UNIVERSIDADE DE SÃO PAULO
FFCLRP – DEPARTAMENTO DE BIOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ENTOMOLOGIA

Sistemática e Biogeografia de Besouros Curculionídeos (Curculionoidea;
Coleoptera) associados a figueiras (*Ficus*; Moraceae)

Luciano Palmieri Rocha

Tese apresentada à Faculdade de Filosofia, Ciências e
Letras de Ribeirão Preto da USP, como parte das
exigências para a obtenção do título de Doutor em
Ciências, Área: Entomologia.

RIBEIRÃO PRETO – SP

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"A journey of a thousand miles begins with a single step"

- Lao Tzu -

RESUMO

PALMIERI, L. Sistemática e Biogeografia de Besouros Curculionídeos (Curculionoidea; Coleoptera) associados a figueiras (*Ficus*; Moraceae). 2017. Tese de Doutorado. Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, São Paulo.

Um dos mais notáveis exemplos de radiação adaptativa de insetos em classes de plantas é o sistema figueiras - vespas de figo. Embora essa interação tenha sido frequentemente usada como modelo nos estudos de mutualismo e coevolução, outros grupos de insetos relacionados às figueiras têm sido negligenciados. Besouros curculionídeos (Coleoptera: Curculionidae) associados a figueiras representam um desses grupos pouco estudados. Apesar dos relatos escassos na literatura, existem fortes evidências do alto grau de especialização destes besouros às suas plantas hospedeiras. O objetivo geral desta tese foi entender como se deu a diversificação dos curculionídeos sobre as figueiras. Trabalhos anteriores nunca utilizaram uma abordagem filogenética para estudar a sistemática e biogeografia dos curculionídeos de figo e, por isso, este estudo analisa o tempo de diversificação das linhagens destes besouros para reconstruir sua biogeografia histórica. De modo a obter informações das espécies estudadas, foram coletadas 325 amostras de frutos de cerca de 12% do total de espécies de figueiras das regiões Neotropical, Afrotropical e Oriental. Sete coleções entomológicas (AMNH, BMNH, INBIO, MNHN, MZUSP, NMNH, SAMC) foram vistas em busca de espécimes de curculionídeos coletados em figo. Pelo menos 80 espécies de cinco gêneros (*Cetatomus*, *Omophorus*, *Carponinus*, *Curculio* e *Indocurculio*) foram encontradas. A radiação dos curculionídeos de figo ocorreu independentemente pelo menos três vezes ao longo da história dos Curculionidae. O período de diversificação das linhagens de curculionídeos de figo é fortemente congruente com o período de diversificação das linhagens de figueiras durante o fim do Cretáceo/Paleoceno. Acredita-se que fatores como a forte variação no nível dos oceanos e o clima mais quente no passado tiveram grande influência na evolução das espécies. Espera-se que os resultados deste trabalho encorajem estudos futuros sobre a biologia e ecologia dos curculionídeos associados às figueiras e auxiliem no entendimento do papel que os curculionídeos possam ter desempenhado na evolução do sistema *Ficus* - vespas de figo.

Palavras-chave: interação inseto-plantas, evolução, taxonomia, relógio molecular, região Neotropical

ABSTRACT

PALMIERI, L. **Systematics and biogeography of weevils (Curculionoidea; Coleoptera) associated with figs (*Ficus*; Moraceae)**. 2017. Doctoral thesis. Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, São Paulo.

Among the number of examples of broad radiations of insects on plants, the fig – fig wasp system is one of the most remarkable. Although this interaction has frequently been used as a model for studies of mutualism and coevolution, other groups of insects associated with fig trees have received less attention. The weevils (Coleoptera: Curculionidae) associated with figs are one notable example. Being the largest family of animals, weevils achieved great evolutionary success due to their early association with their host plants. Despite few reports in literature, there is strong evidence of the specialization of weevils on figs. The main objective of this thesis was to understand how diversification of Curculionidae took place in fig trees. Previous studies have never addressed the systematics and biogeography of fig weevils under a phylogenetic framework. Therefore, we analyzed the tempo of diversification of Curculionidae lineages that use fig trees as host in order to reconstruct their historical biogeography. To gather information on fig weevils, we collected 325 fruit sets from more than 12% of the total *Ficus* species, from the Neotropical, Afrotropical and Oriental regions. We also examined seven entomological collections (AMNH, BMNH, INBIO, MNHN, MZUSP, NMNH, SAMC) searching for weevil specimens collected on figs. At least 80 weevil species from five genera (*Cetartopus*, *Omophorus*, *Carponinus*, *Curculio*, and *Indocurculio*) were found to be associated with figs. The radiation of curculionids on figs occurred at least three times independently. The tempo of diversification of the crown fig weevils is congruent with the diversification of figs during the Upper-Cretaceous/Lower-Eocene period. We hypothesize that the variation of the sea level and warmer climate in the past had great influence on the evolution of the species. Our results encourage future research on the biology and ecology of these species and will help us to understand the role weevils may have played in the evolution of the fig- fig wasp mutualism.

Keywords: insect-plant interaction, evolution, taxonomy, molecular clock, Neotropical region

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General introduction and theoretical contextualization

1.1. Diversification: why there are so many species of insects and plants

Insects and plants, with about one million described species and 300 thousand described species respectively, comprise the vast majority of terrestrial organisms (Grimaldi & Engel, 2005) and are involved in diverse forms of interactions. Ranging from mutualistic (*e.g.* pollination, seed dispersion; Bronstein et al., 2006) to antagonistic (*e.g.* parasitism, herbivory; Novotny & Basset, 2005), plant-insect interactions have been used as models for many studies that show how these interactions promoted, in a long term evolution, the diversification of many groups of insects (Pellmyr, 1992; Price, 2002). Reciprocal adaptation of plant defenses and insect traits are pointed, for instance, as the main reason of evolutionary success of both plants and herbivorous insects (Ehrlich & Raven, 1964). Some butterflies of Papilionidae family, which includes over 500 species, and its host plants belonging to Aristolochiaceae, with about 400 species, are a well-known example of this evolutionary “arms race” (Fordyce, 2010).

This pattern of diversification is also supported by the fossil record. The great increase in number of insect lineages in the Cretaceous, the same period assumed for the origin of flowering plants (Grimaldi & Engel, 2005), is considered one of the main evidences for the enormous insect-angiosperm co-radiations (Pellmyr, 1992; Farrell, 1998). However, as some lineages of herbivorous beetles are older than their lineages of host plants (Hunt et al., 2007) and the diversity of plants and insects is not correlated in all insect orders (Wiens et al., 2015), insect and angiosperm co-radiation is not seen as an universal explanation for their evolutionary success.

Nevertheless, both ecological and historical evidences helped to establish the current paradigm of speciation of insects as the result of adaptive radiation on new host plants and subsequent reproductive isolation (Percy et al., 2004; Fordyce, 2010). To better understand this paradigm, one should also consider the reverse way in which insect communities affect plant speciation. The influence of insects on the diversification of plants seems to be stronger in plant lineages that are attacked by a larger number of specialist insect species than in lineages attacked by generalist species (Becerra, 2007). In this communities, as specialist

herbivores favor to attack hosts with similar defenses (Becerra, 1997), plants that diversify their defensive strategies may have a larger adaptive value. Plant lineages with more defenses will in turn attract more specialists and, due to a positive feedback, increase the plant diversity (Becerra, 2015).

The number of species on Earth and the factors driving their diversification remains two of the most challenging subjects in biological sciences (Mora et al., 2011; Brown, 2014). As two-thirds of all taxonomists work with vertebrates or plants – which represent less than 12% of the total number of all species – and the remaining one-third works with invertebrates (May, 2010), the first issue is mostly a matter of taxonomic effort. The second issue is related to the existence of too many hypotheses to explain the origins of biodiversity (Brown, 2014), resulting in a lack of consensus about the evolutionary drivers of diversification.

1.2. Weevils: reasons for an explosive radiation

One of the most remarkable groups of herbivorous insects is the weevils (Coleoptera: Polyphaga: Curculionoidea). Comprising 5,800 genera and 62,000 described species, Curculionoidea is indubitably the largest superfamily of animals that can be found in nature (Oberprieler et al., 2007). Indeed, the remarkable species richness of the group can be attributed to Curculionidae, or the true weevils, since this hyperdiverse family contains about 80% of all weevils species (Oberprieler et al., 2007). They occur in all terrestrial ecosystems over the world, and mainly feed upon angiosperms, but also on other plant groups and lichens, eating from living tissues to dead and decaying plant materials (Zimmerman, 1994).

This extraordinary success of weevils elicits many questions. Why are they so diverse? Why can they thrive on most terrestrial habitats? Why can they feed on a wide range of plants? The answers are not simple and possibly involve three main explanations. First, the presence of a well-developed rostrum is the most striking feature of any weevil (even though some weevil lineages have secondarily lost their rostrum) and the fact that most Curculionoidea use it as an excavating ovipositor has been long seen as a key adaptation (LeConte, 1867). The majority of insects do not have an elongated sclerotized ovipositor able to reach more inaccessible parts of the substrate in which they oviposit. The Curculionoidea, however, circumvented this disadvantage by using their elongated rostrum in a way analogous to the parasitic hymenopteran ovipositor (Anderson, 1995).

Having this “reach beyond the reach” enabled the weevils to develop an entirely endophytic larva, which is the second possible explanation for their great success. The weevil larva spends its entire life feeding inside the substrate, where the chances of being preyed,

parasitized or suffering from desiccation are reduced (Anderson, 1995). This characteristic allowed the weevils to have a larva totally specialized in feeding, while dispersal and reproduction could be carried out by the adults, reducing the competition between these life forms and giving the Curculionoidea an adaptive advantage over other Coleoptera groups (Anderson, 1995).

Ultimately, the great evolutionary success of weevils can be attributed to the early association with their host plants, the conifers in the Jurassic and, later on, the angiosperms in the Cretaceous. The first weevils to appear in the fossil record were the Nemonychidae in the late Jurassic, on the same period when conifers were the predominant floral element (Oberprieler et al., 2007). The extant nemonychids are a relictual group and their larvae develop in male conifer strobili, feeding on pollen. Contrary to other basal clades of Phytophaga that also feed on conifers – and which the larvae enter the cones to forage on the pollen sacs – the female nemonychids use their rostrum to open the sporophylls and lay their eggs directly inside the cone (Zimmerman, 1994). This behavior apparently granted to the nemonychids an early advantage compared to others conifer herbivorous (Oberprieler et al., 2007).

The weevil families Anthribidae, Attelabidae, Belidae and Caridae were also in some way or another primitively associated to conifers (Oberprieler et al., 2007). Anthribidae and Attelabidae then developed mycetophagy, Belidae radiated more recently to angiosperms and Caridae kept the association with conifers (Oberprieler et al., 2007). All these families combined represent, however, a little more than 10% of the weevil diversity (approximately 6,800 species from a total of 62,000). It was during the mid-Cretaceous that the last two and most derived families, Brentidae and Curculionidae, appeared in the fossil record. Around 100 million years ago, both groups emerged about the same time flowering plants began to rapidly diversify (Oberprieler et al., 2007). The evolutionary success of all Coleoptera and particularly of the weevils ensued then as a result of a series of key adaptations to the new opening niches triggered by the increasing number of angiosperms species (Farrell, 1998).

1.3. Fig trees: a natural nursery for a huge community of insects

Fig trees (Moraceae: *Ficus* L.) are one of the largest genus of plants. With around 755 described species, they can be found in all tropical regions worldwide (Berg, 1989). The largest diversity of the genus is found in the Indo-Australian region (*ca.* 511 spp.), followed by the Neotropical region (*ca.* 132 spp.) and Afrotropical region (*ca.* 112 spp.) (Berg & Corner, 2005). A unique and distinctive characteristic of the genus is the presence of an

especial type of inflorescence, the syconium or fig. Ecologically functioning as a fruit, the fig is a globular, urn-shaped receptacle that conceals hundreds of male and female flowers on its inner surface, and in which the only way of communication between the flowers and the exterior is an apical ostiole (Verkerke, 1989).

Figs are pollinated exclusively by minute wasps belonging to Agaonidae (Hymenoptera: Chalcidoidea), in one of the most extremes cases of mutualism (Cruaud et al., 2012). The life cycle of the fig-wasp is finely synchronized with the development of the fig (Jansen-González et al., 2012), and it is didactically divided in five stages or phases (Galil & Eisikowitch, 1968). On the pre-floral stage or A phase, the fig is immature and the uniloculate female flowers are still in development. Next, the female stage (B phase) is marked by the anthesis of pistillate, when volatile substances will start to be released to attract the pollinating wasps (Grison-Pigé et al., 2002; Souza et al., 2015). On this phase, wasps carrying pollen reach the fig cavity through the ostiole and then pollinate the pistillate flowers while laying eggs in the ovaries of some of them. While staminate flowers, wasp larvae and fig seeds are developing, the fig is on the interfloral stage or C phase. Subsequently, on the male stage (D phase), both staminate flowers and wasp offspring are fully developed at the same time. Male wasps are the first to emerge from their galls (*i.e.* ovary flowers that were oviposited). Male pollinating fig wasps are wingless and have their activities restricted to the fig cavity. They use their telescopic abdomen to copulate with females still inside their galls. Shortly afterwards, the female wasps emerge from their galls and, loaded with pollen (some species actively storing pollen in their thoracic “pockets”, Galil & Eisikowitch, 1969; Ramírez, 1969), exit the fig by a hole made by the male wasps and fly in search for receptive figs. Finally, on the post-floral stage (E phase), the figs ripen and become attractive to frugivorous vertebrates which will consume them and disperse their seeds (Shanahan et al., 2001).

Other insects also exploit this complex mutualistic relationship between figs and fig-wasps. The non-pollinating-fig-wasps (Chalcidoidea: Eurytomidae, Pteromalidae and Torymidae) use the syconium to reproduce without pollinating the flowers (Weiblen, 2002). Although they adopted diverse feeding strategies (Compton, 1993; Elias et al., 2012), these wasps generally have a long ovipositor that can be inserted from outside the fig through its wall, allowing them to compete with the pollinating wasps for female flowers (Cook & Rasplus, 2003). The intricate relationship between fig trees, pollinating wasps and non-pollinating-wasps have been largely scrutinized, showing that the syconium has a long history of diversification with an extremely diverse and specialized community of wasps (Kerdelhué et al., 2000; Jousset et al., 2003; Weiblen, 2004; Cook & Segar, 2010; Cruaud et al., 2011).

1.4. Other insects on figs: a neglected fauna

Much attention has been drawn to the mutualism between fig and fig-wasps and its interrelations with non-pollinating fig wasps (Weiblen 2002). Other non-wasp insects and their relationship with fig trees, however, have been neglected by researchers. Several other species related to figs have been described in relatively few studies, generally in a preliminary form. Their impact on the fig-fig wasp mutualism is poorly known. Flies (Lachaise et al., 1982; Compton & Disney, 1991), moths (Sugiura & Yamazaki, 2004), bugs (Slater, 1972) and beetles (Paarmann et al., 2001; Frank & Nadel, 2012) can be listed among them.

One group of beetles in particular, the weevils, has representatives specialized in figs. The genus *Curculio* (Curculioninae), for example – which the largest radiation was on *Castanea* (Fagaceae) and *Corylus* (Betulaceae) on Palearctic region and innumerable species of *Quercus* (Fagaceae) on Nearctic region – has a second route of radiation on Afrotropical *Ficus* (Perrin, 1992). On that region, 35 species were reported as being exclusively associated with figs (Perrin, 1998; Rasplus et al., 2003). In addition, two species of *Omophorus* (Molytinae) are associated with Afrotropical and Oriental fig trees (Compton, 1993; Wang et al., 2011) while 17 species of *Ceratopus* (Curculioninae) are associated with Neotropical fig trees (Lima, 1956a; Pakaluk & Carlow, 1994).

Despite the few reports, there is indeed a strong evidence of an association between weevils and fig trees. However, data are limited and scattered on the literature, and the biology and evolution of this group of weevils remain undescribed. Studying the evolution of figs and fig-weevils will encourage future studies on the biology and ecology of this insect-plant association and will also help to understand the implications the weevils may have had on the origin and maintenance of fig-wasp-fig mutualism. Ultimately, understanding the processes that affect the diversification of insects is crucial to understand the origins of their diversity and maybe also their fates in the face of global change.

1.5. Thesis structure and objectives

The main objective of this thesis is to understand how the diversification of Curculionidae took place in fig trees. I aim to reconstruct the historical biogeography of Curculionidae beetles associated with *Ficus* at some regions of its distribution (Neotropical, Afrotropical and Oriental) addressing the following questions: (1) which species of Curculionidae are associated with *Ficus* and what is their level of specialization? (2) What

was the scenario of adaptive radiation of fig-weevils? (3) Do the biogeographic connections of weevils, wasps and fig trees form a pattern?

This thesis is divided in five sections. Here I presented a general introduction contextualizing all the subjects related to this work. In the second section, I will discuss the evolution of Curculionoidea and its tempo of diversification in order to better understand the phylogenetic position of fig-weevils. The findings will also be compared with the current scenarios of diversification of fig trees and fig-wasps to check for shared patterns. The taxonomy of Neotropical *Ceratopus* will be reviewed at the third section, while the phylogenetic relationships and historical biogeography of the genus will be reconstructed at the forth section. In the fifth section, the radiations of the Afrotropical and Oriental Curculionini on *Ficus* will be discussed. Finally, on the last section, I will make final considerations and discuss possible implications of fig-weevils on the fig-fig-wasp mutualism.

Evolutionary history of weevils and their adaptive radiation on fig trees

2.1. Introduction

Insects and flowering plants are unquestionably the most diverse group of terrestrial organisms (Grimaldi & Engel, 2005). They interact mostly by pollination and herbivory, which in a long term evolution promoted the diversification of many groups of insects (Mitter et al., 1988; Pellmyr, 1992). Recent studies show that some host plant lineages are older than their insect-associated lineages (Percy et al., 2004; Mckenna et al., 2009; Fordyce, 2010), reinforcing the current paradigm that speciation of insects are the result of adaptive radiation on new host plants and subsequent reproductive isolation (Farrell, 1998; Percy et al., 2004; Fordyce, 2010).

One of the most remarkable group of herbivorous insects are the weevils (Coleoptera: Polyphaga: Curculionoidea). Comprising about 5,800 genera and 62,000 described species, Curculionoidea is indubitably the largest superfamily of animals (Oberprieler et al., 2007). Among other factors such as the presence of a well-developed rostrum and an entirely endophytic larva, their early association with their host plants is one of the reasons of the great evolutionary success of weevils (Anderson, 1995).

One of these associations that have been neglected by researchers is the relationship between a particular group of weevils and fig trees. Although the fig-wasps (Agaonidae; Chalcidoidea; Hymenoptera) and fig trees (*Ficus*; Moraceae) system (Cook & Rasplus, 2003) has been repeatedly used as models to study mutualism and coevolution (Weiblen, 2002; Cook & Rasplus, 2003; Cook & Segar, 2010; Cruaud et al., 2011, 2012), insects as flies (Lachaise et al., 1982; Compton & Disney, 1991), moths (Sugiura & Yamazaki, 2004), bugs (Slater, 1972) and beetles (Paarmann et al., 2001; Frank & Nadel, 2012) are examples of fig-associated-insects described in relatively few studies, generally in a preliminary form.

Despite being few, all reports show strong evidences of an association between weevils and fig trees. The cosmopolitan genus *Curculio* (Curculioninae), for example – which the largest radiation was on *Castanea* (Fagaceae) and *Corylus* (Betulaceae) on Palearctic and innumerable species of *Quercus* (Fagaceae) on Nearctic – has a second route of radiation on

used only when the association between the weevil and its host fig tree could be determined indubitably. In total, we have gathered information of 316 fig samples from 90 species of fig trees (> 10% of the approximately 755 *Ficus* species, Appendix 2) from five of the six subgenera of *Ficus* (Berg & Corner, 2005).

The DNA sequences from each individual weevil were obtained using a nondestructive method (specimen kept as voucher): first, the samples were incubated overnight in a proteinase K solution at 56 °C and then, the extraction took place using the Qiagen® DNeasy kit following the manufacture's protocol. All vouchers used for DNA sampling are in possession of the author.

Due to the inexistence of previous studies using molecular data of fig-weevils and also because there was not enough time to develop our own set of primers, the strategy used to reconstruct the phylogenetic relationships of the fig-weevils was based on information available in literature. The positioning of the fig-weevils species inside the Curculionidae family is uncertain and thus, to have means for comparison, we choose the same set of genes and methodology of published studies on other Curculionoidea (Hughes & Vogler, 2004; Mckenna et al., 2009; Jordal et al., 2011; Toju et al., 2013; Gunter et al., 2015).

Accordingly, we combined the sequences from six genes: two mitochondrial protein-coding genes, Cytochrome b (Cytb) and Cytochrome c oxidase subunit I (COI); the large subunit 28S of ribosomal rDNA (28S); two nuclear protein-coding genes Arginine kinase (ArgK) and Elongation factor 1- α (EF1- α). We also included public sequences downloaded from GenBank of the small subunit 18S of ribosomal rDNA (18S). PCR was performed in a 25 μ l of reactional volume containing 4 μ l of genomic DNA, 14.375 μ l of ultra-pure water, 2.5 μ l 10x Qiagen® Dream Taq green buffer, 1 μ l of supplementary MgCl₂ (25 mM), 0.125 μ l of each dNTP (2.5 mM), 1.25 μ l of each primer (10 μ M, except for the COI cocktail, for which we used the PCR conditions described in Germain *et al.* 2013; Appendix 3), and 0.125 μ l of 5 units Qiagen® Dream Taq DNA Polymerase (0.625 unit).

The standard PCR reaction started with a 3 min step at 94 °C for releasing the polymerase, followed by 35-40 cycles of 30 sec at 94 °C for denaturation, 30-45 sec of annealing (see Table 1 for details), 1 min at 72 °C for elongation and a final extension of 10 min at 72 °C. The PCR products for each gene were sequenced by Eurofins®.

Initially all overlapping sequences were assembled and cleaned using the software Geneious® 8.1.8 (www.geneious.com, Kearse et al., 2012) and the resulting sequences were subsequently queried at BLAST® (<http://blast.ncbi.nlm.nih.gov>) for possible paralogous copies or DNA contamination. Afterwards all sequences were aligned by MAFFT v7.221 (Kato & Standley, 2013) using the G-INS-i option for accuracy; the alignments were then

checked by eye and any mistakes were manually edited. Finally the alignments of the four coding genes (rDNA excluded) were translated to amino acids using Mega v6.06 (Tamura et al., 2013) to detect premature stop codons. The large intron unalignable region of Elongation factor and the gap rich regions of 28S were removed from the alignments because no homology could be established among the bases of these regions, what may lead to inaccuracy on the taxon placement. All new sequences obtained in this study will be deposited in GenBank.

2.2.2. *Phylogenetic analyses and divergence time estimate*

In order to determine the phylogenetic relationships of the fig-weevils among Curculionoidea we analyzed a data set combining (1) only the most complete sequences from all fig-weevils collected (14 spp.), (2) public sequences from GenBank for all Curculionoidea families *sensu* Oberprieler (2007, 2014) (168 spp.; Mckenna *et al.*, 2009; Jordal *et al.*, 2011; Gunter *et al.*, 2015; Appendix 4) and (3) public sequences from GenBank of five species of Chrysomelidae and one Cerambycidae beetle used as outgroup. Our final matrix is composed of 188 terminals. The data set was split into four partitions, one with mitochondrial genes Cytb and COI, another one containing the rDNA (18S + 28S) and two other partitions for the nuclear genes. The best nucleotide-substitution model of each partition was determined by the Akaike information criterion (Akaike, 1973) using MrAIC v1.4.6 (Nylander, 2004) together with PhyML v3.0 (Guindon et al., 2010). The phylogenetic analyses were carried out with RAxML v8.2.8 (Stamatakis, 2014) using the maximum likelihood optimality criterion to build the trees (default setup adopting a GTRCAT mixed/partitioned model and 1000 bootstrapping). We also performed a phylogenetic analysis in a Bayesian framework on MrBayes v3.2.6 (Ronquist et al., 2012), using the same partition scheme as in the RAxML analyses. We set up the default analysis with variable rates across partitions parameters and used default priors to initiate the run. The Metropolis coupled Markov chain Monte Carlo (MCMCMC) searches were conducted with four chains, one cold and three progressively heated. The temperature of the heated chains was set on 0.02 to allow the swap frequencies from 20% to 70%, improving the mix of the chains. The searches ran for 200 million generations (sampling all the values every 20,000 generations) with the first 70 million generations (35%) discarded as burn-in.

The times of divergence among Curculionoidea were estimated using the Bayesian relaxed molecular clock method in two parallel runs on BEAST 2 v2.4.1 (Drummond et al., 2006; Bouckaert et al., 2014). Before setting up the analysis on BEAST, we estimated the number of relaxed clock models best applied to our data employing ClockstaR (Duchêne et

al., 2014). Substitution models were unlinked among partitions with the trees and clocks models linked; we also specified a Yule process of speciation with a random starting tree prior. Because many of the oldest curculionid fossils can be misidentified (Oberprieler et al., 2007) and estimations of the ages of the groups can largely diverge (Gunter et al., 2015; Legalov, 2015), in our analysis the most conservative reliable calibration possible was applied as the maximum bounds and the most phylogenetic-near-accurate groups as the minimum bounds (as suggested by Phillips 2015). The geological ages follow the International Chronostratigraphic Chart (Cohen et al., 2013). We used the earliest known ancestor of modern Coleoptera from the early-Permian (Artinskian-Kungurian Stages, 290.1 to 283.5 Ma), belonging to the extinct suborder Protocoleoptera Crowson (Beckemeyer & Engel, 2008; Kukalová-Peck & Beutel, 2012; Kirejtshuk et al., 2013), to constrain the maximum bounds of all our calibration nodes, setting it to 286 Ma which is the oldest estimate for Coleopterida (Hess et al., 2016). Uniform age calibrations priors for the minimum bounds were assigned to seven nodes (Table 2.1). The first phylogenetic-accurate calibration was taken for the Brentidae family –*Axelrodiellus raptus* Zherikhin & Gratshev 2004 from lower Cretaceous (Aptian) – set the minimum age as 113 Ma, same age used to calibrate the stem node of Curculionidae based on *Arariperhinus monnei* Santos, Mermudes & Fonseca 2011 from the same deposit. For the clade composed by the subfamilies Entiminae and Cyclominae we used *Dorotheus guidensis* Kuschel 1959 (Maastrichtian) to set the minimum prior age of 66 Ma. The stem group node for the family Dhyrophthorinae was based on *Sipalinus* Marshall 1943 (Lutetian-Bartonian) and given the minimum age of 37.8 Ma, also used as the age to constrain the Scolytinae family based on a preserved specimen on amber of contemporary *Hylastes* Erichson 1836. Lastly, we calibrated the crown node of Platypodinae at 28.1 Ma using two preserved specimens on amber from a younger deposit, the contemporary *Tesserocerus* Saunders 1837 and *Cenocephalus* Chapuis, 1865 (upper Rupelian). We also applied a prior enforcing the monophyly of Dhyrophthorinae, Entiminae/Cyclominae and Scolytinae to reduce the time of convergence of the Markov chains. The MCMCMC setup for the divergence time estimate analysis was the same as the one used on MrBayes, except that we ran the analysis for 300 million generations with the first 60 million generations (20%) discarded as burn-in. We used Tracer 1.6 (Rambaut *et al.* 2014) to verify the convergence and stationarity of the chains, evaluating the effective sample size (ESS) scores and the stability of results after multiple runs. The log files from the two independent runs were combined using LogCombiner v2.4.1 and the maximum clade credibility tree with mean heights was built using TreeAnnotator 2.4.1. The final consensus tree was obtained from 24,000 trees and the

chronogram was visualized and edited using FigTree v1.4.2. All analyses were conducted on a 150 core Linux Cluster at CBGP, Montferrier-sur-lez, France.

Table 2.1. Fossil calibrations applied as constraints.

Family/Subfamily	Taxon	Deposit	Age (Ma)	Uniform prior used	References
Platypodinae	<i>Cenocephalus</i>	Dominican amber	33.9–28.1	286–28.1	Bright & Poinar, 1994
Platypodinae	<i>Tesserocerus</i>	Dominican amber	33.9–28.1	286–28.1	Bright & Poinar, 1994
Scolytinae	<i>Hylastes</i>	Baltic amber	47.8–37.8	286–37–8	Gunter <i>et al.</i> , 2015
stem Dryophthorinae	<i>Sipalinus</i>	Baltic amber	47.8–37.8	286–37–8	Gunter <i>et al.</i> , 2015
Cyclominae/Entiminae	<i>Dorotheus guidensis</i>	Maastrichtian	72.1–66.0	286–66.0	Kuschel G., 1959
stem Curculionidae	<i>Arariperhinus monnei</i>	Santana	125.0–113.0	286–113.0	Santos <i>et al.</i> , 2011
Brentidae	<i>Axelrodiellus ruptus</i>	Santana	125.0–113.0	286–113.0	Zherikhin & Gratshev, 2004

2.3. Results

2.3.1. Host association, species occurrence and DNA sequence data

We were able to rear five genera of fig-weevils from syconia of *Ficus* species collected (Appendix 2). *Ceratopus* remained the only genus of weevil associated with fig trees in the Neotropics, for which we gathered data from more than a thousand individuals belonging to 27 species. Although this association was already described (Lima, 1956; Pakaluk & Carlow, 1994), the number of species found here is far superior to the 17 described species in the last checklist for the genus (O’Brien & Wibmer, 1982; Wibmer & O’Brien, 1986). Our analyses confirm that *Ceratopus* is exclusively Neotropical, occurring from Mexico to southern parts of Brazil (Section 4).

The genus *Omophorus* (Metatygini; Molytinae) appears to have only two species associated with African fig trees. Both *O. indispositus* Boheman 1845 and *O. stomachosus* Boheman 1835 were found feeding on different species of figs in several localities. The remaining four species of *Omophorus* found were related to Oriental figs. This is a small

genus with only six species described but with a wide range of distribution, extending from South Africa to the northern islands of the Philippines (Williams, 1932).

