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ORIGINAL RESEARCH ARTICLE

Accessory copulatory structures in the bursa of male acanthocephalans

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Abstract. Two major categories of accessory copulatory structures in the inner surface of the bursa designed to facilitate copulatory activity are identified from 28 species in 10 families and 5 orders of Acanthocephala. Paired lateral suckers are known in the bursa of 9 species of Echinorhynchidae and Pomphorhynchidae. Single, rings or clusters of sensory discs, cups, tubercles, knobs, or papillated receptors are identified in the remaining species of all families: Arhythmacanthidae, Echinorhynchidae, Heteracanthocephalidae, Neoechinorhynchidae, Polymorphidae, Pomphorhynchidae, Rhadinorhynchidae, Transvenidae, and Quadrigyridae in 4 orders. The suckers, similar in all 9 species, are designed to clasp the female posterior trunk to insure effective transfer of sperms. Marks of suckers on the trunk of females indicate their tight adherence to the female trunk during copulation. The sensory structures are presumably connected to the genital and bursal ganglia for species and sex recognition and for successful delivery of sperm with minimal loss. The sensory structures are often distributed circumpenially or circumbursally, or both in identifiable species-specific patterns. Many other studied species of acanthocephalans did not exhibit sensory structures in the bursa. Those that did, like the ones reported in this study, may have a higher selective advantage in the efficacy of their copulatory activity.

Keywords: Copulatory and sensory structures; Bursa; Acanthocephala.

Structuri copulatorii accesorii în bursa acanthocefalilor masculi

Rezumat. Două categorii majore de structuri copulatorii accesorii, aflate în suprafaţa interioară a bursei, destinate să faciliteze activitatea copulatoare, sunt identificate la 28 de specii din 10 familii şi 5 ordine de Acanthocephala. Ventuzele laterale pereche sunt cunoscute în bursa a 9 specii de Echinorhynchidae şi Pomphorhynchidae. Receptori singulari, inele sau ciorchini de discuri senzoriale, cupe, tuberculi, butoane sau receptori papilari sunt identificaţi în speciile rămase din toate familiile: Arythmacanthidae, Echinorhynchidae, Heteracanthocephalidae, Neoechinorhynchidae, Polymorphidae, Pomphorhynchidae, Rhadinorhynchidae, Transvenidae şi Quadrigyridae, în 4 ordine. Ventuzele, asemănătoare la toate cele 9 specii, sunt adaptate pentru a fixa corpul posterior feminin, pentru a asigura transferul eficient al spermei. Marcajele ventuzelor de pe corpul femelelor indică aderenţa lor strânsă la corpul feminin în timpul copulării. Structurile senzoriale sunt, probabil,

conectate la ganglionii genitali şi bursali pentru recunoaşterea speciilor şi sexului şi pentru livrarea cu succes a spermei, cu pierderi minime. Structurile senzoriale sunt adesea distribuite circumpenian sau circumbursal sau ambele, în modele tipice speciilor identificabile. Multe alte specii de acantocefali studiaţi nu au prezentat structuri senzoriale în bursă. Cele care le-au avut, ca şi cele raportate în acest studiu, pot avea un avantaj de selecţie mai mare în eficacitatea activităţii lor de copulare.

Cuvinte cheie: Structuri copulatorii şi senzoriale; Bursă; Acanthocefali.

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Introduction

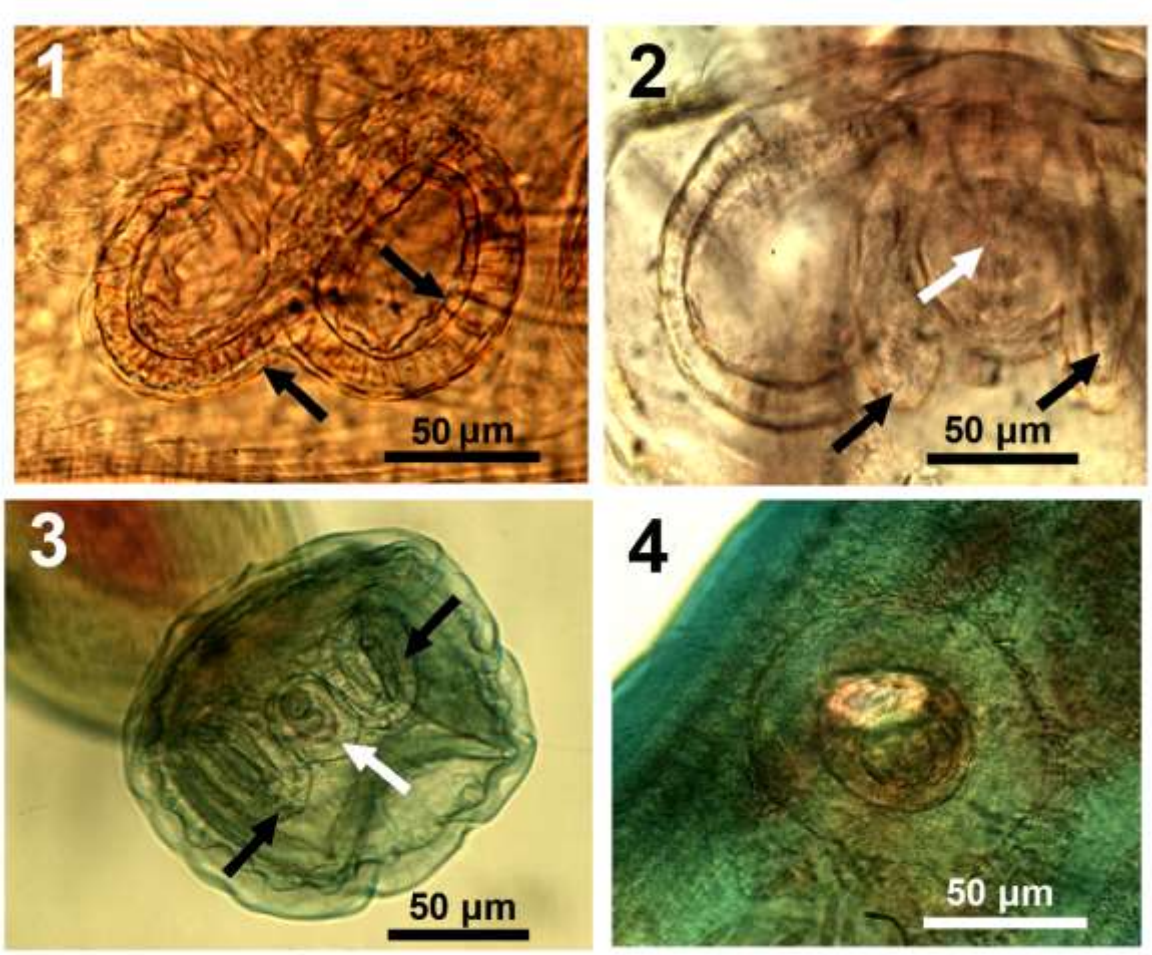
Accessory copulatory structures in the bursa of male acanthocephalans, when present, have not been routinely reported in the descriptions of new species. The taxonomic literature in the pre-SEM times would have missed the sensory receptors because of their minute size. Line drawings in old descriptions would not have documented them if they were not captured microscopically in the first place. Their distribution, morphology, and complexities are apparently species specific and they add considerable diagnostic value to species recognition above and beyond the conventional characters of taxonomic significance. The sensory receptors are part of the extensive nervous system of male acanthocephalans which includes, in addition to the cephalic ganglion, the genital ganglion and the bursal ganglion (Meyer, 1932; Dunagan and Miller, 1977; 1979). The nerves of the bursal and genital ganglia innervate the bursa coordinating its eversion and withdrawal (Dunagan and Miller, 1977; Crompton, 1985) and may also be associated with the function of sensory receptors for recognition and facilitation of copulation. The taxonomic value of accessory structures of the bursa have been documented in the many examples included in this report but this is only a utilitarian use by us, taxonomists, unrelated to their evolutionary history and selective advantages. We are certain that more of these accessory structures will become known once old descriptions are revised or new descriptions are made using SEM or comparable tools. We are also aware that a good number of species do not have accessory copulatory structures as we have studied many using SEM and found none. The present report will provide a new way to look at acanthocephalan systematics using a novel diagnostic tool. It should also stimulate research into the untapped field of the structural-functional relationship of the neurological connection between accessory copulatory structures and the genital and bursal ganglia.

