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Stephen S. Curran

Gulf Coast Research Laboratory, stephen.curran@usm.edu

Robin M. Overstreet

Gulf Coast Research Laboratory, robin.overstreet@usm.edu

Vasyl V. Tkach

University of North Dakota

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PHYLOGENETIC AFFINITIES OF *PLAGIOCIRRUS* VAN CLEAVE AND MUELLER, 1932 WITH THE DESCRIPTION OF A NEW SPECIES FROM THE PASCAGOULA RIVER, MISSISSIPPI

Stephen S. Curran, Robin M. Overstreet, and Vasyly V. Tkach*†,

Department of Coastal Sciences, The University of Southern Mississippi, 703 East Beach Drive, Ocean Springs, Mississippi 39564.
e-mail: stephen.curran@usm.edu

ABSTRACT: *Plagiocirrus loboides* n. sp. (Digenea: Opecoelidae) is described from *Fundulus nottii*, *F. dispar blairae*, *F. chrysotus*, and *Notemigonus crysoleucas* from the Pascagoula River in Mississippi. *Plagiocirrus loboides* differs from *P. primus* Van Cleave and Mueller, 1932, by having a longer postcecal space (14–25% of body length vs. about 7%); a more anterior vitellarium (extending at least to the middle of the ventral sucker vs. to its posterior margin); and larger eggs (51–71 μm long by 23–34 μm wide vs. 40–55 μm long by 30–35 μm wide). *Plagiocirrus loboides* differs from *P. testeus* Fritts, 1959, by having a long postcecal space (vs. <5% of body length); irregular, oblique, contiguous testes (vs. strongly lobed, well separated, tandem testes); and a more extensive vitellarium. *Plagiocirrus loboides* differs from both congeners by having an ovary comprised of 3 or 4 distinct lobes rather than having an entire ovary. *Plagiocirrus wuyienensis* Wang, 1981, from *Hemimyzon zebroidus* in Fujian Province, China, is herein considered a species inquirenda because it has a Y-shaped excretory bladder. Molecular phylogenetic analysis of 28S rDNA gene fragments from *P. loboides* and 17 digenean species demonstrates that *Plagiocirrus* belongs in Opecoelidae.

Plagiocirrus Van Cleave and Mueller, 1932, was erected for *P. primus* Van Cleave and Mueller, 1932, a freshwater trematode parasitizing the golden shiner, *N. crysoleucas* (Mitchill, 1814) (Cypriniformes: Cyprinidae), in Oneida Lake, New York. Van Cleave and Mueller (1932) placed *Plagiocirrus* in Allocreadiidae, a catchall family for freshwater digeneans of fish at that time. They considered *Plagiocirrus* similar to *Plagioporus* Stafford, 1904, also a notional allocreadiid at the time. The initial treatment of *Plagiocirrus* in Allocreadiidae contributed to a legacy of classification problems in Xiphidiata. Van Cleave and Mueller (1934) advocated a diagnosis of Allocreadiidae based on adult features that was more accommodating than diagnoses followed by most of their contemporary workers. Along with *Plagiocirrus* and *Plagioporus*, Van Cleave and Mueller (1934) included *Lissorchis* Magath, 1917, *Macroderoides* Pearse, 1924, *Triganodistomum* Simer, 1929, and *Alloglossidium* Simer, 1929 in Allocreadiidae, with *Alloglossidium* accepting species previously placed in *Plagiorchis* Lühe, 1899 from freshwater fishes in North America. They justified this classification on the basis that: (1) they could find no distinguishing familial characters separating *Lissorchis* from Allocreadiidae; and (2) members in *Macroderoides*, *Triganodistomum*, and *Alloglossidium*, all previously included in Plagiorchidae (e.g., Magath, 1917; Pearse, 1924; Simer, 1929), had I-shaped excretory bladders like allocreadiids, whereas, according to Van Cleave and Mueller (1934), true plagiorchids all had Y-shaped excretory bladders. Van Cleave and Mueller (1934) considered *Plagiocirrus*, *Plagioporus*, *Lissorchis*, and *Triganodistomum* to be closely related within Allocreadiidae because their members all had sinistral genital pores. Many subsequent workers initiated a greater understanding of the freshwater fish digeneans by emphasizing the importance of larval features when classifying genera. Hopkins (1933, 1934) recognized that the North American papillose allocreadiids and at

