

## Redescription of *Cystobranchnus virginicus* Hoffman, 1964, and *Cystobranchnus salmositicus* (Meyer, 1946) (Hirudinida: Piscicolidae) from Freshwater Fishes in North America

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**ABSTRACT:** The freshwater fish leeches of North America are poorly studied taxonomically, and criteria for genus assignment, especially for the morphologically similar genera *Piscicola* and *Cystobranchnus*, are based on the better-studied European fauna. In this study, we redescribe the internal anatomy of *Cystobranchnus virginicus* and *Piscicola salmositica* from North America on the basis of serial sections. Cords of conducting tissue connecting the bursa to the ovisacs and accessory gland cells on the atrial cornu are present in both *C. virginicus* and *P. salmositica*. Presence or absence of accessory gland cells should not be used as a character to distinguish *Piscicola* from *Cystobranchnus*. *Piscicola* is characterized by the presence of an external copulatory area and cords of conducting tissue arising from a ventral mass of vector tissue. *Cystobranchnus* is characterized by the absence of an external copulatory area and the presence of cords of conducting tissue arising from the posterior portion of the bursa. These observations confirm the previous placement of *Cystobranchnus salmositicus*.

**KEY WORDS:** redescription, *Piscicola salmositica*, *Cystobranchnus salmositicus*, *Cystobranchnus virginicus*, leeches, freshwater, Piscicolidae, suborder Rhynchobdellida, North Carolina, British Columbia, Canada, United States.

Descriptions of fish leeches in the family Piscicolidae have been based primarily on external morphology and occasionally on obvious features of the internal anatomy determined by stained whole mounts, cleared unmounted specimens, or dissections. These descriptions often lack detail to support reliable species determinations and certainly are inadequate for defining genera. Attempts to hypothesize relationships among leeches on the basis of morphology have been hindered by inadequate knowledge of internal anatomy, especially of the reproductive and coelomic systems. This information in most cases can only be obtained by serial transverse sections, which can be tedious and, unfortunately, are not attempted often.

There are few critical reviews of the North American freshwater fish leech fauna, and large geographic areas of the United States and Canada have not been surveyed for leeches. Meyer (1940, 1946a) described many new species from a large number of specimens collected in Illinois, U.S.A. Three more recent reviews included keys to known North American fish leech species (Klemm, 1982, 1991; Sawyer, 1986), but they were based primarily on collections from the upper Midwest and eastern United States, and all noted that the paucity of information on

the internal anatomy of North American fish leeches made taxonomic decisions difficult. As a result, current definitions of many genera are based on the internal anatomy of specimens from Europe simply because so little data exist for North American species. In particular, attempts to identify characters that will distinguish between the genera *Cystobranchnus* Diesing, 1859, and *Piscicola* Blainville, 1818, have been hindered by incomplete knowledge of the internal anatomy of North American species. In this study, we redescribe the male and female reproductive systems of *Cystobranchnus virginicus* Hoffman, 1964, and *Piscicola salmositica* Meyer, 1940, from North America; use the data to clarify the diagnosis of *Piscicola* and *Cystobranchnus*; and confirm the transfer of *P. salmositica* to *Cystobranchnus*.

### MATERIALS AND METHODS

Specimens of *C. virginicus* were collected in the Valley River, in western North Carolina, U.S.A., near Murphy (35°10'N; 84°00'W) in late May 1999 and 2000. Gravel redds (nests) of the river redhorse, *Moxostoma carinatum* (Cope, 1870), were agitated with a shovel, and leeches were collected in a dip net held immediately downstream. Leeches were also collected from the underside of rocks in the area of the fish redds. Leeches were observed alive with a dissecting microscope and then relaxed in weak ethanol and fixed in Bouin's fluid or 10% formalin. Three individuals were cut into 6- $\mu$ m transverse serial sections along their entire length, using standard paraffin-embedding

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techniques. The holotype (United States National Museum [USNM] 30843) and the single paratype (USNM 30844) of *C. virginicus* were examined with a dissecting microscope.

