Morphological aspects of marine monogeneans (Platyhelminthes) parasitic on the gills of *Auxis thazard* (Lacépède) (Scombridae) from Rio de Janeiro, Brazil

Cristina Dias Mogrovejo [1], Herman Lent [1] & Cláudia Portes Santos [1, 2]

[1] *Laboratório de Avaliação e Promoção da Saúde Ambiental, Fundação Oswaldo Cruz. Avenida Brasil 4365, Manguinhos, 21045-900 Rio de Janeiro, Rio de Janeiro, Brasil. E-mail: cpsantos@ioc.fiocruz.br*

[2] *Correspondent author.*

ABSTRACT. *Allopseudaxine macrova* (Unnithan, 1957), *Churavera triangula* (Mamaev, 1967), *Hexostoma auxisi* Palombi, 1943 and *H. keokeo* Yamaguti, 1968 are studied based on light and scanning electron microscopy. The previously proposed synonymy of *H. auxisi* and *H. keokeo* is not accepted. Morphological changes between juvenile and adult hexostomatids are highlighted.
KEY WORDS. *Allopseudaxine macrova*, *Churavera triangula*, *Hexostoma auxisi*, *Hexostoma keokeo*, Monogenea.

RESUMO. *Allopseudaxine macrova* (Unnithan, 1957), *Churavera triangula* (Mamaev, 1967), *Hexostoma auxisi* Palombi, 1943 e *H. keokeo* Yamaguti, 1968 são estudadas sob microscopias de luz e eletrônica de varredura. A sinonímia previamente proposta para *H. auxisi* e *H. keokeo* não é aceita. Mudanças morfológicas entre hexostomatídeos jovens e adultos são enfocadas.
PALAVRAS CHAVE. *Allopseudaxine macrova*, *Churavera triangula*, *Hexostoma auxisi*, *Hexostoma keokeo*, Monogenea.

Investigations on the parasites of *Auxis thazard* (Lacépède, 1800) (Scombridae) off Rio de Janeiro revealed the presence of 23 species of helminths and one arthropod among a total of 15,749 parasites examined (Mogrovejo & Santos 2002a).

Among the monogeneans, the species *Allopseudaxine macrova* (Unnithan, 1957), *Churavera triangula* (Mamaev, 1967), *Hexostoma auxisi* Palombi, 1943, *H. keokeo* Yamaguti, 1968, *Caballerocotyla lenti* Mogrovejo & Santos, 2002 and *Sibitrema* sp. were found. Of these species, only *C. lenti* has been previously studied using scanning electron microscopy (Mogrovejo & Santos 2002b) and a species os *Sibitrema* was not possible to identify at this moment. The other four are studied below and aspects of their ultrastructure commented upon.

MATERIAL AND METHODS

Specimens of *Auxis thazard* were obtained fresh from fishermen off Rio de Janeiro, Brazil, and examined for parasites. For light microscopy, the worms recovered alive, were fixed in 70% ethanol under slight pressure, stained in Mayer's paracarmine, differentiated in acid-alcohol, dehydrated in an ethanol series, cleared in beech-wood creosote and mounted in Canada balsam. Drawings were made with the use of a drawing tube. Measurements are presented in millimetres with means in parenthesis.

For scanning electron microscopy (SEM), specimens were fixed for 1h in a solution containing 4% paraformoldehyde and 2.5% glutaraldeyde in 0.2M cacodylate buffer (pH 8.3). Samples were washed in PBS and post-fixed for 1h in a solution of 1 % osmium tetroxide in 0.1 M cacodylate buffer. They were then dehydrated through an ethanol series, critical-point dried and sputter-coated with gold. They were examined using a JSM-8500 scanning electronic microscope. The micrographs were adjusted using Adobe Photoshop 7. Voucher specimens have been deposited in the helminthological collections of Institute Oswaldo Cruz, Brazil (CHIOC) as *Hexostoma auxisi*: 36209 a-b, *Hexostoma keokeo*: 36210 a-b and Allopseudaxine macrova: 36.211 a-b. A voucher of *Hexostoma keokeo* (labelled as *H. auxisi*) (BMNH No. 1993.5.18.3) was borrowed from The Natural History Museum, London.