least 1 nonpapillose species, *Allocreadium isoporium* (Looss, 1894), from Europe, had ophthalmoxiphidiocercariae. DeGiusti (1962) confirmed that *A. lobatum* Wallin, 1909, from North America, also had an ophthalmoxiphidiocercaria. McMullen (1935) demonstrated that *Macroderoides typicus* (Winfield, 1929) and *Alloglossidium corti* (Lamont, 1921) had xiphidiocercariae and transferred the genera back into Plagiorchidae. Similarly, noting that *Lissorchis fairporti* Magath, 1917 possessed a xiphidiocercaria, McMullen (1937) considered Lissorchiidae to be a valid family in Plagiorchioidea. Wallace (1939) demonstrated that a tailless cercaria, identified as *Cercaria mutabile* Cort, 1918, lacking an oral stylet, developed into the adult of *Triganodistomum mutabile* (Cort, 1918). Wallace (1941) compared the cercaria of *T. mutabile* with that of *L. fairporti*, which had a tail and oral stylet, and concluded that the overall morphology was otherwise similar and that the 2 species belonged in Lissorchiidae based on similarity of larval and adult features and despite some cercaria differences. Consequently, *Triganodistomum* and *Lissorchis* were thereafter segregated from *Plagiocirrus* and *Plagioporus* and grouped in Lissorchiidae (Wallace, 1941). Manter (1947) tentatively created Plagioporidae in Opecoelidae for *Plagioporus* and a number of other notional allocreadiids he recognized belonged in Opecoelidae on the basis that they had cotylomicrocercous cercariae. The larval stages of *Plagiocirrus* continued to elude collectors and the genus remained in Allocreadiidae.

For a time, Yamaguti (1954) included *Plagiocirrus* in Zooconidae presumably because the vitellarium consisted of relatively small, paired groups of follicles. Later, Yamaguti (1958) returned *Plagiocirrus* to Allocreadiidae placing it in the new subfamily Urorchiinae with *Urorchis* Ozaki, 1927, a genus with species in Japanese freshwater fishes. Gibson and Bray (1982) advocated a concept of Plagioporidae in Opecoelidae modified from Manter (1947) that included all opecoelids that had a distinct cirrus sac and a canalicular seminal receptacle; however, these authors dealt mainly with European genera and did not classify *Plagiocirrus*. Shimazu (1990) on the other hand partially followed the classification of Yamaguti (1971) and advocated that *Plagiocirrus*, *Urorchis*, and *Pseuduororchis* Yamaguti, 1971 belonged in Urorchiinae, but placed the subfamily

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* Department of Biology, University of North Dakota, P.O. Box 9019, Grand Forks, North Dakota 58202-9010.

† Institute of Parasitology, Polish Academy of Sciences, 51/55 Twarda Street, 00-818 Warszawa, Poland.

within Opecoelidae. Shimazu (1990) considered the key adult features defining Urorchiinae to be a submedian sinistral genital pore, a canalicular seminal receptacle, and preovarian ootype complex. Cribb (2005) did not recognize Urorchiinae as a distinct subfamily because the distinguishing features between Urorchiinae and Plagioporinae were ambiguous; consequently, he considered *Plagiocirrus* to belong in Plagioporinae, which he tentatively considered a senior subjective synonym for Urorchiinae.

Plagiocirrus primus infects golden shiners over a widespread range in North America from Ontario in the north (see Gibson, 1996), to Florida in the south (Williams and Rogers, 1984; Williams and Dyer, 1992). In addition, the species was reported from the fantail darter *Etheostoma flabellare* Rafinesque, 1819 (Perciformes: Percidae), in Wisconsin and the large-scaled sucker *Catostomus macrocheilus* Girard, 1856 (Cypriniformes: Catostomidae), in Idaho (Fischthal, 1947, 1952; Fritts, 1959; Anthony, 1963). Yamaguti (1971) also reported *P. primus* from the common carp *Cyprinus carpio carpio* Linnaeus, 1758 (as *Cyprinus carpio*), the northern pike *Esox lucius* Linnaeus, 1758 (Esociformes: Ecocidae), and *E. flabellare* (as *Catonotus flabellaris lineolatus*) from unspecified localities in the United States. Fritts (1959) reported both *P. primus* and a second species *P. testus* Fritts, 1959 from *C. macrocheilus* in Idaho. A third species, *P. wuyienensis* Wang, 1981, was described from a river loach *Pseudogastromyzon zebroidus* (Nichols) (= *Hemimyzon zebroidus* Nichols, 1925) (Cypriniformes: Balitoridae) from freshwater in Fujian Province, China (Wang, 1981). However, *P. wuyienensis* was described and illustrated with a Y-shaped excretory bladder (Wang, 1981); consequently, we consider it a species inquirenda. In the same publication, Wang (1981) described *Stenakron megalobramae* Wang, 1981, as a fellodistomid from the Black Amur bream *Megalobrama terminalis* (Richardson, 1846) (Cypriniformes: Cyprinidae), also from freshwater in Fujian. Cribb (2005) considered *S. megalobramae* to be a species inquirenda because of its superficial description. We note that *S. megalobramae* conspicuously resembles species in *Plagiocirrus* and occurs in a cyprinid, but the uterus does not occupy the posttesticular space, and therefore we hesitate to consider it a member of *Plagiocirrus*.

During April through June of 2003, and again in May of 2005, various fishes from the Pascagoula River were sampled for trematodes. A new species belonging in *Plagiocirrus* occurred in the intestine of 3 species of topminnows and 1 individual golden shiner. The species is herein described and partial 28S rDNA gene sequences from the species are compared to those of other Xiphidiata to test existing hypotheses regarding the systematic position of *Plagiocirrus*.

