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ORIGINAL ARTICLE

The internal female genitalia of *Aulacigaster* (Diptera: Aulacigastridae)—description and phylogenetic analysis

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Abstract

The internal female genitalia of 22 species of *Aulacigaster* are studied, covering all species groups as defined by Rung and Mathis (Smithson. Contrib. Zool., 633, 1–132, 2011). The method of embedding freshly dissected material in polyvinyl lactophenol with an admixture of chlorazol black E is far superior to results achieved by maceration in potassium hydroxide and allows the detection of delicate structures previously unnoticed. A general description of the internal female genitalia introduces all relevant structures and their variation within the genus. Establishing the presence and the multi-chambered condition of the ventral receptacle in Aulacigastridae constitutes a major result. Another important finding pertains to the presence of a ventral sclerotized ring in the posteroventral wall of the vagina, which had not been described in Aulacigastridae before. The variable characters are coded across the studied species and are subjected to a phylogenetic analysis in combination with the character set of Rung and Mathis (Smithson. Contrib. Zool., 633, 1–132, 2011). The resulting topology is used to reconstruct ancestral character states and to discuss intrafamilial character state changes at and above the species group level. Information extracted from the internal female genitalia corroborates and adds resolution to the results of previous analyses that employed characters of the external morphology and the male terminalia. Brief per species descriptions and additional illustrations are placed in the electronic supplementary material.

Keywords Schizophora · Morphology · Reproduction · Ventral receptacle · Spermathecae

Introduction

Most taxonomic descriptions of flies lack information on the internal female genitalia, or only mention structures that are strongly sclerotized, such as the spermathecae or sclerites associated with the vagina wall. More detailed studies, however, have shown that the morphology of the internal female genitalia is a potential source of additional taxonomically and phylogenetically informative characters (e.g., Sturtevant 1925, 1926; Roháček 1996; Meier et al. 1997; Caloren and Marshall 1998; Kotrba and Baptista 2002; Roháček and Barber 2008; Kotrba and Mathis 2009; Puniamoorthy et al. 2010). It is possible that these characters could contribute to resolve the phylogeny of the acalyptrate Schizophora (McAlpine 1989), a subject that has remained controversial despite massive efforts employing new tools such as DNA analysis (Wiegmann et al. 2011; see Wiegmann and Yeates 2017 for further references).

A particularly interesting structure in this regard is the ventral receptacle, an unpaired organ that arises from the anteroventral portion of the vagina. The unique and widespread occurrence of a ventral receptacle in acalyptrate Schizophora has led Hennig (1973) and McAlpine (1989) to suggest that this structure might be a synapomorphy uniting this group. But the presence of the ventral receptacle has remained undocumented for most of the ca. 65 acalyptrate families.

Where it has been studied, the condition of the ventral receptacle is found to be very diverse, ranging from one-chambered to tubular or multi-chambered, from membranous

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to strongly sclerotized, from an inconspicuous pouch to a large and sometimes oddly shaped vessel (Kotrba 1994, 2000). Some distinctive forms occur repeatedly. For example, the multi-chambered condition occurs in several taxa that are not considered to be closely related, such as Diopsidae (Kotrba 1993; Carr et al. 2006), Tephritidae (Solinas and Nuzzaci 1984; Fritz and Turner 2002), Sepsidae (Puniamoorthy et al. 2010), Opomyzidae (Kotrba and Baptista 2002), Tanypezidae (Lonsdale 2013), and the unplaced genus *Mallochianamyia* (Wheeler 2000). Unraveling the evolution and the functional and phylogenetic significance of this organ in its various configurations requires a broad comparative study with extensive taxon sampling.

One of the families in which the presence and condition of the ventral receptacle has not yet been recorded is the Aulacigastridae (tree sap flies). This is a relatively small family of acalyptrate flies associated with slime fluxes of deciduous trees (Mathis and Freidberg 1994) or with the phytotelmata of bromeliads (Rung and Mathis 2011). Aulacigastridae are found in the Afrotropical, Holarctic, Neotropical, and Oriental Regions, and are most diverse in the Neotropics. According to the latest revisions (Rung and Mathis 2011), the family includes about 55 species in only two genera, *Aulacigaster* Macquart, 1835 and the rare *Curiosimusca* Rung et al., 2005.

Most of what is known about the internal female genitalia of Aulacigastridae regards the number and condition of the spermathecae. Sturtevant (1926) reported three telescoped, sclerotized spermathecae attached to two short ducts in *Aulacigaster leucopeza* (Meigen, 1830). For the same species, McAlpine (1983) provided a sketch of a barrel-shaped spermatheca with transversally wrinkled walls and with apical and basal introverts. Mathis and Freidberg (1994) provided a detailed illustration of the three spermathecae of *Aulacigaster mcalpinei* Mathis and Freidberg, 1994 (their fig. 23). Papp (1998) provided another sketch of a spermatheca of *A. leucopeza* and Papp (2008) showed a more detailed illustration for *Aulacigaster africana* Barraclough, 1993 (his fig. 2). Rung and Mathis (2011) illustrated the spermathecae of *Aulacigaster bromeliae* Rung and Mathis, 2011 and reported that the spermatheca of this species, together with *Aulacigaster lopesi* Rung and Mathis, 2011, *A. mcalpinei*, *Aulacigaster neoleucopeza* Mathis and Freidberg, 1994, and *Aulacigaster tibanae* Rung and Mathis, 2011 have "ventral digitiform projections".

Sturtevant (1926) was the only author to observe the presence of accessory glands in *A. leucopeza*: "there are two parovaria, with ducts that are slightly longer than the spermathecal ducts. The parovaria themselves are about as long as their ducts..."

None of the authors cited above observed the presence of a ventral receptacle in *Aulacigaster*. Sturtevant (1926) explicitly specified for *A. leucopeza* that "No ventral receptacle was observed in the eight specimens dissected. Sections have also failed to show any ventral receptacle."

The present study aimed to complement the available knowledge on the internal female genitalia of *Aulacigaster* based on the study of 22 species, covering all species groups as defined by Rung and Mathis (2011). A general description of the internal female genitalia introduces all relevant structures and their variation in the genus. The variable characters are coded across the studied species and entered into a phylogenetic analysis in combination with the character set of Rung and Mathis (2011). The resulting topology is used to reconstruct ancestral character states, i.e., character states that might be employed for comparative analyses above the family level, and to discuss intrafamilial character state changes at and above the species group level. Brief per species descriptions and additional illustrations are placed in the electronic supplementary material.

Methods

Taxon sampling

The taxon sampling was limited by the availability of material. It includes 13 of the 26 species studied by Rung and Mathis (2011) and 9 additional species. The species are listed with their respective authors, collection data, and preservation (fresh, pinned, or ethanol-preserved) in Table 1. All specimens were identified by the second author. The species *Aulacigaster* sp. could not be identified. It keys out to *Aulacigaster irwini* Rung and Mathis, 2011 within the *femorata* group without matching this species. In the resulting data set, all species groups previously outlined in Rung and Mathis (2011) are represented, and all, with the exception of the *grimaldii* group, are represented by more than one species.

Dissection and morphological evaluation

In fresh specimens, it was generally not very difficult to extract the female terminalia together with the adhering internal genitalia. Pinned or ethanol-preserved specimens, on the other hand, had to be dissected in potassium hydroxide (KOH). This process tends to be more difficult and more destructive to the rest of the specimen. The results are less satisfying, especially regarding the unsclerotized parts, which may become distorted, entangled, ruptured, or entirely lost (Fig. 1). Still, such material can generally be used for a crude evaluation of the most important characters, such as the condition of the spermathecae, sclerotization of the vagina wall, and the presence of a ventral receptacle.

After dissection, the female structures were embedded on a microscope slide in polyvinyl lactophenol with an admixture of chlorazol black E. KOH treated specimens were embedded after neutralization with acetic acid. Polyvinyl lactophenol slowly and progressively macerates the soft tissue components of fresh specimens, while chlorazol black E is enriched in unsclerotized cuticular structures, staining them blue.

Table 1 Material studied. Collection data, number and preservation of specimens. The species appear in the same order as in Rung and Mathis (2011)