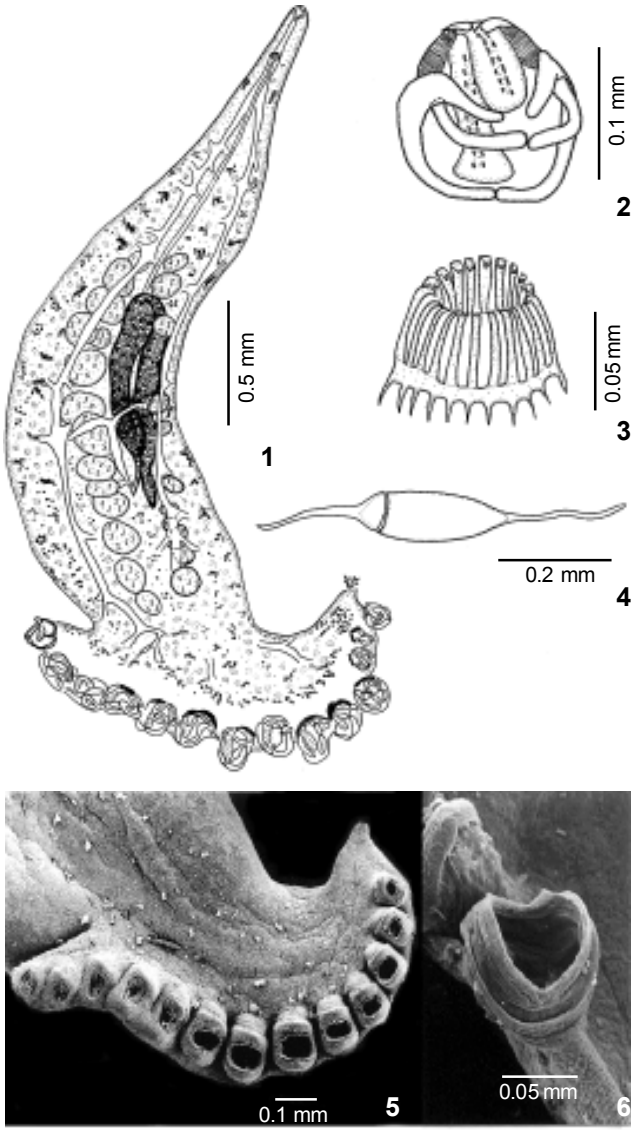
RESULTS

Axinidae

Allopseudaxine macrova (Unnithan, 1957) Yamaguti, 1963

Figs 1-6

Based on 11 specimens. Body 2.15-4.50 (3.75) long; maximum width 0.46-1.34 (0.83) (Fig. 1). Anterior suckers 0.02-0.04 × 0.02-0.03 (0.03 × 0.03). Oral aperture ventro-terminal; pharynx 0.03-0.06 × 0.02-0.05 (0.04 × 0.03); oesophagus 0.25-0.86 (0.43) long; intestinal bifurcation close to genital atrium. Caeca with lateral diverticula, reach close posterior extremity of body. Haptor with single row of 11-17 (14) clamps; caudal digitiform



Figs 1-6. *Allopseudaxine inacrowa*: (1) body in ventral view; (2) clamp with median sclerite, U-shaped perforated, articulated with lateral sclerites; (3) genital corona; (4) egg; (5) haptor in ventral view, clamps dissimilar in size; (6) detail of clamp.

