

## Research Note

# The Structural–Functional Relationship of the Para-Receptacle Structure in Acanthocephala

OMAR M. AMIN,<sup>1,3</sup> RICHARD HECKMANN,<sup>2</sup> AND MICHAEL D. STANDING<sup>2</sup>

<sup>1</sup> Institute of Parasitic Diseases, P.O. Box 28372, Tempe, Arizona 85285-8372, U.S.A. (e-mail: omaramin@aol.com) and

<sup>2</sup> Department of Integrative Biology, Brigham Young University, Provo, Utah 84602 U.S.A. (e-mail: Richard\_heckmann@byu.edu)

**ABSTRACT:** The para-receptacle structure (PRS) was first described in *Neoechinorhynchus* (*Neoechinorhynchus*) *qatarensis* Amin, Saoud, and Alkuwari, 2002 (Neoechinorhynchidae), as a prominent vesicle connected proximally to the anterior body wall and distally to the posterior wall of the proboscis receptacle then extending into the body cavity of males and females as a posterior conduit tube. The PRS has since been observed in *Neoechinorhynchus* (*Neoechinorhynchus*) *golvani* Salgado-Maldonado, 1978 (a new subgeneric diagnosis), and described in a member of another eoacanthocephalan family, *Acanthogyrus* (*Acanthosentis*) *parareceptaculis* Amin, 2005 (Quadrigrigidae). In the latter species, a similar structure was also observed at the posterior end of the trunk. Our optical and transmission electron microscopy (TEM) observations in specimens of *N. qatarensis* show the PRS as a tandemly arranged set of nucleated cells delimited by a double-walled membrane and a muscular wall. It appears to be contractile with a secretory function related to changes in the hydrostatic pressure that may be associated with the extrusion and retraction of the proboscis in worms with weakly developed proboscis receptacle musculature. This is the first detailed study of the PRS and of its proposed function in the only 3 acanthocephalan species from which it has been identified to date. It is considered as an archaic structure present only in a few representatives of some primitive taxa having only a single-walled proboscis receptacle wall.

**KEY WORDS:** Para-receptacle structure (PRS), hydrostatic skeleton, Acanthocephala.

During the course of the description of *Neoechinorhynchus* (*Neoechinorhynchus*) *qatarensis* Amin, Saoud, and Alkuwari, 2002 (Neoechinorhynchidae), from the marine blue-barred flame parrot fish, *Scarus ghobban* Forsskål, 1775, in Qatari waters of the Arabian Gulf, a new and unique structure (the para-receptacle structure, PRS) associated with the proboscis receptacle was discovered in male and female worms (Amin et al., 2002). The PRS was later observed in many adult male and female specimens of *Neoechinorhynchus* (*Neoechinorhynchus*) *golvani*

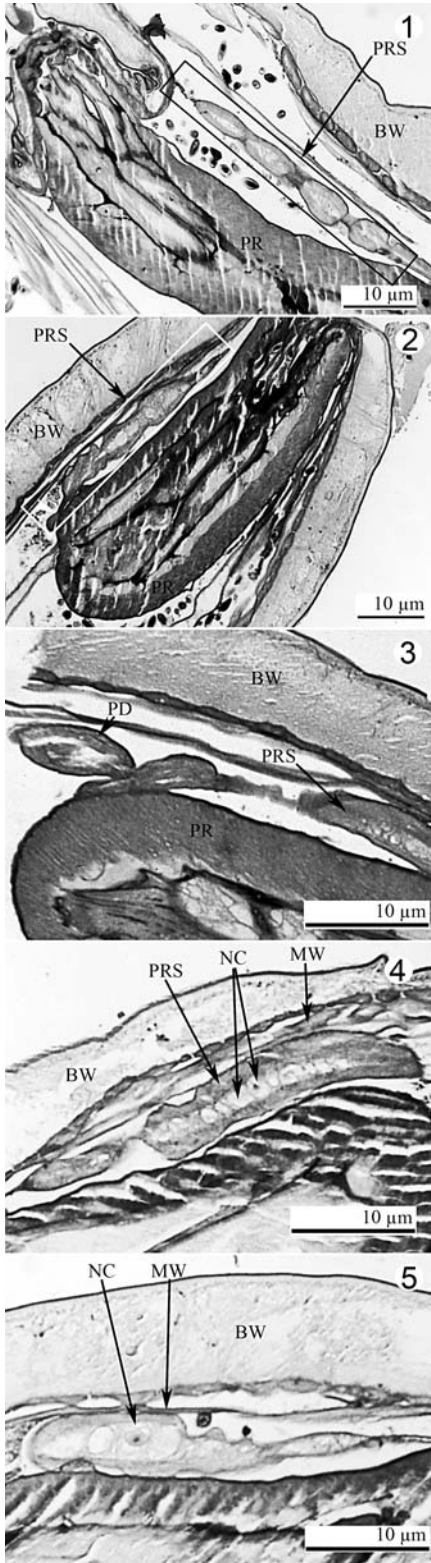
Salgado-Maldonado, 1978, originally described from the estuarine fish *Cichlasoma fenestratum* (Günther, 1862) in the Catemaco Lagoon, Vera Cruz, Mexico (Salgado-Maldonado, 1978). In Quadrigrigidae, it was also described in *Acanthogyrus* (*Acanthosentis*) *parareceptaculis* Amin, 2005, from the freshwater sand loach, *Cobitis biwae* Jordan and Snyder, 1901, in the Daido River, the first major tributary of the Seta River outflow of Lake Biwa, Shiga Prefecture, Japan (Amin, 2005).

