

Taxonomic utility of female copulation organs in Sericini chafers (Coleoptera, Scarabaeidae), with special reference to asymmetry

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Abstract

Female genitalia are widely underrepresented in taxonomic studies. Here we investigate the morphological variation among female copulation organs for a group of scarab beetles (Sericini) with similar ecology, external morphology and copulation mechanics. We examined traits qualitatively and quantitatively based on 80 and 18 species (genus *Pleophylla*), respectively. Additionally we explored whether female genitalia are affected by asymmetry. The vast diversity of sclerotised structures including their shapes illustrated the high taxonomic and phylogenetic utility of female genitalia in this group. The morphometric analysis of *Pleophylla*, confirmed that sclerotisations in the ductus bursae are very suitable for species-level taxonomic purposes. Stable interspecific variation is more hardly discernable in other parts such as the vaginal palps (shape and size) or the other membranous structures such as the shape of the bursa copulatrix. Asymmetric genitalia that arose multiple times independently among insects are found in most of the examined Sericini species. Asymmetries regarded either the bursa copulatrix, or both the bursa copulatrix and ductus bursae and comprised sclerotised and non-sclerotised structures being most common in modern Sericini. Here, highly asymmetric sclerotised structures are linked with strong asymmetry of the male copulation organs. Widespread asymmetry among megadiverse Sericini with a complex male-female genital asymmetry suggests that the shift to asymmetry is phylogenetically rather conserved. From the range of hypotheses, sexual selection seems to be the most reasonable to explain the evolution and stability of asymmetry in chafer genitalia.

Contents

Introduction	167
Material and methods	168
Results	169
<i>General morphology and inter-specific variation</i>	
<i>in female genitalia of Sericini</i>	169
<i>Case study - Interspecific variation among</i>	
<i>Pleophylla species</i>	173
<i>Asymmetry</i>	174
Discussion	174
<i>Utility of female genitalia for taxonomy and</i>	
<i>systematics</i>	174

<i>Symmetry and asymmetry</i>	175
Acknowledgements	176
References	176

Introduction

Male genitalia are considered one of the most important and useful species-diagnostic characters in insect systematics. In many insects, genitalia often provide the only way to reliably distinguish species using morphology. The diversity of male genitalia and their pattern of morphological variation is a well-documented phenomenon in evolutionary biology (Hosken and Stockley, 2004). Sexual characters often show particularly great ‘species-specific’ variation and can play an important role in reproductive isolation and speciation processes (Wojcieszek and Simmons, 2013). This diversity of shapes is often linked to evolutionary factors and selection processes that might contribute to a rapid and divergent evolution of genital morphology (Mayr, 1963; Eberhard, 1985, 1996; Arnqvist and Thornhill, 1998; Hosken and Stockley, 2004).

Despite this crucial role of male genitalia for taxonomy, it has been repeatedly pointed out that the poor knowledge of female genitalia (in particular in terms of infraspecific and interspecific variation but also of degree of asymmetry) is seriously hampering a more comprehensive understanding of genital evolution (Mendez and Cordoba-Aguilar, 2004; Cordoba-Aguilar, 2010; Ah-King *et al.*, 2014). Increasingly, studies report species-specific variation in female genital morphology and its coevolutionary divergence with male genital morphology (Yassin and Orgogozo, 2013; Simmons, 2014).

Symmetry and asymmetry are an essential part of the pattern of that huge variation (Huber, 2010; Schilt-huizen, 2013). Asymmetries are an interesting phenomenon in otherwise bilaterally symmetric organisms

with the potential impact to improve our understanding of fundamental evolutionary processes like the evolution of development and the selection for morphological novelties caused by behavioural changes. Asymmetry has arisen multiple times independently among insects (Schilthuizen, 2013). Several hypotheses have been proposed to explain the evolution of asymmetric structures in genitalia (Huber, 2010; Schilthuizen, 2013). Proposed advantages of asymmetric genitalia due to mating position (Huber *et al.*, 2007; Huber, 2010) do not appear likely for scarab chafers, as all species have the same mating position (male above female and a constant mating angle). Functional differences in the left and the right side can also be rejected, since both parameres in the Coleoptera form a functional unit as a clasping organ and do not perform different tasks (Sharp and Muir, 1912). Therefore, sexual selection may be the best explanation for the evolution of shape divergence between the right and left side of genitalia among chafers.

Here we investigate the infra- and interspecific morphological variation among female copulation organs for a group of scarab beetles (Scarabaeidae: Sericini) with similar ecology, external morphology and copulation mechanics, and explore generally, whether and to what degree female genitalia are affected by asymmetry which has been widely reported so far only in male beetle specimens (Huber *et al.*, 2007; Ahrens and Lago, 2008; Breeschoten *et al.*, 2013). Furthermore, we are interested if and which character traits are useful to be utilized in systematics and species' taxonomy.