Specimens of *P. salmositica* were obtained in November 1972 from 1 of the type localities, Soos Creek hatchery near Auburn, Washington, U.S.A. (47°15'N; 122°20'W). One individual was cut into 6- $\mu$ m transverse serial sections along its entire length, using standard paraffin-embedding techniques. Additional specimens of *P. salmositica* were collected 14 October 2003 from returning chinook, coho, and chum salmon (*Oncorhynchus tshawytscha* (Walbaum, 1792);, *Oncorhynchus kisutch* (Walbaum, 1792); and *Oncorhynchus keta* (Walbaum, 1792), respectively) at the Nitinat hatchery on the Nitinat River on southwest Vancouver Island, British Columbia, Canada (48°50'N; 124°40'W). Individuals were observed alive with a dissecting microscope and then relaxed in weak ethanol and fixed in 10% formalin. One individual was cut into 6- $\mu$ m transverse serial sections along its entire length, using standard paraffin-embedding techniques. The holotype (USNM 20803), a stained whole mount, and 33 paratypes of *P. salmositica* in alcohol (USNM 42696) were also examined.

***Cystobranchus virginicus* Hoffman, 1964  
(Figs. 1–7)**

**Emended description**

Body up to 35.0 mm total length, including suckers; urosome up to 4.0 mm in width when fully engorged. Body smooth, thin-walled, transparent, and extremely flaccid, almost entirely lacking in musculature. Eleven pairs of small pulsatile vesicles apparent on lateral margins of urosome. Pigmentation consists of faint brownish-orange transverse bands most obvious on trachelosome (Fig. 1). There are 5 pigment bands on the trachelosome, 3 on the posterior portion of the clitellum, and usually only 1 or 2 obvious on the anterior portion of the urosome. Suckers small, oral up to 1.0 mm in diameter, caudal up to 1.5 mm in diameter. Oral sucker with faint brownish-orange pigment band and 2 pairs of black pigment concentrations that resemble eyes (Fig. 2). These fade rapidly after death, even in the absence of fixatives. Caudal sucker with brownish-orange pigment bands radiating from the urosome and sucker juncture to near the sucker margin, terminating in 10 black pigment concentrations that resemble ocelli (Fig. 3). These ocelli also fade rapidly on death. Eyes on the oral sucker and ocelli on the caudal sucker are much more ephemeral than those typically observed in the Piscicolidae, which often persist even after fixation. Nonetheless, *C. virginicus* should be considered to have 2 pairs of eyes on the oral sucker and 10 ocelli on the caudal sucker.

Male reproductive system with 6 pairs of testisacs, large, looping ejaculatory ducts, and large bursa. Accessory gland cells (prostate gland) weakly developed, but present dorsally on atrial cornu. A single

cord of conducting tissue emerges dorsally from the posterior portion of the bursa in an area where the interior bursa wall consists of highly folded columnar epithelium (Figs. 4, 5, 7). Cord of conducting tissue continues posteriorly, bifurcates around each side of the common oviduct, and fuses into a single cord posterior to common oviduct. Posteriorly, in region of paired ovisacs, conducting tissue cord bifurcates again, and a cord lies in close association with each ovisac for most of their length (Figs. 4, 5). External, ventral copulatory area absent.

