* subgenus, *Neolema*. He synonymized numerous forms. His untimely death prevented a more complete revision of the Neotropical Criocerinae.

More recently, White (1993) revised the North American Criocerinae. He elevated *Neolema* to generic status and recognized *Oulema* as a valid New World genus. Before this, the validity of *Oulema*, as distinct from *Lema*, was not universally accepted (Mohr, 1985). In addition to *Neolema's* status change, White (1993) described twelve new species, made several new combinations, and numerous reassignments. In accordance with *Neolema's* status change, we have elevated several species within Monrós' subgenus *Neolema* to the generic level. We believe these generic reassignments will yield better resolution for future systematic and phylogenetic investigations.

Seeno & Wilcox (1982) included *Lema* (*Pachylema*) Monrós in the Central American fauna. However, this group is endemic to southeastern Brazil and northern Argentina, and thus is not included in the present study. The New World subgenus *Hapsidolemoides* Monrós is considered artificial and so is not recognized here (Schmitt, 1988). Therefore, the genera and subgenera we recognize herein as constituting the Central American Criocerinae include *Lema* (*Quasilema*), *Neolema*, *Oulema*, and *Metopoceris*. These genera agree for the most part with those proposed by Monrós (1960), Seeno & Wilcox (1982), and White (1993). However, the Central and South American faunas have not yet been completely reconciled with these changes, and consequently, a comprehensive revision of the New World Criocerinae is warranted.

Morphological characters

The Criocerinae can be diagnosed chiefly by their glabrous, shiny appearance, and by the very narrow, often medially or basally constricted, unmargined pronotum. The pronotum often has something of an hourglass shape. The pronotum and head are equal in width, but are much narrower than the elytra. The pygidium is concealed beneath the elytra. Differences in aedeagal morphology provide the strongest evidence that the generic designations used here are natural groups. Aedeagi are described in the generic diagnoses below. The system used to name elytral striae, and their intervals is as follows. Counting should begin, backward as it were, from the margin with the tenth stria. This approach will automatically exclude scutellar stria and so avoid the short rows of punctures present in some species behind the humerus. Characters of the head, elytral punctation, tarsal claws, aedeagal morphology, pronotal shape, color, and color patterns are all important in distinguishing the genera of the Criocerinae. The diagnostic characters of genera are as follows:

Lema – with species of *Lema*, the head is deeply constricted behind the also deeply notched eyes; the antennae, their tubercles widely spaced, are usually shorter than one-half the body length, with the apical segment becoming progressively broader. The prothoracic constriction is pronounced and medial. Elytra have color patterns of longitudinal or transverse stripes, sometimes in conjunction with spots. The ninth stria always has complete punctation. The aedeagus in dorsal aspect has a single, medial fold overlaying the lateral folds (Fig. 1b). The members of *Lema* have the bases of their tarsal claws connate, or touching.

Metopoceris – the diagnostic characters of *Metopoceris* species consist principally of the brilliant metallic sheen of their robust, large bodies, which range in size from 10-16 mm. The pronotum is hourglass shaped and unmargined. The antennae and femora are short and robust. In contrast to the members of *Lema, Neolema*, and *Oulema*, all of which have connate tarsal claws, members of *Metopoceris* have their claws entirely separate (free) all the way to their bases, as do members of the Old World genus *Crioceris*.

