

Research on Chrysomelidae 4

Edited by

Pierre Jolivet, Jorge Santiago-Blay & Michael Schmittor



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Diversity of Alticinae in Oaxaca, Mexico: A preliminary study (Coleoptera, Chrysomelidae)¹

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Abstract

This is a preliminary study of the diversity of the Flea Beetles (Alticinae) of the Mexican state of Oaxaca based on fieldwork by the author in 1991, 1997, and 2010, the literature, and specimens in several institutional collections. The number of genera and species for Mexico as well as for Oaxaca increased significantly from previous studies. There are now 625 species in 90 genera recorded from Mexico with 275 species in 68 genera recorded from Oaxaca. There are 113 species known only from the state of Oaxaca and another 38 species known only from Oaxaca and the surrounding states. Oaxaca has a relatively high diversity as well as a high percentage of endemism. This study also demonstrates the effects of how even a small amount of fieldwork together with extracting specimen data from institutional collections can significantly increase the total faunistic and diversity knowledge of an area. A complete list of the genera and species known from Oaxaca is included.

Resumen

Este es un estudio preliminar de la diversidad de los Escarabajos Pulga (Alticinae) del estado mexicano de Oaxaca basado en el trabajo de campo por el autor en 1991, 1997, y 2010, la revisión de la literatura y el estudio de varios especímenes en colecciones institucionales. El número de géneros y especies para México y para Oaxaca aumentó significativamente a partir de estudios anteriores. En la actualidad hay 625 especies en 90 géneros registrados para México con 275 especies en 68 géneros registrados de Oaxaca. Hay 113 especies conocidas sólo del estado de Oaxaca, y otras 38 especies conocidas sólo de Oaxaca y los estados circundantes. Oaxaca tiene una diversidad relativamente alta, así como un alto porcentaje de endemismo. Este estudio también demuestra como una pequeña cantidad de trabajo de campo junto con

¹ Contribution to the 8th International Symposium on the Chrysomelidae, held August 23, 2012, in Daegu, South Korea

la extracción de datos de especímenes de colecciones institucionales pueden aumentar significativamente el conocimiento sobre la fauna total y la diversidad biológica de una región. Se incluye una lista completa de los géneros y especies conocidas de Oaxaca.

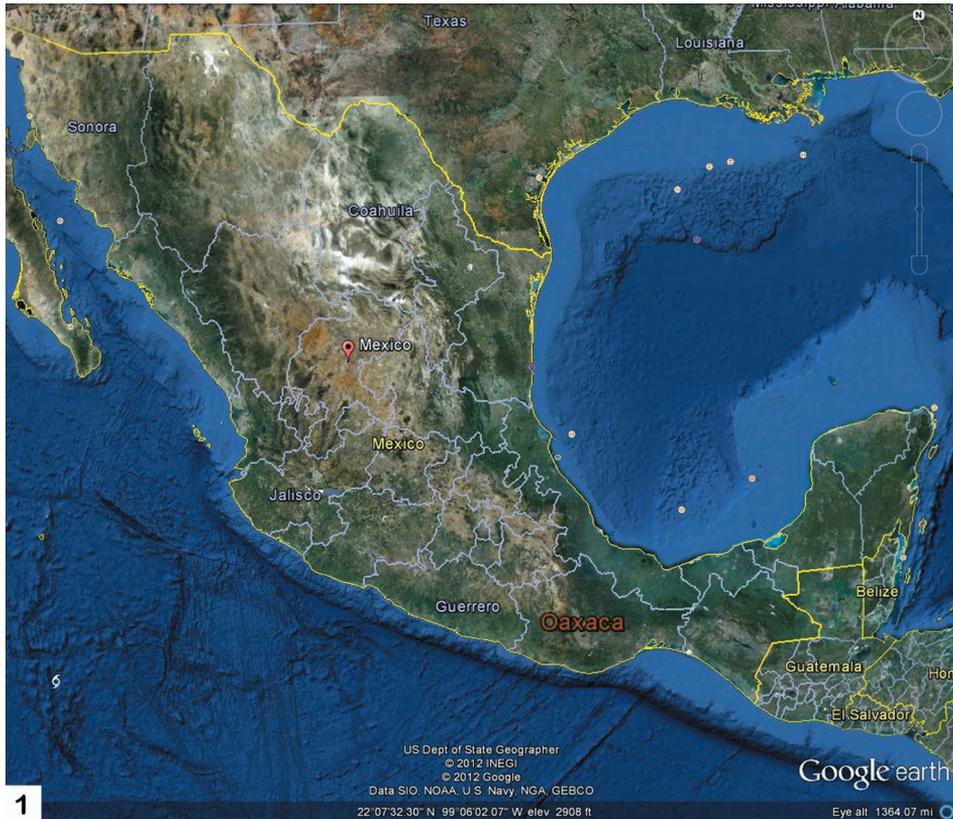
Keywords

Mexico, Oaxaca, diversity, Coleoptera, Chrysomelidae, Alticinae, endemism, fieldwork, collections

Introduction

Although Mexico is the fourteenth largest country in the world (ca. 2,000,000 km²) it is the fifth most biodiverse country and is one of the 25 biodiversity hotspots (Mittermeier 1988; Mittermeier et al. 1999).

Oaxaca is one of the most mountainous and rugged areas in Mexico and it is geologically complex as well (Ferrusquía-Villafranca 1993). Its southern and central areas are composed of the Sierra Madre del Sur mountain range, one of the major ranges in Mexico. However, the mountains of Oaxaca are actually composed of several less extensive ranges. The primary one is the Sierra Madre de Oaxaca that is a mountain range just north of Sierra Madre de Sur, but converging with it. It begins in the state of Veracruz at Pico de Orizaba and extends in a southeasterly direction for 300 km until reaching the Isthmus of Tehuantepec. Mountain peaks in the Sierra Madre de Oaxaca average 2,500 m in elevation, with some peaks exceeding 3,000 m. The Sierra Madre de Oaxaca also can be split into many smaller sierras, each with unique environments and human inhabitants, including Sierra de Juárez (this study area) and Sierra Mazateca (to the northwest) (Maps 1, 2). Of special interest to this study is its home base in Ixtlán de Juárez, a mountain community for the environmentally aware. Here, locals have developed a special eco-tourism project where guests are taken on guided tours through the area's attractive forests. Within the same hour, you can experience a hot, dry climate, and then ascend the mountains to a cold damp region (Map 3). The Sierra de Juárez is a range of mountains in Oaxaca state, Mexico between latitudes 17°20'–17°50'N and longitudes 96°15'–97°00'W, with an area of about 1,700 km². The range is separated from the Sierra de Zongolica to the north by the Santo Domingo River, flowing through the Tecomavaca Canyon. It stretches south-eastward to the Cajones River and the Sierra de Villa Alta. The mountains are in the district of Ixtlán de Juárez in the Sierra Norte de Oaxaca region. It is named after Mexico's only indigenous president, Benito Juárez, who was born here in 1806 in the small village of San Pablo Guelatao (Map 4). These mountains climb from 500 m to 3,250 m, with many large and deep ravines. They are formed of folded sedimentary rocks with series of younger granitic intrusions that date from the Palaeozoic to Cenozoic, with the majority being Mesozoic. The climate is subtropical in the lower regions and temperate and subhumid above 1000 m, with average temperature from 16°–20° C. There is regular frost in the higher mountains. Annual rainfall, fed by the trade winds from the Caribbean Sea, ranges from 700 mm to 4000 mm or more. The Valle Nacional River originates in the Sierra de Juárez, one of the major tributaries of the Papaloapan River (Map 4).



Map 1. Mexico and southern USA, depicting the position of the state of Oaxaca.

The Sierra de Juárez is one of Oaxaca State's wettest areas and richest in forest diversity, with perhaps 2000 of the 8000 or more plant species that are found in the state. It is mostly covered by montane cloud forest, but includes tropical evergreen forests and forests of pine, pine-oak and oak. The cloud forest forms a band from 1,000–1,400 m in height up to 2,250 meters along the northern and eastern slopes. The cloud forest climate is cool (14°–20° C) and has mean annual rainfall that exceeds 2,000 mm and is sometimes much higher. The dominant trees are 20–30 m tall and include evergreen and deciduous species, palms, tree ferns, heather shrubs, vines, and moisture-loving herbs (the facts above were taken from Wikipedia).

As evident from Maps 1 and 2 Oaxaca is positioned in a rather unique biogeographical part of Mexico and, in fact, Central America. It is bordered north and west by the rather dry and deep Rio Balsas (an almost west-east) transect of the state of Guerrero. To the north and east is the southern-most aspect of one of the other major mountain ranges of Mexico, the Sierra Madre Oriental, at that point in Veracruz. The eastern most part of Oaxaca spans the Isthmus of Tehuantepec that borders the state of Chiapas. The Isthmus of Tehuantepec is not mountainous and is distinctly the narrowest part of Mexico where the states of Oaxaca, Veracruz, Chiapas and Tabasco converge. This geographical con-



Map 2. Oaxaca with the surrounding states and demonstrating the strong geographical constriction of the Isthmus of Tehuantepec.

striction certainly has an effect on the diversity and distribution of the fauna and flora. Presumably to the south the biodiversity is predominantly Neotropical.

For Mexico's phanerogamic flora the highest diversity is found along a belt originating in Chiapas, traversing Oaxaca, and continuing to central Veracruz in the east and to Sinaloa and Durango on the west and cloud and evergreen forests are the most diverse per unit area, endemism is prevalent, and Oaxaca has a higher number of species than any state (Rzedowski 1993).

Llorente-Bousquets et al. (1993) report that based on butterflies (Papilionoidea) the two most species-rich areas in Mexico are the Sierra de Juárez (the area of the present study) and Los Tuxtlas (Veracruz), with the highest numbers of species in Oaxaca (40), Chiapas (41), and Veracruz (41). In a survey of 20 different groups of arthropods (8,599 species), the most diverse states were Veracruz (2072), Chiapas (1306), Oaxaca (1256), Guerrero (1124) (Llorente-Bousquets et al. 1996).

The current study is part of a series of publications on the diversity of Alticinae (Flea Beetles) in Mexico (Furth 1998, 2004, 2006, 2007, 2009; Savini et al. 2001). Be-



Map 3. Google Earth view of the Sierra de Juárez mountains with the current study's home base of Ixtlán de Juárez and some of the collecting localities from the 2010 field trip, especially Santa Catarina Lachatao.

sides elucidating the biological diversity of Mexico based on this taxon of herbivores, it provides an example of how the historical literature, historical collecting based on specimens in institutional collections, and new fieldwork can be combined to relatively rapidly assess such diversity. Although the historical literature is a fixed entity, when more institutional collections are examined or surveyed for historical collecting records and when more targeted fieldwork is conducted (even for short periods), there is a significant increase in diversity knowledge very quickly.

Furth and Savini (1996, 1998) listed all Alticinae known from Central America with their known distribution. Furth (2004) published the first accounting of Alticinae diversity in Mexico based primarily on the historic literature as well as some specimens from collections at the USNM, a few borrowed specimens from other collections, and some from very brief collecting by the author in 1991, 1993, 1995, and 1997. At that time there were 501 species in 85 genera listed from Mexico of which 96 species in 33 genera were recorded from Oaxaca. This made Oaxaca the third most diversity state behind Veracruz (182 species) and Guerrero (124 species), and just ahead of Durango

relas (84), Tabasco (81), and Chiapas (81). It is noteworthy that of these seven most diverse states, five are surrounding Oaxaca.

As discussed in Furth (2004) in Mexico there is a major biogeographic transition zone between the Nearctic and Neotropical Regions and biogeographic affinities may also vary greatly depending on the group considered. Also levels of endemism vary greatly depending on the group considered and, of course, depending on the relative knowledge of the group. As with any country some vertebrate and plant groups are well known, whereas most insect groups are not. Aspects of Mexican biogeography and endemism were also discussed in Furth (2004) with some examples from other groups provided. Biogeography and endemism will be treated below relative to the data from this study regarding Oaxaca and surrounding states.

Materials and methods

The data for this study was taken from three primary sources: first, from previously published literature, especially Furth (2004), Furth (2006) that included original published literature, including Furth and Savini (1996); second, from museum specimens borrowed from a variety of collections as follows: University of California, Berkeley (UCB); University of California, Davis (UCD), California Academy of Sciences (CAS); California Department of Food and Agriculture (CDFA); Brigham Young University (BYU); American Museum of Natural History (AMNH); U. S. National Museum of Natural History (USNM); Texas A. & M. University (TAMU); The Natural History Museum, London (NHM); the F. C. Bowditch Collection, Museum of Comparative Zoology, Harvard University (MCZ); Naturhistorisches Museum Basel (Switzerland) (NHMB); Zoologisches Staatssammlungen München (Germany) (ZSMC); third, from fieldwork by the author in 1991 (19-23 August) around Oaxaca City and in the Sierra de Juárez, 1997 (22-23 July) around Oaxaca City and southwest along Route 190, and 2010 (29 July-4 August) around Oaxaca city and in the Sierra de Juárez.

The Appendix is a combination of older records from the literature, a few collections (USNM, MCZ, NHMB), and new collection records from 8 other institutions above and the author's field work (DGF 1991, 1997, 2010).

Examination and determination of the specimens was made using a Leica MZ APO binocular dissecting microscope. The digital photos of Figure 10 were produced by Karolyn Darrow using the Visionary Digital™ imaging system and Adobe Photoshop™.

The fieldwork was primarily based out of the Universidad de la Sierra Juárez (USJI) (Figure 11). Alticinae were collected by general and/or host plant-targeted sweeping with a 15 inch diameter aerial insect net using an aspirator. The majority of the field sites were in the vicinity of Santa Catarina Lachatao (SCL) and daily trips were accompanied by Prof. Atilano Contreras Ramos (UNAM), Prof. Jose Arturo Casasola (USJI), and various members of the SCL community (Figure 12). After the fieldwork extensive collection examination and curation was done at the Colección Nacional de Insectos, Instituto de Biología, UNAM (Figure 13, 14)

Results

As a result of a week of fieldwork in Sierra de Juárez of Oaxaca, Mexico, in 2010 and subsequent determination of the specimens collected, as well as examination of several institutional collections, the number of known species of Alticinae of Mexico increased from 524 (Furth 2006) to 625 (Figure 1) - an increase of over 19%. At the generic level there was only one genus added to the overall fauna of the country (Figure 2). Also resulting from the new fieldwork and collections examined, the number of recorded species for the state of Oaxaca increased from 121 (Furth 2006, 122 were reported but one found later to be in error) to 275 (Figure 3) – an increase of almost 79% and the number of Oaxacan genera rose from 37 to 68 (Figure 4) – an increase of 84%. At both

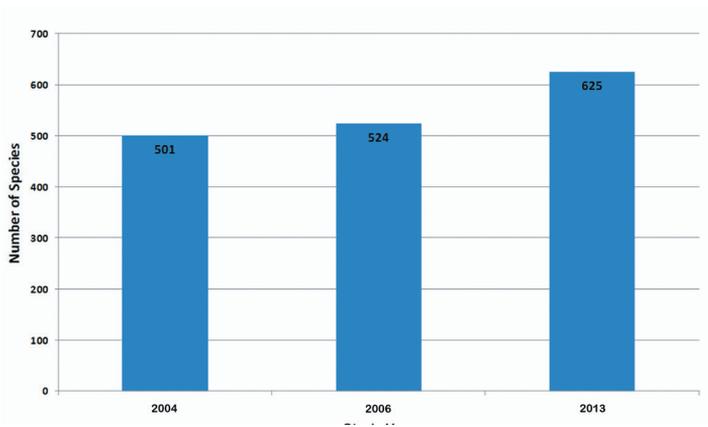


Figure 1. The total number of Alticinae species recorded from Mexico from Furth (2004, 2006) and the current study.

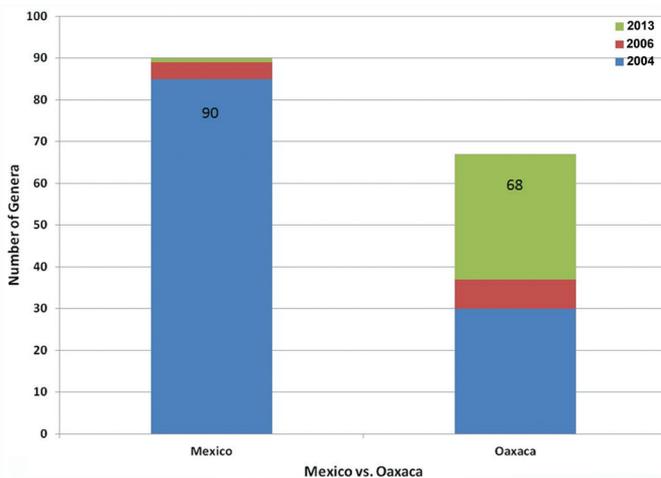


Figure 2. The total number of genera recorded from Mexico and Oaxaca based on Furth (2004, 2006) and the current study.

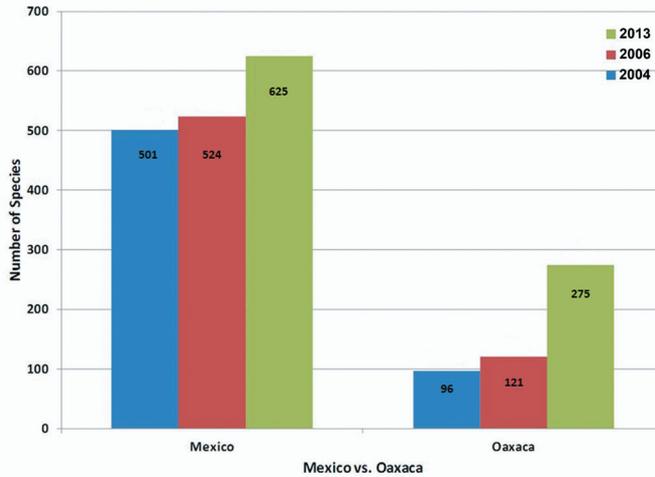


Figure 3. The changes in number of species recorded from Mexico versus Oaxaca only, based on Furth (2004, 2006) and the current study.

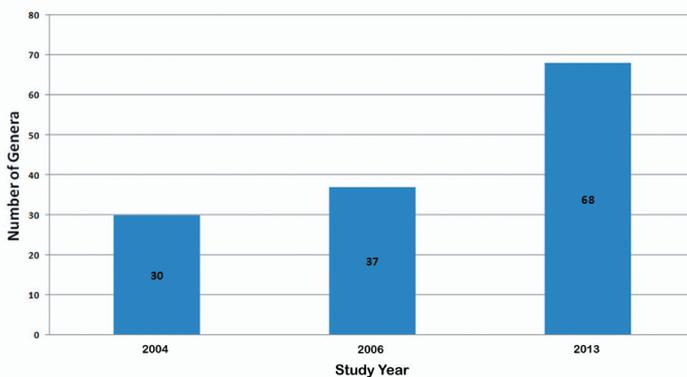


Figure 4. The changes in number of genera of Alticinae recorded only from Oaxaca from Furth (2004, 2006) and the current study.

the species and generic levels in Oaxaca these increases were significantly more than the increases from Furth (2004) to Furth (2006) for species of 96 to 121 (26%) (Figure 3) and for genera from 30 to 37 (23%) (Figure 4).

As for the endemism of Oaxaca as demonstrated by the Alticinae, Figure 5 shows that in Furth (2004, 2006) there were 9 and 11 species, respectively, recorded only from the state of Oaxaca, but as a result of the 2010 fieldwork there are 113 species – an increase of almost 930% from Furth (2006). Many of these (81 species or 72 %) currently only have morphospecies names and probably a significant proportion of these are new to science (see Appendix for OM species numbers). If the endemism is examined at a somewhat broader perspective, i.e., including species recorded in Oax-

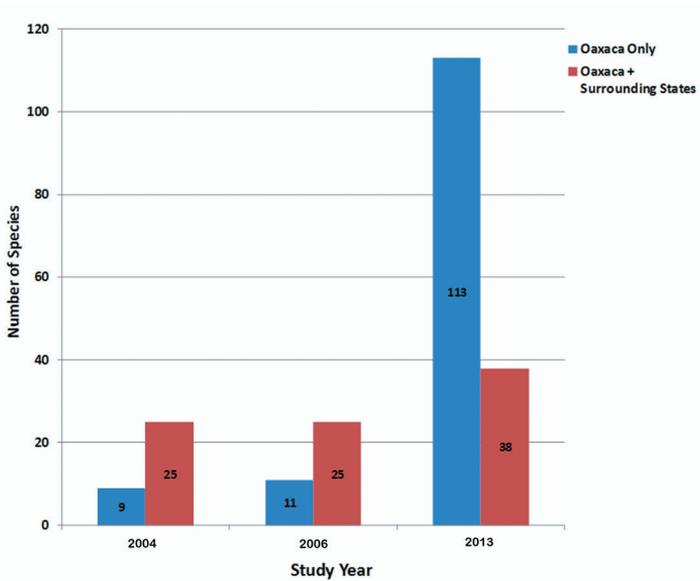


Figure 5. A comparison of the number of endemic species of Alticinae from Oaxaca only and from Oaxaca plus the surrounding states as recorded in Furth (2004, 2006) and the current study.

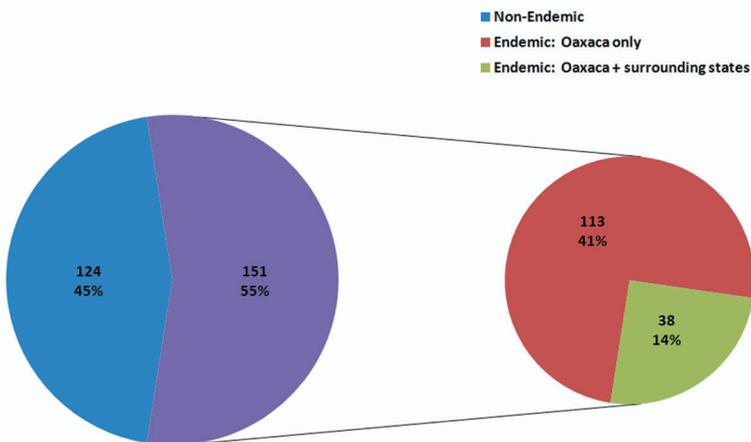


Figure 6. Endemic and non-endemic species numbers and percentages as recorded from the current study.

aca as well as the surrounding states (those bordering Oaxaca, plus Tabasco) then the endemic species from the 2010 data is a less dramatic increase from Furth (2004, 2006) or 25 to 38 – an increase of 52% (Figure 5). This means that of the 275 species recorded from the 2010 fieldwork from Oaxaca, 155 (55%) species are endemic at

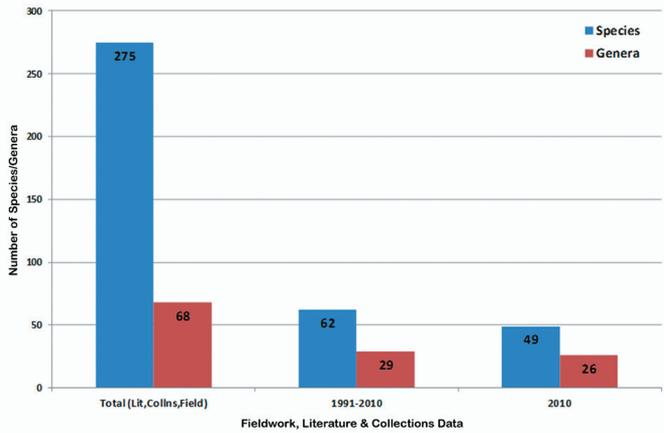


Figure 7. A comparison of the numbers of species/genera recorded from total evidence (literature, collections, author fieldwork), from all fieldwork (1991, 1997, 2010), and from the 2010 field trip alone.

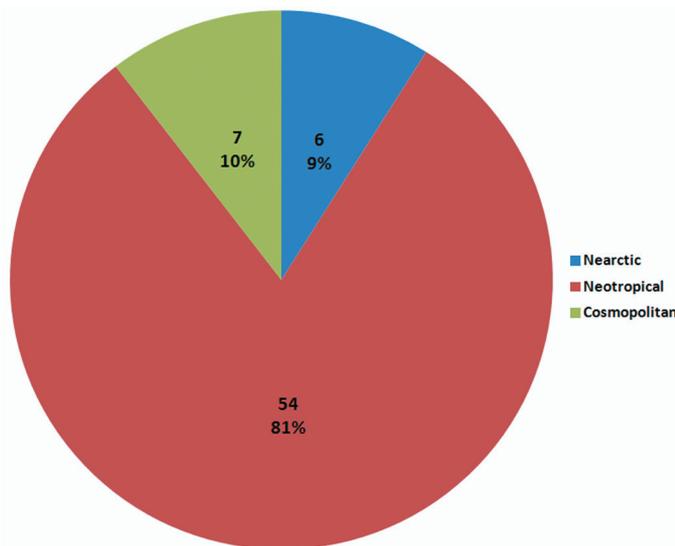


Figure 8. The biogeographic affinity of Alticinae genera of Oaxaca. Cosmopolitan genera are those found in several biogeographic regions.

some level; 41% are restricted endemics known only from Oaxaca and 14% are more broadly endemic; known also from surrounding states (Figure 6).

Figure 7 shows the numbers of species (62) and genera (29) collected by the author during fieldwork from different trips to Oaxaca (1991, 1997, 2010). Of these the 2010 collecting trip alone resulted in 49 species and 26 genera. The 1991 collecting trip was 5 days, the 1997 trip 2 days, and the 2010 trip 7 days. Thus, the 2010 trip alone produced 79% of the species and 90% of the genera (Figure 7).

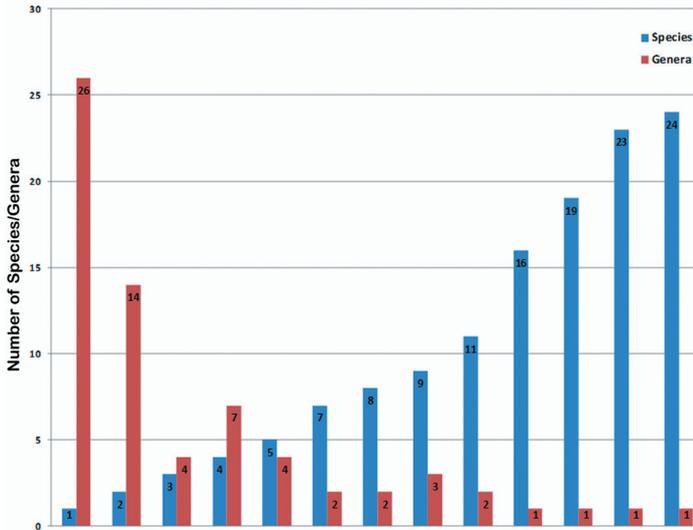


Figure 9. The number of species per genus of recorded Oaxacan Alticinae.

Examining the biogeographical affinities of the Alticinae diversity of Oaxaca at the generic level, i. e., the biogeographic affinities of the 68 genera recorded, there are 6 (9%) genera of Nearctic affinity, 7 (almost 10%) of Cosmopolitan affinity, and 54 (81%) of Neotropical affinity (Figure 8).

Another way to look at the diversity of the Oaxacan Alticinae is to examine the number of species per genus. As shown in Figure 9 of the 68 genera recorded from Oaxaca there is a high number of genera (26) with only one species and a high number of species (16 + 19 + 23 + 24) or 82 from only one genus, with a trend towards more species from fewer genera.

Figure 10 illustrates a few representatives of Alticinae genera and species that demonstrate presumed endemism and significant affinities of the biogeographical elements and distributional extensions in Oaxaca. *Sphaeronychus* OM sp. 2. (Figure 10A) represents one of two probable new species in a genus known from Brazil (25 species), one each from Ecuador and Peru, and only 2 known species from Central America. *Allochroma* OM sp. 1 (Figure 10B) is a probably new species representing a Neotropical genus with 11 known species from Mexico (Furth 2006), another 18 from Central America, and about 7 from South America. *Deuteraltica* OM sp. 1 (Figure 10C) is an undescribed species of a monotypic genus only known from Mexico, Guatemala, and El Salvador (Furth and Savini 1996). *Hypolampsis* OM sp. 2 (Figure 10D) is a probably new species of a very large genus (possibly the largest Neotropical genus of Alticinae) with only 4 known species from Mexico (Furth 2006), another 15 known from elsewhere in Central America (Furth and Savini 1996), and more than 60 from South America. *Disonycha nigrita* Jacoby (Figure 10E) is new to Mexico from the south, previously known only from Guatemala and El Salvador. *Trichaltica zapotensis*

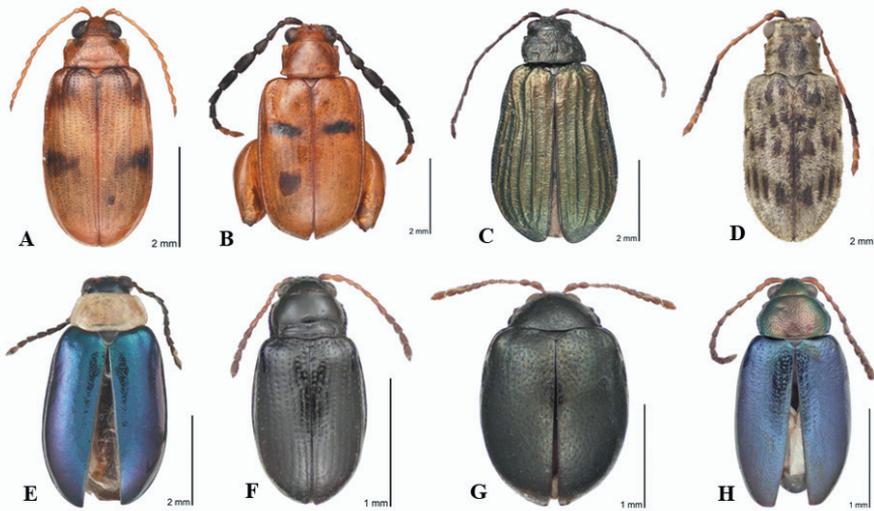


Figure 10. Examples of newly recorded Alticinae from the current study: **A** *Sphaeronychus* OM sp. 2 **B** *Allochroma* OM sp. 1 **C** *Deuteraltica* OM sp.1 **D** *Hypolampsis* OM sp. 2 **E** *Disonycha nigrita* **F** *Trichaltica zapotensis* **G** New Genus **H** *Phyllotreta aeneipennis*.

(Jacoby) (Figure 10F) is new to Mexico from the south, only previously only known from Guatemala and originally described as a species of *Crepidodera*. New Genus OM A (Figure 10G) is almost certainly a new genus probably of Neotropical affinity. *Phyllotreta aeneicollis* Crotch (Figure 10H) is a Nearctic element, new to Mexico from the north, previously only known from southeastern, south central, southwestern USA.

Discussion

As indicated in the Introduction and evident from Maps 1–4, Mexico is geographically positioned rather uniquely between North America and South America and with a large diversity of landscapes, climates, and microhabitats; these are reflected in its diversity of flora and fauna. The southern state of Oaxaca is an interesting reflection of this diverse Mexican geography and its habitats with its own set of special features.

The data in Furth (2004, 2006) were compiled primarily from researching the historical and more recent literature as well as from searching and determination of a few collections (i. e., the USNM, MCZ, and NHMB). The author's previous studies of Alticinae diversity of Mexico were published (2004, 2006) and were based on the literature and examination of primarily two research collections. The current study resulted from more extensive examination of collections from a variety of institutional research collections and a single, brief field trip to one area of Oaxaca. This multi-faceted strategy of reviewing the literature, then searching and examining historical research collections at a larger variety of institutions, as well as increased fieldwork is



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Figure 11. View of the cloud forest environment of Sierra de Juárez mountains from the Universidad de la Sierra Juárez campus.



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Figure 12. A view of typical Bromeliad-dominant cloud forest (many trees covered by *Tillandsia usneoides* (Linnaeus) (L.) Bromeliaceae) around Santa Catarina Lachatao with some of the 2010 collecting team (right to left: Jose Arturo Casasola, Atilano Contreras-Ramos, a local guide, Diana X. Munn).

demonstrated well by the current study. Primarily as a result of the rather brief 2010 fieldwork coupled with the study of at least eight additional institutional collections the number of species known from all of Mexico as well as from Oaxaca increased dramatically, 19% and 79%, respectively. After all examination of historical collections or “indoor collecting”, as it is sometimes called, is the result of many different collecting events (and methods) over many years by different collectors. Also targeted fieldwork by an expert produces significant increase in the known fauna in a relatively short time. The efficacy of the combination of these two aspects (examining new collections and new collecting) is demonstrated by the significant increase in Oaxacan Alticinae diversity by 79% for species and 84% for genera.

The three expert field collecting trips by the author in 1991, 1997, and 2010 were of different lengths and, in the case of 1991, at somewhat different seasons. In each case at least one day was spent collecting in the general vicinity of Oaxaca City, but the 1991 and 2010 field trips overlapped considerably geographically. Therefore, the increase in recorded Alticinae diversity for Oaxaca is due to the addition of a significant number of institutional collections examined as well as the intensive 7 days of fieldwork in 2010.

As mentioned above in the Introduction several previous studies of various members of the flora and fauna have demonstrated the high levels of endemism in southern Mexico, especially in Oaxaca. Again, the current study with its increased examination of institutional collections and additional targeted field collecting demonstrated a very large increase (almost 10 times) in apparent endemic species when limited to those only recorded from the state of Oaxaca. Of course, some of this is the result of the fact that many of these species could not be determined to species; therefore, only recorded as Oaxaca, and may either be new to science or previously rarely collected and they may in fact have somewhat broader distribution outside Oaxaca. However, when endemism is extended to the states directly surrounding Oaxaca, a more conservative and probably more realistic demonstration of Oaxacan Alticinae species endemism is revealed of 55%. Of these 41% (113 species) are currently known to be restricted to the state of Oaxaca and 14% (38 species) are known from Oaxaca and the surrounding states.

As mentioned above, one of the objectives of this study is to demonstrate how a variety of research strategies provides a comprehensive account of the diversity for particular region through a combination of researching historical literature, examination of historical collections, and fieldwork. Figure 7 illustrates this on the left-hand histogram through the total results of this study of Oaxacan species and genera of Alticinae. However, to demonstrate the effectiveness of targeted expert fieldwork the middle histogram bars show the 1991 (5 days), 1997 (2 days) and 2010 fieldwork by the author combined and those on the right-hand show the Alticinae diversity captured only for the more extensive (7 day) trip in 2010. The 2010 fieldwork produced 79% of the species and 90% of the genera collected during the author’s fieldwork. However, this may also reveal something about seasonality for collecting Alticinae in Oaxaca; that is, it is best earlier in the season (July rather than August), especially because the majority of collecting in 1991 was in the Sierra de Juárez, like in 2010.



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Figure 13. Entrance to the Instituto de Biología (UNAM) where the Mexican National Insect Collection is housed.



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Figure 14. Compactors of the Mexican National Collection (UNAM, IB).

As demonstrated in Figure 9 it is interesting to review the *Alicinae* taxa of Oaxaca and to note how many species are represented in each genus. For 26 genera (38%) there is only a single species known, whereas there is one genus that has 24 species (9%) and 82 species



Figure 15. Guelaguetza festival, 2010, in Oaxaca City, performers of the indigenous ethnic group pictured here are from the Pinotepa Nacional people from southern Oaxaca.

(30%) in 4 genera (6%) are represented by single genera. At this time it is not evident the exact cause of this, yet it is still of interest to see this U-shaped curve of species to genera.

Biogeographically it is not surprising that 81% of the genera of Alticinae in Oaxaca show a Neotropical affinity. Other Coleoptera groups also show a strong Neotropical affinity in Mexico overall such as for the species of Curculionidae (41%) (Anderson and O'Brien 1996) and Carabidae at the generic level (40%) are Neotropical (Ball and Shpeley 2000). The geographic position of the state of Oaxaca that includes the extreme “bottleneck” like constriction of the relatively flat Isthmus of Tehuantepec is apparently very important biogeographically and apparently even serves as a kind of transition zone between the more southern Neotropical fauna and the more northern Nearctic fauna. It is probably here that the strong Neotropical influence begins to filter northwards as indicated in Furth (2004) within the southern, more tropical climates of Veracruz and Guerrero. As reported in Furth (2006) the high species diversity in all of Mexico generally is in the southern states of Veracruz (198), Guerrero (141), Oaxaca (122), Chiapas (81), and Tabasco (81). This diversity is certainly influenced strongly by the Neotropical affinities of the taxa. As a result of the current study Oaxaca has jumped to first place among Mexican states as the most Alticinae-diverse, with 275 species – a combination of more extensive examination of collections and the 2010 fieldwork.

In this particular study the 2010 expert fieldwork was done only in one relatively small area of this large tropical state (Oaxaca), i. e., Sierra de Juárez. Given the fact that Oaxaca has many other kinds of habitats and geography (see Maps) one would expect the actual Alticinae diversity to be significantly greater. When other areas of Oaxaca are sampled and even more research collections examined this fact will certainly be realized.

The flora and fauna of Oaxaca is truly diverse demonstrated here by the Alticinae, but the people and culture of Oaxaca is also especially diverse and endemic as can be experienced in the annual festival celebrating this cultural diversity – the Guelaguetza (Figure 15).

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Appendix

List of genera and species names, species authors for Alticinae currently known from Oaxaca. Also listed in the columns are the known distribution in Mexican states outside of Oaxaca (see list below) for these standard state abbreviations), the sources for any records found by the author in various institutional collections (see Methods for abbreviations), and records from the author’s fieldwork indicated as DGF1991, DGF1997, DGF2010. OM sp.1 indicates a morphospecies name (one that cannot currently be associated with any already described species) found by the author in Oaxaca, Mexico, i.e., OM. Taxon names with a “?” afterwards have some question as to the correct determination of this species. A species with a “?” after the state acronym means that there is some question as to the certainty of the locality from that state. Author names for genera can be found in Furth and Savini (1996, 1998). The references for this Appendix are listed separately.

Taxon	Distribution	Literature source	Collections source	Author fieldwork
<i>Acallepitrix</i> OM sp. 1			UCB	
<i>Acanthonycha</i> OM sp. 1			UCB	
<i>Acrocyum sallei</i> Jacoby	OAX	Jacoby 1885		
<i>Alagoasa acutangula</i> (Jacoby)	CHIS, COL, DGO, GRO, JAL, MEX, MOR, NL, OAX, VER	Jacoby 1886	MCZ, USNM, UCB	
<i>Alagoasa bipunctata</i> (Chevrolat)	CHIS, DF, OAX, SLP, VER, YUC	Jacoby 1886	MCZ, USNM, UCB	
<i>Alagoasa ceracollis</i> (Say)	CHIS, DGO, MOR, OAX, VER	Jacoby 1885	USNM	
<i>Alagoasa chevrolati</i> (Baly)	OAX, VER, YUC	Furth and Savini 1996	MCZ	
<i>Alagoasa chypeata</i> (Jacoby)	CHIS, DGO, HGO, MICH, OAX, TAB, VER	Jacoby 1892	USNM, UCB	
<i>Alagoasa decemguttatus</i> (Fabricius)	CHIH, CHIS, COL, DGO, GRO, JAL, MEX, MOR, NAY, OAX, QROO, SON, TAB, VER	Jacoby 1886, Pal-lister 1953	MCZ, USNM, UCB, UCD, BYU	
<i>Alagoasa extrema</i> (Harold)	MOR, OAX, TAB, VER	Jacoby 1886	MCZ, USNM	
<i>Alagoasa fimbriata</i> (Forster)	GRO, MICH, MOR, OAX	Jacoby 1886	MCZ, USNM	
<i>Alagoasa hoegei</i> (Jacoby)	OAX, VER	Jacoby 1886		
<i>Alagoasa infirma</i> (Jacoby)	OAX, VER	Jacoby 1886		
<i>Alagoasa lateralis</i> (Jacoby)	COL, GRO, JAL, MEX, MICH, MOR, NAY, OAX	Jacoby 1886	MCZ, USNM	
<i>Alagoasa longicollis</i> (Jacoby)	OAX	Jacoby 1886		
<i>Alagoasa seriata</i> (Baly)	GRO, MOR, OAX, PUE, VER	Jacoby 1886	MCZ, NHMB, UCB	
<i>Alagoasa tehucana</i> Bechyne	JAL, PUE	Bechyne 1955	USNM, BYU, CAS, UCB	
<i>Alagoasa violaceomarginata</i> (Jacoby)	OAX	Jacoby 1886		

Taxon	Distribution	Literature source	Collections source	Author fieldwork
<i>Alagoasa virgata</i> (Harold)	CHIH, CHIS, COL, DGO, GRO, JAL, MEX, MOR, NAY, PUE, OAX, SIN, SLP, TAB, VER	Jacoby 1886, Pallister 1953	MCZ, USNM, NHMB	
<i>Alagoasa</i> OM sp. 1			UCB	
<i>Alagoasa</i> OM sp. 2			UCB, UCD	
<i>Alagoasa</i> OM sp. 3			UCB	
<i>Allochroma balyi</i> Clark	OAX	Jacoby 1886	BYU	
<i>Allochroma godmani</i> Jacoby	OAX, VER	Jacoby 1886		
<i>Allochroma hoegei</i> Jacoby	DGO, OAX, VER	Jacoby 1886	MCZ	
<i>Allochroma semipunctatum</i> Jacoby	OAX	Jacoby 1886		
<i>Allochroma</i> OM sp. 1			BYU	
<i>Altica bimarginata</i> (Say)	DGO, GRO, OAX, VER	Jacoby 1884	MCZ	
<i>Altica patruelis</i> Harold	DF, GRO, GTO, MEX, MICH, OAX ?, PUE, TAB, VER	Jacoby 1884	MCZ, USNM	
<i>Altica rugicollis</i> Jacoby	CHIH, OAX	Jacoby 1884	MCZ	
<i>Altica</i> OM sp. 1			UCB	
<i>Asphaera abdominalis</i> (Chevrolat)	AGS, CHIH, CHIS, COAH, COL, DF, DGO, GRO, GTO, HGO, JAL, MEX, MICH, MOR, NL, OAX, SIN, SLP, TAMPS, VER, ZAC	Jacoby 1885, Jacoby 1892, Pallister 1953	MCZ, USNM, CAS, UCB	
<i>Asphaera abdominalis</i> var.			UCB	
<i>Asphaera cyanopsis</i> Harold	DF, DGO, OAX, SLP, TAB, VER	Jacoby 1885	MCZ, USNM	
<i>Asphaera icteridera</i> (Harold)	CHIS, DGO, GRO, MOR, OAX, VER	Jacoby 1885, Pallister 1953	USNM	DGF 2010
<i>Asphaera mexicana</i> (Harold)	CHIS, DGO, GRO, MICH, MOR, NAY, OAX, VER	Jacoby 1886	MCZ, USNM, UCB, UCD	
<i>Asphaera polita</i> Jacoby	OAX, TAB, VER	Jacoby 1885	MCZ	
<i>Asphaera reichei</i> (Harold)	CHIS, DF, OAX, SLP, VER	Jacoby 1885	USNM	
<i>Asphaera</i> OM sp. 1			AMNH	
<i>Asphaera</i> OM sp. 2			USNM	
<i>Blepharida bryanti</i> Furth	CHIS, OAX	Furth 1998		
<i>Blepharida flavocostata</i> Jacoby	GRO, MEX, MICH, MOR, OAX, PUE	Furth 1998		
<i>Blepharida godmani</i> Jacoby	CHIS, OAX, VER	Furth 1998		
<i>Blepharida melanoptera</i> (Fall)	MICH, OAX, SON	Furth 1998		
<i>Blepharida mexicana</i> Jacoby	OAX, VER	Furth 1998		
<i>Blepharida punctatissima</i> Jacoby	CHIS, OAX, VER	Furth 1998		

Taxon	Distribution	Literature source	Collections source	Author fieldwork
<i>Blepharida quatuordecimpunctata</i> Jacoby	CHIS, OAX, VER	Furth 1998		
<i>Blepharida rhois</i> (Forster)	CHIH, COAH, DGO, GRO, HGO, NL, OAX, PUE, QRO, SLP, TAMP	Furth 1998		
<i>Blepharida trifasciata</i> Jacoby	OAX	Furth 1998		
<i>Blepharida unami</i> Furth	OAX, PUE	Furth 1998		
<i>Blepharida verdea</i> Furth	GRO, MOR, OAX	Furth 1998		
<i>Cacoscelis flava</i> Clark	OAX, TAMP	Jacoby 1884	USNM	
<i>Cacoscelis varians</i> (Jacoby)	OAX, TAB, VER	Jacoby 1891		
<i>Capraitia conspurcata</i> (Jacoby)	CHIS, DF, DGO, GRO, GTO, HGO, MEX, MICH, MOR, OAX, PUE, VER	Jacoby 1886	MCZ, USNM	DGF 2010
<i>Capraitia maculata</i> (Harold)	CHIS, GRO, JAL, MEX, MOR, OAX, VER, YUC	Jacoby 1886	MCZ	
<i>Centralaphthona fulvipennis</i> ? Jacoby	VER ?		MCZ, UCB	
<i>Centralaphthona mexicana</i> Jacoby	COAH, DGO, GRO	Jacoby 1885, Jacoby 1891	MCZ: BYU	
<i>Centralaphthona obscuripennis</i> (Jacoby)	GRO, MOR		USNM, CAS	DGF 1991, DGF 2010
<i>Centralaphthona semipunctata</i> Jacoby	JAL, VER	Jacoby 1891	MCZ, UCB	DGF 1991
<i>Chaetocnema balyi</i> Jacoby	COAH, DF	Jacoby 1892	MCZ, CAS, CDEFA, BYU, UCB	
<i>Chaetocnema capitata</i> Jacoby	DGO, GTO	Jacoby 1885	MCZ	DGF 2010, DGF 1991
<i>Chaetocnema cephalotes</i> Jacoby	PUE, SIN		NHMB, AMNH, UCB	
<i>Chaetocnema confinis</i> Crotch	DF		USNM, BYU, CDEFA	
<i>Chaetocnema fulvicornis</i> Jacoby	DGO, GRO, GTO	Jacoby 1885		DGF 2010
<i>Chaetocnema fulvilabris</i> Jacoby	GRO, MOR, VER	Jacoby 1892	UCB, USNM	
<i>Chaetocnema minuta</i> Melsheimer			CAS, UCB	DGF 2010
<i>Chaetocnema</i> OM sp. 1				DGF 2010
<i>Chaetocnema</i> OM sp. 2			BYU	
<i>Chaetocnema</i> OM sp. 3				DGF 2010
<i>Chaetocnema</i> OM sp. 4			BYU	
<i>Chrysogramma septempunctata</i> Jacoby	DGO, MOR, OAX, PUE	Furth and Savini 1996	USNM	

Taxon	Distribution	Literature source	Collections source	Author fieldwork
<i>Chrysogramma trifasciata</i> Jacoby	OAX	Jacoby 1891		
<i>Cyrsylus recticollis</i> Jacoby	CHIS, TAB, VER	Jacoby 1892	USNM, TAMU	
<i>Deuteraltica longicornis</i> (Jacoby)	CHIS		USNM, TAMU	
<i>Deuteraltica</i> OM sp. 1			TAMU	
<i>Dibolia championi</i> Jacoby	OAX, VER	Parry 1974	USNM	
<i>Dinaltica</i> OM sp. 1			BYU	
<i>Dinaltica</i> OM sp. 2			USNM	
<i>Dinaltica</i> OM sp. 3			BYU	DGF 2010
<i>Dinaltica</i> OM sp. 4				DGF 2010
<i>Diphaltica nitida</i> (Jacoby)	CHIS, DF, DGO, MICH, OAX, TAB, VER	Jacoby 1884	MCZ, USNM	
<i>Diphaltica</i> OM sp. 1			CAS	
<i>Diphaulaca aulica</i> (Olivier)			UCB	DGF 1997
<i>Diphaulaca aulica cordobae</i> Barber	CHIS, GRO, GTO, HGO, JAL, MEX, MICH, MOR, NAY, OAX, PUE, QROO, SLP?, TAB, TAMP, VER, YUC	Jacoby 1884	MCZ, USNM, BYU, UCB	
<i>Diphaulaca wagneri</i> Harold	CHIS, GRO, OAX, YUC	Barber 1941	NHMB	DGF 2010
<i>Disonycha discoidea abbreviata</i> Melsheimer	DGO, MEX, MOR, OAX	Jacoby 1884	MCZ	
<i>Disonycha antennata</i> Jacoby	COL, DGO, GRO, JAL, MEX, MICH, MOR, OAX, VER	Jacoby 1884, Blake 1955	USNM	
<i>Disonycha brevilineata</i> Jacoby	DGO, GRO, JAL, MOR, OAX	Jacoby 1884, Jacoby 1902, Blake 1955	MCZ, CAS	
<i>Disonycha brunneofasciata</i> Jacoby	GRO, PUE, SLP	Blake 1955	USNM, UCB	
<i>Disonycha caroliniana</i> (Fabricius)	DGO, NL, OAX, SIN, VER	Jacoby 1884	USNM	
<i>Disonycha collata</i> (Fabricius)	CHIH, COAH, DF, DGO, GTO, JAL, MEX, MICH, MOR, OAX, PUE, TAB, VER, YUC	Jacoby 1884, Pallister 1953	MCZ, USNM	
<i>Disonycha dorsata</i> Harold	MOR, OAX, TAB, VER, YUC	Jacoby 1884	MCZ, USNM, BYU	
<i>Disonycha figurata</i> Jacoby	AGS, CHIH, CHIS, COAH, COL, DF, DGO, GRO, GTO, JAL, MEX, MICH, MOR, NAY, OAX, SIN, TAB, VER, YUC	Jacoby 1884, Pallister 1953, Blake 1955	MCZ, USNM, NHMB, UCB	

Taxon	Distribution	Literature source	Collections source	Author fieldwork
<i>Disonycha fumata fumata</i> LeConte	BC, CHIH, CHIS, DGO, GRO, HGO, JAL, MEX, MICH, MOR, NL, OAX, PUE, SLP, SON, TAB, VER, ZAC	Blake 1955	USNM	
<i>Disonycha glabrata</i> (Fabricius)	BC, BCS, CAMP, CHIS, COL, DGO, GRO, JAL, MOR, NAY, OAX, PUE, SIN, SON, TAB, TAMPS, YUC, VER	Jacoby 1884, Blake 1955	MCZ, USNM, CAS, UCB, UCD	DGF 2010
<i>Disonycha guatemalensis</i> Jacoby	CHIS, GRO, MOR, OAX, VER?	Blake 1955	USNM	DGF 2010
<i>Disonycha boegei</i> Jacoby	VER, OAX	Jacoby 1884		
<i>Disonycha leptolineata</i> <i>texana</i> Schaeffer	DGO, GRO, JAL, MOR, NL, OAX, QROO, TAMPS, YUC	Blake 1955	USNM, CAS	
<i>Disonycha maculipes</i> Jacoby	CHIS, VER	Jacoby 1891	USNM, AMNH, CAS, UCB	
<i>Disonycha militaris</i> Jacoby	TAB, VER, YUC	Jacoby 1884	USNM, UCB	
<i>Disonycha nigrita</i> Jacoby			UCB	DGF 2010
<i>Disonycha pluriligata</i> LeConte	BC, CHIH, DGO, JAL, NAY, SIN, SLP, SON, VER	Furth and Savini 1996	MCZ, UCB	
<i>Disonycha politula</i> Horn	AGS, CAMP, CHIH, DF, DGO, GRO, GTO, HGO, JAL, MEX, MOR, OAX, PUE, QRO, SLP, SON, TAMPS, VER, ZAC	Jacoby 1891, Pallister 1953	MCZ, USNM	
<i>Disonycha quinquelineata</i> (Latreille)	CHIS, COL, GRO, OAX, QROO, TAB, TAMPS, VER	Jacoby 1884, Blake 1955	MCZ, USNM	
<i>Disonycha scriptipennis</i> (Jacoby)	CHIS, COL, DGO, GRO, MOR, NAY, OAX, YUC	Jacoby 1891	USNM, NHMB	
<i>Disonycha subaenea</i> Jacoby	DGO, GRO, MOR, OAX	Jacoby 1884	MCZ, USNM	
<i>Disonycha teapensis</i> Blake	OAX, SLP, TAB	Blake, 1955	NHMB	
<i>Disonycha</i> OM sp. 1			C DFA	
<i>Distigmoptera suturalis</i> (Jacoby)	GRO, OAX	Jacoby 1892	NHMB	
<i>Dysphenges</i> OM sp. 1				DGF 2010
<i>Egleraltrica</i> OM sp. 1			BYU, UCB	
<i>Epitrix cucumeris</i> (Harris)	DGO, GRO, MOR, PUE, VER	Jacoby 1891	MCZ, USNM, ZSMC, C DFA, UCB	DGF 1991, DGF 2010
<i>Epitrix fasciata</i> Blatchley	CHIH, DGO, NL, TAMPS	Maes and Staines 1991	USNM	DGF 2010

Taxon	Distribution	Literature source	Collections source	Author fieldwork
<i>Epitrix robusta</i> Jacoby	GRO	Jacoby 1891		DGF 2010, DGF 1997
<i>Epitrix rufula</i> Weise	DE, GRO, MOR	Jacoby 1891	USNM, UCB	DGF 2010
<i>Epitrix</i> OM sp.1			UCB	
<i>Epitrix</i> OM sp.2				DGF 2010
<i>Epitrix</i> OM sp.3			CDFA	DGF 2010
<i>Epitrix</i> OM sp.4				DGF 2010
<i>Genaphthona transversicollis</i> (Jacoby)	CHIS, JAL, OAX, PUE		USNM, BYU, CDFA, UCB	DGF 1997
<i>Glenidion flexicaulis</i> Schaeffer	TAMPS, YUC		USNM, TAMU	
<i>Glyptina nivialis</i> Horn	MOR		USNM	DGF 2010
<i>Heikertingerella</i> OM sp. 1				DGF 2010
<i>Heikertingerella</i> OM sp. 2			BYU	
<i>Heikertingerella</i> OM sp. 3			UCB	
<i>Heikertingerella</i> OM sp. 4			UCB	
<i>Hemiphyrnus elongatus</i> Jacoby	OAX, TLAX, VER	Jacoby 1884		
<i>Hemiphyrnus sulcatipennis</i> (Jacoby)	GRO, MEX, OAX	Jacoby 1891	NHMB, BMNH	
<i>Hemiphyrnus sydneyae</i> Gilbert & Andrews			BYU	
<i>Hemiphyrnus tenuicornis</i> Jacoby	HGO, OAX	Jacoby 1891	MCZ	
<i>Hypolampsis</i> OM sp. 1			UCB	
<i>Hypolampsis</i> OM sp. 2			BYU, USNM	
<i>Hypolampsis</i> OM sp. 3			BYU	
<i>Hypolampsis</i> OM sp. 4			BYU	
<i>Hypolampsis</i> OM sp. 5			USNM	
<i>Iphitroides nigrocinctus</i> Jacoby	GRO	Jacoby 1891	CAS	
<i>Kuschelina laeta</i> (Perbosc)	TAMPS, VER	Heikertinger and Csiki 1940	MCZ, USNM, UCB	
<i>Kuschelina modesta</i> (Jacoby)	CHIH, CHIS, DE, DGO, GRO, GTO, HGO, MEX, MOR, OAX, PUE, SLP, TLAX, VER	Jacoby 1886, Pal- lister 1953	MCZ, USNM	DGF 2010
<i>Leptophysa hirtipennis</i> (Jacoby)	OAX, VER		USNM	
<i>Longitarsus columbicus</i> ? Harold	GRO		MCZ	DGF 2010
<i>Longitarsus mexicanus</i> Csiki	DE, DGO, GRO, GTO, HGO, MEX, MICH, MOR, PUE	Jacoby 1891	MCZ, NHMB, USNM, UCB	DGF 1997
<i>Longitarsus varicornis</i> Suffrian	TAB, VER	Jacoby 1885, Jacoby 1891	UCB	
<i>Longitarsus</i> OM sp. 1			BYU	

Taxon	Distribution	Literature source	Collections source	Author fieldwork
<i>Longitarsus</i> OM sp. 2			UCB	DGF 1997
<i>Longitarsus</i> OM sp. 3			CAS	
<i>Longitarsus</i> OM sp. 4			UCB	
<i>Longitarsus</i> OM sp. 5			UCB	
<i>Longitarsus</i> OM sp. 6			USNM	
<i>Luperaltica longicornis</i> (Jacoby)	CHIS, COL?, MOR?, OAX?		USNM	
<i>Luperaltica sylvia</i> (Bechyne & Bechyne)			USNM, UCB	DGF 1997, DGF 2010
<i>Luperaltica viridipennis</i> (Jacoby)	OAX	Jacoby 1884		
<i>Luperaltica</i> OM sp. 1			USNM	
<i>Luperaltica</i> OM sp. 2			BYU	
<i>Luperaltica</i> OM sp. 3			BYU, USNM	
<i>Luperaltica</i> OM sp. 4			BYU	
<i>Lupraea frontalis</i> (Jacoby)	OAX	Jacoby 1885	USNM	
<i>Lupraea fulvicollis</i> ? Jacoby	VER		MCZ	DGF 2010
<i>Lupraea guatemalensis</i> (Jacoby)	CHIS, GRO, MOR, VER	Jacoby 1891	BYU, CDFA, USNM, UCB	
<i>Lupraea semifulva</i> (Jacoby)	CHIS, OAX		USNM	
<i>Lupraea smithi</i> (Jacoby)	GRO, MOR	Jacoby 1891	USNM	DGF 2010
<i>Lupraea</i> OM sp. 1			BYU	
<i>Lupraea</i> OM sp. 2			UCB	
<i>Lupraea</i> OM sp. 3			UCD	
<i>Lysathia jacobyi</i> (Csiki)	DF, GTO, OAX, TAB, ZAC	Jacoby 1891	USNM	
<i>Lysathia occidentalis</i> (Suf-frian)	YUC		ZSMC, UCB	
<i>Macrohaltica patruelis</i> (Harold)	DF, DGO, GTO, MEX, MICH, MOR, OAX, PUE, VER	Jacoby 1884	USNM	
<i>Macrohaltica</i> OM sp. 1			UCB	
<i>Margaridisa managua</i> ? (Bechyné)	DGO, SLP		USNM	DGF 2010
<i>Monomacra cupreata</i> (Jacoby)	OAX	Jacoby 1891		
<i>Monomacra hoegei</i> (Jacoby)	OAX, VER	Jacoby 1884		
<i>Monomacra mexicana</i> (Jacoby)	OAX, VER	Jacoby 1884		
<i>Monomacra tibialis</i> (Olivier)	OAX		USNM	
<i>Monomacra violacea</i> (Jacoby)	CHIS, VER		USNM, BYU, CAS, UCB	DGF 2010
<i>Monomacra</i> OM sp. 1			UCB	
<i>Monomacra</i> OM sp. 2			USNM	
<i>Neothona</i> sp.	JAL, MICH, OAX, VER		USNM	
<i>Neothona</i> OM sp. 1				DGF 2010

Taxon	Distribution	Literature source	Collections source	Author fieldwork
<i>Neothona</i> OM sp. 2			USNM, UCB	
<i>Nesaecrepida infuscata</i> (Schaeffer)	CAMP, COL, GRO, JAL, MICH, OAX, TAB, TAMPS, VER		USNM, ZSMC, UCB	
<i>Notozona histrionica</i> Baly	OAX, VER	Furth and Savini 1996	MCZ	
<i>Omophoita aequinoctialis aequinoctialis</i> (Linnaeus)	CHIS, HGO, MICH, OAX, QROO, SLP, TAB, TAMPS, VER	Furth and Savini 1996	USNM, AMNH, BMNH, UCB, UCD	
<i>Omophoita cinctipennis</i> (Chevrolat)	JAL, OAX, PUE, SLP, VER	Jacoby 1885	USNM	
<i>Omophoita octomaculata</i> (Crotch)	OAX, TAB, TAMPS, VER	Jacoby 1886	ZSMC	
<i>Omophoita quadrinotata centraliamericana</i> Bechyne	OAX, TAB, VER	Bechyne 1955	USNM, BMNH	
<i>Omophoita reticollis</i> (Baly)	CHIS, HGO, OAX, TAB, TAMPS, VER	Jacoby 1885, 1891	USNM	
<i>Palaeothona chiriquiensis</i> Jacoby				DGF 2010
<i>Palaeothona rubroviridis</i> Blake	DGO	Blake 1950		DGF 2010
<i>Palaeothona rugifrons</i> (Jacoby)	VER	Jacoby 1885	BYU, UCB, USNM	
<i>Palaeothona</i> OM sp. 1			CDFA	
<i>Palaeothona</i> OM sp. 2			USNM	
<i>Palaeothona</i> OM sp. 3			BYU	
<i>Palaeothona</i> OM sp. 4			BYU	
<i>Palaeothona</i> OM sp. 5			UCB	
<i>Palaeothona</i> OM sp. 6			BYU, CDFA	
<i>Pedilia inornata</i> (Jacoby)	OAX, VER	Duckett 1993 in litt.		
<i>Phrynocephala capitata</i> Jacoby	CHIS?, GRO, JAL, OAX, TAB?	Jacoby 1884	USNM, UCB	
<i>Phrynocephala deyrollei</i> Baly	AGS, CHIH, DGO, GRO, GTO, MICH, MOR, OAX, PUE, SLP ?	Jacoby 1884, Pal-lister 1953	USNM, UCB	DGF 2010
<i>Phrynocephala pulchella</i> Baly	CHIS, COL, DGO, GTO, JAL, MICH, MOR, OAX, VER	Jacoby 1884	USNM, NHMB	
<i>Phydania bicolor</i> Horn	OAX, TAMPS		USNM	
<i>Phydania nigriventris</i> Jacoby	GRO, OAX, SLP, SON	Jacoby 1891	USNM	DGF 2010
<i>Phyllotreta aeneicollis</i> Crotch				DGF 1997, DGF 2010
<i>Phyllotreta pusilla</i> Horn	AGS, BC?, CHIH, DE, DGO, HGO, MOR, OAX, ZAC	Chittenden 1923	USNM	DGF 1997

Taxon	Distribution	Literature source	Collections source	Author fieldwork
<i>Phyllotreta</i> OM sp. 1				DGF 2010
<i>Phyllotreta</i> OM sp. 2			UCB	
<i>Physimerus scabrosus</i> (Clark)	DGO, OAX, VER	Jacoby 1886	MCZ	
<i>Physimerus</i> OM sp. 1			CAS	
<i>Platiprosopus pallens</i> (Fabricius)	GRO, HGO, MOR, OAX, PUE, VER	Furth and Savini 1996	USNM	
<i>Plectrotetra clarki</i> Baly	DF, DGO, HGO, MOR, OAX, PUE, SIN, SLP, TAMPS, VER	Jacoby 1884	MCZ, USNM	
<i>Plectrotetra guatemalensis</i> Jacoby	MOR	Jacoby 1891	BMNH	
<i>Plectrotetra inaequalis</i> Jacoby	OAX, TAMPS, VER	Jacoby 1884	USNM	
<i>Plectrotetra multipunctata</i> Jacoby	DGO?, MEX, MOR, OAX, VER	Jacoby 1891	MCZ, USNM	
<i>Plectrotetra submetallica</i> Jacoby	OAX, VER	Jacoby 1884		
<i>Prasona viridis</i> Baly	VER	Jacoby 1886	USNM	
<i>Prasona</i> OM sp. 1			BYU, USNM	
<i>Propiasus fulvus</i> (Jacoby)	GRO	Jacoby 1892	USNM	
<i>Pseudorthygia nigritarsis</i> Jacoby	GRO, OAX, TAMPS	Jacoby 1891	USNM	DGF 2010
<i>Pylliodes convexior</i> LeConte	BCS	Horn 1895, Furth and Savini 1998		DGF 1997
<i>Resistenciana ornata</i> (Jacoby)	PUE, VER	Jacoby 1884	MCZ, BYU, USNM	
<i>Rhinotmetus modestus</i> Jacoby	GRO, MOR	Jacoby 1892	MCZ	DGF 1991
<i>Rhinotmetus</i> OM sp. 1			BYU	
<i>Rhinotmetus</i> OM sp. 2				DGF 1991
<i>Rhinotmetus</i> OM sp. 3				DGF 1991
<i>Scelidopsis rufofemorata</i> Jacoby	TAMPS, VER	Jacoby 1888	USNM, CAS	
<i>Sphaeronychus</i> OM sp. 1			BYU	
<i>Sphaeronychus</i> OM sp. 2			BYU, UCB, USNM	
<i>Stegnea</i> OM sp. 1			TAMU	
<i>Strabala rotunda</i> Blake	CHIS, COL, DF, GRO, JAL, NAY, NL, SLP, TAMPS, VER, YUC	Blake 1953	USNM, NHMB, ZSMC, UCB	
<i>Strabala rufa</i> Illiger	CHIS, COL, DGO, GRO, OAX, PUE, TAB, VER	Jacoby 1884, 1891		
<i>Syphrea burgessi</i> (Crotch)	MOR, OAX, TAMPS		USNM	
<i>Syphrea cyaneipennis</i> (Jacoby)	GRO, HGO, JAL, SLP, TAB, TAMPS	Jacoby 1891	USNM, BYU, CDFA	
<i>Syphrea flavicollis</i> (Jacoby)	BCS, GRO, GTO, JAL, MOR, OAX, PUE	Jacoby 1884, Riley, Clark and Gilbert 2001	MCZ, USNM	

Taxon	Distribution	Literature source	Collections source	Author fieldwork
<i>Syphrea parvula</i> (Jacoby)	JAL, TAB, VER, YUC	Jacoby 1891	USNM, BYU, UCB	
<i>Syphrea smithi</i> (Jacoby)	OAX, TAB, TAMPS	Jacoby 1891	USNM	
<i>Syphrea sublaevipennis</i> (Jacoby)	OAX, VER	Jacoby 1891	MCZ	
<i>Syphrea teapensis</i> (Jacoby)	OAX, SLP, TAB, VER	Jacoby 1891	USNM	
<i>Syphrea</i> OM sp. 1			BYU	DGF 2010
<i>Syphrea</i> OM sp. 2			BYU	
<i>Syphrea</i> OM sp. 3			CAS	
<i>Syphrea</i> OM sp. 4			BYU	
<i>Syphrea</i> OM sp. 5			BYU	
<i>Syphrea</i> OM sp. 6				DGF 2010
<i>Syphrea</i> OM sp. 7			USNM	
<i>Syphrea</i> OM sp. 8			BYU	
<i>Syphrea</i> OM sp. 9			TAMU, USNM	
<i>Systema abbreviata</i> Jacoby	PUE	Jacoby, 1902	CDEFA	
<i>Systema blanda</i> Melsheimer	BC?, CHIH, JAL, MICH, NL, SIN, SLP?, SON, TAB, VER	Pallister 1953	USNM, UCB	
<i>Systema championi</i> Jacoby	GRO, MOR, OAX, VER		USNM	
<i>Systema contigua</i> Jacoby	CHIS, GRO, GTO, HGO, OAX, SON?, TAMPS, VER?, ZAC	Jacoby 1884	USNM, CDEFA, UCB	DGF 2010
<i>Systema gracilentata</i> Blake	NL	Blake 1933, Furth and Savini 1998		DGF 2010
<i>Systema nigroplagiata</i> Jacoby	AGS, CHIH, DE, DGO, GTO, GRO, JAL, MICH, MOR, OAX, PUE, VER	Jacoby 1884, Pallister 1953	MCZ, USNM	DGF 2010
<i>Systema pectoralis</i> Clark	CHIS, GTO, OAX, VER	Jacoby 1884	MCZ	
<i>Systema puncticollis</i> Jacoby	OAX	Jacoby 1884		
<i>Systema s-littera</i> (Linnaeus)	CHIS, GTO, TAB, VER	Jacoby 1884	MCZ, USNM, UCB	
<i>Systema semivittata</i> Jacoby	BCS, GRO, GTO, HGO, MEX, MOR, NL, OAX, SIN	Jacoby 1884	MCZ, USNM, NHMB	DGF 2010
<i>Systema subcostata</i> Jacoby	MICH, MOR, VER	Jacoby 1884	USNM, CDEFA, UCB	DGF 2010
<i>Systema sulphurea</i> Jacoby	CHIH, DGO, GRO, MOR, OAX	Jacoby 1891	MCZ, USNM, BYU	DGF 1997
<i>Systema thoracica</i> Jacoby	CAMP, HGO, PUE, QROO, TAB, VER	Jacoby 1884	MCZ, USNM, UCB	
<i>Systema variabilis</i> Jacoby	CHIH, CHIS, COL, DGO, GRO, GTO, MICH, MOR, NAY, OAX, VER	Jacoby 1884, Pallister 1953	MCZ, USNM, ZSMC, UCB	
<i>Systema</i> OM sp. 1			UCB	DGF 2010

Taxon	Distribution	Literature source	Collections source	Author fieldwork
<i>Systema</i> OM sp. 2				DGF 2010
<i>Systema</i> OM sp. 3			UCB	
<i>Systema</i> OM sp. 4			UCB	
<i>Systema</i> OM sp. 5			UCB	
<i>Systema</i> OM sp. 6			UCB, USNM	
<i>Systema</i> OM sp. 7			UCB	
<i>Systema</i> OM sp. 8			UCB	
<i>Systema</i> OM sp. 9			BYU	
<i>Systema</i> OM sp. 10			UCB	
<i>Trichaltica zapotensis</i> (Jacoby)			BYU, CDFA, TAMU, UCB, USNM	
<i>Trichaltica</i> OM sp. 1			CDFA, TAMU	DGF 2010
<i>Walterianella inscripta</i> (Jacoby)	OAX, SLP, VER	Jacoby 1886	MCZ, USNM	
<i>Walterianella sublineata</i> (Jacoby)	OAX, TAB, VER, YUC	Jacoby 1886	MCZ, USNM, UCD	
<i>Walterianella</i> OM sp. 1			UCB	
New Genus A ? OM sp. 1			USNM	
New Genus B ? OM sp. 1			UCB	

Mexican States (Abbreviations):

Aguascalientes (AGS); Baja California (BC); Baja California Sur (BCS); Campeche (CAMP); Chiapas (CHIS); Chihuahua (CHIH); Coahuila (COAH); Colima (COL); Distrito Federal (DF); Durango (DGO); Guanajuato (GTO); Guerrero (GRO); Hidalgo (HGO); Jalisco (JAL); Mexico (MEX); Michoacan (MICH); Morelos (MOR); Nayarit (NAY); Nuevo Leon (NL); Oaxaca (OAX); Puebla (PUE); Queretaro (QRO); Quintana Roo (QROO); San Luis Potosi (SLP); Sinaloa (SIN); Sonora (SON); Tabasco (TAB); Tamaulipas (TAMPS); Tlaxcala (TLAX); Veracruz (VER); Yucatan (YUC); Zacatecas (ZAC).