MATERIALS AND METHODS

Fish were captured from oxbows associated with the Pascagoula River, in the Upper Pascagoula River Wildlife Management Area, George County, Mississippi, with the use of a kick net or 3-m-long seine with 7-mm mesh. Fish were immediately transported live to the Gulf Coast Research Laboratory, in Ocean Springs, Mississippi, where they were

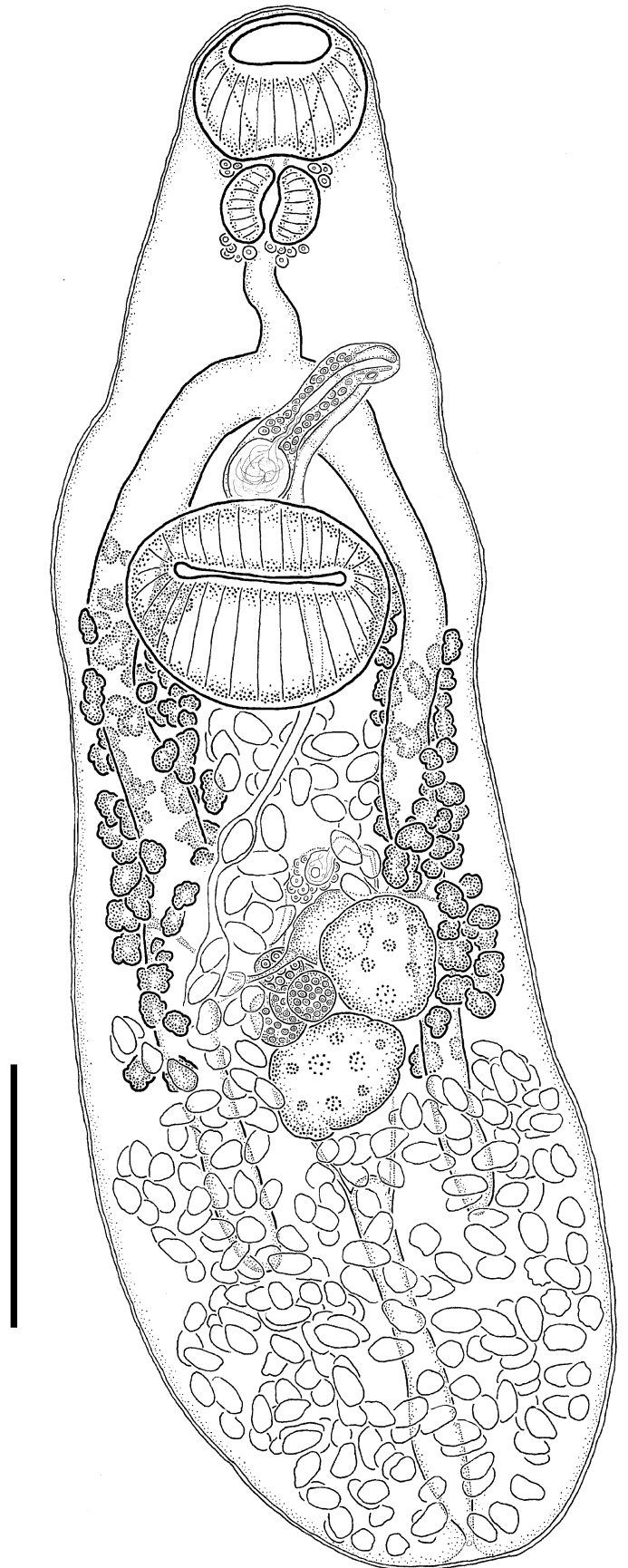
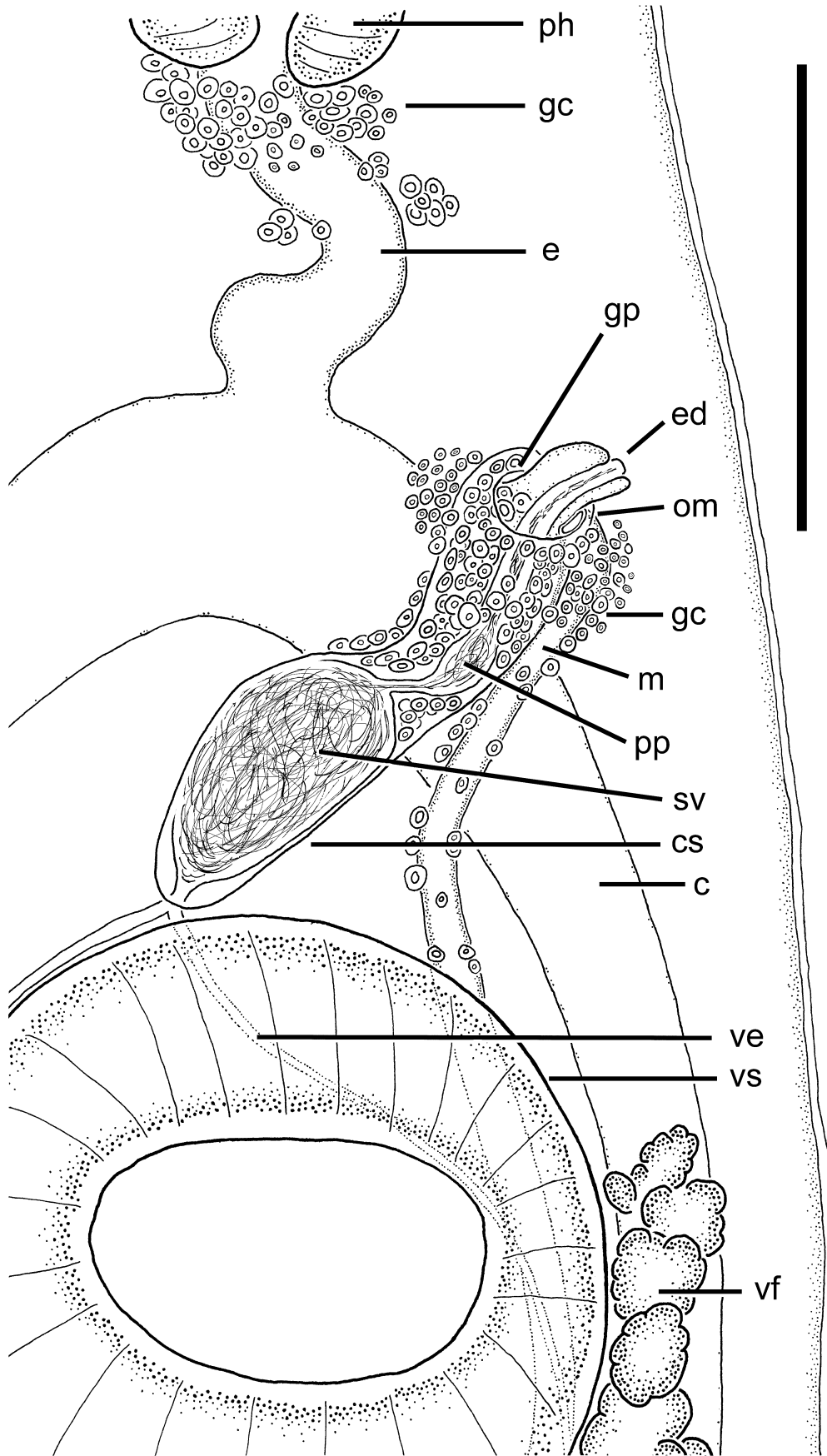