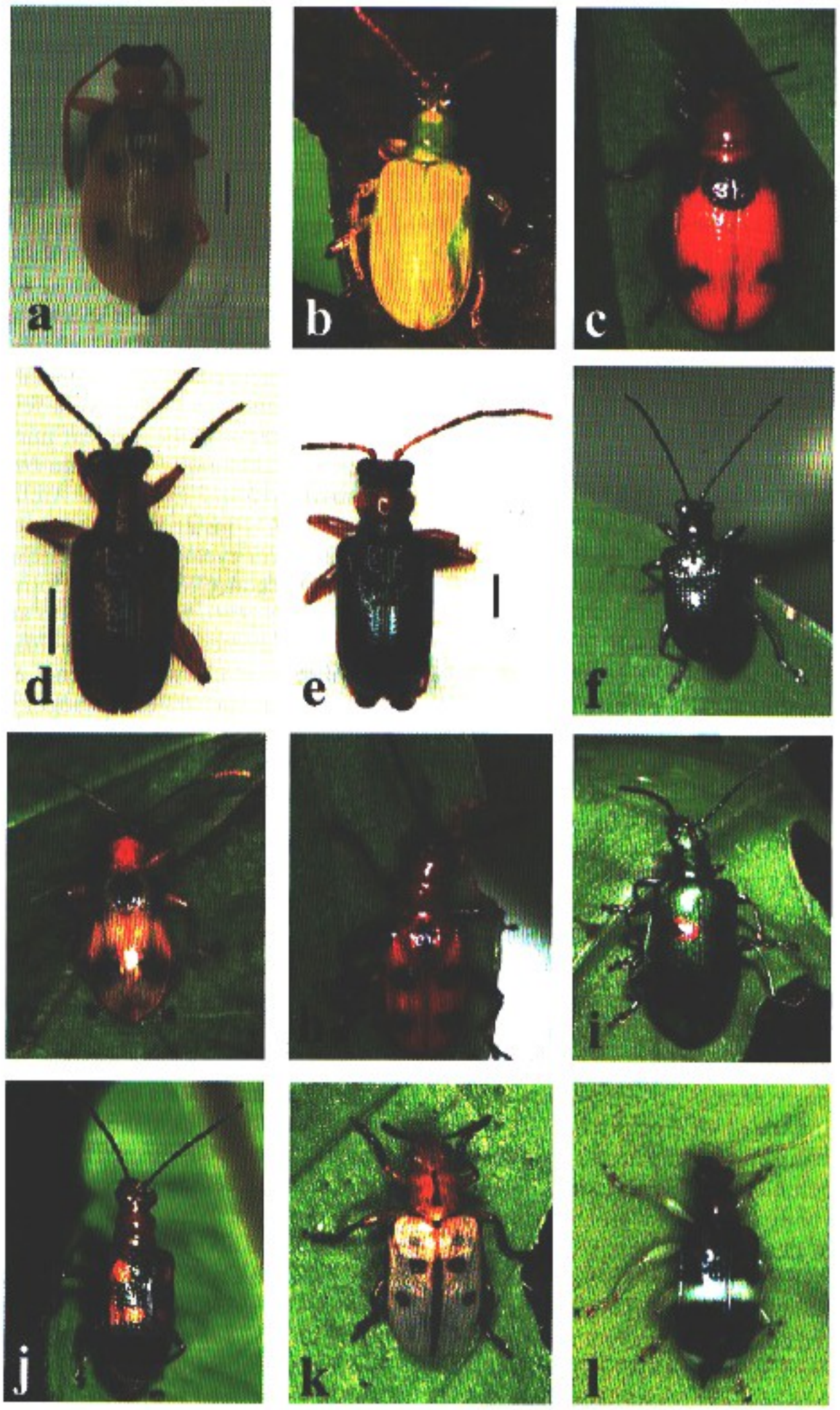

Fig. 2. a. Lema suffriani; b L. immaculipennis; c, L. nigromaculata; d. Neolema approximata*; e. N. praeclara*; f. N. foveipennis*; g. L. insularis*; h. N. hexastigma*; i. Metopoceris gemmans*; j. N. dorsalis; k. L. regularis*; l. L. bitaeniata**. Scale bar: 2 mm; *included in the COX 1 molecular phylogeny (Figs. 3 and 4).

ability serves to thwart predators (Gahan, 1900; Schmitt, 1988). Adult criocerines evidently have joined in several mimetic complexes with other leaf beetles, especially with the diabroticines of the leaf beetle subfamily Galerucinae (Gahan, 1891; Balsbaugh & Fauske, 1991). Adults release glandular secretions when handled (Pasteels *et al.,* 1984, 1988). However, nothing is known about the chemical composition of these adult secretions, or whether they are repellent, or deterrent to any natural enemy. Interestingly, glandular secretions occur in many adult members of the subfamily Chrysomelinae (Termonia & Pasteels, 1999). The convergence, or possible parallelism, in strategies of defensive adult secretions between these two major leaf beetle families forms the basis of a much-needed, comparative

study, one that would likely be informative about phylogenetic constraints on sequestration, and on *de novo* synthesis strategies. The larvae of the Criocerinae are protected by fecal shields. Shields have been shown to contain a combination of plant-derived, primary, and, secondary metabolites that act as deterrents (Morton & Vencl, 1998; Vencl & Morton, 1999)

Host associations and biogeography

There are no published host records for the Criocerinae of the CRP region. What little is known about host associations is largely based on records presented here for the first time, and less so on host records for species outside the study area, but whose ranges include the CRP region. Regrettably, few reliable published records exist for the most Criocerinae found anywhere in Central America.

