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PARACREPTOTREMA BLANCOI N. GEN., N. SP. (DIGENEA: PLAGIORCHIFORMES: ALLOCREADIIDAE) IN THE OLOMINA, PRIAPICHTHYS ANNECTENS (OSTEICHTHYES: POECILIIDAE), FROM THE ÁREA DE CONSERVACIÓN GUANACASTE, COSTA RICA

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ABSTRACT: A new species of allocreadiid digenean is described from *Priapichthys annectens* (Regan) (Osteichthyes: Poeciliidae) in the Área de Conservación Guanacaste, Costa Rica. A new genus, *Paracreptotrema*, is proposed to accommodate this species as well as *Fellodistomum mendezi* Songandares-Bernal, 1955, which was previously described from another poeciliid, *Brachyrhaphis episcopi*, in Panama. *Paracreptotrema* differs from all other nominal genera of Allocreadiidae by a combination of its symmetrical testes, restricted vitellaria, and the lack of oral lappets (muscular ‘papillae’) or other such appendages. *Paracreptotrema blancoi* n. sp. resembles *Creptotrema creptotrema* Travassos, Artigas and Pereira, 1928, but differs in lacking ventral oral lappets and in having vitellaria extending posteriorly only to the level of the testes. It can be distinguished from *P. mendezi* n. comb. in having a relatively larger and more posteriorly placed acetabulum, vitellaria that are more restricted anteriorly, smaller testes, and a uterus that does not extend to the posterior end of the body. The genus seems to be restricted to poeciliids in Central America.

The parasites of freshwater fishes in Central America remain poorly known. Previous studies include surveys of some fish species in Nicaragua (Watson, 1976; Aguirre-Macedo et al., 2001a, 2001b) and reports of individual parasite species (Songandares-Bernal, 1955; Caballero and Brenes, 1957; Bravo-Hollis and Arroyo, 1962; Vidal-Martínez et al., 2001; Choudhury et al., 2002; Scholz et al., 2004). Of these, the reports of the digeneans *Acanthostomum gnerii* Szidat, 1954, 2 *Crassicutis* spp., and, most recently, *Wallinia chavarriae* Choudhury, Hartvigsen-Daverdin and Brooks, 2002, seem to be the only records of endohelminths inhabiting freshwater fish from Costa Rica (Caballero and Brenes, 1957; Bravo-Hollis and Arroyo, 1962; Choudhury et al., 2002; Rodríguez-Ortiz, García-Prieto, and Pérez-Ponce de León, 2004; Rodríguez-Ortiz, García-Prieto, Herrera-Vázquez, and Pérez-Ponce de León, 2004). During June, 1998, freshwater fishes from the Área de Conservación Guanacaste (ACG) in northwestern Costa Rica were examined as part of an ongoing biodiversity inventory of the eukaryotic parasites of vertebrates in this area (<http://brooksweb.zoo.utoronto.ca>). During that study, a hitherto undescribed species of digenean was collected in the poeciliid *Priapichthys annectens* (Regan), which is described here.

MATERIALS AND METHODS

Eight *P. annectens* were caught on 28 June 1998, in the Rio Orosí, in the ACG, Guanacaste Province, Costa Rica. Fish were caught by electroshocking, brought to the laboratory at the Centro de Investigación de the ACG's Santa Rosa field station, and examined within 1–4 hr of capture. Digeneans were collected live, washed briefly in 0.6% saline, killed by rapid immersion in steaming hot water, followed immediately by fixation in alcohol-formalin-acetic acid, or killed and fixed simultaneously in steaming hot 5% formalin (VanCleave and Mueller, 1932) accompanied by brief, vigorous shaking. They were stained with acetocarmine, dehydrated, and mounted in balsam on slides or between 2 coverslips. The double coverslip preparations were permanently held

in aluminum Cobb slides. Specimens were examined using bright-field and differential interference contrast optics on Olympus BX 50 and BX 51 microscopes. Illustrations were made with a drawing tube attached to the microscope.