In its first report, the PRS in *N. qatarensis* was described as “prominent along the ventral side of proboscis receptacle, connecting anteriorly to body wall with duct adheres to side of posterior end of proboscis receptacle, then proceeding posteriorly as a tubular structure [Plate 1 (6) in Amin et al., 2002]. PRS with thick well-defined two-layer membrane enveloping a single strand of cellular structures in tandem may have glandular function.” The PRS appeared very similar in all 3 species from 2 families of Eoacanthocephala Van Cleave, 1936. Questions regarding its fine structure, function, and evolutionary significance needed to be answered.

The work presented herein confirms the above account through detailed optical and transmission electron microscopy (TEM) studies of *N. qatarensis*, provides considerable additions and refinements, and proposes a structural–functional relationship. Available materials from the other 2 species from which the PRS has been reported were whole-mounted and, thus, could not be used in the ultrastructural studies.

Thirty specimens of *N. N. qatarensis* were fixed in 8% formalin, embedded in paraffin, sectioned to a thickness of 6 µm, stained with hematoxylin and eosin for optical observations with a compound microscope at magnifications of ×100, ×400, and ×1,000. All specimens sectioned were mature males and females sampled from about 750 adult worms collected from 12 infected, of 45 examined, *S. ghobban* in Qatari waters of the Arabian Gulf between 1983 and 1987 (type material: U.S. National Parasite Collection, USNPC, 91177–91179; sections: USNPC 98841). Images were

<sup>3</sup> Corresponding author (e-mail: omaramin@aol.com)