As noted, our host records are based on observations of beetles in the act of attacking their food plants. As far as can be determined from the limited data presently at hand, most species evidently have quite specialized diets, feeding on only one or two, usually congeneric members of a single host plant family. The host associations of the CRP Criocerinae are given in Table 1. All members of Monrós' (1960) subgenus *Neolema* have herein been elevated to generic status, in accordance with White's (1993) revision. Appendix 1 summarizes adult characteristics, habitat, host plant use, biogeography, and taxonomic considerations for each species collected, or predicted to occur in the CRP region. A key to the species of Criocerinae in the CRP area is provided in Appendix 2. The current estimate of the number of Criocerinae species in the CRP region is 55.

Members of the genus *Neolema* predominantly attack plants in the Commelinaceae, as do all but one species of *Oulema,* host plant leaves the piper-feeding *O. pumila*. Recorded for the first time in the Neotropics, several species attack *Calathea* (Marantaceae). Species of *Lema* feed predominantly on the Solanaceae, but a closely related clade attacks *Dioscorea*. The first host reports for the genus *Metopoceris* are presented here (Table 1). *Metopoceris gemmans* was observed feeding on foliage of two solanaceous shrubs, one a species in the genus *Acnistus*, in Costa Rica, and the other, in the genus *Witheringia*, in Panama.

Utilizing the locality data from Jacoby (1880, 1888), Monrós (1960), Blackwelder (1946), and from our collecting effort, we categorized the ranges of CRP species by region (Table 3). Although most species are rare, some are locally abundant throughout much of the study area. A few species, such as *Neolema dorsalis, N. sexpunctata, Lema confusa, L. trabeata,* and *L. insularis,* have distributions from South America to Mexico, and into North America. Some species are very common in collections, while the vast majority are not. In the field, members of many species are found individually, or in tiny groups, on rare and inconspicuous host plants growing in remote habitats at elevations around 500 m.

A comparison of diversities between regions can be calculated using the Simpson Similarity Index (Simpson, 1947):

$$S = 100 \times C / N_i$$

in which, S: similarity; C: number of shared species; and N_i: number of species in the region with fewer species. The Simpson Similarity Index comparing South America and CRP regions yields 40%, while the Index comparing Mesoamerica with the CRP region, equals 94%.

At the species level, the CRP region is astonishingly diverse compared to other regions (Table 4). Dispersal, vicariance, climatic fluctuations, and continued tectonic and volcanic activity have certainly conspired to generate the relatively richer CRP criocerine fauna. How these processes produced their combinatorial effect is an intensely debated area of research.

Phylogenetic considerations

We wish to examine here the degree to which a representative sampling of sequence variation for the CO I gene contains evidence of the evolutionary history of the New World Criocerinae. The alignment of 21 sequences revealed a total of 249 variable sites. Eleven MP trees of length 328 were obtained, whose strict consensus is shown in Figure 3. *Neolema, Lema* and *Oulema* formed a fairly well-supported clade, within which there was embedded a strongly supported clade consisting of all *Lema* species except *L. fulvipes*. Although Monrós (1960) did not place it in the subgenus *Neolema*, *L. fulvipes*' morphology, host association, and molecular affinities justify its inclusion therein. The ML tree gives similar results, but resolution among the *Neolema* taxa is different from the MP trees. However, the differing topologies have little bootstrap support, so the differences between methods cannot be considered significant.

Indeed, understanding of the history of host plant use is not affected by these differences in results (Fig. 4). MP reconstructions of host plant use revealed that the in-group ancestor may have used any of the host plants, but that there was an early bifurcation into commelinoid-using and *Dioscorea*-using lineages. A small group of Solanaceae-using species (*L. bitaeniata, L. trivittata, L. trilinea*) is nested within the *Dioscorea*-users. According to the ML tree and one MP tree, there may have been one host shift from *Dioscorea* to Solanaceae with one reversal, or two independent shifts from *Dioscorea* to Solanaceae. Other MP trees suggest that there was a single shift from *Dioscorea* to Solanaceae with no reversal.

Table 1. Criocerinae of Costa Rica and Panama and their host plants.

