

Functional and phylogenetic components in cercarial nervous systems

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Key words: cercarial sensory receptors, chaetotaxy, cholinergic network, ultrastructure, site-specificity, taxon-specificity, functional morphology

Abstract. Studies involving comparisons of taxa that vary in their degree of relatedness may allow the distinction of functional and phylogenetic components in cercarial sensory systems. In this study, cercariae of allocreadiids *Bunodera* Railliet, 1896 and *Crepidostomum* Braun, 1900, lecithodendriid *Allassogonoporus* Olivier, 1938 and opecoelid *Allopodocotyle* Pritchard, 1966 were compared as regards ultrastructure and chaetotaxy of sensory receptors as well as neuromorphology. Cercariae were treated with acetylthiocholine iodide and silver nitrate and some were processed for scanning and transmission electron microscopy. The types of cercarial sensory receptors differed in the presence of a tegumentary sheath, a dome-like base and a tegumentary collar, number of cilia (0, 1, 2 or more), cilium length (short, moderately long or long) and tegumentary collar length (low to moderately low, high or very high). Chaetotaxic patterns were consistent at the family level in all taxa studied. Irregular cholinergic nerve networks were identified. The present study indicates that the major categories of cercarial sensory receptors are nonciliated (including sheathed and subtegumentary types) and ciliated (including unciliated and ciliated types) receptors. It also allows the distinction of functional and phylogenetic components in the sensory systems of the cercariae studied. Functional components were reflected in the numbers of sensory receptors associated with each nerve region and in the ultrastructure and site-specificity of receptor types. Phylogenetic components included taxon-specific chaetotaxic patterns and receptor types.

In the past, the investigation of cercarial sensory systems had emphasised the morphology and distribution of receptor types. Ultrastructural studies have revealed the existence of several types of receptors across the larval tegument of Digenea (Halton et al. 1997). These types suggest that different forms are associated with distinct functions. Site-specificity of receptor types also supports this idea in several species (Bogéa and Caira 2001a, b, c). Functional components are more strongly implicated when similar site-specific patterns are found among distant related taxa, particularly in those that share life-cycle strategies.

Sensory receptor types found to be restricted to groups of related species, on the other hand, would suggest that there might be at least some phylogenetic components to these features. Besides, it is clear that the distribution of receptors on the cercarial tegument is phylogenetically based, with chaetotaxic patterns recognised at the family, genus and species levels (Richard 1971, Bayssade-Dufour 1979, Bayssade-Dufour et al. 1993). What remains unclear, however, is whether receptor types and/or distribution patterns of receptor types may be taxon-specific.

Studies involving comparisons among taxa that vary in their degree of relatedness, therefore, emerge as a strategy that may allow distinction between functional and phylogenetic components in cercarial sensory systems. In the present work, the cercarial neuromorphology and the distribution of sensory receptors were compared among species of the order Plagiorchiiformes.

Species representing four genera were selected based on a phylogenetic perspective. This allowed comparisons involving: (1) close relatives with very similar transmission modes, (2) distant relatives with similar transmission modes and (3) distant relatives with different transmission modes. Thus, species belonging to confamilial genera *Bunodera* Railliet, 1896 and *Crepidostomum* Braun, 1900 (Allocreadiidae) allowed the first set of comparisons. Both taxa are probably sister-genera (Caira 1989) and have free-swimming ophthalmoxiphidiocercariae, with total length varying from 200 to 300 µm. The second set of comparisons included the two allocreadiids and a species of *Allassogonoporus* Olivier, 1938 (Lecithodendriidae), which has a free-swimming virgulate xiphidiocercaria of approximately 100 µm length. The third set of comparisons involved the above mentioned taxa and a species of *Allopodocotyle* Pritchard, 1966 (Opecoelidae), which has a non-swimming cotylomicrocercous cercaria of approximately 350 µm length.

MATERIALS AND METHODS

Cercariae of *Bunodera* sp. and *Crepidostomum* sp. were obtained from naturally infected fingernail clams *Musculium partumeium* (Say), *Pisidium casertanum* (Poli) and *Pisidium aequilaterale* (Lea) (Bivalvia: Sphaeriidae). Cercariae of *Allassogonoporus* sp. and *Allopodocotyle* sp. were obtained from naturally infected freshwater snails *Fluminicola virens* (Lea) (Gastropoda: Hydrobiidae) and *Juga plicifera* (Lea)

(Gastropoda: Pleuroceridae), respectively. Bottom sediment was collected with the aid of dip nets and sieved through ¼" mesh aluminium screening. The molluscs were transported to the laboratory in vials containing pond water and screened for cercariae according to standard procedures.