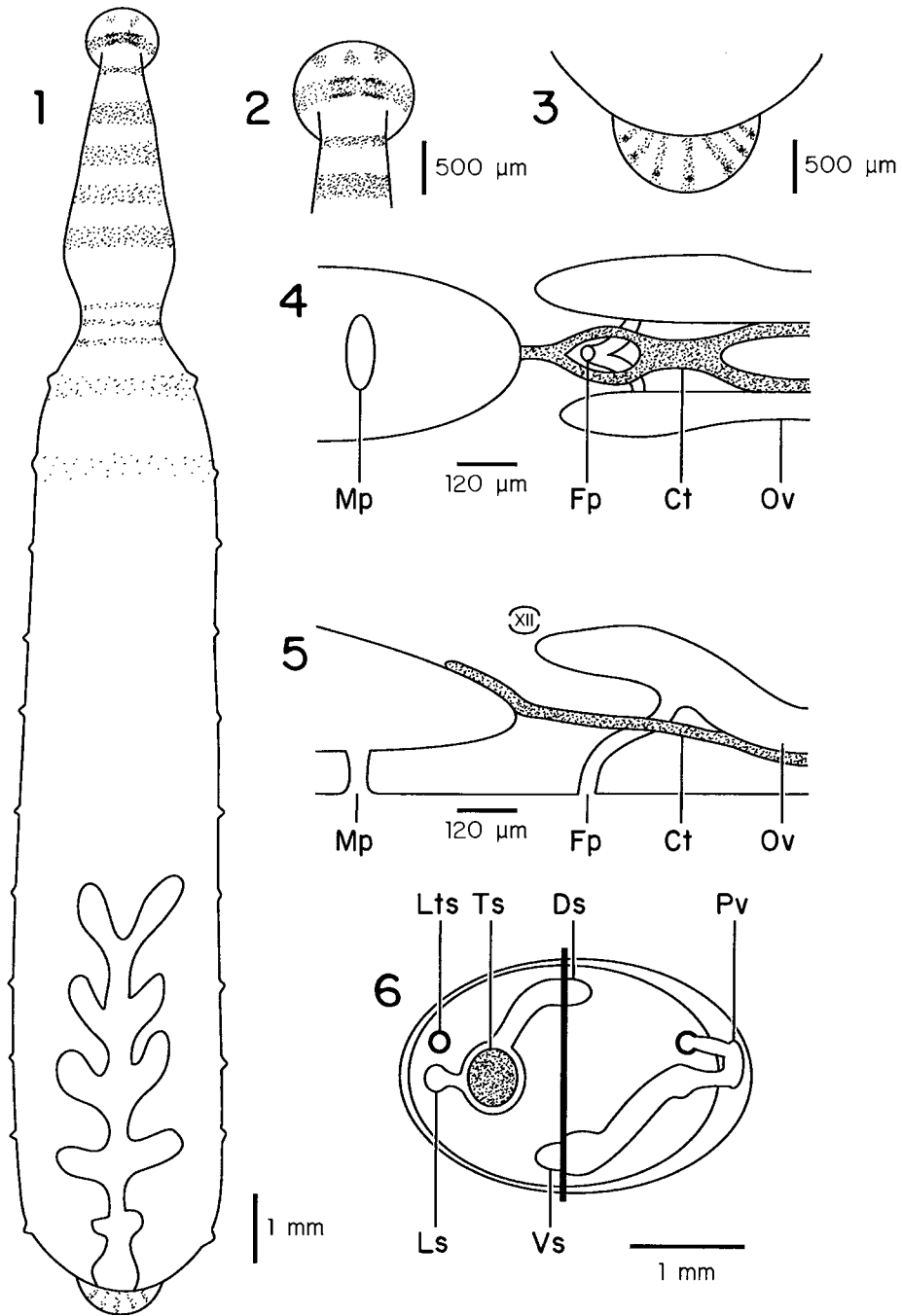
Coelomic system (Fig. 6) consists of 11 pairs of small pulsatile vesicles, dorsal and ventral sinuses, and both thin-walled and thick-walled lateral sinuses. At each urosome ganglion ventral sinus expands laterally, joins with thin-walled lateral sinus and also with pulsatile vesicle. Posterior portion of pulsatile vesicle connects with thick-walled lateral sinus. Testicular sinuses present intersegmentally and connect with thin-walled lateral sinus and with dorsal sinus (Fig. 6).

No mycetome is associated with the esophagus.

