Female genitalia are moderately informative for phylogenetic inference and not concerted with male genitalia in Saprininae beetles (Coleoptera: Histeridae)

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Abstract. Our study – the first of its kind within Histeridae (Coleoptera) – deals with the female genitalia in the subfamily Saprininae, with respect to their variation and utility for phylogenetic reconstruction. Morphology of female genitalia (chiefly characters of the spermatheca, and variation of the gonocoxites, articulating sclerites and gonostyli) is herein described and depicted. We perform parsimony-based morphological phylogenetic analyses of Saprininae using combined datasets that comprise somatic, male and female genitalia characters. We assess phylogenetic utility of female genital characters and test for their correlation with male genital characters. We found that female genitalia are generally moderately informative for phylogenetic reconstruction. The somatic and male genitalia characters (on average) possess higher phylogenetic signal; female genitalia provide better support and diagnoses for particular clades (emphasizing their importance for systematics), in which the evolution of female genitalia can be linked to environmental factors imposed by the mode of life. Regardless of the degree of informativeness, structures of female genitalia represent an additional interesting and important source of information for systematics and taxonomy. We did not find any evidence of correlated evolution between male and female genitalia.

Introduction

The insect genitalia (the reproductive structures that are explicitly involved in copulation and internal interaction of male and female genital parts) form a highly composite organ that varies considerably between orders, families, genera and species, and thus frequently serve as an important tool for classification and identification (Eberhard, 1985). Specifically, male genitalia have been recognized as being morphologically the most diverse and variable organismal structures (Eberhard, 1985) that are well studied across insects, primarily due to their use in species differentiation (Tuxen, 1970). Song & Bucheli (2010) demonstrate that, among the genital traits, the overwhelming proportion of the studies in insects is focused on male genitalia (92.9%), whereas female genitalia remain largely unknown (7.1%). There are three main explanations of why female genitalia are so understudied. One explanation suggests

that poor sclerotization of female genitalia makes their investigation challenging. For example, more strongly sclerotized terminal abdominal segments are more often studied in females (at least in some families) than the membranous and poorly visible parts between gonopore and ovaries (P. Jałoszyński, personal communication). Another explanation is that researchers generally consider that female genitalia lack variation (Eberhard, 1985) or, at least, evolve substantially more slowly than male genitalia, and are thus uninformative for systematics. The latest investigation (Ah-King *et al.*, 2014) points to the bias that reflects now outdated assumptions about the unimportance of female genitalia (due to the lack of variation) in studying evolutionary processes. Increasing numbers of recent studies provide considerable evidence that female genitalia are indeed variable at different taxonomic levels, including closely related species (e.g. Iwan & Kamiński, 2016), or even at populational level (Polihronakis, 2006). Yet those studies emphasize that our poor knowledge of female genitalia hinders a comprehensive understanding of processes driving genital evolution in general (Ronn *et al.*, 2007; Puniamoorthy *et al.*, 2010).

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Like other insects, beetles also have a history of lack of research on female genitalia, with only a few works conducting assessments at broad phylogenetic scales. For example, Miller (2008) and Miller & Bergsten (2012) utilized female genitalia characters to reconstruct the phylogeny of the beetle families Noteridae and Gyrinidae. Lawrence *et al.* (2011), in their phylogeny of the families of Coleoptera based on the morphological characters of adults and larvae, used 14 characters of female genitalia, mainly those of the ovipositor. Two studies on the family Carabidae (Bils, 1976; Deuve, 1993) stress the utility of female tract characters for the higher classification of the family. Liebherr & Will (1998) showed that female genitalia exhibit a moderate degree of informativeness for inferring the Carabidae tree. Özgül-Siemund & Ahrens (2015) provided compelling evidence of high taxonomic and phylogenetic importance of female genitalia in Sericini chafers (Scarabaeidae). A notable exception to the rule of neglecting the female genitalia within Coleoptera is the superfamily Tenebrionoidea, with numerous studies concluding that 'the comparative morphological studies of the female genitalia are extremely useful for determining the phylogenetic relationships among the members of this superfamily' (Iwan & Kamiński, 2016 and the references therein).

In the present study, we provide the first study of female genitalia in the beetle subfamily Saprininae (Coleoptera: Histeridae) by giving a comprehensive description of genital morphology and assessing the utility of genital traits for phylogenetic inference. We evaluate the degree of coevolution between male and female genitalia in the Saprininae and strive to disentangle the evolutionary mechanisms that drive morphological changes in female and male genitalia within this subfamily. Although our paper treats mainly the sclerotized terminal abdominal segments of the female genitalic system, the coevolution of genitalia happens when there is a correspondence between pairs of structures, which may be functional, mechanical or developmental. We therefore argue that the correspondence between terminal male abdominal segments (e.g. eighth and ninth sternites or tergites; coded in most morphological studies of Coleoptera as 'male genitalia'), and our characters of terminal female abdominal segments (mostly those of the ovipositor; likewise coded in most morphological studies as 'female genitalia') cannot be, *a priori*, ruled out or refuted. Therefore, we conduct statistical tests to test our hypothesis of their possible coevolution. Our primary reason to test for correlation between characters is based on the evidence from other organisms that evolution of female and male genitalia might be correlated due to different biological mechanisms.

The monophyletic subfamily Saprininae is the second-largest subfamily of the Histeridae, itself a worldwide radiation of 620+ species from 73 genera and subgenera, with many more still awaiting discovery. The members of the subfamily are mostly generalist predators preying on larval Diptera or Coleoptera, but several inquilinous as well as psammophilous lineages appeared during their evolutionary history (Lackner, 2014). They are characterized by the presence of a distinctive sensory apparatus situated inside their antennal clubs (for more details on this, as well as on the general diagnosis of the subfamily see Lackner, 2010) and by the opened antennal cavities, not covered by prosternal 'alae'. Their morphology has recently been reviewed by Lackner (2010) and research attention has been growing in the recent years (see, e.g., Lackner, 2014 and the references therein). A morphology-based phylogeny of the subfamily (*loc. cit.*) will be complemented by a molecular one (T. Lackner *et al.*, in preparation).

Despite being underrepresented overall, morphological studies of histerid female genitalia were conducted by Vienna (1980), Mazur (1981) and Yélamos (2002). More detailed descriptions, especially those of spermatheca, can be found in De Marzo & Vienna (1982) and Ôhara (1994). Specifically, the gonocoxites and spermatheca of Saprininae and allies were reviewed by Ôhara (2003, 2017), Mazur *et al.* (2005) and Mazur & Ôhara (2003). Other authors (e.g. Vomero, 1973, 1977; Dégallier, 1981 or Tishechkin & Lackner, 2012) have also contributed to investigation of gonocoxites and spermatheca through their taxonomic works. Several characters of female genitalia were included into the phylogenetic datasets by Caterino & Vogler (2002) and Ślipiński & Mazur (1999), and Caterino & Tishechkin (2014) included 31 female genitalia characters in their phylogenetic study of the tribe Exosternini (Histerinae).

Our present paper addresses the informativeness of the female genitalia characters and compares their correspondence with the male genitalia in Saprininae evolution. Along these lines, we decided to re-examine the classification of the Saprininae spermathecae, as proposed by De Marzo & Vienna (1982), with denser sampling of included subfamily representatives. The new analysis of the morphological characters of the subfamily presented here is not meant to represent a substantial reanalysis of the group's phylogeny. For more informative conclusions on the deeper Saprininae phylogeny, we urge the reader to await the upcoming molecular evidence analysis (T. Lackner *et al.*, in preparation).

Material and methods

Morphological techniques and taxa examined

Dried female specimens of most type species from genera and subgenera of the Saprininae, as well as several other nontype members of the subfamily (used when female specimens of type species were not available) and representatives of taxa purported to be closely related to the Saprininae (labelled as outgroup; see below and Table 1 for details), have been treated according to the standard methods (see, e.g., Lackner, 2014), with special regard to the spermatheca. The amount of time the spermatheca spends in the KOH and/or in the lactic acid is essential for successful preparation. Internal structures (and even the outside pouch and the spermathecal gland) of the spermatheca can be easily damaged and it becomes challenging to determine the shape or position of the invaginations (see later). The effect of 'too much KOH' can be seen, for example, on the drawing 3J of Ôhara (2003), where the spermathecal gland is destroyed, most probably because of long exposure to KOH. One possible way of avoiding this is to remove spermatheca from genitalia after a *c.* 30-min exposure to KOH and to treat it with lactic

acid for several minutes only. When removing the female genital apparatus, great care needs to be taken with regard to the spermatheca. It often becomes disconnected from the rest of the female terminalia and remains stranded in the abdominal cavity where, owing to its minute size, it is located only with great difficulty. One possible method to avoid this is to detach the entire abdomen, or at least tergites IV–VII, but even then there is no guarantee the spermatheca will not be lost.

Selection of taxa. Every higher taxonomic unit of the Saprininae (subgenus or genus; sensu Lackner, 2014, 2016a,b; Lackner & Ratto, 2014; Lackner & Gomy, 2014; Vienna, 2015; Lackner & Leschen, 2017; Tishechkin & Lackner, 2017) has been represented by their type species, with the single exception of *Xerosaprinus (Auchmosaprinus) laciniatus* (Casey), type species of the subgenus *Auchmosaprinus* Wenzel of the genus *Xerosaprinus* Wenzel (see Table 1 for details). The female genitalia of *X. (A.) laciniatus* had been drawn (Fig. S1E), but the specimen itself was destroyed before coding. For the sake of the completion of the genitalia drawings, the gonocoxite, gonostylus and articulating sclerite of *X. (A.) laciniatus* are depicted among the online supplementary files, but the taxon itself is missing from the phylogenetic analysis. In several other cases, the female specimens of the type species were unavailable for the morphological examinations of female genitalia, but were included in the phylogenetic analysis (see Table 1 and morphological matrix for details). No member of *Saprinosternus* Lackner, a subgenus of *Phoxonotus* Marseul, was available for study. The Palaearctic taxon *Euspilotus (Neosaprinus) perrisi* (Marseul) has been included although it is not the type species of the subgenus *Neosaprinus* Bickhardt. This subgenus is chiefly spread in the Nearctic and Neotropical realm and there is compelling reason to suspect that it does not belong to this subgenus (T. Lackner, unpublished data). The genus *Saprinus* Erichson was represented by two species [*S. (S.) maculatus* (P. Rossi) and *S. (S.) semistriatus* (Scriba) – the type species of the genus)] because of the presumed heterogeneity of the genus. Digital photographs of male genitalia were taken with a Nikon 4500 Coolpix camera (Japan) and edited in Adobe Photoshop CS5 (San José, California). Genitalia drawings based on the photographs or direct observations were produced with the aid of a Hakuba klv-7000 light box (Japan). Beetle terminology follows that of Ôhara (1994) and Lackner (2010). Apart from the representative figures of female genitalia included in this paper, the remaining figures are included in separate plates available as online supplementary files. The character list and matrix are likewise available as online supporting information.