Sericini is a highly diverse group with nearly 4000 species in about 200 genera (Ahrens, 2006b), which is traditionally placed into the subfamily of Melolonthinae (Smith, 2006). The monophyly of Sericini has been proven in several studies (Ahrens, 2006b; Ahrens and Vogler, 2008; Ahrens *et al.*, 2011) and the relationships of the major lineages are roughly understood. Adults of Sericini are polyphagous herbivores and feed on a variety of plant species. The larvae, known also as 'white grubs', feed on roots and underground stems of living plants (Ritcher, 1966). Some species of Sericini are economically important crop pests. Most of the more derived species show a very significant asymmetry in male copulatory organs (Ahrens and Lago, 2008).

The earliest documented studies on female internal reproductive organs of scarab beetles are those of Stein (1847) and Tanner (1927). Subsequently, many additional species were characterized (*e.g.* Willimzik, 1930; Heymons, 1930; Williams, 1945; Krause, 1947;

Dajoz, 1972; Dupuis 2005; Imelda Martinez and Trotta-Moreu, 2010). While female genitalia are frequently used for taxonomic purposes in dung beetles (Zunino, 1971, 1972; Martin-Piera, 1992), most previous works on melolonthines (and other pleurostict scarabaeidae) studied the morphology only for a limited number of species in detail without applying comparative criteria valuable for taxonomic purposes (*e.g.* Straus-Dürckheim, 1828; Menees, 1963; Watt, 1971; Berberet and Helms, 1972; Barratt and Campbell, 1982; Stringer, 1988). There are a handful of studies that focused on variation in the female genital morphology in pleurostict scarabs and used it for species taxonomy and systematics (*e.g.* Coca-Abia and Martin-Piera, 1991; Coca-Abia *et al.*, 1993; Coca-Abia and Robbins, 2006, Micó and Galante, 2000; Riley and Wolfe, 1995; Ahrens, 2000, 2001, 2006a,b, 2007; Ahrens and Fabrizi, 2009; Woodruff and Sanderson, 2004; Polihronakis, 2007; Zorn, 2011); however no one have accounted so far for issues of asymmetry.

Material and methods

The samples of females of approximately 80 species were mainly taken from dried museum specimens that required softening in hot water for dissection of the genitalia. Additionally we included ethanol preserved specimens for a few taxa. Samples were drawn from basal and more derived lineages of Sericini (Ahrens and Vogler, 2008) and included the genera *Astaena*, *Paratriodonta*, *Omolaplia*, *Pleophylla*, *Heteroserica*, *Leuroserica*, *Leucoserica*, *Sericania*, *Cycloserica*, *Nepaloserica*, *Taiwanoserica*, *Serica*, *Pachyserica*, *Gynaecoserica*, *Lasioserica*, *Amiserica*, *Neoserica* (s.str.), *Chryso-serica*, *Calloserica*, *Eumaladera*, and *Maladera*.

For all species, at least two specimens were examined. Every specimen was provided with an identification number in order to be able to associate individuals and images. Sampling was based on specimens from the following museums: Ditsong (formerly Transvaal) Museum Pretoria (TMSA), South African National Collection of Insects, Pretoria (SANC), Royal Museum for Central Africa Tervuren (RMCA), Museum für Naturkunde Berlin (ZMHB) and Zoological Research Museum A. Koenig Bonn (ZFMK).

The dissection and preparation of female genitalia followed the preparation methods of Konstantinov (1998) and involved particular attention in order to avoid destruction of soft tissue parts. After softening the specimen, the abdomen was carefully removed

from the body with a fine forceps. The pleuron was cut to allow an opening of sternites and tergites including the pygidium. The genitalia were carefully detached from the latter. After dissection of the genitalia the abdomen was glued onto a piece of paper with the pinned specimen. Dissected genitalia were relaxed in 10 % potassium hydroxide (KOH) for about 15 to 20 minutes (depending on the condition of the tissues) in order to remove soft tissues from the genitalia. All female genitalia were stored in glycerol in a microvial attached to the pinned specimen. In a final step, genitalia were stained with chlorazol-black before being soaked in acetic acid for several minutes.

For digital imaging, genitalia were mounted on microscope slides using a standardized amount (one drop) of glycerin or glycerin-gelatin as a mounting medium. Digital images of female genitalia were taken in dorsal view with a Zeiss discovery V20 stereomicroscope using a unique magnification (23.5×).

For the genus *Pleophylla* we investigated as an example study case inter- and infraspecific variation more in detail using a much wider infraspecific sampling for the 18 included species. For this objective, additional *Pleophylla* specimens were collected during fieldwork in South Africa, which were identified partly by matching with confidently identified male specimens using a mitochondrial DNA marker (*Cox1* 3-prime end) (data not shown here). The shape of the sclerotised area of the ductus bursae (dorsal view) was analyzed quantitatively using Standard Eigenshape analysis (Lohmann, 1983; McLeod, 1999) as implemented in the program PAleontological STatistics, PAST.v2.10 (Hammer *et al.*, 2001). Outlines were digitized using TpsDIG v2.10 (Rohlf, 2006) with a graphic tablet as closed outlines by 100 semi-landmarks drawn manually. The start and end points that defined homology across specimens (Lohmann and Schweitzer, 1990) and the distance between semi-landmarks were identical. The scores with 95% of cumulative variation were used for subsequent Canonical Variate analysis (CVA) to investigate the morphospace and possible groupings of the specimens.