FIGURE 1. *Plagiocirrus loboides* n. sp. (Digenea: Opecoelidae) from the intestine of *Fundulus notti*, ventral view of holotype. Scale bar = 275 μ m.



necropsied within 3 days and examined for trematodes. Collected worms were placed briefly in 0.85% saline solution, then either fixed with near-boiling tap water and placed in 5% neutral buffered formalin solution or directly into 95% ethanol. Specimens in formalin were later rinsed in distilled water, stained with aqueous Van Cleave's hematoxylin, and partially dehydrated in a graded ethanol series. Drops of lithium carbonate saturated in 80% ethanol, plus a small amount of butylamine, were added to make the specimens basic. They were subsequently dehydrated through a graded ethanol series then cleared in clove oil and mounted in Canada balsam on glass slides. Drawings were made with the aid of a drawing tube. Measurements presented in the description are in micrometers and given as the mean followed by the range, when appropriate. Morphological terms used in the description followed those used by Manter (1970). Museum specimens were examined at the United States National Parasite Collection, Beltsville, Maryland (USNPC), and at the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska (HWML). These included *Plagiocirrus primus*, holotype (USNPC No. 8565), 3 voucher specimens (HWML 23690); and *Plagiocirrus testeus*, holotype and 1 paratype (USNPC No. 55622).