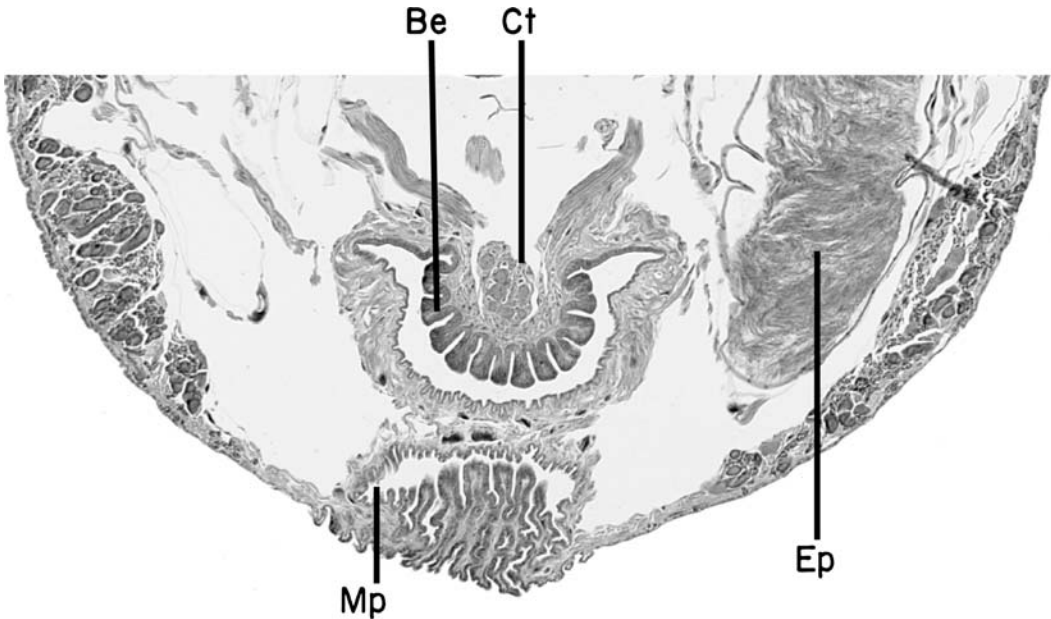
*Specimens deposited:* Specimens of *C. virginicus* from the Valley River, North Carolina, U.S.A., were fixed in 10% formalin, preserved in 70% ethanol, and deposited in the USNM, Division of Invertebrate Zoology (USNM 1024419).

**Remarks and biology**

Comparison of our specimens from the Valley River, North Carolina, U. S. A., with the holotype and paratype of *C. virginicus* clearly indicates that the specimens from the Valley River are *C. virginicus*. The original description of *C. virginicus* by Hoffman (1964) is accurate in most respects and is adequate to allow identification of the species. His drawing of the entire leech (Hoffman, 1964, fig. 1) is excellent and with the exception of pigmentation, shows most of the important features of the gut and external morphology. The emended description presented here adds information regarding the pigmentation, provides details of the coelomic system, and documents the presence of conducting tissue and accessory gland cells on the male atrium, which were not mentioned by Hoffman (1964). Serial sections also document the absence of mycetomes associated with the esophagus. Mycetomes are diverticula in the region of the esophagus that are filled with bacteria believed to aid in digestion of blood. Hoffman (1964) mentioned the presence of esophageal glands, referring to what are now called salivary gland cells associated with the proboscis.



**Figures 1–6.** *Cystobranchus virginicus*. 1. Dorsal view, drawn from life, specimen collected in the Valley River, North Carolina, U.S.A. 2. Oral sucker showing pigmentation and eyes. 3. Caudal sucker showing pigmentation. 4. Terminal portions of male and female reproductive systems, ventral view. 5. Terminal portions of male and female reproductive systems, lateral view. 6. Coelomic system, right side segmental, left side intersegmental. Ct, conducting tissue cords; Ds, dorsal sinus; Fp, female gonopore; Ls, thin-walled lateral sinus; Lts, thick-walled lateral sinus; Mp, male gonopore; Ov, ovisac; Pv, pusatile vesicle; Ts, testisac; Vs, ventral sinus.



**Figure 7.** *Cystobranchus virginicus*, transverse histological section through the posterior bursa. Be, highly invaginated epithelium of bursa wall; Ct, conducting tissue cord; Ep, epididymis portion of vas deferens; Mp, male gonopore.