Results

General morphology and inter-specific variation in female genitalia of Sericini

The female reproductive organ is composed of the accessory gland, the vaginal palps, the vagina, the ductus

bursae, bursa copulatrix, spermatheca, spermathecal gland, and the median oviduct (Fig. 1). The accessory glands open into the cloaca with a common, slightly tubuliform duct which represents one of the functionally most outstanding synapomorphies of the Sericini (Ahrens, 2006b). Although their exact function is not completely understood, several authors have attributed a lubrication function during copulation and oviposition. In many cases, bacteria have been reported to occur within these glands, which produce male attracting pheromones in Melolonthinae (Berberet and Helms, 1927; Stringer, 1988). Accessory glands are absent in the more ancestral lineages of Scarabaeoidea, but they are present in the herbivorous pleurostict lineages (Ahrens, 2006b). They are morphologically quite uniform among Sericini and not very stable in overall shape. However, the common duct is much shorter in *Pleophylla* (and possibly also in other ancestral lineages not examined yet) with the single glands being less compact in shape.

The symmetrical vaginal palps have a sensory function during copulation (Konstantinov, 1998) and are situated in the dorsal wall of the vagina composed by a single sclerite on each side; they appear to be quite uniform in shape for the whole tribe, except for a few lineages. For example, they are more elongate and sharply pointed at the apex in the *Maladera castanea* group, (Fig. 3Ii, Mm, Nn), while they appear (Fig. 2E-T) to be slightly smaller in *Pleophylla* than in other groups of Sericini.

The bursa copulatrix, as a bag-like, membranous terminal extension of the female copulatory organ that receives the sperm (being ejected from the endophallus of male copulatory organ) during copulation, shows a high infraspecific variation in shape and colour depending on the female's reproductive stage at time of the collection (but also according to dissection, preparation, and preservation of the specimen) and only minor interspecific variation. The ductus bursae is in many cases also strongly sclerotised (Figs 2-3) and was therefore already used in previous studies to differentiate between female specimens of very similar and sympatrically occurring species in *Gastroserica*, *Chrysoserica*, and *Gynaecosserica* (Ahrens, 2000; 2001; Ahrens and Fabrizi, 2009) or for phylogenetic reconstructions of the genera *Pachyserica* and *Serica* (Ahrens, 2006a; 2007). The ductus bursae may also have additional sacs (*e.g.* in *Pachyserica*, *Serica*, *Nepaloserica*), or more undefined lateral three-dimensional extensions (*e.g.* in *Pleophylla*). Although these more or less sclerotised structures are highly informative for taxonomic and systematic purposes, they are three-dimensional and therefore difficult

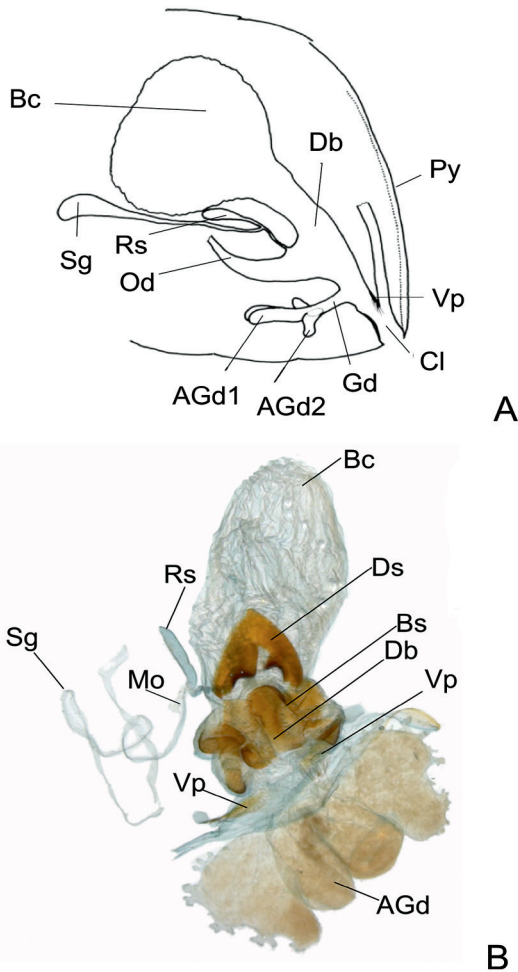


Fig. 1. Schematic overview on general morphology of female genitalia in Sericini. A: lateral view (schematic); B: dorsal view (*Pleophylla* sp.). Abbreviations: Py- Pygidium, Cl- Cloaca, Gd- accessory glands duct, Bc- Bursa copulatrix, Rs- Receptaculum seminis, Sg- Spermathecal gland, Mo- Median oviduct, Vp- Vaginal palps, AGd- Accessory glands, Db- Ductus bursae, Bs- Basal sclerite, Ds- Distal sclerite.

to capture in images for comparative examination, especially for morphometric approaches. In almost all observed cases (except some *Pleophylla*) sclerotised structures in the ductus bursae were asymmetric.

