Title

From Soup to Systematics: Exploring the Phylogenetics and Extraordinary Evolution of Head and Genitalia of Nannocoiris Reuter (Hemiptera: Dipsocoromorpha) & Taxonomic Revision of Guapinannus Wygodzinsky (Hemiptera: Dipsocoromorpha)

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From Soup to Systematics: Exploring the Phylogenetics and Extraordinary Evolution of Head and Genitalia of *Nannocoris* Reuter (Hemiptera: Dipsocoromorpha) & Taxonomic Revision of *Guapinannus* Wygodzinsky (Hemiptera: Dipsocoromorpha)

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Dedication

This Thesis is dedicated to my future self who will look back with fondness and to the little girl who could only dream of the person I am now.

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Introduction to Thesis

Systematics and phylogenetics allows us to explore the evolution of biodiversity on our planet and illuminate relationships between organisms. The Tree of Life in its entirety is vast and beyond the reach of our knowledge thus far, however with every small branch that is reconstructed we see a little more and understand a little better the complexity of the relationships between all living things. Dipsocoromorpha, also known as minute litter bugs, is one such small branch of this vast tree, and with ~430 described species is one of the smallest and least studied infraorders of Heteroptera. They are minute in size as well (1-2 mm) and found primarily in cryptic microhabitats such as leaf litter in tropical regions (Emsley 1969; Henry 2009; Knyshov et al. 2016; Leon and Weirauch 2016; Schuh and Slater 1995; Weirauch and Fernandes 2015; Weirauch and Štys 2014). The natural history of these insects is poorly documented, they are rarely collected, and phylogenetic relationships between the ~60 genera are largely unknown (Weirauch and Štys 2014). In this study we curated specimens from bulk samples already housed in various natural history collections which has proven to be an effective method for exploring biodiversity, phylogenetic relationships, and evolution. The study focuses on two genera in the largest and most diverse, both morphologically and with respect to species numbers, family of Dipsocoromorpha, the Schizopteridae. The first two chapters investigate phylogenetic relationships within *Nannocoris* Reuter 1891 and infer the evolutionary history of exaggerated traits in this genus. The final chapter is a taxonomic revision of *Guapinannus* Wygodzinsky, 1951 (Hemiptera: Schizopteridae).

The first chapter investigates the interspecific evolution of head length based on the first phylogenetic hypothesis of the group and ancestral state reconstruction. The diversity of head length within this genus is unique ranging from approximately one sixth the body length to more than a quarter the body length. The reach of this assumed predator is affected by the length of the rostrum which is correlated to the length of the head. While little to nothing is known about their biology the lack of sexual dimorphism in this trait suggests that the head evolution may be driven by natural selection.

The second chapters continues the story of *Nannocoris* with the investigation of a perhaps more unbelievable exaggerated trait, the extreme diversity of the length of the male and female genitalia. Studying genitalia has helped naturalist study the patterns of morphological evolution for more than a century. *Nannocoris* has demonstrated vast diversity in the length of the male intromittent organ (vesica) ranging from 1/8th the body length to 17 times the body length making this male genial organ one of if not the longest in the animal kingdom relative to the bug's body size. We investigate the diversity and correlation between the male and female genitalia of *Nannocoris* using the same phylogenetic hypothesis obtained in the previous chapter. We reconstruct the ancestral states of the length of the male vesica and the female spermathecal duct, and calculate phylogenetic independent contrasts to test for correlation between the male and female genitalia. The male and female genitalia are found to be strongly correlated. Coupled with the incredible interspecific diversity in length of both genitalic structures make this an intriguing system for future evolutionary studies.

The final chapter of this thesis is the taxonomic revision of the genus *Guapinannus*. This monotypic genus was described based on a single female specimen from Costa Rica. Residue samples from museums all over the world were sorted and more than 250 specimens of *Guapinannus* representing 19 new species were obtained including the first time male specimens. Genitalic and wing venation characters were documented through digital imaging, scanning electron and confocal micrographs in the hopes that this revision will help to properly place *Guapinannus* within Dipsocoromorpha .

Chapter I

Heads up: evolution of exaggerated head length in the minute litter bug genus *Nannocoris* Reuter (Hemiptera: Schizopteridae)

Abstract

Evolutionary biologists have long been intrigued by exaggerated morphologies tied to sexual or natural selection. In insects, relatively few studies have investigated the evolution of such traits at the genus level and above and have used phylogenetic methods to do so. We here investigate the interspecific evolution of head length in the minute litter bug genus *Nannocoris* Reuter based on the first phylogenetic hypothesis of the group (25 ingroup species, five gene regions, 3409 bp) and ancestral state reconstruction. Head lengths in this speciose genus range from approximately one sixth of the total body length to more than a quarter of the body length, while the head and mouthpart (rostrum) lengths are correlated. Different species therefore possess a markedly different reach of the rostrum when extended. The analyses show that head length evolution in *Nannocoris* is plastic, with head length elongations and reductions occurring in several clades, derived from ancestors with moderately elongate heads. Evidence is provided that exaggerated head lengths evolved through elongation of either the genal (*pricei* group) or the tip (*arimensis* group) region of the head. The biology of species in the genus *Nannocoris* is unknown, but given the lack of sexual dimorphism of head lengths, we speculate that head evolution in this genus may be driven by natural selection, potentially in the context of prey capture.

Introduction

Exaggerated morphologies, such as the long neck of giraffes (Lamarck 1809), grotesquely elongated forelegs of male harlequin beetles (Zeh et al. 1992) , tweezer-shaped mouthparts of ant lions (Griffiths 1980), or bulbous hind legs of flea beetles (Ge et al. 2011) have long intrigued biologist. Starting with the study of long-spurred orchids and their sphingid moth pollinators made famous by (Darwin 1862), evolutionary biologists have investigated patterns of exaggerated traits and the selective pressures that may have driven their evolution. Lavine et al. (2015) reviewed the literature on studies on this topic and identified three areas of context in which disproportionately developed body parts have evolved: as a result of reproductive competition, caste-differentiation in social insects, and adaptations to food resources. Male competition and sexual selection are thought to have played an important role in shaping exaggerated male-specific features in insects and other organisms and phenomena such as the hypertrophic mandibles in lucanid beetles and stalked eyes in diopsid flies have been studied in detail (Cotton et al. 2004; Panhuis and Wilkinson 1999). Similarly, caste-specific exaggerated traits including the defensive morphologies in termite soldiers have attracted significant attention (Cornette et al. 2008; Eggleton 2011; Koshikawa et al. 2002; Miura 2001). Greatly elongated and armed raptorial appendages in praying mantises (Loxton and Nicholls 1979; Maldonado et al. 1967) and long mouthparts in flies, true bugs, sphingid moths, and weevils have been shown to be involved in feeding success and have likely evolved as adaptations to prey capture or other specialized feeding strategies (Brozek and Herczek 2004; Karolyi et al. 2012; Wasserthal 1997; Wcislo and Eberhard 1989). The majority of these studies have concentrated on microevolutionary patterns and processes while investigating selection pressures that have shaped intraspecific variability of these traits within a species (Forsyth and Alcock 1990; Ge et al. 2011; Kelly 2006; Koshikawa et al. 2002; Kral et al. 2000, 2000). In contrast, fewer studies have documented macroevolutionary patterns of exaggerated traits, i.e. have explored evolutionary transitions using species-level phylogenetic hypotheses and ancestral state reconstructions. Among these, most have focused on vertebrates where they have explored exaggerated traits in the context of sexual selection (Wiens 1999, 2001), with fewer studies using insects as their model systems (Baker and Wilkinson 2001). From a survey of the literature it appears that studies investigating the disproportional development of body parts in non-sexually selected systems in insects are rare (Lavine et al. 2015; Refki et al. 2014). We here use the minute litter bugs genus *Nannocoris* Reuter 1891 (Hemiptera: Dipsocoromorpha: Schizopteridae) as a model to investigate the evolutionary history of an exaggerated trait, a dramatically elongated head, that has likely been shaped by natural selection.

Nannocoris is part of the true bug infraorder Dipsocoromorpha, a group of minuscule (1-2 mm body length) insects primarily found in cryptic microhabitats such as leaf litter in tropical regions (~430 described spp. worldwide): (Emsley 1969; Henry 2009; Knyshov et al. 2016; Leon and Weirauch 2016; Schuh and Slater 1995; Weirauch and Fernandes 2015; Weirauch and Štys 2014). The natural history of minute litter bugs is poorly documented, but there is some indication that species are raptorial and feed on other tiny arthropods (Esaki and Miyamoto 1959). The number of undescribed species and phylogenetic relationships between and within the 56 genera are largely unknown (Weirauch and Štys 2014). *Nannocoris* currently comprises 12 described species from the New World (11 Neotropical, one Nearctic). Phylogenetic relationships within the genus are unknown. As part of an US National Science Foundation ARTS grant that focuses on the systematics and evolution of Dipsocoromorpha, we have assembled and examined >660 specimens of *Nannocoris* and estimate that the actual number of species in this genus is between 40 and 50. Sexual dimorphism of body shape and size in *Nannocoris* is limited compared to other groups of Schizopteridae (e.g., Knyshov et al. 2016), but females in some species are coleopteroid (i.e., have hardened, elytriform forewings) and males in many, but not all, species feature a pit-like structure on their vertex that is connected to a complex gland of unknown function with median reservoir and paired glandular areas (unpublished data). *Nannocoris* species display two intriguing morphological features that are unknown in other minute litter bugs: the male vesica (part of the intromittent organ) shows interspecific length variation ranging from very short to many times the bug's body length (Frankenberg and Weirauch, in prep.) and the head in most, but not all species of *Nannocoris* is moderately to extremely elongated and pointed, reaching about one quarter of the total body length in extreme cases (Fig. 1a-d, g, h). The labium of *Nannocoris* spp. is slender and about twice as long as the head, with variation between species mirroring head length variation. Based on preliminary examination of different *Nannocoris* spp. with long heads, we observed that the elongated head shape is either due to the expansion of the gena or the extension of the most anterior part of the head that we here refer to as the tip region. It is unclear if these head length differences evolved stepwise and directional from short to extremely elongated heads, or if transitions were more complex and involved multiple elongations and/or reductions.

Most species of *Nannocoris* occur in wet tropical and subtropical forests. Compared to some other groups of Schizopteridae that are almost exclusively collected from leaf litter using various leaf and soil litter extraction methods (Emsley 1969), more than one third of the specimens of *Nannocoris* that we have examined are derived from yellow pan traps, Malaise traps and flight intercept traps. This suggests that at least some of these minute litter bugs are active fliers. We have swept and beaten *Nannocoris* individuals from herbaceous vegetation during our own field work and therefore suspect that certain species of *Nannocoris* are associated with living plants above the ground (Weirauch lab, unpublished observations). It is unknown if minute litter bugs are ambush predators or if they actively pursue their prey. Assuming that the rostrum (labium plus enclosed mandibular and maxillary stylets) in *Nannocoris* is extended in front of the head during predation similar to feeding behaviors in other true bugs such as Triatominae (Lent and Wygodzinsky, 1979), the elongation of the head and labium observed in some species might drastically increase the reach of the rostrum during predation.

The aims of the present study are threefold. First, we present the first molecular phylogenetic analysis that investigates species-level relationships in the genus *Nannocoris*. Ingroup taxon sampling included 25 described and undescribed species across the geographic range of the genus and contained species with extremely short and long heads. Second, we document *Nannocoris* head features with emphasis on the ratio of head length to head height and contribution of gena and tip region to head length, as well as some unusual head shapes and the male-specific pit-like structure. Third, we use the molecular phylogenetic hypothesis and morphological traits to infer head length evolution across the genus.

Materials and methods

Material acquisition, identifications, specimen vouchering, and imaging. We have sorted and examined ~660 specimens of *Nannocoris* from more than 20 countries in North, Central, and South America. About 440 of these specimens were ethanol-preserved and retrieved from passive-trapping residue samples held at various natural history collections; the remaining specimens were point- or slide-mounted specimens on loan from insect collections. For the molecular component of this project, we DNA-extracted and sequenced 25 described and undescribed species of *Nannocoris*. About 60 additional specimens, some representing other putative species, were extracted. However, initial PCR amplification attempts failed and we disregarded these samples. This is a common issue with specimens derived from residue samples with high insect mass to preservation fluid ratio and sometimes low-percentage ethanol when collected. Because phylogenetic relationships of *Nannocoris* to other genera of Schizopteridae are not well established beyond the phylogenetic hypothesis by Weirauch and Štys (2014) that included poor taxonomic sampling for Schizopterinae, we included a large number of outgroup taxa in our analyses, i.e. 32 non-*Nannocoris* Schizopteridae (Schizopterinae: *Ptenidiophyes* Reuter 1891 – 1 sp., *Schizoptera* Fieber 1860 – 3 spp., *Pinochius* Carayon 1949 – 5 spp., *Hoplonannus* McAtee and Malloch 1925 – 3 spp., *Voragocoris* Weirauch 2012 – 2 spp., *Membracioides* McAtee and Malloch 1925 – 4 spp., and one of undescribed genus (here referred to as "nr *Nannodictyus*"), seven Hypselosomatinae, and six Ogeriinae; two Dipsocoridae and one species of Ceratocombidae are also included. Fourteen of these taxa had been sequenced in the Weirauch lab prior to this project and their sequences were downloaded from GenBank, 18 taxa were sequenced for this project, as were all *Nannocoris* species. Voucher specimens belong to and are deposited in eight natural history collections (see Table 1).

For species identification, we compared material to holotypes and paratypes (or their photographs) where possible (holotypes of *Nannocoris capitata* Uhler 1894, *Nannocoris cavifrons* McAtee and Malloch 1925, *Nannocoris flavomarginata* McAtee and Malloch 1925, *Nannocoris nasua* McAtee and Malloch 1925, *Nannocoris pricei* Emsley 1969, and *Nannocoris schwarzi* McAtee and Malloch 1925; paratype of *Nannocoris descolei* Wygodzinsky 1952). Where types could not be located or where we were unable to examine or photograph specimens, original species descriptions and illustrations were used for identifications (Emsley 1969; McAtee and Malloch 1925; Reuter 1891; Uhler 1894; Wygodzinsky 1952). Unsurprisingly given the overwhelming numbers of undescribed species in many schizopterid groups (Emsley 1969; Knyshov et al. 2016; Weirauch and Frankenberg 2015), the majority of taxa included in our phylogenetic analysis represent undescribed species: we are confident with our identifications of *Nannocoris tuberculifera* Reuter 1891, *N. pricei*, and *N. cavifrons*, and we place two additional terminals as new species “near” a described species (*N.* nr. *flavomarginata*, *N.* nr. *arimensis*). The remaining terminals represent additional undescribed species recognized by a combination of diagnostic characters of the wing venation, coloration, head shape and length, variation in the size and shape of the male-specific pit-like gland opening on the head, and male genitalic structures. Emsley (1969) described three species of *Nannocoris* from one valley in the Northern mountain range of Trinidad, suggesting that multiple congeneric species can be sympatric. Compared to other Schizopteridae, sexual dimorphism is limited in *Nannocoris* (Fig. 1n) and we are therefore confident that we have associated males and females correctly (only females in some species are submacropterous or even coleopteroid). A taxonomic revision of *Nannocoris* is forthcoming (Weirauch and Frankenberg, in prep.).

All specimens examined were associated with a matrix-code label that features unique combinations of a prefix and eight-digit number. Specimens are databased in the PBI instance of the Arthropod Easy Capture database that is served from the American Museum of Natural History (AMNH) https://research.amnh.org/pbi/locality/; records are publicly available through the Heteroptera Species Pages (http://research.amnh.org/pbi/heteropteraspeciespage). Specimens, images, and vouchers are also associated with a lab internal “ED” number that is used to track specimens in the lab, this number is provided together with the USI number and GenBank accession number for molecular vouchers (Tab. 1). Habitus images (dorsal, lateral, and ventral perspectives) of morphological and molecular voucher specimens were taken. Specimens were stabilized with KY jelly in a watch glass, submerged in ethanol, and imaged using a Leica DFC 450 C Microsystems setup with Planapo 1.0× and 2.0× objectives. Images were compiled and edited using the Leica Application Suite (LAS) V4.3.

Molecular protocols and phylogenetic reconstruction. Specimens were digested in Proteinase K solution for a 24-36 hour period. Qiagen DNeasy Blood and Tissue kit was used for all DNA extractions. We targeted four regions of three ribosomal genes, 16S rDNA (using a new primer designed in the Weirauch lab: 5’-AAATTAGTYCTGCCCAATGATTTAT-3’, forward and 5’-TGTAAAAYTTTAATGGTCGAACAGA-3’, reverse), 18S rDNA (18S-3f, 18S-Bi), and the D2 (D2-Fa, D2-Ra) and D3-D5 (D3-Fa, D5-Ra) regions of 28S rDNA (see Weirauch and Munro 2009 for additional primer information), as well as part of one protein-coding gene, CO1 (using the LCO and HCOoutout primers from Folmer et al. [1994] and Prendini et al [2005]). PCR products were cleaned using Bioline Sureclean according to protocol and sequenced at Macrogen USA. Sequences were assembled and edited using Sequencher 4.8™. Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to verify that all sequences belong to Heteroptera and Schizopteridae. Sequences were trimmed using Mesquite 3.04 Maddison and Maddison 2011) and MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2005) was used to align individual gene regions with the E-INS-i algorithm. Sequences were then concatenated using Sequence Matrix v.1.7.8 (Vaidya et al. 2011) with external gaps coded as question marks which resulted in an alignment with 3,409 sites. The aligned, trimmed, and concatenated matrix is deposited in TreeBASE and is available under [URL will be added during proof stage]. The concatenated dataset was partitioned by gene region and analyzed using RAxML-HPC2 on XSEDE v8.0.24 (Stamatakis 2014) at CIPRES (http://www.phylo.org/). We used a representative of the Ceratocombidae (*Ceratocombus_Xylonannus*_sp_Thai_76) to root the topology. The alignment was partitioned by gene regions and in addition by codon position for CO1; GTR was determined to be the best fitting substitution model for all partitions. Random seed was set to 12345. We used RAxML rapid bootstrapping with subsequent maximum likelihood searches and six distinct models/data partitions with joint branch length optimization. All other parameters were set to default.

Head measurements and ancestral state reconstruction. Head length and height in lateral view were measured using ImageJ (Abramoff et al. 2004) from habitus images generated for this project (see above and Fig. 1) for all *Nannocoris* species and the sistergroup of *Nannocoris* in our analysis, a species of the undescribed genus “nr *Nannodictyus*”. We measured: 1) the length of the head from the apex of the clypeus to the posterior margin of the head which typically coincides with the posterior margin of the eye; 2) the height of the head at the midpoint of the eye, between the gula ventrally and the vertex dorsally; and 3) the length of the “tip” of the head as defined by the apex of the clypeus and the “point of convergence” between the clypeus, mandibular plate, and buccula (poc in Fig. 1b); the latter was subtracted from the total length for a value describing the length of the posterior portion of the head where the gena is most variable in length between species. Measurements are provided in Table 2. We also measured the length of the rostrum and investigated if the length of the head and of the rostrum are correlated using linear regression (see Supplemental Material 1). We only measured and coded the molecular voucher specimens, since preliminary investigations (data not shown) indicated that intraspecific variation of head dimensions is negligible. Both males and females are represented in this analysis; although head shapes can vary slightly between conspecific males and females (Fig. 1n), preliminary measurements (data not shown) suggested that male and female head measurements (length and width) do not differ significantly. Ancestral states were reconstructed for two characters (Fig. 1a, b): a) the total head length divided by the head height, for a ratio that describes head elongation; and b) the ratio of the anterior (tip to poc; tip region) length to posterior (poc to posterior margin of head; genal region) length, to describe the relative contribution of these two head regions to the overall length of the head. In Figure 4a, smaller values and colder colors describe shorter heads, warmer colors longer heads, while in Figure 4b smaller values and colder colors describe larger contribution by the posterior or genal region, warmer colors larger contribution by the anterior or tip region of the head. The two characters were coded as continuous characters as this coding most closely approximates the observed variation as optimized on the best likelihood tree derived from the phylogenetic analysis. Using the trace character history analysis function in Mesquite we reconstructed ancestral states for each continuous character using parsimony optimization.

Results

Phylogenetic analysis and putative species groups in *Nannocoris*. The best likelihood tree derived from the phylogenetic analysis of the concatenated molecular dataset is presented in Figure 2, with Figure 3 providing a close up of the *Nannocoris* clade. Schizopteridae are recovered as monophyletic with high bootstrap support (BS; 99%), the schizopterid subfamilies Hypselosomatinae and Schizopterinae are monophyletic (100% BS and 77%, respectively), while "Ogeriinae" are recovered as paraphyletic. Within Schizopterinae, the *Schizoptera* clade is recovered (100% BS), a clade formed by *Pinochius* and members of the *Corixidea* genus group here represented by species of *Hoplonannus*, *Membracioides*, and *Voragocoris*, and one comprising a species of the undescribed genus "nr *Nannodictyus*" and *Nannocoris* (BS for both below 50%). Support for the placement of *Nannocoris* in the overall phylogeny of Schizopterinae is therefore weak, but *Nannocoris* is recovered as monophyletic with highest support (100% BS).

Within *Nannocoris*, several clades are well supported (clade A and several species groups, i.e. the *tuberculifera*, *pricei*, and *arimensis* groups), while other relationships will need to be further tested and stabilized using additional taxa and data. We here name species groups based on observed clades with acceptable branch support (>60% BS) that will facilitate the discussion in this publication, but will also guide the ongoing taxonomic project; however, several species remain unplaced due to low support values. *Nannocoris tuberculifera* (originally described from Venezuela by Reuter [1891]; the specimen sequenced here is from Peru) and an undescribed species from Costa Rica form a clade (100% BS) and are the sister group to all remaining species of *Nannocoris* (clade A); we refer to this taxon as the *tuberculifera* group (clade 1) with a pronounced swelling on the posterior part of the vertex as a putative diagnostic feature (Fig. 1f). The morphospecies from Costa Rica (*Nannocoris*_sp_CR_364) also constitutes the first documentation of a species with coleopteroid males in *Nannocoris*. Both species have a moderately elongate head and concave pit-like gland openings (Fig. 1f) and display similar genitalic features particularly in shape and orientation of the anophoric process (not shown). The next diverging lineage within the highly supported clade A (97% BS) is the *pricei* group (clade 2) that comprises three species from North America, Trinidad, and Peru. Although head shapes range from very long (*Nannocoris*_sp_Trin_2408, Fig. 1n) to short (*Nannocoris*_sp_USA_7516), support for this group is fairly high (83%BS). It includes *N. pricei* that was originally described from Trinidad (Emsley 1969) and is recognized by the absence of the pit-like structure on the vertex, but rather its presence on the pronotal collar (posteriorly on the vertex in the other two species). Putative diagnostic feature for this species group are the comparatively more posterior position of the male-specific pit-like gland opening (more anterior in other species groups), the similarly shaped reservoir of the spermatheca, and the short (~one coil) male vesica that features a “bend” in its proximal half. All of these characters taken together make us fairly confident in this relationship.

