WILEY

Cladistics

Cladistics (2022) 1–26

10.1111/cla.12510

Female terminalia morphology and cladistic relations among Tok-Tok beetles (Tenebrionidae: Sepidiini)

Marcin J. Kamiński[a,b]*, Olivia M. Gearner[b], Marcin Raś[a], Elliot T. Hunsinger[b], Amelia L. Smith[b], Paloma Mas-Peinado[c,d], Jennifer C. Girón[b,e], Aleksandra G. Bilska[a], Werner P. Strümpher[f], Christopher C. Wirth[b], Kojun Kanda[g], Kali Swichtenberg[b], Dariusz Iwan[a] and Aaron D. Smith[b]

[a]*Zoological Museum, Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 64, 00-679, Warszawa, Poland;* [b]*Purdue University, Department of Entomology, 901 W. State Street, West Lafayette, IN, 47907, USA;* [c]*Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid, Spain;* [d]*Centro de Investigación en Biodiversidad y Cambio Global CIBC-UAM, Facultad de Ciencias, Universidad Autónoma de Madrid, c/Darwin 2, 28049, Madrid, Spain;* [e]*Natural Science Research Laboratory, Museum of Texas Tech University, Lubbock, TX, USA;* [f]*Ditsong National Museum of Natural History, 432 Paul Kruger Street, Pretoria 0002, Republic of South Africa;* [g]*USDA Systematic Entomology Laboratory, c/o Smithsonian Institution, National Museum of Natural History, Washington, DC, USA*

Accepted 20 May 2022

Abstract

Tok-tokkies are one of the most iconic lineages within Tenebrionidae. In addition to containing some of the largest darkling beetles, this tribe is recognized for its remarkable form of sexual communication known as substrate tapping. Nevertheless, the phylogenetic relationships within the group remain poorly understood. This study investigates the usefulness of female terminalia morphology for delimiting Sepidiini and reconstructing relationships among it. Data on the structure of the ovipositors, genital tubes and spicula ventrali have been generated for >200 species representing 28 Pimeliinae tribes. This dataset was used in a comparative analysis at the subfamilial level, which resulted in recognition of several unique features of tok-tokkie terminalia. Additionally, new features linking phenotypically challenging tribes also were recovered (Cryptochilini + Idisiini + Pimeliini). Secondly, 23 characters linked to the structure of female terminalia were defined for tok-tok beetles. Cladistic analysis demonstrates the nonmonophyletic nature of most of the recognized subtribes. The morphological dataset was analysed separately and in combination with available molecular data (CAD, Wg, cox1, cox2, 28S). All obtained topologies were largely congruent, supporting the following changes: Palpomodina Kamiński & Gearner **subtr.n.** is erected to accommodate the genera *Namibomodes* and *Palpomodes*; *Argenticrinis* and *Bombocnodulus* are transferred from Hypomelina to Molurina; 153 species and subspecies previously classified within *Psammodes* are distributed over three separate genera (*Mariazofia* Kamiński **nom.n.**, *Piesomera* **stat.r.**, *Psammodes* **sens.n.**). *Psammodes sklodowskae* Kamiński & Gearner **sp.n.** is described. Preliminary investigation of the ovipositor of *Mariazofia basuto* (Koch) **comb.n.** was carried out with the application of microcomputed tomography, illuminating the muscular system as a reliable reference point for recognizing homologous elements in highly modified ovipositors.

Key words: systematics, classification, ovipositor, microcomputed tomography

Introduction

The morphology of the female terminalia is considered essential in reconstructing phylogenetic relationships among darkling beetles (Doyen, 1994; Doyen

*Corresponding author:
E-mail address: kaminskientomo@gmail.com

and Tschinkel, 1982; Iwan and Kamiński, 2016; Tschinkel and Doyen, 1980), yet detailed comparative analyses of the ovipositors and/or genital tubes even at tribal levels are scarce (e.g. Banaszkiewicz, 2006; Bouchard and Yeates, 2001; Matthews and Bouchard, 2008). Furthermore, many recently published descriptions of new species and genera within Tenebrionidae lack data on female terminalia (Ferrer, 2002; Fouquè, 2013; Kamiński, 2011; Kamiński et al., 2021). This discordance is probably driven by multiple factors, from practical ones such as the relative complexity of dissecting the female terminalia, which could often lead to partial specimen destruction, to the subjective opinions that the ovipositors/genital tubes are not informative among the studied groups (Kamiński pers. obs.). Furthermore, the morphology of the female genitalia of many species of darkling beetles is not easily interpreted under the current terminological framework (Kamiński et al., 2021). Namely, it is assumed that the plesiomorphic ovipositors of darkling beetles are composed of four coxite lobes arranged in a single line, with a large gonostylus situated on top of the apical lobe. Although this bauplan is the basis for defining homology among the whole family, it can be problematic in the case of some strongly modified groups (Doyen and Tschinkel, 1982; Tschinkel and Doyen, 1980).

The Sepidiini Eschscholtz (Tenebrionidae: Pimeliinae), commonly known as the tok-tok beetles, are a good example of a group with strongly modified ovipositor (Kamiński et al., 2019). Most of the known species of this group possess 3-lobed coxites, whereas the gonostyli are absent (Kamiński et al., 2021). Although Sepidiini were included in the only phylogenetic study available for the subfamily (Doyen, 1994) that has considered data on female terminalia, the ovipositor structure of the tribe remained insufficiently investigated, and the paper lacked direct indication of homologous structures (figs 101 and 104 in Doyen, 1994). Only recently, Kamiński et al. (2021) had incorporated the 3-lobed ovipositor of tok-tok beetles into the tenebrionid terminological framework, and highlighted the potential value of some characters for intertribal classification promoting this study.

Tok-tok beetles are a morphologically diverse tribe of darkling beetles, composed of >1000 species distributed throughout the African continent and Southern Palaearctic (Kamiński et al., 2019). As a consequence of their unique tapping behaviour, which is assumed to be a form of sexual communication (Gearner et al., 2021; Lighton, 1987, 2019), they are one of the most conspicuous beetle groups in the region (Matthews et al., 2010). To date, 59 genera of Sepidiini have been described (Gearner et al., 2021). However, recently conducted molecular-based studies have revealed the need to designate several previously unrecognized phylogenetic groups at the genus level—suggesting that the current number of genera is an underestimation (Gearner et al., 2021; Kamiński et al., 2021). Owing to the interesting morphology and the large body size of some of the species, tok-tok beetles attract the attention of collectors; however, taxonomic contributions to the group are still scarce. The main reason for this is the lack of clear morphological definitions for the majority of taxa (Penrith, 1986), which concerns all taxonomic levels—from species to subtribes (Gearner et al., 2021). Furthermore, the aforementioned phylogenetic studies of tok-tok beetles were focused mainly on Molurina Solier and left the remaining subtribes scarcely sampled or not included at all (i.e. Sepidiina Eschscholtz). As a result, Sepidiini remains a taxonomically challenging group.

The morphological variability of female terminalia of tok-tok beetles remains almost completely uninvestigated. Although Doyen (1994) analysed terminalia of the representatives of four of five currently recognized subtribes (i.e. Hypomelina Koch: *Brinckia* Koch and *Iugidorsum* Louw; Molurina: *Moluris* Latreille and *Phrynocolus* Lacordaire; Sepidiina: *Sepidium* Fabricius; and Trachynotina Koch: *Cyrtoderes* Dejean and *Somaticus* Hope), he did not mention any major differences among them, besides uniqueness of the ovipositors of *Sepidium*. He concluded that the following features can be used to distinguish Sepidiini from the remaining Pimeliinae: (1) oblique baculus of coxites (lateral view); (2) elongated paraprocts; (3) strongly sclerotized 4th lobes of coxites; and (4) reflexed arm of spiculum ventrale. This study aims to test these conclusions and evaluate the usefulness of the morphology of the female terminalia for phylogenetic studies within the tok-tok beetles by combining dense taxon sampling and the application of cladistic methods. Furthermore, a detailed investigation of the muscular system of the ovipositor with the usage of the microcomputed tomography (microCT) has been performed on representatives of tok-tok beetles (*Mariazofia basuto* **comb.n.**). The presented data can be implemented in future projects as an additional reference point for establishing homology within Pimeliinae, especially between strongly modified ovipositors.

Material and methods

Workflow design

In order to accomplish the above stated goals, the three following levels of analyses were conducted.

Comparative study of female terminalia morphology within Pimeliinae. This analysis aimed to test the reliability of the diagnostic characters proposed by Doyen (1994) for the

sepidinoid beetles. Although the works of Doyen (1994) and Watt (1992) provide valuable insights on the morphology of the female terminalia in the context of the whole family Tenebrionidae, they lack data on several crucial structures, such as proctigers, which hereby turned out to be extremely useful for taxonomic considerations within tok-tok beetles. In order to fill those gaps, and explore further possibilities utilizing female terminalia characters to inform relationships and classification of pimeliinae, dissections of adult female specimens were performed, and the morphology of the ovipositors, genital tubes, spicula ventrali and proctigers was studied for the following pimelinoid tribes: Adelostomini (13 specimens), Adesmiini (6), Akidini (2), Anepsiini (1), Asidini (9), Branchini (1), Cnemodinini (1), Coniontini (1), Cryptochilini (1), Cryptoglossini (2), Edrotini (2), Elenophorini (3), Epitragini (1), Erodiini (3), Evaniosomini (1), Idisiini (1), Leptodini (1), Nycteliini (3), Nyctoporini (1), Pimeliini (4), Praociini (4), Sepidiini (126), Stenosini (2), Tentyriini (5), Thinobatini (1), Trilobocarini (1), Vacronini (1) and Zophosini (2). Taxonomic details on dissected taxa are presented in Appendix S1. In the majority of cases the species analysed here are not aligned with the ones included by Doyen (1994); the accumulated material extends our understanding of morphological variability of female terminalia within the above-stated Pimeliinae tribes.

Because one of the main goals of this study is to examine the diversity of female terminalia forms in Sepidiini, this tribe was the most intensively sampled group. Specimens representing 35 genera and all five currently recognized subtribes were included in the analysis (Appendix S1). Sepidiina is the most under-represented subtribe—two of eight known genera are included (Kamiński et al., 2019). This is mainly due to the lack of unambiguous diagnostic characters for the majority of currently recognized genera and the absence of reliable identification tools for species-level taxa. However, at the same time Sepidiina is the most characteristic and well-defined lineage within tok-tok beetles (Koch, 1958). For the remaining subtribes, the majority of the genera, which were not included in this study, currently are represented by species known only from relatively small series, with no female specimens present in the studied materials (e.g. *Miripronotum* Louw, *Psammoryssus* Kolbe, *Stridulomus* Koch, *Triangulipenna* Louw and *Uniungulum* Koch). For diverse genera such as *Ocnodes* Fåhraeus, *Psammodes* Kirby and *Somaticus* Hope, species that spanned the morphological variability of these genera were chosen to test the monophyly of these groups. (Appendix S1).

Dissections were conducted on material from the following collections: California Academy of Sciences, San Francisco, USA (CASC); Ditsong National Museum of Natural History, Pretoria, South Africa (TMSA); Museum and Institute of Zoology of the Polish Academy of Sciences (MIZ PAS); Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN-CSIC); Purdue University, West Lafayette, USA (PERC); and United States National Museum of Natural History, Washington DC, USA (USNM).

In order to isolate the terminalia and associated structures, the abdomen of specimens to be studied were removed and cleared overnight in 10% potassium hydroxide. Reproductive systems were dissected using entomological needles and scalpels. To increase the visibility of certain features, some samples were stained with Chlorazol Black dissolved in glycerin or water. In the majority of cases the dissected structures then were transferred to a drop of glycerin on a microscope slide for imaging. However, in some cases the ovipositors were dried and later photographed in order to increase contrast and visualize sutures between subsequent coxite lobes. The analysed material was preserved in microtubes filled with glycerin and pinned beneath the specimens. Dissecting procedures are in Kamiński (2021a). Images of morphological details were taken using different imaging systems at MIZ PAS, TMSA and PERC. Raw images can be downloaded from Harvard Dataverse (Kamiński, 2021b).

Nomenclature follows that of Bouchard et al. (2011, 2021).

Cladistic analysis of female terminalia features within Sepidiini. In order to assess the phylogenetic informativeness of female terminalia morphology within tok-tok beetles, a cladistic analysis based solely on features linked to ovipositor, genital tubes, spiculum ventrale and proctiger was performed. Dissections described in the previous section were used to code data into a character matrix (MorphoBank http://morphobank.org/permalink/?P4101). Morphological terminology follows Doyen (1966), Tschinkel and Doyen (1980) and Kamiński et al. (2021). To avoid potential inconsistency problems associated with analysis of large numbers of taxa (Kim, 1996), a preselection process to incorporate species or operational taxonomic units (OTUs) representing the morphological diversity of Sepidiini was undertaken. The process was driven by the observed variability of female terminalia and available molecular data (Gearner et al., 2021; Kamiński et al., 2021). For example, all analysed representatives of the *Dichtha* clade (see Kamiński et al., 2021) were observed to possess similarly structured ovipositors (3-lobed; merged lobe 1 and 2 strongly emarginate basally), female genital tubes (multibranched spermatheca), spicula ventrali (Y-shaped) and proctigers (widely indented medially), and therefore only two of 11 studied species of this group—*Amiantus octocostatus* Péringuey and *Dichtha cubica* (Guérin-Méneville)—were included in the cladistic analysis. Additionally, as molecular data for selected species already were available, the selected OTU also could be included in the combined phylogenetic analysis of morphological and molecular data (see next section). *Stips gebieni* (Hesse) of Adelostomini was used as an outgroup. According to Doyen (1994), adelostomid beetles share some unique features of the female terminalia with Sepidiini (e.g. spiculum ventrale with reflexed arms), which is used here as a justification for the outgroup selection. Moreover, preliminary phylogenomic data renders Adelostomini sister to Sepidiini (Smith pers. comm.). Furthermore, *Stips* possesses four visible coxite lobes, which enables direct comparisons with the sepidiinoid terminalia. However, the ovipositors of many other Pimellinae are strongly modified, which at this point disrupts clarity of any potential phylogenetic conclusions within Sepidiini. Therefore, representatives of other investigated Pimeliinae tribes were not included in the cladistic analysis.

The data matrix was created in Mesquite v.3.61 (Maddison and Maddison, 2019) and later exported to TNT v.1.5 (Goloboff and Catalano, 2016) and MrBayes v.3.2.7 (Ronquist et al., 2012) for phylogenetic analyses. Maximum parsimony analyses were implemented with implicit enumeration, with equal weight assigned to all characters (states treated as unordered), and conducted locally. Branch support was determined using Bremer supports (Bremer, 1994) in TNT. The performance of the characters in the parsimony analysis was verified by calculating the consistency (ci) and retention indices (ri) in Mesquite, whereas the values of the consistency (CI) and retention (RI) indices for the whole matrix were obtained in TNT by using the *stats.run* script. Bayesian analyses were run through the CIPRES portal (Miller et al., 2010) using the Mkv + G model (Lewis, 2001) and flat priors. Two searches were performed with four chains each for 20 million generations with a 0.5 burn-in. Trees were sampled every 1000 generations. Branch support for Bayesian analyses was inferred based on the posterior probability (PP) distribution of tree topologies. Posterior probabilities were mapped onto the strict consensus cladogram from the parsimony analyses. Resulting trees were studied in Winclada 1.61 (Nixon, 2002) and FigTree 1.4.4 (Rambaut, 2009).

Combined analysis of female terminalia traits and molecular data. The majority of the molecular dataset was assembled from previous studies (Gearner et al., 2021; Kamiński et al., 2021). Seven additional species were sequenced to increase

subtribal coverage: *Somaticus* sp. (TB17016) [Trachynotina], *Namibomodes* sp. (TB15893), *Palpomodes physopterus* (Gebien) (TB22641), *Synhimba* sp. 1 (TB20962), *Synhimba* sp. 2 (TB22637) [all representing Oxurina], *Trachynotidus rufozonatus* (TB23057) and *Trachynotidus* sp. (TB22658) [representing Hypomelina]. *Stenocara* sp. (Adesmiini) and *Stips* sp. (Adelostomini), both members of the subfamily Pimeliinae (Bouchard et al., 2011), were used as outgroups.

Six nonoverlapping gene regions from five loci were targeted: nuclear protein coding loci—the carbamoyl-phosphate synthetase domain of *rudimentary* (CAD) (810 bp) and *wingless* (Wg) (435 bp); mitochondrial protein coding loci—cytochrome oxidase subunit I Jerry/Pat (cox1 JP) (828 bp) and barcoding (cox1 BC) (657 bp), and cytochrome oxidase subunit II (cox2) (681 bp); and nuclear ribosomal locus—D1–D3 region of 28S (1042 bp). GenBank accession numbers are presented in Appendix S1.

For newly sequenced taxa, DNA was extracted from ethanol-preserved specimens (95% EtOH) using DNeasy Blood & Tissue Kits (Qiagen, Germantown, MD, USA) following the manufacturer's protocols. Extractions were performed on soft tissue from the head or thorax; no cuticle was ground during the extraction process. Voucher specimens are deposited in the PERC. Polymerase chain reactions (PCRs) were performed using ExTaq (TaKaRa, Moutain View, CA, USA) with thermocycler protocols and primers described in Kanda et al. (2015). PCR cleanup, quantification and sequencing were performed by the University of Arizona's Genetics Core Facility. Cleaned PCR products were sequenced on an Applied Biosystems 3730XL DNA Analyser (Foster City, CA, USA). Assembly of chromatograms was performed as described in Kamiński et al. (2021).