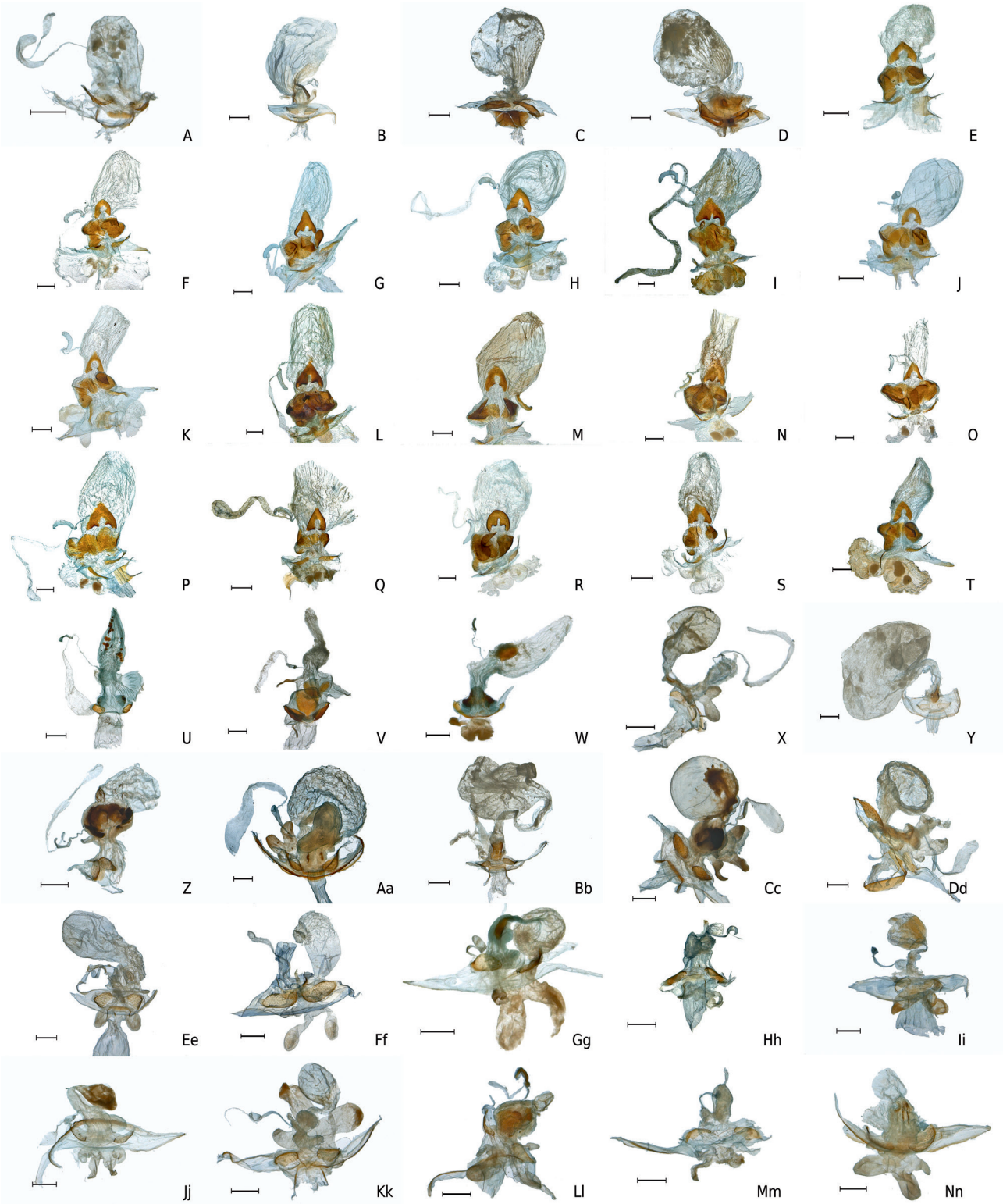
The spermatheca, spermathecal gland and accessory gland have extremely delicate membranes and are rarely fully preserved in dry-pinned museum specimens. The wall of the spermatheca was only slightly sclerotized in most examined taxa and does not maintain a stable shape. Therefore, we could not observe much species-specific shape variation except that of their general dimensions (length and size).

Case study - Interspecific variation among *Pleophylla* species

Sclerotised structures in *Pleophylla* comprise a basal piece of the ductus bursae with a number of small lateral protuberances, and a more distal triangular sclerite (Fig. 1B, Fig. 2E-T). The latter is nearly of only two-dimensional extension and therefore also suitable for comparative analysis in terms of 2D morphometric analysis. At first glance (by-eye inspection), this sclerite demonstrates sufficiently stable variation among many of the species and seems to be, consequently, widely species-specific. The single lobes of the accessory glands are less compact in shape but their external outline is amorphous and irregular, a feature that has so far been found neither in any other Melolonthine chafer lineage, nor in the hypothesized sister group, *Omoloptia* or in any other more ancestral Sericini lineage. Consequently, this character state might constitute a synapomorphy of the genus, however, for a confirmation we need to examine a wider range of taxa of basal Sericini lineages (e.g. *Astaena*, *Triodontella*, *Euronycha*, *Hymenoptia* etc.).