appendix 0.23-0.43 (0.32) long, with two pairs of dissimilar anchors 0.05-0.06 (0.05) and 0.02-0.03 (0.02) long. Clamps of different sizes; largest 0.06-0.33 × 0.05-0.20 (0.19 × 0.16); smallest close to anchors, 0.04-0.11 × 0.03-0.12 (0.08 × 0.08). Median sclerite U-shaped, perforated; lateral sclerites arcuate (Fig. 2). Genital atrium 0.02-0.03 × 0.04-0.05 (0.02 × 0.04), at 0.2-0.5 (0.3) from anterior end; genital corona with 18-21 (19) slightly curved spines of 0.02-0.03 (0.02) in length with bifid tips (Fig. 3). Testes 20-28 (25), pre-, para- and post-ovarian. Ovary 0.85-2.56 × 0.06-0.13 (1.86 × 0.09). Vitelline follicles co-extensive with caeca; vitelline reservoir Y-shaped, 0.23 long. Egg 0.20-0.26 × 0.07-0.09 (0.24 × 0.08), with two polar filaments of equal size, 0.16-0.19 (0.17) long (Fig. 4).

SEM. Asymmetrical haptor with 13 dissimilar clamps disposed in single row; after body constriction, haptor detaches from body proper in oblique projection directed antero-laterally and bearing first two clamps; haptor continues posteriorly in line with margin of one side of body; it's posterior end is curved towards opposite side of body; clamps embedded in body tissue are large in middle of row, decreasing in size towards extremities. Caudal digitiform appendix with two pairs of anchors (Fig. 5). External structure of clamps reveals valves equal in size opening ventro-laterally; delicate membrane, slightly pleated, associated with margin of valves (Fig. 6).

Gastrocotylidae

Churavera triangula (Mamaev, 1967) Lebedev, 1986

Figs 7-13

Based on 12 specimens. Body 2.34-4.71 (3.18) long; maximum width, not including haptor, 0.29-0.59 (0.45) wide (Fig. 7). Anterior suckers 0.02-0.03 × 0.02-0.03 (0.03 × 0.02). Oral aperture ventro-terminal; pharynx 0.02-0.04 × 0.01-0.03 (0.03 × 0.02); oesophagus 0.14-0.30 (0.24) long; intestinal caeca with lateral diverticula reach end of body. Haptor with single row of 13-27 (20) clamps of 0.03-0.05 × 0.03-0.04 (0.04 × 0.03) (Fig. 8); caudal digitiform appendix 0.11-0.28 (0.17) long with 2 pairs of dissimilar anchors 0.04-0.05 (0.05) and 0.02-0.03 (0.03) long. Genital atrium 0.02 × 0.02-0.03 (0.02 × 0.03) at 0.17-0.25 (0.20) from anterior end; genital corona with 15-17 curved spines of 0.014 in length (Fig. 9). Testes 40-45 (42), para- and post-ovarian. Ovary 0.37-0.50 × 0.11-0.15 (0.42 × 0.13). Vitelline follicles co-extensive with caeca; vitelline reservoire 0.12-0.16 (0.14) long. Egg 0.21-0.28 × 0.07-0.09 (0.25 × 0.08), with filaments on both poles 0.13-0.28 (0.22) long (Fig. 10).