Phylogenetic analysis

The character matrix used in a previous analysis (Lackner, 2014) was complemented by the addition of female genitalia characters (character numbers 96–100). Phylogenetic inference was performed under the maximum parsimony (MP) approach in TNT (Goloboff *et al.*, 2008) using the following options: hold 1 000 000; collapse; mult = tbr replic1000; hold 500; best. We ran both the parsimony analyses under equal (EW) and implied weights (IW). Implied weights parsimony was chosen because it was shown to outperform EW parsimony (Goloboff *et al.*, 2017). The IW analyses were run under a range of values of the concavity factor, $k = \{20, 40, 60, 100, 150, 200\}$ to evaluate the sensitivity of the results.

Assessing character support and informativeness

We calculated partitioned Bremer support (PBS) values using the approach of Peña *et al.* (2006) for the inferred set of EW trees to assess the clade support for the three categories of characters: somatic, male and female. Additionally, we calculated the distribution and mean values of consistency index (CI) and retention index (RI) (Farris, 1989) for each of the three character categories and for each female character across the 1500 randomly selected trees from the EW analysis.

To assess the statistical significance of mean values for each index and each character category, we ran pairwise permutation tests (Fay, 2010). These indices were chosen to evaluate homoplasy and grouping capabilities of the characters.

WINCLADA (Nixon, 2002) was used to map synapomorphies onto the most parsimonious tree from the IW analysis using an option showing unambiguous changes only. To assess correlated evolution, we tested the null hypothesis that synapomorphies corresponding to characters of male and female genitalia are distributed independently of each other along each branch in the tree. The synapomorphies of each character category were treated as present/absent regardless of the number of synapomorphies along a branch. The null hypothesis was tested using the multinomial test implemented in the R package EMT (https://cran.r-project.org/web/packages/EMT/index.html). We acknowledge that this statistic is only capable of testing the association between synapomorphies (i.e. character changes) of male and female genitalia over branches, and does not, to the full extent, test for character correlation. Nevertheless, an association of synapomorphies may be taken as good evidence for character correlation, but not vice versa. A character change on one branch that influences a character change on the other branch exhibits a type of correlation that cannot be tested using our approach. Model-based comparative methods can potentially be used to elucidate the correlation between discrete traits, but in the light of recent criticism (Maddison & FitzJohn, 2015) demonstrating high false-positive rates for these methods, we decided not to apply them here.

Results

Descriptive morphology of female genitalia in Saprininae

The female reproductive tract consists of paired ovaries with four ovarioles that connect to lateral oviducts uniting to form a common oviduct and expanded bursa copulatrix (Kovarik & Caterino, 2016), a vagina and the spermatheca [for schematic figures, see Yélamos (1989) or Caterino &

Table 1. Type species of the genera and subgenera of the Saprininae with the characters and character states of their female genitalia.

Genus or subgenus	Taxon	Spermatheca – form, structure	Articulating sclerite	Gonostylus	Gonocoxite, shape
Anapleus – OG Horn	*Anapleus cyclonotus* (Lewis)	SP weakly sclerotized, no invaginations visible	Absent	Well developed, situated in apical third of gonocoxite	Constricted medially, apex dilated
Dendrophilus – OG Leach	*Dendrophilus punctatus* (Herbst)	SP pear-shaped; very weakly sclerotized, no invaginations visible	Present, small, circular	Well developed, situated in apical third of gonocoxite	Rather elongate, apex dentate
Afroprinus – IG Lackner	*Afroprinus cavicola* Lackner	SP cylindrical; invaginations well developed, equal	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Afrosaprinus – IG Vienna	*Afrosaprinus maculatus* Vienna	Female unavailable	Female unavailable	Female unavailable	Female unavailable
Alienocacculus – IG Kanaar	*Alienocacculus neftensis* (Olexa)	SP lost	Absent	Atrophied, situated on apex of gonocoxite	Wider than long, apex simple
Ammostyphrus – IG Reichardt	*Ammostyphrus cerberus* Reichardt	SP subcylindrical; invaginations well developed, equal	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Aphelosternus – IG Wenzel	*Aphelosternus interstitialis* (J.L. LeConte)	SP globular; invaginations weak	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Atavinus – IG Olexa	*Atavinus atavus* Reichardt	SP ovoid; invaginations well developed, equal	Present, boomerang-shaped	Absent	Wider than long, apex simple
Auchmosaprinus – IG Wenzel	*Auchmosaprinus laciniatus* (Casey)	SP globular; invaginations well developed, equal	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex simple (outwardly arcuate)
Australopachylopus – IG Lackner & Leschen	*Australopachylopus lepidulus* (Broun)	SP cylindrical; BI large, AI very weak	Present, boomerang-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex faintly inwardly arcuate
Axelinus – IG Kryzhanovskij	*Axelinus ghilarovi* Kryzhanovskij	SP cylindrical; invaginations well developed, equal	Present, labrum-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Baeckmanniolus – IG Reichardt	*Baeckmanniolus dimidiatus* (Illiger)	SP barrel-shaped; invaginations well developed, equal	Present, boomerang-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Chalcionellus – IG Reichardt	*Chalcionellus amoenus* (Erichson)	SP globular; invaginations well developed, equal	Present, labrum-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Chelyoxenus – IG Hubbard	*Chelyoxenus xerobatis* Hubbard	SP globular; invaginations weak	Present, reduced	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Chivaenius – IG Olexa	*Chivaenius kryzhanovskii* Olexa	SP globular; invaginations well developed, equal	Present, labrum-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Colpellus – IG Reichardt	*Colpellus praecox* (Erichson)	SP ovoid; invaginations well developed, equal	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Ctenophilothis – IG Kryzhanovskij	*Ctenophilothis chobauti* (Théry)	SP ovoid; invaginations well developed, equal	Present, reduced	Absent	Wider than long, apex simple
Dahlgrenius – IG Penati & Vienna	*Dahlgrenius sculpturifer* (Marseul)	SP barrel-shaped; invaginations well developed, equal	Absent	Atrophied, situated on apex of gonocoxite	Wider than long, apex simple
Eopachylopus – IG Reichardt	*Eopachylopus ripae* (Lewis)	SP cylindrical; invaginations well developed, equal	Present, reduced	Rather small, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Erebidus – IG Reichardt	*Erebidus vlasovi* Reichardt	SP lost	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate

Table 1. Continued

Genus or subgenus	Taxon	Spermatheca – form, structure	Articulating sclerite	Gonostylus	Gonocoxite, shape
Eremosaprinus - IG Ross	*Eremosaprinus unguiculatus* (Ross)	SP globular; invaginations weak, visible	Present, reduced	Rather small, situated in apical third of gonocoxite	Elongate, apex simple (outwardly arcuate)
Euspilotus – IG Lewis	*Euspilotus zonalis* Lewis	SP barrel-shaped; invaginations weak	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex faintly inwardly arcuate
Exaesiopus – IG Reichardt	*Exaesiopus grossipes* (Marseul)	SP subcylindrical; invaginations well developed, equal	Present, labrum-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Farabius - IG Reichardt	*Farabius hexeris* Reichardt	SP ovoid; invaginations well developed, equal	Present, boomerang-shaped	Atrophied, situated on apex of gonocoxite	Elongate, apex simple (outwardly arcuate)
Geomysaprinus – IG Ross	*Geomysaprinus goffi* Ross	SP globular; invaginations weak	Present, boomerang-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Gnathoncus – IG Jacquelin du Val	*Gnathoncus rotundatus* (Kugelann)	SP globular; invaginations very weakly developed	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex dentate
Hemisaprinus – IG Kryzhanovskij	*Hemisaprinus subvirescens* (Ménétries)	SP ovoid; invaginations hourglass-like, interconnected	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex simple (outwardly arcuate)
Hesperosaprinus – IG Wenzel	*Hesperosaprinus assimilis* (Paykull)	SP bean-shaped; invaginations weak	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Hypocacculus – IG Bickhardt	*Hypocacculus metallescens* (Erichson)	SP barrel-shape; invaginations well developed, equal	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Hypocaccus – IG C. Thomson	*Hypocaccus rugiceps* (Duftschmid)	SP barrel-shaped; invaginations well developed, equal	Present, labrum-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Iridoprinus – IG Lackner & Leschen	*Iridoprinus myrmecophilus* Lackner & Leschen	Female unavailable	Female unavailable	Female unavailable	Female unavailable
Lophobregmus – IG Wenzel	*Lophobregmus scabriceps* (Casey)	Female unavailable	Female unavailable	Female unavailable	Female unavailable
Malagasyprinus – IG Lackner & Gomy	*Malagasyprinus caeruleatus* (Lewis)	SP globular; invaginations well developed, equal	Present, labrum-shaped	Atrophied, situated on apex of gonocoxite	Almost as wide as long, apex faintly inwardly arcuate
Microsaprinus – IG Kryzhanovskij	*Microsaprinus therondianus* (Dahlgren)	SP globular; invaginations well developed, sub equal	Present, labrum-shaped	Rather small, situated in apical third of gonocoxite	Rather elongate, apex simple (outwardly arcuate)
Monachister – IG Mazur	*Monachister californicus* Mazur	Female unavailable	Female unavailable	Female unavailable	Female unavailable
Myrmetes – IG Marseul	*Myrmetes paykulli* Kanaar	SP globular; invaginations very weakly developed	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex dentate
Nannolepidius – IG Reichardt	*Nannolepidius braunsi* (Bickhardt)	SP lost	??	Absent	Wider than long, apex simple
Neopachylopus – IG Reichardt	*Neopachylopus sulcifrons* (Mannerheim)	SP globular; invaginations well developed, equal	Present, boomerang-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Neosaprinus – IG Bickhardt	*Neosaprinus limatus* (Marseul)	SP lost	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex dentate
Neosaprinus – IG Bickhardt	*Neosaprinus perrisii* (Marseul)	SP globular; invaginations weak	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex dentate

