

# Phylogeny and Systematics of Wood-Degrading Neotropical Bess Beetles (Coleoptera: Passalidae: Passalinae)

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**Abstract.** Passalidae (Coleoptera: Scarabaeoidea) are saproxylophagous beetles that live and feed inside decaying wood. Passalid diversity and biogeography are relatively well-established at the tribal-level, particularly in the New World. However, a robust phylogenetic framework to test the current taxonomic status of the generic groups in the family is lacking. In the New World, Passalidae is represented by the tribes Passalini and Proculini. To test the monophyly of Proculini and Passalini and reconstruct relationships among the New World genera, we used DNA sequences from three genes along with data from 57 morphological characters. Our taxon sampling included 25 of the 26 genera of New World Passalidae across their entire geographic range (The United States of America to Argentina). We recovered Proculini and Passalini as reciprocally monophyletic sister groups. The genus *Passalus* was rendered polyphyletic by the other four genera of Passalini included in our analyses. The genera of Proculini were mostly monophyletic, except for *Odontotaenius*, *Petrejoides*, *Popilius*, *Pseudacanthus*, and *Vindex*. Based on our phylogenetic results and analyses of morphological data, we suggest transferring three species from *Petrejoides* to *Chondrocephalus* (*Chondrocephalus guatemalae* [Reyes-Castillo & Schuster, 1983] **comb.n.**, *Chondrocephalus pokomchii* [Schuster, 1981] **comb.n.**, and *Chondrocephalus reyesi* [Schuster, 1988] **comb.n.**), and one species, *Pseudoarrox caldasi* Reyes-Castillo & Pardo-Locarno, 1995 **comb.n.**, back from *Petrejoides* to *Pseudoarrox*. The genus *Passalus* and the non-monophyletic genera of Proculini require extensive systematic revision.

**Key words.** Proculini, Passalini, Passalidae, New World.

## 1. Introduction

Species of the family Passalidae Leach (Scarabaeoidea) (commonly referred to as “bess beetles”) are relatively large black beetles (13 to 80 mm in length) with striae present on their elytra. There are approximately 930 extant species of Passalidae worldwide, distributed mainly in the Pantropical region (BOUCHER 2006), with a few species present in the Nearctic (REYES-CASTILLO 1970; SCHUSTER 1994). Bess beetles exhibit sub-social behaviors and complex acoustic communications (SCHUSTER 1983). Most members of the family form pairs in chambers that they build inside rotting logs (REYES-CASTILLO 1970; SCHUSTER 1983; REYES-CASTILLO & HALFFTER 1983).

Both larval and adult Passalidae are adapted to a saproxylophagous diet, functioning as wood decomposers in tropical and subtropical forests (CASTILLO & REYES-CASTILLO 2008). Passalidae have been of much interest to biogeographers of the New World tropics (e.g., HALFFTER 1978; MACVEAN & SCHUSTER 1981; SCHUSTER & CANO 2006; GUTIÉRREZ-VELÁZQUEZ et al. 2013). Furthermore, species of Passalidae had been used as ecological indicators for forest conservation (SCHUSTER et al. 2000; CANO & SCHUSTER 2009; KATTAN et al. 2010).

Despite this interest, phylogenetic relationships among the tribes, genera, and species of Passalidae are poorly-

known. Passalidae is a well-established monophyletic group supported by twelve adult and six larval morphological characters (REYES-CASTILLO 1970; FONSECA 1987; NEL & SCHOLTZ 1990; SCHOLTZ 1990; CARLSON 1991; GILLOGLY 2005). The family has been recovered sister to Geotrupidae in recent large-scale molecular phylogenies (e.g., MCKENNA et al. 2015, 2019; ZHANG et al. 2018). Passalidae contains two subfamilies (Aulacocyclinae Kaup, and Passalinae Leach). Aulacocyclinae occur in Asia, Australia, and New Caledonia and the subfamily is comprised of three tribes (Aulacocyclini, Ceracupini, Ceracyclini) (BOUCHER 2006; BOUCHER et al. 2017). Passalinae has a Pantropical distribution and contains five tribes (Passalini, Proculini, Solenocyclini, Leptaulacini, Macrolinini) (BOUCHER 2006). Solenocyclini, Leptaulacini, and Macrolinini have Paleotropical distributions. Proculini and Passalini are endemic to the New World and are the only tribes of Passalidae present in this region – they together comprise about 50% of passalid diversity.

The tribe Passalini includes six genera (JIMÉNEZ-FERBANS & REYES-CASTILLO 2014). Of the six genera of Passalini, the monophylies of *Paxillus* MacLeay, 1819 (17 species), *Passipassalus* Fonseca & Reyes-Castillo, 1993 (5 species), and *Ameripassalus* Jiménez-Ferbans & Reyes-Castillo, 2014 (5 species) are well supported (JIMÉNEZ-FERBANS & REYES-CASTILLO 2014, 2015). *Ptichopus* Kaup, 1869, according to BOUCHER (2006), is a monotypic genus; however, robust phylogenetic studies (e.g., JIMÉNEZ-FERBANS, 2014) suggest the genus comprise at least three species. *Passalus* Fabricius, 1792 (approximately 180 species) and *Spasalus* (approximately 8 species) Kaup, 1869 have been recovered as non-monophyletic (GILLOGLY 2005; BOUCHER 2006; JIMÉNEZ-FERBANS 2014; BEVILAQUA & FONSECA 2020).

Proculini includes 20 genera (SCHUSTER & CANO 2008; BEZA-BEZA et al. 2019). Although the number of genera of Proculini has remained stable since the tribe was established by REYES-CASTILLO (1970), the species composition of some genera (e.g., *Odontotaenius* Kuwert, 1869, *Pseudacanthus* Kaup, 1869, *Petrejoides* Kuwert, 1896, and *Popilius*, 1871 Kaup) remains the subject of debate (e.g., BOUCHER 2006; SCHUSTER & CANO 2008). Additionally, three genera (*Petrejoides*, *Popilius*, and *Odontotaenius*) have been recovered as non-monophyletic (GILLOGLY 2005; BEZA-BEZA et al. 2017). In contrast, the monophylies of *Proculus* Kaup, 1868, *Veturius* Kaup, 1871, *Verres* Kaup, 1871, *Ogyges* Kaup, 1871, and *Yumtaax* Boucher, 2006 are very well supported by morphological and/or molecular studies (SCHUSTER et al. 2003; MARSHALL 2000; BOUCHER 2006; BEZA-BEZA et al. 2017; CANO et al. 2018). While Proculini is one of the best-known insect taxa (both taxonomically and geographically) in Mesoamerica (BOUCHER 2006), a phylogenetic framework (beyond genus level) – which would facilitate the testing of taxonomic hypotheses – is still needed.

Several efforts have been made to resolve the phylogenetic relationships of the major groups in Passalidae (e.g., GILLOGLY 2005; BOUCHER 2006; FONSECA et al. 2011, JIMÉNEZ-FERBANS 2014); however, only two of these studies (BOUCHER 2006, FONSECA et al. 2011) are published in peer-reviewed journals. BOUCHER (2006) published an extensive morphological diagnostic study of the family, including preliminary phylogenies for the tribes, and for the genera of Proculini, and a species-level phylogeny of the genus *Veturius* (Proculini). However, the phylogenetic analyses presented in Boucher (2006) are limited. For instance, some character states are not very well defined, and the tips in the phylogenies do not necessarily represent monophyletic groups (e.g., Passalini, and *Petrejoides* in the Proculini phylogeny); or are not consistent between phylogenies (e.g., Passalini is monophyletic in the tribal-level phylogeny, but paraphyletic in the genus-level phylogeny of Proculini). The phylogeny proposed by FONSECA et al. (2011) had no resolution of generic-level relationships, and their character set was limited to the hindgut.

Under the current phylogenetic concept, Passalini is rendered paraphyletic by Proculini (GILLOGLY 2005; BOUCHER 2006; JIMÉNEZ-FERBANS 2014). Proculini appears nested in the genus *Passalus*, closely related to the subgenus *Pertinax*. Proculini is strongly supported as monophyletic (REYES-CASTILLO 1970; VIRKKI & REYES-CASTILLO 1972; SCHUSTER & REYES-CASTILLO 1981; SCHUSTER 1992; SERRANO et al. 1998; BOUCHER 2006; FONSECA et al. 2011). Nonetheless, the results of previous phylogenetic studies of Passalidae are limited by taxon sampling (e.g., GILLOGLY 2005; BOUCHER 2006), and the delimitation of supra-specific terminals (e.g., BOUCHER 2006); thus, leaving the monophyly of Passalini, and its phylogenetic relationship with Proculini uncertain.

Lower level phylogenetic studies are now standard in Passalidae to establish new taxa in the family (e.g., SCHUSTER et al. 2003; JIMÉNEZ-FERBANS & REYES-CASTILLO 2014; JIMÉNEZ-FERBANS et al. 2016; BEZA-BEZA et al. 2017, CANO et al. 2018, BEZA-BEZA et al. 2019). However, a more comprehensive phylogeny is needed to resolve higher-level relationships in the group. We used nuclear DNA sequences and morphological data to reconstruct the phylogenetic relationships of the genera of Proculini and Passalini. Our phylogeny includes representatives of six of the eight tribes of Passalidae, with an emphasis placed on sampling in Passalini and Proculini. We sampled 20 species of Passalini, including representatives of five of its six genera, and 69 species of Proculini, including representatives from all 20 genera. The aim of this study is to: (1) test the monophyly of the New World Tribes (Passalini and Proculini), (2) test the monophyly of several New World genera, and (3) reconstruct a robust phylogenetic framework for the genera of New World Passalidae.

## 2. Material and methods

### 2.1. Taxon sampling

#### 2.1.1. Specimen determination

Identification for this work was done by comparison with museum material, experts in the group (Edwin Ariza-Marín, Cristian Beza-Beza, Enio Cano, Alan Gillingly, Larry Jiménez-Ferbans, Pedro Reyes-Castillo and Jack Schuster), and the following keys: REYES-CASTILLO (1978, 2010), SCHUSTER & CANO (2005), PARDO-LORCANO (2012), JIMÉNEZ-FERBANS et al. (2014), REYES-CASTILLO & JIMÉNEZ-FERBANS (2016), JIMÉNEZ-FERBANS et al. (2018).

#### 2.1.2. Outgroups

Given the close phylogenetic relationship of Passalidae to Geotrupidae suggested by large scale phylogenetic studies of Coleoptera (McKENNA et al. 2015, 2019; ZHANG et al. 2018), we selected a species of Geotrupidae (*Geotrupes* sp.) to root our phylogenetic analyses. From Passalidae we included representatives from six of the eight tribes of Passalidae. As outgroups within the family Passalidae we included three species of Aulacocyclinae from the genus *Aulacocylus* Kaup, 1868; in Passalinae we included two species of *Pharochilus* Kaup, 1868 as representatives of Macrolinini, *Leptaulax dentatus* (Fabricius, 1792) as a representative of Leptaulacini, and *Erionomus planiceps* (Eschscholtz, 1829) as a representative of Solenocyclini.

#### 2.1.3. Ingroup

We included representatives of 25 of the 26 genera of New World Passalidae (See Table S1). For Passalini, *Ameripassalus*, *Paxillus*, *Passipassalus*, and *Ptichopus* were each represented by one species. We included 20 species of *Passalus*, including representatives for the three subgenera of the genus, *P. (Passalus)*, *P. (Pertinax)*, and *P. (Mitrorhinus)*. Additionally, we included representatives of the three sections (“Neleus”, “Phoroneus”, and “Petrejus”) recognized for *P. (P.)* species (LUEDERWALT 1931; HICKS & DIBB 1935). We were unable to amplify molecular data from any representative of *Spasalus*; thus, we had to exclude it from our analysis.

In total, we included 69 species of Proculini, including representatives of all extant genera (Supp. Table S1). For the genera *Arrox* Zang, 1905, *Pseudoarrox* Reyes-Castillo, 1970, *Spurius* Kaup, 1871, *Tonantzin* Beza-Beza, Jiménez-Ferbans & Clarke, 2019, and *Xylopas-saloides* Reyes-Castillo, Fonseca & Castillo, 1988, we included one species. Of these, *Tonantzin* is a monotypic genus. Additionally, we included one specimen (voucher DDM3251 [Supp. Table S1]) with an unclear generic position within Proculini. The voucher specimen DDM3251 is an undescribed species that has morphological features known from *Vindex* Kaup, 1871 and *Xylopas-saloides*. In

general, the specimen DDM3251 presents the diagnostic characters for *Vindex sensu* Reyes-Castillo (1970) (relatively small size, mesofrontal structure of the “marginatus” type, and enlarged inner tubercles projecting forward beyond the anterior border of the clypeus). However, the frontoclypeal suture (well defined, wide, with irregular punctures, and opaque) fits the description of the character in *Xylopas-saloides*. BOUCHER (2006) recovered both genera as sister groups and morphologically the genera are similar. Thus, we decided to place DDM3251 as an *incertae sedis* species of Proculini.