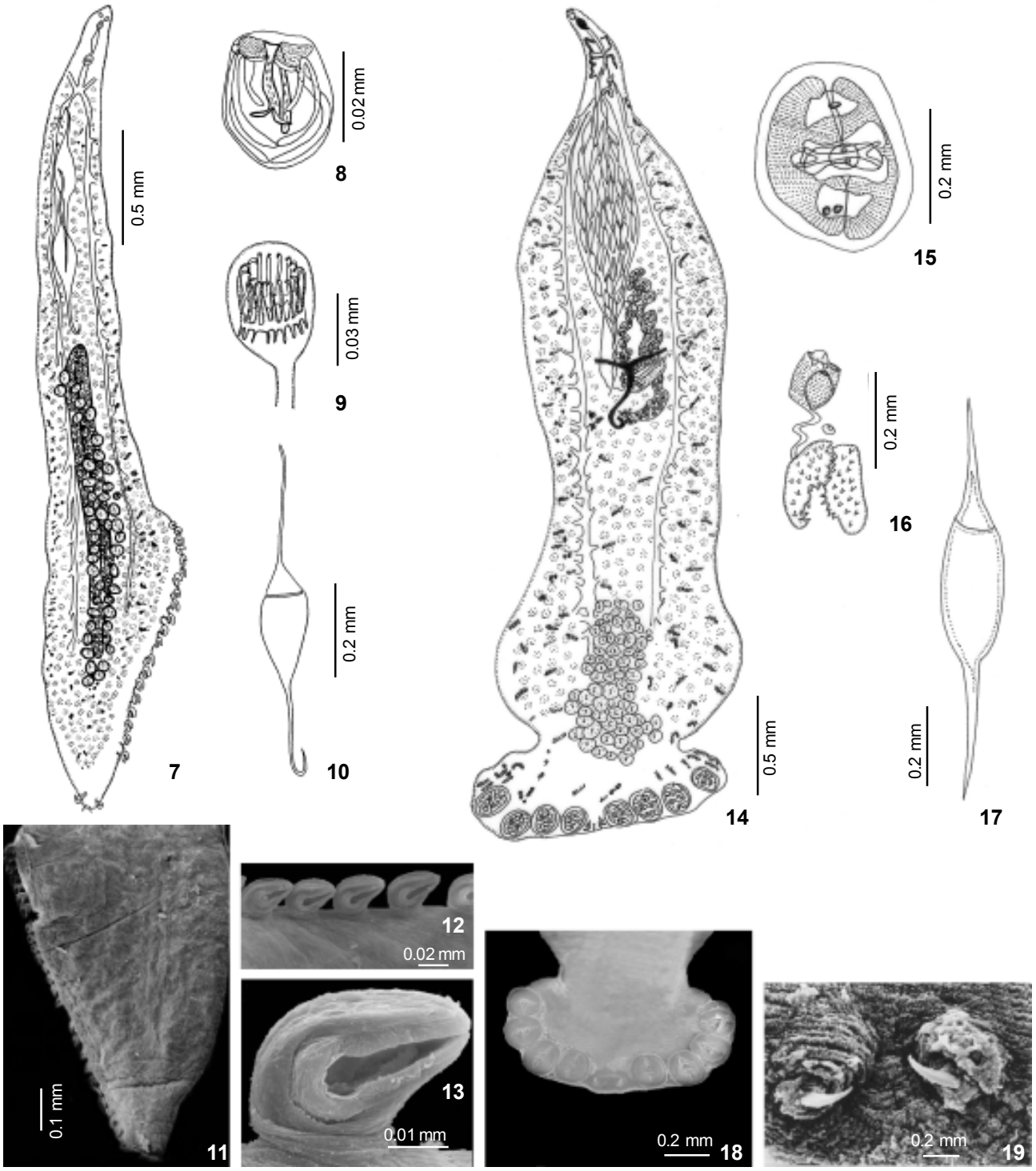
SEM. Haptor asymmetrically triangular. Clamps small, similar in size (Fig. 11), symmetrically disposed in single row, project from body proper on reduced peduncles (Fig. 12); clamp valves different in size: larger upper one covers smaller lower ones, directing clamp aperture downwards (Fig. 13).