Protein-coding genes were manually aligned in Mesquite. 28S was aligned with MAFFT v.7.130b (Katoh and Standley, 2013) using the L-INS-I method. All alignments were concatenated into a single matrix (4453 bp) for phylogenetic analyses (MorphoBank http://morphobank.org/permalink/?P4101). The following species were represented exclusively by morphological data: *Argenticrinis lossowi* (Koch), *Oxura setosa* Kirby, *Psammodes sklodowskae*, *Vieta muscosa* (Gerstaecker) and *Vieta speculifera* Gebien.

Combined (DNA + morphological data), as well as molecular-only, Bayesian analyses were performed in MrBayes v.3.2.7 on XSEDE via CIPRES. Data partitions and models of sequence evolution were assessed with Partitionfinder v.2.0 (Lanfear et al., 2017) implemented on CIPRES. Only models for "mrbayes" were considered. The search was conducted using greedy searches and the Bayesian information criteria (BIC). For the morphological partition the Mkv model (Lewis, 2001) with gamma-distributed rate variation was implemented. For each, combined and molecular-only, analysis, two independent runs with four chains were performed. Analyses were run for 40 million generations, and parameters were sampled every 1000 generations. A burnin fraction of 25% was used, and convergence was determined by the standard deviation (SD) of the split frequencies; runs were considered to have converged at <0.01. Nodes with PP > 0.95 were considered strongly supported, with PP = 0.90–0.94 moderately supported and with PP = 0.70–0.89 weakly supported.

The performance of the characters in the combined analysis was verified by calculating the consistency (ci) and retention indices (ri) in Mesquite. Furthermore, character optimization (Fig. 7) has been carried out with the maximum parsimony method implemented in the same software.

Ovipositor musculature. Based on the results of the preceding analyses, *Mariazofia basuto* (formerly *Psammodes basuto*, see Taxonomy section) was selected to represent tok-tok beetles. This taxon possesses 3-lobed coxites (3:1) and well-developed c4 lobes (12:1), characters which are present in the vast majority of the examined Sepdiini species (MorphoBank http://morphobank.org/permalink/?P4101). Furthermore, the large body size of *M. basuto* (*c.* 20.0 mm) and its ovipositor facilitated the digital segmentation process.

The ovipositor, with primary focus on coxites, of the studied EtOH-preserved female specimen was manually segmented without application of 10% potassium hydroxide. During this process the proctiger plate was detached. Subsequently the dissected ovipositor was dried with hexamethyldisilazane (HMDS) (Bray et al., 1993), and placed in a PCR tube (0.2 ml) for microCT scanning.

MicroCT analyses were performed at MIZ PAS using a SkyScan 1172 system (SkyScan, 2008). During the scanning processes, the X-ray source was set to a voltage of 40 kV and a current of 250 mA. As a result of the elongate shape of the analysed ovipositor, the specimen was scanned using four oversized scans (Iwan et al., 2015; Raś et al., 2018); image pixel size equals 1.66 μm. Reconstruction was performed using NRecon v.1.6.4.7. Primary segmentation was conducted in CTAn v.1.18.4.0+. Specimen anatomy was later investigated in Blender v. 2.91.0.The resulting 3D model is available online (Raś, 2021).

This investigation of coxities musculature is, to the authors' knowledge, the first within Coleoptera (Doyen, 1966; Lawrence et al., 2011; Medvedev, 2001). In the absence of previous nomenclature for particular muscle groups, new terminology is introduced within the present paper (see the Results).

Results

In order to clarify the characters used in the following phylogenetic analyses, a description of the sepidiinoid female terminalia is given here. The ovipositor is the most distal structure of the female reproductive system. It can be fully extended from the body and is composed of two sets of paired sclerites (Fig. 1b): the distal, more strongly sclerotized sclerites are referred to as the coxites (Fig. 1b, c), and the proximal, more membranous sclerites the paraprocts (Fig. 1b, p). Each paraproct also bears a longitudinal sclerotization known as the baculus of the paraproct (Fig. 1b, bp). It should be noted that Doyen (1966) referred to the paraprocts and coxites as valvifer 1 and 2, respectively. However, in subsequent papers he used the term valvifer to refer only to the proximal plate of the coxite and used the term paraproct to refer to the proximal pair of sclerites of the ovipositor (Tschinkel and Doyen, 1980), which is the terminology followed here. In Tenebrionidae, the coxites are usually composed of four visible lobes in ventral view, named c1 to c4 from proximal to distal. However, it is hypothesized that the basal plate of Sepidiini is the product of the fusion of the c1 and c2 lobes (Kamiński et al., 2021). Therefore, here the coxite plates are referred to as c1 + c2 (basal plate), c3, and c4 (apical plate) (Fig. 1b). The apical plate bears latero-ventral sensory setae (ss), whereas the gonostyli are absent. The base of each c1 + c2 plate is strongly sclerotized, forming a transverse or oblique baculus of the coxite (Fig. 1b, bc) that directly articulates to the apex of the

corresponding baculus of the paraproct (Fig. 1b). Each paraproct is proximally attached to abdominal segment 8 by a flexible membrane (Fig. 1c). In Sepidiini, each paraproct (Fig. 1b) is composed of inner (ip) and outer (op) plates separated by the longitudinal baculus (bp). In some other groups of darkling beetles, for example Blaptinae Leach, the inner plate can be largely reduced or absent (Fig. 1e). The anus (an) is located dorsal to the ovipositor, and both these structures are shielded dorsally by the proctiger (Fig. 1b, pr). The proctiger is equipped with a pair of longitudinal lateral baculi (bpr) along the basal two thirds of its length (Fig. 1b). Within Sepidiini the apical margin of the proctiger is variable in shape (Fig. 5q-s). Ovipositor, anus, and proctiger, when retracted within the body cavity, are apically surrounded by abdominal segment 8 (Fig. 1a, s8). Sternite VIII usually forms a single plate (Fig. 5t-w), but sometimes it is formed by two lateral plates (hemisternites) medially joined together by membrane (e.g. Fig. 2f, l). Proximal to the sternite VIII (or hemisternites), a second pair of lateral sclerites form the spiculum ventrale (Fig. 1a, sv). In the majority of the studied Pimeliinae species, including Sepidiini, the spicula ventralia are proximally fused and thus can be divided into arms (distal) and base (proximal). The presence of an unfused base is exclusively reported in the case of studied Cryptochilini, Idisiini, and Pimeliini (Fig. 3d, f, l). Medially, the ovipositor possesses the vulva, which is proximally linked to a sac-like vagina (Fig. 1f, vg). The vagina of Sepidiini is devoid of sclerites or spikes. During the present investigation no eggs or larvae were found inside of the vaginas of the studied specimens. Although some Afrotropical lineages of darkling beetles were proven to be ovoviviparous (Fig. 1d), this mode of reproduction is unlikely for Sepidiini mainly as a consequence of the elongation of the ovipositor. The presence of paired oviducts was found in Sepidiini (Fig. 1f, ov1, ov2). However, both oviducts are merged into a single canal before entering the vagina distally

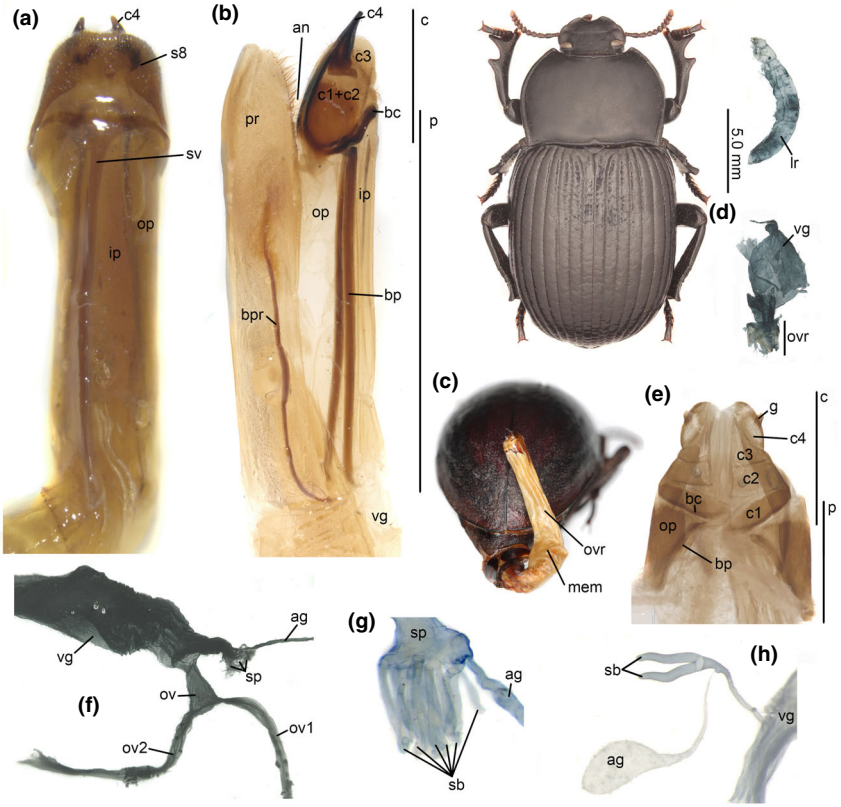


Fig. 1. Female terminalia morphology of selected species representing Tenebrionidae. Ovipositor in (a, c, e) ventral and (b) lateral views; (d) adult, larva, and female terminalia of ovoviviparous darkling beetle species; (f–h) genital tubes. (a) *Trachynotinus rufozonatus* (Sepidiini); (b) *Huilamus welwitschi* (Sepidiini); (c) *Tibiocnodes lucidus* (Sepidiini); (d, e) *Sebastianus projectus* (Platynotini); (f) *Psammoryssus titanus* (Sepidiini); (g) *Psammodes sklodowskae* (Sepidiini); (h) *Lepidochora* sp. (Adelostomini). Abbreviations: ag: accessory gland, an: anus, bc: baculus of coxities, bp: baculus of paraproct, bpr: baculus of proctiger, c: coxities, c1-c4: subsequent plates of coxites (c1+c2 fused plates 1 and 2), g: gonostylus, ip: inner plate of paraproct, lr: larva, op: outer plate of paraproct, mem: flexible membrane, ov: oviduct (median/merged channel), ovr: ovipositor, ov, ov1/2: oviducts, p: paraproct, pr: proctiger, s8: sternite 8, sb: ramifications of spermatheca sp: spermatheca, ss: sensory setae, sv: spiculum ventrale (composed of arms and base), vg: vagina.

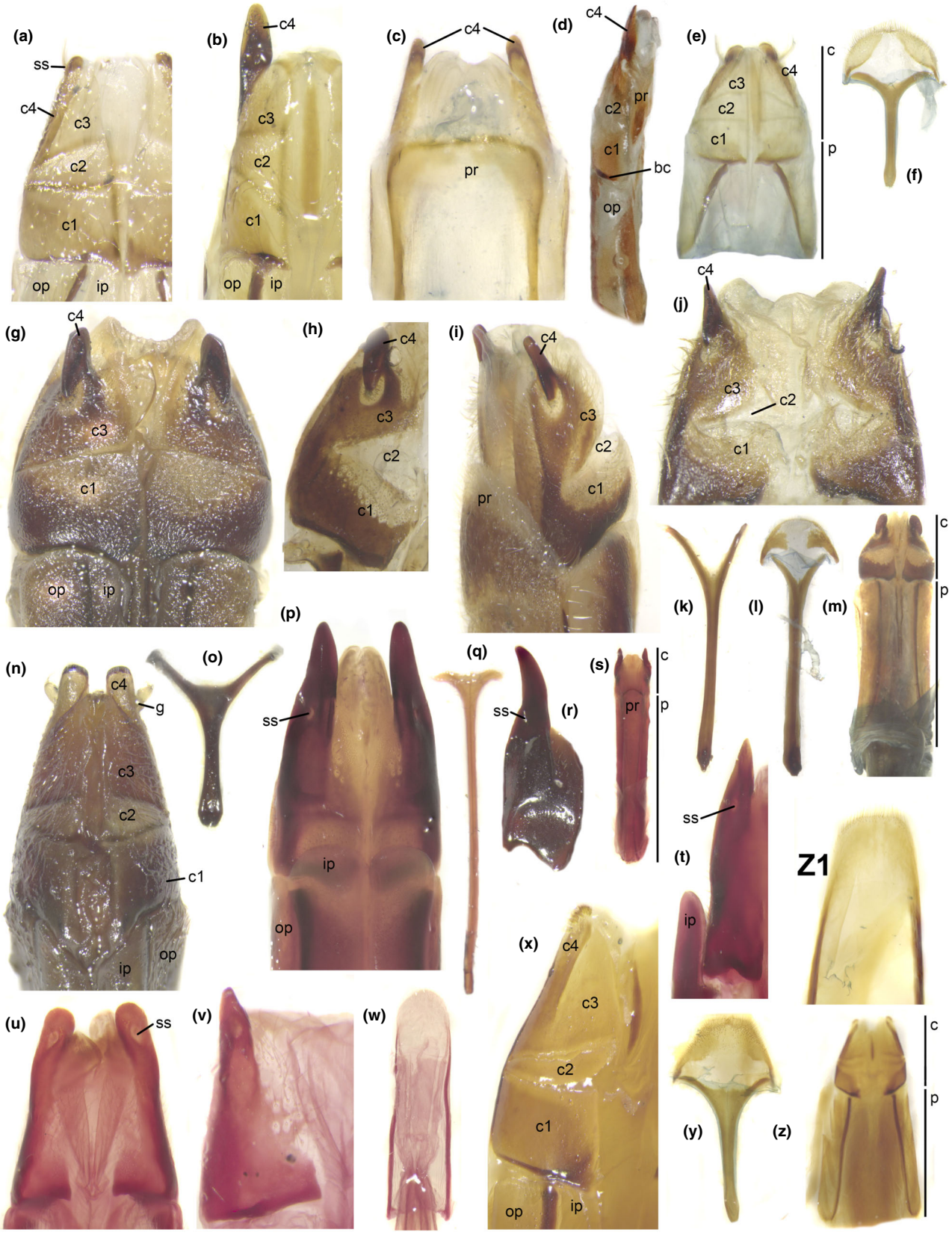

(a)
ss
c4
c3
c2
c1
op
ip
(b)
c4
c3
c2
c1
op
ip
(c)
c4
pr
(d)
c4
pr
c2
c1
bc
op
(e)
c4
c3
c2
c1
c
p
(f)
(g)
c4
c3
c1
op
ip
(h)
c4
c3
c2
c1
(i)
c4
c3
c2
c1
pr
(j)
c4
c3
c2
c1
(k)
(l)
(m)
c
p
(n)
c4
g
c3
c2
c1
ip
op
(o)
(p)
ss
ip
op
(q)
(r)
ss
(s)
c
pr
p
(t)
ss
ip
Z1
(u)
ss
(v)
(w)
(x)
c4
c3
c2
c1
op
ip
(y)
(z)
c
p

Fig. 2. Female terminalia morphology of selected species representing Pimeliinae. Ovipositor in (a, b, e, g, h, j, m, n, p, r-v, x, z) ventral, (c) dorsal, and (d, i) lateral views; (f, k, l, o, q, y) spiculum ventrale; (w, z1) proctiger. (a) *Lycantrope* sp. (Adelostomini); (b, d, f) *Stips costata* (Adelostomini); (c) *Lepidochora* sp. (Adelostomini); (e) *Gophanus* sp. (Adelostomini); (g, k, m) *Epiphysa ciliata* (Adesmini); (h, i, j, k) *Stenocara longipes* (Adesmini); (n, o) *Akis spinosa* (Akidini); (p) *Stenomorpha subvittta* (Asidini); (q, r) *Stenomorpha obovata* (Asidini); (s) *Stenomorpha suturalis* (Asidini); (t) *Branchus* sp. (Branchini); (u, v, w) *Eusattus reticulatus* (Coniontini); (x, y, z, z1) *Asbolus verrucostus*(Cryptoglossini). For the abbreviations see Fig. 1.

(Fig. 1f, ov). The spermatheca (sp) is present near the proximal end of the vagina. In Sepidiini the spermatheca is composed of several smaller tubes (multibranched spermatheca; Fig. 1g, sb), whereas in Adelostomini it splits over into two smaller ducts (Fig. 1g, h). Laterally, the spermatheca connects with the spermathecal accessory gland (Fig. 1g, h, ag). Together, vagina, oviducts, spermatheca and spermathecal accessory gland form the genital tubes. Table 1 provides logical definitions for each of the structures here discussed, along with their term equivalency in the ontology for the Anatomy of the Insect SkeletoMuscular System (AISM; Girón et al., 2021).

Comparative analysis of female terminalia within Pimeliinae

Sepidiinoid beetles differ from the remaining investigated Pimeliinae tribes by having (Table 2): laterally flattened ovipositors (Fig. 1b) and elongate proctigers (Fig. 1b) with the apical margin variably shaped (apical margin weakly sclerotized and rounded in other investigated tribes). These character states were not observed in any other Pimeliinae examined (Figs 2–4). Additional reliable diagnostic characters for Sepidiini are: medially elevated base of paraproct (Fig. 5a,b, d–f, h–o) (feature shared by Asidini, Branchini, Erodiini, Thinobatini, Vacronini and Zophosini); medially elevated basal margin of coxite plate c1 (Fig. 5e, f) (shared by Erodiini, Thinobatini, Vacronini, and Zophosini); and ventrally oriented coxite plate c4 (Fig. 5k–m) (feature co-occurring in Erodiini and *Stips* and *Lepidochora* of Adelostomini). The elongation of the paraprocts (Table 2) and sclerotization of coxite plate c4 seem to be common among the investigated pimeliinoid tribes (Figs 2–4), although these features can be highly variable at the tribal levels (e.g. Adelostomini; Fig. 5a–e).