Table 1. Continued

Genus or subgenus	Taxon	Spermatheca – form, structure	Articulating sclerite	Gonostylus	Gonocoxite, shape
Nessus – IG Reichardt	Nessus rubripes (Erichson)	SP subcylindrical; invaginations well developed, equal	Present, labrum-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Notosaprinus – IG Kryzhanovskij	Notosaprinus irinus (Marseul)	SP globular; BI very weakly developed, AI large	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex simple (outwardly arcuate)
Orateon – IG Lackner & Ratto	Orateon praestans Lackner & Ratto	SP globular; invaginations well developed, equal	Present, reduced	Absent	Wider than long, apex simple
Pachylopus – IG Erichson	Pachylopus dispar Erichson	SP globular; invaginations well developed, equal	Present, boomerang-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Parahypocaccus – IG Vienna	Parahypocaccus weyerichi Vienna	Female unavailable	Female unavailable	Female unavailable	Female unavailable
Paramyrmetes – IG Bruch	Paramyrmetes foveipennis Bruch	Female unavailable	Female unavailable	Female unavailable	Female unavailable
Paraphilothis – IG Vienna	Paraphilothis mirabilis Vienna	Female unavailable	Female unavailable	Female unavailable	Female unavailable
Paravolvulus – IG Reichardt	Paravolvulus ovillum (Solskij)	SP lost	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Phaonius – IG Reichardt	Phaonius pharao Marseul	SP ovoid; invaginations weak	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex inwardly arcuate
Philothis – IG Reichardt	Philothis arcanus Reichardt	SP cylindrical; invaginations well developed, equal	Absent	Absent	Wider than long, apex simple
Philoxenus – IG Mazur	Philoxenus desertorum Mazur	SP globular; invaginations well developed, equal	Present, reduced	Well developed, situated in apical third of gonocoxite	Elongate, apex simple (outwardly arcuate)
Pholioxenus – IG Reichardt	Pholioxenus phoenix (Reichardt)	SP globular; invaginations well developed, equal	Present, labrum-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Phoxonotus – IG Marseul	Phoxonotus tuberculatus Marseul	SP bean-shaped; invaginations weak	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Pilisaprinus – IG Kanaar	Pilisaprinus verschureni (Thérond)	SP globular; invaginations well developed	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex simple (outwardly arcuate)
Platysaprinus – IG Bickhardt	Platysaprinus latimanus (Schmidt)	SP bean-shaped; invaginations weak	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex inwardly arcuate
Priscosaprinus – IG Wenzel	Priscosaprinus pectoralis (J.L. LeConte)	SP globular; invaginations well developed, equal	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Psammoprinus – IG Gomy & Vienna	Psammoprinus namibiensis Mazur	SP globular; invaginations well developed, equal	Absent	Absent	Wider than long, apex simple
Reichardtia – IG Wenzel	Reichardtia pedator (Sharp)	SP semiglobular; invaginations hourglass-like, interconnected	Present, boomerang-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex faintly inwardly arcuate
Reichardtiolus – IG Kryzhanovskij	Reichardtiolus duriculus (Reitter)	SP cylindrical; invaginations well developed, equal	Present, labrum-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Saprinillus – IG Kryzhanovskij	Saprinillus paromaloides Kryzhanovskij	SP lost	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate
Saprinodes – IG Lewis	Saprinodes falcifer Lewis	SP lost	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex simple (outwardly arcuate)

Table 1. Continued

Genus or subgenus	Taxon	Spermatheca – form, structure	Articulating sclerite	Gonostylus	Gonocoxite, shape
Saprinosternus – IG Lackner	*Saprinosternus venustus* (Erichson)	Female unavailable	Female unavailable	Female unavailable	Female unavailable
Saprinus – IG Erichson	*Saprinus semistriatus* (Scriba)	SP globular; invaginations large, oriented following two different axes	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex faintly inwardly arcuate
Sarandibrinus – IG Lackner & Gomy	*Sarandibrinus araceliae* Lackner & Gomy	SP lost	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex faintly inwardly arcuate
Satrapister – IG Bickhardt	*Satrapister nitens* Bickhardt	Female unavailable	Female unavailable	Female unavailable	Female unavailable
Styphrus – IG Motschulsky	*Styphrus corpulentus* Motschulsky	SP globular; invaginations well developed, equal	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex simple (outwardly arcuate)
Terametopon – IG Vienna	*Terametopon levissimestriatus* Vienna	SP lost	Absent	Absent	Wider than long, apex simple
Tomogenius – IG Marseul	*Tomogenius incisus* (Erichson)	SP globular; invaginations very weakly developed	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex dentate
Toxometopon – IG Reichardt	*Toxometopon rubricatus* (Lewis)	SP globular; invaginations well developed, equal	Present, labrum-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex faintly inwardly arcuate
Turanostyphrus – IG Tishechkin	*Turanostyphrus kizilkumis* Tishechkin	SP lost	??	Rather small, situated in apical third of gonocoxite	Elongate, apex simple (outwardly arcuate)
Vastosaprinus – IG Wenzel	*Vastosaprinus ciliatus* (J.L. LeConte)	Female unavailable	Female unavailable	Female unavailable	Female unavailable
Xenonychus – IG Wollaston	*Xenonychus tridens* (Jacquelin-Duval)	SP globular; invaginations well developed, equal	Present, labrum-shaped	Rather small, situated in apical third of gonocoxite	Elongate, apex faintly inwardly arcuate
Xenophilothis – IG Kryzhanovskij	*Xenophilothis choumovitchi* (Thérond)	SP cylindrical; invaginations well developed, equal	Absent	Absent	Wider than long, apex with tiny notch
Xerosaprinus – IG Wenzel	*Xerosaprinus lubricus* (J.L. LeConte)	SP lost	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Rather elongate, apex simple (outwardly arcuate)
Zorius – IG Reichardt	*Zorius funereus* (Schmidt)	SP lost	Present, labrum-shaped	Well developed, situated in apical third of gonocoxite	Elongate, apex inwardly arcuate

OG, outgroup; IG, ingroup; SP, spermatheca; AI, apical invagination; BI, basal invagination; violet typeset, nontype species of the genus (female of type species not available).
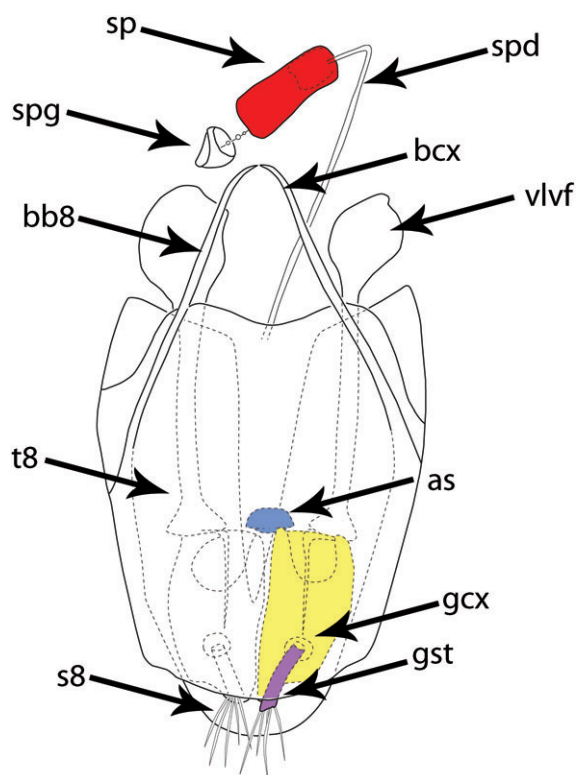
Fig. 1. Female genitalia of *Afroprinus cavicola* Lackner, dorsal view, schematic: s8, eighth sternite; gst, gonostylus; gcx, gonocoxite; as, articulating sclerite; t8, eighth tergite; bb8, basal baculi of the eighth sternite; vlvf, valvifer; bcx, bursa copulatrix; spd, spermathecal duct; spg, spermathecal gland; sp, spermatheca. [Colour figure can be viewed at wileyonlinelibrary.com].

Tishechkin (2014), who in fig. 217 provided the most accurate and detailed schematic depiction of the female genitalia in Histeridae (Histerinae: Exosternini) to date]. Although the present contribution deals solely with the spermatheca (for the lesser part) and with the variation found on the gonocoxites, gonostyli and articulating sclerites (the major part), a schematic drawing of the female genitalia is provided for clarity (Fig. 1). Because of the general uniformity, only several spermathecae representing the most common types are illustrated and provided with a discussion. The major part of the study is thus devoted to the variation found on the gonocoxites, articulating sclerites and gonostyli. As already mentioned, only representative figures of gonocoxites, articulating sclerites and gonostyli are included in the paper, with the remainder vailable online as a supplementary file (Figs S1–S6).