Hexostomatidae

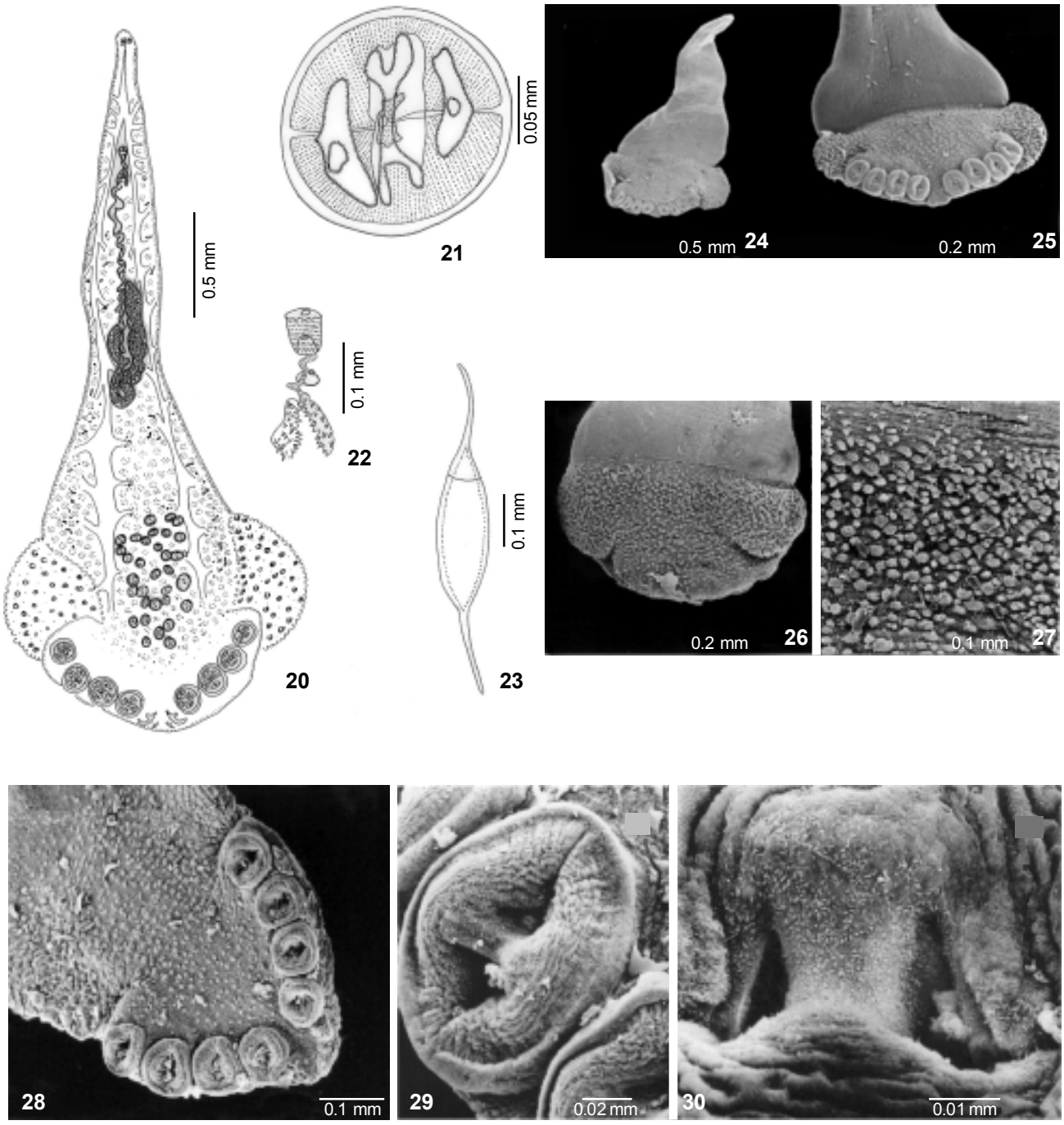
Hexostoma auxisi Palombi, 1943

Figs 14-19

Based on 10 specimens. Body 3.69-9.32 (7.10) long, constricted in the region between body proper and haptor; maximum width, not including haptor, 0.539-2.80 (1.66) (Fig. 14). Anterior end of body slender; one pair of suckers 0.023-0.037 × 0.184-0.037 (0.030 × 0.027). Pharynx 0.018-0.690 × 0.014-0.046 (0.029 × 0.032); oesophagus 0.308-0.893 (0.570) long; caeca with lateral diverticula extends to haptor. Haptor sym-



Figures 7-19. (7-13) *Churavera triangular*: (7) general ventral view with typical triangular shape haptor; (8) clamp with median sclerite with bifid tip; (9) detail of genital corona; (10) egg; (11) detail of haptor in ventral view; (12) row of pedunculated clamps; (13) detail of clamp. (14-19) *Hexostoma auxisi*: (14) ventral view of body with median constriction; (15): sucker-like clamp with median sclerite X-shaped; (16) vagina with two indented valves; (17) egg; (18) haptor with four pairs of clamps and anchors; (19) detail of small anchors.