Taxon	*Range*	*Host*	*Source*
Lema Fabricius			
bicincta Lacordaire	M, P*	*Dioscorea sp.**	*fvv*
biannularis Clark	M, CR, P	*Acnistus arborescens**	*fvv*
bitaeniata Chevrolat	M, G, CR, P	*Witheringia sp.**	*fvv*
dimidiaticornis de Borre			
flavofasciata Clark			
picturata Lacordaire			
incerta Jacoby			
sexsignata Jacoby			
troberti Lacordaire			
bouchardi Baly	CA	*Dioscorea sp.**	*fvv*
problematica Jacoby (synonomized by Blackwelder and Jacoby)			
centralis Clavareau	P		
apicornis Jacoby	P		
chiriquensis Jacoby	CR*, P	*Solanum sp.**	*fvv*
forreri Jacoby			
championi Jacoby	CA	*Solanum nigrum**	*fvv*
confusa Chevrolat	CA, P	*Physalis pubescens*	*fvv*
bivittata Lacordaire			
fasciaticollis Pic			
caerulea Lacordaire	G, C	*Commelina spp.**	*fvv*
coerulea Clavareau			
elegans Jacoby	CR, P, C	*Witheringia solanacea**	*fvv*
equestris Lacordaire	CA		
fulvipes Jacoby	M, P*	*Commelina rufipes**	*dmw*
godmani Jacoby	CA	*Commelina diffusa**	*fvv*
hamata Lacordaire	P, C	*Dioscorea sp.**	*fvv*
immaculipennis Jacoby	P	*Dioscorea mexicana**	*fvv*
modesta Jacoby			
incisa Jacoby	P		
insularis Jacoby	P	*Dioscorea mexicana**	*fvv*
obliterata Jacoby	P	*Dioscorea mexicana**	*fvv*
obscura Fabricious	A, B, P*	*Commelina erecta*	*fvv*
quadraticollis Jacoby	CA, SA	*Calathea spp.**	*fvv*
propinqua Jacoby			
nigromaculata Jacoby	CA	*Dioscorea sp.**	*fvv*
nupta Lacordaire	M, G, C		
stigmula Jacoby	CR		
vuripes Lacordaire			
regularis Jacoby	P	*Dioscorea sp.**	*dmw*
brunnea Jacoby	M		
semisepta Lacordaire	B, G		
impunctata Pic			
sexplagiata Jacoby	P		
subapicalis Baly	G, CR, P*	*Dioscorea sp.**	*fvv,dmw*
suffriani Jacoby	CR, P	*Witheringia solanacea**	*fvv*
trabeata Lacordaire	CA	*Witheringia solanacea**	*fvv*
trivittata Say	NA, CR	Solanaceae	
transversofasciata Jacoby	CR, P	*Solanum sp.**	*fvv*
Neolema Monrós			
approximata Jacoby	CA	*Commelina diffusa**	*fvv*
bispilota Monrós (*bipustulata* Jacoby)	M, CR, P	*Commelina spp.**	*fvv*
dorsalis Olivier	CA, SA	*Commelina spp.*	*fvv*
ancoralis Baly	P		
maculifrons Clark	CR, CR, V		
nigricornis Fabricious	G		
septempunctata Lacordaire	CR		
eremita Jacoby	M, P*	*Commelina erecta**	*fvv*
exarata Clark	M, P*	*Commelina diffusa**	*fvv*
hoegei Jacoby			
flavipennis Jacoby	M, CR, P*	*Commelina spp.**	*fvv*
foveipennis Jacoby	CA	*Calathea latifolia**	*fvv*
gundlachiana (Suffrian)	Cuba, P*	*Campelia zanonia**	*fvv*
intermedia Suffrian			
hexastigma Lacordaire	M, CR, P	*Commelina spp.**	*fvv*

scutaria Jacoby	G		
plumbea Chevrolat	CR, P	*Tripogandra serrulata**	*fvv*
		*Commelina diffusa**	
maculifrons Clark	CR		
praeclara Baly	SA, CR*, P*	*Campelia zanonia**	*fvv*
relucens Jacoby	M, P*	*Commelina erecta**	*fvv*
ricana Pic	CR		
sallaei Jacoby	CA	*Tripogandra serrulata**	*fvv*
		*Commelina spp.**	
sexpunctata Olivier	N, CA	*Commelina diffusa*	
albini Lacordaire			
sexmaculata Germar			
(synonomized by Blackwelder and Monrós)			
Oulema des Gozis			
pumila Vencl and Aiello	P*	*Peperomia sp.**	*aa*
Metopoceris Heinze			
championi (Jacoby)	P, C		
gemmans (Guérin)	M to C	*Acnistus sp.**	*hps,fvv,gk,dmw*
		Witheringia sp.	
chiriquensis Bowditch			
cullizona Clark			
intermedia Jacoby			
nullicedo Lacordaire			
flohri (Jacoby)	M, G, CR		
unidentified			
Lema	P	*Dioscorea*	*fvv*
Lema	CR		*fvv*
Neolema	CR*, P	*Commelina spp.**	*fvv*
Neolema	P, E	*Calathea insignis*	*fvv*
Neolema	P, E	*Campelia zononia*	*fvv*