Materials and methods

Microscopy

Specimens of *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919 and *Acanthocephalus dirus* (Van Cleave, 1931) Van Cleave and Townsend, 1936 (figures 1-4) were made from whole mounts selected from 3,540 and 73 specimens, respectively, collected from the northern hogsucker *Hypentelium nigricans* (lesueur) in the West Fork of Drake's Creek, north of Franklin, Simpson County, Kentucky (36°44′N 86°35′W) between February, 1977 and November, 1981 by Gleason (1984) and from 2,300 male *A. dirus* from *Catostomus commersoni* Lacépède in Wisconsin (Amin, 1984). Fish were collected by electrofishing and seining and specimens were dissected from the host, placed in distilled water until distended, fixed in alcohol-formalin-acetic acid (AFA), stained in Semichon's Carmine, and counter stained with Fast Green (Doyle and Gleason, 1991) or distended in cold water, fixed in 70% ethanol, stained in Semichon's carmine, dehydrated without counter stain, cleared in xylene and mounted in Canada balsam (Amin, 1984). Whole mounts were made using standard techniques. Color 35mm Kodachrome slides were created by Lester Doyle and Larry Gleason and given to Omar Amin on June, 6, 1990. Comparable figures in black and white were published one year later

by Doyle and Gleason (1991). Gleason's specimens of *P. bulbocolli* and *A. dirus* showing salient bursal characteristics were deposited in the University of Nebraska State Museum's Harold W. Manter Laboratory collection (HWML Coll. Numbers 31697-31699).



Figures 1-4. The bursal suckers in species of *Acanthocephalus* and *Pomphorhynchus*. **1.** Two suckers in the bursa of a male *A. dirus* with arrows pointing the lining and enveloping connective membranes. **2.** Two suckers on the bursa of another *A. dirus* male showing the penis (white arrow) and the two lateral lobes (black arrows). **3.** Two bursal suckers (black arrows) and the penis (white arrow) of a male *P. bulbocolli.* **4.** A crater in the tegument of a female specimen of *P. bulbocolli* outlined by the dent left by the attachment of a bursa.

SEM

Figures of accessory bursal structures of other specimens featured in this work (figures 5-34) are based on SEM studies of material examined from Vietnam and other geographical locations as annotated in table 1. Specimens that had been fixed and stored in 70% ethanol were processed for SEM following standard methods (Lee, 1992). These included critical point drying (CPD) in sample baskets and mounting on SEM sample mounts (stubs) using conductive double-sided carbon tape. Samples were coated with gold and palladium for 3 minutes using a Polaron #3500 sputter coater (Quorum (Q150 TES) www.quorumtech.com) establishing an approximate thickness of 20 nm. Samples were placed and observed in FEI Helios Dual Beam Nanolab 600 (FEI, Hillsboro, Oregon) Scanning Electron Microscope with digital images obtained in the Nanolab software system (FEI, Hillsboro, Oregon) and then transferred to a USB for future reference. Samples were received under low vacuum conditions using 10 KV, spot size 2, 0.7 Torr using a GSE detector. References (table 1) should be consulted for more collection and processing details of specimens processed with SEM or other specimens referenced.

Table 1. Accessory copulatory structures in bursa of male acanthocephalans

Species*	Host	Geography	Description	Reference	Our figures
Arhythmacanthidae (Echinorhynchida)					
Acanthocephaloides irregularis Amin, Oguz, Heckman, Tepe, Kvach, 2011	*Proterorhinus marmoratus* (Pallas) *Syngnathus abaster* Risso *Parabiennius zvonimiri* (Kolombatovic) *Ponticola eurycephalus* (Kessler)	Black Sea, Ukraine	Circumbursal ring of sensory discs	Amin et al. (2011a)	5, 6
Heterosentis holospinus Amin, Heckmann, Ha, 2011	*Plotosus lineatus* (Thunberg)	Halong Bay, Vietnam	One Circumbursal circle of sensory bulbs	Amin et al. (2011b)	7-9
Echinorhynchidae (Echinorhynchida)					
Acanthoceplalus clavula (Dujardin, 1845) Grabda-Kazubska & Chubb, 1968	*Salmo trutta* L., Coregonus clupeoides, Cavier & Valenciennes, Thymallus *thymallus* (L.); *Esox lucuis* L. *Rutilus rutilus* (L.), *Anguilla anguilla* (L.) *Perca fluviatilis* L.	Llyn Tegid, UK	**Two well marked** muscular suckers**	Grabda-Kazubska & Chubb (1968)	
Acanthocephalus dirus (Van Cleave, 1931) Van Cleave & Townsend, 1936	*Catostomus commersoni* Lacépède	Pike River, Wisconsin	**Two lateral suckers**	This paper; Doyle & Gleason (1991)	1, 2
Acanthocephalus lucidus Van Cleave, 1925	*Salvelinus l. leucomaenis*	N. Honshu, Japan	Ring of circumbursal papillae and 2 suckers	Nagasawa (2014)	
Acanthocephalus lucii (Müller, 1776) Lűhe, 1911	*Perca fluviatilis* L.; *Esox lucius* L.	UK, Germany, Finland	Elevated center and 1 ring of receptors	Amin et al. (2011c)	10
Acanthocephalus ranae (Schrank, 1788) Lűhe, 1911	*Bufo bufo* (L.); *Hyla arborea* (L.); *Rana dalmatina* Bonaparte; *R. macrocanis* Boulenger; *R. ribidunda* Pallas; *R. travasensis* Baran & Atatűr	Denzili, Antalya, Bucak-Burdur, Bursa, Sakarya, Turkey	Rings of sensory discs	Heckmann et al. (2011)	11, 12