Figures 20-30. *Hexostoma keokeo*: (20) ventral view of body with papillated lobes near haptor; (21) sucker-like clamp; (22) detail of vagina; (23) egg; (24) ventral view of body with lateral lobes and haptor; (25) clamps horizontally disposed in adult; lateral lobes and haptor in ventral view with papillae; (26) dorsal view of body, haptor and lobes covered with papillae; (27) detail of dome shaped papillae; (28) juvenile with papillate tegument, prehaptoral lobes absent and clamps disposed in almost vertical rows; (29) detail of sucker-like clamp with two semi-circular muscular pads; (30) detail of median sclerite of clamp with ciliated-like sensory receptors.

metrical, with 4 pairs of sessile clamps disposed almost in horizontal line. Clamps sucker-like, 0.231-0.351 × 0.218-0.332 (0.298 × 0.263), composed of three sclerites imbedded in two muscular pads; median sclerite "X"-shaped, perforated (Fig. 15). Two pairs of anchors terminal, 0.100-0.136 (0.114) and 0.027-0.046 (0.036) in length. Testes 50-70 (56), post-ovarian, at posterior end of body; vas deferens sinuous; muscular copulatory organ 62-73 (69) long. Ovary tubular, handle-like, 2.42-4.08 × 0.54-0.77 (3.55 × 0.69); seminal receptacle 0.33-0.52 × 0.11-0.19 (0.41 × 0.15). Vitelline follicles extend into haptor; vitelline reservoire Y-shaped. Enlarged uterus package with eggs. Vagina 0.082-0.100 × 0.087-0.114 (0.087 × 0.104), composed of two symmetrical indented valves (Fig. 16). Egg fusiform 0.123-0.277 × 0.064-0.123 (0.209 × 0.087), with two polar filaments of equal size, 0.141-0.215 (0.172) long (Fig. 17).

SEM. Haptor with smooth tegunment, without glands; clamps similar in size, typically disposed side by side in two almost horizontal rows separated by anchors (Fig. 18) whose tips project through tegument (Fig. 19).

Hexostoma keokeo Yamaguti, 1968

Figs 20-28

Based on five specimens. Body 2.717-6.747 (4.91) long; maximum width, not including haptor, 0.831-2.671 (1.88) (Fig. 20). Anterior end of body slender; one pair of suckers 0.018-0.032 × 0.018-0.028 (0.023 × 0.023). Pharynx 0.018-0.046 × 0.018-0.023 (0.031 × 0.020); oesophagus 0.21-0.97 (0.56) long. Caeca with lateral diverticula reach to haptor. Haptor papillate 0.667-1.380 (1.040) long by 1.307-3.172 (2.330) wide with two lateral papillate lobes, 0.385-1.124 × 0.360-0.893 (0.737 × 0.602) (Fig. 20). Four pairs of clamps, 0.123-0.285 (0.197) × 0.132-0.304 (0.199), aligned horizontally. Clamps sessile, sucker-like; oval muscular pads enclose 3 sclerites (Fig. 21). Two pairs of terminal anchors, 0.016-0.028 (0.024) and 0.073-0.109 (0.085) in length. Testes 20-32 (22) post-ovarian. Bulbous muscular cirrus, 0.046-0.092 × 0.032-0.055 (0.072 × 0.044). Ovary tubular, sinuous 0.617-2.66 (1.78) long; vagina typical for genus, 0.064-0.087 × 0.055-0.085 (0.074 × 0.069) (Fig. 22). Egg fusiform 0.173-0.215 × 0.069-0.087 (0.198 × 0.075); filaments on each pole, equal in size, 0.078-0.132 (0.113) long (Fig. 23).