Species	Collecting data	Specimens
The *bromeliae* group		
A. bromeliae Rung and Mathis, 2011	BRAZIL, Rio de Janeiro, 1999, A. Baptista	1 in ethanol
A. korneyevi Rung and Mathis, 2011	COSTA RICA, Heredia, St. Domingo, INBIO (9° 58.4′ N, 84° 5.6′ W), 19 Jun 2003 D. & W.N. Mathis (Paratype)	1 pinned
A. vespertina Rung and Mathis, 2011	ECUADOR, Puerto Orellana, Tiputini Biodiversity Station, 12–26 Aug 1999, W.N. Mathis, A. Baptista & M. Kotrba	1 fresh
A. tibanae Rung and Mathis, 2011	BRAZIL, São Paulo, Praia do Estaleiro (23° 20.5′ S, 44° 53′ W), 29 Mar 2010, D. & W.N. Mathis	1 pinned
The *ecuadoriensis* group		
A. melanoleuca (Hennig, 1956)	COSTA RICA, Puntarenas, Piedras Blancas (24 km W, 8° 47′ N, 83° 15′ W, 200 m), Oct 1990, P. Hanson	1 pinned
A. stenoptera Rung and Mathis, 2011	PERU, Madre de Dios, Manu, Rio Manu, Pakitza (5 km E), Aguajal, 19 Sep 1988, A. Freidberg	2 pinned
The *femorata* group		
A. femorata Rung and Mathis, 2011	PERU, Madre di Dios, Manu, Rio Manu, 250 m, Pakitza (12° 7′ S, 70° 58′ W), 9–23 Sep 1988, A. Freidberg (Paratypes)	2 pinned
A. bella Rung and Mathis, 2011	BOLIVIA, La Paz, Mapiri (15° 18.6′ S, 68° 13′ W; 720 m), 15 Mar 2001, W.N. Mathis	1 pinned
A. sp.	ECUADOR, Puerto Orellana, Rio Tiputini (0° 38.2′ S, 76° 8.9′ W), 12–26 Aug 1999, W.N. Mathis, A. Baptista & M. Kotrba	2 fresh
A. formosa Rung and Mathis, 2011	ECUADOR, Puerto Orellana, Rio Tiputini (0° 38.2′ S, 76° 8.9′ W), 12–26 Aug 1999, W.N. Mathis, A. Baptista & M. Kotrba (Paratype)	1 pinned
A. peruana Rung and Mathis, 2011	PERU, Madre di Dios, Manu, Rio Manu, 250 m, Pakitza (12° 7′ S, 70° 58′ W), 9–23 Sep 1988, A. Freidberg (Paratype)	1 pinned
The *grimaldii* group		
A. grimaldii Rung and Mathis, 2011	COSTA RICA, Puntarenas, Rincon (3 km SW, 9° 55′ N, 84° 13′ W, 10 m), Oct-Dec 1990, P. Hanson ECUADOR, Puerto Orellana, Tiputini Biodiversity Station, 6 Feb 1999, T.L. Erwin	2 pinned
The *minuta* group		
A. minuta Hennig, 1969	PANAMA, Madden Forest, 7–16 Mar 1961, S.B. Pipkin	1 pinned
A. *gaimarii* Rung and Mathis, 2011	BOLIVIA, La Paz, Mapiri (5 km W; 15° 17.8′ S, 68° 15.6′ W; 750 m), 16 Mar 2001, A. Freidberg, S.D. Gaimari (Paratypes)	2 pinned
The *plesiomorphica* group		
A. erika Rung and Mathis, 2011	ECUADOR, Puerto Orellana, Tiputini Biodiversity Station, 12–26 Aug 1999, W.N.Mathis, A. Baptista & M. Kotrba	1 fresh
A. albifacies Rung and Mathis, 2011	GUYANA, Kanuku Mts., Moco Moco River (3° 18.2′ N, 59° 38.9 W), 29 Apr 1995, W.N. Mathis	1 pinned
A. rufifemur Rung and Mathis, 2011	ECUADOR, Puerto Orellana, Tiputini Biodiversity Station (0° 38.2′ S, 76° 8.9′ W), 12–26 Aug 1999, W.N.Mathis, A. Baptista & M. Kotrba	1 fresh, 1 pinned
A. *belize* Rung and Mathis, 2011	PERU, Madre di Dios, Manu, Rio Manu, Pakitza (12° 7′ S, 70° 58′ W; 250 m), 9–23 Sep 1988, A. Freidberg	1 pinned
A. trifasciata Rung and Mathis, 2011	ECUADOR, Puerto Orellana, Tiputini Biodiversity Station, 12–26 Aug 1999, W.N. Mathis, A. Baptista & M. Kotrba	4 fresh, 1 pinned
The *leucopeza* group		
A. leucopeza (Meigen, 1830)	BRD, Berlin, Tiergarten, 28 July 1999, A. Baptista & M. Kotrba	4 fresh
A. mcalpinei Mathis and Freidberg, 1994	USA, Washington DC, 26 Aug 1993, W.N. Mathis & A. Freidberg	2 fresh
A. neoleucopeza Mathis and Freidberg, 1994	USA, MD, Anne Arundel Co., SI Environ.Res.Center, 4 Aug 1994, W.N. Mathis	1 in ethanol

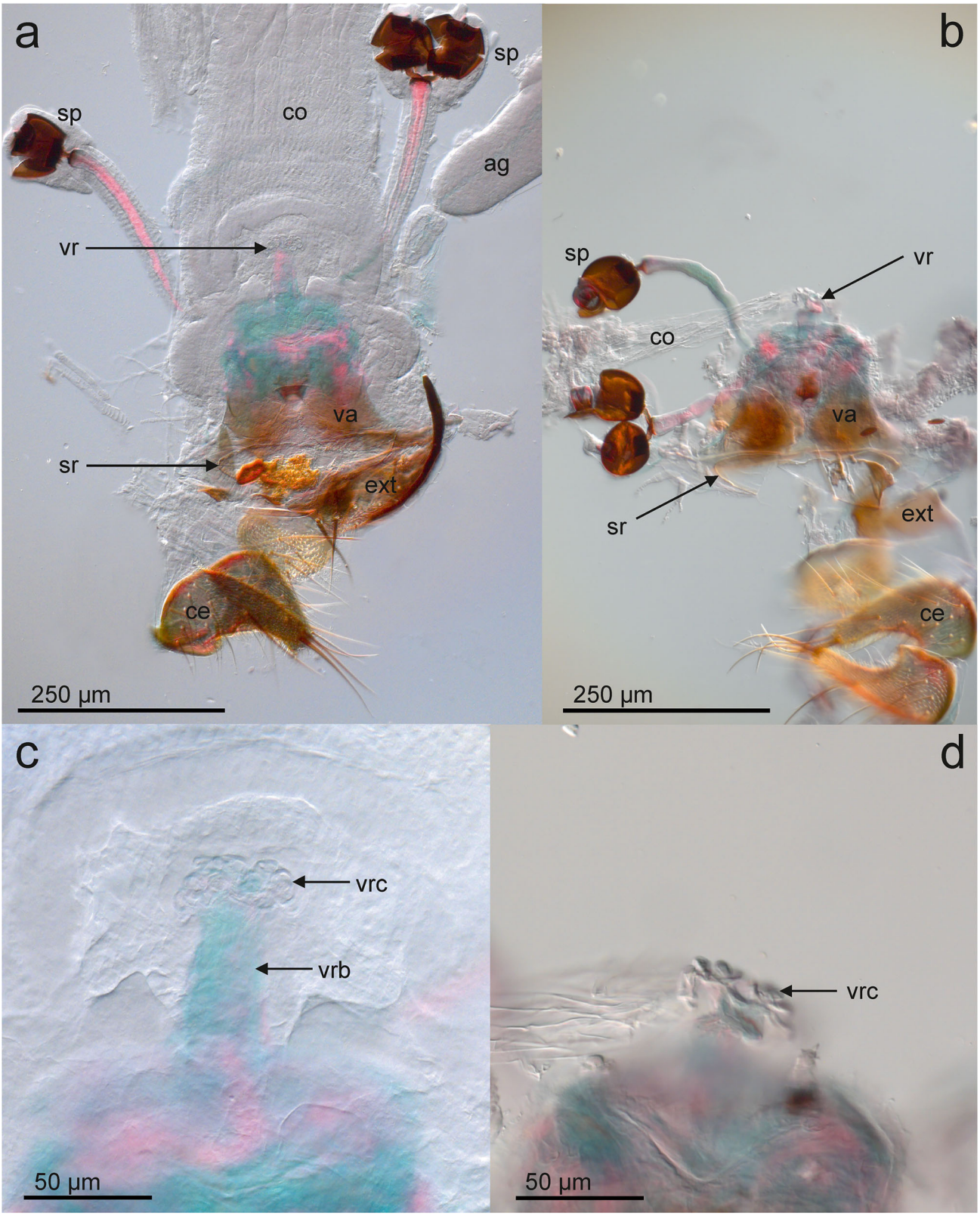


Fig. 1 Internal female genitalia of *Aulacigaster rufifemur*, ventral aspect, comparison of dissections of fresh specimen (**a**, **c**), and pinned specimen (**b**, **d**), the latter of which was treated with KOH. After maceration in KOH, unsclerotized structures are collapsed and distorted. **a**, **b** Overviews. **c**, **d** Details of ventral receptacle. *ag* accessory gland, *ce* cercus, *co* common oviduct, *ext* fragments of external sclerotized structures, *sp* spermatheca, *sr* sclerotized ring, *va* vagina with extensive sclerotized areas, *vr* ventral receptacle, *vrc* ventral receptacle chambers, *vrb* ventral receptacle base. *Bar*: **a**, **b**: 250 µm; **c**, **d**: 50 µm

Table 2 Characters and their states used in the phylogenetic analysis

	Partition 1—external characters and male genitalia																							Partition 2—internal female genitalia																	
Character no.	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	4	4	4
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2
Species																																									
Aulacigaster																																									
grimaldi	1	2	1	2	1	1	1	1	1	1	1	3	2	2	1	2	2	1	1	2	1	1	1	1	1	1	1	1	1	2	1	1	4	2	2	2	3	2	1	1	1
bromeliae	1	1	1	1	1	1	1	2	2	2	2	4	1	2	1	1	2	1	1	2	1	1	1	1	2	1	4	3	1	2	1	1	3	3	1	3	3	1	2	2	1	1
korneyevi	1	1	1	1	1	1	1	2	2	2	2	4	1	2	1	1	2	1	1	2	1	1	1	1	2	1	4	3	1	2	1	1	3	3	2	3	2	3	2	?	?	1
vespertina	1	1	1	1	1	1	1	2	2	2	2	4	1	2	1	1	2	1	1	2	2	1	1	1	2	1	4	3	1	2	1	1	3	3	1	3	3	3	2	2	1	1
tibanae	1	1	1	1	1	1	1	2	2	2	2	4	1	2	1	1	2	1	1	2	2	1	1	1	2	1	4	3	1	2	1	1	3	3	1	3	3	1	1	?	?	1
erika	1	3	1	1	1	1	1	1	1	1	1	3	1	2	1	1	2	1	1	2	1	3	1	1	2	1	2	2	1	2	1	1	1	1	3	1	1	2	2	2	4	3
albifacies	1	3	1	1	1	1	1	1	1	1	1	3	1	2	1	1	2	1	1	2	1	3	1	1	2	1	2	2	1	2	1	2	1	2	3	1	1	2	2	2	2	3
rufifemur	1	3	1	1	1	1	1	1	1	1	1	3	1	2	1	1	2	1	1	2	1	3	1	1	2	1	2	2	1	2	1	1	1	1	2	1	1	3	2	2	1	3
belize	1	3	1	1	1	1	1	1	1	1	1	3	1	2	1	1	2	1	1	2	1	3	1	1	2	1	2	2	1	2	1	1	1	1	2	1	1	3	2	2	2	3
trifasciata	1	3	1	1	1	1	1	1	1	1	1	3	1	2	1	1	2	1	1	2	1	3	1	1	2	1	3	2	1	2	1	2	2	2	3	1	2	2	2	2	4	3
melanoleuca	1	1	1	4	2	1	2	1	1	1	3	3	1	1	–	1	1	1	2	2	1	3	1	2	2	1	2	1	1	2	1	2	1	1	2	1	2	2	2	2	3	3
stenoptera	1	1	1	4	2	1	2	1	1	1	1	3	1	1	–	1	2	2	2	2	2	1	1	2	1	1	1	1	2	1	2	2	1	4	2	2	1	3	2	1	1	2
femorata	1	1	1	3	1	2	1	1	1	1	1	3	1	2	1	2	2	2	2	2	2	1	2	1	1	1	1	1	1	1	1	1	5	2	3	1	3	2	1	1	2
bella	1	1	1	3	1	2	1	1	1	1	1	3	1	2	1	2	2	2	2	2	2	1	2	1	1	1	1	1	?	1	1	1	4	2	1	1	2	1	1	1	2
sp.	1	1	1	3	1	2	1	1	1	1	1	3	1	2	1	2	2	2	2	2	2	1	2	1	1	1	2	1	2	1	1	1	1	2	2	1	3	2	1	1	1
formosa	1	1	1	3	1	2	1	1	1	1	1	3	1	2	1	2	2	2	2	2	2	1	2	1	?	2	?	1	?	1	2	1	1	4	2	2	1	2	2	1	1	2
peruana	1	1	1	3	1	2	1	1	1	1	1	3	1	2	1	2	2	2	2	2	2	1	2	1	1	2	1	1	2	1	2	2	1	4	2	2	1	2	2	1	1	2
minuta	1	1	1	4	1	1	2	1	1	1	1	3	1	1	–	2	2	2	2	2	2	1	3	1	1	1	1	1	1	2	1	1	4	2	1	3	2	2	1	?	2
gaimarii	1	1	1	4	1	1	2	1	1	1	1	3	1	1	–	2	2	2	2	2	2	1	3	1	1	1	1	2	1	2	1	1	4	2	1	3	2	2	1	1	2
leucopeza	2	2	2	1	1	1	1	1	1	1	1	3	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1