Species*	Host	Geography	Description	Reference	Our figures
Echinorhynchus baeri Kostylew, 1928	*Salmo trutta* L.	Murat River, Turkey	Rings of sensory Knobs	Amin et al. (2016)	13
Echinorhynchus bothniensis Zdzitowiecki & Valtonen, 1987	*Osmerus eperlanus* L.	Lake Keitele & Bothnian Bay	**Bursal Suckers**	Wayland (2013)	
Echinorhynchus truttae Schrank, 1788	*Salmo trutta* L.	River Carron, Scotland	**Bursal Suckers**	Wayland (2013)	
Echinorhynchus veli (George & Nadakal, 1978)	*Brachirus orientalis* (Bloch & Schneider)	Veli Lake, Kerala, India	Many sensory papillae	Sheema et al. (2016)	
Metacanthocephaloides zebrini Yamaguti, 1959	*Zebrias zebrinus* (Timminick & Schlegel), *Suggrundus meerdervoorti* (Sleeker), *Pleuronichthys cornutus* (Timminick & Schlegel), *Paralichthys olivaceus* (Timminick & Schlegel)	Inland Sea, Japan	Many small tubercles	Yamaguti (1959)	
Pseudoacanthocephalus bufonis (Shipley, 1903) Petrochenko, 1956	*Duttaphrynus melanostictus* (Schneider) *Rana cancrivora* Gravenhorst) *Takydromus sexlineatus* Daudin	Indonesia	Circumbursal receptors	Kennedy (1982)	
Pseudoacanthocephalus nguyenthileae Amin, Ha, Heckmann, 2008	*Rana guentheri* Boulenger, *R. taipehensis Denburgh, Bufo melanostictus Schneider, Paa verrucospinosa* (Bourret), *Polypedatos mutus* (Smith), *Naja atra* Cantor, *Hemidactylus frenatus* Dumeril & Bibron	Vinh Phue, Vietnam	Many nucleated sensory pits and **2 thick suckers**	Amin et al. (2008)	
Heteracanthocephalidae (Echinorhynchida)					
Aspersentis megarhynchus (Von Linstow, 1892) Golvan, 1960	*Notothenia coriiceps* Richardson	Galindez Island, Antarctica	Ring of beady receptors	Amin et al. (2020)	14, 15

Species*	Host	Geography	Description	Reference	Our figures
Moniliformidae (Moniliformida)					
Moniliformis kalahariensis Meyer, 1931	*Atelerix frontalis* Smith	Limpopo, South Africa	Many complex sensory Discs	Amin et al. (2014)	31-34
Neoechinorhynchidae (Neoechinorhynchida)					
Neoechinorhynchus zabensis Amin, Abdullah, Mhaisen, 2003	*Capoeta damascina* (Velenciennes) *C. trutta* (Heckel)	Zab River, Iraq	Many sensory papillae	Amin et al. (2003)	
Polymorphidae (Polymorphida)					
Corynosoma constrictum Van Cleave, 1918	*Oidemia americana* Sawainson	Yellowstone Lake, Wyoming, USA	**Two mascular pouches lateral to cirrus**	Van Cleave, 1945	
Polymorphus minutus (Goeze, 1782) Lűhe, 1911	–	Europe	Circumpenial and circumbursal circles of receptors	Whitfield (1969) in Brown (1987)	
Pomphorhynchidae (Echinorhynchida)					
Pomphorhynchus bulbocolli linkins in Van Cleave, 1919	*Hypentelium nigricans* (Lesueur)	Kentucky, USA	**Two lateral suckers**	This paper; Doyle and Gleason (1991)	3, 4
Pomphorhynchus laevis Zoega in Müller, 1776	*Barbus barbus* (L.)	River Severn, UK	Two circumpenial and 2 Circumbursal circles of Receptors	Brown (1987)	

Species*	Host	Geography	Description	Reference	Our figures
Rhadinorhynchidae (Echinorhynchida)					
Rhadinorhynchus dorsoventrospinosus Amin, Heckmann, Ha, 2011	*Decapterus kurroides* Bleeker	Halong Bay, Vietnam	Inner clusters and outer circles of cup-like receptors	Amin et al. (2011d)	16
Rhadinorhynchus hiansi Soota & Bhattacharya, 1981	*Sarda orientalis* Temminck & Schlegel	Nha Trang, Vietnam	Outer clusters & inner sensory receptors	Amin et al. (2020)	17-19
Rhadinorhynchus laterospinosus Amin, Heckmann, Ha, 2011	*Balistes* sp.	Halong Bay, Vietnam	Circles of sensory papillae clusters	Amin et al. (2011d)	20-22
Rhadinorhynchus oligospinosus Amin, Heckmann, 2017	*Scomber japonicus* Houttuyn *Trachurus murphyi* Nichols	Peruvian Pacific	Many circumpenial and outer circles of sensory papillae	Amin & Heckmann (2017)	23-26
Transvenidae (Echinorhynchida)					
Paratrajectura longcementglandatus Amin, Heckmann, Ali, 2018	*Nemipterus japonicus* Bloch *Otolithes ruber* Block & Schneider	Arabian Gulf	Two rings of sensory papillae	Amin et al. (2018)	27, 28
Quadrigyridae (Gyracanthocephala)					
Acanthogyrus (Acanthosentis) fusiformis Amin, Chaudhary, Heckmann, Ha, Singh, 2019	*Arius* sp.	Gulf of Thailand	Rings of circumpenial & circumbursal sensory discs	Amin et al. (2019)	29-30

* Species are recorded in alphabetically listed families (orders).
** Suckers are bolded.

Results

An examination of descriptions of the bursa of many species of acanthocephalans has revealed the presence of copulatory accessory structures in at least 28 species in 10 families and 5 orders. Host and acanthocephalan species, geographical distribution, brief description of accessory structures, references to literature and figure numbers are summarized in table 1. Nine species had well defined paired suckers on the inner surface of the bursa including 2 species also with sensory papillae or other sensory structures. Suckers were only found in species in the order Echinorhynchida; 6 in the family Echinorhynchidae and 2 in the family Pomphorhynchidae and 1 in the family Polymorphidae. The remaining species had sensory structures in simple random patterns or more often in various specific designs and distribution in circumpeneal, circumbursal, or other species-specific arrangements. The family Echinorhynchidae included the largest number of species (12) with accessory copulatory structures. We suspect that many more species may have accessory sensory structures that have been overlooked or dismissed and thus remain unreported or are yet to be discovered.

Discussion

The suckers

The suckers on the internal surface of acanthocephalan bursa make up accessory copulatory structures facilitating the clasping ability of males to females during copulatory activity for effective transfer of sperms and release of cement to form the cement plug. They can be seen at the level of the tip of the everted penis but are also readily observed in retracted bursae. The form and position of suckers can occasionally be seen on the cement plugs that apparently have hardened before the withdrawal of the bursa (Doyle and Gleason, 1991). We have observed and documented single or multiple cement plugs attached to the posterior end of females; see for example figure 9 in Amin et al. (2011b) of the posterior end of a female specimen of *Heterosentis holospinus* with double cement plugs showing the outline of bursal rim. Cement plugs have also been seen attached to trunk sides of females and males of various species with evidence of scarring indicating indiscriminate copulation. Abele and Gilchrist (1977) coined the famous phrase "homosexual rape" to describe such occurrences. "Bursal rays support the bursa but do not limit the ability of the terminal portions to flare up" (Doyle and Gleason, 1991); even though they may not be consistently evident in individuals of the same species, or inter-specifically. Overall, the presence and form of suckers on the bursa of males is species-specific and has undeniable taxonomic importance. Suckers may be seen using light microscopy but they can be more readily observed using SEM which was not available in most of the earlier descriptions. Re-examination of old material or revisions of old descriptions may reveal the true extent of their presence. Naturally, some species like some that we have studied, simply don't have them.