Genomic DNA was extracted from 2 ethanol-fixed specimens of the new *Plagiocirrus* species, under the protocol of Tkach and Pawlowski (1999). A 1,227-bp-long fragment of the nuclear 28S rDNA gene was amplified by PCR from each specimen and sequenced on an ABI Prism 3100[™] automated capillary sequencer following protocols described in Curran et al. (2006). One obtained sequence (GenBank EF523477) was aligned with those of 15 other xiphidiatans: *Plagiorchis vespertilionis* (Müller, 1784) (AF151931), *Brachycoelium salamandrae* (Froelich, 1789) (AF151935), *Prosthenhystera obesa* (Diesing, 1850) (EF032690), *A. lobatum* (EF032693), *Megalogonia ictaluri* Surber, 1928 (EF032694), *Crepidostomum cornutum* (Osborn, 1903) (EF032695), *Nagmia floridensis* Markell, 1953 (EF032691), *Phyllodistomum lacustri* (Loewen, 1929) (EF032692), *Gorgodera cygnoides* (Zeder, 1800) (AF151938), *Opecoeloides furcatus* (Bremser in Rudolphi, 1819) (AF151937), *Macvicaria mormyri* (Stossich, 1885) (AF184256), *Gaevskajatrema perezi* (Mathias, 1926) (AF184255), *Peracreadium idoneum* (Nicoll, 1909) (AY222209), *Saccocoeloides* sp. (EF032696), and *Hapladena nasonis* Yamaguti, 1970 (AY222265). *Lissorchis kritskyi* Barnhart and Powell, 1979 (EF032689) (Lissorchiidae) and *Diplomonorchis leiostomi* Hopkins, 1941 (AY222252) (Monorchiidae) were included for outgroup comparison. Sequences were all previously published by Tkach et al. (2001), Olson et al. (2003), or Curran et al. (2006). Sequences were aligned with the use of Clustal W and further aligned by eye with MacClade ver. 4.03 (Maddison and Maddison, 2000). The full alignment was deposited with European Bioinformatics Institute and is available by anonymous FTP from FTPEBI.AC.UK in directory/pub/databases/emb/align and from the EMBLALIGN database through SRS at <http://srs.ebi.ac.uk> (instructions on retrieval can be found at <http://www3.ebi.ac.uk/Services/webin/help/webin-align/alignSRShelp.html>) under accession ALIGN_001133; exclusion sets were added as comments. Regions that could not be aligned unambiguously were excluded from the analysis. Fragments in the alignment were localized at the 5' end of the nuclear 28S rDNA gene and included the D1-D3 variable domains. Fragments ranged in length from 1,225 bp in *A. furcatus* to 1,253 bp in *H. nasonis*. After gap introduction the alignment measured 1,276 bases long. Of these, 106 ambiguously aligned positions were excluded from the analysis, resulting in a total of 500 variable positions out of 1,170 unambiguous positions. A maximum likelihood (ML) analysis was performed with the use of PAUP* ver. 4.0b10 (Swofford, 2002). Nodal support was calculated based on 1,000 bootstrap replicates with 100 replicates at each step.

DESCRIPTION

Plagiocirrus loboides n. sp.

(Figs. 1–3)

Diagnosis (description based on 21 mature specimens): Body elongated, tapering in anterior third, plump, 1,525 (1,319–2,442) long, 539

(393–685) wide. Tegument relatively thin, lacking spines. Eyespot remnants lacking. Oral sucker, 172 (151–206) long, 187 (162–236) wide, with truncated base. Ventral sucker 280 (217–346) long, 307 (257–368) wide. Ratio of oral sucker to ventral sucker width 1:1.4–1.7. Prepharynx, 11–27 long. Pharynx 92 (78–112) long, 114 (89–145) wide. Esophagus slightly longer than pharynx. Forebody 481 (372–730), representing 25–34% of body length. Intestine bifurcating in forebody, closer to ventral sucker than to oral sucker, with ceca terminating blindly in hindbody. Postcecal space 328 long (201–513), 14–25% of body length.

Testes oblique, intercecal in mid-hindbody, with irregular outline, contiguous; anterior testis sinistral, 123 (85–161) long, 119 (78–189) wide; posterior testis, 143 (105–173) long, 146 (102–245) wide. Vasa efferentia joining together at proximal end of cirrus sac; cirrus sac claviform, 245 (142–363) long, 59 (45–74) at widest part, containing subspherical to elongated seminal vesicle, prostatic complex and ejaculatory duct; duct entering small genital atrium, everted in some individuals; proximal portion of cirrus sac usually dorsal to ventral sucker, sometimes entirely in forebody. Genital pore opening submedian, sinistral, approximately at level of intestinal bifurcation.

Ovary contiguous with anterior or both testis, comprised of 3 or 4 distinct lobes; overall size 96 (78–156) long, 98 (61–129) wide. Oviduct extending in anterior direction. Ootype and Mehlis' gland located anterior to ovary. Seminal vesicle canalicular. Laurer's canal surrounded by glandular cells at distal end, opening on left side of body on dorsal surface. Uterus looping throughout hindbody; proximal portion usually containing sperm; distal portion a metraterm; metraterm dorsal relative to cirrus sac, joining with dorsal wall of genital atrium. Eggs numerous, operculate, 60 (51–71) long, 27 (23–34) wide measured from metraterm or distal uterus, embryonated, without eye-spotted miracidia. Vitellarium follicular; follicles grouping in 2 lateral bunches surrounding ceca; bunches usually extending from level of middle of ventral sucker to that of middle of posterior testis (near mid-hindbody); anterior extent of vitellarium occurred at anterior margin of ventral sucker in 2 of 21 specimens. Vitelline reservoir medial, ventral relative to other components of female complex.

Excretory vesicle I-shaped, extending to level between posterior testis and cecal terminus, with 2 slender anterolateral secondary collecting ducts extending anteriorly. Pore terminal.