The *cavifrons* group (73% BS; clade 3) within the poorly supported clade B (51% BS) includes *N. cavifrons* (originally described from Guatemala; voucher specimen from Honduras) and two putatively closely related species from Guyana and Colombia. All species feature a relatively short and convex head (Fig. 3) and males display both a process on tergite 8 and on the anophore (data not shown), a situation that appears to be uncommon in *Nannocoris*. We recovered *Nannocoris* 3021 (from Colombia) as the weakly supported sister taxon to this clade; despite similarities in head shape and male genitalia the notched shape of the pit-like opening set this species apart from the *cavifrons* group and we refrain from including it in a species group in the present study.

Clade C also contains a terminal (*Nannocoris*_sp_3906; Fig. 1i) that we did not include in a species group; prior analyses had placed this taxon in various positions in the phylogenetic hypothesis, suggesting that the available sequence data cannot resolve the phylogenetic position of this rouge taxon, and support for uniting this taxon with Clade D is weak (<50% BS). This undescribed species from Colombia is characterized by a short and high head and a notch-like pit on the head (Fig. 1i) similar to species in the *cavifrons* group. Including morphological characters and additional taxa in future analyses might resolve the phylogenetic position of this species.

Clade 4 (80% BS), here refered to as the small pit species group, contains three species from Colombia and Brazil and share the similar medium head length (e.g., Fig. 1c, l), similar pit morphology, and c-shaped spermathecal reservoir. Three additional species were recovered as closely related to this species group, but we refrain from naming this larger clade due to low support values.

Clade E contains the two final species groups, the *nasua* and *arimensis* groups. The *nasua* group (61% BS) is represented in our analysis by four taxa, two of which resemble *Nannocoris nasua* McAtee and Malloch, but are not conspecific with that species. Head shapes are moderately elongated (e.g., Fig. 1d, j, k). While species in this group display relatively large pit-like gland openings of varying shapes, they are similar in position and size, and males share a long and straight anophoric process (data not shown). The *arimensis* group (84% BS) consists of five morphospecies characterized by relatively long heads with the apex pointing obliquely dorsad. This group contains the species with the longest head known to us (*Nannocoris*_sp_Bra_0961; Fig. 1h), but also species with some of the most unique head shapes, e.g., *Nannocoris*_sp_Guat_6270, for which a conspecific is shown as Fig. 1g and where the apex of the head is expanded to appear shovel-shaped in dorsal view. Males also possess a distinctive s-shaped bifurcating anophoric process (data not shown) and a long vesica with many coils. Females possess a long spermathecal duct with many coils and a similarly shaped spermathecal reservoir.

***Nannocoris* head features.** Head measurements are provided in Table 2, documenting the wide range of overall head length, head height, length of the anterior (tip) and posterior (genal) parts of the head, as well as ratios and overall body length. In the following, we briefly describe other features pertaining to the head morphology in *Nannocoris* that could prove valuable for future investigations into the systematics of this genus. In addition to the differences in head length between species, the shape and size of different head structures shows species-specific differences. Notable examples are an undescribed species (*Nannocoris*_sp_Col_0873) from Columbia where the dorsal surface of the post-clypeal head is dramatically expanded dorsally, resulting in a grotesquely inflated head (Fig. 1e). The length and shape of the clypeus varies between species, as does the length and height of the bucculae, and shape and extension of the gena (Fig. 1). The dorsal surface of the frons and vertex can be slightly concave (Fig. 1f) or convex (Fig. 1c, d). The eye size shows considerable differences between species (e.g., Fig. 1d, h). A type of “male-specific organ” sensu (Hill 2014; Knyshov et al. 2016), the pit-like structures on the head of males found in many, but not all species of *Nannocoris* show diversity with respect to diameter, depth, and shape, as well as position on the head or anterior part of the pronotum (Fig. 1i-m). Based on preliminary observations, the pit-like structure in male *Nannocoris* is the opening of a complex gland embedded in the head that we here call “vertex gland” (Fig. 1m). Documented here is a selection of notch-like (Fig. 1i), v-shaped (Fig. 1k), wide (Fig. 1j) and pinhole-shaped (Fig. 1l) pit-like openings of this vertex gland. These features do not appear to vary within species, but almost certainly will be relevant for species delimitation.

Reconstruction of head length evolution across *Nannocoris* (Fig. 4a,b). Based on the ancestral state reconstructions of head ratios on the best likelihood tree generated here, the most recent common ancestor (MRCA) of *Nannocoris* likely had a moderately elongate head (ratio of head length to width of between 1.3 and 1.4; Fig. 4a). This is in part due to the fact that a species of the undescribed genus “nr *Nannodictyus*” was recovered as sister species to *Nannocoris*, a taxon characterized by a somewhat elongated head (ratio of 1.1-1.3) compared to most other Schizopterinae. Head length increased slightly in the MRCA of clade A, but remained fairly stable across the backbone of the phylogeny of *Nannocoris*, with ratios between 1.4 and 1.8 reconstructed for clades A to E. The most dramatic shifts in head length occurred within specific species groups. The MCRA of the *pricei* groups was reconstructed at a ratio of 1.4 to 1.6, but head lengths vary widely within the group, from some of the longest (ratio of 2.0 to 2.1) to some of the shortest (0.8 to 1.0 ratio) observed in all of *Nannocoris*. The MRCA of the *cavifrons* group is similarly reconstructed as having possessed a moderately elongated head (1.2 to 1.4 ratio), while the head became shorter in two species within the group. Several of the species and species groups diverging within clade C (*Nannocporis*_sp_Col_3906, small pit group, *nasua* group) initially retained the moderately elongate head (to a maximum of a 1.8 ratio). Head length extended in parallel within the small pit, *nasua*, and *arimensis* groups. We recovered drastic secondary length reductions of the head in both the *flavomarginata* and *nasua* groups (e.g., *Nannocoris*_sp_Col_0998 with a ratio of 1.3 to 1.4 and *Nannocoris*_sp_Nic_6312 with a ratio of 1.1 to 1.3). The most extreme head extension and length reductions were recovered in the *arimensis* group: from a MRCA with a head that was twice as long as high (ratio 2.0 to 2.1), that ratio increased to 2.2 to 2.4 in *Nannocoris*_sp_Bra_0961, and dropped to 1.2-1.4 in *Nannocoris*_sp_Pan_6833.

To evaluate the contribution of the anterior and posterior portions of the head to the overall head length, we show the ancestral state reconstruction of the ratio of the anterior and posterior portions of the head in a mirror tree (Fig. 4b). Warm colors visualize a larger contribution of the anterior portion of the head to the overall head length, while cool colors indicate a larger contribution of the posterior part of the head. The ratio of the anterior to posterior portion of the head in the MRCA of *Nannocoris* ranged around 0.3, indicating that the clypeal/buccal area accounts for roughly 1/3 of the overall head length. Ratios ranging between 0.2 and 0.4 are observed in the majority of the diverging clades. This includes species with drastically elongated heads such as *Nannocoris*_sp_Trin_2408, where much of the head elongation appears to be due to an elongation of the posterior portion of the head, a pattern also found within the *flavomarginata* and *nasua* groups *(Nannocoris*_sp_Nic_6292 *and Nannocoris*_sp_Nic_6312). This is dramatically different in the *arimensis* group, where the ratio increases to 0.6 in *Nannocoris*_sp_Bra_0961, indicating that in this species the head elongation is in great part due to an extension of the clypeal/buccal region.

Discussion

The degree of head length variation that we here document for species of *Nannocoris* appears to be rare in insects, in particular at low taxonomic levels. In Hemiptera, head length can vary dramatically across taxa in higher taxonomic groupings, such as the Hydrometridae, where species of *Heterocleptes* (Villiers 1948) feature relatively short heads, while the head in species of *Hydrometra* Latreille 1796 is very long (Andersen 1982). The situation is similar in triatomine Reduviidae, where the monotypic genus *Alberprosenia* Martinez & Carcavallo 1977 is diagnosed by its short head, while the head is long in species of *Rhodnius* Stål 1859 (Lent and Wygodzinsky 1979). However, head length in Hemiptera does not typically vary drastically between species at lower taxonomic levels, i.e. within a given genus. Exceptions are the extreme head-length differences observed in the seed bug genus *Myodocha* Latreille 1807 (Henry et al. 2015) (see their plate 16.3 Fig. 31-35) or in the lantern bug genera *Enchophora* Metcalf 1938 and *Prichtus* (Caldwell 1945) where striking length variation coincides with a diversity of different head shapes (O'Brien 1988) (see their figures 63-67 and 102-117). Outside Hemiptera, a literature search revealed few well-documented examples of substantial head modifications between species within a genus, with the most widely investigated being *Curculio* Linnaeus 1758 (Coleoptera: Curculionidae) weevils and *Cyrtodiopsis* Frey 1928 (Diptera: Diopsidae) diopsid flies: while the specifies-specific and variable length of the rostrum in *Curculio* species is tied to gaining access to different types of seeds for oviposition (Bonal et al. 2011; Toju and Sota 2006), the variable eye-stalk lengths in diopsid flies have been shown to play an important role in male-male competition (Panhuis and Wilkinson 1999). Exaggerated features in insects have rarely been explored in a phylogenetic context at the genus level, mostly due to the lack of species-level phylogenetic hypotheses for the taxa in question. It is therefore unknown if the evolution of exaggerated features in *Myodocha, Enchophora*, or *Curculio* has progressed linearly from short to long, or if longer body parts have evolved multiple times independently. Even in *Curculio*, where a species-level phylogeny is available for a subset of species (Hughes and Vogler 2004), the rostrum length has not been explored in the context of that hypothesis. Our model of head evolution in *Nannocoris* presents a unique system for studying evolutionary modification for two reasons. First, our phylogenetic hypothesis and ancestral state reconstruction document that head elongations and reductions did not evolve as a simple transition from shorter to longer heads. Instead, the backbone of the phylogenetic hypothesis is characterized by average head length and transitions from short to long head lengths and back occurred separately in several species groups. Second, this study revealed that head elongation is reached through different avenues in different clades: while the long head in species of the *arimensis* group is largely due to the elongation of the tip region of the head, those in the *pricei* group are due to an elongation of the genal part of the head, providing further evidence of the plastic nature of head length evolution in this genus.

Given that head length is not sexually dimorphic, we speculate that the evolution of head length may be driven by ecology, possibly tied to prey capture or feeding mechanism, similar to scenarios proposed in other groups of insects (Lavine et al. 2015). Although feeding behaviors of Schizopteridae are virtually undocumented, the stout labium found in most schizopterid taxa suggests predatory habits across the group, similar to the situation in predatory Reduviidae and other carnivorous Heteroptera (Miller 1956). We assume that Nannocoris species are also carnivorous, even though the rostrum in *Nannocoris* is long and slender, and its length is correlated with different head lengths observed in the genus (see Supplemental Materials). This combination of a long and slender rostrum and variable head length is reminiscent of the situation observed in the reduviid subfamily Triatominae: among Reduviidae, only members of the vertebrate blood-feeding Triatominae are capable of moving the rostrum in front of the head

(Lent and Wygodzinsky 1979), a modification that may facilitate access to the blood source while being perched next to or underneath a vertebrate host, rather than on top of it. Intriguingly, Triatominae show a greater degree of head length variation than any other reduviid subfamily: the head plus extended rostrum, and thus the “reach” of the tip of the mouthparts, in long-headed taxa such as *Eratyrus* Stål 1859 is much greater than it is in short-headed taxa such as *Cavernicola* (Barber 1937). In both Triatominae and *Nannocoris* species, the length of the head appears to be correlated with the length of the rostrum. Feeding behaviors under natural conditions are unknown for Triatominae and species of *Nannocoris*, but we speculate that an extended reach of the head plus rostrum may be a selective advantage for members in both groups. Feeding ecology may therefore have played a role in the evolution of head lengths in both taxa. Although molecular diagnostics of gut contents now provides data on predatory associations beyond direct feeding observations, the minuscule size of all *Nannocoris* species may render both approaches difficult.

Other features of the head in *Nannocoris* are no less enigmatic: the variation in head shapes observed within *Nannocoris* is unparalleled in Dipsocoromorpha, but male-specific organs on the head, pronotum, and forewing have evolved multiple times across the minute litter bugs (unpublished data). Preliminary observations have shown that the majority of these male-specific organs are associated with complex glands (i.e. glands consisting of, reservoirs, and/or gland ducts), suggesting that they may be involved in male courtship behaviors or other male-specific activities. We have not examined gland structures associated with the pit-like openings on the vertex in *Nannocoris* in a comparative way, but both external and internal components of these vertex glands will likely offer excellent species-diagnostic features for a future taxonomic revision of *Nannocoris*. Based on their occurrence in all species groups, we consider the vertex glands with pit-like openings as a putative synapomorphy of *Nannocoris*; the pit-like structure on the pronotum in *N. pricei* requires further investigation.

Taxonomic revisions of schizopterid genera are daunting, mostly because of the overwhelming number of undescribed species in virtually any genus-level taxon revised in recent times (Hill 2014; Knyshov et al. 2016). Molecular phylogenies have the potential to assist in partitioning large genus-level revisions into several smaller and more feasible projects. As an example, a phylogeny of the *Schizoptera* group of genera (Leon and Weirauch, 2017a) has facilitated taxonomic revisions of genera and subgenera (Leon and Weirauch 2016, 2017b). We envision that this first molecular phylogeny of the genus *Nannocoris* that includes about 50% of the predicted species diversity from across the geographic range of the genus will similarly encourage taxonomic revisionary work on this genus. The proposed species groups and their diagnostic features are tentative, due to a combination of limited taxon sampling and, in some cases poor branch support. Future analyses that include a more substantial set of taxa and combine molecular and morphological datasets should test the proposed species group hypotheses and assess the value of the proposed diagnostic features. In addition, higher-level phylogenetic relationships of Schizopterinae are still poorly understood and the exact phylogenetic position of *Nannocoris* remains uncertain. Weirauch and Štys (2014) found support for a sister group relationship between *Nannocoris* and *Pinochius* and this is primarily why we included a substantial number of *Pinochius* species in our analysis. However, the monophyletic *Pinochius* was recovered as sister taxon to the *Corixidea* group of genera, while an undescribed Oriental genus that somewhat resembles but is not congeneric with *Nannodictyus* Štys 1982 and that we here refer to as "nr *Nannodictyus*" is recovered as sister taxon of *Nannocoris*. While *Pinochius*, the *Corixidea* genus group, and *Nannocoris* are supported with 100% bootstrap support, relationships between these clades and "nr *Nannodictyus"* are unresolved. Additional taxonomic and character sampling, or the use of combined morphological and molecular analyses has the potential to settle this phylogenetic conundrum.

In conclusion, we emphasize that this study has opened the door for subsequent research on an intriguing system likely shaped by natural selection and one of the most extraordinary examples of exaggerated trait variation within a single genus seen in the animal kingdom.

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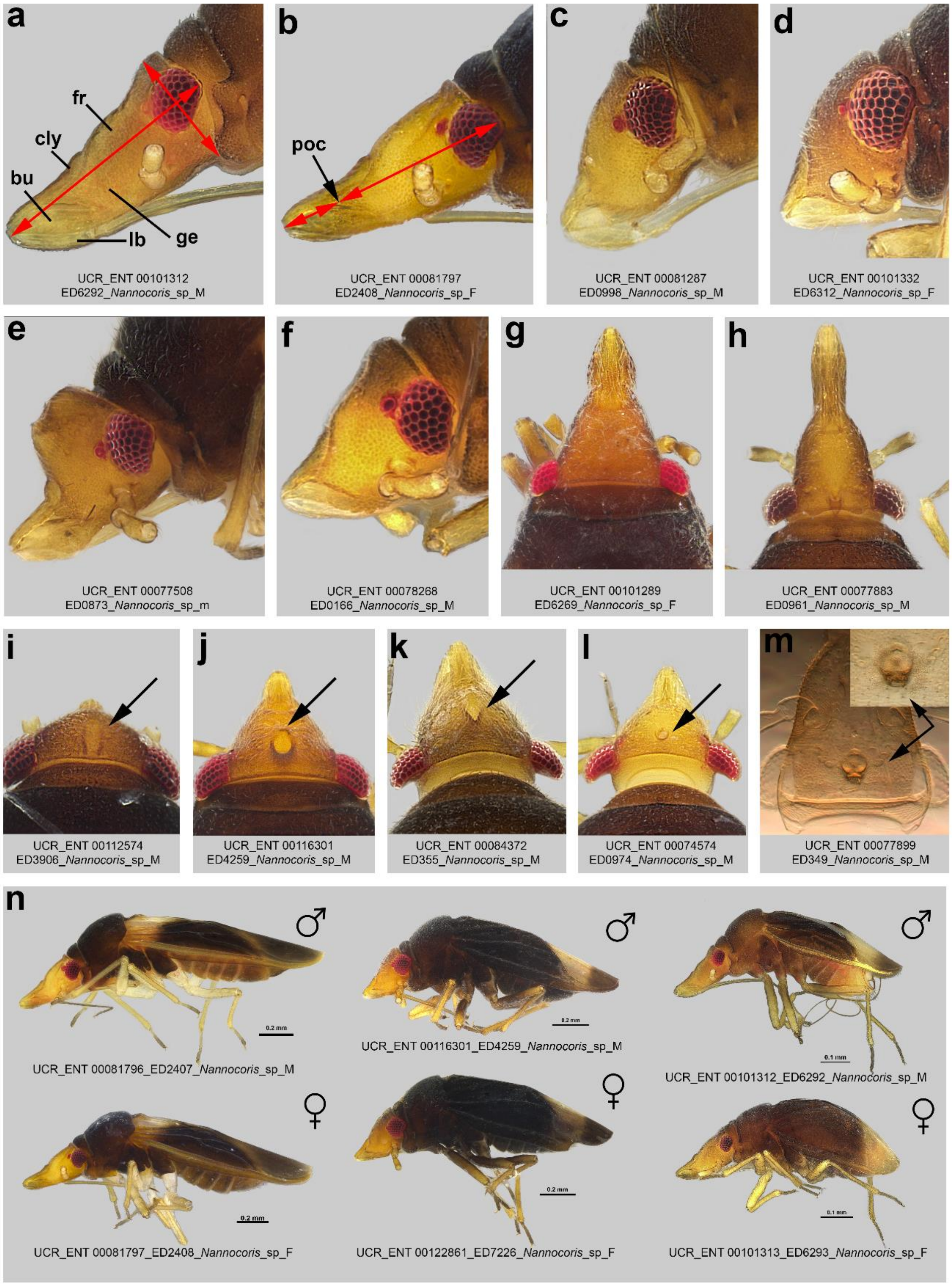
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ED6312_*Nannocoris*_sp_F
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UCR_ENT 00101313_ED6293_*Nannocoris*_sp_F

Figure 1-1. ***Nannocoris*** **head morphology**

Nannocoris spp: head morphology and habitus in lateral view. **a**: Head in lateral view, illustrating head morphology and length and height measurements; bu, buccula; cly, clypeus; fr, frons; lb, labium; ge, gena. **b**: Head in lateral view, illustrating length measurements; poc, point of convergence of the (clypeus, gena, and buccula). Red arrows indicate head length and head height measurements (a) anterior and posterior lengths taken of head (b). **a-d**: Head of *Nannocoris* spp. in lateral view, illustrating variation in head length. **e-h**: Head of *Nannocoris* spp. in lateral view, illustrating variation in head shape. **i-m**: Head of *Nannocoris* spp. in dorsal view, showing variation in size and shape of male-specific pit-like structures; (i-l) are images of specimens in ethanol while (m) is of a cleared, slide-mounted specimen where the associated gland is visible (inset). **n**: *Nannocoris* spp. in lateral view, documenting the lack of sexual dimorphism with regard to length of the head

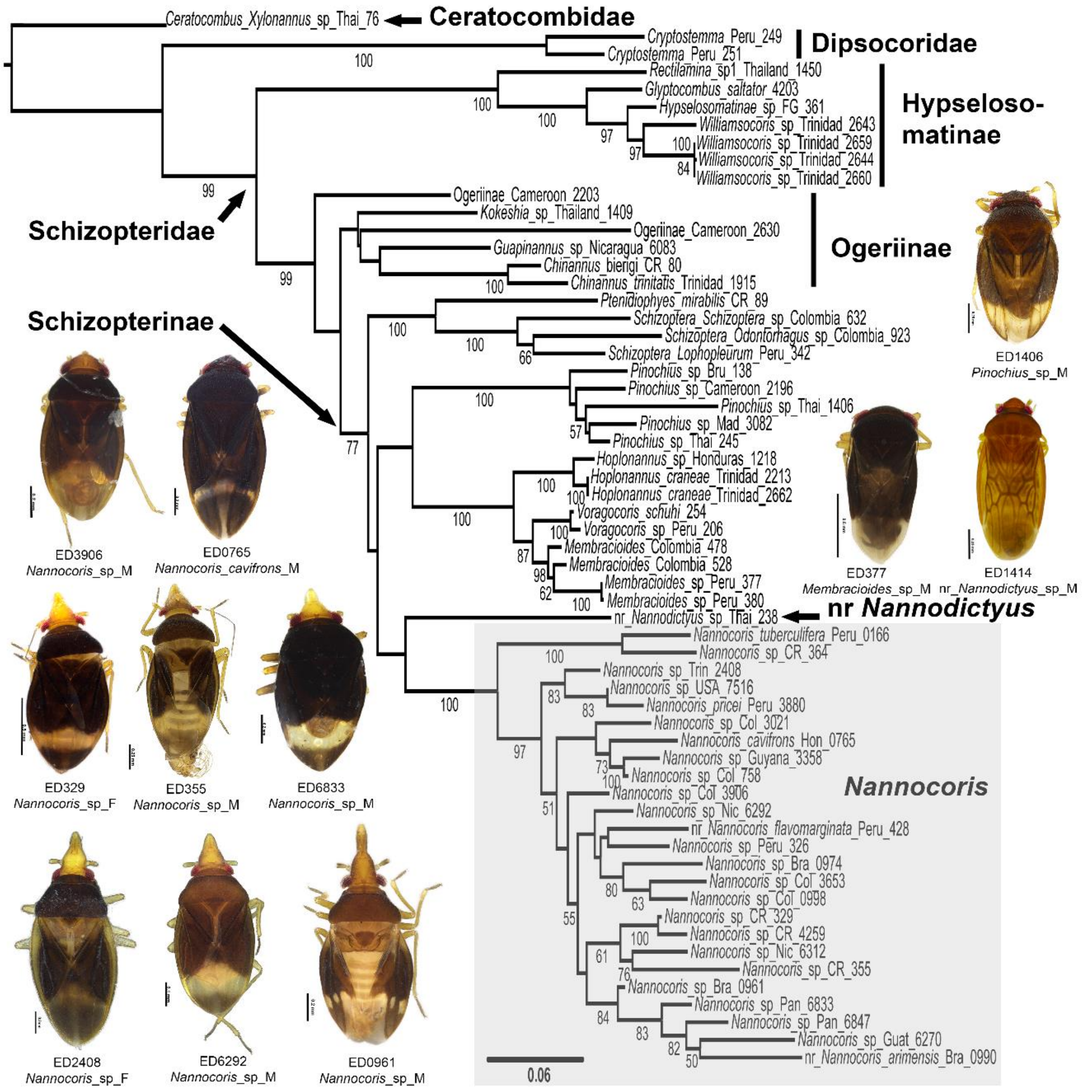


Figure 1-2. Phylogenetic reconstruction of *Nannocoris* and other Dipsocoromorpha

Phylogenetic reconstruction of *Nannocoris* and other Dipsocoromorpha derived from the maximum likelihood analysis of the combined 16s rDNA, 18s rDNA, and 28s rDNA (D2 & D3-5) and CO1 gene regions. *Nannocoris* is recovered as monophyletic (grey box). Numbers indicate bootstrap values (>50%). Dorsal habitus images illustrate selected terminals.