We report 9 species of acanthocephalans with bursae equipped with two well-developed suckers each, including 2 species also with sensory receptors, *Acanthocephalus lucii* and *Pseudoacanthocephalus nguyenthileae* (table 1).

1. The suckers in *Acanthocephalus clavula* were described as "two well-marked muscular pockets" visible in the retracted bursa of the line drawing of a male specimen (figure 1a of Grabda-Kazubska and Chubb, 1968).
2. Doyle and Gleason (1991) provided black/white microscope images of 2 suckers on the inner surface of the bursa of *Acanthocephalus dirus* from *Hypentelium nigricans* in Kentucky (their figure 7) that we have previously observed in the same acanthocephalan species from *Catostomus commersoni* (figures 1, 2), among other hosts, from Wisconsin but never reported it. We have re-examined a representative sample of males in our extensive collection of over 4,500 specimens of *A. dirus* including about 2,300 males (Amin, 1984). We reconfirm our earlier observations of paired bursal suckers lined with a layer of connective tissue and adjoining the penis laterally in

all individuals. The conical penis in our specimens was located between the suckers, included a stylet (figure 2), not noted by Doyle and Gleason (1991) in their specimens, and adjoined ventrally by 2 lateral lobes. These 2 lateral lobes, when inflated, would appear similar to the "vesicles" shown by Brown (1987, figure 3 of fully everted bursa) adjoining the penis of *Pomphorhynchus laevis.* The function of these lobes is unknown at this time and remains to be investigated.

3. In 2 populations of males of *Echinorhynchus bothniensis* from Lake Keitele and Lake Pulmankijarvi in Finland, suckers were reported and their diameter measured (137-219, 135-191) but no line drawings, images, or verbal description were given by Wayland (2013).
4. In *Echinorhynchus truttae* from River Carron, central Scotland, suckers were reported and their diameter measured (123-197) but no line drawings, images, or verbal description were given by Wayland (2013).
5. The two suckers of *Acanthocephalus lucidus* were clearly marked in the retracted bursa of the type male (figure 1 of Van Cleave, 1925) but no reference to them was made in the brief original description. Nagasawa (2014), however, described a "Bursa with papillae, 0.32 X 0.48" and his line drawing of an entire *A. lucidus* male (figure 1A) showed 1 circle of papillae at the distal end of the bursa, bursal rays, and 2 well defined suckers near the proximal end of the bursa that were not referenced in the text.
6. *Pseudoacanthocephlus nguyenthileae* is the only other acanthocephalan that we report with both 2 suckers and sensory structures on the same bursa. The line drawing in Amin et al. (2008, figure 9) showed a bursa with randomly distributed sensory structures described as "uni-nucleated sensory pits" and two well defined suckers adjoining the penis on both sides.
7. Van Cleave (1945) described 2 lateral suckers (termed pouches) lateral to the cirrus of *Corynosoma constrictum* (his line drawings figures 2, 3) from Wyoming.
8. Suckers were also reported in the bursa of *Pomphorhynchus bulbocolli*. Doyle and Gleason (1991) provided the first black/white microscope images of the 2 suckers of the bursa of *P. bulbocolli* at the same level as the tip of the everted penis. Our colored images of the same suckers (figure 3) appeared lined with a layer of connective tissues; they were stained with Semichon's acetic carmine and counterstained with fast green.
9. In *Pomphorhynchus sphaericus*, the line drawing by Gil De Pertierra et al. (1996, figure 2A) shows 2 prominent suckers on the inner surface of the bursa on both sides of the penis.

The sensory structures

Male acanthocephalans possess a much more extensive nervous system than females. Aside from the cephalic ganglion in the receptacle of both sexes, males possess the genital ganglion and the bursal ganglion (Meyer, 1932; Dunagan and Miller, 1977; 1979). The sensory and motor nerves of the bursal and genital ganglia innervate the bursa, coordinating its eversion and withdrawal in coordination with bursal muscles, penis, and Saefftigen's pouch (Dunagan and Miller, 1977; Crompton, 1985). This organization must also be associated with the function of sensory receptors for species and sex recognition and facilitation of copulation; an aspect that has not been investigated. The taxonomic value of accessory structures of the bursa have been documented in the many examples included in this report but this is only a utilitarian use by us, taxonomists, unrelated to their evolutionary history and selective advantages. We present but a sample and there is no doubt that more of these accessory structures will be revealed once old descriptions are revised or new descriptions are made using SEM or comparable tools.

1. The sensory discs in the circumbursal ring of *Acanthocephaloides irregularis* are each elevated with central dome (figures 5, 6).