The other three genera of fig-weevils belong to the Curculionini tribe: *Curculio*, *Carponinus* Heller 1925 and *Indocurculio* Pajni, Singh & Gandhi 1994. We found at least 50 species of *Curculio* occurring on Afrotropical figs. We added 15 associations between *Curculio* and figs to the 35 already described (Perrin, 1992; Rasplus et al., 2003).

Curculio, *Carponinus* and *Indocurculio* occur in the figs in the Oriental region. As most of the larvae took more than 60 days to develop, we obtained only a few adult individuals. We collected 12 Curculionini morphospecies, eight of which were already described and associated with Philippine figs by Williams (Williams, 1932; Pelsue, 2009) and other four being apparently new to science. The Curculionini fig-weevils are overspread on Afrotropical and Oriental fig trees (Section 5).

In total, we extracted DNA from 41 species and 122 specimens of fig-weevils. The amplifications were successful for at least two genes in 87% of the samples. The average completeness of our final matrix is 58%. If only the mitochondrial and rDNA genes are taken into account, this value increases to 74%; on the other hand, if only the nuclear genes are considered, the value decreases to 31%. The final alignment was represented by 8619 bp for the fig-weevils and all Curculionoidea families. The individual length of each gene region was 498 bp for Cytb; 1510 bp for COI; 2142 bp for 18S (GenBank sequences only); 2634 bp for 28S; 1034 bp for EF1- α ; and 801 bp for ArgK. The evolution model selection by MrAIC was GTR + I + Γ for all partitions.

2.3.2. *Curculionoidea* phylogeny

Both Maximum Likelihood and Bayesian inference estimated the same topology for the Curculionoidea phylogeny (Figure 2.1), although Maximum Likelihood analysis presented stronger support for the nodes. Overall the same groups as those of previous phylogenetic reconstructions of Curculionoidea (Mckenna et al., 2009; Haran et al., 2013; Gillett et al., 2014; Gunter et al., 2015) were found, though the positioning and node support of some clades varied consistently. Curculionoidea was recovered as monophyletic and divided in seven strong supported family clades: (1) Anthribidae; (2) Nemonychidae; (3) Belidae; (4) Attelabidae; (5) Caridae; (6) Brentidae; and (7) Curculionidae *sensu lato* (Oberprieler, 2014). The relationships between the some clades (*i.e.*, Anthribidae, Nemonychidae, Attelabide and Belidae) are not strongly supported and their positioning is uncertain. Caridae was recovered as sister group of the clade formed by Brentidae + Curculionidae, and Brentidae was recovered as sister group of Curculionidae. The formation of those clades is consistent with

Figure 2.1. cont.

Curculioninae and Molytinae were not recovered as monophyletic and their status as subfamilies remains uncertain. We also retrieved Brachycerinae as sister group of all remaining Curculionidae, though with an extremely low support. Bagoinae grouped with Brachycerinae outside the broad nose species in all our analysis, but also with low branch support.

2.3.3. *Radiation of fig-weevils and divergence time estimate*

The radiation of weevils on figs occurred inside the major clade of Curculionidae at least three independent times (Figure 2.1). The most ancient radiation occurred within Curculionini, which overspread on Afrotropical and Oriental *Ficus* during Upper Cretaceous (around 100-70 Ma) and then shift to temperate Fagales during the Eocene (around 50 Ma, see Section 5 for details). The other two radiations occurred around the same epoch during the Eocene (also around 50 Ma). One of these events happened in the Metatygini tribe, with *Omophorus* colonizing figs over Afrotropical and Oriental regions. The other one occurred within the ancestral lineages of Ceratopodini colonizing the Neotropical figs.

Our divergence time estimate for Curculionoidea lineages were considerably older than most of the previous estimates for the group (Table 2.2), agreeing with previous data only in the age of Scolytinae subfamily (Mckenna et al., 2009; Jordal et al., 2011; Gunter et al., 2015). Although we used a combined set of sequences of previous studies, we applied a more relaxed age constrain to the maximum boundaries in our analysis, which brought the origin of Curculionoidea to early Triassic. However, considering the uncertainty of the molecular clock, our results were congruent with the ages inferred by Toussaint et al. (2016) for Curculionoidea and Curculionidae, which recovered the origin of those groups at 226 and 160 Ma respectively, and the age inferred by Misof et al. (2014) for Curculionidae, which estimated the origin of the family as approximately 180 Ma.

2.4. Discussion

2.4.1. *Curculionoidea phylogeny*

We combined sequences of three independent multi-gene phylogenetic reconstructions of Curculionoidea (Mckenna et al., 2009; Jordal et al., 2011; Gunter et al., 2015), adding to these sequences information about the fig-weevils that we collected. Our objectives were to check the phylogenetic affinity of the fig-weevils and to verify which groups reflect a more natural classification within Curculionoidea. Despite the incompleteness of our sequence matrix and the non-overlap of all gene regions among the studies used to construct our data

set, our analyses were very congruent with the results of previous studies, either on morphological (Kuschel, 1995; Marvaldi & Morrone, 2000) and modern NGS mitogenomes (Haran et al., 2013; Gillett et al., 2014).

Among the seven well-supported family clades composing Curculionoidea, Anthribidae and Nemonychidae appear as paraphyletic families in Mckenna et al. (2009). Our results, however, found both families as well-supported monophyletic groups. Gunter et al. (2015) show Anthribidae as monophyletic and also support Urodontinae as the sister group of the remaining Anthribidae. The results of Gillett et al. (2014) and Gunter et al. (2015) suggest that the antribids may represent the most ancestral lineage of Curculionoidea; our

Table 2.2. Distribution of mean age estimates inferred by BEAST for Curculionoidea, showing the 95% upper and lower Highest Posterior Distribution limits between parentheses. The asterisk shows nodes constrained by fossils.

Group/ Taxon	Median age (max - min HPD)
Curculionoidea	247.2 Ma (358.5 - 160.2)
Stem Anthribidae (Urodontinae included)	230.6 Ma (331.4 - 153.0)
Crown Anthribidae (Urodontinae included)	169.1 Ma (258.2 - 96.9)
Stem Attelabidae	201.5 Ma (294.5 - 128.6)
Crown Attelabidae	159.2 Ma (239.4 - 92.9)
Stem Nemonychidae/Belidae	173.8 Ma (257.3 - 106.3)
Crown Nemonychidae	59.8 Ma (114.1 - 18.6)
Crown Belidae	132.8 Ma (199.4 - 79.0)
Stem Caridae	213.2 Ma (305.4 - 141.8)
Crown Caridae	38.8 Ma (74.5 - 10.4)
Stem Brentidae/Curculionidae <i>sensu lato</i> *	198.8 Ma (285.9 - 133.9)
Crown Brentidae	152.6 Ma (216.7 - 113.0)
Crown Curculionidae <i>sensu lato</i>	182.0 Ma (270.7 - 126.6)
Crown Dryophthorinae*	168.7 Ma (242.7 - 111.7)
Crown Platypodinae*	143.8 Ma (220.9 - 100.3)
Crown Entiminae + Cyclominae*	111.3 Ma (164.7 - 66.7)
Crown Conoderinae	100.7 Ma (150.0 - 62.9)
Crown Cossoninae	92.7 Ma (147.4 - 65.6)
Crown Scolytinae*	87.2 Ma (132.0 - 50.3)
Crown Cryptorhynchinae	80.1 Ma (163.1 - 74.6)
Crown Lixinae	72.9 Ma (115.9 - 35.6)
Crown Ceutorhynchinae	72.4 Ma (138.7 - 53.5)
Stem Curculionini	98.5 Ma (147.6 - 57.9)
Crown Curculionini	67.0 Ma (101.0 - 39.0)
Stem Metatygini	97.0 Ma (151.9 - 52.0)
Crown Metatygini	48.1 Ma (83.1 - 20.4)
Stem Ceratopodini	88.0 Ma (134.9 - 52.5)
Crown Ceratopodini	48.2 Ma (75.1 - 25.5)

analysis remain inconclusive regarding this observation. We recovered Nemonychidae as sister group of Belidae for the first time. The strong likelihood support of Nemonychidae/Belidae clade, however, may reflect the small number of taxa of nemonychids in our analysis, given that we only included in Nemonychidae two species of Cimberidinae. Other studies that included nemonychids and belids did not recovered this association (Marvaldi et al., 2002; Mckenna et al., 2009).

We also recovered a monophyletic Attelabidae as all previous phylogenetic studies that included the family (Marvaldi et al., 2002; Mckenna et al., 2009; Haran et al., 2013; Gillett et al., 2014), failing, however, to recover the traditional placement of the family as sister-group of Caridae + Brentidae + Curculionidae. Gunter et al. (2015) found a Attelabidae + Caridae clade as sister of Brentidae + Curculionidae mostly due to the clustering of *Car Blackburn* (Caridae, Carinae) and *Metopum* Agassiz (Attelabidae, Rhynchitinae), although the support for this phylogenetic scheme was weak.

Our results show that Belidae form a monophyletic group, which was also found in other studies (Kuschel, 1995; Marvaldi & Morrone, 2000; Mckenna et al., 2009; Gunter et al., 2015). The sequences for Belinae extracted from Gunter et al. (2015), however, did not clustered with the ones extracted from McKenna et al. (2009), which causes our results to remain inconclusive about the relationships of Belidae subfamilies.

The small Caridae family was also recovered as monophyletic and sister group of Brentidae + Curculionidae with strong branch support. This result is congruent with most morphological works (Kuschel, 1995; Marvaldi & Morrone, 2000; Marvaldi et al., 2002) and also with the molecular analysis of McKenna et al. (2009). The only exception is Gunter et al. (2015) that found Caridae clustered with Attelabidae. Contrastingly, in Kuschel (1995) Caridae is nested in Brentidae and in Oberprieler et al. (2007) Caridae is placed as sister-group of the “higher” weevils.

Brentidae is recovered as monophyletic and sister-group of Curculionidae *sensu lato* in all phylogenetic reconstructions of weevils including ours. Within the family we found a monophyletic and well-supported Brentinae but still inconclusive results regarding the other subfamilies. Other studies that included a reasonable number of Brentidae sequences (Mckenna et al., 2009; Gunter et al., 2015) also recovered Brentinae as monophyletic, although only weakly supported in McKenna et al. (2009).

A broad definition of Curculionidae (Oberprieler et al., 2007; Oberprieler, 2014) is well supported and recovered as monophyletic, as it was found in all phylogenetic reconstructions we used for comparisons (Kuschel, 1995; Marvaldi & Morrone, 2000; Marvaldi et al., 2002; Mckenna et al., 2009; Jordal et al., 2011; Haran et al., 2013; Gillett et

al., 2014; Gunter et al., 2015). Most molecular studies agree in separating Curculionidae in two main groups: (1) a basal grade with pedotectal male genitalia including Brachycerinae, Dryophthorinae and Platypodinae and (2) a major clade formed by the Curculionidae *sensu stricto* comprising two other clades with pedal male genitalia ((Entiminae + Cyclominae + Hyperini + Gonipterini) (Ceutorhynchinae + Conoderinae + Cossoninae + Cryptorhynchinae + Lixinae + Scolytinae + Curculioninae + Molytinae)). The only exception to this configuration is Bagoini, which has a pedal type of genitalia but cluster within the pedotectal grade (Mckenna et al., 2009; Gillett et al., 2014; Gunter et al., 2015; our results). Although both Brachycerinae and Bagoini are always associated with the basal lineages of Curculionidae in molecular studies, their affinity with Dryophthorinae and Platypodinae and their phylogenetic positioning as sister of the remaining Curculionidae must be regarded as uncertain.

We recovered with strong support Platypodinae nested in Dryophthorinae and our results are compatible with the comprehensive mitogenome analysis of Gillett et al. (2014). Other previous studies using molecular data, instead, recovered Platypodinae as sister-group of Dryophthorinae (Mckenna et al., 2009; Haran et al., 2013; Gunter et al., 2015). If morphological data is added to the analyses, however, Platypodinae clusters within the pedal genitalia clade as sister of Scolytinae (Kuschel, 1995; Marvaldi & Morrone, 2000; Marvaldi et al., 2002; Jordal et al., 2011), which probably is a homoplasy resulted from the reduced structures on the cephalic capsule on both subfamilies.

Our results were also consistent with previous analyses regarding the two clades of species with pedal type of male genitalia (excluding Bagoini). The broad-nose weevils Entiminae and Cyclominae, and the tribes Hyperini and Gonipterini form a monophyletic group in all molecular reconstructions (Mckenna et al., 2009; Haran et al., 2013; Gillett et al., 2014; Gunter et al., 2015). Remarkably, all the studies, including ours, found extreme low support of the inner nodes of this clade, thus precluding further discussion on the relationship between the nodes. Haran et al. (2013) is the only exception due to the recovery of well-supported internal nodes of Entiminae/Cyclominae clade and the establishment of the monophyly of larval ectophagy; their work, however, only used eight terminals for this clade.

The second monophyletic lineage of Curculionidae *sensu stricto* contains the remaining subfamilies of weevils Conoderinae, Cossoninae, Curculioninae, Molytinae, and Scolytinae. The clade composed by these subfamilies appears in all molecular reconstructions, either the ones using traditional SANGER methods (Mckenna et al., 2009; Jordal et al., 2011; Gunter et al., 2015, this study) or the ones using modern NGS technology (Haran et al., 2013; Gillett et al., 2014). None of these studies resolved the relationships between the internal

nodes of this clade – even the comprehensive mitogenome analysis elucidated the lower level nodes of Curculionidae (Gillet et al. 2014). In fact, the arrangement of higher level subfamilies of Curculionidae in natural groups is one of the largest problems in modern taxonomy (Crowson, 1955; Oberprieler et al., 2007; Franz & Engel, 2010). Gunter et al. (2015) state that the difficulty in resolving relationships within Curculionidae *sensu stricto* relies on insufficient taxon sampling rather than on the rapid radiation of these clades. If we imagine, however, a research group trying to resolve the classification of Curculionidae through the analysis of a massive dataset including 10,000 terminals (of over 48,000 species), they would barely reach 20% of the total diversity of the family independent of the method. Our study can be used as another example: although we made a considerable compilation of a large number of sequences available to the present (188 spp.), it only represents a very small proportion (less than 0.5%) of the diversity of Curculionoidea. Thus, taxon sampling has been and may continue to be the main reason for lack of resolution for a long time. We agree with the suggestion of Franz and Engel (2010) that researchers working on the taxonomy of Curculionoidea should focus on small generic and tribal units. Once the phylogenetic relationships at this level are satisfactory, research should advance to the next level and so forth, as if we were adding bricks to an immense wall.

2.4.2. *Timing Curculionoidea diversification: treading among uncertainty*

We found a older origin of Curculionoidea than previous studies dating the *tempo* of diversification of the group (Mckenna et al., 2009; Gunter et al., 2015). While we recovered a Midle-Triassic origin (247 Ma) for the superfamily, other studies indicate a Midle/Upper-Jurassic origin (167-155 Ma), making our estimation about 80 Ma older. For Curculionidae, our estimation put their crown diversification in the Lower Jurassic (180 Ma), while the current origin of the family is presumed to have occurred in Lower-Cretaceous (136-124 Ma). As we compiled molecular sequences of previous studies, it was surprinsing to found this discrepant age in our study. Our results, however, are congruent with other studies broaching the divergence time estimates of Coleoptera and insect lineages: Toussaint et al. (2016) recovered the origin of Curculionoidea and Curculionidae at 226 and 160 Ma respectively while Misof et al. (2014) inferred approximately 180 Ma for the origin of Curculionidae (divergence age of *Dendroctonus ponderosae* Hopkins, the single curculionid used in their analysis).

We attribute the discrepancies among our results and the results of McKenna et al. (2009) and Gunter et al. (2015) to methodological differences regarding the molecular clock

setup. To calibrate the minimum bounds of our analysis, we used the same Mesozoic and Cenozoic fossils reviewed by Gunter et al. (2015). However, our analysis differed at the calibration of the maximum bound in which we applied the most ancient and reliable fossil of Coleopterida, following the recommendations of Phillips (2015). Another disparity between our divergence time estimates and McKenna (2009) and Gunter et al. (2015) lies on the type of prior distributions; while they used a lognormal distribution and we applied a uniform distribution for all constrained nodes. This setup certainly reduced the influence of constraints on the calculation of node ages, allowing a larger variation. Both methodological variations in maximum bound and prior distribution applied here were already pointed as responsible for affecting the results of divergence time analyses (Sanders & Lee, 2007; Ho & Phillips, 2009).

An older origin of Curculionoidea would imply that their association with flowering plants might not represent the major factor acting in their diversification as previously thought (McKenna et al., 2009). In fact, some lineages of phytophagous beetles, other than weevils, have been recovered as older than their lineages of host plants (Toussaint et al., 2016). In this case, other factors might be proven to have equal impact on weevil diversification. The association with bacteriocyte symbionts (Toju et al., 2013), which helps some species to digest nutrient-poor substrates, or efficient aggregation pheromones (Ambrogi et al., 2009), which trigger social behavior in some species that increases their chances of reproduction, should be also investigated under a phylogenetic framework. In a megadiverse group such as Curculionoidea, with more than 62,000 species overspread worldwide, the odds of having a single major factor to explain their enormous diversification are very low.

On the other hand, even if a Triassic origin of Curculionoidea is true, the influence of their association with Angiosperms can still not be ruled out as one of the main factors influencing the diversification of the group. As a matter of fact, the association of weevils and fig trees itself demonstrates how host association with flowering plants drives the evolution of the associated species (Section 4 and Section 5). Unfortunately, the lack of resolution on higher phylogenetic level preclude us to extrapolate our results and to hypothesize about the factors responsible for the incredible evolutionary success of Curculionoidea. Any attempt of doing so would incur in storytelling without strong empirical support (Franz & Engel, 2010). For this reason, we present our results only as an independent insight on the time of divergence of Curculionoidea and its major lineages, hoping that it may contribute for a better understanding of the evolutionary history of the group.

2.4.3. Adaptive radiation of weevils on fig trees

The radiation of weevils on figs occurred at least three independent times along the history of Curculionidae. However, our phylogeny was not well-resolved to recover the affinity of fig-weevils within the family. Considering that *Ceratopus* is exclusively associated with Neotropical figs while *Omophorus* and several species of Curculionini are associated with Afrotropical and Oriental ones, the presence of endemic species on each region suggests a strong biogeographical influence on their diversification. The origin of the three weevil lineages associated with figs varied along the time, and their stem appear to be older than the initial diversification of figs (around 75 Ma; Cruaud et al., 2012). Nonetheless, their crown group diversification is remarkably congruent with the diversification of their hosts during the Eocene on each specific region (Section 4 and Section 5).

We suggest that the wide distribution of fig-weevils species (e.g., *Omophorus stomachosus* occurring from Israel to South Africa) and their relative host specificity (e.g., *Ceratopus* species specialized in *Ficus* sections) evolved *via* host-tracking (Morand & Krasnov, 2010). The ancestral of each lineage of fig-weevil probably shifted to figs and then followed their hosts as they increased their distribution range inside each biogeographic region. As the effect of this prolonged association with weevils is still unknown, the investigation of the evolution of the interaction between these organisms will encourage future research on their biology and ecology and will also help us to understand the implications weevils may have had on the fig-wasp-fig mutualism. Understanding the processes that affect the diversification of insects is crucial to understand the origins of biodiversity.

Taxonomy of *Ceratopus* – The Neotropical fig-weevil

3.1. Introduction

Ceratopus Schönherr, 1843 is a small genus of weevils including 17 species exclusively distributed in the Neotropical region (O'Brien & Wibmer, 1982; Wibmer & O'Brien, 1986). They can be distinguished from other weevils by the presence of a strong femoral tooth on every leg. The margin of the tooth is never serrate as in other Curculionidae groups (e.g., Camarotini and Prionobrachiini) and its inner part has a hollow region to fit the correspondent tibia when the insect reposes. They also present divaricate and appendiculate tarsal claws, an ascendant mesepimeron not seen in dorsal view, and a compact club with asymmetric sutures between the club segments.

Ceratopus is associated with the two sections of Neotropical fig trees (i.e., *Americana* and *Pharmacosycea*) (Lima, 1956; Pakaluk & Carlow, 1994). Their larvae develop inside the figs, feeding mainly on the fig wall, and reaching the fig cavity at the last larval instar. Contrary to other insects that develop inside figs (e.g., Pyralidae moths), they do not cause too much damage to the syconia and infested figs frequently produce fig wasps and seeds. This strategy may have evolved to avoid abortion of single fruits, yet some crops can present 100% of infested fruits (Palmieri, 2012). Generally, only one larva is found in each fig but two, three or more larvae may also occur together. When the larva reaches the last instar, it migrates to the soil where it digs a hole and pupates inside an earthen-cocoon. The adults emerge in approximately 30 days, depending on external temperature (L. Palmieri, unpublished data). Although the larva of *C. helicostylis* Hustache, 1940 was described by Bondar (1947) as feeding on the pulp of the fruits of another Moraceae, *Helicostylus poeppigiana* Trécul, no further details of the association was given.

The genus was first included in the Ceratopodini tribe by Lacordaire (1863). Since the establishment of the tribe, a number of genera have been transferred to other tribes or subfamilies and several new species have been described. In the most recent catalogue of Curculionoidea, six genera (*Aetiomerus* Pascoe, 1886; *Anthomelus* Hustache, 1920; *Neoanthomelus* Hustache, 1933; *Catiline* Champion, 1906; *Ceratopus*, and *Stelechodes* Faust,

1899) are considered valid (Alonso-Zarazaga & Lyal, 1999). However, *Neoanthomelus* does not seem to be a valid genus and both Madagascan *Anthomelus* and New Guinean *Stelechodes* do not likely belong to the tribe (Caldara et al., 2014). Moreover, although Ceratopodini is included in the Curculioninae subfamily, its affinity and phylogenetic status are uncertain, and an overall revision of all these genera is required (Caldara et al., 2014).

Here we present a review of *Ceratopus* including detailed host associations as well as data on the species distribution. We also include the description of 18 new species.

3.2. Material and methods

3.2.1. Specimen collection

As larvae of *Ceratopus* can be easily found developing inside near ripe and ripe figs (D-F Phases), we performed unsystematic walks in several localities (Appendix 2) of the Neotropics in order to find fig trees in the adequate reproductive stage. After collection, the figs were put in a cloth-covered plastic box with a thin layer of soil and checked periodically for the emergence of adult beetles (ca. 35 days), which were then fixed in 95% ethanol. In order to improve our distribution map, we included data on host association and localities found on the labels of *Ceratopus* specimens analyzed at the natural history collections visited; these data were used only when the association between the weevil and its host fig tree could be determined indubitably. The species we reared from figs are labeled as “fresh material”; otherwise, the collection in which the specimens are deposited is designated. *Ceratopus* types and specimen depositories, as well as the curators of the collections consulted are described in Appendix 1.

3.2.2. Morphological study

The adult *Ceratopus* is easily recognizable, especially if one looks at the large femoral tooth and divaricate-appendiculate tarsal claws. However, to separate the species can be an extremely tricky task. They are small and have the same yellowish-brown color and, when preserved in alcohol, look very similar. Concordantly, despite the small size of the genus – only 17 valid species (O’Brien & Wibmer, 1982; Wibmer & O’Brien, 1986)– many mistakes were made since its description in the nineteenth century. Males and females were described as distinct species; and the same species were described numerous times by different authors (Champion, 1902; Hustache, 1938; Bondar, 1947).

There are some key characteristics that help the separation of *Ceratopus* species. The shape and size of the antennal club varies considerably among *Ceratopus* species. The ratio (C_1/F_1) between the length of the club (from the basis of last segment to the apex of the club) and the length of the funicle (from the insertion on the scape to the division between the seventh funicular segment and the club) is a good estimate of the antennal club size. The shape of the mesoventrite lobe (between the mesocoxae) may also vary and it is a valuable diagnostic character. It can be flat with straight margin in some species, varying to round or bifurcate margin in others. It can also be protruding round or bilobate. Another remarkable characteristic is the size and vestiture pattern of elytral callus at the fifth interstriae (located at the apical third of the elytra right at the junction of the fifth and sixth stria). The callus may be absent or protruding in various levels, from acute to round. Finally, the features in male fifth ventrite as the presence of a median cluster of setae, a straight or bilobate protruding apex are also diagnostic characteristics.

Images were made with a Leica MZ16 stereoscope attached with a DFC320 digital camera. Leica Application Suite (LAS) V3.6 imaging software was used in order to merge image series comprising 10-15 focal planes. Specimens found in the collections visited were photographed using Canon EOS-D500 with macro lens setup. The final images were then enhanced using GNU Image Manipulation Program v2.8.16 (<http://gimp.org>). Data present on the labels (as host association and geographic location) were recorded. When absent, the geographical coordinates were obtained by inserting the collection site on Google Earth v. 7.1.2.2041. When the determination of coordinates by this method was doubtful, the localities were discarded. Morphological terminology follows mostly Lyal, C.H.C. (Ed.) Glossary of Weevil Characters at the International Weevil Community Website (<http://weevil.info/glossary-weevil-characters>, accessed 21/11/2015).

3.3. Taxonomy

3.3.1. Redescription of *Ceratopus*

Ceratopus Schöenherr, 1843, p. 120.

= *Acanthobrachium* Boheman, 1859, p. 128.

Type-species: *Ceratopus bisignatus* Boheman, 1843

Diagnosis: Club connate, oval, covered with small hair-like sensilla, sutures between club segments asymmetric, never straight (Figure 3.1 A); Rostrum long, semi-filiform, longer

than the length of the head and prothorax combined, shorter in males, sometimes with longitudinal striae from the antenna insertion to the basal area; Scutellar shield triangular, small, ascending, sometimes with vestiture thinner than the rest of the body; Femora large, armed with a strong tooth, larger in mid and hind legs, margin of the tooth never serrate (Figure 3.2 A and B), inner part of the tooth lacking vestiture, the apex of the tooth usually exceeding the width of tibia, especially on metafemur; Tibial apex uncinata, sometimes unarmed (Figure 3.2); Tarsal claws largely divaricate, appendiculate (Figure 3.1 D).

Redescription: Length 2.5-8.5 mm. Body finely densely punctuated, always covered with dense and small oblong scales, various in color, brownish-yellow, brown, red, cinereous or black, never with metallic tinges; Body shape rhomboidal in dorsal view, elongate to semi-piriform in lateral view. *Head.* Antenna geniculated, with 11 segments; Scape glabrous, clavate, with only a few bristles at the apex; Funicle setose, the first segment thicker and slightly longer than the others six; Club connate, oval, covered with small hair-like sensilla, sutures between club segments asymmetric, never straight; Antennal insertion lateral, more apical in males; Scrobe long, directing ventrally, fitting the straight part of the scape; Epistome gently sinuate; sometimes with median dorsal projections over the mandible (Figure 3.2 B and C); Rostrum long, semi-filiform, longer than the length of the head and prothorax combined, longer in females, sometimes exceeding the body length (Figure 3.5 and 3.6), usually with longitudinal striae from the antenna sockets to the basal area; Mouth-parts phanerognathous; Eyes longitudinally oval, flat, following the natural shape of the head, more close at the basis; Forehead sometimes with a small fovea. *Prothorax and elytra.* Prothorax larger than longer, sub-parallel; usually constricted at the basis; bearing a weak longitudinal dorsal line, usually noticed only by changes in the vestiture; Noto-sternal suture incomplete, visible in front of each procoxa; Postocular lobes reduced, small, with a apical comb of minute setae; Scutellar shield small, usually triangular, ascending, with vestiture thinner than the rest of the body; Elytra with 10 regular punctuated striae, basis never advancing over the prothorax, semi-convex, with emarginated apex, covering the pygidium, sometimes slightly abbreviated; Humerus slightly protruding, with an angle almost straight; Interstriae flat, usually with a protruding callus at the apical part of interstria five. *Pleural and sternal sclerites.* Mesepimeron large, frontward, ascending, not visible on dorsal view (hidden by the humerus); Mesanepisternum triangular, suture between mesepimeron and mesanepisternum straight; Metanepisternum not covered by elytra, Metepimeron fused to the metanepisternum, not visible; Mesoventrite lobe (between the mesocoxa) presenting various features important to separate the species; Metaventrite flat, with a strong transversal carina in front of each metacoxa, not reaching the metanepisternal suture, sometimes forming a ridge. *Legs.*

Procoxae round, contiguous; Mesocoxae round, separated by a distance inferior to the diameter of mesocoxa; Metacoxae large transversely oval, extending until the metanepisternum, Trochanter reduced, triangular, with at least one strong hair-like setae (completely different from the vestiture); Pro, Meso and Meta femora large, armed with a strong tooth, larger in meso and metafemora, margin of the tooth never serrate, usually with a row of scales, inner part of the tooth glabrous, with a hollow region to fit the tibia when in repose, the apex of the tooth at the metafemora usually exceeding the width of metatibia; Tibial apex usually uncinat, sometimes reduced, bearing an oblique apical comb of setae (Figure 3.2); Tarsi pseudotetramerous; Tarsal claws strongly divaricate, appendiculate. *Abdominal ventrites*. Ventrite one larger than the others with a lobe projecting between the metacoxae, usually median depressed in males; Ventrite two larger than ventrites three, four and five, the other ventrites about the same size; Ventrite five sometimes presenting a median tuft of hair-like setae in males.

All the species presented hereafter have the characteristics described above, being different only if stated otherwise.

Figure 3.1. Details of *Ceratopus* morphology. (A) Head of *C. crassipes* male, arrow indicating the asymmetric suture between club segments. (B and C) Apex of the rostrum of *Ceratopus* sp24 and *Ceratopus* sp25, arrows indicating the projected epistome over the mandibles. (D) Close up of *Ceratopus* sp2 tarsal claw.