Indented specific names are synonomies proposed by Monrós (1960), or by the other authors, as noted. Distributions are compiled from the literature and from our fieldwork. A: Argentina; B: Brazil; CA: Central America; C: Colombia; CR: Costa Rica; G: Guatemala; M: Mexico; N: Nicaragua; NA: North America; P: Panama; SA: South America; *: newly recorded; *aa:* Annette Aiello; *gk:* Gwen Keller; *hps:* Henry P. Stockwell; *fvv:* Fredric V. Vencl; *dmw:* Donald M. Windsor

Table 2. Host plant families of the Criocerinae of Costa Rica and Panama

Plant family	*No. of species*	*% total*
monocots		
Commelinaceae	17	31
Marantaceae	3	5
Dioscoreaceae	11	20
dicots		
Piperaceae	1	1
Solanaceae	10	18
Unknown	14	24

Discussion

Of the species whose hosts are presently known, a third of them feed on members of the Commelinaceae, while about 18% are found on members of the Solanaceae. This relative weighting of host associations between the two plant families parallels that found in South America for the Criocerinae there. However, the radiation onto the Dioscoreaceae is much more robust in Mesoamerica than previously thought. We also report here the first unambiguous records of Criocerinae on the Marantaceae for the New World. By one estimate, the order Zingiberales, of which the Marantaceae is a member, is sister to the Commelinaceae (Chase *et al.*, 1993). This close relationship may indicate that the spread of the *Dioscorea*- and commelinoid-associated criocerines most likely pre-dated the Gondwana break-up, since these groups are also hosts in the Old World. The current affiliation of the Criocerinae with these older plant lineages supports the hypothesis that the Criocerinae have retained associations that were formed very much earlier, perhaps in the late Mesozoic.

We also discovered that *Metopoceris gemmans* feeds on more recently evolved, solanaceous hosts (Gothan & Weyland, 1973), even though this beetle is considered to be relictual, and basal within the Cricerinae (Monrós, 1960). According to our phylogeny, *Metopoceris'* nearest extant, New World relatives are monocotyledon-feeders. Whether or not *Metopoceris* represents a relictual group that once had a broader distribution, prior to the late Meso-

Table 3. Biogeography of the Criocerinae of Costa Rica and Panama

	Costa Rica	*Panama*	*Both*	*Disjunct*	*Central America*	*South America*
No. of species	5	7	4	2	15	22
% species	9	13	7	4	27	40

Table 4. Comparison of species diversity and relative species-area richness (SARI) for particular geographic areas

Geographic Unit	*n*	*Area (km²)*	SARI*
USA	40	7828,080	5×10^{-6}
Florida	20	151,670	1×10^{-4}
CRP	55	126,374	4×10^{-4}