Spermatheca

The spermatheca [an organ that stores and maintains the viability of sperm until fertilization; itself an invagination of the eighth abdominal segment – Snodgrass (1935)] in the Histeridae is known to exhibit two basic forms:

1 Consisting of four to nine sessile receptacles lacking a distinct spermathecal gland (Histerinae: Histerini; for illustrations see De Marzo & Vienna, 1982, figs 1–4).
2 Consisting of a single receptacle with a distinct spermathecal gland (Fig. 1) – this type is also found in the Saprininae. In this second type, the spermatheca can be variously sclerotized. For example, it is very weakly sclerotized in *Dendrophilus* (Fig. 2A), a taxon that is believed to be the sister taxon to the Saprininae subfamily.

In the Saprininae, the spermatheca is always strongly sclerotized and variously shaped: in many cases it is globular (Fig. 2B), but can likewise be barrel-shaped (Fig. 2C), bean-shaped (Fig. 2D), subcylindrical (Fig. 2E), cylindrical (Fig. 1) or ovoid (Fig. 2F). Numerous subcategories subtending the ones described exist within the subfamily. We have done our best to parse this variation into discrete character states, but were unable to distinguish among many different forms and decided to code the spermathecal characters more conservatively. It is positioned beneath the apex of bursa copulatrix.

De Marzo & Vienna (1982), in their classic study of the spermatheca in the Histeridae, divided the variation of the spermatheca within the Saprininae into six categories, based on the presence/absence or position or shape of the basal and apical invaginations of the spermatheca [these invaginations form a muscle, the contraction of which causes the sperm to be transferred to the bursa copulatrix; Rodríguez (1994)] (Fig. 2E). According to their observations, categories 1–6 are all found within *Saprinus*, whereas all the other Saprininae higher taxa they examined belong exclusively to their sixth category. According to our observations, the amount of variation regarding the shape, position, absence or presence of both invaginations is impossible to categorize and De Marzo & Vienna's (1982) categorization is therefore regarded as arbitrary and rejected in the present paper.

Despite numerous ambiguities, and the large amount of variation, some general observations are presented here:

1 Taxa, which were recovered to be near the root of the Saprininae phylogeny (see Lackner, 2014), have both their invaginations very weakly developed, in several cases almost absent. This applies to *Tomogenius*, *Gnathoncus* and *Myrmetes*. This contradicts the observations of De Marzo & Vienna (1982), who included *Gnathoncus* in their sixth category ('both invaginations well-developed, oriented following a common axis'), as well observations of Mazur & Ôhara (2003), who in fig. 3B depicted the spermatheca of *Gnathoncus vietnamicus* Kryzhanovskij, 1972 with both basal and apical invaginations, albeit small and weakly developed.
2 Taxa representing mostly diverse and widely known groups which, according to the published phylogeny (Lackner, 2014), appeared in the evolution of the subfamily next (e.g. *Euspilotus*, *Geomysaprinus*, *Chelyoxenus*, *Aphelosternus, Phoxonotus, Eremosaprinus*) have their basal and apical invaginations rather weakly developed; albeit more pronounced than the taxa in the first group. De Marzo & Vienna (1982) only examined *Euspilotus (Neosaprinus) perrisi* from
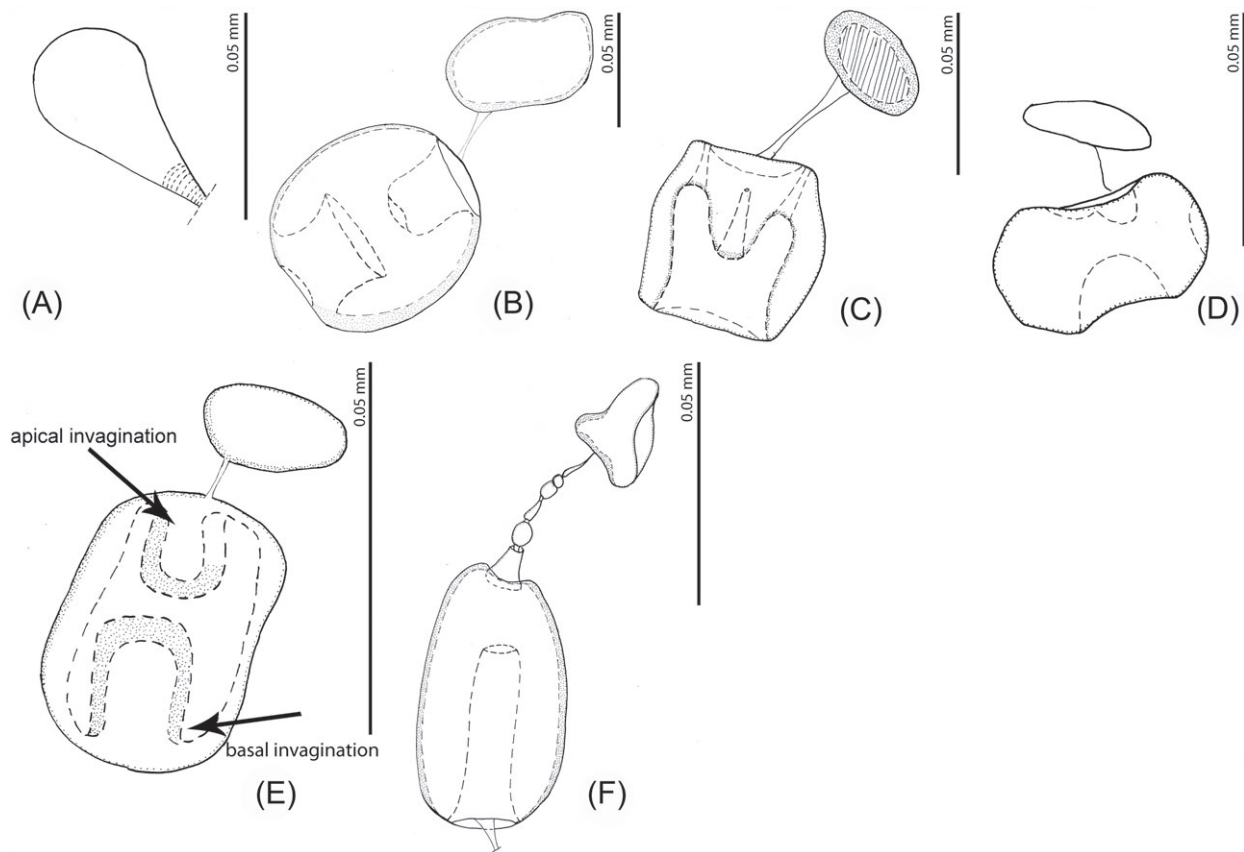
Fig. 2. Spermatheca of the selected representatives of Dendrophilinae and Saprininae. (A) *Dendrophilus (Dendrophilus) punctatus punctatus* (Herbst) (Dendrophilinae); (B) *Neopachylopus sulcifrons* (Mannerheim) (Saprininae); (C) *Notosaprinus irininus* (Marseul) (Saprininae); (D) *Euspilotus (Platysaprinus) latimanus* (Schmidt) (Saprininae); (E) *Philothis (Atavinus) atavus* Reichardt (Saprininae); (F) *Australopachylopus lepidulus* (Broun) (Saprininae).

among these taxa and placed it again in their sixth category ('both invaginations well-developed, oriented following a common axis'). Dégallier (1981) depicted spermathecae of several *Euspilotus* species – without, however, showing the internal structures.

3 Basal and apical invaginations vary widely within *Saprinus*. As mentioned earlier, De Marzo & Vienna (1982) placed the *Saprinus* species they examined into all of their six categories. The great heterogeneity of basal and apical invaginations regarding their shape and orientation can be further confirmed by examining illustrations provided by Ôhara in Mazur & Ôhara (2003; figs 7C, 11A–B), Ôhara (2003; figs 3J, 6) and Mazur *et al.* (2005), as well as our own observations. So, according to Ôhara (2003) (*loc. cit.*), the apical invagination can be nonexistent, as in *S. (S.) splendens* (Paykull) [this would fit into De Marzo & Vienna's (1982) category I]. Both invaginations can be oriented on the same axis and be actually interconnected, as in *S. (S.) optabilis* Marseul [this would loosely fit into De Marzo & Vienna's (1982) category VI], or even be so large that they actually seem to steer clear of each other and are oriented following two different axes, as in *S. (S.) subustus* Marseul [this would fit

into De Marzo & Vienna's (1982) category IV]. The basal and apical invaginations can vary considerably within *Saprinus*, and extremes at both ends are very different.

4 Almost all taxa that were recovered in the previous analysis (Lackner, 2014) as members of a large, albeit poorly resolved, clade can be further characterized by the spermathecal structure. Both their basal and apical invaginations are well developed and pronounced, often (Fig. 2E) equally sized, never interconnected and always oriented along the common axis. Notable exceptions to this rule are the spermathecae of *Australopachylopus lepidulus* (Broun), whose apical invagination is weakly developed but whose basal invagination is rather large (Fig. 2F), and that of *Reichardtia pedator* (Sharp), where both invaginations are large, hourglass-like and interconnected. By the shape and configuration of their spermathecae these New Zealand endemics thus resemble species of *Saprinus* or *Hemisaprinus*.