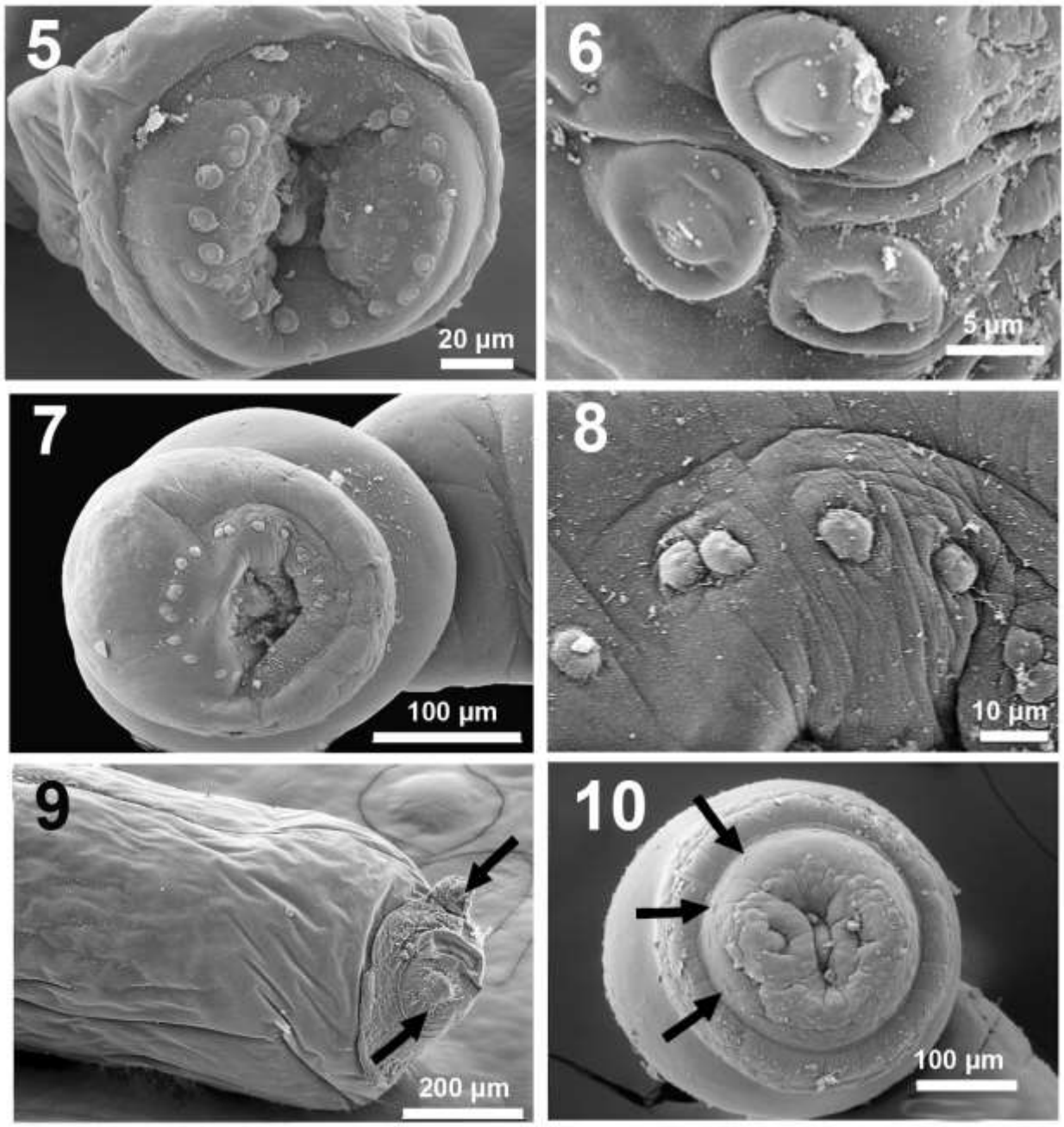
2. The circumbursal ring in the bursa of *Heterosentis holospinus* has mostly single or occasionally double semi-spherical sensory bulbs (figures 7-8); cement plugs (figure 9).
3. In *Acanthocephalus lucidus*, there is one ring of spherical circumbursal sensory papillae and two suckers (Nagasawa, 2014, figure 1). Nagasawa (2014) only mentioned "Bursa with papillae" but made no reference to the suckers. In the original description of *A. lucidus*, Van Cleave (1925) showed a retracted bursa (his figure 1 of the type male) which had what appears to be 2 identical suckers.
4. The muscular bursa of *Acanthocephalus lucii* has one ring of small elevated ovoid sensory receptors and a large convoluted raised center (figure 10).
5. The closely related species, *Acanthocephalus ranae* had at least 3 irregular rings of elevated dome-like sensory papillae (figures 11, 12).
6. In *Echinorhynchus baeri*, there appears to be only one ring of small, slightly elevated ovoid sensory receptors (figure 13).
7. The bursa of *Echinorhynchus veli* is studded with many irregularly distributed circumbursal and circumpenial circles of sensory knobs not clearly separated. The authors (Sheema et al., 2017; figures 8, 9) described the bursa as having "many papillae. The papillae may be sensory in function as suggested by Heckmann et al. (2011) in *A. ranae*" but Sheema et al. (2017) mistakenly mentioned that "*A. lucii*… devoid of papillae (Amin et al. 2011a or 2011b)."
8. The bursal cap of *Metacanthocephaloides zebrini* was described as "with a number of short lobular projections… with the non-muscular portion… provided inside with numerous small tubercles" (Yamaguti, 1959). The retracted bursa in his figure no. 8 was not informative (Yamaguti, 1959).
9. Kennedy's (1982) figure 2 of the bursa of *Pseudoacanthocephalus bufonis* depicted many small spheroid sensory structures randomly organized in 1 zone at the posterior margin of the bursa. Kennedy's (1982) description of sensory structures in *P. bufonis* by a line drawing (no text reference) is the only one of the many descriptions made by other observers of this species that made no reference to these structures.
10. The bursa of *Pseudoacanthocephlus nguyenthileae* showed many randomly distributed nucleated sensory pits and 2 suckers (line drawing figure 9 in Amin et al., 2008).
11. In *Aspersentis megarhunchus*, 1 beady ring of small ovoid circumpenial receptors was observed (figures 14, 15); work is still in progress.
12. *In Moniliformis kalahariensis*, a number of large and compound sensory discs are prominent on the bursa (figures 31-33, arrow) with comparable but somewhat smaller discs in the vicinity (figure 34) that may also be involved in the copulatory sensory function.
13. The line drawings of *Neoechinorhynchus zabensis* (figure 7 in Amin et al., 2003) show many randomly distributed sensory papillae throughout the everted bursa.
14. Brown (1987) stated that Whitfield (1969) "speculated that the circumbursal and circumpenial receptors of *Polymorphus minutus* were touch or chemosensory receptors." Whitfield's (1969) Ph.D. thesis could not be made available for further details. "It seems likely that the primary function of these receptors…. is to recognize and correctly align mates prior to and during copulation, a species-specific function" (Brown, 1987).
15. The bursa of 2 species of *Pomphorhynchus*, *P. bulbocolli* from North America (Doyle and Gleason, 1991) and *P. sphaericus* from Argentina (Gil De Pertierra et al., 1996) had only suckers, 2 each (see Suckers above). The bursa of the European species *Pomphorhynchus laevis* had 2 concentric circles of circumbursal and 2 similar circles of circumpenial round-raised sensory receptors but no suckers (Brown, 1987).
16. Each of 4 species of *Rhadinorhynchus* had its own distinct pattern of circumbursal

and/or circumpenial circles of sensory structures. The taxonomic value of these species-specific differences, like those in *Pomphorhynchus* (above) cannot go unnoticed. In *R. dorsoventrospinosus*, the inner lip of the bursa had an inner circle of elevated saucer-like sensory cups in clusters and outer rings of similar but not clustered sensory cups (figure 16).