The distal sclerite (Fig. 1B) can be subdivided into the following homologous portions: the (distal) apex, the median (basal) sinuation, and the internal and external basal angles. The distal apex is sharply pointed but it can be also rounded in some species. The distal sclerite is generally symmetric, and only rarely asymmetric. Most importantly, species-specific characters seem to be present in the shape of the median sinuation and of the external and internal angles. The median sinuation is in general symmetric and concave, in a few cases it is angled or asymmetric, and sometimes it

Fig. 2. Dorsal view of female copulation organ: A: *Astaena* sp.; B: *Paratriodonta* sp.; C: *Omoloptia nigromarginata*; D: *O. erythroptera*, E: *Pleophylla fasciatipennis*; F: *P. pilosa*; G: *P. Sp9*; H: *P. ferruginea*; I: *P. navicularis*; J: *P. maculipennis*; K: *P. SpA1*; L: *P. SpM-1*; M: *P. SpM-10*; N: *P. SpM-16*; O: *P. SpM-17*; P: *P. SpM-18*; Q: *P. SpM-21*; R: *P. SpM-23*; S: *P. SpM-27*; T: *P. Sp1*; U: *Heteroserica* sp.; V: *H. sp1*; W: *H. sp2*; X: *Leucoserica lateralis*; Y: *Leucoserica arenicola*; Z: *Sericania mela*; Aa: *S. kashmiriensis*; Bb: *Cycloserica excisipes*; Cc: *Nepaloserica mustangia*; Dd: *Taiwanoserica gracilipes*; Ee: *S. nigroguttata*; Ff: *S. brevitaris*; Gg: *Serica* (s.str.) *tukuchiana*; Hh: *S. (s.str.) heydeni*; Ii: *S. (s.str.) murensis*; Jj: *S. (s.str.) brunnea*; Kk: *S. (s.str.) tibetana*; Ll: *S. (s.str.) schoenmanni*; Mm: *S. minshanica*; Nn: *S. (s.str.) kingdoni*. Scale bar: 0.5 mm.