Figure 3.2. Details of *Ceratopus* morphology. (A) *C. dorytomoides* male leg, arrows indicating the reduced uncus and the inner margin of femoral tooth. (B) *Ceratopus* sp2 female leg, arrows indicating well developed uncus and the inner margin of femoral tooth. (C) *Ceratopus* sp2 male leg, arrow indicating the absent uncus.

3.3.2. Description of the species

Ceratopus bisignatus Boheman, 1843, p. 121. (Lectotype, BMNH, male, Jalapa (Xalapa), Veracruz, Mexico, ex. coll. Chevrolat, examined). (Figure 3.3 A and 3.5 a, A). syn. *Ceratopus subfasciatus* Champion, 1902 (BMNH, examined); *Acanthobrachium gounellei* Hustache, 1940 (MNHN, examined); *Ceratopus gounellei* Hustache, 1940.

Material examined: **(Fresh material)** BRAZIL, São Paulo, E.E.Caetetus, Gália. 10.IV.2011. - 22,39107 -49,69008.col. Palmieri, L. ex. *Ficus adhatodifolia* n° EEC002. COSTA RICA, Puntarenas, Monteverde. 08.XII.2013. 10,298784 -84,803901.col. Palmieri, L. ex. *Ficus yoponensis* n° CR040. **(AMNH)** BRAZIL, Bahia. -15.265657° -39.610010°. ex. *Ficus* sp. BRAZIL, Parana, Caviuna. IV.1945. -24.317887° -50.430637°. BRAZIL, Santa Catarina, Corupa. III.1938 - I.1946. -26.388252° -49.291551°. ex. *Ficus* sp. **(BMNH)** HONDURAS, Hondo river. MEXICO, Vera Cruz, Xalapa. COSTA RICA, Cartago, Turrialba PERU, Loreto, Yanamono. 28.IX.1981. -3.383333° -72.750000°. GUATEMALA, Quetzaltenango, Cerro Zunil. **(MNHN)** BRAZIL, Minas Gerais, Diamantina. ex. *Ficus* sp. **(MZUSP)** BRAZIL, São Paulo, Itu. 22.I.1961. BRAZIL, Goiás, São Jose do Tocantins. 22.VI.1942. BRAZIL, São Paulo, Castilho. X.1964. BRAZIL, Minas Gerais, Buritis. 31.X.1964. BRAZIL, São Paulo, Barueri. 20.X.1954. **(NMNH)** COSTA RICA. ex. *Ficus crassiusculua*. COSTA RICA. ex. *Ficus insipida*. HONDURAS, San Juan. 14.390841° -88.444454°. VENEZUELA, Rancho Grande, Guamita. 9.169186° -64.671991°.

Diagnostic description: Length 5-6 mm. Brown; club, head, funicle and rostrum reddish-brown. Elytra dappled with whitish and black vestiture, sometimes denser at the apex, scutelar shield the same color as the rest of the body. Interstriae five with a spot of white scales at the callus region sometimes preceded with a small line of black vestiture. Body shape rhomboidal and elongate. Rostrum rough, with longitudinal striae, covered with scales until antennal sockets in males, smooth, slender and glabrous in females. Club oval, small, shorter than the funicle, ratio $C_1/F_1 = 0.5$. Insertion of the antenna at median position in females and more apical in males. Forehead presenting a small fovea in males. Elytral callus slightly protruding rounded. Mesoventrite lobe saddle-like, margin straight. Metatibia with reduced uncus. Male ventrites median depressed.

Distribution: Neotropical, From Mexico to southern Brazil.

Biology: Reared from figs of *Ficus* section *Pharmacosycea*.

Ceratopus bondari Voss, 1940. (Paratype, MNHN, female, Bahia, Brazil, ex. “Gameleira branca” (common name for *Ficus* sp. in Portuguese), coll. Bondar, examined). (Figure 3.3 B-3.5 b, B). syn. *Acanthobrachium fici* Hustache, 1940 (MNHN, examined); *Ceratopus fici* Hustache, 1940, *Ceratopus ficusae* (Hustache) Bondar, 1947.

Material examined: (**Fresh material**) BRAZIL, São Paulo, Ribeirão Preto. 15.V.2014. -21,162095 - 47,860818.col. Palmieri, L. ex. *Ficus adhatodifolia* n° SP006. (MNHN) BRAZIL, Bahia. 11.III.1938. ex. *Ficus* sp. (MZUSP) BRAZIL, Goiás, Jataí. I.1964. BRAZIL, Santa Catarina, Timbo. VIII.1956. BRAZIL, Maranhão, Imperatriz. VIII.1960.

Diagnostic description: Length 6-6.5 mm. Brown; body uniformly colored. Body uniformly covered with whitish and brownish vestiture. Interstriae five with a spot of white scales at the callus region sometimes preceded with a small patch of black vestiture, shorter than the one of *C. bisignatus*. Body shape rhomboidal and elongate. Rostrum rough, with longitudinal striae, covered with scales until antennal sockets, presenting weak sexual dimorphism. Antennal insertion after the median part of the rostrum toward the apex, more apical in males Club oblong, ratio $C_1/F_1 = 0.75$. Elytral callus slightly protruding rounded. Mesoventrite lobe saddle-like, margin straight. Reduced uncus in all legs. Male ventrites median depressed.

Distribution: Brazil.

Biology: Reared from figs of *Ficus* section *Pharmacosycea*.

Notes: Voss (1940) and Hustache (1940) described this species under different names in the same year. The description of Voss, however, was published in May while the one of Hustache in December. For the principle of priority the name *C. bondari* must be applied.

Ceratopus sampsoni Bondar, 1947, p. 288 (Lectotype, AMNH, male, Bahia, Brazil, ex. *Ficus* sp. type n° 1567, examined). (Figure 3.3 C-3.5 c1, c2, C).

Material examined: (**Fresh material**) BRAZIL, Acre, Sena Madureira. 20.XI.2014. 154m. - 9.098156327 -68.6975334.col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus schultesi* n° AC200. BRAZIL, São Paulo, Gália. 05.V.2011. 685m -22.373365 -49.669503.col. Palmieri, L. ex. *Ficus obtusifolia* n° EEC017. BRAZIL, São Paulo, Gália. 28.XI.2010. 674m -22.383058 -49.673687.col. Palmieri, L. ex. *Ficus citrifolia* n° EEC064. BRAZIL, São Paulo, Gália. XI.2011. 678m -22.377423 -49.671087.col. Palmieri, L. ex. *Ficus trigona* n° FCA016. BRAZIL, São Paulo, Ribeirão Preto. - 21.162908 -47.858862.col. Palmieri, L. ex. *Ficus citrifolia* n° SP001. (**AMNH**) BRAZIL, Santa Catarina, Corupa. XI.1945. -26.388252 -49.291551. TRINIDAD, Arima Valley. VI.1950 - III.1951. 10.634112 -61.260012. (**NMNH**) EL SALVADOR, San Salvador. VI.1970. 13.729608 -89.237092. MEXICO, Cuernavaca, Mor. XI.1944. 18.877900 -99.188528. ex. *Ficus* sp. MEXICO, Tampico. 22.334496 -97.917436. MEXICO, Taxco, Gro. I.1945. 18.551449 -99.630690. ex. *Ficus* sp. PANAMA, Barro Colorado. 9.157577 -79.828360. ex. *Ficus* sp. PANAMA, Culebra. 8.313545 -81.617661. ex. *Ficus* sp. VENEZUELA, Rancho Grande, La Trilha, Aragua. IX.1964. 9.154918 -64.639981. ex. *Ficus* sp. (**UCR**) COSTA RICA. ex. *Ficus* sp.

Diagnostic description: Length 6-6.5 mm. Reddish-brown; club, funicle and apical third of the rostrum deep brown. Elytra with a weak transversal band of white vestiture on its median third. Interstriae five with a small tuft of white scales at the callus region. Males with a very distinct median black spot at fifth ventrite. Body shape rhomboidal and semi-piriform. Rostrum with longitudinal striae covered with scales, smooth from the antenna sockets to the apex. Club slightly shorter than the funicle, ratio $C_l/F_1 = 0.9$. Elytral callus flat, not protruding. Mesoventrite lobe margin acutely-bilobate.

Distribution: Neotropical, From Mexico to southern Brazil.

Biology: Reared from figs of *Ficus* section *Americana*.

Notes: This species has a wide distribution and its morphology do not vary among the populations.

Ceratopus mixtus Champion, 1902, p. 124. (Holotype, BMNH, male, Chontales, Nicaragua, examined). (Figure 3.3 D-3.5 d, D).

Material examined: (**Fresh material**) BRAZIL, São Paulo, E.E.Caetetus, Gália. -22.416793 -49.703601.col. Palmieri, L. ex. *Ficus adhatodifolia* n° EEC129. COSTA RICA, Puntarenas, Monteverde. 08.XII.2013. 10.303852 -84.792567.col. Palmieri, L. ex. *Ficus crassiuscula* n° CR039. (**NMNH**) GUATEMALA. IV.1937. HONDURAS, Choluteca. VII.1977. 13.300785 -87.194110. HONDURAS, Siquatepeque. 14.599056 -87.833054. HONDURAS, Taulabe. VI.1980. 14.667198 -87.987234. HONDURAS. XI.1934. MEXICO, Puente Nacional. 18.065059 -94.474216. MEXICO, San Andres. 19.274149 -99.016531. MEXICO, Taladro. X.1979. 20.628877 -103.329915. MEXICO, Tapanatepec. II.1953. 16.349411 -94.193931. MEXICO, Veracruz, Lake Catemaco, Coyame. 18.437526 -95.024557. PANAMA, Barro Colorado. XI.1934. 9.154198 -79.849201. ex. *Ficus* sp. PANAMA, El Cermen. V.1939. 8.740604 -79.841846. PANAMA, Paraiso. 9.031187 -79.624678. PANAMA, Porto Belo. 9.548800 -79.648884. PANAMA. III.1957. VENEZUELA, Rancho Grande. 9.165619-64.616828. (**MZUSP**) BRAZIL, Sao Paulo, Barueri. 1962. BRAZIL, Santa Catarina, Timbo. XII.1956. (**MNH**) BOLIVIA. COSTA RICA, Cartago, Turrialba. PERU, Loreto. II.1992. -3.975049-73.556398 ex. *Ficus insipida*. (**AMNH**) BRAZIL, Parana, Caviuna. -24.273907 -50.482352. BRAZIL, Santa Catarina, Corupa. -26.388252 -49.291551.

Diagnostic description: Length 7-8.5 mm. Brown, uniformly tinted; apical third of the rostrum darker. Body uniformly covered with yellowish-white vestiture, denser at the callus, elytra dappled with light brown scales. Rostrum covered with scales until antenna sockets in males, glabrous in females. Body shape rhomboidal and elongate. Rostrum with longitudinal striae in males, smooth in females. Club conic, shorter than the funicle, ratio $C_l/F_l = 0.6$. Prothorax with a median longitudinal line of converging vestiture. Interstriae five with a protruding callus toward the apex. Mesoventrite lobe protruding, rounded-bilobate. Ventrites one and two median depressed. Tibial uncus very small, reduced in all legs.

Distribution: From tropical Mexico to the southern Brazil.

Biology: Reared from figs of *Ficus* section *Pharmacosycea*.

Notes: Type lacking antenna and the abdomen.

Ceratopus maculicollis Champion, 1910, p 182. (Holotype, BMNH, male, Managua, Nicaragua, examined). (Figure 3.3 E-3.5 e, E).

Material examined: (**Fresh material**) COSTA RICA, San José, Curridabat. 24.XI.2013. 1229m. 9.921782 -84.032053.col. Palmieri, L. ex. *Ficus costaricana* n° CR006. COSTA RICA, San José, San José. 25.XI.2013. 1213m 9.937612 -84.048661.col. Palmieri, L. ex. *Ficus jimenezii* n° CR007. COSTA RICA, San José, San José. 22.XI.2013. 1200m 9.937612 -84.048661.col. Palmieri, L. ex. *Ficus costaricana* n° CR018.

Diagnostic description: Length 5-5.5 mm. Light-brown. Body spotted with small dark-brown vestiture. Head, prothorax, scutellar shield, legs and the basis of alternate interstria

irregularly variegated with yellowish-brown scales. Rostrum covered with scales until antenna sockets. Body shape rhomboidal and obovate. Rostrum with longitudinal striae. Club large, oval, about the same size of funicle, ratio $C_1/F_1 = 1.0$. Callus flat, inconspicuous. Mesoventrite lobe not protruding, straight. Tibial uncus very small, reduced in all legs.

Distribution: Nicaragua and Costa Rica.

Biology: Reared from figs of *Ficus costaricana*.

Ceratopus rufirostris Champion, 1902, p 125. (Holotype, BMNH, female, Amula (?), Guerrero, Mexico, examined). (Figure 3.3 F-3.5 F).

Diagnostic description: Length 7 mm. Reddish-brown. Densely covered with vestiture, except for the antenna and the rostrum. Dorsal region and legs with yellowish vestiture, ventral region and coxae covered with white vestiture. Elytra with intermixed white scales, forming two incomplete transverse bands, one basal and other at the median third. Body shape rhomboidal and elongate. Rostrum very long, almost the size of the body, completely smooth. Club small, shorter than the funicle, ratio $C_1/F_1 = 0.35$. Scutelar shield rounded. Callus flat, inconspicuous. Uncus at the pro and mesotibia large, reduced at metatibia.

Distribution: Mexico.

Biology: Unknown.

Notes: To the present known only by the type specimen.

Ceratopus dorytomoides Champion, 1902, p 124. (Holotype, BMNH, male, Cerro Zunil, Quetzaltenango, Guatemala, examined). (Figure 3.3 G-3.5 g, G). syn. *Acanthobrachium helicostylis* Hustache, 1940 (MNHN, examined); *Ceratopus helicostylis* Hustache, 1940.

Material examined: (Fresh material) BRAZIL, Acre, Sena Madureira. 20.XI.2014. 180m. - 9,108434469 -68,72432364.col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus donnell-smithii* n° AC201.BRAZIL, Acre, Xapuri. 17.XI.2014. 208m. -10,76471597 -68,39307988.col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus trigona* n° AC168.BRAZIL, Acre, Xapuri. 17.XI.2014. 209m. - 10,76379991 -68,39336746.col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus citrifolia* n° AC172.BRAZIL, Acre, Xapuri. 17.XI.2014. 213m. -10,76555198 -68,39483094.col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus lauretana* n° AC176.BRAZIL, Acre, Xapuri. 18.XI.2014. 247m. - 10,56619126 -68,54520447.col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus donnell-smithii* n° AC190.BRAZIL, Mato Grosso, Sto. Antônio do Leverger. 23.VIII.2012. -15,826094 -56,073998.col. Farache, F.H.A ex. *Ficus crocata* n° MT001.BRAZIL, Rio de Janeiro, Barra Mansa. 28.X.2014. 515m

-22,663832 -44,18566.col. Palmieri, L. ex. *Ficus eximia* n° BM012.BRAZIL, São Paulo, Araraquara. II.2011. -21,785788 -48,195269. col. Farache, F.H.A ex. *Ficus obtusifolia* n° SP005.BRAZIL, São Paulo, Gália. 25.VIII.2011. 674m -22,380795 -49,673392. col. Palmieri, L. ex. *Ficus eximia* n° EEC001.BRAZIL, São Paulo, Gália. 05.V.2011. 685m -22,373365 -49,669503. col. Palmieri, L. ex. *Ficus obtusifolia* n° EEC017.BRAZIL, São Paulo, Gália. XI.2011. 678m -22,377423 -49,671087. col. Palmieri, L. ex. *Ficus trigona* n° EEC062.BRAZIL, São Paulo, Gália. 28.XI.2010. 674m -22,383058 -49,673687. col. Palmieri, L. ex. *Ficus citrifolia* n° EEC064.BRAZIL, São Paulo, Ribeirão Preto. 01.IV.2010. -21,162908 -47,858862. col. Palmieri, L. ex. *Ficus citrifolia* n° SP001.BRAZIL, São Paulo, Ribeirão Preto. 13.X.2010. 566m -21,170502 -47,851238. col. Palmieri, L. ex. *Ficus eximia* n° SP002.BRAZIL, São Paulo, Sta. Rita do Passa Quatro. 19.IX.2013. -21,706312 -47,551053. col. Palmieri, L. ex. *Ficus citrifolia* n° SP004.COSTA RICA, Limón, Guápiles. 26.XI.2013. 276m. 10,211811 -83,777812.col. Palmieri, L. ex. *Ficus colubrinae* n° CR010.COSTA RICA, Limón, Guápiles. 26.XI.2013. 298m. 10.203363° -83.781168°.col. Palmieri, L. ex. *Ficus colubrinae* n° CR013. COSTA RICA, Limón, Guápiles. 28.XI.2013. 315m. 10.205848° -83.810556°.col. Palmieri, L. ex. *Ficus colubrinae* n° CR016. COSTA RICA, Puntarenas, Monteverde. 08.XII.2013. 935m. 10.262430° -84.839523°.col. Palmieri, L. ex. *Ficus jimenezii* n° CR036. COSTA RICA, Puntarenas, Monteverde. 08.XII.2013. 888m. 10,419212 -84,92183.col. Palmieri, L. ex. *Ficus jimenezii* n° CR038.COSTA RICA, San José, Curridabat. 24.XI.2013. 1229m 9,921782 -84,032053.col. Palmieri, L. ex. *Ficus costaricana* n° CR006. COSTA RICA, San José, Curridabat. 23.XI.2013. col. Palmieri, L. ex. *Ficus jimenezii* n° CR019. COSTA RICA, San José, San José. 22.XI.2013. 1200m. 9,939032 -84,049877.col. Palmieri, L. ex. *Ficus jimenezii* n° CR017.(AMNH) BRAZIL, Bahia, Água Preta. -15.265657° -39.610010°. ex. *Amora Vermelha*. BRAZIL, Santa Catarina, Corupa. XI.1944. -26.456452° -49.296242°. BRAZIL, Bahia. ex. *Amoreira vermelha* ou *Pau-de-Leite*. (NMNH) COSTA RICA, Guanacaste. 10.513868° -85.369146°. ex. *Ficus insipida*?? ECUADOR, Pichilingue. MEXICO, Cuernavaca, Mor. 18.948244° -99.176060°. MEXICO, Tampico. 22.265231° -97.892057°. MEXICO, Taxco, Gro. 18.564818° -99.624409°. MEXICO, Urapan. 19.423790° -102.088501°. PANAMA, Barro Colorado. 9.150769° -79.848566°. PANAMA, Culebra. 8.295860° -81.618750°. PANAMA, Trinidad river. 8.190778° -81.555057°. VENEZUELA, Rancho Grande. 9.206679° -64.718763°. (MZUSP) BRAZIL, Mato Grosso, Xingu. 1961. BRAZIL, Sao Paulo, Itu. 1959. BRAZIL, Sao Paulo, Mongagua. 1967. VENEZUELA, Rancho Grande. 1968. BRAZIL, Sao Paulo, Mongagua. 1967. BRAZIL, Mato Grosso, Xingu. 1961. BRAZIL, Sao Paulo, Itu. 1959. VENEZUELA, Rancho Grande. 1968. BRAZIL, Sao Paulo, Barueri. 13.I.1962.

Diagnostic description: Length 5-5.5 mm. Light brown, uniformly tainted. Densely covered with intermixed light brown, yellowish and black vestiture, scutelar shield white; scales at the elytra arranged in a tessellate pattern on alternate interstriae. Prothorax with a median longitudinal line of converging vestiture. Interstriae five with a small tuft of white scales at the callus region, sometimes absent. Rostrum covered with scales until antenna sockets. Body shape rhomboidal obovate. Rostrum recurvate, with longitudinal striae, the median stria stronger, ending up on a small fovea in the forehead. Club small, ratio $C_1/F_1 = 0.4$. Funicle one strongly clavate. Scutelar shield small. Callus flat. Mesoventrite lobe slightly protruding, rounded. Ventrite one median depressed in males. Reduced uncus at metatibia, absent in males. Trochanter and the base of the femora densely covered with long hair-like vestiture.

Distribution: From tropical Mexico to southern Brazil.

Biology: Reared from figs of several species of *Ficus* from section *Americana*. Also reported in association with *Helicostylis poeppigiana* (Bondar, 1947).

Notes: This is certainly the most conspicuous species of *Ceratopus*. It has a great intraspecific variation among the populations and was described several times under different names. Molecular data suggest that this species may form a complex of species (see Section 4), with the populations of southern Brazil being isolated from the ones of Central America. So far, it is the only species of the genus reported as associated with a non-*Ficus* host.

Ceratopus longiclava Champion, 1902, p 125. (Holotype, BMNH, female, Bugaba, Panama, examined). (Figure 3.3 I-3.5 i. I). syn. *Ceratopus bilineolatus* Hustache, 1938 (MNHN, examined); *Acanthobrachium bilineolatus* Hustache, 1938.

Material examined: (**Fresh material**) BRAZIL, São Paulo, Gália. 05.V.2011. 685m -22.373365 -49.669503.col. Palmieri, L. ex. *Ficus obtusifolia* n° EEC017. BRAZIL, São Paulo, Gália. 28.XI.2010. 674m -22.383058 -49.673687.col. Palmieri, L. ex. *Ficus citrifolia* n° EEC064. BRAZIL, São Paulo, Ribeirão Preto. 01.IV.2010. -21.162908 -47.858862.col. Palmieri, L. ex. *Ficus citrifolia* n° SP001. COSTA RICA, Limón, Guápiles. 26.XI.2013. 298m. 10.203614 -83.781416.col. Palmieri, L. ex. *Ficus costaricana* n° CR012. COSTA RICA, San José, Curridabat. 24.XI.2013. 1229m 9.921782 -84.032053.col. Palmieri, L. ex. *Ficus costaricana* n° CR006. COSTA RICA, San José, Escazú. 24.XI.2013. 1464m. 9.887748 -84.129109.col. Palmieri, L. ex. *Ficus citrifolia* n° CR003. COSTA RICA, San José, San José. 25.XI.2013. 1213m 9.937612 -84.048661.col. Palmieri, L. ex. *Ficus jimenezii* n° CR007. COSTA RICA, San José, San José. 22.XI.2013. col. Palmieri, L. ex. *Ficus costaricana* n° CR018. (NMNH) NICARAGUA, Jinotega. I.1981. 13.088260 -85.989217. ex. *Ficus* sp. VENEZUELA, Rancho Grande. VI.1967. 9.203944 -64.650241. (MNHN) PARAGUAY.

Diagnostic description: Length 5.5-6 mm. Reddish-brown; apical third of the rostrum and sometimes the ventrites, deep brown. Elytra dappled with reddish-brown, black and white scales. Interstriae five with a strong elongated spot toward the callus, callus apex densely covered with white scales. Body shape rhomboidal and semi-piriform. Rostrum with longitudinal striae covered with scales, smooth from the antenna sockets to the apex. Club slightly longer than the funicle, ratio $C_1/F_1 = 1.05$. Elytral callus softly protruding. Mesoventrite lobe not protruding, margin slightly sinuate. Apical margin of the fifth ventrite emarginated in females. Uncus reduced at the metatibia.

Distribution: Nicaragua, Costa Rica, Panama, Venezuela, Brazil, and Paraguay.

Biology: Reared from figs of *Ficus* section *Americana*.

Notes: The type of *C. bilineolatus* described by Hustache (1928) corresponds to a male of *C. longiclava*.

Ceratopus sp02 sp.nov. (Figure 3.3 H-3.5 h, H).

Material examined: (**Fresh material**) BRAZIL, Acre, Rio Branco. 15.XI.2014. 153m. -9.947464485 - 67.82574439.col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus citrifolia* n° AC129. BRAZIL, Acre, Xapuri. 17.XI.2014. 209m. -10.76485285 -68.39348271.col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus sphenophylla* n° AC167. BRAZIL, São Paulo, Gália. 28.XI.2010. 674m -22.383058 - 49.673687.col. Palmieri, L. ex. *Ficus citrifolia* n° EEC064. BRAZIL, São Paulo, Porto Ferreira. 22.VIII.2013. -21.812244 -47.39952.col. Palmieri, L. ex. *Ficus citrifolia* n° SP003. BRAZIL, São Paulo, Ribeirão Preto. 01.IV.2010. -21.162908 -47.858862.col. Palmieri, L. ex. *Ficus citrifolia* n° SP001. BRAZIL, São Paulo, Sta. Rita do Passa Quatro. 19.IX.2013. -21.706312 -47.551053.col. Palmieri, L. ex. *Ficus citrifolia* n° SP004.

Diagnostic description: Length 5.5-6 mm. Yellowish-brown, with intricate color pattern. Rostrum, antenna, pleural sclerites and mesoventrite light brown; Apical two thirds of elytra black dappled with white scales, excepting from region near the suture, apex of the callus also with white scales. Elytral basis with two small tufts of black scales near the humerus. Scutelar shield white. Body shape rhomboidal and elongated. Rostrum long, delicately rough, glabrous, without longitudinal striae, smoother in females. Club shorter than the funicle, ratio $C_1/F_1 = 0.7$. Elytra apex lightly constricted, strongly emarginated. Elytral callus protruding. Protiba with a strong apical cuticular expansion on its inner surface, forming a tooth at the apical third, sometimes reduced. Metatibial uncus small, absent in males. Mesoventrite lobe flat with a straight margin. Apex of the fifth ventrite emarginated, forming a notch in males.

Distribution: Brazil.

Biology: Reared from figs of *Ficus citrifolia* and *Ficus sphenophylla*. Apparently specialized to *F. citrifolia* in Southeastern Brazil.

Notes: Molecular data suggest that this species may form a complex of species (see Section 4), with considerable morphological variation among the populations of southern Brazil and Amazonia.

***Ceratopus crassipes* Boheman, 1859, p.128. (Paratype, MNHN, male, Turialba, Costa Rica, examined. Holotype not available). (Figure 3.3 J-3.5 j, J). syn. *Acanthobrachium crassipes* Boheman, 1859.**

Material examined: (**Fresh material**) BRAZIL, Rio de Janeiro, Barra Mansa. 28.X.2014. 454m - 22.594797 -44.230837.col. Palmieri, L. ex. *Ficus adhatodifolia* n° BM007. BRAZIL, São Paulo, Gália. 10.IV.2011. -22.39107 -49.69008.col. Palmieri, L. ex. *Ficus adhatodifolia* n° EEC002. (**AMNH**) BRAZIL, Parana, Caviuna. -24.279232 -50.440367. (**MZUSP**) BRAZIL, Sao Paulo, Barueri. 1962. -23.519868 -46.880288. BRAZIL, Sao Paulo, Itu. 1961. -23.24522 -47.266167. (**MNHN**) BOLIVIA.

Diagnostic description: Length 6.5-7 mm. Reddish; Vestiture mostly white; prothorax with two sparse spots of black vestiture above postocular lobes; Elytra with a large apical-lateral region covered with black scales dappled with white. Metafemora also with black scales dappled with white. Apex of elytra and callus with dense white vestiture. Body shape rhomboidal. Rostrum with longitudinal striae and covered with scales until the antenna sockets in males; Female rostrum glabrous, smooth, without striae. Club conic, setose, shorter than the funicle, ratio $C_1/F_1 = 0.75$. Scutelar shield small, rounded, covered with reduced scales. Callus protruding. Mesoventrite lobe protruding, bilobate. Tibial uncus very small, reduced in all legs.

Distribution: Costa Rica, Brazil and Bolivia.

Biology: Reared from figs of *Ficus adhatodifolia* Schott.

Notes: Bondar (1947) describes by mistake this species as *C. bisignatus*.

***Ceratopus sp08* sp.nov. (Figure 3.3 K-3.5 k, K).**

Material examined: (**Fresh material**) BRAZIL, Acre, Sena Madureira. 21.XI.2014. 172m. - 9,025475504 -68,80486325.col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus insipida* n° AC212. COSTA RICA, Puntarenas, Monteverde. 08.XII.2013. 10,298784 -84,803901.col. Palmieri, L. ex. *Ficus yoponensis* n° CR040. (**AMNH**) COSTA RICA. IX.1936. ex. *Ficus insipida*. (**BMNH**) BRAZIL, Bahia, Mucuri. (**NMNH**) COSTA RICA. ex. *Ficus insipida* DOMINICA, Clarke Hall. 15.386637° -61.355140°. HONDURAS, La Ceiba. 15.747539° -86.820686°. PANAMA, Alhajuelo. ex. *Ficus* sp. PANAMA, Ancón. 9.039958° -79.612638°. ex. *Ficus crassiuscula*. PANAMA, Barro Colorado. 9.164442° -79.845664°. ex. *Ficus* sp. PANAMA, Tocumen. 9.116035° -79.395395°. PERU, Loreto. II.1992. ex. *Ficus insipida*.

Diagnostic description: Length 4.5-5 mm. Dark brown, rostrum, antenna, tibia and tarsi reddish. Body uniformly covered by whitish vestiture, presenting three spots of lighter

vestiture at interstria five. One at the callus, a second at the median part of elytra and the last at the basal third of elytra. Body shape rhomboidal elongated. Rostrum with longitudinal striae and covered with scales until the antenna sockets in males; Female rostrum glabrous, smooth, without striae. Club oval, shorter, shorter than the funicle, ratio $C_l/F_1 = 0.5$. Scutelar shield small, covered with reduced scales. Callus inconspicuous. Mesoventrite lobe almost flat, with rounded margin. Tibial uncus small, reduced in males.

Distribution: Honduras, Costa Rica, Panama, Caribe, Peru, and Brazil.

Biology: Reared from figs of *Ficus* section *Pharmacosycea*.

Notes: Molecular data suggest that this species may form a complex of species (see Section 4). This species generally appears an inconspicuous black beetle, but a few differences on density and coloration of vestiture can be noted among the samples.

Ceratopus sp10 sp.nov. (Figure 3.3 L-3.5 L, L).

Material examined: (NMNH) VENEZUELA, Rancho Grande, Aragua. 9.202819 -64.631984. PERU. (BMNH) VENEZUELA, Rancho Grande. 1949.