### 2.2. DNA extraction, PCR amplification, and alignment protocols

We extracted genomic DNA with the Omniprep kit (G Biosciences, St. Louis, MO), following the manufacturer’s protocol with slight modifications. We used leg and thoracic tissue when necessary; legs were manually cut into smaller pieces with a razor blade. We macerated the tissue with a sterile plastic pestle and added 500 µl of genomic lysis buffer, and 5 µl of Proteinase K. Following this treatment, we incubated the samples at 60°C for 14 to 16 hours.

We amplified partial segments of CAD which encodes several enzymes involved in pyrimidine biosynthesis (WILD & MADDISON 2008), and Wingless (WGS), both nuclear protein-coding genes (NPC); and the 28S small ribosomal subunit. We performed all amplifications in a Thermo Hybaid PxE 0.2 thermal cycler, using a PCR core Kit (Qiagen, Hilden, Germany). The primers used for amplification are listed in Table 1. For CAD and 28S, we followed the PCR protocols described by BEZA-BEZA et al. (2019). We amplified WGS using the 550F/AbRZ (Table 1) primer set. The PCR reaction had a final volume of 20 µl with the following final concentrations 1 × CoralLoad PCR buffer, 0.2 µM dNTP mix, 2.5 µM MgCl<sub>2</sub>, 0.75 µM of each primer, and 1.0 µL of DNA (concentration was variable for each sample). We amplified these genes under the following conditions: (1) 94°C for 2 min, (2) 94°C for 30 s, (3) 58°C for 1 min, (4) 72°C for 1 min, steps 2–4 were repeated for 36 cycles, followed by (5) 72°C for 5 min.

All PCR products were run in 1.5x agarose gel, extracted, and purified using the QIAquick kit (Qiagen, Hilden, Germany) as described by BEZA-BEZA et al. (2019). We sent the final eluted products to Eurofin Genomics LLC (Louisville, KY, USA) for standard Sanger sequencing. We assembled the targeted amplicon sequences using Geneious 11.1.5 (<https://www.geneious.com>) from the resulting \*.ab1 files. Individual fasta files were produced for each gene and imported into MEGA X (KUMAR & STECHER et al. 2018) for CAD and WGS, or the MAFFT version 7 web server (KATO et al. 2017) for 28S.

Both NPC genes were aligned in MEGA X (KUMAR & STECHER et al. 2018) first using the ClustalW algorithm, followed by a secondary alignment using the Muscle algorithm (EDGAR 2004) (in MEGA X) as described in

**Table 1.** Primer sequences used for PCR amplification.

Gene	Primer Name	Sequence	Source
28S	rD1.2a	5' -CCC SSG TAA TTT AAG CAT ATT A-3'	WHITING, 2001
28S	rD4.2b	5' -CCT TGG TCC GTG TTT CAA GAC GG-3'	WHITING, 2001
28S	Squirtle	5' -GTG CAC TTC TCC CCC WGT AG-3'	MOORE et al., 2015
28S	rD5b	5' -CCA CAG CGC CAG TTC TGC TTA C-3'	WHITING, 2001
WGS	550F	5' -ATG CGT CAG GAR TGY AAR TGY CAY GGY ATG TC-3'	WILD & MADDISON, 2008
WGS	578F	5' -TGC ACN GTG AAR ACY TGC TGG ATG-3'	WARD & DOWNIE, 2005
WGS	AbRZ	5' -CAC TTN ACY TCR CAR CAC CAR TG-3'	WILD & MADDISON, 2008
WGS	AbR	5' -ACY TCG CAG CAC CAR TGG AA-3'	ABOUHEIF & WRAY, 2002
CAD	439F	5' -TTC AGT GTA CAR TTY CAY CCH GAR CAY AC-3'	WILD & MADDISON, 2008
CAD	668R	5' -ACG ACT TCA TAY TCN ACY TCY TTC CA-3'	WILD & MADDISON, 2008
CAD	688R	5' TGT ATA CCT AGA GGA TCD ACR TTY TCC ATR TTR CA-3'	WILD & MADDISON, 2008

**Table 2.** Alignment statistics for each gene studied.

Alignment	# specimens	# sites	# constant sites	# variable sites	# parsimony-informative sites
CAD	133	717	306	411	364
WGS	136	438	185	253	209
28S	132	1756	1125	631	450
28S_masked	132	1301	873	428	313

BEZA-BEZA et al. (2017) and BEZA-BEZA et al. (2019). The results obtained from MEGA X were saved in fasta format and imported to Mesquite 3.04 (MADDISON & MADDISON 2018). We used Mesquite to assign codon positions by choosing the reading frame that minimized the number of stop codons in the alignment. We then translated the nucleotides to amino acid sequences to help refine the nucleotide alignment. Both NPC genes had an observed proportion of constant sites of 0.42. The summary of the number of taxa, and character information for the individual gene alignments are in Table 2.

For the 28S data, we used two alignments. First, we aligned the complete 28S data in MAFFT version 7 (KATOHI et al. 2017) using the Q-INS-I iterative refinement method to account for ribosomal RNA secondary structures, as described by BEZA-BEZA et al. (2019). Additionally, using the 28S alignment, we created a secondary alignment masking the poorly aligned positions in Gblocks v 0.91b (CASTRESANA 2000). The proportion of observed constant characters for the 28S alignment was 0.87, whereas for the 28S-masked alignment the proportion was 0.67.

### 2.3. Morphological data

We selected 57 adult morphological characters to include in the phylogenetic reconstruction. We used the terminology of BOUCHER (2006) for the cephalic capsule and REYES-CASTILLO (1970) for the rest of the body. These characters were scored for each voucher specimen. Non-applicable characters were scored as N/A, ambiguous characters were coded as “?” (Electronic Supplement File 2).

- Habitus, shape: **(0)** subcylindrical; **(1)** compress dorso-ventrally.
- Frontoclypeus, anterodorsal exposure: **(0)** present; **(1)** absent.
- Clypeus, dorsal exposure: **(0)** weak; **(1)** not exposed (Figs. 1 A–F); **(2)** fully exposed (Fig. 1 G).
- Infra-anterior angles of clypeus: **(0)** indistinct; **(1)** developed, size < mediofrontal tubercles **(2)** developed, size = to mediofrontal tubercles; **(3)** strongly developed, size > mediofrontal tubercles.
- Infra-anterior angles of clypeus, position: **(0)** under the frons (or mediofrontal tubercles) not visible dorsally; **(1)** under the frons visible dorsally.
- Frontoclypeal suture: **(0)** absent (Fig. 1 A); **(1)** present (Figs. 1 B, C).
- Secondary mediofrontal tubercles, number: **(0)** 0 (Figs. 1 D, F); **(1)** 1; **(2)** 2 (Fig. 1 E).
- Internal tubercles: **(0)** absent (Fig. 1 G); **(1)** present (Figs. 1 A–F).
- Secondary internal tubercles: **(0)** absent; **(1)** present.
- Epicranial fossae: **(0)** absent (Fig. 1 G); **(1)** present (Figs. 1 A–F).
- Apex of central tubercle: **(0)** not free (Fig. 1 D); **(1)** free (without reaching the frons border) (Fig. 1 E); **(2)** very free (reaching the frons border).
- Frontal ridges: **(0)** absent (Fig. 1 G); **(1)** present (Figs. 1 A–F).
- Frontal ridge, shape: **(0)** Y-shaped (Fig. 1 B, C); **(1)** V-shaped (Figs. 1 D–F).
- Transversal ridge linking frontal ridges: **(0)** absent (Figs. 1 A, B); **(1)** present (Fig. 1 C).
- Lateroposterior tubercles: **(0)** absent (Fig. 1 G); **(1)** present (Figs. 1 A–F).



**Fig. 1.** Head and anterior part of pronotum of: **A:** *Veturius tuberculifrons*. **B:** *Vindex gonzaloi*. **C:** *Popilius gibbosus*. **D:** *Passalus convexus*. **E:** *Passalus interruptus*. **F:** *Ptichopus angulatus*. **G:** *Aulacocyclus edentulus*. Scale bars: 2 mm. — **Labels:** aa – anterior angles of the fronto-clypeus; cc – exposed clypeus; ct – central tubercle; ef – epicranial fossae; fc – fronto-clypeus; fcs – fronto-clypeal suture; ff – frontal fossae; fr – frontal ridge; it – internal tubercles; lt – latero-anterior tubercles; oc – ocular canthus; pt – postero-lateral tubercles; st – secondary frontal tubercles.



**Fig. 2.** A–B. Head and anterior part of pronotum. A: *Aulacocyclus edentulus*. B: *Passalus interruptus*. C–D: Habitus, ventral view. C: *Passalus interruptus*. D: *Aulacocyclus edentulus*. E–F: Lacinia, ventral view. H–I: Mentum. J–L: Prosternellum. Scale bars: 2 mm. — **Labels:** af – anteromedial fossae; hp – hypostomal process; lk – ligula keel.

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| <p>16. Lateroposterior tubercles, distinctness: <b>(0)</b> weak (Fig. 1 D); <b>(1)</b> marked (Fig. 1 E); <b>(2)</b> very marked (Figs. 1 A, B).</p> <p>17. Lateroposterior tubercles, ridge linking to central tubercle: <b>(0)</b> absent (Fig. 1 E); <b>(1)</b> present (Figs. 1 A, B).</p> <p>18. Frontal fossae, pubescence: <b>(0)</b> absent (Figs. 1 A, B); <b>(1)</b> present (Fig. 1 C).</p> <p>19. Ocular ridges: <b>(0)</b>: unituberculate; <b>(1)</b> bituberculate.</p> <p>20. Ocular canthus, pubescence: <b>(0)</b> absent; <b>(1)</b> present.</p> <p>21. Eyes, reduction: <b>(0)</b> ocular canthus not reaching the middle of the eye (Figs. 1 C–E); <b>(1)</b> ocular canthus reaching the middle of the eyes (Figs. 1 B, G); <b>(2)</b> ocular canthus extending beyond the middle of the eyes (Fig. 1 F).</p> | <p>22. Mandibular apical teeth: <b>(0)</b> two, = size; <b>(1)</b> two, ≠ size (Fig. 1 F); <b>(2)</b> three, = size (Fig. 1 D); <b>(3)</b> three, ≠ size.</p> <p>23. Lacinia apical teeth: <b>(0)</b> unidentate (Fig. 2 E); <b>(1)</b> bidentate, basal thickened (Fig. 2 G); <b>(2)</b> bidentate, basal not thickened (Fig. 2 F).</p> <p>24. Hypostomal process: <b>(0)</b> apex reaching anterior border of mentum (Fig. 2 B); <b>(1)</b> apex not reaching anterior border of mentum (Fig. 2 A).</p> <p>25. Hypostomal process, pubescence: <b>(0)</b> absent; <b>(1)</b> present.</p> <p>26. Hypostomal process, apical groove: <b>(0)</b> present; <b>(1)</b> absent.</p> <p>27. Mentum, median basal region: <b>(0)</b> protruding (Fig. 2 I); <b>(1)</b> flat (Fig. 2 H).</p> |
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28. Mentum, median basal region, anteromedial fossae, number: **(0)** 0 (Fig. 2 A); **(1)** 1; **(2)** 2 (Fig. 2 B).
29. Mentum, basal depression: **(0)** present; **(1)** absent.
30. Mentum, basal depression: **(0)** open in shape of “C”; **(1)** closed in shape of “O”; **(2)** open in shape of “U”.
31. Mentum, basal depression, pubescence: **(0)** absent (Fig. 2 B); **(1)** present (Fig. 2 A).
32. Ligula, keel: **(0)** present; **(1)** absent.
33. Ligula keel, number: **(0)** 1 (Fig. 2 A); **(1)** 2; **(2)** 3 (Fig. 2 B).
34. Antennae, club, number of lamellae: **(0)** 3; **(1)** 4; **(2)** 5; **(3)** 6.
35. Longitudinal prothoracic sulcus: **(0)** erased; **(1)** almost erased; **(2)** marked.
36. Pronotum, marginal sulcus on anterior border: **(0)** present; **(1)** absent.
37. Pronotum, anteromarginal sulcus with respect to anterior border: **(0)** complete (covering the entire border) (Fig. 1 G); **(1)** incomplete (Figs. 1 B–F).
38. Prosternellum, shape: **(0)** rhomboidal acute (Fig. 2 J); **(1)** rhomboidal truncate (Fig. 2 K); **(2)** pentagonal (Fig. 2 L).
39. Prosternellum, position regarding procoxae **(0)** same level (not covered by procoxae) (Fig. 2 C); **(1)** not at same level (covered by procoxae) (Fig. 2 D).
40. Prosternellum, apical pubescence: **(0)** present; **(1)** absent.
41. Mesosternal scar: **(0)** absent (Fig. 2 D); **(1)** present, poorly defined; **(2)** present, clearly defined (Fig. 2 C).
42. Mesepisternum, posterior tip pubescence: **(0)** absent; **(1)** present.
43. Mesepimerum, pubescence: **(0)** absent; **(1)** present.
44. Metasternal disc, delimitation by punctation: **(0)** not bounded (Fig. 2 D); **(1)** bounded in the posterior part, not reaching the middle part; **(2)** bounded in the posterior part and reaching the middle part (Fig. 2 C); **(3)** bounded as in 2 and spanning almost the whole disc.
45. Metasternum, lateroanterior pubescence: **(0)** absent; **(1)** sparse; **(2)** dense (Figs. 2 C, D).
46. Mestasternal fossae, pubescence: **(0)** absent (Fig. 2 D); **(1)** sparse; **(2)** dense (Fig. 2 C).
47. Mestasternal fossae, width relative to that of mesotibia: **(0)** greater (Fig. 2 C); **(1)** smaller (Fig. 2 D); **(2)** the same.
48. Elytral humeri, pubescence: **(0)** absent (Fig. 2 D); **(1)** present, sparse at the base; **(2)** fully pubescent (Fig. 2 C).
49. Elytral striae, pubescence: **(0)** absent; **(1)** present.
50. Elytral epipleurae, pubescence: **(0)** absent (Fig. 2 D); **(1)** present, sparse at the base; **(2)** densely pubescent until basal third (Fig. 2 C).
51. Elytra, anterior vertical face, pubescence: **(0)** absent; **(1)** present.
52. Trochanter, anterior longitudinal sulcus: **(0)** absent; **(1)** present.
53. Marginal groove over anterior ventral edge of the profemora, extension: **(0)** spans nearly the entire edge (reaching the apical pubescence); **(1)** spans halfway from base (not reaching the apical pubescence); **(2)** absent.
54. Marginal groove over anterior ventral edge of profemora, distinctness: **(0)** slight; **(1)** marked.
55. Protibiae, exterior border: **(0)** expanded (Fig. 1 F); **(1)** not expanded (Fig. 2 C).
56. Protibiae, shape: **(0)** with four similar faces; **(1)** with the external face compressed.
57. Meso- and metatibiae, external spines **(0)** absent; **(1)** present, weak; **(2)** present, strong.