Taxonomic summary

Type host: Bayou topminnow, *F. nottii* (Agassiz, 1854) (Cyprinodontiformes: Fundulidae).

Other hosts: Western starhead topminnow, *F. dispar blairae* Wiley and Jones, 1975; golden topminnow, *F. chrysotus* (Günther, 1866); and golden shiner, *Notemigonus crysoleucas* (Mitchill, 1814) (Cypriniformes: Cyprinidae).

Site: Intestine.

Type locality: Pascagoula River oxbow, George County, Mississippi (30°52'49.72"N, 88°45'43.47"W).

Specimens deposited: Holotype (USNPC No. 99674); paratype (USNPC No. 99675); 2 paratypes (HWML 48535, 48536).

Etymology: The Greek adjectival name *loboides* refers to the lobed condition of the ovary in mature specimens measuring >1.5 mm in body length. The specific epithet was given in the form of an adjective to agree with the masculine genus name, which is a mixture of the Greek *Plagio* (oblique) and Latin *cirrus*. We presume the genus name was intended to be a noun representing the oblique cirrus.

Remarks

Cribb (2005), like Manter (1947), considered *Plagiocirrus* probably closely related to *Plagioporus* in Opecoelidae rather than Allocreadiidae on the basis of many shared adult features. We agree with this assessment and note that the metraterm occurs dorsal relative to the cirrus sac and enters the dorsal aspect of the genital atrium in species of *Plagiocirrus* and in members of Opecoelidae, whereas the metraterm enters

←

FIGURE 2. Terminal genitalia and nearby features, ventral view showing pharynx (ph), gland cells associated with esophagus (gc), esophagus (e), genital pore (gp), ejaculatory duct (ed), opening to metraterm (om), gland cells associated with genital pore (gc), metraterm (m), pars prostatica (pp), seminal vesicle (sv), cirrus sac (cs), ceca (c), vas efferens (ve), ventral sucker (vs), vitelline follicle (vf). Scale bar = 200 μ m.