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Review on the use of sexually dimorphic characters in the taxonomy of Diabroticites (Galerucinae, Luperini, Diabroticina)¹

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Abstract

Sexual dimorphism occurs frequently in Chrysomelidae Latreille, 1802 and is particularly variable in subfamily Galerucinae Latreille, 1802. This diversity has been early noted by authors a potential source of taxonomic characters. The section Diabroticites (Luperini Gistel, 1848) is one of the largest assemblies of chrysomelid genera with currently 823 valid species in 17 genera (12 based on dimorphic characteristics), being most diverse in the neotropical region. Apart from a revision work on the type specimens for the section, there are no general taxonomic studies for this group. The occurrence of sexually dimorphic characteristics in the section Diabroticites is revised and their practical taxonomic relevance evaluated. A total of 240 species was studied (145 species with males available), representing 15 out of the 17 genera included in Diabroticites. The analysis of characters was based on the study of specimens in south-american collections, literature and the aid of photos in online databases. Sexual dimorphism occurred in most species analyzed. Dimorphic features were divided in general (i. e., occur in higher taxa) and special characters (those that support the definition of species and genera). Special dimorphism was observed in every tagma, and most modifications occur in antennae. Characters used as diagnostic of genera often do not correspond to the modifications present in species included in them. Many modifications were considered by earlier authors as a single character, probably due to vague definitions. Most generic definitions are, therefore, inaccurate. The study of morphology and the homology assessment of characters are needed to increase understanding of the genera in Diabroticites.

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Keywords

Taxonomy of Coleoptera, rootworms, review, leaf-beetles

Introduction

Sexual dimorphism has always been a subject of great curiosity amongst naturalists. Since Darwin's suggestion of his Theory of Sexual Selection, many explanations have been proposed, and several have been successfully tested, for the existence of often peculiar modifications in males. Even though most beetles lack conspicuous sexual dimorphism (Kawano 2006), there are striking examples in all major Coleoptera taxonomic groups (Eberhard 2009). Most sexually dimorphic characters in beetles are described by strongly positive allometries (Kawano 2006), and also modifications found in antennae, tarsi, posterior legs and ventrites (Crowson 1981). Other less common characteristics include the reduction of the wings (Thayer 1992) and the presence of luminescent (Branham and Wenzel 2003) or stridulatory organs (Jansson and Selander 1977).

In Chrysomelidae Latreille, 1802, sexual dimorphism is thought to occur more frequently at the species level (Jolivet and Verma 2002). Common dimorphic features that are the body size (with females usually bigger than males) and the modification of tarsi, usually related to greater adhesion of males to the females' dorsal surface during copulation (Jolivet and Verma 2002, Hammack and French 2007, Voigt et al. 2008, Nardi et al. 2012).

Within the subfamily Galerucinae Latreille, 1802, sexual dimorphism is particularly variable, as Mohamedsaid and Furth (2011) have illustrated and summarized. This diversity has been early noted by authors as a potential source of taxonomic characters. As Horn (1893) pointed out, many taxonomic issues related to this group could be resolved with the aid of "sexual peculiarities", which could be a useful guide for understanding the relationships between species. Blake (1958) also stated that the use of such characteristics could help the delimitation of genera in problematic groups such as the tribe Luperini Gistel, 1848.

The section Diabroticites Chapuis, 1875 (Luperini) is one of the largest assemblies of chrysomelid genera with over 900 recorded names in 17 genera (12 based on dimorphic characteristics), being most diverse in the neotropical region. Apart from a revision work on the type specimens for the section (Smith and Lawrence 1967), there are no general taxonomic studies for this group. The most recent catalogue mentions 793 species (Wilcox 1972), but a review of the subsequent literature reveals that the group has currently 823 valid species. Table 1 presents an overview on the current composition of Diabroticites. Prior to 1906, Diabroticites included *Diabrotica* Chevrolat, 1837 (almost half of the total species in the section), and three other genera, which were all monotypic: *Ensiforma* Jacoby, 1876, *Pseudodiabrotica* Jacoby, 1892, and *Paratriarius* Schaeffer, 1906. Barber (1947) was the first to investigate male genital characters to understand the relationships within the group and described two more genera in the section, *Acalymma* Barber, 1947 and *Amphelasma* Barber, 1947. In the subsequent years, the remaining 11 genera were

Table 1. Overview on the composition of genera of *Diabroticites*. The total number of analyzed species includes specimens in collections and online type-specimens in MCZ database.

Genus	Dimorphism as diagnostic	Number of species in original description	Current number of species	Number of analysed species (% of genus total)
<i>Acalymma</i> Barber, 1947		6	72	28 (38%)
<i>Amphelasma</i> Barber, 1947		5	11	2 (18%)
<i>Anisobrotica</i> Bechyné & Bechyné, 1969	X	1	5	5 (100%)
<i>Aristobrotica</i> Bechyné, 1956	X	10	17	3 (17%)
<i>Buckibrotica</i> Bechyné & Bechyné, 1969	X	1	1	1 (100%)
<i>Cochabamba</i> Bechyné, 1955		4	10	10 (100%)
<i>Cornubrotica</i> Bechyné & Bechyné, 1969	X	1	2	2 (100%)
<i>Diabrotica</i> Chevrolat, 1837		103	360	114 (31%)
<i>Ensiforma</i> Jacoby, 1876	X	1	9	3 (33%)
<i>Gynandrobrotica</i> Bechyné, 1955	X	23	32	4 (12%)
<i>Isotes</i> Weise, 1922 (= <i>Synbrotica</i> Bechyné, 1956)	X	1(51)	182	38 (20%)
<i>Palmaria</i> Bechyné, 1956	X	1	1	-
<i>Paranapiacaba</i> Bechyné, 1958	X	16	59	14 (23%)
<i>Paratriarius</i> Schaeffer, 1906	X	1	51	11 (21%)
<i>Platybrotica</i> Cabrera & Cabrera Walsh, 2004	X	1	1	1 (100%)
<i>Pseudodiabrotica</i> Jacoby, 1892	X	1	1	-
<i>Zischkaiata</i> Bechyné, 1956		1	9	4 (44%)
Total	12	177	823	240 (28%)

erected, eight of which based on species formerly included in *Diabrotica* (*Anisobrotica* Bechyné & Bechyné, 1969, *Aristobrotica* Bechyné, 1956, *Buckibrotica* Bechyné & Bechyné, 1969, *Cochabamba* Bechyné, 1955, *Cornubrotica* Bechyné & Bechyné, 1969, *Gynandrobrotica* Bechyné, 1955, *Synbrotica* Bechyné, 1956, and *Paranapiacaba* Bechyné, 1958), and most supported primarily on dimorphic features (Table 1). Surprisingly enough, only one genus has a detailed description of genital characters (the most recent genus, *Platybrotica* Cabrera & Cabrera Walsh, 2004).

About 80% of the *diabroticites* species have been described prior to 1895, mainly by Joseph Sugar Baly, Charles J. Gahan and Martin Jacoby (Smith and Lawrence 1967). Most of those descriptions lack detailed morphological information, and usually depict characters relative to color pattern and, sometimes, punctuation. For many of the genera this is also true, with internal characters being almost completely ignored. General morphology has also been vaguely treated, described usually without any aid of illustrations whatsoever. Such scarcity of information and the apparent uniformity in morphology of some *diabroticites* resulted in a difficult taxonomic scenario.

The purpose of this study is to summarize the occurrence of sexually dimorphic characteristics in the section *Diabroticites*, as well as to review these characters chosen by earlier authors to support their definitions of genera and evaluate their practical taxonomic relevance. Systematic research is being conducted on *Diabroticites* by the author, and the first results are reported here.

Methods

The analysis of characters was based on the study of specimens, literature (original descriptions and revision works, when available) and the aid of photos in online databases. A total of 240 species was studied, representing 15 out of the 17 genera included in *Diabroticites*.

Specimens were obtained from south-american collections listed in Table 2, always in comparison to original descriptions – and many types were available. Out of the total species available, only 145 species had males available or known. These taxa were listed in Appendix. The specimens were examined and illustrated using a Zeiss Discovery.V8 stereomicroscope with a camera lucida attached. Final art was done in Adobe Illustrator®. Photographs were taken using a Leica M205C stereomicroscope with an attached magnifying lens and Leica DFC 295 video camera. Image combination was performed with Leica Application Suite V3.6.0, and subsequent edition was done in Adobe Photoshop®.

Most taxonomic literature available for *Diabroticites* was reviewed. The original descriptions of monotypic genera *Palmaria* Bechyné, 1956, and *Pseudodiabrotica*, known only for their type-specimens, which could not be loaned, were the only source of characters for comparison. Revisionary works were available only for genera *Acalymma* (in

Table 2. Institutions that provided specimens for the study.

Acronym	Name	City	Country	Curator
CEAH	Coleção Entomológica Adolph Hempel, Instituto Biológico	São Paulo	Brazil	Sérgio Ide
INPA	Coleção Sistemática de Entomologia, Instituto Nacional de Pesquisas da Amazônia	Manaus	Brazil	Augusto Henriques
FIOC	Fundação Instituto Oswaldo Cruz	Rio de Janeiro	Brazil	Jane Costa von Sydow
IACC	Instituto Agronômico de Campinas	Campinas	Brazil	Édson Possidônio Teixeira
MGAP	Museu Anchieta	Porto Alegre	Brazil	Fernando Meyer
MCNZ	Museu de Ciências Naturais da Fundação Zoo-Botânica do Rio Grande do Sul	Porto Alegre	Brazil	Maria Helena Galileo
DZUP	Coleção de Entomologia Padre Jesus Moure, Universidade Federal do Paraná	Curitiba	Brazil	Lúcia Massuti de Almeida
MZSP	Museu de Zoologia da Universidade de São Paulo	São Paulo	Brazil	Sônia Casari
MNRJ	Museu Nacional, Universidade Federal do Rio de Janeiro	Rio de Janeiro	Brazil	Marcela Monné
MPEG	Museu Paraense Emilio Goeldi	Belém	Brazil	Orlando Tobias Silveira
UFVB	Museu Regional de Entomologia da Universidade Federal de Viçosa	Viçosa	Brazil	Paulo Sérgio Fiuza Ferreira
MLPA	Universidad Nacional de La Plata, Museo de la Plata	La Plata	Argentina	Nora Cabrera

part) (Munroe and Smith 1980, Cabrera 1999, Cabrera and Durante 2003), *Diabrotica* (in part) (Marques 1941, Christensen 1943, Krysan and Smith 1987, Cabrera 2000a, Cabrera 2000b) and *Synbrotica* (in part) (Cabrera 1995).

Due to the peculiarity of genus *Isotes* Weise, 1922, which was described based on a single species later found to be a senior synonym of the type-species of genus *Synbrotica* (at that time with over 100 species), the original description of the latter was also included in the analysis for comparative purposes. As a reference to their original descriptions, both names will be used interchangeably throughout the text, even though *Isotes* is the current valid name.

Characters mentioned in original descriptions and other taxonomic works, when available, were compiled and later compared to specimens. Those characters were then redefined, in order to fulfill uniform homology criteria. The broader studies of Mohamedsaid and Furth (2011) and Mohamedsaid (2004) were used for character comparison with other taxonomic groups.

Also, some taxa that had not enough specimens available in south-american collections were studied by the analysis of photos of type specimens available in the Museum of Comparative Zoology online Type Database, Harvard University, Cambridge, USA.

Results and discussion

Out of the 17 genera that comprise section *Diabroticites*, 12 have sexually dimorphic features as diagnostic characters mentioned in original descriptions by nine different authors (15 papers) in a period of more than 150 years (Table 1). Most genera were established based on a single or on few species, further taxa being later added, frequently not by their original authors. This information is relevant when checking whether the initial concept proposed for the genus was maintained or not.

Most species studied have some kind of sexual dimorphism. Those modifications were categorized in two distinct groups, concerning its level of taxonomic comprehension: general and special dimorphism.

General dimorphism

Characters that are referred to as of general comprehension are those proposed several times in the literature as being important to species definition, but were actually found to be more generalized, i. e., they in fact occur in higher taxa. General characters are found in all, or most, male of *diabroticites* analyzed, and might also support the definition of larger taxonomic groups: **1)** Smaller body size: considered general for Chrysomelidae, observed for most *diabroticites*; **2)** Bigger eyes (relative to the total size of the head): cited often in species descriptions, but actually observed in most *diabroticites*; **3)** Tarsal adhesive disks: structures present in most Chrysomelidae, with variation found among subfamilies and often among tribes, regarding the number of

legs in which they occur and the proportion of the dorsal surface that they occupy (Stork 1980). For Diabroticites, the adhesive disks are present at the first tarsomere in pro- and mesothoracic legs; **4**) Emargination on the posterior margin of ventrite V: this character sometimes supports the definition of the subfamily Galerucinae, and its shape can be diagnostic of tribes, being rounded in Luperini and Galerucini, for example (Bechyné and Bechyné 1962, Wilcox 1965). In diabroticites this emargination is, usually, round; and **5**) Prothoracic tibiae with continuous apex, without spurs: probably constant in the whole tribe (Wilcox 1965).

Special dimorphism

Special characters are those used to describe lower taxonomic ranks, i. e., genera and species, and have been or not mentioned as diagnostic features in original descriptions.

With the exception of two genera, *Amphelasma* Barber, 1947 and *Cochabamba* all genera in Diabroticites are represented with special dimorphic characters. Of all species analyzed, only 47 were found to have special dimorphic characteristics. This is interesting, since the original definitions of diabroticites genera were usually based in sexual dimorphism. In accordance with the pattern observed for the subfamily (Mohamedsaid and Furth 2011), special dimorphism was observed in every tagma in the analyzed taxa of Diabroticites (Table 3).

Out of the 12 genera recognized by dimorphic characters, 4 are monotypic (*Buckibrotica*, *Palmaria*, *Platybrotica* and *Pseudodiabrotica*). The diagnostic characters and its validity will be discussed for each tagma.

Head

Most of the dimorphic characters were found in the head, mainly in the antennae, a proportion which agrees with the general pattern observed in the subfamily (Mohamedsaid and Furth 2011).

Gynandrobrotica was described as showing an “excavated clypeus”, without any further details or illustrations. All four species studied have the same kind of modification, which is better described as the frons being elongated and with a shallow, smooth, round concavity (Figure 1), accompanied by sparse, large punctuation. It is interesting to compare this character with the differently excavated type of frons found in species of *Cerotoma* Chevrolat, 1837 (Figure 2), *Eucerotoma* Laboissiere, 1939 and *Neobrotica* Jacoby, 1887– all of which are usually placed in sister-section Cerotomites Chapuis, 1875. *Gynandrobrotica* has been suggested to be more related to these taxa in some phylogenetic analyses (Eben and Monteros 2004, Gillespie et al. 2008). Other characteristics common to these genera, such as the small eyes, the elongated frons and different shapes of antennomeres I-III should be further studied in order to verify if they are indeed homologues.

Table 3. Location of diagnostic, sexually dimorphic characters mentioned in original descriptions.

Genus	Antennae	Legs	Head (except antennae)	Elytra
<i>Anisobrotica</i> Bechyné & Bechyné	X	X		
<i>Aristobrotica</i> Bechyné	X	X		
<i>Paranapiacaba</i> Bechyné	X	X		
<i>Buckibrotica</i> Bechyné & Bechyné	X			
<i>Cornubrotica</i> Bechyné & Bechyné	X			
<i>Ensiforma</i> Jacoby	X			
<i>Paratriarius</i> Schaeffer	X			
<i>Platybrotica</i> Cabrera & Cabrera Walsh	X			
<i>Isotes</i> Weise (ex <i>Symbrotica</i> Bechyné)		X*		
<i>Palmaria</i> Bechyné		X		
<i>Gynandrobrotica</i> Bechyné			X	
<i>Pseudodiabrotica</i> Jacoby				X

*: present in the original description of *Symbrotica*.



Figure 1. *Gynandrobrotica caviceps* (Baly, 1889), head in frontal view (female, left, male, right).

There are also three other species with distinctive head features in the male: *Isotes onira* (Bechyné & Bechyné), 1961 has an enlarged head from the vertex up to the antennal insertions – Figure 3), *Diabrotica serroazulensis* Bechyné & Bechyné, 1962 has an enlarged labrum, which is as large as half of the frontal length, and *Acalymma cornutum* (Baly, 1886), has a deep cleft in the frons, with lateral projections, and also a labrum with an acute anterior projection that reaches the frons (illustrated in Munroe and Smith 1980).

Antennae

Galerucines commonly display filiform antennae, which can show numerous dimorphic variations (Jolivet and Verma 2002, Mohamedsaid 2004). This is also true for



Figure 2. *Cerotoma variegata* (Fabricius, 1792) head in frontal view (female, left, male, right).



Figure 3. *Isotes onira* (Bechyné & Bechyné, 1961), dorsal view, detail of pronotum and head (female, left, male, right).

Diabroticites, with the main antenna type being filiform and antennomeres mostly subequal in size and shape (Figure 4A).

Non-dimorphic modifications are often related to the length of some antennomeres and sometimes support generic definitions. For instance, the two largest genera in Diabroticites, *Diabrotica* and *Synbrotica* are essentially distinguished by the length of antennomere III, described as being subequal to II in the first (same as observed in genus *Cochabamba* in Figure 4B), and subequal to IV (that is, almost twice as longer as II, as in Figure 4C) in the latter (Bechyné 1956).

Eight genera have diagnostic characters based in their dimorphism of antennae (listed in Table 3). Examples of modified antennae are showed in Figure 4C–F. Because of their variability, dimorphic antennae are the most used structure in descriptions, but their modifications have been scarcely detailed. As a result, there are differ-

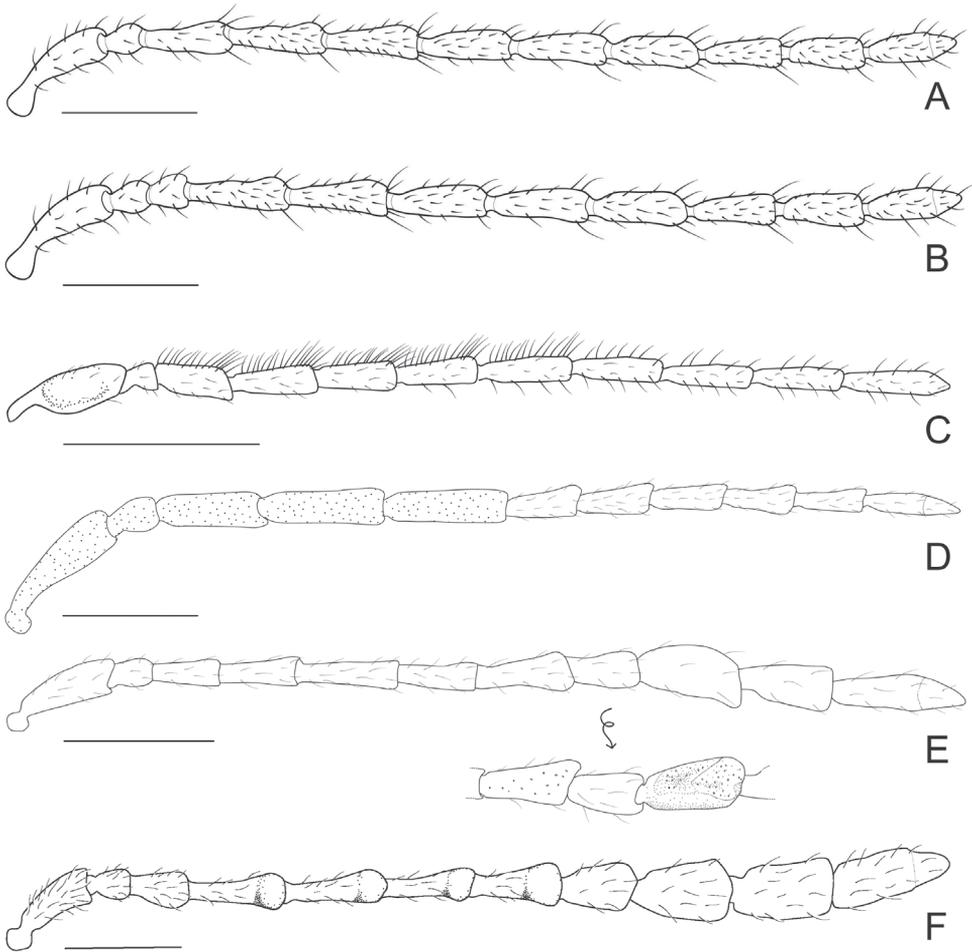


Figure 4. Male modified antennae in lateral view **A** *Isotes borrei* (Baly, 1889) **B** *Cochabamba marginata* (Harold, 1875) **C** *Isotes onira* (Bechyné & Bechyné, 1961) **D** *Aristobrotica angulicollis* (Erichson 1878) **E** *Buckibrotica cinctipennis* (Baly, 1886) (detail in ventral view) **F** *Ensiforma caerulea* Jacoby, 1876. Scale bar = 1 mm.

ent genera proposed on characters depicted in sentences such as “apical antennomeres modified” (*Cornubrotica*), “antennomeres VII and IX of complicated shape” (*Buckibrotica* – Figure 4E), “antennomeres V-VII modified” (*Paratriarius*).

The absence of unified criteria in the understanding of what a “modified” antennomere is has lead, several times, to the establishment of artificial grouping of species, simply because a single “aberrant” antennomere can display an assembly of four different aspects of its morphology. Modifications include change in (in quoting marks, expressions used on original descriptions): **length** – antennomeres considered “elongated” or “shortened” when compared to the usually fixed antennomeres I and III; **width** – antennomeres described as “swollen”, “inflated” (homogeneous modification), “distally expanded” (heterogeneous modification), and dorsoventrally “flattened”; **shape**

– those described as having “lateral projections” or “ventral excavations”; **structure** – antennomeres with “rough punctuation” and different amounts of hairs/sensillae. As variations observed in the species do not always correspond to the diagnosis defined for genera they have been included in, these characteristics do not provide an accurate guide to the identification of taxa in Diabroticites.

For the non-monotypical genera based on antennal dimorphic features, most original definitions do not correspond to their actual characters. In *Anisobrotica*, for example, the “widened” apical antennomeres do not always appear – *A. binisculpta* Bechyné & Bechyné, 1969 only has in common with the other taxa the excavation present in glabrous ventral surface of antennomeres IX–XI (such as excavations observed in apical antennomeres of *A. donckieri* (Baly, 1889) in Figure 5). The same happens with *Paratriarius*, which includes several species that do not show “modified antennomeres V–VII” present in type-species *P. dorsata* (Say, 1824) (illustrated in Wilcox 1965) such as *P. batesi* (Baly, 1859), *P. falvolimbata* (Erichson, 1847), *P. verrucosa* (Jacoby, 1880), *P. alternans* (Weise, 1916), *P. nigrotibialis* (Bowditch, 1911), *P. castanea* (Bowditch, 1911), and also other four species studied by Mohamedsaid and Furth (2011). Instead, these taxa show antennae very similar to the general pattern seen in *Diabrotica*. The two species included in *Cornubrotica* do not show identical antennomeres VIII and IX, although both always have ventral excavations (illustrated in Bechyné and Bechyné 1969 and Moura 2005). In *Paranapiacaba*, the antennal character chosen was, unfortunately, a general one: male antennomeres III–XI uniformly “thickened” (in contrast with slightly slender antennae of females). Nevertheless, antennae do seem to vary uniformly in one genus. In *Aristobrotica*, the pattern of antennomeres III–V “thickened” is constantly repeated, followed by an unmentioned presence of larger punctuation (Figure 4D).

Although Maulik (1936) suggested that, for indo-asian galerucines, the basal antennomeres are more frequently the altered ones, a result that has been corroborated by Mohamedsaid (2004), that feature does not apply to diabroticites analyzed. Also, no obvious topological pattern is seen in the variation of antennomeres (Table 4).

The number of modified antennomeres oscillated between 1 to 6. Antennomere II was recorded as dimorphic only in species of *Aristobrotica*, such as *A. angulicollis* (Erichson, 1878) (Figure 4D) and in *Isotes onira* (Figure 4C). Few modifications occur in antennomeres II and XI. The most affected are antennomeres V to IX. However, there is no indication of an explicit dependency of occurrence between any pair of modified antennomeres. This is the opposite of what has been observed for asian Galerucinae species (Mohamedsaid 2004). Although no pattern is observed, some variation can occur in blocks, i. e., one modified antennomere occurs with one or two adjacent antennomeres also modified.

It seems that most, if not all, antennal variations could be regarded as the result of the presence of punctuation and setae in greater number, either for the production and/or reception of chemical compounds (i. e., pheromones) (Jolivet 2007). A study on *Diabrotica virgifera* Leconte, 1858, for instance, showed that male antennae have a much greater number of sensilla than females, and numerous glandular points linked

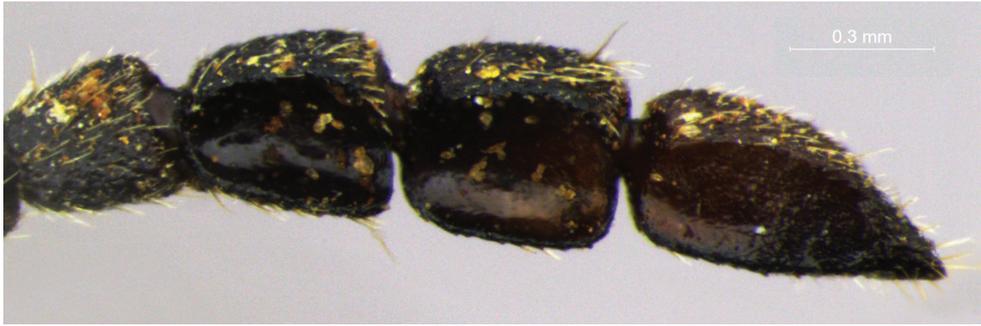


Figure 5. *Anisobrotica donckieri* (Baly, 1889), detail of ventral surface of apical antennomeres, male.

Table 4. Selected diabroticites species representing antennal dimorphic variation. Grey cells indicate modification in the antennomere.

Taxon/antennomere	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
<i>Isotes onira</i> (Bechyné & Bechyné, 1961)											
<i>Ensiforma chiquitoensis</i> (Bechyné, 1958)											
<i>Aristobrotica angulicollis</i> (Erichson, 1878)											
<i>Isotes simplicipennis</i> (Jacoby, 1889)											
<i>Ensiforma asteria</i> (Bechyné & Bechyné, 1962)											
<i>Paratriarius dorsata</i> (Say, 1824)											
<i>Isotes callanga</i> (Bechyné, 1956)											
<i>Platybrotica misionensis</i> Cabrera & Cabrera Walsh, 2004											
<i>Buckibrotica cinctipennis</i> (Baly, 1886)											
<i>Cornubrotica dilaticornis</i> (Baly, 1879)											
<i>Diabrotica samouella</i> Bechyné, 1956											
<i>Anisobrotica donckieri</i> (Baly 1889)											

to the production of chemical compounds allegedly to be attractive to females (Newman Jr et al. 1993). Unfortunately, there is little knowledge on the biology or even on the anatomy of *Diabroticites* to support this view as a more generalized tendency.

Thorax

Although a great diversity of dimorphic characters occur in the thorax of several galerucines (Mohamedsaid and Furth 2011), features reported for *Diabroticites* are limited to elytra, and legs. In elytra, variation occurs only on the apical fifth, and are either depressions or callosities, both which can co-occur with punctuation (as in some species of *Isotes* – Figure 6, and in *Pseudodiabrotica* – the only genus supported on an elytral diagnostic character). Many species in genus *Paratriarius* show elytral dimorphic characters (such as the callosities present in *P. batesi*), and, although such features were never used to originally describe it, they have been used to support the definition of

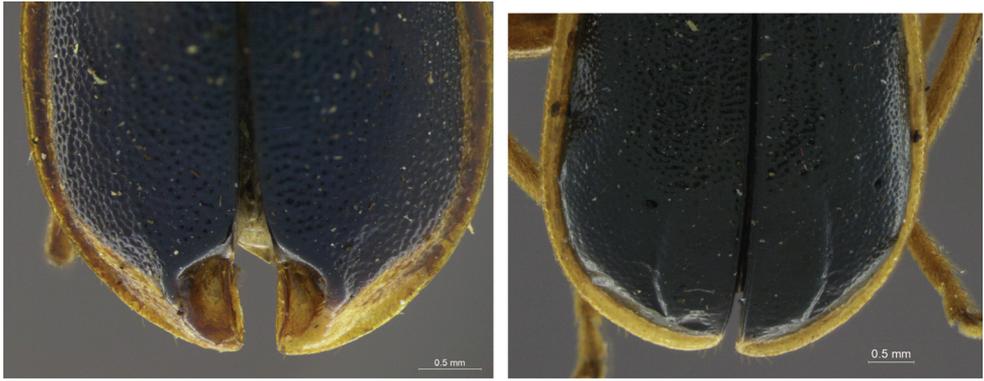


Figure 6. Detail of elytral modifications, left, *Isotes digna* (Gahan, 1891), male, right, *Paratriarius batesi* (Baly, 1859), male.



Figure 7. *Paranapiacaba prolongata* (Jacoby, 1882), detail of ventral surface of tarsomeres of prothoracic leg, male.

genus *Chanhamayia* Bechyné, 1956, now considered to be a subgenus in *Paratriarius* (Smith and Lawrence 1967, Wilcox 1972).

Only metathoracic legs lack alterations in male diabroticites. In accordance with the more general pattern, morphological differences in the pro- and mesothoracic legs



Figure 8. *Zischkaiia serrana* Moura, 2003, ventral view, male.

usually are connected to the augmentation of absolute size in femora and tarsomeres I, the latter which are directly linked to the partial or total covering of the ventral surface by adhesive setae (Figure 7).

Tibiae and femora can also be modified, being greatly enlarged (such as in *Zischkaiia serrana* Moura, 2003 – Figure 8), and frequently with internal margins concave or bearing tubercles, forming the “prehensile organ” (Bechyné 1956). Bechyné’s concept of such structure is based on a combination of multiple adaptations and should be used with caution, since the homology of the “prehensile organs” can be difficult to assess. *Aristobrotica*, for instance, has been described as with one diagnostic feature: the “special build of the median tibiae in male”. The detailed analysis of species included, however, indications that there are at least two distinct types of “prehensile organs” being treated as the same modification. While type-species *A. angulicollis* (Figure 9) bears only a concave mesotibiae with laterally flattened apex, *A. mirapeua* Moura, 1997 and *A. capillosa* Moura, 2011 (both illustrated in their original descriptions) display small projections of the ventral margin of the mesofemora (apical in *A. mirapeua* and basal in *A. capillosa*), and differently shaped tibiae (with a basal concavity in *A. mirapeua* and slightly concave tibiae with apical flattening in *A. capillosa*).

Moreover, general characters have been used to support definition of genera such as *Cornubrotica* and *Synbrotica*, a genus which is now a synonym of *Isotes*. The former



Figure 9. *Aristobrotica angulicollis* (Erichson, 1878), detail of mesothoracic leg, male.

was supposed to be distinguished by pro- and mesothoracic legs of males without emargination, which is rather common in the tribe, and the latter is characterized by antennomere III elongated and a “uniform pilosity covering the ventral surface of tarsomeres in both sexes” (freely translated from the original, in German) – something that does not accurately identify the males in this group, as they normally have distinctive adhesive disks in their tarsomeres.

Abdomen

The most common abdominal modification seen in some galerucines is the presence of processes with different shapes. Although no abdominal characters aid the definition of diabroticites genera, one character was observed for a single species in the group: a central triangular projection, postero-ventrally oriented, in the posterior margin of the ventrite I, in *Zischkaiia serrana* (Figure 10). A similar alteration is observable in *Hemygascelis longicollis* Jacoby, 1896, an asian species that belongs to section Phyllobroticites in subtribe Luperina, a group thought to be a sister group of Diabroticina (illustrated in Mohamedsaid and Furth 2011).

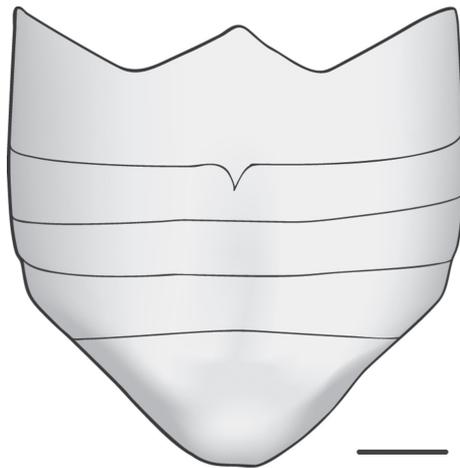


Figure 10. *Zischkaiia serrana* Moura, 2003, schematic abdomen in ventral view, male. Scale bar = 0,5 mm.

Conclusions

The taxonomic history of taxa in section *Diabroticites*, which dates to over 150 years of specimen sampling and species descriptions, has been supported primarily by scarcely detailed descriptions of morphological features, specially coloration and striking “sexual aberrations”. Sexually dimorphic characters have been the foundation for the creation of many genera in this group, albeit with vague definitions and a general disregard for comparative morphology. The use of inappropriate terminology by some authors has caused further confusion, since the habit of inferring homology from homonymy is common in the taxonomy of *Chrysomelidae* (Schmitt 1996). This might have been the origin of the combination of many species into these scarcely defined genera in *Diabroticites*. As a result, most generic original definitions, based on dimorphic characteristics, are not correspondent to their actual assembly of species.

Nevertheless, the establishment of homology theories is far from being trivial, and some variations might even occur in patterns that can actually help the definition of certain taxa, although it is clear that many sexually dimorphic features found in this section are possibly singular and autapomorphic. In this case, a broader morphological study is necessary.

The comparison of the dimorphic characters in *Diabroticites* with their related taxa, such as the Asiatic *Aulacophorites* Chapuis, 1875 (Luperini) and remaining *Galerucinae* is desired, in order to understand the evolution of such characters. There are striking similarities among many modifications found in these groups and thus it should be useful in the evaluation of homology as well. Parallelism might be the more parsimonious choice in many cases, but that is yet to be tested. Understanding the morphology is critical for better character definitions. Also, genital characters, which have been generally ignored, should provide important characters, as well as the account of several non-dimorphic characters, usually regarded as too uninformative, without detailed consideration.

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Appendix

List of taxa with male specimens available in south-american collections.

- Acalymma albidovittatum* (Baly, 1889)
Acalymma bivittulum (Kirsch, 1883)
Acalymma bruchii (Bowditch, 1911)
Acalymma carinipenne (Bowditch, 1911)
Acalymma exigua Bechyné, 1958
Acalymma granulipenne (Bowditch, 1911)
Acalymma incum (Bowditch, 1911)
Acalymma innubum (Fabricius, 1775)
Acalymma isogenum Bechyné & Bechyné, 1968
Acalymma punctatum (Jacoby, 1887)
Acalymma rubeolum Bechyné, 1958
Acalymma thiemei (Baly, 1886)
Acalymma vitigera (Boheman, 1859)
Acalymma vittatum (Fabricius, 1775)
Acalymma xanthographum Bechyné, 1955
Anisobrotica binisculpta Bechyné & Bechyné, 1969
Anisobrotica donckieri (Baly, 1889)
Anisobrotica nordenskiöldi (Jacoby, 1907)
Anisobrotica notaticollis (Baly, 1889)
Aristobrotica angulicollis (Erichson, 1878)
Aristobrotica capillosa Moura, 2011
Buckibrotica cinctipennis (Baly, 1886)
Cochabamba chacoensis (Bowditch, 1911)
Cochabamba chrysopleura (Harold, 1875)
Cochabamba diversicolor (Baly, 1890)
Cochabamba erythrodera (Baly, 1879)
Cochabamba marginata (Harold, 1875)
Cochabamba mera Bechyné, 1956
Cochabamba polychroma Bechyné, 1956
Cochabamba rugulosa (Baly, 1886)
Cochabamba variolosa (Jacoby, 1878)
Cochabamba volxemi (Baly, 1889)
Cornubrotica dilaticornis (Baly, 1879)
Cornubrotica iuba Moura, 2005
Diabrotica alegrensis Bechyné & Bechyné, 1962
Diabrotica amoena (Dalman, 1823)
Diabrotica antonietta Bechyné, 1956
Diabrotica aracatuba Bechyné & Bechyné, 1964
Diabrotica arcuata Baly, 1859

- Diabrotica atrilineata* Baly, 1889
Diabrotica atromaculata Baly, 1889
Diabrotica atosignata Baly, 1890
Diabrotica boggianii Bowditch, 1911
Diabrotica chloropus Harold, 1875
Diabrotica clarki Weise, 1916
Diabrotica confraterna Baly, 1889
Diabrotica consentanea Baly, 1886
Diabrotica cryptochlora Bechyné, 1956
Diabrotica decaspila Baly, 1890
Diabrotica decempunctata (Latreille, 1813)
Diabrotica deliqua Weise, 1921
Diabrotica distincta Jacoby, 1882
Diabrotica egleri Bechyné & Bechyné, 1961
Diabrotica elata (Fabricius, 1801)
Diabrotica emorsitans Baly, 1890
Diabrotica enae Marques, 1941
Diabrotica fallenia Bechyné, 1956
Diabrotica flava (Olivier, 1791)
Diabrotica funerea Bowditch, 1911
Diabrotica fusibilis Bechyné & Bechyné, 1970
Diabrotica gracilentata Erichson, 1847
Diabrotica graminea Baly, 1886
Diabrotica kirbyi Baly, 1890
Diabrotica lamiina Bechyné & Bechyné, 1969
Diabrotica limitata (Sahlberg, 1823)
Diabrotica lutescens Baly, 1890
Diabrotica manaensis Weise, 1921
Diabrotica nitidicollis Baly, 1889
Diabrotica olivacea Jacoby, 1882
Diabrotica panchroma Bechyné, 1955
Diabrotica paranaensis Marques, 1941
Diabrotica pentazyga Bechyné & Bechyné, 1970
Diabrotica piceicornis Baly, 1889
Diabrotica piceosignata Baly, 1890
Diabrotica poecilenta Bechyné, 1958
Diabrotica propylaea Bechyné & Bechyné, 1969
Diabrotica quinquemaculata (Fabricius, 1801)
Diabrotica recki Marques, 1941
Diabrotica rufolimbata Baly, 1879
Diabrotica samouella Bechyné, 1956
Diabrotica schaufussi Baly, 1890
Diabrotica scripta (Olivier, 1808)

- Diabrotica sedata* Baly, 1890
Diabrotica serroazulensis Bechyné & Bechyné, 1962
Diabrotica sharpii Kirsch, 1883
Diabrotica sheba Bechyné, 1958
Diabrotica simulata Baly, 1890
Diabrotica sinuata (Olivier, 1789)
Diabrotica speciosa (Germar, 1824)
Diabrotica stenocoryna Bechyné & Bechyné, 1970
Diabrotica tarcisia Bechyné, 1971
Diabrotica tijuquensis Marques, 1941
Diabrotica transversa Baly, 1890
Diabrotica travassosi Marques, 1941
Diabrotica univittata Jacoby, 1899
Diabrotica viridans Baly, 1889
Diabrotica viridimaculata Jacoby, 1878
Diabrotica viridula (Fabricius, 1801)
Diabrotica wartensis Cabrera & Sosa-Gómez, 2008
Diabrotica westwoodi Baly, 1889
Ensiforma asteria (Bechynné & Bechyné, 1962)
Ensiforma caerulea Jacoby, 1876
Ensiforma chiquitoensis (Bechyné, 1958)
Gynandrobrotica caviceps (Baly, 1889)
Gynandrobrotica equestris (Fabricius, 1787)
Isotes agatha (Bechyné & Bechyné, 1969)
Isotes albidocincta (Baly, 1889)
Isotes bertonii (Bowditch, 1912)
Isotes bicincta (Bowditch, 1912)
Isotes borrei (Baly, 1889)
Isotes brasiliensis (Jacoby, 1888)
Isotes cargona (Bechyné, 1958)
Isotes caryocara (Bechyné, 1956)
Isotes crucigera (Weise, 1916)
Isotes delicula (Erichson, 1847)
Isotes digna (Gahan, 1891)
Isotes eruptiva (Bechyné, 1955)
Isotes ignatia (Bechyné, 1956)
Isotes onira (Bechyné & Bechyné, 1961)
Isotes pollina (Bechyné & Bechyné, 1962)
Isotes puella (Baly, 1886)
Isotes sanguineipennis (Baly, 1891)
Isotes semiflava (Germar, 1824)
Isotes sibylla (Bechyné & Bechyné, 1969)
Isotes taeniolata (Gahan, 1891)

- Isotes ternata* (Bechyné & Bechyné, 1961)
Isotes valentina (Bechyné, 1956)
Isotes varipes (Boheman, 1835)
Paranapiacaba amplexa (Erichson, 1847)
Paranapiacaba biseriata (Gahan, 1891)
Paranapiacaba costalimai (Marques, 1941)
Paranapiacaba decemverrucata (Gahan, 1891)
Paranapiacaba diametralis (Bechyné, 1956)
Paranapiacaba melanospila (Gahan, 1891)
Paranapiacaba morretesi Bechyné & Bechyné, 1969
Paranapiacaba pereirai Bechyné, 1958
Paranapiacaba prolongata (Jacoby, 1882)
Paranapiacaba seraphina (Bechyné, 1956)
Paranapiacaba significata (Gahan, 1891)
Paranapiacaba subirregularis (Bechyné & Bechyné, 1962)
Paranapiacaba teinturieri (Allard, 1894)
Paratriarius batesi (Baly, 1859)
Paratriarius limbatipennis (Baly, 1889)
Platybrotica misionensis Cabrera & Cabrera Walsh, 2004
Zischkaita serrana Moura, 2003

Notes on the ecology of rolled-leaf hispines (Chrysomelidae, Cassidinae) at La Gamba (Costa Rica)¹

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Abstract

A total of 301 adult hispine beetles of the genera *Cephaloleia* and *Chelobasis* were found in rolled leaves of plants of 17 species of Zingiberales (families Costaceae, Heliconiaceae, Maranthaceae, Musaceae, and Zingiberaceae) during a field study at La Gamba, Golfito region, Costa Rica. Of these beetles, *Cephaloleia belti* was recorded from 12 potential host plant species, *C. distincta* from 7, *C. dilaticollis* from 5, *C. Chelobasis bicolor*, *C. championi*, and *C. histrionica* from 3, *Chelobasis perplexa* and *C. instabilis* from 2, whereas *C. trivittata* from only one. Of the plant species, *Heliconia latispatha* had 7 beetle species in its leaf rolls, *Calathea lutea* had 5, *H. imbricata* and *H. rostrata* had 4, *H. stricta* and *Musa paradisiaca* had 3, *H. wagneriana* had 2, while on *H. vaginalis*, *H. danielsiana*, *H. densiflora*, *H. longiflora*, *Calathea crotalifera*, *C. platystachya*, *Goepertia lasiophylla*, *Alpinia purpurata*, *Costus pulverulentus* and *Costus barbatus*, *H. densiflora*, *H. vaginalis*, and *H. danielsiana* only hispines of one species were found.

Cephaloleia belti occurred together with beetles of six other hispine species, whereas *Cephaloleia trivittata* never shared a leaf roll with another hispine species. The remaining beetle species aggregated with one to four other hispines. Adults of *C. belti* and *C. championi* were frequently seen, occasionally also with *C. dilaticollis*, *C. histrionica*, and *Chelobasis perplexa*, to co-occur with the carabid *Calophaena ligata* in the same leaf roll without any sign of interspecific aggression.

A comparison of host choices and the phylogeny of the hispines and of their host plants revealed no signs that beetles used species level phylogenetic relationships within the Zingiberales to select food plants. Obviously, within this plant order, rolled-leaf hispines choose their plant hosts in a nearly opportunistic manner. Seemingly, they use differences among plants at higher taxonomic levels but within the Zingiberales, the availability of young – rolled – leaves might be the actual decisive factor.

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Keywords

Insecta, Coleoptera, Chrysomelidae, *Cephaloleia*, *Chelobasis*, Zingiberales, Costaceae, Heliconiaceae, Maranthaceae, Musaceae, Zingiberaceae, synecology, host plant, Costa Rica

Introduction

Since the nineteenth century it has been known to science that beetles of a (probably monophyletic: McKenna and Farrell 2005) subclade of the traditional Hispinae (hispine Cassidinae) develop as larvae and live as adults inside the tubes formed by rolled leaves of Zingiberales plants (Baly 1885: 8; Maulik 1919: 12). As larvae and adults of these beetles produce characteristic feeding tracks, Wilf et al. (2000) inferred from similar tracks on fossil Zingiberales leaves that this special type of plant-herbivore interaction evolved as early as the late Cretaceous, about 66 Mio years ago. However, García-Robledo and Staines (2008) raised doubt and discussed an origin of this behaviour ca. 20 Mio years later because other insects were found producing similar feeding tracks, e.g. Lepidoptera larvae in the families Pyralidae and Choreutidae and weevils (Coleoptera: Curculionidae) of the genus *Anopsilus* Kirsch, 1869. The use of rolled leaves as habitat by leaf beetles is in the New World restricted to species of the tribes Arescini and Cephaloleiini, whereas in Indonesia also a *Hispidonta* sp. has been observed in rolled leaves of *Musa* and *Zingiber* (see Staines 2004). A remarkable body of publications treat the development, ecology, phylogeny, and taxonomy of these beetles (see Staines 2004; Chaboo 2007 for an extensive literature review). According to Seifert (1982), “the insect fauna associated with *Heliconia* plants is one of the most intensively studied of all (non-cultivated) Neotropical insect-plant associations”. Strong (1983) described concisely the biology of the rolled-leaf hispines, using *Chelobasis bicolor* as the main example. A more detailed description of the natural history of *Cephaloleia*-species, based on field observations and laboratory investigations, is given by García-Robledo et al. (2010). These beetles spend most of their lives inside the rolled leaves of Zingiberales plants, on which they feed. The larvae are flattened and move and feed between the layers of the rolled leaves. Pupation takes place at various places on the host plants. Also the adults of these beetles can move between the layers of the leaf rolls, in many species they are flattened, and all are spineless, in contrast to the majority of hispines that usually live on the surface of leaves as adults.

Our primary aim was to assess the number of rolled-leaf hispines species and their abundances in the area of the biological field station “La Gamba” in the Golfito region of Costa Rica and to compare our findings with those from the “La Selva” biological station (Strong 1977a, b, 1982a, b; García-Robledo et al. 2010; Staines 2011; García-Robledo et al. 2013) and from sites in lowland central Panama (Descampe et al. 2008, Meskens et al. 2008). In addition, we collected data on putative host preferences and inter-specific aggregations of these beetles, including those with the carabid *Calophaena ligata*.

Study site

All field work was performed at the La Gamba biological station, Costa Rica (Puntarenas), 8 km NNW of the city of Golfito, 8°42'61"N, 83°12'97"W, 70 m a.s.l., and ca. 8 km off the coast of the Golfo Dulce. The station is located at the edge of the Piedras Blancas National Park and is run by the Verein zur Förderung der Tropenstation La Gamba (society for the furtherance of the La Gamba tropical field station), based at the University of Vienna (Austria) (www.lagamba.at). Numerous plants of the order Zingiberales grow in the 2200 m²-garden of the station. Most individual plants are accurately identified to species and labelled. Botanists from the department of Tropical Ecology and Animal Biodiversity at the University of Vienna are responsible for the scientific supervision of the station, and the accurate identification of the plants in the garden. The station is situated between secondary and primary forest areas to the west, south, and east, and adjoins agriculturally managed areas, mostly pastures and oil palm plantations, to the north. Several trails through the forest allow access to sites inside the forest, e.g. to clearings where the host plants in this study were most abundant.

Methods

As no other plants at La Gamba formed rolled young leaves, we censused only Zingiberales plants in the station's park and along trails for rolled leaves at 15 day intervals within the months of January through April, 2009. We unrolled 120 rolled leaves and recorded the macrofauna found in them. The hispine leaf beetles and other arthropods were collected from the leaves and taken to the station. We also kept records of findings of hispine larvae, eggs, and feeding tracks. In some cases we took photographs as exemplars. Some of the hispines were killed and mounted for identification, others were stored in ethanol. The whole material is still with the senior author for further examination of the non-hispine species. It will be deposited at the Museo Zoológico of the Universidad de Costa Rica at San Pedro, voucher specimens will be deposited at Zoologisches Forschungsmuseum Alexander Koenig, Bonn (Germany).

We identified the beetles by comparison with identified specimens in the collection at the Instituto Nacional de Biodiversidad (INBio) at Santo Domingo de Heredia and by means of published keys and original descriptions (Baly 1885; Staines 1996, 2009). We identified host plants using the labels in the station's park or by using the keys in Weber et al. (2001). With regard to plant taxonomy and nomenclature, we followed Borchsenius et al. (2012), GRIN (2013), and Tropicos (2013). For the statistical analysis we used the Statistical Package for Social Sciences (SPSS).

Results

We found 301 individuals of nine species of hispines, all from two genera, *Cephaloleia* Chevrolat, 1837 and *Chelobasis* Grey, 1832. These were the Cephaloleiini *Cephaloleia*

belti Baly, 1885, *C. championi* Baly, 1885 (Fig. 1), *C. dilaticollis* Baly, 1858, *C. distincta* Baly, 1885, *C. histrionica* Baly, 1885, *C. instabilis* Baly, 1885, *C. trivittata* Baly, 1885 and the Arescini *Chelobasis bicolor* Gray, 1832 (Fig. 2) and *Ch. perplexa* Baly, 1858. They were collected from 17 identified and at least two unidentified Zingiberales species: *Alpinia purpurata* (Zingiberaceae), *Calathea crotalifera*, *C. lutea*, *C. platystachya*, and *Goepertia lasiophylla* (Marantaceae), *Costus barbatus* and *C. pulverulentus* (Costaceae), *Heliconia danielsiana*, *H. densiflora*, *H. imbricata*, *H. latispatha*, *H. longiflora*, *H. rostrata*, *H. stricta*, *H. vaginalis*, and *H. wagneriana* (Heliconiaceae), and *Musa paradisiaca* (Musaceae). The numbers of the collected beetles and the respective potential host plants are given in Table 1. The beetle records are unequally distributed over their potential host plants. Of all species found in more than 20 individuals and on more than one potential host plant, a marked majority of records are from one or few of their potential host plants.



Figure 1. *Cephaloleia championi* from an unrolled *Heliconia*-leaf at La Gamba. M.Schmitt phot.



Figure 2. *Chelobasis bicolor*, La Gamba. M. Frank phot.

Table I. Numbers of collected hispines and their potential host plants at La Gamba

	<i>C. belti</i>	<i>C. championi</i>	<i>C. dilataticollis</i>	<i>C. distincta</i>	<i>C. histrionica</i>	<i>C. instabilis</i>	<i>C. trivittata</i>	<i>Ch. bicolor</i>	<i>Ch. perplexa</i>
<i>Alpinia purpurata</i> (Zingiberaceae)				14					
<i>Calathea crotalifera</i> (Marantaceae)			8						
<i>C. lutea</i> (Marantaceae)	2	33	15		6				1
<i>C. platystachya</i> (Marantaceae)		3							
<i>Costus barbatus</i> (Costaceae)	1								
<i>C. pulverulentus</i> (Costaceae)							3		
<i>Goepertia lasiophylla</i> (Heliconiaceae)	1								
<i>Heliconia danielsiana</i> (Heliconiaceae)	1								
<i>H. densiflora</i> (Heliconiaceae)	2								
<i>H. imbricata</i> (Heliconiaceae)		1		1	6			1	
<i>H. latispatha</i> (Heliconiaceae)	57		3	3	4	3		4	3
<i>H. longiflora</i> (Heliconiaceae)	4								
<i>H. rostrata</i> (Heliconiaceae)	36		4	1		1			
<i>H. stricta</i> (Heliconiaceae)	12			1				1	
<i>H. vaginalis</i> (Heliconiaceae)	2								
<i>H. wagneriana</i> (Heliconiaceae)	41			3					
<i>Musa paradisiaca</i> (Musaceae)	2		1	1					
Zingiberales indet.	9			1			5		
Totals	170	37	31	25	16	4	8	6	4

The unidentified Zingiberales grew outside the station garden and lacked inflorescences. We could not identify them using Weber et al.'s (2001) key.

We opposed a molecular cladogram of the genus *Cephaloleia* (from McKenna and Farrell 2005) with that of their potential host plants (combined from Marouelli et al. 2010 and Janssen and Bremer 2004) to reveal possible matches between beetle and plant phylogeny and visualise the insect-plant relations as a food-web (Fig. 3). There is no obvious preference of hispines for closely related plants, nor is there an apparent correlation of the phylogenetic relationship of the beetles and that of the plants on which we found them. It becomes clear, however, that the rolled-leaf hispines had preferences for certain Zingiberales species even if they were found on a much broader spectrum of possible food plants.

Rollled-leaf hispines, with the exception of *Cephaloleia trivittata*, co-occurred at La Gamba with at least one other species in the same leaf roll, *C. histrionica* with only one other species. *Cephaloleia belti* was found in the same leaf roll together with *C. championi*, *C. dilaticollis*, *C. distincta*, *C. instabilis*, *Chelobasis bicolor* and *Ch. perplexa*. The remaining hispines shared leaf rolls with two to four other hispine species. The numbers differed considerably, as shown in Table 2. Statistical tests – we used χ^2 – could only be performed for the four most abundant species, as of the remaining species we found too few individuals.

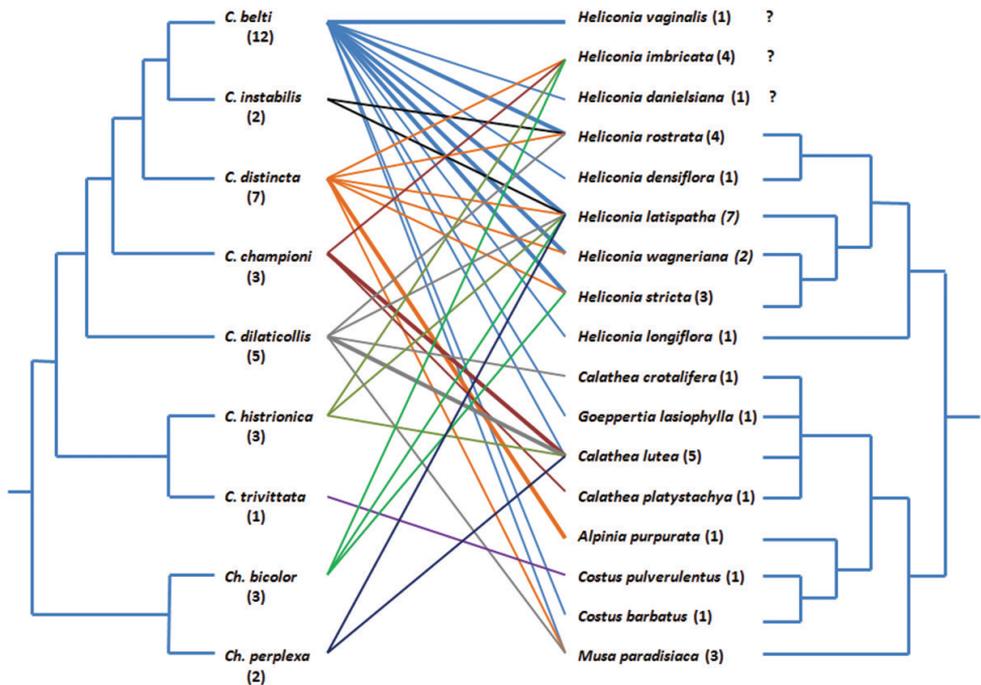


Figure 3. Food web of the rolled-leaf hispines of La Gamba and their possible food plants based on the data in Table 1, drawn by hand using MS Powerpoint. Bold lines indicate more than ten beetle records on the respective plant. Numbers in parentheses give the number of plant or beetle “partners”, respectively. Beetle cladogram after McKenna and Farrell (2005), plant cladogram combined after Marouelli et al. (2010) and Janssen and Bremer (2004). *Heliconia vaginalis*, *H. imbricata*, and *H. danielsiana* were – for some unknown reason - not included in these phylogenetic analyses.