The most actively emerging cercariae were collected and processed as described elsewhere (Bogéa and Caira 2001a). Voucher specimens have been deposited in the Larry R. Penner Parasitology Collection at the University of Connecticut, USA, U.S. National Parasite Collection, USA and in the Instituto Oswaldo Cruz Helminthological Collection at Fundação Oswaldo Cruz, Brazil.

Ultrastructure of the types of sensory receptors, chaetotaxy, site-specific patterns and nerve network arrangements were previously described in detail for the cercariae of all taxa studied (Bogéa and Caira 2001a, b, c). The external ultrastructure of each receptor type found was described based on number of cilia, presence of a tegumentary sheath, a tegumentary dome-like base and a tegumentary collar and on the morphometric parameters cilium length, cilium width, tegumentary collar length and tegumentary collar width. These parameters were statistically compared among similar receptor types found in each species with the Mann-Whitney *U* test for two independent samples at a level of significance of less than 0.005. Total number of sensory receptors and numbers of ventral receptors and of receptors located in each nerve region were compared among all species. All comparisons were performed with the SPSS (Base 10.0 for Macintosh) statistical package.

RESULTS

Ultrastructure

A great heterogeneity of types of sensory receptors was identified in the cercariae of *Bunodera* sp., *Crepidostomum* sp., *Allassogonoporus* sp. and *Allopodocotyle* sp. (Fig. 1). The types were distinguished based on gross morphology seen with scanning and transmission electron microscopes and on statistical analyses. The types of receptors differed in number of cilia (e.g., Figs. 1A, 1J–L), presence of a tegumentary collar (e.g., Fig. 1L), a dome-like base (Figs. 1G–H) and a tegumentary sheath (Figs. 1I, 1N).

Collared receptors were abundant. These receptors have a sensory bulb attached to the tegument by septate extracellular junctional complexes, thickened nerve collars, unsheathed cilia with a central pair of microtubules and a basal body (Figs. 1A–B, 1D).

Similar receptor types were distinguished mostly based on cilium and tegumentary collar lengths (Table 1). The types had either low ($0.23 \pm 0.07 \mu\text{m}$) (e.g., Figs. 1D, 1J–K), moderately low ($0.29 \pm 0.11 \mu\text{m}$) (e.g., Fig. 1C) or high ($0.62 \pm 0.36 \mu\text{m}$) (e.g., Figs. 1A, 1L) tegumentary collars with short ($1.02 \pm 0.46 \mu\text{m}$) (e.g., Figs. 1A–B), moderately long ($3.12 \pm 1.53 \mu\text{m}$) (e.g., Fig. 1C) or long ($4.73 \pm 1.90 \mu\text{m}$) (e.g., Figs. 1D, 1J–L) cilia.

The ultrastructural investigation of the cercarial sensory receptors in the present taxa revealed that some types were restricted to certain species. Thus, types DI

(Fig. 1G) and DII (Fig. 1H) were restricted to *Bunodera* sp. whereas types NI (Fig. 1E) and NII (Fig. 1F) occurred in *Crepidostomum* sp. Types MI (Fig. 1K), MII (Fig. 1L), SI (Fig. 1I), SII (Fig. 1N) and St (Fig. 1M) were found in *Allopodocotyle* sp.

Basic chaetotaxic and site-specific patterns

The basic chaetotaxic maps of the four species were investigated in detail. Approximately 170, 163, 178 and 172 cercarial sensory receptors were found on the tegument of *Bunodera* sp., *Crepidostomum* sp., *Allassogonoporus* sp. and *Allopodocotyle* sp., respectively. *Allassogonoporus* sp., thus, exhibited more sensory receptors than the other species (Table 2). The numbers of ventral receptors were similar (65.9% in *Bunodera* sp., 63.8% in *Crepidostomum* sp., 60.7% in *Allassogonoporus* sp. and 64.0% in *Allopodocotyle* sp.).

Groups of sensory receptors were associated with the nerve regions. Most receptors occurred on the cephalic region (47.65% in *Bunodera* sp., 46.63% in *Crepidostomum* sp., 49.44% in *Allassogonoporus* sp. and 45.35% in *Allopodocotyle* sp.). The numbers of posterior receptors were similar among all taxa (Table 2). *Bunodera* sp. and *Crepidostomum* sp. presented similar numbers of receptors in each nerve region. *Allassogonoporus* sp. exhibited more cephalic and anterior receptors and less ventral sucker receptors whereas *Allopodocotyle* sp. showed more caudal receptors than the other taxa (Table 2).

Fig. 2 illustrates the distribution of the types of sensory receptors throughout the nerve regions in all taxa studied. Type UI was mainly observed around the oral opening whereas type UII was commonly found on the ventral surface of the cercarial body. Type UIII was observed on the body as well as on the cephalic region. Type UIV tended to occur on the dorsal surface of the body.