This leech has an unusual feeding behavior, apparently feeding entirely on the yolk of fish eggs (Light et al., 2005). Specimens collected in the Valley River were obtained by digging up “nests” of *M. carinatum*, and the leeches were clearly engorged with egg material, rather than fish blood. The gut contents were visible through the transparent body of the leech, and the yellow-orange color was the same as that of the fish eggs. In fact, the leeches were at first mistaken for fish eggs because the gut was so engorged with egg material and the body of the leech is so transparent and flaccid that, in the dip net, they looked like bags of egg material.

Richardson (1948) described an identical situation from nests of fallfish, *Semotilus* (= *Leucosoma*) *corporalis* (Mitchill, 1817), in Fisher Creek, Brome Lake, Quebec, Canada, where leeches were “obviously engorged on eggs.” Richardson (1948) identified the leeches as *Piscicola punctata* (Verrill, 1871), although he noted their resemblance to *Piscicola milneri* (Verrill, 1871), an observation also noted by Hoffman (1964) in his description of *C. virginicus*. The feeding behavior noted by Richardson (1948) and his description of the morphology, including the transparent body and rapidly fading eyes and ocelli, match in all respects the morphology of *C. virginicus* on the basis of observations during the study reported

herein. Thus, the leech examined by Richardson (1948) was likely *C. virginicus*, and his report extends the range of the species to Quebec, Canada.

The report of a heavy infestation of *C. virginicus* on white catfish, *Ameiurus catus* (Linnaeus, 1758), in the York River estuary in Virginia (Paperna and Zwerner, 1974) is in error. The leech was later determined by Sawyer (1986) and confirmed by Bureson (1995) to be *Myzobdella lugubris* Leidy, 1851, on the basis of specimens deposited in the USNM (USNM 52722).

The reports by Putz (1972a, b) of *C. virginicus* feeding on longnose dace, *Rhinichthys cataractae* (Valenciennes, 1842) and as a vector for *Cryptobia cataractae* Putz, 1972, should be viewed with caution. No morphological description of the leech was reported, and no specimens were deposited in museums, so it is impossible to confirm the identity of the leech. However, the fish blood feeding behavior and the long-term maintenance of the leeches in the laboratory reported by Putz (1972a, b) suggest that it was not *C. virginicus*. All *C. virginicus* collected by us for feeding experiments died within a few hours of collection. In addition, leeches were not observed on any of the fish collected in the vicinity of *Moxostoma* spp. nests, where leeches were present (Light et al., 2005). Collections by E.M.B. and J.I.W. in April

2004 at Providence Forge, West Virginia, U.S.A. (39°22.025'N; 77°57.463'W), using the same electroshocking fish-collecting method in the exact location and time of year that Putz (1972a, b) sampled, yielded only *Cystobranchnus meyeri* Hayunga and Grey, 1976, on longnose dace. These collections suggest that the leeches on longnose dace examined by Putz (1972a, b) and the vector for *C. cataractae* were *C. meyeri*, which had not been described when Putz (1972a, b) conducted his research.

***Cystobranchnus salmositicus* (Meyer, 1946)  
(=*Piscicola salmositica* Meyer, 1946)  
(Figs. 8–14)**

**Emended description**

Body up to 31 mm total length, including suckers; urosome up to 6.0 mm wide when engorged. Body smooth, lacking tubercles, or papillae. Eleven pairs of large pulsatile vesicles obvious on lateral margins of urosome (Fig. 8). Pigmentation consists of stellate black chromatophores that are distributed evenly on the urosome giving the leech an overall dark gray to black coloration. Oral sucker up to 2.5 mm in diameter with 2 pairs of eyes (Figs. 8, 9). Pigmentation on oral sucker variable; stellate black chromatophores cover most of sucker, but there are often paired unpigmented areas anterior to the eyes. Caudal sucker up to 4.0 mm in diameter with 8 to 10 large ocelli around the lateral and posterior margins. Most ocelli are rod-shaped, but ocelli on lateral margins of sucker appear somewhat crescent shaped. Caudal sucker evenly pigmented with stellate black chromatophores except for unpigmented areas outside of ocelli (Fig. 10).