Ovipositor: Gonocoxite

The female ovipositor consists of a pair of appendages, the gonocoxites, which are attached to the paraprocts
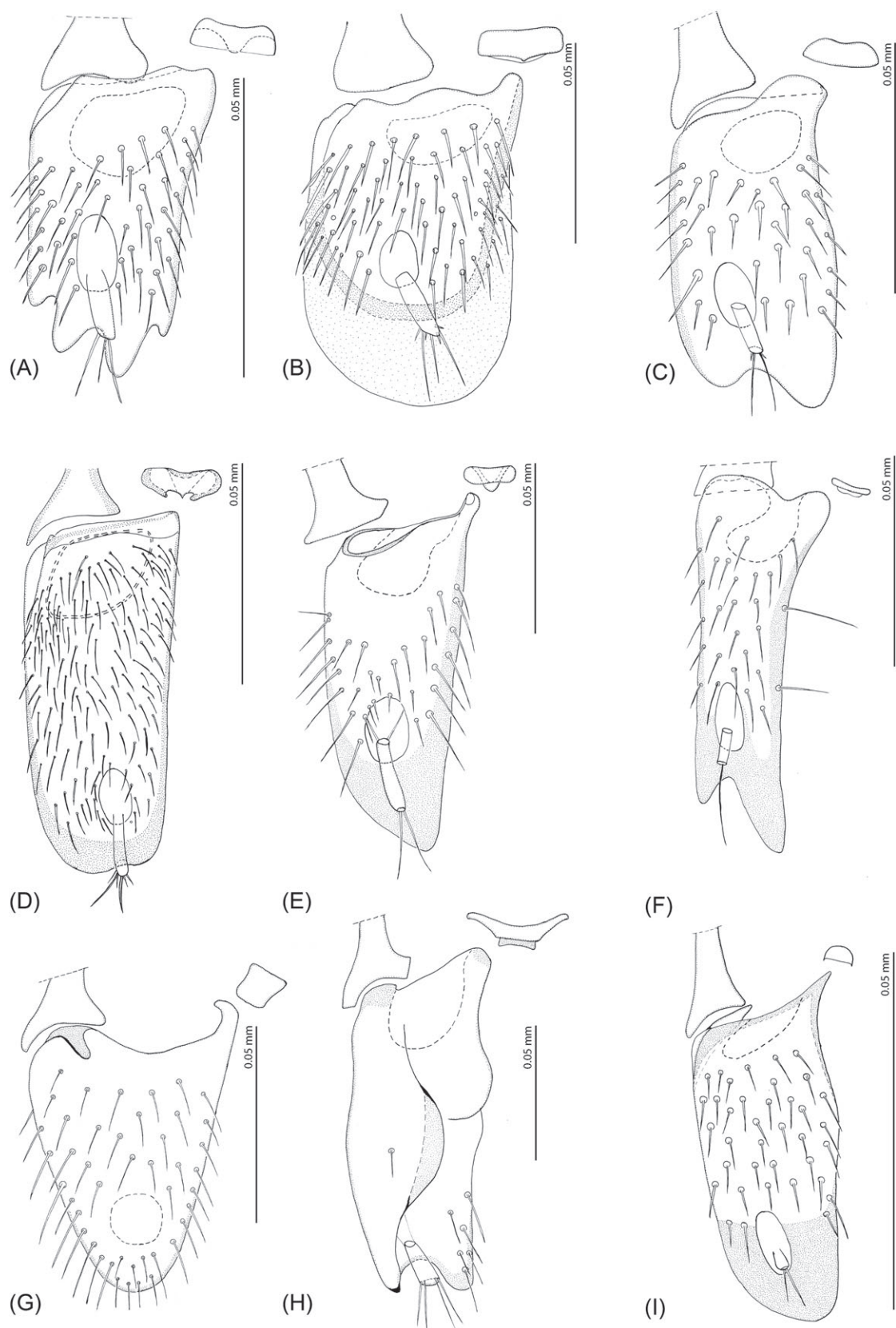
Fig. 3. Legend on next page.

(laterotergites IX), which, in turn, flank the tergite X (or proctiger) lying immediately above the anal opening (Lawrence & Ślipiński, 2013). Each gonocoxite is in the Histeridae divided into two parts: the proximal (also called 'valvifer') and the distal gonocoxite. The valvifers are paddle-shaped and exhibit a large degree of variation and, according to the present observations, are not informative or useful in diagnosing the genera or subgenera of Saprininae. The valvifers could, however, be useful in diagnostics on the species level. In the Saprininae, the variously shaped distal gonocoxite (called simply 'gonocoxite' in the present paper, as the proximal part is referred to as the valvifer) usually bears a gonostylus adorned with setae at its apex (see later). Between the two gonocoxites, an 'articulating sclerite' is found in many taxa. This sclerite can be variously shaped to even atrophied or absent (see later).

Gonocoxites in the Saprininae are variously densely setose, often from the dorsal as well as the ventral side, in most cases slightly to strongly elongate and tapered apically; gonocoxites of many taxa are scoop-like ventrally. In several taxa, most of which were recovered in the previous analysis (Lackner, 2014) close to the root of the tree or placed in subsequent grades and clades before the appearance of a large clade of mostly psammophilous taxa (arenophiles), the gonocoxites are slightly wider, not clearly elongate (Fig. 3A). In these cases, their apices can be simple and outwardly arcuate (Fig. 3B), slightly inwardly arcuate (Fig. 3C) or adorned with apical teeth, dentate (Fig. 3A). The dentate apex marks the taxa placed closest to the root of the tree [*Gnathoncus*, *Myrmetes*, *Tomogenius*, *Euspilotus (Neosaprinus)* – but not *Erebidus*] as well as *Dendrophilus* – a sister taxon to the subfamily (Lackner, 2014). Similar to the spermatheca situation, the gonocoxite shape varies within *Saprinus*: it is distinctly elongate in *S. (S.) semistriatus* (Fig. 3D), whereas it is almost as wide as long in *S. (S.) maculatus* (Fig. 3B).

In cases where the gonocoxite is distinctly elongate, its apex can be simple, rounded (outwardly arcuate) (Fig. 3E) or (variously deeply) inwardly arcuate (Fig. 3F). A distinctly elongate gonocoxite is never dentate apically, at least in the observed taxa.

The third category is formed by the mostly obligate arenophiles that have their gonocoxites distinctly wider than long, or at least as wide as long, in many cases triangular in shape (Fig. 3G). The entire surface area of their gonocoxites is significantly smaller than the gonocoxite of the rest of the examined taxa.

The South African and Namibian taxon *Pachylopus dispar* has highly autapomorphic gonocoxites bearing a median ridge overlapping the surface; furthermore, their dorsal surface is almost asetose (Fig. 3H). This taxon is, furthermore, characterized by high sexual dimorphism, also an autapomorphy in the subfamily.

Ovipositor: gonostylus

Gonostyli are present in higher taxa of the Saprininae, and are usually well developed, rather thin and their apices are adorned with setae (e.g. Fig. 3A–F). In several taxa they can be weakly developed and rather short (Fig. 3I). The position of the gonostylus on the gonocoxite is usually in its apical third, or even closer to the apical margin; in some notable exceptions they can be positioned slightly closer to the middle of the gonocoxite. By the method of outgroup comparison, we can deduce that the gonostyli are well developed and slender in *Anapleus* and *Dendrophilus* (presumed sister taxa) as well as in the taxa closest to the root [*Tomogenius*, *Gnathoncus*, *Myrmetes*, *Erebidus*, *Euspilotus (Neosaprinus)*; Lackner, 2014]. In several psammophilous taxa, the gonostylus is atrophied and moved at the apical margin of gonocoxite (Fig. 4A), but in other specialized arenophiles, the gonostylus may be lacking altogether (Fig. 4B; see Table 1 for details). The number and length of setae on the gonostylus vary within the Saprininae as well. The most common state is the condition where two long and several (usually two to six) microscopic setae are present (Fig. 3D). The long setae can be more numerous, as can the microscopic ones; occasionally the microscopic setae are lacking altogether.

Ovipositor: articulating sclerite

Between the gonocoxites a variously shaped sclerite, a so-called 'articulating sclerite' (a term coined by Caterino & Tishechkin, 2014) is usually present. The gonocoxites are hinged to it, tightly connected by membrane with freedom of motion limited by the sclerite. In most cases, this sclerite is rather small, not surpassing one-tenth of the entire surface of the gonocoxite, and its shape loosely resembles the shape of the Saprininae labrum (compare figures in this paper with those in Lackner, 2010), or a boomerang (Fig. 3H). As with the gonostylus, this articulating sclerite is very reduced to absent in most of the true arenophiles, probably representing a serial homology with that character (see Table 1 for details). This, however, does not apply to two typical psammophiles, both type species of the respective subgenera of the genus *Philothis*: the sclerite is present in *Philothis (Farabius) hexeris* [the type species of the subgenus *Farabius* of the genus *Philothis* (Fig. 4C)] as it is present in *Philothis (Atavinus) atavus* [the type species of the subgenus *Atavinus* of the genus *Philothis* (Fig. 4D)], whereas it is absent in *Philothis (Philothis) generator* [the type species of the nominotypical subgenus (Fig. 4E)]. The articulating sclerite of *Nannolepidius braunsi* is probably absent and what we interpreted as an articulating sclerite was, in fact, the broken right gonocoxite (Fig. 4F). Unfortunately we did not have another specimen to hand for comparison and so the actual presence or shape of this structure remains unresolved. The