Some types of sensory receptors were restricted to specific sites within a species (Fig. 2). In *Bunodera* sp., types DI and DII were concentrated on the dorsal surface of the body and on the tail, and on the lateral surface of the body, respectively (Fig. 2A). Types NI and NII were found only on the tail of *Crepidostomum* sp. whereas type Bi was restricted to its oral opening (Fig. 2B). *Allopodocotyle* sp. exhibited three pairs of receptor type SI, each on the cephalic, anterior, and posterior regions. In addition, types MI and MII were found on the ventral surface of the cephalic region (Fig. 2D).

Similar site-specific patterns of cercarial sensory receptors were observed among the species studied (Figs. 2A–D). For instance, high-collared receptors with a short cilium were mainly observed around the oral opening. Low-collared sensory receptors with a moderately long cilium were observed on the cercarial body as well as on the cephalic region in *Bunodera* sp., *Crepidostomum* sp. and *Allassogonoporus* sp. Low-collared receptors with a short cilium were commonly found on

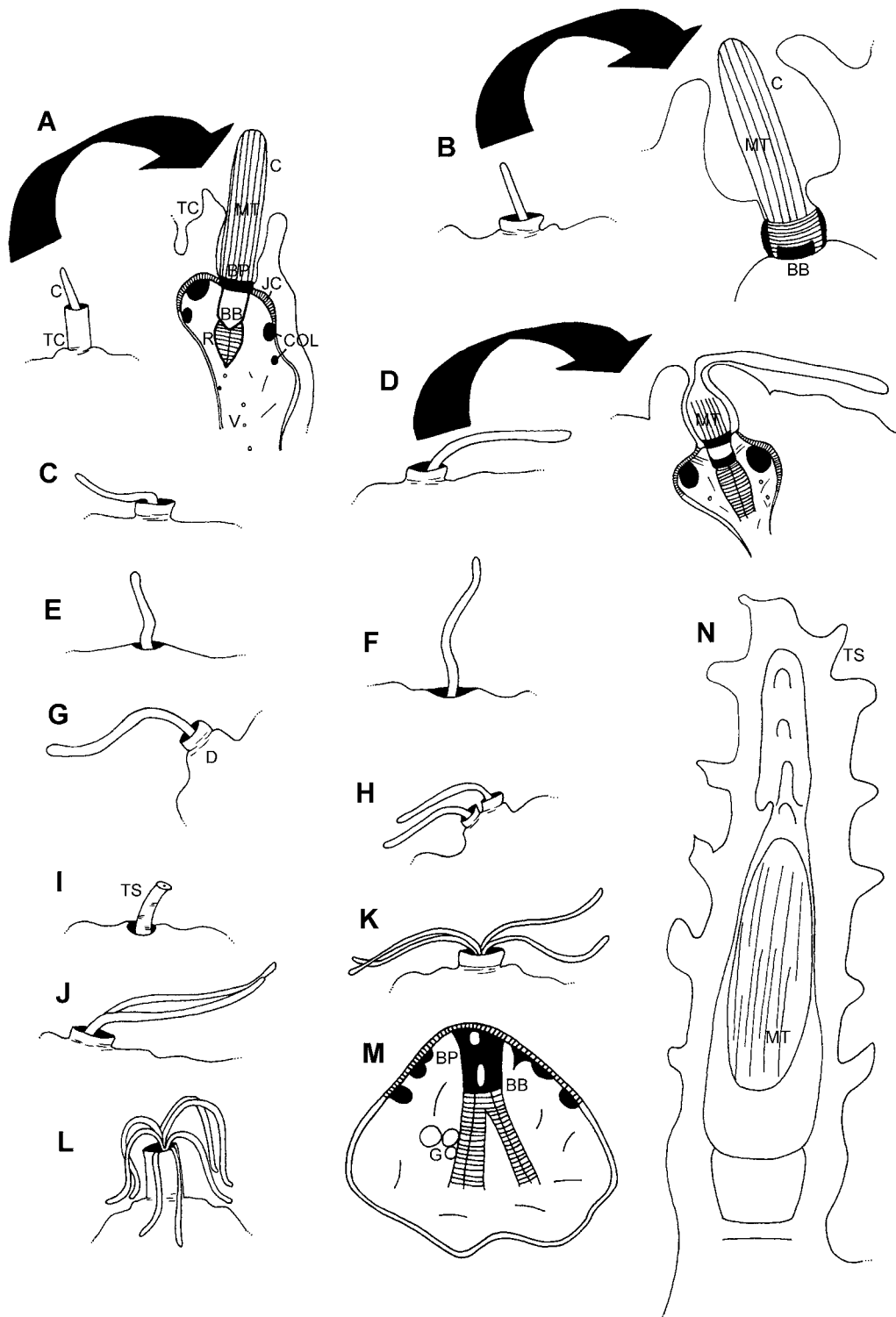


Fig. 1. Types of sensory receptors in cercariae of *Bunodera* sp., *Crepidostomum* sp., *Allassogonoporus* sp. and *Allopodocotyle* sp. as seen with scanning electron microscopy (SEM). Insets show internal ultrastructure as seen with transmission electron microscopy (TEM). **A.** Type UI. **B.** Type UII. **C.** Type UIII. **D.** Type UIV. **E.** Type NI. **F.** Type NII. **G.** Type DI. **H.** Type DII. **I.** Type SI. **J.** Type Bi. **K.** Type MI. **L.** Type MII. **M.** Type St as seen with TEM. **N.** Type SII as seen with TEM. BB – basal body; BP – basal plate; C – cilium; COL – thickening of nerve collar; D – dome-like base; G – granules; JC – extracellular junctional complex; MT – microtubules; TC – tegumentary collar; TS – tegumentary sheath; R – ciliary rootlet; V – vesicles.