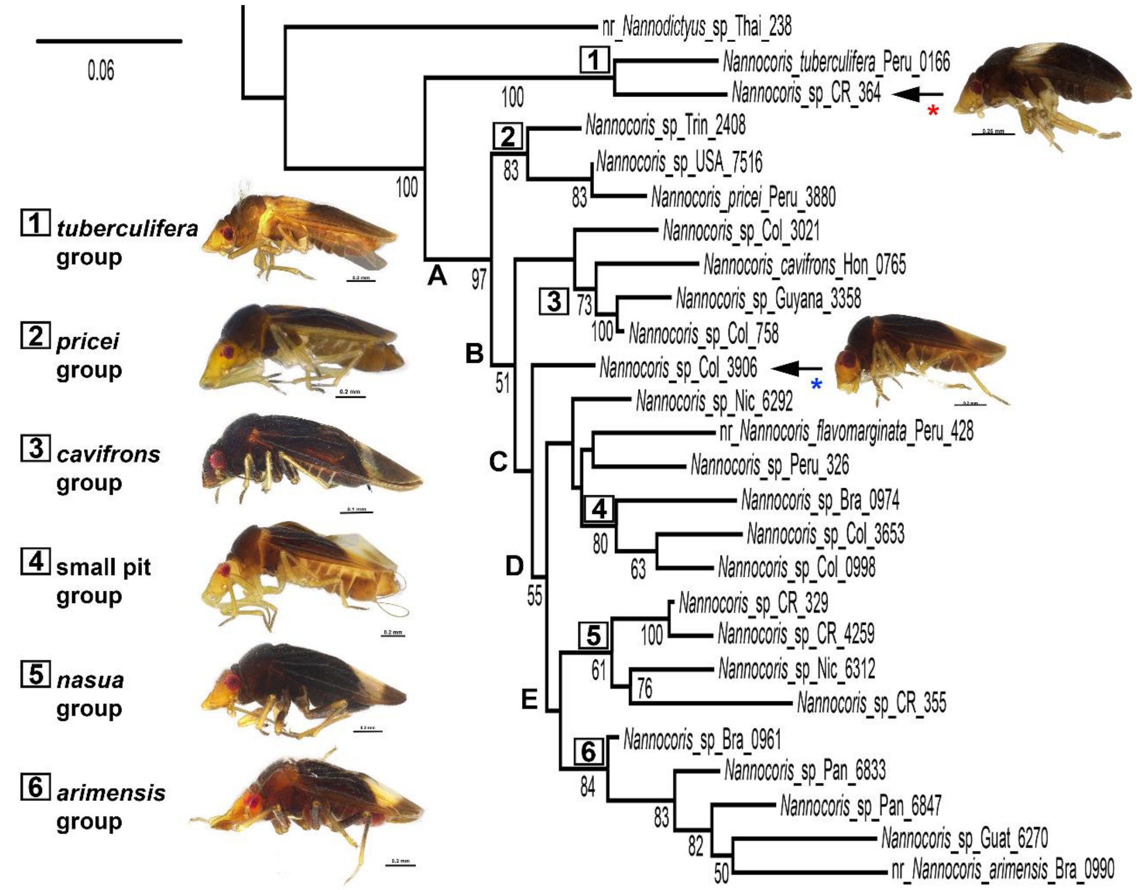


Figure 1-3. Expanded view form Fig. 2 of the *Nannocoris* clade

Letters A-E indicate larger clades and Arabic numbers species groups referred to in the results. *Nannocoris* is sister to an undescribed genus nr *Nannodictyus* (with low support). Species 364 (red *) in the *tuberculifera* group represents the first record of a coleopteroid male in *Nannocoris*. Species 3906 (blue *) in clade C was recovered with low support in various positions in prior matrices

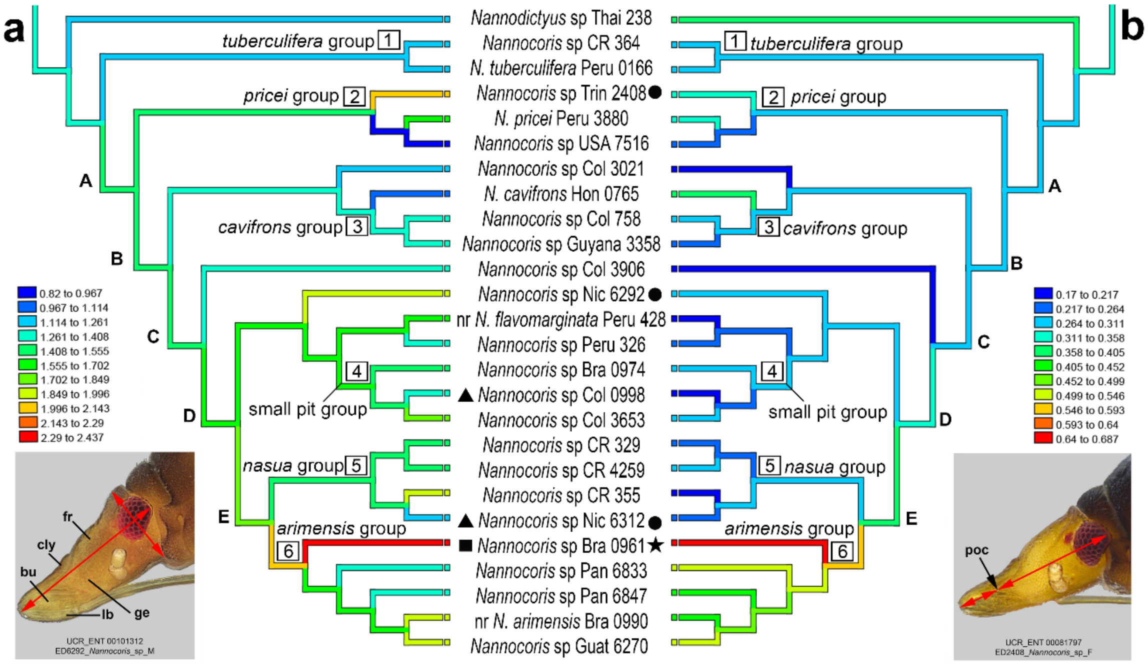


Figure 1-4. Ancestral state reconstructions of head traits across *Nannocoris* spp.

Ancestral state reconstructions (ASR) of head traits across *Nannocoris* spp. based on the best likelihood tree and parsimony reconstruction of continuous characters. Cool colors indicate shorter and warm colors longer lengths. **a**. ASR of the ratio of head length to head height (see inset for measurements). Triangles indicate species with drastic secondary head reductions, the square highlights the species with most extreme overall length. **b**. ASR of the ratio of the length of the anterior portion of head to the length of the posterior portion of head (see inset for measurements). Circles indicates species with overall head length driven by the long posterior portion of the head, the star a species where the overall length is mostly due to the long anterior portion of the head.

species/taxon	USI	ED	dep.	16S rDNA	18S rDNA	28S D2 rDNA	28S D3-D5 rDNA	CO1
Ceratocombus_Xylonannus_sp_Thai_76	UCR_ENT 00057535	76	TIGER	N/A	N/A	KF781241	KF781241	N/A
Cryptostemma_Peru_249	UCR_ENT 00057528	249	UCR	N/A	KF781210	KF781247	KF781247	N/A
Cryptostemma_Peru_251	UCR_ENT 00057530	251	UCR	N/A	KF781211	KF781248	KF781248	N/A
Rectilamina_sp1_Thailand_1450	UCR_ENT 00084928	1450	TIGER	N/A	MF662009	MF662047	N/A	N/A
Glyptocombus_saltator_4203	UCR_ENT 00094273	4203	TAMU	N/A	MF661996	KX810856	KU315295	N/A
Hypselosomatinae_sp_FG_361	UCR_ENT 00084398	361	UCR	N/A	MF661998	MF662020	MF662059	N/A
Williamsocoris_sp_Trinidad_2643	UCR_ENT 00088667	2643	UCR	N/A	MF662014	MF662052	MF662090	N/A
Williamsocoris_sp_Trinidad_2659	UCR_ENT 00088130	2659	UCR	N/A	KU315259	KX810857	KU315296	N/A
Williamsocoris_sp_Trinidad_2644	UCR_ENT 00087659	2644	UCR	N/A	MF662007	MF662044	MF662084	N/A
Williamsocoris_sp_Trinidad_2660	UCR_ENT 00088131	2660	UCR	N/A	MF662008	MF662045	MF662085	N/A
Ogeriinae_Cameroon_2203	UCR_ENT 00087547	2203	UCR	N/A	KT272763	KT224613	KT272754	N/A
Kokeshia_sp_Thailand_1409	UCR_ENT 00063170	1409	TIGER	N/A	KT272760	KT224614	KT272751	N/A
Ogeriinae_Cameroon_2630	UCR_ENT 00088114	2630	UCR	N/A	KT272764	KT224612	KT272755	N/A
Guapinannus_sp_Nicaragua_6083	UCR_ENT 00101103	6083	FMNH	N/A	N/A	MF662018	MF662057	N/A
Chinannus_bierigi_CR_80	UCR_ENT 00004804	80	UCR	N/A	KF781208	KF781243	KF781243	N/A
Chinannus_trinitatis_Trinidad_1915	UCR_ENT 00088673	1915	UCR	N/A	KT272757	KT224609	KT272745	N/A
Ptenidiophyes_mirabilis_CR_89	UCR_ENT 00004805	89	UCR	N/A	KF781223	KF781258	KF781258	N/A
Schizoptera_Schizoptera_sp_Colombia_632	UCR_ENT 00077905	632	SHARKEY	N/A	MF662012	MF662050	MF662088	N/A
Schizoptera_Odontorhagus_sp_Colombia_923	UCR_ENT 00081350	923	SHARKEY	N/A	MF662011	MF662049	MF662087	N/A
Schizoptera_Lophopleurum_Peru_342	UCR_ENT 00082350	342	UCR	N/A	MF662010	MF662048	N/A	N/A
Pinochius_sp_Bru_138	UCR_ENT 00004807	138	UCR	N/A	KF781221	KF781256	KF781256	N/A
Pinochius_sp_Cameroon_2196	UCR_ENT 00087540	2196	UCR	N/A	N/A	KX810859	KU315298	N/A
Pinochius_sp_Thai_1406	UCR_ENT 00081667	1406	TIGER	MF661990	N/A	MF662046	MF662086	N/A
Pinochius_sp_Mad_3082	UCR_ENT 00045001	3082	CAS	MF661989	N/A	N/A	see Suppl. Materials	N/A
Pinochius_sp_Thai_245	UCR_ENT 00057526	245	TIGER	N/A	KF781222	KF781257	KF781257	N/A
Hoplonannus_sp_Honduras_1218	UCR_ENT 00081695	1218	UCR	N/A	MF661993	MF662015	MF662054	N/A
Hoplonannus_craneae_Trinidad_2213	UCR_ENT 00084981	2213	UCR	N/A	KT272759	KT224610	KT272749	N/A
Hoplonannus_craneae_Trinidad_2662	UCR_ENT 00077101	2662	UCR	N/A	MF661997	MF662019	MF662058	N/A
Voragocoris_schuhi_254	UCR_ENT 00055624	254		N/A	KF781228	KF781263	KF781263	N/A
Voragocoris_Peru_206	UCR_ENT 00082340	206	UCR	N/A	MF662013	MF662051	MF662089	N/A
Membracioides_Colombia_478	UCR_ENT 00076304	478	SHARKEY	N/A	MF661999	MF662021	MF662060	N/A
Membracioides_Colombia_528	UCR_ENT 00076278	528	SHARKEY	N/A	MF662000	MF662022	MF662061	N/A
Membracioides_sp_Peru_377	UCR_ENT 00077047	377	HERATY	N/A	MF661994	MF662016	MF662055	N/A
Membracioides_sp_Peru_380	UCR_ENT 00084415	380	UCR	N/A	MF661995	MF662017	MF662056	N/A
nr_Nannodictyus_sp_Thai_238	UCR_ENT 00057524	238	TIGER	N/A	KF781218	KF781254	KF781254	N/A
Nannocoris_tuberculifera_Peru_0166	UCR_ENT 00078268	166	UCR	MF661988	MF662006	MF662043	MF662083	MF662101
Nannocoris_sp_CR_364	UCR_ENT 00084411	364	UCR	MF661978	N/A	MF662029	MF662067	N/A
Nannocoris_sp_Trin_2408	UCR_ENT 00081797	2408	UCR	MF661987	N/A	MF662042	MF662081	N/A
Nannocoris_sp_USA_7516	UCR_ENT 00094252	7516	TAMU	N/A	N/A	N/A	MF662082	N/A
Nannocoris_pricei_Peru_3880	UCR_ENT 00104900	3880	UCR	N/A	N/A	MF662024	MF662063	MF662094
Nannocoris_sp_Col_3021	UCR_ENT 00093297	3021	SHARKEY	MF661981	N/A	MF662032	MF662070	MF662096
Nannocoris_cavifrons_Hon_0765	UCR_ENT 00082365	765	UCR	MF661974	MF662001	MF662023	MF662062	MF662093
Nannocoris_sp_Guyana_3358	UCR_ENT 00074528	3358	UCRC	N/A	N/A	MF662036	MF662075	N/A
Nannocoris_sp_Col_758	UCR_ENT 00077804	758	SHARKEY	N/A	N/A	MF662035	MF662073	N/A
Nannocoris_sp_Col_3906	UCR_ENT 00112574	3906	SHARKEY	N/A	N/A	MF662034	MF662072	N/A
Nannocoris_sp_Nic_6292	UCR_ENT 00101312	6292	FMNH	MF661983	N/A	MF662037	MF662076	N/A
nr_Nannocoris_flavomarginata_Peru_428	UCR_ENT 00086112	428	UCR	MF661992	N/A	MF662053	MF662092	N/A
Nannocoris_sp_Peru_326	UCR_ENT 00077041	326	HERATY	MF661986	MF662005	MF662041	MF662080	MF662100
Nannocoris_sp_Bra_0974	UCR_ENT 00074574	974	UCR	MF661975	N/A	MF662026	MF662065	N/A
Nannocoris_sp_Col_3653	UCR_ENT 00106928	3653	SHARKEY	N/A	N/A	MF662033	MF662071	MF662097
Nannocoris_sp_Col_0998	UCR_ENT 00081287	998	UCRC	MF661980	N/A	MF662031	MF662069	N/A
Nannocoris_sp_CR_329	UCR_ENT 00077040	329	HERATY	MF661976	N/A	MF662027	N/A	N/A
Nannocoris_sp_CR_4259	UCR_ENT 00116301	4259	UCR	MF661979	MF662003	MF662030	MF662068	N/A
Nannocoris_sp_Nic_6312	UCR_ENT 00101332	6312	FMNH	N/A	N/A	MF662038	MF662077	N/A
Nannocoris_sp_CR_355	UCR_ENT 00084372	355	UCR	MF661977	MF662002	MF662028	MF662066	MF662095
Nannocoris_sp_Bra_0961	UCR_ENT 00077883	961	UCR	N/A	N/A	MF662025	MF662064	N/A
Nannocoris_sp_Pan_6833	UCR_ENT 00122798	6833	UCR	MF661984	MF662004	MF662039	MF662078	MF662098
Nannocoris_sp_Pan_6847	UCR_ENT 00122835	6847	UCR	MF661985	N/A	MF662040	MF662079	MF662099
Nannocoris_sp_Guat_6270	UCR_ENT 00101290	6270	FMNH	MF661982	N/A	see Suppl. Materials	MF662074	N/A
nr_Nannocoris_arimensis_Bra_0990	UCR_ENT 00077884	990	UCR	MF661991	N/A	see Suppl. Materials	MF662091	N/A

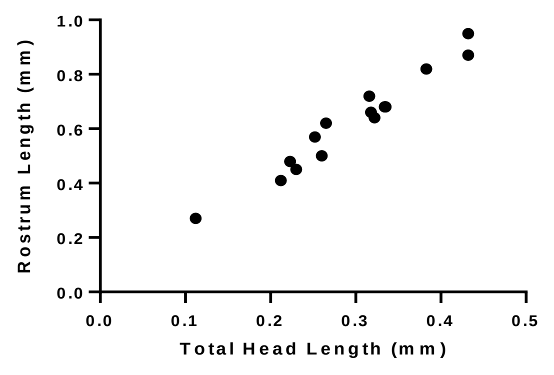
Table 1-1. Voucher specimens

Name in analysis	Head Height (mm)	Head Total Length (mm)	Head Anterior Length (mm)	Head Posterior Length (mm)	Head Length/Height (mm)	Head Anterior/Posterior (mm)	Total Body Length
Nannodictyus_Thai_238	0.183	0.223	0.061	0.162	1.218579235	0.37654321	1.32
Nannocoris_tuberculifera_Peru_0166	0.204	0.247	0.054	0.193	1.210784314	0.279792746	1.31
Nannocoris_sp_CR_364	0.19	0.219	0.052	0.167	1.152631579	0.311377246	1.06
Nannocoris_sp_Trin_2408	0.216	0.432	0.105	0.327	2	0.321100917	1.67
Nannocoris_sp_USA_7516	0.218	0.18	0.038	0.142	0.825688073	0.267605634	1.1
Nannocoris_pricei_Peru_3880	0.19	0.322	0.081	0.241	1.694736842	0.336099585	1.38
Nannocoris_sp_Col_3021	0.2	0.247	0.041	0.206	1.235	0.199029126	1.5
Nannocoris_cavifrons_Hon_0765	0.11	0.112	0.031	0.081	1.018181818	0.382716049	0.6
Nannocoris_sp_Guyana_3358	0.184	0.25	0.053	0.197	1.358695652	0.269035533	1.38
Nannocoris_sp_Col_758	0.19	0.26	0.059	0.201	1.368421053	0.293532338	1.53
Nannocoris_sp_Col_3906	0.17	0.23	0.041	0.189	1.352941176	0.216931217	1.07
Nannocoris_sp_Nic_6292	0.11	0.212	0.047	0.165	1.927272727	0.284848485	0.82
nr_Nannocoris_flavomarginata_Peru_428	0.199	0.318	0.048	0.27	1.59798995	0.177777778	1.42
Nannocoris_sp_Peru_326	0.181	0.252	0.048	0.204	1.392265193	0.235294118	1.24
Nannocoris_sp_Bra_0974	0.219	0.335	0.075	0.26	1.529680365	0.288461538	1.82
Nannocoris_sp_Col_3653	0.186	0.334	0.077	0.257	1.795698925	0.299610895	1.6
Nannocoris_sp_Col_0998	0.195	0.265	0.047	0.218	1.358974359	0.21559633	1.46
Nannocoris_sp_CR_329	0.224	0.316	0.067	0.249	1.410714286	0.269076305	1.83
Nannocoris_sp_CR_4259	0.223	0.318	0.068	0.25	1.426008969	0.272	1.25
Nannocoris_sp_Nic_6312	0.18	0.223	0.044	0.179	1.238888889	0.245810056	1.02
Nannocoris_sp_CR_355	0.195	0.383	0.067	0.316	1.964102564	0.212025316	1.88
Nannocoris_sp_Bra_0961	0.188	0.432	0.17	0.262	2.29787234	0.648854962	1.76
Nannocoris_sp_Pan_6833	0.217	0.283	0.097	0.186	1.304147465	0.521505376	1.8
Nannocoris_sp_Pan_6847	0.231	0.305	0.089	0.216	1.32034632	0.412037037	1.36
Nannocoris_sp_Guat_6270	0.192	0.357	0.12	0.237	1.859375	0.506329114	1.32
nr_Nannocoris_arimensis_Bra_0990	0.175	0.329	0.101	0.228	1.88	0.442982456	1.62

Table 1-2. Head measurements of *Nannocoris* spp.