Data for characters 1–24 (partition 1, external morphology and male terminalia) were taken from Rung, A., & Mathis, W.N. (2011). A revision of the genus *Aulacigaster* Macquart (Diptera: Aulacigastridae). Smithsonian Contributions to Zoology, 633: appendix

A certain degree of compression under the cover slip is desirable as it facilitates discerning delicate structures embedded in layers of soft tissue, such as the ventral receptacle. It has the disadvantage, however, that the sclerotized spermathecal capsules are sometimes crushed. Also, the vagina tends to end up in a dorsoventral aspect, rendering it difficult to discern whether structures are located in the dorsal or ventral vagina wall. Delicate structures such as the ventral receptacle or the basal part of the spermathecal ducts may become obscured by overlaying other structures of the reproductive tract. Most regrettably, in some of the specimens treated with KOH, the specific condition of the ventral receptacle could not be discerned.

The preparations were studied with bright field illumination and differential interference contrast (DIC) using a Zeiss Axioskop2 equipped with Plan-Apochromat × 20, Plan-Apochromat × 40, and Plan-Neofluar × 100 oil objectives as well as a drawing tube and a Jenoptic Progres Gryphax Subra digital camera. The photographs included a digital scale bar calibrated with a stage micrometer. The dimensions of long structures such as the spermathecal ducts were measured in sketches done by drawing tube using a stage micrometer. The plates were created with CorelDRAW X5 and Corel PHOTO-PAINT X5. Some of the photos were stacked by hand from photos at different focal planes. Line-drawings were created with Adobe Illustrator.

Most aulacigastrid species are represented by only a few specimens in collections. For this reason, in many cases, only one or two specimens could be dissected. This means that intraspecific variation was not investigated. In particular, all numerical data in this study refer to measurements of a single specimen. The measurements of the lengths of the two spermathecal ducts and the width of the three spermathecal capsules were averaged.

Phylogenetic analysis

The data matrix contains a total of 42 morphological characters in 2 partitions (Table 2). Partition 1 comprises the characters of the external morphology (chars. 1–20) and male terminalia (chars. 21–24) established by Rung and Mathis (2011). Species not included in their matrix were coded following the respective character definition (Rung and Mathis 2011, pp. 115–116). Partition 2 comprises 18 new characters

from the internal female genitalia (chars. 25–42) defined in the character list below.

Multistate characters of partition 2 were ordered whenever a plausible transformation series was apparent, i.e., if they refer to measurements (chars. 28, 33, 35, 36) or degree of sclerotization (chars. 37, 38). All other multistate characters were treated as unordered.

For the three studied species of the *leucopeza* group (one from the Nearctic and two from the Palearctic region), all character codings were identical. To avoid redundancy, only *A. leucopeza* was included in the matrix.

All analyses were performed using PAUP 4.0 (Swofford 2002). Analyses were carried out based on the combined data set (total evidence) and on each of the two partitions alone. Tree search was exhaustive, using the branch-and-bound command. The initial sets of trees obtained for the three character sets were re-analyzed by applying three iterations of successive weighting based on Farris' consistency-index (Farris 1969).

Since we were unable to obtain exemplars of the putative sister group of *Aulacigaster*, *Curiosimusca*, we used *A. leucopeza* to root the trees. This choice was based on Rung and Mathis's (2011) hypothesis of a sister group relationship between the worldwide *leucopeza* group and the clade formed by the exclusively Neotropical species groups.

Character states were optimized on the single most parsimonious tree obtained from all characters combined, which is our preferred tree. The unambiguous synapomorphies for each branch were obtained with Paup's command "apolist," and we only address unambiguous character changes in the discussion, unless specified otherwise. Characters 1, 3, and 17 at the base of the cladogram were polarized based on the states present in *Curiosimusca* (Rung and Mathis 2011).

Results

General description

Apart from the ovaries and oviducts, which are not lined by cuticle and were not studied, the internal female genitalia

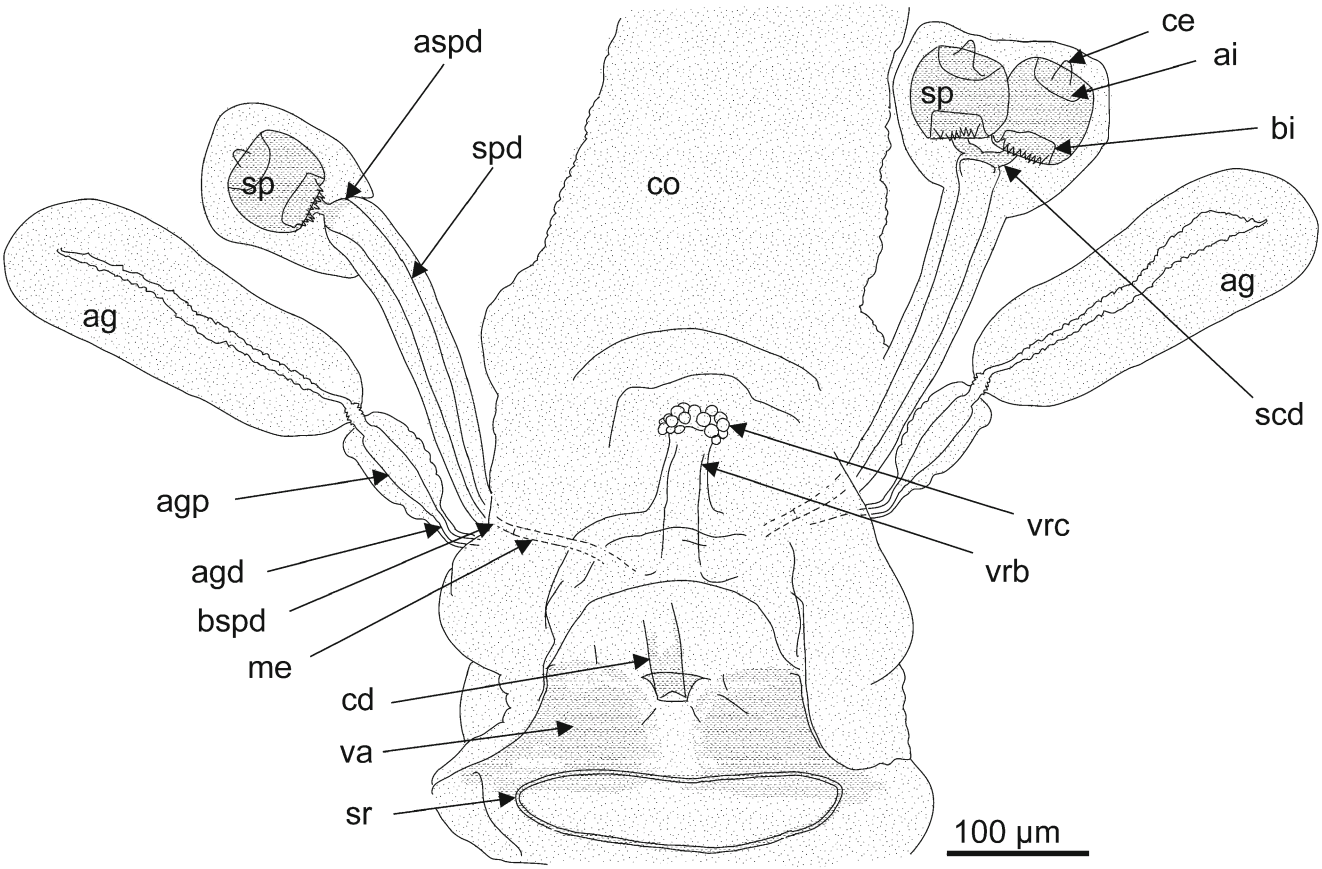


Fig. 2 Internal female genitalia of *Aulacigaster rufifemur*, ventral aspect, schematic drawing for the demonstration of relevant structures. *ag* accessory gland, *agd* accessory gland duct, *agp* accessory gland pump, *ai* apical introvert, *aspd* apical end of spermathecal duct, *bi* basal introvert, *bspd* base of spermathecal duct, *cd* common duct formed by membranous extensions of spermathecal ducts, *ce* central evagination, *co* common oviduct, *me* membranous extension of spermathecal duct, *scd* smooth connecting duct, *sp* spermatheca, *spd* spermathecal duct, *sr* sclerotized ring, *va* vagina with extensive, weakly to moderately sclerotized areas, *vr* ventral receptacle, *vrc* ventral receptacle chambers, *vrb* ventral receptacle base. *Bar*: 100 µm