Table 2. Co-occurrence of rolled-leaf hispinines in the same leaf roll at La Gamba.

Found in the same leaf roll together with:											
	<i>C. belti</i>	<i>C. championi</i>	<i>C. dilatocollis</i>	<i>C. distincta</i>	<i>C. histriónica</i>	<i>C. instabilis</i>	<i>C. trivittata</i>	<i>Ch. bicolor</i>	<i>Ch. perplexa</i>	a	b
<i>Cephaloleia belti</i> (n = 170)	120	1	17	26		10		15	8	Chi ² : 286.7 p: <0.001	Chi ² : 9.878 p: 0.003
<i>Cephaloleia championi</i> (n = 37)	6	17	12		8				1	Chi ² : 6.581 p: 0.087	Chi ² : 1.884 p: 0.227
<i>Cephaloleia dilatocollis</i> (n = 31)	15	11	14	1					1	Chi ² : 0.650 p: 0.778	Chi ² : 3.600 p: 0.080
<i>Cephaloleia distincta</i> (n = 25)	11		1	15		2		2	1	Chi ² : 17.20 p: 0.001	Chi ² : 0.000 p: 1.000
<i>Cephaloleia histriónica</i> (n = 16)		4			12					Chi ² : n.a. p: n.a.	Chi ² : n.a. p: n.a.
<i>Cephaloleia instabilis</i> (n = 4)	4			3						Chi ² : n.a. p: n.a.	Chi ² : n.a. p: n.a.
<i>Cephaloleia trivittata</i> (n = 8)							8			Chi ² : n.a. p: n.a.	Chi ² : n.a. p: n.a.
<i>Chelobasis bicolor</i> (n = 6)	4			3				1		Chi ² : n.a. p: n.a.	Chi ² : n.a. p: n.a.
<i>Chelobasis perplexa</i> (n = 4)	3	1	1	2						Chi ² : n.a. p: n.a.	Chi ² : n.a. p: n.a.

Numbers in the cells indicate the numbers of beetles given in the first column co-occurring with beetles of species given in the same horizontal row, e.g.: 26 of the 170 *Cephaloleia belti* were found together with *C. distincta*. As in several cases beetles of more than two species were found in one leaf roll, the checkums in these cases are higher than the total numbers given in the first column. White cells mark the exclusively conspecific aggregations, pink cells indicate observed records, yellow and blue cells mean that these theoretically possible co-occurrences have not been found in the present study.

Column **a**: Chi² and p-value for an aggregation of the species in the line with conspecifics or with any other species.

Column **b**: Chi² and p-value for an aggregation of the species in the line with the pooled other hispinines.



Figure 4. *Calophaena ligata* on an uncoiled *Calathea lutea*-leaf. M. Schmitt phot.

Individuals of the ground beetle, *Calophaena ligata* Bates, 1883 (Carabidae: Harpalinae, Fig. 4) (29 individuals) were found on *Calathea lutea* exclusively, co-occurring with *Cephaloleia belti* (11), *C. championi* (19), *C. dilaticollis* (7), *C. histrionica* (4), and *Chelobasis perplexa* (1). Four *C. ligata*-individuals were found in a single leaf roll without any hispine company. These beetles always sat on the inner surface of the leaf roll and were never found between two layers of a roll. We could never observe them feeding inside the leaf roll, nor could we find them on an uncoiled Zingiberales leaf at daylight.

Discussion

Our small set of observations show that in the use of host plants there are generalists and specialists among the hispine beetles found in the rolled leaves of Zingiberales at La Gamba. This is in general concordance with earlier investigations (Strong 1977b; Staines 2011; García-Robledo and Horvitz 2012a, b). Our sample is by orders of magnitude smaller than that of Strong (let aside that of García-Robledo et al. 2013), so that comparisons must be judged cautiously. At La Gamba, it seems that in species where we found more than 20 individuals on more than one plant species, the beetles clearly preferred some plants over others, as indicated by their numbers in the sample. We found *Cephaloleia belti* on 12 Zingiberales plants, but only on five *Heliconia*-species (*H. latispatha*, *H. rostrata*, *H. stricta*, *H. vaginalis*, *H. wagneriana*) were there more than 10 individuals, on the remaining seven plants there were only one or two. Similarly, 37 individuals of *Cephaloleia championi* occurred on three plant species, but on *Calathea*

thea lutea there were 33, whereas on *C. platystachya* and *Heliconia imbricata* we found only three and one individual(s), respectively. Other examples are *C. dilaticollis* and *C. distincta*, see Tab. 1. However, due to the many empty cells in Table 1, only for *C. belti* could a statistical test be performed. It revealed a χ^2 of 524.84 and a $p < 0.001$ for the distribution of the beetles over the plant species being caused by chance. Since the findings with only four or fewer beetle individuals cannot conclusively indicate that the beetles used the places where we found them as feeding sites, we address these plants as “potential host plants”. We regularly found feeding traces on the surface of the uncoiled leaves. Some traces could be assigned to certain hispine species, following Strong (1977b). However, not all feeding traces were clearly species-specific, and often we found beetles not actually feeding, and in assemblages of several species. Nevertheless, it seems that the rolled-leaf hispines have the ability to exploit plants of more than one family, in contrast to Strong’s (1977b) suggestion. This is corroborated also by Descampe et al. (2008) who found in Panama that the 8 rolled-leaf hispines in their study attacked 4 to 9 of the 11 species of Heliconiaceae and Marantaceae investigated. Also, Meskens et al. (2008) conclude that the host plant spectrum of the rolled-leaf hispine species is broader than previously thought. García-Robledo and Horvitz’s (2012a, b) found in a choice experiment that *C. dilaticollis* – classified as a generalist – accepted *Alpinia purpurata*-leaves as oviposition sites to the same degree as their native host plant *Renealmia alpinia* (Zingiberaceae). At La Gamba, we found no individual of this species in *Alpinia* leaf rolls. This could mean that in the field interspecific competition could have prevented *C. dilaticollis* from entering the *Alpinia* leaf rolls.

It is evident that of all Zingiberales species at La Gamba, *Heliconia latispatha* harboured the greatest number of rolled-leaf hispine species (7), followed by *Calathea lutea* (5) and *Heliconia imbricata* and *H. rostrata* (4 each). The species richness of *H. latispatha* is well documented (Strong 1977a). However, the markedly highest number of hispines on these *Heliconia*-species were *Cephaloleia belti*. Only on *Calathea lutea* we found two hispine species (*C. championi* and *C. dilaticollis*) in roughly comparable numbers.

Due to limitations in sample size, the outstanding case of *Cephaloleia trivittata* on *Costus pulverulentus* does not convincingly demonstrate a high degree of specialisation, especially since we found five *C. trivittata* individuals on unidentified Zingiberaceae. We can interpret this finding at best as an indication of a possible specialist among the species investigated.

Another remarkable observation is that *Cephaloleia distincta* obviously prefers *Alpinia purpurata* but uses sporadically up to six other Zingiberales species. This could mean that *C. distincta* has a high potential to shift host plants when necessary. Differing from Staines (2011), we did not find *C. distincta* on *Calathea* species, while so far this hispine was not reported from *Alpinia*. *Alpinia purpurata* is introduced to the Neotropics, it is native in Malaysia, New Guinea and possibly other parts of South East Asia (GRIN 2013). García-Robledo and Horvitz (2012a, b) did not use *C. distincta* in their experiments. Nevertheless, as these authors have found *C. distincta* exclusively on *Heliconia mariae*, this beetle is certainly not a generalist, even if we have found single individuals on other Zingiberales spp (see Table 1). Further and more detailed stud-

ies are necessary to decide whether our finding was caused by chance (e.g. by factors occurring only in the season in 2009 when we collected beetles), or if they indicate a special case of shift to a novel host plant by a rolled-leaf hispine.

As Fig. 3 shows, there is seemingly no phylogenetic pattern in the hispine-Zingiberales relations at La Gamba. Even if we consider only those relations based on more than ten beetle records per plant species, indicated by the bold lines in the figure, phylogenetic relatedness of the plants or the beetles involved does not seem to play any role. Otherwise, we would have found that closely related beetles use closely related plants. It may well be that phylogenetic factors determine food plant choice on a broader scale so that a phylogenetic pattern, as McKenna and Farrell (2005) found, becomes obvious only on a more inclusive taxonomic level. Alternatively, these factors affect other aspects of feeding behaviour such as choice of the type of plant tissue exploited or the site on the plant where the beetle or its larva actually feeds. As rolled leaves are a limited resource (Seifert 1982; Strong 1977b - even in an area where Zingiberales are planted, as in the station's garden), their actual availability does certainly influence the beetles' choice of host plants. García-Robledo and Horvitz (2009) found that *Cephaloleia*-individuals of four species (*C. dorsalis*, *C. erichsonii*, *C. fenestrata*, *C. placida*) reacted positively to scents of Zingiberales leaves and discriminated in most of the experiments between different plant species (e.g., their host plant against a non-host plant). In the light of these findings, our results could indicate that the chemical signals of possible host plants do not reflect their phylogenetic relationships. Wink (2003) found several such cases in his investigation on secondary metabolites of Fabaceae, Solanaceae, and Lamiaceae.

We could observe feeding only occasionally. Therefore, it is by no means certain that the associations we report here represent indeed trophic interactions. García-Robledo et al. (2013) studied plant-herbivore networks between rolled leaf hispines and Zingiberales over two years at La Selva. They identified the plants digested by the beetles using a three-locus DNA barcode. Our selection of plant species and the beetle species we recorded do not match exactly those of García-Robledo et al. (2013). Nevertheless, our results concur fairly well with theirs: *Cephaloleia belti* and *C. dilatocollis* appear as generalists feeding on plants of more than one Zingiberales family with a preference for Heliconiaceae and Marantaceae, respectively (Table 1, see also García-Robledo and Horvitz 2012a), *Chelobasis bicolor* is restricted to Heliconiaceae. We found *C. trivittata* on *Costus pulverulentus*, a plant these beetles had not consumed in García-Robledo et al.'s (2013) study. Given the low number of three individuals in our sample, this difference is probably biologically insignificant. It is certainly more important that also the plant-herbivore network presented by García-Robledo et al. (2013) does not show a clear phylogenetic pattern when we applied the beetle relationships as given in McKenna and Farrell (2005) and the plant relationships as presented in Marouelli et al. (2010) and Janssen and Bremer (2004).

Strong (1977b) found a correlation between the number of hispine species exploiting plants of the different Zingiberales families and the number of plant species within these families. We compared his finding with our results and found a good

correspondence. It might be of interest that at La Gamba we found the same number of beetles species (8) as Strong found at La Selva on more *Heliconia*-species (9 instead of 5). This difference could indicate that the hispine diversity at La Gamba is lower than could be expected. Strong (1977a) found in his study of species richness of *Heliconia latispatha* herbivores the ratio of actual and possible feeders among the rolled-leaf hispines is 4:5 at Palmar Sur and 4:6 at Golfito, two study sites in the vicinity of La Gamba. This relation is 7:9 at La Gamba, which lies exactly between the two other values. Nevertheless, we should keep in mind that of the 77 individuals found on *H. latispatha*, 57 belonged to *Cephaloleia belti*. Descampe et al. (2008) report a similar relation from Panama. They found – among others – 289 individuals of *C. belti* and 44 of *C. instabilis* on *Heliconia latispatha*, but none of *C. dilaticollis*. At La Gamba, we had three individuals of the two latter species each on *H. latispatha*.

The other possible meaningful result is that we found four adult beetles of three species in banana leaf rolls. Banana (*Musa x paradisiaca*) was introduced to this area of Central America by man ca. 120 years ago (Vandermeer 1983), *Alpinia* certainly not earlier, as it is used as an ornamental plant by people who worked on banana plantations. The land immediately to the north of the La Gamba station was a banana plantation until the 1980s, when United Brands abandoned the region (Hein 1990: 228). Our observations – albeit minute – could point to a beginning integration of *Musa* spp. into the food web of hispines and Zingiberales. Staines (1996) recorded two *Cephaloleia* species on banana, García-Robledo and Horvitz (2012a, b) found that *C. belti* even preferred feeding on *Musa velutina* over their native host plants in experiments. Nevertheless, in these experiments *C. belti* did not lay eggs on *Musa*-leaves. Since we found only four adults on *Musa*, we do not draw any further conclusion. It would be worth checking banana plants on cultivated areas near the station La Gamba for utilisation by hispines.

Strong (1982a, b) reported that several species of rolled-leaf hispines co-existed harmoniously on the same food plant and even in the same leaf roll. We, too, often found individuals of more than one hispine species inside the same leaf roll. However, it is remarkable that different hispine species tended to co-occur inside the leaf rolls considerably less often than expected by chance (see Table 2). *Cephaloleia belti* showed euryoecious behaviour not only with respect to food plants but also to tolerated allospecifics. We found individuals of this species in assemblages together with six other rolled-leaf hispines, but never with *Cephaloleia histrionica* or *C. trivittata*. Moreover, 120 (71 %) of the 170 individuals discovered, had no other hispine companion in “their” leaf roll (which could be expected by chance only with a probability of less than 0.001). Similarly, 12 (75 %) of the 16 *C. histrionica* lived in leaf rolls as the only hispine. Of course, the low numbers of, e.g. *Chelobasis bicolor* and *Ch. perplexa*, and *Cephaloleia instabilis* allow only tentative conclusions. *Cephaloleia championi*, *C. distincta*, and *Ch. perplexa* showed a medium level of interspecific tolerance, *C. dilaticollis*, *C. instabilis*, *Ch. bicolor* and *C. histrionica* a decreasing lower level, whereas *C. trivittata* could be a rare example not only of monophagy (on *Costus pulverulentus*) but also of interspecific intolerance. That the probability of error for *C. distincta* to be found together with another hispine species is 0.001 is no reliable evidence for a marked inter-

specific intolerance since 11 individuals co-occurred with *C. belti* (so that the p-value for an aggregation of *C. distincta* with any other hispine species is 1.000).

Although it was already reported by Baly in 1885 that *Cephaloleia*-individuals were found “often in company with species of *Calophaena* (Carabidae)” (p. 8), it was to our knowledge not mentioned in the modern papers on the biology of rolled-leaf hispines. We found 29 individuals of *Calophaena ligata* in the lumen of *Calathea-lutea*-leaf-rolls. Obviously, they co-existed harmoniously with the hispines in their leaf rolls (*C. belti*, *C. championi*, *C. dilaticollis*, *C. histrionica*, and *Ch. perplexa*). The genus *Calophaena* belongs to the tribe Harpalini, which comprises many phytophagous species (see, e.g., Lawrence and Britton 1994: 87). One could, therefore, presume that adults of *Calophaena ligata* feed on the plants rather than on hispine beetles. Possibly, they could occasionally prey upon larvae of rolled-leaf hispines. We cannot exclude this possibility, but we regard it as very unlikely because we never saw hispine larvae exposed on the inner surface of a leaf roll (but always feeding between two layers of a rolled-leaf), whereas we never detected one of the carabid beetles in the narrow space between two leaf layers. The adults of *Calophaena ligata* are more than twice as long as, e.g., *C. championi* adults, and their body appendages are long and slender (Fig. 4), in contrast to the stout legs and antennae of the hispines. Although the body of the *Calophaena* beetles is depressed, as compared to epigeal ground beetles, it seems that they would have problems if they intended to hunt between leaf layers of Zingiberales plants. We speculate that the co-occurrence of *Calophaena ligata* and certain rolled-leaf hispines is a result of parallel host plant choice rather than of interspecific beetle attraction or exclusive interspecific tolerance. The syn-ecological relation of rolled-leaf hispines and *Calophaena* ground beetles remains enigmatic. We suspect that there is hardly any direct interaction but that the individuals of these two beetle families meet accidentally.

After all, it is interesting to note that *Calophaena*-individuals have exclusively been found on *Calathea lutea* plants. Daniel Blanke reported in his unpublished diploma thesis (“Autökologie der Laufkäfer der Gattung *Calophaena* – Coleoptera, Carabidae – im Piedras Blancas Nationalpark, Costa Rica”, University of Bonn 2010, supervised by M.S.) that he had found 389 individuals of this species at La Gamba, of which 387 were discovered on *Calathea lutea*. He saw them moving around on lower leaf surfaces and gnawing at the base of leaves at dark. He speculates that the ground beetles take up flavonoids from the plant and use them to produce their aposematic colouration (see Fig. 4). This idea appears plausible since *C. lutea*-leaves are outstandingly rich among Zingiberales in flavonoid content (Williams and Harborne 1977). Possibly, also *Cephaloleia championi*, which shows a similar colour pattern as *Calophaena ligata* (see Fig. 1), prefers *Calathea lutea* over other Zingiberales due to the high content in flavonoids.

The core conclusions from our results are: The rolled-leaf hispines at La Gamba have been found on Zingiberales, as already known from other regions in Central America (La Selva, Panama). However, we did not systematically check other plants. Among the beetles we collected were some with a broader spectrum of potential host plants, above all *Cephaloleia belti*, while other species live on fewer plant species or even only on one (*C. trivittata* on *Costus pulverulentus*). However, the many observations of

few individuals or singletons of a beetle species on several Zingiberales when the majority of their conspecifics was found on one or only a few other Zingiberales underpin the statement of Descampe et al. (2008) that the host plant spectrum of the rolled-leaf hispines is certainly broader than assumed in Strong's earlier publications. Similar conclusions apply to the multi-species assemblages, which we often discovered. However, the high proportion of individuals found without allospecific company, even if the minority obviously is interspecifically tolerant, could mean that the rolled-leaf hispines prefer un-occupied leaf rolls over occupied ones.

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Subsocial Neotropical Doryphorini (Chrysomelidae, Chrysomelinae): new observations on behavior, host plants and systematics¹

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Abstract

A summary of literature, documented observations and field studies finds evidence that mothers actively defend offspring in at least eight species and three genera of Neotropical Chrysomelinae associated with two host plant families. Reports on three *Doryphora* species reveal that all are oviparous and feed on vines in the Apocyanaceae. Mothers in the two subsocial species defend eggs and larvae by straddling, blocking access at the petiole and greeting potential predators with leaf-shaking and jerky advances. A less aggressive form of maternal care is found in two *Platyphora* and four *Proseicela* species associated with Solanaceae, shrubs and small trees. For these and other morphologically similar taxa associated with Solanaceae, genetic distances support morphology-based taxonomy at the species level, reveal one new species, but raise questions regarding boundaries separating genera. We urge continued study of these magnificent insects, their enemies and their defenses, both behavioral and chemical, especially in forests along the eastern versant of the Central and South American cordillera.

Keywords

Maternal care, Subsociality, Coleoptera, Chrysomelidae, Chrysomelinae, Neotropical

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Introduction

Arthropod parents influence the survival prospects of offspring in a multitude of ways. When parents bring resources to larvae, guide larvae to resources or actively shield offspring from predators and parasitoids, they are engaging in subsocial behavior (Alexander 1974, Wilson 1971, 1975). The study of subsocial behavior, additional to its intrinsic appeal, offers a perspective on selective forces at work during the earliest stages of insect social evolution, stages through which eusocial insects passed long ago. Understanding how environmental factors elevate the reproductive success of parents who defend offspring above those who abandon and direct investment toward future offspring remains a considerable challenge (Gilbert and Manica 2010, Tallamy and Wood 1986). This challenge is especially daunting for rare and diverse tropical beetles narrowly associated with rare host plants.

The Coleoptera include numerous examples of independently evolved subsocial behavior where the importance of competition, resource provisioning and defensive parental behavior can be examined (Costa 2006, Jordal et al. 2011). Within the leaf beetles, maternal care of offspring is found in just two of 15 subfamilies, the broad-shouldered leaf beetles (Chrysomelinae) and the tortoise beetles (Cassidinae), groups possibly more vulnerable to predators and parasitoids due to slow-moving and exposed immature stages (Jeffries and Lawton 1984, Cox 1994, Cornell and Hawkins 1995). Chrysomelinae larvae are often aposematic, aggregated (Santiago-Blay et al. 2012), chemically defended by eversible abdominal glands (Pasteels et al. 1994, Dobler et al. 2012) and, in some taxa, guarded by adults capable of secreting toxins obtained by sequestration of plant secondary metabolites (Pasteels et al. 2001, Termonia et al. 2002).

Below we review evidence of restricted host plant use and the presence of defensive maternal behavior in eight species of Neotropical Chrysomelinae. Detailed observations from one of these species suggests that mothers modify leaf resources in advance of bearing live offspring, and later block and herd movement of larvae among leaves on the same food plant. To clarify species relationships among Solanaceae-feeding species, some varying only in minor aspects of elytra color, we present genetic distance estimates obtained from mitochondrial sequence data for eleven described and one undescribed species, including both maternal care and non-care species.

Methods

Tissue samples are preserved in ethanol at -80°C , and pinned adult voucher specimens are stored in the working collections of D.W., both at the Smithsonian Tropical Research Institute, Tupper Research and Conference Center, Panama City, Panama. Field observations reported below come from field notes and photographic records made at diverse occasions in Central and South America over the past 20 years.

DNA extraction, sequencing and analysis

Adult Chrysomelinae were stored in 95% ethanol at -80°C , flight muscle removed and ground in 180 μl ATL tissue lysis buffer (Qiagen Inc., Valencia, CA USA) and 20 μl proteinase K with a sterile pestle, vortexed for 10 s and incubated overnight at 55°C . Following incubation, 200 μl AL lysis buffer (Qiagen Inc.) was added and the sample was heated at 70°C for 10 min, then 200 μl molecular grade ethanol was added to each sample. This mixture was then pipetted into a DNeasy mini spin column and centrifuged at 8000 rpm ($\sim 6000\text{ g}$) for 1 min, then the flow-through and collection tube were discarded. The DNeasy mini spin column was placed in a new 2-ml collection tube and 500 μl wash buffer AW1 (Qiagen, Inc.) was added, the sample was centrifuged for 1 min at 8000 rpm, then the flow-through and collection tube were discarded. Again a new collection tube was used, 500 μl wash buffer AW2 (Qiagen, Inc.) was added and the sample centrifuged for 3 min at 14,000 rpm (20,000 g); the collection tube was then discarded. The mini column was placed in a 1.5-ml tube and 200 μl AE elution buffer (Qiagen, Inc.) was added, the sample was incubated for 2.5 min at room temperature, and the sample centrifuged for 1 min at 8000 rpm ($\sim 6000\text{ g}$). Extractions were held at -20°C between use, and at -80°C for long-term storage. The primers: C1-J-1718F (26-mer; 5'-GGA GGA TTT GGA AAT TGA TTA GTT CC-3') and C1-N-2191 (26-mer; CCC GGT AAA ATT AAA ATA TAA ACT TC-3') (Simon et al. 1994) were used to amplify the mitochondrial cytochrome oxidase 1 gene (COI) in a volume of 20 μl : 1 μl DNA sample, 2 μl 10x buffer (Applied Biosystems Inc., Foster City, CA, USA), 2 μl MgCl_2 (25 μM), 1 μl nucleotide mix (8 mM each), 0.8 μl dimethyl sulfoxide 5%, 1 μl each primer (20 mM), 0.2 U *Taq* DNA polymerase (AmpliTaq, Applied Biosystems Inc.) plus sterile water. The PCR cycling conditions were: 94°C for 2 min, 10 cycles of 94°C for 30 s, 46°C for 30 min, 72°C for 45 min, then 24 cycles of 94°C for 30 s, 48°C for 30 min, 72°C for 45 min, and finally 72°C for 10 min and 10°C for 2 min.

Forward and Reverse sequences were combined and reconciled in Sequencher v5 (Gene Codes Corporation, Ann Arbor, MI, USA) and trimmed, leaving a single 472 bp fragment, which was then translated to amino acids and found free of stop codons. Sequences from ten species were combined with three sequences from GenBank creating an ingroup of 12 species (20 individuals) and a single outgroup species. Where possible we included two separate individuals from the same population. New sequences were deposited in GenBank under accession numbers in Table 1. Evolutionary relationships of the samples were inferred by Bayesian analysis, with 2 million generations, and Maximum Likelihood analysis, with 100 bootstrap pseudo-replications. The ideal partitioning strategy and models of nucleotide substitution were determined using PartitionFinder v.1.0.1 (Lanfear et al. 2012) and this scheme was implemented in both analyses. The strategy was determined with three character sets, one for each codon position of COI. The partitioning scheme divided the dataset in two partitions: Partition (1): first and second codon positions of COI; Partition (2): third codon positions of COI. Pairwise genetic distance estimates were calculated in MEGA version 5.0 (Tamura et al. 2011) using the Kimura 2-parameter model.

Table 1. Apocyanaceae and Solanaceae feeding taxa mentioned in the text, collection and host plant information, life history characteristics, accession numbers and references.

Chrysomelinae species	Location	Host Plant Family ¹	Host Plant Species	Larval Group Defense	Maternal Care	Reproduction	Genbank Accession Numbers	References
<i>Doryphora paykulli</i> (Stål, 1859)	Gamboia, Panama Province, Panama	Apo	<i>Prestonia seemannii</i> Miers (subfamily Apocynoideae)	aggregated	yes	oviparous	-	new observation
<i>Doryphora reticulata</i> (Fabricius, 1787)	Boqueirão Reserve, Minas Gerais State, Brazil	Apo	<i>Prestonia tomentosa</i> R. Br. (subfamily Apocynoideae)	aggregated	yes	oviparous	-	new observation
<i>Doryphora</i> sp. near <i>D. punctatissima</i> (Olivier, 1790)	El Porvenir, Meta Province, Colombia	Apo	<i>Prestonia isibmica</i> Woodson (subfamily Apocynoideae)	aggregated	no	oviparous	-	Eberhard 1981
<i>Eugorycha melanostoma</i> (Stål, 1859)	Serra do Japi, Jundiá, São Paulo State, Brazil	Sol	<i>Solanum</i> sp.	aggregated	no	larviparous	-	Vasconcellos-Neto and Jolivet 1994
<i>Platyphora amabilis</i> (Baly, 1859)	Yasuni, Orellana Province, Ecuador	Sol	<i>Solanum</i> sp.	?	?	?	AY055517	new observation
<i>Platyphora anastomozans</i> (Perry, 1832)	Serra do Japi, Jundiá, São Paulo State, Brazil	Sol	<i>Solanum bullatum</i> Vell., <i>S. mauritianum</i> (Scopoli), <i>S. sancta-catarinae</i> Dunal, <i>S. megalochiton</i> Mart., <i>S. rufescens</i> Sendt.	aggregated	no	larviparous	KF251110, KF251111	Vasconcellos-Neto and Jolivet 1994
<i>Platyphora atilica</i> (Olivier, 1807)	Montagne de Kaw, Roura Commune, French Guiana	Sol	<i>Solanum rugosum</i> Dunal, <i>S. torium</i> Sw.	solitary	no	larviparous	KF251112, KF251113	new observation
<i>Platyphora conivna</i> (Stål, 1858)	Serra do Japi, Jundiá, São Paulo State, Brazil	Sol	<i>Solanum</i> spp.	aggregated ^{2,3}	no	larviparous	-	Medeiros and Vasconcellos-Neto 1994, Vasconcellos-Neto and Jolivet 1994
<i>Platyphora fasciatomaculata</i> (Stål, 1857)	Ijuí, Rio Grande do Sul State, Brazil	Sol	<i>Solanum mauritianum</i>	aggregated	no	larviparous	-	Medeiros et al. 1996
<i>Platyphora microspina</i> Bechyné, 1954	Cerro Campana, Panama Province	Sol	<i>Markea megalandra</i> (Dunal)	aggregated	yes	larviparous	KF251120	new observation
<i>Platyphora nigronotata</i> (Stål, 1857)	Serra do Japi, Jundiá, São Paulo State, Brazil	Sol	<i>Solanum bullatum</i> , <i>S. mauritianum</i> , <i>S. sancta-catarinae</i> , <i>S. megalochiton</i>	aggregated	no	larviparous	KF251121	Medeiros 1991
<i>Platyphora nitidissima</i> (Stål, 1857)	Serra do Japi, Jundiá, São Paulo State, Brazil	Sol	<i>Solanum bullatum</i>	aggregated	no	larviparous	-	Medeiros and Vasconcellos-Neto 1994, Vasconcellos-Neto and Jolivet 1994

Chrysomelinae species	Location	Host Plant Family ¹	Host Plant Species	Larval Group Defense	Maternal Care	Reproduction	Genbank Accession Numbers	References
<i>Platyphora quadrisignata</i> (Germar, 1824)	Serra do Japi, Jundiá, São Paulo State, Brazil	Sol	<i>Solanum variabile</i> Mart., <i>S. fastigiatum</i> Willd.	aggregated ²	no	larviparous	-	Medeiros and Vasconcellos-Neto 1994, Vasconcellos-Neto and Jolivet 1994, Medeiros et al. 1996
<i>Platyphora selva</i> Daccordi, 1994	La Selva Biological Station, Heredia Province, Costa Rica	Sol	<i>Solanum</i> (Witheringia) <i>heteroclitia</i> .	aggregated	yes	larviparous	-	Choe 1989
<i>Platyphora sphaerica</i> (Jacoby, 1903)	Serra de Baturite, Fortaleza, Brazil	Sol	<i>Solanaceae</i> , 2 spp.	solitary	no	larviparous	AY055529	Termonia et al. 2002
<i>Platyphora vinula</i> (Stål, 1858)	Ijuí, Rio Grande do Sul State, Brazil	Sol	<i>Solanum</i> sp. aff. <i>S. megalochiton</i> , <i>S. sancta-catarinae</i>	aggregated	no	larviparous	-	Medeiros et al. 1996
<i>Proseicela antennalis</i> (Kirsch, 1883)	Reventador, Napo Province, Ecuador	Sol	<i>Solanum</i> sp.	?	?	?	KF251114, KF251115	new observation
<i>Proseicela bicruciatata</i> Jacoby, 1880	Yanayacu Biological Station, Napo Province, Ecuador	Sol	<i>Solanum abitaquense</i> S. Knapp	aggregated	yes	larviparous	KF251116, KF251117	new observation
<i>Proseicela crucigera</i> (Sahlberg, 1823)	Serra do Japi, Jundiá, São Paulo State, Brazil	Sol	<i>Solanum decompositiflorum</i> Sendtn.	aggregated	no	larviparous	-	Medeiros 1991, Medeiros and Vasconcellos-Neto 1994
<i>Proseicela flavipennis</i> (Erichson, 1847)	Reventador, Napo Province, Ecuador	Sol	<i>Solanum abitaquense</i> S. Knapp	?	?	?	KF251118, KF251119	new observation
<i>Proseicela</i> sp. n. "Yasuni"	Yasuni, Orellana Province, Ecuador	Sol	<i>Guatresia</i> sp.	aggregated	yes	larviparous	KF251126, KF251127	new observation
<i>Proseicela spectabilis</i> (Baly, 1858)	Río Malo & Reventador, Napo Province, Ecuador	Sol	<i>Solanum abitaquense</i> S. Knapp, <i>Solanum</i> sp. (sect. Dulcamara)	aggregated	yes	larviparous	KF251122, KF251123	new observation
<i>Proseicela vittata</i> (Fabricius, 1781)	Montagne de Kaw, Roura Commune, French Guiana	Sol	<i>Solanum morii</i> S. Knapp	aggregated	yes	larviparous	KF251124, KF251125	new observation
<i>Stilodes modesta</i> Jacoby, 1882	Cerro Campana, Panama Province, Panama	Mal	<i>Banisteriopsis</i> sp.	aggregated	no	oviparous	AY055522	new observation

¹ Apo=Apocynaceae, Ast=Asteraceae, Sol=Solanaceae, Mal=Malpighiaceae²=nocturnally active³=larvae cut and cover themselves with trichomes

Results

Field observations of behavior and natural history

Doryphora paykulli (Stål, 1859)

http://species-id.net/wiki/Doryphora_paykulli

Remarks. According to Blackwelder (1982) the range of this large beetle (19.8 ± 1.5 mm, $n=6$) (Figs 1a–c) extends from Mexico to Nicaragua. However, specimens collected later (L.D. Gomez near San Vito, Costa Rica; H. Stockwell, Cerro Campana, Panama Prv., 30 V 70 and 18 VII 76; D.M.W., Los Santos Prv., Cerro Canajagua, 25 V 92 and Colon Prv., Cerro Galera, 1 V 02; M. Cuignet, Colon Prv. Sta. Rita Ridge Rd. km 2, 2 XI 02; S. Van Bael, Bocas del Toro Prv., Chiriqui Gde., 17 I 04; M. Membache, 1 VI 92, Colon Prv., Gamboa; S. Lankowski, Panama Prv., Parque Metropolitan, 15 IV 07) indicate the species extends to at least 10 km east of the Panama Canal. These records plus observations of a *D. paykulli* adult following a tightly arranged group of larvae moving between leaves on their food plant near Chiriquí Grande, Bocas del Toro Province (S. Van Bael, pers. comm.) documents the presence of the species in the Caribbean as well as in the Pacific lowlands of Panama and provides the first unequivocal record of subsocial habits for the species.

Subsequently, individual *D. paykulli* adults were observed (D.W., S.L.) during late April and early May of 2005 moving slowly and feeding among low vegetation. Pairs of individuals were observed interacting aggressively on the small leaf fragments remaining on the host plant, *Prestonia seemannii* Miers (Apocynaceae) under late dry season conditions along trails in the Parque Metropolitan (elevation 30 m, $8^{\circ}59.24'N$; $79^{\circ}32.797'W$), Panama City. Whether these were contests over resources or precopulatory courtship is unclear, however, analysis of video taken of one of these interactions shows the use of the mesosternal horn in dislodging a competitor, much as described by Eberhard (1981) for the closely related beetle, *Doryphora* sp. near *punctatissima*. Adult females were discovered during mid-May of both 2005 and 2007 in close proximity to eggs attached to the underside of cupped, newly expanded *P. seemannii* leaves (Fig. 1a) on the western slope of Cerro Pelado, Gamboa (elevation 95 m, $9^{\circ}7.29'N$; $79^{\circ}41.78'W$). Eggs measured 3.5×0.9 mm and at first were an opaque, cherry-red, with the chorion becoming transparent and larvae distinguishable as development progressed. Clutches initially contained ten eggs with roughly ten new eggs added each day over the course of 3 to 5 days. The female became noticeably more defensive the second day of oviposition, straddling the eggs and jerking from side to side apparently in response to movement by the observer.

Larval emergence began on days 6 and 7 following first oviposition with clutches ($n=3$) at that time containing from 40–50 eggs. Within 24 hours after larvae began to emerge many of the original eggs were missing, apparently consumed by early-emerging larvae such that clutches were reduced to 8, 15 and 20 surviving larvae accompanied by some intact, opaque, less-developed eggs and opened eggs with red-colored residues of

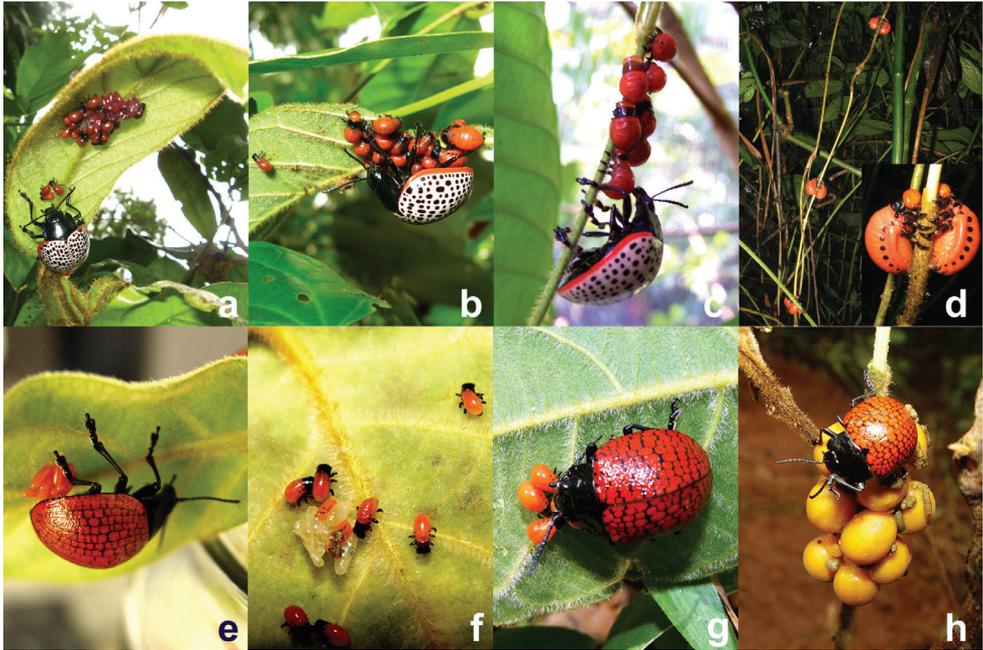


Figure 1. Maternal care providing *Doryphora* species, **a** *D. paykulli* female with eggs and first instar larvae under an apical leaf of *Prestonia seemanii* (photo by S.L.) **b** female straddling a mix of first and second instar larvae (photo by S.L.) **c** *D. paykulli* larvae moving to a new leaf followed by their mother (photo by S. Van Bael) **d** *D. paykulli* larvae stripping the cortex of their host while descending in pairs to pupate, (photo by D.W.) **e** *D. reticulata* ovipositing under apical leaf of *Prestonia tomentosa* in Central Brazil (photo by F.F.) **f** *D. reticulata* larvae on the natal leaf (photo by F.F.) **g** female *D. reticulata* straddling first instar larvae (photo by F.F.) **h** *D. reticulata* female tending fully-developed larvae at the base of the food plant just prior to pupating underground (photo by F.F.).

once-developing larvae visible under close inspection (Fig. 1). One day later an adult was observed (S.L.) returning to a natal group after having fallen from the plant on a detached leaf, then deposit a single egg which was immediately consumed by a nearby larva. First instar larvae (1.5–2.0 mm in length) had small black heads at the time of emergence, these easily distinguished them from second instar larvae appearing, 1 to 2 days later with red head capsules and a larger and more rotund appearance. Larvae expanded rapidly in size following their first meals but did not feed on leaf tissue until after the first molt, 2 to 3 days after emerging from the egg. Mothers at times tightly straddled their aggregated first and second instar larvae on the natal leaf, preventing their advance down the leaf petiole (Fig. 1b). While guarding, mothers reacted aggressively by charging to the edge of the leaf when a thin stick was introduced to the area by an observer. Charges, stamping and shaking continued for at least two minutes after the stimulus was presented and removed. The strongest reaction was given to a camera held approximately 10 cm under and to the side of the natal leaf. The mother seemed to be reacting to the camera lens—suggesting that a mirror held near guarding

mothers might provide a non-invasive means of assessing defensiveness. On one occasion an *Ectatomma tuberculatum* (Olivier) worker was observed to pass by the base of the petiole, eliciting aggressive shaking of the natal leaf and short charges, after which the ant reversed course and departed that portion of the plant. As larvae became larger and began moving between leaves the intensity of the mothers' reactions to foreign stimuli appeared to subside. Increasingly, mothers were seen feeding on leaves and leaf petioles, rather than guarding, as larval development proceeded.

Once the natal leaf was consumed, larvae began moving down the petiole to the stem, where they then moved either up or down in smaller groups to other leaves, sometimes moving as solitary individuals. Mothers, often fed from the pedicel of the leaf just consumed, occasionally accompanied by one or two larvae. Mothers actively trampled upon the backs of larvae still located on the pedicel, in effect pushing them away from the leaf and toward the stem. Mothers on other occasions stepped on and over larvae, rapidly tapping larvae with antennae and tarsi until they reversed direction. After leaving the natal leaf, mothers resumed guarding one of the several larval groups that reassembled. However, some groups continued to split into ever smaller units and moved to adjoining leaves and stems, leaving mothers guarding smaller sets of offspring and spending more time travelling among groups in what seemed to the observer as an effort to herd offspring back together (Fig. 1c). Mothers also increasingly divided their time between guarding and feeding on the cortex of the stem, girdling the vine over distances from a few cm to nearly 1 m. Seven days following eclosion larvae were arranged largely in doublets, girdling stems as intact leaves had disappeared in substantial sections of the plant. Larvae then descended in unguarded pairs to pupation sites by backing down the stem from which all cortex was stripped (Fig. 1d), effectively killing that section of the plant. By day eleven, most larvae had descended the host plant and moved along small above-ground roots into the leaf litter. One mother was last seen guarding two slow-developing larvae high (2 m) on the plant thirteen days after oviposition. Development from first oviposition to larvae wandering on the ground took approximately 20 days. Several larvae collected and placed in a plastic container with moist leaves, molted at day 4 and eclosed as teneral adults on days 18 and 19, a metamorphosis period slightly shorter than the 24 days estimated for *D. sp. near punctatissima* by Eberhard (1981). Development from oviposition to the eclosion of adults in mid-June required 35 days. Monthly visits to the study area throughout the rest of the year were successful in finding solitary, feeding adults on nearby host plants but not in finding signs of additional reproductive activity. Thus, this species seems to have but a single generation per year timed to the period of accelerated leaf growth by its host plant. The nearly synchronous May onset of reproduction in both *D. paykulli* in Panama and *D. sp. near punctatissima* in Colombia (Eberhard 1981) is likely the product of similar climate regimes in the two species' ranges and subsequent effects on host phenology. Finally, the high morphological similarity of these neighboring species, the presence of larval cannibalism in both, but the presence of maternal care in only one, raises intriguing questions regarding the lability of defensive behaviors and underscores the importance of reconstructing phylogenetic relationships for as many *Doryphora* species as possible.

***Doryphora reticulata* Fabricius, 1787**

http://species-id.net/wiki/Doryphora_reticulata

Remarks. Recent observations by F.F. reveal clearly that maternal care is expressed by *Doryphora (Megistomela) reticulata* (Fabr.) in the cerrado of south-central Brazil (Fig. 5) (see also photo in Chaboo 2011). Photographs of this species in the Boqueirão Biological Reserve, Minas Gerais of Brazil (elevation 1200 m; 21°20.76'S; 44°59.49'W) in 2005 clearly show behaviors strikingly similar to that observed in *D. paykulli* in Panama. Females oviposit on the underside of partially-expanded, apical leaves of *Prestonia tomentosa* (Apocynaceae) (Fig. 1e, f). Larvae emerge and are tightly straddle-guarded by the female (Fig. 1g), but unlike *D. paykulli* and *D. sp.* near *punctatissima*, no larval cannibalism of eggs was observed. Larvae guarded by the mother continued to feed on leaves and strip cortex, eventually descending to the ground tended by the mother prior to pupating nearby in the soil (Fig. 1h). Indeed, of the many Chrysomelinae species associated with Solanaceae and other plant families studied at Serra do Japi and other sites near Campinas in Central Brazil (Table 1), *D. reticulata* is the only species in which mothers are known to actively guard their larval brood. Inferences regarding subsocial habits in *Pl. conviva* (Reid et al. 2009) are incorrect according to J. Vasconcellos-Neto (personal communication, 2013). Further, ongoing studies in the eastern lowlands of Bolivia by one of the authors (D.W.) have found no evidence of maternal care occurring in any of 16 species of Doryphorini.

***Platyphora selva* Daccordi, 1994**

http://species-id.net/wiki/Platyphora_selva

Remarks. Within New World Chrysomelinae, reports of subsociality until recently were limited to a single species studied at the La Selva Field station in the Atlantic lowlands of Costa Rica (Choe 1989). However a misidentification of that species (not by the author) lead to erroneous attribution of subsocial behavior to *Labidomera suturalis*, rather than to an unidentified species of *Platyphora*. The species was subsequently described and named *Platyphora selva* by Daccordi without comments on Choe's behavioral observations (Daccordi 1993). As noted by Reid et al. (2009), this first record of subsocial behavior in Neotropical chrysomelines led to a number of reports citing the original paper and repeating the taxonomic error (e.g. Windsor and Choe 1994, Kudô and Hasegawa 2003, Costa 2006).

Choe (1989) observed 18 guarding *Pl. selva* females in two different years, all feeding on *Lycianthes (Witheringia) heteroclita* Sendtm. (Solanaceae) in the Atlantic lowlands of Costa Rica. His observations were remarkable in first describing how females of this species tightly guarded offspring by straddling. By removing mothers from roughly half of the families, he was able to demonstrate that guarding was highly effective in preventing predation by the gigantic ponerine ant, *Paraponera clavata* Fab. The importance of maternal defenses in reducing losses to parasitoids, however, was

not investigated. Further, it was noted that mothers always guarded groups of four or fewer larvae; but eggs of the beetle were never observed during the study. From observations of related taxa (see below) we now suspect that *Pl. selva* is not oviparous, but instead deposits temporally isolated clutches of four larvae. This inference remains to be documented and is based on the habits of the morphologically similar species, *Pl. microspina*, which occurs widely (but rarely) in neighboring Panama. Regrettably, sequence data are not yet available for *Pl. selva*.

***Platyphora microspina* (Bechyně, 1954)**

http://species-id.net/wiki/Platyphora_microspina

Remarks. *Platyphora microspina* was initially observed on Cerro Campana (Parque Nacional Altos de Campana), along a ridgeline approximately 50 m west and up-slope from the Podocarpus trail in July 1999 (elevation 900 m; 8°41.07'N; 79°55.82'W). Large numbers of adult and immature beetles were observed feeding on *Markea megalandra* (Dunal), a woody hemiepiphyte which grows within the canopy of forests at elevations of 1000–2000 m in Western and Central Panama (Correa et al. 2004). Larvae and adults of *Pl. microspina* (Fig. 2a) were largely associated with quick-growing sprouts coming from a portion of the plant damaged earlier by limb fall. A small number of *Pl. microspina* including one female tending three small and partially-sclerotized larvae were moved to a terrarium in an air conditioned laboratory containing host plant cuttings to facilitate observations. Larvae remained physically in contact with one another, often beneath one or more legs of the mother during the first three days. However, as larvae grew in size and spent more time feeding, the mother moved to the side of the group for the remaining 10–12 days of development and feeding (Fig. 2b). Single larvae occasionally left the aggregation, apparently to find new leaves, and through alternative bouts of substrate tapping with the tip of the abdomen—approximately one to two taps per second for two to three minutes—isolated larvae appeared able to call or stimulate their siblings and mother to visit new feeding sites (Fig. 2c). The mother was also observed physically nudging inactive larvae. As the first cohort neared the end of its feeding period the mother deposited another cohort of 4 larvae, briefly leaving 7 larvae of two distinct cohorts and size classes together under the mothers care (Fig. 2d). One day later larvae in the first cohort fell to the base of the terrarium, became inert and later pupated. An additional two cohorts produced by the same mother, each containing 4 larvae, were subsequently observed in the lab. Larvipositions occurred over a span of 28 days, 10 days between cohort 2 and 3, 17 days between cohorts 3 and 4. The larval feeding period for the second cohort lasted 21 days. The non-feeding prepupal period lasted 8 days and the pupal period 7 days. Thus, the interval between larviposition and adult emergence takes approximately 5 weeks in this species. Observations were terminated after the fourth cohort, so reproduction possibly continues for an even longer period in this relatively non-seasonal, premontane forest. The species is dependent upon the continued presence of its hemiepiphytic host plant on Cerro

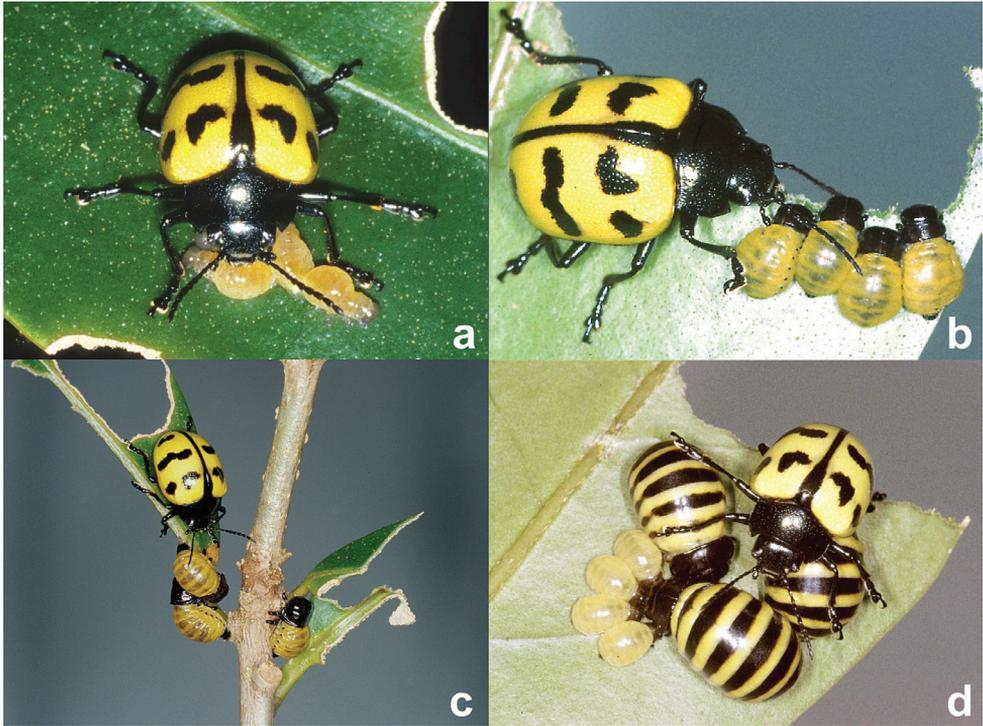


Figure 2. Maternal care providing *Platyphora microspina* in Panama, **a** female with recently deposited larvae (photo by D.W.) **b** female guarding mid-sized larvae (photo by D.W.) **c** female and young larval brood moving among leaves (photo by D.W.) **d** female tending overlapping cohorts of larvae (photo by D.W.).

Campana and similar small refuges along the cordillera passing through Panama. Approximately eight years after these observations were made a single adult specimen resembling *Pl. microspina* was collected at the Cana field station near the Colombian border. One year later at the same site, a group of recently emerged adults of the same species were observed on a woody shrub in the family Solanaceae, 3–5 m to the side of the entrance of to Cana gold mine. Preliminary analysis of its COI gene sequence shows that it is nearly identical to that of *Pl. microspina* on Cerro Campana. Continuing observations at this and similar remote sites, coupled with molecular sequencing, should add considerably to our knowledge of this species and its relationship to *Pl. selva* and similar species in South America.

***Proseicela vittata* (Fabricius, 1781)**

http://species-id.net/wiki/Proseicela_vittata

Remarks. Female *Pr. vittata* (Fig. 3a) were observed by D.W. tending offspring during each of three visits to Montagne de Kaw, French Guiana (elevation 150 m; 4°32.686'N;

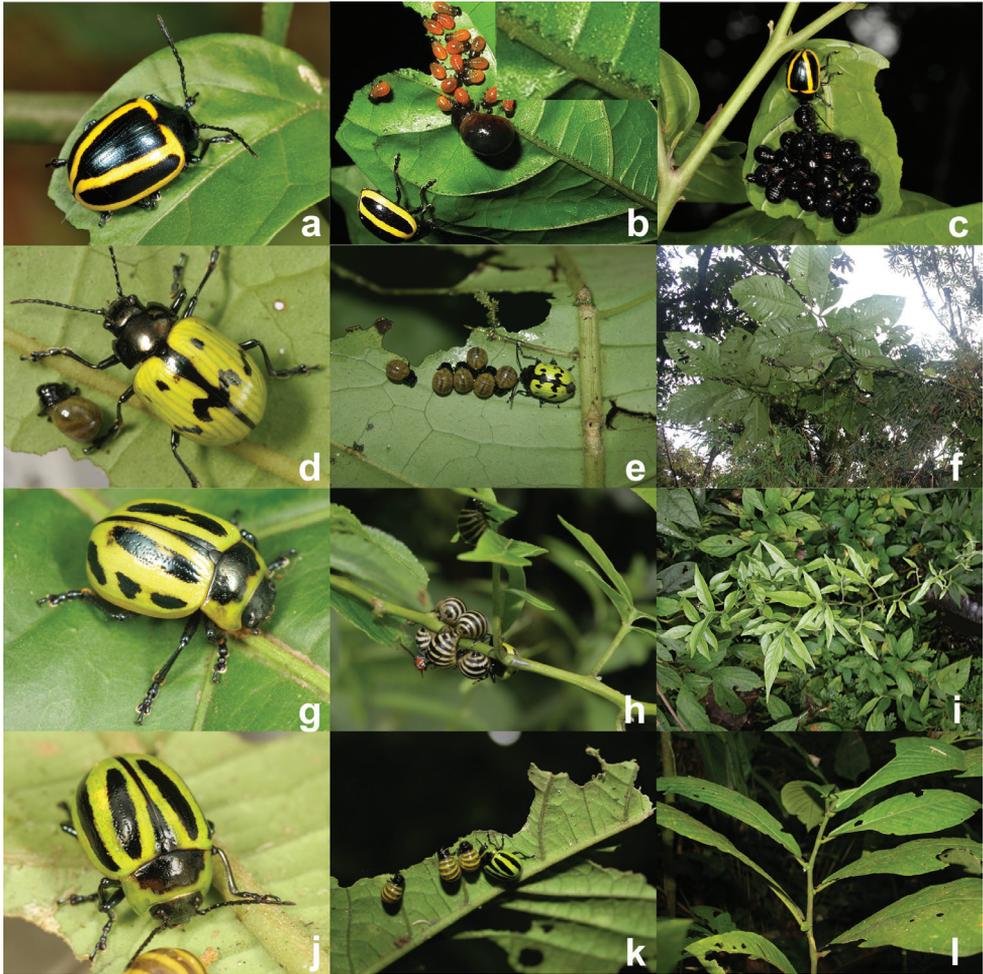


Figure 3. Maternal care providing *Proseicela* species, **a** *Proseicela vittata* adult (Photo by D.W.) **b** *Pr. vittata* female and larvae from two cohorts. Insert shows detail of vein pinching along approximately 1cm of the primary vein (Photo by D.W.) **c** *Pr. vittata* female with late stage larvae (Photo by D.W.) **d** *Pr. bicrucciata* adult female, (photo by G.D.) **e** *Pr. bicrucciata* female tending larvae (photo by G.D.) **f** *Pr. bicrucciata* food plant, *Solanum abitaguense* (photo by G.D.) **g** *Pr. spectabilis* adult (photo by G.D.) **h** *Pr. spectabilis* with nearly full-grown larval brood and tachinid parasitoid (photo by G.D.) **i** *Pr. spectabilis* host plant, *Solanum* sp. (photo by G.D.) **j** *Proseicela* sp. n. adult female (photo by G.D.) **k** the same female tending three feeding larvae feeding on *Cuatresia* sp. (Solanaceae) (photo by G.D.) **l** wider view of the host plant (photo by G.D.).

52°09.151'W), 11–18 April 2010, 25–31 January 2011 and 19 June–2 July 2012. All individuals and family groups were found on a single species of host plant, *Solanum morii* S. Knapp, a 1–3 m shrub with glabrous leaves and pendulous green and white fruit and a colonist of disturbed areas (Plate 121a, Mori et al. 2002). We searched

host plants for this species mainly along logging roads. While numerous individuals and family groups were found on each trip, most groups contained older larvae. Only four females were found tending recently deposited larvae, 11 to 18 in number, which had not begun to feed. The brood tended by one female contained a single large larva feeding and resting beside 17 freshly deposited larvae (Fig. 3b). Within 2 days the single large larva descended alone to pupate, a sign that while broods may overlap in this species (as in *Pl. microspina*) the period of overlap is brief. Normally, individual larvae within cohorts were remarkably similar in size (Fig. 3c). The only exception came if they were observed on day 2 or 3 while molting was in progress. Despite three observation periods per day of approximately 15 min per family, possible predators and parasitoids were rarely observed. And while a *Pachycondyla* ant or a carabid beetle may have been responsible for the abrupt loss of 15 of 17 larvae from one female over night, ongoing predation has yet to be observed in this species.

The 17 larvae belonging to another female were observed to take approximately 30 hours to consume the entire lamina of the natal leaf. While the last of the leaf was being consumed some larvae began to molt while still on the remnants of the natal leaf. The mother maintained a tight grip on the leaf petiole (blocking behavior), but eventually larvae pushed by and began traversing nearby stem and petioles solitarily or in small groups. Commonly families split into two or more separated feeding groups at this stage, with the mother usually remaining with a larger group. Groups often reunited but others remained separated until pupation. The transition from the natal to second leaf appears to be a crucial and dynamic time for larvae and events proceeded differently for most groups. During this period mothers moved actively among different leaves and branches in what seemed to be attempts to herd and reconstitute a single larval group. While we observed what we interpret as herding behavior in most species in this report, its possible importance to group safety and success remains open and in need of experimental study.

***Proseicela bicrucata* Jacoby, 1880**

http://species-id.net/wiki/Proseicela_bicrucata

Remarks. Strong indications of maternal care in *Pr. bicrucata* (Fig. 3d) were gathered by G.D. while walking trails in the montane cloud forest of the Yanayacu Biological Station and Center for Creative Studies, Napo Province, Ecuador (2150 m elevation, 0°36.27'S, 77°53.25'W) during the first week of July 2011. A total of five females were found, each tending small groups of uniform larvae (Fig. 3e), on the undersides of large leaves of *Solanum abitaguense* S. Knapp growing in streamside habitats (Fig. 3f). The group containing the smallest larvae was composed of nine individuals, while groups with larger larvae contained five and six individuals. The group with five larvae had two individuals separated on leaves 20–30 cm in different directions from the central three larvae arranged in a small rosette with heads to the inside and the mother to the side.

***Proseicela spectabilis* (Baly, 1858)**

http://species-id.net/wiki/Proseicela_spectabilis

Remarks. Observations of *Pr. spectabilis* were taken by G.D. while walking the main trail leading to the Cascadas de San Rafael, Reventador, Napo province, Ecuador (1300 m elevation; 0°6.07'S, 77°35.18'W) on July 17, 2011. A single female (Fig. 3g), was perched half above its larvae and half on the branch of its food plant. Six of the seven larvae in the formation were tightly aggregated around the thin stem of the plant, while the seventh larva was on a leaf a few centimeters away. A tachinid fly was present on the dorsal surface of a larva located on the side opposite the mother (Fig. 3h). The fly departed when the observer approached but promptly returned to land on the larva opposite the mother. The host plant (Fig. 3i) was later identified as a nightshade, *Solanum* sp. section *Dulcamara*.

***Proseicela* sp. n. “Yasuni”**

Remarks. A single *Proseicela* adult tending a group of three larvae (Fig. 3j, k) was observed and photographed by G.D. while walking a trail leading to the 50 ha forest dynamics plot, within the Estación Científica Yasuní (ECY), Orellana province, Ecuador (220 m elevation; 0°40.83'S, 76°23.89'W) on 15 July 2011. Following discovery the larvae formed a small rosette with heads to the inside. The female and larvae were attached to the underside of a leaf of *Cuatresia* sp. (Solanaceae) (Fig. 3l). Two other adults of the same species were found nearby, one on a different branch of the same host and the other on an unidentified plant. According to M. Daccordi, this is an undescribed species.