## 2.4. Phylogenetic analysis

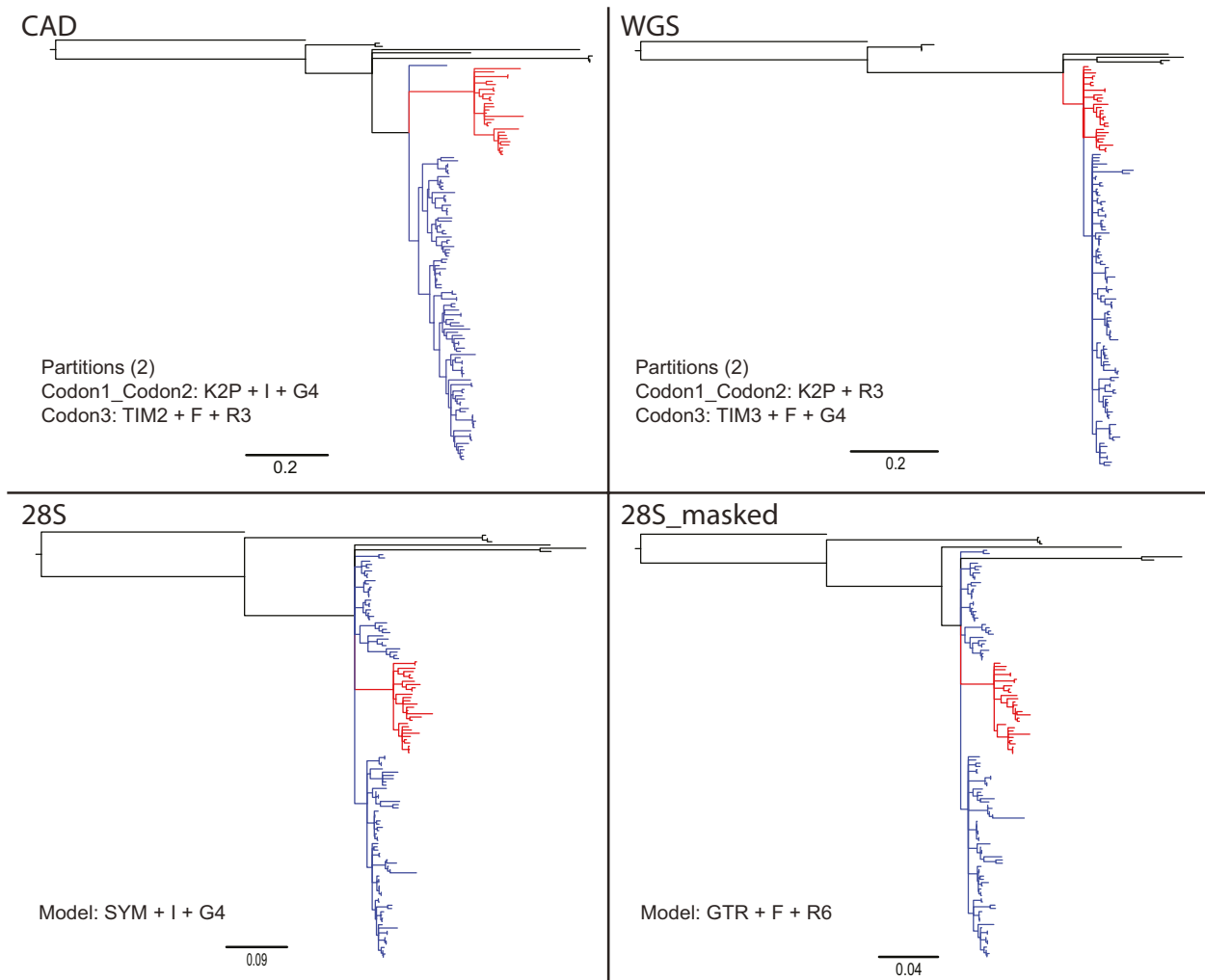
### 2.4.1. Tree reconstruction

We analyzed seven datasets (CAD, WGS, 28S, concatenated, reduced concatenated, total evidence, and reduced total evidence). The concatenated alignment comprised CAD, WGS, and 28S alignment; the reduced concatenated alignment consisted of the CAD, WGS, and 28S-masked alignments. The total evidence and reduced total evidence alignment included the concatenated alignments plus the morphological data set, respectively. All data sets were analyzed using maximum likelihood optimality criteria (ML) in IQTree version 1.6.7.2 (NGUYEN et al. 2015), and 1000 bootstrap pseudoreplicates were used to assess nodal support. We used the IQTree partition and model finder (CHERNOMOR et al. 2016; KALYAANAMOORTHY et al. 2017); all analyses were implemented using the best partition scheme chosen by IQTree (Electronic Supplement File 3).

Additionally, we conducted Bayesian analyses and parsimony analyses for all combined alignments. We used the best-fit partitioning scheme and model suggested by PartitionFinder 1.0 (LANFEAR et al. 2012) for the Bayesian analyses (Electronic Supplement File 3). We implemented all Bayesian analyses using the program Mr. Bayes 3.2.6 (RONQUIST & HUELSENBECK 2003), including four independent runs for 50,000,000 generations. We used Tracer v1.6.0 (RAMBAUT et al. 2013) to assess convergence of stationary distribution of our mcmc chains and discarded the first 20% of the trees in each run as burn-in based on the Tracer plots. We performed all parsimony analyses in PAUP version 4.0a (SWOFFORD 2003). We conducted a complete heuristic search of the most parsimonious trees with 100 random additions, and we conducted a parsimony bootstrap analysis with 1000 pseudoreplicates to assess nodal support.

### 2.4.2. Alternative topology test (Four-cluster Likelihood Mapping)

Based on the recovered conflicting topologies and varied statistical support for the Proculini node, (see sections 3 and 4), we used Four-cluster Likelihood Mapping (FcLM) (STRIMMER & HAESELER 1997) to test alternative hypotheses for the resolution of this node. We conduct-



**Fig. 3.** Majority-rules consensus trees from the ML analyses implemented using each single gene alignment. Black branches represent out-group taxa, branches in red indicate taxa currently classified as Passalini, and branches in blue indicate taxa currently classified as Proculini.

ed Model Finder (KALYAANAMOORTHY et al. 2017), and FcLM analyses of the Concatenated and Reduced Concatenated alignments in IQTree version 1.6.7.2 (NGUYEN et al. 2015). For the four-cluster selection, we considered Old-World Passalinae, Passalini, *Proculus*, and Clade V (See sections 3.2 and 4) as the four clusters, excluding the species of *Aulacocyclus* and *Geotrupes*.

### 3. Results

#### 3.1. Gene coverage and phylogenetic performance

Our data set was comprised of DNA sequences from 140 specimens (~ 100 spp.) of Passalidae (Supp. Table S1), 86% of the specimens had sequences for all three genes, and the remaining 14% had sequences for two of the three genes. Overall, the data set contained data for 97% of all possible taxon by gene combinations. Individual gene coverage was 95% for CAD (133 specimens), 97%

for WGS (136 specimens), and 94% for 28S (132 specimens). The CAD alignment constituted a total of 717 sites, with an average content of 706.5 nucleotides, and T, C, A, G content of 24.2%, 19.9%, 29.6%, 26.4%, respectively. The WGS alignment constituted a total of 438 sites, with an average content of 426.5 nucleotides, and T, C, A, G content of 15.3%, 29.6%, 26.4%, 28.6%, respectively. The 28S alignment constituted a total of 1756 sites, with an average content of 1401.2 nucleotides, and T, C, A, G content of 20.3%, 26.6%, 21.8%, 31.2%, respectively. The 28S masked alignment constituted a total of 1301 sites, with an average content of 1285.7 nucleotides, and T, C, A, G content of 19.6%, 26.9%, 21.7%, 31.8%, respectively.

Maximum likelihood reconstructions for each gene recovered short internal branches within the ingroup (Fig. 3). Based on the recovered tree topology and statistical nodal support, of the amplified genes, CAD was better at recovering lower (between genera) and mid-level (tribal) relationships, than deeper relationships in the tree (Fig. S1). Deep nodes in the ML tree had lower bootstrap support (Fig. 3, Fig. S1) than shallower nodes. These results are consistent with the CAD results of WILD &



MADDISON (2008). In contrast, WGS recovered well supported clades that were equivalent to genera, or closely related genera; but failed to resolve the phylogenetic relationships between such clades (tribal relationships) (Fig. 3, Fig. S2). Both 28S alignments recovered similar topologies and were effective in resolving deep-level relationships (subfamily-level) or very shallow relationships (generic-, or species-level) with strong to moderate statistical support. Strongly supported nodes (MLBS > 85%) usually remained strongly supported in both 28S alignments (Figs. S3, S4); for the nodes with moderate support (50% < MLBS < 85%) there was no clear pattern of increase or decrease of support between the 28S and the 28S-masked alignments. The topologies of the resulting trees from the ML analysis of the individual genes were not identical. However, when topologies disagreed, neither tree showed strong statistical measures of nodal support where relationships differed, suggesting lingering uncertainty in the affected relationships.

When data were combined in the concatenated alignment (Fig. S5) and the reduced concatenated alignment (Fig. S6), the resulting resolution and statistical measures of nodal support both increased. When the concatenated data sets were combined with the morphological data set (Fig. 4, Fig. S7), we observed a marginal increase in statistical measures of nodal support. The combined Bayesian and Parsimony analyses recovered similar topologies to the ML analyses (Figs. S8–S15). Parsimony scores and number of most parsimonious trees for each alignment can be found in Table S2. Strict consensus of the most parsimonious trees for each alignment can be found in Figs. S16–S19.

### 3.2. Tribal level relationships

We recovered a monophyletic *Aulacocyclus*, sister to a clade comprised of all representatives of Passalinae with high statistical support across all analyses (Fig. 4, Figs. S5–S15). Within the clade Passalinae (Fig. 4), all representatives of the New World were consistently recovered as a monophyletic group. This clade had maximal to strong statistical support across all analyses.

Although we consistently recovered the representatives of Aulacocyclus, Passalinae, and all representatives of the New World as monophyletic, and with strong nodal support, the interrelationships of the Old-World representatives of Passalinae were still largely unresolved. However, the interrelationships of the Old-World representatives of Passalinae were outside of the scope of this study. The ML Total Evidence and all Bayesian analyses recovered Passalinae in two clades (1) Old-World taxa and (2) New World taxa (Figs. S7–S11). However, the Old-World clade had poor nodal support across all analyses. The Old-World clade consistently had *L. dentatus* (Leptaulacini) sister to a clade of *E. planiceps* (Solenocyclini) + *Pharochilus* (Macrolinini) (Figs. S8–S11).