Male reproductive system with 6 pairs of large testisacs, convoluted ejaculatory ducts, large atrial cornu with well-developed masses of accessory gland cells dorsally and laterally on atrial cornu and on common atrium (Fig. 14), and a large bursa. Female reproductive system with 2 cords of conducting tissue arising independently from posterior portion of bursa. Cords continue posteriorly, separated for their entire length, and become closely associated with paired ovisacs (Figs. 11, 12). Coelomic system (Fig. 13) consists of 11 pairs of large pulsatile vesicles, dorsal and ventral sinuses, and both thin-walled and thick-walled lateral sinuses. At each urosome ganglion ventral sinus expands laterally, joins with thin-walled lateral sinus and also with pulsatile vesicle. Posterior portion of each pulsatile vesicle connects with thick-walled lateral sinus. Testicular sinuses present intersegmentally and connect with thin-walled lateral sinus and with dorsal sinus (Fig. 13).

No mycetome is associated with the esophagus.

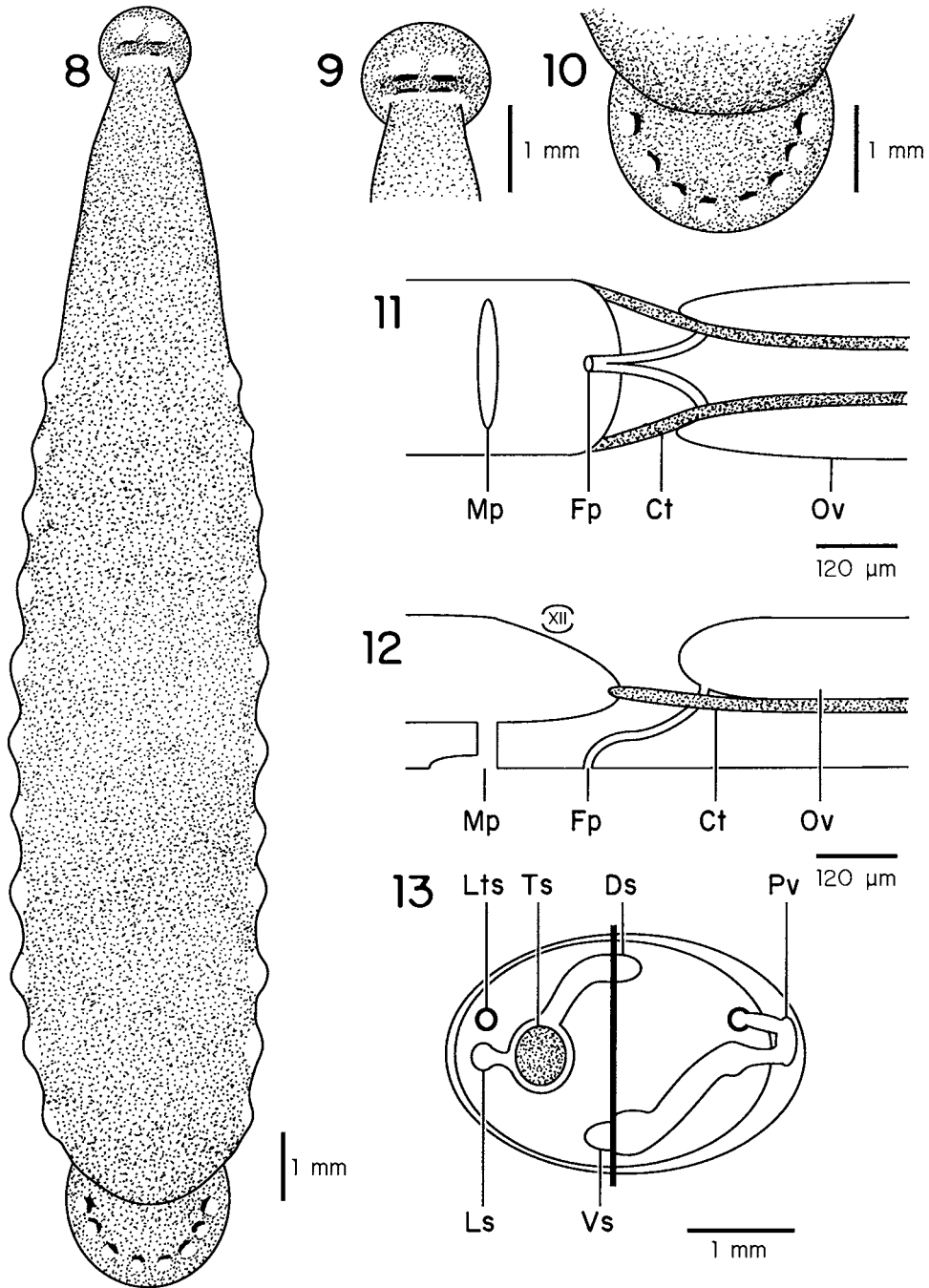
*Specimens deposited:* Specimens of *C. salmositicus* from the Nitinat River, Vancouver Island, British Columbia, Canada, were fixed in 10% formalin, preserved in 70% ethanol, and deposited in the USNM (USNM 1070012).