Observations on other Solanaceae-feeding species

Two additional *Proseicela* species are known from understory Solanaceae at Cascadas de San Rafael, Ecuador. Several *Pr. antennalis* adults (Fig. 4a) were collected by D.W. and J.P. from unidentified Solanaceae, 12 August 2001. Additionally, one *Pr. flavipennis* adult (Fig. 4b) was collected by G.D. at the same site on leaves of *Lycianthes glandulosa* (Ruiz & Pav.) Bitter, 17 July 2011. As none of the individuals in these two species were reproducing, their interactions with offspring remain unknown, however high morphological similarity to adults of other *Proseicela* species in the area suggest they are good candidates to be subsocial. *Platyphora amabilis* (Fig. 4c) adults were observed and collected from a well-armed solanaceous food plant growing in open, roadside habitats at the Estación Científica Yasuní (Fig. 4d) by D.W. during August 2001. Larvae of this species were not observed. *Platyphora aulica* (Fig. 4e) was observed on numerous occasions and collected from *Solanum rugosum* Dunal and *S. torvum* Sw. in the same roadside habitats and the same dates in French Guiana as *Pr. vittata*. An adult female *Pl. aulica* placed in a container with abundant food,

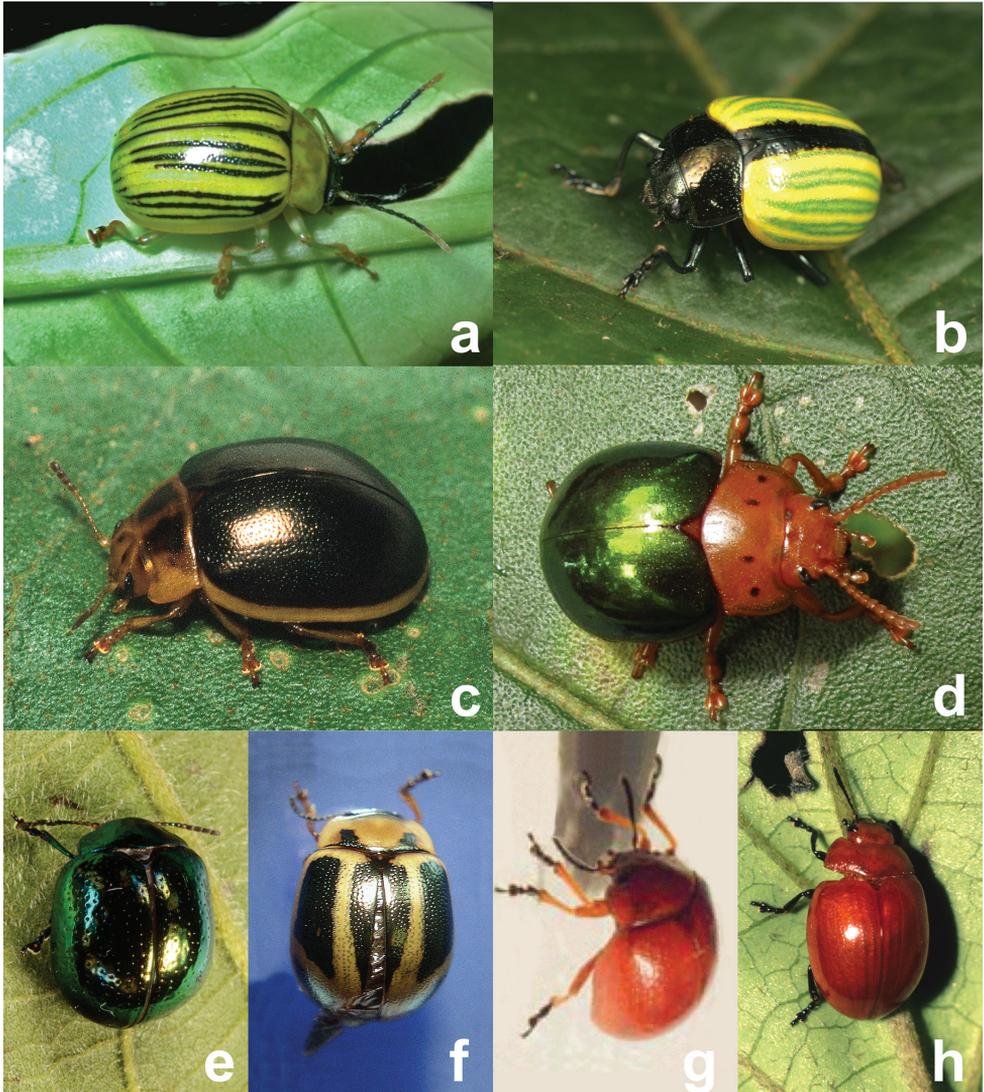


Figure 4. Other Solanaceae associated Chrysomelinae of unknown habits (**a, b, c, g**), known not to provide maternal care (**d, e, f**) and outgroup taxon (**h**), **a** *Proseicela antennalis* (Photo by D.W.) **b** *Proseicela flavipennis* (Photo by G.D.) **c** *Platyphora amabilis* (Photo by D.W.) **d** *Platyphora aulica* (Photo by D.W.) **e** *Platyphora nigronotata* (Photo by D.W.) **f** *Platyphora anastomozans* (Photo by D.W.) **g** *Platyphora sphaerica* (Photo by J.P.) **h** *Stilodes modesta* (Photo by D.W.).

deposited approximately one larva per day. Mothers of this species walk away from their live born larvae, leaving all to feed and develop as solitary individuals. Three additional Solanaceae-feeding species from Brazil are included in the analysis that follows. *Platyphora anastomozans* (Perty) and *Pl. nigronotata* (Stal) specimens were collected from Serra do Japi, Brazil where aspects of their biology has been studied

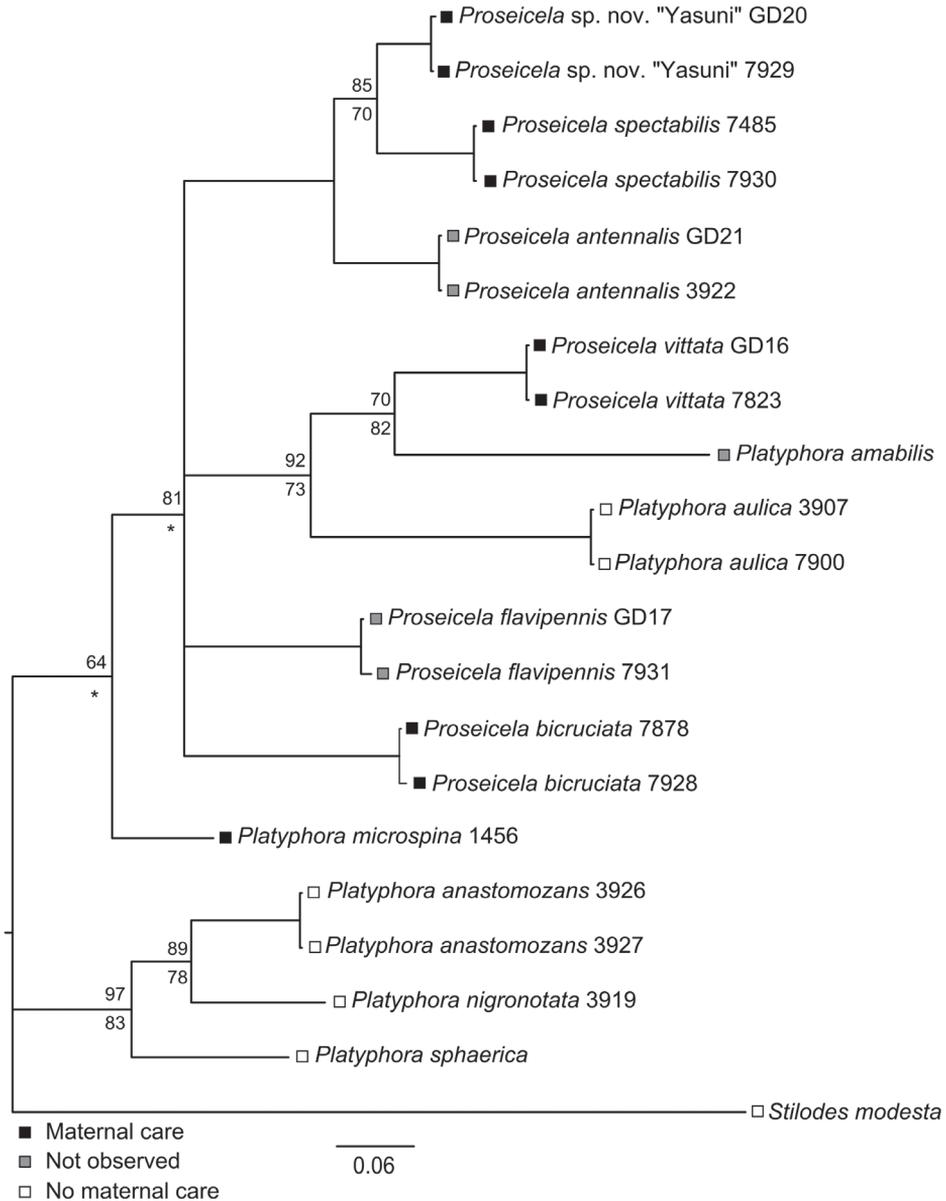


Figure 5. Bayesian Consensus tree of 472 bp COI sequences obtained for 12 species of Central and South American Solanaceae-feeding Doryphorini and one outgroup. For nodes with less than 100% support, Bayesian values are placed above node, Maximum Likelihood bootstrap values below the node, while asterisks (*) indicate nodes with different taxon placement under ML analysis and thus are not strictly comparable.

(Medeiros and Vasconcellos-Neto 1994, Vasconcellos-Neto and Jolivet 1994). *Platyphora sphaerica* Jacoby specimens were observed on several unidentified solanaceous food plant species near Fortaleza by J.P., 3 April 1999.

Species validity and life history characteristics of Solanaceae-feeding taxa

Two lines of evidence support the validity of the *Solanum*-feeding species recognized above. The first is the expert opinion of M. Daccordi who has reviewed each of the species in this report including voucher specimens and finds only one unidentified species, *Proseicela* sp. n. "Yasuni". Nevertheless, while an experienced taxonomist can separate species, the criteria can be subtle and based on few characters. The five Ecuadorean *Proseicela* species differ only in subtle aspects of elytral and pronotal color pattern and for this reason we sought genetic evidence of species limits. The topology of the resulting consensus tree generated by Bayesian Inference (Fig. 5) resolved all taxa as separate entities, with Bayesian support values ranging from 64 to 100%, while Maximum Likelihood bootstrap estimates for nodes common to both trees ranged from 70 to 100%. The smallest pair-wise genetic distance estimates occurred between the pairs, *Pr.* sp. n. "Yasuni", *Pr. spectabilis* (6.4%) and *Pr. antennalis* (7.5%), and between *Pr. spectabilis* and *Pr. antennalis* (9.0%) with all remaining pair-wise distances ranging between 10.2 and 18.6%. Thus all mean distances fall above the 2 to 6% range considered a threshold for distinct species, depending on COI evolution rates within particular clades (Hajibabaei et al. 2006, Wiemers and Fiedler 2007). While the polytomy in our tree does obscure relationships among some *Proseicela* species and clades, the remaining placement of taxa raises questions regarding generic assignments, especially those for *Pl. amabilis* and *Pl. aulica*, the latter seemingly a species which has secondarily lost subsocial habits common among closest taxa.

Aspects of the biology of 20 Neotropical species of Solanaceae-feeding Doryphorini (i.e. excluding *Leptinotarsa* and similar genera) are now known and where the mode of reproduction has been recorded, all (17) are live-bearing or "larviparous" (Table 1). Further, 15 species are reported to have aggregated larvae, while only two species (*Pl. aulica* and *Pl. sphaerica*) have solitary larvae. Maternal care is now known to occur in six species and known not to occur in another eleven species. While five *Proseicela* species are maternal care providers, *Pr. crucigera* in south-central Brazil has gregarious larvae but mothers do not extend care after larviposition (João Vasconcelos-Neto, personal communication, 2013).

Discussion

Because of the caveats to which mitochondrial data are subject (Rubinoff et al. 2006), and limitations in taxon sampling, the relationships of the Apocynaceae and Solanaceae associated taxa to one another and to other Doryphorini associated with eight other host plant families is best left to a multi-gene analysis (Dury et al. in preparation). However, the COI data are sufficient to confirm the validity of 12 Solanaceae feeding species and supports the existence of two major clades, one of which contains non-maternal care providing species distributed primarily in the "cerrado" of central and southern Brazil. The second clade contains five maternal care species, three spe-

cies whose habits are unreported and one species, *Pl. aulica*, which has apparently lost parental care. The latter is particularly interesting in that it is distributed over the northern portion of the Brazilian shield in “cerrado” habitats resembling those occupied by species in the non-maternal care clade. Further, our tree raises doubts about present generic concepts, at least for Solanaceae-feeding taxa. This problem is best seen in the small well-resolved clade containing *Pl. aulica*, *Pl. amabilis* and *Pr. vittata* in Fig. 5. Taking into consideration their non-natural grouping and the fact that a short mesothoracic horn is possessed by all members of the ingroup, but not by other *Platyphora* species (D.W. personal observation), one solution may eventually consist of transferring most, or perhaps all, short-horned, Solanaceae-feeding *Platyphora* species into the genus, *Proseicela*.

New records of maternal care in New World Chrysomelinae are beginning to reveal patterns. The first is that maternal care behavior is primarily defensive in nature. *Doryphora* mothers straddle larvae and take measures to physically confront pedestrian predators. There is no convincing evidence that mothers act to supply or direct offspring to resources, although there are indications that *Pr. vittata* mothers are modifying resources through vein-pinching. *Proseicela* and *Platyphora* mothers closely tend, straddle and herd offspring much as observed in *Doryphora*, but aggressive challenges of nearby insects or artificial stimuli have not been observed. For the most part, mothers remain immobile and close to their larvae (e.g. the tachinid parasitoid on the *Pr. spectabilis* larva, Fig 3h). But, elaborate vein-pinching by *Pr. vittata* mothers on the natal leaf just prior to and after larvaposition brings a dimension to maternal care which is apparently absent in *Doryphora* species. Presently, we do not know whether vein-pinching behavior simply deactivates plant defensive canals (Dussord 2009) or has an unknown effect on plant chemistry. Regardless, it seems the behavior is for the benefit of vulnerable offspring who exclusively feed on that part of the leaf.

As *Doryphora* species are specialist feeders on Apocynaceae it is entirely possible that adults have a defense system based on the dual sequestration of plant amyryns and lycopsamine-type alkaloids (Termonia et al. 2002). Adult *Proseicela* and *Platyphora* species associated with Solanaceae (e.g. *Pl. microspina*), are likely to sequester only plant amyryns as precursors of saponin-defensive secretions but whether the secretions of either of these taxa are employed in defense of larvae remains unknown.

The second pattern beginning to emerge concerns choice of food plants. New World Chrysomelinae are specialist feeders on one of approximately nine different host plant families, yet all maternal care species to date are restricted to only two, Apocynaceae and Solanaceae. Clearly more documentation is desirable, but if this pattern were to continue then it will be important to look for attributes possessed by these two plant families which promote evolution of maternal defenses but which are absent in other families, such as the Malpighiaceae, a family which hosts many poorly known Chrysomelinae species in South America.

We note that all eight maternal care species in the New World occur in distinctly tropical latitudes whereas subsocial *Gonioctena* species occur from Europe (Lengerken 1939, Goidanich 1956) to Japan (Kudô and Ishibashi 1995, 1996; Kudô et al. 1995,

Kudô and Hasegawa 2003). More recently, Reid et al. (2009) documented morphology and aspects of maternal care in the Australian chrysomeline beetle, *Pterodunga mirabile* Daccordi, a viviparous species associated with Proteaceae (Daccordi 2000). Female *Pt. mirabile* adopt a position at the base of the leaf lamina, facing toward tightly-grouped feeding larvae. Whether mothers continue to guard from the leaf petiole during resting periods as in Asian *Gonioctena sibirica* (Kudô and Ishibashi 1996) or approach or even straddle younger offspring as occurs in Neotropical care-providing species remains unclear. We learn additionally from Reid's interesting account that once the leaf is eaten the female moves aside and larvae wander separately to leaves, while other larvae are herded so they rejoin the group.

Mafra-Neto and Jolivet (1996) proposed a tradeoff exists between cannibalism and other forms of parental investment. The rareness of cannibalism in Chrysomelinae was hypothesized to be due to other forms of costly investment in offspring, such as aggregated larvae, although it is not clear how this behavior can be seen as a cost for the parent. We would expect their argument extends to include other forms of investment such as provisioning larvae with trophic eggs. Thus *D. paykulli* is not kind to their hypothesis as mothers invest heavily in both maternal care and through egg cannibalism—whether fertile or not. In contrast, the study of *D. sp.* near *punctatissima* by Eberhard (1981) is friendlier to their proposal as mothers do not defend or herd offspring but larvae consume 20 to 40% of eggs. The extensive guarding by *D. reticulata* mothers and the lack of egg consumption by larvae is again consistent with the idea of a tradeoff. Thus the variation in traits we observe among just three species of *Doryphora* cast some doubt on their hypothesis. Further, we have yet to observe larval cannibalism in the larviparous species of *Platyphora* and *Proseicela* associated with Solanaceae. Indeed, we suggest the nearly synchronous deposition of larvae may largely preclude cannibalism, while the staggered deposition of eggs by *Doryphora* females may facilitate the origin and maintenance of this behavior. Testing the Mafra-Neto and Jolivet (1996) hypothesis will require the study of additional species and forms of maternal investment, its synchronicity and perhaps even a search for hidden factors such as bacterial parasites (e.g. *Wolbachia* and *Spiroplasma*), well-studied manipulators of host reproduction (Werren 1997).

Conclusion

Maternal care behavior appears limited to three genera of Neotropical Chrysomelinae and is not present in all species of these genera. *Doryphora* species are exclusively associated with lianas in the family Apocynaceae, possess a long mesosternal horn, are oviparous and reproduce at the transition from dry to wet seasons. *Proseicela* and some species currently placed within *Platyphora* are associated with Solanaceae host plants, and are live-bearing throughout the year. While extreme rareness remains an impediment to the study of most *Doryphora* species, *Proseicela* and allied species on the eastern slope of the Andes in Ecuador can be found more predictably on moderately common

host plants. Large voids remain in our understanding of the natural history of both groups, including the identity and importance of predators and parasitoids and the diverse ways in which mothers may be influencing the survival of offspring.

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Pachybrachis (Coleoptera, Chrysomelidae, Cryptocephalinae) of Eastern Canada

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Abstract

Seventeen *Pachybrachis* species occurring in eastern Canada [Ontario (ON), Québec (QC), New Brunswick (NB), Nova Scotia (NS), and Prince Edward Island (PE)] are treated by the authors. Two new national records were discovered, both from southernmost Ontario: *P. cephalicus* Fall and *P. luctuosus* Suffrian. Four species were new provincial records: *P. cephalicus* (ON), *P. luctuosus* (ON, QC), *P. obsoletus* Suffrian (NB), *P. peccans* (PE). A fully illustrated key to the *Pachybrachis* of eastern Canada is provided and supported with extensive photographs, distribution maps, and plant associations.

Three species were distributed from southern Ontario into at least one province in the Maritimes (*P. nigricornis* (Say), *P. obsoletus* Suffrian and *P. peccans* Suffrian). Six species were distributed along the shores of the Great Lakes (Erie, Michigan, and Ontario) and rivers (Ottawa, Saguenay and St. Lawrence), but unknown from central and northern ON and QC (*P. bivittatus* (Say), *P. hepaticus hepaticus* (F. E. Melsheimer), *P. othonus othonus* (Say), *P. pectoralis* (F. E. Melsheimer), *P. spumarius* Suffrian and *P. trinotatus* (F. E. Melsheimer)). Seven species were rare, five being found exclusively from southern ON (*P. calcaratus* Fall, *P. cephalicus*, *P. luridus* (Fabricius), *P. subfasciatus* (J. E. LeConte) and *P. tridens* (F. E. Melsheimer)), with two having, in addition, a disjunct population in QC (*P. atomarius* (F. E. Melsheimer) and *P. luctuosus*). One species was found to be the northern most extension of an eastern United States (US) distribution into the eastern townships of QC (*P. m-nigrum* (F. E. Melsheimer)). There were no *Pachybrachis* that could be considered arctic, subarctic, or boreal species; no specimens were found from Labrador and Newfoundland, and all species had southern affinities. *Pachybrachis atomarius*, *P. calcaratus*, *P. luridus*, *P. subfasciatus*, and *P. tridens*, not seen over the last 30–70 years, may be extirpated from eastern Canada.

Résumé

Les auteurs traitent des 17 espèces de *Pachybrachis* présentes dans l'est du Canada [Ontario (ON), Québec (QC), Nouveau-Brunswick (NB), Nouvelle-Écosse (NS), Île-du-Prince-Édouard (PE)]. Deux espèces en provenance de l'extrême sud de l'Ontario sont nouvelles pour le pays : *P. cephalicus* Fall et *P. luctuosus* Suffrian. Quatre espèces représentent de nouvelles mentions provinciales : *P. cephalicus* (ON), *P. luctuosus* (ON, QC), *P. obsoletus* Suffrian (NB) et *P. peccans* (PE). La clé d'identification des espèces de l'est du Canada s'appuie sur de nombreuses illustrations en plus d'inclure des cartes de répartition et les associations avec les plantes.

Trois espèces se répartissent du sud de l'ON jusqu'à au moins l'une ou l'autre des provinces Maritimes (*P. nigricornis* (Say), *P. obsoletus* Suffrian et *P. peccans* Suffrian). Six espèces sont limitées aux rives des Grands-Lacs (Érié, Michigan et Ontario), du fleuve Saint-Laurent et des grandes rivières (des Outaouais, Saguenay) sans atteindre le centre et le nord de l'ON et du QC (*P. bivittatus* (Say), *P. hepaticus* (F. E. Melsheimer), *P. othonus* (Say), *P. pectoralis* (F. E. Melsheimer), *P. spumarius* Suffrian et *P. trinotatus* (F. E. Melsheimer)). Cinq, parmi les sept espèces rares, proviennent exclusivement du sud de l'ON (*P. calcaratus*, *P. cephalicus*, *P. luridus* (Fabricius), *P. subfasciatus* (J. E. LeConte) et *P. tridens* (F. E. Melsheimer)), alors que les deux autres possèdent, en outre, une population disjointe au QC (*P. atomarius* et *P. luctuosus*). Une espèce, présente seulement en Estrie (QC), se trouve à la limite septentrionale de sa répartition dans l'est des États-Unis (*P. m-nigrum* (F. E. Melsheimer)). Aucune espèce, parmi les *Pachybrachis* étudiés, n'est arctique, subarctique ou boréale, mais toutes présentent des affinités méridionales. Aucun spécimen en provenance de Terre-Neuve et du Labrador n'a été observé. Les espèces *Pachybrachis atomarius*, *P. calcaratus*, *P. luridus*, *P. subfasciatus* et *P. tridens* n'ont pas été vues depuis les 30–70 dernières années de sorte qu'elles sont probablement disparues de la faune de l'Est du Canada.

Keywords

Coleoptera, Chrysomelidae, *Pachybrachis*, eastern Canada, taxonomy

Introduction

In the *Catalog of Leaf Beetles of America North of Mexico* Riley et al. (2003) listed *Pachybrachis* (Chrysomelidae: Cryptocephalinae) as one of the genera most in need of taxonomic revision, because many species cannot be identified with confidence. The senior author of the current investigation has been struggling with *Pachybrachis* for over 30 years and recently began a systematic review of all specimens housed in public and private collections, with a goal of a revision of the species found within the eastern United States. This effort then expanded into eastern North America after the junior authors provided access to the major collections in Canada as part of their Canadian Beetles Project. This collaboration has resulted in this paper as a prelude to the more diverse and daunting challenge of the entire eastern North American *Pachybrachis* fauna.

Historical review

When Johann Christian Fabricius (1798) described *Cryptocephalus luridus* from "America borealis", the genus *Cryptocephalus* represented the entire Cryptocephalini tribe of today. The generic concept was even larger for Melsheimer (1806) in his *Cata-*

logue of insects of Pennsylvania, since several of the 28 species listed under *Cryptocephalus* are currently placed in non-cryptocephaline genera (*Glyptoscelis*, *Paria*, etc.) and only six belong to *Pachybrachis*. Chevrolat (1836) split this broad genus into several new genera, including *Pachybrachis*, to which *P. luridus* was the first Nearctic species to be transferred by Suffrian (1852). Because this species was variable and common, it was subsequently described under several names which are all currently considered synonyms: *C. femoratus* Say, 1824; *C. aesculi* Melsheimer, 1847; *P. moerens* Stål, 1857; and the varieties *P. luridus nigrinus* Blatchley, 1910 and *P. luridus festivus* Fall, 1915.

Thomas Say (1824) was one of the first North American entomologists to describe Nearctic insects. Under the old concept of *Cryptocephalus* Geoffroy, 1762, he described *C. bivittatus*, *C. nigricornis*, and *C. othonus*, three species currently placed in *Pachybrachis* Chevrolat, 1836 (Riley et al. 2003).

The same year, John Eatton LeConte (1824) described several new beetle species from Georgia (US) including *Cryptocephalus subfasciatus*, which was transferred to *Pachybrachis* by Haldeman (1849). F. E. Melsheimer (1847) described six new species of *Pachybrachis* (as *Cryptocephalus*) from Pennsylvania (US) that have subsequently been found in eastern Canada: *P. atomarius*, *P. hepaticus*, *P. m-nigrum*, *P. pectoralis*, *P. tridens* and *P. trinotatus*. Haldeman (1849) provided the first key to 16 species of *Pachybrachis*, including seven found in eastern Canada: *P. bivittatus*, *P. carbonarius* (= *P. nigricornis*), *P. luridus*, *P. m-nigrum*, *P. othonus*, *P. subfasciatus*, and *P. trinotatus*.

The beetles collected by the Swiss scientist Louis Agassiz and the Italian explorer John Cabot during the exploration of several regions of Lake Superior (ON) were studied and identified by LeConte (1850) who cited *P. abdominalis* (probably *P. peccans*) and *P. m-nigrum* with no additional information.

The German entomologist Christian Wilhelm Ludwig Eduard Suffrian specialized in Coleoptera, especially Chrysomelidae. He described many North American species, including four in *Pachybrachis* that are found in eastern Canada: *P. luctuosus*, *P. obsoletus*, *P. peccans*, and *P. spumarius* (Suffrian 1852, 1858).

The *Catalogue of the described Coleoptera of the United States* (Melsheimer 1853, revised by S. S. Haldeman and J. L. LeConte) listed 20 species of *Pachybrachis*, eight of which are found in eastern Canada (*P. atomarius*, *P. bivittatus*, *P. carbonarius* (= *P. nigricornis carbonarius*), *P. luridus*, *P. m-nigrum*, *P. othonus*, *P. subfasciatus*, and *P. trinotatus*), and four additional *Pachybrachis* species listed as *Cryptocephalus* (*P. hepaticus*, *P. nigricornis*, *P. obsoletus*, and *P. tridens*).

During the next 50 years, several Canadian collectors explored various areas of Ontario and Québec, but no records show that they were successful in collecting *Pachybrachis*: William Couper in Toronto (ON) and in various localities in the province of Québec (Couper 1855a, 1855b); Robert Bell (1858) in Gaspesia [list revisited by D'Urban (1859a)]; D'Urban (1859b, 1859c, 1860a, 1860b) in Argenteuil County (QC), in the vicinity of Montréal (QC), at Hudson Bay (QC) and in Ottawa (ON); Beadle (1861) in the county of Lincoln (ON); Couper (1864, 1865) in the vicinity of Toronto (ON) and in Québec City (QC); Saunders (1868) in the Saguenay region (QC); Ritchie (1869) on the Island of Montréal (QC); Reed (1869) in the neighbour-

hood of London (ON), listing only the families of collected beetles; Jones (1870) in Halifax (NS); Packard (1870) at Caribou Island (LB); Provancher (1870) at Portneuf (QC); Packard (1872), listing the beetles known to occur in Labrador and later adding spiders, myriapods and other groups of insects (Packard 1888); Couper (1874) in Anticosti Island (QC); Couper (1881, 1882, 1883) in the province of Québec in general; Bell (1881) at Belleville (ON); Harrington (1882) at Ottawa (ON) and at Chelsea, Hull and Montebello (QC); LeConte (1883), studying the specimens collected by J.T. Bell and others in various areas north of Lake Superior (ON); Fletcher (1888), identifying the specimens collected by J.S. Cotter and J.M. Macoun on the south coast and islands of James Bay (QC); Harrington (1890a, b, c), relisting several of the previous reports; Hausen (1893) at Saint-Jérôme (QC); Hanham (1894) in Québec City and vicinity (QC); Harrington (1894a, b) at Ottawa (ON) and Copper Cliff (ON); Evans (1899) at Halifax (NS); Ouellet (1902) at Montréal, Rigaud and Joliette (QC); Evans (1905) at light at Trenton (ON); and Sherman (1910) in Labrador.

Pettit (1868–1872) listed 1290 species in a series of articles on beetles collected at Grimsby (ON), including three *Pachybrachis* species (*P. mollis*, *P. subfasciatus*, and *P. tridens*). His *P. mollis* is now considered a synonym of *P. tridens* (Riley et al. 2003).

The priest Léon Provancher (1877a) was the first Canadian to describe and key out two *Pachybrachis* species (*luridus*, *othonus*) in his *Petite faune entomologique du Canada*, to which was added *P. atomarius* (Provancher 1877b). It should be noted here that *P. luridus* in the key was erroneously transcribed as *P. "lividus"* in the description. This is an evident misspelling since Fabricius never described any insect under such name (Zimsen 1964). Two years later, Provancher (1879) added *P. litigiousus* from Saint-Hyacinthe.

William Couper (1883) listed three species collected in the province of Québec but did not specify the localities from where they were obtained: *P. atomarius*, *P. luridus*, and *P. othonus*. Brodie and White (1883a, b) prepared a checklist of the insects of the Dominion of Canada (= Ontario and Québec) and listed nine names under *Pachybrachis*, but two were misidentified (*P. litigiousus*, and *P. morosus*).

In his list of the Ottawa Coleoptera, Harrington (1884) reported four species found in this locality: *femoratus*, *litigiousus*, *tridens* and *viduatus*. However, his report of *P. femoratus* was most likely based on misidentified specimens of *P. luridus*, his report of *P. litigiousus* was probably based on misidentified specimens of the striped version of *P. nigricornis* (*difficilis*), and *P. bivittatus* was often misidentified as *P. viduatus*, which is a southern US species not found in Canada.

Henshaw (1885, 1887, 1889) updated the aging checklist of Melsheimer (revised by Haldeman and LeConte 1853) including its supplement (Austin 1880). At the beginning of the 20th century, Clavareau (1913) published the first world catalogue of the subfamily Cryptocephalinae, including species in the genus *Pachybrachis*. As regards the eastern Canadian species, *P. bivittatus* was given as a synonym of *P. viduatus* (Fabricius 1801) and *P. m-nigrum* as a synonym of *P. picturatus* (Germar 1824); since, *P. viduatus* and *P. picturatus* are fairly rare species only found in the southern US, these two species were evidently misidentified. The same year, Weise (1913) proposed *P.*

praeclarus as a replacement name for *P. elegans* Blatchley, 1910, since this name was already preoccupied by a *Pachybrachis* species described by Graëlls (1851) from Spain.

Evans (1895a, b) listed several beetle species collected in the district of Sudbury (ON), including *P. femoratus* (= *P. luridus*?) and *P. infaustus* (= *P. atomarius*); however, voucher specimens were not found for this study. In a series of papers on the Chrysomelidae of Ontario and Québec, Wickham (1896) provided a key to nine species of *Pachybrachis*, plus a description and illustration of beetles reported to be *P. viduatus*, but that in actuality were clearly *P. bivittatus*.

In his report on the insects of the Toronto region (ON), Walker (1913) listed *P. atomarius*, *P. femoratus* (= *P. luridus*?), *P. hepaticus*, *P. othonus* and *P. trinotatus*. Gibson (1915, 1919) reported several collection records of *Pachybrachis* in his reports to the Entomological Society of Ontario, but they were all from Manitoba.

During the same period, H. C. Fall (1915) thoroughly revised the Nearctic *Pachybrachis* (*Pachybrachys*), and his revision is still, today, the only available complete taxonomic work on this genus. Out of the 159 recognized species, only six were given with a distribution extending into eastern Canada: *luridus* (ON), *othonus othonus* (MB, ON), *peccans* (MB, NB, ON), *relictus* (ON), *subfasciatus* (ON), and *trinotatus* (ON), with *calcaratus* located in nearby Detroit (USA, Michigan). The well-known *Catalogue of the Coleoptera of America, north of Mexico* of Leng (1920) relisted the species treated by Fall (1915), but the distributions were reduced to a few state records or short statements, with the result that only *P. peccans* was clearly reported from New Brunswick. No relevant information on *Pachybrachis* was added in the supplements, except the record of *P. donneri* from Oregon (Leng and Mutchler 1927, 1933; Blackwelder 1939; Blackwelder and Blackwelder 1948).

Gustave Chagnon (1917) treated the Coleoptera of Québec in the third part of a preliminary list of the insects of this province. Eight species of *Pachybrachis* were collected in Montréal or in neighboring localities: *P. atomarius*, *P. carbonarius* (= *P. nigricornis carbonarius*), *P. femoratus* (= *P. luridus*), *P. infaustus* (= *P. atomarius*), *P. othonus*, *P. tridens*, *P. trinotatus*, and *P. viduatus* (= *P. bivittatus*). In 1918, he published a list of corrections brought to his attention by Fall (Chagnon 1918), noting *P. viduatus* as a wrong determination in Chagnon (1917) and Wickham (1896).

In the thirties, Chagnon published a series of contributions on the most important beetles of the province of Québec in the journal *Le Naturaliste canadien* (Chagnon 1933–1939). The Cryptocephalini were treated in 1937 (Chagnon 1937). Four species of *Pachybrachis* were keyed out (*P. bivittatus*, *P. carbonarius* (= *P. nigricornis*), *P. othonus* and *P. trinotatus*), and an additional four only mentioned (*P. atomarius*, *P. femoratus* (= *P. luridus*), *P. peccans*, *P. relictus* Fall). The year after, Chagnon (1940) grouped together his previous contributions and published them as a separate book. The brother Adrien Robert of the Université de Montréal updated the nomenclature used in the book of Chagnon (1940), but did not otherwise modify its contents (Chagnon and Robert 1962), except that a table was added at the end of the book, giving both the nomenclature used by Chagnon (1940) and the more recent nomenclature. As regards *Pachybrachis*, no changes were noted between the two editions.

The list of Coleoptera collected by Notman (1919) at Cochrane in northern Ontario did not include *Pachybrachis*.

Father Léopold (1934) of the entomology laboratory at the agriculture institute of Oka (QC) published a list of beetles preserved in the collection of the institution. Seven species of *Pachybrachis* were listed, five of which are still valid: *P. atomarius*, *P. difficilis* (= *P. nigricornis*), *P. hepaticus*, *P. peccans*, and *P. tridens*. His *P. pubescens* (= *P. morosus*) and *P. vestigialis* were very likely misidentified.

Hicks (1944, 1945, 1947a, b) collected mainly in southern Ontario and provided several new province records or information on the host plants or biology of species. He reported that *P. calcaratus*, *P. peccans*, *P. othonus* and *P. relictus* were taken by sweeping the vegetation, and *P. obsoletus* was observed on willows (*Salix* sp.).

Latendresse (1963) increased to 36 the number of known chrysomelid species from the Saguenay region on the north shore of the St. Lawrence, in Québec. A century before, Saunders (1868) had found only two species of leaf beetles, but neither collector found *Pachybrachis* species in their survey.

Balsbaugh (1973) studied the geographical variation in *Pachybrachis othonus*, recognized three subspecies, and described *P. othonus sioux* Balsbaugh as a new subspecies. The geographical variation of *P. nigricornis* was treated three years later (Balsbaugh and Tucker 1976), but the distribution of subspecies needs to be clarified in eastern Canada since three have been reported for this region (Riley et al. 2003).

The *Checklist of Chrysomelidae of Canada, United States, Mexico, Central America and the West Indies* of Wilcox (1975) was a working draft which was pretty complete taxonomically, but lacked detailed information on the distribution of species. LeSage (1991) provided the known distribution in Alaska and in Canada by province for all chrysomelid species, including *Pachybrachis*. Laplante et al. (1991) extracted the information for Québec only, and published it as a separate checklist for this province.

Lawson (1976) described and illustrated the structural details of the egg and larval case of *P. bivittatus*. The mature larva of the same species was sketched by Lawson (1991), and LeSage (1985) described and illustrated all its larval instars and egg. This author also treated in detail these life stages for *P. peccans*. Eggs were distinguished by their external ornamentation, the larval instars by their size and by their head and leg chaetotaxy.

Edward G. Riley, Shawn M. Clark, R. Wills Flowers and Arthur J. Gilbert are the authors of the most recent synthesis on American Chrysomelidae (Riley et al. 2002). The reader is referred to this major work for diagnoses and keys to subfamilies, tribes and genera. The North American *Pachybrachis* species have not been assigned to subgenera as the Palaearctic species were in the recent *Catalogue of Palaearctic Coleoptera* (Schöller et al. 2010).

The *Catalogue of the Leaf Beetles of America North of Mexico* by Riley et al. (2003) was the first extensive and complete catalogue ever published on this family of beetles for the North American continent north of Mexico. Consequently, we have followed the nomenclature and classification adopted by these authors. The compiling by Clark et al. (2004) of the known host plants of the Nearctic *Pachybrachis* is the best and most

extensive source of information available on the subject. Both works are essential tools to anybody interested in *Pachybrachis* species and Nearctic leaf beetles in general.

An examination of leaf beetle specimens in the largest beetle collections in Kentucky, inventory work in state nature preserves and other protected areas, and a review of the literature revealed 20 species of *Pachybrachis* present in Kentucky, 14 of which were new state records (Barney et al. 2011). Twenty species of *Pachybrachis* were also reported for Illinois (Barney 1984).

The latest contribution on the eastern Canadian *Pachybrachis* is by Webster et al. (2012), based on extensive collecting of beetles in New Brunswick by the senior author. *Pachybrachis bivittatus* and *P. m-nigrum* were added to the previously known *P. peccans* and *P. pectoralis* for this province.

Material and methods

Provinces

For the purposes of this study, eastern Canada is defined as provinces east of Manitoba: Ontario (ON), Québec (QC), New Brunswick (NB), Nova Scotia (NS), Prince Edward Island (PE), Newfoundland (NF) and Labrador (LB). When not given on labels, counties were found using the gazetteer of CPCGN (1974, 1988) for Ontario, CTQ (1987) for Québec, CPCGN (1994a) for New Brunswick, CPCGN (1993) for Nova Scotia, and CPCGN (1994b) for Prince Edward Island. No specimens were available from Labrador and Newfoundland. In older specimens, for example those collected by Brimley in Prince Edward and Hasting Counties, only the county names were given in the collection data.

Species data

For each species of *Pachybrachis* found in eastern Canada, the following information is provided: name, synonymies, habitus photo, brief description of species recognition characters, distribution and maps, label data, recorded or potential host plants, and comments.

Label data

For each specimen examined the following information is provided: province, county/district, date, label information that may include potential host plants, habitat or collection method, collector, number of males and females, and museum. If a specimen had a H. C. Fall identification label or was found in Fall's personal collection (Fall-MCZ), this information was cited before the museum name. Within a species treatment, data are ordered alphabetically by province, county/district, locale, and then date.

Sex determination

Determination of sex is relatively easy as follows: males (Figure 9a) are generally smaller and less robust than females, with the abdomen flat and more or less concave; in females (Figure 9b) the abdomen is convex beneath, the last segment with a deeply rounded fovea (depression). Singleton females of many species cannot be confidently identified without associated males for dissection.

Size measurement

Ten males (when available) of each species were measured using a Leica Z16 APO microscope equipped with a DFC295 digital color camera and Leica Application Suite software.

There are many species of *Pachybrachis* in the eastern US and Canada that could only be confidently separated and identified via examination of the male reproductive organ (aedeagus). This was accomplished by removing the labels from a pinned (and usually pointed) specimen and placing the pointed, pinned specimen in gently boiling water for one minute. The now relaxed pinned (or separated) beetle was placed in a Petri dish with a small amount of 70% Ethanol. The beetle was held upside down with featherweight forceps and the abdomen pried off with an insect pin. The abdomen was then held by the pygidium with a pin and the aedeagus removed with fine forceps. After drying, the beetle was reattached to a new point using clear nail polish, as were the abdomen and aedeagus.

Plant nomenclature

The scientific and popular names of plants were taken in Fernald (1950), Scoggan (1978) and Marie-Victorin (1995).

Physiographic features

The Carolinian Life Zone is by far the richest zone for the *Pachybrachis* fauna of eastern Canada, several species being exclusively associated to it (e.g. *P. calcaratus* [Map 3], *P. cephalicus* [Map 4], etc.). Some species are primarily present in this zone but also have, small additional northern disjunct populations (e.g. *P. atomarius* [Map 1]). The Carolinian Zone extends into southwestern Ontario between Lakes Huron and Erie. In addition to the usual common trees of the larger Great Lakes-St. Lawrence forest region (e.g. sugar maple [*Acer saccharinum* Marsh.], beech [*Fagus grandiflora* Ehrh.], etc.), the northern limits of several deciduous trees are found in this zone: tulip-tree [*Liriodendron tulipifera* L.], pawpaw [*Asimina triloba* (L.) Dunal], black walnut [*Juglans nigra* L.], etc. (Fox and Soper 1952, 1953, 1954; Shelford 1963; Hosie 1979; Johnson 2012).

The major rivers seem to play an important role in the distribution of some species. For example, *P. bivittatus* [Map 2] is closely spread along the Great-Lakes-St. Lawrence River system and in the Ottawa River valley. The Saguenay River seems a northern limit impassable for all species.

There are two noticeable disjunct refugia. The most important is the well-known Eardley Escarpment located on the south border of Gatineau Park. It corresponds to about 40 km of steep cliffs oriented southwards which are significantly warmer than the Ottawa Valley below and the Laurentian Highlands above. Eastern red cedar (*Juniperus virginiana* L.) is well represented on the cliffs, and the relatively recent discovery of its associated olive hairstreak, *Callophrys grynea* (Hubner), has been an enthusiastically received discovery for butterfly collectors (Hall 1991, NCC 2011a). There are over thirty additional vascular plant species growing exclusively there at their northernmost limits, considerably disjunct from their main distribution south of the Great Lakes (Brunton and Lafontaine 1971).

The second refugium corresponds to a small zone between the islands Île-aux-Allumettes and Île-du-Grand-Calumet, both in Pontiac County (QC), within the Ottawa River. A special flora has been identified there by botanists, but no results were published. On the other hand, Desroches and Laparé (2004) reported the first captures of the ribbonsnake (*Thamnophis sauritus septentrionalis* Rossman) in this refugium. The distribution of *P. luctuosus* corresponds to this pattern [Map 6].

Finally, the influence of Lake Champlain cannot be ignored. Although almost completely lying in the states of Vermont and New York in the United States, it extends about 10 km across the Canadian border. Its microclimate is important enough to allow some plants and animals to cross the border and reach their northernmost limits in the southeastern townships of Québec (DECNY 2012). The very recent discovery of two sycamores (*Platanus occidentalis* L.) in this region - a first record for Québec - is a good example of such distribution (Bibeau-Lemieux 2010, 2011).

Codens of collections examined and referred to in this study are as follow:

The major insect collections (and curators) in eastern Canada and the United States, which contained *Pachybrachis* specimens from eastern Canada, are listed below:

AJGC	Art J. Gilbert Collection (private), Fresno, CA
AMNH	American Museum of Natural History, New York, NY (Lee Herman)
CDFA	California Department of Food and Agriculture, Sacramento, CA (Chuck Bellamy)
CEUM	Collection entomologique de l'Université de Montréal, QC (Louise Cloutier)
CFIM	Collection en Fiducie de l'Insectarium de Montréal, QC (Stéphane LeTirant)
CNC	Agriculture and Agri-Food Canada, Ottawa, ON (Laurent LeSage)
FALL	H. C. Fall Collection, Harvard Museum of Comparative Zoology, Cambridge, MA (Phillip Perkins)

FSCA	Florida State Collection of Arthropods, Gainesville, FL (Paul Skelley)
LEM	Lyman Entomological Museum, McGill, QC (Stéphanie Boucher)
LFC	Laurentian Forestry Center (Insectarium René-Martineau) Québec, QC (Jan Klimaszewski)
LSAM	Louisiana State Arthropod Museum, Baton Rouge, LA (Victoria Bayless)
MCZ	Harvard Museum of Comparative Zoology, Cambridge, MA (Phillip Perkins)
MSUC	Michigan State University Collection, East Lansing, MI (Gary Parsons)
RWIC	Reginald Webster Insect Collection (private), Charters Settlement, NB
OSUC	Ohio State University Collection, Columbus, OH (Creighton Freeman)
UNHM	University of New Hampshire, Durham, NH (Donald Chandler)
USNM	National Museum of Natural History, Washington D.C. (Alex Konstantinov)

Results

Pachybrachis Chevrolat, 1836

<http://species-id.net/wiki/Pachybrachis>

Pachybrachis Chevrolat in Dejean, 1836: 420. Type species: *Cryptocephalus hieroglyphicus* Laicharting, 1781, by subsequent designation of Jacoby 1908: 265.

Pachybrachys: Mannerheim 1843: 311. [incorrect subsequent spelling].

Remarks. There has been some debate as to the correct spelling of the genus *Pachybrachis*. Fall's (1915) monumental work used *Pachybrachys* Chevrolat and cited its general American usage by J. L. LeConte. However, this emendation was unjustified under the rules of the International Code of Zoological Nomenclature (ICZN 1999, Article 32).

Pachybrachis is a member of the subfamily Cryptocephalinae Gyllenhal, 1813, commonly known as the case bearers due to the fact that all known larval stages live in a case constructed of their fecal matter and often plant debris (LeSage 1985). Their cylindrical, compact body characterizes the adults, which usually have the head retracted into the pronotum to the level of the eyes.

In the recent revision of family-group names in Coleoptera (Bouchard et al. 2011), the former tribe Pachybrachini Chapuis, 1874 was relegated to subtribe under the tribe Cryptocephalini Gyllenhal, 1813. Pachybrachina Chapuis, 1874 contains only two genera north of Mexico, *Griburius* and *Pachybrachis*, and is characterized by long filiform antennae, with a marginal bead at the base of pronotum which is not crenulate. Riley et al. (2002) separated the two genera by prosternal characteristics (prosternum broad, as wide as long in *Griburius*, narrower, longer than wide in *Pachybrachis*). Additional generic keys can be found in Blatchley (1910), Chagnon and Robert (1962), Downie and Arnett (1996), and Ciegler (2007).

Useful morphological characters. Fall (1915) provided a very detailed "Review of Structural Characters Useful in Taxonomy," which we will not repeat here. However, there are a few key characters that will be useful to separate the seventeen Canadian

species. These features will be described, detailed and illustrated, most of them being used in the identification key.

Size. The seventeen species can generally be divided into four size classes by average length: very small, <1.75 mm; small, >1.75 mm to 2.35 mm; medium, >2.35 mm to 2.85 mm; and large, >2.85 mm to 3.30 mm. *Pachybrachis hepaticus* is the only species in the very small category, with a mean length of 1.68 mm. *Pachybrachis m-nigrum* (2.59 mm), *P. othonus othonus* (2.63 mm), and *P. luridus* (2.65 mm) are in the medium category. *Pachybrachis trinitatus* (3.09 mm) and *P. bivittatus* (3.12 mm) are the only species with males averaging over 3 mm in length. Small is the largest category, with the remaining eleven species. Mean length and width of males are reported for each species. Females are generally larger, thus accounting for the larger overall sizes reported by Fall (1915).

Antennae. In most species (e.g. *P. atomarius*, Habitus 1; *P. bivittatus*, Habitus 2), the length of antennae equals about 2/3 to 3/4 the length of the body. There are two noticeable exceptions. In *P. hepaticus* (Habitus 5) the antennae do not exceed half of the body length, whereas in *P. trinitatus* (Habitus 17) the antennae equal or exceed the body length.

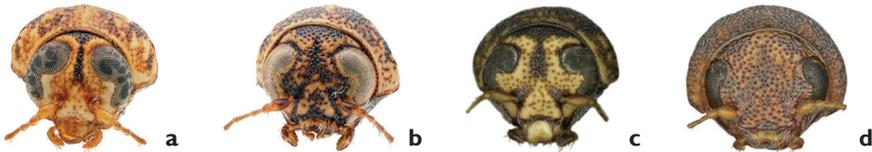


Figure 1. Eyes: **a** close eyes, *P. pectoralis* **b** normal eyes, *P. peccans* **c** normal eyes and yellow face, male *P. atomarius* **d** remote eyes, *P. hepaticus*.

Eyes. The eyes of *P. pectoralis* are close to each other and separated by less than their width (Figure 1a). In most species the distance between the eyes roughly corresponds to their width (e.g. *P. peccans*, Figure 1b). A normal distance between eyes, coupled with the head coloration, can be diagnostic, as in *P. atomarius* that has a largely yellow face (Figure 1c). In *P. hepaticus*, the eyes are very small and markedly remote, separated by much more than their diameter (Figure 1d).



Figure 2. Ocular lines: **a** present, *P. peccans* **b** small, *P. hepaticus* **c** absent, *P. spumarius*.

Ocular lines. Many *Pachybrachis* species have an impressed line, called the ocular line, around the margin of the eyes, and in some species the line diverges from each eye as lines of darker colored punctures between the eyes (e.g. *P. peccans*, Figure 2a).

This character is very consistent within each species, and it is easy to see provided the specimens are properly oriented and lighted. In *P. hepaticus* the ocular lines are very short but distinct above the eyes (Figure 2b). In other species, however, such ocular lines are absent (e.g. *P. spumarius*, Figure 2c).

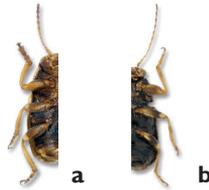


Figure 3. Front femora: **a** not enlarged, *P. hepaticus* **b** thickened, *P. peccans*.

Femora. Except for *P. hepaticus* (Figure 3a), the femora on the forelegs of all species (Figure 3b) are incrassate or thickened in relation to the other femora. This character is difficult to see because in most cases legs are folded and pressed tightly against the body. Consequently, it might be necessary to relax the legs and spread them out to compare the front femora with those of the middle and hind legs. When such preparation is achieved, the larger size of the femora becomes evident (e.g. *P. calcaratus*, Habitus 3).

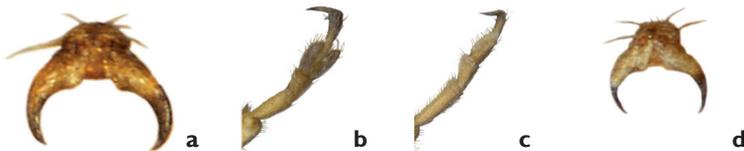


Figure 4. Claws: **a, b** front claws enlarged **c, d** normal.

Claws. In *Pachybrachis*, the tarsal claws are all simple (Figures 4a–d), but claws on the forelegs (Figures 4a, b) of several species are distinctly enlarged relative to the claws on the other legs (Figures 4c, d), as in *P. peccans* (Habitus 12) or *P. pectoralis* (Habitus 13). Due to the position of the legs in dead specimens, this character is often easier to see in lateral view (Figures 4c, 4d) than in front view (Figures 4a, 4b).

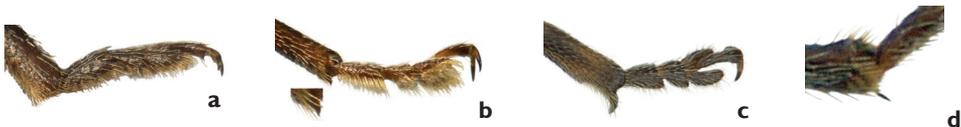


Figure 5. Tibial spurs: **a** absent from front leg, *P. atomarius* **b** minute on front leg, *P. spumarius* **c** large on front leg, *P. calcaratus* **d** small and pointed on middle legs in most species.

Tibial spurs. In *P. atomarius* (Habitus 1), *P. m-nigrum* (Habitus 8), and *P. trinotatus* (Habitus 17), there is no apical spur on front tibia (Figure 5a), but a tuft of large apical setae grouped together may superficially look like a spur. In *P. spumarius* (Figure 5b,

Habitus 14) the front tibial spur is very small, hidden and difficult to see, but the very large and exposed front tibial spur is unique and distinctive of *P. calcaratus* (Figure 5c, Habitus 3). In all species, except *P. hepaticus*, the middle tibiae are armed with small slender apical spur (Figure 5d). In all species studied here, the hind tibiae are unarmed.

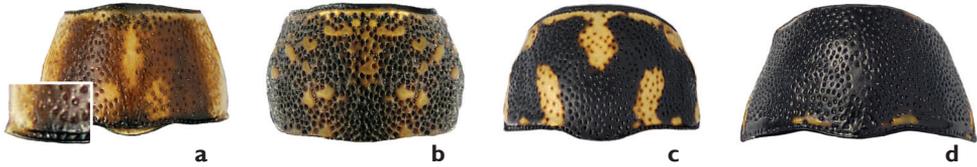


Figure 6. Pronotum: **a** reddish, with close-up of marginal bead, *P. bivittatus* **b** mottled, *P. spumarius* **c** with black M-mark, *P. m-nigrum* **d** almost black, *P. nigricornis carbonarius*.

Pronotum. In *Pachybrachis*, the pronotum is margined at base, the margin usually ornamented with a row of large punctures (Figure 6a, close up). This character is very useful to separate *Pachybrachis* Chevrolat from *Cryptocephalus* Geoffroy or *Bassareus* Haldeman. The last two genera superficially look like *Pachybrachis* but are not margined at the base of the pronotum.

The density and pattern of pronotal punctures can be a useful character. Punctures usually dissipate near the side margins, and are generally a darker color than the background.

The pronotal coloration varies from a common mottled pattern (e.g. *P. spumarius*, Figure 6b), to a black M-mark on a light background (e.g. *P. m-nigrum*, Figure 6c), to an almost entirely black pronotum with only yellow basal and lateral markings (e.g. *P. nigricornis carbonarius*, Figure 6d).



Figure 7. Elytral punctures and coloration: **a** in rows in deep striae, *P. luctuosus* **b** confused in basal half, in rows in apical half, *P. calcaratus* **c** all confused, *P. hepaticus* **d** confused and mottled, *P. spumarius* **e** vitate with marginal vitta interrupted, *P. bivittatus* **f** black, margined with yellow, *P. nigricornis carbonarius* **g** entirely black, *P. luridus*.

Elytra. As on the pronotum, the density and pattern of punctures on the elytra are easily seen and useful characters. The elytral punctures generally form fairly regular deep striae, consisting of one sutural, one marginal and eight discal striae on each elytron, although the first may be somewhat irregular in the basal third (e.g. *P. luctuosus*, Figure 7a). Punctures may be confused in the basal half but with a tendency towards regular rows in the apical half, as in *P. calcaratus* (Figure 7b).

Finally, punctures may be completely confused and not aligned at all in rows (e.g. *P. hepaticus*, Figure 7c).

The elytral color pattern is, of course, a very useful character for the identification of many species. The mottled pattern is common (e.g. *P. spumarius*, Figure 7d). Some species are vittate (= with longitudinal black stripes), sometimes with a lateral vitta interrupted as in *P. bivittatus* (Figure 7e). In some species, the elytra are largely black with only a few yellow markings or with narrow apical and lateral margins (e.g. *P. nigricornis carbonarius*, Figure 7f), or the elytra can be entirely black (e.g. *P. luridus*, Figure 7g).

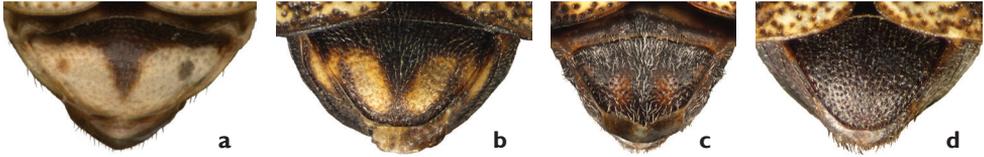


Figure 8. Pygidium: **a** largely yellow, *P. bivittatus* **b** with well-defined yellow spots, *P. cephalicus* **c** with faint reddish spots, *P. spumarius* **d** black, *P. atomarius*.

Pygidium. The coloration of the pygidium can be largely yellow (e.g. *P. bivittatus*, Figure 8a), dark with distinct yellow spots of various sizes (e.g. *P. cephalicus*, Figure 8b), or dark with faint small reddish spots (e.g. *P. spumarius*, Figure 8c). A completely black pygidium is distinctive of *P. atomarius* (Figure 8d).

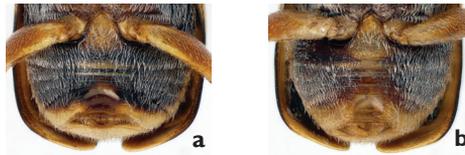


Figure 9. Sexes: **a** male abdomen, ventral view, *P. bivittatus* **b** female abdomen, ventral view, *P. bivittatus*.

Sexes. Males are usually smaller and less robust than females, with their abdomen flat (Figure 9a). In females, the abdomen is convex beneath, the last visible segment having a deep, round, concave depression or fovea (Figure 9b).

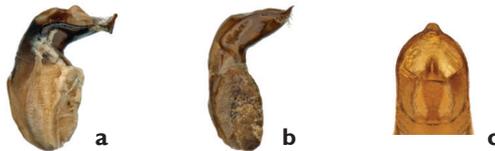


Figure 10. Aedeagus: **a** lateral view, *P. luctuosus* **b** lateral view, *P. spumarius* **c** apex, dorsal view, *P. spumarius*.

Genitalia. In most cases, individuals of each sex can be identified to species using coloration and external morphological features alone. However, an examination of the aedeagus is essential for the determination of superficially similar and variable species, such as *P. cephalicus*, *luctuosus* and *spumarius*.

In *Pachybrachis*, the basal portion of the aedeagus may appear bulbous (e.g. *P. luctuosus*, Figure 10a) or more tubular (Figure 10b), but we don't know yet if this character is reliable and consistent. The apical half is usually considerably bent, sometimes at a right angle, the degree of the curvature being an important diagnostic feature. In lateral view, the tip of the aedeagus may appear straight, sinuous and curved upwards, or sinuous and curved downwards (e.g. *P. spumarius*, Figure 10b). In dorsal view, the tip offers various shapes: small, large, pointed, triangular, lanceolate, nipple-shaped (e.g. *P. spumarius*, Figure 10c), etc.

Although the genitalic features are very constant and most reliable, they have been rarely described and illustrated in *Pachybrachis*. In the following key to the males of the 17 species treated here, the aedeagus is reported for only three species when external morphological characters may not be sufficient. The female genitalia are still unknown for all of them.

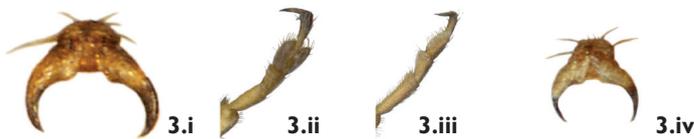
Illustrated key to males



- 1a Front femora not thicker than others (Fig. 1.i); eyes small and remote (Fig. 1.iii); punctures of pronotum and elytra dense and confused (Habitus 5) *hepaticus hepaticus* (F. E. Melsheimer)
- 1b Front femora thicker than others (Fig. 1.ii); eyes narrowly separated or normal (Fig. 1.iv) 2



- 2a Ocular lines between eyes present (Fig. 2.i) 3
- 2b Ocular lines absent (Fig. 2.ii) 6



- 3a Front claws larger (Figs 3.i, 3.ii) than middle or hind claws 4
- 3b Front claws not enlarged; size similar to those in middle and hind legs (Figs 3.iii, 3.iv) 5



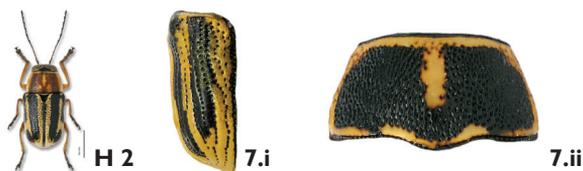
- 4a Eyes very close, separated by less than their width; ocular lines fine to indistinct (Fig. 4.i; Habitus 13).....***pectoralis* (F. E. Melsheimer)**
- 4b Eyes normal, more distant, ocular lines very distinct, with darker punctures (Fig. 4.ii; Habitus 12).....***peccans* Suffrian**



- 5a Last antennomere brownish; pronotum with subrectangular black markings pierced with yellow spots; elytral dark markings diffuse and irregular (Habitus 10)..... ***obsoletus* Suffrian**
- 5b Antennae entirely yellow; pronotum with solid black rectangular markings; elytral dark markings well-defined (Habitus 16)..... ***tridens* (F. E. Melsheimer)**



- 6a Elytra vittate (with longitudinal color stripes) (Fig. 6.i).....7
- 6b Elytra mottled (Fig. 6.ii), spotted (Fig. 6.iii), or mostly to entirely black (Fig. 6.iv).....9



- 7a Elytral punctures confused, outer (marginal) vitta often interrupted, pronotum yellow with darker reddish M-shaped mark (Habitus 2)..... ***bivittatus* (Say)**
- 7b Many elytral punctures arranged in rows (Fig. 7.i); pronotum not reddish, rather yellow with black markings of various sizes and shapes (Fig. 7.ii)..... 8



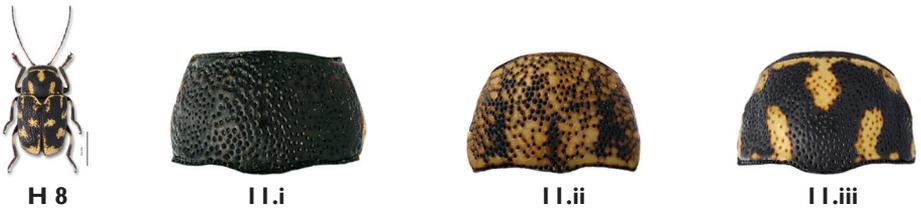
- 8a Pronotum black, margined with yellow; sutural, discal and marginal vittae of elytra distinct (Habitus 11) *othonus othonus* (Say)
- 8b Pronotum yellow with large, black, M-shaped marking; marginal and discal vittae of elytra very variable, usually not distinct (Habitus 9a)
..... *nigricornis difficilis* Fall



- 9a Body entirely black, or sides and apex narrowly margined with yellow in some females (Habitus 9b).....*nigricornis carbonarius* Haldeman
- 9b Pronotum mottled (Fig. 9.i) or with reddish spots (Fig. 9.ii); elytra variable, black (Fig. 9.iii) to mottled (Fig. 9.iv)..... 10



- 10a Elytra almost or entirely black (Fig. 10.i) 11
- 10b Elytra spotted (Fig. 10.ii) or mottled (Fig. 10.iii)..... 13



- 11a Antennae longer than body; pronotum reddish with black M-mark; elytra entirely black (Habitus 17) *trinotatus* (F. E. Melsheimer)
- 11b Antennae shorter than body; pronotum black (Fig. 11.i), mottled (Fig.11. ii), or with M-shaped marking (Fig. 11.iii, H 8 below); if pronotum with M-shaped marking, then elytra mottled, not entirely black..... 12



- 12a Elytra entirely black to streaked with whitish-yellow in outer areas; pronotum with reddish sides and upside-down reddish Y-mark (Habitus 7) ... *luridus* (Fabricius)
- 12b Elytra with large median reddish spots almost joining at suture, with additional smaller apical spots; pronotum entirely black (Habitus 15)
..... *subfasciatus* (J. E. LeConte)



- 13a Elytra yellow with black markings; pronotum with well-defined black M-mark; size larger, 3+ mm (Habitus 8)..... *m-nigrum* (F. E. Melsheimer)
- 13b Elytra (Fig. 13.i) and pronotum mottled (Fig. 13.ii), no discernable pattern; size smaller, less than 3 mm 14



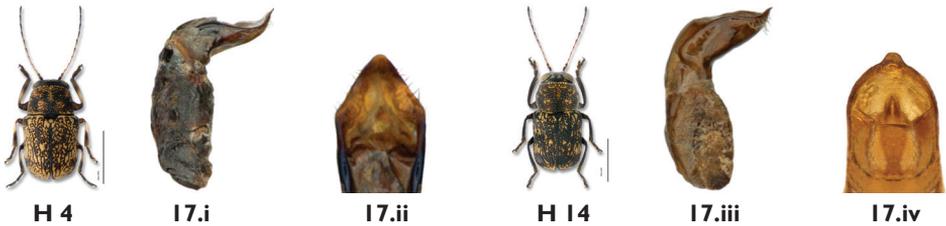
- 14a Front tibia with large, curved spur (Fig. 14.i, Habitus 3) *calcaratus* Fall
- 14b Front tibia with tiny spur (Fig. 14.ii), or without spur (Fig. 14.iii) 15



- 15a Face largely yellow in males (Fig. 15.i); pygidium entirely black (Fig. 15.iii); pronotum and elytra mottled (Habitus 1)..... *atomarius* (F. E. Melsheimer)
- 15b Face largely dark (Fig. 15.ii); pygidium spotted, with spots of some specimens being smaller and fainter than illustrated (Fig. 15.iv) 16



- 16a Elytral punctures regular in deeply impressed striae; size small (2 mm) (Habitus 6); aedeagus with apical diamond-shaped denticle (Figs 16.i, 16.ii)..... *luctuosus* Suffrian
- 16b Elytral punctures confused, not inserted into deep striae (Fig. 16.iii); size larger (2+ mm); aedeagus different (Figs. 17.i, 17.iii)..... 17



- 17a Pronotum mostly fuscous, with relatively few pale markings, densely punctate, darker than elytra (Habitus 4); aedeagus slender, sinuous, and sharper at apex in lateral view (Fig. 17.i); aedeagal tip triangular in dorsal view (Fig. 17.ii)..... *cephalicus* Fall
- 17b Markings of pronotum and elytra numerous; darker areas dark brown to rufous (Habitus 14); aedeagus sinuous and thicker at apex in lateral view (Fig. 17.iii); aedeagal tip nipple-shaped in dorsal view (Figs. 17.iv)..... *spumarius* Suffrian

***Pachybrachis atomarius* (F. E. Melsheimer, 1847)**

http://species-id.net/wiki/Pachybrachis_atomarius

Habitus 1; Map 1; Figures 1c, 5a, 8d

Cryptocephalus atomarius F. E. Melsheimer, 1847:170.