Supplemental material 1-1. Head and Rostrum measurements

Name in analysis	Total Head Length (mm)	Rostrum Length (mm)
Nannocoris_cavifrons_Hon_0765	0.112	0.27
Nannocoris_sp_Nic_6292	0.212	0.41
Nannocoris_sp_Col_3906	0.23	0.45
Nannocoris_sp_Nic_6312	0.223	0.48
Nannocoris_sp_Col_758	0.26	0.5
Nannocoris_sp_Peru_326	0.252	0.57
Nannocoris_sp_Col_0998	0.265	0.62
Nannocoris_pricei_Peru_3880	0.322	0.64
Nannocoris_sp_CR_4259	0.318	0.66
Nannocoris_sp_Bra_0974	0.335	0.68
Nannocoris_sp_Col_3653	0.334	0.68
Nannocoris_sp_CR_329	0.316	0.72
Nannocoris_sp_CR_355	0.383	0.82
Nannocoris_sp_Trin_2408	0.432	0.87
Nannocoris_sp_Bra_0961	0.432	0.95
Nannocoris_sp_Col_3021	0.247	NA
Nannocoris_sp_CR_364	0.219	NA
Nannocoris_sp_Guat_6270	0.357	NA
Nannocoris_sp_Guyana_3358	0.25	NA
Nannocoris_sp_Pan_6833	0.283	NA
Nannocoris_sp_Pan_6847	0.305	NA
Nannocoris_sp_USA_7516	0.18	NA
Nannocoris_tuberculifera_Peru_0166	0.247	NA
Nannodictyus_Thai_238	0.223	NA
nr_Nannocoris_arimensis_Bra_0990	0.329	NA
nr_Nannocoris_flavomarginata_Peru_428	0.318	NA



Pearson r	
r	0.978
95% confidence interval	3 to 0.993
R squared	0.956
P value	
P (two-tailed)	<0.0001
P value summary	****
Significant? (alpha = 0.05	Yes
Number of XY Pairs	15

Supplemental material 1-2. Sequences excluded from GenBank

28S D2 rDNA (< 150 bp)

>Nannocoris_sp_Guat_6270 [organism= Nannocoris sp] specimen_voucher_ED_6270_UCR_ENT_00101290_28S_D2_ribosomal_RNA_gene_partial_sequence

GGATTTTAATCTATATAAATTATTTAAAATCATTATTACTTTGTTTAAACAAGGCTTACAACACTAGTAAG

71 bp

28S D2 rDNA (< 150 bp)

>nr_Nannocoris_arimensis_Bra_0990 [organism= nr Nannocoris arimensis] specimen_voucher_ED_0990_UCR_ENT_00077884_28S_D2_ribosomal_RNA_gene_partial_sequence

CGAGACCGATAGTAAACACGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAGTTAAAAAGTACGTGAAACCGTTTAGGGGTAAACGGAAAA

98 bp

28S D3-D5 rDNA (< 150 bp)

>Pinochius_sp_Mad_3082 [organism= Pinochius sp] specimen_voucher_ED_3082_UCR_ENT_00045001_28S_D35_ribosomal_RNA_gene_partial_sequence

AGGTTATAAAACCTAAAGGCGTAACAAAAGTAAAGTAAGCTGTCCTTTATTAGGGAGAATGGNTATTTAATAGCTCGCACTCCCGGGGCGTTAATAATTCATTGAGAATTAAG

113 bp

Supplemental material 1-3. Concatenated alignment

Not available in this document

Supplemental material 1-4. Partition file

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DNA, 16s = 1-419
DNA, 18s = 420-1483
DNA, D2-D5 = 1484-2670
DNA, CO1 = 2671-3409\3
DNA, CO1 = 2672-3409\3
DNA, CO1 = 2673-3409\3
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Chapter II

Correlated evolution of genitalic structures in animals with exaggerated male and female genitalia

Abstract

Studies on animal genitalia have long shaped our understanding of concepts, patterns and processes of morphological evolution. Coevolution between male and female genitalic structures is predicted to be ubiquitous, regardless of the evolutionary mechanism involved. However, studies that simultaneously evaluate male as well as female genitalic variation in a comparative phylogenetic framework have remained relatively scarce. We here investigate correlated male and female genital evolution in *Nannocoris* Reuter (Hemiptera: Schizopteridae), a minute litter bugs genus that may be unique among arthropods in the degree and variation of its exaggerated genitalic traits: we here show that the length of the male intromittent organ differs dramatically between species, ranging from 1/8th to 20 times the respective body length and that the female spermathecal duct also displays drastic length differences between species. Using a phylogeny of the genus, measurements of male and female genitalic features, and phylogenetically independent contrasts, we find that the length of male intromittent organ and female spermathecal duct are indeed correlated. The mode of evolution that has led to this striking phenotypes is unknown, but we stress that this system has potential for future research in the evolution of male and female genitalic features in general.

Introduction

The study of animal genitalia has a long tradition in the fields of systematics and evolution due to the often stunning morphological differences between closely related species. Genitalia offer both reliable taxonomic features and their often rapid divergence in groups with internal fertilization make them ideal models for the study of evolutionary processes (Eberhard 1985; Arnquist 1998; Genevcius et al. 2017). Male and female genitalic structures are expected to covary to a certain extent in such groups of organisms. Various models involving elements of natural and sexual selection have been invoked to explain observed patterns, including lock-and key mechanisms, sexual antagonism, female cryptic choice, and male-male competition (e.g., Kameda et al. 2009; Bergsten and Miller, 2007; Eberhard and Huber, 2010). Much of the study of genitalic evolution has focused on the typically fairly accessible male genitalic features that have traditionally been thought to show greater between-species divergences than females, but also offer more easily quantifiable traits because of their frequently more rigid properties compared to females (e.g., Ah-King et al., 2014; Brennan and Prum, 2015). Nevertheless, a number of recent studies have shown and summarized evidence that coevolution between male and female genitalic structures may indeed be common (Brennan 2016; Brennan and Prum 2015; Yassin 2016). In insects, this research has largely focused on beetles and flies and has provided evidence for one specific evolutionary mechanism to be responsible for the observed patterns in a given model system, e.g., sexually antagonistic coevolution in seed beetles (Cayetano et al. 2011; J. L. Ronn et al. 2011), lock and key in carabid beetles (Sota and Kubota 1998), and female choice in diopsid flies (Kotrba et al. 2014). Less than a handful of studies have focused on true bugs or Heteroptera (order Hemiptera), where grasping and antigrasping appendages in male and female water striders (Arnqvist and Rowe 2002; Perry and Rowe 2012) as well as structures involved in traumatic insemination in both plant bugs (Tatarnic and Cassis 2010) and bed bugs (Morrow and Arnqvist

2003) are all assumed to have evolved in the context of sexually antagonistic coevolution (Brennan and Prum 2015). Overall, the number of comparative morphological studies examining and evaluating both male and female genitalic features and interpreting findings in the context of a species-level phylogeny across larger taxonomic groups such as entire genera are still relatively rare in insects (Kotrba et al. 2014; Tatarnic and Cassis 2010), but have the potential to establish new model systems to study patterns and processes of genitalic evolution. We here introduce a new potential model system that is characterized by striking exaggerated genitalic features that appear to covary between males and females across a genus of insects.

The system for this study are minute litter bugs in the genus *Nannocoris* Reuter (Insecta: Hemiptera: Schizopteridae), a group of tiny insects (~1-2 mm) with cryptic habits and significant undocumented biodiversity (Frankenberg et al. In review). This group of about 40 described and undescribed species distributed across the New World is attractive for evolutionary research for several reasons. First, our preliminary observations have shown that in some species of *Nannocoris* the male intromittent organ, the sclerotized tube-like vesica, consists of more than 30 tightly wound coils that extend to about 20 times the body length of the respective individual (Fig. 1d-g). These vesicae range among the longest male intromittent organs in relation to body length so far discovered in the animal kingdom (Neufeld and Palmer 2008) and we here refer to them as exaggerated traits (see supplemental video 1a). At rest, they are coiled dorsally on the genital segment (pygophore), but preserved specimens with vesicae in various degrees of unraveling are commonly encountered (Fig. 1f, g). Second, the length of the male intromittent organ is species specific and intraspecific variation is limited. In fact, the range of vesica to body length ratios observed across *Nannocoris* is unparalleled in other true bugs or potentially even other insects and animals: while several species show exaggerated intromittent organs, other species feature a vesica that is only about 1/8th of the body length and consists of about one loop or less (Fig. 1b, c). Third, our preliminary investigations into the female internal genital tract of *Nannocoris* species showed that although consistent with the general structure of a true bug spermatheca, the length of the lightly sclerotized spermathecal duct varies dramatically in females of different species ranging from a short duct (Fig. 1h) to ducts consisting of a tightly wound coil many times the length of the body (Fig. 1j, k) (see supplemental video 1b). Despite their minute size, both male and female genitalic traits can measured relatively easily from digital images of dissected specimens. Fourth, a species-level phylogeny of *Nannocoris* has just become available (Frankenberg et al. In review), inviting comparative analyses of the covariation of vesica and spermathecal duct length in a phylogenetic framework.

We here use this phylogenetic hypothesis, measurements of male vesica and female spermathecal duct length, ancestral state reconstructions both traits as continuous characters, and phylogenetically independent contrasts to investigate if the evolution of vesica and spermathecal duct length in *Nannocoris* is indeed correlated.

Materials and Methods

Dissections, imaging, and measurements. To establish that vesical length does not vary noticeably within a given species, five male specimens of one undescribed species of *Nannocoris* were dissected using standard protocols for Dipsocoromorpha dissections (e.g., Weirauch and Frankenberg, 2015; Knyshov et al. 2016). To generate measurements of male vesicae and female spermathecal ducts for the 14 species included in the comparative analysis (see below), 14 male and 14 female specimens were subsequently dissected. A list of the specimens examined is presented in (Frankenberg et al. In review). Imaging of habitus and genitalic features using light microscopy, confocal laser microscopy, and scanning electron microscopy followed protocols outlined in (Knyshov et al. 2016) and (Weirauch 2012) . The spermathecal duct is held tightly coiled within the body, the male vesica is a tube wound into a tight coil on the dorsum of the male genital segment. Manipulations of vesica and spermatheca are difficult due to their sclerotized nature and minute size in specimens with total body length of about 1mm, and uncoiling these structures to obtain a direct measurement was not an option. We therefore used the more easily identifiable number of loops in a given coil as a proxy for its length. This strategy is sound, because we determined using Image J software (Abràmoff et al. 2004) that the diameter of a single loop in the coil of both vesicae and spermathecal ducts is 0.15mm, regardless of sex, species and specimen sampled. The total number of loops for vesicae and spermathecae for the 14 species are provided as Supplement material 2.

Phylogenetic hypothesis, ancestral state reconstruction, and trait correlation. We utilized a recently generated phylogenetic hypothesis of *Nannocoris* and other Dipsocoromorpha that includes 25 described and undescribed species of *Nannocoris* (Frankenberg et al. In review). We pruned the ingroup of the maximum likelihood topology using Mesquite 3.04 (Maddison and

Maddison 2011) to include only the 14 species of *Nannocoris* for which genitalic data for both males and females were available (the remaining 11 taxa are only known from either male or female specimens). We have established that sexual dimorphism of wing type and body shape is fairly minimal in species of this genus, and our assessment of male and female conspecificity was therefore based on morphological similarity and geographic proximity of the collecting locality. We traced the evolutionary histories of vesical (Fig. 2a, left) and spermathecal duct (Fig. 2a, right) length on the pruned topology using parsimony-based ancestral state reconstruction based on continuous characters. Cooler colors represent shorter lengths, while warmer colors indicate longer vesicae and spermathecal ducts. To assess if the length of vesicae and spermathecal ducts are correlated, we calculated phylogenetically independent contrasts (Felsenstein, 1985) using the PDAP plug-in (Phenotypic Diversity Analysis Programs; Midford et al., 2009) in Mesquite 3.04. (Fig. 2b) that uses a Brownian Motion model of evolution.

Results and Discussion

Phylogenetically independent contrasts of vesical and spermathecal length derived from a species-level phylogeny of 14 *Nannocoris* species indicate that these two traits are strongly correlated (2-tailed t-test, t22 = 3.76–11, P < 0.00) (Fig. 2). This result is in line with coevolutionary theory that predicts that male and female genitalic features involved in internal fertilization co-vary (Bergsten and Miller 2007; Eberhard 2010). Although far from being the first example of coevolution of male and female genitalic structures in insects based on comparative evidence among species (Ilango and Lane 2000; Kotrba et al. 2014; J. Ronn et al. 2007; Yassin and Orgogozo 2013), the *Nannocoris* system is appealing because of its simplicity: both the vesica and spermathecal duct are sclerotized, tube-like structures making it comparatively easy to measure length and facilitating in turn both ancestral state reconstructions and trait correlation.

The *Nannocoris* system is also intriguing because of the dramatic range of observed vesical and spermathecal duct lengths from less than one loop to more than 30 (vesica) or 20 (spermatheca) that includes one of the longest male intromittent organ to body ratios observed in animals. Ancestral state reconstructions of both vesica and spermathecal duct length using continuous characters (Fig. 2a; left and right panel, respectively) show that both structures are relatively short (vesica: 0.5 to 6.4 loops; spermathecal duct: 0.5 to 4.4 loops) in the early diverging lineages of *Nannocoris* (e.g., *Nannocoris* sp. 1 + N. *tuberculifera* and *Nannocoris* sp. 2 + sp. 3). The exaggerated vesicae and spermathecal ducts found in *Nannocoris* species 6 (vesica with >30 loops, spermathecal duct with ~20) and 9 (vesica: 12-15; spermatheca: 10-12) are independently derived from ancestors with shorter vesicae/spermathecal ducts (vesica: 12-15; spermatheca: ~8-10). The short vesicae/spermathecal ducts reconstructed for the common ancestor of *Nannocoris* species 7 + 8 (vesica: 0.5-3.45; spermatheca: 0.5-2.45) that are deeply nested within the phylogeny suggests that the evolution of genital length in this system does not simply progress from short to longer, but that a reversal occurs in at least one clade.

Nannocoris is compelling for evolutionary studies because of the above mentioned reasons, although there are limitations with the current study as well as the system. We have assembled the largest collection to date of archival specimens of *Nannocoris* (~660 specimens from 12 natural history collections) and have discovered >30 undescribed species that will complement the currently described 12 species (Frankenberg et al., in review). Many of the examined *Nannocoris* specimens were retrieved from suboptimally preserved samples and we were unable to amplify genetic data consistently. In addition, many species in our samples are only represented by male or female specimens and were therefore not included in the present analyses. Our proposed hypotheses of coevolution of male and female genitalic structures in *Nannocoris* therefore should be further corroborated using a more extensive species-level phylogenetic framework in the future.

Because Schizopteridae are very small, occur primarily in cryptic microhabitats such as leaf litter or hidden among low herbaceous borders, have greatest species diversity in tropical regions, and few biologists have attempted to study their natural history, basic biology and behaviors including mating behavior of *Nannocoris* species are all but unknown (Emsley 1969; Henry 2009; Schuh and Slater 1995; Weirauch and Fernandes 2015). Systems such as diopsid flies (Cotton et al. 2010; Kotrba et al. 2014) or Lygaeus bugs (Tadler et al. 1999) where female and male behaviors have been studied in detail allow for the formulation of evidence-based hypotheses on the likely mechanism driving genital evolution. In contrast, we are unable to do so for *Nannocoris* given the current lack of basic behavioral data, with natural selection, male-male completion, and female choice all being contenders. The first step towards insights into mechanisms that may have shaped the correlated and exaggerated evolution of male and female genitalic structures will therefore require establishing laboratory cultures of *Nannocoris* species.

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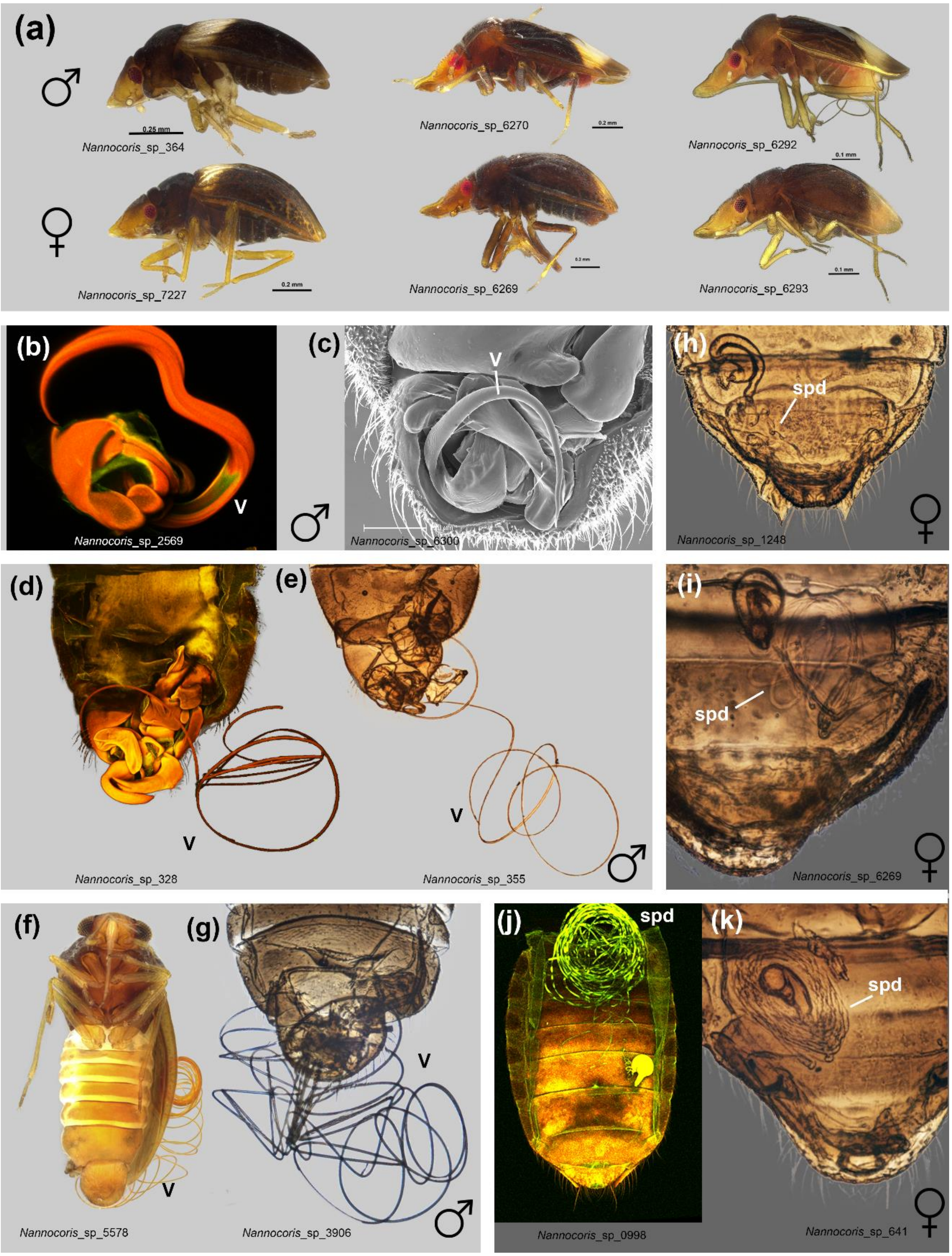

(a)
♂
♀
0.25 mm
Nannocoris_sp_364
Nannocoris_sp_6270
0.2 mm
Nannocoris_sp_6292
0.1 mm
Nannocoris_sp_7227
0.2 mm
Nannocoris_sp_6269
0.3 mm
Nannocoris_sp_6293
0.1 mm
(b)
v
Nannocoris_sp_2569
(c)
v
♂
Nannocoris_sp_6300
(h)
spd
Nannocoris_sp_1248
♀
(d)
(e)
v
v
Nannocoris_sp_328
Nannocoris_sp_355
♂
(i)
spd
Nannocoris_sp_6269
♀
(f)
v
Nannocoris_sp_5578
(g)
v
Nannocoris_sp_3906
♂
(j)
spd
Nannocoris_sp_0998
(k)
spd
Nannocoris_sp_641
♀

Figure 2-1. *Nannocoris* genital morphology

(a) Lateral habitus images of three *Nannocoris* spp. with macropterous males (top) and brachypterous or coleopteroid females (*Nannocoris* sp 1, 13, 6 respectively as seen in Fig. 1). (b) and (c) Short vesicae of one loop or less in two *Nannocoris* spp. (d) and (e) Medium-length vesicae with 5 and 7 loops, respectively in *Nannocoris* sp. and *Nannocoris* sp. 9. (f) and (g) Long vesicae with ~40 and 21 loops, respectively, in *Nannocoris* sp. and *Nannocoris* sp. 5. (h) Short spermathecal duct (less than one loop) in *Nannocoris* sp. 4. (i) Median spermathecal duct (5 loops) in *Nannocoris* sp. 13. (j) and (k) Long spermathecal ducts (15 loops) in unrepresented *Nannocoris* sp. and *Nannocoris* sp. 5 respectively.