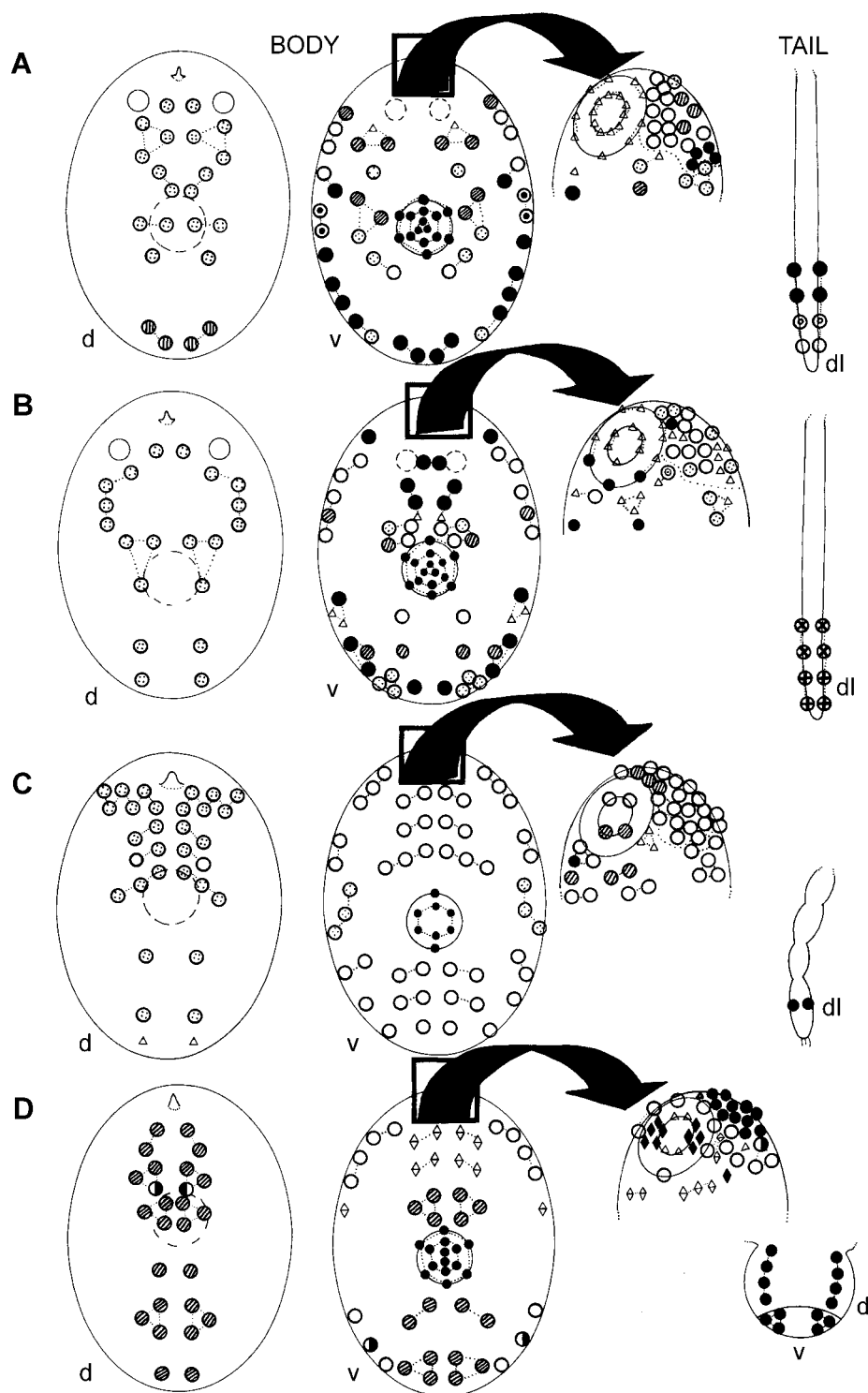


Fig. 2. Sensory receptors in cercariae of *Bunodera* sp., *Crepidostomum* sp., *Allassogonoporus* sp. and *Allopodocotyle* sp. identified using acetylthiocholine method for cholinesterase, silver nitrate impregnation and scanning electron microscopy. Dorsal and ventral views of cercarial body and tail are presented for each species. Insets show receptors of cephalic region. **A.** *Bunodera* sp. **B.** *Crepidostomum* sp. **C.** *Allassogonoporus* sp. **D.** *Allopodocotyle* sp. d – dorsal view; dl – dorsolateral view; v – ventral view. Codes refer to sensory receptor types shown in Fig. 1. Δ – type UI; \bigcirc – type UII; $\textcircled{\text{///}}$ – type UIII; $\textcircled{\text{///}}$ – type UIV; \oplus – type NI; \otimes – type NII; \odot – type DI; \odot – type DII; \bullet – type SI; \odot – type Bi; \blacklozenge – type MI; \blacklozenge – type MII; \bullet – observed with light microscopy.

Table 1. Comparisons of sensory receptor types among cercariae of *Bunodera* sp., *Crepidostomum* sp., *Allassogonoporus* sp. and *Allopodocotyle* sp. as indicated by the Mann-Whitney *U* test. Significant differences ($p < 0.005$) are shown in bold. CL – cilium length; CW – cilium width; TCL – tegumentary collar length; TCW – tegumentary collar width.

Species	CL	CW	TCL	TCW
<i>Bunodera</i> sp.	UI = UII UI < UIII UI < UIV UII < UIII UII < UIV UIII < UIV	UI = UII UI = UIII UI = UIV UII = UIII UII = UIV UIII > UIV	UI > UII UI > UIII UI > UIV UII = UIII UII = UIV UIII = UIV	UI = UII UI = UIII UI = UIV UII = UIII UII = UIV UIII = UIV
<i>Crepidostomum</i> sp.	UI < UII UI < UIII UI < UIV UII < UIII UII < UIV UIII < UIV	UI = UII UI = UIII UI < UIV UII = UIII UII < UIV UIII < UIV	UI > UII UI > UIII UI > UIV UII > UIII UII = UIV UIII < UIV	UI < UII UI < UIII UI < UIV UII = UIII UII = UIV UIII < UIV