Fig. 3. Right gonocoxite (dorsal view) and articulating sclerite of the selected Saprininae taxa. (A) *Myrmetes paykulli* Kanaar; (B) *Saprinus (Saprinus) maculatus* (P. Rossi); (C) *Eopachylopus ripae* (Lewis); (D) *Saprinus (Saprinus) semistriatus* (Scriba); (E) *Styphrus corpulentus* Motschulsky; (F) *Chelyoxenus xerobatis* Hubbard; (G) *Orateon praestans* Lackner & Ratto; (H) *Pachylopus dispar* Erichson; (I) *Eremosaprinus unguiculatus* Ross.
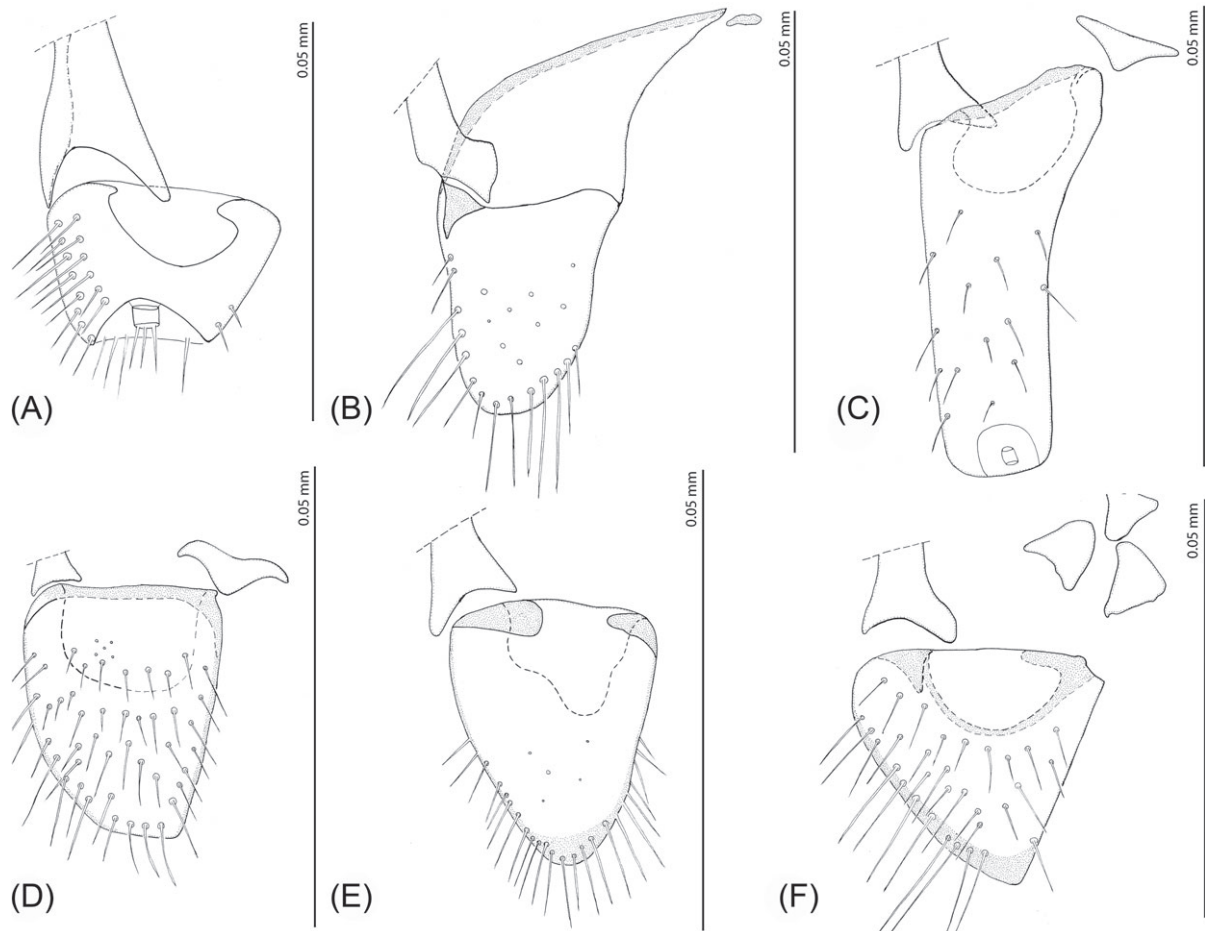
Fig. 4. Right gonocoxite (dorsal view) and articulating sclerite of the selected Saprininae taxa. (A) *Alienocacculus neftensis* (Olexa); (B) *Ctenophilothis chobauti* (Théry); (C) *Philothis (Farabius) hexeris* Reichardt; (D) *Philothis (Atavinus) atavus* Reichardt; (E) *Philothis (Philothis) arcanus* Reichardt; (F) *Nannolepidius braunsi* Reichardt.

female genitalia of the holotype of *Turanostyphrus kizilkumis* were quite damaged when we received them for examination and the articulating sclerite was not to be found in the mounted genitalia. Its presence or absence must be verified by further sampling.

Phylogenetic analyses

The EW analysis yielded 18 004 MP trees ($L = 613$). The strict consensus of the EW trees is poorly resolved (Fig. 5). The IW analyses produced trees of the same length as those of the EW analysis only when $k > 40$. All IW analyses with $k > 40$ yielded the identical well-resolved tree (CI = 0.295, RI = 0.592; Fig. 6).

Character informativeness

The distribution of CI (Fig. 7) and its mean values (Table 2) shows that female characters are moderately homoplastic, occupying the medium range of values. By contrast, the greatest proportion of somatic and male characters is concentrated over either higher or lower CI values. The mean CI is highest for somatic characters (0.558), followed by that for male (0.467) and female characters (0.396).

The distribution of RI (Fig. 8) and its mean values (Table 2) demonstrates a pattern similar to that of CI – the female characters occupy the moderate value range, and somatic and male characters are predominantly concentrated over higher values, but possessing a significant proportion of characters with low RI. Interestingly, the mean RI is higher for female characters (0.68) than for male (0.47) and somatic characters (0.659).

The pairwise permutation test between all character categories for CI and between all character categories (except male vs somatic characters) for RI yielded P-values > 0.05. This result does not allow rejection of the null hypothesis of insignificant differences in mean values between the mentioned character categories. The permutation test between male and somatic characters for RI returned $P = 0.028$, indicating that the mean RI of male characters is significantly different from that of somatic characters.

Fig. 5. The phylogenetic tree of Saprininae. Strict consensus of 18 004 maximum parsimony trees. The values below the branches indicate partitioned Bremer support in the order somatic/male/female.

The multinomial test used for testing the association between characters of female and male genitalia yielded a high *P*-value (0.3465) that does not allow rejection of the null hypotheses of independent character distribution across the branches.

Discussion

Topologies: current versus previous studies and the informativeness of female genitalia

The obtained strict-consensus cladogram (Fig. 5) is less resolved than that of Lackner (2014). The lower resolution of our cladogram can be caused by the added taxa that, due to the impossibility to dissecting some of them, lack numerous characters.

Although their resolution differs, the previously published strict consensus tree (Lackner, 2014) and the present IW phylogeny (Fig. 6) are generally fairly similar. Therefore, in the present discussion, we focus mainly on the informativeness of the female genitalia for the Saprininae phylogeny. The clade containing the inquilinous *Gnathoncus* + *Tomogenius* is present in both strict consensus and IW trees, with rather low PBS (1.7, somatic characters; −1.1, male genitalia characters; 0.4, female genitalia characters). The clade that is for practical reasons only termed here as the '*Iridoprinus* group', containing mostly inquilines of small mammals (*Turanostyphrus*, *Microsaprinus*, *Eremosaprinus*) or ants (*Paramyrmetes*, *Iridoprinus*) or taxa of unknown biology (*Saprinodes*), is supported by a single homoplasious female genitalia synapomorphy (character no. 98:4), but its members contain many missing characters and the relationships among them should probably

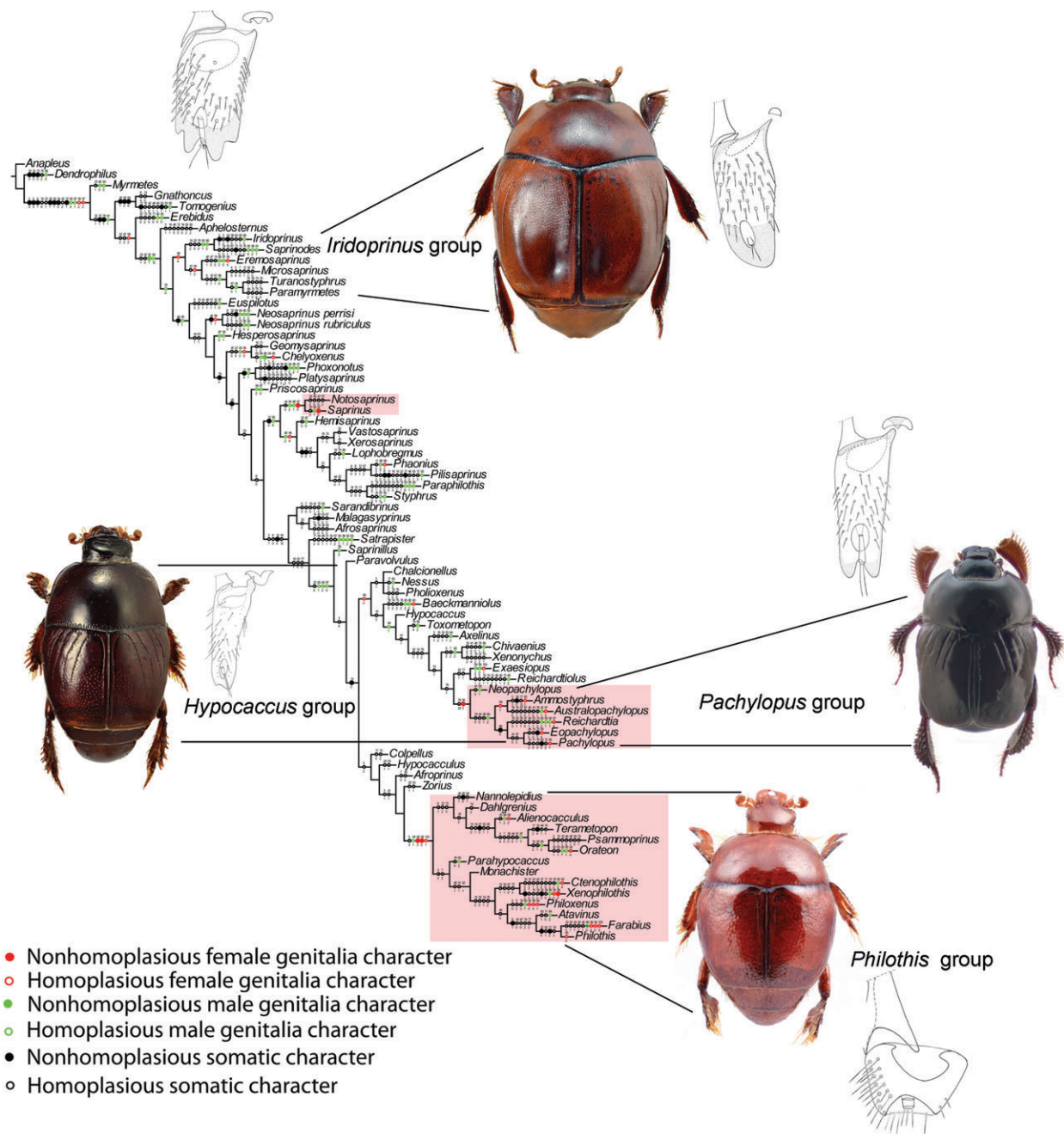Gonocoxite, gonostylus and articulating scleriteof the out-group and Saprininae taxa closest to the tree root.