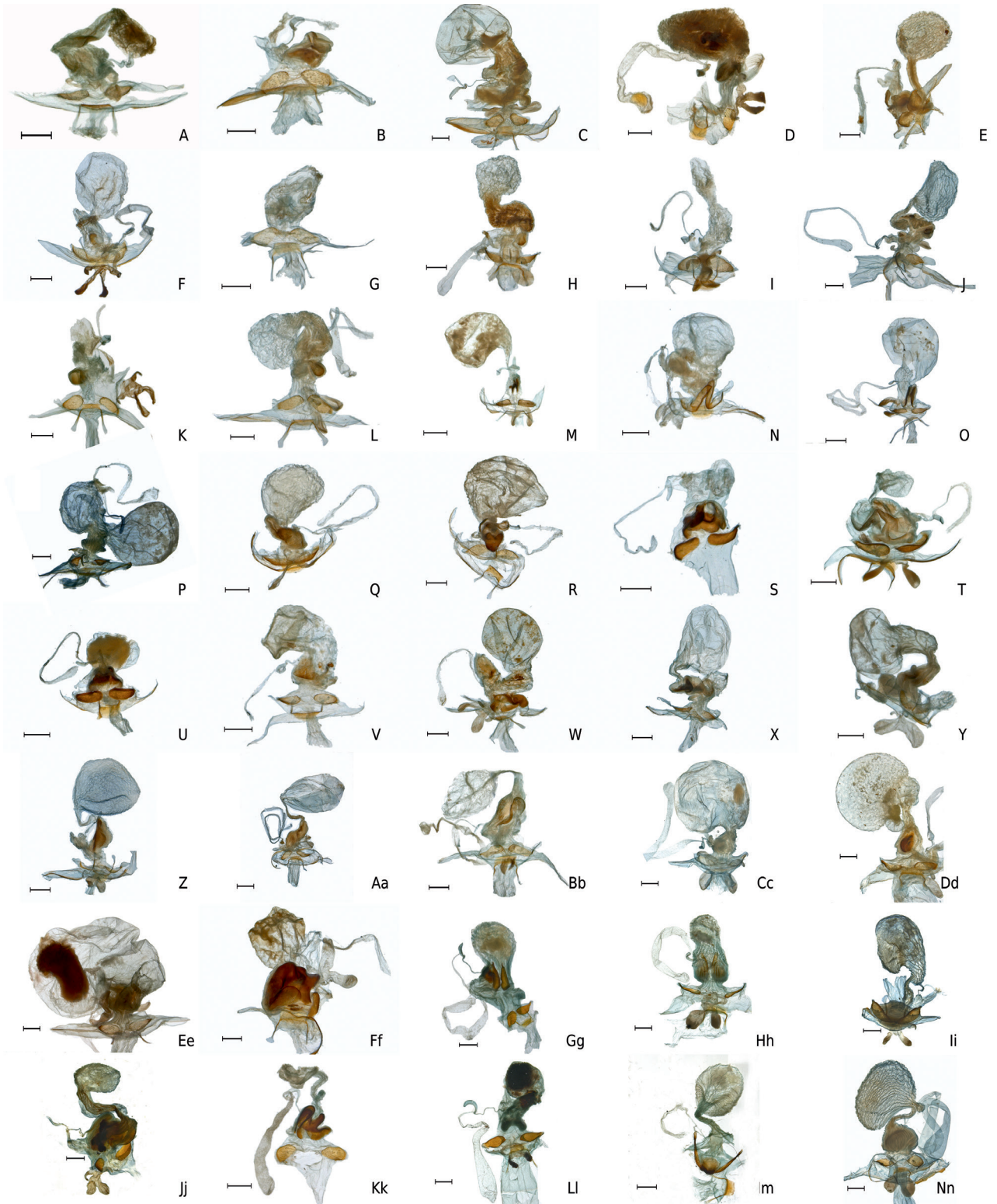


Table 1. Eigenvalues (EV) and variance (var.) of axes of Eigen-shape analysis of *Pleophylla* specimens representing 75% of the cumulative trait variation.

Axis	EV	Var. (%)	Cumulative var. (%)
ES1	0.882	13.02	13.02
ES2	0.627	9.26	22.28
ES3	0.392	5.79	28.07
ES4	0.359	5.30	33.37
ES5	0.241	3.56	36.94
ES6	0.206	3.05	39.99
ES7	0.195	2.88	42.88
ES8	0.188	2.77	45.66
ES9	0.184	2.71	48.37
ES10	0.158	2.34	50.72
ES11	0.148	2.18	52.90
ES12	0.145	2.15	55.05
ES13	0.129	1.91	56.97
ES14	0.122	1.81	58.78
ES15	0.115	1.70	60.48
ES16	0.104	1.53	62.02
ES17	0.094	1.39	63.42
ES18	0.085	1.26	64.69
ES19	0.081	1.20	65.89
ES20	0.076	1.12	67.02
ES21	0.075	1.10	68.13
ES22	0.073	1.08	69.21
ES23	0.070	1.03	70.25
ES24	0.067	0.98	71.24
ES25	0.064	0.95	72.19
ES26	0.060	0.89	73.09
ES27	0.058	0.85	73.95
ES28	0.057	0.84	74.79
ES29	0.056	0.82	75.62

is additionally provided with a thin sclerotised membrane. The depth and width of the median situation show a great variation between the different species.

The shape of the distal sclerites distinguished many, but not all of the species. In the Standard Eigenshape analysis the first 29 Eigenshape (ES) axes represented 75% and the first 67 ES axes 95% of the cumulative variance of the shape of the sclerotised area (Table 1). CVA analysis based on the first 67 ES axes provided a good separation between many species, but not all.

Plots of some species were still partly or also widely overlapping when all species were analyzed together (Fig. 4) showing only little divergence between those species. Clear morphological gaps were almost not visible.

Asymmetry

Asymmetry is found in most of the examined Sericini species, either in the bursa copulatrix only, or in both the bursa copulatrix and ductus bursae. Asymmetry comprises sclerotised and non-sclerotised structures and is only less pronounced in more ancestral lineages with a symmetrical or weakly asymmetrical male aedeagus like *Astaena*, *Pleophylla* and *Triodontella* (Fig. 2A-T). In the genus *Omaloplia*, with strongly asymmetric aedeagus we found only little to no asymmetry in the membranous bursa copulatrix (sclerotised structures are absent in this group, Fig. 2C,D). In most of the modern Sericini (*i.e.* redefined subtribe Sericina, Ahrens, 2006b), sclerotised structures are often developed in the ductus bursae that in most cases were highly asymmetric (Figs 2X-3Nn). This asymmetry is linked to strong asymmetry of the male copulation organ in all cases examined (Ahrens and Lago, 2008). Given that we found female and male asymmetry for almost all species examined, it is possible that male and female asymmetry is present in the majority of the species of the tribe, which includes nearly 4000 species.

Discussion

Utility of female genitalia for taxonomy and systematics

This preliminary study revealed significant taxonomic (and also likely phylogenetic) value of female genital traits despite a very limited sampling from the very diverse group of Sericini beetles. Our observations indicate a vast diversity in the shapes of sclerites of the ductus bursae (see Figs 2-3) that seem suitable for use in species-level taxonomy. Stable interspecific variation is

◀ Fig. 3. Dorsal view of female copulation organ: A: *Pachyserica mamorata*; B: *P. nantouensis*; C: *Pachyserica olafi*; D: *P. albosquamosa*; E: *P. bistrata*; F: *P. horrida*; G: *P. subpilosa*; H: *P. himalayensis*; I: *P. gracilis*; J: *P. jendeki*; K: *P. nepalica*; L: *P. ambiversa*; M: *Gynaecoserica cymosa*; N: *G. variipennis*; O: *G. lobiceps*; P: *G. singhikensis*; Q: *Lasioserica maculata*; R: *L. nobilis*; S: *L. brevopilosa*; T: *Lasioserica petri*; U: *Amiserica patibilis*; V: *Amiserica rejseki*; W: *Amiserica flavolucida*; X: *Amiserica costulata*; Y: *Amiserica krausei*; Z: *Neoserica* (s.str.) *tandaoensis*; Aa: *N.* (s.str.) *ursina*; Bb: *N.* (s.str.) *pseudovulpina*; Cc: *Chrysoerica* sp.; Dd: *C. auricoma*; Ee: *C. stebnickae*; Ff: *Calloserica langtangica*; Gg: *Eumaladera* sp.; Hh: *S. brunnescens*; Ii: *Maladera affinis*; Jj: *Maladera* sp.; Kk: *Maladera* sp809; Ll: *M. cruralis*; Mm: *M. verticalis*; Nn: *M. hongkongica*. Scale bar: 0.5 mm.