<i>Allassogonoporus</i> sp.	UI = UII UI < UIII UI < UIV UII < UIII UII < UIV UIII = UIV	UI = UII UI = UIII UI = UIV UII = UIII UII = UIV UIII = UIV	UI > UII UI > UIII UI > UIV UII = UIII UII = UIV UIII = UIV	UI < UII UI = UIII UI < UIV UII > UIII UII = UIV UIII < UIV
<i>Allopodocotyle</i> sp.	UI = UII UI < MI UI < MII UII < MI UII < MII MI > MII	UI = UII UI > MI UI > MII UII > MI UII > MII MI = MII	UI > UII UI > MI UI = MII UII > MI UII < MII MI < MII	UI = UII UI = MI UI = MII UII = MI UII = MII MI = MII

Table 2. Comparisons in distribution of sensory receptors among cercariae of *Bunodera* sp., *Crepidostomum* sp., *Allassogonoporus* sp. and *Allopodocotyle* sp. as indicated by the Mann-Whitney *U* test. Significant differences ($p < 0.005$) are shown in bold. T – total number of sensory receptors; V – number of ventral receptors; C – number of cephalic receptors; A – number of anterior receptors; S – number of ventral sucker receptors; P – number of posterior receptors; U – number of caudal receptors.

Comparison	T	V	C	A	S	P	U
<i>Bunodera</i> sp.	170	112	81	42	15	24	8
×	≠	=	≠	≠	≠	=	=
<i>Allassogonoporus</i> sp.	178	108	88	56	8	24	2
<i>Bunodera</i> sp.	170	112	81	42	15	24	8
×	=	=	=	=	=	=	≠
<i>Allopodocotyle</i> sp.	172	110	78	40	14	26	14
<i>Bunodera</i> sp.	170	112	81	42	15	24	8
×	=	=	=	=	=	=	=
<i>Crepidostomum</i> sp.	163	104	76	40	15	24	8
<i>Crepidostomum</i> sp.	163	104	76	40	15	24	8
×	≠	=	≠	≠	≠	=	=
<i>Allassogonoporus</i> sp.	178	108	88	56	8	24	2
<i>Crepidostomum</i> sp.	163	104	76	40	15	24	8
×	=	=	=	=	=	=	≠
<i>Allopodocotyle</i> sp.	172	110	78	40	14	26	14
<i>Allassogonoporus</i> sp.	178	108	88	56	8	24	2
×	≠	=	≠	≠	≠	=	≠
<i>Allopodocotyle</i> sp.	172	110	78	40	14	26	14

the ventral surface of the cercarial body whereas those with a long cilium tend to occur mainly on the dorsal surface in all species.