Pachybrachis infaustus Haldeman, 1849: 262.

Pachybrachys atomus Bowditch, 1909: 319.

Recognition. Body largely fuscous, dull, mottled with many yellow spots (Habitus 1); elytral puncturation dense, confused discally, more or less arranged in rows towards rear and sides; face of males predominately yellow; pygidium entirely black, convex (Figure 8d); male size small: length 1.85 ± 0.07 mm, width 1.01 ± 0.03 mm.

Distribution. The distribution in eastern Canada is restricted in southern Ontario to remnants of the Carolinian forest (Johnson 2012; Shelford 1963). In Québec, the distribution is isolated from the main distribution area (Map 1). The unique speci-

men available was probably collected on the Eardley Escarpment, which is a warmer refugium created by cliffs of the Laurentian Highlands oriented southwards (Brunton and Lafontaine 1971).

Material examined. ONTARIO: Essex Co., Ojibway, 7.VIII.1943, S. D. Hicks [6♀, CNC]; same data, except 8.VII.1943, S. D. Hicks [1♀, CNC]; Point Pelee, 9.VII.1931, W. J. Brown [5♂ 1♀, CNC]; Roseland, 24.VI.1944, S. D. Hicks [1♂, CNC]; Lambton Co., Walpole Island [1♂ 1♀, CNC]; Norfolk Co., Normandale, 4.VI.1931, W. J. Brown [1♂ 1♀, CNC]; Turkey Point, 8.VII.1931, W. J. Brown [1♂ 4♀, CNC]; Walsh, 10.VI.1931, W. J. Brown [1♀, CNC]; Ontario Co., Fisher Glen, 12.VI.1931, W. J. Brown [2♂ 6♀, CNC].

QUÉBEC: Pontiac Co., Old Chelsea, 12.VII.1961, J. R. Vockeroth [1♂, CNC].

Host plants. No plant association records were available from specimens examined, and Barney et al. (2011) did not report any either. Clark et al. (2004) presents the known literature, but since adults were usually swept from vegetation, these records cannot automatically be interpreted as real host associations.

Comments. *Pachybrachis atomarius* is one of Fall's (1915) Group C species that have "great variation in the degree of (elytral) maculation." In spite of the extremely variable elytral mottling, ranging from heavily speckled with yellow to almost entirely black, *P. atomarius* males are relatively easy to identify by the combination of the predominately yellow face (Figure 1c) and entirely black, convex pygidium (Figure 8d). The entirely black, convex pygidium character also permits identification of singleton females.

Although *P. atomarius* is a typical eastern North American species distributed from Manitoba to Oklahoma to Atlantic states (Riley et al. 2003; LeSage 1991; Barney, unpublished data), in Ontario it is restricted to the Carolinian Zone in the southernmost part of the province. Its presence in Québec is considerably disjunct from its main distribution area, and this isolation is due to the warmer microhabitat of the Laurentian Highlands cliffs of Eardley Escarpment, which are fully exposed southwards and harbor similarly disjunct insects and plants (Hall 1991; Layberry et al. 1998; NCC 2011a).

Since *P. atomarius* has not been collected in eastern Canada over the last 50 years, it is likely extirpated from the eastern Canadian fauna.

Pachybrachis bivittatus (Say, 1824)

http://species-id.net/wiki/Pachybrachis_bivittatus

Habitus 2; Map 2; Figures 6a, 7e, 8a, 9a, 9b

Cryptocephalus bivittatus Say, 1824: 440.

Pachybrachys albescens Suffrian, 1858: 404.

Recognition. Body very large, primarily yellow (Habitus 2); prothorax suffused with rufous; elytral punctuation confused, with somewhat apparent rows on disc; elytral color pattern bivittate, with outer vitta rarely entire (Figure 7e); pygidium yellow (Figure

8a); male size large: length 3.12 ± 0.16 mm, width 1.64 ± 0.05 mm. These characters allow identification of even singleton females.

Distribution. *Pachybrachis bivittatus* is a transcontinental species found across Canada and the United States (LeSage 1991; Riley et al. 2003). In Ontario, it is most common in the southernmost counties. In Québec, it occurs in the Ottawa Valley and in the St. Lawrence Lowlands. The Saguenay Region is probably its northernmost limit (Map 2).

Material examined. ONTARIO: Carleton Co., Constance Bay, 26.VI.1995, B. F. & J. L. Carr [1♀, CNC]; Elgin Co., New Sarum, 16.VI.1956, W. J. Brown [5♂ 5♀, CNC]; Essex Co., Amherstburg, 6.VI.1936, G. M. Stirrett [2♀, CNC]; Belle River, 26.V.1946, S. D. Hicks [1♂, CNC]; Kingsville, 23.V.1962, Kelyone & Thorpe [1♀, CNC]; same data, except 19.VI.1954, G. B. Wiggins [1♀, ROM]; Pelee Island, 24–27.VI.1935, R. C. Osburn [1♂ 1♀, OSUC]; same data, except 4.VII.1940, W. J. Brown [2♀, CNC]; Point Pelee, 29.VI.1931, W. J. Brown [4♂ 7♀, CNC]; same data, except 1.VI.1933, G. M. Stirrett [3♂, CNC]; same data, except 29.V.1955, S. D. Hicks [3♂ 4♀, CNC]; same data, except 28.VI.1961, Kelton & Brumpton [2♂ 1♀, CNC]; Haldimand Co., Dunnville, 7.VII.1961, W. & W. Plath [1♂, USNM]; same data, except 7.VIII.1961, W. Plath [1♂, USNM]; Kent Co., Thamesville, 15.VI.1930, G. M. Stirrett [1♀, CNC]; Rondeau Park, 5.VI.1985, J. M. Campbell & A. Davies [1♀, CNC]; Lambton Co., Grand Bend, 17.VI.1956, W. J. Brown [1♂ 4♀, CNC]; Lincoln Co., Beamsville, 19.VII.1939, S. D. Hicks [3♂ 3♀, CNC].

QUÉBEC: Argenteuil Co., Carillon, 24.V.1974, E. J. Kiteley [2♂ 1♀, CNC]; Deux-Montagnes Co., La Trappe, 5.VIII.1932, J. Ouellet [1♂, CEUM]; Île-de-Montréal Co., Montréal, F. Knab [1♂, USNM]; same data, except 31.V.1941 [1♀, CEUM]; same data, except 15.VI.1961, M. Larochelle [1♂, CEUM]; same data, except 3–14.VII.1969, E. J. Kiteley [4♂ 6♀, CNC]; same data, except 17.VI, J. Ouellet [1♀, USNM]; Nicolet Co., Bécancour, 24.VI.1967, J. L. Laliberté [1♂, IDM]; Portneuf Co., Neuville, 9.VII.1939, J. Filteau [1♂ 1♀, CEUM]; Sainte-Catherine, 17.VI.1953, J. C. Aubé [1♂ 7♀, LEM]; same data, except 8.VII.1956 [2♀, LEM]; Québec Co., Sainte-Foy, 17.VI.1933, V. Boulet [1♀, CEUM]; Cap-Rouge, 27.VI.1956, J. L. Laliberté [2♂ 2♀, IDM]; Saguenay Co., Rivière Deschênes, 22.VI.1966, A. Franeslier [1♀, CEUM]; Saint-Jean Co., Cantic, 25.VI.1983, on *Salix amygdaloides* Andersson, A. Larochelle [1♂ 3♀, CNC]; Saint-Maurice Co., Pointe-du-Lac, 3.VII.1937 [1♀, CEUM]; Vaudreuil Co., Rigaud, 12.VII.1908, J. Ouellet [1♂, CEUM].

Host plants. *Pachybrachis bivittatus* is typically associated with willows (*Salix* spp., Salicaceae) (Fall 1915; Barney 1984; Downie and Arnett 1996; Clark et al. 2004 for literature review). In Canada, MacNay (1958) reported a light infestation of *P. bivittatus* on the foliage of willow along the river banks north of Coaldale, in Alberta. In eastern Canada, LeSage (personal observations) observed it on Bebb's willow (*Salix bebbiana* Sarg.), sand-bar willow (*S. interior* Rowlee), and stalked willow (*S. petiolaris* J.E. Smith). Larochelle (see above) collected it on the peach-leaved willow (*S. amygdaloides* Andersson). The larvae feed in the litter, on willow leaves, but only when they are decayed (LeSage 1985).

Comments. With its large size, bivittate elytra, and close association with willows, *P. bivittatus* is one of the easiest species to identify.

***Pachybrachis calcaratus* Fall, 1915**

http://species-id.net/wiki/Pachybrachis_calcaratus

Habitus 3; Map 3; Figures 5c, 7b

Pachybrachys calcaratus Fall, 1915: 389

Recognition. Color dull yellow, with diffuse brown markings on pronotum, with more contrasting markings on elytra (Habitus 3); ocular lines absent; front tibiae of male with subapical rectangular tooth on inner margin, due to abrupt narrowing of tibia, and with long stout curved terminal spur (Figure 5c); terminal spur of front tibia of female reduced to narrow spine; male size small: length 2.09 ± 0.07 mm, width 1.106 ± 0.04 mm.

Distribution. *Pachybrachis calcaratus* has been found across the central portion of the United States but only in small numbers (Barney, unpublished data). Within the area of this study, it is restricted to the Carolinian Life Zone in southern Ontario (Map 3).

Material examined. ONTARIO: Essex Co., Roseland, 24.VI.1942, ‘compared with type,’ J. A. Wilcox [1♂, CDFA]; same data, except 24.VI.1944, S. D. Hicks [1♂, CNC].

Host plants. No information was recorded on the specimens collected in southern Ontario. Clark et al. (2004) reported *P. calcaratus* nibbling purple loosestrife, *Lythrum salicaria* L. (Lythraceae), in Ohio.

Comments. *Pachybrachis calcaratus* is another of Fall’s (1915) Group C species that have “great variation in the degree of (elytral) maculation.” The tibial spur (Figure 5c) is a defining character. *Pachybrachis calcaratus* was very likely associated with the Carolinian Life Zone in southern Ontario in the past. However, it has not been seen from there for the last 68 years.

***Pachybrachis cephalicus* Fall, 1915**

http://species-id.net/wiki/Pachybrachis_cephalicus

Habitus 4; Map 4; Figure 8b

Pachybrachys cephalicus Fall, 1915: 419.

Pachybrachys cephalicus var. *dixianus* Fall, 1915: 419.

Pachybrachys cephalicus var. *parvus* Fall, 1915: 419.

Recognition. Pronotum and head generally fuscous, densely punctate and darker than elytra; elytra with puncturation dense and confused (Habitus 4); ocular lines absent; male size small: length 1.94 ± 0.12 mm, width 1.05 ± 0.08 mm.

Distribution. A typical eastern species distributed from Louisiana to New York to Atlantic Coast (Riley et al. 2003), restricted to southern Ontario in eastern Canada (Map 4).

Material examined. ONTARIO: Norfolk Co., Walsingham Forest Station, 28.VII.1982, ex. *Potentilla* or strawberry, L. LeSage [11♂ 14♀, CNC].

Host plants. Cinquefoil (*Potentilla* sp.) and strawberry (*Fragaria* sp.) (both Rosaceae) are the first host associations reported for *P. cephalicus*. The specimens were swept from these two plants growing in a sandy clearing within a dry oak-pine forest (LeSage, personal field notes). Since 1984, the previous Walsingham Forestry Station is part of the St. Williams Dwarf Oak Forest, the largest block of publicly owned forest in the Carolinian Life Zone (NHIC 1998).

Comments. *Pachybrachis cephalicus* is another of Fall's (1915) Group C species that have "great variation in the degree of (elytral) maculation." The fairly large number of examined specimens may be misleading since they all come from only one event. In fact, *P. cephalicus* is very rarely collected in eastern Canada and known from only one locality within the Carolinian Life Zone. This is also a first record of this species for Canada.

***Pachybrachis hepaticus hepaticus* (F. E. Melsheimer, 1847)**

http://species-id.net/wiki/Pachybrachis_hepaticus_hepaticus

Habitus 5; Map 5; Figures 1c, 3a, 7c

Cryptocephalus hepaticus F. E. Melsheimer, 1847: 171.

Cryptocephalus punctatus Haldeman, 1849: 257.

Recognition. Front femora not enlarged in comparison to those of middle and hind legs (Figure 3a); eyes small and remote (Figure 1d); antennae short, less than half body length (Habitus 5); integument densely, diffusely punctate (Figure 7c); elytra tapered to apex; male size very small: length 1.68 ± 0.07 mm, width 0.96 ± 0.07 mm.

Distribution. Transcontinental, extending from California to Maine (Riley et al. 2003). In eastern Canada, *P. hepaticus hepaticus* occurs in southern Ontario from Lake Ontario to the Ottawa River Valley in Québec (Map 5).

Material examined. ONTARIO: Carleton Co., Jockvale, 4.VII.1934, W. J. Brown [1♂, CNC]; Ottawa, 24.VI.1995, [ex. field notes: "pinery forest preserve, on sand dunes..."], B. F. & J. L. Carr [1♀, CNC]; Essex Co., Leamington, 3.VII.1931, W. J. Brown [1♀, CNC]; same data, except 17.VI.1940 [1♂, CNC]; Point Pelee, 24.VI.1931, W. J. Brown [1♂, CNC]; Haldimand- Norfolk Cos., Turkey Point, 24.VII.1984, sweeping in marshy area, L. LeSage [1♀, CNC]; Hasting Co., 10.VII.1938, Brimley [1♀, CNC]; Norfolk Co., Forestville, 15.VI.1931, W. J. Brown [1♂, CNC]; Parry Sound Dist., Scotia Junction, 28.VII.1934, H. W. Wenzel [1♂ 1♀, OSUC]; Prince Edward Co., 22.VI.1919, Brimley [1♀, CNC]; same data, except 28.VI.1921 [1♂, CNC]; same data, except 2.VIII.1925 [1♀, CNC]; Renfrew Co., Arnprior, 20.VII.1941, W. J. Brown [1♀, CNC]; Russell Co., Mer Bleue, 18.VI.1986, W. J. Brown [1♂, CNC]; same data, except 10.VII.1936 [1♀, CNC]; Toronto Co., Toronto, F. Knab [1♀, USNM]; same data, except 26.V.1896, R. J. Crew [1♀, ROM]. Unknown Co., East Ontario, 1885 [1♀, CNC].

QUÉBEC: Deux-Montagnes Co., La Trappe, 30.VI.1931, J. Ouellet [1♂, CEUM]; same data, except 27–29.VI.1933 [21♂ 15♀, CEUM]; same data, except

23.VII.1933 [1♀, CEUM]; same data, except 28.VII.1934 [1♀, CEUM]; same data, except 20.VIII.1936 [1♂, CEUM]; same data, except 26.VIII.1946 [1♀, CEUM]; same data, except 7.VII.1949 [1♂, CEUM]; Gatineau Co., Alcove, 24.VIII.1936, W. J. Brown [1♀, CNC]; Gatineau Park, Meach Lake, 30.VII.1972, A. Davies [1♀, CNC]; Wakefield, 20.VII.1932, W. J. Brown [2♀, CNC]; Wright, 27.VI.1933, G. S. Walley [1♂, CNC]; Labelle Co., Nomingue, 29.VII.1931, J. Ouellet [3♀, CEUM]; same data, except 6–21.VII.1932 [20♂ 13♀, CEUM]; same data, except 4.VII.1933 [2♂ 1♀, CEUM]; same data, except 2.VII.1934 [1♂, CEUM]; same data, except 8.VII.1935 [1♂, CEUM]; same data, except 22.VII.1936 [2♀, MSUC]; same data, except 2.VIII.1936 [1♂, CEUM].

Host plants. No plant association records were available from specimens examined. *Pachybrachis hepaticus hepaticus* may be a polyphagous species, considering the number of plant families listed in Clark et al. (2004): Asteraceae, Euphorbiaceae, Fabaceae, Juncaceae, Salicaceae, and Tamaricaceae.

Comments. Of all the species here studied, the shortest antennae (Habitus 5) and smallest eyes are found in *P. hepaticus hepaticus*. Further investigation may require that a new genus is established for the eastern and western subspecies of *hepaticus*. Fall (1915) cited specimens from Montréal, May 24 (Liebeck Coll.); Toronto (Crew); and Scotia Junction, July 27 (Wenzel), but these specimens could not be located and examined.

Pachybrachis luctuosus Suffrian, 1858

http://species-id.net/wiki/Pachybrachis_luctuosus

Habitus 6; Map 6; Figures 7a, 10a

Pachybrachys luctuosus Suffrian 1858: 401.

Recognition. Color black or piceous; ocular lines absent; pronotum and sides of elytra with few yellow marks; elytral punctures confused in scutellar area, in fairly regular rows in apical half; elytral striae deep and quite regular (Habitus 6); aedeagus with terminal nodule and denticle forming small, 90° diamond shape (Figure 10a); male size small: length 1.87 ± 0.10 mm, width 0.95 ± 0.11 mm.

Distribution. A relatively rare Atlantic species distributed from Alabama to New York in the United States (Riley et al. 2003; Barney, unpublished data). The Parry Sound specimens in Ontario and those of the Île-du-Grand-Calumet in the Ottawa River are two small populations disjunct from the main Atlantic one (Map 6).

Material examined. ONTARIO: Hastings Co., 20.VI.1952, J. F. Brimley [1♂, CNC]; Parry Sound Dist., Hwy. 69, 12 km S Shawanaga, 13.VII.1995, B. F. & J. L. Carr [1♂, CNC]. Leeds Co., 7.VIII.1950, ex. pine, J. F. Brimley [3♂ 12♀, CNC].

QUÉBEC: Pontiac Co., L'Île-du-Grand-Calumet, 3.VIII.1985, on *Pinus resinosa* Ait., Larochelle & Larivière [1♂ 1♀, CNC]; Luskville, 4.VII.1985, on *Quercus rubra* L., Larochelle & Larivière [1♂, CNC].

Host plants. A large series was taken on pine in Leeds Co., ON. *Pachybrachis luctuosus* was first reported from *Pinus virginiana* P. Mill. in Alabama (Balsbaugh and Hays 1972). This record was extended to the northeastern states by Wilcox (1979), and assumed to be valid as well in West Virginia (Clark 2000). Specimens from Larochelle & Larivière had label notations reporting collections from *Pinus resinosa* Ait. and *Quercus rubra* L.

Comments. *Pachybrachis luctuosus* is another of Fall's (1915) Group C species that have "great variation in the degree of (elytral) maculation." Fall commented that he would not be surprised if *P. carolinensis* Fall was a paler form of *P. luctuosus*. Our comparison of aedeagi of specimens identified by Fall as either *luctuosus* or *carolinensis* revealed the same, distinctive form – the subplanar surface with one median subapical denticle. *Pachybrachis carolinensis* appears to be a larger, more yellow variation, but more work needs to be done. *P. luctuosus* is reported for the first time in Canada, and therefore, is also a first record for ON and QC.

***Pachybrachis luridus* (Fabricius, 1798)**

http://species-id.net/wiki/Pachybrachis_luridus

Habitus 7; Map 7; Figure 7g

Cryptocephalus luridus Fabricius, 1798:109.

Cryptocephalus femoratus Say, 1824: 439.

Cryptocephalus aesculi F. E. Melsheimer, 1847:171.

Pachybrachys moerens Stål, 1857: 63.

Pachybrachys luridus var. *nigrinus* Blatchley, 1910:1130.

Pachybrachys luridus var. *festivus* Fall, 1915: 470.

Recognition. Body dull black, densely, coarsely punctured; pronotum black with anterior median line and sides red or reddish yellow, varying to almost entirely red; elytra mottled with yellow, especially toward sides, varying to entirely yellow to entirely black (Habitus 7); front claws of male much enlarged (as in Figure 4a); male size medium: length 2.65 ± 0.23 mm, width 1.45 ± 0.12 mm.

Distribution. Occuring in the eastern half of the United States (Riley et al. 2003) to the Rocky Mountains, but in Canada restricted to the Carolinian Life Zone of southern Ontario (Map 7).

Material examined. ONTARIO: Essex Co., Ojibway, 9.VI.1943, S. D. Hicks [1♀, CNC]; Lambton Co., Grand Bend, 20.VII.1930, G. E. Shewell [1♀, CNC]; Simcoe Co., 19.VI.1939, G. S. Walley [1♀, CNC]; Toronto Co., Toronto, 26.VI.1896, C. T. Hills [2♂ 2♀, LEM]; same data, except 15–30.VI.1927, L. J. Milne [1♀, UNHC]; same data, except F. Knab [29♂ 20♀, USNM]; Toronto, High Park, 4.VI.1897 [3♂ 2♀, ROM]; Unknown Co., Black Creek, 14.VI.1897 [1♀, ROM]; Springfield [2♀, ROM]; Can., G. M. Greene [1♂, USNM].

Host plants. No plant association records were available from Canadian specimens. In the United States, the false indigos (*Baptisia leucantha* T. & G., *B. tinctoria*

(L.) R. Br.) (Fabaceae) were the associations most often cited by authors (Frost 1945, details in Clark et al. 2004). However, these plants are not present in Québec (Marie-Victorin 1995), and extremely rare in southern Ontario (Scoggan 1978). Barney et al. (2011) stated that recently collected specimens in Kentucky were probably from oak (*Quercus* spp., Fagaceae).

Comments. Fall (1915) observed specimens from Ontario: Toronto (Wickham). However, no specimens of *P. luridus* have been collected from the province in the last 68 years, and one of its potential hosts (*Baptisia* spp.) were always extremely rare in southern Ontario (Scoggan 1978). Consequently, *P. luridus* is likely extirpated from the eastern Canadian fauna.

***Pachybrachis m-nigrum* (F. E. Melsheimer, 1847)**

http://species-id.net/wiki/Pachybrachis_m-nigrum

Habitus 8; Map 8; Figure 6c

Cryptocephalus m-nigrum F. E. Melsheimer, 1847:170.

Pachybrachys intricatus Suffrian, 1852:180.

Recognition. Pronotum usually with thick, black, M-shaped marking; elytra yellow with variable black markings, but these usually leaving basal, lateral and sutural margins yellow, in addition to a basal and median yellow spots on each elytron (Habitus 8); male size medium: length 2.59 ± 0.11 mm, width 1.42 ± 0.07 mm.

Distribution. A typical eastern species distributed in the eastern half of the United States (Riley et al. 2003). Its presence in the south of the eastern Townships, in Québec, corresponds to the northernmost limit of this species (Map 8).

Material examined. NEW BRUNSWICK: York Co., 15 km W of Tracy, off Rt 645, 45,6837°N, 65,8809°W, 22.vii.2007, red pine forest, sweeping foliage of *Comptonia peregrina*, R. P. Webster [1?, RWIC].

QUÉBEC: Châteauguay Co., Ormstown, 12.VII.1977, sweeping, E. J. Kiteley [1♀, CNC]; same data, except 30.VII.1978 [1♂, CNC]; Huntingdon Co., Covey Hill, 30.VI.1927, G. S. Walley [1♀, CNC]; same data, except 1.VII.1927, W. J. Brown [1♀, CNC].

Host plants. An old record by Schwarz (1890) concerned *Toxicodendron radicans* (L.) Kuntze) (Anachardiaceae), but this was probably based on misidentified specimens of *P. tridens*. Clark et al. (2004) reviewed the literature. More recently, Webster swept it from *Comptonia peregrina* (Myricaceae) (Webster et al. 2012). Recent surveying in Kentucky barrens/prairies by Barney et al. (2011) cited *P. m-nigrum* collected from and found to feed on St. John's-wort, *Hypericum dolabriforme* Vent. (Clusiaceae) (Barney and Hall (2011)).

Comments. Based upon external morphology, these specimens appear to be *P. m-nigrum*. However, extensive dissections of material from across the eastern half of the US reveal an externally similar species, but with a distinctly different aedeagus, from the midwestern states (Barney, unpublished data).

***Pachybrachis nigricornis* (Say, 1824)**

http://species-id.net/wiki/Pachybrachis_nigricornis

Habitus 9a, 9b, 9c; Map 9; Figures 6d, 7f

Cryptocephalus nigricornis Say, 1824: 436.

Pachybrachys carbonarius Haldeman, 1849: 260.

Pachybrachys autolytus Fall, 1915: 458.

Pachybrachys autolytus var. *difficillis* Fall, 1915: 459.

Pachybrachys autolytus var. *wahsatchensis* Fall, 1915: 459.

Pachybrachys carbonarius var. *janus* Fall, 1915: 462.

Recognition. Pronotum strongly alutaceous and opaque, more finely punctate than elytra. In subspecies *difficilis*, black stripes usually complete and distinct (Habitus 9a), but in some specimens fused together. In subspecies *carbonarius*, elytra largely black (Habitus 9b), margined with yellow, or with yellow at edge of elytra in some females (Habitus 9c); male size small: length 2.20 ± 0.14 mm, width $1.21 \text{ mm} \pm 0.06$ mm (*difficilis*); length 2.10 ± 0.07 mm, width 1.17 ± 0.04 mm (*carbonarius*).

Distribution. The species *P. nigricornis* is distributed across the eastern two-thirds of North America (Riley et al. 2003). The distribution of the subspecies remains a subject of debate (Balsbaugh and Tucker 1976; Barney and Hall 2009) (Map 9).

Material examined. NEW BRUNSWICK: Kent Co., Kouchibouguac National Park, 7.VII.1978, Code 7785K, H. Goulet [3♂, CNC]; same data except Code 7754F [1♂ 1♀, CNC]; same data, except 13.VII.1977, code 5599I, G.A. Calderwood [1♂ 1♀, CNC]; same data, except 4.VIII.1977, code 5779G [1♂, CNC]; same data, except 25.VIII.1977, code 5849Y, S.J. Miller [1♂ 1♀, CNC]; Queens Co., Jemseg, 18.VI.1981, ex. *Potentilla simplex*, L. LeSage & D. Ward [1♀, CNC].

ONTARIO: Carleton Co., Britannia, 14.VI.1949, R. de Ruelle [2♀, CNC]; Constance Bay, 12.VII.1996, lot 3, B. F. & J. L. Carr [1♀, CNC]; Innis Point, 9–16.VII.1985, Interception trap, J. Denis & L. Dumouchel [1♂, CNC]; Ottawa, 17.VII.1912, G. Ouellet [1♀, IDM]; Hasting Co., Trenton, 28.V.2000, Evans [1♂, CNC]; Kenora Co., Berens River, 4–9.VII.1938, W. J. Brown [1♂ 1♀, CNC]; Hwy 17, 15 km east of Borups Corners, 23.VI.1996, lot 2, B. F. & J. L. Carr [1♀, CNC]; Leeds Co., Saint-Lawrence Island National Park, Grenadier Island Center, 27.V.1975, sweeping, E. Sigler [1♀, CNC]; Lennox - Addington Co. Kaladar, 21.VII.1996, lot 3, B. F. & J. L. Carr [1♂, CNC]; Muskoka Co., Bala, 24.VI.1956, W. J. Brown [3♂ 11♀, CNC]; Nipissing Co., Algonquin Provincial Park near Brent, 19.VIII.1980, R. Baranowski [1♀, CNC]; Hwy 11, 30 km north of North Bay, 14.VII.1995, lot 3, B. F. & J. L. Carr [1♂ 1♀, CNC]; Hwy 17, 13 km west of Mattawa, 14.VI.1995, lot 1, B. F. & J. L. Carr [2♀, CNC]; Parry Sound Co., Parry Sound, 12.VII.1961, G. Brumpton [2♂, CNC]; same data, except 12.VII.1995, lot 1, B. F. & J. L. Carr [1♂, CNC]; Hwy 69, 12 km south Shawanaga, 13.VII.1995, Lot 3, B. F. & J. L. Carr [1♀, CNC]; Prescott Co., Alfred Bog, 8.VI.1982, breeding, ex. *Cassandra calyculata*, L. LeSage [4♂ 4♀, CNC]; same data, except 4.VI.1982, ex. Ericaceae [2♂ 4♀, CNC]; Prince

Edward Co., 23.VI.1933, J. F. Brimley [1♀, CNC]; same data, except 1.VII.1936 [1♂, CNC]; same data, except 28.VI.1939 [1♂, CNC]; same data, except 19.VII.1942 [1♂, CNC]; same data, except 11–18.VI.1947 [12♂ 7♀, CNC]; same data, except 9.VI.1948 [8♂ 7♀, CNC]; same data, except 21.VI.1950 [2♀, CNC]; same data, except 17.VI.1953 [8♂ 14♀, CNC]; same data, except 19.VII.1961 [1♂, CNC]; same data, except 2.VII.2000, Evans [1♀, CNC]; Russell Co., Mer Bleue, 13.VI.1932, W. J. Brown [2♂ 1♀, CNC]; same data, except 30.VI.1932 [5♂, CNC]; same data, except 26.VII.1932 [1♂, CNC]; same data, except 30.VI.1934 [1♂, CNC]; same data, except 10.VIII.1936 [1♂, CNC]; same data, except 25.VII.1932, L. J. Milne [6♂ 3♀, CNC]; same data, except 29.VI.1954, E. C. Becker [2♂ 2♀, CNC]; same data, except 3.VII.1981, ex. *Cassandra calyculata*, L. LeSage [1♂ 1♀, CNC]; same data, except 30.VII.1979, sweeping, H. Goulet [1♂, CNC]; Sudbury Co., Sudbury, 1898 [1♀, CNC]; Thunder Bay Co., Manitouwadge, paper yard, 3.VII.1985, T. Baker [1♂ 1♀, CNC]; 59 km north junction of highways 516 & 599, 24.VI.1992, lot 1 B. F. & J. L. Carr [1♂, CNC].

QUÉBEC: Beauce Co., Beauceville, 21.VI.1937, Frère Étienne-Maurice [1♀, CEUM]; Bonaventure Co., Cascapédia, 22.VI.1933, W.J. Brown [1♂ CNC]; same data, except 9.VII.1933 [1♂, CNC]; same data, except 16.VIII.1933 [1♀, CNC]; Châteauguay Co., Ormstown, 12.VII.1977, E. J. Kiteley, sweeping [1♂, CNC]; Deux-Montagnes Co., La Trappe, VII.1933 [1♂ 1♀, *autolycus*, var. *difficillis* Fall, Fall-MCZ]; same data, except 25.V.1929, P. Leopold [4♀, CEUM]; same data, except 2–19.VI.1936 [1♀, CEUM]; same data, except 21.VI.1933, J. Ouellet [5♂ 3♀, CEUM]; same data, except 23.VII.1933 [1♂, CEUM]; same data, except 3–28.VII.1934 [2♂ 1♀, CEUM]; same data, except 3–17.VII.1935 [1♂ 1♀, CEUM]; same data, except 15.VI.1946 [1♂, CEUM]; Gatineau Co., Aylmer, 11.VI.1932, W. J. Brown [6♂ 4♀, CNC]; Gracefield, 22.VI.1937, O. Peck [1♂, CNC]; Kazabazua, 28.VIII.1928, W. J. Brown [1♀, CNC]; Lytton, 1.VII.1981, weeds, side of road, A. Larochelle [1♂, CNC]; Mont King, Parc de la Gatineau 7–14.VII.1997, L. LeSage & C. Lacroix [1♂ CNC]; Huntingdon Co., Saint-Antoine-Abbé, 16.VI.1983, fern, A. Larochelle [2♀, CNC]; Joliette Co., Joliette, 12.VII.1922, J. Ouellet [1♂ 2♀, CEUM]; Labelle Co., Nominingue, 21.VII.1932 [1♂, *autolycus*, var. *difficillis* Fall, Fall-MCZ]; same data, except 12–19.VII.1932, J. Ouellet [4♂ 3♀, CEUM]; same data, except 4.VII.1934, L. Daviault [1♀, CEUM]; Lac-Saint-Jean-Ouest Co., Mistassini, 28.VII.1944, A. Robert [1♂ 1♀, CEUM]; Montcalm Co., Parc du Mont-Tremblant, 22.VI.1956, A. Robert [1♀, CEUM]; same data, except 11.VII.1961 [1♀, CEUM]; Pontiac Co., Beech Grove, 10.VIII.1966, H. Goulet [1♂, CNC]; Portneuf Co., Sainte-Catherine, 3.VII.1956, J. C. Aubé [1♀, LEMC]; Territoires-du-Nouveau-Québec Co., Casa-Berardi, 22–29.VI.1997, interception trap, P. Paquin [2♂, LEM]; same data, except 6–27.VII.1997 [4♂ 2♀, LEM]; same data, except 3–24.VIII.1997 [3♂ 1♀, LEM]; Chemin Selbale, 6–27.VII.1997, interception trap, P. Paquin [1♂ 4♀, LEM].

Host plants. Although over 200 specimens were examined, the only potential host plants recorded by collectors were *Cassandra calyculata* (L.) D. Don (Ericaceae) and *Potentilla simplex* Michx. (Rosaceae). Balsbaugh and Tucker (1976) reported that

they collected series of *carbonarius* on wild strawberry (*Fragaria* sp.) (Rosaceae), and tick-trefoil (*Desmodium* sp.) (Fabaceae) in Alabama. Barney and Hall (2009) reared the same subspecies on *Desmodium paniculatum* L. DC and *Lespedeza virginica* (L.) Britton (Fabaceae) in Kentucky.

Comments. Balsbaugh and Tucker (1976) and Riley et al. (2003) recognized *P. nigricornis* as having four subspecies in North America. Fall (1915) described *P. autolytus* as a separate species with two “varieties,” and he added the variety *janus* to *P. carbonarius*, which he also recognized as a separate species. Specimens from each of the provinces of NB, ON and QC included an assortment of var. *carbonarius*, var. *difficilis*, and the yellow variation of *carbonarius* as per Barney and Hall (2009). In the “Material examined” section above, the QC specimens from Fall’s personal collection (Fall-MCZ) are listed, with an indication of his identification label of *autolytus*, var. *difficillis*.

Pachybrachis obsoletus Suffrian, 1852

http://species-id.net/wiki/Pachybrachis_obsoletus

Habitus 10; Map 10

Pachybrachys obsoletus Suffrian, 1852: 200.

Recognition. Background color yellow, with numerous, usually not sharply outlined black spots on both pronotum and elytra (Habitus 10); eyes distant; ocular lines faint; male size small: length 1.87 ± 0.16 mm, width 0.98 ± 0.08 mm.

Distribution. Species broadly distributed from North Dakota to New Mexico to Atlantic Coast in the United States (Riley et al. 2003), and in Canada from British Columbia to New Brunswick. In eastern Canada, it is found in Ontario, Québec and New Brunswick (Map 10).

Material examined. NEW BRUNSWICK: Kent Co., Kouchibouguac National Park, 21.VII.1977, Code 5680L, D. J. Brown [1♀, CNC]; same data, except 17.VII.1978, Code 7291K, D. B. Lyons [1♀, CNC]; Queens Co., Canning Grand Lake near Flowers Cove, 1.VII.2004, D. Sabine and R. Webster [2♂ 2♀, RWIC]; White’s Cove, Grand Lake, 24.VII.1957, ex. *Salix lucida* [2♂ 2♀, CNC]; Sunbury Co., 9.5 km NE Jct. 101 & 645, 22.VII.2007, R. P. Webster [1♀, RWIC]; York Co., Fredericton, French Lake, 20.VII.1931, C. W. Maxwell [1♀, LEM]; Fredericton, 22.VII.1936, R. E. Balch [1♀, CNC]; French Lake, 2.VI.1928, W. J. Brown [1♀, CNC].

ONTARIO: Carleton Co., Carp, 5.VII.1932, W. J. Brown [1♂, CNC]; Merivale, 16.VII.1936, W. J. Brown [2♀, CNC]; Stittsville, 18.VII.1963, J. F. McAlpine [1♂, CNC]; Stittsville, 18.VIII.1963, Malaise trap [1♀, CNC]; Durham Co., Durham, VI.1969, [1♀, CNC]; Hastings Co., 10.VII.1938, J. F. Brimley [2♀, CNC]; same data, except 16.VII.1950 [2♀, CNC]; same data, except 31.VI.1936 [1♀, CNC]; Marmora, 19.VI.1952, J. R. Vocheroth [1♂, CNC]; same data, except 4.VII.1952, J. R. McGillis [1♀, CNC]; same data, except 18.VIII.1952, E. H. N. Smith [1♀, CNC]; Kent Co., Tilbury, 20.VIII.1947, on willow, S. D. Hicks [1♀, CNC]; Lambton Co.,

Grand Bend, 11.VII.1939, G.E. Shewell [1♀, CNC]; same data except 20.VII.1939 [1♀, CNC]; Lanark Co., Bell's Corners, 6.VI.1942, F.I. Survey 1942, Rec 5436C, White Pine [1♀, CNC]; Leeds Co., Mulcaster Island, Saint-Lawrence Island National Park, 17.VIII.1976, Sweeping *Pinus strobus*, Code 4438, W. Reid [1♀, CNC]; Thwartway Island, Saint-Lawrence Island National Park, 18.VII.1976, Malaise trap, Code 4147-M, W. Reid [3♀, CNC]; Middlesex Co., Coldstream, 22.VI.1922, A. A. Wood [1♂ 5♀, CNC]; Nipissing Co., North Bay, 11.VII.1972, E. J. Kiteley [1♂, CNC]; Peterborough Co., 3.VII.1958, J. F. Brimley [1♀, CNC]; Prescott, Co., Alfred Bog, 7.VI.1982, sweeping vegetation in a bog, L. LeSage [2♂, CNC]; Prince Edward Co., 12.VII.1914, J. F. Brimley [1♀, CNC]; same data, except 29.XI.1914 [1♀, CNC]; same data, except 11.VII.1920 [1♂, CNC]; same data, except 15–30.VII.1922, J. F. Brimley [3♀, CNC]; same data, except 19.VI.1926 [1♀, CNC]; same data, except 6.VII.1935 [1♂, CNC]; same data, except 28.VI.1936 [1♀, CNC]; same data, except 26.VII.1936 [1♀, CNC]; same data, except 17.VIII.1938 [1♀, CNC]; same data, except 9.VII.1941 [1♀, CNC]; same data, except 5.VII.1942 [3♀, CNC]; same data, except 18.VI.1947 [1♀, CNC]; same data, except 29.VIII.1948 [1♀, CNC]; same data, except 29.VI.1949 [1♀, CNC]; same data, except 31.VIII.1949 [1♂, CNC]; same data, except 19.VII.1950 [1♀, CNC]; same data, except VII.1953 [2♂ 4♀, USNM]; same data, except 24.VII.1955 [1♀, CNC]; same data, except 29.VII.1956 [4♂ 10♀, CNC]; same data, except 26.VI.1966 [1♀, CNC]; Renfrew Co., Chalk River, 3.VIII.1937, J. M. Cameron [1♀, LEM]; Russell Co., Mer Bleue, 30.VI.1932, W. J. Brown [1♀, CNC]; same data, except 17.VII.1936 [1♀, CNC]; same data, except 2.VII.1938, G. E. Shewell [1♂, CNC]; Sudbury Co., Sudbury, 4.VIII.1979, R. S. Anderson [1♀, CNC]; Thunder Bay Dist., Black Sturgeon Lake, 1–15.VIII.1956, Lindberg [1♂ 1♀, CNC]; Jarvis Island, 20.VIII.1952, on white pine, J. F. McAlpine [1♂, CNC]; Manitouwadge, 22.VI.1988, T. Baker [1♂, CNC]; same data, except 30.VII.1992 [1♂, CNC]; Toronto Co., Toronto, 27.V.1896, H. R. [1♀, LEM]; Quetico Provincial Park, 8.VIII.1982, C. B. Barr [2♀, LSAM]; Unknown Co., East Ontario [1♀, CNC].

QUÉBEC: Abitibi Co., Duparquet, 7.VIII.1983, ex. *Pinus banksiana* Lamb., A. Larochelle [1♀, CNC]; Berthier Co., Berthierville, 8.VII.1950, A. Robert [1♀, CEUM]; Lanoraie, VII.1935, G. Chagnon [1♀, H. C. Fall, CEUM]; Charlevoix-Est Co., Clermont, 17.VIII.1982, ex. *Pinus strobus* L., A. Larochelle [2♀, CNC]; Port-au-Saumon, 19.VIII.1982, ex. spruce sp., A. Larochelle [1♂, CNC]; Drummond Co., Saint-Cyrille, 10.VII.1982, ex. *Cassandra calyculata*, L. LeSage [1♂ 1♀, CNC]; Gatineau Co., Wakefield, 5.VIII.1974, ex. *Pinus strobus*, R. Sexton [1♀, CNC]; Île-Jésus Co., Île-Jésus, 5.VII.1935, G. Chagnon [1♀, CEUM]; Montcalm Co., Parc du Mont-Tremblant, 13.VIII.1932, A. Robert [1♂, CEUM]; Montgomery Co., Saint-Jean-d'Orléans, 22.VII.1957, J. L. Laliberté [1♀, IDM]; Montmagny Co. Montmagny, 8.VIII.1981, ex. *Pinus strobus* L., A. Larochelle [1♀, CNC]; Pontiac Co., Beech Grove, 15.VIII.1948, S. D. Hicks [3♀, CNC]; Saguenay Co., Grandes-Bergeronnes, 15.VIII.1982, ex. *Pinus resinosa* Ait., A. Larochelle [1♀, CNC]; Stanstead Co., Barnston, 26.VIII.1984, ex. *Thuja* sp., Larochelle & Larivière [1♀, CNC]; Terrebonne

Co., Terrebonne, 1.VII.1933, J. Ouellet [1♀, CEUM]; Vaudreuil Co., Rigaud, 19.VII.1985, ex. *Pinus strobus* L., Larochelle & Larivière [2♀, CNC]; same data except 20.V.1977, sweeping sumac sp., E. J. Kiteley [1♀, CNC]; Saint-Lazare, 6.VIII.1985, ex. *Betula papyrifera* Marsh., A. Larochelle [1♂, CNC].

Host plants. *Pachybrachis obsoletus* may be associated with peat bogs in eastern Canada. Specimens were collected in Alfred Bog (Pope 2011), and Mer Bleue (NCC 2011b; Wikipedia 2011), two well-known bogs of Ontario. The Lanoraie specimens, in Québec, were collected in an ecological preserve, which includes several fens and bogs (MDDEP 2011). Larochelle specimens, also from Québec, had a wide range of potential plant associations. Leather leaf (*Cassandra calyculata* (L.) D. Don.) (Ericaceae) is definitively a host (LeSage, collecting and personal observations), whereas the beetles' presence on white pine (*Pinus strobus*) (Pinaceae) is very likely incidental. Sweeping from willows (*Salix* spp.) (Salicaceae) was reported by Barney et al. (2011). Additionally, *P. obsoletus* was reported as causing light injury to cultivated roses in Saskatoon, Saskatchewan, but the species identification cannot be confirmed (Arnason 1942, 1943; Arnason et al. 1946; King et al. 1944, 1945; Campbell et al. 1989).

Comments. *Pachybrachis obsoletus* has a broad distribution from Manitoba to Oklahoma and eastwards to the Atlantic Ocean, with Alberta and British Columbia disjunct from this main area (Riley et al. 2003). It is reported here for the first time from NB.

Pachybrachis othonus othonus (Say, 1825)

http://species-id.net/wiki/Pachybrachis_othonus_othonus

Habitus 11; Map 11

Cryptocephalus othonus Say, 1825: pl. 28.

Cryptocephalus marginaticollis Randall, 1838: 46.

Recognition. Body robust. Pronotum black, with all margins and narrow median anterior stripe yellow; each elytron black, with rather narrow sub sutural, discal and marginal vittae yellow; legs yellow. Punctures of pronotum larger and denser than those on elytra; elytral punctures in somewhat regular rows on disc and sides (Habitus 11); male size medium: length 2.63 ± 0.12 mm, width 1.56 ± 0.09 mm.

Distribution. A typical eastern species distributed from North Dakota to Texas to the Atlantic Coast in the United States (Riley et al. 2003), and in the south of Ontario and Québec in eastern Canada (Map 11).

Material examined. ONTARIO: Carleton Co., Britannia, 17.VI.1948, S. D. Hicks [1♂, CNC]; same data, except 23.VI.1950, R. de Ruelle [1♀, CNC]; Constance Bay, 14.VII.1950, S. D. Hick [1♀, CNC]; Dirleton, 4.VII.1956, S. D. Hicks [1♀, CNC]; Ottawa, 7.VIII.1914, F. G. Ouellet [1♂, IDM]; same data, except 15.VII.1957, J. E. H. Martin [1♂, CNC]; Essex Co., Leamington, 24.VI.1940, W. J. Brown [1♀, CNC]; Ojibway, 27.VI.1943, S. D. Hicks [1♀, CNC]; same data, except 10.VI.1944 [1♂, CNC]; Roseland, 17–24.VI.1944, S. D. Hicks [1♂ 3♀, CNC]; same

data, except 17.VI.1946 [2♀, CNC]; same data, except 30.VI.–13.VII.1946 [2♂ 9♀, CNC]; Halton Co., Burlington, 1920, G. M. Stirrett [1♂ 1♀, CNC]; Hastings Co., Chatterton, 16.VII.1950, J. F. Brimley [2♂, CNC]; same data, except 2.VII.1951, J. C. Martin [1♀, CNC]; Marmora, 6.VII.1951, J. F. McAlpine [1♂, CNC]; same data, except 29.VIII.1952, C. Boyle [1♂, CNC]; Lanark Co., Bell's Corners, 15–26.VI.1950, S. D. Hicks [1♂ 2♀, CNC]; same data, except 4.VII.1950 [1♀, CNC]; Niagara Co., St. Catharines, Decew Falls, 27.VII.1940, S. D. Hick [1♀, CNC]; Toronto Co., Kingsport, 3.VII.1965, D. D. Munroe [1♂ 1♀, CNC]; Toronto, 26.VI.1896, C. T. Hills [1♂ 2♀, LEM]; same data, except 27.V.1896 [1♂ 2♀, LEM]; same data, except 9.VI.1905, E. C. Oakley [3♀, ROM]; same data, except F. Knab [3♂ 1♀, USNM]; Wentworth Co., Ancaster, 10.VII.1965, J. E. Martin [2♂, CNC].

QUÉBEC: Berthier Co., Berthierville, 27.VII.1938, J. Ouellet [1♀, CEUM]; same data, except 8.VII.1950 [1♀, CEUM]; Lanoraie, 1.VII.1932 [1♂, CEUM]; Chambly Co., Boucherville, 1.VII, J. Ouellet [1♀, CEUM]; Deux-Montagnes Co., Saint-Eustache, 12.VIII.1917, J. Ouellet [2♀, CEUM]; Saint-Placide, 13.VII.1931, J. Ouellet [1♀, CEUM]; Gatineau Co., Aylmer, 21.VII.2009, ex. *Hypericum*, *Lythrum*, *Daucus*, graminées, etc, L. LeSage [1♀, CNC]; Île-de-Montréal Co., Montréal, 14.VII.1904, Beaulieu [1♀, USNM]; Joliette Co., Joliette, 12.VII.1909, J. Ouellet [1♀, CEUM]; same data, except 7–13.VII.1922 [29♂ 46♀, CEUM]; Napierville Co., Saint-Rémi, 1.VII.1920, J. Ouellet [1♂ 1♀, CEUM]; Papineau Co., Montebello, 16.VII.1937, J. Ouellet [1♀, CEUM]; Pontiac Co., Beech Grove, 18.VII.1951, J. F. McAlpine [1♀, CNC]; Yarm, 23.VII.1955, C. H. Mann [1♂, CNC]; Vaudreuil Co., Hudson Heights, 24–30.VII.1956, Lindberg [1♀, CNC]; Rigaud, 5.VII.1920, J. Ouellet [2♂ 1♀, CEUM].

Host plants. No specific plant associations were recorded on labels of specimens examined. Chagnon (1937, 1940) and Chagnon and Robert (1962) gave willow (*Salix* sp.) (Salicaceae) as a host in Québec, but *P. othonus* was reported on a large number of questionable “hosts” by authors (details in Clark et al. 2004). Barney and Hall (2011) reported feeding, mating and oviposition on *Desmodium marilandicum* (L.) (Fabaceae).

Comments. Balsbaugh (1973) and Riley et al. (2003) recognized *P. othonus* as having three subspecies in North America. More information on habitats and hosts are needed on *P. othonus othonus*, which is one of the easiest species to recognize.

Pachybrachis peccans Suffrian, 1852

http://species-id.net/wiki/Pachybrachis_peccans

Habitus 12; Map 12; Figures 1b, 2a

Pachybrachis peccans Suffrian, 1852:192.

Recognition. Ocular lines prominent (Figure 2a); males with enlarged foreleg claws (Figures 4a, 4b); color extremely variable, ranging from yellowish with faint black spots to almost black speckled with small yellow marks (Habitus 12); male size small: length 2.15 ± 0.16 mm, width 1.13 ± 0.09 mm.

Distribution. Transcontinental species, widely distributed from Texas to Yukon to Atlantic Coast (LeSage 1991; Riley et al. 2003). Found in eastern Canada from Ontario to Prince Edward Island (Map 12).

Material examined. NEW BRUNSWICK: Kent Co., Kouchibouguac National Park, 5–6.VII.1977, M. Ivanochko [40♂ 28♀, CNC]; same data, except 5–19.VII.1977, S. J. Miller [2♂ 7♀, CNC]; same data, except 8–13.VIII.1977 [2♂ 1♀, CNC]; same data, except 27.VII.1977, G. A. Calderwood [1♀, CNC]; same data, except 4.VIII.1977 [1♂ 1♀, CNC]; same data, except 16.VI.1978, D. B. Lyons [1♂, CNC]; Kings Co., Mechanic's Lake, 30.VII.1926 [1♂, CNC]; Penobsquis, 21–31.VII.1926, C. A. Frost [1♂ 2♀, CNC]; Queens Co., Canning Grand Lake near Flowers Cove, 1.VII.2004, D. Sabine and R. Webster [1♂, RWIC]; Saint John Co., St. John, 8.VII.1902, W. McIntosh [1♀, USNM]; same data, except 19.VI.1981, sweeping vegetation, D. R. Ward [1♂ 1♀, CNC]; York Co., New Maryland Charters Settlement, 27.VI.2004, R. P. Webster [1♂, RWIC].

NOVA SCOTIA: Annapolis Co., Annapolis Royal, 21.VII.1928, W. J. Brown [1♂ 1♀, CNC]; Inverness Co., Cape Breton Highlands National Park, Grande Falaise, 30 m, 0.5 km north, 9.VI.1983, forest, flood plain, H. Goulet [1♂, CNC]; Kings Co., Kentville, 20.VI.1981, sweeping, D. Ward [1♀, CNC]; Queens Co., Greenfield Queens, 13.VI.1910, P. G. Bolster [1♀, MCZ]; Port Medway & vic. Queen, 7–20.VII.1910 [2♂ 2♀, MCZ].

ONTARIO: Algoma Dist., Lake Superior Provincial Park, Agawa Bay Campground, 8.VII.1970, ROM Field Party [1♂ 2♀, ROM]; Carleton Co., Britannia, 14.VI.1949, R. de Ruelle [1♂, CNC]; Britannia Bay, 3.VI.1959, S. D. Hicks [2♂ 1♀, CNC]; Britannia Heights, 16.VII.1958, S. D. Hicks [1♀, CNC]; same data, except 7.VII.1961, ex. *Populus balsamifera* [17♂ 12♀, CNC]; Constance Bay, 30.VIII.1982, L. J. Milne [1♀, CNC]; Ottawa, 14.VI.1972, F. G. Ouellet [1♀, IDM]; Cochrane Dist., Smoky Falls, Mattagami River, 6.VII.1934, G. S. Walley [1♂, CNC]; Timmins, 16.VI.1982, on plants in gravel, J. Pilny [1♀, CNC]; same data, except Mattagami River, 48°30'N 81°15'W, 16.VI.1982, Pilny & Motz [2♀, CNC]; Essex Co., Belle River, 26.V.1946, S. D. Hicks [1♀, CNC]; Leamington, 4.VI.1937, G. S. Walley [1♂ 3♀, CNC]; Ojibway, 9.VI.1943, S. D. Hicks [2♂ 2♀, CNC]; same data, except 28.V.1944 [1♂ 1♀, CNC]; Pelee Island, VI.24, R. C. Osburn [1♂, OSU]; same data, except 3.VII.1931, W. J. Brown [2♀, CNC]; same data, except 11.VI.1940, ex. *Salix* [6♂ 4♀, CNC]; Point Pelee, 23–29.VI.1931, W. J. Brown [18♂ 18♀, CNC]; same data, except 3.VII.1931 [1♂ 1♀, CNC]; same data, except 29.V.1940 [3♂ 2♀, CNC]; same data, except 1.VII.1940 [1♂ 2♀, CNC]; same data, except 3.VI.1929, L. J. Milne [5♂ 5♀, CNC]; same data, except 1.VI.1933, ex. willow, G. M. Stirrett [2♂ 4♀, 6, CNC]; same data, except 19–20.V.1955, ex. *Salix interior*, S. D. Hicks [2♂ 8♀, CNC]; same data, except 30.V.1929, G. S. Walley [3♂ 3♀, CNC]; same data, except 25.VI.1920, N. K. Bigelow [1♂ 1♀, ROM]; Point Pelee National Park, 6.VI.1981, ex. *Salix* spp., L. LeSage & D. Ward [1♂, CNC]; same data, except 29.VII.1982, L. LeSage [1♂ 1♀, CNC]; Haldimand Co., Dunville, 30.V.1954, R. Plath [1♂ 1♀, USNM]; Hastings Co., Marmora, 1.VII.1952, sweep from *Rubus* spp., G. P. Holland [1♀, CNC]; Kenora Dist., Malachi, 13–14.VII.1947,

W. Y. Watson [1♂ 1♀, ROM]; Willard Lake, 22.VI.1992, lot 1, B.F. & J.L. Carr [1♂, CNC]; Kent Co., Erieau, 26.VI.1932, ex. *Cornus* sp., G. M. Stirrett [5♂ 4♀, CNC]; Rondeau Park, 3.VI.1981, L. LeSage [3♂ 11♀, CNC]; same data, except 4.VI.1981, ex. *Salix* spp. [2♀, CNC]; same data, except 29.V.1985, marsh trail [2♂ 3♀, CNC]; same data, except 28.V.1985, sandy beach [1♀, CNC]; same data, except 7.VI.1981, sifted litter under willows, L. LeSage & D. Ward [4♂ 7♀, CNC]; same data, except 3.VI.1981, sweep willow and grasses, D. Ward [1♂, CNC]; same data, except 5.VI.1985, under willow, J. M. Campbell & A. Davies [1♂, CNC]; Lanark Co., Bell's Corner, 30.VI.1950, S. D. Hicks [1♀, CNC]; Niagara Co., Ridgeway, A. H. Kilman [1♀, ROM]; Nipissing Dist., Algonquin Park, 18.VI.1922, J. McDunnough [2♀, CNC]; same data, except 4.VII.1965, W. F. O. [1♀, CNC]; North Bay, 11.VII.1972, E. J. Kiteley [1♀, CNC]; North Bay, Trout Creek, 25.VI.1984, willow [1♀, CNC]; Norfolk Co., Hemlock, 9.VIII.1945, ex. *Salix*, G. M. Stirrett [1♂ 1♀, CNC]; Turkey Point, 8.VI.1931, W. J. Brown [2♂ 1♀, CNC]; same data, except 24.VII.1984, sweeping in marshy area, L. LeSage [5♂ 1♀, CNC]; Walsingham Forestry Station, 25.VII.1984, sweeping in ditch, L. LeSage [1♂ 3♀, CNC]; Parry Sound Dist., Burk's Falls, 14.VII.1926, F. P. Ide [1♀, CNC]; Scotia Junction, 28.VIII.1934, H. W. Wenzel [1♂, OSU]; Peel Co., Port Credit, 4.VII.1908 [1♂, ROM]; Peterborough Co., Hastings, 2.VI.1934, J. F. Brimley [2♀, CNC]; same data, except 31.VI.1934 [1♀, CNC]; same data, except 9.VI.1935 [1♀, CNC]; same data, except 4.VI.1950 [1♀, CNC]; same data, except 5.IX.1956 [2♀, CNC]; Prince Edward Co., same data, except 9–13.VII.1941 [1♀, CNC]; same data, except 24.VII.1945 [1♀, CNC]; Prince Edward Co., Picton, 22.VI.1985, M. Davis [1♀, CNC]; Rainy River Dist., 13–15.VII.1924, J. F. Brimley [2♀, CNC]; same data, except 24.VIII.1924 [1♀, CNC]; Renfrew Co., Petawawa, 17.VI.1980, ex. *Comptonia peregrina*, L. LeSage [1♀, CNC]; Sudbury Dist., Sudbury 1988 [1♂ 1♀, CNC]; same data, except Wickham [1♀, USNM]; Thunder Bay Dist., Black Sturgeon Lake, 1–15.VIII.1956, Lindberg [7♂ 24♀, CNC]; Manitouwadge, 22.VII.1983, T. Baker [2♀, CNC]; same data, except 7.VII.1991, on weeds near woodpile pine logs, [1♂, CNC]; same data, except 21.VI.1992 [1♀, CNC]; 59 km north of junction of highway 516 & 599, 24.VI.1992, lot 2, B.F. & J.L. Carr [1♀, CNC]; Timiskaming Dist., Elk Lake, 30.VI.1958, G. H. Dieke [1♂, USNM]; Toronto Co., Toronto, F. Knab [10♂ 6♀, USNM]; same data, except 2.VII.1894, [1♀, ROM]; same data, except VII.1933 [2♂ 2♀, ROM]; same data, except 30.V.1896, H. R. [1♂ 1♀, LEM]; same data, except 26.VI.1896, C. T. Hills [1♀, LEM]; same data, except 20.VI.1908, R. J. Crew [1♀, ROM]; same data, except 8.VI.1926, E. C. Oakley [1♂ 2♀, LEM]; same data, except VII.1933, L. J. Milne [2♀, UNHC]; Victoria Co., Fenelon Falls, Lindsay, 17.VI.1959, ex. *Salix* sp., F.I.S. [1♂, CNC]; Unknown Co., Sultan road, 68 km west of junction of highway 144, 26.VI.1996, Lot 2, B.F. & J.L. Carr [1♂ 2♀, CNC].

PRINCE EDWARD ISLAND: Kings Co., Souris, 12.VII.1993, ex. lowbush blueberry, M. E. M. Smith [2♂ 1♀, CNC]; Queens Co., PEI National Park Stanhope Campground, 13.VIII.1991, D. S. Chandler [2♂, UNHC].