*Index of Species Richness (SARI) = n / km² x 100

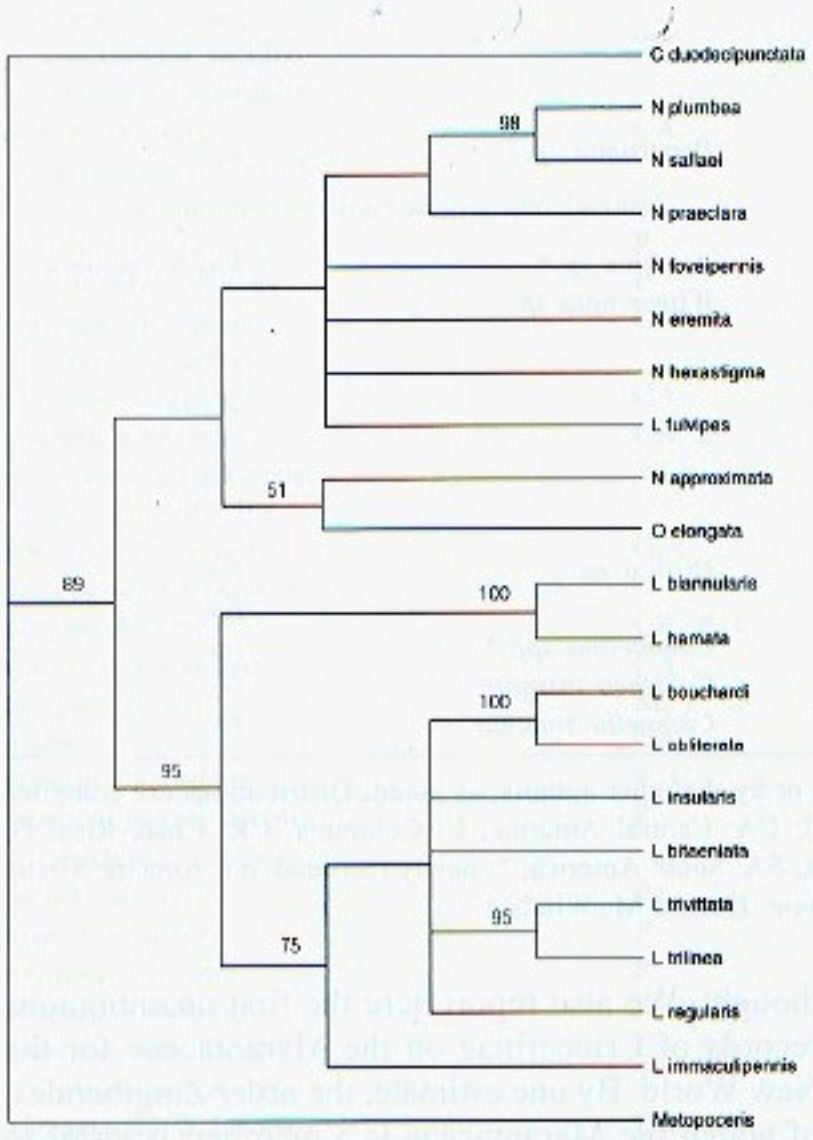

Fig. 3. Strict consensus of eleven maximum parsimony trees of length 328. Bootstrap support of > 50% is indicated by numbers along branches for 1000 replicates. *O. elongata* inhabits LA and TX and was described by White in 1993.

zoic Gondwana break-up, is problematic. Perhaps the genus is an endemic, which recently colonized younger hosts. If so, *Metopoceris* may bracket the Gondwana vicariance, and thus provide an interesting historical-biogeographic subject for research into the rapidity and directionality of host-shifts (Monrós, 1960; Brundin, 1965). The significance of our observation must await more complete revelations about the unknown hosts of its other congeners.

Two new stem borers are reported herein, *Neolema sallaei*, and *N. gundlachiana*. Stem-boring is rare in the subfamily. It appears to be a derived condition because, both boring species are capable of forming shields when forced to feed on the leaf surface. As noted elsewhere, all the proposed sister groups to the Criocerinae, and to the Chrysomelidae in general, are endophytic (Crowson, 1981). One criocerine is found in Piperaceae, *Oulema pumila*, as a leaf miner (Vencl & Aiello, 1997). According to one estimate of angiosperm evolution, the Piperaceae are thought to be a sister taxon to the monocots (Chase *et al.*, 1993). Thus, mining in this instance may well represent an example of a retained, ancestral trophic habit within the Criocerinae.

Costa Rica and Panama appear to have equal numbers of endemic or relictual species. The number of species with ranges in Mesoamerica, versus South America, is nearly equal. However, since the pool of potential colonists from the south is approximately ten times larger than that from the north, the nearly equal representation of species in the CRP region is remarkable. A similar pattern of disproportionate representation for Mesoamerican species was observed in the cassidoid Hispinae (Windsor *et al.*, 1992). This disparity may merely reflect differences in collection effort, but, we believe, may more likely indicate an 'isthmian effect'. Only during the last 25 my has there been sufficient propinquity between Mesoamerica and South America to permit migration over water. The barrier presented by the still unclosed isthmian land bridge, which was not completed until about 3.2 million years ago, may have created bilateral reservoirs, on both sides of the proto-isthmus, that served to collect less vagile colonists long enough for them to diversify, *in situ*, on continuously emergent land masses. Species trying to move across the straights separating East and West Panama, might have had their populations fragmented long enough to diverge from their respective ancestral lineages on the opposite side of the gap, thus enhancing Panama's fauna. Birds show a similar biogeographic pattern. The distributional limits on either side of the isthmian bridge of record this rift in a striking way. Distributions of northern species tend to find a limit at El Valle, while those species of predominantly southern distributions reach, but do not, move further westward at Cerro Azul and Cerro Jeffe on the eastern side of the Isthmian portal. These disjunct patterns indicate a barrier effect that may have helped to produce the remarkably high Panamanian bird diversity (N. Smith, personal communication).

At least we can say that the composition of CRP criocerine fauna is of mixed origin. The geographical affinities of species, such as *Metopoceris*, that occur at higher elevations, where endemism is expected to be the greatest, have older, northern origins. In contrast, the lowlands are dominated by recent, colonial species with southern distributions. Scarab beetles and the herpetofauna of Mesoamerica exhibit similar biogeographic patterns (Thomas, 1993; Savage, 2002). The Simpson Similarity Indices for the CRP criocerines support the view that a combination of mixed migration and vicariance events has contributed to the inordinately high diversity of the CRP region.