Nerve network

The basic arrangements of the nerve networks of the cercariae of *Bunodera* sp., *Crepidostomum* sp., *Allasso-*

gonoporus sp. and *Allopodocotyle* sp. are shown in Fig. 3. They were found to consist of a bilobed cerebral ganglion located at the pharynx level (Figs. 3A–C) or of a large ramified cerebral ganglion located above the pharynx (Fig. 3D). Three pairs of lateral, dorsal and ventral longitudinal nerve cords extended anteriorly and posteriorly from the cerebral ganglion.

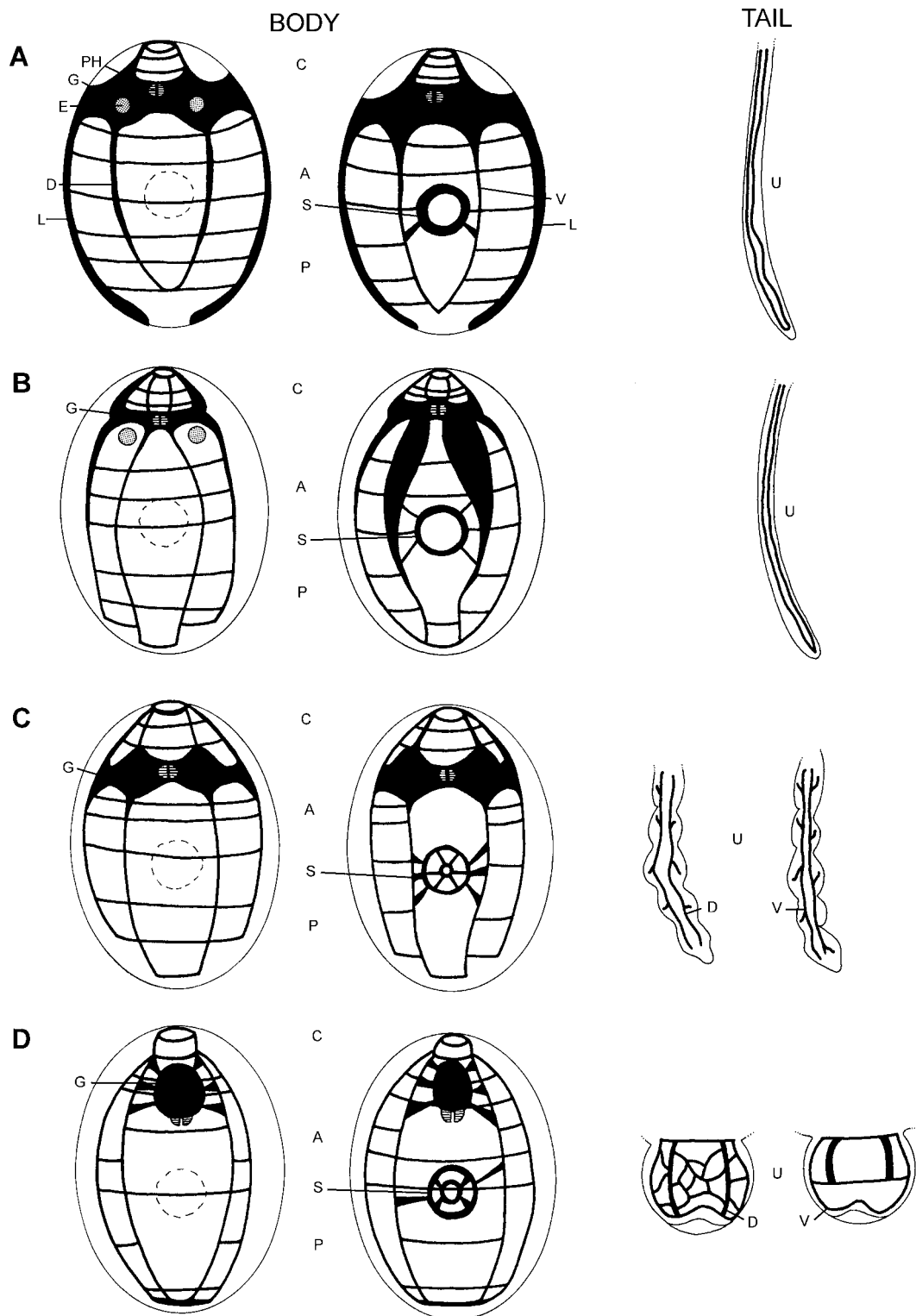


Fig. 3. Arrangement of major transverse commissures and nerve cords in cercariae of *Bunodera* sp., *Crepidostomum* sp., *Allassogonoporus* sp. and *Allopodocotyle* sp. as seen with acetylthiocholine method for cholinesterase. Dorsal and ventral views of cercarial body and tail are presented for each species. **A.** *Bunodera* sp. **B.** *Crepidostomum* sp. **C.** *Allassogonoporus* sp. **D.** *Allopodocotyle* sp. A – anterior transverse commissures; C – cephalic transverse commissures; D – dorsal nerve cord; E – eyespot; G – cerebral ganglion; L – lateral nerve cord; PH – pharynx; P – posterior transverse commissures; S – ventral sucker nerve region; U – caudal nerve cord; V – ventral nerve cord.

Three cephalic, three anterior and three posterior transverse commissures were identified in all species. In *Allopodocotyle* sp., a fourth cephalic transverse commissure was identified (Fig. 3D). Some of the commissures did not surround the cercaria entirely. All species exhibited distinctive rings on the ventral sucker, with *Allassogonoporus* sp. and *Allopodocotyle* sp. presenting many innervations in this region. Fine connections branching from the ventral longitudinal nerve cords to the oral and ventral suckers were also observed.

Two ventral longitudinal nerve cords were observed in the tail of the four species one along each of the lateral margins of the tail. In the cercariae of *Allassogonoporus* sp. and *Allopodocotyle* sp., the two cords were observed branching into fine ramifications (Figs. 3C–D). A complex nerve network was observed on the tail of *Allopodocotyle* sp.

DISCUSSION

Functional components

Different types of sensory receptors were identified in the cercariae of *Bunodera* sp., *Crepidostomum* sp., *Allassogonoporus* sp. and *Allopodocotyle* sp. They are grouped in the following major categories:

- 1.0 – Nonciliated receptors, with no cilia emerging through the tegument:
 - 1.1 Sheathed receptors (types SI and SII): with microtubules surrounded by a short or long, thick, stiff tegumentary sheath.