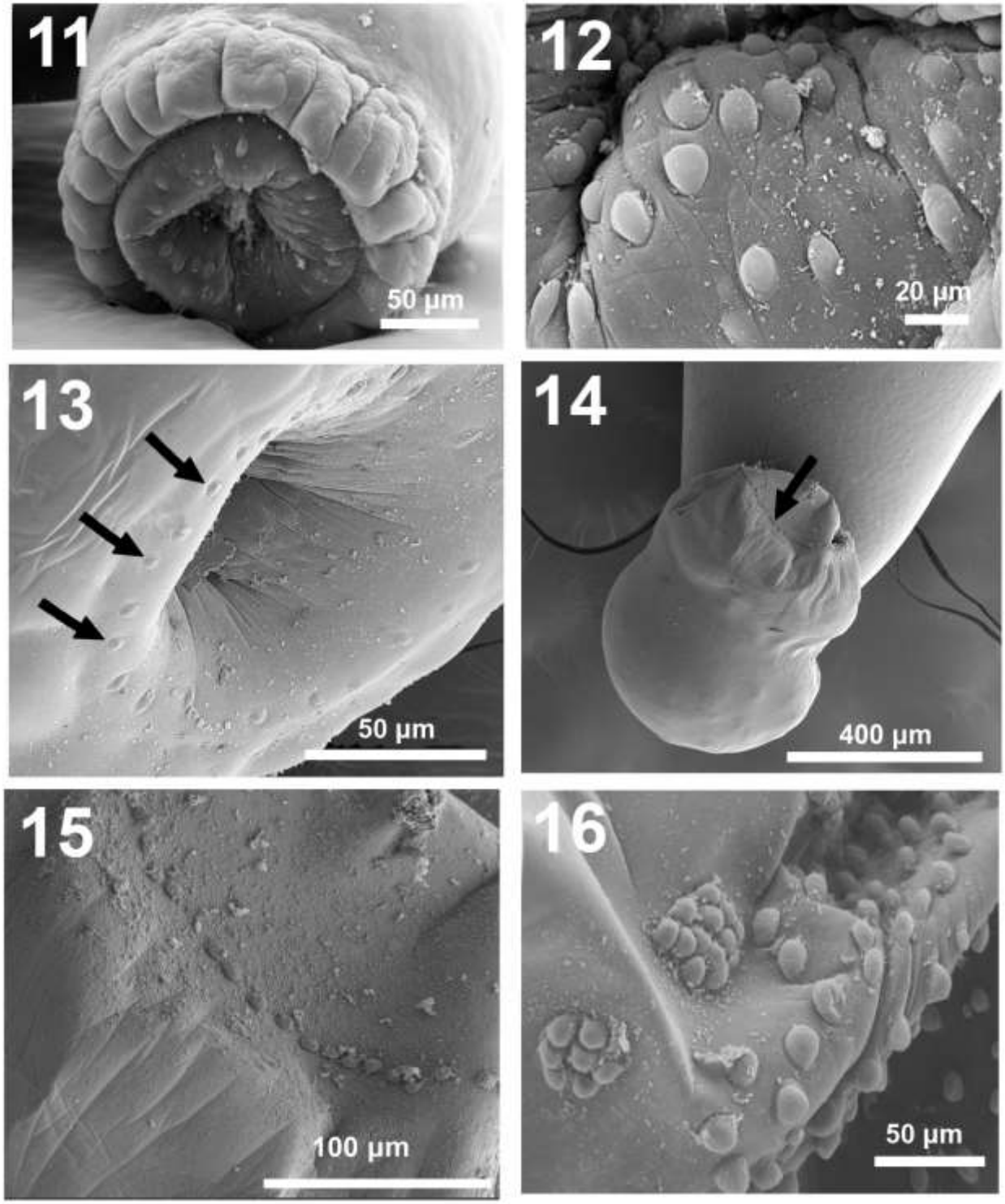
17. The thick bursa of *Rhadinorhynchus hiansi* had many randomly distributed circumpenial flattened sensory receptors encircles by complex clusters of large dome-shaped circumbursal receptors (figures 17-19); the opposite trend observed in *R. dorsoventrospinosus*.
18. In *Rhadinorhynchus laterospinosus*, the thick lipped bursa had many irregular circles of compound circumpenial sensory structures encircled by one ring of similar circumbursal structures in fusiform depressions (figures 20-22).
19. In *Rhadinorhynchus oligospinosus*, there are many random raised round circumpenial papillae contained within a circle encircled by 2 or 3 rings of similar but ovoid and smaller circumbursal papillae (figures 23-26).
20. The thick bursa of *Paratrajectura longcementglandatus* has lobulated lips with raised internal rim having at least 2 rings of circumbursal sensory papillae (figures 27-28).
21. The thick bursa of *Acanthogyrus* (*Acanthosentis*) *fusiformis* also has lobulated lips with raised internal rim having many rings of circumpenial papillae (figures 29, 30). The papilla is saucer-like with raised dome-like center and with its rim almost pinched off the bursal inner surface (figure 30).

Conclusions

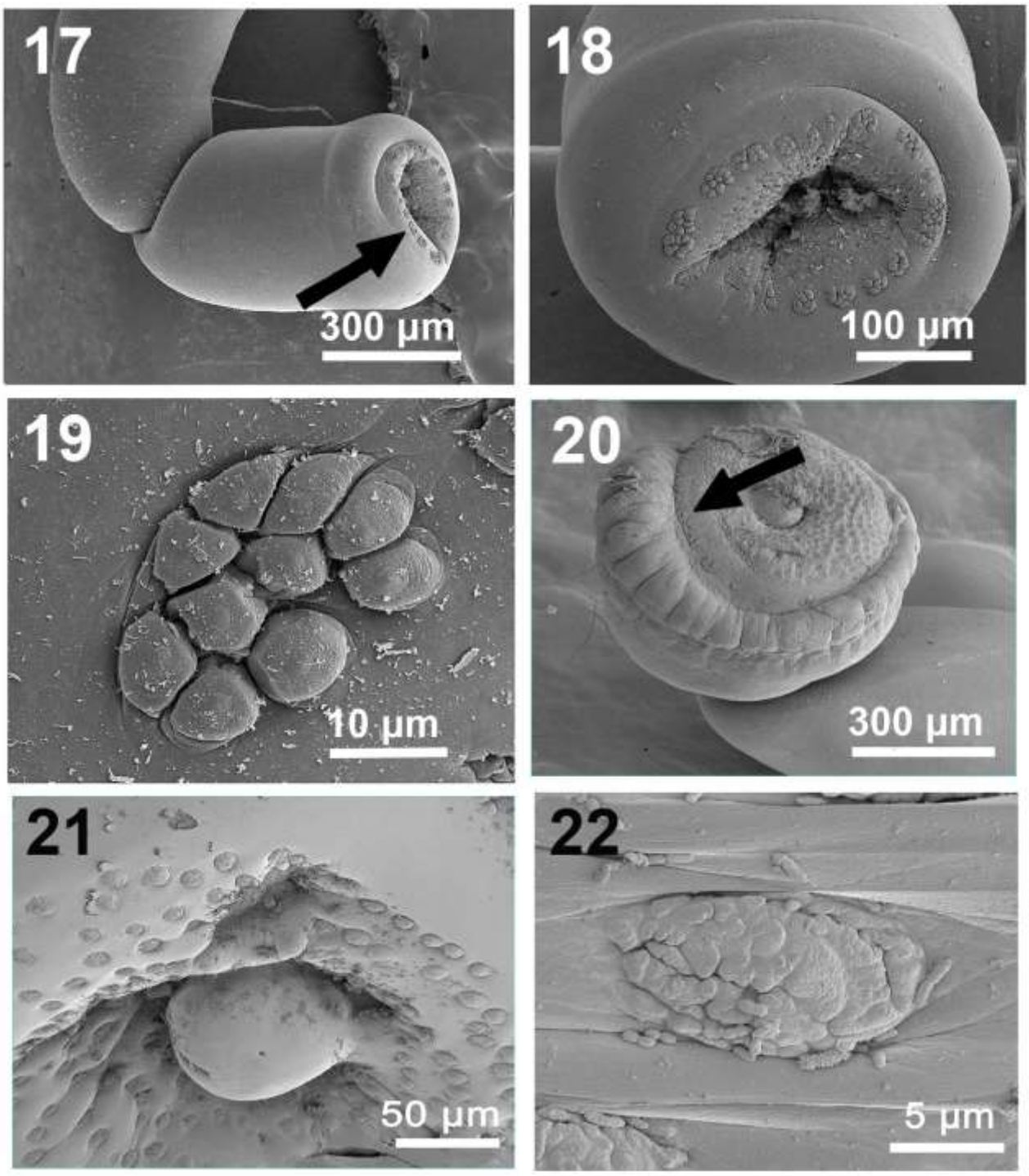
We have established the presence of accessory copulatory structures in a large number of species of acanthocephalans across the taxonomic spectrum. While the anatomy of the suckers appears comparable in all investigated species, that of the sensory structures is not. The structure and distribution of sensory structures vary considerably among the taxa investigated and appear to be species-specific and thus of dramatic taxonomic significance. The coordination of the sensory and motor neurons of the genital and bursal ganglia innervating the bursa, penis, and Saefftigen's pouch synchronizing the eversion and withdrawal of the bursa must take place after effective species and sex recognition have been initiated by the sensory receptors. Brown (1987) summarized the central thesis of our presentation that "the primary function of these receptors…. is to recognize and correctly align mates prior to and during copulation, a species-specific function." The relationship between the structure and organization of the sensory receptors and what we know of the neurological anatomical makeup of acanthocephalan males to date has not been adequately addressed. Dunagan and Miller (1977; 1979), among others, addressed many aspects of the nervous system of the Acanthocephala but the anatomy and functionality of the neurological connections between receptors and ganglia elsewhere have not been investigated. Our research only opens the door to delve into this domain of knowledge that is yet to be explored. The structural-functional relationship between these accessory structures and their utility in the copulatory function which is a primary component of the biology of the organism suggest that they must be the product of parallel evolution. A phylogenetic analysis can pioneer an additional dimension of exploration of this phenomenon which is beyond the scope of this presentation. More of these accessory structures will undoubtedly become known once old descriptions are revised or new descriptions are made using SEM or comparable tools. We are also aware that a good number of species, including ones that we have already studied using SEM, do not have accessory copulatory structures; we found none in quite a few. This leaves open the question: how do species without accessory structures succeed in their copulatory activities in their absence? The field is quite open to investigate this fascinating topic which amazingly remains untapped to date.