Fig. 6. The phylogenetic tree of the Saprininae using implied weights. Clades marked with red show better female genitalic character performance. [Colour figure can be viewed at wileyonlinelibrary.com].

be viewed with suspicion. The triad *Microsaprinus* (*Turanostyphrus* + *Paramyrmetes*) also received rather low PBS (1.7, −1.1, 0.4), and is thus supported mainly by somatic characters. The clade containing *Eremosaprinus* (*Microsaprinus* (*Turanostyprhus* + *Paramyrmetes*)) received support from a single homoplasious synapomorphy of female genitalia (character no. 99:3). The clade including two species of *Neosaprinus* is

supported by a single homoplasious synapomorphy of female genitalia (character no. 98:1). The PBS values (3.1, −2.4, 0.4) indicate that it is mostly somatic characters that support the clade containing another two inquilines of small mammals, *Geomysaprinus* and *Chelyoxenus* (recovered in both our present trees). Their relationship is, however, supported by a single homoplasious synapomorphy of female genitalia (character
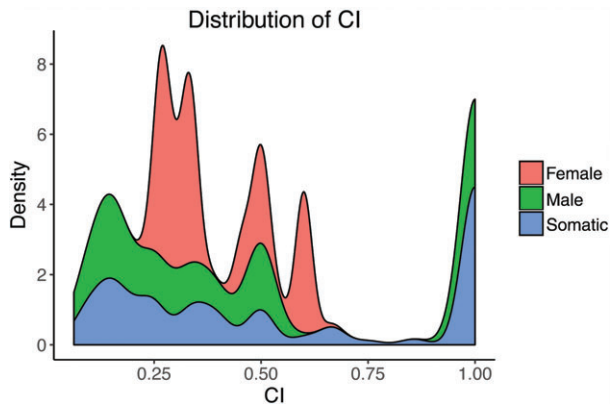
Fig. 7. Distribution of consistency index (CI) across 15 000 maximum parsimony trees and three categories of characters (somatic, male and female). [Colour figure can be viewed at wileyonlinelibrary.com].

Table 2. Mean consistency index (CI) and retention index (RI) for three categories of characters (female, somatic, male) as well as for all female characters individually. The mean values are calculated based on the sample of 1500 equal weights trees.

Characters	Mean CI	Mean RI
Somatic	0.558	0.659
Male	0.467	0.47
Female	0.396	0.68
Female: character no. 96	0.267	0.389
Female: character no. 97	0.601	0.716
Female: character no. 98	0.484	0.757
Female: character no. 99	0.295	0.722
Female: character no. 100	0.333	0.818

no. 96:3). The *Xerosaprinus + Vastosaprinus* (both free-living) clade is likewise recovered in both our present trees, and it received rather low PBS (1.2, −0.8, 0.5), and whereas both somatic and female genitalia characters support the clade, the male genitalia characters argue against it. The PBS for free-living *Notosaprinus + Saprinus* is higher, but a high negative value of male genitalia characters also argues against the clade (4.6, −4.1, 0.5). *Notosaprinus + Saprinus* clade is supported by a single nonhomoplasious synapomorphy of female genitalia (character no. 97:5).

The rather large clade with mostly small taxa, often living in dry or wet sand, is herein, for practical reasons, only termed as the '*Hypocaccus* group' (Fig. 6). It is divided into two major clades (with the exception of *Saprinillus* and *Paravolvulus* which are basal to the rest). The '*Pachylopus* group' (Fig. 6), which is included in one of the two major clades containing *Neopachylopus*(*Ammostyphrus + Australopachylopus*) + *Reichardtia*(*Eopachylopus + Pachylopus*), is present on both the strict consensus tree and the IW tree. It is supported by a single nonhomoplasious synapomorphy of female genitalia (character no. 96:3). Whereas the strict consensus tree depicts the '*Pachylopus* group' (minus *Neopachylopus*) as an unresolved polytomy (PBS: 0, 1.6, −0.6), the IW tree paints a
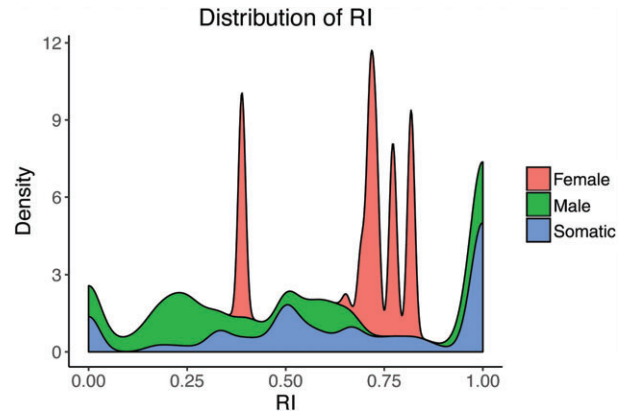

Fig. 8. Distribution of retention index (RII) across 15 000 maximum parsimony trees and three category of characters (somatic, male and female). [Colour figure can be viewed at wileyonlinelibrary.com].

slightly better, albeit still weakly resolved, picture containing two clades, (*Ammostyphrus + Australopachylopus*) and (*Reichardtia* (*Eopachylopus + Pachylopus*)).

The '*Philothis* group', included in the second major clade containing almost exclusively psammophile taxa found in deep dry sand, is supported by two nonhomoplasious synapomorphies and one homoplasious synapomorphy of female genitalia (character no. 98:3; character no. 99:0 and character no. 100:1). It is further divided into two clades. The clade *Nannolepidius*(*Dahlgrenius*)(*Alienocacculus*)(*Terametopon*) (*Psammoprinus + Orateon*)) is recovered in both strict consensus and IW trees, albeit with rather low PBS on the consensus tree (1.1, −1.2, 1.2). Lastly, in both strict consensus and IW trees, a clade containing exclusively strictly psammophilous taxa, *Parahypocaccus*(*Monachister* (*Xenophilothis + Ctenophilothis*)*Philoxenus*(*Atavinus*) (*Philothis + Farabius*)), is resolved, albeit only with low support from two somatic characters. *Parahypocaccus* and *Monachister* are basal to the rest; the support for the subtending clade, (*Ctenophilothis + Xenophilothis*) + *Philoxenus*(*Atavinus*) (*Farabius + Philothis*), is likewise weak (low PBS: 0.7, −1.2, 3.1).

Monophyly of *Philothis* (three subgenera: *Philothis* s.s., *Farabius* and *Atavinus*) was recovered in both analyses, with slightly higher support (PBS: 2.3, −0.5, 0.2). The sister relationship between *Philothis* and its subgenus *Farabius* received even higher PBS values (3.1, −0.4, −0.8), leaning on somatic characters for most support; this was also the case in the study of Lackner (2014) which likewise confirmed the monophyly of this psammophilous taxon.

Comparative evolution of female genitalia

Based on the permutation test of CI and RI mean values, we could not find statistically significant differences between female characters as opposed to male and somatic ones. Most likely this occurred due to a relatively small sample size

of female characters (just four characters). Statistically, this means that given the current sample size, there are no significant differences among the mean values of CI and RI, and more characters are needed to either support or reject such results. However, in reality, scoring more characters of female genitalia can be problematic – we did our best to locate those characters, and we would argue that even a more detailed study with a larger species sample and detainees would face challenges in scoring a substantially larger number of characters. Thus, taking into account these circumstances, we feel it is necessary to compare the given distribution of CI and RI for the female genitalia characters.

The measure of homoplasy using CI shows that female genitalia characters are, on average, more homoplastic than male or somatic ones. The degree of homoplasy can be considered as a rough proxy for the character rate evolution, implying that a higher rate (more changes) corresponds to a higher degree of homoplasy (lower CI values). In this respect, we conclude that the female genitalia characters in Saprininae tend to evolve more slowly than other characters, which corroborates a similar finding in stink bugs (Genevcius *et al.*, 2017). Interestingly, the mean value of RI (index of grouping) in female genitalia (Table 2) is higher than those in somatic and male genitalia characters, thus suggesting that female genitalia bear important phylogenetic signal to define certain clades despite having lower CI than other categories. Specifically, this is true for the clades where female genital characters yield PBS values higher than the other categories. The two main lineages demonstrating this pattern (Fig. 5) are: (i) the clade of *Nannolepidius* and allies (marked as '1' in Fig. 5; PBS for female characters 1.2) including some nested subclades; and (ii) the clade of *Xenophilothis* + *Ctenophilothis* + *Philothis* with subgenera (marked as '2' in Fig. 5; PBS for female characters 1.5) including some nested subclades. It is noteworthy that, generally, the PBS values for female genitalia characters were greater than those for male genitalia.

Interestingly, unlike somatic and male characters, the female genitalia lack perfectly nonhomoplasious characters (CI = 1), although they possess nonhomoplasious as well as homoplasious synapomorphies that are useful in diagnosing the clades. These clades are termed here as 'groups' for practical reasons only (Fig. 6). The '*Philothis* group', containing exclusively specialized psammophilous taxa (except for the termitoxene *Nannolepidius*), is defined by three synapomorphies of female genitalia, among them very reduced to absent gonostylus (Fig. 6). A clade of beach-dwelling psammophiles from the '*Pachylopus* group' is supported by a nonhomoplasious synapomorphy of female articulating sclerite, which is boomerang-shaped (Fig. 6). The '*Hypocaccus* group', containing psammophiles, free-living taxa (*Chalcionellus*, *Hypocacculus*, *Nessus*, *Toxometopon*) and inquilinous *Pholioxenus*, is defined by a homoplasious synapomorphy of female genitalia with a reduced gonostylus (Fig. 6). The homoplasious synapomorphy of elongate gonocoxite in females supports the '*Iridoprinus* group' (Fig. 6).