 - 1.2 Subtegumentary receptors (type St): with a sensory bulb attached to the tegument by septate extracellular junctional complexes, two pairs of thickened nerve collars, a distinctive bifurcated ciliary rootlet, a basal plate and a basal body.
- 2.0 – Ciliated receptors, with one or more cilia:
 - 2.1 Uncollared receptors (types NI and NII): with no tegumentary collar and moderately long or long unsheathed cilia.
 - 2.2 Collared receptors, with a tegumentary collar:
 - 2.2.1 Low-collared receptors (types DI, DII, UII, UIII, UIV, MI and Bi): with a low to moderately low tegumentary collar, with a tegumentary dome-like base or not, short, moderately long or long unsheathed cilia, a central pair of microtubules, a basal body, a sensory bulb attached to the tegument by septate extracellular junctional complexes and two pairs of thickened nerve collars.
 - 2.2.2 High-collared receptors (types UI and MII): with a moderately low to very high tegumentary collar, short or long unsheathed cilia, a central pair of microtubules, a basal body, a sensory bulb attached to the tegument by septate extracellular junctional complexes and two pairs of thickened nerve collars.

Some of these types are similar to types previously described (Koie 1981, Žďárská 1992, Czubaj and Nie-

wiadomska 1996, Niewiadomska and Czubaj 1996, Moravec 2002).

The collared receptor type is abundantly found. Its ultrastructure is similar among the taxa studied, suggesting that these receptors likely respond to the same sensory modality. They are possibly associated with mechanoreception (Bogéa and Caira 2001a).

Similar site-specific patterns of cercarial sensory receptors were observed within a species and among the species studied. Thus, “domed” receptors of *Bunodera* sp., biciliated receptors of *Crepidostomum* sp., and high-collared receptors with multiple cilia of *Allopodocotyle* sp. occurred in particular sites of the cercarial body. Also, the distribution of short- and long-ciliated receptors with low tegumentary collars on the ventral and dorsal surfaces of the cercarial body, respectively, may reflect their different functions.

Functional components were also investigated in the numbers of sensory receptors on each cercarial surface and in the numbers of receptors associated with each nerve region in the four species studied. Independent of their degree of relatedness, all species studied exhibit receptors concentrated on the ventral surface of the cephalic region (Table 2). This similarity may be related to the importance of the oral sucker region in the strategies for host finding in these species (Knight and Pratt 1955, Cannon 1971).