The presence of well-developed gonostyli was observed only in Akidini, Elenophorini, Nyctoporini and Vacronini (Table 2). Coxites divided into four lobes seem to be relatively rare among pimeliinoid beetles and were reported for selected Sepidiini, Adelostomini, Akidini, Elenophorini, Erodiini, Nyctoporini and Vacronini (e.g. Figs 2n, 3m). Likewise, fully fused lobes also are infrequent (Table 2). The coxite plate c2 is membranous and folded under the coxite plate c1 in Adesmiini, Evaniosomini, Nycteliini, Tentyriini, Trilobocarinim and Zophosini (Fig. 2g–j). It can be uncovered by manual manipulation, unlike in other investigated species. Elongation of the base of the spiculum ventrale is present in the majority of the tribes (Table 2). The base is commonly fused with the exception of studied Cryptochilini, Idisiini and Pimelini (Fig. 3d, f, l). In Edrotini, Epitragini and Thinobatini the spiculum ventrale is missing the arms (Fig. 4d, i), whereas in Nycteliini the arms are relatively short when compared to the remaining tribes (Fig. 3q).

Cladistic analysis of female terminalia features within Sepidiini

Morphological matrix. A matrix of 23 characters was constructed for 47 OTUs representing all five currently recognized subtribes of Sepidiini. The complete dataset consists of 13 characters related to the ovipositor, five for the proctiger, four for the spiculum ventrale, and a single character for the genital tubes. Characters 1, 8, 13, 14, 15 and 20 are parsimony-uninformative within the tribe, but constitute the morphological definition of Sepidiini when compared with other lineages of Pimeliinae. The character states, values of ci/ri indexes for morphology-only and combined analyses are provided below, whereas the coded matrix is available at MorphoBank (http://morphobank.org/permalink/?P4101).

Ovipositor (1–13).

1. Ovipositor: (0) at least slightly flattened laterally (length/width ratio of coxites in lateral view >0.30); (1) flattened dorso-ventrally (length:width ratio of coxites in lateral view <0.45) (e.g. Fig. 2d).

All analysed Sepidiini representatives possess somewhat cylindrical ovipositors (1:0), which clearly distinguishes them from the tribe Adelostomini in which only dorso-ventrally flattened ovipositors were observed (see also Doyen, 1994; Kamiński et al., 2021).

2. Coxites: plates in lateral view: (0) narrow, flattened (*Stips gebieni*); (1) pear-shaped (Fig. 5l); (2) cylindrical (Fig. 5e, f). [morphology: ci = 0.67, ri = 0.93; combined: ci = 0.50, ri = 0.78].

A cylindrical lateral outline of the coxites occurs in the studied species of Hypomelina, Molurina and Sepidiina. State 1 was found in the majority of Oxurina (with an exception of *Pterostichula solitudo* Louw) and the whole Trachynotina.

3. Coxites: number of clearly visible plates in ventral view: (0) 3 plates (Fig. 5d); (1) 4 plates (Fig. 2a, b, e).

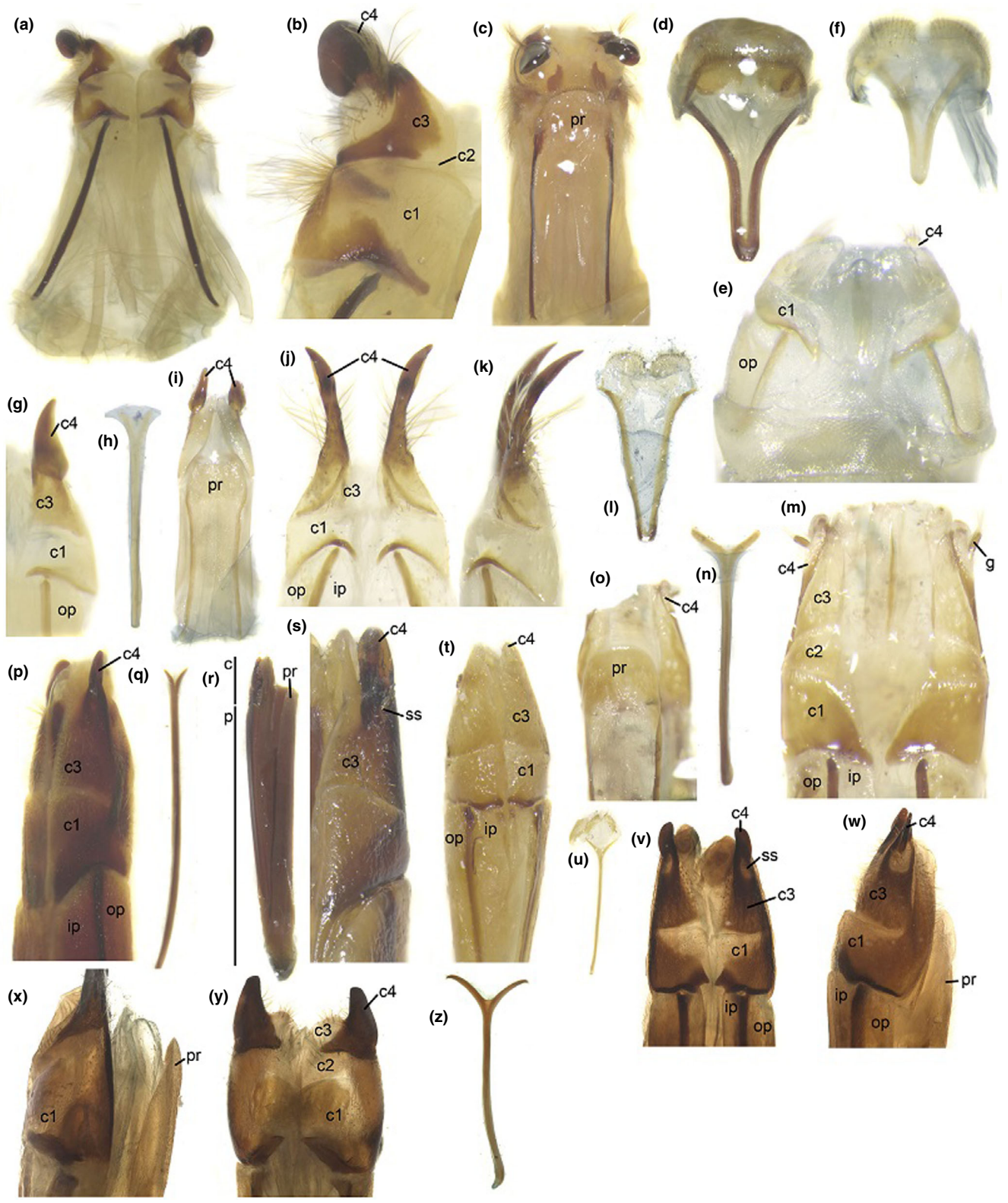


Fig. 3. Female terminalia morphology of selected species representing Pimeliinae. Ovipositor in (a, b, e, g, j, m, s, t, v, y) ventral, (c, i, o) dorsal, and (k, p, r, w, x) lateral views; (d, f, h, l, n, q, u, z) spiculum ventrale. (a–d) *Pimelia* (*Italomelia*) *rugulosa* sublaevigata (Pimeliini); (e, f) *Idisia* sp. (Idisini); (g–i) *Cnemodinus testaceus* (Cnemodinini); (j–l) *Calognathus chevrolati* (Cryptochilini); (m–o) *Nyctoporis carinata* (Nyctoporini); (p–r) *Nyctelia penai* (Nycteliini); (s) *Nyctelia rotundipennis* (Nycteliini); (t, u) *Archinamibia* sp. (Tentyrini); (v, w) *Tentyria wiedemanni* (Tentyrini); (x, z) *Erodius carinatus* (Erodiini); (Y) *Erodius lefranci* (Erodiini). For the abbreviations see Fig. 1.

Table 1
Terms and definitions of morphological structures of the female terminalia in Tenebrionidae and their equivalent terms with their corresponding unique identifiers in the AISM

Term	AISM term	Definition	AISM IRI
Female terminalia		The part of the postabdomen in insect females that contains the reproductive system	N/A
Female reproductive system		The part of the insect female that is composed of the genital tubes, the abdominal segment 8, and the ovipositor	N/A
Ovipositor	Ovipositor	The region of cuticle of the postabdomen that is peripheral to the distal portion of vagina (genital chamber)	AISM:0004077
Sclerite	Sclerite	The region of the insect cuticle that is less flexible than the neighbouring conjunctiva(e) (conjunctiva(e) that the sclerite is continuous with)	AISM:0000003
Coxites	Gonocoxa IX	The paired sclerite of the postabdomen that is attached to the abdominal tergite IX by the abdominal tergite IX-gonocoxa IX conjunctiva and is articulated with abdominal tergite IX.	AISM:0000200
Coxite plate		Each of the ventral subdivisions of the coxites.	N/A
Paraprocts	Gonocoxa VIII	The paired sclerite of the postabdomen that is attached to the abdominal sternite VII by the abdominal sternite VII-gonocoxa conjunctiva and is articulated with abdominal tergite IX. It is attached at the base of gonostylus VIII and adjacent to the gonopore.	AISM:0004068
Baculus of the paraproct		The longitudinal sclerotization along the paraproct	N/A
Gonostyli	Gonostylus IX	The region of the cuticle of the postabdomen that is paired and connected to the anterior region of the gonocoxa IX.	AISM:0004198
Latero-ventral sensory setae		The setae positioned latero-ventrally on the gonostylus IX	N/A
Baculus of the coxite		The transverse scleritization along the proximal margin of gonocoxa IX	N/A
Abdominal segment 8		The abdominal segment of the postabdomen that is attached to the posterior margins of the abdominal segment VII by the abdominal segment VII-abdominal segment VIII conjunctiva and to the anterior margin of the abdominal segment IX by the abdominal segment VIII-abdominal segment IX conjunctiva.	N/A
Membrane	Conjunctiva	The region of the insect cuticle that is more flexible than the neighbouring sclerite (s) (sclerite(s) that the conjunctiva is continuous with)	AISM:0000004
Inner plate of paraproct		The region of the gonocoxa VIII that is medial to the baculus of the paraproct	N/A
Outer plate of paraproct		The region of the gonocoxa VIII that is lateral to the baculus of the paraproct	N/A
Anus		The anatomical space on the postabdomen at the posterior margin of the digestive tract, connected to the epiproct and paraprocti.	AISM:0004197
Proctiger	Epiproct	The sclerite of the postabdomen that is attached to the posterior margin of the abdominal tergite X by the abdominal tergite X-epiproct conjunctiva.	AISM:0004060
Lateral baculi of the proctiger		The longitudinal sclerotization along the epiproct	N/A
Sternite eight	Abdominal sternite VIII	The abdominal sternite of the postabdomen that is attached to the posterior margin of the abdominal sternite VII by the abdominal sternite VII-abdominal sternite VIII conjunctiva. In males it is attached to the anterior margin of the abdominal sternite IX by the abdominal sternite VIII-abdominal sternite IX conjunctiva. In females it is attached to the anterior margin of the gonocoxa.	AISM:0004110
Spiculum ventrale		The sclerite that is laterally attached to the abdominal sternite VIII	N/A
Arms of spiculum ventrale		The paired distal regions of the spiculum ventrale	N/A
Base of spiculum ventrale		The fused proximal region of the spiculum ventrale	N/A
Genital tubes		The part of the insect female that is composed of the genital chamber, oviducts, spermatheca, spermathecal accessory gland, and in some cases bursa copulatrix	N/A
Vulva	Gonopore	The anatomical space in the postabdomen that marks the opening of the reproductive system.	AISM:0004075
Vagina	Genital chamber	The region of the cuticle that is connected to the oviduct, the bursa copulatrix and the gonocoxa VIII, and is adjacent to the gonopore.	AISM:0004069
Oviduct		Each of the tubes extending from the ovarioles to the main oviduct.	N/A
Common oviduct	Oviduct	The region of the cuticle that is continuous with the genital chamber.	AISM:0004083
Spermatheca	Spermatheca	The region of the cuticle that is part of the oviduct and connected to the bursa copulatrix.	AISM:0004078
Spermathecal branch		The region of the cuticle of the spermatheca that is branched.	N/A

Table 1
(Continued)

Term	AISM term	Definition	AISM IRI
Spermathecal accessory gland	Spermathecal gland	The region of the cuticle that is part of the oviduct and attached to the spermatheca.	AISM:0004079
Dorsal longitudinal muscle (dlm)		The dorsal longitudinal muscle of the gonocoxa IX that extends from the antero-dorsal margin to the posterior margin of plate c1 + c2	N/A
Ventral longitudinal muscle (vlm)		The ventral longitudinal muscle of the gonocoxa IX that connects the anterior and the posterior margins of plate c1 + c2	N/A
Proximal dorso-ventral muscle (pdvm)		The dorso-ventral muscle of the gonocoxa IX connecting the proximal regions of the dorsal and ventral sections of c1 + c2	N/A
Distal dorso-ventral muscle (ddvm)		The dorso-ventral muscle of the gonocoxa IX connecting the distal regions of the dorsal and ventral sections of c1 + c2, external to the longitudinal muscles.	N/A
Oblique muscle of c4 (omc4)		The oblique muscle of the gonocoxa IX that extends between the dorso-proximal and the ventro-distal margins of the proximal region of c4	N/A

[morphology: ci = 0.50, ri = 0.50; combined: ci = 0.50, ri = 0.00].

Within Sepidiini coxites with four plates were reported only in *Tarsocnodes* and some *Ocnodes* species (Fig. 5i); plate arrangement observed in these genera supports the hypothesis by Kamiński et al. (2021) concerning homology between sepidinoid basal plate (c1 + c2) and coxite plates 1 and 2. This character directly corresponds to character 51 in Doyen (1994).

4. Coxites: shape of plate 3 in ventral view: (0) >1.15× wider than long (Fig. 5e); (1) <0.8× as wide as long (Fig. 7a). [morphology: ci = 1.00, ri = 1.00; combined: ci = 1.00, ri = 1.00].

Elongation of the 3rd coxite plate is shared between *Argenticrinis* and *Bombocnodulus* (both classified within Hypomelina) and *Psammodes longicornis* Kirby (type species of *Psammodes*) and *Psammodes sklodowskae* Kamiński & Gearner **sp.n.** (see Taxonomy section).

5. Coxites: plate c3: (0) well-developed, not visible laterally, covered by c4 (*Stips gebieni*); (1) well-developed, visible laterally (Fig. 5e); (2) reduced, almost not visible laterally (Fig. 5m). [morphology: ci = 1.00, ri = 1.00; combined: ci = 1.00, ri = 1.00].

A well-developed coxite plate c3 was exclusively observed within Molurina.

6. Coxites: basal margin of basal plate in ventral view: (0) straight or slightly indented ventrally (Fig. 5m); (1) strongly indented ventrally by elevated inner plate of paraproct (Fig. 5d). [morphology: ci = 0.25, ri = 0.85; combined: ci = 0.25, ri = 0.82].

State 1 is shared by Molurina, Sepidiina and Trachynotina.

7. Coxites: baculus of basal plate: (0) not strongly sclerotized or sclerotization restricted to the basal edge (Fig. 5b); (1) strongly sclerotized, with noticeable inner branching (Fig. 7a, *Argenticrinis*). [morphology: ci = 0.50, ri = 0.80; combined: ci = 1.00, ri = 1.00].

State 1 is used here as a reliable supporting diagnostic character for a clade containing *Argenticrinis*, *Bombocnodulus* and *Psammodes* **sens.n.**

8. Coxites: base of basal plate (c1 + c2) in lateral view: (0) straight, transverse; (1) oblique, with section corresponding to inner plate of paraproct more distally projected than section corresponding to outer plate of paraproct (Fig. 5k).

This character directly corresponds to character 56 in Doyen (1994). All of the analysed representatives of Sepidiini possess oblique bases of the basal plate.

9. Coxites: position of sensory field: (0) at the base of plate c4 (Fig. 7a); (1) in the middle of plate c4 (Fig. 5b). [morphology: ci = 1.00, ri = 1.00; combined: ci = 0.50, ri = 0.75].

The majority of the analysed Sepidiini possess sensory fields located near the base of coxite plate c4, except for the following species of the subtribe Hypomelina, *Brinckia australis* Penrith, *B. debilis* (Péringuey), *Iugidorsum cumstriis* Louw, *Sulcipectus* sp., *Trachynotidus rufozonatus* (Fairmaire) and *Pterostichula solitudo* representing Oxurina.

10. Coxites: base of plate 3: (0) without sclerotization (Fig. 5b, d); (1) with short transverse sclerotization (Figs 5j, 6, *Oxura*). [morphology: ci = 1.00, ri = 1.00; combined: ci = 1.00, ri = 1.00].