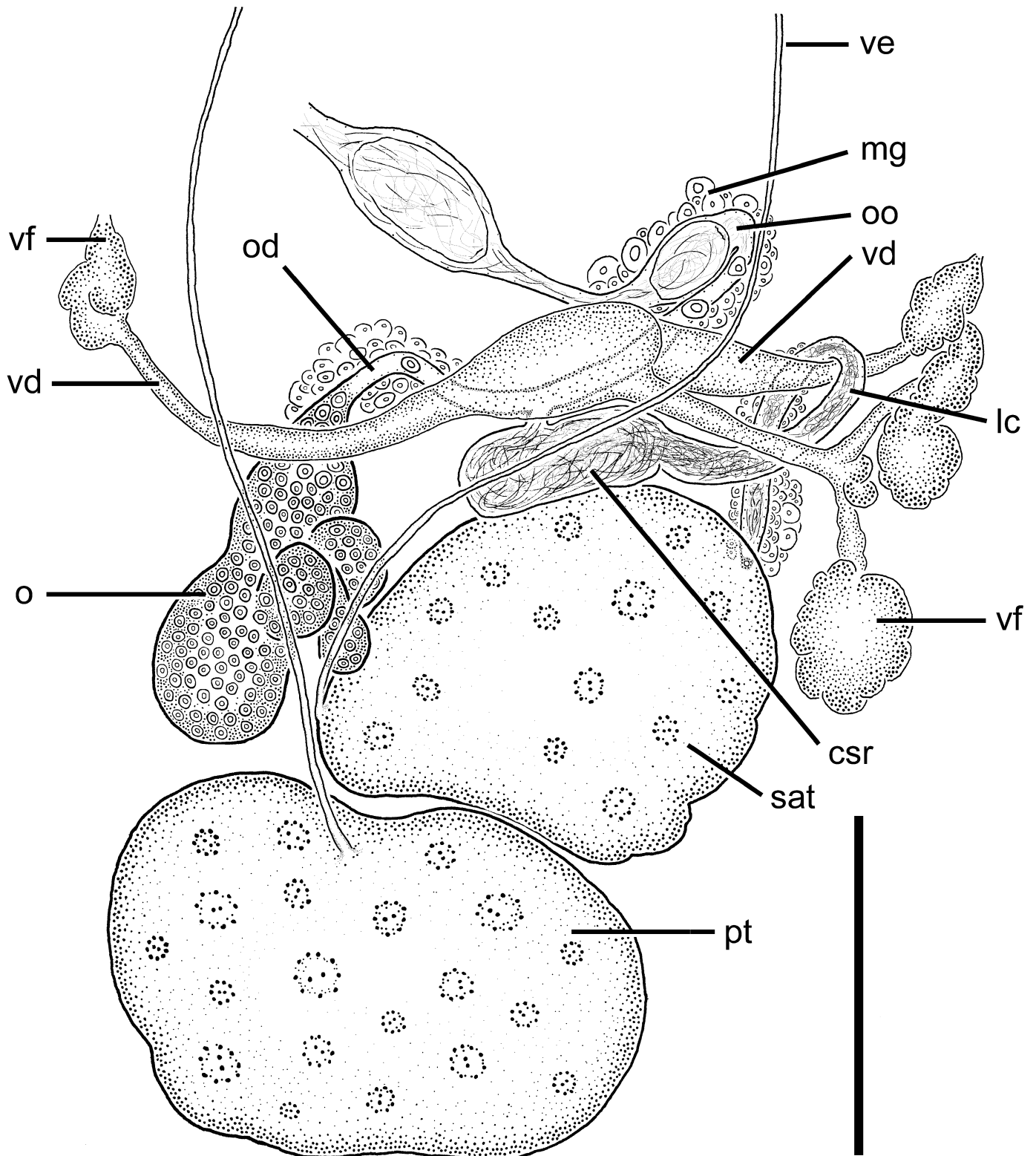


FIGURE 3. Ovarian complex and testes, ventral view showing vas efferens (ve), Mehlis' gland (mg), ootype (oo), vitelline duct (vd), Laurer's canal (lc), vitelline follicle (vf), canalicular seminal receptacle (csr), sinistral anterior testis (sat), posterior testis (pt), ovary (o), ooduct (od). Scale bar = 150 μ m.

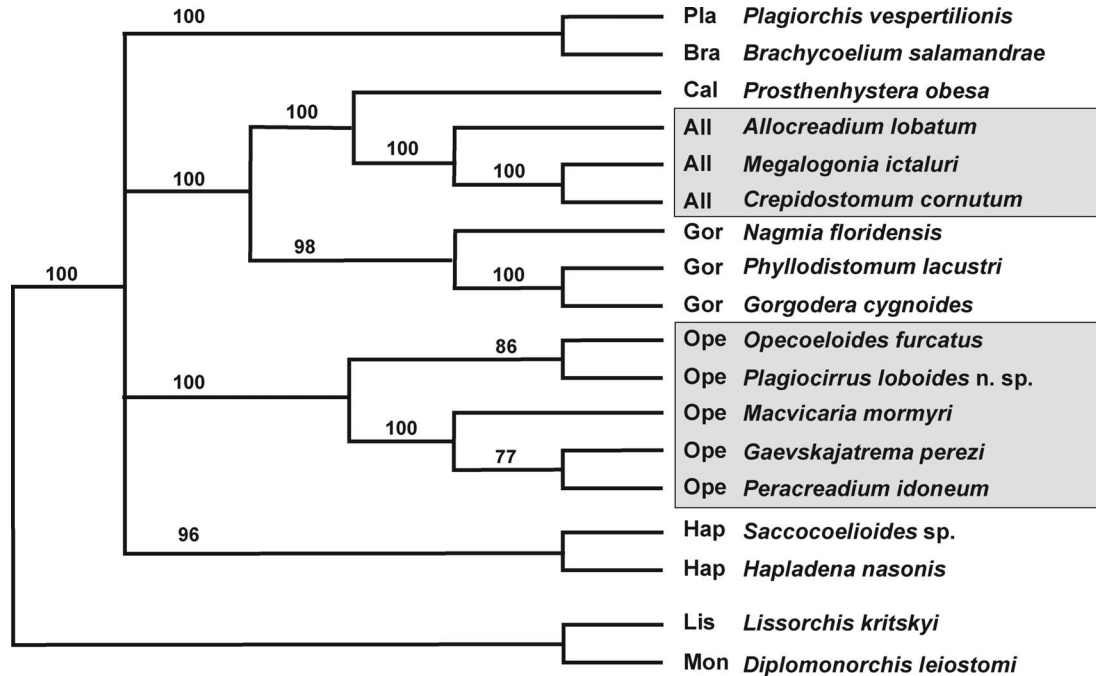


FIGURE 4. Phylogenetic tree based on analysis of partial sequences of 28S rDNA gene with the use of the maximum-likelihood algorithm in PAUP*. Bootstrap support values (1,000 bootstraps with 100 replicates at each step) are shown above internodes. Shaded rectangles demonstrate that *Plagiocirrus* belongs in Opecoelidae and not in Allocreadiidae. Abbreviations: Pla, Plagiiorchiidae; Bra, Brachycoeliidae; Cal, Callodistomidae; All, Allocreadiidae; Gor, Gorgoderidae; Ope, Opecoelidae; Hap, Haploporidae; Lis, Lissorchiidae; Mon, Monorchidae.

the ventral aspect of the genital atrium and occurs ventral relative to the cirrus sac and the cirrus sac overlies the metraterm dorsally in all members of Allocreadiidae. *Plagiocirrus* may be differentiated from *Plagioporus* by having a more extensive uterus that occupies almost the entire hindbody rather than one that is restricted to the portion of the hindbody lying anterior to the anterior testis. *Plagiocirrus* differs further from *Plagioporus* and from all other plagioporine genera by having a relatively reduced vitellarium that is restricted in lateral bands of follicles confined to the middle and anterior regions of the hindbody. The vitellarium is configured in 2 other ways within the subfamily: (1) vitellarium restricted to lateral bands of follicles that extend from the ventral sucker to the posterior end of the body, and (2) vitellarium consisting of lateral bands that extend in both the forebody and hindbody. *Plagiocirrus loboides* n. sp. belongs in *Plagiocirrus* because the vitellarium is confined to lateral bands of follicles that extend from the middle of the posterior testis to at least the middle of the ventral sucker. The vitellarium extends anteriorly to the anterior margin of the ventral sucker in 2 of 21 specimens but the vitellarium never extends into the forebody like in *Plagioporus*.