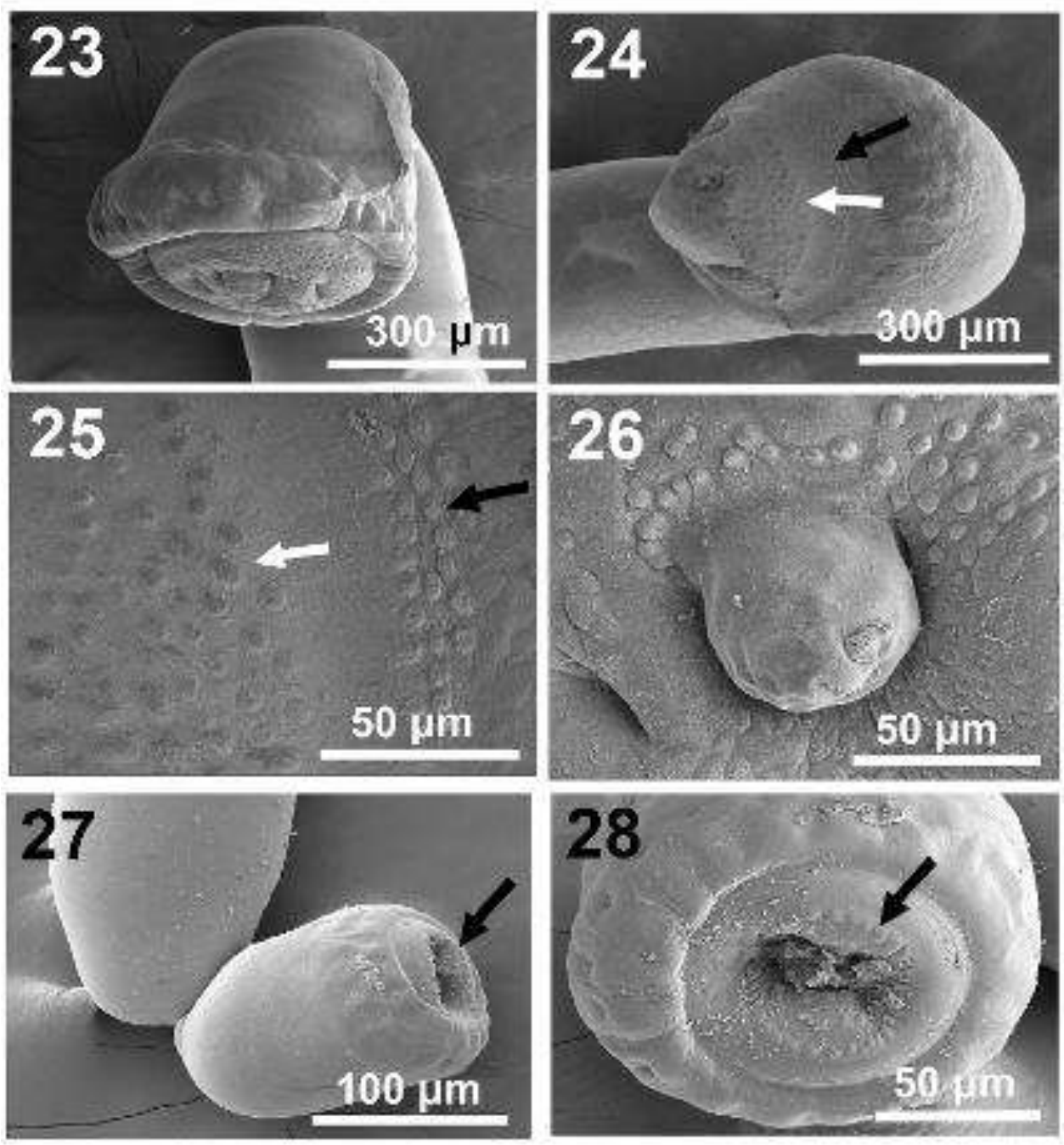
Figures 5-10. Circumbursal sensory receptors in *Acanthocephaloides irregularis*, *Heterosentis holospinus*, and *Acanthocephalus lucii*. **5, 6.** One ring and a few magnified sensory discs in a male *A. irregularis* bursa. **7, 8.** One ring and a few magnified sensory bulbs in a male *H. holospinus*. **9.** Double cement plugs (arrows) on the posterior end of a female *H. holospinus* indicating multiple copulation. **10.** One ring of small sensory receptors (arrows) around elevated center of *A. lucii* bursa.