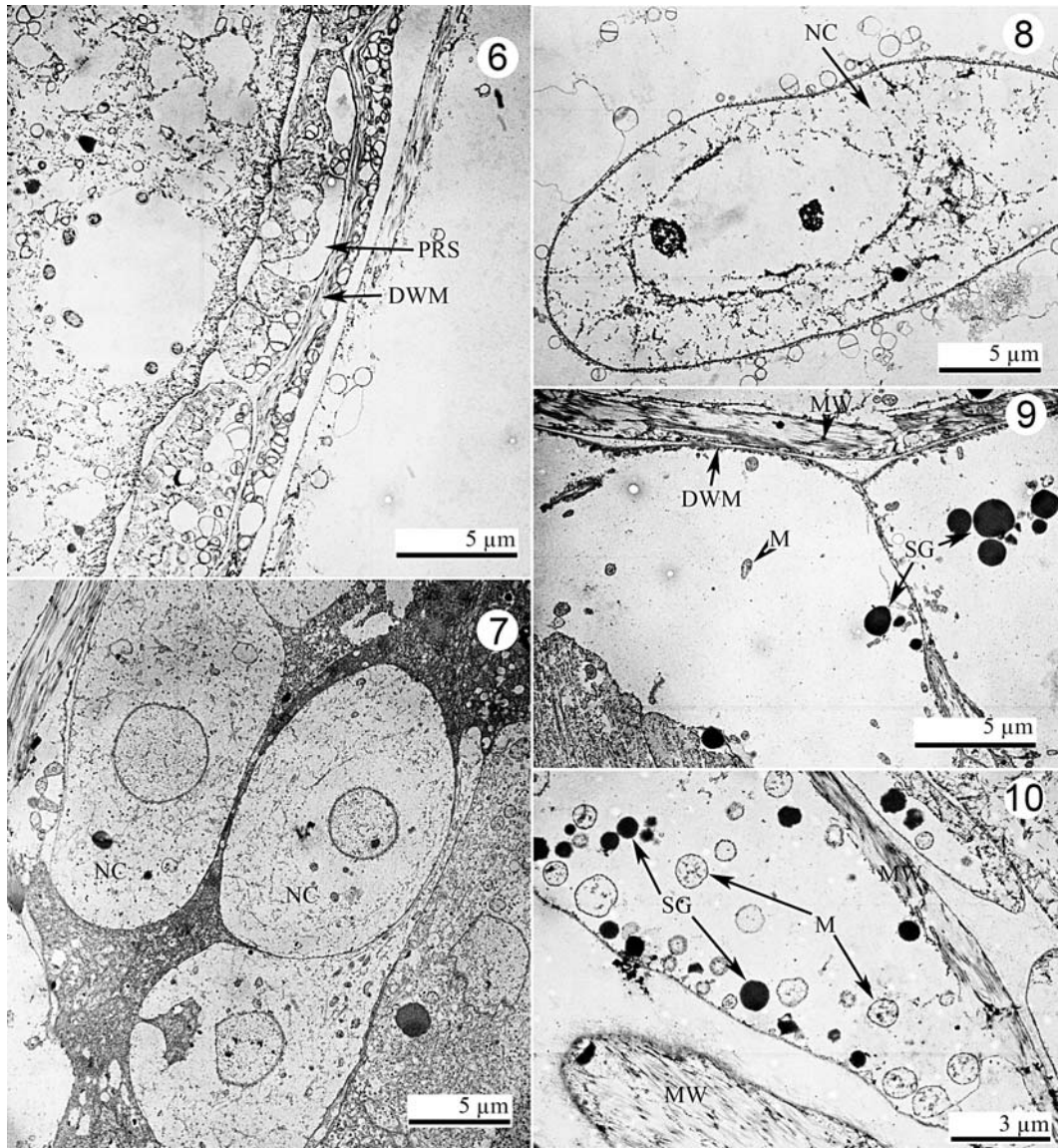
captured with a 35-mm camera. For TEM studies, specimens previously fixed in formalin were dehydrated in an ascending series of ethanol solutions to 70% and then stored until needed. Samples were then rehydrated for postfixation in 1% buffered osmium tetroxide, dehydrated in an ascending series of ethanol followed by 100% acetone. Specimens were embedded in Spurr's resin and sectioned with a diamond knife to a thickness of 80 to 100 nm. After poststaining with Reynolds lead citrate and 5% urinal acetate in 50% ethanol, sections were examined in an FEI Technai T-12 TEM. Images were recorded on film.

Many whole-mounted adult male and female *N. N. golvani* from the Salgado-Maldonado (S-M) collection were examined microscopically. The specimens were collected from *C. fenestratum* (type host) in the Catemaco Lagoon, Vera Cruz, Mexico, in 1975 (type specimens: La Colección Helmintológica del Instituto de Biología II-113), and in subsequent years through 2005. An additional 100 *N. N. golvani* adults recently collected from *Cichlasoma pearsi* (Hubbs, 1936) in El Vapor Lagoon, which is associated with the larger coastal lagoon Laguna de Terminos, Campeche, Mexico, were examined. Additionally, 19 sexually mature adults of *A. A. parareceptaculis* (10 males, 9 females) collected from 7 of 58 examined *C. biwae* from 2 of the 7 sites surveyed (along the Daido and Kusano rivers, Shiga Prefecture, Japan) in the autumns of 1997 and 2003 and the spring of 2000 (type material: Lake Biwa Museum 20-40, 2-69 to 2-72, and 4-27 to 4-29) were examined.