The following museum specimens were examined (accession numbers are preceded by the museum abbreviations and the number of specimens examined are in parentheses): *C. creptotrema*: CHIOC 6086 (4), 6087 (1), 6088 (1), 6089 (19), 32.110b (2), 32.110c (1), 32.110d (1), 32.110e (2), 32.110f (1), 32.110g (1), 32.110h (1), 32.110i (1); *Creptotrematina dissimilis* (de Freitas, 1941): CHIOC 10.719a (1), 10.720 (2). *Creptotrematina dispar* (de Freitas, 1941): CHIOC 15.357 (1); *Creptotrema paraensis* Vicente, Santos and Souza, 1978: CHIOC 31.205 (holotype); *Creptotrema funduli* Mueller, 1934: USNPC 32543 (cotypes, numerous specimens); *Creptotrematina aguirrepequenoii* Jiménez, 1973, ex. *Astyanax mexicanus* (Filippi) San Marcos River, Texas (loan from Dr. Norman Dronen, Texas A&M University, College Station, Texas) (1); *Fellodistomum mendezi* Songandares-Bernal, 1955, USNPC 37482 (holotype and paratype); *Paracreptotrematina limi*: ex. *Umbra limi* (Kirtland), Brokenhead River, Manitoba, Canada, voucher specimens (>20) in AC's collection. USNPC is the U.S. National Parasite Collection, Beltsville, Maryland; HWML is the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska; CHIOC refers to Coleção Helminológica, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; and CNHE is the Colección Nacional de Helmintos, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City. Measurements are given in micrometers (μm) and are expressed as mean \pm standard deviation followed by the range in parentheses. Measurements are from 20 specimens unless otherwise mentioned.

DESCRIPTION

Paracreptotrema n. gen.

Diagnosis: Body aspinose, slightly flattened, widest in mid region, with bluntly rounded ends. Oral lappets or lobes lacking. Oral sucker smaller than acetabulum. Pharynx well developed. No discernible prepharynx. Esophagus short. Ceca reaching testes, overlapping anterior region of testes. Testes symmetrical, entire. Cirrus sac elongate, containing folded seminal vesicle, pars prostatica, short ejaculatory duct. Ovary sinistral or dextral, posterolateral to acetabulum, entire. Seminal receptacle postovarian. Laurers canal present. Vitellaria extending along and overlapping ceca ventrally, from level of esophagus to level of testes. Uterus looping between testes, loop extending posterior to testes or not, but not filling posttesticular region. Eggs few, large, operculate. Excretory bladder I-shaped, extending to level of testes.

Taxonomic summary

Type species: *P. blancoi*.

Other species: *P. mendezi* (Songandares-Bernal, 1955) n. comb. (Syn. *Fellodistomum mendezi* Songandares-Bernal, 1955).

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Etymology: The genus is named after the fact that it resembles *Creptotrema* Travassos, Artigas and Pereira, 1928 and may be closely related to it.

Remarks

The assigning of genera to Allocreadiidae remains somewhat subjective, especially in the absence of life cycle data (Gibson, 1996; Caira and Bogéa, 2005), but we place *Paracreptotrema* in Allocreadiidae because it possesses all the adult characteristics of the family described by Caira and Bogéa (2005). These characteristics include, most notably, the aspinose tegument, the unarmed cirrus, well developed cirrus sac, the absence of an external seminal vesicle, the tubular excretory vesicle restricted to the hindbody, and the general disposition of the vitellaria and gonads. *Paracreptotrema* also resembles the allocreadiid genus *Creptotrema* closely in having a similar size and fusiform shape, a conspicuous acetabulum, similarly placed gonads, mainly lateral vitellaria, and few large eggs. The most recent revision of Allocreadiidae (Caira and Bogéa, 2005) recognizes 15 genera. To this list can be added *Auriculostoma* Scholz, Aguirre-Macedo and Choudhury, 2004 (Scholz et al. 2004). *Paracreptotrema* can be readily distinguished from 10 of these 16 genera of Allocreadiidae, viz., *Creptotrema* Travassos, Artigas and Pereira, 1928; *Creptotrematina* Yamaguti, 1954; *Paracreptotrematina* Amin and Myer, 1982; *Bunoderella* Schell, 1964; *Trematichthys* Vaz, 1932; *Crepidostomum* Braun, 1900; *Bunoderina* Miller, 1936; *Allobunoderia* Yamaguti, 1971; *Bunoderia* Railliet, 1896; and *Auriculostoma* by the absence of oral lappets ('muscular papillae') or lobes (Caira and Bogéa, 2005). It differs from the other 6 allocreadiid genera listed by Caira and Bogéa (2005) (*Margotrema* Lamothe-Argumedo, 1970; *Culaeatrema* Lasee, Font and Sutherland, 1988; *Caudouterina* Martin, 1966; *Polylekithum* Arnold, 1934; *Pseudoalloeceadium* Yamaguti, 1971; and *Alloeceadium* Looss, 1900) by a combination of symmetrical testes and more restricted vitellaria.