The presence of a transverse sclerotization was exclusively observed for the following Oxurina

Table 2
Summary of morphological variability of female terminalia within the studied Pimeliinae tribes

	Coxites										Proctiger		Spiculum			
Tribe	Ovipositor flattened dorso-ventrally	Paraproct longer than coxites	Base of paraproct	Coxites 4-lobed	All lobes fused	c2 membranous	Basal edge of c1	c4 rotated inwards	c4 bent ventrally	Gonostylus present	Proctiger short	Apical edge of proctiger	Spiculum elongate	Spiculum with fused base	Spiculum with elongate arms	Spermatheca
Sepidiini	No	Yes	Elevated			medially	Variable	No	No	Elevated					medially	No
Yes	No	No	Variable	Yes	Yes	Yes										Multibranched
Adelostomini	Yes	Variable	Straight	Yes	No	No	Straight	No	Variable	No	Yes	Rounded	Variable	Yes	Yes	With 2 ducts
Adesmiini	Yes	Yes	Straight	No	No	Yes	Straight	Yes	No	No	Yes	Rounded	Yes	Yes	Yes	With 3 ducts
Akidini	Yes	No	Elevated			laterally	Yes	No	No	Elevated					laterally	No
No	Yes	Yes	Rounded	No	Yes	Yes	Not									investigated
Anepsiini	Yes	No	Straight	No	No	No	Straight	No	No	No	Yes	Rounded	No	Yes	Yes	Not investigated
Asidini	Yes	Yes	Elevated			medially	No	Variable	No	Straight	Yes	No	No	Yes	Rounded	Yes
Yes	Yes															Multibranched
Branchini	Yes	Yes	Elevated			medially	No	Yes	No	Straight	Yes	No	No	Yes	Rounded	Yes
Yes	Yes	Coiled														
Cnemodinini	Yes	Yes	Straight	No	No	No	Straight	Yes	No	No	Yes	Rounded	Yes	Yes	Yes	Coiled
Coniontini	Yes	Yes	Straight	No	Yes	No	Straight	Yes	No	No	Yes	Rounded	-	-	-	Coiled
Cryptochilini	Yes	Yes	Rounded	No	No	No	Rounded	Yes	No	No	Yes	Rounded	Yes	No	Yes	Multibranched
Cryptoglossini	Yes	Yes	Straight	Yes	No	No	Straight	No	No	No	Yes	Rounded	Yes	Yes	Yes	Coiled
Edrotini	Yes	Yes	Straight	No	No	?	Straight	No	No	No	Yes	Rounded	Yes	Yes	No	Not investigated
Elenophorini	Yes	Yes	Elevated			laterally	Variable	No	No	Elevated					laterally	No
No	Variable	Yes	Rounded	Yes	Yes	Yes	Not									investigated
Epitragini	Yes	Yes	Straight	No	No	?	Straight	No	No	No	Yes	Rounded	Yes	Yes	No	Not investigated
Erodiini	Yes	Yes	Elevated			medially	Yes	No	No	Elevated					medially	No
Yes	No	Yes	Rounded	Yes	Yes	Yes	Not									investigated
Evaniosomini	Yes	Yes	Straight	No	No	Yes	Straight	No	No	No	Yes	Rounded	Yes	Yes	Yes	Not investigated
Idisiini	Yes	No	Straight	No	No	No	Straight	No	No	No	Yes	Rounded	No	No	Yes	Not investigated
Leptodini	Yes	Yes	Straight	Yes	No	No	Straight	No	No	Yes	Yes	Rounded	Yes	Yes	Yes	Not investigated
Nycteliini	Yes	Yes	Elevated			laterally	No	No	Yes	Elevated					laterally	Yes
No	No	Yes	Rounded	Yes	Yes	No										Multibranched
Nyctoporini	Yes	Yes	Straight	Yes	No	No	Straight	No	No	Yes	Yes	Rounded	Yes	Yes	Yes	Multibranched
Pimeliini	Yes	Yes	Straight	No	No	No	Straight	Yes	No	No	Yes	Rounded	No	No	Yes	Not investigated
Praociini	Yes	Yes	Variable	No	No	Yes	Variable	Yes	No	No	Yes	Rounded	Yes	Yes	No	Multibranched
Stenosini	Yes	Yes	Straight	No	No	No	Straight	No	No	Yes	Yes	Rounded	No	Yes	Yes	Not investigated
Tentyriini	Yes	Yes	Straight	No	No	Yes	Straight	Yes	No	No	Yes	Rounded	Yes	Yes	Yes	Not investigated
Thinobatini	Yes	Yes	Elevated			medially	No	No	?	Elevated					medially	No
No	No	Yes	Rounded	Yes	Yes	No	Not									investigated
Trilobocarini	Yes	Yes	Straight	No	No	Yes	Straight	Yes	No	No	Yes	Rounded	Yes	Yes	Yes	Not investigated
Vacronini	Yes	Yes	Elevated			medially	Yes	No	No	Elevated					medially	No
No	Yes	Yes	Rounded	Yes	Yes	Yes										Multibranched
Zophosini	Yes	Yes	Elevated			medially	No	No	No	Elevated					medially	Yes
No	No	Yes	Rounded	Yes	Yes	Yes	Not									investigated

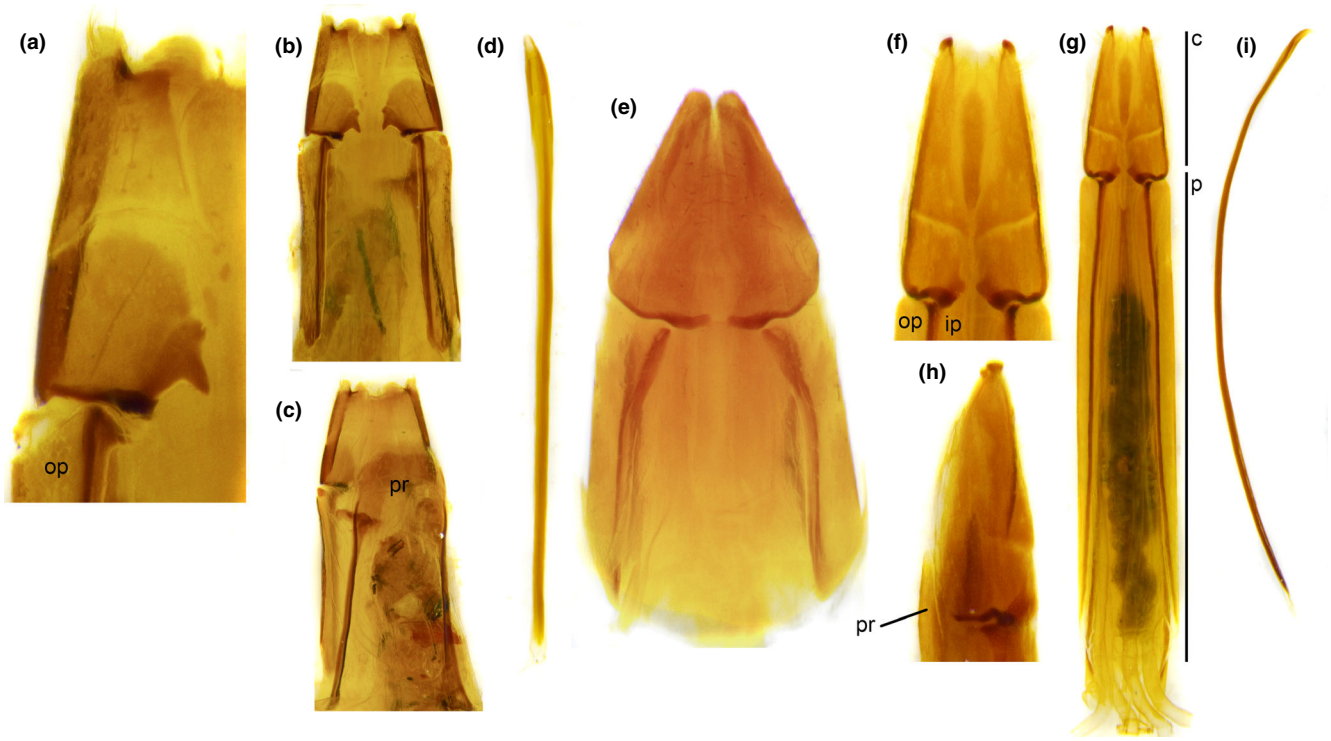


Fig. 4. Female terminalia morphology of selected species representing Pimeliinae. Ovipositor in (a, b, e–g) ventral, (c) dorsal, and (h) lateral views; (d, i) spiculum ventrale. (a–d) *Edrotes ventricosus* (Edrotini); (e) *Telabis* sp. (Edrotini); (f–i) *Phegoneus pectoralis* (Epitragini). For the abbreviations see Fig. 1.

representatives: *Decoriplus costimargo* Louw, *D. striatulus* Louw, *Oxura setosa* Kirby, *Pterostichula kung* Koch and *Synhimba pruinosum* Koch.

11. Coxites: apical margin of basal plate (c1 + c2): (0) without sclerotization (Fig. 5b, d); (1) with elongate transverse sclerotization (Fig. 5o). [morphology: ci = 0.25, ri = 0.40; combined: ci = 1.00, ri = 1.00].

The presence of sclerotization is synapomorphic for Sepidiina.

12. Coxites: apical plate (c4): (0) reduced, thin (Fig. 5a); (1) thick, well-developed (Fig. 5d). [morphology: ci = 0.33, ri = 0.71; combined: ci = 0.33, ri = 0.60].

Thin lobes were observed in the following species: *Namibomodes* sp., *Palpomodes* sp. and *Pterostichula solitudo* (all representing Oxurina); and *Brinckia australis*, *B. debilis*, *Iugidorsum cumstriis* and *Sukcipectus* sp. (all representing Hypomelina).

13. Paraproct length: (0) <1.5× longer than coxite; (1) >2.0× longer than coxite.

Although all investigated Sepidiini representatives possess elongate paraprocts (>2× longer than coxites), this feature reaches its extremes in *Tarsocnodes* (Fig. 6).

Genital tube (14).

14. Spermatheca: (0) with two ducts (Fig. 1h); (1) multibranched (Fig. 1g).

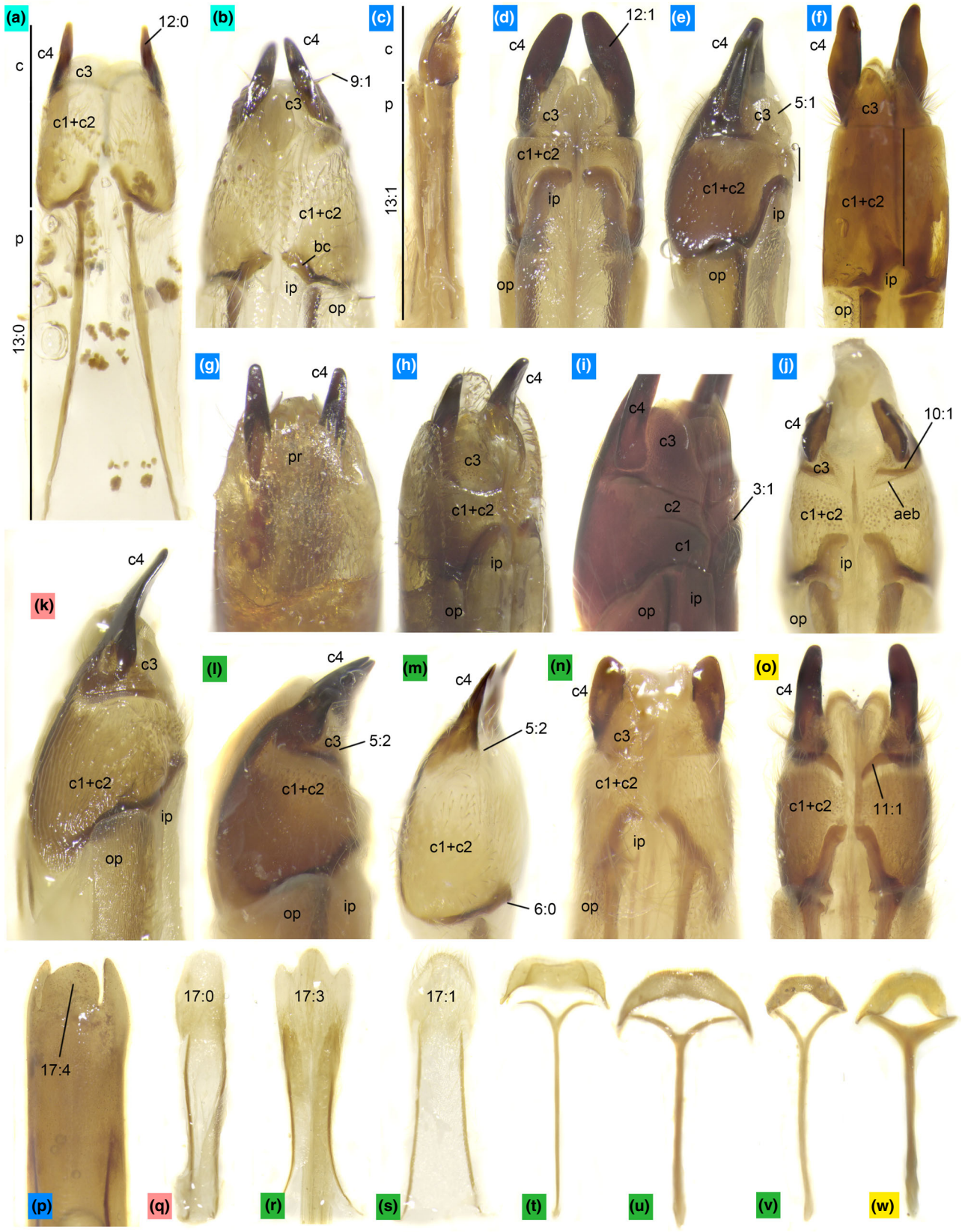
All of the studied Sepidiini species possess multibranched spermathecae. This observation is convergent with data presented by Doyen (1994) for Sepidiini.

Proctiger (15–19).

15. Proctiger, extension: (0) apical margin not reaching tip of coxite plate c3; (1) apical margin reaching tip of coxite plate c3.

In all of the studied Sepidiini, the apical margin of the proctiger reaches the tip of coxite plate c3.

Fig. 5. Female terminalia diversity within tok-tok beetles. Ovipositor in (a, b, d, f, j, n, o) ventral, (g) dorsal, and (c, e, h, i, k–m) lateral views; (t–w) spiculum ventrale; (p–s) proctiger. (a) *Brinckia debilis*; (b) *Sulcipectus* sp.; (c) *Argenticrinis lossowi*; (d, e) *Distretus* sp.; (f) *Ocnodes kruegeri*; (g, h) *Tibiocnodes lucidus*; (i) *Tarsocnodes molossa*; (j) *Synhimba pruinosum*; (k) *Somaticus serratus*; (l) *Decoriplus costimargo*; (m) *Palpomodes physopterus*; (n) *Namibomodes serrimargo*; (o) *Vieta muscosa*; (p) *Mariazofia ponderosa*; (q) *Ethmus* sp.; (r) *Oxura setosa*; (s, t) *Pterostichula* sp.; (u) *Oxura* sp.; (v) *Decoriplus costimargo*; (w) *Vieta speculifera*. Color codes: cyan Hypomelina, blue Molurina, green Oxurina, yellow Sepidiina, red Trachynotina. Character numbers and their states directly correspond to the list presented in the results section of this publication. For the abbreviations see Fig. 1.


(a)
12:0
c
c4
c3
c1+c2
p
13:0
(b)
c4
9:1
c3
c1+c2
bc
ip
op
(c)
c
p
13:1
(d)
12:1
c4
c3
c1+c2
ip
op
(e)
c4
5:1
c3
c1+c2
ip
op
(f)
c4
c3
c1+c2
ip
op
(g)
c4
pr
(h)
c4
c3
c1+c2
ip
op
(i)
c4
c3
3:1
c2
c1
ip
op
(j)
c4
10:1
c3
aeb
c1+c2
ip
op
(k)
c4
c3
c1+c2
ip
op
(l)
c4
c3
5:2
c1+c2
op
ip
(m)
c4
5:2
c1+c2
6:0
(n)
c4
c3
c1+c2
ip
op
(o)
c4
c1+c2
11:1
(p)
17:4
(q)
17:0
(r)
17:3
(s)
17:1
(t)
(u)
(v)
(w)

16. Proctiger: apical area: (0) without sclerotized, parallel, longitudinal fibres (Fig. 5q–s); (1) with clearly visible longitudinal fibres (Fig. 6, *Vieta*). [morphology: ci = 1.00, ri = 1.00; combined: ci = 1.00, ri = 1.00].

State 1 is shared by all studied Sepidiina species and *Cyrtoderes* sp. of Trachynotina.

17. Proctiger: apical margin (Fig. 6): (0) rounded, not sclerotized (Fig. 5q); (1) rounded, strongly sclerotized (Fig. 6, *Decoriplus*); (2) pointed (Fig. 6, *Brinckia*); (3) indented, with mid-section elevated (Figs 5r, 6, *Somaticus*); (4) indented, with mid-section even with lateral sections (Fig. 6 *Tibiocnodes*). [morphology: ci = 0.57, ri = 0.85; combined: ci = 0.80, ri = 0.93].

The observed diversity of the shape of the apical margin of the proctiger is unexpectedly high. To the best of the authors' knowledge, this is the first time that this character has been considered as a phylogenetic and diagnostic feature within darkling beetles (Doyen, 1994; Doyen and Tschinkel, 1982; Tschinkel and Doyen, 1980). State 3 is characteristic for all studied Trachynotina species; however, it is also present in *Oxura setosa* (Oxurina). State 4 is shared between Molurina, *Argenticrinis* and *Bombocnodulus* (both classified within Hypomelina).

18. Proctiger: mid-section of indented edge: (0) not applicable (in species with rounded (17:0,1) or pointed (17:2) apical margin of proctiger); (1) as wide or wider than lateral sections (Fig. 6, *Tibiocnodes*); (2) narrower than lateral sections (Fig. 6, *Tarsocnodes*). [morphology: ci = 1.00, ri = 1.00; combined: ci = 0.66, ri = 0.95].

State 2 is shared by the examined *Ocnodes* and *Tarsocnodes* species.