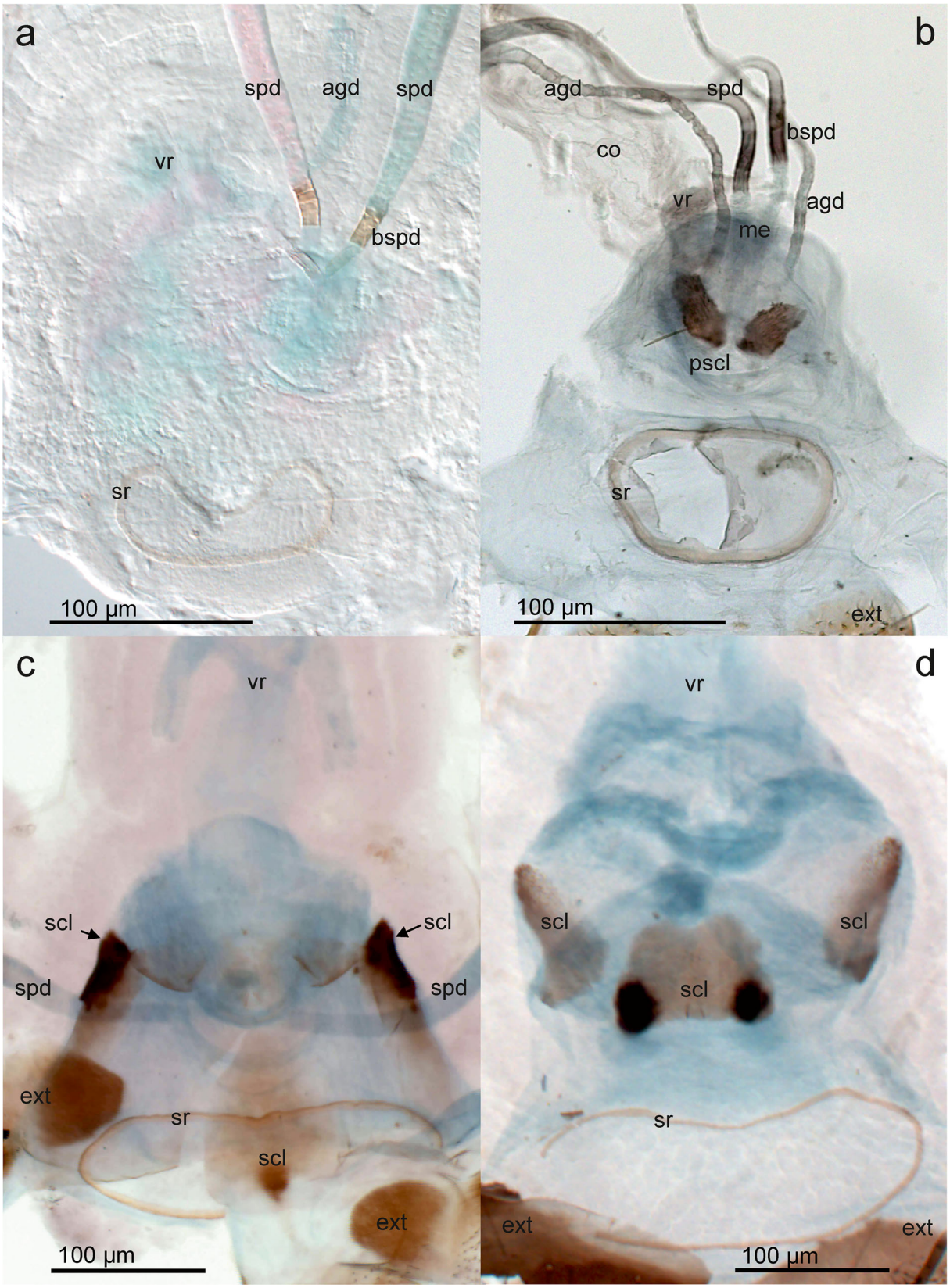


Fig. 3 Internal female genitalia of *Aulacigaster* species exemplifying diversity of sclerotization of the vagina wall. **a** *A. neoleucopeza*. **b** *A. peruana*. **c** *A. trifasciata.* **d** *A. vespertina. agd* accessory gland duct, *bspd* base of spermathecal duct, *co* common oviduct, *ext* fragments of external sclerotized structures, *me* membranous extension of spermathecal duct, *pscl* pair of small sclerites, *scl* sclerotization of vagina wall, *spd* spermathecal duct, *sr* sclerotized ring, *vr* ventral receptacle. *Bar*: 100 µm

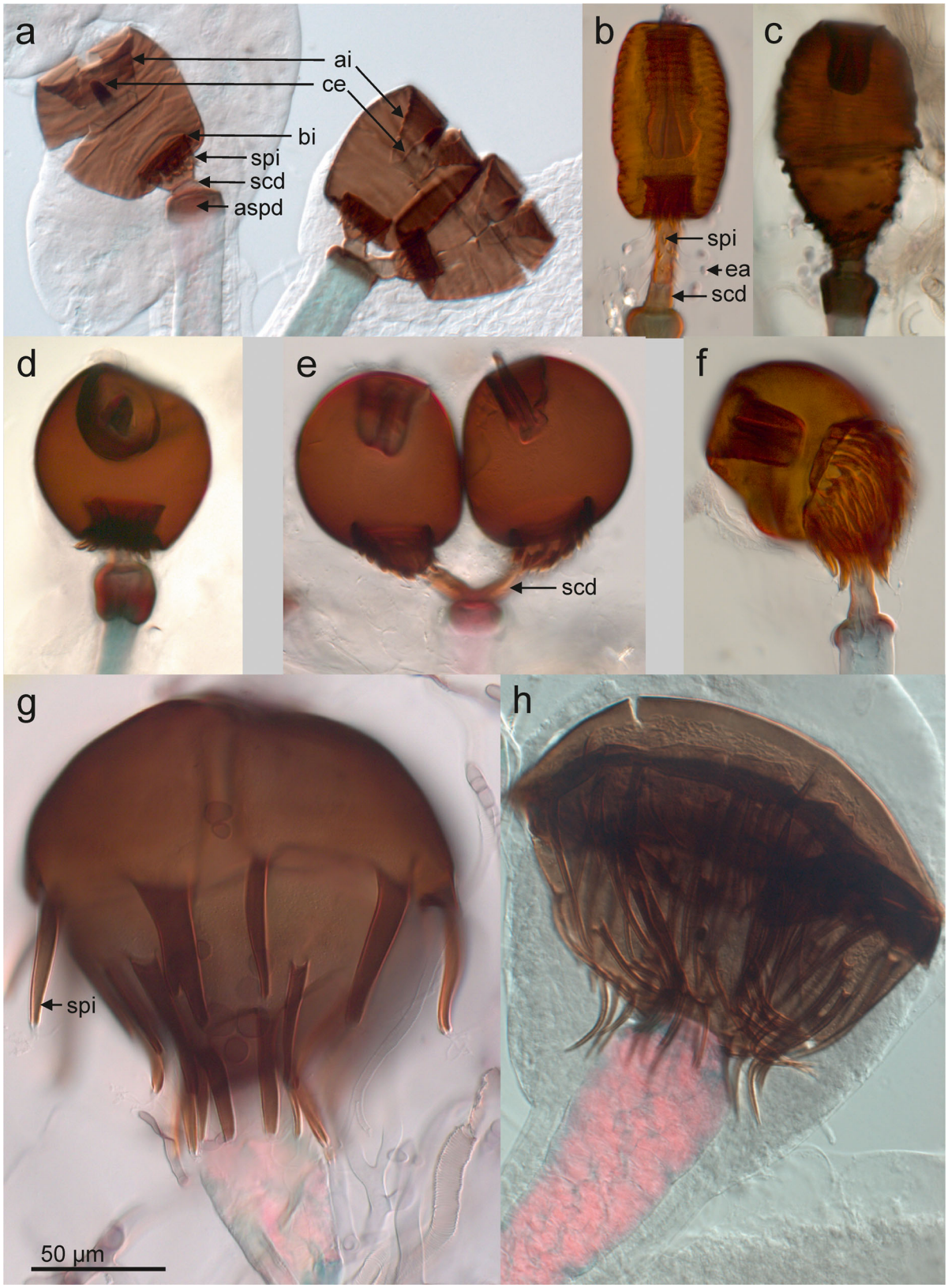


Fig. 4 Spermathecae of *Aulacigaster* species exemplifying diversity of spermathecal shapes. **a** *A. neoleucopeza*. **b** *A. stenoptera*. **c** *A. femorata*. **d** *A. belize*. **e** *A. albifacies*. **f** *A. trifasciata*. **g** *A. tibanae*. **h** *A. vespertina*. *ai* apical introvert, *aspd* apical end of spermathecal duct, *bi* basal introvert, *ce* central evagination, *ea* end apparatus of spermathecal gland cells, *spi* spinules, spines, *scd* smooth connecting duct. All figures to same scale. *Bar*: 50 µm

comprise a short vagina with a sclerotized ring embedded in its posteroventral wall and facultative additional sclerotized areas, three spermathecae, paired accessory glands, and the ventral receptacle (Figs. 1 and 2).

The vagina is dorsoventrally compressed and more or less warped, especially in species with extensive sclerotized areas in the vagina wall. There is always a delicate sclerotized ring embedded in its posteroventral wall. In some species, this structure spans only part of the diameter of the vagina, with the narrowest and most delicate form (ca. 40 μm width) occurring in *Aulacigaster grimaldii*, up to a width of 130 μm (Fig. 3a, b; char. 25: 1). In others, it extends across the entire width of the vagina, from 190 to 260 μm (Fig. 3c, d; char. 25: 2), the largest width occurring in *Aulacigaster melanoleuca* and *Aulacigaster vespertina*. Because the sclerotized but delicate ring is sometimes positioned closely adjacent to sternum 8, it is very easily ruptured or entirely lost in dissections.