Diagnostic description: Length 8-8.5 mm. Deep reddish-brown, uniformly tinted, covered with yellowish vestiture, denser at the apex of elytra; Fifth interstriae with four alternate small patches of denser scales, the last patch being at the callus, also with a small patch of black vestiture before the callus. Body shape rhomboidal and elongated. Rostrum very long, longer than half of the length of the body in both sexes, without longitudinal striae and covered with scales until the antenna sockets in males, smooth, longer and slender on females;. Club oval, small, shorter than the funicle, ratio $C_l/F_1 = 0.45$. Scutelar shield small, rounded, covered with scales of the same color as the elytra. Callus large, protruding. Apex of elytra slightly constricted.

Distribution: Venezuela and Peru.

Biology: Unknown.

Notes: Three specimens analyzed, one male and two females.

Ceratopus sp11 sp.nov. (Figure 3.3 M-3.6 a).

Material examined: (NMNH) MEXICO, Alpuyeca. IX.1944 18.774405° -99.252374°. ex. *Ficus radulina* = *F. insipida* subsp. *insipida*

Diagnostic description: Length 4.5-5 mm. Reddish-brown, uniformly tainted, densely covered by whitish vestiture. Body shape rhomboidal obovate. Rostrum with longitudinal striae and covered with scales until the antenna sockets in males. Club oval, small, ratio $C_1/F_1 = 0.5$. Callus flat, inconspicuous.

Distribution: Mexico.

Biology: Associated with figs of *Ficus insipida*.

Notes: Eight specimens in the collection, all males from the same locality.

Ceratopus sp12 sp.nov. (Figure 3.3 N-3.6 b, B).

Material examined: (NMNH) PANAMA, Barro Colorado. 9.160006° -79.852686°. ex. *Ficus* sp.

Diagnostic description: Length 5-5.5 mm. Reddish-brown, the lateral part of pronotum, the humeral region and the forefemora yellowish tainted. Densely covered with intermixed white, yellowish and black vestiture, scutelar shield white; scales at apical two thirds of elytra arranged in a black/white pattern on alternate interstriae. Prothorax with a median longitudinal line of converging vestiture. Interstriae five with a small tuft of white scales at the callus region. Rostrum covered with scales until antenna sockets. Body shape rhomboidal obovate. Rostrum recurvate, with longitudinal striae in both sexes. Club large, oblong, ratio $C_1/F_1 = 0.7$. Scutelar shield small. Callus protruding.

Distribution: Panama.

Biology: Reared from figs of *Ficus* sp.

Notes: Seven specimens in the collection, males and females. The morphology of this species suggests that it may be associated with figs of section *Americana*.

Ceratopus sp13 sp.nov. (Figure3.3 O-3.6 c).

Material examined: (NMNH) PANAMA, Canal zone. IV.1944. PANAMA, Barro Colorado. IV.1944 9.157458° 79.627237.

Diagnostic description: Length 6.5-7 mm. Deep brown, antennae and tarsi reddish-brown. Densely covered with intermixed whitish, yellowish and brown vestiture. Body shape rhomboidal and elongated. Rostrum with longitudinal striae and covered with scales until the antenna sockets in males. Club oblong, ratio $C_l/F_l = 0.75$. Scutelar shield small, rounded, covered with scales of the same color as the elytra. Callus very large, greatly protruding. Elytra apex slightly constricted, strongly emarginated.

Distribution: Panama.

Biology: Unknown.

Ceratopus sp14 sp.nov. (Figure3.3 P-3.6 d, D).

Material examined: (MZUSP) VENEZUELA, Rancho Grande. 16.XI.1968. (NMNH) COSTA RICA, Puntarenas, Monteverde. 10.302554° -84.814480°. COSTA RICA, Turrialba. 9.950825° -83.722683°. DOMINICA, Springfield est. 15.305337° -61.361708°. PANAMA, Cerro Campana. 8.707374° -79.907354°. VENEZUELA, Rancho Grande. 9.148913° -64.643316°.

Diagnostic description: Length 6-7 mm. Reddish-brown, uniformly tainted, covered with brownish vestiture. The lateral parts of pronotum, the humeral region, and a median transversal band at the elytra covered by yellowish vestiture. Body shape rhomboidal and elongated. Rostrum very long and slender, longer than the elytra in females, without longitudinal striae. In Males rostrum thicker and covered with scales until the antenna sockets. Club oblong, large, ratio $C_l/F_l = 0.6$. Funicle segment one as long as segments two, three and four combined. Scutelar shield small, rounded, covered with scales of the same color as the elytra. Callus unobscured, not protruding. Mesoventrite lobe narrow, rounded.

Distribution: Costa Rica, Panama, Dominica and Venezuela.

Biology: Unknown.

Ceratopus sp15 sp.nov. (Figure 3.4 A-3.6 e, E).

Material examined: COSTA RICA, Limón, Guápiles. 26.XI.2013. 276m. 10,211811 -83,777812.col. Palmieri, L. ex. Ficus colubrinae n° CR010. COSTA RICA, Limón, Guápiles. 28.XI.2013. 234m. 10,226810° -83.781837°.col. Palmieri, L. ex. Ficus colubrinae n° CR014. COSTA RICA, Limón, Guápiles. 28.XI.2013. 313m. 10,202939 -83,78525.col. Palmieri, L. ex. Ficus costaricana n° CR015. COSTA RICA, Puntarenas, Monteverde. 08.XII.2013. 935m. 10,262430° -84.839523°.col. Palmieri, L. ex. Ficus jimenezii n° CR036. COSTA RICA, Puntarenas, Monteverde. 08.XII.2013. 888m 10,419212 -84,92183.col. Palmieri, L. ex. Ficus jimenezii n° CR038. COSTA RICA, Puntarenas, Ojochal. 04.XII.2013. 9,074618° -83.653941°.col. Palmieri, L. ex. Ficus pertusa n° CR035. COSTA RICA, San José, Escazú. 24.XI.2013. 1478m. 9,888814 -84,129128.col. Palmieri, L. ex. Ficus pertusa n° CR001. COSTA RICA, San José, San José. 22.XI.2013. 1200m. 9,939032 -84,049877.col. Palmieri, L. ex. Ficus jimenezii n° CR017. COSTA RICA, San José, San José. 22.XI.2013. col. Palmieri, L. ex. Ficus costaricana n° CR018. COSTA RICA, San José, Zurquí. 24.XI.2013. 10,02613 -84,01141.col. Palmieri, L. ex. Ficus pertusa n° CR005. (INBIO) COSTA RICA.

Diagnostic description Length 3.5-4 mm. Reddish-brown; forehead, rostrum and median part of pronotum black. Body uniformly covered with yellowish vestiture dappled with light brown and black scales. Elytra with intricate color pattern of vestiture, humeral region covered with yellowish scales, the rest of the elytra covered with darker brownish scales; a diffuse pattern of alternate patches of white scales is transversely distributed at the median part of the elytra. Rostrum with longitudinal striae covered with scales, smooth from the antenna sockets to the apex in males, smoother in females. Body shape rhomboidal. Club oblong, large, longer than the funicle, ratio $C_l/F_l = 1.25$. Callus flat, not protruding. Mesoventrite lobe slightly longitudinal depressed, margin straight. Metaventrite and ventrites one median depressed in males, with elongated vestiture. Trochanters and the basis of the femora covered with elongate hair-like scales. Tibial uncus very small, reduced in all legs.

Distribution: Costa Rica.

Biology: Reared from figs of *Ficus* section *Americana*.

Ceratopus tessellatus Champion, 1902, p.125 (Holotype, BMNH, male, Chiriqui volcano, Chiriqui, Panama, examined). (Figure 3.4 B-3.6 m, M). Syn. *Acanthobrachium costatum* Brèthes, 1910; *Ceratopus costatus* Klima, 1935.

Material examined: (Fresh material) BRAZIL, Acre, Sena Madureira. 19.XI.2014. 174m. -9,155433644 -68,61739947.col. Cruaud, Farache, Pereira & Rasplus ex. Ficus mariae n° AC197. BRAZIL, Acre, Sena Madureira. 22.XI.2014. 180m. -9,331057062 -68,33324675.col. Cruaud, Farache, Pereira & Rasplus ex. Ficus sp. n° AC230. COSTA RICA, San José, Curridabat. 24.XI.2013. 1229m 9,921782 -84,032053.col. Palmieri, L. ex. Ficus costaricana n° CR006. COSTA RICA, San

José, Curridabat. 23.XI.2013. col. Palmieri, L. ex. *Ficus jimenezii* n° CR019. COSTA RICA, San José, San José. 25.XI.2013. 1213m 9,937612 -84,048661.col. Palmieri, L. ex. *Ficus jimenezii* n° CR007. COSTA RICA, San José, San José. 22.XI.2013. 1200m. 9,939032 -84,049877.col. Palmieri, L. ex. *Ficus jimenezii* n° CR017. COSTA RICA, San José, San José. 22.XI.2013. col. Palmieri, L. ex. *Ficus costaricana* n° CR018.

Diagnostic description: Length 4.5-5 mm. Light brown, uniformly tainted. Densely covered with intermixed light brown, yellowish and black vestiture; scales at the elytra arranged in a tessellate pattern on alternate interstriae, scutelar shield white; apical third of elytra an interstria one densely covered by yellowish vestiture. Rostrum covered with scales until antenna sockets. Body shape rhomboidal obovate. Rostrum rough, with weak longitudinal striae. Club almost of the same size as funicle, ratio $C_l/F_1 = 0.77$. Scutelar shield small. Callus slightly protruding. Mesoventrite lobe almost flat, with slightly sinuate margin. Ventrite one median depressed in males. Reduced uncus at metatibia, absent in males. Trochanter and the base of the femora densely covered with long hair-like vestiture. Male pygidium with two lateral tufts of elongate serdae.

Distribution: Costa Rica, Panama, and Brazil.

Biology: Reared from figs of *Ficus* section *Americana*.

Ceratopus sp18 sp.nov. (Figure3.4 C-3.6 g, G).

Material examined: (Fresh material) COSTA RICA, Puntarenas, Monteverde. 08.XII.2013. 1400m. 10.312713° -84.815733°.col. Palmieri, L. ex. *Ficus pertusa* n° CR037. COSTA RICA, San José, Escazú. 24.XI.2013. 1478m 9,888814 -84,129128.col. Palmieri, L. ex. *Ficus pertusa* n° CR001. COSTA RICA, San José, Zurquí. 24.XI.2013. 10,02613 -84,01141.col. Palmieri, L. ex. *Ficus pertusa* n° CR005. (INBIO) COSTA RICA.

Diagnostic description: Length 5.5-6 mm. Reddish-brown; apical third of the rostrum deep brown. Vestiture of the body with isolate small patches of whitish scales. Elytra with two big apical spots of black vestiture, extending laterally from the fifth to the eighth interstriae. Body shape rhomboidal and semi-piriform. Rostrum with longitudinal striae covered with scales, smooth from the antenna sockets to the apex. Club shorter than the funicle, ratio $C_l/F_1 = 0.8$. Elytral callus softly protruding. Mesoventrite lobe straight.

Distribution: Costa Rica.

Biology: Reared from figs of *Ficus pertusa* in Costa Rica.

Ceratopus sp19 sp.nov. (Figure 3.4 D-3.6 h, H).

Material examined: (**Fresh material**) COSTA RICA, San José, Escazú. 24.XI.2013. 1478m. 9,888814 -84,129128.col. Palmieri, L. ex. *Ficus pertusa* n° CR001. COSTA RICA, San José, Escazú. 24.XI.2013. 1464m. 9,887748 -84,129109.col. Palmieri, L. ex. *Ficus citrifolia* n° CR003. COSTA RICA, San José, Zurquí. 24.XI.2013. 10,02613 -84,01141.col. Palmieri, L. ex. *Ficus pertusa* n° CR005. COSTA RICA. XI.2013. col. Palmieri, L. ex. *Ficus* sp. n° CR_misc. (**INBIO**) COSTA RICA.

Diagnostic description: Length 5.5-6 mm. Yellowish-brown. Body densely covered by yellowish-brown vestiture, with a transversal band of lighter vestiture at the median part of elytra. Body shape rhomboidal and elongated. Rostrum with longitudinal striae covered with scales, smooth from the antenna sockets to the apex in males, smoother in females. Club large, as long as the funicle, ratio $C_l/F_l = 1$. Elytra apex lightly constricted, strongly emarginated. Elytral callus inconspicuous. Tibial uncus small, reduced in all legs. Mesoventrite lobe squared with a straight margin. Trochanter and the base of the femora densely covered with long hair-like vestiture.

Distribution: Costa Rica.

Biology: Reared from figs of *Ficus citrifolia* and *Ficus pertusa* in Costa Rica.

Ceratopus sp20 sp.nov. (Figure 3.4 E-3.6 I, I).

Material examined: (**Fresh material**) BRAZIL, São Paulo, E.E.Caetetus, Gália. col. Palmieri, L. ex. *Ficus citrifolia* n° EEC074. (**INBIO**) COSTA RICA.

Diagnostic description: Length 5.5 mm. Light reddish-brown, uniformly tainted. Body densely covered by yellowish vestiture. Ventriles three, four and five and the basal two thirds of elytra covered with brown vestiture, The division between elytral light and dark vestiture marked by a single row of whitish scales. Body shape rhomboidal, elongated. Rostrum weakly striate, half smooth. Club conical, small, ratio $C_l/F_l = 0.5$. Scutelar shield rounded, covered with small colorless scales. Callus inconspicuous. Mesoventrite lobe protruding, straight.

Distribution: Costa Rica and Brazil.

Biology: Reared from figs of *Ficus citrifolia*.

Ceratopus sp21 sp. nv. (Figure 3.4 F-3.6 j, J).

Material examined: (**Fresh material**) BRAZIL, Rio de Janeiro, Barra Mansa. 28.X.2014. 515m - 22,663832 -44,18566. col. Palmieri, L. ex. *Ficus eximia* n° BM012.

Diagnostic description: Length 5-5.5 mm. Light brown, uniformly tainted. Densely covered with intermixed light brown, yellowish and black vestiture, scales at the elytra arranged in a tessellate pattern on alternate interstriae. Interstriae five with a small tuft of white scales at the callus region. Rostrum covered with scales until antenna sockets in males, females only at the basal half. Body shape rhomboidal piriform. Rostrum recurvate, with longitudinal striae, the median stria stronger, ending up on a small fovea in the forehead in males. Club small, ratio $C_1/F_1 = 0.5$. Scutelar shield small. Callus flat. Mesoventrite lobe slightly protruding, rounded. Ventrite one median depressed in males. Mesocoxa sockets with thicker distal edges. Trochanter without strong hair-like setae.

Distribution: Brazil.

Biology: Reared from figs of *Ficus eximia* in Brazil.

Notes: Several specimens, males and females. Similar to *C. dorytomoides* in general aspect, but presenting unique morphological features when looked in detail.

Ceratopus sp22 sp.nov. (Figure 3.4 G-3.6 k).

Material examined: (**Fresh material**) BRAZIL, Acre, Xapuri. 18.XI.2014. 214m. -10,55825109 - 68,5155757. col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus pastasana* n° AC194.

Diagnostic description: Length 5 mm. Mostly brown. Scape, scutelar shield and median region of ventrite one and two yellowish -brown. Body densely covered by small, oblong whitish vestiture. Elytra with a large median spot of dark-brown vestiture, extending toward the basis along interstria one, two and three. Vestiture of scutelar shield extremely reduced. Rostrum covered with vestiture until antenna sockets, with a single longitudinal stria. Club oval, small, ratio $C_1/F_1 = 0.6$. Elytral callus softly protruding. Mesoventrite lobe straight. Ventrite one and two median depressed, ventrite two with long hair-like setae around the edges of the depression. Ventrite five with a median patch of hair-like setae. Trochanters with a patch of 4-5 hair-like setae. Tibial uncus reduced.

Distribution: Brazil.

Biology: Reared from figs of *Ficus pastasana*.

Notes: One male. Known only by the type specimen.

Ceratopus sp23 sp.nov. (Figure 3.4 H-3.6 l, L).

Material examined: (**Fresh material**) BRAZIL, Acre, Rio Branco. 15.XI.2014. 152m. -9,947109092 -67,82555941. col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus obtusifolia* n° AC131. BRAZIL, Acre, Sena Madureira. 19.XI.2014. 163m. -9,11519506 -68,61374689. col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus sphenophylla* n° AC19. BRAZIL, Acre, Sena Madureira. 20.XI.2014. 180m. -9,108434469 -68,72432364. col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus donnell-smithii* n° AC201. BRAZIL, Acre, Xapuri. 17.XI.2014. 209m. -10,76485285 -68,39348271. col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus sphenophylla* n° AC167.

Diagnostic description: Length 3-3.5 mm. Yellowish-brown; head, rostrum and antenna darker. Body uniformly covered with yellowish vestiture dappled with light brown scales. Elytra with a diffuse pattern of alternate patches of darker and light scales. Rostrum covered with scales until antenna sockets. Body shape rhomboidal. Rostrum with longitudinal striae. Club oval, large, longer than the funicle, ratio $C_l/F_1 = 1.3$. Callus flat, not protruding. Mesoventrite lobe straight. Metaventrite and ventrites one median depressed, with reduced vestiture. Trochanters and the ventral surface of femoral tooth covered with elongate hair-like scales. Tibial uncus very small, reduced in all legs.

Distribution: Brazil.

Biology: Reared from figs of *Ficus* section *Americana*.

Ceratopus sp24 sp.nov. (Figure 3.4 I-3.6 f).

Material examined: (**Fresh material**) BRAZIL, Acre, Sena Madureira. 20.XI.2014. 198m. -9,134144112 -68,72991881. col. Cruaud, Farache, Pereira & Rasplus ex. *Ficus boliviana* n° AC202.

Diagnostic description: Length 8 mm. Deep brown. Dorsal part of the body covered by yellowish and gray vestiture, dappled with small patches of white scales, forming an alternate pattern on each interstriae. Ventral part of the body densely covered with whitish vestiture. Rostrum covered with vestiture until antenna sockets, delicate on its ventral surface. Club oval, small, ratio $C_l/F_1 = 0.5$. Rostrum without longitudinal striae, finely rough, somewhat

flattened. Epistome with an abrupt median projection. Scutelar shield reduced. Elytral callus inconspicuous. Mesoventrite lobe strongly protruding, saddle shaped. Metaventrite carina acute, with a protruding ridge over the metacoxae. Metaventrite and ventrite one median depressed. Metatibial uncus reduced.

Distribution: Brazil.

Biology: Reared from figs of *Ficus boliviana*.

Notes: One male. Known only by the type specimen.

Ceratopus sp25 sp.nov. (Figure 3.4 J-3.6 n).

Material examined: (**Fresh material**) ECUADOR, Napo. 05.XII.2014. -0,57025 -77,8728.col. Cruaud A. & Rasplus J.Y. ex. *Ficus cuatrecasana* n° JRAS06132.

Diagnostic description: Length 6 mm. Deep brown. Dorsal part of the body covered by black vestiture, irregularly intermixed with light yellowish-brown scales. Callus with a small tuft of golden scales. Ventral part of the body densely covered with whitish vetiture. Rostrum covered with thick vestiture, including its ventral surface. Club small, ratio $C_1/F_1 = 0.6$. Rostrum thick, blunt at the end, with strong longitudinal striae. Epistome median projected over the mandibles, margin of the projection sinuate. Scutelar shield round, reduced. Elytral callus softly raised. Mesoventrite lobe protruding rounded.

Distribution: Ecuador.

Biology: Associated with fruits of *Ficus cuatrecasana*.

Notes: One male. Known only by the type specimen.

Ceratopus sp26 sp.nov. (Figure 3.4 L-3.6 O).

Material examined: (**Fresh material**). BRAZIL, Bahia, Boa Nova. 18.II.2016. S14°20'56.4" W040°12'59.4" col. Anderson Machado. ex. *Ficus caatingae* (**MZUSP**) BRAZIL, Minas Gerais, Serra do Caraça. 5.XII.1972.

Diagnostic description: Length 5.5 mm. Reddish-brown, uniformly tainted. Body covered with light yellowish-brown vestiture, with an intricate dorsal color pattern. Scutelar shield white. Elytra with a few patches of white scales along interstria one; also with two

strong oblique spots of alternate brown and black scales on its apical third, extending from interstria three to eight. The spots are well delimited, surrounded by white scales. Callus densely covered by white scales at apex. Rostrum covered with vestiture until antenna sockets. Club very large, ratio $C_1/F_1 = 1.1$. Funicle one strongly clavate, larger than others. Rostrum with longitudinal striae, smooth at the apical third. Elytral callus subtly protruding, acute (thorn-shaped). Mesoventrite lobe gently bilobate.

Distribution: Brazil.

Biology: Reared from figs of *Ficus caatingae*.

Ceratopus fulvus Hustache, 1929 (Holotype, MNHN, male, Guadeloupe, examined).

Biology: unknown

Notes: The type for this species is glued in a card and not well preserved, making it impossible to observe the diagnostic characteristics without damaging the specimen. For this reason, we could not make a description neither associate it with any of the species found.

Ceratopus litura Chevrolat, 1877 is a synonym for *Embates litura* Chevrolat, 1877.

Notes: This species do not represent a *Ceratopus* and was transferred to the genus *Embates* (Baridinae) by Kuschel (1955).

3.4. Discussion

We presented a review of the genus *Ceratopus* including 18 new species and synonymizing six others. Therefore, the present study expanded the 17 valid species of *Ceratopus* (O'Brien & Wibmer, 1982; Wibmer & O'Brien, 1986) to 27 species. Some species of the genus have strong sexual dimorphism, with males and females varying their morphology considerably, which may have led to misidentification and confusion in the past (Champion, 1902; Hustache, 1940; Bondar, 1947). Our species hypothesis are based on the whole life cycle of the beetles, since we reared the specimens from the larval stage for several species, reducing the chances of misassociation between males and females. Our morphological species delimitation was strongly supported by molecular results (Section 4),

as all species included in the phylogenetic analysis were recovered as monophyletic. Some of the species, however, seem to be composed by a complex of species or subspecies (*e.g.*, *Ceratopus dorytomoides*, *Ceratopus* sp8; Section 4).

The association with fig trees had a great influence on the evolution of the genus, confirming our earlier observation that *Ceratopus* species are specific to each section of Neotropical *Ficus* (*i.e.* *Americana* and *Pharmacosycea*; Palmieri, 2012). In spite of some species being generalists within each section of fig, *Ceratopus* do not utilize hosts from more than one section. This pattern of host association is reflected on the molecular phylogeny of the genus (Section 4) and has morphological support as well. The species associated with section *Pharmacosycea* have as a simpler pattern of vestiture, often composed by a single color covering the entire body that differs only at the callus region. In this group the mesoventrite lobe is more developed, protruding, sometimes bilobate. On the other hand, the species associated with section *Americana* are generally smaller with an intricate pattern of vestiture covering their bodies. Many species on this group present a setose trochanter and long clubs often exceeding the length of the funicle.

Certainly the number of species of *Ceratopus* is far larger than the number presented here. We examined around 30 sufficient fig samples (+200 figs each) of Neotropical fig trees and obtained *Ceratopus* individuals from all those crops. The number of species will surely increase with more sampling of figs and maybe even other Moraceae species. Lastly, we recommend a meticulous analysis of all Ceratopodini under both morphological and molecular phylogenetic framework. This will improve our understanding about the phylogenetic affinity of the tribe, helping us to solve the intricate problem of Curculionidae classification.

Figure 3.3. Dorsal view of *Ceratopus*. (A) *C. bisignatus*. (B) *C. bondari*. (C) *C. sampsoni*. (D) *C. mixtus*. (E) *C. maculicolis*. (F) *C. rufirostris*. (G) *C. dorytomoides*. (H) *Ceratopus* sp2. (I) *C. longiclava*. (J) *C. crassipes*. (K) *Ceratopus* sp8. (L) *Ceratopus* sp10. (M) *Ceratopus* sp11. (N) *Ceratopus* sp12. (O) *Ceratopus* sp13. (P) *Ceratopus* sp14. Scale bars representing 1 mm.

Figure 3. 4. Dorsal view of *Ceratopus*. (A) *Ceratopus* sp15. (B) *C. tessellatus*. (C) *Ceratopus* sp18. (D) *Ceratopus* sp19. (E) *Ceratopus* sp20. (F) *Ceratopus* sp21. (G) *Ceratopus* sp22. (H) *Ceratopus* sp23. (I) *Ceratopus* sp24. (J) *Ceratopus* sp25. (L) *Ceratopus* sp26. Scale bars representing 1 mm.

Figure 3.5. Lateral habitus of *Ceratopus*. Males are indicated by small letters; females indicated by capital letters. (a, A) *C. bisignatus*. (b, B) *C. bondari*. (c, C) *C. sampsoni*, (c1) rostrum detail, (c2) ventral view showing the black spot at the 5th ventrite. (d, D) *C. mixtus*. (e, E) *C. maculicollis*. (F) *C. rufirostris*. (g, G) *C. dorytomoides*. (h, H) *C. sp2*. (i, I) *C. longiclava*. (j, J) *C. crassipes*. (k, K) *Ceratopus* sp8. (l, L) *Ceratopus* sp10. Scale bars representing 1 mm.

Figure 3.6. Lateral habitus of *Ceratopus*. Males are indicated by small letters; females indicated by capital letters. (a, A) *Ceratopus* sp11. (b, B) *Ceratopus* sp12. (c, C) *Ceratopus* sp13. (d, D) *Ceratopus* sp14. (e, E) *Ceratopus* sp15. (F) *Ceratopus* sp24. (g, G) *Ceratopus* sp18. (h, H) *Ceratopus* sp19. (i, I) *Ceratopus* sp20. (j, J) *Ceratopus* sp21. (k, K) *Ceratopus* sp22. (l, L) *Ceratopus* sp23. (m, M) *C. tessellatus*. (n) *Ceratopus* sp25. (O) *Ceratopus* sp26. Scale bars representing 1 mm.

Systematics and Biogeography of *Ceratopus*

4.1. Introduction

Ceratopus Schönherr, 1843 (Curculionidae, Curculioninae) is a small genus of weevils that includes 17 species exclusively distributed in the Neotropical region (O'Brien & Wibmer, 1982; Wibmer & O'Brien, 1986). They distinguish from other weevils by the presence of a strong femoral tooth on every leg. The margin of the tooth is never serrate as in other Curculionidae groups (*e.g.*, Camarotini and Prionobrachiini) and its inner part has a hollow region to fit the correspondent tibia when the insect rests. They also have divaricate and appendiculate tarsal claws, an ascendant mesepimeron and a compact antennal club with asymmetric sutures between segments.

Ceratopus species are associated with the two Neotropical sections of fig trees (*i.e.*, *Americana* and *Pharmacosycea*) (Lima, 1956; Pakaluk & Carlow, 1994), and their larvae develop inside figs, feeding mostly on the fig wall, but sometimes reaching the fig lumen. Although they are specialized in both sections of figs, they do not present a species-specific association with their hosts (see Section 3 for details).

The genus was first included in the Ceratopodini tribe by Locardaire (1863). Since the establishment of the tribe, a number of genera have been transferred to other tribes or subfamilies and several new species have been described. In the most recent catalogue of Curculionoidea, six genera (*Aetiomerus* Pascoe, 1886; *Anthomelus* Hustache, 1920; *Neoanthomelus* Hustache, 1933; *Catiline* Champion, 1906; *Ceratopus*, and *Stelechodes* Faust, 1899) are considered valid for the tribe (Alonso-Zarazaga & Lyal, 1999); the inclusion of *Anthomelus*, *Neoanthomelus* and *Stelechodes* within Ceratopodini, however, is subject of debate (Caldara et al., 2014). Although *Ceratopus* species present characteristics of Curculioninae, such as long and slender rostrum and ascendant mesepimeron, they also show characteristics of Molytinae, as rostrum with an enlarged basis, body densely covered by oblong scales and protruding interstria. Thus, despite the inclusion of Ceratopodini in the Curculioninae subfamily, its phylogenetic positioning is uncertain and a comprehensive phylogenetic analysis of all Ceratopodini is still needed (Caldara et al., 2014).

Ceratopus was never analyzed under a phylogenetic framework. In fact, since the description of most of the species at the end of 19th and beginning of 20th century (Schoenherr & Gyllenhaal, 1843; Champion, 1902; Hustache, 1938, 1940), few studies were addressed to their classification. Among more recent studies on the tribe, it can be mentioned the morphological description of the larvae (Pakaluk & Carlow, 1994) and an identification key based on morphological characters of all Neotropical Curculionidae (Marvaldi & Lanteri, 2005). Here we analyze data from five genes of *Ceratopus* species in order to explore their phylogenetic relationships. Additionally, we reconstruct the historical biogeography of *Ceratopus* under a phylogenetic framework trying to understand how the lineages evolved and how they are distributed along Neotropics.

4.2. Material and Methods

4.2.1. Species distribution

As larvae of *Ceratopus* can be easily found developing inside near ripe and ripe figs (D-F Phases), we performed unsystematic walks in several localities (Appendix 2) of Neotropics in order to find fig trees in the adequate reproductive stage. After collection, the figs were put in a cloth-covered plastic box with a thin layer of soil and checked periodically for the emergence of adult beetles (*ca.* 35 days), which were then fixed in 95% ethanol. In order to improve our distribution map, we included data on host association and localities found on the labels of *Ceratopus* specimens analyzed at the natural history collections visited (Appendix 1); these data were used only when the association between the weevil and its host fig tree could be determined indubitably.