QUÉBEC: Abitibi Co., Saint-Vital-de-Clermont, 9.VIII.1983, sweeping weeds in *Pinus banksiana* Lamb. forest, A. Larochelle [1♂, CNC]; Argenteuil Co., Saint-Philippe-

d'Argenteuil, 21.VI.1983, ex. *Betula populifolia* Marsh., Larochelle [1♂, CNC]; Arthabaska Co., Blandford, 21.VI.1980, ex. *Salix* sp., A. Larochelle [1♀, CNC]; Bellechasse Co., Saint-Étienne, 1.VI.1980, J. C. Aubé [3♂ 2♀, LEM]; Berthier Co., Berthierville, 4.VI.1944, A. Robert [1♀, CEUM]; Bonaventure Co., Carleton, 29.VII.1981, waste land on weeds, A. Larochelle [1♂, CNC]; Cascapédia, 11–14.VI.1933, W. J. Brown [7♂ 12♀, CNC]; Port-Daniel, 30.VII.1981, field on weeds, A. Larochelle [1♂, CNC]; Brome Co., Knowlton, 10–12.VII.1928, G. H. Fish [2♂ 1♀, CNC]; Champlain Co., La Tuque, 8.VIII.1981, ex. *Myrica asplenifolia* L., A. Larochelle [1♀, CNC]; Chicoutimi Co., Jonquière, 27.VI.1970, ex. *Populus tremuloides*, C. Chantz [1♂, AJGC]; Deux-Montagnes Co., La Trappe, 25.V.1929, P. Leopold [1♀, CEUM]; same data, except 27.VI.1933, J. Ouellet [3♂ 1♀, Det. by H. C. Fall, CEUM]; same data, except 2.VII.1933 [2♂ 1♀, CEUM]; same data, except 28.VI.1935 [14♂ 19♀, CEUM]; same data, except 23.VII.1950 [2♂ 2♀, CEUM]; Saint-Placide, 4.VI.1933 [1♀, CEUM]; Gaspé-Est Co., Gaspé, 7.VII.1931 [1♂, Fall-MCZ]; same data, except 25.VII.1954, W. J. Brown [1♂ 2♀, CNC]; Percé, 30.VII.1981, roadside, on weeds, A. Larochelle [1♀, CNC]; Val-d'Espoir, 18.VIII.1939, J. Ouellet [1♀, CEUM]; Gatineau Co., Lytton, 1.VII.1981, ex. *Salix* sp., A. Larochelle [1♀, CNC]; Île-de-Montréal Co., Montréal, 28.VI, J. Ouellet [1♀, CEUM]; same data, except 2.VII.1917 [1♂, CEUM]; same data, except 30.V.1940, A. Robert [2♂ 3♀, CEUM]; same data, except 15.VI.1951, M. Larochelle [1♀, CEUM]; same data, except 17.VI.1980, E. J. Kiteley [1♀, CNC]; same data, except 30.V.1981 [1♂, CNC]; Joliette Co., Joliette, 15.VII.1924 [1♂, Fall-MCZ]; same data, except, 15.VII.1917, J. Ouellet [3♀, CEUM]; same data, except 7–13.VII.1922 [2♂ 2♀, CEUM]; same data, except 7–15.VII.1924 [15♂ 5♀, CEUM]; same data, except 12.VI.1930 [1♂, CEUM]; Kamouraska Co., Sully, 24–26.VI.1936, J. Ouellet [33♂ 45♀, CEUM]; same data, except 1–2.VII.1936 [17♂ 26♀, CEUM]; same data, except 9.VII.1936 [25♂ 24♀, CEUM]; same data, except 13–14.VII.1936 [18♂ 22♀, CEUM]; same data, except 21.VII.1936 [2♂ 4♀, CEUM]; Labelle Co., Lac Sagouay, 19.VI.1981, ex. *Salix* sp., A. Larochelle [1♀, CNC]; Nominigüe, 25.VIII.1930, J. Ouellet [1♀, CEUM]; same data, except 12–24.VII.1932 [22♂ 37♀, CEUM]; same data, except 24.VII.1933, A. Robert [1♂ 2♀, CEUM]; same data, except 19.VI.1934, L. Daviault [1♂, CEUM]; same data, except 21.VII.1932 [1♀, Fall-MCZ]; Lévis Co., Lauzon, 29.VI.1932, J. Ouellet [1♀, CEUM]; Montcalm Co., Parc du Mont-Tremblant, 15.VIII.1954, A. Robert [1♂ 1♀, CEUM]; same data, except 27.VI.1971, E. J. Kiteley [1♂, CNC]; Nicolet Co., Blandford, 21.VI.1980, A. Larochelle [1♀, CNC]; Papineau Co., Montebello, 3.VII.1937, J. Ouellet [1♀, CEUM]; Portneuf Co., Lac Sergent, 2.VII.1961, J. L. Laliberté [1♀, IDM]; Saint-Augustin, 17.VI.1967, J. L. Laliberté [1♂, IDM]; same data, except 22.VI.1977 [1♂, IDM]; Sainte-Catherine, 8–14.VII.1956, J. C. Aubé [7♂ 4♀, LEM; 2♂ 2♀, AMNH, 1♂, USNM]; same data, except 19.VI.1957 [1♂, LEM]; same data, except 11.VI.1960 [1♀, LEM]; same data, except 9–15.VII.1960 [1♂ 1♀, LEM; 1♀, USNM]; same data, except 26.VII.1961 [2♀, LEM]; same data, except 5.VIII.1961 [1♀, LEM]; same data, except 15.VII.1956, J. L. Laliberté [1♂ 2♀, IDM]; Québec Co., Cap-Rouge, 27.VI.1959, J. C. Aubé [1♂, LEM]; same data, except 24.VI.1981, D. R. Ward [1♀, CNC]; Lac Beauport, 11–23.VII.1956, J. L. Laliberté [1♂ 2♀, IDM]; Québec, 15.VI.1957, J. C. Aubé [1♂, LEM; 1♂, AMNH]; Saguenay Co.,

Grandes-Bergeronnes, 20.VII.1981, field on weeds, A. Larochelle [1♀, CNC]; Rivière Barthélemy, 22.VII.1981, field on weeds, A. Larochelle [1♂, CNC]; Tadoussac, 23.VII.1932, A. F. Winn [3♀, CEUM]; Saint-Jean Co., Saint-Jean-sur-Richelieu (as “St. Johns”), F. Knab [1♂, USNM]; Saint-Maurice Co., Pointe-du-Lac, 11.VII.1926, J. L. Laliberté [1♀, IDM]; same data, except 5.VIII.1927 [1♂ 1♀, IDM]; same data, except 26.VII.1928 J. L. Laliberté [1♀, IDM]; same data, except 20.VII.1936 [2♀, IDM]; Soulanges Co., Rivière-Beaudette, 10.VIII.1985, Larochelle & Larivière [1♀, CNC]; Témiscamingue Co. Laniel, 5–6.VI.1963, W. Gagné [1♂, CNC]; Notre-Dame-du-Nord, 36.VI [2♂, CEUM]; Vaudreuil Co., Rigaud, 13.VII.1973, sweeping *Salix* sp., E. J. Kiteley [3♂ 3♀, CNC]; same data, except 7.VI.1985, willow in flower (*Salix* sp.), Larochelle & Larivière [1♂, CNC]; same data, except 7.VI.1985, old field birch (*Betula populifolia* Marsh.), Larochelle & Larivière [1♀, CNC]; Saint-Lazare, 20.VI.1983, ex. *Salix fragilis* L., A. Larochelle [1♀, CNC]; Mont Lyall 1500 ft, 31.VII.1933, W. J. Brown [2♂ 4♀, CNC].

Host plants. Large series of *P. peccans* were reportedly taken on *Populus balsamifera* L. and *Salix* spp. (Salicaceae). Other plant associations recorded on labels were *Populus tremuloides* Michx.; lowbush blueberry (*Vaccinium angustifolium* Ait.) (Ericaceae); *Rubus* spp. (Rosaceae); and *Comptonia peregrina* (L.) (Myricaceae). Larvae were reared on dead leaves of sand-bar willow (*Salix interior* Rowlee) by LeSage (1985). Larochelle specimens from Québec had a wide range of potential plant associations. Several additional potential hosts are listed by Clark et al. (2004) from their literature review.

Comments. *Pachybrachis peccans* was the most commonly examined species (over 900 specimens; Table 1). It has also the largest distribution, being found from Nova Scotia to Yukon (Map 12). *P. peccans* is reported here for the first time from PE.

As one of several species in North America having distinct ocular lines, enlarged claws, and varying degrees of maculation, there is much potential confusion with other species. Fall (1915) stated, “While *peccans* varies toward *melanostictus* in its darker individuals, it approaches so closely to *diversus* and *abdominalis* in some of its paler forms as to make distinction purely discretionary.” At this time, we believe *diversus* is a more southern species not found in Canada, and *abdominalis* and *melanostictus* are not found in eastern Canada.

***Pachybrachis pectoralis* (F. E. Melsheimer, 1847)**

http://species-id.net/wiki/Pachybrachis_pectoralis

Habitus 13; Map 13; Figure 1a

Cryptocephalus pectoralis F. E. Melsheimer, 1847:171.

Pachybrachis sobrinus Haldeman, 1849: 262.

Pachybrachis oculatus Suffrian, 1852:178.

Pachybrachis sticticus Blatchley, 1910:1130.

Recognition. Form, especially of the male, narrower than usual; color dull yellow, maculate with brown or black (Habitus 13); surface not or scarcely shining; eyes narrowly separated; ocular lines present (Figure 1a); front tibiae sinuate on inner margin

beyond middle; front claws of male obviously, though not greatly, enlarged; male size small: length 1.92 ± 0.07 mm, width 0.96 ± 0.04 mm.

Distribution. Eastern species distributed from Nebraska to Texas to Atlantic Coast in the United States (Riley et al. 2003). In eastern Canada, *P. pectoralis* has been found in southern Ontario and the Ottawa Valley in Québec (Map 13).

Material examined. ONTARIO: Hastings Co., 10.vii.1938, J. F. Brimley [2♀, CNC]; same data, except 5.VIII.1957 [1♀, CNC]; Muskoka Dist., Norway Point Lake of Bays, 30.XI.1922, J. McDunnough [1♀, CNC]; Prince Edward Co., 3.VIII.1938, J. F. Brimley [3♂ 3♀, CNC]; Kawartha Gull Lake, 2.VIII.1943, J. F. Brimley [2♂, CNC]; same data, except 19.VII.1950 [1♀, CNC]; Toronto Co., Toronto, 15–30.VI.1927, L. J. Milne [1♀, CNC]; Unknown Co., East Ontario [1♂, CNC].

QUÉBEC: Gatineau Co., Mont King, Parc de la Gatineau, 19.VIII.1996, L. LeSage [8♂ 13♀, CNC]; same data, except 6.IX.1996 [1♀, CNC]; same data, except 28.V.1997 [1♀, CNC]; same data, except 21.VII.1997 [1♂ 3♀, CNC]; Pontiac Co., Luskville, 4.VII.1985, ex. *Quercus rubra* L. Larochelle & Larivière [1♀, CNC]; same data except 30.VII.1985, ex. *Quercus alba* L. [1♂, CNC]; Vaudreuil Co., Rigaud, A. Robert [2♂ 2♀, CEUM]; same data, except 20.VIII.1984, ex. *Quercus rubra* L., Larochelle & Larivière [2♂, CNC]; same data except 19.VIII.1985, ex. *Quercus rubra* L., Larochelle & Larivière [1♀, CNC].

Host plants. No information is available from the specimens examined. According to Blatchley (1924a, b), Fall (1915), Barney (1984) and Clark (2000), *P. pectoralis* is associated with common locust (*Robinia pseudoacacia* L.) (Fabaceae). Larochelle's specimens were found on *Quercus alba* L. and *Q. rubra* L. Additional potential hosts are given in Clark et al. (2004).

Comments. No specimens from New Brunswick and Nova Scotia, from where this species was reported by LeSage (1991) and Riley et al. (2003), were located, although the material of all the important collections of eastern Canada was examined. The Ottawa River Valley is the northernmost limit of *P. pectoralis*, in Québec. This species may extend further north to the Maritime Provinces along the Atlantic Coast, but it has not yet been recorded from Maine (Riley et al. 2003; Majka et al. 2011).

Pachybrachis spumarius Suffrian, 1852

http://species-id.net/wiki/Pachybrachis_spumarius

Habitus 14; Map 14; Figures 2b, 5b, 6b, 7d, 8c, 10b, 10c

Pachybrachis spumarius Suffrian, 1852:179.

Pachybrachis roboris Fall, 1915: 420.

Recognition. Elytra and pronotum with small yellow spots and diffuse rufous mottled marks; prothoracic puncturation dense, extending to side margins (Habitus 14); ocular lines absent; aedeagus tubular with terminal nodule (Figure 10 b) or nipple-shaped

apex when seen from above (Figure 10c); male size small: length 1.91 ± 0.13 mm, width 1.06 ± 0.05 mm.

Distribution. Eastern species distributed from southern Saskatchewan to Texas to Atlantic Coast (Riley et al. 2003), and present in southern Ontario and Québec in eastern Canada. The Ottawa Valley and the south of eastern Townships, in Québec, are probably the northernmost distribution limit of *P. spumarius* (Map 14).

Material examined. ONTARIO: Carleton Co., Britannia, 28.VI.1931, L. J. Milne [1♂ 1♀, UNHC]; same data, except 19.VII.1949, R. de Ruelle [1♀, CNC]; Britannia Heights, 16.VII.1958, S. D. Hicks [1♂, CNC]; Constance Bay, 10.VII.1941, W. J. Brown [1♀, CNC]; Carp, 5.VII.1932, W. J. Brown [1♂, CNC]; Essex Co., Ojibway, 24.VI.1945 [1♀, CNC]; Pelee Island, 3.VII.1931, W. J. Brown [1♂ 1♀, CNC]; Roseland, 26.VI.1944, S. D. Hicks [1♀, CNC]; Hasting Co., 2.IX.1934, J. F. Brimley [1♂ 1♀, CNC]; same data, except 25.VII.1954 [1♂, CNC]; Leeds Co., Lindsay Island, Saint Lawrence Island National Park, 15.VII.1976, ex. *Betula papyrifera*, W. Reid [2♂, CNC]; Mermaid Island, Saint Lawrence Island National Park, 23.VII.1976, W. Reid [1♀, CNC]; Lennox & Addington Co., 16.VII.1939, J. F. Brimley [1♀, CNC]; same data, except 6.IX.1948 [1♀, CNC]; same data, except 10.VII.1949 [3♀, CNC]; Lincoln Co., DeCew Falls, 27.VII.1940, S. D. Hicks [1♀, CNC]; Norfolk Co., Turkey Point Provincial Park, 24.VII.1984, sweeping in mixed forest, L. LeSage [3♂, CNC]; Walsingham, 11.VII.1956, W. J. Brown [6♂ 7♀, CNC]; Walsingham Forest Station, 25.VII.1984, ex. *Rhus typhina*, L. LeSage [45♂ 47♀, CNC]; Northumberland Co., 2.IX.1950, J.F. Brimley [1♀, CNC]; Prince Edward Co., 10.VII.1935, J. F. Brimley [3♂ 3♀, CNC]; same data, except 21–25.VII.1937 [5♂ 9♀, CNC]; same data, except 3.VII.1938 [1♀, CNC]; same data, except 13.VII.1941 [1♂, CNC]; same data, except 16.VII.1947 [6♂ 2♀, CNC]; same data, except 5–19.VII.1950 [3♂ 3♀, CNC]; same data, except 5.VII.1953 [1♀, CNC]; same data, except 7.VII.1954 [1♂ 1♀, CNC]; Clearwater Bay, 30.VII.1996, sweeping miscellaneous vegetation, B. F & J. L. Carr [2♀, CNC]; Toronto Co., Toronto, 15.VIII.1908, R. J. Crew [1♀, ROM]; Kelly Lake, 26.VII.1933, L. J. Milne [1♂, UNHC]; Victoria Co., Coboconk, 2.VIII.1948, J. F. B. [2♂ 1♀, CNC]; Unknown Co., East Ontario [1♂ 1♀, CNC].

QUÉBEC: Bagot Co., Saint-Pie, 1.VII.1985, ex. *Acer rubrum* L., Laroche & Lari-vière [1♂, CNC]; Châteauguay Co., Ormstown, 12.VII.1977, sweeping sumac, E. J. Kiteley [3♂ 2♀, CNC]; Deux-Montagnes Co., La Trappe, 19–22.VII.1945, J. Ouellet [1♂ 1♀, CEUM]; same data, except 7.VII.1946, [1♀, CEUM]; same data, except 13–15.VII.1949 [2♂ 4♀, CEUM]; same data, except 8–23.VII.1950, ex. Virginia sumac [27♂ 35♀, CEUM]; same data, except 1–8.VIII.1950, ex. Virginia sumac [9♂ 5♀, CEUM]; same data, except 27.VII.1951 [6♂ 7♀, CEUM]; Gatineau Co., Mont-King, Parc de la Gatineau, 19.VII.1981, P. Bélanger [1♂ 2♀, LFC]; Kazabazua, 3.IX.1967, H. J. Teskey [1♀, CNC]; Missisquoi Co., Phillipsburg, 15–19.VII.1969, J. L. Laliberté [1♂ 1♀, IDM]; same data, except 29.VII.1972 [3♂ 4♀, IDM]; same data, except 22.VI.1975 [2♂, IDM]; Pontiac Co., Luskville, 30.VII.1985, ex. *Quercus alba* L., Laroche & Lari-vière [1♀, CNC]; Témiscamingue Co., Laniel, 14.VIII.1932, W.J. Brown [1♀, CNC]; Vaudreuil Co., Hudson Heights, 24–30.VII.1956, Lindberg [1♂ 1♀, CNC]; Rigaud, 26.VII.1902, F. Knab [1♂ 1♀, USNM]; same data, except 25.VII.1939, A. Robert [1♂,

CEUM]; same data, except 15.VIII.1972, on sumac, E. J. Kiteley [3♂, CNC]; same data, except 13.VII.1973, sweeping sumac [1♂ 3♀, CNC]; same data, except 22.VII.1974, on sumac [3♂ 2♀, CNC]; same data, except 27.VIII.1977, on sumac [1♂ 1♀, CNC]; same data, except 16.VIII.1984, ex. *Rhus typhina* L., Larochelle & Larivière [1♀, CNC]; Saint-Lazare, 9.VIII.1982, UV light in a pine plantation, A. Larochelle [1♀, CNC].

Host plants. Several specimens were recorded as being collected from *Rhus typhina* L., Virginia sumac, or just sumac (Anacardiaceae), which are various names for the same plant. Barney and Hall (2011) reported collecting specimens in abundance on *R. copallina* L. and *R. glabra* L., and observed feeding, mating and oviposition on these species in the laboratory. Larochelle cited *Acer rubrum* L. (Aceraceae) and *Quercus alba* L. (Salicaceae) as potential hosts on his labels. *Betula papyrifera* Marsh. (Betulaceae) was also reported here but cannot be confirmed as a plant association.

Comments. *Pachybrachis spumarius* is the second most commonly collected species in eastern Canada and can often be found in large series on *Rhus* spp. (Table 1).

Pachybrachis subfasciatus (J. E. LeConte, 1824)

http://species-id.net/wiki/Pachybrachis_subfasciatus

Habitus 15; Map 15

Cryptocephalus subfasciatus J. L. LeConte, 1824:173.

Pachybrachys biguttatus Suffrian, 1852:167.

Pachybrachys impurus Suffrian, 1852:186.

Pachybrachys xanthias Suffrian, 1852:199.

Pachybrachys impurus var. *umbrosus* Fall, 1915: 379.

Recognition. Color dark, pronotum with sides narrowly yellow; elytra with red or yellow, more or less broad, irregular or indented transverse median fascia often interrupted at suture, and with red or yellow apical spot (Habitus 15); Disc of pronotum often, and head more rarely, variegated with reddish yellow; male size small: length 2.21 ± 0.09 mm, width 1.18 ± 0.06 mm.

Distribution. Eastern species distributed from Kansas to Louisiana to Atlantic Coast in the United States (Riley et al. 2003), restricted to the Carolinian Zone in southern Ontario in eastern Canada (Map 15).

Material examined. ONTARIO: Essex Co., Leamington, 6.VII.1931, G. S. Walley [1♀, CNC]; same data, except 9.VI.1937 [1♀, CNC]; Hastings Co., 19.VII.1938, J. F. Brimley [2♀, CNC]; Lennox & Addington Co., 16.VII.1938, J. F. Brimley [1♂, CNC]; Norfolk Co., Normandale, 5.VI.1931, W. J. Brown [1♀, CNC]; Walsingham, 3.VI.1944, W. J. Brown [3♀, CNC]; same data, except 11.VII.1956 [2♀, CNC]; Northumberland Co., Hamilton, 15.VII.1981, M. Sanborne [1♂, CNC]; Toronto Co., Toronto, 15.VI.1896 [3♀, LEM]; same data, except 30.V.1897, C. T. Hills [1♂ 1♀, LEM].

Host plants. No records are available from the specimens examined. Downie and Arnett (1996) reported *P. subfasciatus* from black walnut (*Juglans nigra* L.) (Juglandaceae).

Comments. *Pachybrachis subfasciatus* is another possible example of a species once living in the Carolinian Zone which may have been extirpated from the Canadian fauna. No specimens were collected in the last 55 years. If its association with black walnut is correct, this may explain its rarity, or even extinction, since Fox and Soper (1953) reported this tree occurring naturally only in rich woods in southernmost Ontario and considered recent trees above these limits to have been planted.

***Pachybrachis tridens* (F. E. Melsheimer, 1847)**

http://species-id.net/wiki/Pachybrachis_tridens

Habitus 16; Map 16

Cryptocephalus tridens F. E. Melsheimer, 1847:172.

Cryptocephalus flavicornis F. E. Melsheimer, 1847:172.

Pachybrachys mollis Haldeman, 1849: 263.

Recognition. Color pale yellow with broad, sharply limited, black markings; antennae (usually) and legs entirely yellow (Habitus 16). Lustre dull. Eyes separated by about twice length of basal antennomere in male, and by two and one-half to three times length of this antennomere in female. Ocular lines fine. Front claws of male not enlarged (as in Figure 4c); male size small: length 1.93 ± 0.10 mm, width 1.01 ± 0.04 mm.

Distribution. *Pachybrachis tridens* is an eastern species distributed from Manitoba to Texas to the Atlantic Coast in the United States (Riley et al. 2003), and restricted to the Carolinian Zone of southern Ontario in eastern Canada (Map16).

Material examined. ONTARIO: Prince Edward Co., 21–28.VI.1950, J. F. Brimley [3♂ 5♀, CNC].

Host plants. No records are available from the specimens examined. Poison ivy (*Toxicodendron radicans* (L.) Kuntze) (Anacardiaceae) was given as the preferred host by Fall (1915), Blatchley (1924a), Wilcox (1954, 1979), Furth (1985), and others. A complete list of citations is found in Clark et al. (2004).

Comments. Since *P. tridens* has not been collected in the last 55 years, it can be considered as extirpated from the Canadian fauna. Formerly, it was probably restricted to the Carolinian Life Zone, which is now reduced to minute remnants. For this reason, the Manitoba record reported by LeSage (1991) and Riley et al. (2003), and the Québec record cited by Riley et al. (2003) are questionable. No specimens were available to confirm them.

***Pachybrachis trinotatus* (F. E. Melsheimer, 1847)**

http://species-id.net/wiki/Pachybrachis_trinotatus

Habitus 17; Map 17

Cryptocephalus trinotatus F. E. Melsheimer, 1847:170.

Recognition. Pronotum red with heavy, sharply defined, black M-mark, and with pale anterior and lateral margins; elytra entirely black (Habitus 17); male size large: length 3.09 ± 0.13 mm, width 1.75 ± 0.09 mm.

Distribution. *Pachybrachis trinotatus* is an eastern species distributed from Kansas to the Atlantic Coast in the United States (Riley et al. 2003), and in Ontario and Québec in eastern Canada (Map 17).

Material examined. ONTARIO: Carleton Co., Stittsville, 26.VII.1961, G. Brumpton [1♂, CNC]; Essex Co., Leamington, 27.VI-3.VII.1931, W. J. Brown [1♂ 1♀, CNC]; Hamilton Co., Ancaster, 2.VII.1958, J. E. H. Martin [1♂, CNC]; Hastings Co., 10.VII.1938, J. F. Brimley [1♀, CNC]; same data, except 24.VII.1960, J. F. Brimley [1♂ 1♀, CNC]; Marmora, 2.VII.1952, C. Boyle [1♀, CNC]; Lambton Co., Grand Bend, 10.VII.1939, G. E. Shewell [1♂ 1♀, CNC]; Lanark Co., Bell's Corners, 15.VII.1954, S. D. Hicks [3♂ 2♀, CNC]; same data, except Lanark, Kerr Lake 13.VII.1975 [1♂, CNC]; Lincoln Co., DeCew Falls, 29.VI.1940, S. D. Hicks [1♂, CNC]; same data, except 27.VII.1940 [1♂ 1♀, CNC]; same data, except VIII.1941 [1♂, CNC]; Northumberland Co., Hamilton, 14-19.VII.1984, M. Sanborne [1♀, CNC]; Prince Edward Co., 21.VII.1937, beaten from oak, J. F. Brimley [1♀, CNC]; same data, except 4.VII.1946 [1♀, CNC]; same data, except 11.VIII.1947 [1♀, CNC]; same data, except 14.VII.1948 [1♀, CNC]; same data, except 10.VIII.1948 [1♀, CNC]; same data, except 20.VI.1949 [1♂, CNC]; same data, except 6.VII.1949 [1♀, CNC]; same data, except 17.VII.1950 [5♂ 3♀, CNC; 1♂, AMNH; 2♂ 1♀, St. John's wort blossom, FSCA]; same data, except 7.VII.1955 [1♂, CNC]; same data, except 29.VII.1956 [1♀, CNC]; same data, except 27.VII.1962 [1♂ 1♀, CNC]; Renfrew Co., Hwy 512 15 km W Eganville, 5.VII.1996, B. F. & J. L. Carr [1♂, CNC]; Simcoe Co., Craighurst, 30.VIII.1963, G. G. E. Scudder [1♀, CNC]; Tiny Township, Cawaja Beach, 17.VII.1968, J. C. E. Riotte [1♂ 1♀, ROM]; Toronto Co., Toronto, Kelly Lake, 13-26.VII.1933, L. J. Milne [2♂ 1♀, UNHC]; Victoria Co., Coboconk, 7.VIII.1940, S.D. Hicks [1♀, CNC]; Wellington Co., Guelph, VII.1924, D. C. B. Duff [1♂, ROM].

QUÉBEC: Huntingdon Co., Covey Hill, 1.VII.1927, W. J. Brown [1♂, CNC]; Île-de-Montréal, Montréal, 10.VII.1977, sweeping field, E. J. Kiteley [1♂, CNC]; Missisquoi Co., Phillipsburg, 31.VII.1972, J. L. Laliberté [1♂, IDM]; Québec Co., Québec, 26.VII.1902, F. Knab [1♂ 1♀, USNM]; Vaudreuil Co., Hudson Heights, 24-30.VII.1956, Lindberg [1♀, CNC]; Rigaud 29.VI.1907, J. Ouellet [1♂, CEUM]; same data, except 18.VIII.1921 [2♂, CEUM]; same data, except 23.VII.1974, E. J. Kiteley [2♀, CNC].

Host plants. No information was available from specimens examined. Barney and Hall (2011) reported handpicking specimens from St. John's wort, *Hypericum punctatum* L. (Clusiaceae), and observed feeding, mating and oviposition on *H. punctatum*, *H. perforatum* L. and *H. dolabrisforme* Vent. in the lab. Following Banks (1912), New Jersey tea, *Ceanothus americanus* L. (Rhamnaceae) was often given by authors as a host for *P. trinotatus* (complete citation in Clark et al. 2004).

Comments. With its black elytra and reddish pronotum ornamented with a large, black, M-shaped marking, *P. trinotatus* is very easily distinguished from all other Canadian

species of the genus (*Habitus* 17). It is widely distributed in southern Ontario but is found only in the Ottawa River Valley and south of the eastern Townships in Québec (Map 17). Both areas very likely represent its northernmost distribution limit in this province.

Conclusion

According to the *Catalog of Leaf Beetles of America North of Mexico* (Riley et al. 2003), there are 17 species of *Pachybrachis* in the eastern provinces. This study verified 15 of those species (all except *P. praeclarus* and *P. relictus*), and discovered two new national and provincial records, both from southernmost Ontario: *P. cephalicus* and *P. luctuosus*. *Pachybrachis obsoletus* is new to NB, and *P. peccans* is new to PE.

The *P. relictus* records cited by Fall (1915) (ON: Toronto, Blaisdell Coll.; Scotia Junction, July 27, Wenzel) cannot be confirmed, and there is no evidence *P. praeclarus* ever existed in eastern Canada.

A review of the distribution and abundance of the seventeen *Pachybrachis* species reveals four general groups: (1) species distributed from Ontario into at least one province in the Maritimes (*P. nigricornis*, *P. obsoletus* and *P. peccans*); (2) species distributed along the shores of the Great Lakes (Erie, Michigan and Ontario) and rivers (Ottawa, Saguenay and St. Lawrence), but unknown from central and northern ON and QC (*P. bivittatus*, *P. hepaticus hepaticus*, *P. othonus othonus*, *P. pectoralis*, *P. spumarius* and *P. trinotatus*); (3) rare species exclusively from southern ON (*P. calcaratus*, *P. cephalicus*, *P. luridus*, *P. subfasciatus* and *P. tridens*) and/or with an additional disjunct population in QC (*P. atomarius* and *P. luctuosus*); and (4) species having the northernmost extension of an eastern US distribution into the southeastern Townships of QC (*P. m-nigrum*). There are no *Pachybrachis* that could be considered arctic, subarctic, or boreal species; no specimens were found from Labrador or Newfoundland; and all species had southern affinities.

Pachybrachis bivittatus, *P. hepaticus* and *P. peccans* are transcontinental species extending from the Atlantic to the Pacific Oceans. They are common across eastern Canada, and have been collected rather recently (1990s – present).

A large group of species found in this study share a similar eastern United States *Pachybrachis* distribution, occurring from the Atlantic coastal states into the Great Plains: *P. atomarius*, *P. luridus*, *P. m-nigrum*, *P. nigricornis*, *P. obsoletus*, *P. othonus othonus*, *P. pectoralis*, *P. spumarius*, *P. subfasciatus*, *P. tridens*, and *P. trinotatus* (Riley et al. 2003; Barney, unpublished data). *Pachybrachis nigricornis*, *P. obsoletus*, *P. othonus othonus*, and *P. pectoralis* have all been collected within the last 20 years and probably have viable populations, but *P. atomarius*, *P. calcaratus*, *P. luridus*, *P. subfasciatus*, *P. tridens*, and *P. trinotatus* have not been collected in over 30 years (*luridus* in over 70 years) and may be considered extirpated from eastern Canada.

The remaining species, *P. calcaratus*, *P. cephalicus*, and *P. luctuosus*, were from the relatively small, southern Carolinian Ecozone, but their North American distribution is not as well defined or widespread as the others (Riley et al. 2003; Barney, unpublished data). *Pachybrachis calcaratus* has not been collected in Canada since 1944 and

Table 1. List of *Pachybrachis* species recorded from eastern Canada, with number of specimens examined per province. No specimens were found bearing a label from Newfoundland or Labrador.

	ON	QC	NB	NS	PE	Total
<i>Pachybrachis atomarius</i> (F. E. Melsheimer)	32	1	0	0	0	33
<i>Pachybrachis bivittatus</i> (Say)	59	43	0	0	0	102
<i>Pachybrachis calcaratus</i> Fall	2	0	0	0	0	2
<i>Pachybrachis cephalicus</i> Fall*	25**	0	0	0	0	25
<i>Pachybrachis hepaticus hepaticus</i> (F. E. Melsheimer)	19	91	0	0	0	110
<i>Pachybrachis luctuosus</i> Suffrian*	17**	3**	0	0	0	20
<i>Pachybrachis luridus</i> (Fabricius)	66	0	0	0	0	66
<i>Pachybrachis m-nigrum</i> (F. E. Melsheimer)	0	4	1	0	0	5
<i>Pachybrachis nigricornis</i> (Say)	143	82	11	0	0	236
<i>Pachybrachis obsoletus</i> Suffrian	87	22	14**	0	0	123
<i>Pachybrachis othonus othonus</i> (Say)	59	94	0	0	0	153
<i>Pachybrachis peccans</i> Suffrian	337	483	93	9	5**	927
<i>Pachybrachis pectoralis</i> (F. E. Melsheimer)	15	36	0	0	0	51
<i>Pachybrachis spumarius</i> Suffrian	179	142	0	0	0	321
<i>Pachybrachis subfasciatus</i> (J. E. LeConte)	18	0	0	0	0	18
<i>Pachybrachis tridens</i> (F. E. Melsheimer)	8	0	0	0	0	8
<i>Pachybrachis trinotatus</i> (F. E. Melsheimer)	53	11	0	0	0	64
Total per province	1119	1012	119	9	5	2264

*New national record for Canada, **New provincial record

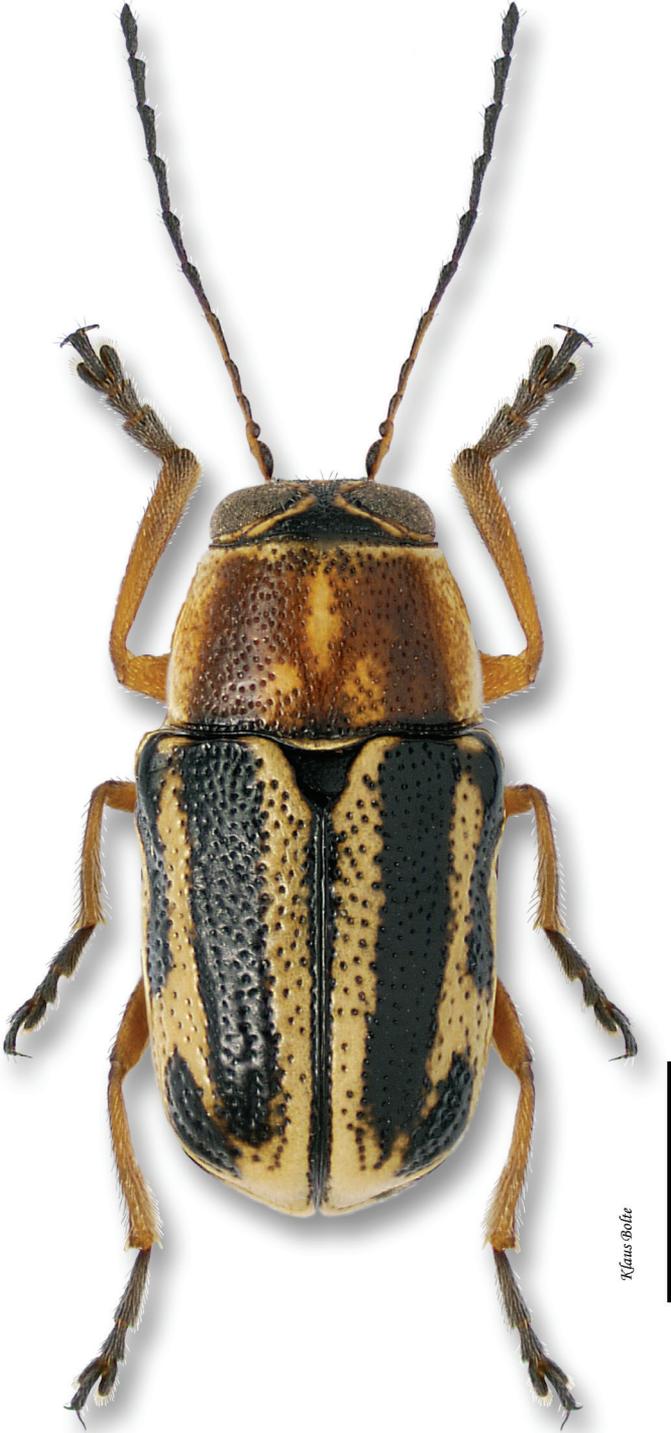
is very likely extirpated from Canadian fauna. *Pachybrachis cephalicus* may survive in its refugium in the Walsingham Forest (ON). *Pachybrachis luctuosus* was collected recently in southern Ontario and is probably still surviving there, but we have no recent information on the disjunct population in the Ottawa Valley.

Of course, any faunal survey such as this is only as good as the naturalists and collectors out in the field. There have been five major collectors of eastern Canadian *Pachybrachis*: J. Ouellet, 680 specimens during 1900s to 1940s; J. F. Brimley, 257 specimens during 1910s to 1950s; W. J. Brown, 234 specimens during 1920s to 1950s; S. D. Hicks, 120 specimens during 1940s and 1950; and L. LeSage, 224 specimens during 1980s to 2000s. This demonstrates that 57% of all eastern Canada *Pachybrachis* ever collected were found by four collectors between 1900 and 1959. The loss of habitat appears to be accompanied by a loss of people monitoring the habitats. Hopefully, the species cited above as possibly being extirpated from eastern Canada are still out there waiting to be rediscovered.

One of the consequences of global warming of the climate is that many plant and animal species will move northward. Woodall et al. (2009) stated that the process of northward migration of trees in the eastern United States is currently underway. According to Diffenbaugh et al. (2008) and Woodall et al. (2009), the relaxed cold limitations and a greater accumulation of degree-days should favor several herbivores, but native *Pachybrachis* species and their host plants could benefit as well of expected warmer conditions.

Legends for habitus

Habitus 1. Dorsal habitus of *Pachybrachis atomarius*. Scale bar, 1 mm.



Habitus 2. Dorsal habitus of *Pachybrachis bivittatus*. Scale bar, 1 mm.



Habitus 3. Dorsal habitus of *Pachybrachis calcaratus*. Scale bar, 1 mm.



Habitus 4. Dorsal habitus of *Pachybrachis cephalicus*. Scale bar, 1 mm.



Klaus Bolte

Habitus 5. Dorsal habitus of *Pachybrachis hepaticus hepaticus*. Scale bar, 1 mm.



Habitus 6. Dorsal habitus of *Pachybrachis luctuosus*. Scale bar, 1 mm.



Habitus 7. Dorsal habitus of *Pachybrachis luridus*. Scale bar, 1 mm.



Habitus 8. Dorsal habitus of *Pachybrachis m-nigrum*. Scale bar, 1 mm.



Habitus 9a. Dorsal habitus of *Pachybrachis nigricornis difficilis*. Scale bar, 1 mm.



Habitus 9b. Dorsal habitus of *Pachybrachis nigricornis carbonarius*. Almost black. Scale bar, 1 mm.



Habitus 9c. Dorsal habitus of *Pachybrachis nigricornis carbonarius*. Yellow at edge of elytra. Scale bar, 1 mm.



Habitus 10. Dorsal habitus of *Pachybrachis obsoletus*. Scale bar, 1 mm.



Habitus 11. Dorsal habitus of *Pachybrachis othonus othonus*. Scale bar, 1 mm.



Habitus 12. Dorsal habitus of *Pachybrachis peccans*. Scale bar, 1 mm.



Habitus 13. Dorsal habitus of *Pachybrachis pectoralis*. Scale bar, 1 mm.



Habitus 14. Dorsal habitus of *Pachybrachis spumarius*. Scale bar, 1 mm.



Habitus 15. Dorsal habitus of *Pachybrachis subfasciatus*. Scale bar, 1 mm.

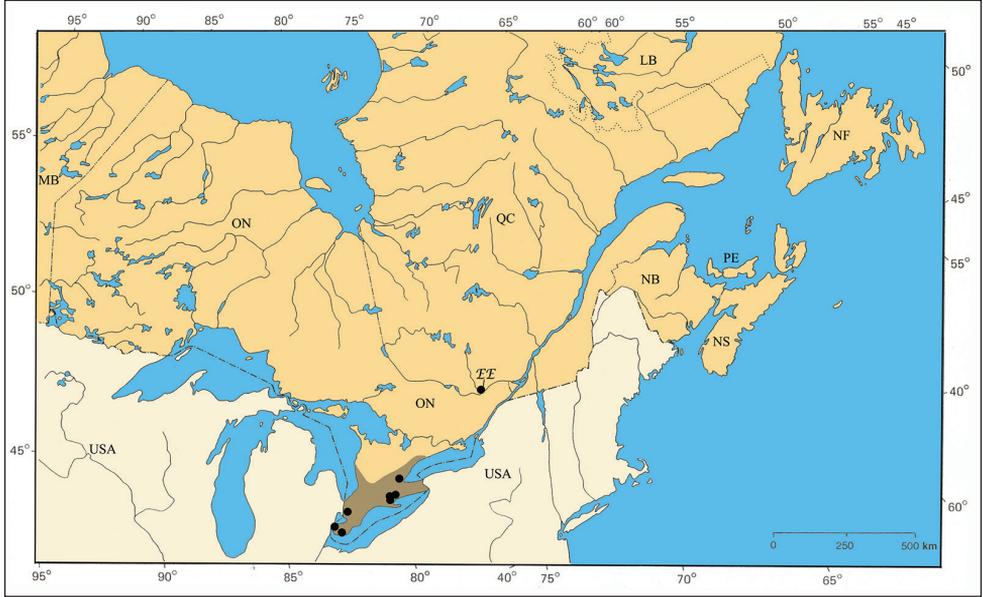


Habitus 16. Dorsal habitus of *Pachybrachis tridens*. Scale bar, 1 mm.

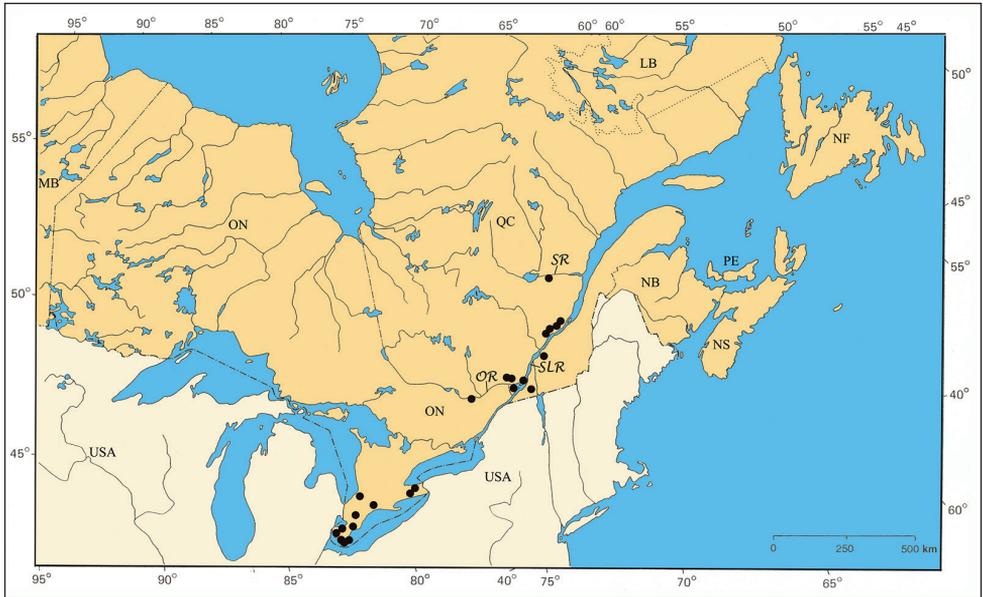


Habitus 17. Dorsal habitus of *Pachybrachis trinotatus*. Scale bar, 1 mm.

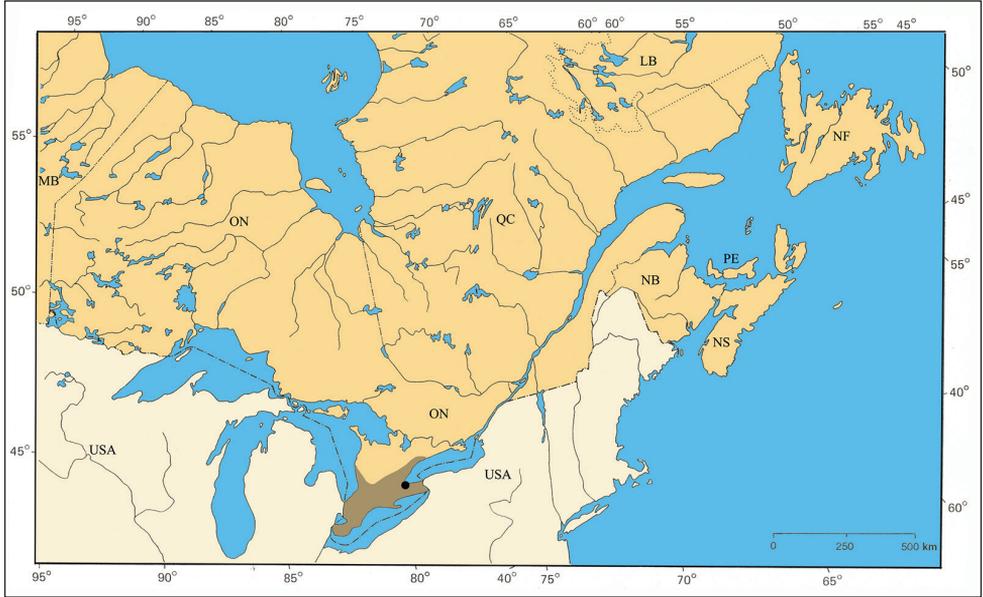
Map



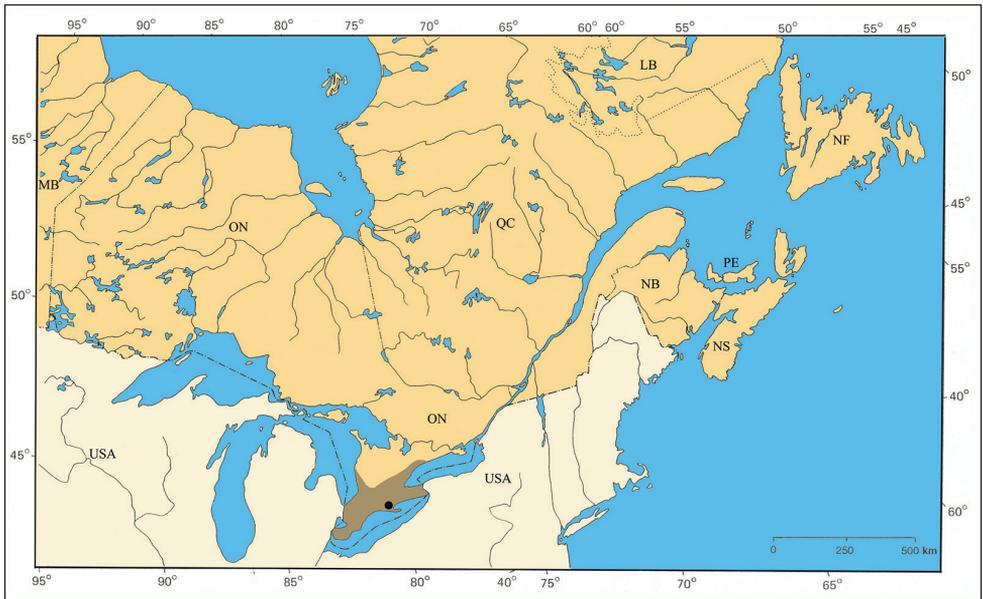
Map 1. The known distribution of *Pachybrachis atomarius* in eastern Canada. Carolinian Zone in dark beige; EE – Eardley Escarpment.



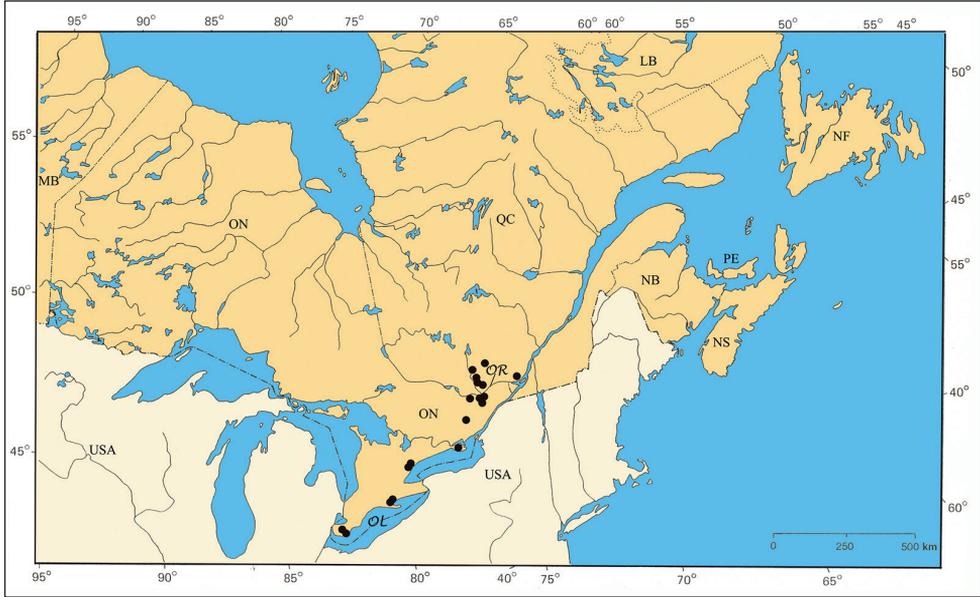
Map 2. The known distribution of *Pachybrachis bivittatus* in eastern Canada. OR – Ottawa River; SLR – St. Lawrence River; SR – Saguenay River.



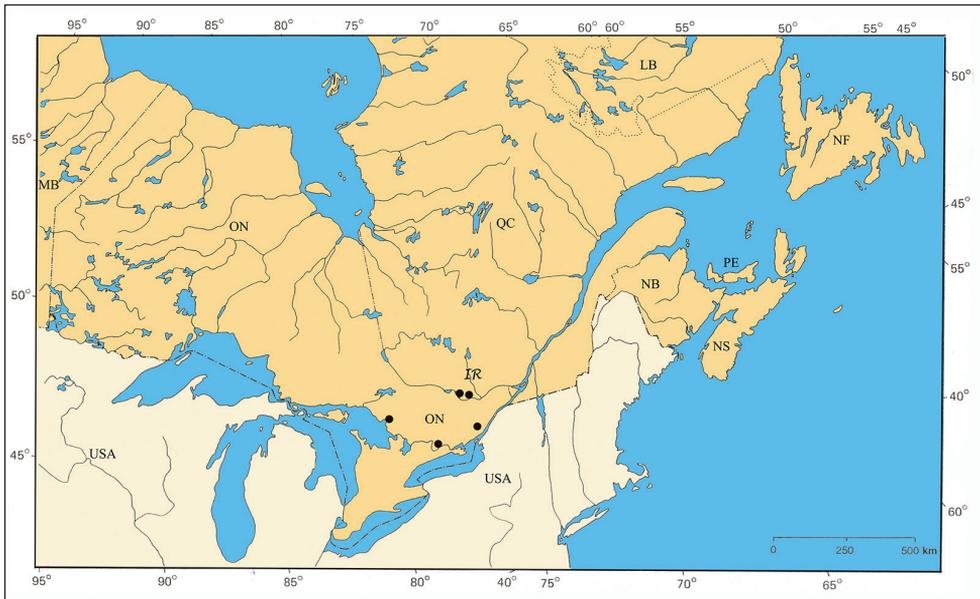
Map 3. The known distribution of *Pachybrachis calcaratus* in eastern Canada. Carolinian Zone in dark beige.



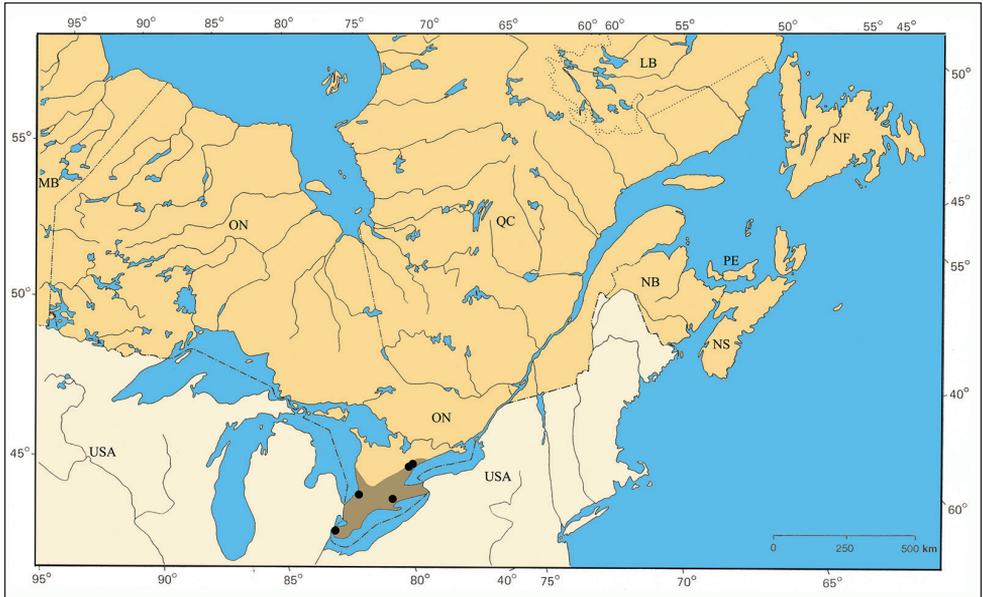
Map 4. The known distribution of *Pachybrachis cephalicus* in eastern Canada. Carolinian Zone in dark beige.



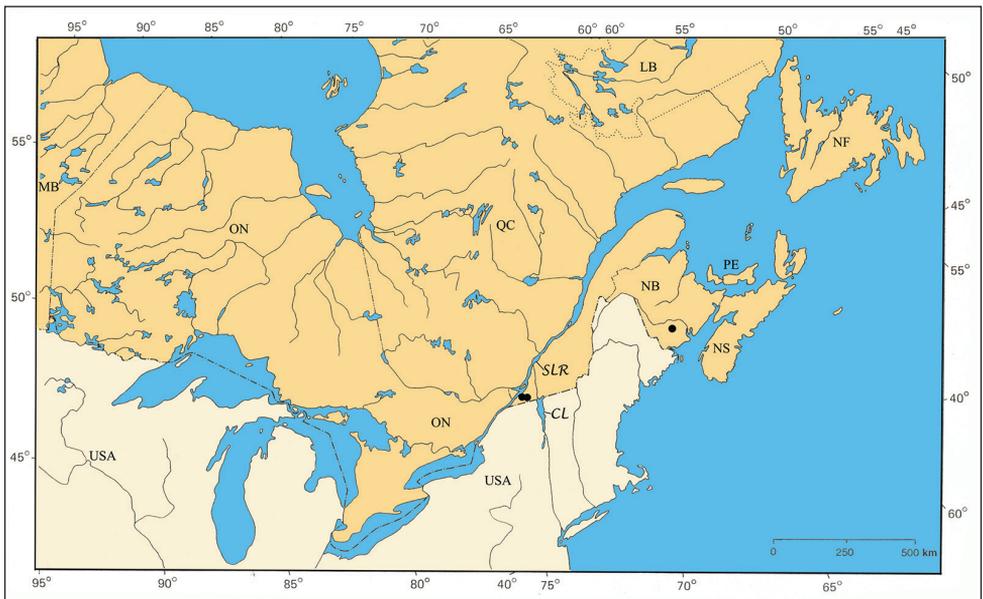
Map 5. The known distribution of *Pachybrachis hepaticus hepaticus* in eastern Canada. OL – Ontario Lake; OR – Ottawa River.



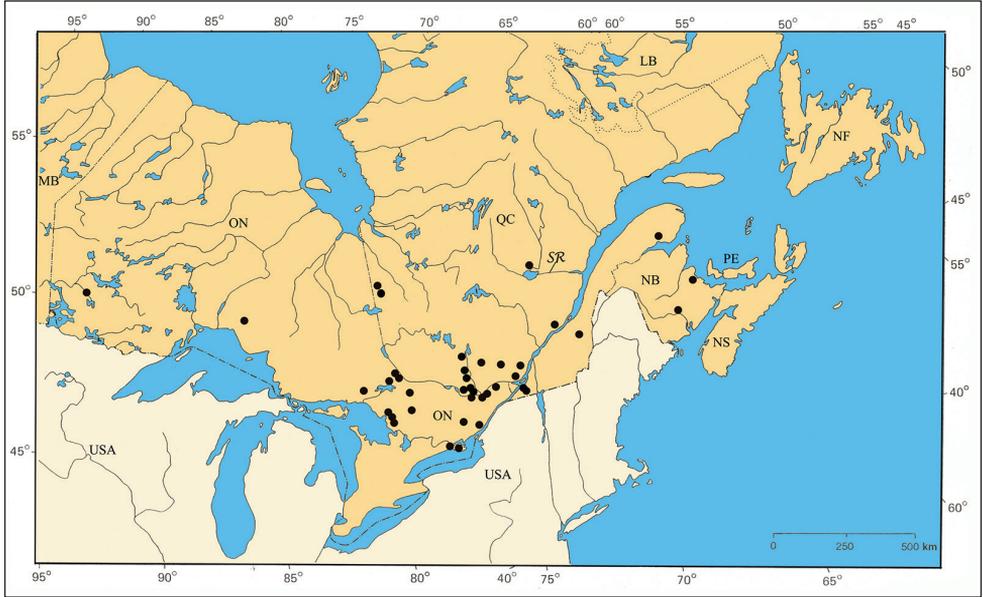
Map 6. The known distribution of *Pachybrachis luctuosus* in eastern Canada. IR – Île-du-Grand-Calumet in Ottawa River.



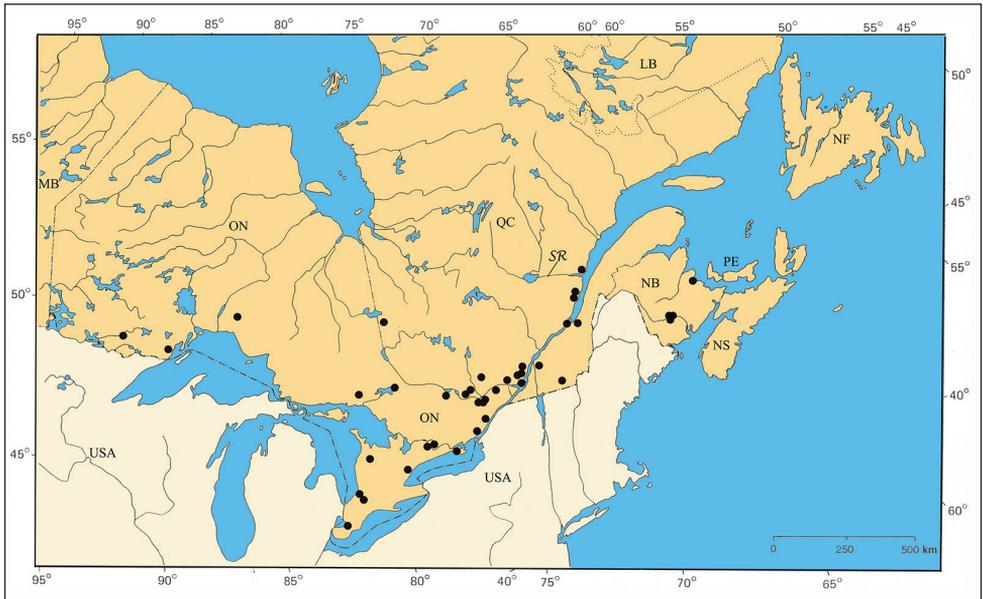
Map 7. The known distribution of *Pachybrachis luridus* in eastern Canada. Carolinian Zone in dark beige.



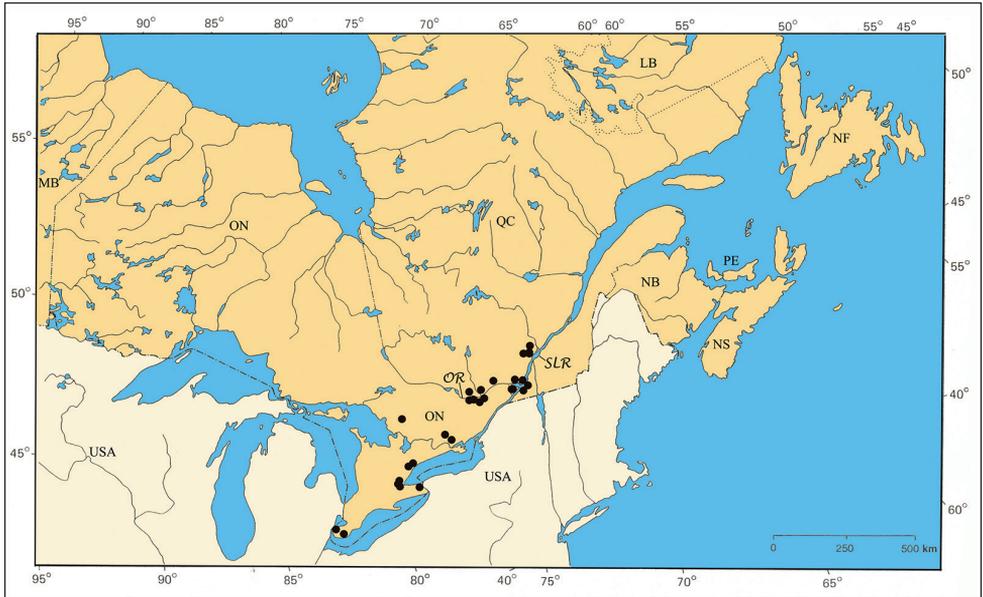
Map 8. The known distribution of *Pachybrachis m-nigrum* in eastern Canada. CL – Champlain Lake; SLR – St. Lawrence River.



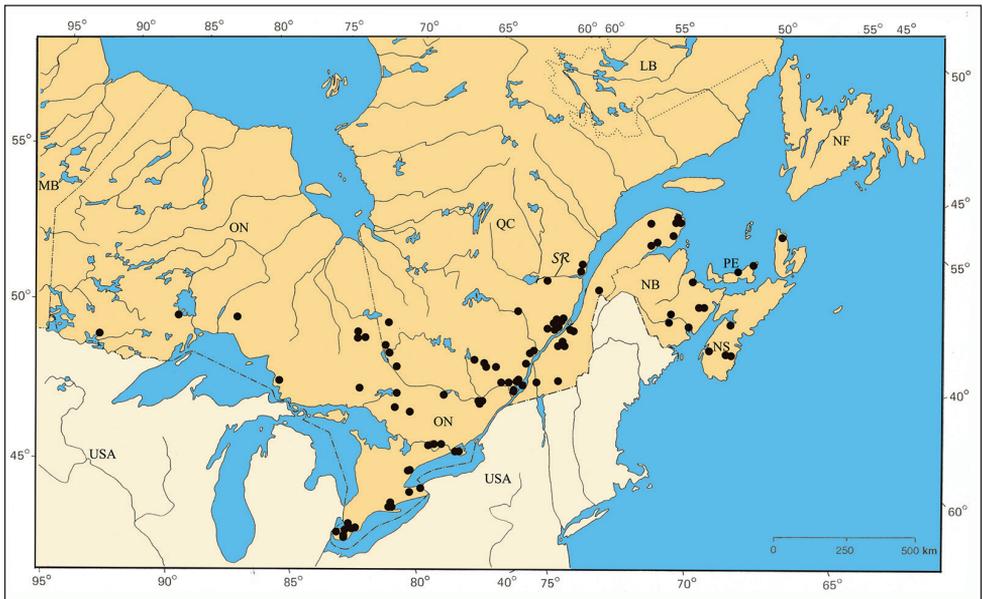
Map 9. The known distribution of *Pachybrachis nigricornis* in eastern Canada. SR – Saguenay River.



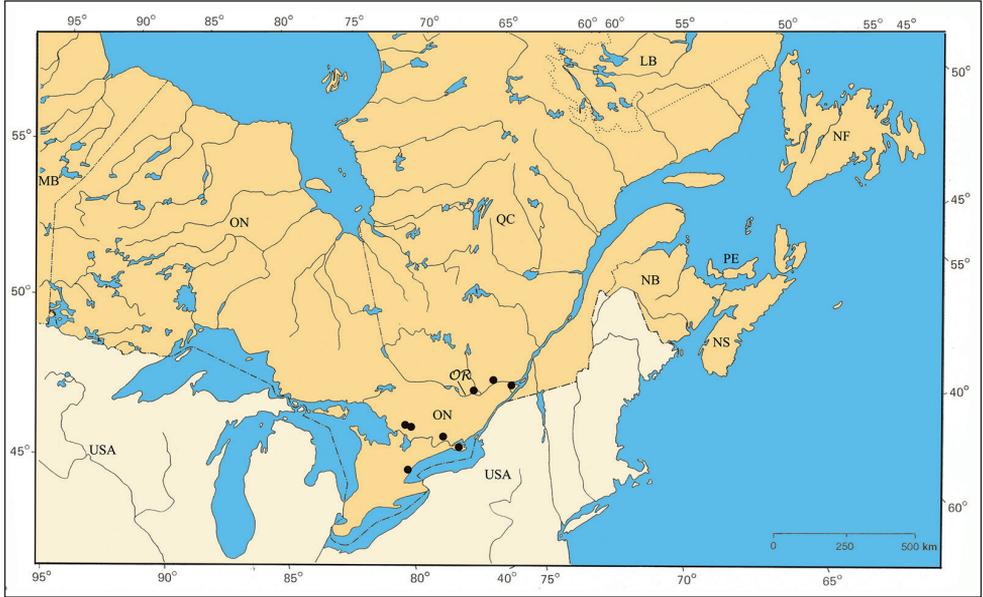
Map 10. The known distribution of *Pachybrachis obsoletus* in eastern Canada. SR – Saguenay River.