19. Proctiger: sclerotization of apical margin: (0) absent (e.g. Fig. 6, *Decoriplus*); (1) present (Fig. 6, *Somaticus*). [morphology: ci = 1.00, ri = 1.00; combined: ci = 1.00, ri = 1.00].

State 1 is shared by the studied Sepidiina species, and *Cyrtoderes* and *Somaticus* of Trachynotina.

Spiculum ventrale (20–23).

20. Spiculum ventrale: base: (0) short, length of each arm equal/subequal to base (Fig. 2f); (1) elongate, length of base largely exceeding length of each arm (Fig. 5t–w).

All of the analysed Sepidiini species have elongate bases of spiculum. Within Pimeliinae, and many other tenebrionids, this feature is apparently linked with the elongation of the ovipositor (Medvedev, 2001).

21. Spiculum ventrale: (0) Y-shaped, each arm forming an obtuse angle with base (e.g. Fig. 6, *Tarsocnodes*); (1) T-shaped, arms inclined at *c*. 90° to base (Fig. 6, *Somaticus*). [morphology: ci = 0.33, ri = 0.78; combined: ci = 0.25, ri = 0.57].

State 1 is shared by several representatives of Trachynotina and *Vieta speculifera* (Sepidiina), with the exception of included *Ethmus* species. This finding contradicts the diagnostic character proposed by Doyen (1994), who concluded that in Sepidiini the spiculum arms are reflexed (i.e. with tips pointing basally, as in *Somaticus*, Fig. 6).

22. Spiculum ventrale: with subparallel arms (inclined at *c*. 160° to base): (0) no, spiculum structured differently (Fig. 5t–w); (1) yes (Fig. 6). [morphology: ci = 0.50, ri = 0.75; combined: ci = 0.50, ri = 0.88].

State 1 shared is by *Ocnodes*, *Tarsocnodes* and *Tibiocnodes*.

23. Arms of spiculum ventrale: (0) thin (Figs 5t–v, 6, *Brinckia*); (1) thick (Figs 5w, 6, *Moluris*). [morphology: ci = 0.33, ri = 0.91; combined: ci = 0.17, ri = 0.74].

State 1 is shared by the analysed representatives of *Dithcha* clade, *Psammophanes*, *Psammotyria*, *Toktokkus*, *Tuberocnodes*, and the majority of *Psammodes* (clade A), Sepidiina and Tachynotina (with the exception of *Ethmus*).

Morphology-based analysis. The heuristic search with characters equally weighted yielded 12 most parsimonious trees of 45 steps, with a CI of 0.644 and a RI of 0.908. Topological differences between the maximum parsimony (MP) trees concerned the shallow nodes (mostly the clade including Sepidiina + Trachynotina). The strict consensus tree (CI = 0.652, RI = 0.909) with Bremer support values is presented in Fig. 6. Branch support for the majority of recovered nodes was low.

Only Sepidiina was recovered as monophyletic in the MP analysis. Trachynotina was recovered paraphyletic with the majority of representatives clustered within a single clade additionally containing Sepidiina, whereas the genus *Ethmus* was recovered separately in a clade containing representatives of Hypomelina, Molurina and Oxurina. However, *Ethmus* did not cluster within any other major lineage in that clade (Fig. 6). The close affinity of Sepidiina and Trachynotina (excluding *Ethmus*) is supported by the specific structure of the ovipositor and proctiger; i.e. apical margin of the basal plate of the coxites (c1 + c2) with elongate transverse sclerotization (character state 11:1), basal margin of the same plate oblique (6:1) and proctiger with sclerotized apical margin (19:1).

Representatives of Oxurina were recovered in three different clades. The first contains *Oxura* (the type genus of the subtribe), *Decoriplus*, *Synhimba* and *Pterostichula kung* (Fig. 6). This grouping is supported by a unique structure of coxite plate c3—base with short transverse sclerotization (10:1). The second clade with Oxurina representatives contains *Palpomodes* and *Namibomodes* (Fig. 6), and is differentiated by a thin/reduced apical plate of coxites (c4; 12:0). The last Oxurina representative, *Pterostichula solitudo*, was recovered in a clade containing the majority of sampled Hypomelina, i.e. *Brinckia*, *Iugidorsum*, *Sulcipectus* and *Trachynotidus*; this grouping was supported by a

synapomorphic placement of the sensory field, which is situated in the middle of plate c4 (9:1).

The remaining Hypomelina, *Argenticrinis* and *Bombocnodulus*, were recovered deeply nested within the clade containing all the Molurina taxa included in these analyses. Specifically, they were in a subclade together with *Psammodes longicornis* (type species of the genus *Psammodes*). This relationship is morphologically well-supported by synapomorphies of the coxites and proctiger, i.e. elongate, nonreduced plate c3 of the coxites (4:1, 5:1), baculus of the basal plate of coxites (c1 + c2) strongly sclerotized, with noticeable inner branching (7:1), and proctiger with indented apical margin, with middle section wider than and even with lateral sections (17:4, 18:1).

As a result of the abovementioned relationship between *Argenticrinis, Bombocnodulus* and *Psammodes longicornis*, Molurina was rendered paraphyletic. Nevertheless, all of the included Molurina (+*Argenticrinis, Bombocnodulus*) species are united by a common structure of the ovipositor and proctiger (synapomophies 5:1, 17: 1, 18:1). Two major subclades were recognized within the Molurina (+*Argenticrinis, Bombocnodulus*) clade. The first contains *Amiantus*, *Chiliarchum*, *Dichtha*, *Moluris*, *Psammophanes*, *Psammotyria*, *Tibiocnodes*, *Tuberocnodes*, and selected representatives of *Ocnodes* and *Psammodes*, i.e. *O. rowleianus*, *P. caelatus*, *P. longipes*, *P. basuto* and *P. striatus* (Fig. 6). This subclade is supported by the specific structure of the coxites; i.e. oblique base of the basal plate (6:1). The second subclade is composed of the remaining *Ocnodes* species (*O. kruegeri*, *O. ovulus*, *O. scrobricollis*) + *Tarsocnodes*, and the already described grouping of *Argenticrinis, Bombocnodulus* and *Psammodes longicornis* (Fig. 6). This subclade is defined by the strongly sclerotized baculus of the basal plate of coxites (7:1).

Bayesian analyses were less conclusive (Appendix S2). Although the taxonomic composition of the major clades was largely congruent with the results of the MP analysis (Molurina + *Argenticrinis–Bombocnodulus*, Hypomelina + *Pterostichula solitudo*), the relationships between them remained unresolved. The branch support values were variable (Fig. 6).

Combined analysis of female terminalia traits and molecular data

As in the morphological analysis, Sepidiina was recovered as monophyletic in the combined dataset (Fig. 7), and placed as sister to Trachynotina, with strong support for the relationship (Sepidiina: PP = 0.98, Sepidiina + Trachynotina: 1.00). Additionally, Trachynotina and Hypomelina were both recovered as monophyletic (Trachynotina: 1.00, Hypomelina: 1.00), but it should be noted that owing to a lack of molecular data *Ethmus* and *Pterostichula solitudo* were not included in this analysis. Oxurina was again recovered as paraphyletic, with the clade containing *Namibomodes* and *Palpomodes* sister to the remaining Sepidiini, excluding other Oxurina, and the rest of the Oxurina taxa recovered as a clade sister to all other Sepidiini (including *Namibomodes* + *Palpomodes*). Support for *Namibomodes* + *Palpomodes* was strong (1.00), however, support was low for the placement of this clade in the tree (0.54). Clustering and placement of the remaining Oxurina taxa was strongly supported (1.00, 1.00). *Argenticrinis* was recovered as sister to *Psammodes longicornis* (1.00) within the clade containing all Molurina taxa.

The relationship between *Argenticrinis* and *P. longicornis* was supported by the structure of the coxites, specifically, the elongated plate c3 and the strongly sclerotized baculus of the basal plate with a noticeable inner branching (synapomorphies 4:1, 7:1). The included Molurina taxa + *Argenticrinis* share a well-developed plate c3 of the coxites and the apical margin of the proctiger is rounded and strongly sclerotized (5:1, 17:1). The clade containing *Somaticus* (Trachynotina) was supported by the presence of an elongate transverse sclerotization of the apical margin of the basal coxite plate, and the apical edge of the proctiger, which has a sclerotized margin (11:1, 19:1). In the *Vieta* clade (Sepidiina), the apical area of the proctiger possessed clearly visible longitudinal fibres (16:1). Hypomelina was supported by two synapomorphies: the sensory field of the coxites is situated in the middle of plate c4 and the apical edge of the proctiger is pointed (9:1, 17:2). Finally, the clade containing members of Oxurina, excluding *Namibomodes* and *Palpomodes*, share a short transverse sclerotization on the base of plate c3 of the coxites (10:1).

The phylogeny generated from the molecular dataset alone (Fig. 7b) recovered the same topology as the combined dataset, but resolved the polytomies found in the combined tree, which means that even though morphological features can support certain clades and represent synapomorphies, they might dilute the resolution of molecular data.

Ovipositor musculature

MicroCT analysis revealed the existence of five different pairs of muscles within the coxites of the studied specimen. Four were located within the basal plate c1 + c2 (Fig. 8), namely: the dorsal longitudinal muscle (dlm), which extends from the antero-dorsal margin to the posterior margin of plate c1 + c2 (this muscle is probably homologous with M25 in Medvedev, 2001); the ventral longitudinal muscle (vlm) that connects the antero-ventral margin and the postero-ventral margins of plate c1 + c2 (probably homologous with M23 in Medvedev, 2001); the proximal dorso-ventral muscle (pdvm) connecting the bases of the dorsal and ventral

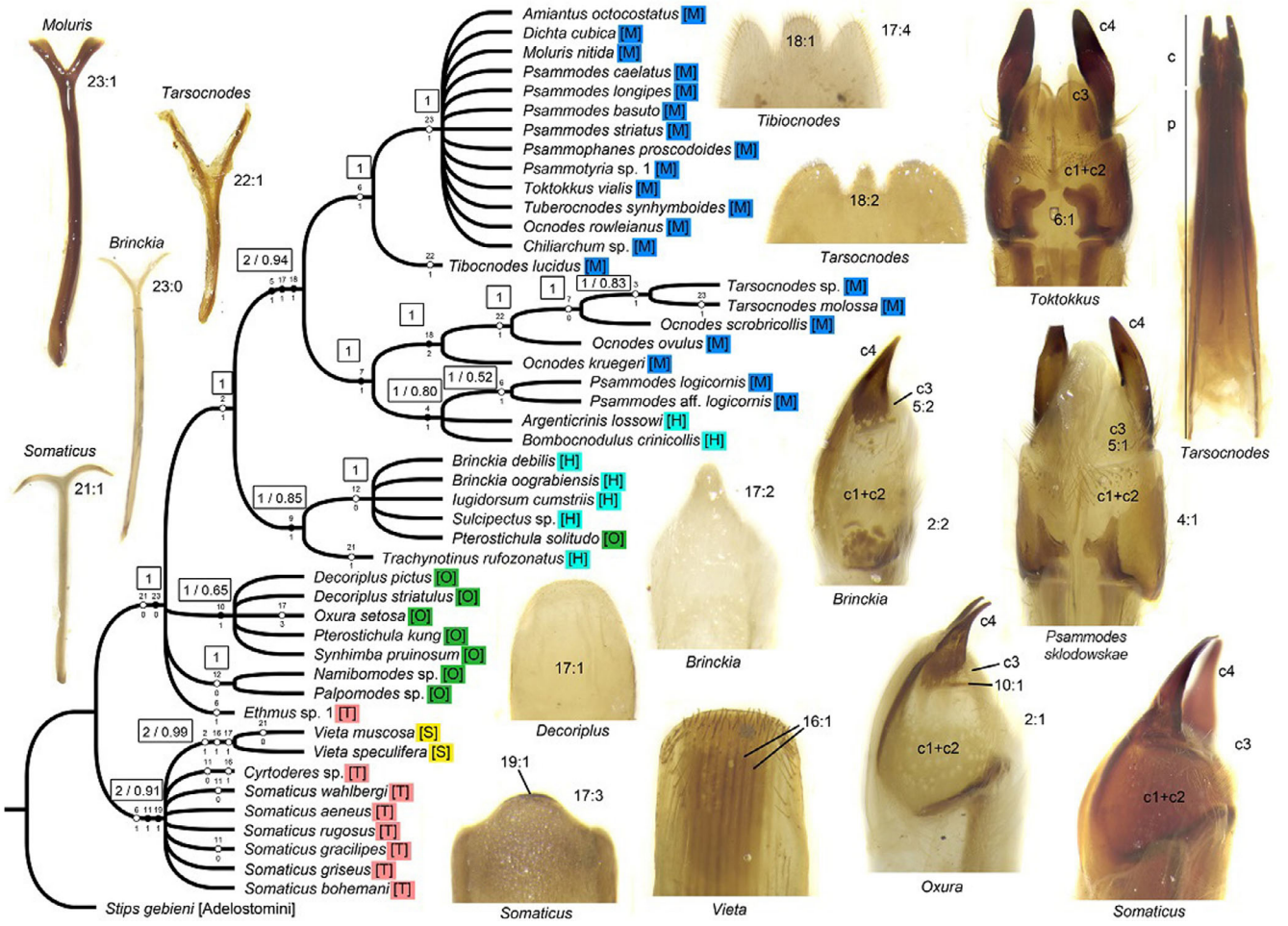


Fig. 6. Phylogeny of Sepidiini recovered solely based on morphological traits associated with female terminalia. Strict consensus tree derived from the maximum parsimony analysis is presented. Node values respectively represent Bremer support (integers) and Bayesian posterior probability (decimals). Non-homoplastic changes of character states are represented with black circles, while the homoplastic ones with white circles. Character numbers and their states directly correspond to the list presented in the results. [H] Hypomelina; [M] Molurna; [O] Oxurina; [S] Sepidiina; [T] Trachynotina. For the abbreviations see Fig. 1.

sections of c1 + c2; and the distal dorso-ventral muscle (ddvm), located on the dorso-medial part of of c1 + c2. The dorso-ventral muscle is external to the longitudinal muscles. The last of the recovered muscles is the oblique muscle of c4 (omc4), which extends between the dorso-proximal and the ventro-distal margins of the base of c4 (Fig. 8).

Discussion

Terminology for the morphology of the female terminalia

The terminology to refer to the female terminalia in this paper partly overlaps with the general terminology applied to Insecta, as depicted in the ontology for the Anatomy of the Insect SkeletoMuscular system (AISM; see Table 1). However, a Coleoptera-specific ontology probably would be more appropriate. For some terms in the AISM, even though their definitions are generally compatible with Coleoptera, their labels do not match commonly used beetle anatomy terms. As the beetle-specific ontology currently is being developed (Girón pers. comm.), the present study should be considered as a reliable starting point for development of logical definitions linked to the female terminalia, especially as it highlights important nomenclatural inconsistencies between the most commonly referenced papers for Tenebrionidae (i.e. Doyen, 1966; Tschinkel and Doyen, 1980).