Based on the described pattern, we conclude that the female genitalia are generally moderately informative, at least to the extent studied in this paper, regarding the phylogenetic reconstruction in Saprininae. The somatic and male genitalia characters (on average) possess higher phylogenetic signal. Specifically, the permutation test between male and somatic characters showed that somatic characters have a significantly higher grouping capability. Nevertheless, female genitalia provide better support and diagnosis for the particular clades outlined earlier. This result is consistent with some previous studies of beetle female genitalia (Liebherr & Will, 1998; Jensen *et al.*, 2009). In contrast to this, other studies report high informativeness of female genitalia for phylogenetic reconstruction (e.g. Özgül-Siemund & Ahrens, 2015). This discordance of the results can be explained by differences in the selective regimes operating across different taxa. Regardless of the degree of informativeness, the structure of female genitalia represents an additional interesting and important source of information for systematics and taxonomy.

The PBS values and synapomorphies indicate that characters of female and male genitalia show similar distribution pattern over the tree: they tend to support shallow or very deep, but not intermediate, splits of lineages. Additionally, PBS values indicate that somatic characters phylogenetically outperform both male and female genitalia characters. Despite these similarities, we did not find statistical support for an association between synapomorphies of male and female genitalia; the high *P*-value from the multinomial test does not allow rejection of the null hypothesis of their independent distribution across the tree branches. As the association between synapomorphies is a just a subset of the character correlation phenomenon, we acknowledge that we cannot completely rule out the hypothesis of a correlated evolution between male and female genitalia. The correlation may exist in the lack of association between the synapomorphies across the branches, which is difficult to test using available comparative methods (see Materials and Methods). However, as any evidence of correlation has not been found in the current study, we conclude that female and male genitalia are not correlated in the Saprininae. Interestingly, both patterns – the correlated (Özgül-Siemund & Ahrens, 2015; Cayetano *et al.*, 2011) and independent (Polihronakis *et al.*, 2016) evolution of male-female genitalia – have been found in insects. The common driving force of the correlated evolution between male and female genitalia consists of sexual conflict, female choice and lock-and-key mechanism (Cordero-Rivera, 2017). The lack of correlation, at least with regard to the present investigation, does not support the possibility that sexual conflict, lock-and-key mechanism or coevolution via female choice influences genital morphology in the Saprininae. At the same time, we have evidence that genital morphology in the Saprininae can be linked to environmental factors. The small size of the gonocoxite, absence (or reduction) of gonostyli, and absence (or reduction) of articulating sclerites are primarily observed in highly specialized psammophilous genera (mainly genera present in the '*Philothis* group'). We hypothesize that these characters might be associated with the psammophile life mode. The reduction of these structures putatively facilitates oviposition and prevents them from damage when females lay eggs into deep, fine-grained sand. Thus, based on the aforementioned evidence, we speculate that the evolution of female and male

genitalia in Saprininae is probably shaped by the combination of natural selection and sexual selection without coevolution. However, we acknowledge that differentiation (if possible) among the specific selective mechanism influencing evolution of genitalia in Saprininae requires further research.

Conclusions

The present paper describes and compares the characters of the female genital apparatus (spermathecae and characters of the ovipositor) for the majority of type species and (sub)genera of the histerid subfamily Saprininae (Coleoptera: Histeridae). We perform combined phylogenetic analyses of somatic, male and female genitalia characters to assess the utility of female genitalia. Although the female genitalia of the Saprininae overall show moderate phylogenetic signal and slower mean evolutionary rate in comparison to other characters, structures of female genitalia represent an additional interesting and important source of information for systematics and taxonomy. This result corroborates some previous studies of female genitalia in beetles and insects. We did not find any sign of correlation between male and female genital characters but acknowledge that a more detailed investigation is needed to test it. On average, the female genitalic characters are more homoplastic than the male or somatic characters. Our results were probably influenced by the relatively small size of the sample of female genitalia characters, and we acknowledge that parsing, for example, characters of spermatheca into discrete states was, due to the high variability found, rendered very difficult. The PBS values for the female genitalia were generally greater than those of the male genitalia. Our results show that the spermathecae in the Saprininae had the largest amount of variation and were therefore coded more conservatively than the characters of the ovipositor. After careful examination of spermathecae of a substantial number of higher Saprininae taxa (mostly type species of all genera and subgenera; as of 2018), we decided to deem the classification of spermathecae, sensu De Marzo & Vienna (1982), as arbitrary and reject it in the present paper. The characters of ovipositor, on the other hand, showed more stable categories, with the taxa closest to the root of the subfamily possessing dentate apical margins of their gonocoxites (a condition also found in the outgroup taxon *Dendrophilus*), whereas most 'derived' psammophilous taxa showed a substantial reduction of the gonocoxite size, and absence of both articulating sclerites and gonostyli. We hypothesize that the reduction and absence of structures are linked to their life mode and are an adaptation to facilitate the oviposition into fine-grained sand.

According to the results of our morphology-based analysis, the relationships among the subfamily members largely resemble those published by Lackner (2014) and thus we do not feel able to present our results as a substantial reanalysis of the subfamily. Particular clades that were almost exclusively supported by the female genitalia are termed here as 'groups' for practical reasons only. Most of our characters and their states are novel for the Saprininae morphology studies and we depict them

all, either in the paper or in supplementary files online. We likewise acknowledge that denser sampling, especially among species-rich genera (*Saprinus*, *Dahlgrenius*, *Euspilotus*), would be desirable for the future studies.

Supporting Information

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

Figure S1. Right gonocoxite (dorsal view) and articulating sclerite of the selected Dendrophilinae and Saprininae taxa. (A) *Afroprinus cavicola* Lackner (Saprininae); (B) *Ammostyphrus cerberus* Reichardt (Saprininae); (C) *Anapleus cyclonotus* Lewis (Dendrophilinae: Anapleini); (D) *Aphelosternus interstitialis* (J.L. LeConte) (Saprininae); (E) *Xerosaprinus (Auchmosaprinus) laciniatus* (Casey) (Saprininae); (F) *Axelinus ghilarovi* Kryzhanovskij (Saprininae); (G) *Hypocaccus (Baeckmanniolus) dimidiatus dimidiatus* (Illiger) (Saprininae); (H) *Hypocacculus (Colpellus) praecox* (Erichson) (Saprininae); (I) *Dahlgrenius sculpturifer* (Marseul) (Saprininae).

Figure S2. Right gonocoxite (dorsal view) and articulating sclerite of the selected Dendrophilinae and Saprininae taxa. (A) *Dendrophilus (Dendrophilus) punctatus punctatus* (Herbst) (Dendrophilinae: Dendrophilini); (B) *Erebidus vlasovi* Reichardt (Saprininae); (C) *Australopachylopus lepidulus* (Broun) (Saprininae); (D) *Euspilotus (Euspilotus) zonalis* Lewis (Saprininae); (E) *Exaesiopus grossipes* (Marseul) (Saprininae); (F) *Geomysaprinus (Geomysaprinus) goffi* Ross (Saprininae); (G) *Gnathoncus rotundatus* (Kugelann) (Saprininae); (H) *Hemisaprinus subvirescens* (Ménétries) (Saprininae); (I) *Euspilotus (Hesperosaprinus) assimilis* (Paykull) (Saprininae).

Figure S3. Right gonocoxite (dorsal view) and articulating sclerite of the selected Saprininae taxa. (A) *Hypocacculus (Hypocacculus) metallescens* (Erichson); (B) *Hypocaccus (Hypocaccus) rugiceps* (Duftschmid); (C) *Chalcionellus amoenus* (Erichson); (D) *Chivaenius kryzhanovskii* Olexa; (E) *Malagasyprinus caeruleatus* (Lewis); (F) *Microsaprinus therondianus* (Kryzhanovskij); (G) *Neopachylopus sulcifrons* (Mannerheim); (H) *Euspilotus (Neosaprinus) limatus* (Marseul); (I) *Euspilotus (Neosaprinus) perrisi* (Marseul).

Figure S4. Right gonocoxite (dorsal view) and articulating sclerite of the selected Saprininae taxa. (A) *Hypocaccus (Nessus) rubripes* (Erichson); (B) *Notosaprinus irininus* (Marseul); (C) *Paravolvulus ovillum* (Solskyi); (D) *Saprinus (Phaonius) pharao* (Marseul); (E) *Philoxenus desertorum* Mazur; (F) *Pholioxenus phoenix* (Reichardt); (G) *Phoxonotus tuberculatus* Marseul; (H) *Pilisaprinus verschureni* (Thérond); (I) *Euspilotus (Platysaprinus) latimanus* (Schmidt).

Figure S5. Right gonocoxite (dorsal view) and articulating sclerite of the selected Saprininae taxa. (A) *Geomysaprinus (Priscosaprinus) pectoralis* (J.L. LeConte); (B) *Terametopon (Psammoprinus) namibiensis* Mazur; (C) *Reichardtia pedator* (Sharp); (D) *Reichardtiolus duriculus* (Reitter); (E) *Saprinillus paromaloides* Kryzhanovskij; (F) *Saprinodes falcifer* Lewis; (G) *Sarandibrinus araceliae* Lackner & Gomy; (H) *Terametopon (Terametopon) levissimestriatus* Vienna; (I) *Tomogenius incisus* (Erichson).

Figure S6. Right gonocoxite (dorsal view) and articulating sclerite of the selected Saprininae taxa. (A) *Hypocacculus (Toxometopon) rubricatus* (Lewis); (B) *Turanostyphrus kizilkumis* Tishechkin; (C) *Xenonychus tridens* (Jacquelin du Val); (D) *Xenophilothis choumovitchi* Thérond; (E) *Xerosaprinus (Xerosaprinus) lubricus* (J.L. LeConte); (F) *Zorius funereus* (Schmidt).

Appendix S1. Saprininae.

Acknowledgements

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