SEM. Adult body slender anteriorly, widening towards posterior region of body. Pre-haptoral hemispherical lobes project laterally from body; tegunment covered by numerous papillae; haptor proper also covered with papillae, with four symmetrical pairs of clamps aligned horizontally in two groups, separated by anchors (Fig. 24). Lateral lobes in ventral view have larger number of papillae than central part of haptor; clamps horizontally disposed only in adult (Fig. 25). In dorsal view, papillae also cover lobes and entire posterior region of body and haptor (Fig. 26). Detail of dome shaped papillae reveals absence of sensilla (Fig. 27). Juveniles with papillate tegument readily visible; prehaptoral lobes absent; clamps disposed in different, symmetrical, almost vertical rows (Fig. 28). Shape of clamps, in both adult and juveniles, remain identical; external border entire; folded tegument invests two semi-circular muscular pads and median sclerite; two cavities present between median and lateral sclerites (Fig. 29). Clamp tegument covered with ciliated-like sensory receptors (Fig. 30).

DISCUSSION

In addition to the Monogenea, the parasite fauna reported from *Auxis thazard* off Rio de Janeiro included the trematodes *Annulocystis auxis* (Taschenberg, 1879), *Colocyntotrema auxis* Yamaguti, 1951, *Dinurus euthynni* Yamaguti, 1934, *Lecithochirium microstomum* Chandler, 1935, *Rhipidocotyle pentagonum* (Ozaki, 1924) and *Tergestia laticollis* (Rudolphi, 1819); the larval tetraphyllidean (*Scolex pleuronectis* Müller, 1788) and trypanorhynch cestodes; the nematodes *Oncophora melanocephala* (Rudolphi, 1819), *Prospinitectus exiguus* Crites, Overstreet & Maung, 1993, *Anisakis typica* Diesing, 1860 larvae, *Contracaecum* sp. larvae, *Raphidascaris* sp. larvae and *Goezia* sp. larvae; the acanthocephalans *Rhadinorhyncus tenuicornis* (Linton, 1891), *R. pristis* (Rudolphi, 1902) and *Bolbosoma* sp. larvae; and the copepod *Caligus bonito* Wilson, 1905 (Mogrovejo & Santos 2002a).

Among the Monogenea found, *Allopseudaxine macrova* has also been reported from *A. thazard* by Mamaev (1968) in the South China Sea and by Murugesh (1995) in the Bay of Bengal, Arabian Sea and off Hawaii, while *Churavera triangula* have been reported from the same host in the South China Sea (Mamaev 1967, Lebedev 1986) and in the Pacific Ocean (Pozdnyakov 1990). When the surface topography of the clamps of these two gastrocotylines is compared differences in the shape, position and insertion of clamps on the haptor were noted. In *A. macrova*, the clamps have valves of a similar size which are deeply embedded in the haptor and open ventrolaterally, while, in C. triangula, the small clamps are pedunculate and have valves of a markedly different size which direct their aperture downwards. These are the first reported observations of these species using scanning electron microscopy.

Hexostoma auxisi was described by Palombi (1943) from the gills of *A. thazard* off Genova, Italy. Subsequently, it has been reported from the same host in the Pacific Ocean (Pozdnyakov 1990). Yamaguti (1968) described *H. keokeo* from the same host off Hawaii, differentiating it from *H. auxisi* by the presence of a pair of lateral, papillate lobes on the haptor. He also mentioned that juveniles did not present these lobes and that their clamps were disposed in vertical rows. Murugesh (1995) proposed that *H. keokeo* be considered a synonym of *H. auxisi*, indicating that the diagnostic character used, the presence/absence of papillate lobes on the haptor, could be only used to differentiate juveniles from adults. The juveniles and adult specimens of *H. keokeo* collected off Rio de Janeiro pre-