In many of the species, the vagina lacks any additional sclerotized structures (Fig. 3a; char. 26: 1). In *Aulacigaster formosa* and *Aulacigaster peruana*, a pair of small sclerites occurs near the base of the ventral receptacle. (Fig. 3b; char. 26: 2). In some other species, the vagina has extensive additional sclerotized areas, which vary in their exact location, extent, distinctness, and degree of sclerotization. A large portion of the vagina wall may be weakly to moderately sclerotized with rather ill-defined borders (Fig. 1; char. 27: 2). In *Aulacigaster trifasciata*, there is a pair of well-defined lateral plates, strongly sclerotized anteriorly and diverging posteriorly, plus an unpaired median portion posterior to these (Fig. 3c; char. 27: 3). In other species, e.g., *A. vespertina*, there is a pair of well-defined lateral lobes that diverge anteriorly, plus an median two-lobed plate with strongly sclerotized areas posteriorly (Fig. 3d; char. 27: 4). Although the latter two conditions (char. 27: 3 and 4) are similar in their general composition, each comprising a pair of well-defined and strongly sclerotized lateral structures plus a median sclerotized element, they are distinct in their shapes and were therefore coded as separate states. It was not possible to determine with certainty whether these structures are positioned in the ventral or dorsal vagina wall.

The width of the three sclerotized spermathecae varies from about 50 μm to about 200 μm (char. 28: 1–3). They are most often roundish to barrel-shaped (Fig. 4a, d–h; char. 29: 1), but sometimes elongate (Fig. 4b; char. 29: 2). Their wall is transversely wrinkled (Fig. 4a–c; char. 30: 1) or smooth (Fig. 4d–h; char. 30: 2). The spermathecae generally have an apical invagination (apical introvert, Fig. 4a). In a few species, this is particularly long, exceeding half the length of the spermathecal capsule, typically with the deepest part somewhat expanded into an unsclerotized bulb (Fig. 4b; char. 31: 2). The deepest part of the apical introvert in turn bears a cone-shaped central evagination (Fig. 4a), and this, too, is particularly long in a few species (Fig. 4b, e, f; char. 32: 2). Invariably part of the base of the spermathecae is adorned with hollow projections of varying numbers and sizes, from tiny spinules (Fig. 4a–c; char. 33: 1) to long and narrow spines (Fig. 4g, h; char. 33: 3). Each of these projections is associated with the delicate cuticular end apparatus of one of the surrounding spermathecal gland cells (Fig. 4b). In most species, the spiny basal area of the spermathecae is invaginated (basal introvert, Fig. 4a, d; char. 34: 1). In a few other species, it is enlarged and only slightly invaginated at its margin (Fig. 4e, f; char. 34: 2) or particularly large, not invaginated, and amply adorned with larger spines (Fig. 4g, h; char. 34: 3). In yet some other species, the basal introvert is extended into a cylindrical neck that protrudes from the base of the spermatheca (Fig. 4b; char. 34: 4). The neck is undoubtedly formed by part of the introvert, as it is ornamented with the same small spinules. The condition in *Aulacigaster femorata* is unique, with a large basal area whose tiny spinules are in a peculiar spiral arrangement (Fig. 4c; char. 34: 5; present in both specimens dissected).

The base of the spermathecae is generally connected to the spermathecal duct by a very short (Fig. 4a–d; char. 35: 2) to slightly longer (Fig. 4e, f; char. 35: 3) smooth connecting duct. This duct is clearly distinguishable from the neck portion of the basal introvert by its smooth wall as opposed to the spiny ornamentation of the spermathecal base (see above). Its degree of sclerotization is generally consistent with the sclerotization of the apical end of the spermathecal duct. In very few species, the spermathecae seem to insert directly at the spermathecal ducts without a distinct connecting duct (Fig. 4g, h; char. 35: 1).

The spermathecal ducts generally grow wider apically toward an abrupt end, which receives the smooth connecting ducts from the spermathecae. One spermathecal duct bears a single spermatheca, the other bears two. The spermathecal ducts vary largely in their length from about 180 μm to about 870 μm (char. 36). In many species, they appear rather stout (Fig. 1), but in a few species they are longer and narrower, and in yet some others they are very long and wide. Their apical and/or basal ends are sometimes more or less distinctly sclerotized (chars. 37, 38). The spermathecal ducts open into the vagina dorsomedially, generally via a short delicate membranous extension, which traverses the vagina wall. These extensions are distinct from the thick-walled spermathecal ducts by their delicate cuticular lining. In some species, these basal extensions are quite long, and in some species they merge in the middle to form a common duct that may also receive the accessory gland ducts. Because these delicate structures are difficult to discern and interpret, especially in specimens treated with KOH, this feature was not incorporated into the matrix. To ensure homology, the length of the spermathecal ducts was measured excluding any basal membranous extensions and excluding the smooth connecting ducts leading to the spermathecae.

Due to their very delicate nature, the accessory glands were lost in several dissections, and were torn, overstretched, or

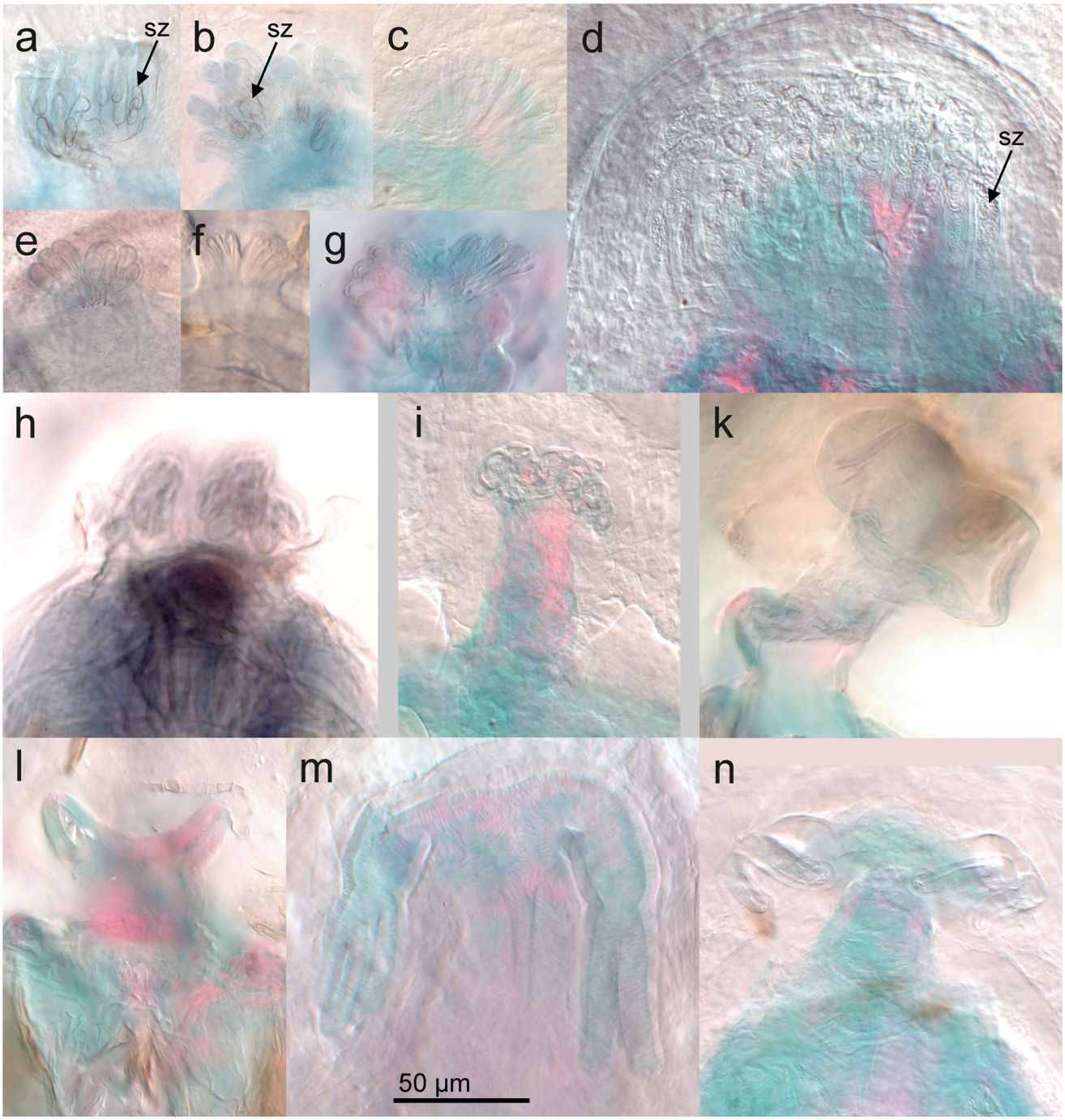


Fig. 5 Ventral receptacle of *Aulacigaster* species exemplifying diversity of ventral receptacle shapes. **a** *A. leucopeza.* **b** *A. neoleucopeza.* **c** *A. mcalpinei.* **d** *A. vespertina.* **e** *A.* sp. **f** *A. grimaldii.* **g** *A. stenoptera.* **h** *A. melanoleuca*. **i** *A. rufifemur.* **k** *A. belize.* **l** *A. albifacies*. **m** *A. trifasciata.* **n** *A. erica. sz* spermatozoa. All figures to same scale. *Bar*: 50 µm

entangled in others. Where they could be studied, they show little variation. The accessory glands have elongated reservoirs, which are lined by delicate cuticle and embedded in a thick layer of glandular epithelium (Figs. 1 and 2). Gland reservoirs and ducts together are slightly to considerably longer than the spermathecae and their ducts together. The ducts alone are about one-half to two-thirds as long as the spermathecal ducts. They are wider apically, forming a muscular pump region. Their base may be slightly sclerotized (char. 39: 1). It opens into the dorsal wall of the vagina next to the spermathecal ducts.