Optical studies of hematoxylin and eosin-stained sections of *N. N. qatarensis* show the PRS as an oblong capsule enclosing a column of nucleated core (NC) cells that appear lighter colored compared with the darker walls of the PRC (Figs. 1, 2). The PRC

←

**Figures 1–5.** Details of the para-receptacle structure (PRS) of *Neoechinorhynchus N. qatarensis* using light microscopy. **1.** Para-receptacle structure (PRS) between the proboscis receptacle (PR) and the body wall (BW) attached to the anterior trunk near the inverted proboscis. Note constrictions in the PRS. **2.** A general view of the joint attachment of the PRS and the posterior duct to the wall of the PR (arrow). Constrictions in the PRS indicate contractibility of the structure. The anterior attachment of the PRS to the BW is not shown. **3.** Higher magnification of the attachment site showing the common insertion of the PRS and the posterior duct (PD) into the wall of the PR near its posterior end. **4.** Segment of a PRS showing the linear arrangement of the nucleated core cells (NC), surrounding membranes, muscular wall (MW), and BW. **5.** Segment of a contracting PRS showing NC, surrounding membranes, MW, and BW.



**Figures 6–10.** Transmission electron microscopy (TEM) of the para-receptacle structure (PRS) of *Neoechinorhynchus N. qatariensis*. **6.** View of a PRS showing the double-walled enveloping membranes (DWM). **7.** A cluster of nucleated cells (NC) in a congested section of a contracting PRS. **8.** Double-nucleated core cell with many secretory globules at its surface. **9.** The interface between 2 nucleated cells showing a number of secretory globules (SG), mitochondria (M), DWM, and myofibers of the surrounding muscular wall (MW). **10.** The posterior end of 1 nucleated cell with many M and SG indicating high activity. Note myofibers of MW.

extends from its anterior attachment site at the anterior tip of the body wall (BW) to near the posterior end of the proboscis receptacle (PR) on the ventral side. Its posterior attachment site to the PR is also shared by the anterior end of its posterior duct (PD), which extends into the body cavity as a conduit tube

(Figs. 1, 2). The PRS and PD are both inserted into the receptacle wall at the same point then pass through it as a canal opening past the wall into the receptacle cavity (Fig. 3). The PRS includes a single central column of NC encapsulated in a surrounding double membrane and muscular wall (MW) (Figs. 4,

5 in part). The PRS often showed constrictions that varied in extent and location in different specimens (Figs. 1, 2). The PD lacks the NC structure characteristic of the PRS.

Optical observations at the same magnification showed the PRS in *N. N. qatarensis*, *N. n. golvani*, and *A. A. parareceptaclis* to be very similar. The additional presence of a similar structure at the posterior end of a male *A. parareceptaclis* is documented in Amin (2005) but needs further study pending the availability of new unmounted specimens.

TEM studies show the NC of the PRS to be encapsulated in a thin, double-walled membrane (DWM) (Fig. 6). Clusters of NCs with prominent spherical nuclei may bulge between contracted sections of the PRS (Fig. 7), indicating the contractile nature of the structure. During such activity periods, NCs appear to produce and process secretory globules (SG) often seen at their outer surface (Fig. 8). These globules appear similar to those found in the body wall of *Neoechinorhynchus cylindratus* (Van Cleave, 1913) Van Cleave, 1919 by Amin et al. (1993), and glycogen granules were also found in the same sites by Amin and Larsen (1989). Double-nucleated NCs are occasionally present (Fig. 8). The DWM is tightly enclosed within a well-defined MW made up of longitudinal and circular myofibers (Fig. 9), where the NCs demonstrate mitochondria (M) (round structures) with well-defined cristae and a large number of SG, especially during contractile activity (Fig. 10). The demonstrated contraction of the PRS appears to produce hydrostatic pressure using fluids drawn from the body cavity via the PD into the PR cavity perhaps aided by a secretory process of NCs to affect the eversion of the proboscis in primitive worms that have only a weak, single-walled PR.

The presence of the PRS in marine, estuarine, and freshwater fish in geographically distinct localities with no common geological history suggests its independent evolution in a few representatives of Neoechinorhynchidae and Quadrigyridae. Worms in these 2 families have been considered the most primitive acanthocephalans based on 1) reduced proboscis and proboscis armature, 2) syncytial cement glands, 3) embryonic development, 4) absence of armature in embryonic larvae, 5) primitive intermediate host, and 6) single-walled proboscis receptacle. One or more of these arguments were advanced by Meyer (1933), Van Cleave (1941, 1949, 1952), and Petrochenko (1956). Petrochenko (1956) placed these 2 families at the bottom of his phylogenetic tree. It is thus here proposed that the rarely reported PRS similarly represents another primitive structure in the eoacan-