Terminalia of Pimeliinae

This study demonstrates the usefulness of the morphology of female terminalia for reconstructing

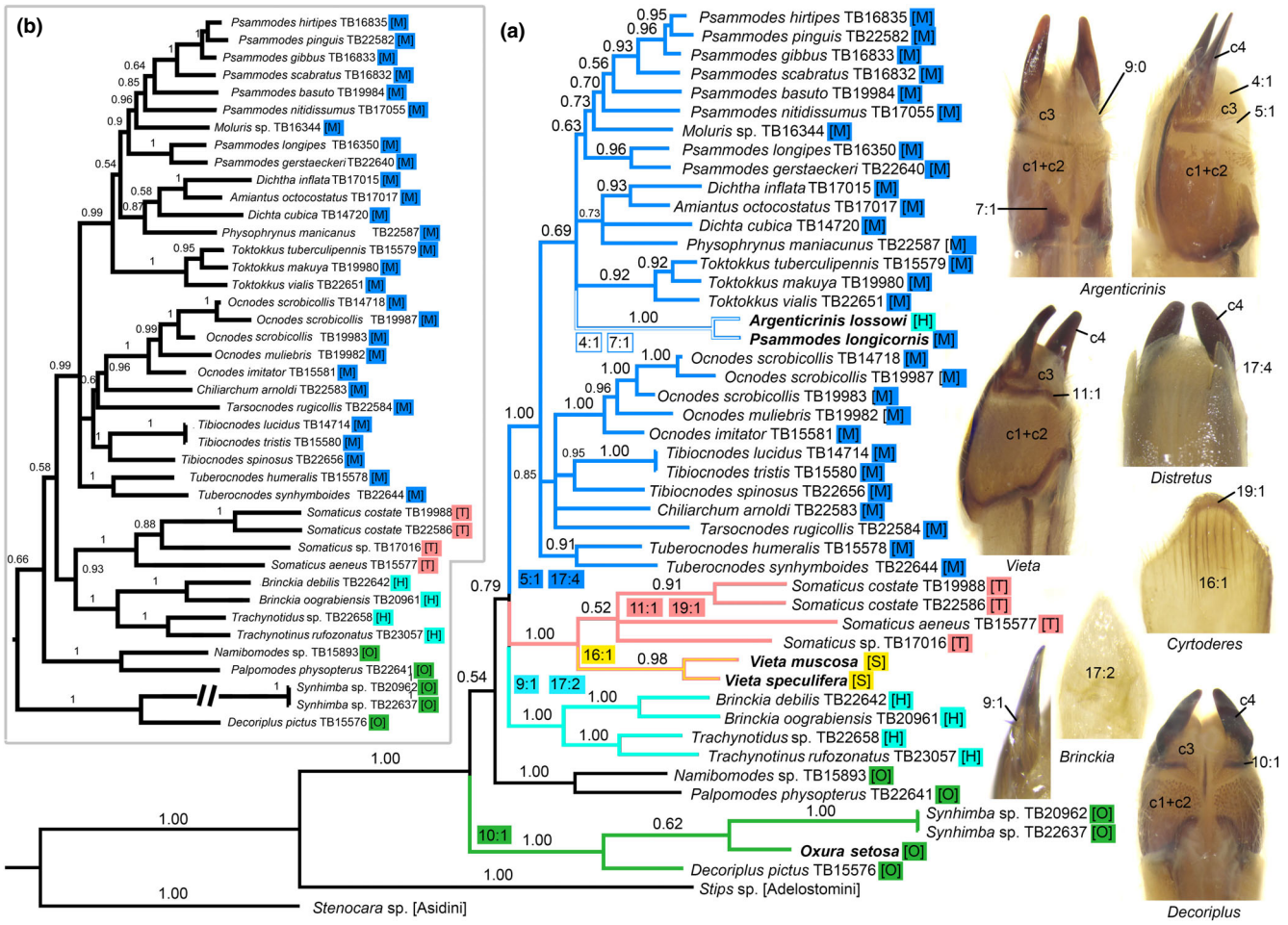


Fig. 7. Phylogenetic relationships among tok-tok beetles. Topologies recovered based on (a) combined analysis of female terminalia and molecular data; and (b) solely based on molecular data. In both cases, the majority rule consensus of post-burn-in trees obtained in Bayesian analysis is presented. Posterior probabilities are displayed above branches. Character optimizations were analyzed using the maximum parsimony method in Mesquite. Character numbers and their states directly correspond to the list presented in the results. For the abbreviations see Fig. 1.

phylogenetic relations among darkling beetles. Although this observation has been made already by previous contributors who investigated higher-level relationships within the family (Doyen and Tschinkel, 1982; Iwan and Kamiński, 2016; Kergoat et al., 2014; Watt, 1974), up to now, specific data on pimelinoid beetles have remained scarce (see Watt, 1992). The structures of the investigated ovipositors, spermathecae, proctigers and spicula ventralia proved to be extremely diverse not only in tok-tok beetles, but also in other groups of pimeliinoid beetles. For example, within Adelostomini two major lineages differentiated by the structure of the coxites were distinguished. The first consists of *Lepidochora* and *Stips* and is characterized by elongate apical coxite lobes c4 (Fig. 2b), whereas in the other, which groups the remaining Adelostomini, the lobes c4 are not extending beyond the outline of coxites (Fig. 2a, e). This cursory observation highlights the potential informativeness of the morphology of the female terminalia within this group, and should be used as a reference by future revisionary workers. Up to now, the ovipositor and genital tubes of Adelostomini have remained almost fully uninvestigated (however, see Doyen, 1994).

The material analysed here reveals that to some extent the morphology of the female terminalia is stable among the studied tribes. This enables the presentation of new and unambiguous diagnostic features for these taxa. This is well-illustrated in three morphologically diverse tribes Adesmiini, Asidini and Nyctelini. The ovipositors of the representatives of Adesmiini, regardless of the high distinctiveness of the beetle forms (*Adesmia*, *Epiphysa*, *Onymacris* and *Stenocara*), are characterized by the enlarged basal plates of coxites c1 + c2, the membranous and folded coxite c3 lobes (Fig. 2g–j), and the presence of three spermathecal ducts. Asidini, regardless of their

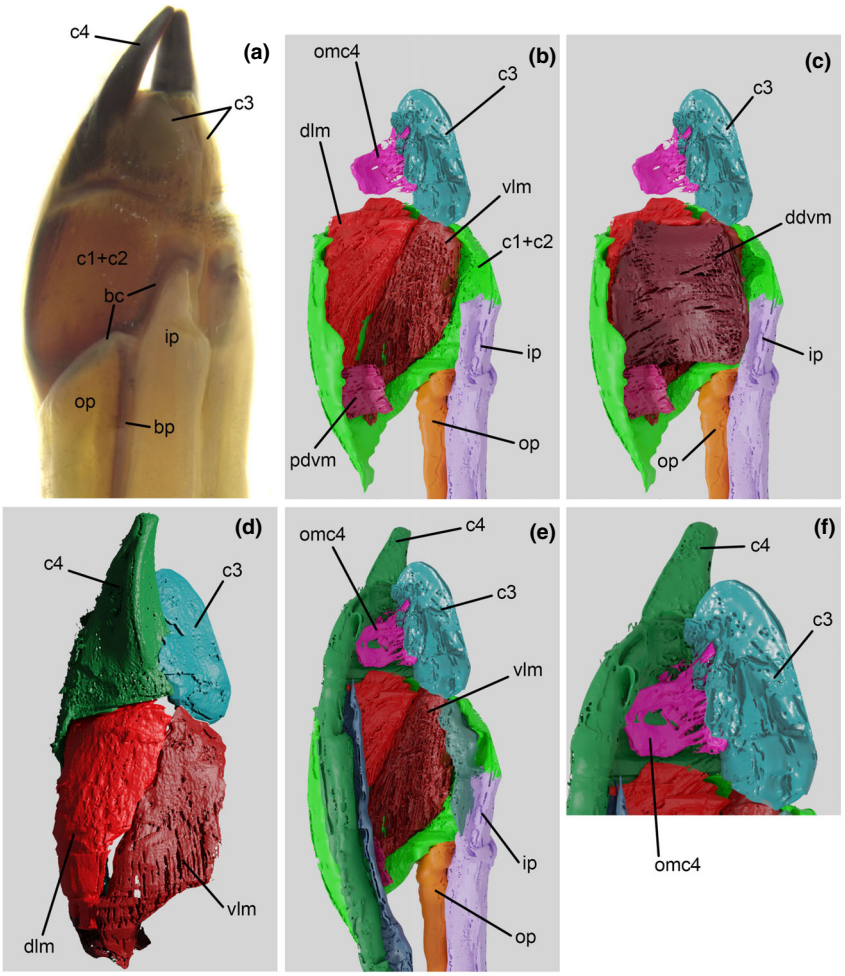


Fig. 8. Musculature of the apical part of the ovipositor of *Mariazofia basuto*. (a) ventro-lateral image of the ovipositor; (b–f) digital, lateral disarticulations of the coxites illustration placement of different muscle groups. Abbreviations: bc: baculus of basal plate; bp: baculus of paraproct; c1–c4: subsequent plates of coxites; ip: inner plate of paraproct; dlm: dorsal longitudinal muscle; vlm: ventral longitudinal muscle; pdvm: proximal dorso-ventral muscle; ddvm: distal dorso-ventral muscle; omc4: oblique muscle of c4; op: outer plate of paraproct.

geographical origin (Europe, South Africa, North and South America), are easily recognizable from most Pimeliinae by the strong sclerotization of the coxites, which also are mostly fully fused into a single plate. Furthermore, the inner plate of the paraproct characteristically covers a large portion of the basal plate of the coxites (Fig. 2p). Finally, the representatives of Nycteliini are well-characterized by having short arms of the spiculum ventrale and rhomboidal coxite plate c1 (Fig. 3p–s).

Furthermore, the analysed data revealed similarities of female terminalia among some seemingly unrelated tribes. The spicula ventralia of studied Cryptochilini (Sub-Saharan Africa), Idisiini (East Asia) and Pimeliini (Palaearctic) are characterized by unfused bases (Fig. 3d, f, l). However, it has to be mentioned that not all Pimeliini share this feature (e.g. *Podhomala* Solier; see Chigray, 2019). Nevertheless, outside the above-mentioned tribes this feature has been reported only for *Morica* Dejean of Akidini (Doyen, 1994; Doyen and Tschinkel, 1982; Tschinkel and Doyen, 1980; Watt, 1992). Until now, Cryptochilini, Idisiini and Pimeliini were not considered to be closely related (Endrödy-Younga, 1989; Medvedev, 1973; Watt, 1992). However, different authors, while discussing unrelated features, unknowingly provided support for this relationship. Namely, over the course of history, externally open procoxal cavities were reported for *Idisia* (of Idisiini), *Platyope* (Pimeliini), *Cryptochile* and *Pachynotelus* (of Cryptochilini) (Doyen, 1994; Watt, 1992). This feature previously has been linked with the close association of the pro- and pterothoraces in these species and treated as a secondary adaptation in contrast to the primarily opened cavities of Zolodininae Watt, which were interpreted as plesiomorphic among Tenebrionidae (Watt, 1992). Furthermore, data analysed here show that studied Pimeliini and Cryptochilini are further linked by the

strongly rotated apical lobes of coxites, similarly structured coxite plates and baculi of coxite plate c1 + c2 (Fig. 3a, b, e, j, k). The ovipositor of *Idisia* is highly reduced and thus cannot be used in this comparison (Fig. 5e; Watt, 1992). Nevertheless, the similar structure of the spicula ventralia is interpreted here as evidence of a close phylogenetic relationship between Cryptochilini, Idisiini and Pimeliini. This affiliation, and basal placement of those tribes within Pimeliinae, also is strongly supported by genomic data (Smith et al., in prep.).

By contrast, the observed diversity of female terminalia of pimeliinoid beetles sometimes leads to problems with homology recognition. The greatest challenge concerns the ovipositor, especially in cases where the four coxite lobes are not fully visible (e.g. Figs 2–4). Some previous authors have evaded this problem by developing new nomenclature systems for describing more challenging terminalia (e.g. Pérez Vera, 2014). However, this approach prevents the incorporation of such data into analyses concerning a wider spectrum of taxa. Examination of the proportions of the plates of closely related tribes and the position of key reference points, such as sensory fields or baculi, might be helpful when assessing the homology of structures in problematic groups (e.g. Banaszkiewicz, 2006; Kamiński et al., 2019). It is hypothesized here that the musculature of the ovipositor can also be used as a reliable navigation point. Although preliminary in this aspect, this study reveals the specific connections of particular muscles and different chitinous elements of the coxities (Fig. 8). The nomenclature introduced herein, as well as the three-dimensional models, provides a basis for future studies. To our knowledge, the data presented here constitute the first description of the muscular system of coxities (Doyen, 1966; Eggs et al., 2018; Ernst et al., 2013; Medvedev, 2001; Scudder, 1961).

Terminalia of Sepidiini

The results of this study refute the diagnostic characters proposed by Doyen (1994) for Sepidiini. Although the oblique baculus of coxites, elongated paraprocts and strongly sclerotized apical lobes were present in all of the studied tok-tok beetle species, these features have also been reported for many other unrelated pimeliinoid taxa (Table 2). In addition, the morphology of the arms of the spiculum ventrale is too variable to be used as a reliable diagnostic character at the tribal level (Figs 5t–w, 6). Instead, the analysed data, including previous contributions (Doyen, 1994), suggest that Sepidiini is the only pimeliinoid tribe in which representatives possess laterally flattened ovipositors and elongate proctigers (Fig. 1b). Furthermore, the diversity of the forms of the latter structure is surprisingly high when compared to other tribes of Pimeliinae. For example, no other tribe of Pimeliinae is currently known to possess medially indented proctigers (Figs 2–4; Doyen, 1994). Although the evolutionary drivers leading to this increased diversity remain unknown, it can be hypothesiszed that, at least in Molurina, these modifications enable the proctiger to enfold, and thus protect, the elongate ovipositor (Fig. 5g, h).

The analysed data reveal that the ovipositors of tok-tok beetles are ventrally directed (Fig. 5a–m). Although this feature was also reported for Erodiini and for *Stips* and *Lepidochora* of Adelostomini, it seems to be quite rare among Pimeliinae (Table 1). In the majority of the investigated tribes, the apical lobes of the coxites are rotated inwards. This is well-visualized by the ventrally located sensory fields (Fig. 5e). Such arrangement impedes the recognition of homologous structures in different pimelinoid beetles, as the area homologous to c3 often is rotated (see Kamiński et al., 2021).

The present study shows no direct indications as to which Pimeliinae tribes have a close relationship with the tok-tok beetles. On account of the inflexed spiculum arms of some of the species and the above-mentioned ventral orientation of the ovipositor, the close affiliation between tok-tok beetles and Adelostomini might be considered (Fig. 2a–f). Nevertheless, both tribes can be separated easily by the differently structured spermathecae (multibranched in Sepidiini, with two ducts in Adelostomini) and the orientation of the baculi of the basal coxite lobe c1 (oblique in Sepidiini, straight in Adelostomini), and no external characters are currently known to link these tribes together (Doyen, 1994). The morphology-based phylogenetic analyses conducted by Doyen (1994) recovered Sepidiini close to Akidini and Ceratanisini Gebien. Judging solely on the morphology of the female terminalia, this relationship seems unlikely as no common features were found to link these three tribes (Figs 2–4; Doyen, 1994).

Within Sepidiini, the morphology of the female terminalia proved to be extremely informative for reconstructing phylogenetic relationships (Fig. 6) and produced topologies that were largely congruent with molecular data (Fig. 7). Several phylogenetically informative features were identified on the apical parts of the ovipositor and proctiger. These structures are often exposed in nondissected specimens, making the female terminalia an easily accessible resource for identification purposes. Our analyses of female terminalia data reveal the need for further research into the taxonomy of Sepidiini, as several previously unrecognized evolutionary lineages were identified. One of the most unexpected findings concerns the recovery of a well-supported clade containing the type species of the

genus *Psammodes* (*P. longicornis*), and the following two genera of Hypomelina (Figs 6, 7a)—*Argenticrinis* and *Bombocnodulus*. In order to restore the monophyly of Molurina, *Argenticrinis* and *Bombocnodulus* are transferred into this subtribe. However, the position of *P. longicornis* creates a cascade of taxonomic and nomenclatural problems.

With about 150 species, *Psammodes* is the most diverse genus of tok-tok beetles (Kamiński et al., 2019). Although the monophyly of this genus already has been questioned by previous authors (Gearner et al., 2021; Kamiński et al., 2021), this study is the first to revise the morphology of the type species. When taking into consideration the results of the combined (morphology + DNA) analyses, representatives of *Psammodes* were recovered in three separate clades (Fig. 7). One containing *P. longicornis* (hereinafter referred to as *Psammodes* **sens.n.**), a second clade containing *P. longipes* + *P. gerstaeckeri*, and a third clade containing species with the convex forms traditionally attributed to *Psammodes* (Fig. 7). An examination of Molurina species, including many type specimens, resulted in recognition of the morphological features delimiting these lineages (for details see Taxonomy section). This information was used to divide the species previously attributed to *Psammodes* into these three lineages. Only three species were included in *Psammodes* **sens.n.**, 12 were included in the *P. longipes* + *P. gerstaeckeri* clade, and >140 with the most third lineage. Owing to the inclusion of *Pimelia scabra* Fabricius, the second group is hereinafter referred to as *Piesomera* Solier (Kamiński et al., 2019). Although, the third lineage contains *Psammodes caffra* Fåhraeus, 1870, which is the type species of *Parmularia* Koch, this generic name cannot be used as it is a junior homonym of *Parmularia* Macgillivray (Bryozoa: Cheilostomida). Therefore, a new replacement name—*Mariazofia* Kamiński **nom.n.**—is hereby introduced. *Piesomera* and *Mariazofia* possess similarly structured female terminalia (3:1, 12:1), however, the latter genus can be easily distinguished by the presence of confined abdominal setal patches in males (Fig. 9a–e). This feature groups *Mariazofia* together with the genus *Moluris*—which also is reflected in the molecular analyses (Fig. 7). All new combinations and related nomenclatural acts are presented in the Taxonomy section.

The subtribe Oxurina was recovered as polyphyletic in all phylogenetic analyses. Specifically, a clade composed of *Palpomodes* + *Namibomodes* was recovered outside the core Oxurina clade (Figs 6, 7). This result was not unexpected, as the morphological distinctiveness of *Palpomodes* and *Namibiodes* from other Sepidiini was already postulated by Koch (1952, 1958). To accommodate these findings, a new subtribe Palpomodina Kamiński & Gearner **subtr.n.** is erected. Detailed diagnosis of this taxon is provided in the Taxonomy section. Furthermore, the morphological analyses recovered *Pterostichula solitudo* within the main Hypomelina clade. Based on female terminalia (especially proctiger morphology) this species clearly groups with *Brinkia*, *Iugidorsum*, *Sulcipectus* and *Trachynotidus*. However, at the moment it is not clear if it should be accommodated in a separate monotypic genus. Detailed investigation of other *Pterostichula* species should be conducted as, besides the data presented here, no information is available on the morphology of female terminalia within this genus. As a result of these uncertainties, no taxonomic decisions are introduced.

This study is the first to include members of Sepidiina within a phylogenetic framework (Gearner et al., 2021; Kamiński et al., 2021). This subtribe was recovered as being closely related to Trachynotina (Figs 6, 7). This relation is well-supported by structures of the female terminalia (synapomorphies 11:1, 19:1; homoplasy 6:1). The recovery of a close affinity between Sepidiina and Trachynotina is interesting, as representatives of both of these subtribes strongly differ in terms of external morphology. In the morphology-based analyses, Trachynotina was rendered polyphyletic, as the genus *Ethmus* was recovered close to Hypomelina, Molurina and Oxurina (Fig. 6). However, no direct external characters are currently known to link *Ethmus* with the representatives of the abovementioned subtribes (Koch, 1958). Therefore, the recovered position of this genus is believed to be biased by the relatively apomorphic structure of its female terminalia. Furthermore, due to the unavailability of EtOH-preserved specimens, the position of *Ethmus* could not be verified by molecular data (Fig. 7b). As a result, no taxonomic decisions are made in the context of this genus.