Alternatively, the other three ML and parsimony analyses did not recover the Old-World members of Pas-

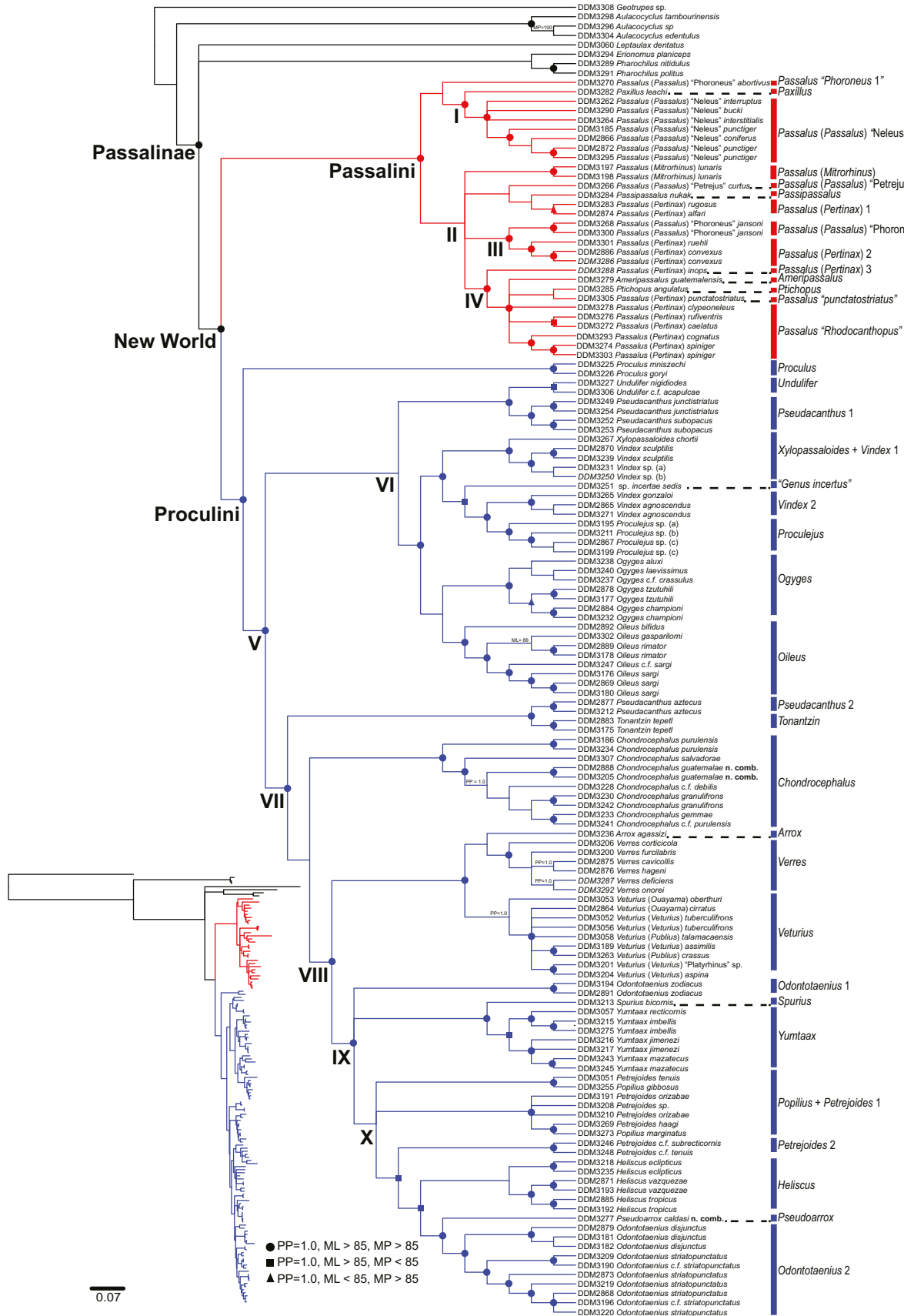
salinae as a monophyletic group. Instead, ML analysis of the Concatenated, Reduced Concatenated, and Reduced Total Evidence alignments, recovered a polytomy involving three taxa or clades: (1) *L. dentatus*, (2) a clade of *E. planiceps* sister to *Pharochilus*, and (3) a clade of the New World Passalidae (Fig. 4, Supp. Figs. S5, S6). In contrast, all MP analyses recovered a polytomy including four taxa or clades: (1) *L. dentatus*, (2) *E. planiceps*, (3) *Pharochilus*, and (4) a clade comprised of the New World Passalidae (Figs. S12–S15).

The New World clade contained representatives of two tribes: Passalini and Proculini. In most cases, both tribes were monophyletic with strong statistical support. All analyses recovered a monophyletic Passalini with strong or maximal nodal support. For Proculini, all Bayesian, all MP, and ML analyses of the Reduced Total Evidence alignment recovered the tribe as monophyletic with strong nodal support. However, ML analyses of the Total Evidence alignment recovered Proculini monophyletic but with poor statistical support (Supp. Fig. S7). The Reduced Concatenated alignment (Supp. Fig. S6) recovered a polytomy in the New World Clade comprised of (1) *Proculus* (member of Proculini), (2) Passalini, and (3) a clade of the remaining genera of Proculini. Furthermore, the ML analysis of the Concatenated alignment (Fig. S5) did not recover a monophyletic Proculini. Instead, the analysis recovered an early divergent *Proculus* (Proculini), sister to a clade comprised of Passalini + Clade V (Fig. 4, Fig. S5). Nevertheless, the sister relationship of Passalini with Clade V had poor statistical support (61% MLBS) (Fig. S5).

### 3.3. Circumscription and generic status within Passalini

Within Passalini, we only assessed the monophyly of the genus *Passalus*, which was rendered polyphyletic, or at least paraphyletic, with the remaining genera of Passalini mixed in the different clades of *Passalus* (Fig. 4). Of the three subgenera of *Passalus*, two were non-monophyletic: The subgenus *Passalus* was rendered polyphyletic, and the subgenus *Pertinax* was rendered at least paraphyletic. Regarding the species section within *P. (Passalus)*, the group “Neleus” was monophyletic, the group “Phoroneus” was polyphyletic, and the monophyly of section “Petrejus” was not tested.

In general, we recovered Passalini as a polytomy or a collection of poorly-supported clades. Within the recovered topologies, we consistently recovered three well supported clades in the tribe (Fig. 4). (1) Clade I which was recovered with maximal statistical nodal support across all combined analyses and is comprised of *Paxillus leachi* MacLeay, 1819 and all representatives of *P. (Passalus)* section “Neleus”. In all instances, *Paxillus leachi* was recovered as an early-divergent taxon and all the representatives of *P. (Passalus)* section “Neleus” formed a clade. The “Neleus” clade (Fig. 4) included *Passalus (Passalus) interruptus* (L., 1758), the type spe-



**Fig. 4.** Cladogram (majority rule consensus tree) showing 1000 maximum likelihood bootstrap replicates reconstructed from the Reduced Total Evidence alignment. Nodes supported by only one analysis have support indicated above the subtending branch. Names on the right of the phylogeny are generic-level clades. The small tree on the left is the majority rule consensus tree with branch lengths. — **Symbols:** ● strong nodal support in results from all three phylogenetic methods; ■ strong support for ML and BB analyses and moderate support for MP; ▲ strong support for MP and BB analyses and moderate support for ML. — **Colors:** Black – outgroup taxa; red – taxa currently classified as Passalini; blue – taxa currently classified as Proculini.

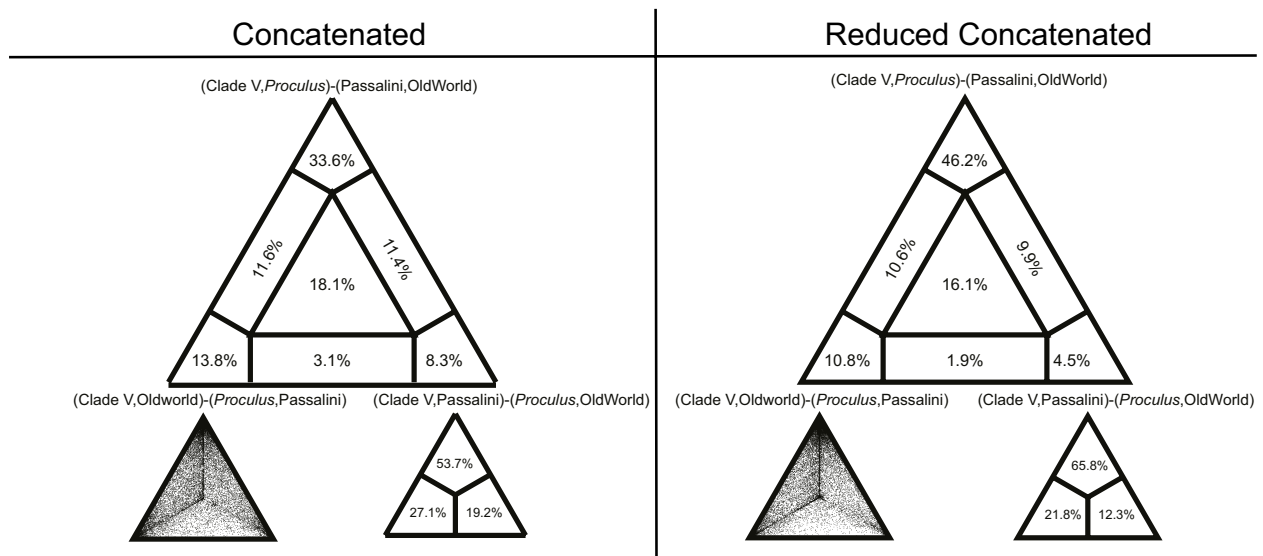


Fig. 5. Four-cluster Likelihood Maps for alternative topologies of the New World clades.

cies of the genus; thus, the group “Neleus” should be considered *Passalus sensu stricto*. (2) Clade III (Fig. 4) is comprised of *Passalus (Passalus) jansonii* (Bates, 1886), *Passalus (Pertinax) rühli* (Kuwert, 1891), and *Passalus (Pertinax) convexus* Dalman, 1817. (3) Clade IV (Fig. 4) is comprised of the mainly Mesoamerican species *Passalus (Pertinax) inops* Truqui 1857, *Ameripassalus guatemalensis* (Kaup, 1869), *Ptichopus angulatus* (Percheron, 1835), *Passalus (Pertinax) punctatostriatus* Percheron, 1835, and all representatives of *Passalus (Pertinax)* of the group “Rhodocanthopus” *sensu* JIMÉNEZ-FERBANS *et al.* (2016). The phylogenetic position of *Passalus (Passalus) abortivus* Percheron, 1835, *Passalus (Mitrorhinus) lunaris* (Kaup, 1869), *Passalus (Passalus) curtus* (Kaup, 1869), *Passalus (Pertinax) alfari* (Pangella, 1905), *Passalus (Pertinax) rugosus* Gravely, 1918, and *Passipassalus nukak* Jiménez-Ferbans & Reyes-Castillo, 2015 remain unresolved.

### 3.4. Circumscription and generic status within Proculini

Nine of the 20 currently valid genera of Proculini were recovered as monophyletic (Electronic Supplement File 6). Five genera were represented by one specimen only, and their monophyly could not be addressed (Electronic Supplement File 6). Our analyses recovered *Vindex*, *Pseudacanthus*, *Chondrocephalus* Kuwert, 1896, *Odontotaenius*, *Petrejoides* and *Popilius* as non-monophyletic (Fig. 4).

We recovered Proculini in three main clades, *Proculus*, Clade VI, and Clade VII (Fig. 4). All topologies (except for the ML Concatenated analysis, see results above) recovered *Proculus* as an early-divergent branch sister to Clade V, which is comprised of Clade VI + Clade VII (Fig. 4). Statistical support for Clade V and Clade VII was strong across all analyses. Clade VI received moderate statistical support in ML and parsimony analyses, and strong nodal support in Bayesian analyses.

Clade VI mainly comprised strictly montane genera that are exclusive to Mesoamerica (*Undulifer* Kaup, 1869, Part of *Pseudacanthus*, *Vindex*, *Xylopassaloides*, *Proculejus* Kaup, 1868, *Oileus* Kaup, 1869, and *Ogyges*). The recovered topology for Clade VI had an early divergent clade comprised of *Undulifer* + *Pseudacanthus* 1, and this clade sister to [(*Vindex* + *Xylopassaloides* + *Proculejus*) + (*Ogyges* + *Oileus*)] (Fig. 4). Clade VII was comprised by the remaining genera of Proculini + part of *Pseudacanthus* (Fig. 4). All analyses recovered Clade VII with strong statistical nodal support including an early divergent clade comprised of *Tonantzin* + *Pseudacanthus* 2, sister to *Chondrocephalus* + Clade VIII (Fig. 4). However, the sister relationship of *Chondrocephalus* with Clade VIII was recovered with low nodal support in all analyses.