4.2.2. Phylogenetic analyses and divergence time estimate

The data set for the phylogenetic reconstructions contained 41 sequences of fresh specimens of *Ceratopus* plus 24 available sequences of the genus obtained from GenBank. We also added nine out groups, totalizing 75 terminals. The out groups were composed by six species with our most complete sequences of *Curculio* (Curculionini, Curculioninae; Section 5) and three species of *Omophorus* (Metatygini, Molytynae; Section 2) selected as a far group. The sequences were combined into five partitions, one with mitochondrial genes Cytochrome b (Cytb) and Cytochrome c oxidase subunit I (COI), another one containing the ribosomal rDNA (28S) and three other partitions for the nuclear genes Arginine Kinase (ArgK), Elongation factor 1- α (EF1- α) and Phosphoglycerate mutase (Pglym). For the extraction methods and amplification setups see section 2.2.1 and Appendix 3. The best nucleotide-

substitution model of each partition was determined by the Akaike information criterion (AIC) using MrAIC v1.4.6 (Nylander, 2004) together with PhyML v3.0 (Guindon et al., 2010). The phylogenetic analyses were carried out with RAxML v8.2.8 (Stamatakis, 2014) using the maximum likelihood optimality criterion to build the trees (default set up adopting a GTRCAT mixed/partitioned model and 1000 bootstrap). After the analysis the rogue taxa were pruned out of the tree using RogueNaRok (Aberer et al., 2013) to improve the support of the clades. All analyses were conducted on CIPRES (Miller et al., 2010).

As no *Ceratopus* fossil is available and the phylogenetic positioning of the genus is uncertain, which prevent us to use an external calibration point, the time of divergence of the stem *Ceratopus* was discussed according to the results of the fossil calibrated Chronogram obtained in our analysis of Curculionoidea (section 2.3.3 and Table 2.3). The ages used were 88.0 Ma (134.9-52.5) for the stem *Ceratopus* and 48.2 Ma (75.1-25.5) for the age of diversification of crown *Ceratopus*.

4.2.3. Areas definition and biogeographical inference

The Neotropical region is far from being a biogeographical unit (Amorim & Pires, 1996; Nihei & De Carvalho, 2007; Morrone, 2014a), and thus we divided *Ceratopus* distribution within eight areas of endemism based on the regionalization of the Neotropical region proposed by Morrone (2014b; Figure 4.1). The following areas were defined: (A) Mexican transition zone; (B) Antillean subregion; (C) Mesoamerican dominion; (D) Pacific dominion; (E) Boreal Brazilian dominion; (F) South Brazilian dominion; (G) Chacoan dominion; and (H) Parana dominion. The choice of those areas of endemism is justified by their congruence on the distribution of several taxa of insects, plants and vertebrates (Morrone, 2014a).

To reconstruct the geographical range evolution of *Ceratopus*, we applied the models DIVALIKE and DIVALIKE+J of the BioGeoBEARS package (Matzke, 2013a) implemented on R (R Development Core Team, 2015). We applied only the Dispersion Vicariance analysis (DIVA), which does not require the time to be present (Ree & Sanmartín, 2009; Almeida, 2016), because the time of divergence among *Ceratopus* lineages could not be independently inferred. We ran the models multiple times, varying parameters as presence and absence of sympatric speciation, strict vicariance or jump dispersal to see which model would present higher likelihood value. As many species of *Ceratopus* presented a wide geographical distribution (Section 3), and sometimes the museum labels presented confusing information, we also took a more conservative approach regarding the species distribution. We ran the

biogeographical analysis considering only the collection sites of the fresh specimens, disregarding any area association beyond this.

Figure 4.1. Areas of endemism for the Neotropical region used in this study. Biogeographical regionalization as proposed by Morrone (2014b).

4.3. Results

4.3.1. Species distribution

Ceratopus is the only genus of weevils associated with the fruits of fig trees in the Neotropics. We collected more than 200 fruit sets of Neotropical figs and were able to rear more than a thousand individuals from 27 species of *Ceratopus* (see Section 3 for details). Although this association was already described (Lima, 1956; Pakaluk & Carlow, 1994), the present study increases in 59% the number of 17 described species in the last checklist for the genus (O'Brien & Wibmer, 1982; Wibmer & O'Brien, 1986). The analysis of the collections shows that most species of *Ceratopus* have a wide distribution, ranging from the tropical areas of Mexico to the southern Atlantic Forest of Brazil (Figure 4.2). The results of the molecular reconstructions, however, show that some of the species may in fact represent species complexes or subspecies, which led us to make an interpretation about *Ceratopus* species distribution more carefully (see discussion below).

4.3.2. Phylogenetic reconstructions

The tree topology is well resolved with strong support for the relationships of the main lineages within the genus (Figure 4.3). Species associated to sect. *Pharmacosycea* (Figure 4.3 B) clustered inside the major clade of species associated with sect. *Americana* (Figure 4.3). Although specialized on figs, *Ceratopus* host range within each *Ficus* section shows that their association is not species specific, with each species of *Ceratopus* associated with overlapping host figs along the tree (see host associations on Figure 4.3).

The three major clades found here with molecular data have morphological support. Clade A is composed by large beetles of coarse aspect and thick rostrum when compared with other species of *Ceratopus*. Both of the species that compose this clade present a median projection of the epistome over the mandibles (Section 3, Figure 3.1). Clade B, or *Pharmacosycea* clade, has also some exclusive characteristics, such as a simple pattern of vestiture and a single color often covering the entire body, being different only at the callus region. In this group the mesoventrite lobe is more developed, protruding, sometimes bilobate. Lastly, at Clade C, which contains the vast majority of species, the beetles are smaller with an intricate pattern of vestiture covering their bodies. Many species of this group present setose trochanter and long clubs often exceeding the length of the funicle (Section 3).

Figure 4.2. Distribution of *Ceratopus*. Circles indicate the occurrence of the genus according the information found at the museum collections (AMNH, MZUSP, NMNH, MNHN, and INBIO). Triangles indicate collection sites of fresh specimens.

Despite morphological congruence, our data suggest that some *Ceratopus* species may be formed by species complex or subspecies. *Ceratopus dorytomoides*, for instance, presented a slightly morphological variation among the populations of Central America and Southern Brazil. Those differences are reflected on the phylogram which shows that those populations may no longer be in contact. The same phenomenon can be observed in *Ceratopus* sp2, *Ceratopus* sp8 and *C. tessellatus* (Figure 4.3).

C

B

A

Figure 4.3. Phylogram showing phylogenetic relationships of *Ceratopus* (RAxML). The numbers indicate the support of the nodes (bootstrap). Following the name of *Ceratopus* species are the host *Ficus* and the collection site of the specimen. Clades A and C represent groups of species associated with *Americana* section; Clade B is a group of species associated with *Pharmacosycea* section (gray shade). BR = Brazil, ac – Acre, sp – Sao Paulo, rj – Rio de Janeiro; CR = Costa Rica, li – Limon, pt – Puntarenas, sj – San Jose, hr – Heredia; EC = Ecuador, np – Napo, pc – Pichincha; PN = Panama, bc – Barro Colorado, ch – Chiriqui, bt – Bocas del Toro.

4.3.3. Biogeography

None of the models according to DIVA geographical range reconstructions elucidated the ancestral distribution of *Ceratopus*. Both biogeographical approaches, the one with all distribution information and the more conservative one, could not detect any specific trend among areas. The model with the highest likelihood was achieved using a more conservative approach with no sympatric speciation and widespread vicariance (Table 4.1). Still, a specific trend among the areas could not be traced and the ancestral reconstruction of areas remains inconclusive (Figure 4.4). Our results also show that dispersal might have played an important role on *Ceratopus* diversification, once the models that best fit our data (DIVALIKE+J, with higher likelihood) have a strong dispersal component (Table 4.1). The results of a more complex scenario regarding all distribution information of *Ceratopus* are show on Appendix 5.

Table 4.1. Comparison of the best fitting models of DIVA analysis calculated on BioGeoBEARS. (d) Dispersion. (e) Extinction. (J) Jump dispersion/ founder event. (Lnl) Likelihood log.

	d	e	J	Lnl
Model 1	0.017	0	0.144	-44.37
Model 2	0.0109	0	0.1784	-45.69
Model 3	0.0131	0	0.0715	-47.97
Model 4	0.0683	0.009	0	-60.43
Model 5	0.0744	0	0.1292	-87.65
Model 6	0.0735	0	0.1406	-87.66
Model 7	0.0838	0	0.0302	-88.95
Model 8	0.0875	0	0.0269	-89.44
Model 9	0.0905	0	0	-89.72

Figure 4.4. Simplified cladogram showing the ancestral area reconstruction of *Ceratopus* (DIVA) using a more conservative scenario of distribution. The pie charts at nodes represent the likelihood of each biogeographic region calculated with BioGeoBEARS. The mix of colors on the charts represents uncertainty. Areas of endemism extracted from Morrone (2014b). For a more complex scenario refer to Appendix 5.

4.4. Discussion

We present the first phylogenetic reconstruction of *Ceratopus*. Our results show that the host association with figs had great influence on the evolution of the genus, confirming our earlier observations that *Ceratopus* species are specific to each section of Neotropical *Ficus* (i.e. *Americana* and *Pharmacosycea*; Palmieri, 2012). The two lineages of weevils are not, however, sister groups as we previously thought, since the species associated with *Pharmacosycea* hosts are nested inside a larger clade of species associated with *Americana* hosts. Although it has long been suggested that host associations play a major rule on the diversification of Curculionoidea (Farrell, 1998; Marvaldi et al., 2002; Mckenna et al., 2009), those associations should be interpreted with caution. Any generalization concerning the evolution of the groups should be made under rigorous methodological approaches to avoid

anecdotal conclusions (Franz & Engel, 2010; Gunter et al., 2015). Curculionoidea is a megadiverse group with an extremely complex evolutionary history (Oberprieler et al., 2007), and to better understand this history researchers should focus on small taxonomic unities as tribes or genera (Franz & Engel, 2010). In this sense, we will discuss the radiation of *Ceratopus* on figs compiling the best evidence available to formulate our hypothesis.

Our divergence time estimate shows that the stem lineages of *Ceratopus* are considerably older 88.0 Ma (134.9-52.5, section 2) than the stem lineages of Neotropical figs, section *Americana* 32.3 Ma (46.1-22.1; Cruaud et al., 2012). In fact, *Ceratopus* apparently is older than the estimate ages for *Ficus* 72.0 Ma (88.2-59.6, Zerega et al., 2005) and 74.9 Ma (101.9-60.0, Cruaud et al., 2012). We conclude that it is unlikely that the ancestral *Ceratopus* were associated with figs.

The age of diversification of the crown group of *Ceratopus* 48.2 Ma (75.1-25.5), considering the uncertainty of the method, is congruent with the ages of diversification of *Ficus* sec. *Americana* and its pollinator *Pegoscapus*, 32.3 Ma and 38.2 Ma respectively (Cruaud et al., 2012). At this time, mid to late Eocene, the global temperature was warmer than today (Zachos et al., 2001) and a belt of boreotropical flora facilitated floristic dispersal of many lineages of plants from North to South America via proto-Greater Antilles and GAARlandia (Antonelli et al., 2009). This route of dispersal helped many Moraceae, including fig trees, to reach the Neotropical region (Zerega et al., 2005; Cruaud et al., 2012; Fiaschi et al., 2016), and it was probably around this time that *Ceratopus* adapted to use figs as hosts. We can hypothesize three different scenarios for the beginning of the association between *Ceratopus* and figs: (1) the ancestral lineages of beetles dispersed to South America from North America together with their hosts; (2) they reached South America through temperate faunistic exchanges via Antarctica as other groups of insects (Cruaud et al., 2011; Almeida et al., 2012) and then adapted to figs; (3) they were already at the Neotropical region diversifying in association with different hosts since the break of Gondwana and shifted to figs when they arrived in South America at the end of Eocene (Cruaud et al., 2012).

Although our biogeographical results do not allow us to establish the more likely scenario, there is evidence toward hypothesis three. The fact that all *Ceratopus* species are exclusively Neotropical and associated only with the two endemic sections of Neotropical figs, suggests that the association occurred after, or at the same time, the fig lineages diversified in South America. The other genera of Ceratopodini (*Aetiomerus* and *Catiline*) are also endemic to the Neotropics and not associated with figs, indicating that Ceratopodini may have had time to diversify before their association with figs. The other two Ceratopodini-like forms (*Anthomelus* and *Stelechodes*) are reported from Madagascar and Papua New-Guinea,

components of Madagascan and Australian regions, showing that a post-Gondwana brake origin for the tribe is plausible but also corroborating hypothesis two. However, a more recent diversification of Ceratopodini with a host shift occurring after the radiation of *Ceratopus* on figs may not be ruled out yet.

After adapting to figs, *Ceratopus* diversified with their hosts within the Neotropical region during the Miocene. It is interesting to note that the species associated with section *Pharmacosycea* cluster inside the clade of species associated with *Americana*, which brings insights about the evolution of Neotropical figs. The positioning of *Pharmacosycea* as sister of all fig trees is regarded as uncertain (Cruaud et al., 2012) and it is still a controversial subject on fig phylogeny. In this context, our results are an independent evidence suggesting that diversification of *Pharmacosycea* could be more recent than *Americana* one.

Our results allowed us to hypothesize different scenarios of radiation of *Ceratopus* on fig trees. Although we used the best evidence to reconstruct the ancestral distributions, the best scenario remained inconclusive. Most of the species we used in our molecular analysis have a widespread distribution and are present along the entire Neotropical region (Section 3). Areas of endemism represents the functional unity of a biogeographical analysis (Harold, 1994; De Carvalho, 2016), thus widespread taxa represents a serious methodological problem (Nelson & Platnick, 1981; Harold, 1994; Nihei, 2016), once they bring uncertainty and decrease the resolution of the analysis. Lack of strong empirical support weakens predictive power and makes even harder the task of understanding how the geographical range evolution occurs. We believe that a more comprehensive sampling, including other genera of Ceratopodini associated with different Neotropical hosts, may help elucidate the intricate biogeography of the tribe and their associated hosts.

Historical biogeography of Curculionini

5.1. Introduction

Curculionini is a cosmopolitan tribe of weevils including approximately 15 genera and about 740 species, with the greatest contribution for the tribe diversity coming from *Curculio* L. (ca. 500 species; Caldara et al., 2014). They are commonly distinguished from other weevils mainly by a very long and slender rostrum; seven-segmented funiculus; contiguous forecoxa; a large and ascending mesepimeron; teeth-armed femora; broad flat tarsi; divaricate and appendiculate tarsal claws; exposed pygidium (male); and, their most striking feature, small triangular, vertically moving mandibles (Pelsue & O'Brien, 2011). None of these morphological traits, however, constitute true synapomorphies to the group, and some genera lack one or several of those characteristics. Traditionally, the tribe is divided into three subtribes: Curculionina, Pseudobalaninina, and Timolina (Heller, 1925). More recently, three additional subtribes were proposed – Archariina, Erganiina and Labaninina (Pelsue & O'Brien, 2011) – but this division is not based on strong, well-supported synapomorphies, making the delimitation of subtribes not clear. In fact, Caldara et al. (2014) pointed out that both a revision and a phylogenetic treatment are necessary to define monophyletic groups and to understand the relationships among genera of Curculionini.

The majority of the species of Curculionini are associated with host-plants belonging to the order Fagales (e.g., Betulaceae, Fagaceae and Juglandaceae; Hughes & Vogler, 2004). Other species of the tribe are associated with fig trees (Moraceae; Rasplus et al., 2003) and other genera of plants like *Diospyros* L., *Eclea* L. (Ebanaceae; Caldara et al., 2014) and *Camellia* (Theaceae; Toju et al., 2013); some Curculionini species induce galls or live as inquiline on galls in oaks and willows (Fagaceae and Salicaceae respectively; Hughes & Vogler, 2004). Females of these weevils use their long rostrum to excavate an oviposition site, where for the larvae develop inside the host tissue (flower buds, fruits or galls) until it is ready to pupate. In fact, the rostrum is probably a key adaptation related to the evolutionary success of Curculionidae (Anderson, 1995; Oberprieler et al., 2007). For the genus *Curculio*, it has been shown that the imbalance between the length of the rostrum and the thickness of

the host fruit wall determine the strength of local selection (Toju & Sota, 2006a), which in a long term evolution would have contributed to the increase of species diversity. The huge polymorphism of *Curculio*, especially in relation to rostrum length, provides an unambiguous example of adaptive radiation (Hughes & Vogler, 2004; Toju & Sota, 2006b), for which two major routes have been pointed out: the Afrotropical species of *Curculio* are believed to have radiated on tropical fig trees while a second lineage radiated on temperate Fagales (Perrin, 1992). The African fig feeding species of *Curculio* are suggested to be a sister group of all seed feeding species (Hughes & Vogler, 2004).

None of those hypotheses, however, were tested under a phylogenetic framework. In fact, most of the systematic and taxonomic studies with Curculionini were published more than one hundred years ago (Pelsue & O'Brien, 2011). Among the more recent work with the tribe, it is worth mentioning a molecular phylogenetic reconstruction of Holarctic *Curculio* (Hughes & Vogler, 2004); a study of diversification of endosymbiosis in Curculionini (Toju et al., 2013); and the morphologic review of some Palearctic and Oriental genera of Curculionini (Pelsue & Zhang, 2000b, 2002, 2003, Pelsue, 2001, 2004, 2005, 2009). Here we combined molecular data from fig feeding Curculionini we collected during field expeditions and data from the literature to explore the phylogenetic relationships within the tribe. We reconstruct the historical biogeography of Curculionini under a phylogenetic framework trying to understand how the lineages have evolved and are distributed worldwide. We hope to contribute to a better understanding of Curculionini evolution and to facilitate future works dealing with the classification of the tribe.

5.2. Material and Methods

5.2.1. Species distribution

To reconstruct the historical biogeography of Curculionini we gathered information about fresh fig-weevils we collected during field expeditions (section 2.2.1 for details) and combined it with public sequences of GenBank. We searched for sequences of all the 15 genera that compose the tribe according to Caldara et al., (2014). We inquire GenBank database for the following genera: *Curculio* (ca. 500 described species); *Archarius* Gistel (ca. 10); *Carponinus* Heller (ca. 50); *Ergania* Pascoe (17); *Indocurculio* Pajni, Singh & Gandhi (10); *Koreoculio* Kwon & Lee (2); *Labaninus* Morimoto (14); *Pagumia* Kwon & Lee (1); *Pimelata* Pascoe (1); *Pseudoculio* Pelsue & O'Brien (7); *Shigizo* Morimoto (14); *Pycnochirus* Berg (ca. 2); *Timola* Pascoe (ca. 30); *Aviranus* Fairmaire (ca. 20); and *Pseudobalaninus* Faust (ca. 40).

5.2.2. Phylogenetic analyses and divergence time estimate

For the phylogenetic reconstructions, we grouped the sequences of Afrotropical and Oriental fig-weevils (16 spp.; Genera: *Curculio*, *Carponinus* and *Indocurculio*) and public sequences of Holarctic Curculionini (40 spp.; Hughes & Vogler, 2004; Toju *et al.*, 2013; Genera: *Archarius*, *Curculio* and *Koreoculio*), along with four species used as out groups, totalizing 60 terminals. Thus we included sequences from five of the 15 genera of the tribe. The outgroup was composed by two species with the most complete sequences of *Ceratopus* Schoenherr (Ceratopodini, Curculioninae) and two species of *Omophorus* Schoenherr (Metatygini, Molytinae) selected as a far group.

The data set was combined into four partitions, one with mitochondrial genes Cytochrome b (Cytb) and Cytochrome c oxidase subunit I (COI) coupled together, another one containing the ribosomal rDNA (28S) and two other partitions with the nuclear genes Elongation factor 1- α (EF1- α) and Phosphoglycerate mutase (Pglym). For the extraction methods and amplification setups see section 2.2.1 and Appendix 3. The best nucleotide-substitution model of each partition was determined by the Akaike information criterion (Akaike, 1973) using MrAIC v1.4.6 (Nylander, 2004) together with PhyML v3.0 (Guindon *et al.*, 2010). The phylogenetic analyses were carried out with RAxML v8.2.8 (Stamatakis, 2014) using the maximum likelihood optimality criterion to build the trees (default setup adopting a GTRCAT mixed/partitioned model and 1000 bootstrap). After the analysis, the rogue taxa were pruned out of the tree using RogueNaRok (Aberer *et al.*, 2013) to improve the support of the clades.

The times of divergence among Curculionini lineages were estimated using the Bayesian relaxed molecular clock method with two parallel runs as implemented in BEAST 2 v2.4.4 (Drummond *et al.*, 2006; Bouckaert *et al.*, 2014). Before setting up the analysis on BEAST, we estimated the number of relaxed clock models that best applied to our data employing ClockstaR (Duchêne *et al.*, 2014). Substitution models were unlinked among partitions while the trees and clocks models were linked; we also specified a Yule process of speciation with a random starting tree prior. As many of the oldest weevil fossils can be misidentified (Oberprieler *et al.*, 2007) and estimations of the ages of the groups can largely diverge (Gunter *et al.*, 2015; Legalov, 2015), in our analysis the most conservative reliable calibration possible was applied as the maximum bounds and the most phylogenetic-near-accurate groups as the minimum bounds (as suggested by Phillips 2015). We used the split between Brentidae and Curculionidae families to constrain the maximum bounds of two calibration nodes, setting 113 Ma as the estimate age of diversification of stem Curculionidae

based on the brentid *Axelrodiellus ruptus* Zherikhin & Gratshev, 2004 from lower Cretaceous (Aptian) and the curculionid *Arariperhinus monnei* Santos, Mermudes & Fonseca, 2011 from the same deposit. The first phylogenetic-accurate calibration was taken from *Curculio havighorstensis* Zherikhin, 1995 from lower Eocene (Ypresian) to set the minimum prior age of 47.8 Ma to the stem node of all Holarctic Curculionini. The second calibration was set at the crown node of Nearctic *Curculio* based on an abundance of extinct species of *Curculio*-like-forms found in the Florissant formation from upper Eocene (Priabonian; Legalov, 2015) – establishing the minimum age as 33.9 Ma. The MCMCMC searches ran for 50 million generations (sampling all the values every 3,000 generations) with the first 35% discarded as burn-in. We used Tracer 1.6 (Rambaut *et al.* 2014) to verify the convergence and stationarity of the chains, evaluating the effective sample size (ESS) scores and the stability of results after multiple runs. The log files from the two independent runs were combined using LogCombiner v2.4.4 and the maximum clade credibility tree with mean heights was built using TreeAnnotator 2.4.4. The final consensus tree was obtained from 21,000 trees and the Chronogram was visualized and edited using FigTree v1.4.2. All analyses were conducted on CIPRES (Miller *et al.*, 2010).

5.2.3. Areas definition and biogeographical inference

The Curculionini, especially *Curculio*, are composed by wide-range distribution species. In our analysis we divided the Curculionini distribution in five areas based on the biogeographical domains of the Earth, which also represent areas of endemism for several groups of species (Morrone, 2015). We defined: (1) Afrotropical region; (2) Oriental region; (3) Western Palearctic region; (4) Eastern Palearctic region; and (5) Nearctic region. The choice of large operational area units in our analysis is justified by the paleogeographical history of the continents. We also divided the Palearctic region in Western and Eastern to verify any specific biogeographical trends between those areas and the remaining.

For the biogeographic reconstructions, we used BioGeoBEARS package (Matzke, 2013a) implemented on R (R Development Core Team, 2015). BioGeoBEARS employs six event-based analytical models to reconstruct the historical biogeography of the taxa (Matzke, 2013b). To choose the model that better describes the geographical range evolution of Curculionini, we compared the Akaike Information Criterion (AIC) of the six probabilistic models implemented in the package. The Dispersal-Extinction-Cladogenesis (DEC) model (Ree *et al.*, 2005; Ree & Smith, 2008) implements likelihood methods for geographic range evolution on phylogenetic trees, inferring the rates of dispersal, local extinction and ancestral ranges. It uses the time-scale of the chronogram to estimate the probability of changes

between ancestral distributions along the branches. For this, DEC uses tree topology, branch lengths and clade distributions to test biogeographical parameters (*i.e.*, migratory events or local extinctions) and to maximize the probability of those parameters to fit the data. A second model available is a likelihood version of the Dispersal-Vicariance Analysis (DIVA; Ronquist, 1997). Originally, DIVA is a parsimony-based method that optimizes ancestral distributions on top of the phylogenetic tree nodes by favoring vicariance events in relation to dispersal and extinction events (Ronquist, 1997). On its implementation on BioGeoBEARS, however, the maximum likelihood optimality criterion is used to optimize the ancestral distributions instead of parsimony, thus originating the name DIVALIKE (Matzke, 2013b). BayArea (Landis et al., 2013), another method emulated, uses Bayesian inference to sample ancestral distributions along phylogenetic branches while estimating and comparing biogeographical parameters values of any given biogeographic model. In BioGeoBEARS, the sampling of distributions and the estimation of parameters of BayArea are made, once again, in a maximum likelihood framework, resulting in the name BayAreaLIKE (Matzke, 2013b). Finally, BioGeoBEARS also implement a “+J” version of all of these methods (DEC+J; DIVALIKE+J; BayAreaLIKE+J), which specifically include the founder-event speciation, a process that improve the accuracy of the models and is often left out of most inference methods (Matzke, 2014). The implementation of all those methods in a common likelihood framework allows a direct comparison of how well the different biogeography models represent the data (Matzke, 2013b).

5.3. Results and discussion

5.3.1. Species data

We collected at least 3 different genera of Curculionini from Afrotropical and Oriental figs: *Curculio*, *Carponinus* and *Indocurculio*. In the Afrotropical region the only Curculionini genus of fig-weevils registered was *Curculio*; at the Oriental figs, we also found *Curculio* and at least three other genera: *Carponinus*, *Indocurculio* and an unidentified form of Curculionini. Most of the larvae collected from the Oriental region took more than 60 days to develop, which led us to fix the larva and obtain only few adult individuals. Therefore, this unidentified form of Curculionini may represent a fourth genus. From the data gathered from GenBank we could obtain good sequences for only three genera, the Holarctic *Curculio*, *Archarius* and *Koreoculio*. In summary, our data set comprises five of the 15 genera of the tribe and 56 species (<10% of the total number of species). The other genera *Ergania*,

Labaninus, *Pagumia*, *Pimelata*, *Pseudoculio*, *Shigizo*, *Pycnochirus*, *Timola*, *Aviranus* and *Pseudobalaninus* are absent from our analysis.

5.3.2. Time and phylogenetic relationships of Curculionini

We recovered five well-supported clades within the Curculionini (Figure 5.1). The topology of the Curculionini tree indicates a great influence of the resource each group of species utilizes on their diversification. The fig-feeding African species of *Curculio* (A) were recovered as sister group of all other clades, confirming previously observations (Hughes & Vogler, 2004). The second clade to appear is the *Indocurculio* group (B), feeding mostly upon Oriental sections of fig trees (*e.g.* sect. *Conosycea* and sect. *Sycomorus*). The adaptation to figs, however, is not monophyletic and seems to occur at least two times in the history of the tribe: once in the ancestral Curculionini giving origin to the clades mentioned above and a second time in a possible recolonization of figs, section *Sycomorus*, by *Carponinus* group (C). The next clade, the *glandium* group (D), is formed by Palearctic species that consume a wide variety of plants and plant-parts from temperate families (*e.g.*, Betulaceae, Elaeagnaceae, Theaceae). The clade formed by species of the *Archarius*, group (E), which feeds on galls induced by other insects (Toju et al., 2013). The most iconic group of Curculionini, the acorn-associated *Curculio*, forms the *elephas* group (F), containing Holarctic species that feed mostly upon seeds of Fagaceae plants. Regarding *Curculio*, our results show that the genus is not monophyletic and must pass through a taxonomic scrutiny for a possible restructuration. We could recover, however, the two previously described European species groups *elephas* and *glandium* (Hughes & Vogler, 2004). We also are the first to recover the Nearctic curculios as monophyletic (Figure 5.1).

The inferred divergence ages indicate that the diversification of Curculionini began in the Upper Cretaceous around 100 Ma ago (Table 5.1). This result matches our previous dating estimate for the stem node of Curculionini (mean age of 98.5 Ma) established on the analyses of a larger data set from all Curculionoidea (Table 2.3, section 2.3). Most Curculionini lineages currently recognized as subtribes (Archariina, Curculionina and Pseudobalaninina) began to diverge during this initial period, and the differentiation of these major lineages occurred in a time span of about 25 Ma. It is interesting to note that, taking into account the uncertainty in the estimation of divergence times, the time of diversification of the fig-feeding curculionids (crown Clade B) and a possible recolonization of figs by *Carponinus* (stem Clade C) coincides with the age estimated to the diversification of fig trees and fig wasps (74.9 Ma and 75.1 Ma respectively; Cruaud et al., 2012). There is also a striking coincidence between the time of diversification of the Afrotropical group (crown Clade A) and the time

estimated to the diversification of the African fig section *Galoglychia* and its pollinators (around 30 Ma for both lineages; Cruaud et al., 2012). The *tempo* of diversification of temperate weevil species is also congruent with the diversification of Fagaceae, which had increased their range of distribution at the beginning of Cenozoic (72-66 Ma) and were overspread at the Holarctic region at that time (Manos & Stanford, 2001). The ancestral Curculionini was probably associated with early lineages of fig trees once the fig-wasp mutualism was already in existence in Eurasia during the Cretaceous (Cruaud et al., 2012). One evidence to support this hypothesis is that all basal lineages have figs as their main host (Figure 5.1). The first host shift seems to have occurred a little later still during Upper Cretaceous (88 Ma), between the fig-feeding species and the lineages that gave origin to the temperate groups; it was during this time that the ancestral *Curculio* took the second pathway of specialization shifting hosts from figs to Fagales (Perrin, 1992). Another host shift, showed by the split between clades E and F, occurred at the same time the Fagaceae clade diversified (around 76 Ma), when the ancestral of *Archarius* and *Koreoculio* adapted to feed upon the galls of other insects (Figure 5.1 and Table 5.1).

Table 5.1. Distribution of mean age estimates inferred by BEAST for the main nodes of Curculionini, showing the upper and the lower highest posterior distribution limits. The clades are represented on Figure 5.1.