The ventral receptacle arises from the anteroventral portion of the vagina (Figs. 1 and 2). It is embedded in a thick layer of epithelium and musculature. Its length, measured from its base

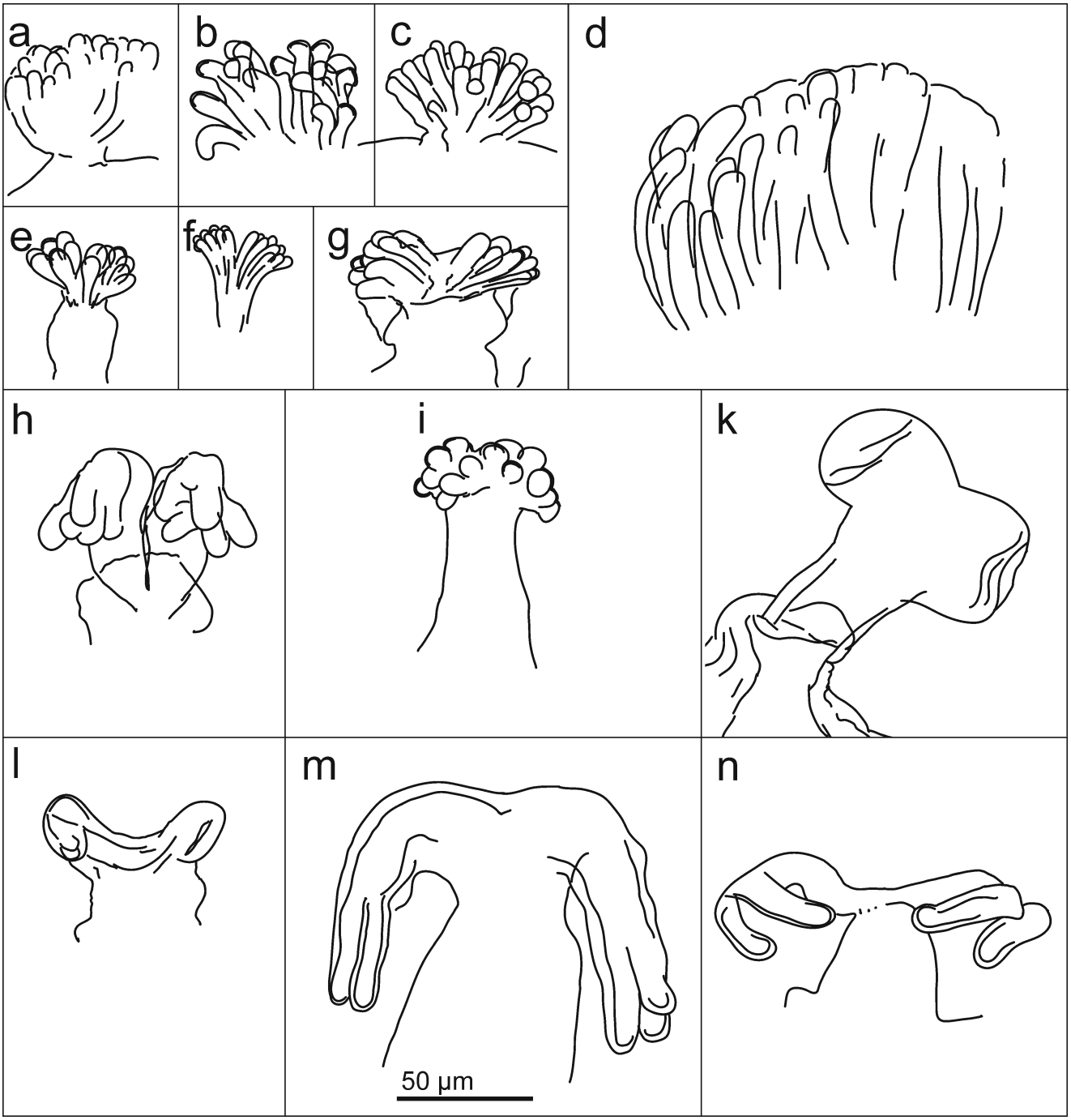


Fig. 6 Ventral receptacle of *Aulacigaster* species exemplifying diversity of ventral receptacle shapes, line drawings highlighting the very delicate structures in Fig. 5. **a** *A. leucopeza.* **b** *A. neoleucopeza.* **c** *A. mcalpinei.* **d** *A. vespertina.* **e** *A.* sp. **f** *A. grimaldii.* **g** *A. stenoptera.* **h** *A. melanoleuca*. **i** *A. rufifemur.* **k** *A. belize.* **l** *A. albifacies*. **m** *A. trifasciata.* **n** *A. erica.* All figures to same scale. *Bar*: 50 μm

to its anterior border, varies from about 30 μm to about 130 μm (char. 40). In none of the studied species, the ventral receptacle is sclerotized, and in many it is small and hard to discern. Most often it consists of a cluster of delicate elongate cuticular chambers fanning out anteriorly from an inconspicuous base (Figs. 5a–f and 6a–f; char. 41: 1, 42: 1). In *A. vespertina*, the chambers are particularly numerous, long, and delicate (Figs. 5d and 6d). In some other species, the base of the ventral receptacle is stouter and apparently thick-walled (Figs. 5g and 6g; char. 42: 2), sometimes obscuring morphological details. In yet another set of species, the ventral receptacle is quite large with a substantial cylindrical base (Figs. 5h–n and 6h–n char: 42: 3). In these species, the apical portion of the ventral receptacle shows remarkable variation: In *A. melanoleuca*, the anterior portion bends dorsally and bears two lateral clusters of about five large but delicate

Table 3 Number of most parsimonious trees retained, included characters, and tree statistics after analysis of partitions 1 and 2 separately and combined (total evidence), with weighted and unweighted characters. The number of steps, consistency index (excluding uninformative characters), and retention index are given for the unweighted characters optimized over one of the most parsimonious trees

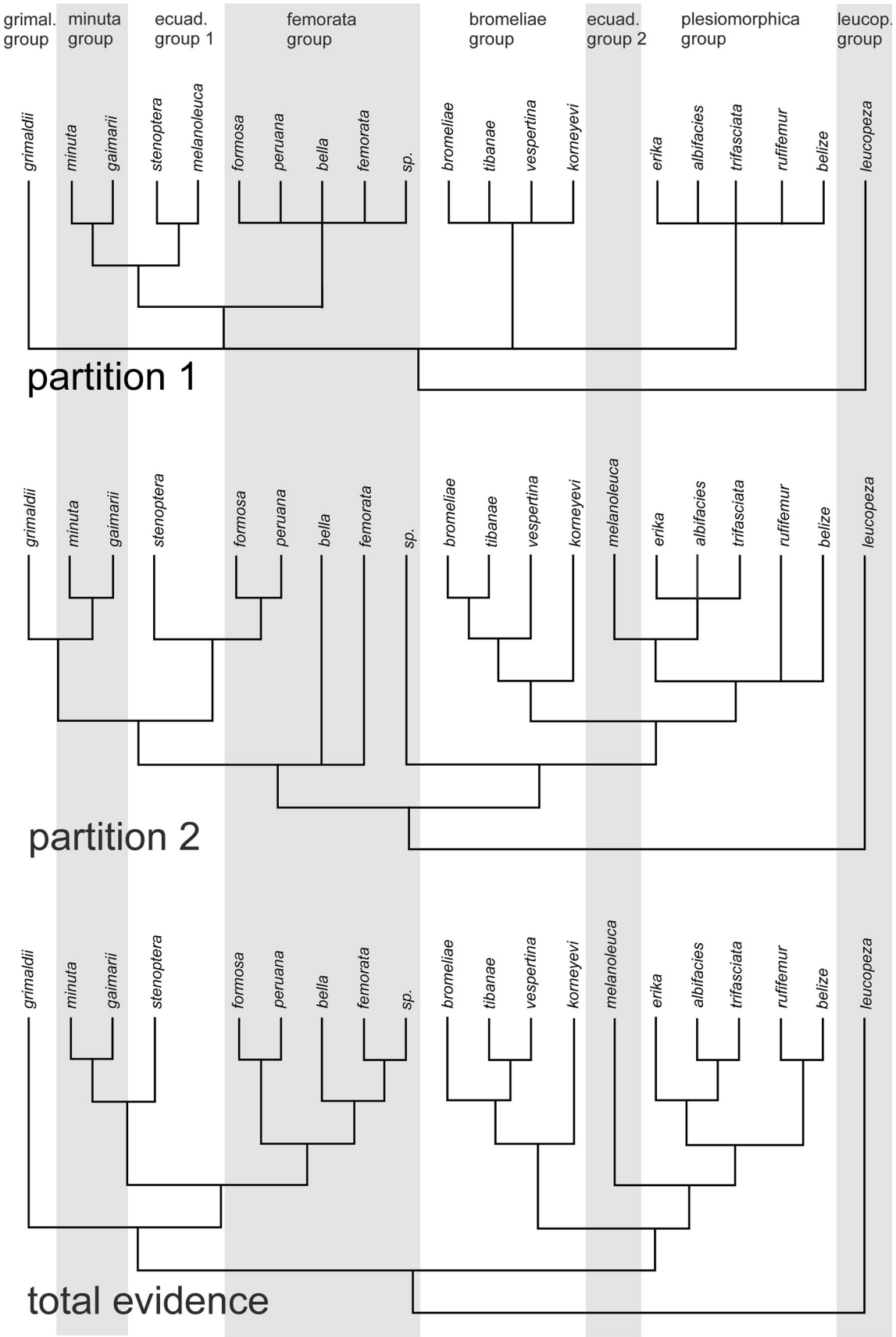
	Partition 1		Partition 2		Total evidence	
	Unweighted	Weighted	Unweighted	Weighted	Unweighted	Weighted
Trees retained	14	11	304	3	30	1
Characters	24	24	18	18	42	42
Ordered characters	0	0	6	6	6	6
Tree length	34	34	55	56	100	100
Consistency index	0.77	0.77	0.58	0.57	0.58	0.58
Retention index	0.91	0.91	0.81	0.8	0.8	0.8

chambers each (Figs. 5h and 6h; char. 41: 3). This character state was coded separately because the interpretation of its homology was uncertain. In *Aulacigaster rufifemur*, the apical portion is multi-chambered, but the chambers are not elongate but roundish and rather robust (Figs. 5i and 6i). In *Aulacigaster belize*, the apical portion is a long sac that apically bears two large lateral lobes (Figs. 5k and 6k; char. 41: 2). In *Aulacigaster albifacies*, the structure is shorter, but the condition at the apical end appears to be similar to the one in *A. belize* (Figs. 5l and 6l). In *A. trifasciata* and *Aulacigaster erica*, the apical portion bifurcates into two laterally directed portions, each side terminating in 2–3 prominent tubular and comparatively thick-walled chambers (Figs. 5m, n and 6m, n; char. 41: 4). The tubular chambers are directed dorsally in *A. erica* and posteriorly in *A. trifasciata*. In some dissections from fresh material, spermatozoa can be discerned within the chambers of the ventral receptacle (Fig. 5a, b, g).