Plagiocirrus loboides is most similar to *P. primus* but differs by having a longer postcecal space measuring 14–25% of total body length compared with about 7%, a vitellarium that extends further anteriorly (at least to the middle of the ventral sucker) compared with extending to the posterior margin of the ventral sucker, and slightly longer eggs that measure 51–71 μm long by 23–34 μm wide compared with 40–55 μm long by 30–35 μm wide. In addition, the ovary in the new species is situated at the same level as the anterior testis rather than pretesticular. *Plagiocirrus loboides* differs from the other species in the genus, *P. testeus*, by having a longer post-cecal space, measuring 14–25% of total body length compared with less than 5%. Additionally, the testes of *P. loboides* are oblique, contiguous, and irregular whereas they are tandem, well-separated, and lobed in *P. testeus*. Moreover, the vitellarium is more extensive in *P. loboides* extending anteriorly from the region of the testes to at least the middle of the ventral sucker compared with being confined between the ovarian level and the posterior margin of the ventral sucker. *Plagiocirrus loboides* is most easily distinguished from its congeners by having an ovary comprised of 3 or 4 lobes rather than an entire one. Seventeen of 21 mature specimens of *P. loboides* measuring >1.4 mm in body length possessed a lobed ovary. Only 4

of 21 mature specimens measuring 1.3–1.4 mm long had a subspherical, entire ovary. Because the ovary is lobed in all specimens measuring more than 1.4 mm long, we consider this a useful feature for identifying *P. loboides*.

Neither Van Cleave and Mueller (1932) nor Fritts (1959) illustrated or described a bipartite seminal vesicle for species they described in *Plagiocirrus*. Mueller (1934) illustrated and described a simple, undivided seminal vesicle in specimens of *P. primus* from Oneida Lake. Yamaguti (1971) considered the presence of a bipartite seminal vesicle to be an important generic level feature for *Plagiocirrus*. Yamaguti (1971) refigured the holotype of *P. primus* (USNPC No. 8565), including what he termed a bipartite internal seminal vesicle in the drawing. We compared our specimens to the holotype of *P. primus*, 3 voucher specimens of *P. primus* (HWML 23690), as well as the holotype and 1 paratype of *P. testeus* (USNPC No. 55622) and concluded that all the species shared the same condition of having either a subspherical or a slightly elongate seminal vesicle rather than a bipartite one because a muscular sphincter differentiating 2 distinct portions was absent (Fig. 2).

Cribb (2005) considered the presence of a canalicular seminal receptacle an important feature characterizing Plagioporinae, but noted that a canalicular seminal receptacle had never been reported or observed in species of *Plagiocirrus*. Additionally, Cribb (2005) believed all members of Plagioporinae lacked a uterine seminal receptacle. We observed a canalicular seminal receptacle in *P. loboides* (Fig. 3) and noted that copious amounts of sperm were also present in the proximal uterus of every live specimen we observed.

Molecular analysis

Maximum-likelihood analysis of partial 28S rDNA gene sequences including 2 outgroup species (*L. kritskyi* + *D. leiostomi*) and an ingroup consisting of *P. loboides* and members of 7 digenean families resulted in a phylogenetic tree showing 4 main clades as a polytomy: (1) Haploporidae; (2) Opecoelidae; (3) Gorgoderidae + Callodistomidae + Allocreadiidae; and (4) Plagiiorchiidae + Brachycoeliidae (Fig. 4). The 4 clades and almost all subclades were characterized by very high bootstrap support values (Fig. 4). This analysis unequivocally places *Plagiocirrus* in the Opecoelidae, thus supporting the original viewpoint by

Shimazu (1990) that the genus belonged in Opecoelidae. Our analysis did not corroborate the notion advocated by Cribb (2005) that *Plagiocirrus* belongs in the subfamily Plagioporinae. *Plagiocirrus* branched most closely with the opecoeline *O. furcatus* rather than with the 3 plagioporine species *M. mormyri*, *G. perezi*, and *P. idoneum* (Fig. 4). Our results suggest that relationships among genera in Plagioporinae as perceived by Cribb (2005) in the most recent review of Opecoelidae might need reevaluation. The idea that *Plagiocirrus* may belong in Urorchiinae as advocated by Yamaguti (1971), but as a member of Opecoelidae rather than Allocreadiidae as promoted by Shimazu (1990), must be revisited.

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