Clade VIII was recovered with strong statistical support in all analyses; and comprised all representatives of *Arrox*, *Verres*, *Veturius*, *Petrejoides*, *Odontotaenius*, *Spurius*, *Yumtaax*, *Popilius*, *Heliscus* Zang, 1905, and *Pseudodarrox*. We recovered two clades within clade VIII: (1) *Arrox* + *Verres* + *Veturius* and (2) Clade IX (Fig. 4). We recovered Clade IX with strong nodal support, but the internal topology of the clade varied in different analyses. Clade IX was comprised of two strongly supported clades (1) *Spurius* + *Yumtaax*, (2) Clade X (Fig. 4), plus *Odontotaenius* 1. Clade X was consistently monophyletic with strong nodal support and comprised of members of *Petrejoides* 1, *Petrejoides* 2, *Popilius*, *Heliscus*, *Pseudodarrox*, and *Odontotaenius* 2 (Fig. 4).

### 3.5. Four-cluster Likelihood Mapping (FCLM)

Due to the conflicting topologies recovered by different analyses, we assessed the monophyly of Proculini using FcLM, testing for alternative phylogenetic hypotheses. The FcLM of the New World node showed a higher like-

likelihood for the sister relationship of *Proculus* and clade V in analyses of the Concatenated (33.6%) and Reduced Concatenated (46.2%) alignments (Fig. 5). A sister relationship of Passalini with Clade V was least likely for both alignments (Concatenated: 8.3%, Reduced Concatenated: 4.5%) (Fig. 5).

## 4. Discussion

### 4.1. Tribal relationships among the tribes of Passalinae and the phylogenetic status of the New World Tribes

Following BOUCHER (2006), Passalinae comprises five tribes, Leptaulacini, Macrolinini, and Solenocyclini which are distributed in the old world; and Passalini and Proculini which are exclusive to the New World. FONSECA et al. (2011) suggested a similar classification with two key differences: (1) The groups are considered subfamilies rather than tribes, and (2) Solenocyclinae includes both Solenocyclini and Leptaulacini *sensu* BOUCHER (2006). To facilitate the discussion, we will consider the groups as tribes, as suggested by BOUCHER (2006), as this is currently the most widely used system of taxonomy for passalid workers.

In their phylogenetic analysis, BOUCHER (2006) recovered the tribes of Passalinae in two main clades, the Old World and the New World clades. Within the Old-World Clade Solenocyclini and Leptaulacini were recovered sister to each other, and this clade sister to Macrolinini. Passalini and Proculini were recovered sister to each other (BOUCHER, 2006). In contrast, FONSECA et al. (2011) did not recover the Old World and the New World clades. Instead, he recovered Macrolinini sister to Passalini, and these together were sister to Proculini. Solenocyclinae *sensu* FONSECA et al. (2011) was sister to (Proculinae + (Macrolinae + Passalinae)) (FONSECA et al., 2011).

In general, our Bayesian analyses supported the hypothesis of monophyly for the Old-World tribes; however, this clade lacked nodal support. ML and MP could not resolve the relationships between these taxa. Nonetheless, we consistently recovered strong statistical support for a clade comprised of all New World Passalidae. This contradicts the results of FONSECA et al. (2011), who recovered Macrolinini sister to Passalini. Our data therefore support the monophyly and a single origin of the New World Passalidae, but the interrelationships of the Old-World tribes (which were not a focus of our study) remain uncertain.

For the Two New World tribes, previous phylogenetic analyses have recovered conflicting results for the monophyly of Passalini (e.g. BOUCHER 2006; FONSECA et al. 2011). Most analyses (GILLOGLY 2005; BOUCHER 2006; JIMÉNEZ-FERBANS 2014) have recovered a paraphyletic Passalini. Both GILLOGLY (2005) and JIMÉNEZ-FERBANS

(2014) found that Passalini was rendered paraphyletic by Proculini, with the latter tribe nested in *Passalus* and closely related to *P. (Pertinax)*. BOUCHER (2006), provides two phylogenetic hypotheses for the circumscription of Passalini. In the tribal level phylogeny, BOUCHER (2006) considered Passalini monophyletic, in the genus level phylogeny *Passalus* is rendered paraphyletic by Proculini; yielding similar results to GILLOGLY (2005) and JIMÉNEZ-FERBANS (2014). BOUCHER (2006) considered supra-specific taxa as terminals, and the monophyly of each terminal was determined before the phylogenetic analysis on the basis of “autapomorphies”. This methodological constraint is why the author considered Passalini monophyletic in the tribal level phylogeny, conflicting with the results of the generic level phylogeny of Proculini. However, there were relatively few Passalini included in BOUCHER (2006) and GILLOGLY (2005), and JIMÉNEZ-FERBANS (2014) included terminals representing only 6 of the 20 genera of Proculini. Taxon sampling in these phylogenies was therefore likely insufficient to clarify the relationship between these two tribes. In contrast, FONSECA et al. (2011) recovered a monophyletic Passalinae *sensu* FONSECA et al. (equivalent to Passalini *sensu* BOUCHER) based on a phylogenetic analysis of hindgut morphology.

Our analyses strongly support Passalini as a monophyletic group. Additionally, the topologies we recovered support the polyphyly of *Passalus*, as suggested by GILLOGLY (2005), BOUCHER (2006), and JIMÉNEZ-FERBANS (2014). However, the significant difference between our results and previously published phylogenies is that *Passalus* is polyphyletic with respect to the other genera of Passalini, excluding Proculini. Taking into consideration our findings and the limitations of previous phylogenies, we consider the evidence to support a monophyletic Passalini, but with a great need to redefine the generic boundaries within the tribe.

In Contrast, Proculini is strongly supported as a monophyletic group by adult and larval morphology (e.g., REYES-CASTILLO 1970; SCHUSTER & REYES-CASTILLO 1981; SCHUSTER 1992), cytotaxonomy (VIRKKI & REYES-CASTILLO 1972; SERRANO et al 1998), and phylogenetic analyses (e.g., BOUCHER 2006; FONSECA et al. 2011). Our analyses largely support the monophyly of Proculini (Fig. 4, 5), except for the ML analyses of the concatenated data set, which recovered a paraphyletic Proculini (Fig. S5). According to the ML analysis of the concatenated data set, *Proculus* is an early divergent taxon sister to a clade comprised of Passalini and the rest of Proculini (61% MLBS). Long branch attraction or noisy data from the 28S unalignable regions might be causing the alternative topology suggested by the ML analysis of the concatenated data set. When we removed the unalignable 28S regions (reduced concatenated data set) *Proculus*, Passalini, and Clade V formed a polytomy (Fig. S6). Furthermore, when morphology was added (total evidence, reduced total evidence analyses), a monophyletic Proculini was recovered (Fig. 4, Fig. S7). Proculini was the only clade considered to have a clean

synapomorphy (the anterodorsal exposure of the fronto-clypeus), based on the 57 morphological characters evaluated here. This morphological character is used to define the tribe by REYES-CASTILLO (1970) and BOUCHER (2006). Additionally, the FcLM analyses favored the topology where Proculini was monophyletic (Fig. 5). Thus, we consider the overall evidence to overwhelming support a monophyletic Proculini, consistent with REYES-CASTILLO (1970), BOUCHER (2006), and FONSECA et al. (2011).

#### 4.2. Circumscription and generic status of the genus *Passalus*

In our results, the limited sampling within *Passalus* and the difficult delimitation of the genus blur the relationships of the other genera of Passalini. *Passalus* Fabricius (1792) was created for three species, of which, only *P. interruptus* is currently considered within the genus. Except for the separation made by MACLEAY (1819) when the author described *Paxillus*, all other species described in Passalidae belonged to *Passalus*. Subsequently, KAUP (1868, 1871) divided the family into several subfamilies and genera. KUWERT (1891, 1896, 1898) and ZANG (1905) also divided Passalidae into several groups, both authors without following clear criteria (REYES-CASTILLO 1970) for group definitions. Later, GRAVELY (1918) restructured *Passalus* by synonymizing many genera proposed by KAUP, KUWERT and ZANG, and developed a clearer delimitation of the genus. Subsequently, LUEDERWALDT (1931, 1934) divided *Passalus* into three subgenera that are still used today, and REYES-CASTILLO (1970) redefined *Passalus*, indicating that it should be recognized by bidentate lacinia, rhomboid prosternellum, unbroken anterior tibia, lateral apical spines of middle and posterior tibia of equal size, and elongated hypostomal process separated from the labium. However, authors of the group (e.g., REYES-CASTILLO 1970; JIMÉNEZ-FERBANS et al. 2014; BEVILAQUA & FONSECA 2020) pointed out that *Passalus* is a heterogeneous taxon that contains several genera; our results support this idea.

*Passalus* is the New World passalid genus with the least well resolved delimitation, and our results suggest, as expected, that it is not monophyletic. To solve this problem, a phylogenetic analysis must be carried out that includes most of the species of the genus, including representatives of its three sub-genera and groups of species. Likewise, other authors (e.g. JIMÉNEZ-FERBANS et al. 2014, 2015, 2016; BEVILAQUA & FONSECA 2020) have suggested the need for a more robust study of the phylogenetic relationships within *Passalus*. Recently, BOUCHER (2015) revalidated several genera that were synonymous with *Passalus*. However, the author supports the revalidation of the genera with the establishment of homologies never tested in the context of a phylogenetic analysis. Furthermore, BOUCHER (2015) does not offer a clear delimitation of the genera and the species that they would contain.

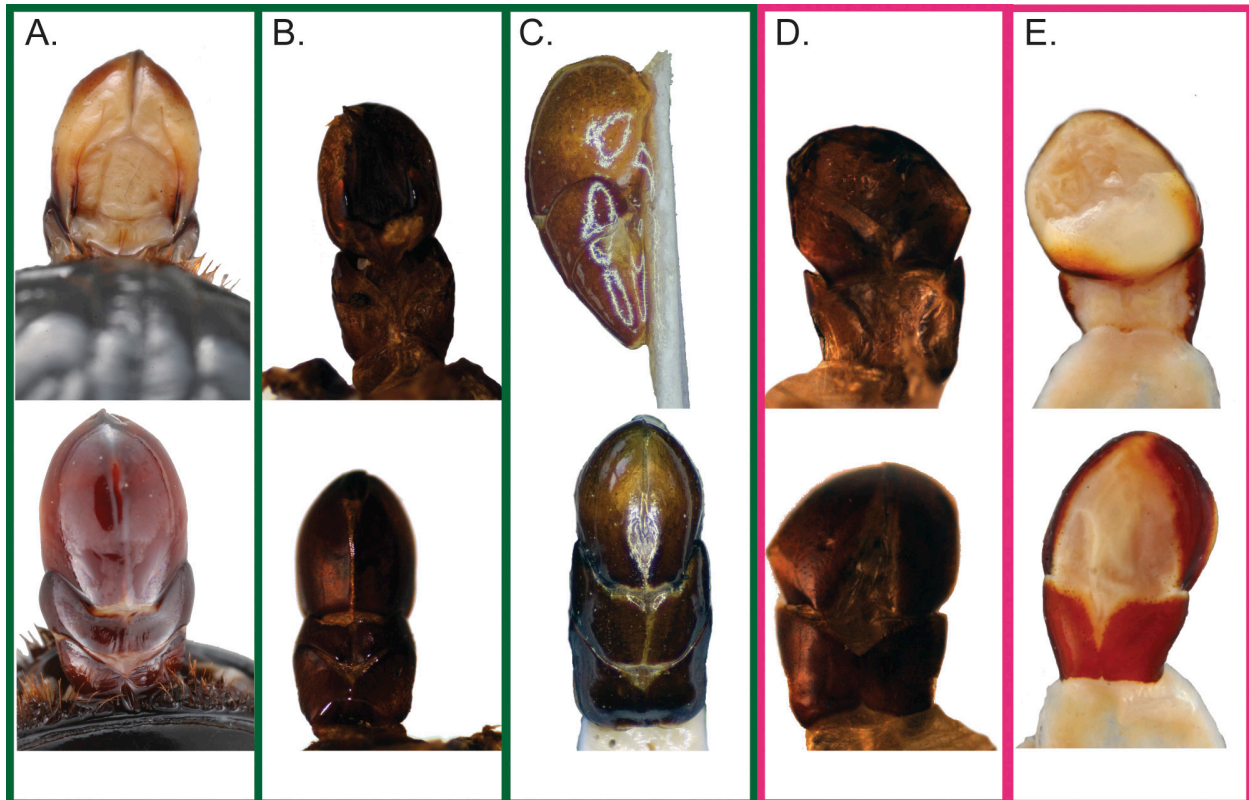
#### 4.3. Circumscription and generic status within Proculini

REYES-CASTILLO (1970) was the first author to suggest Proculini as a tribe. In his original conception, the author included 19 genera. Subsequent to this work, REYES-CASTILLO (1974) synonymized *Prosoclitus* Bates, 1886 to *Pseudacanthus*, and REYES-CASTILLO et al. (1987) described *Xylopassaloides*; changing the circumscription of the genera but maintaining the number of genera at 19. BOUCHER (2006) made the last major delimitation of the genera in Proculini, synonymizing *Coniger* Zang, 1905 with *Heliscus*, and *Publius* Kaup, 1871 with *Veturius*, revalidating *Arrox*, and describing *Yumtaax*. Additionally, BOUCHER (2006) proposed several new combinations of species within the tribe. BOUCHER (2006) redefined the genera of Proculini based on genus-level “autapomorphies” (character states were defined as generic autapomorphies *a priori* of a phylogenetic analysis). According to BOUCHER (2006) the generic delimitations proposed by REYES-CASTILLO (1970) were based mainly on “homoplasies”, and the genera needed to be reorganized. Again, after the treatment of BOUCHER (2006) the generic concepts changed but the number of genera remained 19. Lastly, BEZA-BEZA et al. (2019) described *Tonantzin*, an endemic genus from Mexico. Currently, the tribe contains 20 valid genera (BOUCHER 2006; SCHUSTER & CANO 2008; BEZA-BEZA et al. 2019).