Although the usefulness of female terminalia morphology for alpha-taxonomy was not directly tested in this paper, the analysed data suggest that at least in some of the groups the structure of the ovipositor seems to be variable between closely related species. Particularly good examples of this phenomenon are the taxonomically challenging genera *Mariazofia* of Molurina and *Vieta* of Sepidiina. In both cases, the coxite plate c4 seems to be the most variable structure. Future alpha-taxonomic and revisionary works also should consider this finding.

Taxonomy

This section summarizes the taxonomic decisions proposed in this paper. A revised key to the subtribes of Sepidiini is available at MorphoBank (http://morphobank.org/permalink/?P4101).

Subtribe Molurina Solier.

Type genus. *Moluris* Latreille.

Fig. 9. Selected representatives of Molurina sens. nov. (a–e, j) abdominal ventrites; (k) sculpture of elytral disc; (f–i, l–p) dorsal habitus. (a, f) *Mariazofia subgranulata*; (b, g) *Mariazofia pinguis*; (c) *Mariazofia ponderosa*; (d, h) *Piesomera scaber*; (e, n) *Psammodes longicornis*; (i, j, k) *Piesomera brunnea*; (l) *Argenticrinis haackei*; (m) *Bombocnodulus crinicollis*; (n) *Psammodes longicornis*; (e, o) *Psammodes probes*; (p) *Psammodes sklodowskae*.

Diagnosis. This subtribe can be unambiguously distinguished from the remaining subtribes of Sepidiini by a unique structure of the proctiger (17:4) – apical margin indented, with mid-section even with lateral sections (Fig. 5g). Furthermore, Molurina is the only lineage among the tribe whose representatives possess well-developed 3rd lobes of the coxites (5:1). An alternative definition of Molurina, based solely on external features, was recently provided by Gearner et al. (2021).

Genera included (28 genera, 579 species and subspecies). *Amiantus* Fåhreus, *Argenticrinis* Louw, *Arturium* Koch, *Bombocnodulus* Koch, *Brachyphrynus* Fairmaire, *Chiliarchum* Koch, *Dichtha* Haag-Rutenberg, *Distretus* Haag-Rutenberg, *Euphrynus* Fairmaire, *Glyptophrynus* Fairmaire, *Huilamus* Koch, *Mariazofia* Kamiński **nom.n.**, *Melanolophus* Fairmaire, *Moluris* Latreille, *Ocnodes* Fåhreus, *Piesomera* Solier **stat.r.**, *Phrynocolus* Lacordaire, *Phrynophanes* Koch, *Physophrynus* Fairmaire, *Psammodes* Kirby, *Psammophanes* Lesne, *Psammorhyssus* Kolby, *Psammotyria* Koch, *Stridulomus* Koch, *Tarsocnodes* Gebien, *Toktokkus* Kamiński & Gearner, *Tibiocnodes* Gearner & Kamiński, and *Tuberocnodes* Gearner & Kamiński.

Genus *Mariazofia* Kamiński nom.n. (replacement name).

urn:lsid:zoobank.org:act:F0918966-5EF5-40F0-B7DB-EE517B1E6404.

= *Parmularia* Koch, 1955: 35 homonym of *Parmularia* Macgillivray (Bryozoa: Cheilostomida).

Type species. *Psammodes caffra* Fåhraeus (by monotypy).

Diagnosis. *Mariazofia* is closest to *Moluris* by having confined abdominal setal patches in males (Fig. 9a–c). Both genera also share similar structure of female terminalia (Fig. 5d, e, h). *Mariazofia* can be distinguished from *Moluris* by having prognathous head (hypognathous in *Moluris*) and nonconvex pronotal disc (Koch, 1958).

Etymology. Named in honour of the daughters of the first author—Maria Antonina Kamińska (born 9 June 2016 in Warsaw, Poland) and Zofia Irena Kamińska (born3 November 2018 in Flagstaff, USA). Gender: feminine.

Species and subspecies included (141). *Mariazofia algoensis* (Péringuey) **comb.n.**, *M. asperulipennis* (Fairmaire) **comb.n.**, *M. atrata* (Haag-Rutenberg) **comb.n.**, *M. barbata* (Fåhraeus) **comb. n.**, *M. basuto* (Koch) **comb.n.**, *M. batesi* (Haag-Rutenberg) **comb.n.**, *M. bennigseni* (Kraatz) **comb.n.**, *M. brunnipes* (Haag-Rutenberg) **comb.n.**, *M. caelata* (Péringuey) **comb.n.**, *M. caffra* (Fåhraeus) **comb.n.**, *M. caraboides* (Haag-Rutenberg) **comb.n.**, *M. carinata* (Haag-Rutenberg) **comb.n.**, *M. clara* (Haag-Rutenberg) **comb.n.**, *M. collaris* (Haag-Rutenberg) **comb.n.**, *M. colorata* (Haag-Rutenberg) **comb.n.**, *M. comata* (Haag-Rutenberg) **comb.n.**, *M. convexa* (Solier) **comb.n.**, *M. coriacea* (Gerstaecker) **comb.n.**, *M. costalis* (Haag-Rutenberg) **comb.n.**, *M. dejeani* (Solier) **comb.n.**, *M. depressicollis* (Haag-Rutenberg) **comb.n.**, *M. devexa* (Fåhraeus) **comb.n.**, *M. difficilis* (Haag-Rutenberg) **comb.n.**, *M. diluta* (Haag-Rutenberg) **comb.n.**, *M. dimidiata* (Haag-Rutenberg) **comb.n.**, *M. dohrni* (Haag-Rutenberg) **comb.n.**, *M. eberlanzi* (Koch) **comb.n.**, *M. ethologa* (Koch) **comb.n.**, *M. expleta* (Quedenfeldt) **comb.n.**, *M. farta* (Péringuey) **comb.n.**, *M. ferruginea* (Haag-Rutenberg) **comb.n.**, *M. flagrans* (Péringuey) **comb.n.**, *M. fragilis* (Haag-Rutenberg) **comb.n.**, *M. fritschi* (Haag-Rutenberg) **comb.n.**, *M. funesta* (Haag-Rutenberg) **comb.n.**, *M. gariesa* (Péringuey) **comb.n.**, *M. gibba coelata* (Solier) **comb.n.**, *M. gibba gibba* (Linnaeus) **comb.n.**, *M. gibba gravida* (Solier) **comb.n.**, *M. gibba hemisphaerica* (Solier) **comb.n.**, *M. gibba nigrocostata* (Haag-Rutenberg) **comb.n.**, *M. gibba solieri* (Gebien) **comb.n.**, *M. gibba unicolor* (Fabricius) **comb.n.**, *M. glabra* (Koch) **comb.n.**, *M. glabrata biena* (Koch) **comb.n.**, *M. glabrata glabrata* (Harold) **comb.n.**, *M. grandis* (Solier) **comb.n.**, *M. granulata* (Solier) **comb.n.**, *M. granulifer* (Haag-Rutenberg) **comb.n.**, *M. guillarmodi* (Koch) **comb.n.**, *M. haagi* (Gebien) **comb.n.**, *M. herero* (Péringuey) **comb.n.**, *M. hirtipennis* (Haag-Rutenberg) **comb.n.**, *M. hirtipes* (Laporte) **comb.n.**, *M. hirta* (Bertoloni) **comb.n.**, *M. hottentottus* (Péringuey) **comb.n.**, *M. incongruens* (Péringuey) **comb.n.**, *M. infernalis* (Harold) **comb.n.**, *M. intermedia* (Péringuey) **comb.n.**, *M. janitor* (Koch) **comb.n.**, *M. kamagasa* (Péringuey) **comb.n.**, *M. kirschi* (Haag-Rutenberg) **comb.n.**, *M. kubub* (Péringuey) **comb.n.**, *M. kuisip* (Péringuey) **comb.n.**, *M. laevicollis* (Solier) **comb.n.**, *M. lanuginosa* (Haag-Rutenberg) **comb.n.**, *M. lethargica* (Péringuey) **comb.n.**, *M. memnonia* (Haag-Rutenberg) **comb.n.**, *M. mimipinguis* (Koch) **comb.n.**, *M. moschleri* (Haag-Rutenberg) **comb.n.**, *M. muata* (Harold) **comb.n.**, *M. nigrisaxicola* (Koch) **comb.n.**, *M. nitens* (Fåhraeus) **comb.n.**, *M. nitidicollis* (Haag-Rutenberg) **comb.n.**, *M. nitidipennis* (Fairmaire) **comb.n.**, *M. nitidissima* (Haag-Rutenberg) **comb.n.**, *M. obsulcata* (Haag-Rutenberg) **comb.n.**, *M. ovata* (Solier) **comb.n.**, *M. ovipennis* (Haag-Rutenberg) **comb.n.**, *M. perfida* (Péringuey) **comb.n.**, *M. picea* (Haag-Rutenberg) **comb.n.**, *M. pilifer* (Haag-Rutenberg) **comb.n.**, *M. pilosella* (Haag-Rutenberg) **comb.n.**, *M. pilosipennis* (Haag-Rutenberg) **comb.n.**, *M. pilosa* (Thunberg) **comb.n.**, *M. pinguis* (Solier) **comb.n.**, *M. plicata* (Solier) **comb.n.**, *M. plicipennis* (Gemminger) **comb.n.**, *M. ponderosa* (Fåhraeus) **comb.n.**, *M. procera* (Fåhraeus) **comb.n.**, *M. procustes* (Westwood) **comb.n.**, *M. profana* (Péringuey) **comb.n.**, *M. propinqua* (Quedenfeldt) **comb.n.**, *M. protensa* (Haag-Rutenberg) **comb.n.**, *M. pubescens* (Solier) **comb.n.**, *M. pustulifer* (Haag-Rutenberg) **comb.n.**, *M. quadricostata* (Fåhraeus) **comb.n.**, *M. rauca* (Haag-Rutenberg) **comb.n.**, *M. reflexi-margo* (Gebien) **comb.n.**, *M. retrospinosa* (Haag-Rutenberg) **comb.n.**, *M. rotundicollis* (Haag-Rutenberg) **comb.n.**, *M. rufofasciata* (Haag-Rutenberg) **comb.n.**, *M. rufonervosa* (Haag-Rutenberg) **comb.n.**, *M. rufostriata* (Haag-Rutenberg) **comb.n.**, *M. rugulosipennis* (Haag-Rutenberg) **comb.n.**, *M. rugulosa* (Solier) **comb.n.**, *M. rustica* (Péringuey) **comb.n.**, *M. scabrata gariepina* (Koch) **comb.n.**, *M. scabrata scabrata* (Solier) **comb.n.**, *M. scabriuscula* (Haag-Rutenberg) **comb.n.**, *M. segnis* (Haag-Rutenberg) **comb.n.**, *M. semipilosa* (Haag-Rutenberg) **comb.n.**, *M. semivillosa* (Haag-Rutenberg) **comb.n.**, *M. solitaria* (Péringuey) **comb.n.**, *M. spiculosa* (Haag-Rutenberg) **comb.n.**, *M. splendens* (Haag-Rutenberg) **comb.n.**, *M. steinhelli* (Haag-Rutenberg) **comb.n.**, *M. striatopilosa* (Haag-Rutenberg) **comb.n.**, *M. subaenea* (Harold) **comb.n.**, *M. subcostata* (Solier) **comb.n.**, *M. subgranulata* (Haag-Rutenberg) **comb.n.**, *M. tenuipes* (Fåhraeus) **comb.n.**, *M. timarchoides* (Haag-Rutenberg) **comb.n.**, *M. togatus* (Koch) **comb.n.**, *M. tomentosus* (Solier) **comb.n.**, *M. trachysceloides* (Haag-Rutenberg) **comb.n.**, *M. transvaalensis* (Haag-Rutenberg) **comb.n.**, *M. tricostata* (Fåhraeus) **comb.n.**, *M. tumidipennis* (Haag-Rutenberg) **comb.n.**, *M. undulata* (Haag-Rutenberg) **comb.n.**, *M. uniformis litoralis* (Koch) **comb.n.**, *M. uniformis rugigaster* (Koch) **comb.n.**, *M. uniformis uniformis* (Haag-Rutenberg) **comb.n.**, *M. valida* (Kratz) **comb.n.**, *M. velutina* (Haag-Rutenberg) **comb.n.**, *M. ventricosa* (Fåhraeus) **comb.n.**, *M. villosostriata* (Haag-Rutenberg) **comb.n.**, *M. villosula* (Haag-Rutenberg) **comb.n.**, *M. vittata* (Solier) **comb.n.**, *M. volvula* (Haag-Rutenberg) **comb.n.**, *M. zschokkei* (Koch) **comb.n.**

Genus *Piesomera* Solier stat.r.

Type species. *Pimelia scabra* Fabricius (by monotypy).

Diagnosis. *Piesomera* can be distinguished from all other Molurina by the following combination of characters (Fig. 9d, h): basal pronotal margination complete, prosternal process deflated, epipleuron with a distinct groove in median part, male setal patch large, covering several ventrites, and elytral surface covered with microtubercles.

Species and subspecies included (12). *P. blapsoides* (Haag-Rutenberg) **comb.n.**, *P. brunnea brunnea* (Olivier) **comb.n.**, *P. brunnea rufocastaneus* (Haag-Rutenberg) **comb.n.**, *P. compta* (Haag-Rutenberg) **comb.n.**, *P. diabolica diabolica* (Koch) **comb.n.**, *P. diabolica tactilis* (Koch) **comb.n.**, *P. egregia* (Haag-Rutenberg) **comb.n.**, *P. gerstaeckeri* (Haag-Rutenberg) **comb.n.**, *P. longipes* (Haag-Rutenberg) **comb.n.**, *P. producta* (Haag-Rutenberg) **comb.n.**, *P. scabra* (Fabricius) **comb.r.**, *P. setipennis* (Haag-Rutenberg) **comb.n.**

Genus *Psammodes* Kirby sens.n.

=*Psammodophysis* Péringuey, 1899: 296; type species: *Psammodophysis probes* Péringuey, 1899 (subsequent designation by Kamiński et al., 2019).

Type species. *Psammodes longicornis* Kirby (by monotypy).

Diagnosis. According to the morphological data analysed here *Psammodes* together with *Argenticrinis*, and *Bombocnodulus* forms a distinct lineage within Molurina (Figs 6, 7a). This grouping is clearly distinguishable from the remaining Molurina by having elongated 3rd coxite lobe (4:1), strongly sclerotized (with noticeable inner branching) baculus of the basal plate (7:1) and elongated basal coxites (c1 + c2)—which unambiguously separates it from *Mariazofia*. Furthermore, *Psammodes* also is easily distinguishable by its prominent eyes (Fig. 9o).

From *Argenticrinis, Psammodes* can be separated by not having pronotal and elytral surfaces covered with very dense, long, silver-coloured setae (Louw, 1979), and by having elevated edges of the pronotal disc (Fig. 9l). From *Bombocnodulus* (Fig. 9m), *Psammodes* differs by having rounded prosternal apophysis (in lateral view)—flat and sunk between procoxae, not following the curvature of procoxa in *Bombocnodulus* (Penrith, 1986).

Species Included (3). *Psammodes longicornis* Kirby, *P. probes* Péringuey, *P. sklodowskae* Kamiński & Gearner **sp.n.**

***Psammodes sklodowskae* Kamiński & Gearner sp.n.**

urn:lsid:zoobank.org:act:295C23D4-9354-4258-90B9-5D2A9EB3A766.

Type material. Holotype (TMSA), male: "S. Afr.: Namaqualand/Soutpan Dunes/31.15 S-17.52 E", "27.8.1989; E-Y: 2671\costal dunes, night Endrody & Klimaszewski". Paratypes, 5 males and 5 females (TMSA): same data as holotype; 5 males and 4 females: same data but with "31.7.1989".

Diagnosis. *P. sklodowskae* can be easily distinguished from its congeners by the presence of a deep circular depression in the middle of the pronotal disc (Fig. 9p). The other members of *Psammodes* have a shallow depression on the pronotal disk; however, these species can be distinguished from *P. sklodowskae* by the presence of costa on the elytra (Fig. 9n–p).

Description. Length 20.0–24.0 mm, width of pronotum 6.0–8.0 mm and elytra 7.0–11.0 mm. **Head**: Prognathous. Frons with densely distributed punctures (0.2–1.0 diameters apart); frontoclypeal suture visible only laterally; epistoma sparsely punctate (punctures 2–5 diameters apart), projecting anteriorly, extending well beyond the epistomal ridges; apical margin of labrum slightly emarginate medially, margin densely covered with yellowish, acuminate setae. Eye comma-shaped, with reduced ventral part; strongly projecting laterally. Mentum trapezoidal, with anterior margin straight. Submentum trapezoidal, situated in deep transverse pit. Gula with sparse, transverse rugosities. Last segment of maxillary palpi narrow. Antenna slender, moderately covered in recumbent acuminate narrow setae; besides antennomere 2 all remaining antennomeres elongate; antenna noticeably longer than pronotum. **Prothorax**: pronotum cordiform; widest in apical part. Disc densely punctate (punctures 0.1–0.2 diameters apart); with large circular depression in middle; only lateral margins complete. Hypomeron glabrous at margins. Prosternal process flat, slightly rounded in lateral view. **Pterothorax**: Scutellar shield densely covered with punctures. Elytra slender, evenly convex, widest in basal third; covered in short yellowish setae, projecting from small sparsely distributed tubercles (1–2 diameters apart); sharply extending laterally at epipleura. Epipleura—the only portion of elytra visible ventrally—impunctate and glabrous; evenly flat across whole length. Meso- and metaventrites medially densely covered with goldish setae (males). Metepisternal suture partly visible (anterior half). **Legs**: slender in both sexes; covered with sparsely distributed setae. **Abdomen**: Ventrites medially densely (male) or sparsely (female) covered with goldish setae. Ventrite 5 not marginated. **Terminalia**: Ovipositor as in Fig. 6. Aedeagus elongate as in other Molurina.