Paracreptotrema blancoi n. sp.

(Figs. 1–4)

Description: Body 688 ± 95 (500–850) long. Anterior end rounded; body widening gradually, reaching maximum width of 349 ± 47.6 (250–450) at level of acetabulum, terminating in narrower posterior end. Oral sucker 126.7 ± 15.8 (100–155) long, 126.2 ± 13.7 (100–155) wide, with 2 papillae on posterior margin (not visible on all specimens), several papillae along outer edge; opening subterminal, anteroventrally directed. Ventral oral lappets or lobes lacking. Acetabulum 153.2 ± 15.3 (120–175) long, 174 ± 21.4 (130–205) wide, strongly muscular, sunken, visible externally by its rounded opening; tegument of acetabulum with fine striations radiating outward from acetabular opening. Oral sucker length: acetabulum length 1:1.2 (1.2–1.21) and oral sucker width: acetabulum width 1:1.4 (1.2–1.7). Prepharynx absent. Pharynx muscular, well developed, 50 ± 6.3 (40–60) long, 57 ± 7.7 (45–75) wide. Esophagus short, winding, 27.5 ± 9.7 (15–45). Cecal bifurcation short distance anterior to acetabular margin. Cecae extending posterior to acetabulum, terminating blindly at level of anterior region of testes, 204 ± 15.5 (140–280) from posterior end. Ovary 86.2 ± 17.3 (55–110) long, 69.2 ± 15.9 (35–62.5) wide, rounded, entire, dextral or sinistral, immediately postacetabular, overlapping with anterior border of testis. Mchlis' gland comprised of loose aggregate of gland cells. Seminal receptacle postovarian, muscular. Laurer's canal long, extending laterally, opening dorsal to lateral edge of testis. Uterus short, mostly pretesticular with short loop extending between testes, metraterm weakly developed. Eggs few, well developed, ovoid, operculate, 55.4 ± 2.3 (52.5–62.5) long ($n = 18$), 38.5 ± 2.3 (32.5–42.5) wide ($n = 18$). Vitellaria follicular, in 2 ventrolateral fields, extending from level of pharynx or esophagus to anterior margin of testes, follicles overlapping cecal field ventrally, row of follicles occasionally present along inner margin of ceca, follicles consisting of granular vitelline cells; vitelline ducts emptying into conspicuous median vitelline reservoir filled with vitellocalcyl cells. Testes 2, symmetrical, rounded or ellipsoidal, entire, left testis 132 ± 24.6 (90–170) long, 94.7 ± 15.8 (70–120) wide, posterior margin 134.2 ± 27.8 (95–190) from posterior end of body; right testis 134 ± 20.2 (89–167) long $\pm 95.1 \pm 18$ (74–115) wide, posterior margin 133.7 ± 26.8 (85–185) from posterior end of body. Cirrus sac elongate, median, dorsal, maximum width 52 ± 7.7 (35–62.5) ($n = 18$), containing folded tubular seminal vesicle, conspicuous

ovoid glandular pars prostatica, curved unarmed ejaculatory duct; posterior end of cirrus sac 57.1 ± 20.53 (12.5–102.5, $n = 18$) anterior to posterior margin of acetabulum. Genital pore median, at or immediately posterior to cecal bifurcation. Excretory vesicle I-shaped, tubular, narrow, reaching anteriorly to slightly beyond level of posterior margin of testes. Excretory pore terminal.

Taxonomic summary

Type host: *Priapichthys annectens* (Regan, 1907) (Osteichthyes: Poeciliidae).

Site of infection: Intestine.

Prevalence of infection: Seven of 8 infected (87.5%). 3–10 worms per fish. Mean abundance: 7.43 ± 3.1 .

Type locality: Río Orosí, ACG, Guanacaste Province, Costa Rica. Universal Transverse Mercator (UTM) Coordinates: 3804 3300. Locality is ~675 m above sea level.

Specimen deposition: Holotype: USNPC 97785. Paratypes, CNHE 5315 (3 specimens); USNPC 97786–97789 (10 specimens).

Etymology: The species is named in honor of Roger Blanco, Associate Director for Research at the ACG, for his untiring efforts in ensuring that the ACG remains one of Costa Rica's premier biodiversity conservation areas and a vital natural laboratory.