Our analyses recovered at least 23 lineages that could have generic level status (Fig. 4). The difference in the number of generic level clades compared to the number of current valid genera is due to the non-monophyly of *Vindex*, *Pseudacanthus*, *Odontotaenius*, *Petrejoides* and *Popilius* recovered by our analyses (Fig. 4), and the uncertain generic placement of an undescribed species related to *Vindex* and *Proculejus* (Fig. 4). However, to facilitate revision of the genera of the tribe (including synonyms and splits) a thorough taxonomic revision of the non-monophyletic genera, with a strong phylogenetic component, is needed. We considered our data to support the necessity of taxonomic changes, but our taxonomic sampling is insufficient to efficiently circumscribe these new groups. Below, we discuss our phylogenetic findings for the genera of Proculini recovered non-monophyletic and those with new combinations.

##### 4.3.1. *Pseudacanthus* Kaup, 1869

The modern concept of *Pseudacanthus* was proposed by REYES-CASTILLO (1970), but it has undergone significant changes since that circumscription. Furthermore, there is an ongoing debate about the species composition of the genus. BOUCHER (2006) recognizes three species (*Proculejus obesus* [Bates, 1886], *Undulifer nigridiodes* [Hincks, 1949], and *Undulifer violetae* [Reyes-Castillo & Castillo, 1986]) formerly considered *Pseudacanthus* as belonging to other genera. However, the transfer of *U. nigridiodes* and *U. violetae* to *Undulifer* by BOUCHER (2006) was not followed by all workers in the group



**Fig. 6.** Aedeagus of *Pseudacanthus* spp. A. dorsal (top) and ventral (bottom) view of *Ps. junctistriatus*. B. dorsal (top) and ventral (bottom) view of *Ps. subopacus*. C. lateral (top) and ventral (bottom) view of *Ps. zuninoi*. C. Dorsal (top) and ventral (bottom) view of *Ps. mexicanus*. D. dorsal (top) and ventral (bottom) view of *Ps. aztecus*. — **Colors:** Green – species of *Triaenurgus*; pink – species of *Pseudacanthus sensu stricto*.

(e.g., SCHUSTER & CANO 2008; REYES-CASTILLO & CHAMÉ-VÁZQUEZ 2014). Our evidence supports the transfer of *U. nigidiodes* and *U. violeatae* made by BOUCHER (2006) (see in section 4.3.2). Thus, we considered *Pseudacanthus* as proposed by BOUCHER (2006). This treatment of the genus comprises 7 species and is distributed in the mountains of Mexico and Guatemala (BOUCHER 2006; REYES-CASTILLO & CHAMÉ-VÁZQUEZ 2014).

Nonetheless, our analyses recovered *Pseudacanthus sensu* Boucher as polyphyletic – split into two clades (Fig. 4). Both species recovered in *Pseudacanthus* 1 are distributed southeast of the Isthmus of Tehuantepec while *Pseudacanthus aztecus* (Truqui, 1857) is distributed northwest of the Isthmus of Tehuantepec. SCHUSTER (1992) suggested differences in larval morphology between species of *Pseudacanthus* from different sides of the Isthmus of Tehuantepec. Two main groups of larvae exist in Proculini, the “*Chondrocephalus*” and “*Vindex*” groups (SCHUSTER 1992). SCHUSTER (1992) described the larvae of three species of the genus, *Pseudacanthus mexicanus* (Truqui, 1857), *U. violeatae*, and *Pseudacanthus subopacus* (Bates, 1886). *Pseudacanthus mexicanus* (northwest of the Isthmus of Tehuantepec) had a “*Chondrocephalus*” type larva, while the other two species (southwest of the Isthmus of Tehuantepec) had a “*Vindex*” type larva. SCHUSTER (1992) suggested that the circumscription of *Pseudacanthus* should be re-considered and the genus probably needs to be split. Our analyses yielded results

similar to SCHUSTER (1992). *Pseudacanthus* 1 is more closely related to genera that have larvae of the “*Vindex*” type (Clade V). While *Ps. aztecus* is most closely related to genera that have larvae of the “*Chondrocephalus*” type. However, we must consider that we used a different species (*Ps. aztecus*) from northwest of the Isthmus of Tehuantepec than the one used by SCHUSTER (1992) (*Ps. mexicanus*). Nonetheless, *Ps. mexicanus* and *Ps. aztecus* are very closely related (REYES-CASTILLO 1970).

*Pseudacanthus* 1, which is distributed southeast of the Isthmus of Tehuantepec, comprises the two species formerly considered to belong to the genus *Triaenurgus* Bates, 1886; which later was synonymized with *Pseudacanthus* (KUWERT 1891; REYES-CASTILLO 1970). We suspect *Triaenurgus* is a valid genus. However, the species composition of *Triaenurgus* needs further study and we consider genera revalidation/description when a group meets all three of the following criteria: 1. Broad context evidence that generic status of the group is granted, 2. A complete list of the species composition of the new group, and 3. Official circumscription criteria (description) of the genus. Our data for *Triaenurgus* only meet criteria 1; taking this into account, we prefer to leave the revalidation of the genus as a suggestion of further exploration.

Aedeagus characters, in addition to the evidence from phylogenies and larval morphology, also suggest the species formerly grouped in *Triaenurgus* (distributed south-

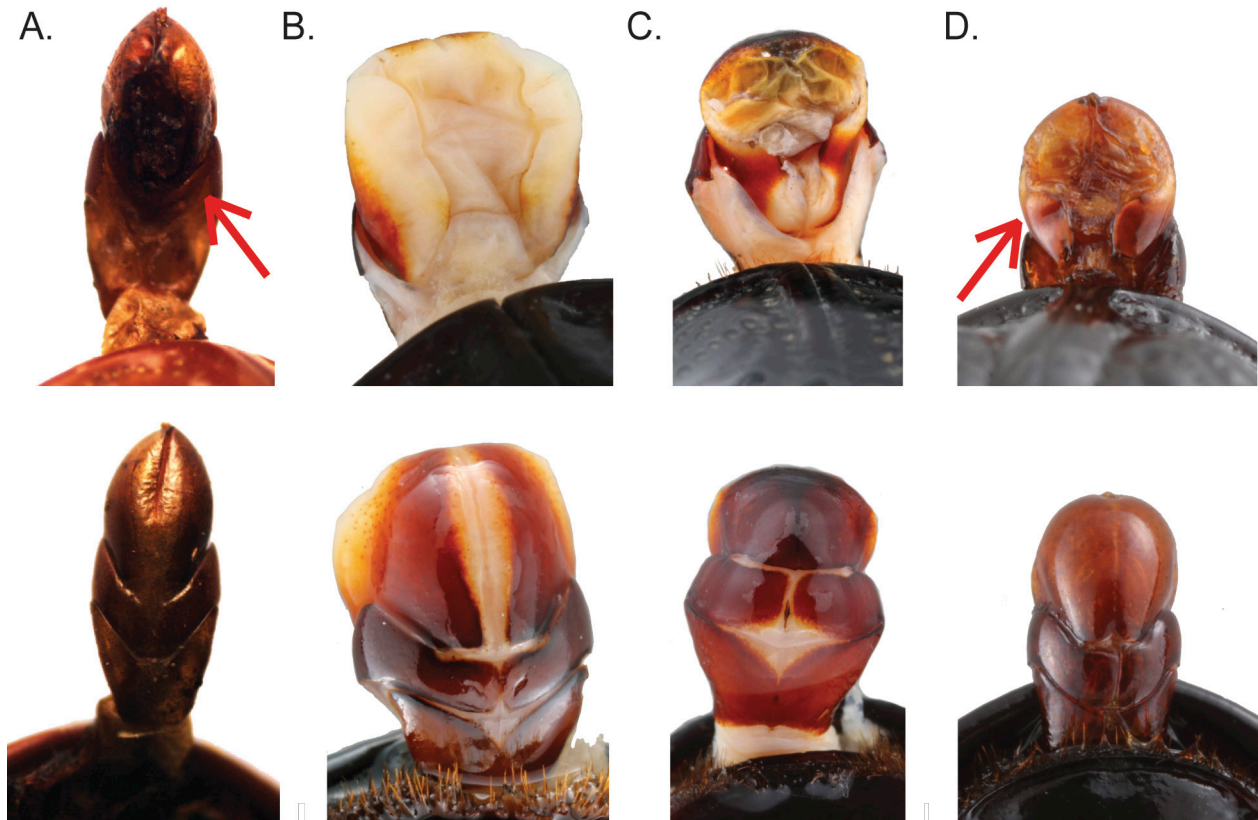


Fig. 7. Dorsal (top) and ventral (bottom) views of the aedeagus of *Undulifer* spp. A. *U. incisus*, B. *U. c.f. acapulcae* C. *U. nigridiodes*, D. *U. violetae*. The red arrow indicates basal sclerotization of the phallus.

east of the Isthmus of Tehuantepec) are different from at least *Ps. aztecus* and *Ps. mexicanus* (distributed northwest of the Isthmus of Tehuantepec) (Fig. 6). *Ps. subopacus* and *Pseudacanthus junctistriatus* (Kuwert, 1891) have the phallus with the ventral side fully sclerotized, the ventral sclerotization expands to the dorsal side like a hood, leaving only the middle and proximal part of the dorsal side membranous; the parameres and phallobase have a distinct membrane between them (Figs. 6 A–C). In addition to the species included in this study, *Pseudacanthus zuninoi* Reyes-Castillo & Chamé-Vázquez, 2014 has the same aedeagus configuration (Fig. 6C) as *Ps. junctistriatus* and *Ps. subopacus*. While *Ps. aztecus* and *Ps. mexicanus* have the phallus globose (as described by BEZA-BEZA et al. 2017), the median part of the ventral side membranous, and the dorsal side completely membranous. The parameres appear completely fused with each other and to the phallobase (Figs. 6 D, E).

Our data suggest *Pseudacanthus sensu* BOUCHER should be split into at least two lineages (perhaps genera) *Pseudacanthus sensu stricto* and *Triaenurgus*. Based on the male genitalic characters *Ps. mexicanus* and *Ps. aztecus* should be considered *Ps. sensu stricto*, while *Ps. junctistriatus*, *Ps. subopacus* and *Ps. zuninoi* are part of the *Triaenurgus* lineage. *Pseudacanthus truquii* Kuwert, 1891 and *Pseudacanthus solidus* (Arrow, 1907) cannot be placed in any of these lineages with confidence, because they are only known from their type material (REYES-CASTILLO & CHAMÉ-VÁZQUEZ 2014).

#### 4.3.2. *Undulifer* Kaup, 1896 *sensu novo*

REYES-CASTILLO (1970) listed *Undulifer incisus* (Truqui, 1857) and *Undulifer acapulcae* Kuwert, 1897 as the two members of this genus. REYES-CASTILLO & CASTILLO (1981) listed the same two species for *Undulifer*. However, BOUCHER (2006) stated that REYES-CASTILLO (1970) did not issue an opinion on the existence of one or two species of the genus “Dans son premier examen du genre, REYES-CASTILLO (1970) n’a pas émis d’opinion tranchée sur l’existence d’une, ou bien de deux, espèces (l’espèce type *incisus* (Truqui), du Mexique et *salvadorae* Kuwert, décrit de El Salvador et jamais repris depuis.” Perhaps, BOUCHER (2006) was referring to the species status of *Undulifer salvadoris* Kuwert, 1897 which REYES-CASTILLO (1970) explicitly synonymized with *U. incisus* “En el presente trabajo se ha incluido en la sinonimia de *U. incisus* a *U. salvadoris*, basándose en el estudio comparado de la genitalia masculina de los tipos de ambas especies.” In addition to this confusion, BOUCHER (2006) transferred *U. nigridiodes* and *U. violetae* from *Pseudacanthus* to *Undulifer*. The changes suggested by BOUCHER (2006) to *Undulifer* were criticized by SCHUSTER & CANO (2008) based on morphological inconsistencies of the *Undulifer* concept proposed by BOUCHER (2006) and the two transferred species. In the case of SCHUSTER & CANO (2008), the authors were unclear about the number of species in the genus “Two species listed; however, probably only one.” (SCHUSTER & CANO 2008).