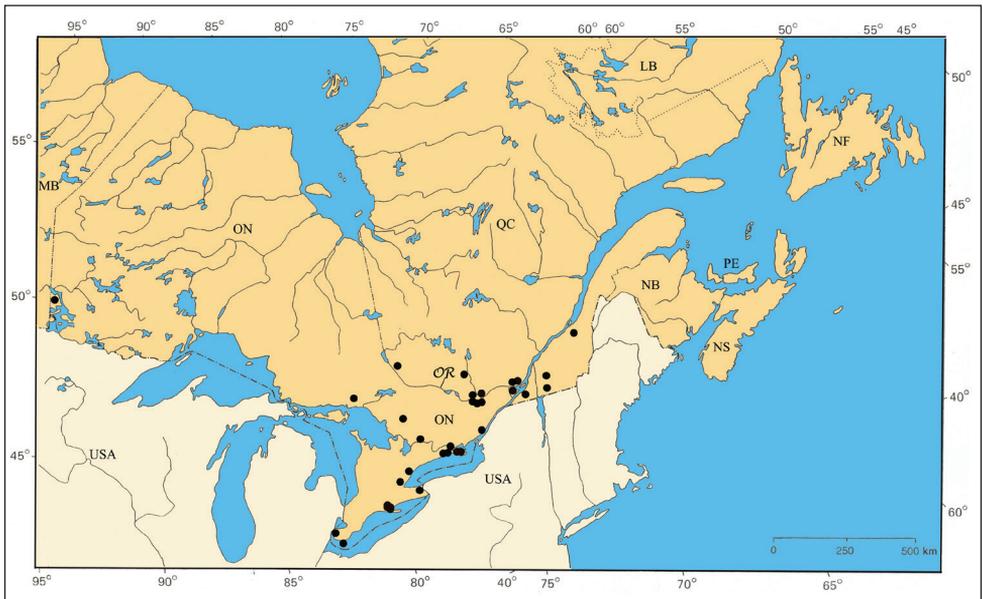
Map 11. The known distribution of *Pachybrachis othonus othonus* in eastern Canada. OR – Ottawa River; SLR – St. Lawrence River.



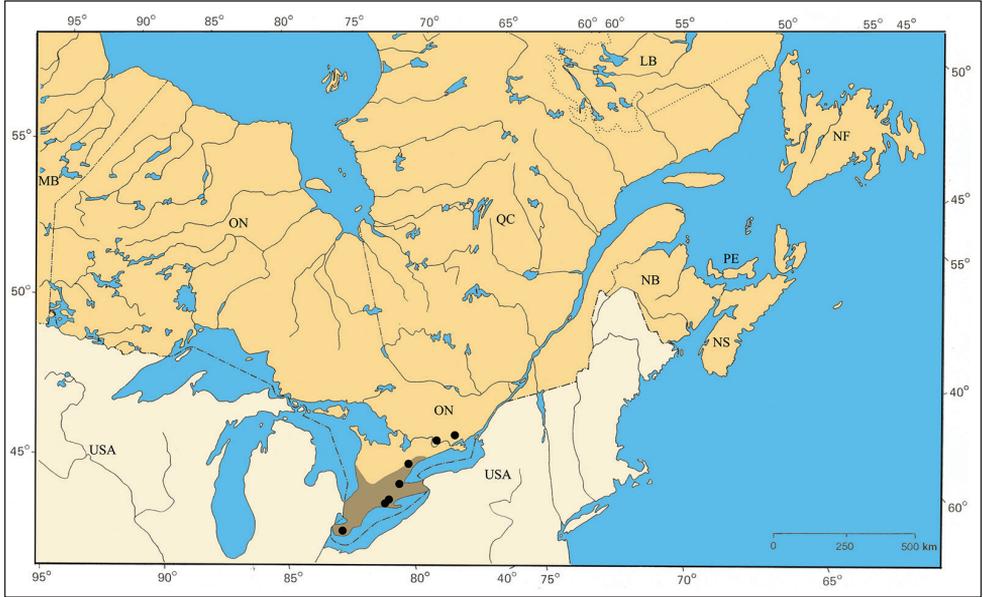
Map 12. The known distribution of *Pachybrachis peccans* in eastern Canada. SR – Saguenay River.



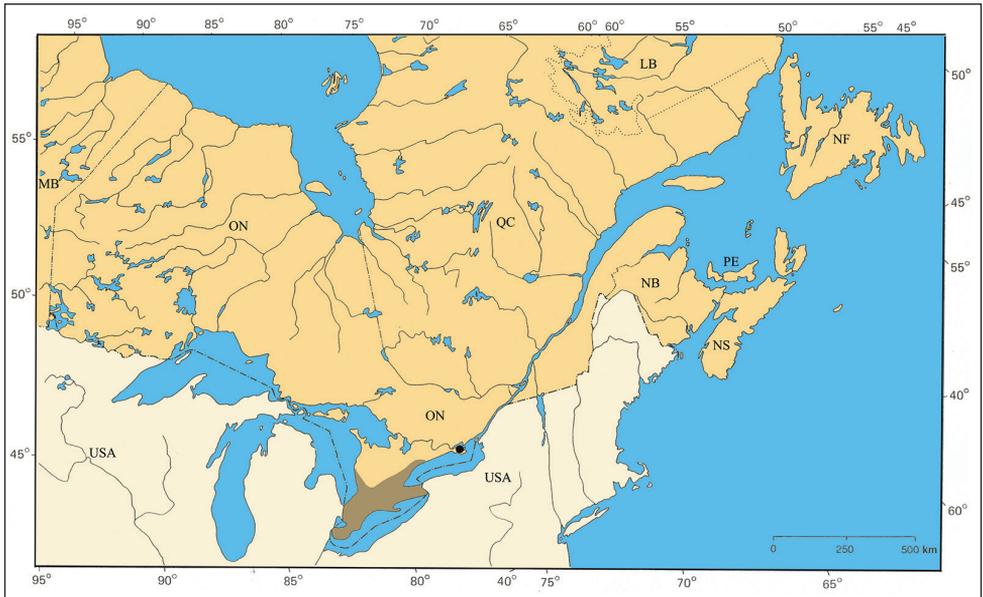
Map 13. The known distribution of *Pachybrachis pectoralis* in eastern Canada. OR – Ottawa River.



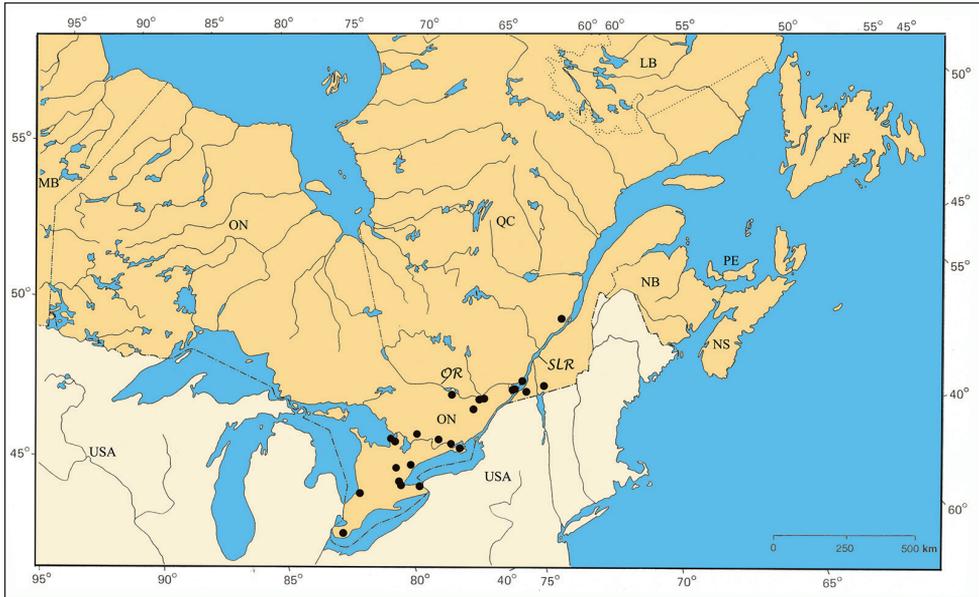
Map 14. The known distribution of *Pachybrachis spumarius* in eastern Canada. OR – Ottawa River.



Map 15. The known distribution of *Pachybrachis subfasciatus* in eastern Canada. Carolinian Zone in dark beige



Map 16. The known distribution of *Pachybrachis tridens* in eastern Canada. Carolinian Zone in dark beige.



Map 17. The known distribution of *Pachybrachis trinotatus* in eastern Canada.

Acknowledgements

The assistance of the curators of the collections listed above was greatly appreciated. They are thanked for allowing us to study their *Pachybrachis* holdings. Robert Bouchrier (Agriculture Canada, Lethbridge Research Centre, Biological Control) forwarded to us historical specimens identified in the past as "*P. obsoletus*." Klaus Bolte (Bio Imaging) is credited for all habitus photos, as well as photos of various structures, and Henri Goulet (ECORC) is credited for some close-ups of heads. André Larochelle and Marie-Claude Larivière generously provided host plant information preserved in their field notes. The comments and suggestions of Shawn M. Clark and an anonymous reviewer of a manuscript version of the present communication are most appreciated.

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Endemism patterns in the Italian leaf beetle fauna (Coleoptera, Chrysomelidae)

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Abstract

In this contribution the results of a zoogeographical analysis, carried out on the 123 endemic leaf beetle species (Coleoptera: Chrysomelidae) occurring in Italy and its immediately adjacent regions, are reported. To assess the level of faunistic similarity among the different geographic regions studied, a cluster analysis was performed, based on the endemic component. This was done by calculating the Baroni Urbani & Buser's similarity index (BUB). Finally, a parsimony analysis of endemism (PAE) was used to identify the most important areas of endemism in Italy.

Keywords

Coleoptera, Chrysomelidae, Italy, Alps, Apennines, Corsica, Sardinia, Sicily, endemic species, cluster analysis, parsimony analysis of endemism

Introduction

Even if there is not general agreement on whether conservation strategies should focus on hotspots of richness, extinction threat, endemism or rarity, since the correlation among these factors and their role as biodiversity indicators are still controversial (Bonn et al. 2002; Jetz et al. 2004; Lamoreux et al. 2006; Orme et al. 2005; Prendergast et al. 1993;

Reid 1998), the individuation of areas with great endemic species concentration is very important for biogeographical and conservation purposes (Brooks et al. 2006; Burlakova et al. 2011; Whittaker et al. 2005; Wilson et al. 2006). It is important not only for the intrinsic value of the particular species, but also for the funding for projects, since the idea of something exclusive and unique also appeals politicians (Riddle et al. 2011) and lay people (Meuser et al. 2009).

In this contribution, the results of a zoogeographical analysis carried out on the endemic species of leaf beetles (Coleoptera: Chrysomelidae) occurring in Italy, and the immediately adjacent regions, are reported.

Considering the biogeographic purpose of this contribution, we have preferred to follow the “traditional” subdivision into subfamilies as proposed by Lawrence and Newton (1995), and recently also adopted in Löbl and Smetana (2010), for the taxonomy of the Chrysomelidae but Cryptocephalinae and Clytrinae are treated as separated subfamilies. The subfamily Bruchinae was not considered for this study.

Leaf beetles, with 37.000–40.000 described species that are widespread in all the zoogeographical regions, are one of the most species rich families of phytophagous insects (Biondi and D’Alessandro 2012; Jolivet and Verma 2002; Schmitt 1996). In Italy and Corsica there are at least 830 species (D’Alessandro and Biondi 2011; Löbl and Smetana 2010), not including Bruchinae, of which about 15% show varying levels of endemism.

Distribution patterns in Chrysomelidae are very diverse, varying from cosmopolitan or sub-cosmopolitan species, to species that are strictly and locally endemic. Leaf beetles live in any habitat that has vegetation; because of the species richness of this beetle family and its well documented chorological and ecological information, it is highly representative of the overall biodiversity of a given ecosystem. For these reasons, Chrysomelidae have to be considered an effective instrument for environmental analysis (cf. Hilty and Merenlender 2000). Moreover, this beetle family comprises many species that show high levels of ecological and biological specialization, at least in temperate regions, and a significant trend towards differentiation and endemism in general.

Materials and methods

In this paper we consider an “endemic species” as “a species showing a distribution restricted to a geographical area, delimited by natural elements, and independent of administrative borders” (cf. Biondi 2006a); whereas an “area of endemism” is “a geographic region comprising the distributions of two or more monophyletic taxa, that exhibit a phylogenetic and distributional congruence, and have their respective relatives occurring in other such defined regions” (cf. Harold and Mooi 1994). Finally, by “subendemic species” we mean “an endemic species not occurring exclusively in a single area”; and “exclusively endemic species”, or “strictly local endemic species”, as “endemic species occurring exclusively in a single area”.

Study region

The region studied for this research includes the continental, peninsular and insular parts of Italy, and includes Corsica, as delimited in Fig. 1. The Alps were subdivided into sectors using the SOIUSA method, proposed by Marazzi (2006), which provided the following: the Central-Eastern Alps, North-Eastern Alps, North-Western Alps, South-Eastern Alps, and South-Western Alps (Fig. 1). We refer to the “faunal provinces” proposed by Minelli et al. (2006) for the peninsular and insular parts of Italy, showing the following areas: the Apulian Province, Central Apennines, Northern Apennines, Padanian Province, Sardinia, Sicily, and the Southern Apennines (Fig. 1). Finally, Corsica and the small Tyrrhenian Islands (Capri, Ischia, Pontine Islands, and Tuscany Islands) were added by us (Fig. 1). Distribution maps were constructed by ESRI ArcGis 10.0 software.

Database

Our database of the endemic species of leaf beetles (Coleoptera: Chrysomelidae), used for the statistical analyses, consisted of: a) records obtained by a critical bibliographic screening (Baviera 2007; Biondi et al. 1994; Biondi 2006c; Kapp 2007; Kippenberg 2008; Leonardi 2007; Moncoutier 2011; Montagna 2011; Sassi 2006, 2011); b) material from entomological collections; and c) information obtained from specialists. The complete list of endemic species, expressed as presence/absence in the different regions studied, is reported in Tab. I. Subspecies were not considered, because their status is often not well defined and also not universally accepted.

The terminology and typology used for the distribution types of endemic and subendemic Italian species follows Vigna Taglianti et al. (1999) and Stoch and Vigna Taglianti (2006), but with the following modifications: a) “3900.22 SISA Sicilian-S-Apennine Endemic” replaces “SISC Sicilian-S-Calabrian Endemic”; b) “3900.23 APSI - Apennine-Sicilian Endemic” was added by us.

Data on host plants were obtained through careful bibliographic screening, integrated with personal observations. Regarding the trophic range, we refer to Biondi (1996), and the following terminology is used:

“monophagous”: species with adults feeding on one or two systematically closely related plant genera;

“oligophagous”: species with adults feeding on plant genera from one or two systematically closely related families; and

“polyphagous”: species with adults feeding on many botanical species that are not closely related systematically.

Finally, the term “herb” refers to species where adults are associated with herbaceous plants, while by “arb/shr” refers to species where adults are associated with trees and/or shrubs.



Figure 1. Study region and geographical sectors researched with: CE Alps – Central-Eastern Alps; NE Alps – North-Eastern Alps; NW Alps – North-Western Alps; SE Alps – South-Eastern Alps; and SW Alps – South-Western Alps.

Cluster analysis

The level of faunistic similarity between the different geographic regions studied was assessed, based on the endemic component, by performing a cluster analysis which used Operational Geographic Units (OGUs) (see Fig. 1) as reference. This was done by calculating the Baroni Urbani & Buser's similarity index (BUB) (Baroni Urbani and Buser 1976) on a presence-absence binary matrix of the endemic species. As clustering algorithm, the WPGMA (Weighted Pair-Group Method using Arithmetic averages) was used (McQuitty 1966), and the analysis was done using the MVSP (Kovach 1999) and NTSys (Rohlf 2008) statistical packages. The BUB similarity index is expressed by the formula $[(\sqrt{ad})+a]/[(\sqrt{ad})+a+b+c]$, where: a is the total number of co-presences; b is the total number of species present in OGU 1 but absent in OGU 2; c is the total number of species present in OGU 2 but absent in OGU 1; d is a total number of co-absences. This index was preferred because we regard the knowledge of the distribution of the endemic leaf beetle fauna in the study area to be good. Thus, we think that co-absences in this analysis represent a highly informative element (Biondi 1988a, 2006b).

Parsimony analysis of endemism

Parsimony analysis of endemism (PAE) was used to identify the most important areas of endemism in Italy (Morrone 1994; Rosen 1988; Rosen and Smith 1988). To increase the reliability of the analysis a selection was made from the data matrix, including only those species that have been reported to live in two or more contiguous OGUs. As geographic reference we used a grid of 50×50 km quadrats superimposed on our study region. Species occurring in only one, or in non-contiguous OGUs, were excluded from this analysis as they are uninformative. PAE was implemented using the MIX programme (1000 randomization, seed = 25) in the PHYLIP package (Felsenstein 1989), with the Camin–Sokal optimization technique (Camin and Sokal 1965), allowing for extinction but not the possibility of new colonization events. For the strict consensus tree (Margush and McMorris 1981; Sokal and Rohlf 1981) the CONSENSE program in the PHYLIP package (Felsenstein 1989) was used.

Abbreviations. Distribution types - ALAP: Alpine-Apennine; ALPE: Eastern Alpine; ALPS: Southern Alpine; ALPW: Western Alpine; ALSE: South-Eastern Alpine; ALSW: South-Western Alpine; APPC: Central Apennine; APPE: Apennine; APPN: Northern Apennine; APPS: Southern Apennine; APSI: Apennine-Sicilian; AWWA: Western Alpine-Northern Apennine; CORS: Corsican; ITAL: Italian; PADA: Padanian; SACO: Sardinian-Corsican; SARD: Sardinian; SICI: Sicilian; SISA: Sicilian-Southern Apennine; TYRR: Tyrrhenian.

Geographical sectors - APUL: Apulian Province; CAPE: Central Apennines; CEALP: Central-Eastern Alps; COR: Corsica; NAPE: Northern Apennines; NEALP:

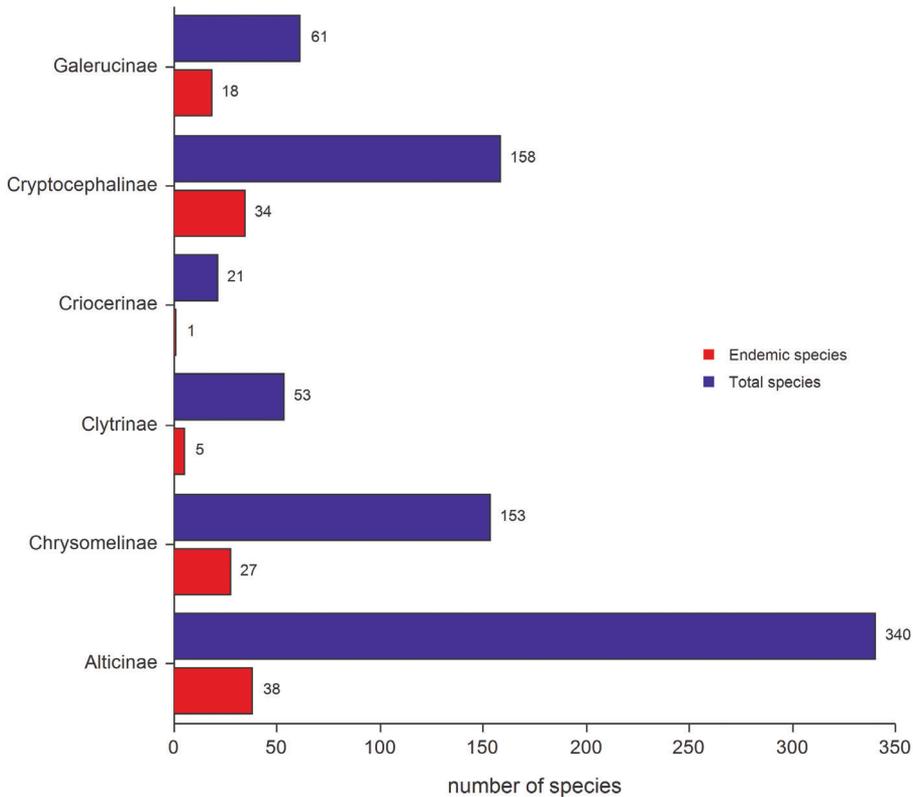


Figure 2. Total number of species and endemic species for the leaf beetle subfamilies occurring in the study area.

North-Eastern Alps; NWALP: North-Western Alps; PAD: Padanian Province; SAPE: Southern Apennines; SAR: Sardinia; SEALP: South-Eastern Alps; SIC: Sicily; SWALP: South-Western Alps; and STI: small Tyrrhenian Islands.

Results

We found 123 endemic species of Chrysomelidae occurring in the study region (Table 1), which represent about 15% of the total leaf beetle fauna in Italy. This percentage is very high if compared to the total percentage for the endemic component of the terrestrial and inland water fauna in Italy, which is 10%. Seen in the European context, the latter percentage represents a high value (Stoch 2000, 2008).

The percentage of endemic species is not proportionate to the different subfamilies of Chrysomelidae (Fig. 2). Alticinae, for example, display the greatest endemic species richness (38), but only represent 11.18% of the entire Italian flea beetle fauna (340 species). In some of the other subfamilies the percentage of endemic species is higher

Subfamily	Species	NEALP	CEALP	SEALP	NWALP	SWALP	PAD	NAPE	CAPE	SAPE	APUL	SIC	SARD	CORS	TYRR	DISTR
Alticinae	<i>Neorepidodera spectabilis</i> (J. Daniel)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	ALPW
Alticinae	<i>Orestia apennina</i> Weise	0	0	0	0	0	1	1	1	1	0	0	0	0	0	APPE
Alticinae	<i>Orestia brandstetteri</i> Kapp	0	0	0	0	0	0	0	1	0	0	0	0	0	0	APPC
Alticinae	<i>Orestia carnica</i> Leonardi	0	0	1	0	0	0	0	0	0	0	0	0	0	0	ALSE
Alticinae	<i>Orestia carniolica</i> Weise	0	0	1	0	0	0	0	0	0	0	0	0	0	0	ALSE
Alticinae	<i>Orestia coiffaiti</i> Doguet	0	0	0	0	0	0	0	0	0	0	0	0	1	0	CORS
Alticinae	<i>Orestia electra</i> Gredler	0	1	1	1	0	0	0	0	0	0	0	0	0	0	ALPS
Alticinae	<i>Orestia heikertingeri</i> Leonardi	0	0	1	1	0	0	0	0	0	0	0	0	0	0	ALPS
Alticinae	<i>Phyllorreta ziegleri</i> Lohse	1	1	0	0	0	0	0	0	0	0	0	0	0	0	ALPE
Alticinae	<i>Psylliodes biondii</i> Leonardi	0	0	0	0	0	0	0	1	0	0	0	0	0	0	APPC
Alticinae	<i>Psylliodes canepari</i> Leonardi	0	0	0	0	0	0	0	0	1	0	1	0	0	0	SISA
Alticinae	<i>Psylliodes feroniae</i> Leonardi	0	0	0	0	0	0	0	1	1	1	0	0	0	0	APPE
Alticinae	<i>Psylliodes forellae</i> Leonardi	0	0	0	0	0	0	1	0	0	0	0	0	0	0	APPN
Alticinae	<i>Psylliodes leonhardi</i> Heikertinger	0	0	0	0	0	0	1	0	1	0	1	0	0	0	APSI
Alticinae	<i>Psylliodes parodii</i> Leonardi	0	0	0	0	0	0	1	1	0	0	0	0	0	0	APPE
Alticinae	<i>Psylliodes raffii</i> Leonardi	0	0	0	0	0	0	1	1	1	0	1	0	0	0	APSI
Alticinae	<i>Psylliodes solaris</i> Leonardi	0	0	0	0	1	0	1	0	0	0	0	0	0	0	AWNA
Alticinae	<i>Psylliodes springeri</i> Leonardi	0	0	0	0	0	0	0	1	0	0	0	0	0	0	APPC
Chrysomelinae	<i>Chrysolina bourdonnei</i> Daccordi & Ruffo	0	0	0	0	0	0	0	1	1	0	0	0	0	0	APPE
Chrysomelinae	<i>Chrysolina osellai</i> Daccordi & Ruffo	0	0	0	0	0	0	1	0	0	0	0	0	0	0	APPN
Chrysomelinae	<i>Chrysolina platypoda</i> Bechyné	0	0	0	0	1	0	0	0	0	0	0	0	0	0	ALSW
Chrysomelinae	<i>Chrysolina schatzmayri</i> (G. Müller)	0	0	0	0	0	1	0	0	0	0	0	0	0	0	PADA
Chrysomelinae	<i>Chrysolina sirenensis</i> (Meier)	0	0	0	0	0	0	0	1	1	1	0	0	0	0	APPE
Chrysomelinae	<i>Chrysolina stachydis</i> (Gene)	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO
Chrysomelinae	<i>Chrysolina suffriani</i> (Fairmaire)	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO

Subfamily	Species	NEALP	CEALP	SEALP	NWALP	SWALP	PAD	NAPE	CAPE	SAPE	APUL	SIC	SARD	CORS	TYRR	DISTR
Chrysomelinae	<i>Chrysolina variolosa</i> (Pezagna)	0	0	0	0	0	0	0	0	1	0	1	0	0	0	SISA
Chrysomelinae	<i>Gonioctena gobanzi</i> (Reitter)	0	1	1	0	0	0	0	0	0	0	0	0	0	0	ALPE
Chrysomelinae	<i>Gonioctena holdhausi</i> (Leedet)	1	1	1	1	0	0	0	0	0	0	0	0	0	0	ALPS
Chrysomelinae	<i>Gonioctena lineata</i> (Gené)	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO
Chrysomelinae	<i>Gonioctena ibeae</i> Baviera	0	0	0	0	0	0	0	0	0	0	1	0	0	0	SICI
Chrysomelinae	<i>Hydrobasa suffriani</i> (Küster)	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO
Chrysomelinae	<i>Oreina canavesei</i> Bontems	0	0	0	1	0	0	0	0	0	0	0	0	0	0	ALPW
Chrysomelinae	<i>Oreina collucens</i> (K. Daniel)	0	0	0	1	1	0	0	0	0	0	0	0	0	0	ALPW
Chrysomelinae	<i>Oreina elongata</i> (Suffrian)	1	1	1	1	1	0	1	1	0	0	0	0	0	0	ALAP
Chrysomelinae	<i>Oreina genei</i> (Suffrian)	0	0	0	1	1	0	1	0	0	0	0	0	0	0	AWNA
Chrysomelinae	<i>Oreina linurata</i> (Scopoli)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	ALSE
Chrysomelinae	<i>Oreina melancholica</i> (Heer)	1	1	1	1	1	0	0	0	0	0	0	0	0	0	ALPS
Chrysomelinae	<i>Oreina petiolerii</i> (Bassi)	0	0	0	1	1	0	0	0	0	0	0	0	0	0	ALPW
Chrysomelinae	<i>Oreina sybilla</i> (Binaghi)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	APPC
Chrysomelinae	<i>Timarcha apuana</i> Daccordi & Ruffo	0	0	0	0	0	0	1	0	0	0	0	0	0	0	APPN
Chrysomelinae	<i>Timarcha cornuta</i> Bechyné	0	0	0	0	0	0	0	0	0	0	0	0	1	0	CORS
Chrysomelinae	<i>Timarcha fracassii</i> Meier	0	0	0	0	0	0	0	1	0	0	0	0	0	0	APPC
Chrysomelinae	<i>Timarcha pimeloides</i> Herrich-Schaeffer	0	0	0	0	0	0	0	0	1	1	1	0	0	1	TYRR
Chrysomelinae	<i>Timarcha sardea</i> Villa & Villa	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO
Chrysomelinae	<i>Timarcha sicelidis</i> Reiche	0	0	0	0	0	0	0	0	0	0	1	0	0	0	SICI
Clytrinae	<i>Coptocephala raffrayi</i> (Desbrochers des Loges)	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO
Clytrinae	<i>Labidostomis centromaculata</i> Gené	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO
Clytrinae	<i>Labidostomis syriaca</i> Lacordaire	0	0	0	0	0	0	0	0	0	0	0	0	1	0	CORS
Clytrinae	<i>Lachnaia caprai</i> Grasso	0	0	0	0	0	0	0	0	0	0	1	0	0	0	SICI
Clytrinae	<i>Snamagdina ferulae</i> Gené	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO

Subfamily	Species	NEALP	CEALP	SEALP	NWALP	SWALP	PAD	NAPE	CAPE	SAPE	APUL	SIC	SARD	CORS	TYRR	DISTR
Critocerinae	<i>Oulema magistretorium</i> (Ruffo)	0	0	0	0	0	0	1	1	1	0	0	0	0	0	APPE
Cryptocephalinae	<i>Cryptocephalus albolineatus</i> Suffrian	1	1	1	1	1	0	0	0	0	0	0	0	0	0	ALPS
Cryptocephalinae	<i>Cryptocephalus alnicola</i> Costa	0	0	0	0	0	0	0	0	0	0	0	1	0	0	SARD
Cryptocephalinae	<i>Cryptocephalus atrifrons</i> Abelle	0	0	0	0	1	0	0	0	0	0	0	0	0	0	ALSW
Cryptocephalinae	<i>Cryptocephalus barri</i> Burlini	0	0	1	0	0	0	0	0	0	0	0	0	0	0	ALSE
Cryptocephalinae	<i>Cryptocephalus biondii</i> Sassi & Regalin	0	0	0	0	0	0	0	0	0	0	0	1	1	1	TYRR
Cryptocephalinae	<i>Cryptocephalus cognatus</i> Costa	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO
Cryptocephalinae	<i>Cryptocephalus czwalinae</i> Weise	0	0	0	0	0	0	1	1	1	1	0	0	0	0	APPE
Cryptocephalinae	<i>Cryptocephalus daccordii</i> Biondi	0	0	0	0	0	0	0	0	1	0	0	0	0	0	APPS
Cryptocephalinae	<i>Cryptocephalus equiseti</i> Costa	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO
Cryptocephalinae	<i>Cryptocephalus eridani</i> Sassi	0	1	1	1	1	1	1	0	0	0	0	0	0	0	ALAP
Cryptocephalinae	<i>Cryptocephalus etruscus</i> Suffrian	0	0	0	0	0	1	1	1	1	1	0	0	0	1	ITAL
Cryptocephalinae	<i>Cryptocephalus falzoni</i> Burlini	0	0	0	0	0	0	0	0	1	0	1	0	0	0	SISA
Cryptocephalinae	<i>Cryptocephalus grobmannii</i> Suffrian	0	0	0	0	0	0	0	0	1	0	1	0	0	0	SISA
Cryptocephalinae	<i>Cryptocephalus henningi</i> Sassi	0	0	0	0	1	0	1	0	0	0	0	0	0	0	AWNA
Cryptocephalinae	<i>Cryptocephalus informis</i> Suffrian	0	0	0	1	1	1	1	1	0	0	0	0	0	0	ALAP
Cryptocephalinae	<i>Cryptocephalus leonbardei</i> Breit	0	0	0	0	0	0	0	1	1	0	0	0	0	0	APPE
Cryptocephalinae	<i>Cryptocephalus loxtianus</i> Burlini	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO
Cryptocephalinae	<i>Cryptocephalus paganensis</i> Pic	0	0	0	0	0	0	0	1	0	0	0	0	0	0	APPC
Cryptocephalinae	<i>Cryptocephalus plantaris</i> Suffrian	0	0	0	0	0	0	0	0	0	0	1	0	0	0	SICI

Subfamily	Species	NEALP	CEALP	SEALP	NWALP	SWALP	PAD	NAPE	CAPE	SAPE	APUL	SIC	SARD	CORS	TYRR	DISTR
Galerucinae	<i>Luperus leonardii</i> Fogato	0	0	1	1	1	1	1	1	1	1	0	0	0	0	ALAP
Galerucinae	<i>Luperus maculicornis</i> Desbrochers	0	0	0	0	0	0	0	0	0	0	0	0	1	0	CORS
Galerucinae	<i>Luperus pygmaeus</i> Joannis	0	0	0	0	0	1	1	1	1	1	0	0	0	1	ITAL
Galerucinae	<i>Luperus ragusai</i> Laboissière	0	0	0	0	0	0	0	0	1	0	1	0	0	0	SISA
Galerucinae	<i>Luperus revulterei</i> Perris	0	0	0	0	0	0	0	0	0	0	0	1	1	0	SACO
Galerucinae	<i>Luperus vitaliei</i> Ragusa	0	0	0	0	0	0	0	0	0	0	1	0	0	0	SICI

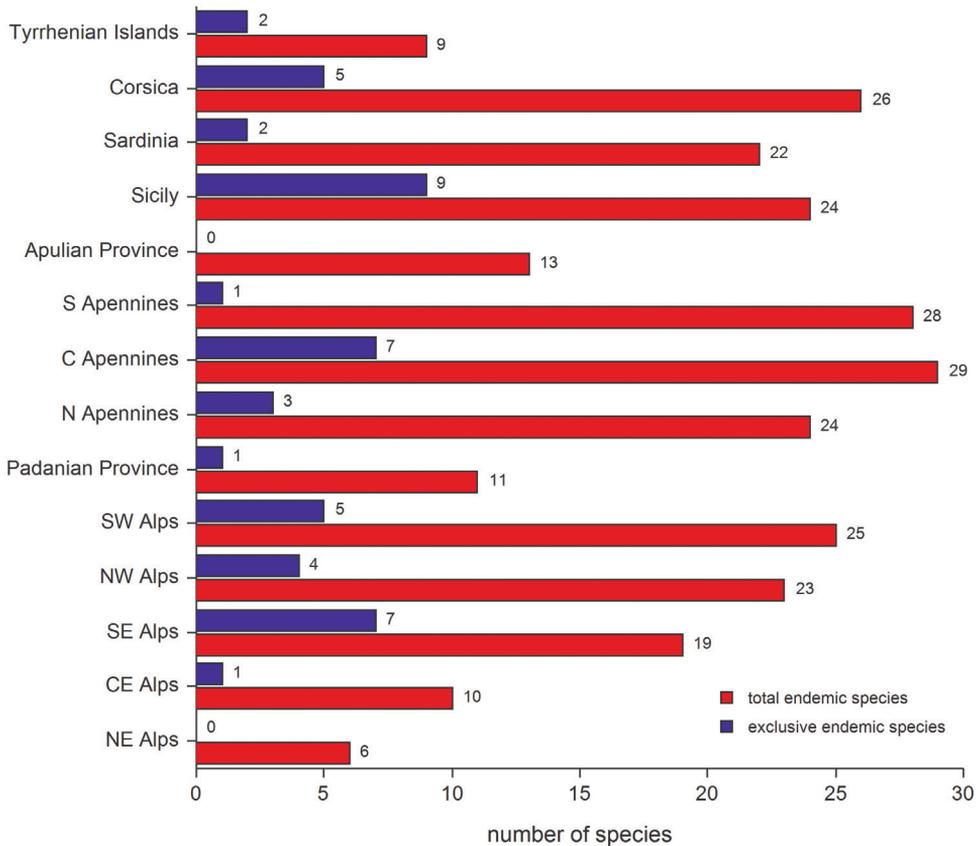


Figure 3. Number of endemic and exclusively endemic species occurring in all geographic sectors studied, depicting the: Apulian Province; South-Western, North-Western, South-Eastern, Central-Eastern and North-Eastern Alps; Central, Northern, and Southern Apennines; Corsica; Padanian Province; Sardinia; Sicily; and the small Tyrrhenian Islands.

than the total average of 14.82% for the Chrysomelidae in Italy. Examples include: the Galerucinae with 29.51% (18 endemic species from a total of 61); Cryptocephalinae with 21.52% (34 endemic species from a total of 158); and Chrysomelinae with 17.65% (27 endemic species from a total of 153). Besides the Alticinae, lower endemic species percentages are also found in Clytrinae with 9.43% (5 endemic species from a total of 53) and Criocerinae with 4.76% (1 endemic species from a total of 21). No endemic species of Cassidinae, Donaciinae, Eumolpinae, Hispinae or Lamprosomatinae were found in the study area.

Regions with the greatest richness of endemic leaf beetle species are (Fig. 3): the Central Apennines (29 species), Southern Apennines (28), Corsica (26), South-Western Alps (25), Northern Apennines (24), Sicily (24), North-Western Alps (23) and Sardinia (22). Areas with the poorest endemic species richness are: the North-Eastern Alps (6), Tyrrhenian Islands (9), Central-Eastern Alps (10), Padanian Province (11),

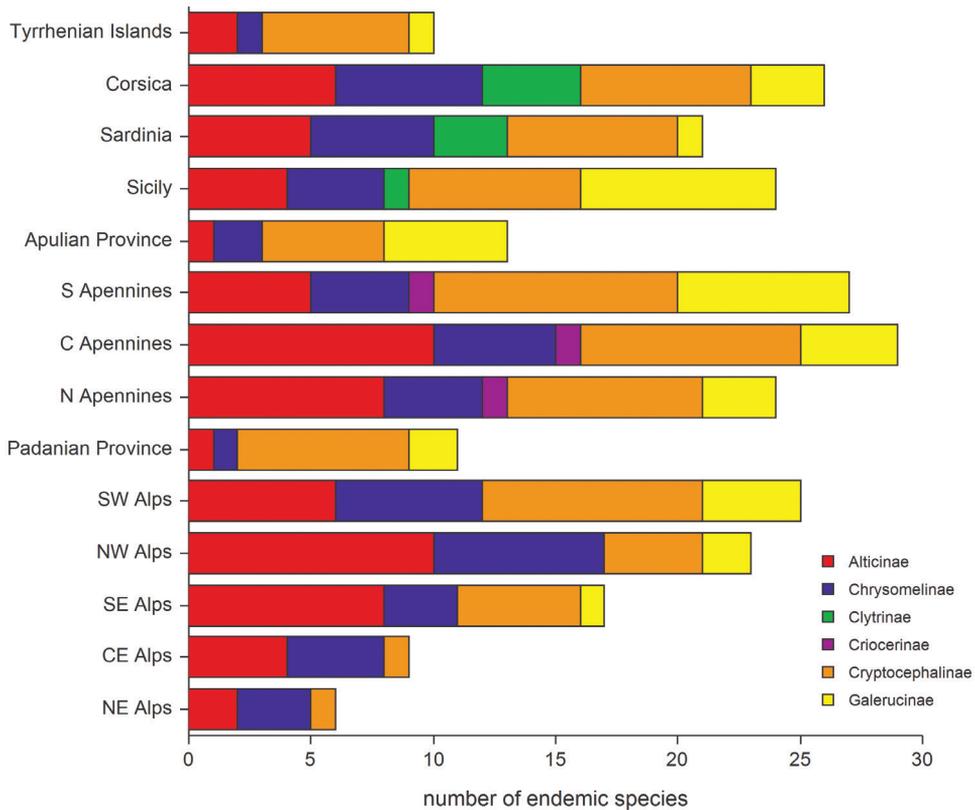


Figure 4. Number of endemic species for leaf beetle subfamilies occurring in the geographical sectors studied.

and Apulian Province (13). However, if we only consider the local exclusively endemic species for every region, the greatest endemic species richness can be found in Sicily (9 species), the Central Apennines (7), and South-Eastern Alps (7) (Fig. 3). Local exclusively endemic species can provide important information about past and present isolation conditions for a given geographical region. At this point it is noteworthy that the number of exclusively endemic species for Corsica (5), and especially for Sardinia (2), is very low. However, if we consider the Sardinian-Corsican area as a whole, the number increases to 17 species, demonstrating the common history shared by these two islands and their intensive faunistic exchange. The Apulian Province and the North-Eastern Alps have no exclusively endemic species. The former probably because the Apulian Province was never isolated geographically or ecologically; the latter because the North-Eastern Alps, besides a poor endemic species richness, display ecological continuity that has possibly promoted horizontal range expansions into other adjacent Alpine sectors, thus hampering local endemization.

The endemic component of the different subfamilies for all the regions studied (Fig. 4) is, in general, significantly correlated with an increase in altitude or the meas-

ure of insularity; in the largest islands these two factors often fulfill a synergistic role. Regarding the altitude, the highest number of endemic species occur between 400 m and 1800 m a.s.l. with the majority found between 700 m and 800 m; the species from the plains have been added to the low and medium altitude species (Fig. 9). Endemization associated with lower altitudes generally occurs in insular areas, particularly Sardinia, Corsica and the small Tyrrhenian islands; whereas endemization associated with higher altitudes mainly occurs in the Central Apennines, where the alternation of catathermic and hypsothermic phases during the Pleistocene glaciations played an important role in the isolation and differentiation of the montane fauna. The number of endemic species and area are not significantly correlated (Fig. 8).

Based on the recognized distribution types for the Italian endemic fauna, as proposed by Vigna Taglianti et al. (1999) and Stoch and Vigna Taglianti (2006), and partially modified by us (see “Materials and methods” above), we can conclude that the Sardinian-Corsican type (SACO) in which Cryptocephalinae and Chrysomelinae are dominant, and the Western Alpine type (ALPW) where Alticinae are clearly in the majority, represent the distributions with the greatest abundance of endemic species. However, other distribution types that are well represented are the: Sicilian (SICI), where the absence of Alticinae has to be emphasized; Sicilian-Southern Apennine (SISA), where the Galerucinae are more abundant; Apennine (APPE) and Central Apennine (APPC), where Alticinae and, to a lesser extent, Chrysomelinae, Cryptocephalinae and Galerucinae, are more plentiful (Fig. 5). Distribution types with poor representation are the: Padanian (PADA), with *Chrysolina schatzmayri* (G. Müller) being the only species; and the Southern Apennines (APPS), with *Cryptocephalus dacordii* Biondi and *Luperus calabricus* Laboissière, being the only species - the latter also occurring in the Apulian Province (Fig. 5).

The results of the cluster analysis are depicted in a dendrogram (Fig. 10). In broad terms, it shows an “Alpine block” and an “Apennine-insular block” which are distinctly separated. The Alpine block is, in turn, subdivided into the Eastern Alps [(NE Alps - CE Alps) SE Alps] and Western Alps (NW Alps - SW Alps); whereas the Apennine-insular block shows a clear separation between the Apennines [(Apulian Province - S Apennines) C Apennines (Padanian Province - N Apennines)], and the small Tyrrhenian islands (Tyrrhenian Is.), Sicily (Sicily) and the Corsican-Sardinian region (Corsica-Sardinia). Within the Apennine block, the central and southern sectors (the Apulian Province included) show a higher faunistic similarity among them than with the northern sector (Padanian Province included). Finally, the position of Sicily reflects its close geographic proximity and ecological continuity with the Apennines.

Host plant families preferred by the endemic species occurring in the study region are, among herbaceous plants, the Asteraceae (13.97%), Poaceae (7.35%), Lamiaceae (5.88%) and Brassicaceae (5.15%); whereas among arboreal and shrubby plants, the Fagaceae (8.09%) and Rosaceae (5.88%) (Fig. 6) are dominant. The endemic leaf beetle species studied show an increase in trophic specialization. This is demonstrated by a high prevalence of monophagous (Herb: 50.00%; Arb/Shr: 44.44%) and, to a lesser extent, oligophagous elements (Herb: 34.48%; Arb/Shr: 33.33%), while species as-

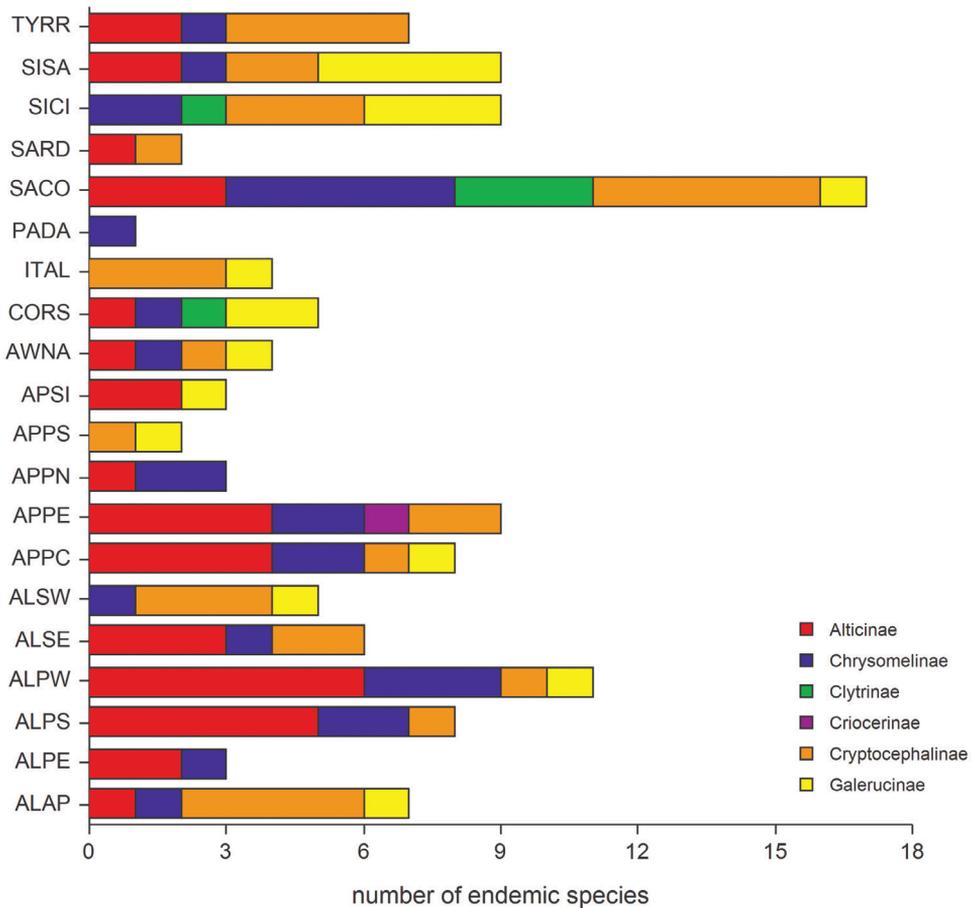


Figure 5. Number of endemic species in the different leaf beetle subfamilies, based on their distribution (for abbreviations see the text).

sociated with herbaceous plants show the lowest percentage (15.52%) of polyphagous elements (Fig. 7).

The results of the parsimony analysis of endemism (PAE) are reported in Fig. 11. This analysis reveals that the most significant region, displaying the richest endemism, is the Alps:

- the CE Alps (Western Tauern and Eastern Tauern Alps) (quadrats C15, D13-14), are mainly characterized by sharing the endemic species *Neocrepidodera simplicipes* (Kutschera) and *Phyllotreta zieglerti* Lohse;
- the SE Alps (Julian Alps, Venetian Prealps, Dolomites, Carnic Alps) (E13-16, F11-12, F14), are characterized by the presence of *Aphthona juliana* Springer, *Neocrepidodera obirensis* (Ganglbauer), *Orestia carnica* Leonardi, *O. carniolica* Weise and *Pachybrachis fraudulentus* G. Müller;

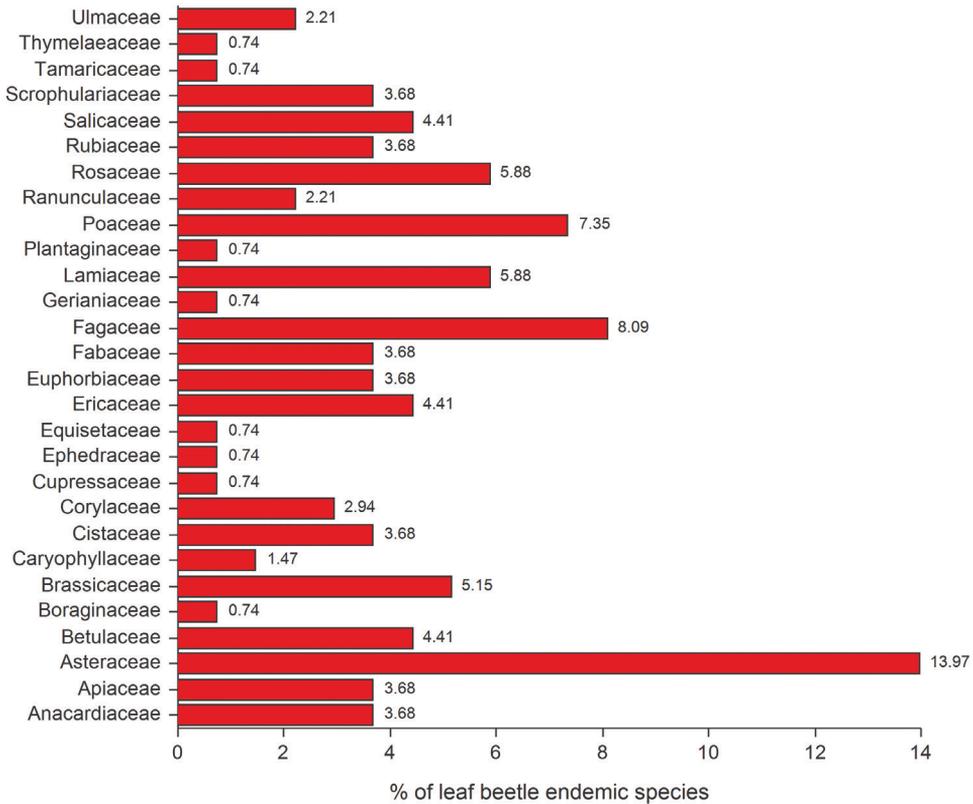


Figure 6. Percentage of endemic species associated with specific plant families.

- the Central Alps (Rhaetian and Bergamasque Alps) (E9, F7-9, G8), are mainly characterized by the presence of *Cryptocephalus barii* Burlini, but also by *Orestia heikertingeri* Leonardi;
- the NW Alps (Pennine, Cottian and Graian Alps) (F5, G4-6, H4, I3-4), are mainly characterized by the presence of *Cryptocephalus tardus* Weise, *Neocrepidodera adelinae* (Binaghi), *N. basalis* (K. Daniel), *N. nobilis* (J. Daniel), *Oreina canavesei* Bontems and *Pachybrachis alpinus* Rapilly, but also by *Orestia heikertingeri*; and
- the SW-Alps (Maritime and Ligurian Alps) (J4-5, K5), are characterized by the presence of *Arima buai* Havelka, *Cryptocephalus atrifrons* Abeille, *Cryptocephalus zoiai* Sassi and *Neocrepidodera ligurica* (J. Daniel).

In the remaining peninsular and insular regions, the following were also detected by the PAE (Fig. 11):

- many strictly local endemic species such as *Cryptocephalus paganensis* Pic, *Longitarsus springeri* Leonardi, *Luperus fiorii* Weise, *Oreina sibylla* (Binaghi), *Orestia*

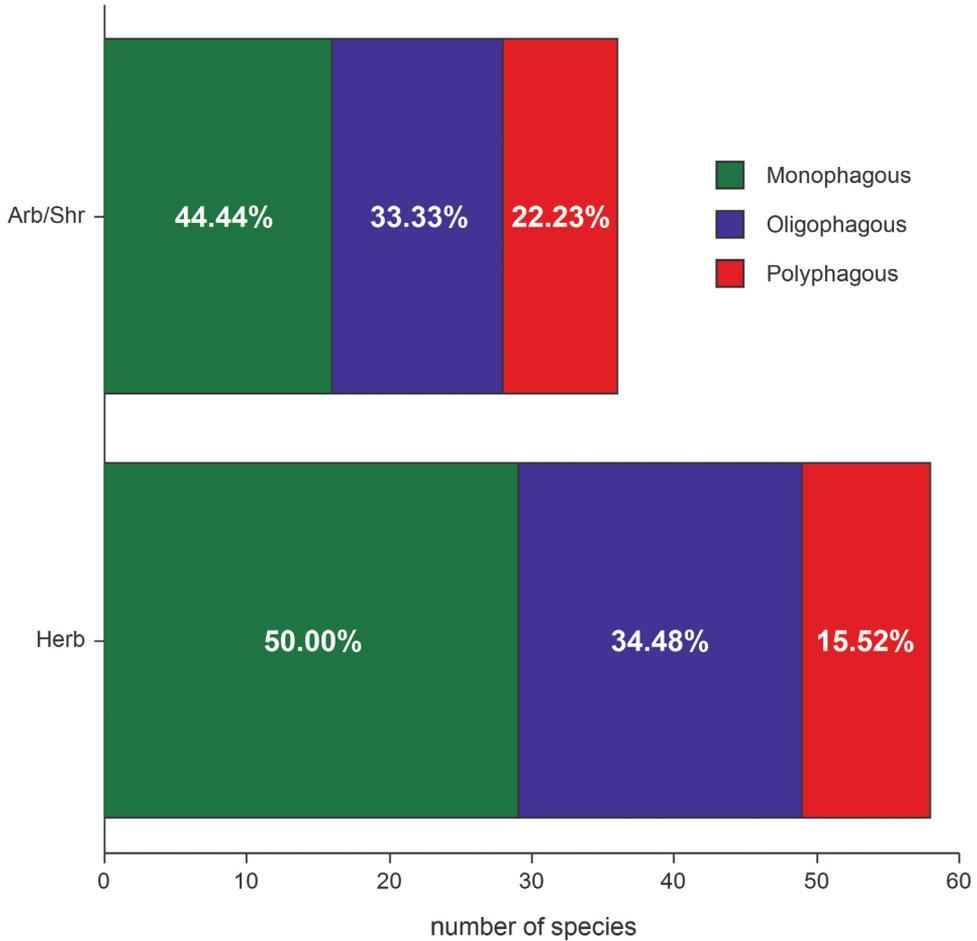


Figure 7. Percentage of endemic species within particular trophic categories.

- *brandstetteri* Kapp, *Psylliodes biondii* Leonardi, *Psylliodes springeri* Leonardi and *Timarcha fracassii* Meier occur in the Central Apennines (N14-16, O15-16);
- the exclusively endemic species *Luperus vitalei* Ragusa, *Calomicrus rottenbergi* Ragusa, *Galeruca nebrodensis* Ragusa and *Gonioctena theae* Baviera occur in Northern Sicily (Madonie and Nebrodi) (X16-17); and
- Western Corsica (N7, O7, P7) is characterized by the presence of *Galeruca corsica* (Joannis), *Labidostomis syriaca* Lacordaire, *Luperus maculicornis* Desbrochers, *Orestia coiffaiti* Doguet and *Timarcha cornuta* Bechyné.

Finally, in addition to the areas of endemism determined by the PAE in Fig. 11 we have also added the regions with restricted endemic species, represented by a single quadrat, namely: the Apuan Alps (J9) with *Timarcha apuana* Daccordi & Ruffo and *Chrysolina osellai* Daccordi & Ruffo; the Giglio Island (N10) with *Pachybrachis sassii*

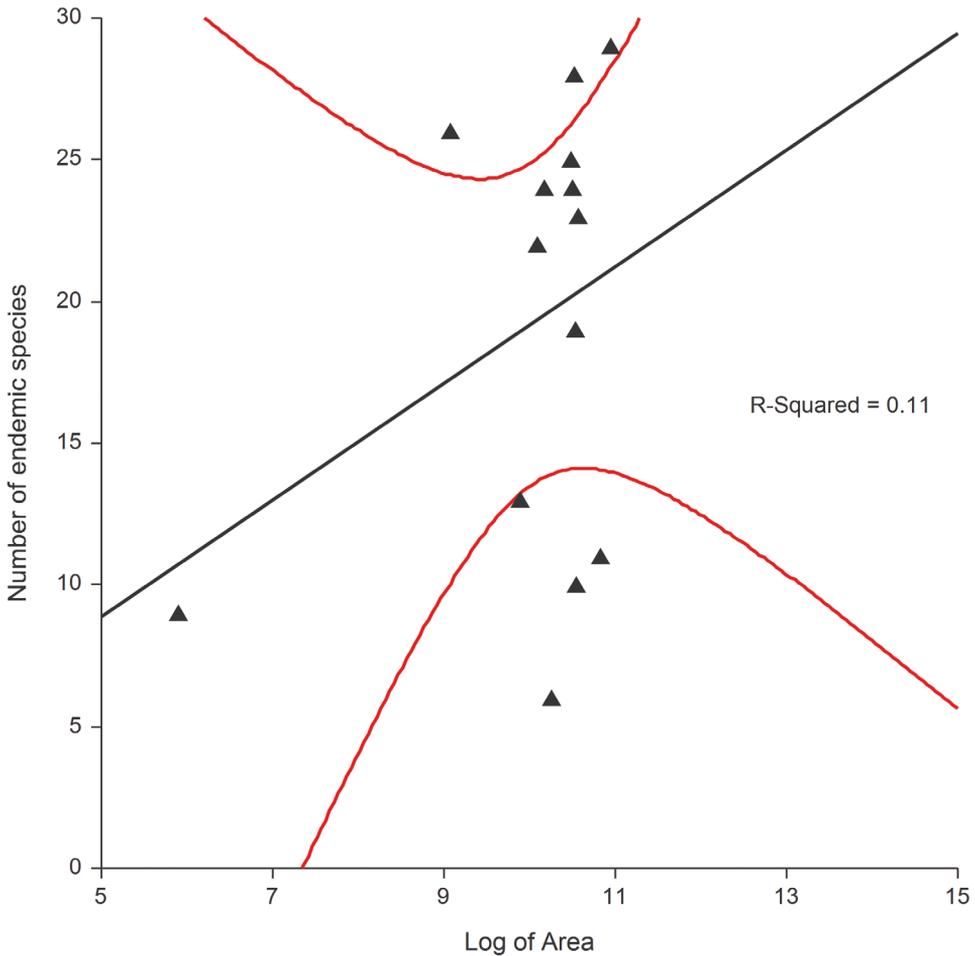


Figure 8. Number of endemic species to logarithm of area relationship.

Montagna; the Pontine Islands (Q14) with *Pachybrachis burlinii* Daccordi & Ruffo; the Aegadian Islands (X13) with *Pachybrachis osellai* Daccordi & Ruffo; and finally Gennargentu (S8), with *Cryptocephalus alnicola* Costa.

Discussion

Chrysomelidae contribute significantly to the Italian endemic fauna, with 123 known endemic species representing 14.82% of the entire leaf beetle fauna for the country.

In the Alps, and particularly in the Apennines, the majority of endemic and sub-endemic species most likely originated as a result of the range shifts caused by the cyclic climatic changes during the Pleistocene glaciations. These climatic changes strongly influenced the recent biogeographic history of the faunas currently occur-

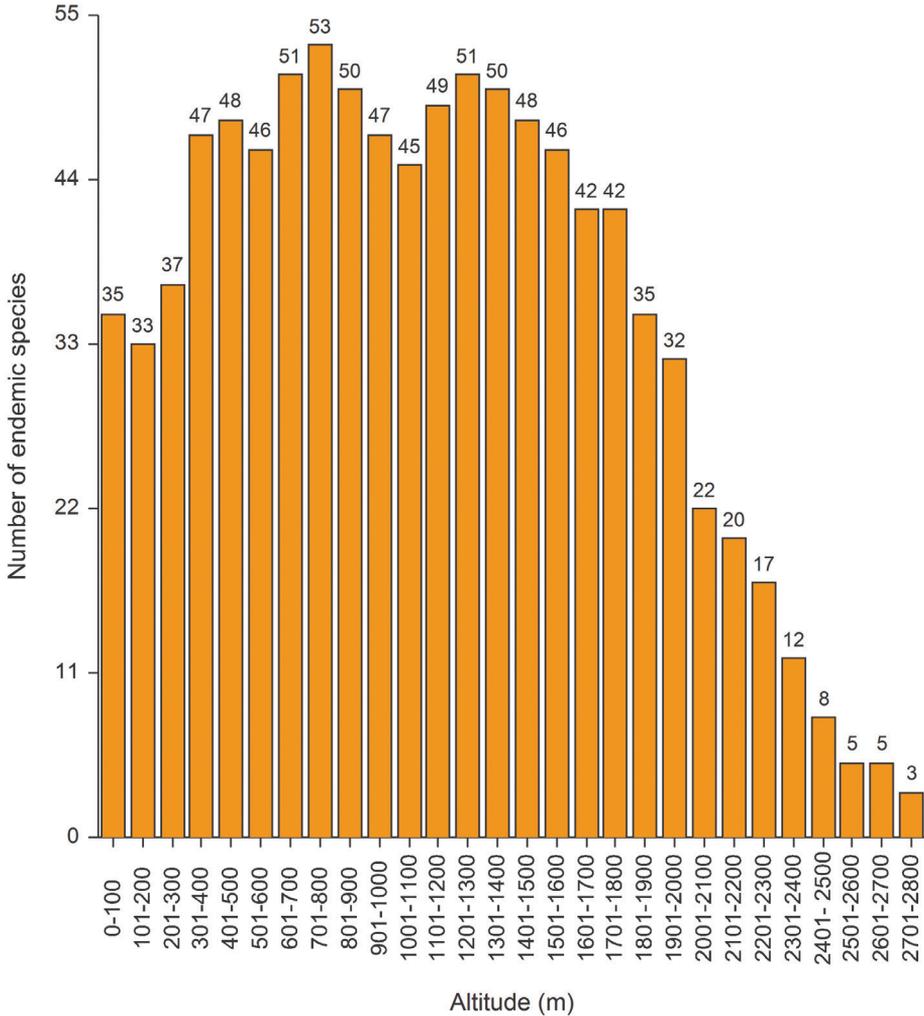


Figure 9. Number of endemic species associated with particular altitudinal intervals.

ring in the high altitude montane systems of Europe (Triponez et al. 2011). Italy, in particular, represented one of the most important ice-age refugia for Mediterranean and Alpine species in Europe during the Pleistocene (Schmitt 2009; Taberlet et al. 1998). In the Alps the largest concentrations of strictly endemic species are in the south-eastern and western sectors, areas that were at lower altitudes and not covered by ice during glaciation (cf. Schmitt 2009). These ice-free areas in some sectors of the Alps, as also in the Central Apennines, most likely served as centres of glacial survival from where these species performed only altitudinal shift, but no major horizontal range expansions to other deglaciating areas. On the other hand, endemic species currently confined only to high alpine habitats in the inner Alps, such as *Neocrepidodera nobilis*, *N. obirensis*, *N. simplicipes*, *N. spectabilis* (J. Daniel), *Oreina peirolerii* (Bassi),

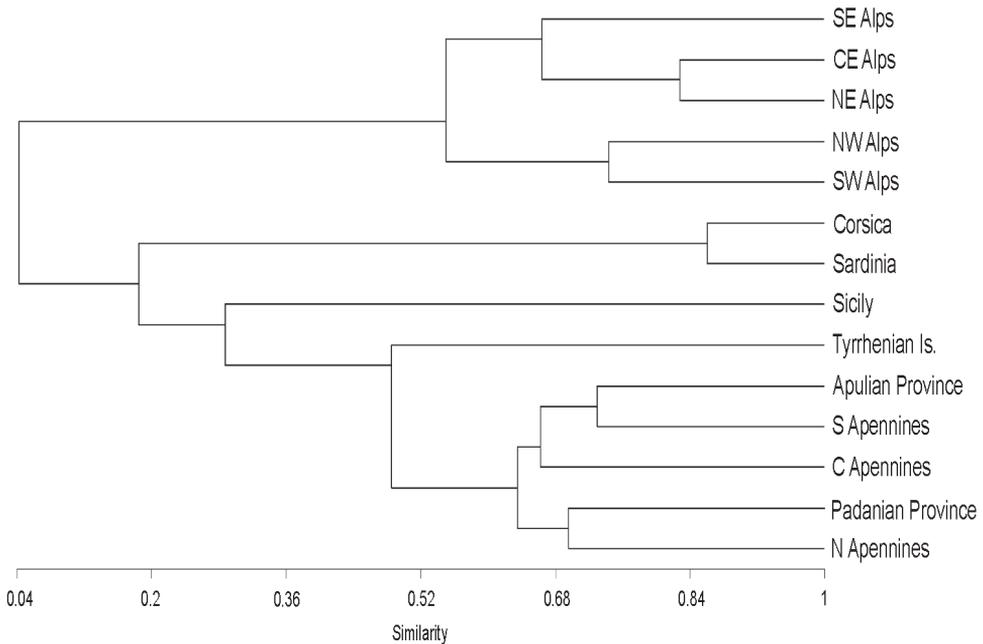


Figure 10. Dendrogram of endemic faunal similarity among the regions studied.