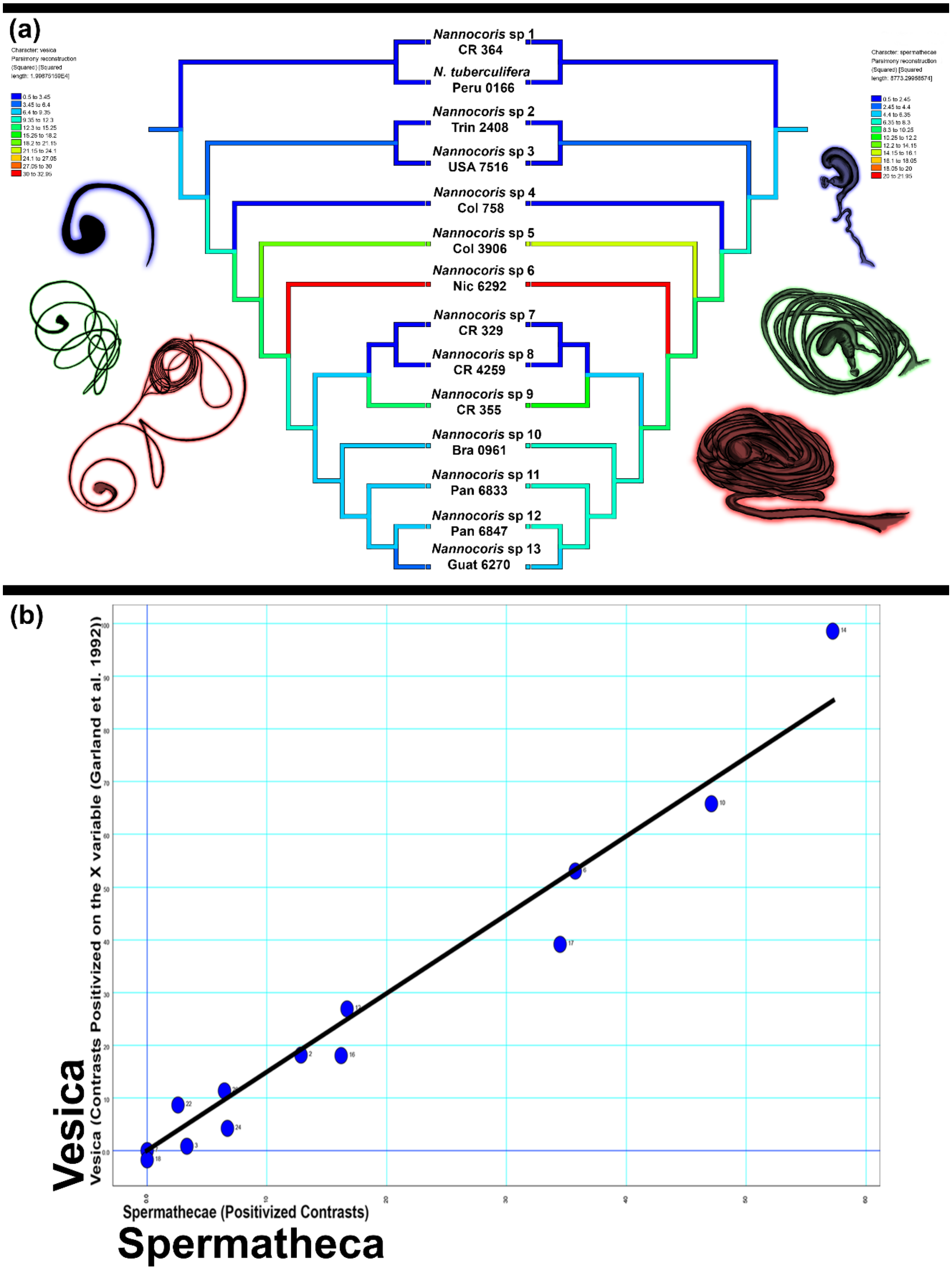

(a)
Character: vesica
Parsimony reconstruction
(Squared) [Squared
length: 1.99875159E4]
0.5 to 3.45
3.45 to 6.4
6.4 to 9.35
9.35 to 12.3
12.3 to 15.25
15.25 to 18.2
18.2 to 21.15
21.15 to 24.1
24.1 to 27.05
27.05 to 30
30 to 32.95
Character: spermathecae
Parsimony reconstruction
(Squared) [Squared
length: 8773.29956574]
0.5 to 2.45
2.45 to 4.4
4.4 to 6.35
6.35 to 8.3
8.3 to 10.25
10.25 to 12.2
12.2 to 14.15
14.15 to 16.1
16.1 to 18.05
18.05 to 20
20 to 21.95
Nannocoris sp 1
CR 364
N. tuberculifera
Peru 0166
Nannocoris sp 2
Trin 2408
Nannocoris sp 3
USA 7516
Nannocoris sp 4
Col 758
Nannocoris sp 5
Col 3906
Nannocoris sp 6
Nic 6292
Nannocoris sp 7
CR 329
Nannocoris sp 8
CR 4259
Nannocoris sp 9
CR 355
Nannocoris sp 10
Bra 0961
Nannocoris sp 11
Pan 6833
Nannocoris sp 12
Pan 6847
Nannocoris sp 13
Guat 6270
(b)
Vesica
Vesica (Contrasts Positivized on the X variable (Garland et al. 1992))
Spermathecae (Positivized Contrasts)
Spermatheca
100
90
80
70
60
50
40
30
20
10
0.0
0.0
10
20
30
40
50
60
14
10
6
17
12
2
16
22
21
24
3
18

Figure 2-2 Ancestral state reconstruction of length of male vesicae and female spermathecal ducts and regression of their phylogenetic independent contrasts

(a) Reconstructed ancestral states of length of the male vesicae (left) and female spermathecal ducts (right) across *Nannocoris* based using parsimony reconstruction of continuous characters. Cool colors indicate shorter lengths, warm colors longer lengths. (b) Positivized independent contrasts of male vesicae vs. female spermathecal duct length shows strong correlation (2-tailed t-test, t22 = 3.76–11, $P < 0.00$).

Supplemental Material

Supplemental material 2-1.

Vesica video (not available in this document)

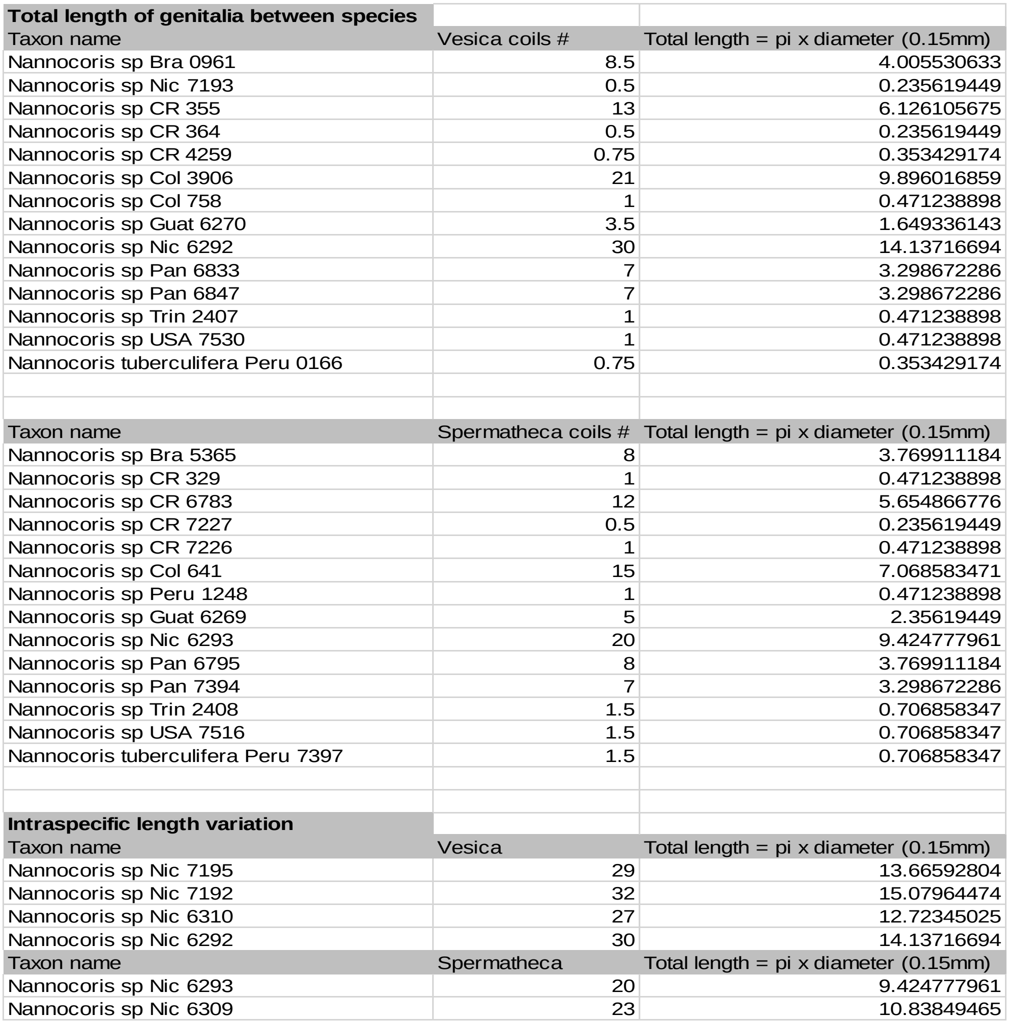
Nannocoris sp 7195 (same species as Nannocoris sp 6 in Fig. 1) is used to demonstrate the coiled behavior and length of the male vesica (~30 loops). Vesica was manipulated using minuten pin and shot using cell phone camera mount for dissecting microscope.

Supplemental material 2-2.

Spermathecal duct video (not available in this document)

Nannocoris sp 641 (Nannocoris sp 5 in Fig. 1) is used to demonstrate the coiled behavior and length of the female spermathecal duct (~15 loops). Video was taken with confocal laser microscopy, following protocols outlined in (Knyshov et al. 2016) and (Weirauch 2012)

Video taken by Alexander Knyshov



Total length of genitalia between species		
Taxon name	Vesica coils #	Total length = pi x diameter (0.15mm)
Nannocoris sp Bra 0961	8.5	4.005530633
Nannocoris sp Nic 7193	0.5	0.235619449
Nannocoris sp CR 355	13	6.126105675
Nannocoris sp CR 364	0.5	0.235619449
Nannocoris sp CR 4259	0.75	0.353429174
Nannocoris sp Col 3906	21	9.896016859
Nannocoris sp Col 758	1	0.471238898
Nannocoris sp Guat 6270	3.5	1.649336143
Nannocoris sp Nic 6292	30	14.13716694
Nannocoris sp Pan 6833	7	3.298672286
Nannocoris sp Pan 6847	7	3.298672286
Nannocoris sp Trin 2407	1	0.471238898
Nannocoris sp USA 7530	1	0.471238898
Nannocoris tuberculifera Peru 0166	0.75	0.353429174
Taxon name	Spermatheca coils #	Total length = pi x diameter (0.15mm)
Nannocoris sp Bra 5365	8	3.769911184
Nannocoris sp CR 329	1	0.471238898
Nannocoris sp CR 6783	12	5.654866776
Nannocoris sp CR 7227	0.5	0.235619449
Nannocoris sp CR 7226	1	0.471238898
Nannocoris sp Col 641	15	7.068583471
Nannocoris sp Peru 1248	1	0.471238898
Nannocoris sp Guat 6269	5	2.35619449
Nannocoris sp Nic 6293	20	9.424777961
Nannocoris sp Pan 6795	8	3.769911184
Nannocoris sp Pan 7394	7	3.298672286
Nannocoris sp Trin 2408	1.5	0.706858347
Nannocoris sp USA 7516	1.5	0.706858347
Nannocoris tuberculifera Peru 7397	1.5	0.706858347
Intraspecific length variation		
Taxon name	Vesica	Total length = pi x diameter (0.15mm)
Nannocoris sp Nic 7195	29	13.66592804
Nannocoris sp Nic 7192	32	15.07964474
Nannocoris sp Nic 6310	27	12.72345025
Nannocoris sp Nic 6292	30	14.13716694
Taxon name	Spermatheca	Total length = pi x diameter (0.15mm)
Nannocoris sp Nic 6293	20	9.424777961
Nannocoris sp Nic 6309	23	10.83849465

Supplemental material 2-3. Table of total length of genitalia between *Nannocoris* species and intraspecific length variation

Top table is the total length and number of loops for all 14 males and 14 females. Bottom table are the intraspecific length variation of four male vesicae and two female spermathecal ducts from one undescribed species of *Nannocoris* (*Nannocoris* sp 6 in Fig. 1).

Chapter III

Taxonomic revision of *Guapinannus* Wygodzinsky, 1951 (Hemiptera: Schizopteridae), with description of 19 new species

Abstract

Dipsocoromorpha is one of the least studied and smallest infraorders within Heteroptera (Hemiptera) with only ~350 described species. These small insects are only about 1-2 mm in length and live in cryptic habitats making them rare and difficult to collect. Their biodiversity in natural history collections is severely underrepresented given the large number of species that are estimated to thrive in these habitats and have yet to be described. We here examine the genus *Guapinannus* Wygodzinsky, 1951 (Hemiptera: Schizopteridae) which was described based on only one female specimen from Costa Rica. Thorough examination of residue samples from various museums we have located more than 250 specimens of *Guapinannus*, representing 19 new species that we here describe. We also for the first time describe males of this genus. Male and female genitalic characters and wing venation and structure are documented using digital imaging, scanning electron and confocal micrographs. This revision of *Guapinannus* does not only document the array of biodiversity that has until now remained hidden, but also provides character data to eventually reconstruct the phylogeny of Dipsocoromorpha including this genus that is not currently placed in a subfamily.

Introduction

Guapinannus Wygodzinsky was described as a monotypic genus of Schizopteridae based on the single female holotype of *Guapinannus bierigi* Wygodzinsky, 1951 from Guapiles in Costa Rica (Wygodzinsky 1951). Males and additional specimens or species have remained undocumented. Wygodzinsky (1951) diagnosed this genus by its distinctive wing venation, pointing out that the fracture in the forewing margin somewhat resembles the situation in the African *Humpatanannus* Wygodzinsky, 1950. He stated that *Guapinannus* is similar to *Tropistotrochus* Reuter, 1891 in having strongly flattened and widened forewings and the general outline of wing veins, but failed to provide a differential diagnosis to separate the two genera. Reuter's (1891) original description of monotypic genus *Tropistotrochus* based only on the female holotype from Blumenau in southern Brazil and his simple line drawings provide little detail. Wygodzinsky (1951) and Emsley (1969) were unable to study the holotype of *Tropistotrochus* and we have since confirmed that this specimen that was deposited in the Helsinki Museum of Zoology is lost https://kotka.luomus.fi/view?uri=http://id.luomus.fi/GV.44336. Given that no new information has become available on this genus and that the single specimen from Brazil that we have examined is easily accommodated within *Guapinannus*, we here treat *Tropistotrochus* as a nomen dubium.

As part of US National Science Foundation-funded project on Dipsocoromorpha we have assembled and examined >9,000 specimens of Schizopteridae with the majority originating from the Neotropical region. Among those were 262 specimens including 65 males that we classified as *Guapinannus* based on general habitus and wing venation. All represent undescribed species that we here describe as 19 new species.

Emsley (1969) in his discussion of intergeneric relationships within Schizopteridae pointed out that females of *Guapinannus* and *Peloridinannus* Wygodzinsky, 1951 are unusual among non-hypselosomatine Schizopteridae in possessing well-developed ovipositors. Because these two genera did not fit into his concepts of Hypselosomatinae, Schizopterinae, and the paraphyletic "Ogeriinae", he treated their subfamily placement as incertae sedis. Weirauch and Frankenberg (2015) in their taxonomic revision of *Peloridinannus* agreed with the assessment that the two genera are likely closely related and speculated that they may form one of the early diverging lineages within the "Ogeriinae" + Schizopterinae clade, a hypothesis that was recently confirmed by a combined morphological and molecular analysis Knyshov et al. (in prep.). We here follow Emsley (1969) and Weirauch and Frankenberg (2015) in leaving *Guapinannus* unplaced until phylogenetic relationships of this lineages have further consolidated and the classification is revised accordingly.

Materials and Methods

Material

Twenty-eight point-mounted specimens were loaned from INBIO (Instituto Nacional de Biodiversidad, Costa Rica), EAPZ (Escuela Agricola Panamericana El Zamorano, Honduras), and UCR (Entomology Research Collection, University of California, Riverside). The slide-mounted holotype of *Guapinannus bierigi* was imaged at the AMNH (American Museum of Natural History, New York, USA). The remaining specimens were separated from ethanol-preserved bulk samples belonging to FMNH (Field Museum of Natural History, Chicago, USA), MHNG (Museum d'histoire naturelle de Geneva, Genève, Switzerland), MTEC (Montana State University, Bozeman, USA), and gifts from Bob Anderson.

Methods

Databasing. A matrixcode label with specimen identifier was associated with each specimen consisting of a prefix and eight-digit number unique to each specimen. These numbers were used to database each specimen in the PBI instance of the Arthropod Easy Capture database served from the AMNH http://research.amnh.org/pbi/databases/locality_database.html. Google Earth was used to georeference specimens where label data did not include geographic coordinates.

Maps. Coordinates for individual species were downloaded from the PBI database and imported into SimpleMappr http://www.simplemappr.net/.

Dissections. For examination of genitalic structures, the abdomens of selected males and females was removed and immersed in warm ~ 10% KOH solution for approximately 5 to 10 minutes to remove soft tissues. The female abdomen was then stained with Chlorazol Black E to enhance visibility of the spermathecal, males were not stained. The abdomen was temporarily slide mounted using glycerin on microscope slides after being rinsed in deionized water and ethanol.

Slide-mounting. Dissected specimens were permanently slide-mounted following protocols outlined in Noyes (1982) and Platner et al. (1999).

Digital habitus imaging. (Figs 2-4). In preparation for habitus imaging, ethanol-preserved specimens were mounted in a watch glass on the surface of a small droplet of hand sanitizer and the watch glass then filled with 70% ethanol. Dry-mounted specimens were cleaned with a minuten pin before imaging. Dorsal, lateral, and ventral habitus images were taken using a Leica DFC 450 C Microsystems setup with Planapo 1.0X and 2.0X objectives and the Leica Application Suite (LAS) V4.3. Zerene Stacker was used to assemble composite images. Images were edited and assembled into habitus image plates using Photoshop CS5.

Scanning electron microscopy. (Figs 5-6). One male of *G. clava* **sp. n.** (ED 5470) was dissected, mounted on a stub with an adhesive carbon sticker, coated with gold-palladium using a Cressington 108 auto sputter coater, and examined and documented using a Hitachi S-4700 electron microscope at the Central Facility for Advanced Microscopy and Microanalysis at the University of California, Riverside (UCR).

Imaging of slide. (Fig. 1). Temporary and permanently slide-mounted specimens were imaged using a GT Vision imaging system on a Zeiss Axioskop 2 compound microscope (UCR) or a Leica setup at the AMNH (*G. bierigi* holotype).

Confocal imaging. (Fig. 7, K). One male of *G. clava* **sp. n.** (ED5472) was dissected and temporarily mounted on a microscopic slide in glycerin. The specimen was examined and imaged using a Leica SP5 Inverted confocal microscope with lasers of 488 nm and 543 nm and detectors set to diapasons of 500–535 nm and 555–700 nm.

Male genitalic illustrations. (Fig. 8). Overview illustrations of the male genitalia were prepared using a Nikon Eclipse 80i with camera lucida.

Species descriptions. Characters and character states were coded using a character matrix in WinClada ver. 1.00.08 (Nixon 2002) and species descriptions written to text files using the command “describe”. Species descriptions were edited and the re-description of the genus assembled based on individual species descriptions.

Measurements. The total length was measured from images using the measure tool in Image J for one male and one female per species where available. Total length was measured from the apex of the head to the tip of the wing. The head in many specimens is strongly pointed ventrad making total length measurements variable and unreliable. We therefore also measured from the posterior margin of the pronotum to the tip of the wing. Additional measurements are the greatest width across the pronotum and greatest width across the forewing. Measurements are provided in Table 1.

Terminology. Morphological terminology follows a combination of sources, most importantly Wygodzinsky (1951), Emsley (1969) and Hill (2013, 2014). Homologies of wing veins and cells are straightforward between different species of *Guapinannus*, but are difficult to assess across other schizopterid taxa due to the unusual number and orientation of veins and cells in *Guapinannus* spp. We derived cell names from their anterior bordering veins (e.g., medial and cubital cells) or followed the terminology proposed by other authors (e.g., Emsley 1969) to tentatively propose homology with other schizopterids (e.g., basal, discal, and trapezoidal cells).

Results

Taxonomy

Guapinannus Wygodzinsky, 1951

Figs 1-9

Type species: *Guapinannus bierigi* Wygodzinsky, 1951

Diagnosis. Recognized among Schizopteridae by the relatively small eyes, four-segmented labium, flat and wide forewings, unique forewing venation with long fracture in the costal margin, prominent veins proximally, and in most species with articulating processes on the clavus and corium, 2-2-3 tarsal formula in both sexes, and well-developed ovipositor.

Re-description: Male: Total body length 0.66–2.06 mm; length from posterior margin of pronotum to posterior wing margin (0.51–1.49 mm). **General coloration**: uniformly pale or a mix of pale and darker brown or reddish brown. **Vestiture**: Dorsum with dense setae, setae long or short, setae on posterior margin of hemelytron long or extremely long. **Structure**: **Head**: Eye less than one quarter head height, one third of head height to almost half as high as head; ocellus adjacent to eye, at 11 o’clock position seen in lateral view and about twice the size of one ommatidium; four-segmented labium with segments 1, 2 and 3 of similar length and 4 about 2.5-3 times as long as any other segment; muscle scars present on the frons and vertex either small or large. **Thorax:** Collar in dorsal view narrow; proepisternal lobe inflated; separation of anterior and posterior lobes (dorsal view) marked by depression; anterior pronotal lobe with muscle scars, margin concave or straight; posterior margin slightly concave to almost straight; pits on pronotum small medium-sized, or large, evenly distributed or localized; scutellum in dorsal view abruptly or gradually narrowed to tip, tip pointed, rounded, or slightly inflated. **Wings:** with outline of forewings elongate oval or broad anteriorly; membrane contributing about one third or less than one third to forewing length; posterior distal margin rounded or square at anterior distal end; clavus, twice as long as wide; vein-tracing areoles on proximal part of wing absent or present, when present either small, large or combination of small and large, along margins of cells dc and rc or of dc, rc, m, scc1; Sc uniformly broad or wider at R; R flattened in distal part; distal process of R pronounced or weakly developed; AN1/2 clavus process pronounced or weakly developed, shape of apex (dorsal view) acute and pointing dorsad; AN1/2 process on corium strongly inflated, weakly inflated, or absent, with or without notch articulating with clavus process, AN1 corium process rounded or acute, with or without median notch; scc1 about 1.5 times as wide as long or two times as long as wide; with single cell except in *G. dispar* **n. sp.** (Fig. 2); proximal part of Cu outlining bc clearly defined; rc about 3 times as long as wide except in *G. dispar*, slightly or much wider anteriorly; dc about 4 or 5 times as long as wide; tc relative length and width more than three times as long or three times as long as wide; distal margin of rc, dc, and tc cells double s-shaped, s-shaped, or angular s-shaped (Fig. 2, *G. dispar*); m almost triangular, trapezoidal, or rectangular (Fig. 2, *G. dispar*); cub almost reaching wing margin or distant form wing margin (except *G. dispar* elongate Fig); dc2 horse-head-shaped or dog-head shaped; M beyond Cu short or longer. **Legs**: males and females with tarsal formula 223, with claws long (except *G dispar* short). **Genitalia**: Pygophore without lobe on right side, with small or large lobe, or with slightly extended right margin. Vesica with between half and one loop, about one half loop, or one loop, surpassing right pygophore margin or not reaching margin, at midpoint thick, very thick, or more slender, tapering to blunt or acute tip, tip itself blunt, bottle-opener shaped, slightly s-shaped, rounded, or fairly straight. Anophore ridge with process present or absent, process short or long, with single lobe curved or relatively straight, or with multiple lobes, tip pointed, blunt, or combination of pointed and blunt. Right paramere with anterior lobe curved or straight, lobe tapering towards apex or broad throughout; posterior lobe broad, narrow, thumb-shaped, or duck-head shaped. Left paramere with anterior lobe straight or curved, tapering towards apex or broad throughout, posterior lobe broad or narrow. **Female** as male, some species without inflated AN1 corium process. Genitalia with well-developed ovipositor, some without inflated AN1 corium process. Reservoir of spermatheca kidney-shaped with short spermathecal duct (Fig. 7, A).