Our analyses consistently recovered *U. c.f. acapulcae* and *U. nigridiodes* sister to each other (Fig. 4). Thus, our phylogenetic analyses at least support the transfer of *U. nigridiodes* from *Pseudacanthus* to *Undulifer*. The male genitalia of *U. nigridiodes* (Fig. 7C) and *U. violeatae* (Fig. 7D) are more like *Undulifer* species (Figs. 7 A–B) than any of the forms described for *Pseudacanthus* (Fig. 6). Three of the four species have the phallus with the ventral side completely sclerotized, and the dorsal side membranous except for the posterior part, where the ventral sclerotization expands to the sides. The ventral sclerotization of the phallus in *U. incisus* expands to the dorsal side like a hood, similar to *Pseudacanthus* spp. of the *Triaenurgus* lineage; however, the phallus of *U. incisus* also has the posterior sclerotizations of the *Undulifer* spp. (Fig. 7 A). This last character is clear in *U. nigridiodes* and *U. violeatae*, but less notable in *U. c.f. acapulcae* (Fig. 7). Thus, we also considered the transfer of *U. violeatae* by BOUCHER (2006) as valid, but not for the reasons he mentioned in his work.

A loose end in the genus is the status of *U. acapulcae* which seems to be omitted by BOUCHER (2006) and SHUSTER & CANO (2008). We did not find any transfer or synonym status for this species in the literature; thus, we still consider the species valid and part of *Undulifer*. Additionally, we compared specimens identified by REYES-CASTILLO as *U. incisus* and *U. acapulcae* and they are different species based on differences in the male genitalia (Figs. 7 A–B). Based on the overall evidence, *Undulifer sensu novo* includes: *U. incisus* (type species), *U. acapulcae*, *U. nigridiodes*, and *U. violeatae*. All species are strictly montane, and the genus is distributed in Mexico and Guatemala. We believe a reconsideration of the genus is necessary, and the male genitalic characters suggested in this work might serve as a morphological character to circumscribe the genus—rather than the configuration of the fronto-clypeus as proposed by REYES-CASTILLO (1970) and BOUCHER (2006).

#### 4.3.3. *Vindex-Xylopassaloides-Proculejus* complex

*Vindex* is a strictly montane genus from Mesoamerica comprised of five described species (ARIZA-MARÍN et al. 2019). Of these species, two are distributed northwest of the Isthmus of Tehuantepec, while three are distributed southeast of the Isthmus of Tehuantepec. BOUCHER (2006) suggested two female genitalic characters as synapomorphies for the group. However, ARIZA-MARÍN et al. (2019), pointed out that the autapomorphies proposed by BOUCHER (2006) only applied to *Vindex agnoscendus* (Percheron, 1841), *Vindex gonzaloi* Reyes-Castillo & Castillo, 1993, and an undescribed species from Nicaragua (which were the three species dissected by BOUCHER [2006]). While *Vindex sculptilis* Bates, 1886, and *Vindex chimalapensis* Ariza-Marín, Reyes-Castillo, Moctezuma & Sánchez-Huerta, 2019 have female genitalia more similar to *Xylopassaloides* (ARIZA-MARÍN et al. 2019).

Our analyses consistently recovered *Vindex* at least paraphyletic, split into two generic level clades: *Vindex*

1 which was recovered sister to *Xylopassaloides chortii* Schuster, 1993, and *Vindex* II which was recovered in a clade comprised of an early divergent undescribed species of undetermined generic status, sister to *Vindex* 2 + *Proculejus* (Fig. 4). *Vindex agnoscendus*, the type species of *Vindex*, was recovered within *Vindex* 2 which should be considered as *Vindex sensu stricto*. *Vindex* 1 comprised *V. sculptilis* and two undescribed species from Guatemala, *Vindex* 2 comprised *V. agnoscendus* and *V. gonzaloi*; our topology is consistent with the observations made by ARIZA-MARÍN et al. (2019) in female genitalia. Our data suggest that *Vindex* 1 should be transferred to *Xylopassaloides*. The female genitalia could be useful in making these decisions.

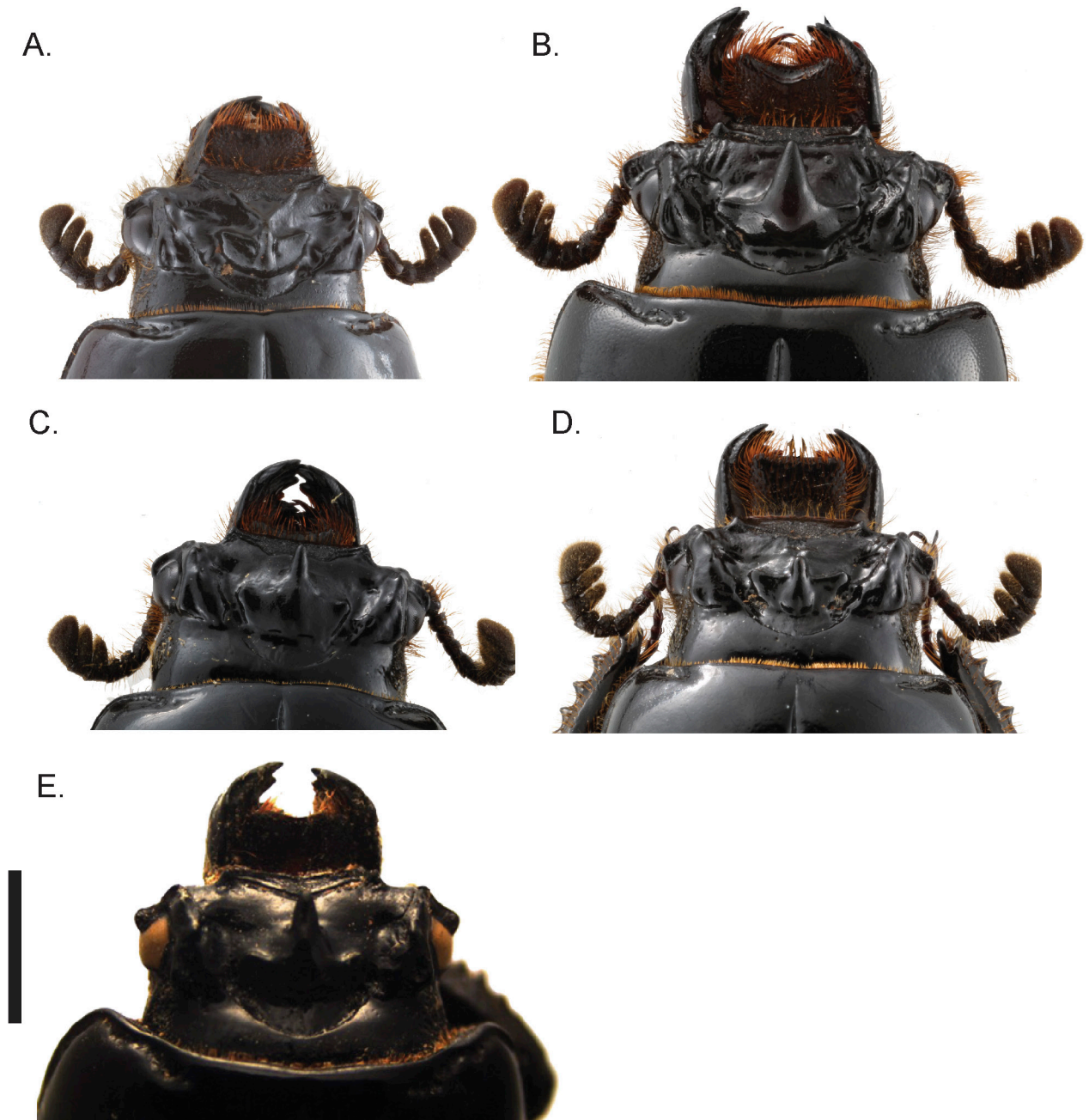
#### 4.3.4. *Chondrocephalus* Kuwert, 1896 *sensu novo*

This is a strictly montane genus, endemic to Nuclear Mesoamerica (REYES-CASTILLO 1970; BOUCHER 2006; SHUSTER & CANO 2008). The species composition of *Chondrocephalus* is uncertain, BOUCHER (2006) listed 6 species and SCHUSTER & CANO (2008) listed 5 valid species and many to be described. The difference between BOUCHER (2006) and SCHUSTER & CANO (2008) is the generic placement of *Chondrocephalus salvadorae* (Schuster, 1989). *Chondrocephalus* is diagnosed by a granular texture of the clypeo-frons, and the oblique clypeo-frontal suture (Figs. 8, 9) (REYES-CASTILLO 1970; BOUCHER 2006). BOUCHER (2006) noted *C. salvadorae* (Fig. 8B) had the diagnostic character of *Chondrocephalus* and transferred the species from *Petrejoides*. In contrast, SCHUSTER & CANO (2008) considered *C. salvadorae* to belong to an undescribed genus including four other species described in *Petrejoides*; although they do not mention what the other four species of this new genus were. BEZA-BEZA et al. (2017) generated a molecular phylogeny for the genus *Yumtaax* and the authors recovered *C. salvadorae* in a clade with *Chondrocephalus purulensis* Bates, 1886, and *Petrejoides guatemalae* Reyes-Castillo & Schuster, 1983, but they did not discuss the generic placement of either species in *Chondrocephalus*.

Here we confirm the findings of BOUCHER (2006) and BEZA-BEZA et al. (2017) and reject the hypothesis of SCHUSTER & CANO (2008) regarding the generic placement of *C. salvadorae*. Furthermore, to consider *Chondrocephalus* monophyletic we had to transfer *Pe. guatemalae* from *Petrejoides* to *Chondrocephalus*. We consistently recovered the representatives of *Petrejoides guatemalae* as monophyletic, but nested within *Chondrocephalus* (Fig. 4).

The transfer of *Pe. guatemalae* to *Chondrocephalus* is also justified on morphological basis (Fig. 8C); *Pe. guatemalae* has the diagnostic characters for *Chondrocephalus* (Figs. 8, 9). This morphological configuration is also true for another two species originally described in *Petrejoides* (*Petrejoides reyesi* Schuster, 1988 [Fig. 8E] and *Petrejoides pokomchii* Schuster, 1991 [Fig. 8D]). We proposed to transfer *Pe. guatemalae* and the other

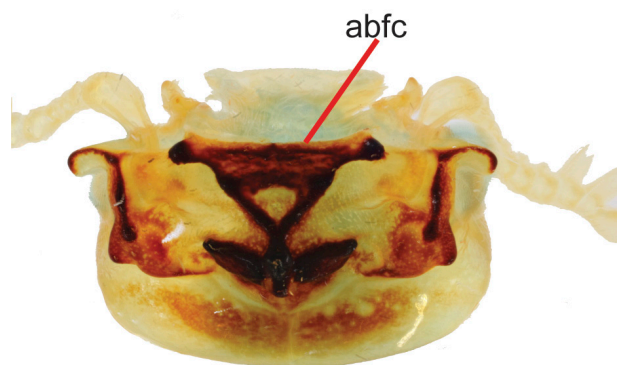




**Fig. 8.** Dorsal view of the head for **A:** *C. granulifrons*, **B:** *C. salvadorae*, **C:** *C. guatemalae*, and **D:** *C. pokomchii*. **E:** *C. reyesi*. Scale bar 3 mm.

two species; resulting in *Chondrocephalus guatemalae* (Reyes-Castillo & Schuster, 1984) **comb.n.**, *Chondrocephalus reyesi* (Schuster, 1988) **comb.n.** and *Chondrocephalus pokomchii* (Schuster, 1991) **comb.n.** These latter species share the diagnostic character configuration of the clypeo-frons with other members of *Chondrocephalus* (Fig. 8).

Additionally, we proposed the notable difference between the anterior border of the clypeo-frons and the rest of this plaque as another diagnostic character of the genus (Fig. 9). This character resembles what BOUCHER (2006) considered an exposed clypeus for *Aulacocyclus*. But the marked difference in *Chondrocephalus s.n.* might be due to the difference in texture that is granular



**Fig. 9.** Cleared head of *C. granulifrons*, showing the anterior border of the fronto-clypeus (abfc).

for the clypeo-frons and smooth for the anterior border. *Chondrocephalus s.n.* includes the following species: *Chondrocephalus granulifrons* (Bates, 1886) [type species], *Chondrocephalus debilis* (Bates 1886), *C. purulensis*, *Chondrocephalus granulum* Kuwert, 1897, *Chondrocephalus gemmae* Reyes-Castillo & Castillo, 1986, *C. guatemalae* **comb.n.**, *C. reyesi* **comb.n.**, *C. salvadorae*, and *C. pokomchii* **comb.n.**

#### 4.3.5. *Odontotaenius* Kuwert, 1896

This genus with 13 valid species, has one of the widest distributions within Proculini, ranging from southern Canada to the Andes Mountains in South America (SCHUSTER & CANO 2008; PARDO-LOCARNO 2012). *Odontotaenius* was revalidated by REYES-CASTILLO (1970) who proposed the genus to be diagnosed by the presence of a fronto-clypeal suture, and a dentate anterior border of the fronto-clypeus. Later BOUCHER (2006) argued that a subset of characters used by REYES-CASTILLO (1970) to separate *Odontotaenius* from other genera were “symplesiomorphies” and the genus was not monophyletic. BOUCHER (2006) transferred *Od. decipiens* and *Od. yucatanus* from *Heliscus* to *Odontotaenius* and proposed male and female genital characters as autapomorphies to circumscribe the genus.