O. melancholica (Heer), and *Cryptocephalus tardus*, may instead have survived *in situ* in the small ice-free areas topping the ice shield, the so-called “nunataks”, during the catathermic periods of the glaciations.

The Apennines have played an important role as a centre for differentiation and currently host 50 endemic species of leaf beetles, 30% of which are shared with the Alps, and 26% with Sicily. Some species, such as *Luperus fiorii*, *Oreina sibylla*, *Psylliodes biondii* and *P. springeri*, are faunistic elements of northern origin that found suitable ecological conditions for their survival at the highest altitudes of montane systems, such as Sibillini, Laga, Gran Sasso and Maiella, during the inter- and post-glacial hypsothermic periods. In other Apennine sectors, during the “climatic optimum” after the last glaciation, the increase in dominance of woody vegetation strongly reduced the presence of high altitude refugia, so causing the definitive disappearance of the cold-adapted species that had found refuge on isolated mountain massifs (cf. D’Alessandro and Biondi 2007; La Greca 2002).

Some endemic species of the Central and Southern Apennines, for example *Chrysolina sirentensis* (Meier), probably had a trans-adriatic origin instead (Bourdonné and Doguet 1991; Daccordi and Ruffo 1979, 1988, 2005; D’Alessandro and Biondi 2007). This distributional pattern is due to the effect of marine transgressions that occurred during the glacial phases, which allowed the formation of terrestrial bridges from the Balkan Peninsula and Central Italy and vice versa, particularly promoting the transit of submontane mesophilous elements (Gridelli 1950).

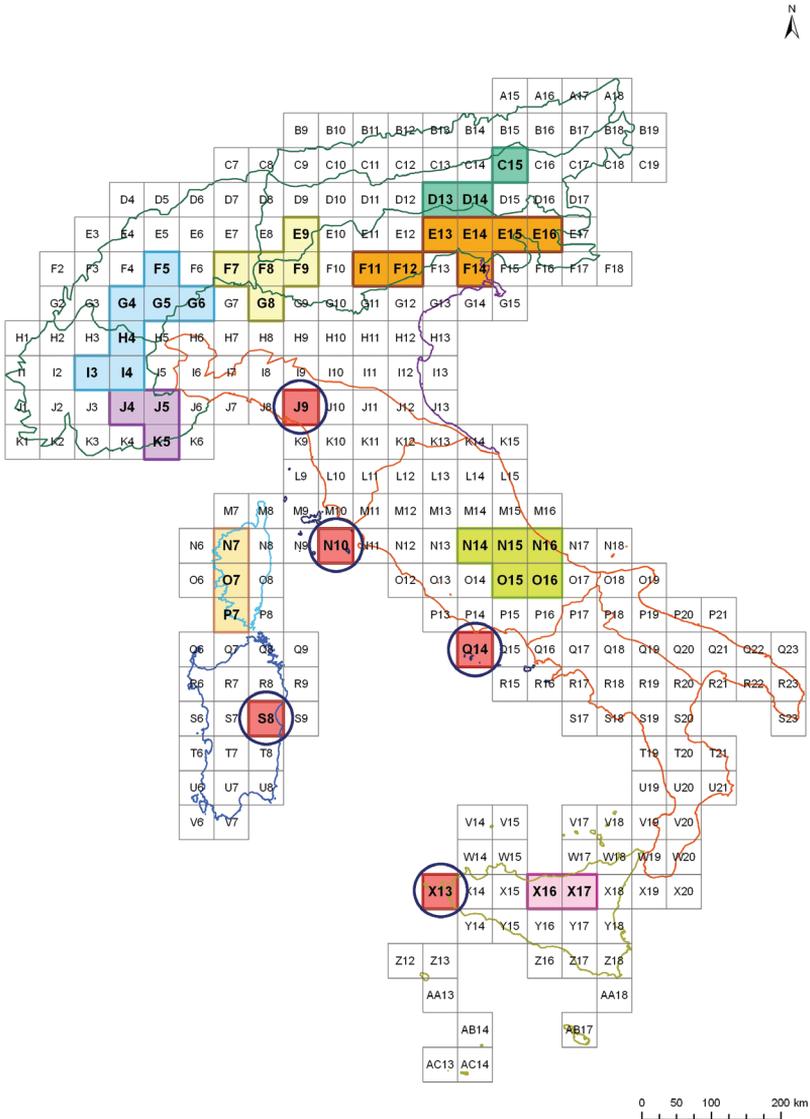


Figure 11. Areas of endemism identified by the parsimony analysis of endemicity (PAE) and single quadrats highlighting strictly local endemisms in red.

Other endemic Apennine species seem to belong to more ancient stock, generally pre-quaternary, that includes both Paleo-Mediterranean and possible Tertiary Alpine elements (Ruffo 1971). Among them are:

- *Longitarsus laureolae* Biondi, occurring in the Southern Apennines and North-Western Sicily. This species is very closely related to the West Mediterranean *L. candidulus* (Foudras), and *L. leonardii* Doguet endemic to the Pyrenees (Biondi 1988b);

- *Chrysolina osellai*, which is endemic to the Apuan Alps and belongs to a Paleo-Mediterranean species group with possible Pyrenean affinities (Daccordi and Ruffo 2005); and
- *Longitarsus springeri*, a species that is taxonomically distinct from the other congeneric European and Mediterranean entities (Leonardi 1975).

Among the species studied, only *Dibolia alpestris* Mohr shows a disjunct Alpine-Central Apennine distribution. Considering the absence of significant diagnostic phenotypic characters between these populations, it is possible to suppose that this species only reached the Central Apennines during the last glaciation.

The results from the PAE also show that the most important determining factor for the individualization of the areas of endemism, both in the Alps and in the Apennines, is the altitude. As reported above, this fact is surely due to historical events, and mainly the Pleistocene glaciations that promoted important differentiation phenomena as a consequence of relictual conditions, but also due to the subsequent role of high altitude montane environments in conserving and supporting animal populations that would otherwise have disappeared at lower altitude, because of human pressure.

Sicily has 14 subendemic species, the equivalent of 62.5% of the entire endemic leaf beetle component. Of these 13 are shared with the Southern Apennines and only one, *Pachybrachis testaceus* Perris which is of probable Paleo-Mediterranean origin, is shared with Sardinia and Corsica. The nine exclusively endemic species include some pre-Quaternary elements, such as *Timarcha sicelidis* Reiche which belongs to an ancient group (Miocene?) and possibly has affinities with *T. cornuta*, endemic to Corsica, and *T. sardea* Villa & Villa, endemic to Sardinia and Corsica (Daccordi and Pistarino 2001). No phylogeographical data are available for *T. sicelidis* occurring in Northern Sicily and the Hyblean area, but its biogeographical history could most likely be analogous to the history of other taxa, such as the tenebrionid beetles *Pimelia grossa* Fabricius and *P. rugulosa* Germar (Stroscio et al. 2011). The current distribution of these two species reflects the extensive geological changes over the Plio-Pleistocene period, that have deeply influenced the origin and distribution of the Sicilian fauna. Most of the exclusively endemic species, such as *Calomicrus rottenbergi*, *Galeruca sicana* (Reiche), *G. nebrodensis*, *Gonioctena theae*, *Lachnaia caprai* Grasso and *Luperus vitalei*, are limited to Northern Sicily. Only *Cryptocephalus plantaris* Suffrian has a limited distribution in southern Sicily and Malta (Sassi and Zoia 2002); its origin can probably be traced back to marine regressions of the Pleistocene, when Malta and the Hyblean region were occasionally in contact (cf. Bonfiglio et al. 2002), or due to a more recent colonization of Sicily from the South.

The Sardinian-Corsican leaf beetle fauna comprises a great number of exclusively endemic species (24), including some pre-Quaternary elements of probable Miocene origin, such as *Timarcha cornuta* and *T. sardea* (Daccordi and Pistarino 2001; Daccordi and Ruffo 1988).

Finally, among the possible Paleo-Mediterranean elements, we find the following: *Aphthona wagneri* Heikertinger, occurring in Corsica and on the small Tyrrhenian Islands

(Tuscany Islands); *Aphthona perrisi* Allard and *Cryptocephalus biondii* Sassi & Regalin occurring in Sardinia, Corsica and on the Tuscany Islands; the above-mentioned *Pachybrachis testaceus*, occurring in Sardinia, Corsica, Sicily and on the circum-Sicilian islands.

In conclusion we can affirm that endemization phenomena in this beetle family seem mainly due to factors as philopatry, trophic specialization, meiopterism and adaptation to high altitudes, often in combination with vicariance and colonization events, which have contributed to create reproductively isolated units in the course of the time (cf. Piper and Compton 2010).

About endemization as consequence for adaptation to high altitudes, it can be due to historical events, mainly the Pleistocenic glaciations, that promoted important differentiation phenomena as consequence of relictuality conditions, but can also be due to the subsequent role of high montane environments in conservation, supporting animal populations that would otherwise have disappeared at lower altitude because of human pressure.

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Tempo and mode of evolutionary radiation in Diabroticina beetles (genera *Acalymma*, *Cerotoma*, and *Diabrotica*)

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Abstract

Adaptive radiation is an aspect of evolutionary biology encompassing microevolution and macroevolution, for explaining the principles of lineage divergence. There are intrinsic as well as extrinsic factors that can be postulated to explain that adaptive radiation has taken place in specific lineages. The Diabroticina beetles are a prominent example of differential diversity that could be examined in detail to explain the diverse paradigms of adaptive radiation. Macroevolutionary analyses must present the differential diversity patterns in a chronological framework. The current study reviews the processes that shaped the differential diversity of some Diabroticina lineages (i.e. genera *Acalymma*, *Cerotoma*, and *Diabrotica*). These diversity patterns and the putative processes that produced them are discussed within a statistically reliable estimate of time. This was achieved by performing phylogenetic and coalescent analyses for 44 species of chrysomelid beetles. The data set encompassed a total of 2,718 nucleotide positions from three mitochondrial and two nuclear loci. Pharmacophagy, host plant coevolution, competitive exclusion, and geomorphological complexity are discussed as putative factors that might have influenced the observed diversity patterns. The coalescent analysis concluded that the main radiation within Diabroticina beetles occurred between middle Oligocene and middle Miocene. Therefore, the radiation observed in these beetles is not recent (i.e. post-Panamanian uplift, 4 Mya). Only a few speciation events in the genus *Diabrotica* might be the result of the Pleistocene climatic oscillations.

Keywords

Coalescence time, Diabroticina, host plants range, macroevolution, pharmacophagy, phylogeny

Introduction

Why does a clade have more species than others within the same lineage? This is a common question in evolutionary biology that has been pondered for almost a century (Simson 1953, Givnish and Sytsma 1997, Schluter 2000, Harmon et al. 2003). The phylogenetic pattern of cladogenesis that often is accompanied by ecological and/or morphological disparity among lineages is known as “evolutionary radiation”. The study of the processes and patterns in evolutionary biology are structured in three hierarchical levels of complexity: population level (microevolution), species level (speciation and biodiversity), and supraspecific level (macroevolution). The latter level usually explores patterns of diversity between and within monophyletic lineages. In phylogenetic analysis, macroevolution is also viewed as the origin of mayor phenotypic characters or character complexes (i.e. key characters) that permit a lineage to undergo an adaptive radiation (Nitecki 1990). In this sense, macroevolution assesses the level of morphological divergence and their presumed adaptive outcome. Macroevolution can be focused on taxon patterns such as lineage richness (differential species diversity), and/or character patterns (Cracraft 1982). Macroevolutionary analyses should present such patterns in a chronological framework; otherwise, they are meaningless for explaining biodiversity scenarios. The optimal way to do so is to explain the pattern in the light of a cladistic hypothesis for the group in question. Consequently, macroevolutionary conclusions must be linked to a phylogenetic hypothesis for three main reasons. First, only monophyletic lineages have evolutionary meaning. Second, cladogenesis is inferred; therefore, biodiversity can be quantified. Third, the origin of key characters can be established; thus, they can be correlated to biodiversity differences.

Nevertheless, there are examples in which lineage radiation is not necessarily correlated with the evolution of phenotypic characters (Farrell and Mitter 1994, Janson et al. 2008, Tilmon 2008, Ramamurthy and Gaur 2012). Observed diversity patterns, such as radiation or large-scale macroevolutionary trends, are the balance between speciation and extinction rates that can be modulated also by extrinsic factors (e.g. environmental complexity). It is also important to consider that speciation is not necessarily an adaptive process (Cracraft 1985). The changes of biodiversity at different dimensions can be the outcome of differential sorting of species. Environmental factors may determine the range of resource use between populations; therefore, affecting reproduction and mortality rates. In fact, extrinsic physical factors, such as geological or climatic history, possibly have a stronger effect in modulating global speciation and extinction rates than the emergence of key features in taxa.

The family Chrysomelidae is the most species rich lineage of Coleoptera with nearly 40,000 described species. All species feed on plants, and most of the species are specialists on a certain host (Jolivet and Hawkeswood 1997). Diabroticina beetles are a neotropical lineage. One species, *Diabrotica virgifera virgifera* LeConte, however, has recently been introduced to Europe where it has quickly become a pest on corn (*Diabrotica virgifera virgifera*) (www.eppo.int). Females lay their eggs in the ground and larvae feed on roots of the host plant, whereas adults feed on leaves and pollen. The

subtribe Diabroticina encompasses 933 species (sensu Seeno and Wilcox 1982) distributed primarily in Mesoamerica and Brazil (Branson and Krysan 1981). It is divided in the sections Diabroticites, Cerotomites, Phyllecthrites and Trachyscelites. Webster (1895) concluded that the center of origin of these beetles was Mesoamerica based on the diversity and species richness. However, the information on the natural history of Diabroticina is scarce, and for many species nonexistent. Most of what we know about these insects has been derived from less than 70 species, most of them belonging to the genera *Acalymma* and *Diabrotica*. All the 72 recognized species of *Acalymma* are specialists on Cucurbitaceae. The *Diabrotica*, the most diverse genus within the subtribe, has been traditionally divided in three groups (i.e. polyphagous fucata with more than 300 species, oligophagous virgifera that encompass 24 species, and signifera with only 11 species). Signifera is endemic to South America, no pest species have been reported, and their biology is almost unknown.

The aim of the present study is to review the processes that shaped the speciation pattern of some lineages encompassed in Diabroticina beetles (i.e. genera *Acalymma*, *Cerotoma*, and *Diabrotica*), and to set those processes within a reliable time framework. To reach this objective we have performed phylogenetic and coalescent analyses based on DNA sequences from mitochondrial and nuclear loci. In a previous study we applied a molecular clock hypothesis on the evolutionary scenario for host-range expansion in Diabroticinas (Eben and Espinosa de los Monteros 2008). For that scenario, however, only a few nodes were dated. Moreover, we used a strict-clock model on trees that became ultrametric after pruning the lineages that did not pass the constant-rate test. The novelty of the present study is that the phylogeny is entirely dated. These dates are inferred based on more reliable coalescent models that can independently handle unlinked molecular partitions, and account for rate-variations among lineages. Furthermore, here we discuss, in addition to host-range interactions, other processes that may affect the speciation rate of these insects.

Materials and methods

Diabroticina beetles and molecular markers

DNA sequences for 44 lineages of chrysomelid beetles were used for this study (Table 1). Most taxa were chosen because they occur sympatrically in Mexico that has been proposed as the putative centre of origin for Diabroticites. Twenty-four species were collected in the field and sequenced by us. The remaining taxa encompassed in the dataset were selected based on DNA sequences availability.

Three mitochondrial-genome regions (i.e. COI, 12S, and 16S) were sequenced to provide the adequate level of variability for reconstructing the phylogeny of this group. To complement the molecular dataset we downloaded supplementary data available in GenBank. This database provided us with two additional nuclear fragments (i.e. 28S and ITS2) and 20 extra Diabroticina species. The concatenated matrix, therefore,

included sequences from five loci (three mitochondrial, and two nuclear), encompassing 44 taxa and 2,718 nucleotide positions. Sequences for the 28S and complementary sequences for the cytochrome oxidase subunit I (COI) gene were taken from studies by Gillespie et al. (2003, 2004). Sequences of the internal transcribed spacer 2 (ITS2) were taken from Clark et al. (2001). The use of alternative sources of molecular data has the inconvenience that taxon sampling differs among authors. As a consequence the full matrix contains missing entries (Table 1). The core ingroup encompassed 34 Diabroticite lineages. As putative outgroups we included five species from the genus *Cerotoma* (Cerotomites), and two species from the genus *Trichobrotica* (Phyllecthrites). To root the phylogenetic hypothesis we employed the sequences published by Swigonova and Kjer (2004) for *Schematiza flavofasciata*. We selected the outgroup species based on the phylogeny presented by Gillespie et al. (2003). The entire species list and the GenBank accession numbers for the molecular markers are provided in Table 1.

DNA extraction, PCR, and sequencing techniques

A small amount of tissue (i.e. 2 or 3 legs) was ground in Chelex 5% (w/v solution) for total genomic DNA extraction following the method suggested by Singer-Sam et al. (1989). Oligonucleotides specifically designed for beetles were used for DNA amplification. PCR assays were conducted in Peltier-effect thermocyclers (ABI GeneAmp PCR system 2400) using the following parameters: one initial cycle at 95° C for 120 s, followed by 30 cycles of 95° C for 20 s, 50° C for 20 s, 72° C for 60 s, with one final cycle at 72° C for 180 s. All PCR reactions were conducted along with positive and negative controls to detect potential false positives due to contamination. Successful amplifications were purified using the UltraClean™ 15 DNA Purification kit (MoBio Laboratories Inc.). Purified PCR products were subjected to cycle sequencing using the ABI Prism BigDye® Terminator v 3.1 Cycle Sequencing Kit, following the protocol suggested in the kit instructions. The excess of *Taq* dideoxy terminators was removed with Centri-Sep spin columns (Princeton Separations) in a variable speed microcentrifuge at 2 500 rpm for 2 min. Final purifications were dried down in a vacuum centrifuge and suspended in 25 µl of the loading solution. Sequencing products were subjected to capillary electrophoresis in the ABI Prism 310 DNA Sequencer (Perkin Elmer). Sequence files were analyzed with the aid of the program SEQUENCHER v 4.0 (Gene Codes Corp., Ann Arbor, MI). Fragments were sequenced on both DNA strands to ensure accurate data collection.

Phylogenetic reconstruction and temporal scenario

The phylogenetic hypothesis was reconstructed using Bayesian inference (BI). We used an Akaike Information Criterion (Alfaro and Huelsenbeck 2006) in jMODELTEST v 2.0.2 (Posada 2008) to select an appropriate model of nucleotide substitution for each

Table 1. Diabroticina specimens used and GenBank accession numbers for the molecular markers.

Taxa	COI	12S rRNA	16S rRNA	28S rRNA	ITS2
<i>Acalymma albidovittatum</i> Baly	AY242447*	AY243713*			
<i>Acalymma bivittatum</i> (Fabricius)	AY242443*	AY243709*			
<i>Acalymma blandulum</i> LeConte	AF278543^	AF278558^			
<i>Acalymma blomorum</i> Munroe & Smith	AY533582	AY533610	AY533637	AY243710*	
<i>Acalymma fairmairei</i> (Fabricius)	AY533583	AY533611	AY533638	AY243708*	
<i>Acalymma innubum</i> (Fabricius)	AY533585	AY533613	AY533640		
<i>Acalymma trivittatum</i> Mannerheim	AY533584	AY533612	AY533639	AY243711*	
<i>Acalymma vittatum</i> (F.)	AY533586	AY533614	AY533641	AY646317*	AF278557^
<i>Amphelasma cavum</i> (Say)	AY533590	AY533618	AY533645		
<i>Amphelasma nigrolineatum</i> Jacoby	AY242488*	AY243754*			
<i>Amphelasma sexlineatum</i> Jacoby	AY242489*	AY243755*			
<i>Cerotoma arcuata</i> Olivier	AY242494*	AY243760*			
<i>Cerotoma atrofasciata</i> Jacoby	AY533587	AY533615	AY533642		
<i>Cerotoma fascialis</i> Erickson	AY646323*				
<i>Cerotoma ruficornis</i> Olivier	AY646322*				
<i>Cerotoma trifurcata</i> (Forster)	AF395803				
<i>Diabrotica adelpha</i> Harold	AF278552^	AY243735*	AF278567^		
<i>Diabrotica amecameca</i> Krysan & Smith	AY533578	AY533606	AY533634		
<i>Diabrotica balteata</i> LeConte	AY533569	AY533597	AY533625	AY243731*	AF278568^
<i>Diabrotica barberi</i> Smith & Lawrence	AF278544^	AF278559			
<i>Diabrotica biannularis</i> Harold	AY242466°	AY243732*			
<i>Diabrotica cristata</i> (Harris)	AY533580	AY533608	AF278560^		
<i>Diabrotica decempunctata</i> Latreille	AY242467°	AY243733*			
<i>Diabrotica dissimilis</i> Jacoby	AY533577	AY533605	AY533633		
<i>Diabrotica lemniscata</i> LeConte	AF278546^	AF278561^			
<i>Diabrotica limitata</i> (Sahlberg)	AY242481°	AY243747*			
<i>Diabrotica longicornis</i> (Say)	AF278547^	AF278562^			
<i>Diabrotica nummularis</i> Harold	AY533568	AY533596	AY533624		
<i>Diabrotica porracea</i> Harold	AY533571	AY533599	AY533627	AY243737*	AF278563^
<i>Diabrotica scutellata</i> Baly	AY533567	AY533595	AY533623		
<i>Diabrotica sexmaculata</i> Baly	AY533566	AY533594	AY533622		
<i>Diabrotica speciosa</i> Germar	AY533579	AY533607	AY533635	AY646319*	AF278569^
<i>Diabrotica tibialis</i> Baly	AY533576	AY533604	AY533632	AY243746*	
<i>Diabrotica undecimpunctata</i> <i>duodecimnotata</i> Harold	AY533572	AY533600	AY533628		
<i>Diabrotica undecimpunctata</i> <i>howardi</i> Barber	AY533573	AY533601	AY533629	AY243738*	AF278570^
<i>Diabrotica undecimpunctata</i> <i>undecimpunctata</i> Barber	AF278556^	AF278571^			

Taxa	COI	12S rRNA	16S rRNA	28S rRNA	ITS2
<i>Diabrotica virgifera virgifera</i> LeConte	AY533575	AY533603	AY533631	AY243734*	AF278564
<i>Diabrotica virgifera zea</i> Krysan & Smith	AY533574	AY533602	AY533630	AF278565	
<i>Diabrotica viridula</i> (Fabricius)	AY533570	AY533598	AY533626	AY243748*	AF278566
<i>Paratriarius curtisii</i> Baly	AY533591	AY533619			
<i>Paratriarius subimpressa</i> Jacoby	AY242461°	AY243727*			
<i>Trichobrotica nymphaea</i> (Jacoby)	AY242440°	AY243706*			
<i>Trichobrotica sexplagiata</i> Jacoby	AY533581	AY533509	AY533636		
<i>Schematiza flavofasciata</i> Guér	AY515035+	AY507265+	EF197976	AY243786*	AY514312+

^: Clark et al. 2001; **bold**: Eben and Espinosa de los Monteros 2008; °: Gillespie et al. 2003; *: Gillespie et al. 2004; +: Swigonova and Kjer 2004.

locus and the concatenated dataset. Table 2 presents the best-fit models selected and the specific parameters that were incorporated as prior information in the BI analyses. These were performed on each molecular marker as well as on the combined dataset using MRBAYES v. 3.2.1 (Ronquist and Huelsenbeck 2003). Two sets of analyses were performed for the combined dataset. The first used a single model for the entire combined loci dataset (the “unpartitioned” analyses) and the second set of analyses employed partition-specific DNA evolution models of each gene. For each dataset, two parallel Markov chain Monte Carlo (MCMC) analyses were executed simultaneously, and each was run for a minimum of 20 million generations, sampling every 1 000 generations. A majority consensus tree was calculated, showing nodes with a posterior probability (*PP*) of 0.5 or more. Bayesian posterior probability values were calculated from the sampled trees remaining after 25% burn-in samples were discarded (Ronquist and Huelsenbeck 2003) to only include trees after the $-\ln L$ scores reached an asymptote. The consensus tree was drawn with FIGTREE v 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>). We used Bayes factors to determine whether applying partition-specific models significantly improved explanation of the data (Nylander et al. 2004).

Times for potential isolation events within the Diabroticina beetles' phylogeny were calculated using the software BEAST v 1.7.5. (Drummond and Rambaut 2007). The trees inferred from the phylogenetic analyses were used to constrain specific monophyletic groups. For dating we used the five data partitions (i.e. 12S, 16S, 28S, COI, and ITS2), each with independent evolutionary models as chosen by jMODELTEST. Substitution rates of the five genes were unlinked to be estimated independently. However, calibrating the tree is difficult and involves making a number of assumptions (Burleigh 2012). Based on both, fossil records and secondary calibrations inferred from isozyme studies, Metcalf (1986) suggested that the splitting event between the genus *Diabrotica* and the genus *Acalymma* occurred approximately 45 million years ago (Mya). As far as we know, this is the most reliable published date for the divergence time within the Diabroticina lineage. We, therefore, used Metcalf's data to estimate nucleotide evolution rates for each molecular marker. Based on the mean genetic dis-

Table 2. Molecular markers best-fit evolutionary model, model parameters, and mean likelihood for trees inferred from Bayesian analyses.

Maker	Model	Nucleotide frequency	Γ	Rate matrix	p-inv	lnL HM
12S rRNA	TPM1uf + Γ	0.380, 0.050, 0.117, 0.453	0.477	1.00, 5.29, 1.76, 1.76, 5.29, 1.00	nr	-2158.86
16S rRNA	TVM + Γ	0.409, 0.161, 0.080, 0.350	0.245	0.94, 4.25, 3.70, 0.83, 4.25, 1.00	nr	-2622.66
28S rRNA	TPM2 + I	0.250, 0.250, 0.250, 0.250	nr	2.50, 9.48, 2.50, 1.00, 9.48, 1.00	0.759	-2001.79
COI	GTR + Γ + I	0.341, 0.133, 0.104, 0.422	0.384	0.80, 6.38, 2.05, 0.92, 17.7, 1.00	0.416	-7899.90
ITS2	TPM1uf + Γ	0.299, 0.180, 0.210, 0.312	0.411	1.00, 3.86, 1.64, 1.64, 3.86, 1.00	nr	-1891.23
Total evidence	GTR + Γ + I	0.313, 0.157, 0.171, 0.359	0.625	1.32, 6.07, 4.10, 1.02, 10.2, 1.00	0.535	-17525.05

lnL HM = harmonic mean of the normal logarithm for the tree likelihood score; nr = not relevant in the best-fit model.

tance between the genera *Diabrotica* and *Acalymma* we decided to set the follow nucleotide substitution rates: 0.00253 my^{-1} for the 12S, 0.0024 my^{-1} for 16S, 0.0006 my^{-1} for 28S, 0.004 my^{-1} for COI, and 0.002 my^{-1} for ITS2. The “relaxed clock (uncorrelated)” model was used with a lognormal distribution of rates. The Markov chain Monte Carlo was run 10 times for 10 million generations, and parameters were sampled every 1000th generations. The program TRACER v 1.5 (Rambaut and Drummond 2007) was used for assessing stationarity of the MCMC, effective sample sizes (ESSs), and posterior intervals spanning the 95% highest posterior density. The single runs were combined with LogCombiner implemented in the BEAST package. Trees were summarized using TREEANOTATOR v 1.6.1, and displayed in FIGTREE v 1.3.1.

Finally, we compared the rate and timing of diversification events among the major lineages of *Diabroticina*. The average time between nodes was used as a straightforward measurement of speciation time. It was obtained directly from the chronogram inferred with BEAST. We also calculated the D and S indexes that describe diversification rate (Good-Avila et al. 2006). The former is based on a pure-birth model for the rate of diversification, whereas the latter assumes a constant rate of speciation but uses the phylogenetic information in the tree (i.e. branch length). Both indexes give the diversification rate in species per million years, and allow for simple comparisons among groups. To assess changes in diversification rates within genera we computed the γ statistic (Pybus and Harvey 2000). Under a constant speciation rate, γ has a standard normal distribution. However, it becomes negative when speciation has occurred more

frequently early in the lineage history (deceleration of diversification rate), or positive when speciation has occurred more frequently toward the present (acceleration of diversification rate). This statistic was computed with the aid of the package APE v3.0-8 (Paradis et al. 2004) written in R language.

Results

Phylogeny

The topologies of the best-scoring trees obtained for the individual partitions were congruent with the concatenated tree, with most nodes having good support (Figure 1). The Bayes factor indicated that the BI tree obtained with the data partitioned by DNA region was more informative ($2\ln = 7.48$), although this difference was not necessarily significant (Kass and Raftery 1995). Phylogenetic relationships of major groupings represented in our study are supported with *PP* values ≥ 0.95 and their phylogenetic relationships were largely consistent with previous phylogenetic studies (Clark et al. 2001, Gillespie et al. 2003, Eben and Espinosa de los Monteros 2004). Likewise, the phylogenetic analyses in MrBayes inferred from the individual markers yielded congruent inter-specific relationships with strong support (not shown, available upon request from the authors), including the same general interrelationships within *Diabrotica*. Our study suggests that the genera *Amphelasma* and *Paratriarius* are paraphyletic, with *P. curtisii* being more closely related to the virgifera group; whereas *A. nigrolineatum* is intermixed with *Diabrotica* species that belong to the fucata group (Figure 1). Two species of *Amphelasma* (i.e. *A. nigrolineatum*, *A. sexlineatum*) in addition to the two species of *Paratriarius* (i.e. *P. curtisii*, *P. subimpresa*) were found in different clades; therefore, these genera apparently are not monophyletic. Those species, nonetheless, are more closely related to the genus *Diabrotica* than to any other genus. From now on, therefore, we will refer to *Diabrotica sensu lato*, including all the species encompassed in the genus *Diabrotica*, the two species of *Paratriarius* and the two species of *Amphelasma*. The third species of *Amphelasma* included in this study (*A. cavum*) was recovered far from the other members of this genus, as the sister taxon of the genus *Acalymma* forming a highly supported clade (*PP* value = 1).

In the strict sense, the proposal for subdividing the *Diabrotica* species into smaller groups (i.e. virgifera and fucata) was not supported in our study (Figure 1). Among the strongly supported relationships for *Diabrotica sensu lato* are the following: a) *Diabrotica sensu lato* was found to be monophyletic (*PP* = 1.0) and sister to the *Amphelasma cavum*-*Acalymma* spp. clade; b) *Diabrotica sensu lato* is split into three distinct monophyletic clades; c) all the species belonging to the so-called virgifera group are encompassed in Clade I in a well supported apical clade (*PP* = 1.0); d) in spite of that, the virgifera group is not supported as monophyletic group, since *P. curtisii* is included within this major clade; e) in Clade I, sister to the virgifera species is a monophyletic clade formed by two fucata beetles (i.e. *D. dissimilis* and *D. sexmaculata*); f) the

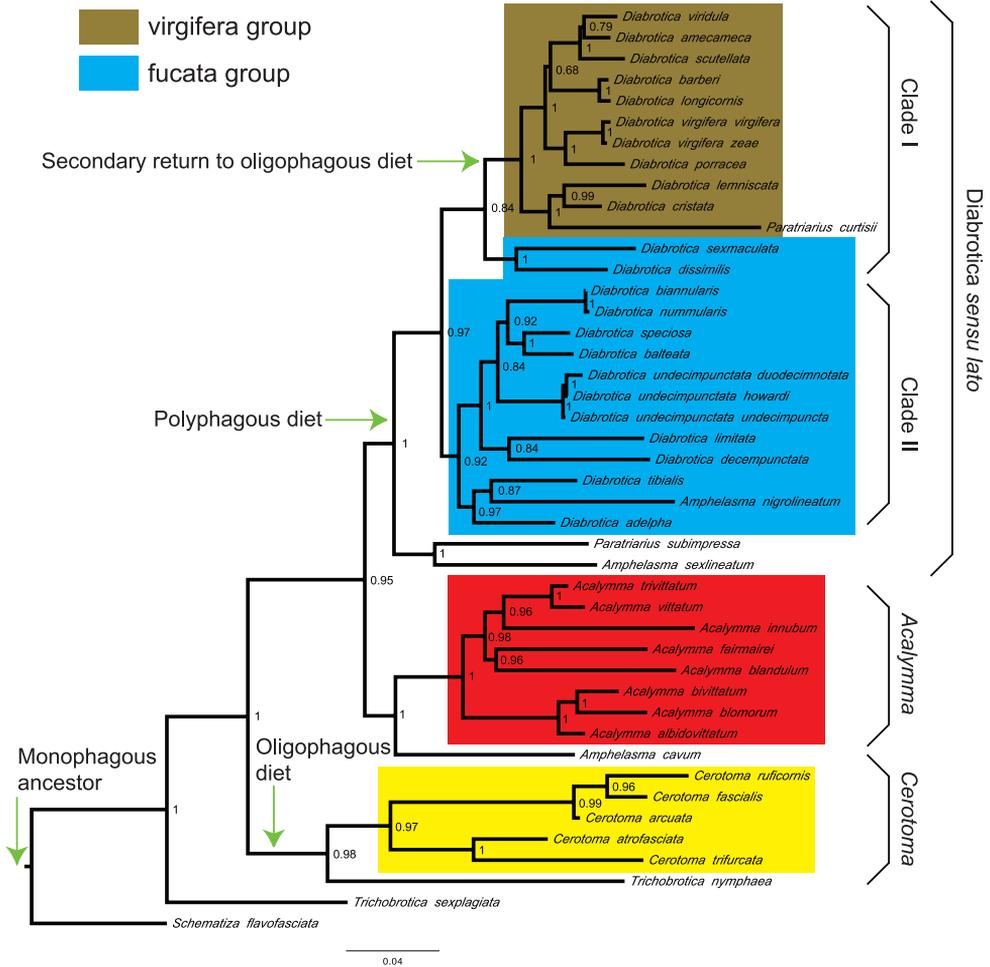


Figure 1. Phylogenetic tree recovered from Bayesian inference showing posterior provability values at the nodes. The genera *Acalymma* and *Cerotoma* are recovered as monophyletic lineages. *Diabrotica*, however, is paraphyletic unless some species of *Amphelasma* and *Paratriarius* are renamed as *Diabrotica*. The general evolutionary scenario for changes in diet spectrum is mapped in the phylogeny.

heterogeneity and instability of Clade I is demonstrated by the low support scored by BI ($PP = 0.84$); g) with moderate support ($PP = 0.92$) Clade II encompasses most of the species usually placed within the so-called fucata group (*D. adelpha* and *D. tibialis*, nonetheless, are found to be more closely related to *A. nigrolineatum* than to the remaining fucata species); h) at the base *Diabrotica sensu lato*, and sister to clades I-II, we recovered a highly supported ($PP = 1.0$) monophyletic clade form by *A. sexlineatum* and *P. subimpressa*. The polyphyly of the fucata group has been acknowledged before. Fucata was created as a convenience group for hosting a large number of highly variable species that did not fit into the virgifera or signifera group. Notwithstanding, the fucata and virgifera group could be easily rescued. Small adjustments, such as renam-

ing *Paratriarius* to *Diabrotica*, and reassigning species from fucata into virgifera, would reconcile the observed phylogenetic pattern with the traditional taxonomy.

The other two genera survey within our analysis showed phylogenetic patterns more consistent with the taxonomic schemes. All the species of *Acalymma* form a strongly supported monophyletic clade ($PP = 1.0$). The *Acalymma* clade is divided in two monophyletic groups: one presents a strong interrelationship between *A. bivittatum*-*A. blomorum*, and *A. albidovittatum*; whereas the other showed the next cladistic structure ((*A. blandulum*, *A. fairmairei*), (*A. trivittatum*, *A. vittatum*), *A. innubum*). The genus *Cerotoma* is also monophyletic ($PP = 0.97$), and forms a sister genus to the Phyllechthrites species *Trichobrotica nymphaea*. This clade, formed by *Cerotoma* spp.-*T. nymphaea*, is the most basal monophyletic group, and is the sister group of the “*Acalymma*-*Diabrotica*” clade (Figure 1).

Divergence time estimates

The comparison of all coalescence analyses revealed high convergence among the inferred parameters, and ESSs were larger than 200 for all of them. Analyses of divergence time estimation using the calibration method resulted in very similar divergence estimates, for both the concatenated (Figure 2, Table 3) and the individual matrices (not shown; but available upon request). Likewise, the BEAST analyses based on the concatenated dataset, using the coalescent method assuming constant population size, exponential growth or logistic growth yielded similar time estimates for the different nodes of major Diabroticina clades. These results can thus be considered as robust (Figure 2, Table 3).

The mean value for divergence times indicated that the split between Cerotomites and the other Diabroticina subtribes occurred at *ca.* 60 Mya (95% confidence limits 55 - 68 Mya; Figure 2, node A). The split of the common ancestor of *Acalymma* and *Diabrotica sensu lato* was dated at *ca.* 45 Mya (95% confidence limits 37–53 Mya; Figure 2, node B). The important radiation events within the genera *Cerotoma*, *Diabrotica*, and *Acalymma*, began almost simultaneously; our estimates place these events at *ca.* 32, 31, and 27 Mya respectively (Figure 2, nodes C, D, and E). Another meaningful evolutionary episode in the history of the genus *Diabrotica* took place around 17 Mya. During that time, a reduction in the diet breadth of Diabroticina took place, resulting in a secondary specialization on host plants, switching from the polyphagous fucata to the oligophagous virgifera group (node F). Several speciation events were dated during the Pleistocene; being the most recent, the divergence between *D. nummularis* and *D. biannularis* that occurred *ca.* 500 000 years in the past (node G).

Based on the coalescent analysis we deduced that the main radiation within Diabroticina beetles occurred between middle Oligocene and middle Miocene (Figure 2). *Cerotoma* shows the slowest radiation rate with an average time between nodes of 13 My (Table 4). *Acalymma* has an intermediate radiation rate. This genus underwent on average one evolutionary splitting event almost every 7 My. Finally, *Diabrotica sensu*

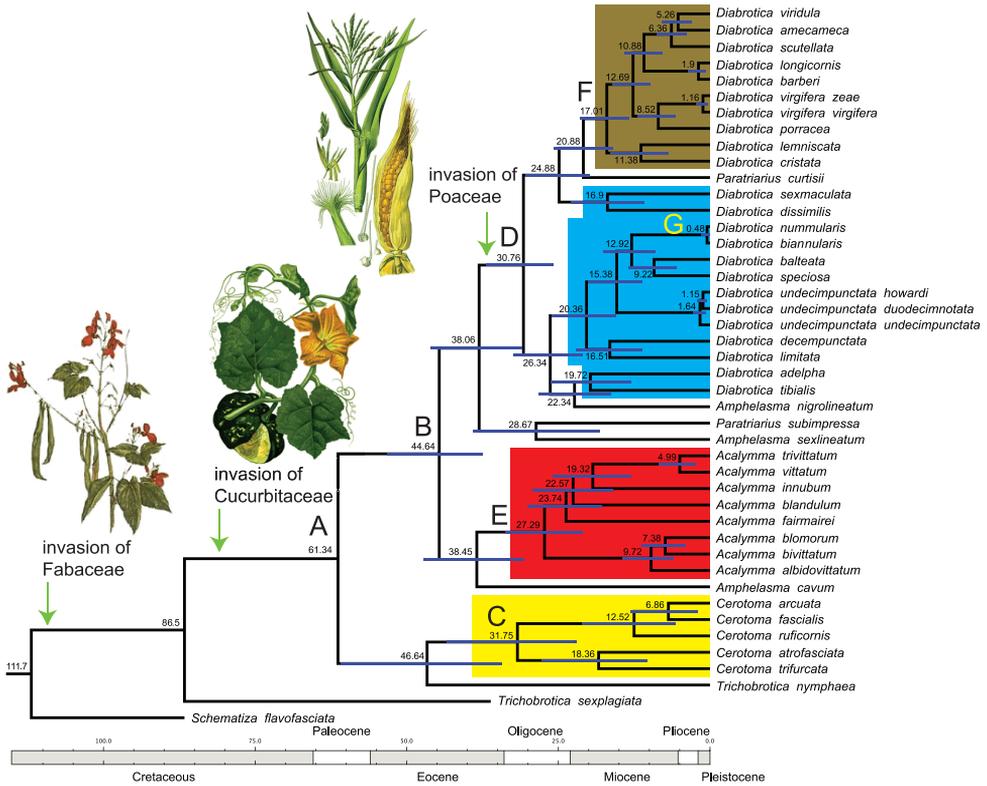


Figure 2. Chronogram inferred from a coalescence analysis. The blue lines at the nodes indicate the 95% confidence range for the estimated split times. Letters A to G pinpoint at key nodes in the evolutionary history of Diabroticina beetles (see Table 3 for further detail). The evolutionary scenario for the acquisition of main plant hosts is presented.

Table 3. Chronology for key events during the evolutionary history of Diabroticina beetles.

Node *	Event	Time Inferred	95% confidence limits
A	Split between Cerotomites / Diabroticites	61.34 Mya	54.92– 67.76 Mya
B	Split between <i>Diabrotica</i> and <i>Acalymma</i>	44.64 Mya	37.41– 53.17 Mya
C	Basal radiation within Cerotomites	31.75 Mya	21.96– 43.34 Mya
D	Basal radiation within <i>Diabrotica</i>	30.76 Mya	25.76– 36.88 Mya
E	Basal radiation within <i>Acalymma</i>	27.29 Mya	21.02– 33.82 Mya
F	Basal radiation within vigifera group	17.01 Mya	13.41– 21.10 Mya
G	most recent speciation event	0.48 Mya	0.01– 1.45 Mya

* as presented in Figure 2

lato is the most specious clade; consequently, this group presents the highest radiation rate. The average time between internodes is 5 My. If we compare the number of lineages through time *Cerotoma* and *Diabrotica sensu lato* show a relatively constant incre-

ment in diversity. *Acalymma* instead, displays a fast increment in diversity between 27 and 19 My in the past, followed by a 10 My stasis period, and then new radiation events during the last 9 My.

Discussion

Diversity patterns change across geography, geological time, and phylogenetic level. Key characters (e.g. flight ability, host specialization, pharmacophagy, etc.) are the central concept of the adaptationist approach that explains how lineages can radiate through those different levels. Species or lineages move into “unoccupied” or new adaptive zones, and thanks to those key characters the lineage goes through a process in which the rate of speciation depends on characteristics of that zone. If we analyze such an adaptationist approach to explain biodiversity based on its theoretical principles we might conclude the following: a) lineages radiate and adapt to different life strategies; b) those strategies are what we call “adaptive zones”; c) the main evolutionary force that mediates speciation (and extinction) rates is Natural Selection; d) this evolutionary force interacts with the fore mentioned key characters, resulting in further lineage adaptation and differentiation. Key characters are usually considered as intrinsic features of the lineage. Nonetheless, speciation rate is regulated by both intrinsic and extrinsic factors. Next we discuss a series of “key features” that may be responsible for modelling the biodiversity patterns observed in Diabroticina beetles.

Evolutionary radiation: Pharmacophagy

Most wild species of Cucurbitaceae contain bitter, toxic secondary compounds known as cucurbitacins. These tetracyclic triterpenoids are synthesized from mevalonic acid. Cucurbitacins are the bitterest natural molecules known and protect the plants against many herbivores (Torkey et al. 2009). Diabroticina beetles, however, have overcome the deterrent effects of cucurbitacins. Moreover, *Diabrotica* spp. are the most sensitive insects to these phagodeterrents known. With the exception of *Amphelasma cavum*, all Diabroticina species studied to date feed compulsively as soon as they get in contact with cucurbit tissue. (Eben et al. 1997, Eben and Espinosa de los Monteros 2008). Once ingested, cucurbitacins are fixed in the beetle's tissues, and may protect the insect against predators and pathogens (Ferguson and Metcalf 1985, Nishida and Fukami 1990, Tallamy et al. 1998, 2000, Gámez-Virués and Eben 2005b). Pharmacophagous lineages, therefore, should have a higher survival and reproduction rate.

To gain access to the compounds, adapted beetles have developed curious behaviours. Morchete (*Cucurbita okeechobeensis* ssp. *martinezii*) leaves frequently show a semicircular cut along their edges. Field observations have demonstrated that a coccinellid beetle (*Epilachna tridecimlineata*) is responsible for this damage. This vein cutting behaviour impedes the coagulation of sticky phloem sap around the insect's mouthparts.

In continuation, the insect starts to feed on the tissues inside the circle. Once the trench is finished *Diabroticina* beetles begin to feed alongside the coccinellid from the tissue inside the semicircle (Eben and Gámez-Virués 2007). Cucurbitacins are such sequestered in the hemolymph, and fixed in the insect tissue including exoskeleton, and gonads (Ferguson and Metcalf 1985). Thus pharmacophagy not only gives protection to the adults, the bitter substances are also transferred to the eggs repelling potential predators (Brust and Barbercheck 1992). The “chemical defense” obtained by pharmacophagy, however, is questionable. Brust and Barbercheck (1992) observed no differences in the predation rate on eggs laid by females of *D. undecimpunctata howardi* fed on bitter and non-bitter cucurbits. The bitter eggs, nonetheless, hatched first. This might give the larvae a head start for finding food sources. Gámez-Virués and Eben (2005a), based on laboratory experiments, report that *Repipta flavicans* Stål (Hemiptera: Reduviidae) preyed on adults of *A. blomorum* regardless of the beetles’ diet. Surprisingly, the cucurbitacins contained in the beetles’ tissue were sequestered by the assassin bug; furthermore, these “bitter” bugs lived longer than the bugs fed on non-bitter insects.

Although the hypothesis of sequestering cucurbitacins for the insect’s protection is very appealing, there is data suggesting that *Diabroticina*s do not receive any fitness benefit from this behavior. On the contrary, some experiments have shown that the metabolic costs are high. For instance, the larvae fed on cucurbitacin containing diet have a lower growth rate than those fed on a cucurbitacin free diet (Ferguson et al. 1985, Hirsh and Barbercheck 1996, Eben and Barbercheck 1996). Detoxification costs, apparently, are correlated with feeding habits and host range. The oligophagous species *D. v. virgifera* had higher costs, than polyphagous taxa (e.g. *D. balteata*, *D. undecimpunctata*), but lower than monophagous species like *A. vittatum* (Ferguson et al. 1985).

So far, the available evidence for an advantage of pharmacophagy is inconsistent. More observations and experiments are essential to shed light on the role of pharmacophagy in the evolutionary fate of these beetles.

Evolutionary radiation: Beetle/host-plant "coevolution"

A lineage that occupies heterogeneous environments (e.g. geographical, ecological, climate, host species) might speciate more rapidly than others that inhabit homogeneous environments. When such is the case, the difference between closely related lineages showing disparate diversities is not due to the expression of key adaptation. It is just the consequence of exploiting environments of different complexity (Cooper and Westneat 2009, Aguilar-Medrano et al. 2011). Therefore, clades that have the ability for using a more diverse environment should have the highest biological diversity. Many species of insects spend their entire live cycle on a single plant. Host plants, therefore, represent the only environment known by those species. Insect/host-plant interactions play a significant role in the long-term evolution of insect lineages, and have exerted reciprocal influences on one another’s diversification (Farrell and Mitter 1994). Detailed co-evolutionary scenarios can be reached after comparing the cladogenetic pat-

terns between interacting lineages. Currently, there are some robust phylogenies for the plants associated with Diabroticina beetles [e.g. Cucurbitaceae (Schaefer et al. 2009), Angiosperms (Magallón and Castillo 2009)]. Unfortunately, the available information of specific hosts used by Diabroticinas is too vague (e.g. beans, corn, etc), and in many cases nonexistent. Therefore, general evolutionary scenarios for diet spectrum and main host invasion are presented in Figures 1 and 2.

Host plant selection depends on the insect's perception of the rate between stimulant and deterrent compounds in the plants. In the genus *Chrysolina*, host changes are preceded by exploring other closely related plant species (Termonia et al. 2001). Similar behaviour are expected in other polyphagous Diabroticinas. Based on molecular phylogenies several authors have inferred evolutionary scenarios for host plant use in Diabroticina beetles (Szalanski et al. 2000, Clark et al. 2001, Eben and Espinosa de los Monteros 2004). These beetles show a wide range of host breadth: from monophagous species (e.g. *Acalymma* spp., *D. scutellata*, *I. teraspilota*, *P. curtisii*) to polyphagous species that may use more than 300 host species from over 50 families (e.g. *D. balteata*, *D. speciosa*). Our scenario (see Eben and Espinosa de los Monteros 2004 for more details) shows that the basal lineages of Diabroticina feed exclusively on one plant family (i.e. Fabaceae); therefore, the ancestral condition is monophagy (Figure 1). Although some lineages have discarded Fabaceae as hosts, the monophagous state was upheld within the Diabroticina lineage, and the oligophagous condition independently evolved twice. Early in the evolution of these beetles Cucurbitaceae was incorporated within their host range, and has been maintained in most species (Figure 2). The genus *Cerotoma* was characterized by a slow increment in the number of hosts, reaching a polyphagous spectrum in some of the apical lineages. Nonetheless, a secondary regression to oligophagy within this genus was inferred. At the base of the *Diabrotica* clade a fast acquisition of hosts was observed. Throughout the evolution of Diabroticina the use of Poaceae species has independently occurred at least three times. Secondary regressions to oligophagy, monophagy, and also polyphagy were observed in the genus *Diabrotica*. On the other hand the least diverse genus *Acalymma* retained the ancestral condition of monophagy. This scenario supports the idea that the Diabroticina beetles are one example of behavioural plasticity; furthermore, it contradicts the generalist to specialist trend commonly assumed to be the result from the evolutionary process (Kelley and Farrell 1998, Janz et al. 2001).

Ferguson et al. (1985) demonstrated that polyphagous species (e.g. *Diabrotica sensu lato*) sequester less cucurbitacin than monophagous lineages (e.g. *Acalymma* spp). Some studies concluded that the optimization of metabolic pathways for the detoxification of cucurbitacins might be the explanation for the secondary monophagy on cucurbits (Andersen and Metcalf 1987, Metcalf and Lampman 1989). Apparently, leaf-beetles show a high ecological plasticity that enables them to switch hosts depending on their availability (Pasteels and Rowell-Rahier 1991). Consequently, the trophic niche that was available after such physiological adaptations could have favored the expansion and subsequent cladogenesis in the New World Diabroticinas.

Evolutionary radiation: Competitive exclusion

One more process that may have favored the rapid radiation within *Diabroticina* is the result of ecological interactions with closely related lineages. Taxa that diversify to a large extent during their evolutionary history may fill available ecological space, pushing less fit individuals towards alternative adaptive zones leading to subsequent ecological diversification within subclades. Several evidences have supported such evolutionary pathways. It has been documented that strong competition occurs among the population members of some genera of lizards. In the absence of other sympatric species such competition, apparently, is responsible for the members of these taxa to experience ecological release and niche shifts (Smith 1981, Sites et al. 1992, Knox et al. 2001).

In Mexico, *Diabroticina* beetles are rarely found feeding on cucurbit leaves (either wild or cultivated; Eben and Barbercheck 1996, Gámez-Virués and Eben 2005b). These insects, however, are abundant inside the male flowers feeding on pollen and possibly waiting for potential mates. Field observations in central Veracruz frequently found more than 20 male insects sitting inside a single flower (Gámez-Virués and Eben 2005b). Similar observations have been reported for other species of *Diabroticina* in South America (Cabrera-Walsh 2005). Although, in Mexico cucurbit plants are present all year long, the number of flowers per plants is small (less than five per day). Flowers, thus, represent a limited resource for *Diabroticina* beetles.

An untested hypothesis states that males may be searching for food sources rich in cucurbitacins, because these secondary compounds are transferred to the females within the spermatophors (Eben 2012). Chemical analysis of pollen from *Cucurbita moschata* listed high concentrations of beta-alanine, asparagine, and alanine; however, the presence of secondary compounds like cucurbitacins has not been registered (Mullin et al. 1994). These authors also reported that the amino acids present in the pollen triggered phagostimulant pathways via chemoreceptor cells. When pollen from different plants was offered to adults of several species of *Acalymma* and *Diabrotica*, most insects showed a significant preference for the pollen of Cucurbitaceae (Eben and Van Loon, unpublished data). An interesting result was that species of *Diabroticina* that have been reported as cucurbit specialists fed on the same amounts of pollen from other plant species like corn. Poaceae has a pollen composition based mainly on proline, alanine, and GABA (Mullin et al. 1994). The preference for certain flowers, therefore, may be mediated by the occurrence of one, or the combination of several amino acids, instead of secondary compounds. It is possible that other factors like scents, form, and color of the flowers are determinant for selecting a specific plant host (Andersen and Metcalf 1987). It is probable, therefore, that those “feeding leks” promote competition pressure and different fitness among the individuals.

Differential selection (natural or sexual) intensities are the causal agent for variable rates of evolution and speciation (Cracraft 1992). However, there is considerable debate about the importance of biotic interactions such as competition in structuring the distribution and abundances of species populations and therefore communities (Mayr 1997). This is a process that has spatial and temporal scales at the level of local

populations and their speciation and extinction rates, and thus in structuring phylogenetic diversity. Without doubt, the need for specific studies centering on the effect of intra-specific competition on morphological, physiological, ecological, or behavioral shifts in insects must be considered a priority.

Evolutionary radiation: Geomorphological complexity

One of the primary determinants of speciation rate is extrinsic, in that it largely interlocks processes external to the lineages that are differentiating. Spatial and long-term temporal variation in geological complexity influences the rate at which populations become isolated and therefore differentiated. Since the evolutionary synthesis, geographic isolation has been regarded as a main factor in promoting taxonomic differentiation within most terrestrial lineages (Mayr 1997).

Mesoamerica, the putative center of origin for Diabroticina beetles, is one of the most complex biogeographical areas in the world (Ferrari et al. 1999, 2000, Morrone 2002). This complexity reflects the confluence of Neotropical and Nearctic ecosystems and a long history of geological activity, stretching from the Late Oligocene to the present (Guzman-Speziale et al. 2005). Throughout this period, movements of the Cocos, North American, Pacific and Caribbean Plates created barriers and land-bridges that have fragmented and merged the distribution, or allow long distance dispersal, of terrestrial populations (Zeh et al. 2003, Ornelas et al. 2013). During the Miocene intensive tectonic processes took place that were responsible for modifying the topographic landscape of Mesoamerica. The Trans Mexican Volcanic Belt and Sierra Madre del Sur were formed at this time (Ferrari 2004). The Pliocene, also, was marked by a number of significant tectonic events. One such event was the joining of the plates of North and South America. This had a significant impact on flora and fauna (Behrensmeyer et al. 1992). Pleistocene climate fluctuations had a deep effect on Middle American populations (Hewitt 2000). The existence of several refuges has been postulated in Mexico (Toledo 1982, Ceballos et al. 2010). Neotropical montane forests experienced extremely complex glacial-interglacial dynamics. The available data describe different scenarios concerning the effect of climatic fluctuations on the genetic structure and population history of species distributed in these habitats (see Ramírez-Barahona and Eguiarte 2013, and references within). Ornelas et al. (2013) documented temporal and spatial genetic divergence of 15 species (including seed plants, birds and rodents), and related them to the evolutionary history of the naturally fragmented cloud forests in Mesoamerica. Their results showed shared phylogeographic breaks that correspond to the Isthmus of Tehuantepec, Los Tuxtlas, and the Chiapas Central Depression. However, the identified barriers are apparently lineage-specific revealing a complexity that seems to be the result of differences among taxa in ecological niche requirements and dispersal capabilities. It is likely that within these habitats there existed multiple successive opportunities for populations to diverge in isolation.

The identification of biogeographic breaks needs to be considered in a temporal framework, which allows comprehension of some of the present day diversity patterns for *Diabroticina* beetles. Temporal consideration in biogeographic analyses has been neglected in historical biogeography (Avice 2000). However, new methods involving mt DNA analysis could lead to an improvement in the identification of historical scenarios.

Tempo of evolution

The use of divergence times has been severely criticized due to the presence of different rates of evolution in different taxonomic groups or even in individual genes (Nabholz et al. 2009). This study shows that it is possible to identify temporal congruence, in spite of the different evolutionary rates and divergence sequence within the taxa. Although many of the individual lineages show a sympatric distribution in the present, the individual divergences represent different evolutionary histories. The heterogeneity in those lineages reflects different responses to the same climatic, geological and ecological events that have modelled the actual configuration of their genetic structure, distributions, and biodiversity (Avice 2000). Unfortunately, for most cases, the description of genetic divergence and biodiversity patterns has been established without a temporal framework (Liebherr 1994, Vandergast et al. 2008). Consequently, the difficulties of setting an adequate temporal frame are limited by a gap in the understanding of the evolutionary history of the groups. The use of fossil data, which provides a better estimate of minimum divergence times (Oaks 2011), is confounded in several taxa given the paucity or absence of a fossil record. Nevertheless, a rough estimation based on each taxon's evolutionary pattern could be useful in the establishment of a temporal framework in the diversity inference of biota (Daza et al. 2010).

While the origin of *Diabroticinas* could be set sometime during the Cretaceous (Figure 2), our data suggest that the diversification of the *Diabroticites* probably started *ca.* 62 Mya. This would be just after the Cretaceous-Paleogene boundary, a harsh climatic period of Earth's history associated with a global biodiversity turnover. The initial radiation process can be attributed to the acquisition of Cucurbitaceae as a new plant host, and consequently to the origin of pharmacophagy. The split and further diversification of the main lineages, however, did not start until the Eocene/Oligocene boundary (*ca.* 34 Mya). Interestingly, this concurs with the inferred radiation date for other non-related lineages [e.g. Neotropical trogons (31 Mya, Moyle 2005), Neotropical parrots (35 Mya, Schweizer et al. 2011), the Microphyla subsection in the genus *Bursera* (30 Mya, De Nova et al. 2012), *Commiphora* the second most specious genera in Burseraceae (from 30 Mya, Becerra et al. 2012, to 33 Mya, De Nova et al. 2012)]. Metcalf and Lampman (1989) proposed the "inter-cropping theory" in order to explain the high diversity in some lineages of *Diabroticites*. This theory is based on the prehispanic agricultural tradition of intermixed cropping with corn, beans, cucurbits and chilies. The ancestral *Diabroticina* invaded those rich spots loaded with potential new hosts. Then, fast switches within the feeding niche led the ancestral species into

different adaptive peaks. So, favoring an explosive speciation process that gave origin to the high species diversity observed in this beetle group.

Such Mesoamerican agricultural practices, however, originated *ca.* 10,000 years ago (Smith 1997). The start of the radiation for the three genera surveyed in this study was estimated between 32 and 27 Mya. More precisely, our data shows that the diversification within *Cerotoma* occurred between 18 and 7 Mya, for *Acalymma* between 24 and 5 Mya, whereas for *Diabrotica* between 22 and 0.5 Mya. These radiation periods are incompatible with an explosive radiation mediated by agricultural techniques. There is no doubt that mixed cropping systems and monocultures have privileged the dispersal of rootworm beetles, and in some cases allowed a species to become an important pest. It is not probable, however, that such a level of speciation took place in a short time. Although species sampling is limited in comparison to the number of extant lineages, the radiation observed in Diabroticina lineages is not recent (i.e. post-Panamanian uplift, 4 Mya). At least five apical nodes indicate recent speciation events in the genus *Diabrotica*. These splits occurred between 1.9 and 0.5 Mya, and might be the result of the Pleistocene climatic oscillation.

As expected, the rate of diversification changed considerably among the mayor lineages of Diabroticina (Table 4). *Cerotoma* was the slowest lineage showing one cladogenetic event every 13.3 My. Whereas, Clade I within the genus *Diabrotica* speciated nearly three times faster (i.e. average splitting time 4.6 My). The rate of diversification inferred in the genus *Cerotoma* is $D = 0.057$ species per My or approximately a third of the rate observed in Clade II in *Diabrotica* ($D = 0.187$ species per My). An increment in the diet spectrum going from the oligophagous species of *Cerotoma*, to the polyphagous species of the fucata group encompassed in the different clades of the genus *Diabrotica* might explain such changes in the diversification rate. Other factors, nonetheless, could also be involved within the complex dynamics of species formation. When the phylogenetic pattern is included a similar scenario is observed. Calculations set the highest rate within Clade I ($S = 0.071$), and the lowest within the *Cerotoma* lineage ($S = 0.034$). Regardless of the fact that the generation time in these insects is significantly smaller than the generation time of many plants, the S values obtained for Diabroticinas are at least one order of magnitude smaller than those observed in some genera of plants that have undergone rapid events of speciation (e.g. *Agave sensu lato*, $S = 0.320$; Good-Avila et al. 2006). Incomplete sampling of taxa, however, can artificially bias the average time between internodes, as well as the diversification rate indexes (D , and S). A useful approach, thus, is to evaluate the timing of speciation within the lineage. For all the main lineages of Diabroticina the γ statistic took negative values (Table 4). This would suggest that the speciation rates have been slowing down toward the present. None of the γ values are smaller than -1.645; therefore, the null hypothesis of constant birth-death process cannot be rejected at the 5% confidence level (one-tailed test, Pybus and Harvey 2000).

The evolutionary history and biodiversity patterns in the Diabroticina beetles is very complex and has been the result not only of recent climatic oscillation, but the combination of several intrinsic and extrinsic factors. Our data support the conclusion that these insects have gone through a series of dispersion and speciation events

Table 4. Comparative rate and timing of speciation among *Diabroticinas*.

Lineage	average time	S ^a	D ^a	γ^b
<i>Cerotoma</i>	13.29 My	0.034	0.057	-0.273
<i>Acalymma</i>	7.62 My	0.046	0.111	-1.252
<i>Diabrotica sensu lato</i>	5.02 My	0.068	0.154	-1.510
Clade I	4.63 My	0.071	0.152	-0.992
Clade II	5.33 My	0.062	0.187	-0.818

a Good-Avila et al. 2006

b Pybus and Harvey 2000

that have been the result of events occurred in Mesoamerica since the Eocene until the present. Unfortunately, we did not obtain samples from species belonging to the South American signifera group. Those samples are essential for understanding the biogeographic and diversification history of the genus *Diabrotica*, and for testing the hypothesis that the invasion of South America is a recent event posterior to the Panamanian uplift. Finally, the species sampling must be increased especially for the species rich South American genera in order to corroborate the ideas presented here.

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