Distribution. Species of *Guapinannus* are documented from Central and South America, ranging from Veracruz in Mexico to the southern part of Brazil. The greatest species diversity is found in Central America and the northern areas of South America. Specimens for which habitat or macrohabitat information was provided on the lab had been collected in tropical wet or moist forest, including cloud forest and mixed hardwood forest, from elevation ranging from 5 meters above sea level in Panama to more than 200 meters in Chiapas, Mexico.

Collecting method. The majority of specimens for which collection method was recorded were collected using leaf litter sifting and Berlese and Winkler extraction methods. A small number of specimens are derived from Malaise and flight intercept traps.

Guapinannus anaticulus **n. sp.**

Figs 2, 7, 8, 9

Diagnosis: Recognized by the short, slender and straight vesica, and the duck-head shaped posterior lobe of the right paramere.

Description: Male: Total body length 1.45 mm; length from posterior margin of pronotum to posterior wing margin 1.23 mm. **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with dense setae, setae long; setae on posterior margin of hemelytron extremely long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum medium-sized, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip pointed. Wings with outline of forewings elongate oval; membrane contributing about one third of forewing length, posterior distal margin rounded, vein-tracing areoles on proximal part of wing absent; Sc uniformly broad; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, slightly wider anteriorly; dc about 5 times as long as wide; tc relative length and width more than three times as long; distal margin of rc, dc, and tc cells double s-shaped; m almost triangular; cub almost reaching wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with between half and one loop, surpassing right pygophore margin, thick at midpoint, tapering to blunt tip, close to apex slightly s-shaped. Anophore ridge with process, process short, with single lobe curved, tip blunt. Right paramere with anterior lobe curved, tapering, posterior lobe duck-shaped. Left paramere with anterior lobe straight, tapering, posterior lobe broad.

Female: unknown.

Specimens examined: Holotype: GUATEMALA: San Marcos: Bojonal Road, 14.94786°N 91.8837°W, 1618 m, 04 Jun 2015, R. Anderson, 1;m (UCR).

Etymology: Named for the duck-shaped posterior lobe of the right paramere after “anus” meaning duck

Guapinannus artus **n. sp.**

Figs 2, 8, 9

Diagnosis: Recognized by small lateral lobe on right side of pygophore, long and narrow dc, and the narrow body.

Description: Male: Total body length 1.29 mm, length from posterior margin of pronotum to posterior wing margin 1.04 mm. **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with dense setae, setae long; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum large, in distinct clusters; scutellum in dorsal view abruptly narrowed to tip, tip rounded. Wings with outline of forewings broad anteriorly; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing absent; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, much wider anteriorly; dc about 5 times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m almost triangular; cub almost reaching wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore with small lobe on right side; vesica with between half and one loop, not reaching right pygophore margin, relatively slender at midpoint, tapering to acute tip, close to apex rounded. Anophore ridge with process, process short, with single lobe curved, tip blunt. Right paramere with anterior lobe curved, tapering, posterior lobe narrow. Left paramere with anterior lobe straight, tapering, posterior lobe narrow.

Female: as in male and generic description.

Specimens examined: Holotype: MEXICO: Veracruz: or Unknown Co.: Road to Ruiz Cortinez, 18.5229°N 95.15441°W, 1114 m, 05 Jun 2016, R.S. Anderson, 1;m (UCR).

Paratypes: MEXICO: Veracruz: or Unknown Co.: Road to Ruiz Cortinez, 18.5229°N 95.15441°W, 1114 m, 05 Jun 2016, R.S. Anderson, 1;f (UCR). Road to Ruiz Cortinez,, 18.52241°N 95.14957°W, 1170 m, 04 Jun 2016, R.S. Anderson, 1;f (UCR). San Andres Tuxtla Co.: None, 18.52303°N 95.15453°W, 1109 m, 05 Jun 2016, Manuel Barrios, 2;m (UCR).

Etymology: Named for narrow dc cell of the forewing in dorsal view after “artus” Latin for narrow.

Guapinannus auriculus **n. sp.**

Figs. 2, 7, 8, 9

Diagnosis: Recognized by the large lateral lobe on right side of pygophore and shorter dc.

Description: Male: Total body length 1.23 mm; length from posterior margin of pronotum to posterior wing margin 0.9 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with dense setae, setae long; setae on posterior margin of hemelytron extremely long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum large, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip rounded. Wings with outline of forewings broad anteriorly; membrane contributing about one third of forewing length, posterior distal margin rounded, vein-tracing areoles on proximal part of wing absent; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, much wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m almost triangular; cub almost reaching wing margin; dc2 dog-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore with large lobe on right side; vesica with between half and one loop, not reaching right pygophore margin, thick at midpoint, tapering to acute tip, close to apex rounded. Anophore ridge with process, process short, with single lobe curved, tip pointed. Right paramere with anterior lobe curved, tapering, posterior lobe narrow. Left paramere with anterior lobe straight, tapering, posterior lobe narrow.

Female: as in male and generic description.

Specimens examined: Holotype: MEXICO: Veracruz: San Andres Tuxtla Co.: None, 18.52303°N 95.15453°W, 1109 m, 05 Jun 2016, Manuel Barrios, 4;m (UCR).

Paratypes: MEXICO: Veracruz: or Unknown Co.: hills above Ruiz Cortinez,, 18.5304°N 95.14127°W, 1239 m, 07 Jun 2016, R.S. Anderson, 1;f (UCR). San Andres Tuxtla Co.: None, 18.52303°N 95.15453°W, 1109 m, 05 Jun 2016, Manuel Barrios, 8;m, 13;f (UCR).

Etymology: Named for the large lobe on the right side of the pygophore after "auricula" Latin for lobe.

Guapinannus bierigi Wygodzinsky, 1951

Figs 1, 9

Diagnosis: Recognized among species of *Guapinannus* by the distance of the cub from the margin of the wing.

Re-description: Female: Total body length 1.45 mm, length from posterior margin of pronotum to posterior wing margin unknown; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with sparse setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin unknown; pits on pronotum medium-sized, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip pointed. Wings with outline of forewings broad anteriorly; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing absent; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium absent, without notch articulating with clavus process, elongate, with median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, slightly wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells s-shaped; m almost triangular; cub removed from wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. Genitalia: as in generic description

Male: unknown.

Specimens examined: Holotype: COSTA RICA: Limon: Guapiles, 10.2167°N 83.7833°W, 268 m, 05 Feb 1946 - 13 Feb 1946, A. Bierig, 1;f (AMNH).

Guapinannus castigates **n. sp.**

Figs 2, 8, 9

Diagnosis: Recognized by slightly extended right pygophore margin, areoles, vesica relatively short and thick.

Description: Male: Total body length 1.48 mm; length from posterior margin of pronotum to posterior wing margin 1.27 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with dense setae, setae long; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum medium-sized, evenly distributed; scutellum in dorsal view gradually narrowed to tip, tip pointed. Wings with outline of forewings elongate oval; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing present; areoles small, distributed along dc, rc, m, and scc1; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 2 times as wide as long; rc about 3 times as long as wide, slightly wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m trapezoidal; cub almost reaching wing margin; dc2 dog-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore with slightly extended right margin; vesica with about half loop, not reaching right pygophore margin, thick at midpoint, tapering to acute tip, close to apex rounded. Anophore ridge without process; Right paramere with anterior lobe curved, tapering, posterior lobe narrow. Left paramere with anterior lobe straight, tapering, posterior lobe narrow.

Female: unknown.

Specimens examined: Holotype: MEXICO: Chiapas: or Unknown Co.: Palenque, 15.63333°N 92.65°W, 02 Jul 1983 - 30 Jul 1983, S. B. Peck, J. Kukalova-Peck, R. S. Anderson, 1;m (FMNH).

Etymology: Named for the stout vesica after "castigatus" Latin for stout.

Guapinannus clava **n. sp.**

Figs 2, 5-9

Diagnosis: Recognized by the broad habitus, dark coloration, and areoles on all veins.

Description: Male: Total body length 1.54 mm; length from posterior margin of pronotum to posterior wing margin 1.16 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with sparse setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum medium-sized, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip pointed. Wings with outline of forewings broad anteriorly; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing present; areoles large, distributed along dc, rc, m, and scc1; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 2 times as wide as long; rc about 3 times as long as wide, much wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m trapezoidal; cub almost reaching wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with about half loop, not reaching right pygophore margin, thick at midpoint, with blunt apex, close to apex rounded. Anophore ridge with process, process long, with single lobe curved, tip pointed. Right paramere with anterior lobe curved, tapering, posterior lobe broad. Left paramere with anterior lobe straight, tapering, posterior lobe narrow.

Female: as in male and generic description.

Specimens examined: Holotype: PANAMA: Bocas del Toro: Almirante, at dam on Nigua Creek, 9.25°N 82.65°W, 23 Mar 1959, H. S. Dybas, 1;m (FMNH).

Paratypes: COSTA RICA: Puntarenas: 5 km SW Finca Las Cruces, La Fila, 8.78937°N 82.98133°W, 1433 m, 21 Mar 1973, J. A. Wagner, J. B. Kethley, 1;m (FMNH). PANAMA: Bocas del Toro: Almirante, at dam on Nigua Creek, 9.25°N 82.65°W, 23 Mar 1959, H. S. Dybas, 2;m, 7;f (FMNH); 25 Mar 1959, H. S. Dybas, 1;m (FMNH). Almirante, trail to dam on Nigua Creek, 9.3003°N 82.40214°W, 5 m, 23 Mar 1959, H. S. Dybas, 1;f (FMNH); 23 Mar 1959, H. S. Dybas, 2;f, 5;u, 1;m (FMNH). Bocas del Toro: Almirante, trail to dam on Nigua Creek, 9.29792°N 82.4211°W, 31 Mar 1959, H. S. Dybas, 2;m (FMNH).

Etymology: Named for the club-shaped vesica after “clava” Latin for club (noun in apposition).

Guapinannus dispar **n. sp.**

Figs 2, 9

Diagnosis: Recognized by the additional vein dividing scc1, slender and elongate cub and tc.

Description: Female: Total body length 0.66 mm; length from posterior margin of pronotum to posterior wing margin 0.51 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with sparse setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars small. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin almost straight; pits on pronotum large, evenly distributed; scutellum in dorsal view gradually narrowed to tip, tip pointed. Wings with outline of forewings broad anteriorly; membrane contributing less than one third of forewing length, posterior distal margin rounded, vein-tracing areoles on proximal part of wing absent; Sc wider at widest part of wing; distal process of R weakly developed; AN1/2 process on clavus absent; AN1/2 process absent; scc1 about 1.5 times as wide as long; rc about 4 times as long as wide, much wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells angular s-shaped; m rectangular; cub removed from wing margin; dc2 dog-head-shaped; M beyond Cu short. Legs: As in generic description with claws short. Genitalia: as in generic description

Male: unknown.

Specimens examined: Holotype: BRAZIL: Mato Grosso: Jacare Alto Xingu, 10.54341°S 53.75637°W, 01 Nov 1965, Alvarenga & Werner, 1;f (FMNH).

Etymology: Named for the characteristic wing venation after "dispar" Latin for different.

Guapinannus falcis **n. sp.**

Figs. 2, 7, 8, 9

Diagnosis: Recognized by the dense vestiture and sickle-shaped vesica.

Description: Male: Total body length 1.56 mm; length from posterior margin of pronotum to posterior wing margin 1.27 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with dense setae, setae long; setae on posterior margin of hemelytron long. **Structure: Head:** Eye about one third as high as head; muscle scars unknown; **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum large, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip pointed. Wings with outline of forewings broad anteriorly; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing absent; Sc uniformly broad; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, slightly wider anteriorly; dc about 5 times as long as wide; tc relative length and width more than three times as long; distal margin of rc, dc, and tc cells double s-shaped; m almost triangular; cub almost reaching wing margin; dc2 dog-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with between half and one loop, surpassing right pygophore margin, relatively slender at midpoint, tapering to acute tip, close to apex slightly s-shaped. Anophore ridge with process, process short, with single lobe curved, tip pointed. Right paramere with anterior lobe curved, broad throughout, posterior lobe broad. Left paramere with anterior lobe straight, tapering, posterior lobe broad.

Female: as in male and generic description.

Specimens examined: Holotype: COSTA RICA: Heredia: 6 km ENE Vara Blanca, 10.18333°N 84.11667°W, 2000 m, 16 Mar 2002, INBio-OET-ALAS, 1;m (INBIO).

Paratypes: COSTA RICA: Alajuela: Upala Co.: Bijagua, San Miguel, Finca Inti-Aura, 10.75778°N 85.00939°W, 300 m, 16 Apr 2011 - 20 May 2011, I. Chacon, 1;m (INBIO). Heredia: Sarapiqui Co.: P.N.B. Carrillo 5 km. E Vara Blanca, 10.17226°N 84.11213°W, 2100 m, 20 Apr 2002, INBio-OET-ALAS, 1;f (INBIO). P.N.B. Carrillo 6 km. ENE Vera Blanca, 10.15°N 83.91667°W, 2000 m, 17 Feb 2002 - 21 Feb 2002, INBio-OET-ALAS, 1;f (INBIO); 10 Mar 2002, INBio-OET-ALAS, 1;f, 1;m (INBIO). 6 km ENE Vara Blanca, 10.18333°N 84.11667°W, 2000 m, 16 Mar 2002, INBio-OET-ALAS, 1;f (INBIO); 22 Mar 2002, INBio-OET-ALAS, 4;f (INBIO).

Etymology: Named for sickle shaped vesica after “flax” Latin for sickle.

Guapinannus lutosus **n. sp.**

Figs 3, 8, 9

Diagnosis: Recognized by the thick Sc margin and s-shaped vesica.

Description: Male: Total body length 1.16 mm; length from posterior margin of pronotum to posterior wing margin 0.89 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with sparse setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum medium-sized, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip pointed. Wings with outline of forewings broad anteriorly; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing absent; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, slightly wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells s-shaped; m trapezoidal; cub almost reaching wing margin; dc2 dog-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with between half and one loop, not reaching right pygophore margin, thick at midpoint, tapering to acute tip, close to apex slightly s-shaped. Anophore ridge with process, process short, with single lobe curved, tip pointed. Right paramere with anterior lobe curved, tapering, posterior lobe narrow. Left paramere with anterior lobe straight, tapering, posterior lobe narrow.

Female: as in male and generic description.

Specimens examined: Holotype: NICARAGUA: Region Autonoma del Atlantico Sur: 13km WNW Rama, 12.19472°N 84.33667°W, 190 m, 18 Apr 2011, J. T. Longino, 1;m (FMNH).

Paratypes: NICARAGUA: Region Autonoma del Atlantico Sur: 13km WNW Rama, 12.19472°N 84.33667°W, 190 m, 18 Apr 2011, J. T. Longino, 3;f, 1;m Carduus occidentalis Nutt. (Asteraceae), 1;m (FMNH). Rivas: Matagalpa: RN Cerro Musún, 12.95944°N 85.225°W, 01 May 2011, Unknown, 1;m (FMNH).

Etymology: Named for the broad Sc margin of the forewing after "lutosus" Latin for thick.

Guapinannus minutus **n. sp.**

Figs 3, 9

Diagnosis: Recognized by the small size, inflated scutellum, well-defined proximal part of Cu, and dark coloration (female-based).

Description: Female: Total body length 1.14 mm; length from posterior margin of pronotum to posterior wing margin 0.90 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with dense setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum medium-sized, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip slightly inflated. Wings with outline of forewings elongate oval; membrane contributing about one third of forewing length, posterior distal margin rounded, vein-tracing areoles on proximal part of wing absent; Sc uniformly broad; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium weakly inflated, without notch articulating with clavus process, elongate, with median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, slightly wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m almost triangular; cub almost reaching wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. Genitalia: as in generic description.

Male: unknown.

Specimens examined: Holotype: COSTA RICA: Heredia: Sarapiqui Co.: P.N. Braulio Carrillo, 16 Km SSE La Virgen, 10.26784°N 84.084°W, 1050 m, 20 Feb 2001 - 23 Feb 2001, INBio-OET-ALAS, 1;f (INBIO).

Etymology: Named for the small body size after “minutus” Latin for small.

Guapinannus orbiculatus **n. sp.**

Figs. 3, 8, 9

Diagnosis: Recognized by the elongate body shape, absence of areoles, pale coloration, and one-loop vesica.

Description: Male: Total body length 1.26 mm; length from posterior margin of pronotum to posterior wing margin 0.9 mm; **General coloration** uniformly pale. **Vestiture:** Dorsum with sparse setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye about one third as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum medium-sized, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip rounded. Wings with outline of forewings elongate oval; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing absent; Sc uniformly broad; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, slightly wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m trapezoidal; cub almost reaching wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with between half and one loop, not reaching right pygophore margin, thick at midpoint, tapering to acute tip, close to apex rounded. Anophore ridge with process, process short, with single lobe curved, tip pointed. Right paramere with anterior lobe curved, tapering, posterior lobe broad. Left paramere with anterior lobe straight, tapering, posterior lobe narrow.

Female: as in male and generic description.

Specimens examined: Holotype: PANAMA: Bocas del Toro: Bocas del Toro: Almirante, trail to dam on Nigua Creek, 9.29792°N 82.4211°W, 31 Mar 1959, H. S. Dybas, 1;m (FMNH).

Paratypes: PANAMA: Bocas del Toro: Bocas del Toro: Almirante, trail to dam on Nigua Creek, 9.29792°N 82.4211°W, 31 Mar 1959, H. S. Dybas, 1;m, 4;f, 2;juvu (FMNH). Chiriqui: Near Nueva California W. of Finca Palo Santo, 8.5°N 82.41667°W, 1524 m, 10 Mar 1959, H. S. Dybas, 1;m (FMNH).

Etymology: Named for the rounded vesica after “orbiculatus” Latin for round.

Guapinannus plurilobus **n. sp.**

Figs 3, 7, 8, 9

Diagnosis: Recognized by multilobed anophore process, with combination of blunt and acute apices.

Description: Male: Total body length 1.14 mm; length from posterior margin of pronotum to posterior wing margin 0.8 mm; **General coloration** uniformly pale. **Vestiture:** Dorsum with sparse setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum medium-sized, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip rounded. Wings with outline of forewings broad anteriorly; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing absent; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, elongate, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, slightly wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m almost triangular; cub almost reaching wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with one loop, not reaching right pygophore margin, relatively slender at midpoint, tapering to blunt tip, close to apex rounded. Anophore ridge with process, process long, with multiple lobes, tip blunt. Right paramere with anterior lobe curved, broad throughout, posterior lobe broad. Left paramere with anterior lobe straight, tapering, posterior lobe narrow.

Female: as in male and generic description.

Specimens examined: Holotype: PERU: Amazonas: Pacaya–Samiria National Reserve, 5.2012°S 74.59319°W, 07 Nov 1980, CI. Vaucher, 1;m (MHNG).

Paratypes: PERU: Amazonas: Pacaya–Samiria National Reserve, 5.2012°S 74.59319°W, 07 Nov 1980, CI. Vaucher, 4;m, 5;f (MHNG). unknown: Unknown Co.: Unknown, 9.18997°S 75.01515°W, no date provided, Unknown, 1;f (MHNG).

Etymology: Named for the multiple lobes on the ananphore process after "pluri" and "lobus" Latin for multiple lobes.

Guapinannus policis **n. sp.**

Figs 3, 7, 8, 9

Diagnosis: Recognized by the wide thumb-shaped posterior right paramere lobe.

Description: Male: Total body length 1.03 mm; length from posterior margin of pronotum to posterior wing margin ambiguous 0.7 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with sparse setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars small. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum large, evenly distributed; scutellum in dorsal view gradually narrowed to tip, tip pointed. Wings with outline of forewings broad anteriorly; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing absent; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, much wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m trapezoidal; cub almost reaching wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with between half and one loop, not reaching right pygophore margin, relatively slender at midpoint, tapering to acute tip, close to apex rounded. Anophore ridge with process, process short, with single lobe curved, tip pointed. Right paramere with anterior lobe curved, broad throughout, posterior lobe thumb-shaped. Left paramere with anterior lobe straight, tapering, posterior lobe narrow.

Female: as in male and generic description.

Specimens examined: Holotype: COLOMBIA: Cauca: PNN Gorgona, Antigua Laguna, 2.96667°N 78.18333°W, 70 m, 18 Dec 2000 - 03 Jan 2001, H. Torres, 1;m (UCR).

Paratypes: COLOMBIA: Cauca: PNN Gorgona, Alto el Mirador, 2.96666°N 78.18333°W, 180 m, 06 Oct 2000 - 23 Oct 2000, R. Duque, 1;f (UCR).

Etymology: Named for the thumb-shaped posterior lobe of the right paramere after “policis” Latin for thumb.

Guapinannus robustus **n. sp.**

Figs 3, 7, 8, 9

Diagnosis: Recognized by the slight extensions of the right pygophore margin and very stout vesica.

Description: Male: Total body length 1.49 mm; length from posterior margin of pronotum to posterior wing margin 1.1 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with dense setae, setae long; setae on posterior margin of hemelytron extremely long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum medium-sized, evenly distributed; scutellum in dorsal view gradually narrowed to tip, tip rounded. Wings with outline of forewings elongate oval; membrane contributing about one third of forewing length, posterior distal margin rounded, vein-tracing areoles on proximal part of wing present; areoles small, distributed along dc, rc, m, and scc1; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, slightly wider anteriorly; dc about 5 times as long as wide; tc relative length and width more than three times as long; distal margin of rc, dc, and tc cells double s-shaped; m trapezoidal; cub almost reaching wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore with slightly extended right margin; vesica with about half loop, not reaching right pygophore margin, very thick at midpoint, tapering to blunt tip, close to apex slightly s-shaped. Anophore ridge with process, process short, with single lobe relatively straight, tip pointed. Right paramere with anterior lobe curved, broad throughout, posterior lobe broad. Left paramere with anterior lobe straight, tapering, posterior lobe narrow.

Female: unknown.

Specimens examined: Holotype: MEXICO: Chiapas: or Unknown Co.: RSV A El Triunfo, Est. El Trrunfo, 16.20286°N 91.86044°W, 1950 m, 08 Aug 1996 - 11 Aug 1996, A. R. Gillogly, 1;m (UCR).

Etymology: Named for the extremely thick vesica after "robustus" Latin for robust.