sented papillae but had no constriction between the end of body proper and the haptor, and were thus readily distinguishable from *H. auxisi*, which has a definite constriction and smooth haptor, lacking papillae. The papillate lobes of *H. keokeo* only appear when they are adult and change the original orientation of clamps from vertical to horizontal, in agreement with the observations of YAMAGUTI (1968). It is worth noting that the papillate tegument is readily visible, even in juveniles, but that the prehaptoral lobes are completely absent in the latter, and that the clamps are disposed in different, symmetrical and almost vertical rows. As the papillate lobes grow, the central region of haptor extends, pushing the clamps into a horizontal arrangement. The original description (YAMAGUTI 1968), based on light microscopy, indicated that only adults had papillate lobes, and this study has shown that although juveniles have no papillate lobes, a papillate tegument is present on both adult and juvenile haptors.

The examination of Murugesh's material (BMNH No. 1993.5.18.3) indicated that it is in fact H. keokeo and not *H. auxisi*. Thus her references to *H. auxisi* in contributions between 1992 and 1995 (*e.g.* MURUGESH 1995, MURUGESH & MADHAVI 1995) in India are erroneous. Consequently, her synonymy of *H. auxisi* and *H. keokeo* is not here considered valid.

ACKNOWLEDGEMENTS

To Dr David I. Gibson, The Natural History Museum, London, for the loan of material and comments on the manuscript. To CNPq and FAPERJ for financial support.

REFERENCES

LEBEDEV, B.I. 1986. **Monogenea suborder Gastrocotylinea.** Akademiya Nauka, Leningrad, 200p.

MAMAEV, Y.L. 1967. Pseudaxine triangula sp. n. and Metapseudaxine ventrosicula gen. et sp. n., and their position in the system of Monogenoidea. **Zoologicheskii Zhurnal**, Moscow, **46**: 993-998.

————. 1968. Helminths of tunafish in the South Chines Sea, p. 5-27. *In*: K.I. SKRJABIN & Y.L. MAMAEV (Eds). **Helminths of Animals of the Pacific Ocean.** Moscow, Neuka, 158p.

MOGROVEJO, C. & C.P. SANTOS. 2002a. Biological tags and environmental factors in southwestern Atlantic waters, p. 229-235. *In*: S.P.A. MONDUZZI (Ed.). **Proceedings of the 10th International Congress of Parasitology.** Bologna, Medimond Inc., 660p.

————. 2002b. *Caballerocotyla lenti* sp. nov., a Capsalid Monogenean from *Auxis thazard* (Scombridae) from off the Southeastern Coast of Brazil. **Memórias do Instituto Oswaldo Cruz**, Rio de Janeiro, **97** (7): 1067-1071.

MURUGESH, M. 1995. Monogenetic trematodes from scombrid fishes of the Visakhapatnam coast, Bay of Bengal. **Journal of Natural History**, London, **29**: 1-26.

MURUGESH, M. & R. MADHAVI. 1995. Some new and known species of the genus *Didymocystis* Ariola, 1902 (Trematoda: Didymozoidae) from scombrid fishes of the Visakhapatnam coast, Bay of Bengal. **Systematic Parasitology**, Dordrecht, **31**: 11-24.

PALOMBI, A. 1943. Notizie helminthologiche. VIII. - Le specie italiane del genere *Hexostoma* Raphinesque (= *Hexacotyle* Blainville). **Annales Museium Zoologie Reversidad**, University Napoli, Napoli, **7**: 1-8.

POZDNYAKOV, S.E. 1990. **Helminths of scombrid-like fishes of the world's oceans.** Vladivostok, Dal'nevostochnogo Otdeleniya Akademiya Nauka, 185p.

YAMAGUTI, S. 1968. **Monogenetic trematodes of Hawaian fishes.** University of Hawaii Press, Honolulu, 288p.

Received in 09.IX.2003; accepted in 29.IV.2004.