Node	Mean age Ma (95% HPD)	Upper HPD (min. age)	Lower HDP (max. age)
Stem Afrotropical Curculio	100.0	72.4	137.2
Crown Afrotropical Curculio	35.0	18.0	57.7
Split of clades AB\CDEF ¹	86.1	63.7	117.2
Split of clades CD\EF ²	80.8	61.2	111.3
Split of clades E\F ³	74.6	55.0	102.0
Crown <i>Indocurculio</i>	76.2	54.9	105.5
Stem <i>Carponinus</i>	71.4	51.2	98.1
Crown <i>Carponinus</i>	28.6	18.4	42.4
Crown <i>Archarius</i>	59.3	42.4	81.9
Crown <i>elephas</i> group ⁴	50.5	37.8	69.2
Crown <i>glandium</i> group	49.2	34.8	69.4

1 Estimate age of the shift from *Ficus* to Fagales.

2 Node assigned uniform calibration of minimum and maximum ages of 47.8 - 113 Ma.

3 Estimate age of the shift from Fagales to Insect galls.

4 Node assigned uniform calibration of minimum and maximum ages of 33.9 - 113 Ma.

Figure 5.1. Chronogram showing the phylogenetic relationships and time of diversification of Curculionini. Stars indicate bootstrap values (RAxML) > 70% and posterior probability (BEAST) > 95%. The color of the squares at the tip of each taxon indicates the present distribution. The host genus for the studied taxa is presented between brackets. Pie charts at the main nodes show the likelihood of ancestral areas as inferred by DIVALIKE+J (BioGeoBEARS). The clades (A) Afrotropical *Curculio*; (B) *Indocurculio* group; (C) *Carponinus* group; (D) *glandium* species group; (E) *Archarius* group; (F) *elephas* species group, are discussed on the text.

5.3.3. *Curculionini* Biogeography

According to BioGeoBEARS analysis, the model with the greater likelihood to explain the present distribution of *Curculionini* was DIVALIKE+J (Table 5.2). The second model with greater likelihood was BAYAREALIKE+J, almost equally possible and probably predicting similar results. Both models favor vicariance events allowing the addition of the founder-event speciation to improve the accuracy of the model. For this reason, to recreate the scenarios of geographical range evolution of *Curculionini*, we will restrict our hypothesis to vicariance events keeping long-range dispersal explanations to a minimum. The top three best fitting models, however, included the +J parameter, showing that long range or jump dispersal may have played an important role on the evolution of *Curculionini*. Our data suggest (Figure 5.1; pie charts) that the split between the African lineages of *Curculionini* and the rest of the tribe may have occurred by vicariance in the early Upper Cretaceous (100 Ma), when the populations who lived in the Southern islands of Tethys ocean lost connection with the populations who lived in North-Eastern Gondwana or at the islands connected to the northeast part of the proto-African continent. The break in the continuous distribution occurred probably by the rise of the sea level during this period (Haq et al., 1987). Sea levels around the early Upper Cretaceous raised more than 100 meters in an interval of only 15 Ma (Figure 5.2 A; Haq et al., 1987), and this event caused great part of the regions that today form Eastern-Europe, Southwest-Asia and North-Africa to be submerged, isolating the North regions of Laurasia from the dividing Gondwanaland (Baraboshkin et al., 2003). Thus, the sea level rise broke the continuous formed by tropical islands connecting Southern Laurasia and Northeastern Gondwana and may have isolated the ancestral *Curculionini* lineages that lived on the islands from those living on the continent. In fact, there is three other genera of *Curculionini* endemic to the Afrotropical region (e.g. *Timola*, *Aviranus* and *Pseudobalaninus*; Caldara et al., 2014), which reinforces that the separation of the African lineages may have happened early in the Upper Cretaceous. However, this evidence may also suggest a Gondwanan origin for the tribe once those genera are encountered only in continental Africa (*Timola*) and Madagascar (*Aviranus* and *Pseudobalaninus*). Yet, our results (Figure 5.1) favor that the initial diversification of *Curculionini* (Crown age interval 72.4-137.2 Ma, Table 5.1) occurred in Laurasia together with the first lineages of fig trees (Crown *Ficus* age interval 60.0-101.9; Cruaud et al., 2012); then they expanded their range of distribution southward to occupy the North-eastern part of Gondwanaland before the sea level rise in the Upper Cretaceous (Figure 5.2 A). The evidences to support our hypothesis are: (1) the vast majority of the *Curculionini* diversity is found in Palearctic and Oriental regions (Caldara et al., 2014); (2) *Curculionini* does not occur in South America except for the genus *Megaoculis* Pelsue &

O'Brien (2 spp.), which apparently does not fit into the tribe (Caldara et al., 2014); (3) all basal lineages of Curculionini are associated with figs; (4) there is fossil and molecular support for a Laurasian presence of figs during Upper Cretaceous (Zerega et al., 2005); (5) there is also congruence in the early split between the African figs section *Sycomorus* together with its pollinator *Ceratosolen* and the Asian *Sycidium* together with its pollinator *Kradibia* (Cruaud et al., 2012), even though the dates of divergence between fig/pollinator are a little more recent than the age of divergence in Curculionini; and (6) there is no Curculionini associated to Neotropical species of figs. However, a Gondwanan origin of Curculionini may still not be ruled out.

Table 5.2. Comparison among models of BioGeoBEARS. The parameters and the AIC of the chosen model are marked in bold.

Model	Likelihood log	Number of parameters	D	e	J	AIC	AIC wt.
DIVALIKE+J	-48	3	0	0	0.031	102	0.39
BAYAREALIKE+J	-48.08	3	0	$< 10^{-9}$	0.027	102.2	0.36
DEC+J	-48.42	3	0	$< 10^{-9}$	0.03	102.8	0.25
BAYAREALIKE	-59.83	2	0.0006	0.0009	0	123.7	$< 10^{-6}$
DIVALIKE	-63.15	2	0.0011	0	0	130.3	$< 10^{-7}$
DEC	-67.81	2	0.0009	0.0004	0	139.6	$< 10^{-9}$

The sea level was kept at its maximum height during the next 25 Ma (from 100 Ma to approximately 75 Ma; Figure 5.2) and other two main lineages of Curculionini arose at this time, one associated with the tropical component of the paleo flora (the ancestral of clade B; Figure 5.1) and another associated with the subtropical component (the ancestral of clades C, D, E and F; Figure 5.1). The tropical lineages remained associated with figs, diversifying together with its host until the present time (Clade B; Figure 5.1), and the subtropical lineages shifted their hosts and adapted to live in a wide variety of temperate families (Clade C, D, and F; Figure 5.1). During this long time of geological and climatic stability, both ancestral lineages expanded their range of distribution tracking their host plants (Figure 5.2 B). The fig-feeding species expanded southward advancing to Sundaland and probably Australia; and the seed-feeding species took a bidirectional route of expansion toward Europe and North America via the Bering Land Bridge (Figure 5.1 pie charts; Figure 5.2 B). The four evidences to support this scenario are (1) the striking diversification of the genus *Ficus* beginning at

Figure 5.2. Historical Biogeography of Curculionini, showing the main events on the geographical evolution of the tribe based on DIVALIKE+J results. The positions of the continents during the different periods of Earth history are not represented. Sea level variation adapted from Haq et al., 1987. For A, B, C and D descriptions refer to the text.

approximately 75 Ma (Cruaud et al., 2012) coinciding with the time of diversification of Asian fig-feeding lineages (stem *Indocurculio* 76.2 Ma and stem *Carponinus* 71.4 Ma; Table 5.1); (2) the wide distribution of the genus *Quercus* (Fagaceae) in boreal-tropical deciduous forest that extended throughout the northern hemisphere at the beginning of the Tertiary (Manos & Stanford, 2001); (3) the monophyly of the seed-feeding group indicating that the host shift from figs to Fagales occurred only once within the tribe (Figure 5.1); and (4) the presence in the fossil record of *Curculio elegans* (Menat, France) and *Curculio havighorstensis* (Havighost, Germany), from Paleocene and lower Eocene respectively (Legalov, 2015), indicating that the genus *Curculio* had already diversified at this time. Later on, while expanding West toward Europe, another host shift occurred and the *Archarius* group adapted to feed upon insect galls and started to differentiate over Palearctic hosts (Figure 5.1 and Figure 5.2 B).

Our data show the split between Palearctic and Nearctic species occurring at 50.5 Ma ago (37.8-69.2 Ma; Table 5.1), in a time known as Eocene climatic optimum (Zachos et al., 2001). At that time, the greenhouse effect was at its maximum and the temperatures on Earth were significantly higher than today (Hansen et al., 2008), with no ice occurring on the poles (Zachos et al., 2001). Many tropical families of plants increased their distributions and advanced over higher latitudes (Donoghue, 2008), which may be exemplified by the dispersal of fig trees to South America through North Atlantic bridges from Eurasia (Cruaud et al., 2012). On the other hand, the climatic optimum may have caused a retraction on the distribution of temperate lineages, as it is easier to move than to adapt to new climatic conditions (Donoghue, 2008). In this scenario, a vicariant break might have occurred between the Palearctic and Nearctic lineages of *Curculio*, which became isolated due to a climatic barrier that restricted their hosts' distribution during the early Eocene (Figure 5.1 and Figure 5.2 C). The greenhouse effect started to weaken around 45-40 Ma and the global temperature started to decrease (Zachos et al., 2001) as Antarctica returned to the polar position and started to accumulate ice (Boger, 2011). The climatic barriers for the temperate species plants began to fade and the populations started to disperse southward once again. This event coincides with the increase on the diversification of seed-feeding lineages of Curculionini around 40 Ma (Figure 5.1). As Earth became colder and the temperatures started to lower, the populations of temperate Fagales started to diversify once again at early Oligocene (Manos & Stanford, 2001). It was also at this time that the ancestral lineages of *Carponinus* (crown Calde C) started to move south, avoiding the cold climate, and diversify on tropical islands of Oriental region (e.g., The Philippines) at the end of Oligocene (Table 5.1 and Figure 5.1). This ecological dispersal southward during the Oligocene-Miocene transition is more striking

at the Nearctic region, where the Curculionini expanded their distribution until the mountains of Central America (Figure 5.2 D). Two evidences to support this scenario are (1) the abundance of *Curculio* in fossil record on Florissant deposits (Colorado, USA; Legalov, 2015); (2) the absence of *Curculio* in South America, which was not connected to North America at Oligocene.

5.4. Conclusion

Our results present multiple evidences of strong influence of host association on the evolution of Curculionini. We show that the cosmopolitan distribution of the tribe today is the result of a long and intricate history of range expansion and break of distributions. Curculionini and their most ancient host, the fig trees, seemed to have arisen during the Upper Cretaceous (Cruaud et al., 2012) and diversified together for a period of 20 Ma until the ancestral lineages of *Curculio* shifted to the early forms of Fagales. The two pathways of radiation, however, are not as simple as previously thought (Perrin, 1992). Instead of a double radiation of a single taxon (i.e. *Curculio*), our results show that several lineages of Curculionini are involved with the radiation to tropical and temperate environments. We were the first to combine a large number of sequences of different genera of Curculionini in a single phylogenetic analysis, especially of the groups associated with figs. Although we have strong evidence to build our radiation on figs scenarios, an older origin of Curculionini independent of figs cannot be ruled out. Our biogeographic hypothesis would be stronger if other groups of Curculionini, as the Australian fig-feeding *Curculio* (Zimmerman, 1994) and the African-endemic *Timola*, could have been included. Considering that adding at least those two groups would help test the Curculionini for a Gondwanan origin or an early fig association, we suggest that future studies include those groups as well as species from Madagascar and India.

Our results also show that the genus *Curculio* is not monophyletic and that it should go under meticulous taxonomic scrutiny, both morphological and molecular. It is very easy to identify and separate Curculionini from other Curculionidae, but to separate them into species is a very laborious task. We believe that the lack of synapomorphies to define Curculionini groups occurs until today because different monophyletic groups were being compared, which mislead the creation of well-supported synapomorphies. We hope our study will help to define the groups within Curculionini for future systematic studies of the tribe.

Final thoughts and future steps

The main objective of this thesis was to understand how the diversification of Curculionidae took place in fig trees. Our results present multiple evidences of a strong influence of host association on the evolution of fig-weevils. We also show that the fig-weevils have a long history of adaptive radiation on fig trees. Although their initial diversification is a little older than the diversification of figs, once weevils adapted on figs, they started to follow up their hosts in a similar pattern of radiation.

I believe that a few steps should be taken to improve our understanding of the biogeography of fig-weevils genera. First, in the Neotropics, a more comprehensive sampling of fresh specimens of *Ceratopus* will be crucial to elucidate the diversification of the lineages within the genus. An ideal scenario would include other Ceratopodini as well in the analyses, but their biology, being obscure, makes that a hard task. Secondly, for Curculionini, it would be best to gather a more complete sampling of Afrotropical and Australian specimens. Some Afrotropical endemic subtribes of Curculionini (*e.g.*, Timolina), for instance, could help clarify a probable Gondwanan origin of the tribe. Finally, a better sampling of *Omophorus*, a small genus with only six species described but presenting a vast continuous distribution area from South Africa to the Philipinnes, would probably result in the discovery of new species and maybe even break the current species in many others. That way, the pattern of diversification of *Omophorus* could be compared with the one of fig adapted Curculionini to check wether they are congruent, since both groups co-occur in the same sections of Afrotropical and Oriental figs.

The current paradigm states that the non-pollinating wasps exert the higher pressure over the fig-fig wasps mutualism. Although fig-weevils are not as specialized as other fig parasites (*e.g.*, the non-pollinating fig wasps), they also share an evolutionary history with fig trees. I consider that more detailed studies quantifying the real impact of fig-weevils on the *Ficus*-wasp relationship could bring insights about their role on the evolution of the mutualism.

Generally, as in any host-parasite association, fig parasites should trigger on its host defensive traits to decrease infestation. Fig trees, however, are dependent of a nursery pollination system where several pollinating wasps use their fruits to develop; hence, any

adaptation on the trees to deter fig parasites could also harm the pollinators. In this sense, fig defenses result of millions of years of delicate tuning between protection against parasites and preservation of pollinators. Regretably for the trees, it seems that weevils “discovered” a way of overcoming those defenses and exploiting the system without being penalized by it. The female weevil lays its eggs at the beginning of the interfloral stage, exactly at the same time the pollinating wasp larvae start to develop inside the fig. Possibly, this behavior may trick the figs to keep the weevil larva as one of the pollinators. Weevil larvae are much less aggressive than the larvae of other occasional fig herbivorous (*e.g.*, Pyralidae moths and Dynastinae beetles; Palmieri pers. obs.), since it has to avoid fig abortion by the plant in order to stay a long time inside the figs. Although fig trees severely attacked by other herbivorous such as flies and moths frequently abort a large part of their fruits, this response does not happen in the case of severe infestation by weevil larvae (Palmieri pers. obs.). Our observations suggest that weevil larvae can deceive fig trees to avoid abortion. However, the mechanisms of how this association occurs remain unclear.

The investigation of all the issues discussed above will bring even more complexity to an already extremely complex system. I hope that the results of this study will encourage future research on the biology and ecology of fig-weevils, which will help us to understand their role in the evolution of the fig fig-wasp mutualism.

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Appendix 1. List of the natural history collections visited.

Collection	City/Country	Acronym	Curator
American Museum of Natural History	New York/USA	AMNH	Lee Herman
British Museum of Natural History	London/UK	BMNH	Maxwell Barclay
Instituto nacional de Biodiversidad	San Jose/Costa Rica	INBIO	Angel Solis
Iziko: South African Museum	Cape Town/South Africa	SAMC	Simon Van Noort
Museu de Zoologia da USP	São Paulo/Brazil	MZUSP	Sérgio Vanin
Muséum national d'Histoire naturelle	Paris/France	MNHN	Hélène Perrin
National Museum of Natural History	Washington D.C./USA	NMNH	Lourdes Chamorro

Appendix 2. List of *Ficus* species collected.

Subgenera	Section	Species	Samples (n°)	Country
Ficus	Ficus	<i>F. ischnopoda</i> Miquel	1	Laos
Pharmacosycea	Oreosycea	<i>Ficus</i> sp.	1	Philippines
	Pharmacosycea	<i>F. adhatodifolia</i> Schott	21	Brazil
		<i>F. crassiuscula</i> Standley	1	Costa Rica
		<i>F. insipida</i> Willdenow	34	Brazil, Costa Rica
		<i>F. maxima</i> P. Miller	1	Brazil
		<i>F. obtusiuscula</i> Miquel	1	Brazil
		<i>F. piresiana</i> Váz. Avila& Berg	2	Brazil
		<i>F. tonduzii</i> Standley	1	Costa Rica
		<i>F. yoponesis</i> Desvaux	2	Costa Rica
		<i>F. heteropleura</i> Blume	3	Philippines
Sycidium	Paleomorphe	<i>F. parietalis</i> Blume	1	Philippines
		<i>F. tinctoria</i> G. Forster	1	Philippines
		<i>F. virgata</i> Blume	2	Philippines
	Sycidium	<i>F. ampelas</i> Burman f.	1	Philippines
		<i>F. exasperata</i> Vahl	1	Guinea
		<i>F. heteropoda</i> Miquel	1	Philippines
	Sycidius	<i>F. ulmifolia</i> Lamarck	1	Philippines
		<i>F. benguetensis</i> Merrill	3	Philippines
		<i>F. botryocarpa</i> bot. Miquel	1	Philippines
		<i>F. carpenteriana</i> Elmer	1	Philippines
		<i>F. fistulosa</i> Blume	7	Philippines
		<i>F. minahassae</i> Teijsmann&D.Vriese	4	Philippines
		<i>F. nota</i> (Blanco) Merrill	4	Philippines
		<i>F. psuedopalma</i> Blanco	2	Philippines
		<i>F. satterthwaitei</i> Elmer	1	Philippines
		<i>F. septica</i> Burman f.	11	Philippines
Sycomorus	Sycomorus	<i>F. sur</i> Forsk.	9	Malawi, South Africa
		<i>F. sycomorus</i> Linnaeus	4	Malawi, Zambia
		<i>F. vallis-choudae</i> Delile	2	Ivory Coast
		<i>F. variegata</i> syc. Blume	6	Philippines
		<i>F. variegata</i> var. Blume	1	Philippines
		<i>F. americana</i> Aublet	1	Costa Rica
		<i>F. arpazusa</i> Casar.	2	Brazil
		<i>F. boliviana</i> C. C. Berg	3	Brazil
		<i>F. cabalina</i> Standley	1	Brazil
		<i>F. castelviana</i> Dugand	1	Brazil
Urostigma	Americana	<i>F. cervantesiana</i> Standley&Williams	1	Ecuador
		<i>F. citrifolia</i> P. Miller	27	Brazil, Costa Rica
		<i>F. coerulescens</i> (Rusby) Rossberg	1	Brazil
		<i>F. colubrinae</i> Standley	5	Costa Rica
		<i>F. costaricana</i> (Liebmann) Miquel	4	Costa Rica
		<i>F. crocata</i> Miquel	3	Brazil
		<i>F. cuatrecasana</i> Dugand	1	Ecuador
		<i>F. donnell-smithii</i> Standley	2	Brazil
		<i>F. eximia</i> Schott	8	Brazil
		<i>F. goldmanii</i> Standl.	2	Costa Rica
		<i>F. gomeleira</i> Kunth & C.D. Bouché	1	Brazil
		<i>F. guianensis</i> Desvaux	1	Brazil
		<i>F. hartwegii</i> Miquel	1	Ecuador
		<i>F. hirsuta</i> Schott	1	Brazil
		<i>F. jimenezii</i> Standley	6	Costa Rica
		<i>F. krukovichii</i> Standley	1	Brazil
		<i>F. lauretana</i> Vázquez Avila	5	Brazil
		<i>F. marie</i> Berg, Emygdio&Carauta	1	Brazil
		<i>F. maroma</i> Castellanos	1	Brazil
		<i>F. nymphaeifolia</i> P. Miller	1	Brazil
		<i>F. piresiana</i> Váz. Avila& Berg	2	Brazil
		<i>F. tonduzii</i> Standley	1	Costa Rica
		<i>F. yoponesis</i> Desvaux	2	Costa Rica
		<i>F. heteropleura</i> Blume	3	Philippines
		<i>F. parietalis</i> Blume	1	Philippines
		<i>F. tinctoria</i> G. Forster	1	Philippines
		<i>F. virgata</i> Blume	2	Philippines
		<i>F. ampelas</i> Burman f.	1	Philippines
		<i>F. exasperata</i> Vahl	1	Guinea
		<i>F. heteropoda</i> Miquel	1	Philippines
		<i>F. ulmifolia</i> Lamarck	1	Philippines
		<i>F. benguetensis</i> Merrill	3	Philippines
		<i>F. botryocarpa</i> bot. Miquel	1	Philippines
		<i>F. carpenteriana</i> Elmer	1	Philippines
		<i>F. fistulosa</i> Blume	7	Philippines
		<i>F. minahassae</i> Teijsmann&D.Vriese	4	Philippines
		<i>F. nota</i> (Blanco) Merrill	4	Philippines
		<i>F. psuedopalma</i> Blanco	2	Philippines
		<i>F. satterthwaitei</i> Elmer	1	Philippines
		<i>F. septica</i> Burman f.	11	Philippines
		<i>F. sur</i> Forsk.	9	Malawi, South Africa
		<i>F. sycomorus</i> Linnaeus	4	Malawi, Zambia
		<i>F. vallis-choudae</i> Delile	2	Ivory Coast
		<i>F. variegata</i> syc. Blume	6	Philippines
		<i>F. variegata</i> var. Blume	1	Philippines

Appendix 2. Cont.

Subgenera	Section	Species	Samples (n°)	Country
Urostigma	Americana	<i>F. obtusifolia</i> Kunth	7	Brazil, Costa Rica
		<i>F. partasana</i> C. C. Berg	1	Brazil
		<i>F. pertusa</i> Linnaeus fil.	19	Brazil, Costa Rica
		<i>F. schultesi</i> Dugand	4	Brazil, Ecuador
		<i>F. sphenophylla</i> Standley	5	Brazil
		<i>F. trigona</i> Linnaeus fil.	12	Brazil
		<i>F. tubulosa</i> Pelissari & Romaniuc	3	Brazil
		<i>F. ypsilophlebia</i> Dugand	1	Brazil
	Conosycea	<i>F. benjamina</i> Linnaeus	3	Philippines
		<i>F. callophyla</i> Blume	2	Philippines
		<i>F. chrisolepes</i> Miquel	1	Philippines
		<i>F. crassiramea</i> Miquel	2	Philippines
		<i>F. forstenii</i> Miquel	1	Philippines
		<i>F. microcarpa</i> Linnaeus	3	Philippines
	Galoglychia	<i>F. artocarpoides</i> Warb.	1	Uganda
		<i>F. burkei</i> Miquel	7	South Africa, Zambia
		<i>F. burtt-davyi</i> Hutch.	1	South Africa
		<i>F. bussei</i> Mildbr. & Burret	2	Malawi
		<i>F. chirindensis</i> C. C. Berg	1	Uganda
		<i>F. fischeri</i> Mildbr. & Burret	1	Zambia
		<i>F. glumosa</i> Delile	4	South Africa
		<i>F. lutea</i> Vahl	4	Guinea, Ivory Coast
		<i>F. natalensis</i> Hochst.	1	Uganda
		<i>F. ovata</i> Vahl	1	Uganda
		<i>F. pertesii</i> Warb.	1	Namibia
		<i>F. polita</i> Vahl	4	South Africa
		<i>F. recurvata</i> De Wildeman	2	Ivory Coast
		<i>F. sansibarica</i> Warb.	1	South Africa
		<i>F. stuhlmannii</i> Warb.	2	South Africa
		<i>F. thonningii</i> Blume	1	Guinea
		<i>F. trichopoda</i> Baker	3	South Africa
	Urostigma	<i>F. caulocarpa</i> Miquel	1	Philippines
		<i>F. ingenes</i> Miquel	1	Ivory Coast

Appendix 3. PCR and sequencing primers used in this study.

Primer name [sense]	Annealing temp (n° of cycles)	Primer sequence (5'→3')	References
Cytb			
CB-N-11367 [fw]	45 °C	ATTACACCTCCTAATTTATTAGGAAT	10
CB-J-10933 [rv]	(40)	TATGTACTACCATGAGGACAAATATC	10
COI			
C1-J-2183 [fw]	50 °C	CAACATTATTTTGTATTTTGG	10
L2-N-3014 [rv]	(35)	TCCAATGCTACTAATCTGCCATATTA	10
LCO1490puc_t1 [fw]	45-51 °C	TGTAACACGACGGCCAGTTTCAACWAATCATAAAGATATTGG	2
LCO1490Hem1_t1 [fw]	(5+35)	TGTAACACGACGGCCAGTTTCAACTAAYCATAARGATATYGG	4
HCO2198puc_t1 [rv]		CAGGAAACAGCTATGACTAACTTCWGGRTGWCCAAARAATCA	2
HCO2198Hem2_t1 [rv]		CAGGAAACAGCTATGACTAAACYTCAGGATGACCAAAAAAYCA	4
HCO2198Hem1_t1 [rv]		CAGGAAACAGCTATGACTAAACYTCDGGATGBCCAAARAATCA	4
28S			
C2DF [fw]	57 °C	CGTGTGCTTGATAGTGCAGC	5
C2DR [rv]	(35)	TTGGTCCGTGTTTCAAGACGGG	1
ZR1 [rv]	55 °C	GTCTTGAAACACGGACCAAGGAGTCT	6
rD5b [fw]	(35)	CCACAGCGCCAGTTCTGCTTAC	11
ArgK			
ArgKforB4 [fw]	52 °C	GAYTCCGGWATYGGWATCTAYGCTCC	8
ArgKrevB1 [rv]	(40)	TCNGTRAGRCCCATWCGTCTC	3
PgIym			
PGIymFc [fw]	52 °C	GATGCCWAYTTTRAGCGAAA	7
PgIymRc [rv]	(35)	TACCATGGGCAGCAATAAGAA	7
EF1-α			
S149 [fw]	52 °C	ATCGAGAAGTTCGAGAAGGAGGCYCARGAAATGGG	9
A754 [rv]	(40)	CCACCAATTTTGTAGACATC	9

Primer sense given between brackets, [fw] forward sense, [rv] reverse sense. Original references provided at the last column.

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Appendix 4. GENBANK Sequences used in this study.

Family	Subfamily	Tribe	Genus		Cytb	COI	18S	28S	EF1a	ArgK	Pglym
CURCULIONIDAE	Cyclominae	Aterpini	<i>Acalonoma</i>	<i>pusilla</i>	–	KF016342	–	KF016474	–	–	–
CURCULIONIDAE	Cyclominae	Aterpini	<i>Acalonoma</i>	<i>pusilla</i>	–	KF016349	–	–	–	–	–
CURCULIONIDAE	Cyclominae	Amycterini	<i>Acantholophus</i>	sp.	–	–	FJ867727	–	–	FJ859915	–
CURCULIONIDAE	Molytinae	Cleogonini	<i>Achelocis</i>	sp.	–	KF016270	–	KF016405	–	–	–
CURCULIONIDAE	Molytinae	Trachodini	<i>Acicnemis</i>	<i>meriones</i>	–	KF016263	–	KF016398	–	–	–
CURCULIONIDAE	Molytinae	Trachodini	<i>Acicnemis</i>	sp.	–	KF016327	–	KF016462	–	–	–
CURCULIONIDAE	Cyclominae	Aterpini	<i>Aesiotes</i>	<i>notabilis</i>	–	FJ867836	FJ867728	FJ867654	FJ867852	FJ859916	–
BRENTIDAE	Apioninae	Rhadinocybini	<i>Alissapion</i>	sp.	–	KF016291	–	KF016427	–	–	–
CURCULIONIDAE	Scolytinae	Hylesinini	<i>Alniphagus</i>	<i>aspericollis</i>	–	HQ883655	–	–	–	HQ883885	–
CURCULIONIDAE	Scolytinae	Xyleborini	<i>Amasa</i>	<i>truncata</i>	–	KF016317	–	–	–	–	–
CURCULIONIDAE	Scolytinae	Xyleborini	<i>Ambrosiodmus</i>	<i>aegir</i>	–	–	FJ867730	–	–	–	–
CURCULIONIDAE	Scolytinae	Xyleborini	<i>Ambrosiodmus</i>	<i>compressus</i>	–	KF016319	–	KF016455	–	–	–
CURCULIONIDAE	Molytinae	Amorphocerini	<i>Amorphocerus</i>	<i>rufipes</i>	–	HQ883664	–	HQ883575	HQ883736	HQ883893	–
CURCULIONIDAE	Curculioninae	Eugnomini	<i>Ancyttalia</i>	<i>tarsalis</i>	–	KF016354	–	KF016486	–	–	–
CURCULIONIDAE	Scolytinae	Xyleborini	<i>Anisandrus</i>	<i>dispar</i>	–	HQ883695	–	HQ883606	HQ883763	HQ883926	–
BRENTIDAE	Brentinae	Brentini	<i>Ankleineella</i>	<i>subscripta</i>	–	–	–	KF016424	–	–	–
CURCULIONIDAE	Curculioninae	Anthonomini	<i>Anthonomus</i>	<i>grandis</i>	–	JQ894358	EU215423	EU215423	JQ894487	JX151002	–
BRENTIDAE	Apioninae	Antliarhinini	<i>Antliarhis</i>	<i>zamia</i>	–	FJ867838	FJ867731	FJ867656	FJ867853	–	–
CURCULIONIDAE	Cyclominae	Aterpini	<i>Aoplocnemis</i>	<i>rufipes</i>	–	KF016268	–	KF016403	–	–	–
BRENTIDAE	Apioninae	Apionini	<i>Apion</i>	<i>cf.comosum</i>	–	KF016386	–	KF016526	–	–	–
BRENTIDAE	Apioninae	Apionini	<i>Apion</i>	sp.	–	–	–	–	HQ883698	–	–
ATTELABIDAE		Apoderini	<i>Apoderus</i>	<i>coryli</i>	–	HQ883609	–	HQ883528	–	HQ883842	–
ATTELABIDAE		Apoderini	<i>Apoderus</i>	<i>jekelii</i>	–	HQ883610	–	HQ883529	HQ883697	HQ883843	–
BRENTIDAE	Eurhynchinae		<i>Aporhina</i>	<i>australis</i>	–	FJ867840	FJ867732	FJ867657	–	FJ859917	–
ANTHRIBIDAE	Choraginae		<i>Araecerus</i>	sp.	–	KF016281	–	KF016416	–	–	–
BELIDAE	Oxycoryninae	Aglycyderini	<i>Aralius</i>	sp.	–	–	FJ867733	–	FJ867854	FJ859918	–
CURCULIONIDAE	Molytinae	Trypetidini	<i>Araucarietius</i>	<i>viridans</i>	–	FJ867839	FJ867735	FJ867658	FJ867855	FJ859919	–
CURCULIONIDAE	Cossoninae	Araucariini	<i>Araucarius</i>	<i>major</i>	–	–	–	–	HQ883711	HQ883860	–
CURCULIONIDAE	Cossoninae	Araucariini	<i>Araucarius</i>	<i>minor</i>	–	–	–	–	–	–	–
CURCULIONIDAE	Cossoninae	Araucariini	<i>Araucarius</i>	<i>minor</i>	–	FJ867835	FJ867736	FJ867659	–	–	–

Appendix 4. Cont.