The numbers of cercarial sensory receptors associated with each nerve region, on the other hand, differ in all species (Table 2). Besides, they differ among the species, with *Allassogonoporus* sp. exhibiting more cephalic and anterior receptors and less ventral sucker receptors and *Allopodocotyle* sp. presenting more caudal receptors than the other taxa. These results support the idea that sensory receptors tend to accumulate in regions that are actively involved in host recognition and penetration (Richard 1971, Bayssade-Dufour 1979, Haas and Haberl 1997). These regions may vary according to the type of cercaria and its transmission mode.

Phylogenetic components

The present study extends the chaetotaxic investigations to *Bunodera* sp., *Crepidostomum* sp., *Allassogonoporus* sp. and *Allopodocotyle* sp. Different maps obtained for each species suggest taxon-specific patterns (Fig. 2). These maps also confirm the diagnostic characters previously established for the families Allocreadiidae, Lecithodendriidae and Opecoelidae (Richard 1971, Bayssade-Dufour 1979, Bayssade-Dufour et al. 1993).

Some of the types of sensory receptors detected seem species-specific. For example, “domed” receptors were only observed in the cercaria of *Bunodera* sp. (Fig. 2A). Uncollared receptors with a short or a moderately long cilium and low-collared receptors with two long cilia were restricted to the cercaria of *Crepidostomum* sp. (Fig. 2B). Collared receptors with multiple cilia and sheathed and subtegumentary receptors were exclusively found in *Allopodocotyle* sp. (Fig. 2D). Studies on

sensory receptors in cercariae of different families are particularly encouraged to verify whether taxon-specific types could be used as synapomorphies for the taxa involved.

The total numbers of sensory receptors and the numbers of ventral receptors are rather uniform among the species studied. The free-swimming cercariae of *Bunodera* sp. and *Crepidostomum* sp. and the non-swimming cercaria of *Allopodocotyle* sp. exhibit similar total numbers of sensory receptors whereas the free-swimming cercaria of *Allassogonoporus* sp. has slightly more receptors than the other species (Table 2). The numbers of ventral receptors, however, are similar among all species. Because the total length of these cercariae varies considerably, the total number of sensory receptors and the number of ventral receptors appear relatively independent of cercarial size. Further investigations are required to determine whether the number of ventral receptors reflects an optimal design associated with constraints of a free-living mode of existence.

The numbers of cercarial sensory receptors associated with each nerve region seem to be more similar between closely related cercariae than among those more distantly related. The allocreadiid cercariae of *Bunodera* sp. and *Crepidostomum* sp. exhibit more similar numbers of receptors between each other than in relation to those detected in lecithodendriid *Allassogonoporus* sp. and opacoelid *Allopodocotyle* sp. (Table 2). These results suggest that there are phylogenetic correlates involved in the distribution of cercarial sensory receptors throughout each nerve region.

Cholinergic components are demonstrated in the cerebral ganglion, nerve cords and transverse commissures of the cercariae studied. They are particularly

associated with the innervations of cercarial sensory receptors (Bogéa T. and Brito-Gitirana L., UFRJ, Rio de Janeiro, Brazil, unpubl.). Similar arrangements have been determined in cholinergic nerve networks of other cercariae (Nezlin et al. 1992).

The investigation of cholinergic nerve networks has been useful to identify evolutionary trends in the organisation of platyhelminth nervous systems. It has been suggested that the general trend would involve an increased cephalisation followed by a reduction of nerves in the posterior body (Shishov 1991). Different platyhelminth orthogons had been considered homologous, being derived from the cord-type of Acoela and Catenu- lida which is characterised by a meshwork of nerve fibres (Kotikova 1991 after Reuter and Gustafsson 1995). The present cercarial orthogons are classified as irregular because many transverse commissures do not surround the cercaria entirely. This arrangement would have derived directly from the cord-type.

Acknowledgements. I am particularly indebted to Dr. J.N. Caira for invaluable support and for comments on an earlier version of this paper. I thank all individuals that helped in the collection of molluscs. I am grateful to Dr. K. Tiekotter for his assistance as well as for providing me with infected snails. Special thanks go to J. Romanow for expert assistance and to Drs. M. Cantino, C. Henry, E. Joikinen, L. Khairallah and C. Schaefer for good advice. I am indebted to Dr. L. Brito-Gitirana for comments on a version of the present paper and for granting access to laboratory facilities. This investigation was supported by Doctoral fellowships of the University of Connecticut, USA, and the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), Brazil, (no. 200142/94-1) and by Research grants of CNPq (nos. 300509/00-6 and 150021/03-8) awarded to T.B.

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Received 13 April 2004

Accepted 28 July 2004