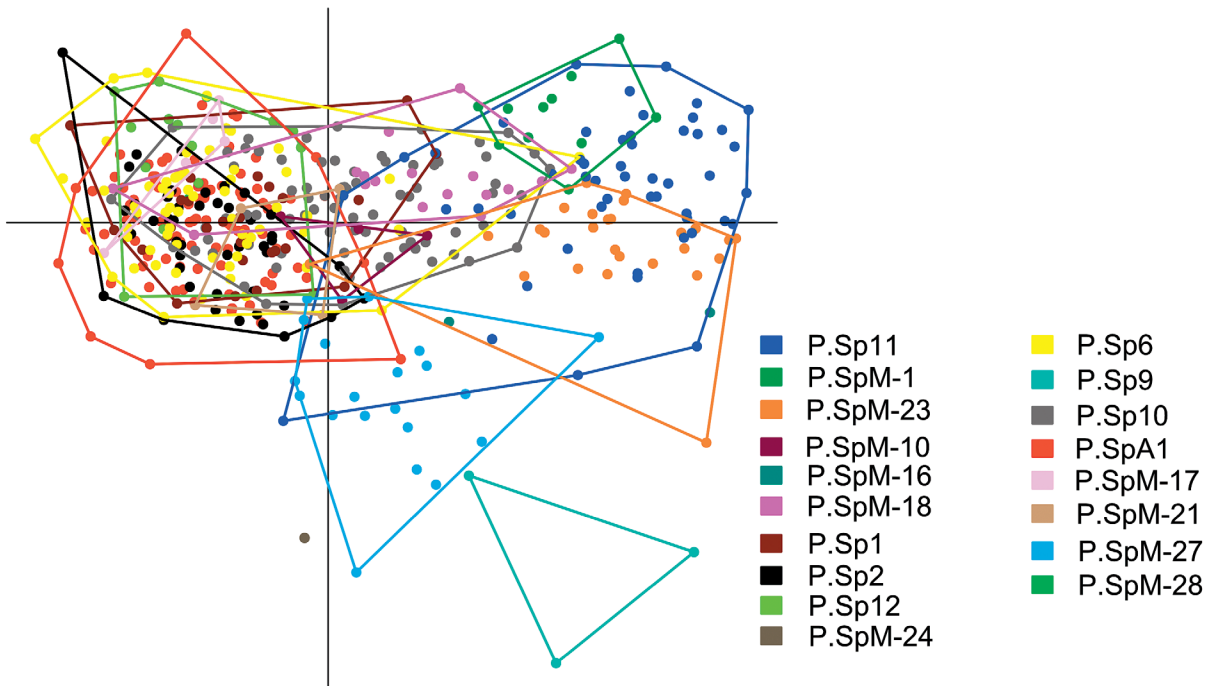


Fig. 4. Results of shape analysis of the genus *Pleophylla*: Plot of axes 1 and 2 of Canonical Variate Analysis from Eigenshape analysis.

rarely discernable in the shape and size of vaginal palps and all other membranous structures such as the shape of the bursa copulatrix (see the problems mentioned above). The same applies for the spermatheca and the spermathecal gland, which can vary in size and proportions, but to verify robust differences among the species requires well-preserved specimens and a larger sampling per species to detect also species-constant characteristics. The more detailed examination and quantitative shape analysis of the genus *Pleophylla* consisting of many very similar species that are difficult to distinguish by external morphology showed the importance of investigating both, species-specific male and female genitalia in order to revalidate morphology. *Pleophylla* appears to be a young radiation and most male specimens differ very significantly in the shape of parameres (Warnock, 2009). Their female genitalia are species-specific in most but not all of the species, but for many pair-wise species comparisons they provide a useful trait to be used to distinguish between the species (Fig. 4).

Symmetry and asymmetry

Based on evidence in various insect groups, Huber *et al.* (2007) concluded that asymmetries in insect taxa

tend to evolve first in male genitalia, while female asymmetries evolve later or not at all. The evolutionary sequence of genital asymmetry (male-first or male-only asymmetry) is crucial to understand patterns of asymmetry and to infer explanations for asymmetry (Schilthuizen, 2013). In most scarab beetles, male external genitalia are symmetrical (D'Hotman and Scholtz, 1990). The male genitalia of the presumptive sister group of Sericini (*i.e.* Ablaberini) as well of all more ancestral lineages (*i.e.* Southern World Melolonthinae; Ahrens and Vogler, 2008) are symmetrical, too. However, the morphological inaccessibility and lack of hard tissues means that female genitalia morphology is generally less well-studied and rarely used for taxonomic questions (Eberhard, 1985; Huber, 2010; Ah-King *et al.*, 2014), also in these mentioned groups. Therefore, some patterns of apparent 'male first' evolution may actually reflect a lack of knowledge of the female genitalia rather than a real evolutionary trend, which is a general problem in research on insect genitalia (Brennan *et al.*, 2007; Huber *et al.*, 2007), or might indicate the need to date (and investigate) such events at a much earlier stage when asymmetry in external genitalia was not apparent yet.