Etymology. Named in honour of Maria Skłodowska-Curie, two-time Nobel prize winner, born on 7 November 1867 in Warsaw, died on 4 July 1934 in Passy, Haute-Savoie.

Subtribe Palpomodina Kamiński & Gearner subtr.n.

urn:lsid:zoobank.org:act:07DC4A21-DA50-43BB-8752-BB6789719251.

Type genus. *Palpomodes* Koch.

Diagnosis. Convex and ovate to subcordiform elytra is characteristic for Palpomodina when compared to

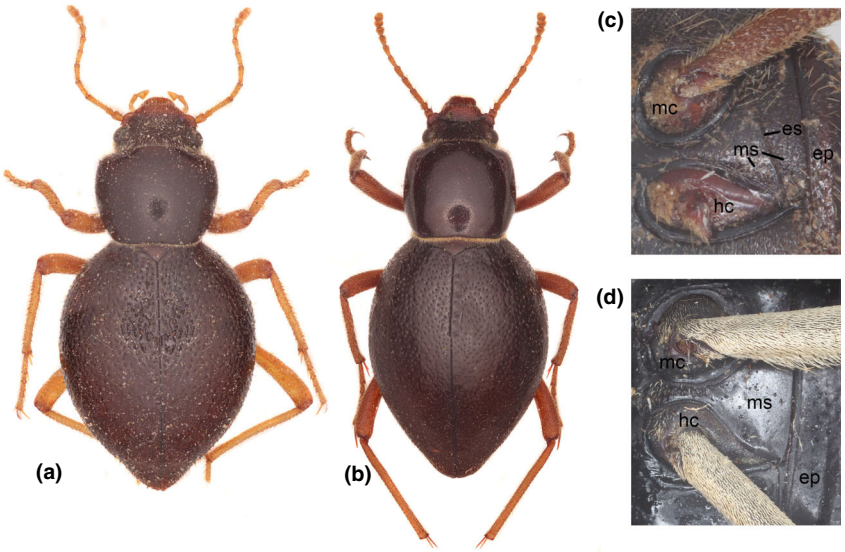


Fig. 10. Representatives of Palpomodina subtr. nov. and reference taxa. (a, b) dorsal habitus; (c, d) metasternum. (a, c) *Namibomodes zarcoi*; (b) *Namibomodes serrimargo*; (d) *Dichtha cubica*. Abbreviations: ep: epipleuron, es: episternal suture, hc: hind coxa, mc: mesocoxa, ms: metasternum.

other subtribes of Sepidiini (Fig. 10). From Hypomelina, the newly designated subtribe can be distinguished by having the sensory fields of the coxites situated at the base of lobe 4 (9:0)—situated in the middle of lobe 4 in Hypomelina; and rounded apical margin of proctiger (17:1)—pointed in Hypomelina. The structure of the proctiger also differentiates Palpomodina from Molurina—the apical margin is indented, with the mid-section even with lateral sections in Molurina (Fig. 5g). Furthermore, both those subtribes can be distinguished by the structure of spiculum ventrale—thin in Palpomodina, thick in Molurina (Fig. 5d, m). The new subtribe differs from Oxurina by not having a short transverse sclerotization on the base of the coxite lobe 3 (10:0), whereas it can be separated from Sepidiina and Trachynotina by the lack of the sclerotized apical margin of the proctiger (19:0), absence of sclerotization of the apical margin of basal plate (11:0) and Y-shaped spiculum ventrale (21:0). Furthermore, from Sepidiina it differs by the absence of pronotal appendages and by having enlarged mesotrochantin—punctiform or absent in Sepidiina. From Trachynotina it also can be differentiated by the lack of helotatic eyes. Additionally, from Hypomelina, Molurina and Trachynotina the newly designated subtribe can be distinguished by having deep and complete episternal suture of metasternum (Fig. 10c, d); this is fine to obsolescent or abbreviated in the other subtribes (Koch, 1955, 1958).

Notes. Tools for species and generic identification are available in Koch (1952, 1958, 1962) and Louw (1979).

Genera included (2 genera, 8 species and subspecies). *Namibomodes* Koch, *Palpomodes* Koch.

Acknowledgements

Funding was provided by the National Science Centre, Poland (OPUS-19 Project 2020/37/B/NZ8/02496) and National Science Foundation (DEB-1754630/2009247). The authors are grateful to Gustavo E. Flores (Argentina) for his help with identification of some South American taxa included in this study; and Magdalena Kowalewska-Groszkowska (Poland) for her assistance with laboratory procedures. Furthermore, the authors thank the following curators for the trustful loan of the specimens used in this study: Ruth Müller (TMSA), Mercedes París (MNCN-CSIC), Floyd Shockley (USNM) and Christopher C. Grinter (CASC). We also thank Riaan Stals (South African National Collection of Insects, Pretoria) for his comments on the previous manuscript versions.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer.

Conflict of interest

None declared.

References

Banaszkiewicz, M., 2006. Comparative study of female genitalia in Pedinini (sensu Iwan 2004) (Coleoptera: Tenebrionidae: Pedinini), with notes on the classification. Ann. Zool. 56, 59–77.

Bouchard, P. and Yeates, D.K., 2001. Phylogenetic relationships of the Australasian Coelometopini (Coleoptera: Tenebrionidae); a quantitative cladistic analysis with a review of biology. Org. Divers. Evol. 1, 17–43.

Bouchard, P., Bousquet, Y., Davies, A.E., Alonso-Zarazaga, M.A., Lawrence, J.F., Lyal, C.H.C., Newton, A.F., Reid, C.A.M., Schmitt, M., Ślipiński, S.A. *et al*., 2011. Family-group names in Coleoptera (Insecta). ZooKeys 88, 1–972. https://doi.org/10.3897/zookeys.88.807

Bouchard, P., Bousquet, Y., Aalbu, R.L., Alonso-Zarazaga, M.A., Merkl, O. and Davies, A.E., 2021. Review of genus-group names in the family Tenebrionidae (Insecta, Coleoptera). ZooKeys 1050, 1–633. https://doi.org/10.3897/zookeys.1050.64217.

Bray, D.F., Bagu, J. and Koegler, P., 1993. Comparison of hexamethyldisilazane (HMDS), Peldri II, and critical-point drying methods for scanning electron microscopy of biological specimens. Microsc. Res. Tech. 26, 489–495. https://doi.org/10.1002/jemt.1070260603.

Bremer, K., 1994. Branch support and tree stability. Cladistics 10, 295–304.

Chigray, S.N., 2019. Revision of the Palaearctic Pimeliini (Coleoptera, Tenebrionidae): The genus *Podhomala* Solier, 1836. Entomol. Rev. 99, 924–948. https://doi.org/10.1134/s0013873819070066.

Doyen, J.T., 1966. The skeletal anatomy of *Tenebrio molitor* (Coleoptera: Tenebrionidae). J. Entomol. Soc. Am. 5, 102–150.

Doyen, J.T., 1994. Cladistic relationships among Pimeliinae Tenebrionidae (Coleoptera). J. NY Entomol. Soc. 101, 443–514.

Doyen, J.T. and Tschinkel, W.R., 1982. Phenetic and cladistic relationships among tenebrionid beetles (Coleoptera). Syst. Entomol. 7, 127–183.

Eggs, B., Birkhold, A.I., Röhrle, O. and Betz, O., 2018. Structure and function of the musculoskeletal ovipositor system of an ichneumonid wasp. BMC Zool. 3, 12. https://doi.org/10.1186/s40850-018-0037-2.

Endrödy-Younga, S., 1989. Restructuring of the tribe Cryptochilini (Coleoptera: Tenebrionidae: Tentyriinae). Ann. Transvaal Mus. 35, 109–145.

Ernst, A., Miko, I. and Deans, A., 2013. Morphology and function of the ovipositor mechanism in Ceraphronoidea (Hymenoptera, Apocrita). J. Hymenopt. Res. 33, 25–61. https://doi.org/10.3897/jhr.33.5204.

Ferrer, J., 2002. Révision des espèces asiatiques du genre *Mesomorphus* Seidlitz, 1893 (Coleoptera: Tenebrionidae: Opatrinae: Opatrini). Ann. Zool. 524, 533–557.

Fouquè, R., 2013. Revision of the genus *Herbertfranziella* Kaszab and description of the new genus *Nepalofranziella* of Himalayan Dichillina (Coleoptera: Tenebrionidae: Stenosini). Stuttgart. Beitr. Naturk. A, NS 6, 183–197.

Gearner, O.M., Kamiński, M.J., Kanda, K., Swichtenberg, K. and Smith, A.D., 2021. Discovery of new genera challenges the subtribal classification of Tok-Tok beetles (Coleoptera: Tenebrionidae: Sepidiini). Insect Syst. Divers. 5, 1–10. https://doi.org/10.1093/isd/ixab006.

Girón, J. C., Mikó, I., González Montaña, L., Tarasov, S. and Matentzoglu, N. 2021. Ontology for the Anatomy of the Insect SkeletoMuscular system (AISM). https://zenodo.org/record/5715345#.YbOoVb3MJaQ

Goloboff, P.A. and Catalano, S.A., 2016. TNT version 1.5, including a full implementation of phylogenetic morphometrics. Cladistics 32, 221–238.

Iwan, D. and Kamiński, M.J., 2016. Toward a natural classification of opatrine darkling beetles: Comparative study of female terminalia. Zoomorphology 135, 453–485.

Iwan, D., Kamiński, M.J. and Raś, M., 2015. The last breath: A μCT-based method for investigating the tracheal system in Hexapoda. Arthropod Struct. Dev. 44, 2018–2227.

Kamiński, M.J., 2011. A new species of *Atrocrates* Koch, 1956 from South Africa (Coleoptera: Tenebrionidae: Platynotina). Ann. Zool. 61, 641–645.

Kamiński, M.J., 2021a. Techniques for dissecting adult darkling beetles. *Harvard Dataverse*, https://doi.org/10.7910/DVN/8ZQVYW

Kamiński, M.J., 2021b. Atlas of female terminalia morphology of Pimeliinae (Coleoptera). *Harvard Dataverse*, https://doi.org/10.7910/DVN/GZKRNS

Kamiński, M.J., Kanda, K., Lumen, R., Ulmer, J.M, Wirth, C.C., Bouchard, P., Aalbu, R., Mal, N. and Smith, A.D., 2019. A catalogue of the tribe Sepidiini Eschscholtz, 1829 (Tenebrionidae, Pimeliinae) of the world. ZooKeys 844, 1–121.

Kamiński, M.J., Gearner, O.M., Kanda, K., Swichtenberg, K., Purchart, L. and Smith, A.D., 2021. First insights into the phylogeny of Tok-tokkie beetles (Tenebrionidae: Molurina, Phanerotomeina) and examination of the status of the *Psammodes vialis* species-group. Zool. J. Linn. Soc. 191, 883–901. https://doi.org/10.1093/zoolinnean/zlaa052.

Kanda, K., Pflug, J.M., Sproul, J.S., Dasenko, M.A. and Maddison, D.R., 2015. Successful recovery of nuclear protein-coding genes from small insects in museums using illumina sequencing. PLoS ONE 10, e0143929. https://doi.org/10.1371/journal.pone.0143929.

Katoh, K. and Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

Kergoat, G.J., Soldati, L., Clamens, A.L., Jourdan, H., Jabbour-Zahab, R., Genson, G., Bouchard, P. and Condamine, F.L., 2014. Higher level molecular phylogeny of darkling beetles (Coleoptera: Tenebrionidae). Syst. Entomol. 39, 486–499.

Kim, J., 1996. General inconsistency conditions for maximum parsimony: Effects of branch lengths and increasing numbers of taxa. Syst. Biol. 45, 363–374.

Koch, C., 1952. Die Tenebrioniden des südlichen Afrikas XIII Vorstudien zu einer Monographie der Molurini, 3. (Col. Tenebrionidae). Entomol. Arbeit. 3, 214–349.

Koch, C., 1955. Monograph of the Tenebrionidae of southern Africa vol I (Tentyriinae, Molurini, Trachynotina: *Somaticus* Hope). Transvaal Mus. Mem. 7, 242.

Koch, C., 1958. Tenebrionidae of Angola. Publ. Cultur. Comp. Diamant. Angola 39, 11–231.

Koch, C., 1962. The Tenebrionidae of southern Africa XXXII new psammophilous species from the Namib Desert. Ann. Transvaal Mus. 24, 107–159.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. and Calcott, B., 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34, 772–773.

Lawrence, J.F., Ślipiński, A., Seago, A.E., Thayer, M.K., Newton, A.F. and Marvaldi, A.E., 2011. Phylogeny of the Coleoptera based on morphological characters of adults and larvae. Ann. Zool. 61, 1–217.

Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50, 913–925. https://doi.org/10.1080/106351501753462876.

Lighton, J.R.B., 1987. Cost of tokking: The energetics of substrate communication in the Tok-Tok beetle, *Psammodes striatus*. J. Comp. Physiol. A 157, 11–20.

Lighton, J.R.B., 2019. Knock-knock, who's there: Sex-coding, competition and the energetics of tapping communication in the Tok-Tok beetle, *Psammodes striatus* (Coleoptera: Tenebrionidae). bioRxiv 509257, preprint: Not peer reviewed. https://doi.org/10.1101/509257

Louw, S., 1979. A partial revision of the subtribes Oxurina and Hypomelina (Coleoptera: Tenebrionidae: Molurini). Cimbebasia 5, 95–177.

Maddison, W.P. and Maddison, D.R., 2019. Mesquite: A modular system for evolutionary analysis. Version 3.61, Available at: http://www.mesquiteproject.org

Matthews, E.G. and Bouchard, P., 2008. Tenebrionid Beetles of Australia: descriptions of tribes, keys to genera, catalogue of species. Australian Biological Resources Study, Canberra, p. 410.

Matthews, E.G., Lawrence, J.F., Bouchard P., Steiner, W.E. and Ślipiński, S.A., 2010. 11.14. Tenebrionidae Latreille, 1802. In: Kristensen, N.P., Beutel, R.G. (Eds.) Handbook of Zoology. Arthropoda: Insecta. Volume 2: Coleoptera, beetles. Morphology and systematics (Elateroidea, Bosthichiformia, Cucujiformia partim). DeGruyter, Berlin, pp. 574–659

Medvedev, G.S., 1973. Polozhenie rodov *Leichenum* Dej. I *Idisia* Pask. (Coleoptera, Tenebrionidae) v sisteme i opisanie novogo roda iz severnykh Karakumov [position of the genera *Leichenum* Dej. And *Idisia* Pasc. (Coleoptera, Tenebrionidae) in the system and a description of a new genus from northern Karakums]. Entomol. Obozr. 52, 644–650 [in Russian; English translation in entomological review 52, 428–433].

Medvedev, G.S., 2001. Evolyucia i sistema Zhukov-chernotelok triby Blaptini (Coleoptera, Tenebrionidae). (evolution and system of darkling beetles of the tribe Blaptini (Coleoptera, Tenebrionidae) – Meetings in memory of N. A. Cholodkovsky, vol. 53. Russian entomological society, Sankt-Petersburg, 331 pp. [in Russian]

Miller, M.A., Pfeiffer, W. and Schwartz, T., 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), IEEE, New Orleans, LA, pp. 1–8.

Nixon, K.C., 2002. WinClada, version 1.00.08. Available at: http://www.cladistics.com

Penrith, M.-L., 1986. Revision of the genera *Bombocnodulus* Koch and *Brinckia* Koch (Coleoptera: Tenebrionidae: Molurini). Afr. Entomol. 49, 55–85.

Pérez Vera, F., 2014. L'ovipositeur et sa signification dans la taxonomie de la tribu des Asidini. Bull. Soc. Entomol. Fr. 119, 181–190.

Rambaut, A., 2009. FigTree version 1.3.1, Available at: http://tree.bio.ed.ac.uk/software/figtree

Raś, M., Iwan, D. and Kamiński, M.J., 2018. The tracheal system in post-embryonic development of holometabolous insects: A case study using the mealworm beetle. J. Anatom. 232, 997–1015. https://doi.org/10.1111/joa.12808.

Raś, M. 2021. The apical part of the ovipositor of *Mariazofia basuto*. https://doi.org/10.7910/DVN/2LDFNC, Harvard Dataverse, V1

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. and Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Scudder, G.G.E., 1961. The comparative morphology of the insect ovipositor. Trans. R. Entomol. Soc. Lond. 113, 25–40.

SkyScan, 2008. Instruction Manual, Skyscan 1172 Desktop MicroCT. Skyscan, Belgium, p. 54.

Tschinkel, W.R. and Doyen, J.T., 1980. Comparative anatomy of the defensive glands, ovipositors and female genital tubes of tenebrionid beetles (Coleoptera). Int. J. Insect Morphol. Embryol. 9, 321–368.

Watt, J., 1974. A revised subfamily classification of Tenebrionidae (Coleoptera). NZ J. Zool. 11, 381–452.

Watt, J.C., 1992. Relationships of *Actizeta* and Cnemeplatiini (Coleoptera: Tenebrionidae). Syst. Entomol. 17, 287–299.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1: Taxonomic details of dissected female specimens.

Appendix S2: The majority rule consensus of post-burn-in trees obtained in Bayesian analysis of morphological data.