Characters of the internal female genitalia, used in the phylogenetic analysis (partition 2)

25. Ventral sclerotized ring width: 1. up to 130 μm; 2. more than 190 μm.
26. Pair of small sclerites near base of ventral receptacle: 1. absent; 2. present.
27. Vagina sclerotization (other than chars. 25 and 26): 1. absent; 2. extensive, weakly to moderately sclerotized areas; 3. well-defined, one pair of lateral plates strongly sclerotized anteriorly and diverging posteriorly, plus an unpaired median portion posterior to these; 4. well-defined, one pair of large lateral plates diverging anteriorly plus a median two-lobed plate with strongly sclerotized areas posteriorly.
28. Spermathecae width: 1. 46–58 μm; 2. 63–82 μm; 3. 120–200 μm.
29. Spermathecae shape: 1. roundish to barrel-shaped; 2. distinctly elongate.
30. Spermathecae (other than basal introvert): 1. transversely wrinkled; 2. smooth.
31. Apical introvert length: 1. not exceeding one-half of spermathecal capsule length; 2. exceeding one-half of spermathecal capsule length.
32. Central evagination: 1. cone-shaped, relatively short; 2. particularly long and narrow, digitiform.
33. Basal spinules on spermatheca: 1. up to 12 μm; 2. roughly 20 μm; 3. 45–60 μm.
34. Basal part of spermathecal capsule that bears spinules: 1. invaginated and not protruding or only weakly protruding ("basal introvert"); 2. large and only slightly invaginated at the margin; 3. large and not invaginated; 4. forming a cylindrical neck partially invaginated but also protruding from the base of the spermatheca; 5. large with spirally arranged spinules.
35. Smooth connecting ducts: 1. absent; 2. very short; 3. longer.
36. Spermathecal ducts length: 1. 180–290 μm; 2. 314–476 μm; 3. more than 660 μm.
37. Apical end of spermathecal ducts: 1. distinctly sclerotized; 2. slightly sclerotized; 3. weakly or not sclerotized.
38. Base of spermathecal ducts: 1. distinctly sclerotized; 2. slightly sclerotized; 3. weakly or not sclerotized.
39. Base of accessory glands ducts: 1. slightly sclerotized; 2. not sclerotized.
40. Ventral receptacle length: 1. 30–50 μm; 2. 60–130 μm.
41. Ventral receptacle shape: 1. multi-chambered; 2. two-lobed sac; 3. two apical portions, each with ca. five chambers; 4. two apical portions, each with 2–3 tubular chambers.

Fig. 7 Comparison of the results obtained from the analysis of partitions 1 and 2 separately and combined (total evidence). **a** Strict consensus cladogram of 11 trees obtained with partition 1 (34 steps, consistency index 0.77, retention index 0.91). **b** Strict consensus cladogram of 3 trees obtained with partition 2 (56 steps, consistency index 0.57, retention index 0.80). **c** Single most parsimonious tree obtained with all characters combined (100 steps, consistency index 0.58, retention index 0.8). All trees and cladograms were obtained with branch and bound search after successive weighting, with characters 28, 33, 35–38 ordered. Species-groups highlighted by alternating vertical gray and white zones


grimal. group
minuta group
ecuad. group 1
femorata group
bromeliae group
ecuad. group 2
plesiomorphica group
leucop. group
grimaldii
minuta
gaimarii
stenoptera
melanoleuca
formosa
peruana
bella
femorata
sp.
bromeliae
tibanae
vespertina
komeyevi
erika
albifacies
trifasciata
rufifemur
belize
leucopeza
partition 1
partition 2
total evidence

42. Ventral receptacle base: 1. inconspicuous; 2. stout; 3. large, cylindrical.

Phylogenetic analysis

The statistics of the trees obtained from the analysis of the two character partitions separately and combined are summarized in Table 3. In all analyses, successive weighting was applied and provided increased tree resolution that was generally compatible with groupings supported without such weighting. Therefore, we have placed greater credence in, and centered our discussion on, the results obtained with successive weighting. Figure 7 shows the strict consensus cladograms obtained with the separate partitions in comparison with the single most parsimonious tree obtained with all characters combined (total evidence), which is our preferred tree. Note that in each of the topologies, the clade formed by the exclusively Neotropical species groups is a result of rooting of the tree with *A. leucopeza*.

Character analysis

Figure 8 shows the characters optimized on our preferred tree. The consistency indices for the individual characters are listed in Table 4. In our analysis, two of the characters in partition 1 are invariant and three are not parsimony-informative, because some of the species studied by Rung and Mathis (2011) are not represented in our sampling. We chose to keep these characters in our matrix to facilitate comparison with the previous analysis.

Discussion

In spite of the considerably altered species set, the results of the analysis of partition 1 (Fig. 7a), comprising characters of the external morphology and male terminalia as defined by Rung and Mathis (2011), are largely consistent with the phylogenetic hypothesis proposed by these authors (Rung and Mathis 2011, p. 118, their fig. 202). All species groups represented by more than one species were rendered monophyletic. The *femorata*, *minuta*, and *ecuadoriensis* groups together form a clade in which the *minuta* and *ecuadoriensis* groups are sister groups. Only the branch linking *A. grimaldii* with the base of this major clade is not recovered in the present analysis.

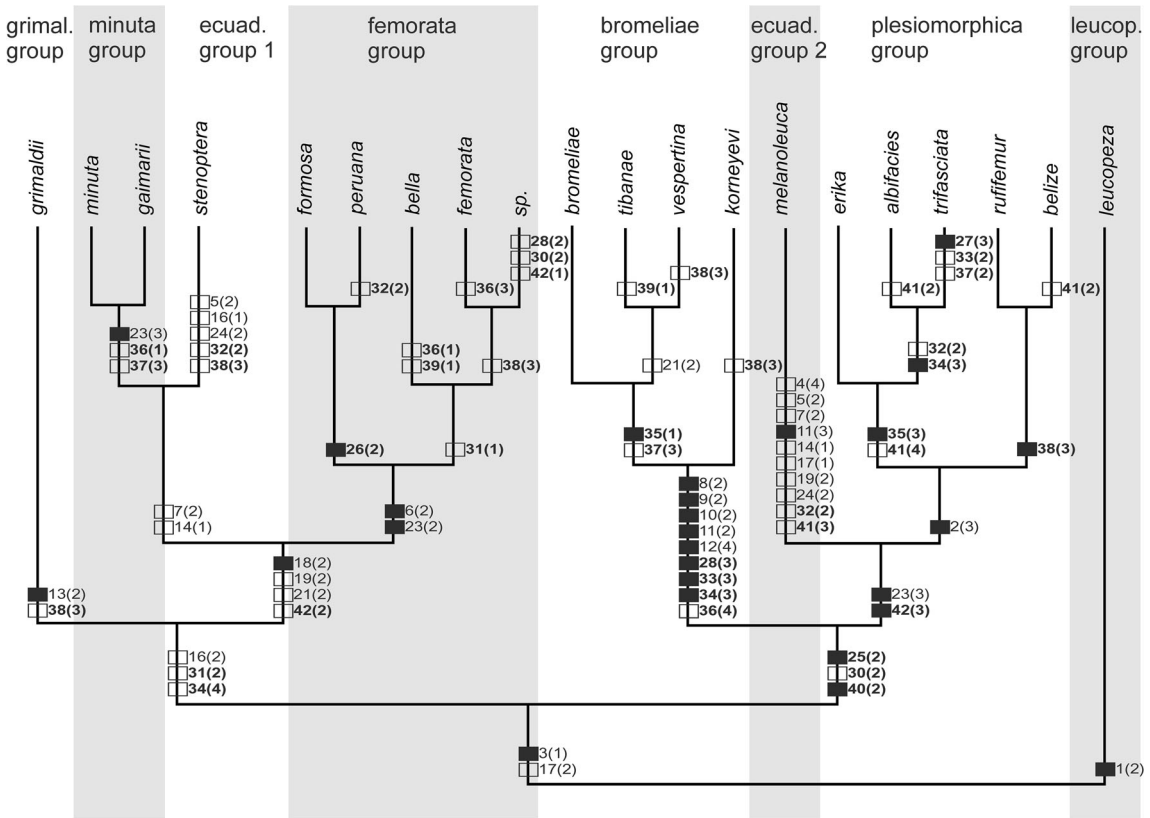


Fig. 8 Character optimization on single most parsimonious tree obtained with all characters combined (100 steps, consistency index 0.58, retention index 0.8), chosen to represent the phylogenetic relationships among the species-groups of *Aulacigaster*. Only unambiguous synapomorphies are indicated. *Empty squares*, homoplasious synapomorphies; *filled squares*, non-homoplasious synapomorphies. Partition 2 characters printed in bold face

Table 4 Character states and consistency index (CI) for the individual characters unweighted and optimized over our preferred tree (Fig. 8)

Partition 1				Partition 2			
Character	States		CI	Character	States		CI
1	1, 2	Uninformative	1.00	25	1, 2		1.00
2	1, 2, 3		0.67	26	1, 2		1.00
3	1, 2	Uninformative	1.00	27	1, 2, 3, 4		1.00
4	1, 2, 3, 4		0.75	28	1, 2, 3	Ordered	0.50
5	1, 2		0.50	29	1, 2		0.33
6	1, 2		1.00	30	1, 2		0.50
7	1, 2		0.50	31	1, 2		0.50
8	1, 2		1.00	32	1, 2		0.25
9	1, 2		1.00	33	1, 2, 3	Ordered	0.67
10	1, 2		1.00	34	1, 2, 3, 4, 5		0.80
11	1, 2, 3		1.00	35	1, 2, 3	Ordered	1.00
12	3, 4		1.00	36	1, 2, 3	Ordered	0.33
13	1, 2	Uninformative	1.00	37	1, 2, 3	Ordered	0.29
14	1, 2		0.50	38	1, 2, 3	Ordered	0.22
15	1	Invariant	1.00	39	1, 2		0.33
16	1, 2		0.50	40	1, 2		1.00
17	1, 2		0.50	41	1, 2, 3, 4		0.75
18	1, 2		1.00	42	1, 2, 3		0.67
19	1, 2		0.50				
20	2	Invariant	1.00				
21	1, 2		0.50				
22	1, 3		1.00				
23	1, 2, 3		1.00				
24	1, 2		0.50				

Part of the clades found by Rung and Mathis (2011) are independently recovered also by analysis of partition 2 with characters of the internal female genitalia only (Fig. 7b). These are specifically the monophyly of the *bromeliae*, *minuta*, and *plesiomorphica* groups (the latter with the inclusion of *A. melanoleuca*), and a major clade combining the members of the *femorata*, *grimaldii*, *minuta*, and *ecuadoriensis* groups. Differences are the disintegration of the *femorata* and *ecuadoriensis* groups, the displacement of *A. melanoleuca*, the close association of *A. grimaldii* with the *minuta* group, and the finding of a second major clade combining the *bromeliae* and the *plesiomorphica* groups.