Family	Subfamily	Tribe	Genus		Cytb	COI	18S	28S	EF1a	ArgK	Pglym
CURCULIONIDAE	Curculioninae	Curculionini	<i>Archarius</i>	<i>alvobittatus</i>	–	AB573481	–	AB573444	AB573551	–	AB573582
CURCULIONIDAE	Curculioninae	Curculionini	<i>Archarius</i>	<i>esakii</i>	AB573523	AB573489	–	AB573452	AB573558	–	AB573589
CURCULIONIDAE	Curculioninae	Curculionini	<i>Archarius</i>	<i>murakamii</i>	AB573516	AB573480	–	AB573443	AB573550	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Archarius</i>	<i>parasiticus</i>	AB573526	AB573492	–	AB573455	AB573560	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Archarius</i>	<i>pictus</i>	AB573514	AB573478	–	AB573441	AB573548	–	AB573580
CURCULIONIDAE	Curculioninae	Curculionini	<i>Archarius</i>	<i>roelofsi</i>	AB573537	AB573502	–	AB573466	–	–	–
BRENTIDAE	Brentinae	Arrhenodini	<i>Arrhenodes</i>	<i>funnebris</i>	–	–	–	–	–	FJ859921	–
ATTELABIDAE	Attelabinae	Attelabini	<i>Attelabus</i>	sp.	–	FJ867834	–	FJ867660	FJ867856	FJ859922	–
Chrysomelidae	Aulacoscelidinae		<i>Aulacoscelis</i>	<i>appendiculata</i>	–	FJ867833	–	–	–	–	–
CURCULIONIDAE	Platypodinae	Platypodini	<i>Austroplatypus</i>	<i>incompertus</i>	–	FJ867832	–	FJ867661	FJ867857	FJ859923	–
CURCULIONIDAE	Brachycerinae	Bagoiini	<i>Bagoiini</i>	sp.	–	–	–	FJ867662	–	FJ859978	–
CURCULIONIDAE	Brachycerinae	Bagoiini	<i>Bagous</i>	<i>americanus</i>	–	–	FJ867738	–	–	FJ859924	–
CURCULIONIDAE	Brachycerinae	Bagoiini	<i>Bagous</i>	sp.	–	KF016322	–	KF016458	–	–	–
CURCULIONIDAE	Conoderinae	Baridini	<i>Baris</i>	sp.1	–	KF016279	–	–	–	–	–
CURCULIONIDAE	Baridinae	Baridini	<i>Baris</i>	sp.2	–	–	–	FJ867664	–	–	–
BELIDAE	Belinae	Pachyurini	<i>Basiliobelus</i>	<i>flavovittatus</i>	–	FJ867831	FJ867739	FJ867665	FJ859977	FJ859925	–
CURCULIONIDAE	Scolytinae	Xyleborini	<i>Beaverium</i>	<i>insulindicus</i>	–	KF016316	–	KF016453	–	–	–
CURCULIONIDAE	Molytinae	Cryptorhynchini	<i>Bepharus</i>	<i>ellipticus</i>	–	–	FJ867740	FJ867666	–	FJ859926	–
CURCULIONIDAE	Cyclominae	Hipporhinini	<i>Bronchus</i>	sp.	–	FJ867830	FJ867741	FJ867667	–	FJ859927	–
CARIDAE	Carinae		<i>Caenominurus</i>	<i>topali</i>	–	–	FJ867742	FJ867668	–	–	–
CURCULIONIDAE	Curculioninae	Camarotini	<i>Camarotus</i>	sp.	–	FJ867829	–	FJ867669	–	–	–
CURCULIONIDAE	Scolytinae	Scolytini	<i>Camptocerus</i>	<i>aeneipennis</i>	–	HQ883676	–	HQ883587	HQ883745	HQ883907	–
CURCULIONIDAE	Scolytinae	Scolytini	<i>Camptocerus</i>	<i>auricomus</i>	–	–	–	HQ883586	–	HQ883906	–
CURCULIONIDAE	Molytinae	Ithyporini	<i>Camptorhinus</i>	<i>interstitialis</i>	–	KF016267	–	KF016402	–	–	–
CARIDAE	Carinae		<i>Car</i>	<i>cf.condensatus</i>	–	KF016271	–	–	–	–	–
CARIDAE	Carinae		<i>Car</i>	<i>pini</i>	–	–	FJ867743	FJ867670	FJ867858	FJ859928	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Carponinus</i>	<i>hilaris</i>	AB573531	–	–	AB573460	AB573564	–	AB573595
CURCULIONIDAE	Entiminae	Leptopiini	<i>Catasarcus</i>	<i>impressipennis</i>	–	KF016266	–	–	–	–	–
CURCULIONIDAE	Entiminae	Leptopiini	<i>Catasarcus</i>	sp.	–	FJ867847	FJ867744	FJ867671	–	FJ859929	–
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Cenocephalus</i>	sp.	–	HQ883682	–	HQ883593	HQ883751	HQ883912	–
BRENTIDAE	Brentinae	Cyphagogini	<i>Cerobates</i>	<i>ophthalmicus</i>	–	–	–	KF016503	–	–	–
CURCULIONIDAE	Baridinae	Ceutorhynchini	<i>Ceutorhynchus</i>	sp.	–	HQ165521	–	FJ867672	FJ867859	FJ859930	–
CURCULIONIDAE	Cossoninae	Araucariini	<i>cf.Xenocnema</i>	sp.	–	HQ883642	–	HQ883557	HQ883722	–	–
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Chaetastus</i>	<i>montanus</i>	–	HQ883683	–	HQ883594	HQ883752	HQ883913	–

Appendix 4. Cont.

Family	Subfamily	Tribe	Genus		Cyto B	COI	18S	28S	EF1a	ArgK	Pglym
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Chaetastus</i>	<i>tuberculatus</i>	–	HQ883684	–	HQ883595	HQ883753	HQ883914	–
CURCULIONIDAE	Entiminae	Sitonini	<i>Chlorophanus</i>	<i>sibiricus</i>	–	HQ883651	–	HQ883566	HQ883727	HQ883882	–
CURCULIONIDAE	Cyclominae	Aterpini	<i>Chrysolopus</i>	<i>spectabilis</i>	–	KF016299	–	KF016435	–	–	–
CURCULIONIDAE	Cyclominae	Aterpini	<i>Chrysolopus</i>	<i>spectabilis</i>	–	–	FJ867745	–	–	FJ859931	–
NEMONYCHIDAE	Cimberidinae	Cimberidini	<i>Cimberis</i>	<i>pilosa</i>	–	FJ867848	FJ867746	FJ867673	FJ867860	FJ859932	–
CURCULIONIDAE	subfamilia incerta	Phrynixini	<i>Cisolea</i>	sp.	–	–	–	KF016508	–	–	–
CURCULIONIDAE	Scolytinae	Scolytini	<i>Cnemonyx</i>	sp.	–	–	–	HQ883588	–	HQ883908	–
CURCULIONIDAE	Scolytinae	Xyleborini	<i>Cnestus</i>	<i>solidus</i>	–	KF016318	–	KF016454	–	–	–
CURCULIONIDAE	Molytinae	Conotrachelini	<i>Conotrachelus</i>	<i>nenuphar</i>	–	KR486585	AF250100	FJ867674	–	–	–
CURCULIONIDAE	Cossoninae	Araucariini	<i>Coptocorynus</i>	sp.1	–	HQ883630	–	HQ883545	HQ883713	–	–
CURCULIONIDAE	Cossoninae	Araucariini	<i>Coptocorynus</i>	sp.2	–	HQ883631	–	HQ883546	HQ883714	HQ883862	–
CURCULIONIDAE	Coptonotinae	Coptonotini	<i>Coptonotus</i>	<i>cyclopus</i>	–	HQ883624	–	–	–	HQ883856	–
CURCULIONIDAE	Cossoninae	Cossonini	<i>Cossonus</i>	<i>linearis</i>	–	HQ883629	–	–	HQ883712	HQ883861	–
CURCULIONIDAE	Cossoninae		<i>Cossonus</i>	sp.	–	KF016385	–	KF016525	–	–	–
CURCULIONIDAE	Curculioninae	Cranopoeini	<i>Cranopoeus</i>	sp.	–	KF016330	–	KF016463	–	–	–
CURCULIONIDAE	subfamilia incerta	Phrynixini	<i>Crossixus</i>	<i>punctatus</i>	–	KF016378	–	KF016517	–	–	–
CURCULIONIDAE	Platypodinae	Platypodini	<i>Crossotarsus</i>	<i>minusculus</i>	–	HQ883669	–	HQ883579	HQ883739	HQ883899	–
CURCULIONIDAE	Platypodinae	Platypodini	<i>Crossotarsus</i>	sp.	–	KF016325	–	KF016461	–	–	–
CURCULIONIDAE	Curculioninae	Cryptoplini	<i>Cryptoplus</i>	sp.	–	KF016302	–	KF016438	–	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>aino</i>	AB573517	AB573482	–	AB573445	AB573552	–	AB573583
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>breviscapus</i>	AB573529	AB573495	–	AB573458	AB573562	–	AB573593
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>camelliae</i>	AY327726	AY327685	–	–	AY327652	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>camelliae</i>	AY327756	AY327724	–	–	–	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>camelliae</i>	AB573541	AB573506	–	AB573470	AB573573	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>caryae</i>	AY327732	AY327692	–	–	AY327657	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>caryae</i>	AY327746	AY327709	–	–	AY327671	–	AY327644
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>caryae</i>	AY327747	AY327710	–	–	AY327672	–	AY327645
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>cerasorum</i>	AB573524	AB573490	–	AB573453	AB573559	–	AB573590
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>confusor</i>	–	AY327701	–	–	AY327665	–	AY327638
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>confusor</i>	AY327740	AY327703	–	–	AY327667	–	AY327639
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>convexus</i>	AB573510	AB573474	–	AB573438	AB573545	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>dentipes</i>	AB573544	AB573509	–	AB573473	AB573576	–	AB573606
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>distinguendus</i>	AB573542	AB573507	–	AB573471	AB573574	–	AB573604
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>elaeagni</i>	–	AB573484	–	AB573447	AB573554	–	AB573584

Appendix 4. Cont.

Family	Subfamily	Tribe	Genus		Cytb	COI	18S	28S	EF1a	ArgK	Pg1ym
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>elephas</i>	AY327742	AY327705	–	–	AY327669	–	AY327640
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>elephas</i>	AY327745	AY327708	–	–	AY327670	–	AY327643
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>elephas</i>	AY327755	AY327723	–	–	AY327682	–	AY327650
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>funebri</i>	AB573536	AB573501	–	AB573465	AB573569	–	AB573600
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>glandium</i>	AY327748	AY327711	–	–	AY327673	–	AY327646
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>glandium</i>	AY327749	AY327713	–	–	AY327674	–	AY327647
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>hachijoensis</i>	AB573512	AB573476	–	AB573440	AB573547	–	AB573578
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>hilgendorfi</i>	AB573538	AB573503	–	AB573467	AB573570	–	AB573601
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>hirashimai</i>	AB573519	AB573485	–	AB573448	AB573555	–	AB573585
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>humeralis</i>	AY327729	AY327688	–	–	–	–	AY327632
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>humeralis</i>	AY327738	–	–	–	–	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>humeralis</i>	–	–	–	–	AY327683	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>iowensis</i>	–	AY327697	–	–	–	–	AY327636
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>kojimai</i>	AB573528	AB573494	–	AB573457	–	–	AB573592
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>koreanus</i>	AB573521	AB573487	–	AB573450	AB573556	–	AB573588
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>lateritius</i>	AB573533	AB573498	–	AB573462	AB573566	–	AB573597
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>longidens</i>	AY327744	AY327707	–	–	–	–	AY327642
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>longidens</i>	AY327752	AY327718	–	–	AY327678	–	AY327649
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>maculanigra</i>	AB573522	AB573488	–	AB573451	AB573557	–	AB573588
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>morimotoi</i>	AB573530	AB573496	–	AB573459	AB573563	–	AB573594
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>nasicus</i>	–	AY327695	–	–	AY327660	–	AY327635
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>nasicus</i>	–	AY327702	–	–	AY327666	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>niveopictus</i>	–	–	FJ867750	FJ867675	FJ867861	FJ859933	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>nucum</i>	–	AY327696	–	–	AY327661	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>nucum</i>	–	AY327725	–	–	–	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>ochrofasciatus</i>	AB573525	AB573491	–	AB573454	–	–	AB573591
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>okumai</i>	AB573534	AB573499	–	AB573463	AB573567	–	AB573598
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>pardalis</i>	AY327753	AY327720	–	–	AY327679	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>pellitus</i>	AY327727	AY327686	–	–	AY327653	–	AY327631
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>pellitus</i>	AY327741	AY327704	–	–	AY327668	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>pellitus</i>	AY327754	AY327721	–	–	AY327680	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>pellitus</i>	AY327758	–	–	–	AY327684	–	AY327651
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>proboscideus</i>	AY327736	AY327698	–	–	–	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>proboscideus</i>	AY327739	AY327700	–	–	AY327664	–	–

Appendix 4. Cont.

Family	Subfamily	Tri be	Genus		Cytb	COI	18S	28S	EF1a	ArgK	Pglym
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>pyrrhoceras</i>	AY327728	AY327687	–	–	AY327654	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>pyrrhoceras</i>	AY327734	AY327694	–	–	AY327659	–	AY327634
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>rai</i>	AB573513	AB573477	–	–	–	–	AB573579
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>robustus</i>	AB573540	AB573505	–	AB573469	AB573572	–	AB573603
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>salicivorus</i>	–	AY327719	–	–	–	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>scutellaris</i>	–	AY327712	–	–	–	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>scutellaris</i>	–	AY327715	–	–	–	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>sikkimensis</i>	AB573543	AB573508	–	AB573472	AB573575	–	AB573605
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	sp.1	AB573527	AB573493	–	AB573456	AB573561	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	sp.2	AB573539	AB573504	–	AB573468	AB573571	–	AB573602
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	sp.27	–	AY327722	–	–	AY327681	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	sp.55	AY327757	–	–	–	–	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>sp.Africa8</i>	AY327733	AY327693	–	–	AY327658	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>styracis</i>	AB573518	AB573483	–	AB573446	AB573553	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>sulcatulus</i>	AY327731	AY327690	–	–	AY327656	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>sulcatulus</i>	AY327737	AY327699	–	–	AY327663	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>sulcatulus</i>	AY327735	–	–	–	AY327662	–	AY327637
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>undulatus</i>	AY327759	–	–	–	–	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>venosus</i>	AY327730	AY327689	–	–	AY327655	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>venosus</i>	–	AY327691	–	–	–	–	AY327633
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>venosus</i>	AY327750	AY327714	–	–	AY327675	–	AY327648
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>venosus</i>	AY327751	AY327716	–	–	AY327676	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>victoriensis</i>	AY327743	AY327706	–	–	–	–	AY327641
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>victoriensis</i>	–	AY327717	–	–	AY327677	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Curculio</i>	<i>yanoi</i>	AB573535	AB573500	–	AB573464	AB573568	–	AB573599
BRENTIDAE	Brentinae	Cyladini	<i>Cylas</i>	<i>formicarius</i>	–	FJ867849	–	FJ867676	FJ867862	FJ859934	–
CURCULIONIDAE	Baridinae	Zygopini	<i>Cylindrocopturus</i>	sp.	–	–	FJ867751	FJ867677	–	FJ859935	–
BELIDAE	Belinae	Agnesiotidini	<i>Cyrotypus</i>	sp.	–	KF016367	–	KF016505	–	–	–
CURCULIONIDAE	Scolytinae	Hylesinini	<i>Dactylipalpus</i>	<i>grouvellei</i>	–	HQ883656	–	HQ883570	HQ883731	HQ883886	–
CURCULIONIDAE	Scolytinae	Tomicini	<i>Dendroctonus</i>	<i>micans</i>	–	HQ883680	–	HQ883591	HQ883749	–	–
CURCULIONIDAE	Scolytinae		<i>Dendroctonus</i>	<i>ponderosae</i>	–	JQ308492	AF308337	AF308385	AF308428		
CURCULIONIDAE	Scolytinae	Tomicini	<i>Dendroctonus</i>	<i>pseudotsugae</i>	–	FJ867851	FJ867752	FJ867678	–	FJ859936	–
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Diapus</i>	<i>unispineus</i>	–	HQ883685	–	HQ883596	HQ883754	HQ883915	–
CURCULIONIDAE	Cossoninae	incertae sedis	<i>Dobionus</i>	<i>araucarinus</i>	–	HQ883632	–	–	–	–	–

Appendix 4. Cont.

Family	Subfamily	Tribe	Genus		Cytb	COI	18S	28S	EF1a	ArgK	Pglym
CURCULIONIDAE	Platypodinae	Platypodini	<i>Doliopygus</i>	<i>rhodesianus</i>	–	HQ883670	–	HQ883580	HQ883740	HQ883900	–
NEMONYCHIDAE	Cimberidinae	Doydirhynchini	<i>Doydirhynchus</i>	<i>austriacus</i>	–	–	–	–	FJ867863	FJ859937	–
CURCULIONIDAE	Scolytinae	Dryocoetini	<i>Dryocoetes</i>	<i>autographus_sp</i>	–	–	FJ867754	HQ883565	–	HQ883880	–
CURCULIONIDAE	Dryophthorinae	Stromboscerini	<i>Dryophthoroides</i>	sp.	–	KF016380	–	KF016520	–	–	–
CURCULIONIDAE	Dryophthorinae	Dryophthorini	<i>Dryophthorus</i>	sp.	–	KF016368	–	KF016506	–	–	–
CURCULIONIDAE	Dryophthorinae	Dryophthorini	<i>Dryophthorus</i>	sp.	–	KF016372	–	KF016511	–	–	–
CURCULIONIDAE	Brachycerinae	Eirrhiniini	<i>Echinocnemus</i>	sp.1	–	KF016392	–	–	–	–	–
CURCULIONIDAE	Brachycerinae	Eirrhiniini	<i>Echinocnemus</i>	sp.2	–	–	FJ867755	FJ867679	FJ867864	FJ859938	–
CURCULIONIDAE	Entiminae	Leptopiini	<i>Ecrizothis</i>	<i>boviei</i>	–	KF016297	–	KF016433	–	–	–
BRENTIDAE	Brentinae	Brentini	<i>Ectocemus</i>	<i>decemmaculatus</i>	–	KF016289	–	KF016425	–	–	–
CURCULIONIDAE	Molytinae	Trypetidini	<i>Eisingius</i>	<i>chusqueae</i>	–	–	FJ867756	FJ867680	FJ867865	–	–
CURCULIONIDAE	Curculioninae	Storeini	<i>Emplesis</i>	<i>tessellata</i>	–	KF016352	–	KF016484	–	–	–
CURCULIONIDAE	Molytinae	Cryptorhynchini	<i>Enteles</i>	<i>vigorsii</i>	–	KF016373	–	KF016512	–	–	–
CURCULIONIDAE	Curculioninae	Acalyptini	<i>Epamoebus</i>	sp.1	–	KF016346	–	KF016479	–	–	–
CURCULIONIDAE	Curculioninae	Acalyptini	<i>Epamoebus</i>	sp.2	–	KF016345	–	KF016478	–	–	–
Protocucujidae			<i>Ericmodes</i>	<i>sylvaticus</i>	–	FJ867850	–	FJ867681	FJ867866	FJ859939	–
CURCULIONIDAE	subfamilia incerta	unplaced	<i>Ethadomorpha</i>	<i>australis</i>	–	KF016264	–	KF016399	–	–	–
ATTELABIDAE	Rhynchitinae	Rhynchitini	<i>Eugnaptus</i>	<i>angustatus</i>	–	FJ867846	FJ867757	–	FJ867867	–	–
ATTELABIDAE	Attelabinae	Euopini	<i>Euops</i>	<i>cf.parvoarmatus</i>	–	KF016335	–	KF016467	–	–	–
ATTELABIDAE	Attelabinae	Euopini	<i>Euops</i>	<i>coxalis</i>	–	KF016262	–	KF016397	–	–	–
ANTHRIBIDAE	Anthribinae		<i>Eupanteos</i>	<i>ornatus</i>	–	–	–	KF016502	–	–	–
BRENTIDAE	Eurhynchinae		<i>Eurhynchus</i>	<i>acanthopterus</i>	–	KF016376	–	–	–	–	–
BRENTIDAE	Eurhynchinae		<i>Eurhynchus</i>	<i>laevior</i>	–	FJ867845	FJ867758	FJ867682	–	FJ859940	–
CURCULIONIDAE	subfamilia incerta	Hyperini	<i>Eurychirus</i>	<i>bituberculatus</i>	–	KF016278	–	KF016413	–	–	–
CURCULIONIDAE	Molytinae	Cryptorhynchini	<i>Euthyrhinus</i>	<i>meditabundus</i>	–	KF016280	–	KF016415	–	–	–
CURCULIONIDAE	Entiminae	Ottistirini	<i>Eutinophaea</i>	sp.	–	KF016310	–	KF016447	–	–	–
CURCULIONIDAE	Entiminae	Leptopiini	<i>Evas</i>	<i>cf.argenteiventris</i>	–	KF016305	–	KF016441	–	–	–
CURCULIONIDAE	Molytinae	incertae sedis	<i>Exeiratus</i>	<i>bigranulatus</i>	–	KF016375	–	KF016514	–	–	–
CURCULIONIDAE	Molytinae	Cryptorhynchini	<i>Exithius</i>	<i>cariosus</i>	–	KF016383	–	KF016523	–	–	–
CURCULIONIDAE	Scolytinae	Hylesinini	<i>Ficicis</i>	sp.	–	KF016320	–	KF016456	–	–	–
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Genyocerus</i>	<i>exilis</i>	–	HQ883686	–	HQ883597	HQ883755	HQ883916	–
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Genyocerus</i>	<i>serratus</i>	–	HQ883687	–	HQ883598	HQ883756	HQ883917	–
CURCULIONIDAE	subfamilia incerta	Hyperini	<i>Gerynassa</i>	sp.	–	KF016344	–	KF016476	–	–	–
CURCULIONIDAE	subfamilia incerta	Gonipterini	<i>Gonipterus</i>	<i>pulverulentus</i>	–	KF016391	–	–	–	–	–

Appendix 4. Cont.

Family	Subfamily	Tribe	Genus		Cytb	COI	18S	28 S	EF1a	ArgK	Pglym
CURCULIONIDAE	Curculioninae	Gonipterini	<i>Gonipterus</i>	sp.	–	–	FJ867759	FJ867683	–	–	–
CURCULIONIDAE	Curculioninae	Mecinini	<i>Gymnetron</i>	<i>tetrum</i>	–	–	–	FJ867684	–	FJ859941	–
CURCULIONIDAE	Scolytinae	Ctenophorini	<i>Gymnochilus</i>	<i>reitteri</i>	–	HQ883644	–	–	–	HQ883873	–
CURCULIONIDAE	Entiminae	Eupholini	<i>Gymnopholus</i>	sp.	–	–	FJ867760	FJ867685	FJ867868	–	–
CURCULIONIDAE	Curculioninae	Cryptoplini	<i>Haplonyx</i>	sp.	–	FJ867844	FJ867761	FJ867686	–	FJ859942	–
CURCULIONIDAE	Molytinae	Hylobiini	<i>Heilipodus</i>	<i>argentinicus</i>	–	FJ867843	–	FJ867687	FJ867869	–	–
CURCULIONIDAE	Brachycerinae	Eirrhiniini	<i>Himasthlophallus</i>	<i>flagellifer</i>	–	HQ883654	–	HQ883569	HQ883730	–	–
CURCULIONIDAE	Baridinae	Conoderini	<i>Homoeometamelus</i>	sp.	–	HQ883643	–	HQ883558	HQ883723	HQ883872	–
BRENTIDAE	Brentinae	Trachelizini	<i>Hormocerus</i>	<i>fossulatus</i>	–	KF016294	–	KF016429	–	–	–
CURCULIONIDAE	Scolytinae	Hylastini	<i>Hylastes</i>	<i>opacus</i>	–	HQ883660	–	HQ883927	HQ883732	–	–
CURCULIONIDAE	Scolytinae	Hylesinini	<i>Hylesinus</i>	<i>varius</i>	–	HQ883657	–	–	–	HQ883887	–
CURCULIONIDAE	Molytinae	Hylobiini	<i>Hylobius</i>	<i>piceus</i>	–	HQ883665	–	HQ883576	–	HQ883894	–
CURCULIONIDAE	Scolytinae	Hylastini	<i>Hylurgops</i>	<i>rugipennis</i>	–	HQ883659	FJ867763	–	–	HQ883889	–
CURCULIONIDAE			<i>Hypera</i>	<i>postica</i>	–	–	FJ867764	–	–	FJ859943	–
CURCULIONIDAE	Curculioninae	Hyperini	<i>Hypera</i>	<i>postica</i>	–	HQ165561	JX239440	JX239440	–	–	–
CURCULIONIDAE	Scolytinae	Xyloterini	<i>Indocryphalus</i>	<i>pubipennis</i>	–	HQ883693	–	HQ883604	–	HQ883922	–
CURCULIONIDAE	Scolytinae	Ipini	<i>Ips</i>	<i>acuminatus</i>	–	HQ883661	FJ867766	HQ883572	HQ883733	HQ883890	–
BELIDAE	Belinae	Belini	<i>Isacantha</i>	sp.	–	FJ867842	FJ867767	FJ867688	FJ867870	FJ859944	–
BRENTIDAE	Ithycerinae		<i>Ithycerus</i>	<i>noveboracensis</i>	–	–	FJ867726	–	FJ867871	–	–
BRENTIDAE	Brentinae	Trachelizini	<i>Ithysten</i>	<i>hollandiae</i>	–	–	–	KF016430	–	–	–
BRENTIDAE	Brentinae	Brentini	<i>Kleinella</i>	<i>barbata</i>	–	KF016290	–	KF016426	–	–	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Koreoculio</i>	<i>antennatus</i>	AB573515	AB573479	–	AB573442	AB573549	–	AB573581
CURCULIONIDAE	Curculioninae	Curculionini	<i>Koreoculio</i>	<i>minutissimus</i>	AB573511	AB573475	–	AB573439	AB573546	–	AB573577
CURCULIONIDAE	Molytinae	Lixini	<i>Larinus</i>	sp.	–	HQ883622	–	HQ883541	HQ883707	HQ883854	–
CURCULIONIDAE	Entiminae	Leptopiini	<i>Leptopius</i>	sp.	–	KF016285	–	KF016420	–	–	–
BRENTIDAE	Apioninae	Rhadinocybini	<i>Lissapion</i>	sp.	–	KF016292	–	–	–	–	–
CURCULIONIDAE	Brachycerinae	Eirrhiniini	<i>Lissorhoptrus</i>	sp.	–	–	FJ867769	FJ867689	FJ867872	FJ859946	–
CURCULIONIDAE	Cyclominae	Rhithirini	<i>Listronotus</i>	<i>cryptops</i>	–	FJ867812	–	FJ867690	–	–	–
CURCULIONIDAE	Baridinae	Lixini	<i>Lixus</i>	sp.1	–	HQ883623	–	–	HQ883708	HQ883855	–
CURCULIONIDAE	Baridinae	Lixini	<i>Lixus</i>	sp.2	–	–	–	HQ883562	–	–	–
CURCULIONIDAE	Molytinae	Cleogonini	<i>Lybaeba</i>	sp.	–	KF016301	–	KF016437	–	–	–
CURCULIONIDAE	Cossoninae	Pentarthrini	<i>Macroscytalus</i>	sp.	–	HQ883634	–	HQ883548	–	HQ883864	–
CURCULIONIDAE	Molytinae	Magdalinini	<i>Magdalis</i>	sp.	–	–	–	FJ867691	FJ867873	FJ859947	–
CURCULIONIDAE	Entiminae	Ottistirini	<i>Maleuterpes</i>	<i>spinipes</i>	–	KF016314	–	KF016451	–	–	–

Appendix 4. Cont.