Our analyses included *Odontotaenius striatopunctatus* (Percheron, 1835) (type species), *Odontotaenius disjunctus* (Illiger, 1800), and *Odontotaenius zodiacus* (Truqui, 1857). We recovered the genus as polyphyletic (Fig. 4). We recovered *Od. zodiacus* (*Odontotaenius* 1) as an independent lineage, and *Odontotaenius* 2 (*Odontotaenius sensu stricto*) as a strongly supported clade comprised of *Od. striatopunctatus* and *Od. disjunctus* (Fig. 4). The recovered topology in our analyses (Fig. 4) strongly rejects the possibility of the two *Odontotaenius* clades as sister taxa; thus, we suggest the split of *Odontotaenius* into at least two genera.

KUWERT (1896, 1897) created *Odontotaenius* placing *Od. striatopunctatus*, *Odontotaenius brevioripennis* (Kuwert, 1891), and *Odontotaenius haberi* Kuwert, 1897 in the genus. While *Od. zodiacus* was placed by KUWERT (1896, 1897) within the genus *Passalotaenius* Kuwert, 1896 along with *Odontotaenius cuspidatus* (Truqui, 1857) and *Heliscus falsus* (Kuwert, 1987). REYES-CASTILLO (1970) recognized *Odontotaenius* as homogenous and a natural group, including seven species. Later CASTILLO et al. (1988) described *Odontotaenius cerastes* Castillo, Rivera-Cervantes & Reyes-Castillo, 1988, where the authors proposed two “phyletic lines” within the genus. One phyletic line included *Od. striatopunctatus*, *Od. brevioripennis*, *Od. haberi*, *Odontotaenius striatulus* (Dibb, 1940), and *Od. disjunctus*; and a second group comprised *Od. cuspidatus*, *Od. zodiacus*, and *Od. cerastes* (REYES-CASTILLO 1970; CASTILLO et al. 1988).

Morphologically, *Od. cerastes* and *Od. zodiacus*, can be distinguished from other *Odontotaenius* by pubescent frontal fossae, and the absence of the characteristic dentation in the middle of the anterior border of the fronto-

clypeus of *Odontotaenius* (CASTILLO et al. 1988; SCHUSTER 1994). Given the morphological evidence discussed in the literature and our phylogenetic analyses, it is very likely that *Passalotaenius* is a valid genus that includes at least two species (*Od. zodiacus*, and *Od. cerastes*), and perhaps also *Od. cuspidatus*.

#### 4.3.6. *Petrejoides*-*Popilius* complex

*Petrejoides* and *Popilius* have unstable taxonomic histories (REYES-CASTILLO 1970; GILLOGLY 2005; BOUCHER 2006; BEZA-BEZA et al. 2017). The most recent concept of *Petrejoides* was provided by BOUCHER (2006); in this concept the author included 23 species (two undescribed). BOUCHER (2006) included a mix of species from *Petrejoides sensu* REYES-CASTILLO (1970) (including posterior species descriptions), and most Mesoamerican species of *Popilius sensu* REYES-CASTILLO (1970).

Our analyses recovered *Petrejoides sensu* Boucher as polyphyletic. Based on our phylogenetic analyses we propose to transfer three species from *Petrejoides sensu* BOUCHER to *Chondrocephalus* (see section 4.3.4), and we transfer *Petrejoides caldasi* (Reyes-Castillo & Pardo-Locarno, 1995) back to *Pseudoarrox*, resulting in *Pseudoarrox caldasi* Reyes-Castillo & Pardo-Locarno **comb.n.** (See section 4.3.7). Even with the proposed changes, the generic concept of *Petrejoides* is still problematic. Consistent with our results BEZA-BEZA et al. (2017) recovered the genus as polyphyletic.

Our data conflicts on the placement of some species of *Petrejoides* which bounced around between different positions inside Clade X (Fig. 4; Supp. Figs. S5–S15). We mainly considered two groups within the genus; *Petrejoides* 1 comprised of *Petrejoides tenuis* Kuwert, 1897, *Petrejoides haagi* (Kaup, 1868), *Petrejoides orizabae* Kuwert, 1897, and an undescribed species from Mexico; and *Petrejoides* 2, which comprises two undescribed species similar to *Petrejoides subrecticornis* (Kuwert, 1897) (which was not included in this study).

*Petrejoides* 1 is not monophyletic. ML and MP analyses recovered *Petrejoides* 1 split into two clades in a polytomy including (1) *Pe. tenuis* (Part of *Petrejoides* 1) + *Popilius gibbosus* Burmeister, 1847, (2) the rest of the species of *Petrejoides* 1 + *Popilius marginatus* (Percheron, 1835), and (3) *Petrejoides* 2 + *Heliscus* + *Pseudoarrox* + *Odontotaenius* 2 (Fig. 4). However, Bayesian analyses recovered all members of *Petrejoides* 1 in a clade mixed in with *Popilius*, and this clade sister to *Petrejoides* 2 + *Heliscus* + *Pseudoarrox* + *Odontotaenius* 2 (Figs. S8–S11). The Bayesian relationships had low nodal support.

We never recovered *Petrejoides* 1 and *Petrejoides* 2 sister to each other. Furthermore, *Pe. subrecticornis* and related species, including *Petrejoides chocoensis* Reyes-Castillo & Pardo-Locarno, 1996 and other undescribed species, deserve generic status (clade *Petrejoides* 2: Fig. 4). Our data suggest *Petrejoides* is perhaps the most challenging group in Proculini, and the genus needs re-circumscription and a comprehensive revision.

On the other hand, our analyses included two species from *Popilius*, we never recover those species as sister taxa. The species of *Popilius* were always recovered sister to other species of *Petrejoides* 1. *Popilius gibbosus* was always recovered sister to *Pe. tenuis* and *Po. marginatus* was always recover sister to *Pe. haagi*. An extensive taxonomic revision of *Popilius* with a robust phylogenetic study including most species of *Petrejoides* and *Popilius* might be needed to untangle the relationships among these genera.

#### 4.3.7. *Pseudoarrox* Reyes-Castillo, 1970 *sensu novo*

This genus was considered monotypic by BOUCHER (2006), endemic to the Talamanca mountains in Panama and Costa Rica. In the original description of *Pseudoarrox* REYES-CASTILLO (1970) described the genus as monotypic. Later, REYES-CASTILLO & PARDO-LOCARNO (1995) described *Pseudoarrox caldasi* in *Pseudoarrox* increasing the number of species to two. However, BOUCHER (2006) transferred the *Pse. caldasi* to *Petrejoides*, suggesting the characters considered to place the species in *Pseudoarrox* by the original authors of the species were due to evolutionary convergence with *Pseudoarrox karreni* Reyes-Castillo, 1970; without any information to substantiate such a claim.

Our data strongly rejects *Pse. caldasi* as a member of *Petrejoides sensu stricto* (Fig. 4). Instead *Pse. caldasi* is closely related to *Odontotaenius* 2. In his phylogeny BOUCHER (2006) recovered *Pseudoarrox sensu* BOUCHER sister to *Odontotaenius*; which is consistent with the placement of *Pse. caldasi* in all our analyses. Given the uncertainty of *Petrejoides* as a monophyletic genus, the original character set that placed *Pse. caldasi* in *Pseudoarrox*, we do not accept the transfer suggested by BOUCHER (2006) and transfer the species back to its original genus. *Pseudoarrox sensu novo* includes: *Pse. karreni* (type species) and *Ps. caldasi comb.n.* Its distribution is now extended to the mountains of Costa Rica and Panama, and the biogeographic region of Chocó in western Colombia.

## 5. Conclusions

The presented phylogeny resolved the phylogenetic relationships between the two tribes present in the New World. This had been a long-standing question for the group. Furthermore, our data revealed taxonomic issues involving generic concepts within Passalini and Proculini and provided suggestions for how to resolve them. The resolution of such problems will refine some of the already interesting evolutionary and biogeographic patterns observed in the family. For example, *Vindex* and *Pseudacanthus*, two montane restricted genera of Proculini, occur on both sides of the Isthmus of Tehuantepec.

However, our analyses indicate that both genera are not monophyletic and revealed geographically-cohesive clades for each genus, revealing higher degrees of endemism than previously thought. The phylogeny presented in this work therefore resolves longstanding taxonomic questions involving Neotropical Passalidae (especially Proculini) and is useful for interpreting patterns of evolution in Passalidae. However, the findings in this work point to the necessity of more robust analyses for the genera found non-monophyletic.

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## Authors' contributions

C.F.B-B has done conceptualization, methodology, formal analysis, investigation, data curation, writing-original draft preparation, visualization, project administration. L.J-G. and D.D.M. have done conceptualization, methodology, investigation, writing-review and editing together.

## Electronic Supplement Files

at <http://www.senckenberg.de/arthropod-systematics>

ASP\_78-2\_Beza-Beza\_et\_al\_Electronic\_Supplements.zip

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**File 1:** Supplementary Table S1.docx — **Table S1.** Specimen Data, and Genbank accession numbers. **Symbols:** \* sequences obtained from BEZA-BEZA et al. 2019.

**File 2:** Morphological Matrix.xlsx

**File 3:** Partition Strategies.docx

**File 4:** Supplementary Figures.pdf — **Fig. S1.** Majority Rule Consensus cladogram from 1000 Bootstrap replicates ML analysis, for CAD. Number above branches represent bootstrap support values. — **Fig. S2.** Majority Rule Consensus cladogram from 1000 Bootstrap replicates ML analysis, for WGS. Number above branches represent bootstrap support values. — **Fig. S3.** Majority Rule Consensus cladogram from 1000 Bootstrap replicates ML analysis, for 28S. Number above branches represent bootstrap support values. — **Fig. S4.** Majority Rule Consensus from 1000 Bootstrap replicates ML analysis, for 28S\_masked. Number above branches represent bootstrap support values. — **Fig. S5.** Majority Rule Consensus tree from 1000 Bootstrap replicates ML analysis of the Concatenated alignment. Number above branches represent bootstrap support values. — **Fig. S6.** Majority Rule Consensus from 1000 Bootstrap replicates ML analyses of the reduced concatenated alignment. Number above branches represent bootstrap support values. — **Fig. S7.** Majority Rule Consensus tree from 1000 Bootstrap replicates ML analysis of the Total Evidence alignment. Number above branches represent bootstrap support values. — **Fig. S8.** Majority rule consensus Bayesian tree with posterior probabilities support thicken lines of Concatenated alignment. — **Fig. S9.** Majority rule consensus Bayesian tree with posterior probabilities support thicken lines of reduced Concatenated alignment. — **Fig. S10.** Majority rule consensus Bayesian tree with posterior probabilities support thicken lines of Total Evidence alignment. — **Fig. S11.** Majority rule consensus Bayesian tree with posterior probabilities support thicken lines of Reduced Total Evidence alignment. — **Fig. S12.** Majority rule consensus tree of 1000 bootstrap replicate parsimony analysis of the Concatenated alignment. Number above branches represent bootstrap support values. — **Fig. S13.** Majority rule consensus tree of 1000 bootstrap replicate parsimony analysis of the reduced Concatenated alignment. Number above branches represent bootstrap support values. — **Fig. S14.** Majority rule consensus tree of 1000 bootstrap replicate parsimony analysis of the Total Evidence alignment. Number above branches represent bootstrap support values. — **Fig. S15.** Majority rule consensus tree of 1000 bootstrap replicate parsimony analysis of the Reduced Total Evidence alignment. Number above branches represent bootstrap support values. — **Fig. S16.** Strict consensus tree of the most parsimonious trees from parsimony analysis of the Concatenated alignment. — **Fig. S17.** Strict consensus tree of the most parsimonious trees from parsimony analysis of the reduced Concatenated alignment. — **Fig. S18.** Strict consensus tree of the most parsimonious trees from parsimony analysis of the Total Evidence alignment. — **Fig. S19.** Strict consensus tree of the most parsimonious trees from parsimony analysis of the Reduced Total Evidence alignment.

**File 5:** Supplementary Table S2.docx — **Table S2.** Tree scores from Parsimony analyses of the four combine alignments.

**File 6:** Monophyletic Proculini genera.docx