Guapinannus sinuosus **n. sp.**

Figs 3, 8, 9

Diagnosis: Recognized by Sc margin broad anteriorly and more slender posteriorly, double s-shaped margin of rc, dc, tc cells, and s-shaped vesica.

Description: Male: Total body length 1.49 mm; length from posterior margin of pronotum to posterior wing margin 0.9 mm; **General coloration** uniformly pale. **Vestiture:** Dorsum with sparse setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum small, evenly distributed; scutellum in dorsal view gradually narrowed to tip, tip pointed. Wings with outline of forewings elongate oval; membrane contributing about one third of forewing length, posterior distal margin rounded, vein-tracing areoles on proximal part of wing absent; Sc uniformly broad; distal process of R weakly developed; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, slightly wider anteriorly; dc about 5 times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m almost triangular; cub almost reaching wing margin; dc2 dog-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with between half and one loop, not reaching right pygophore margin, thick at midpoint, tapering to acute tip, close to apex slightly s-shaped. Anophore ridge with process, process short, with single lobe curved, tip pointed. Right paramere with anterior lobe curved, tapering, posterior lobe broad. Left paramere with anterior lobe straight, tapering, posterior lobe narrow.

Female: as in male and generic description.

Specimens examined: Holotype: MEXICO: Chiapas: or Unknown Co.: Palenque, 15.63333°N 92.65°W, 02 Jul 1983 - 30 Jul 1983, S. B. Peck, J. Kukalova-Peck, R. S. Anderson, 1;m (FMNH).

Paratypes: MEXICO: Chiapas: or Unknown Co.: El Bosque (6.6 mi SW), 17.01667°N 92.78333°W, 1463 m, 29 Aug 1973, A. F. Newton, 7;f (FMNH). Playón de la Gloria, 16.16028°N 90.90139°W, 160 m, 26 Jun 2008, Unknown, 1;m (MTEC).

Etymology: Named for the s-shaped vesica after “sinuousus” Latin for bending.

Guapinannus tatumbia **n. sp.**

Figs 4, 8, 9

Diagnosis: Recognized by elongate body shape, inflated scutellum, and vesica not surpassing pygophore margin.

Description: Male: Total body length 1.51 mm; length from posterior margin of pronotum to posterior wing margin 1.27 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with dense setae, setae long; setae on posterior margin of hemelytron extremely long. **Structure: Head:** Eye about one third as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum small, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip rounded. Wings with outline of forewings broad anteriorly; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing absent; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, much wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m trapezoidal; cub almost reaching wing margin; dc2 dog-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with between half and one loop, not reaching right pygophore margin, thick at midpoint, tapering to acute tip, close to apex fairly straight. Anophore ridge without process; Right paramere with anterior lobe curved, broad throughout, posterior lobe broad. Left paramere with anterior lobe straight, tapering, posterior lobe narrow.

Female: as in male and generic description.

Specimens examined: Holotype: HONDURAS: Francisco Morazan: Tatumbia Montaña del Aguacate, 14°N 87.08333°W, 14 Mar 1996, R. Cave, 1;m (EAPZ).

Paratypes: HONDURAS: Francisco Morazan: Tatumbia Montaña del Aguacate, 14°N 87.08333°W, 14 Mar 1996, R. Cave, 5;m, 5;f (EAPZ). Olancho: La Union Parq Nac La Maralla, 15.07949°N 86.76175°W, 14 Sep 1994, R. Cordero, 1;f (EAPZ).

Etymology: Named for the type locality “Tatumbia Montana” in Honduras (noun in apposition).

Guapinannus tenuis **n. sp.**

Figs 4, 7, 8, 9

Diagnosis: Recognized by the s-shaped vesica, very narrow Sc margin, and double-s vein.

Description: Male: Total body length 2.06 mm; length from posterior margin of pronotum to posterior wing margin 1.49 mm; **General coloration** uniformly pale. **Vestiture:** Dorsum with sparse setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye about one third as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin straight, posterior margin slightly concave; pits on pronotum large, in distinct clusters; scutellum in dorsal view abruptly narrowed to tip, tip rounded. Wings with outline of forewings elongate oval; membrane contributing about one third of forewing length, posterior distal margin rounded, vein-tracing areoles on proximal part of wing absent; Sc uniformly broad; distal process of R weakly developed; AN1/2 process on clavus very pronounced; AN1/2 process on corium absent, with notch articulating with clavus process, elongate, with median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, much wider anteriorly; dc about 5 times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells s-shaped; m almost triangular; cub removed from wing margin; dc2 dog-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with between half and one loop, not reaching right pygophore margin, thick at midpoint, tapering to acute tip, close to apex slightly s-shaped. Anophore ridge with process, process short, with single lobe curved, tip pointed. Right paramere with anterior lobe straight, tapering, posterior lobe broad. Left paramere with anterior lobe straight, tapering, posterior lobe broad.

Female: unknown.

Specimens examined: Holotype: COSTA RICA: Unknown: Costa Rica, 9.74277°N 83.75944°W, 1167 m, no date provided, Unknown, 1;m (INBIO).

Etymology: Named for the slender vesica after "tenuis" Latin for slender.

Guapinannus tergus **n. sp.**

Figs 4, 8, 9

Diagnosis: Recognized by the round, almost full-loop vesica, anophore ridge long and pointed, areoles only on two cells.

Description: Male: Total body length 0.67 mm; length from posterior margin of pronotum to posterior wing margin 0.52 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with sparse setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum medium-sized, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip pointed. Wings with outline of forewings broad anteriorly; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing present; areoles small, distributed along dc and rc; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, much wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m trapezoidal; cub almost reaching wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with between half and one loop, not reaching right pygophore margin, thick at midpoint, tapering to acute tip, close to apex rounded. Anophore ridge with process, process long, with single lobe curved, tip pointed. Right paramere with anterior lobe curved, broad throughout, posterior lobe narrow. Left paramere with anterior lobe straight, broad throughout, posterior lobe narrow.

Female: unknown.

Specimens examined: Holotype: ECUADOR: Pastaza: 25 km N Puyo, 1.2855°S 78.02291°W, 1000 m, 13 Jul 1976, S. B. Peck, 1;m (FMNH).

Paratypes: ECUADOR: Pastaza: Pastaza, 1.43583°S 78.00194°W, 13 Jul 1976, S. B. Peck, 4;m (FMNH).

Etymology: Named for the long ridge-like process on the anophore after “tergus” Latin for ridge (noun in apposition).

Guapinannus trilobus **n. sp.**

Figs 4, 7, 8, 9

Diagnosis: Recognized by the multiple-lobed, pointed anophore processes.

Description: Male: total body length 1.4 mm; length from posterior margin of pronotum to posterior wing margin 1.03 mm; **General coloration** mix of pale and darker brown or reddish brown. **Vestiture:** Dorsum with sparse setae, setae long; setae on posterior margin of hemelytron extremely long. **Structure: Head:** Eye almost half as high as head; muscle scars large. **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum large, evenly distributed; scutellum in dorsal view abruptly narrowed to tip, tip pointed. Wings with outline of forewings broad anteriorly; membrane contributing about one third of forewing length, posterior distal margin squarish, vein-tracing areoles on proximal part of wing absent; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, much wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m almost triangular; cub almost reaching wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with between half and one loop, not reaching right pygophore margin, relatively slender at midpoint, tapering to acute tip, close to apex rounded. Anophore ridge with process, process long, with multiple lobes, tip pointed. Right paramere with anterior lobe curved, broad throughout, posterior lobe broad. Left paramere with anterior lobe curved and tapering, posterior lobe broad.

Female: as in male and generic description.

Specimens examined: Holotype: MEXICO: Veracruz: San Andres Tuxtla Co.: None, 18.52303°N 95.15453°W, 1109 m, 05 Jun 2016, Manuel Barrios, 1;m (UCR).

Paratypes: MEXICO: Veracruz: or Unknown Co.: Road to Ruiz Cortinez, 18.5229°N 95.15441°W, 1114 m, 05 Jun 2016, R.S. Anderson, 1;m, 2;f (UCR).

Etymology: Named for the three lobes on the anophore process after “trilobus” Latin for three lobes.

Guapinannus uncus **n. sp.**

Figs 4, 8, 9

Diagnosis: Recognized by the elongate ovoid body shape and the shape of the apex of the vesica.

Description: Male: Total body length 1.19 mm; length from posterior margin of pronotum to posterior wing margin 0.97 mm; **General coloration** uniformly pale. **Vestiture:** Dorsum with sparse setae, setae short; setae on posterior margin of hemelytron long. **Structure: Head:** Eye almost half as high as head; muscle scars unknown; **Thorax:** As in generic description, with anterior pronotal margin concave, posterior margin slightly concave; pits on pronotum small, evenly distributed; scutellum in dorsal view gradually narrowed to tip, tip pointed. Wings with outline of forewings elongate oval; membrane contributing about one third of forewing length, posterior distal margin rounded, vein-tracing areoles on proximal part of wing absent; Sc wider at widest part of wing; distal process of R pronounced; AN1/2 process on clavus very pronounced; AN1/2 process on corium strongly inflated, without notch articulating with clavus process, rounded, without median notch; scc1 about 1.5 times as wide as long; rc about 3 times as long as wide, slightly wider anteriorly; dc about four times as long as wide; tc relative length and width three times as long or less; distal margin of rc, dc, and tc cells double s-shaped; m trapezoidal; cub almost reaching wing margin; dc2 horse-head-shaped; M beyond Cu short. Legs: As in generic description with claws long. **Genitalia** (Fig. 8): Pygophore without lobe on right side; vesica with between half and one loop, surpassing right pygophore margin, thick at midpoint, with bottle-opener-shaped apex, close to apex rounded. Anophore ridge with process, process short, with single lobe curved, tip pointed. Right paramere with anterior lobe curved, broad throughout, posterior lobe broad. Left paramere with anterior lobe straight, tapering, posterior lobe broad.

Female: unknown.

Specimens examined: Holotype: BELIZE: unknown: Belize, 17.18988°N 88.49765°W, no date provided, Unknown, 1;m (FMNH).

Paratypes: BELIZE: Orange Walk: Rio Bravo Cons. Area 3 Well Tran (Near Res. Station), 18.06667°N 88.55°W, 10 Sep 1995, P. W. Kovarik, 1;m (UCR).

Etymology: Named for the hook-shaped vesica after “uncus” Latin for hook (noun in apposition).

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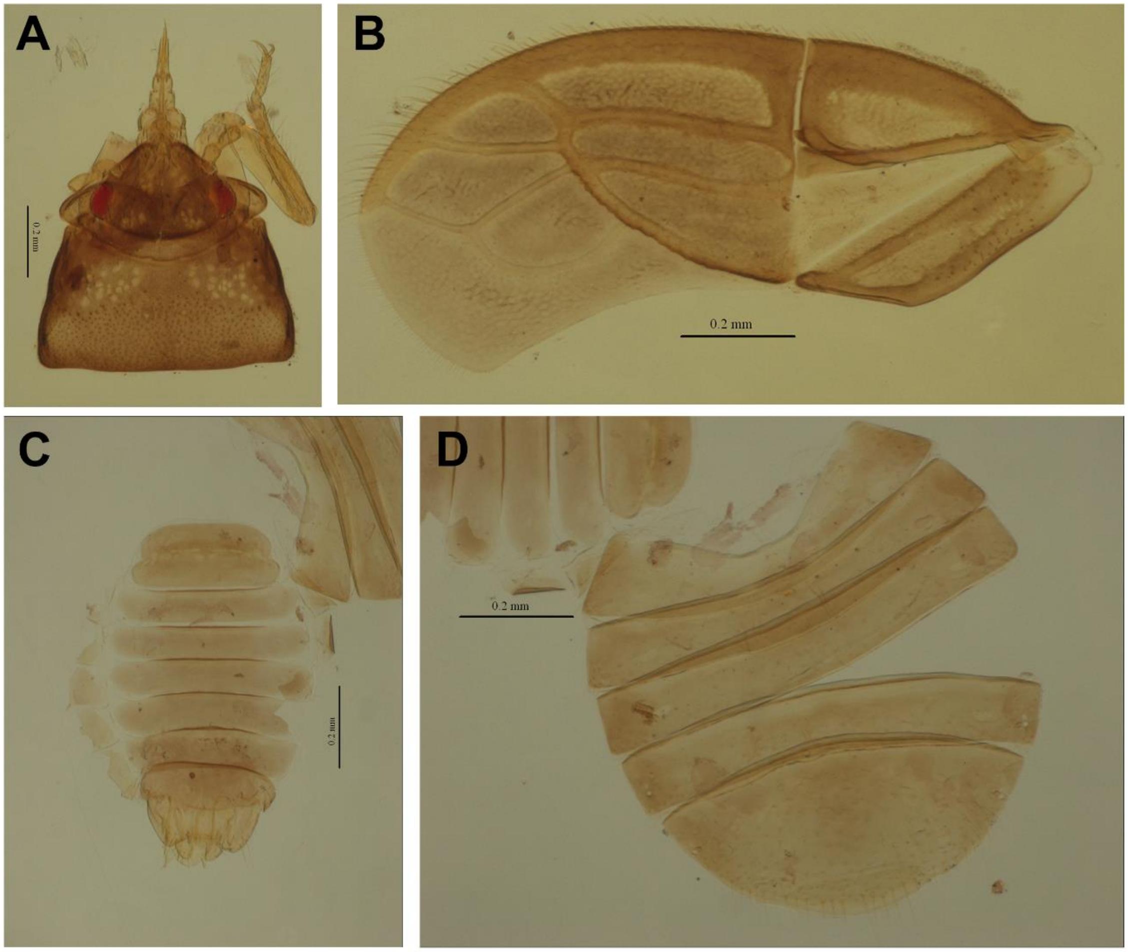
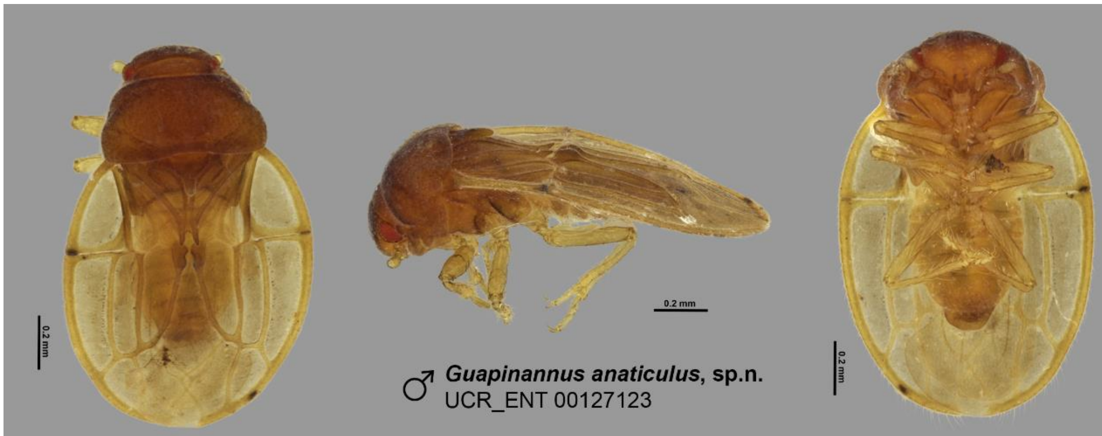


Figure 3-1. ***Guapinannus biergi*, holotype**

A: Head with rostrum extended forward and pronotum showing muscle scars on anterior lobe, dorsal view. B: Left forewing, dorsal view. C-D: Abdomen dorsal view and ventral view respectively.



♂ ***Guapinannus anaticulus*, sp.n.**
UCR_ENT 00127123

♂ ***Guapinannus artus, sp.n.***
UCR_ENT 00125959

♂ ***Guapinannus auriculus, sp.n.***
UCR_ENT 00125965

♂ ***Guapinannus castigatus, sp.n.***
UCR_ENT 00101251

♂ ***Guapinannus clava, sp.n.***
UCR_ENT 00097823

♀ ***Guapinannus dispar, sp.n.***
UCR_ENT 00090847

♂ ***Guapinannus falcis, sp.n.***
UCR_ENT 00014616

Figure 3-2. Habitus of Guapinannus n. spp.

Habitus of *Guapinannus anaticulus* **n. sp.**, *G. artus* **n. sp.**, *G. auriculus* **n. sp.**, *G. castigates* **n. sp.**, *G. clava* **n. sp.**, *G. dispar* **n. sp.**, and *G. falcis* **n. sp.** Orientation: all dorsal, *G. anaticulus* **n. sp.**, dorsal, (left), lateral (middle), and ventral (right) views.

♂ ***Guapinannus lutosus*, sp.n.**
UCR_ENT 00102045

♀ ***Guapinannus minutus*, sp.n.**
UCR_ENT 00014609

♂ ***Guapinannus orbiculatus,* sp.n.**
UCR_ENT 00090718

♂ ***Guapinannus plurilobus*, sp.n.**
UCR_ENT 00089589

♂ ***Guapinannus policis*, sp.n.**
UCR_ENT 00109003

♂ ***Guapinannus robustus*, sp.n.**
UCR_ENT 00094282

♂ ***Guapinannus sinuosus*, sp.n.**
UCR_ENT 00102050

Figure 3-3. Habitus of Guapinannus n. spp.

Dorsal habitus of *Guapinannus lutosus* **n. sp.**, *G. minutus* **n. sp.**, *G. orbiculatus* **n. sp.**, *G. plurilobus* **n. sp.**, *G. policis* **n. sp.**, *G. robustus* **n. sp.**, and *G. sinuosus* **n. sp.**

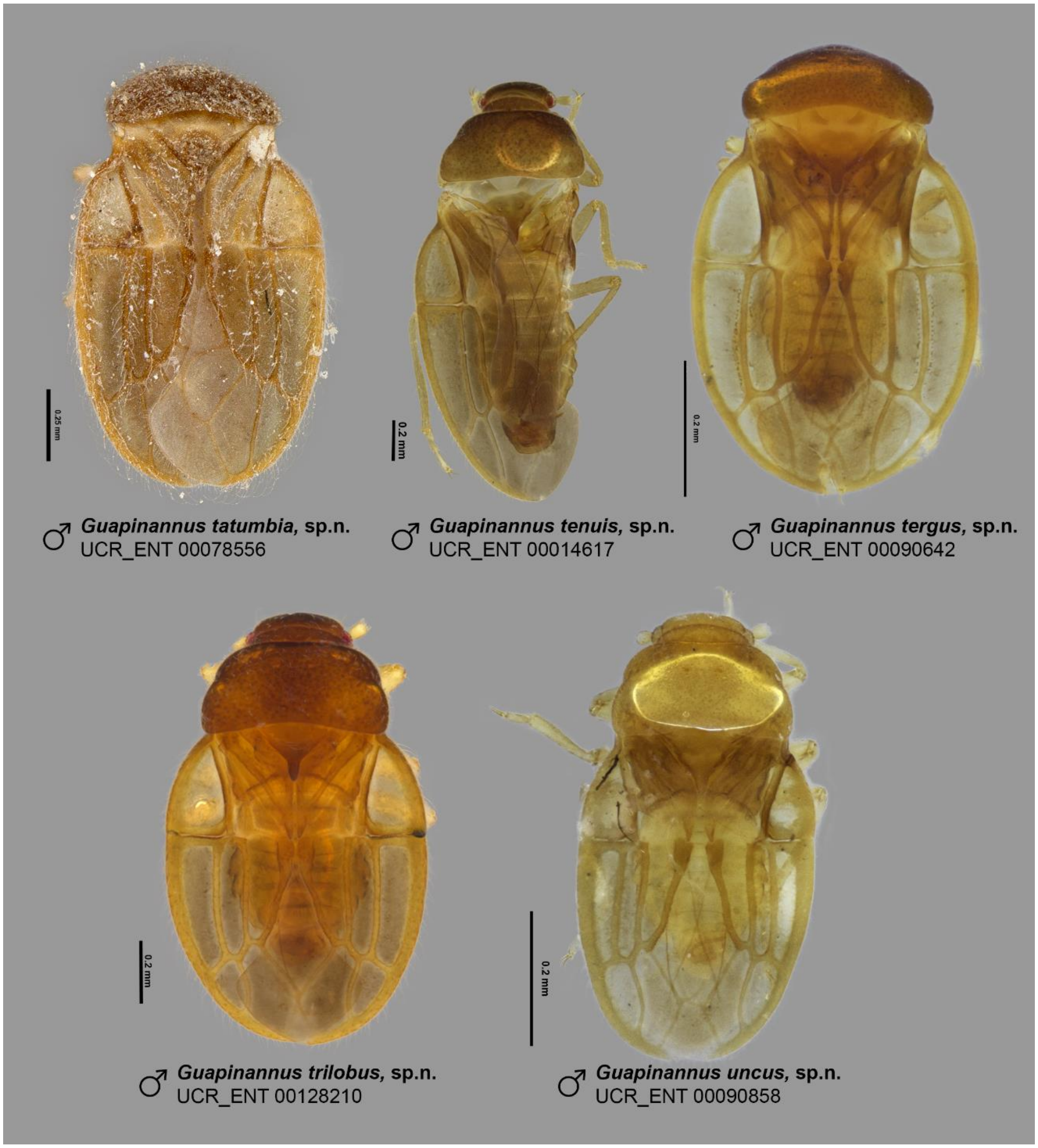


Figure 3-4. Habitus of Guapinannus n. spp.