thocephalans. More recent phylogenetic research, however, suggests that the other ancient group, Archiacanthocephala, is the most basal group within the phylum; see Monks (2001) and Garcia-Varela et al. (2002). Structures like the PRS, however, have never been reported in the Archiacanthocephala and were not observed in any of the various archiacanthocephalans studied microscopically by O.M.A. These include species of the genera *Mediorhynchus* Van Cleave, 1916, *Moniliformis* Travassos, 1915, *Macracanthorhynchus* Travassos, 1917, *Oligacanthorhynchus*, Travassos, 1915, and *Prosthorrhynchus* Travassos, 1915.

The evolution of more advanced taxa with stronger, double-walled PR would eliminate the need for such a structure. It is not here proposed that all acanthocephalans with single-walled proboscides had PRS at some time in their evolutionary history. However, some clearly did. Of these, a few relic taxa did retain it. Accordingly, the PRS is herein considered as an archaic structure demonstrating the evolution of hydrostatic skeletons in the primitive Quadrigyridae and Neoechinorhynchidae.

#### LITERATURE CITED

- Amin, O. M.** 2005. Occurrence of the subgenus *Acanthosentis* Verma and Datta, 1929 (Acanthocephala: Quadrigyridae) in Japan, with the description of *Acanthogyrus (Acanthosentis) alternatospinus* n. sp. and *A. A. parareceptaclis* n. sp. from Lake Biwa drainage fishes and a key to the species of the subgenus. *Systematic Parasitology* 60:125–137.
- Amin, O. M., and D. Larsen.** 1989. Acanthocephala from lake fishes in Wisconsin: a biochemical profile of *Neoechinorhynchus cylindratus* (Neoechinorhynchidae). *Transactions of the American Microscopical Society* 108:309–315.
- Amin, O. M., M. F. A. Saoud, and K. S. R. Akuwari.** 2002. *Neoechinorhynchus qatarensis* sp. n. (Acanthocephala: Neoechinorhynchidae) from the blue-barred flame parrot fish, *Scarus ghobban* Forsskål, 1775, in Qatari waters of the Arabian Gulf. *Parasitology International* 51:171–176.
- Amin, O. M., F. H. Whittaker, K. M. Klueber and J. Hoffpawr.** 1993. Ultrastructural changes in the body wall of *Neoechinorhynchus cylindratus* (Acanthocephala) associated with reproductive activity. *Transactions of the American Microscopical Society* 112: 208–216.
- Garcia-Valera, M., M. P. Cummings, G. Pérez Ponce de León, S. L. Gardner, and J. P. Lacleste.** 2002. Phylogenetic analysis based on 18S ribosomal RNA gene sequences supports the existence of Class Polyacanthocephala (Acanthocephala). *Molecular Phylogenetics and Evolution* 23:288–292.
- Meyer, A.** 1933. Acanthocephala Pages 333–582 in H. G. Bronn, ed. *Klassen und Ordnungen des Tierreichs*. Vol. 4. Akademische Verlagsgesellschaft M.B.H., Leipzig, Germany. (In German.)

- Monks, S.** 2001. Phylogeny of the Acanthocephala based on morphological characters. *Systematic Parasitology* 48: 81–116.
- Petrochenko, V. I.** 1956. *Acanthocephala* of Domestic and Wild Animals. Vol. 1. Izdatel'stvo Akademii Nauk SSSR, Moscow, Russia. 435 pp. (In Russian: English translation by Israel Program for Scientific Translations Ltd., Jerusalem, Israel 1971, 465 pp.)
- Salgado-Maldonado, G.** 1978. Acanthocephalos de peces IV. Descripción de dos especies nuevas de *Neoechinorhynchus* Hamann, 1892 (Acanthocephala: Neoechinorhynchidae) y algunas consideraciones sobre este género. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoológica* 49(1):35–37. (In Spanish.)
- Van Cleave, H. J.** 1941. Relationships of the Acanthocephala. *American Naturalist* 75:31–47.
- Van Cleave, H. J.** 1949. Morphological and phylogenetic interpretations of the cement glands in the Acanthocephala. *Journal of Morphology* 34:157–427.
- Van Cleave, H. J.** 1952. Specialization and formation of genera in Acanthocephala. *Systematic Zoology* 1:62–83.