Combining partitions 1 and 2 roughly doubles the number of informative characters. After successive weighting, the analysis results in a single most parsimonious tree (Figs. 7c and 8), our preferred tree, on which we base our discussion below.

The following characters are unambiguous at the base of the tree, allowing us to infer their ancestral states at this level (Fig. 8): Vagina with a ventral sclerotized ring of moderate size (up to 130 μm; char. 25: 1), lacking other sclerotized areas (chars. 26: 1, 27: 1); three sclerotized spermathecae, about 45–60 μm wide (char. 28: 1), barrel-shaped (char. 29: 1), transversely wrinkled (char. 30: 1), with apical and basal introverts; apical introvert not exceeding half the length of the spermathecal capsule (char. 31: 1), bearing a central cone-shaped central evagination (32: 1); basal introvert (char. 34: 1) adorned with tiny spinules (char. 33: 1); spermathecae connected to their respective ducts via short smooth connecting ducts (char. 35: 1); base of spermathecal ducts slightly sclerotized (char. 38: 2); base of accessory glands ducts not sclerotized (39: 2); ventral receptacle small (up to 50 μm; char. 40: 1), unsclerotized, multi-chambered (char. 41: 1), with delicate elongate chambers fanning out anterolaterally from an inconspicuous base (char. 42: 1). Moreover, although not coded and included in the analysis, the ancestral state of the accessory glands includes tubular gland reservoirs and membranous ducts with a distinct apical pump region; ducts and gland reservoirs together slightly to considerably longer than the spermathecae and their ducts together.

Although we were not able to study the only other genus of the family, *Curiosimusca*, we hypothesize that many of the listed character states likely also apply to the ground plan of Aulacigastridae.

On our preferred tree, the *bromeliae*, *femorata*, *minuta*, and *plesiomorphica* groups are rendered monophyletic. The

ecuadoriensis group is disintegrated, due to the displacement of *A. melanoleuca.*

The tree is divided into two major clades. The first one includes the *femorata* and *minuta* groups, *A. grimaldii* and *A. stenoptera*. It corresponds to a respective clade in Rung and Mathis (2011), which was established based on one synapomorphy (char. 16: 2, reverted in *A. stenoptera*). Now it receives further support from two spermathecal characters: (1) the apical introvert is particularly long, exceeding half the length of the spermathecal capsule (char. 31: 2). This synapomorphy is reverted in *A. bella* + *A. femorata* + *A.* sp. (2) The basal introvert, distinct by its ornamentation with tiny spinules, is extended into a cylindrical neck that protrudes from the base of the spermatheca (char. 34: 4). This synapomorphy is reverted in *A.* sp. *Aulacigaster femorata* shows an autapomorphic condition with the entire basal part of the spermatheca everted (char. 34: 5).

The second major clade includes the *bromeliae* and *plesiomorphica* groups and *A. melanoleuca*. This clade is a new finding, supported by three synapomorphies from the internal female genitalia: (1) The sclerotized ring in the ventral vagina wall has evolved into a much wider, transverse loop (char. 25: 2). (2) The spermathecae have lost their ornamentation with transverse wrinkles (char. 30: 2). Convergently, the spermathecae are smooth also in *A.* sp. within the *femorata* group. (3) The ventral receptacle is enlarged (char. 40: 2). Moreover, the vagina has evolved considerable sclerotized areas anterior of the sclerotized ring. The optimization of this character at the base of this clade is ambiguous and therefore not shown in Fig. 8. That the diffuse sclerotization (char. 27: 2) of the ancestrally unsclerotized vagina wall (char. 27: 1) preceded the evolution of well-defined strongly sclerotized structures, as found in the *bromeliae* group (char. 27: 4) and, convergently, in *A. trifasciata* (char. 27: 3), appears more plausible than the other way around. In this case, the diffuse sclerotization (char. 27: 2) would constitute an additional synapomorphy supporting this major clade.

The relationships within the first major clade match the findings of Rung and Mathis (2011), with the exception that *A. melanoleuca* is removed, leaving *A. stenoptera* in place of the *ecuadoriensis* group. *Aulacigaster grimaldii* is positioned next to the base. A clade combining the *femorata* and *minuta* groups and *A. stenoptera* was already established based on three synapomorphies (chars. 18: 2, 19: 2, and 21: 2, the latter is convergent in *A. tibanae* and *A. vespertina*). This clade now receives further support from one synapomorphy in the ventral receptacle, which has a stout and apparently thick-walled base (char. 42: 2, reverted in *A.* sp.). Other than this, the characters of partition 2 contribute little to the resolution on species group level in this clade. The monophyly of the *femorata* group (based on chars. 6: 2, 23: 2), the *minuta* group (based on char. 23: 3), and a sister group relationship between the *minuta* group and *A. stenoptera* (based on chars. 7: 2, 14: 1) are recovered in concordance with, and based on the characters of, Rung and Mathis (2011). The *minuta* group receives additional support from two, albeit highly homoplastic characters regarding the length of the spermathecal ducts (char. 36: 1) and their sclerotization (char. 37: 3).

Within the second major clade, the *bromeliae* group is strongly supported by nine synapomorphies. In addition to the characters already established by Rung and Mathis (2011; chars. 8: 2, 9: 2, 10: 2, 11: 2, 12: 4), this clade receives further support from four spermathecal characters. (1) The spermathecal capsules are much enlarged (char. 28: 3). (2) Their basal part is not invaginated to form a basal introvert (char. 34: 3). It is (3) ornamented with very prominent hollow spines, 45 μm long or longer (char. 33: 3). The basal spinules are also elongated in *A. trifasciata*, but only moderately so (char. 33: 2). (4) The spermathecal ducts are particularly long (char. 36: 3) and particularly wide. Very long spermathecal ducts also occur convergently in *A. femorata*, but in that species they are narrow. The well-defined sclerotized areas of the vagina wall, with one pair of large lateral plates diverging anteriorly plus a median two-lobed plate (char. 27: 4), likely constitute another synapomorphy for the *bromeliae* group, but the optimization of this character is ambiguous (see above).

A surprising element of our results is the displacement of *A. melanoleuca* from the first major clade to the second. Therein, its close association with the *plesiomorphica* group is supported by two synapomorphies. (1) Strongly developed gonopodal setae are present (char. 22: 3, optimized as a convergence in Rung and Mathis (2011)) and (2) the ventral receptacle has evolved a larger base (char. 42: 3).

Because a close association of *A. melanoleuca* with *A. ecuadoriensis* was established by Rung and Mathis (2011), the new placement of *A. melanoleuca* likely affects the entire *ecuadoriensis* group. But the issue may not be finally resolved. In our preferred tree, the branch leading to *A. melanoleuca* is relatively long when compared with the other terminal branches. It has ten autapomorphies, of which nine are homoplastic, possibly indicating that the present placement of *A. melanoleuca* needs further testing within the context of a larger taxon sampling. However, moving the taxon back to its previous position next to *A. stenoptera* adds three more steps to the length of the tree, and is therefore less parsimonious.

The *plesiomorphica* group is recovered based on one synapomorphy (char. 2: 3) established by Rung and Mathis (2011). Within this species group, the ventral receptacle undergoes the most profound changes. In most of the species, the characteristic compartmentation into many comparatively small cuticular chambers is reduced in favor of a few larger compartments. The shape of the compartments varies from round *A. rufifemur* (Fig. 5i), to tubular in *A. erica* and *A. trifasciata* (Fig. 5m, n), to sac-like in *A. belize* (Fig. 5k). It would be very interesting to explore the functional

significance and the selective forces that brought about this remarkable diversification. In the acalyptrate Schizophora, the ventral receptacle has a pivotal function in reproduction. This is the site where the eggs are fertilized, and in some taxa, spermatozoa are stored for prolonged periods of time prior to fertilization (Nonidez 1920; Schwartz 1965; Solinas and Nuzzaci 1984; Kotrba 1993; Pitnick et al. 1999; Fritz and Turner 2002; Carr et al. 2006; Pattarini et al. 2006; Kotrba 2016; Kotrba et al. 2016). In some of our dissections of fresh material, spermatozoa are visible within the chambers of the ventral receptacle (Fig. 5a, b, d). In *A. vespertina*, in which the ventral receptacle has particularly long chambers, the coiled spermatozoa contained therein appear likewise particularly long (Fig. 5d). This is reminiscent of findings in the diopsid genus *Diasemopsis*, where the implications for sperm storage, sperm competition, and cryptic female choice have been extensively discussed (Kotrba et al. 2016). More detailed studies of the morphology and evolution of the ventral receptacle in the *plesiomorphica* group could contribute to our principal understanding in this respect.

Conclusions

Establishing the presence and the multi-chambered condition of the ventral receptacle in Aulacigastridae constitutes a major result of this study. Another important finding pertains to the presence of the ventral sclerotized ring, which had not been described in Aulacigastridae before. Like the ventral receptacle (see "Introduction" section), the presence of a sclerotized ring constitutes a feature, which also occurs in a few other acalyptrate families that are not considered to be closely related, such as Canacidae and Tethinidae (Hardy and Delfinado 1980), Diopsidae (Kotrba 1993), and Anthomyzidae (Roháček 1996, Roháček and Barber 2008). Whether the recurrence of these structures is due to convergence or homology remains to be clarified. We hope that these and other characters established for the ground plan of the studied clade will ultimately contribute to resolve the suprafamilial relationships of the acalyptrate Schizophora.

The fact that the information extracted from the internal female genitalia has corroborated and added additional resolution to the results of previous analyses that employed characters of the external morphology and the male terminalia (Rung and Mathis 2011), indicates that the study of these organs can indeed help resolve phylogenetic issues.

Here we have only evaluated some of the more obvious aspects of this character set. If a greater number of fresh specimens were available, it should be possible to extract even more detailed information, establish the degree of intraspecific variation, and reliably resolve relationships also within species groups. More, and more detailed studies of these structures may therefore prove highly rewarding.

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