The Criocerinae are considered to be among the most basal of the estimated 21 Chrysomelidae subfamilies (Monrós, 1960; Schmitt, 1985, 1988; Farrell, 1998). Although the group is much older, the oldest unambiguous criocerine fossil dates from the

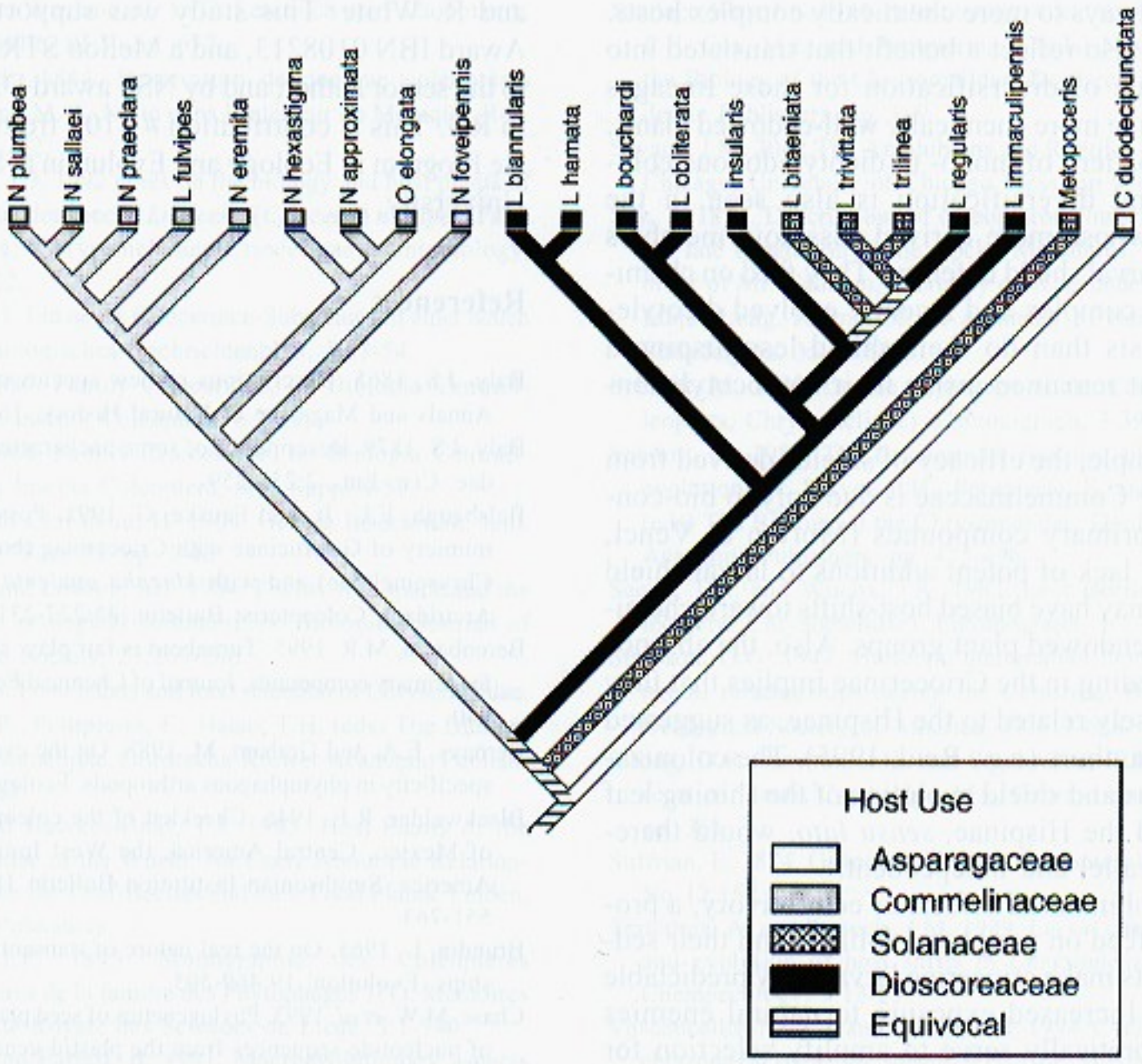

Fig. 4. Evolution of host plant use by Neotropical Criocerinae. Ancestral states of host use mapped on to ML tree using parsimony. All topologies obtained in the current analyses suggest that beetles went from feeding on *Dioscorea* to Solanaceae. The ancestral *Lema-Neolema* may have fed on Commelinaceae, Solanaceae, or *Dioscorea*.