**Remarks**

Comparison of specimens collected from Vancouver Island, British Columbia, Canada, with the holotype and paratypes of *P. salmositica* confirm that the specimens from Canada are the same species. The original description of *P. salmositica* by Meyer (1946b), based on preserved specimens from California and Washington, U.S.A., was complete and accurate in almost all respects, omitting only information on the presence or absence of accessory gland cells on the atrial cornu, the presence or absence of mycetomes, and erroneously reporting the absence of conducting tissue. Bielecki (1997) also interpreted the absence of accessory gland cells on the atrium in the figure of Meyer (1946b) as true absence of these glands, but Meyer (1946b) made no mention of accessory gland cells, much less state their absence. Bielecki (1977) also seems to have confused the ovaries drawn by Meyer (1946b) for conducting tissue. Bielecki (1977) stated that plate III, figure 3 in Meyer (1946b) illustrates conducting tissue connecting the ovaries with the atrium, but this interpretation is incorrect. Nonetheless, *C. salmositicus* does possess conducting tissue.

All live specimens examined from Vancouver Island had 8 ocelli on the caudal sucker, and all had the body and suckers covered by stellate-shaped black chromatophores. Meyer (1946b) noted between 8 and 10 ocelli on the caudal sucker, with most specimens from California having 10 ocelli and most specimens from Washington having 8 ocelli. Meyer (1946b) also noted that most specimens from California had stellate chromatophores, whereas most specimens from Washington lacked such pigmentation; but Meyer (1946b) only observed preserved specimens, and his observations on the absence of pigmentation on most Washington specimens may have been a fixation artifact. None of the 33 paratypes examined comprising USNM 42696, all of which were collected in California, displayed pigmentation.

The number of individuals of *C. salmositicus* in the Nitinat River, and perhaps in many other Pacific Northwest salmon rivers, is 1 of the highest concentrations of piscicolid leeches anywhere. At the hatchery, the returning fish are anesthetized slightly



**Figures 8–13.** *Cystobranchus salmositicus*. **8.** Dorsal view, drawn from life, specimen collected from the Nitinat Hatchery, Vancouver Island, Canada. **9.** Oral sucker showing pigmentation and eyes. **10.** Caudal sucker showing pigmentation and ocelli. **11.** Terminal portions of male and female reproductive systems, ventral view. **12.** Terminal portions of male and female reproductive systems, lateral view. **13.** Coelomic system, right side segmental, left side intersegmental. Ct, conducting tissue cords; Ds, dorsal sinus; Fp, female gonopore; Ls, thin-walled lateral sinus; Lts, thick-walled lateral sinus; Mp, male gonopore; Ov, ovisac; Pv, pusatile vesicle; Ts, testisac; Vs, ventral sinus.



**Figure 14.** *Cystobranchus salmositicus*, transverse histological section through atrial cornu. Ac, atrial cornu; Ag, accessory gland cells; Dv, dorsal blood vessel; Gt, gut; Vd, vas deferens.

with clove oil and lifted to sorting troughs with a large lift auger. The water from the auger is diverted into a large round tank, and the fish slide down aluminum sorting troughs. The round tank held thousands of leeches that had become dislodged from the fish during the lifting process from the previous day's operations. On 14 October 2003, only 1 raceway of returning fish was processed; but by the end of the operation, many thousands more leeches had been added to the effluent tank, and the sorting trough also contained an abundance of leeches that had detached as the fish slid down the trough.