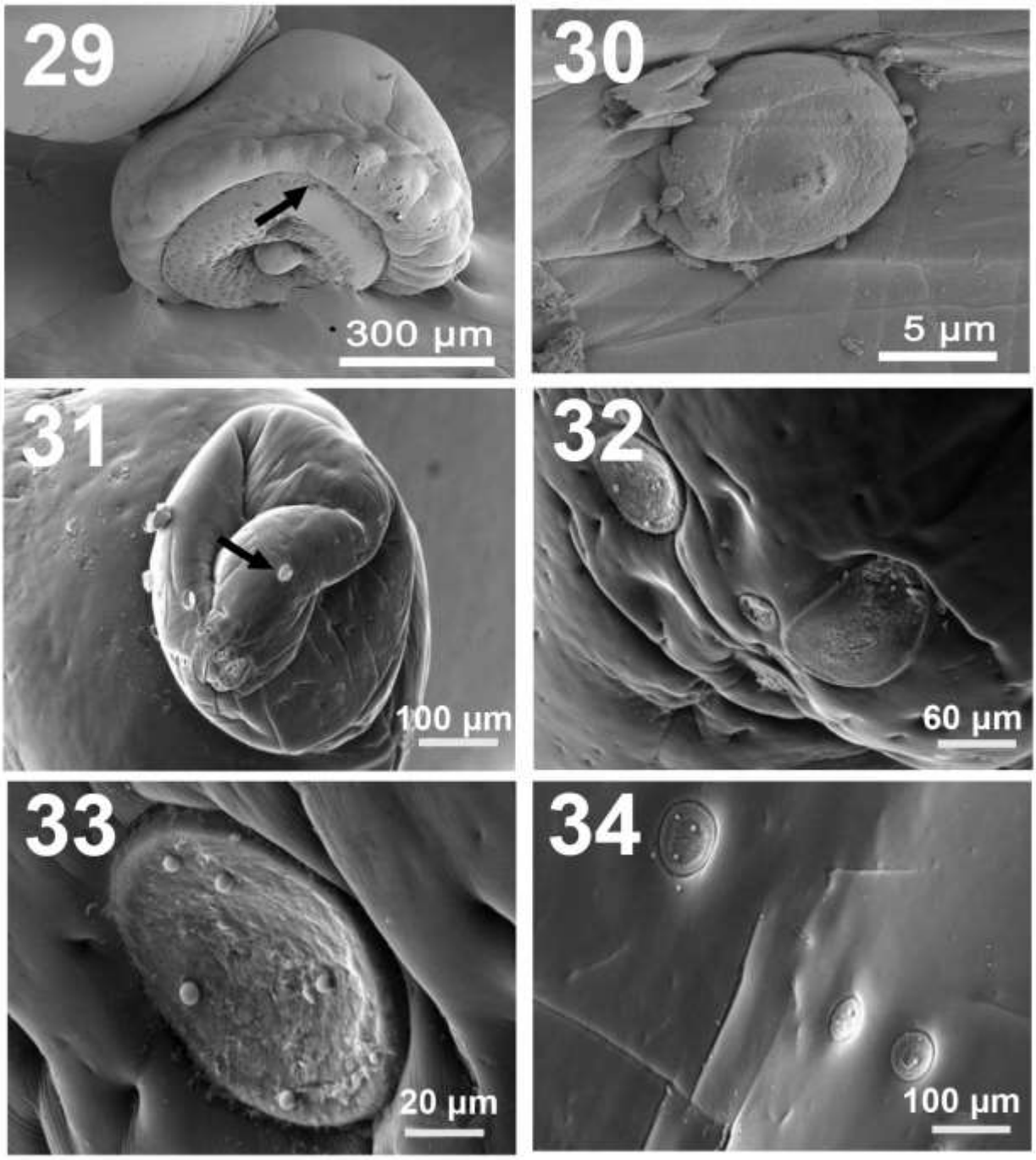
Figures 11-16. Rings of sensory receptors on the bursae of *Acanthocephalus ranae*, *Echinorhynchus baeri*, *Aspersentis megarhynchus*, and *Rhadinorhynchus dorsoventrospinosus.* **11, 12.** Rings of sensory discs, mostly circumbursal, and magnified discs on the muscular bursa of *A. ranae*. **13.** One ring of slightly elevated circumbursal sensory structures (arrows) in *E. baeri*. **14, 15.** The position (arrow) and a higher magnification of one ring of beady sensory structures in the bursa of a male *A. megarynchus.* **16.** Inner clusters and outer rings of sensory cups on the bursa of a male *R. dorsoventrospinosus.*



Figures 17-22. Sensory receptors on the bursae of *Rhadinorhynchus hiansi*, and *Rhadinorhynchus laterospinosus*. **17-19**. In *R. hiansi*, the outer clusters of receptors and inner single receptors are shown in the lateral (arrow) and face views of a bursa (Figs. 17, 18). The organization of sensory units of one cluster is shown in Fig. 19. **20-22.** In *R. laterospinosus*, one circle of circumbursal papillae (Fig. 20, arrow) and many circumpenial papillae surrounding the penis (Figs. 20, 21) are shown. A high magnification of one compound papillae complex (Fig. 22) is deeply embedded in an epidermal depression.



Figures 23-28. Receptors in *Rhadinorhynchus oligospinosus* and *Paratrajectura longcementglandatus*. **23-26**. The distribution of single circumbursal (black arrows) and circumpenial receptors (white arrows) on the bursa of *R. oligospinosus* are shown (Figs. 24, 25). A different perspective (Fig. 23) and a high magnification of circumpenial receptors encircling the penis (Fig. 26) are also shown. **27, 28.** A lateral and a face view of the bursa of *P. longcementglandatus* showing two rings of sensory papillae (arrows).



Figures 29-34. Sensory discs on the bursae of *Acanthogyrus* (*Acanthosentis*) *fusiformis* and *Moniliformis kalahariensis*. **29, 30.** In *A.* (*A.*) *fusiformis*, rings of circumbursal (arrow) and circumpenial discs (Fig. 29) are apparent. A high magnification of one sensory disc (Fig. 30) shows its characteristic raised center and elevation from the epidermis. **31-34.** In *Moniliformis kalahariensis*, large complex sensory discs are sporadically distributed throughout the bursa (Fig. 31) showing detail under different magnifications (Figs. 32, 33). Similar discs in the vicinity (Fig. 34) are also observed.

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Postscript

Accounts of four other acanthocephalan species with accessory copulatory structures (ACS) on the bursa have become available since the submission of the original manuscript. There is no doubt ACS are present in other species of acanthocephalans, including previously described ones, that we will not become aware of.

- Amin O.M., Heckmann R.A., Halajian A, El-Naggar A.M., Tavakol S. 2013 (Parasitol. Res. 112: 3873-3882) described large suction cup-like sensory structures (figure 3) on the bursa of male *Leptorhynchoides polycristatus* Amin, Heckmann, Halajian, Elnaggar, Tavakol, 2013 (Rhadinorhynchidae) from *Acipenser stellatus* Pallas in the Caspian Sea.
- Amin O.M. 2019 (Acta Parasitol. 65: 77-89) described circles of concentric rings of sensory papillae on the bursa of *Rhadinorhynchus trachuri* Harada, 1935 (Rhadinorhynchidae) from *Onchorhynchus kisutch* in the Pacific off California (figure 14).
- Amin O.M., Heckmann R.A., Dallarés S., Constenla M., Rubini S. 2020 (J. Helminthol. in press) described the bursa of *Centrorhynchus globocaudatus* (Zeder, 1800) Lühe, 1911 (Centrorhynchidae) from *Falco tinnunculus* and *Buteo buteo* in Italy as being "large with oblong sensory pits in round elevated rims… at its posterior end" (figures 20-23).
- Huston D.C., Smales L.R. 2020 (Syst. Parasitol. July; doi.org/10.1007/s11230-020-09923-7) described the bursa of *Spinulacorpus biforme* (Smales, 2014) (=*Rhadinorhynchus biforme* Smales, 2014) (Spinulacorpidae) from a trumpeter in Australia as having "a circle of outer papillae and a cluster of inner papillae" (figure 2C).