Eocene, some 54 mya (Santiago-Blay, 1994). The approximate age of the first appearance of their host plants can also be estimated from the fossil record (Gothan & Weyland, 1973). The Criocerinae appear to have diverged from their cycad and fern-feeding ancestors probably by the mid-Cretaceous, whence they colonized the nascent radiation of the monocotyledonous angiosperms (Schmitt, 1988; Farrell, 1998). Schmitt (1988) arranged the Criocerinae into three monophyletic lineages, the *Lilioceris, Crioceris*, and *Lema +Oulema* clades. He proposed a dichotomous phylogeny with the *Lilioceris* clade forming an early split and the *Crioceris-Lema* clades forming a later split. The splits were broadly contemporaneous with the appearance of their respective host plant families, the Liliaceae, and Asparagaceae, and later, the Solanaceae and Poaceae in the fossil record (Gothan & Weyland, 1973).

When we considered the combined phylogenetic and morphological evidence, two facts emerged. Firstly, all *Dioscorea*- and Solanaceae-feeders had *Lema*-type aedeagi, that is, with a pronounced central fold, while all the *Calathea* (Marantaceae) and Commelinaceae-feeders had either the *Neolema* configuration, that is, convergent lateral folds obscuring the central fold, or, the *Oulema*-type fold pattern, with the lateral folds laying in parallel. Secondly, the molecular and morphological data supported the monophyly of the *Lema* clade. Certainly, the phylogenetic results support removal of *L. fulvipes* from *Lema*, and its placement within *Neolema*. Nonetheless, there is also strong support for the monophyly of *Neolema*.

The step-wise phylogenetic ordering evident in the radiation of the NW Criocerinae, as revealed in our preliminary molecular phylogeny, is in basic agreement with the coevolutionary model proposed by Ehrlich and Raven (1964). Their model predicts that: *(1)* the evolution of traits that influence ecological interactions between host plants and their insect herbivores are responsible for present day diversities of both groups; *(2)* host shifts may be due to chemical similarities, or 'bridges', that present lower thresholds to recognition and acceptance; and *(3)* host plant-herbivore associations should be conservative, whereby older plant lineages will be associated with older insect lineages. For example, the shift from the Dioscoreaceae to the Solanaceae may have been enabled by the steroidal sapogenins that members of both families share. The relatively greater diversity of the putatively younger *Dioscorea*- and *Solanum*-feeding clades when compared to the much older commelinoid-feeding habit is interesting. It may be partly related to changes in shield chemistry since

shifts are always to more chemically complex hosts. But it may also reflect a benefit that translated into a higher rate of diversification for those lineages colonizing the more chemically well-endowed plants. A similar pattern of mono- to dicotyledonous colonization and diversification is also seen in the Hispinae, whose more derived cassidoid members also have larval shield defenses. They feed on chemically more complex and recently evolved dicotyledonous hosts than do their shield-less hispinoid cousins that remained inside their monocotyledonous hosts.

For example, the efficacy of shields derived from hosts in the Commelinaceae is due only to bio-concentrated primary compounds (Morton & Vencl, 1999). The lack of potent additions to larval shield chemistry may have biased host-shifts toward chemically well-endowed plant groups. Also, the absence of palm feeding in the Criocerinae implies that they are not closely related to the Hispinae, as suggested by several authors (*e.g.*, Reid, 1995). The colonization patterns and shield evolution of the shining leaf beetles and the Hispinae, *sensu lato*, would therefore be parallel and independent.

The combinatorial effects of ectofolivory, a propensity to feed on peripheral foliage, and their sedentary habits make criocerine larvae very predictable resources. Increased exposure to natural enemies would theoretically serve to amplify selection for defense. The formidable array of chemical shield and adult defenses found within the Criocerinae is likely a response to the numerous selection pressures that abound in exposed leaf surface feeding niche. The evolution of diet-dependent, shield-based chemical defenses supports the idea that, at the macro-evolutionary level, criocerines may have been constrained to colonize only new hosts that afforded them with more effective chemicals for their shield defenses. This adaptation, in addition to a key innovation, the dorsal anus, allowed the Criocerinae to enter the new, unexploited leaf surface adaptive zone, where they enjoyed reduced competition and, 'enemy free space' (Ehrlich & Raven, 1964; Jeffries & Lawton, 1984; Bernays & Graham, 1988). These factors may, in part, account for the polyphagy at the host family level, and for the dietary conservatism at the specific level. Based on the evidence presented here, it seems likely that the Criocerinae might have escaped predation via a key innovation, the larval shield defense. If so, the 'escape-and-radiate' coevolutionary model may also have major implications for the third trophic level.

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