#### DISCUSSION

There has been much debate regarding characters that distinguish the genera *Cystobranchus* and

*Piscicola*. Epshtein et al. (1994) include 4 species in *Cystobranchus*: *Cystobranchus mammillatus* (Malm, 1863); *Cystobranchus verrilli* Meyer, 1940; *C. salmositicus* (Meyer, 1946); and *C. meyeri*. They were apparently unaware of the existence of *C. virginicus* and did not list it in their analyses. They listed 6 species in *Piscicola*: *Piscicola geometra* (Linnaeus, 1758); *Piscicola fasciata* Kollar, 1842; *Piscicola respirans* Troschel, 1850; *P. punctata*; *P. milneri* (Verrill, 1874); and *Piscicola pojmanskae* Bielecki, 1994. Epshtein (1969) and Epshtein et al. (1994) defined *Cystobranchus* as having a long bursa, well-developed conducting tissue, and a copulatory area located on the bursa, but lacking accessory gland cells on the atrium. *Piscicola* were defined as having a long bursa, well-developed conducting tissue,

accessory gland cells on the atrium, and a copulatory area located on the clitellum (Epshtein et al., 1994). Thus, the only difference between *Cystobranthus* and *Piscicola*, as interpreted by Epshtein et al. (1994), is the presence or absence of accessory gland cells on the atrium and the location of the copulatory area.

Bielecki (1997) provided a thorough discussion of the characters used to define *Cystobranthus* and *Piscicola*. He properly differentiated conducting tissue and vector tissue by restricting conducting tissue to the long strands or cords of tissue that lie in close proximity to the ovisacs and vector tissue as a ventral mass of tissue associated with the copulatory area. According to Bielecki (1997), *Cystobranthus* has conducting tissue connecting the ovisacs with the atrium (=bursa), whereas *Piscicola* has conducting tissue connecting the ovisacs with a ventral mass of vector tissue. In addition, *Piscicola* is characterized by a spermatheca, mycetomes, and accessory gland cells on the atrium, but none of these structures are present in *Cystobranthus*.

Both *C. virginicus* and *C. salmositicus* have cords of conducting tissue connecting the ovisacs to the bursa (=atrium of Bielecki [1997]). This supports the inclusion of both species in *Cystobranthus*, as suggested by both Epshtein et al. (1994) and Bielecki (1997). However, both species also have accessory gland cells on the atrium, a character of *Piscicola* sensu Epshtein et al. (1994) and Bielecki (1997). However, those authors developed criteria primarily on the basis of European species because the internal anatomy of North American species is inadequately known. We are in the process of collecting and redescribing all the North American species of *Piscicola* and *Cystobranthus*, and it seems prudent to postpone the assignment of final diagnostic characters until that analysis is complete. However, it is now clear that presence or absence of accessory gland cells is not a good criterion for separating the 2 genera. The best criterion is the presence of cords of conducting tissue connecting the ovisac and the bursa in *Cystobranthus* in contrast to cords of conducting tissue connecting ovisacs with a ventral mass of vector tissue in *Piscicola*. In addition, mycetomes are present in *Piscicola* but absent in *Cystobranthus*, and *Piscicola* has an external ventral copulatory area, whereas *Cystobranthus* has a copulatory area on the internal wall of the bursa.

Given this sense of *Cystobranthus* and *Piscicola*, *P. salmositica* is placed within *Cystobranthus* as suggested by Epshtein et al. (1994) and Bielecki (1997), and the combination *C. salmositicus* (Meyer, 1946) is confirmed.

## ACKNOWLEDGMENTS

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Balance on Hand, 1 January 2004 . . . . .	\$27,859.33
Receipts . . . . .	\$718.53
Contributions from Members of the Helminthological Society of Washington . . . . .	\$1,080.00
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