Remarks

The generic name reflects the resemblance of *P. blancoi* to *C. creptotrema*, from which it differs in lacking ventral oral lappets and in possessing vitellaria extending posteriorly only to the level of the testes. *Paracreptotrema blancoi* closely resembles *F. mendezi*, which was described "based chiefly on a single favorable specimen" (Sogandares-Bernal, 1955), in *Brachyrhaphis episcopi*, also a poeciliid, from Gatun Lake, Panama. The morphology of both species differs in some notable ways from the diagnosis of *Fellodistomum* Stafford 1904, and of *Fellodistomidae* Nicoll 1909. As part of the diagnosis for the family *Fellodistomidae*, Bray (2002) mentions a Y- or V-shaped excretory vesicle "with anterior arms reaching about the level of pharynx" and a uterus with numerous small eggs. Neither is true for *Paracreptotrema*, which has a tubular excretory bladder reaching to the level of the testes and a uterus with few large eggs. Sogandares-Bernal (1955) described the excretory bladder of *F. mendezi* as Y-shaped. Examination of the holotype and paratype of *F. mendezi* failed to confirm this shape; the holotype seems to have a tubular excretory bladder. Diagnostic characters of *Fellodistomum* include a bipartite seminal vesicle, wide pars prostatica, sinistrally submedian genital pore, and a multilobate ovary. None of these features is present in *P. blancoi* or *F. mendezi*. Furthermore, species of *Fellodistomum* are parasitic in the bile duct and gall bladder of marine teleosts (Yamaguti, 1971; Bray, 2002). On the basis of this knowledge and the resemblance of *Paracreptotrema* to *Creptotrema*, we contend that neither *P. blancoi* nor *F. mendezi* belong in *Fellodistomidae*. As a result, we transfer *F. mendezi* to *Paracreptotrema*, and it becomes *P. mendezi* n. comb. *Paracreptotrema blancoi* can be distinguished from *P. mendezi* in having a relatively larger and more posteriorly placed acetabulum, vitellaria that do not overlap the pharynx, a rounded ovary as opposed to a subtriangular ovary, smaller testes, and a uterus that does not extend to the posterior end of the body.

DISCUSSION

We tentatively assign *Paracreptotrema* to Allocreadiidae, where it joins *Creptotrema* and a diverse collection of aspinose plagiorchiiiforms (Caira and Bogéa, 2005; see Remarks section for *Paracreptotrema*). In the absence of a phylogenetic analysis that includes all allocreadiids, it is not possible at present to judge whether the absence of lappets and the restriction of the vitellaria are derived states for *Paracreptotrema*, implying that it may be a more recently evolved clade closely related to *Creptotrema*, or whether 1 or both of these character states are plesiomorphic. This situation is reminiscent of *Culaeatrema inconstans* Lasee, Font and Sutherland, 1988, originally described by Lasee et al. (1988) to represent a monotypic genus and later

FIGURES 1–4. *P. blancoi*. (1) Ventral view (some selected oocytes and cells of the testes illustrated, 1 egg overlying the seminal receptacle omitted). Bar = 100 μ m. (2) Anterior end showing tegumental papillae. Bar = 100 μ m. (3) Cirrus sac (curved ejaculatory duct mainly in en face view). Bar = 50 μ m. (4) Female reproductive complex (e, egg in proximal uterus). Bar = 50 μ m.

shown to be a species of *Bunodera* that had lost its muscular oral lappets over evolutionary time (Brooks, 1992). Caira and Bogéa (2005) chose to recognize *Culaeatrema* Lasee, Font and Sutherland, 1988, until a more extensive analysis of the Allocreadiidae is available.

Paracreptotrema blancoi and *P. mendezi* are both parasites of poeciliid fishes, and, at present, the genus is known only from Central America. It is possible that such parasites will be found in these and other poeciliids elsewhere in the Neotropical region. Within the ACG, *P. blancoi* seems to be an exclusive parasite of *P. annectens* in Río Orosí, which is an Atlantic drainage. None of the other 369 individuals, belonging to 21 species of fishes, examined in the ACG during this phase of the work (June 1998, Choudhury et al., 2002; data not shown) was infected with this species. In addition, none of the 47 individuals of this poeciliid that were examined in other rivers of the Atlantic drainage portion of the ACG during June 1998 and March 2001 was found infected with *P. blancoi*.

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