Our results presented here on symmetry/ asymmetry of female genitalia of Sericini are based exclusively

on a qualitative level. We refrained from a quantitative assessment of asymmetry of female genitalia (under the current sampling) for two major reasons: 1) From dissections of multiple specimens (at least 2 per species), we realised that the inclination and shape (degree of expansion) of bursa copulatrix (due to mounting artefacts and status of female in reproduction cycle) varied within species to a quite large extent, which would not allow the disentangling between true shape variation and artefacts or life cycle bias. This would strongly hamper the estimate of interspecific variation of asymmetry. 2) Asymmetric structures comprise mainly the shape of the ductus bursae and the bursa copulatrix. Asymmetry of the latter is hard to measure from shape (see 1) while more significant asymmetric structures like sclerotisations within the ductus bursae are difficult to homologize. Consequently, in many cases we would not know what we compare exactly.

Nevertheless, our findings have shown that the megadiverse Sericini has achieved a complex and long evolutionary history of male-female genital asymmetry. This might indicate that the shift to asymmetry is phylogenetically highly conservative. It seems that selection is stabilising the once acquired asymmetry in many different lineages within such a large group. Conclusions from patterns of asymmetry variation in male versus female genitalia should be drawn with caution. The bursa copulatrix and other soft tissue parts may be damaged easily during preparation, which might strongly bias the morphological observations. On the other hand, the endophallus has not yet been studied comparatively in scarab beetles in general, nor in Sericini. Therefore our knowledge on the 'true' copulating structures is to be considered to be still very fragmentary. For this reason, we limited our investigation here to a qualitative (and subjective) assessment of degree of apparent symmetry of the corresponding external male genitalia that are introduced only partly in the female reproductive tract in most chafers (Eberhard, 1993; Krell, 1996). Nevertheless, we may conclude from the current observations that more asymmetric external male genitalia seem to be linked with more asymmetric female genitalia.

A first step to investigate the correlation between male and female asymmetry more thoroughly, in particular for beetles, would be to focus studies on the shape of the male endophallus in everted state (rather than only the sclerotized structures of it; e.g. Tarasov and Solodovnikov, 2011) and to establish female genitalia, where possible, also as a standard diagnostic feature for species taxonomy. The latter would widen the

extent of the morphological knowledge on female genitalia and would allow more easily for an observation of male-female asymmetry correlation. Modern DNA taxonomy approaches might greatly enhance this task given the frequent difficulty of identification of female specimens (e.g. Ahrens *et al.*, 2007). Beyond facilitating a more integrative taxonomic approach (Padial *et al.*, 2010; Yeates *et al.*, 2011), this would also allow a better observation of infraspecific variation and help to find exceptions from the 'default' male-female asymmetry of the group, especially where asymmetry is only weak. In this context, interesting study cases might be lineages with reversal to symmetric aedeagi, such as encountered in certain Asian lineages of Sericini (e.g. *Oxyserica*, *Nipponoserica*, some taxa of *Maladera*). We would need to investigate the state of symmetry of these lineages' female genitalia, and whether males constitute 'crypto-asymmetric' lineages (externally symmetric, internal structures and/or endophallus asymmetric), as found for the dynastine beetle genus *Cyclocephala* (Breeschoten *et al.*, 2013).

A second step to investigate the evolution of genital asymmetry further would be to place developments of asymmetry (male and/or female) in a robust phylogenetic context (e.g., Breeschoten *et al.*, 2013). That requires, however, in the case of the Sericini, a very comprehensive sampling, as shifts from symmetry to asymmetry seem to occur at quite early stages of the evolution of the group. Probably, the reversal from (apparent) asymmetry to symmetry (or cryptic asymmetry) would be the more interesting case to investigate (see above), while groups like *Cyclocephala* (Breeschoten *et al.*, 2013), or *Schizonycha* (Pope, 1960), where within one genus symmetric and asymmetric external male genitalia occur, seem to be more suitable and promising to discover mechanisms that lead to asymmetry in the genitalia (in chafer beetles). The gradual shift from symmetric to asymmetric forms found by Breeschoten *et al.* (2013), if reconfirmed also for other groups, might be a very important key to understand better the mechanisms of selection leading to asymmetry and of conserving it.

Here we have reported asymmetry in the female genitalia for the first time among scarab beetles (for a large number of species). Circumstances for this group of beetles suggest that only sexual selection may have driven asymmetric male-female genital co-evolution in this group, but evidence for such a hypothesis is still not available. Therefore, the study the evolution of asymmetric genitalia (Schilthuizen and Gravendeel, 2012; Schilthuizen, 2013) should be one major focus

for future investigations of genital evolution and the evolutionary drivers of their morphological diversity.

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