Family	Subfamily	Tribe	Genus		Cytb	COI	18S	28S	EF1a	ArgK	Pglym
CURCULIONIDAE	Cyclominae	Notiomimetini	<i>Mandalotina</i>	<i>cf.atranotata</i>	–	KF016334	–	–	–	–	–
CURCULIONIDAE	Entiminae	Leptopiini	<i>Mandalotus</i>	sp.	–	KF016396	–	KF016537	–	–	–
CURCULIONIDAE	Platypodinae	Mecopelmini	<i>Mecopelmus</i>	<i>zeteki</i>	–	HQ883663	–	HQ883574	HQ883735	HQ883892	–
CURCULIONIDAE	Conoderinae	Conoderini	<i>Mecopus</i>	<i>rufipes</i>	–	–	–	KF016504	–	–	–
CURCULIONIDAE	Curculioninae	Storeini	<i>Melanterius</i>	sp.	–	–	FJ867770	FJ867692	FJ867874	FJ859948	–
CURCULIONIDAE	Molytinae	Cleogonini	<i>Melanterius</i>	sp.	–	KF016269	–	–	–	–	–
ATTELABIDAE	Rhynchitinae	Rhynchitini	<i>Merhynchites</i>	sp.	–	FJ867841	FJ867772	FJ867693	FJ867875	–	–
CURCULIONIDAE	Entiminae	Leptopiini	<i>Merimnetes</i>	sp.	–	KF016394	–	KF016534	–	–	–
CURCULIONIDAE	Curculioninae	Eugnomini	<i>Meriphus</i>	<i>fullo</i>	–	KF016355	–	KF016487	–	–	–
CURCULIONIDAE	Curculioninae	Eugnomini	<i>Meriphus</i>	sp.	–	–	FJ867771	–	–	FJ859980	–
CURCULIONIDAE	Cossoninae	Cossonini	<i>Mesites</i>	<i>fusiformis</i>	–	–	–	HQ883549	–	HQ883865	–
CURCULIONIDAE	Dryophthorinae	Orthognathini	<i>Mesocordylus</i>	<i>bracteolatus</i>	–	–	FJ867773	–	FJ867876	FJ859949	–
CURCULIONIDAE	Platypodinae	Platypodini	<i>Mesoplatypus</i>	sp.	–	HQ883671	–	HQ883581	HQ883741	HQ883901	–
CURCULIONIDAE	Baridinae	Conoderini	<i>Metialma</i>	sp.	–	HQ883617	–	HQ883536	HQ883703	HQ883849	–
ATTELABIDAE	Rhynchitinae	Auletini	<i>Metopum</i>	sp.	–	KF016336	–	KF016468	–	–	–
ATTELABIDAE	Rhynchitinae	Auletini	<i>Metopum</i>	sp.	–	KF016275	–	KF016410	–	–	–
CURCULIONIDAE	Scolytinae	Ctenophorini	<i>Microborus</i>	<i>boops</i>	–	HQ883646	–	HQ883560	HQ883724	HQ883874	–
CURCULIONIDAE	Cossoninae	Pentarthrini	<i>Microcossonus</i>	sp.1	–	HQ883635	–	HQ883550	HQ883716	HQ883866	–
CURCULIONIDAE	Cossoninae	Pentarthrini	<i>Microcossonus</i>	sp.2	–	HQ883640	–	HQ883556	HQ883721	HQ883871	–
CURCULIONIDAE	Molytinae	Lixini	<i>Microlarinus</i>	sp.1	–	–	FJ867774	–	–	FJ859950	–
CURCULIONIDAE	Molytinae	Lixini	<i>Microlarinus</i>	sp.2	–	–	–	–	FJ867877	–	–
BRENTIDAE	Apioninae	Rhadinocybini	<i>Micronotapion</i>	<i>gibbiceps</i>	–	KF016374	–	KF016513	–	–	–
CURCULIONIDAE	Conoderinae	Ceutorhynchini	<i>Mogulones</i>	<i>larvatus</i>	–	KF016284	–	KF016419	–	–	–
CURCULIONIDAE	Conoderinae	Baridini	<i>Myctides</i>	<i>barbatus</i>	–	KF016300	–	KF016436	–	–	–
CURCULIONIDAE	Entiminae	Cyphicerini	<i>Myloцерus</i>	sp.1	–	KF016341	–	KF016473	–	–	–
CURCULIONIDAE	Entiminae	Cyphicerini	<i>Myloцерus</i>	sp.2	–	KF016393	–	KF016533	–	–	–
CURCULIONIDAE	Curculioninae	Otidoccephalini	<i>Myrmex</i>	<i>floridanus</i>	–	FJ867813	–	FJ867694	–	FJ859951	–
BRENTIDAE	Apioninae	Rhadinocybini	<i>Nanomymnacyba</i>	sp.	–	KF016293	–	KF016428	–	–	–
CURCULIONIDAE	Entiminae	Naupactini	<i>Naupactus</i>	<i>leucoloma</i>	–	KF016309	–	KF016446	–	–	–
CURCULIONIDAE	Entiminae	Naupactini	<i>Naupactus</i>	<i>xanthographus</i>	–	–	FJ867775	FJ867695	FJ867878	FJ859952	–
CURCULIONIDAE	Cyclominae	Aterpini	<i>Nemestra</i>	<i>incerta</i>	–	KF016287	–	KF016422	–	–	–
CURCULIONIDAE	Molytinae	Mesoptiliini	<i>Neolaemosaccus</i>	sp.	–	KF016307	–	KF016443	–	–	–
CURCULIONIDAE	Brachycerinae	Erihrinini	<i>Notaris</i>	<i>acridulus</i>	–	KF016321	–	KF016457	–	–	–
CURCULIONIDAE	Molytinae	Mesoptiliini	<i>Notomagdalis</i>	sp.	–	KF016308	–	KF016444	–	–	–

Appendix 4. Cont.

Family	Subfamily	Tribe	Genus		Cytb	COI	18S	28S	EF1a	ArgK	Pglym
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Notoplatypus</i>	<i>elongatus</i>	–	HQ883688	–	HQ883599	HQ883757	HQ883918	–
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Notoplatypus</i>	<i>elongatus</i>	–	FJ867814	FJ867776	–	FJ867879	FJ859953	–
CURCULIONIDAE	Brachycerinae	Ocladiini	<i>Ocladius</i>	sp.2	–	FJ867815	FJ867777	FJ867696	FJ867880	–	–
CURCULIONIDAE	Molytinae	incertae sedis	<i>Opsittis</i>	sp.	–	KF016382	–	KF016522	–	–	–
CURCULIONIDAE	Curculioninae	Rhamphini	<i>Orchestes</i>	sp.1	–	KF016381	–	KF016521	–	–	–
CURCULIONIDAE	Curculioninae	Rhamphini	<i>Orchestes</i>	sp.2	–	–	–	KF016536	–	–	–
CURCULIONIDAE	Conoderinae	Baridini	<i>Orchidophilus</i>	<i>aterrimus</i>	–	KF016329	–	–	–	–	–
CURCULIONIDAE	Entiminae	Celeuthetini	<i>Oribius</i>	<i>albivarius</i>	–	KF016315	–	KF016452	–	–	–
CURCULIONIDAE	Curculioninae	Eugnomini	<i>Orpha</i>	<i>flavicornis</i>	–	KF016303	–	KF016439	–	–	–
Chrysomelidae	Orsodacninae		<i>Orsodacne</i>	<i>atra</i>	–	FJ867817	–	FJ867697	–	–	–
CURCULIONIDAE	Molytinae	Orthorhinini	<i>Orthorhinus</i>	sp.	–	–	–	KF016519	–	–	–
CURCULIONIDAE	Entiminae	Otiiorhynchini	<i>Otiiorhynchus</i>	<i>auropunctatus</i>	–	HQ883652	–	HQ883567	HQ883728	HQ883883	–
CURCULIONIDAE	Molytinae	Cryptorhynchini	<i>Ouoroporopterus</i>	<i>squamiventris</i>	–	KF016282	–	KF016417	–	–	–
BELIDAE	Oxycoryninae	Oxycorynini	<i>Oxycraspedus</i>	<i>cribricollis</i>	–	FJ867811	FJ867778	FJ867698	FJ867881	FJ859954	–
CURCULIONIDAE	Cossoninae		<i>Oxydema</i>	sp.	–	KF016312	–	KF016450	–	–	–
CURCULIONIDAE	subfamilia incerta	Gonipterini	<i>Oxyops</i>	<i>bilunaris</i>	–	KF016389	–	KF016529	–	–	–
CURCULIONIDAE	subfamilia incerta	Gonipterini	<i>Oxyops</i>	sp.	–	KF016369	–	KF016507	–	–	–
CURCULIONIDAE	Curculioninae	Gonipterini	<i>Oxyops</i>	<i>vitiosa</i>	–	–	–	–	FJ867882	FJ859955	–
Oxypeltidae			<i>Oxypeltus</i>	<i>quadriscopiosus</i>	–	FJ867816	FJ859984	FJ867699	FJ867883	FJ859956	–
Megalopodidae	Palophaginae		<i>Palophagoides</i>	<i>vargasorum</i>	–	FJ867810	–	FJ867700	FJ867884	–	–
CURCULIONIDAE	subfamilia incerta	Gonipterini	<i>Pantoreites</i>	<i>arctatus</i>	–	KF016304	–	KF016440	–	–	–
CURCULIONIDAE	Entiminae	Pachyrhynchini	<i>Pantorhytes</i>	<i>stanleyanus</i>	–	KF016311	–	KF016448	–	–	–
Cerambycidae	Parandrinae		<i>Parandra</i>	sp.	–	–	–	–	FJ859975	FJ859979	–
CURCULIONIDAE	Molytinae	incertae sedis	<i>Paratranes</i>	<i>monopticus</i>	–	–	–	KF016445	–	–	–
CURCULIONIDAE	Cyclominae	Aterpini	<i>Pelolorhinus</i>	<i>variegatus</i>	–	KF016261	–	–	–	–	–
CURCULIONIDAE	Curculioninae	Derelomini	<i>Perelleschus</i>	<i>carludovicae</i>	–	FJ867809	–	FJ867701	FJ867885	FJ859981	–
CURCULIONIDAE	Molytinae	Paipalesomini	<i>Peribleptus</i>	<i>dealbatus</i>	–	KF016326	–	–	–	–	–
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Periommatius</i>	sp.	–	HQ883689	–	HQ883600	HQ883758	–	–
CURCULIONIDAE	Molytinae	Cryptorhynchini	<i>Perissops</i>	<i>mucidos</i>	–	KF016265	–	KF016400	–	–	–
CURCULIONIDAE	Entiminae	Leptopiini	<i>Perperus</i>	sp.	–	KF016296	–	KF016432	–	–	–
CURCULIONIDAE	Conoderinae	Campyloscelini	<i>Phaenomerus</i>	<i>auriceps</i>	–	KF016387	–	KF016527	–	–	–
ANTHRIBIDAE	Anthribinae		<i>Phloeobius</i>	<i>gigas</i>	–	KF016339	–	KF016472	–	–	–
CURCULIONIDAE	Scolytinae	Hylesinini	<i>Phloeoborus</i>	sp.	–	HQ883658	–	HQ883571	–	HQ883888	–
CURCULIONIDAE	Scolytinae	Phloeosinini	<i>Phloeosinus</i>	<i>punctatus</i>	–	HQ883668	–	–	–	HQ883898	–

Appendix 4. Cont.

Family	Subfamily	Tribe	Genus		Cytb	COI	18S	28S	EF1a	ArgK	Pglym
CURCULIONIDAE	Scolytinae	Phloeotribini	<i>Phloeotribus</i>	<i>spinulosus</i>	–	–	–	HQ883585	–	HQ883905	–
CURCULIONIDAE	Baridinae	Phytobiini	<i>Phytobius</i>	sp.	–	–	–	FJ867702	–	FJ859957	–
CURCULIONIDAE	Molytinae		<i>Pissodes</i>	<i>strobi</i>	–	U77976	–	KT799825	–	–	–
CURCULIONIDAE	Scolytinae	Ipini	<i>Pityogenes</i>	<i>bistridentatus</i>	–	HQ883662	–	HQ883573	HQ883734	HQ883891	–
CURCULIONIDAE	Platypodinae	Platypodini	<i>Platypus</i>	<i>incompertus</i>	–	HQ883673	–	–	–	HQ883903	–
CURCULIONIDAE	Platypodinae	Platypodini	<i>Platypus</i>	sp.	–	KF016276	–	KF016411	–	–	–
CURCULIONIDAE	Platypodinae	Platypodini	<i>Platypus</i>	<i>jansonii</i>	–	HQ883672	–	HQ883582	HQ883742	HQ883902	–
CURCULIONIDAE	Entiminae	Polydrusini	<i>Polydrusus</i>	<i>cervinus</i>	–	HQ883653	–	HQ883568	HQ883729	HQ883884	–
CURCULIONIDAE	Entiminae	Leptopiini	<i>Polyphrades</i>	sp.1	–	KF016298	–	KF016434	–	–	–
CURCULIONIDAE	Entiminae	Leptopiini	<i>Polyphrades</i>	sp.2	–	KF016390	–	KF016530	–	–	–
CURCULIONIDAE	Molytinae	Amorphocerini	<i>Porthetes</i>	<i>hispidus</i>	–	HQ883666	–	HQ883577	HQ883737	HQ883895	–
CURCULIONIDAE	Scolytinae	Premnobiini	<i>Premnobius</i>	<i>cavipennis</i>	–	HQ883694	–	HQ883605	HQ883762	HQ883925	–
Cerambycidae	Prioninae		<i>Prionopus</i>	<i>reticularis</i>	–	FJ859985	FJ859983	–	FJ859976	FJ859982	–
CURCULIONIDAE	Cossoninae	Onycholipini	<i>Pselactus</i>	sp.	–	–	–	HQ883552	–	HQ883868	–
CURCULIONIDAE	Molytinae	Psepholacini	<i>Psepholax</i>	<i>leoninus</i>	–	KF016379	–	KF016518	–	–	–
CURCULIONIDAE	Molytinae	Psepholacini	<i>Psepholax</i>	<i>mastersii</i>	–	KF016343	–	KF016475	–	–	–
CURCULIONIDAE	Molytinae	Psepholacini	<i>Psepholax</i>	sp.	–	HQ883626	–	HQ883542	HQ883709	HQ883857	–
CURCULIONIDAE	Scolytinae	Phloeosinini	<i>Pseudochramesus</i>	<i>acuteclavatus</i>	–	–	–	–	–	HQ883897	–
CURCULIONIDAE	Cossoninae	Onycholipini	<i>Pseudostenoscelis</i>	sp.	–	HQ883636	–	HQ883551	HQ883717	HQ883867	–
BRENTIDAE	Apioninae	Rhadinocybini	<i>Pterapion</i>	sp.	–	FJ867808	FJ867779	FJ867703	FJ867886	FJ859958	–
ANTHRIBIDAE	Anthribinae	Ptychoderini	<i>Ptychoderes</i>	<i>nebulosus</i>	–	–	FJ867780	–	FJ867887	FJ859959	–
CURCULIONIDAE	Scolytinae	Ctenophorini	<i>Pycnarthrum</i>	<i>hispidum</i>	–	HQ883647	–	–	–	HQ883875	–
CURCULIONIDAE	Scolytinae	Ctenophorini	<i>Pycnarthrum</i>	sp.	–	HQ883648	–	HQ883561	–	HQ883876	–
CURCULIONIDAE	Dryophthorinae	Rhynchophorini	<i>Rhabdoscelus</i>	<i>obscurus</i>	–	KF016277	–	KF016412	–	–	–
CURCULIONIDAE	Molytinae	Aedemonini	<i>Rhadinomerus</i>	sp.	–	KF016272	–	KF016407	–	–	–
CURCULIONIDAE	Cyclominae	Aterpini	<i>Rhadinomus</i>	<i>lacordairei</i>	–	KF016350	–	KF016482	–	–	–
CURCULIONIDAE	Curculioninae	Rhamphini	<i>Rhamphus</i>	<i>acaciae</i>	–	KF016395	–	KF016535	–	–	–
CURCULIONIDAE	Baridinae	Ceutorhynchini	<i>Rhinoncus</i>	<i>pericarpus</i>	–	HQ883620	–	HQ883539	–	HQ883852	–
CURCULIONIDAE	Cyclominae	Aterpini	<i>Rhinoplethes</i>	<i>foveatus</i>	–	KF016273	–	KF016408	–	–	–
BRENTIDAE	Apioninae	Rhinorhynchidiini	<i>Rhinorhynchidius</i>	<i>australasiae</i>	–	KF016286	–	–	–	–	–
BRENTIDAE	Apioninae	Rhinorhynchidiini	<i>Rhinorhynchidius</i>	sp.	–	FJ867807	FJ867782	FJ867704	–	–	–
BELIDAE	Belinae	Belini	<i>Rhinotia</i>	<i>bimaculata</i>	–	KF016384	–	–	–	–	–
BELIDAE	Belinae	Belini	<i>Rhinotia</i>	sp.	–	FJ867806	FJ867784	FJ867705	FJ867888	FJ859960	–
BELIDAE	Oxycoryninae	Oxycorynini	<i>Rhopalotria</i>	sp.	–	–	FJ867785	FJ867706	FJ867889	FJ859961	–

Appendix 4. Cont.

Family	Subfamily	Tribe	Genus		Cytb	COI	18S	28S	EF1a	ArgK	Pglym
NEMONYCHIDAE	Rhinorhynchinae	Mecomacerini	<i>Rhynchitomacerinus</i>	<i>kuscheli</i>	–	FJ867805	–	FJ867708	FJ867890	FJ859962	–
CURCULIONIDAE	Dryophthorinae		<i>Rhynchophorus</i>	<i>ferrugineus</i>	GU581640	GU581628	EF125057	KF311668	KT748805	KT748862	–
CURCULIONIDAE	Cossoninae	Rhyncolini	<i>Rhyncolus</i>	sp.1	–	HQ883633	–	HQ883547	HQ883715	HQ883863	–
CURCULIONIDAE	Cossoninae	Rhyncolini	<i>Rhyncolus</i>	sp.2	–	HQ883639	–	HQ883555	HQ883720	–	–
BRENTIDAE	Apioninae	Myrmacielini	<i>Rhynolaccus</i>	<i>formicarius</i>	–	KF016313	–	–	–	–	–
CURCULIONIDAE	Platypodinae	Schedlarini	<i>Schedlarius</i>	<i>mexicanus</i>	–	HQ883625	–	–	–	–	–
CURCULIONIDAE	Brachycerinae	Raymondionimini	<i>Schizomicrus</i>	<i>caecus</i>	–	FJ867824	FJ867787	FJ867709	FJ867891	–	–
CURCULIONIDAE	Scolytinae	Ctenophorini	<i>Scolytodes</i>	<i>acuminatus_sp</i>	–	–	FJ867788	–	–	HQ883877	–
CURCULIONIDAE	Scolytinae	Scolytplatypodini	<i>Scolytplatypus</i>	<i>mikado</i>	–	–	FJ867789	–	–	–	–
CURCULIONIDAE	Scolytinae	Scolytplatypodini	<i>Scolytplatypus</i>	<i>entomoides</i>	–	HQ883679	–	–	HQ883748	–	–
CURCULIONIDAE	Baridinae	Conoderini	<i>Scolytproctus</i>	sp.	–	HQ883618	–	HQ883537	HQ883704	HQ883850	–
CURCULIONIDAE	Scolytinae	Scolytini	<i>Scolytus</i>	<i>intricatus</i>	–	HQ883677	–	HQ883589	HQ883746	HQ883909	–
CURCULIONIDAE	Scolytinae	Scolytini	<i>Scolytus</i>	<i>scolytus</i>	–	HQ883678	–	HQ883590	HQ883747	HQ883910	–
CURCULIONIDAE	Curculioninae	Curculionini	<i>Shigizo</i>	<i>rhombiformis</i>	AB573520	AB573486	–	AB573449	–	–	AB573586
CURCULIONIDAE	Curculioninae	Tychiini	<i>Sibinia</i>	sp.	–	HQ883649	–	HQ883563	HQ883725	HQ883878	–
CURCULIONIDAE	Entiminae	Sitonini	<i>Sitona</i>	<i>discoideus</i>	–	–	–	KF016449	–	–	–
CURCULIONIDAE	Entiminae	Sitonini	<i>Sitona</i>	<i>hispidulus</i>	–	–	–	FJ867710	–	–	–
CURCULIONIDAE	Dryophthorinae	Sitophilini	<i>Sitophilus</i>	<i>granarius</i>	–	–	FJ867790	FJ867711	–	FJ859963	–
CURCULIONIDAE	Dryophthorinae	Sitophilini	<i>Sitophilus</i>	<i>zeamais</i>	–	KJ397812	–	–	AY131129	–	–
CURCULIONIDAE	Curculioninae	Smicronychini	<i>Smicronyx</i>	sp.1	–	KF016331	–	KF016464	–	–	–
CURCULIONIDAE	Curculioninae	Smicronychini	<i>Smicronyx</i>	sp.2	–	FJ867825	FJ867791	FJ867712	FJ867892	FJ859964	–
CURCULIONIDAE	Dryophthorinae	Rhynchophorini	<i>Sparganobasis</i>	<i>subcruciata</i>	–	KF016274	–	KF016409	–	–	–
CURCULIONIDAE	Entiminae	Tropiphorini	<i>Spartecerus</i>	sp.	–	FJ867826	FJ867792	FJ867713	FJ867893	–	–
CURCULIONIDAE	Dryophthorinae	Sphenophorini	<i>Sphenophorus</i>	<i>brunnipennis</i>	–	–	FJ867793	FJ867714	FJ867894	FJ859965	–
BELIDAE	Belinae	Pachyurini	<i>Sphinctobelus</i>	<i>quadrimaculatus</i>	–	–	–	KF016469	–	–	–
CURCULIONIDAE	Cossoninae	Rhyncolini	<i>Stenancylus</i>	sp.1	–	–	–	FJ867715	–	–	–
CURCULIONIDAE	Cossoninae	Rhyncolini	<i>Stenancylus</i>	sp.2	–	HQ883641	–	–	–	–	–
CURCULIONIDAE	Brachycerinae	Erihlinini	<i>Stenopelmus</i>	<i>rufinasus</i>	–	–	FJ867794	FJ867716	FJ867895	FJ859966	–
CURCULIONIDAE	Cyclominae	Listroderini	<i>Steriphus</i>	<i>major</i>	–	KF016351	–	KF016483	–	–	–
CURCULIONIDAE	Cyclominae	Listroderini	<i>Steriphus</i>	sp.	–	KF016324	–	KF016460	–	–	–
CURCULIONIDAE	Curculioninae	Storeini	<i>Storeus</i>	sp.	–	KF016353	–	KF016485	–	–	–
CURCULIONIDAE	Dryophthorinae	Stromboscerini	<i>Stromboscerini</i>	sp.	–	FJ867828	FJ867795	FJ867717	–	–	–
CURCULIONIDAE	subfamilia incerta	incertae sedis	<i>Syagrius</i>	sp.	–	KF016377	–	KF016516	–	–	–
CURCULIONIDAE	Molytinae	Psepholacini	<i>Sympiezoscelus</i>	<i>spencei</i>	–	FJ867827	FJ867796	FJ867718	–	FJ859967	–

Appendix 4. Cont.

Family	Subfamily	Tribe	Genus		Cyto B	COI	18S	28S	EF1a	ArgK	Pglym
CURCULIONIDAE	Curculioninae	Rhamphini	<i>Tachygonus</i>	<i>lecontei</i>	–	FJ867823	–	–	–	FJ859968	–
CURCULIONIDAE	Entiminae	Tanymecini	<i>Tanymecus</i>	sp.	–	FJ867822	FJ867797	FJ867719	–	FJ859969	–
CURCULIONIDAE	Brachycerinae	Eirrhini	<i>Tanysphyrus</i>	<i>lemnæ</i>	–	–	–	FJ867720	–	FJ859970	–
ANTHRIBIDAE	Anthribinae		<i>Telala</i>	sp.	–	KF016371	–	KF016510	–	–	–
CURCULIONIDAE	Platypodinae	Platypodini	<i>Teloplatus</i>	sp.	–	HQ883674	–	HQ883583	HQ883743	HQ883904	–
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Tesserocerus</i>	<i>dewalquei</i>	–	HQ883692	–	HQ883603	HQ883761	HQ883921	–
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Tesserocerus</i>	<i>ericus</i>	–	HQ883691	–	HQ883602	HQ883760	HQ883920	–
CURCULIONIDAE	Platypodinae	Tesserocerini	<i>Tesserocranulus</i>	<i>nevermanni</i>	–	HQ883690	–	HQ883601	HQ883759	HQ883919	–
CURCULIONIDAE	Molytinae	incertae sedis	<i>Thaumastophasis</i>	sp.	–	–	–	KF016477	–	–	–
CURCULIONIDAE	Scolytinae	Tomicini	<i>Tomicus</i>	<i>piniperda</i>	–	HQ883681	–	HQ883592	HQ883750	HQ883911	–
CURCULIONIDAE	Scolytinae	Tomicini	<i>Tomicus</i>	<i>piniperda</i>	–	–	–	FJ867721	–	FJ859971	–
ANTHRIBIDAE	Anthribinae	Platystomini	<i>Toxonotus</i>	<i>cornutus</i>	–	FJ867821	FJ867798	–	–	FJ859972	–
BRENTIDAE	Brentinae	Trachelizini	<i>Tracheloschizus</i>	<i>dichrous</i>	–	KF016295	–	KF016431	–	–	–
CURCULIONIDAE	Platypodinae	Platypodini	<i>Trachyostus</i>	<i>schaufussi</i>	–	HQ883675	–	HQ883584	HQ883744	–	–
CURCULIONIDAE	Molytinae	incertae sedis	<i>Tranes</i>	<i>insignipes</i>	–	KF016283	–	KF016418	–	–	–
CURCULIONIDAE	Molytinae	incertae sedis	<i>Tranes</i>	<i>lyterioides</i>	–	FJ867820	FJ867799	FJ867722	FJ867896	FJ859973	–
CURCULIONIDAE	Molytinae	incertae sedis	<i>Tranes (Platyphaeus)</i>	<i>lyterioides</i>	–	KF016328	–	–	–	–	–
ANTHRIBIDAE	Anthribinae	Anthribini	<i>Trigonorhinus</i>	<i>tomentosus</i>	–	FJ867819	FJ867800	FJ867723	–	–	–
CURCULIONIDAE	Scolytinae	Xyloterini	<i>Trypodendron</i>	<i>lineatum</i>	–	–	–	–	–	HQ883923	–
CURCULIONIDAE	Cossoninae	Pentarthrini	<i>Tychiodes</i>	sp.	–	–	FJ867801	FJ867724	–	FJ859974	–
ANTHRIBIDAE	Urodontinae		<i>Urodontellus</i>	sp.	–	KF016338	–	KF016471	–	–	–
ANTHRIBIDAE	Urodontinae		<i>Urodontus</i>	<i>mesemoides</i>	–	FJ867818	FJ867802	FJ867725	FJ867897	–	–
CURCULIONIDAE	subfamilia incerta	Viticiini	<i>Vitidis</i>	<i>bidentatus</i>	–	KF016332	–	KF016465	–	–	–
CURCULIONIDAE	subfamilia incerta	Hyperini	<i>Xeda</i>	<i>amplipennis</i>	–	KF016288	–	KF016423	–	–	–
CURCULIONIDAE	Scolytinae	Dryocoetini	<i>Xylocleptes</i>	<i>bispinus</i>	–	–	–	–	–	HQ883881	–
CURCULIONIDAE	Scolytinae	Xyloterini	<i>Xyloterinus</i>	<i>politus</i>	–	–	–	–	–	HQ883924	–
CURCULIONIDAE	Baridinae	Ceutorhynchini	<i>Zacladus</i>	<i>geranii</i>	–	HQ883621	–	HQ883540	HQ883706	HQ883853	–
ATTELABIDAE	Rhynchitinae	Rhynchitini		sp.	–	–	FJ867786	FJ867707	–	–	–
CURCULIONIDAE	Curculioninae			sp.	–	HQ883650	–	HQ883564	HQ883726	HQ883879	–
CURCULIONIDAE	Curculioninae	Curculionini		sp.	AB573532	AB573497	–	AB573461	AB573565	–	AB573596
CURCULIONIDAE	Entiminae	Leptopiini		sp.	–	KF016306	–	KF016442	–	–	–
CURCULIONIDAE	Entiminae	Ottistirini		sp.	–	KF016333	–	KF016466	–	–	–
CURCULIONIDAE	Molytinae			sp.	–	HQ883667	–	HQ883578	HQ883738	HQ883896	–
ANTHRIBIDAE				sp.1	–	HQ883607	–	HQ883527	–	–	–

Appendix 4. Cont.

Family	Subfamily	Tribe	Genus		Cytb	COI	18S	28S	EF1a	ArgK	Pglym
BRENTIDAE	Brentinae			sp.1	–	HQ883613	–	HQ883532	HQ883699	HQ883845	–
CURCULIONIDAE	Conoderinae	Baridini		sp.1	–	HQ883611	–	HQ883530	–	HQ883844	–
CURCULIONIDAE	Conoderinae	Campyloscelini		sp.1	–	HQ883615	–	HQ883534	HQ883701	HQ883847	–
CURCULIONIDAE	Cossoninae	Onycholipini		sp.1	–	HQ883637	–	HQ883553	HQ883718	HQ883869	–
CURCULIONIDAE	Molytinae	Cryptorhynchini		sp.1	–	HQ883627	–	–	–	–	–
ANTHRIBIDAE				sp.2	–	HQ883608	–	–	HQ883696	HQ883841	–
BRENTIDAE	Brentinae			sp.2	–	HQ883614	–	HQ883533	HQ883700	HQ883846	–
CURCULIONIDAE	Conoderinae	Baridini		sp.2	–	HQ883619	–	HQ883538	HQ883705	HQ883851	–
CURCULIONIDAE	Conoderinae	Campyloscelini		sp.2	–	HQ883616	–	HQ883535	HQ883702	HQ883848	–
CURCULIONIDAE	Molytinae	Cryptorhynchini		sp.2	–	HQ883628	–	HQ883543	HQ883710	HQ883858	–
CURCULIONIDAE	Cossoninae	Onycholipini		sp.3	–	HQ883638	–	HQ883554	HQ883719	HQ883870	–

Appendix 5. Ancestral area reconstruction of *Ceratopus* (DIVA) using a more complex scenario of distribution. The pie charts at nodes represent the likelihood of each biogeographic region calculated with BioGeoBEARS. The black color on the charts represents uncertainty. Areas of endemism extracted from Morrone (2014b).