Dorsal habitus of *Guapinannus tatumbia* **n. sp.**, *G. tenuis* **n. sp.**, *G. tergus* **n. sp.**, *G. trilobus* **n. sp.**, and *G. uncus* **n. sp.**

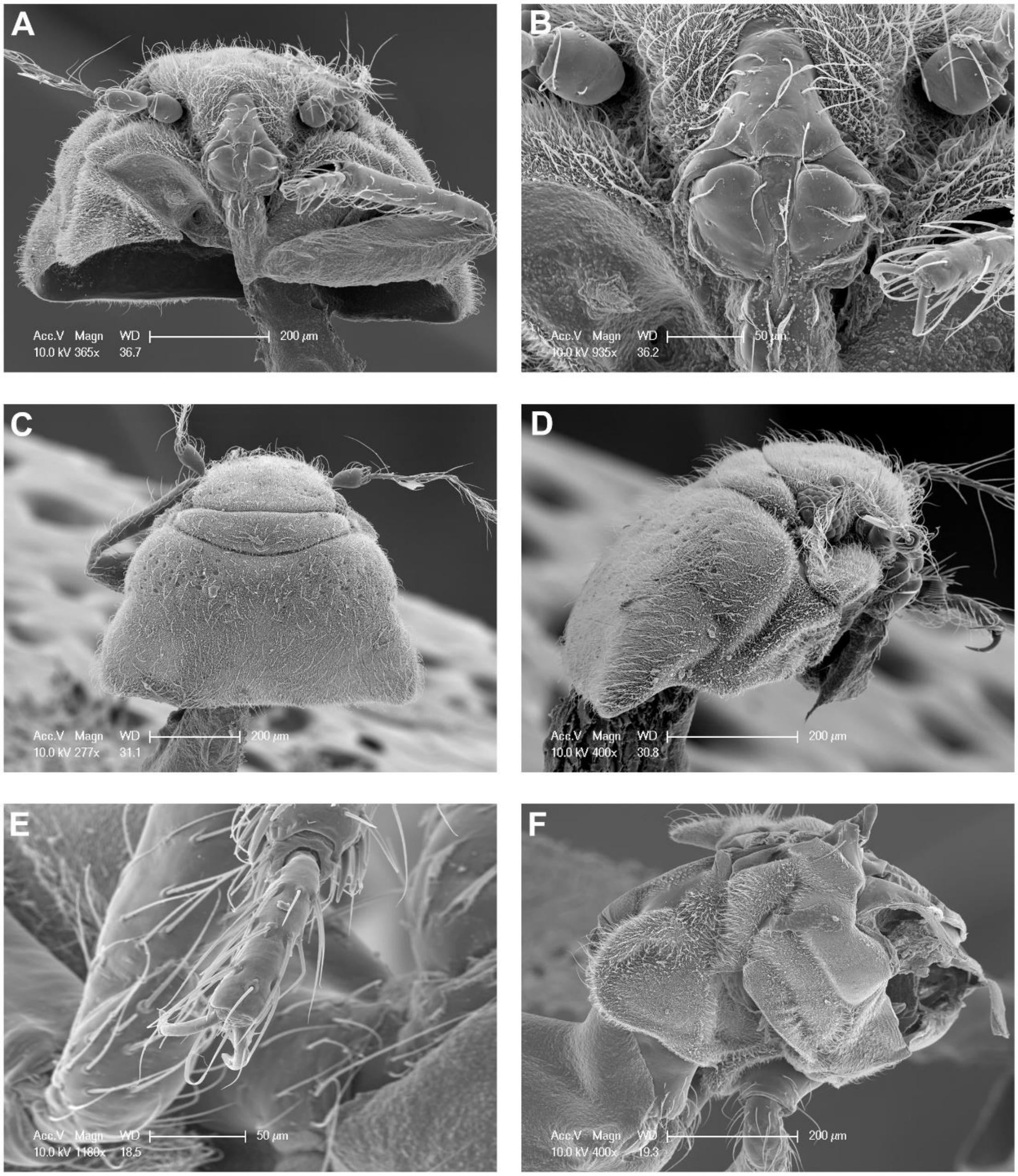

A
Acc.V Magn WD 200 µm
10.0 kV 365x 36.7
B
Acc.V Magn WD 50 µm
10.0 kV 935x 36.2
C
Acc.V Magn WD 200 µm
10.0 kV 277x 31.1
D
Acc.V Magn WD 200 µm
10.0 kV 400x 30.8
E
Acc.V Magn WD 50 µm
10.0 kV 1180x 18.5
F
Acc.V Magn WD 200 µm
10.0 kV 400x 19.3

Figure 3-5. Scanning electron micrographs of head, thoracic structures and tarsi of the male of *Guapinannus clava* n. sp. (UCR_ENT 00090715)

A: Head, frontal view. B: Head (expanded), frontal view. C: Head and pronotum, dorsal view, showing collar and vestiture on pronotum. D: Head and pronotum, lateral view, showing collar and vestiture on pronotum. E: Hind left leg, ventral view, showing claws and taromeres. F: Thorax, lateral view, of ventral portion of head and prothorax, showing scutellum.

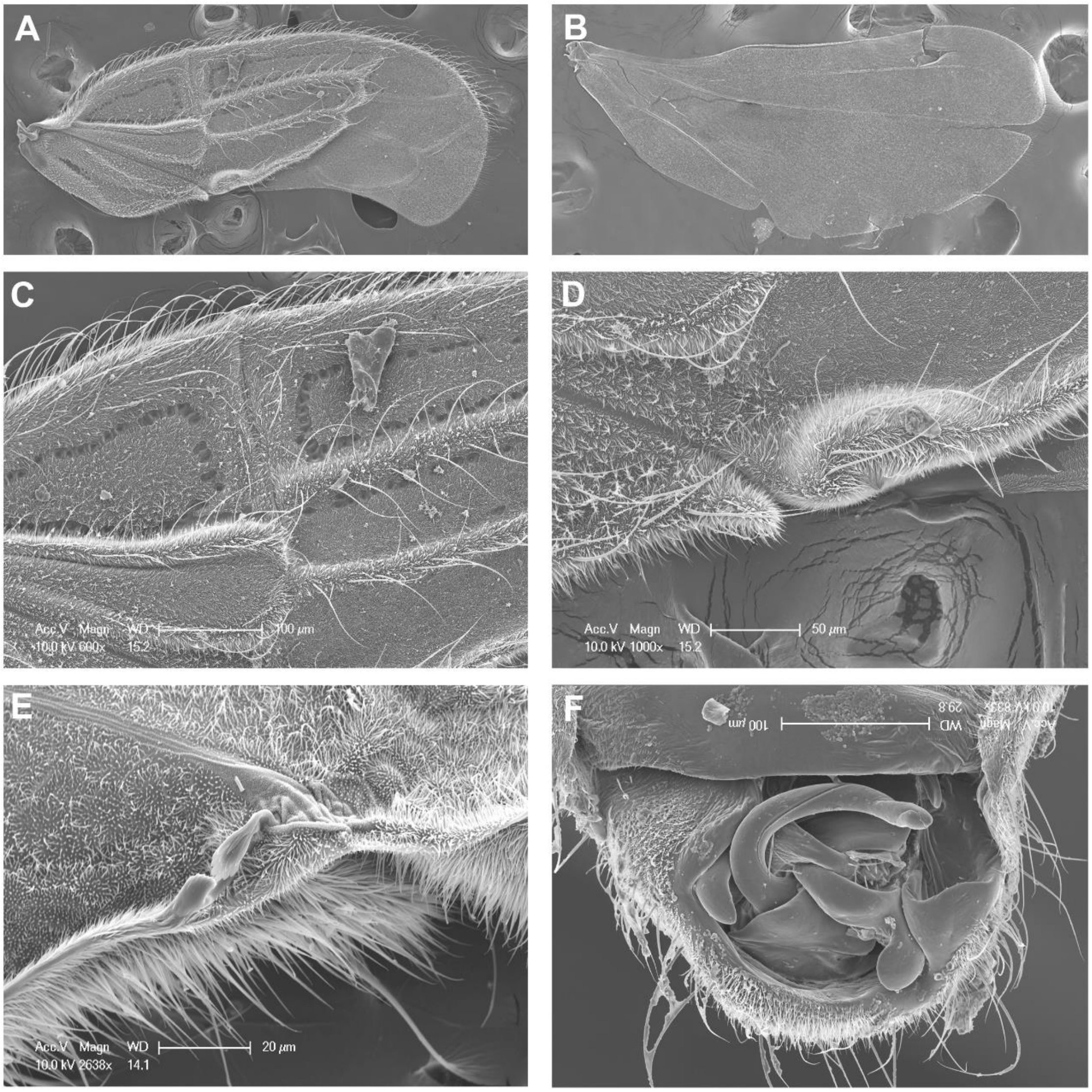


Figure 3-6. Scanning electron micrographs of wings and genitalic structures of male of *Guapinannus clava* n. sp. (UCR_ENT 00090715)

A: Right forewing, dorsal view. B: Right hindwing, dorsal view. C: Right forewing, showing vein tracing areoles, dorsal view. D: Right forewing, showing AN1/2 process on clavus; pronounced AN1/2 process on corium, dorsal view. E: Right forewing, showing wing coupling, dorsal view. F: Pygophore and genitalic structures, dorsal view.

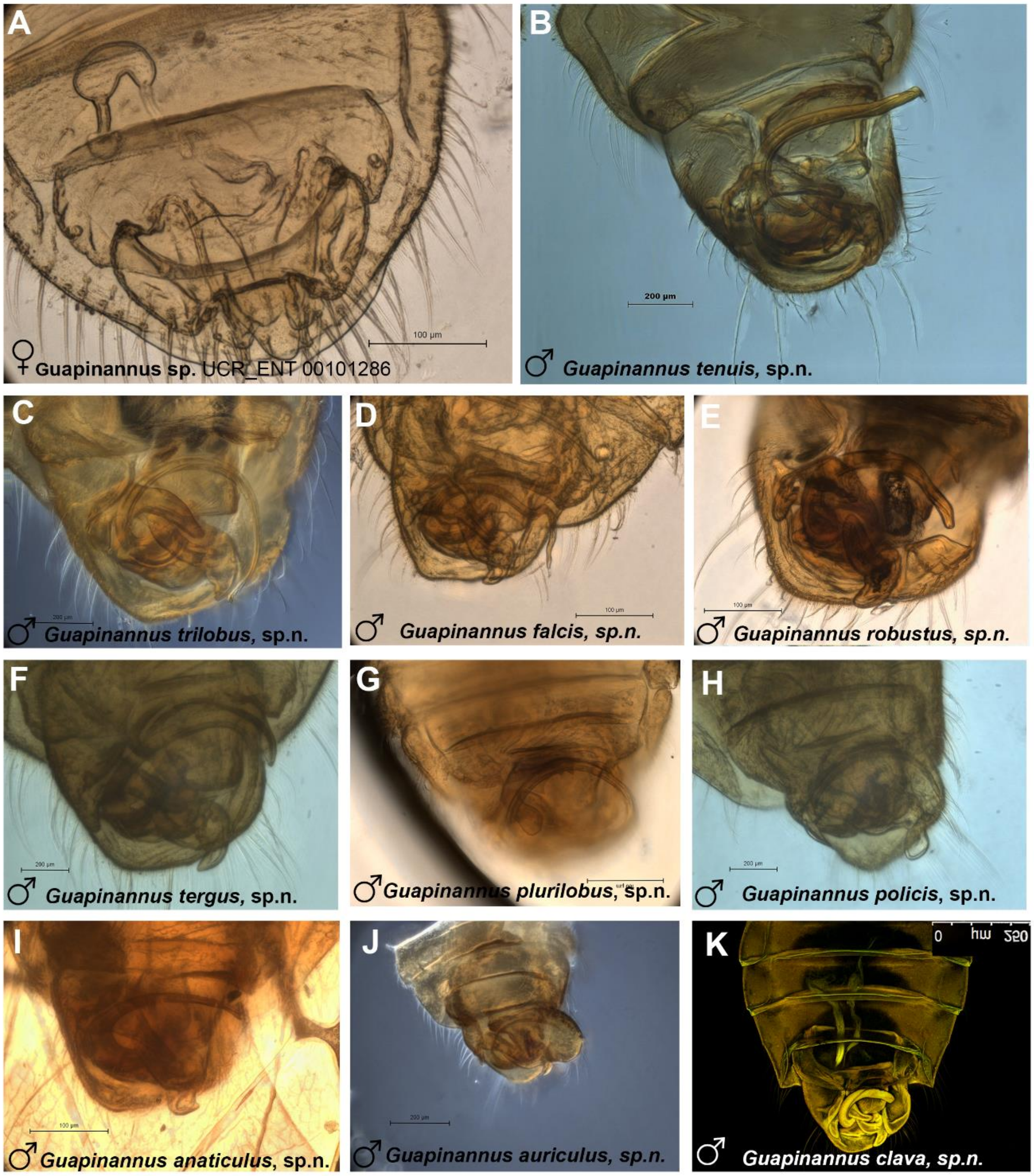
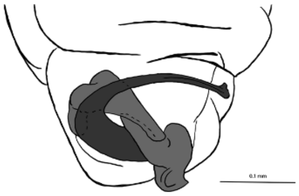
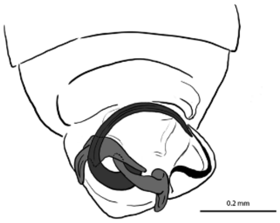

A
100 µm
♀ Guapinannus sp. UCR_ENT 00101286
B
200 µm
♂ Guapinannus tenuis, sp.n.
C
200 µm
♂ Guapinannus trilobus, sp.n.
D
100 µm
♂ Guapinannus falcis, sp.n.
E
100 µm
♂ Guapinannus robustus, sp.n.
F
200 µm
♂ Guapinannus tergus, sp.n.
G
♂ Guapinannus plurilobus, sp.n.
H
200 µm
♂ Guapinannus policis, sp.n.
I
100 µm
♂ Guapinannus anaticulus, sp.n.
J
200 µm
♂ Guapinannus auriculus, sp.n.
K
0 µm 250
♂ Guapinannus clava, sp.n.

Figure 3-7. Female and male genitalic and pre-genitalic abdominal structures in *Guapinannus* spp. (all dorsal view)

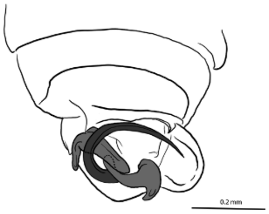
A–J are light compound microscopic images, K is a confocal image. A: Female *Guapinannus* sp. (UCR_ENT 00101286), genitalic structures including ovipositor and spermathecal gland, and spermathecal duct. B: *G. tenuis* **n. sp.**, male genitalia showing short anophore process and vesica tapering to acute tip, and slightly s-shaped. C: *G. trilobus* **n. sp.**, male genitalia showing anaphore ridge with three lobes, and rounded vesica. D: *G. falcis* **n. sp.**, male genitalia showing sickle shaped vesica. E: *G. robustus* **n. sp.**, male genitalia showing very thick vesica. F: *G. tergus* **n. sp.**, male genitalia showing long, pointed anophore ridge. G: *G. pluribus* **n. sp.**, male genitalia showing large multiple lobes on anophore ridge. H: *G. policis* **n. sp.**, showing right paramere with thumb-shaped posterior lobe. I: *G. anaticulus* **n. sp.**, male genitalia showing right paramere with duck-shaped posterior lobe. J: *G. auriculus* **n. sp.**, male genitalia showing large lobe on the right side of pygophore. K: *G. clava* **n. sp.**, male genitalia showing thick, club-like vesica.



***G. anaticulus*, sp. n.**

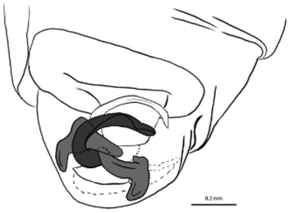


***G. artus*, sp. n.**

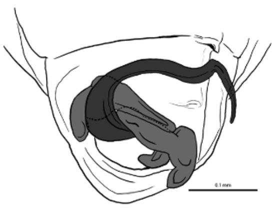


***G. auriculus*, sp. n.**

***G. castigatus*, sp. n.**

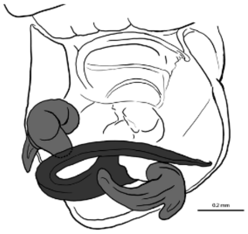


***G. clava*, sp. n.**

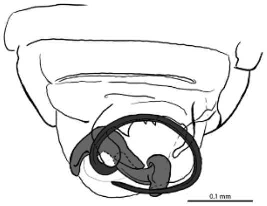


***G. falcis*, sp. n.**

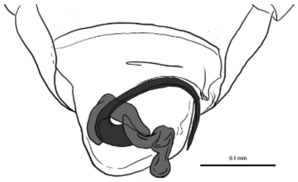
***G. lutosus*, sp. n.**



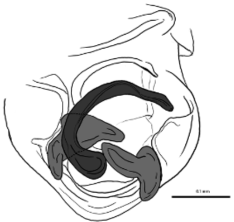
***G. orbiculatus*, sp. n.**



***G. plurilobus*, sp. n.**



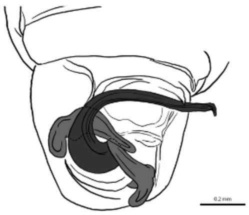
***G. policis*, sp. n.**



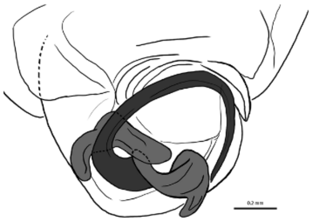
***G. robustus*, sp. n.**

***G. sinuosus*, sp. n.**

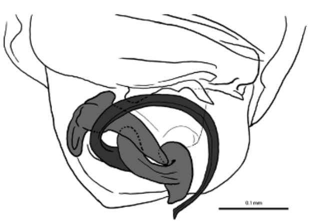
***G. tatumbia*, sp. n.**



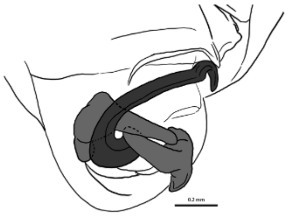
***G. tenuis*, sp. n.**



***G. tergus*, sp. n.**



***G. trilobus*, sp. n.**



***G. uncus*, sp. n.**

Figure 3-8. Male genitalic features of *Guapinannus* spp.

Dorsal view, showing species-diagnostic differences in vesica, right and left parameres, and anophore process.

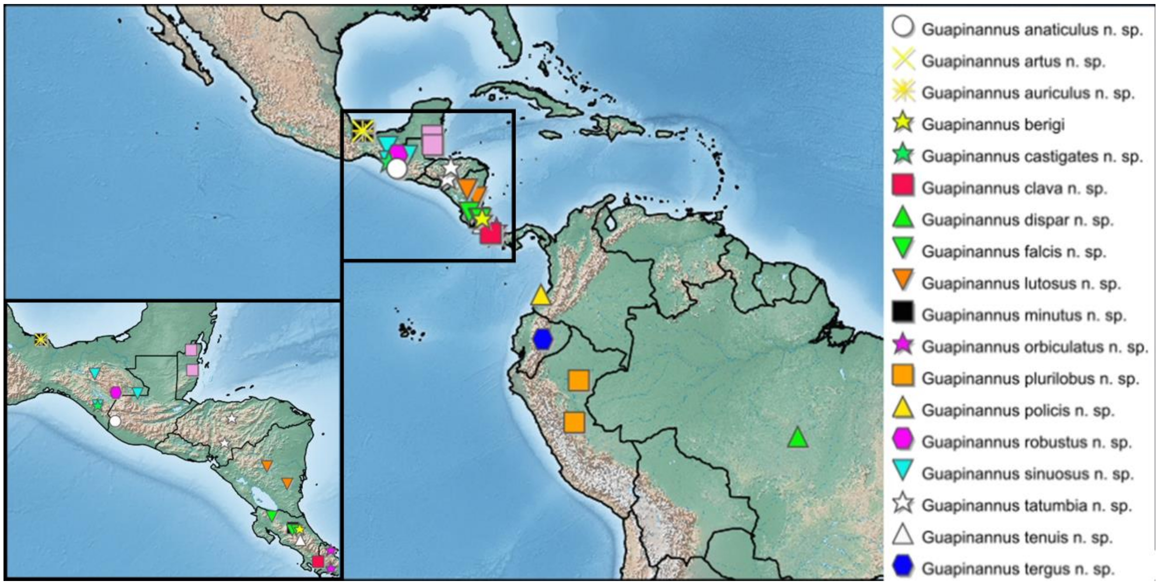


Figure 3-9. Known distributions of *Guapinannus* spp.

ED / USI	Sp. name	Total body length (mm)	wing length (mm)	Gratest width acorss pronotum	Greatest width across wings
UCR_ENT 00127123	*Guapinannus anaticulus*	1.45	1.23	0.63	0.81
UCR_ENT 00125959	*Guapinannus artus*	1.29	1.04	0.54	0.75
UCR_ENT 00125965	*Guapinannus auriculus*	1.23	0.9	0.55	0.75
AMNH_IZC 00150354	*Guapinannus berigi*	1.45 (wygodzinsky)	NA	0.67	NA
UCR_ENT 00101251	*Guapinannus castigatus*	1.48	1.27	0.66	0.88
UCR_ENT 00097823	*Guapinannus clava*	1.54	1.16	0.66	0.87
UCR_ENT 00090847	*Guapinannus dispar*	0.66	0.51	0.28	0.42
UCR_ENT 00014616	*Guapinannus falcis*	1.56	1.27	0.65	0.87
UCR_ENT 00102045	*Guapinannus lutosus*	1.16	0.89	0.56	0.69
UCR_ENT 0014609	*Guapinannus minutus*	1.14	0.9	0.52	0.71
UCR_ENT 00090718	*Guapinannus orbiculatus*	1.26	0.9	0.53	0.68
UCR_ENT 00089590	*Guapinannus plurilobus*	1.14	0.8	0.44	0.67
UCR_ENT 00109003	*Guapinannus policis*	1.03	0.7	0.47	0.63
UCR_ENT 00094282	*Guapinannus robustus*	1.49	1.1	0.28	NA
UCR_ENT 00102050	*Guapinannus sinuosus*	1.49	0.9	0.54	NA
UCR_ENT 00078556	*Guapinannus tatumbia*	1.51	1.27	0.62	0.88
UCR_ENT 00014617	*Guapinannus tenuis*	2.06	1.49	0.69	NA (missing wing)
UCR_ENT 00090642	*Guapinannus tergus*	0.67	0.52	0.28	0.42
UCR_ENT 00128210	*Guapinannus trilobus*	1.4	1.03	0.61	0.85
UCR_ENT 00090858	*Guapinannus uncus*	1.19	0.97	0.27	0.35

Table 3-1. Total body and wing lengths

Conclusion

The variation in head length of *Nannocoris* appears to be rare in insects and given that the length is not sexually dimorphic we can speculate that the evolution of this character may be driven by natural selection and possibly tied to prey capture or a mechanism for feeding. We see striking variation in the length of the genitalia of both males and females making it likely that this character is driven by sexual or natural selection and an excellent model for studying coevolution of male and female genitalic structures.

The taxonomic revision of the monotypic genus *Guapinannus* reveals the first males described for this genus and adds 19 new species, resulting in a total of 20 species for this genus. This is an even more dramatic increase in species numbers than seen in recent revisions of other schizopterid genera, such as *Peloridinannus* Wygodzinsky or *Chinannus* Wygodzinsky. Until now part of the "insect soup" of uncurated bulk samples in museums, this study shows that specimens and therefore biodiversity are indeed hidden away in museum collections, but deserve to be brought to light.