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PTEROBDELLA OCCIDENTALIS N. SP. (HIRUDINIDA: PISCICOLIDAE) FOR *P. ABDITOVESICULATA* (MOORE, 1952) FROM THE LONGJAW MUDSUCKER, *GILLICHTHYS MIRABILIS*, AND STAGHORN SCULPIN, *LEPTOCOTTUS ARMATUS*, AND OTHER FISHES IN THE EASTERN PACIFIC

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KEY WORDS

Pterobdella
Hirudinida
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ABSTRACT

Pterobdella occidentalis n. sp. (Hirudinida: Piscicolidae) is described from the longjaw mudsucker, *Gillichthys mirabilis* Cooper, 1864, and the staghorn sculpin, *Leptocottus armatus* Girard, 1854, in the eastern Pacific, and the diagnosis of *Pterobdella abditovesiculata* (Moore, 1952) from the ʻoʻopu ʻakupa, *Eleotris sandwicensis* Vaillant and Sauvage, 1875, from Hawaii is amended. The morphology of both species conforms with the genus *Pterobdella* in possessing a spacious coelom, well-developed nephridial system, and 2 pairs of mycetomes. Originally described as *Aestabdella abditovesiculata*, *P. occidentalis* (present along the U.S. Pacific Coast), can be distinguished from most congeners by its metameric pigmentation pattern and diffuse pigmentation on the caudal sucker. Based on mitochondrial gene sequences, including cytochrome c oxidase subunit I (*COI*) and NADH dehydrogenase subunit I (*ND1*), *P. occidentalis* forms a distinct polyphyletic clade with *Pterobdella leiostomi* from the western Atlantic. Based on *COI*, *ND1*, and the *18S* rRNA genes, other leech species most closely related to *P. occidentalis* include *Pterobdella arugamensis* from Iran, Malaysia, and possibly Borneo, which likely represent distinct species, and *Pterobdella abditovesiculata* from Hawaii, one of only a few endemic fish parasites in Hawaii. Like *P. abditovesiculata*, *P. arugamensis*, and *Petrobdella amara*, *P. occidentalis* is often found in estuarine environments, frequently infecting hosts adapted to a wide range of salinity, temperature, and oxygen. The physiological plasticity of *P. occidentalis* and the longjaw mudsucker host, and the ease of raising *P. occidentalis* in the lab, make it an excellent candidate for the study of leech physiology, behavior, and possible bacterial symbionts.

Piscicolid leeches (Hirudinida) are sanguivorous, clitellate annelids found as external parasites almost exclusively on fishes, with 1 genus present on mysid shrimp (Burreson et al., 2012a). The family comprises 60 genera and 120 species (Burreson, 2020) present in freshwater, estuarine, and marine habitats, including the deep sea (Goffredi et al., 2012; Utevsky et al., 2019). Piscicolid leeches are also described and/or reported from aquaculture and public aquaria (Burreson et al., 2019) where they cause disease and injury (see Azmey et al., 2020, 2022). More than 20 piscicolid species have been identified from fishes along the Pacific Coast of the United States (Love and Moser, 1983; Burreson, 2007; Burreson et al., 2012a, 2012b, 2019; Burreson and Passarelli, 2015), including *Pterobdella abditovesiculata* (Moore, 1952), present on bony fish in nearshore/estuarine environments (Burreson, 1976).

Pterobdella abditovesiculata was originally described as *Johanssonia abditovesiculata* Moore, 1952, from the whitespotted puffer, *Arothron hispidus* (Linnaeus, 1758), in the Honolulu Aquarium, Hawaii (Moore, 1952). Subsequently, this species was redescribed and transferred to *Aestabdella* by Burreson (1976) based on specimens from the staghorn sculpin, *Leptocottus armatus* Girard, 1854, collected in Yaquina Bay, Oregon. Williams and Burreson (2006) synonymized *Aestabdella* with *Pterobdella* based, in part, on *18S* rRNA sequences obtained from *P. abditovesiculata* collected by the late William Font (Southeastern Louisiana University) from the ʻoʻopu ʻakupa, *Eleotris sandwicensis* Vaillant and Sauvage, 1875, from Hilo, Hawaii.

Table I. Hosts and localities of *Pterobdella occidentalis* n. sp. on the Pacific Coast of the United States.

Host	Locality	Source
Leptocottus armatus, staghorn sculpin	Yaquina Bay, Oregon; Tomales Bay, Colorado Lagoon, Newport Bay, California	Burreson, 1976
	Wallapa Bay, Washington; Morro Bay (common), Mugu Lagoon, Santa Margarita River estuary, California (common)	Present study
Psettichthys melanostictus, Pacific sand sole	Yaquina Bay, Oregon	Burreson, 1976
Hypsopsetta guttulata, diamond turbot	Newport Bay, California	Burreson, 1976
	San Diego Bay, California	Present study
Gymnothorax mordax, moray eel	Tijuana Slough, California	Burreson, 1976
Gillichthys mirabilis, longjaw mudsucker	Morro Bay (rare), Mugu Lagoon, Ballona Wetland, Colorado Lagoon, Anaheim Bay, Santa Ana River Mouth Wetland, Upper Newport Bay, Aqua Hedionda Lagoon, Batiquitos Lagoon, San Elijo Lagoon, San Dieguito Wetland, Los Penasquitos Wetland, Rose Creek Mission Bay, San Diego River, Fomosa Slough, south San Diego Bay wetlands, Tijuana Estuary, California	Present study
Fundulus parvipinnis, California killifish	South San Diego Bay, California (rare)	Present study
Quietula y-cauda, shadow goby	Colorado Lagoon, Long Beach, California (rare)	Present study
Clevelandia ios, arrow goby	South San Diego Bay, California (rare)	Present study
Paralichthys californicus, California halibut (juv.)	South San Diego Bay, California (rare)	Present study
Acanthogobius flavimanus, yellowfin goby	Santa Margarita River, San Diego (common); F Street/G Street Marsh, San Diego, California	Present study

During genetic studies of possible bacterial symbionts of presumptive *Pterobdella abditovesiculata* from longjaw mudsuckers, *Gillichthys mirabilis* Cooper, 1864, and staghorn sculpins collected along the U.S. Pacific Coast, genetic sequences of the leech were found to be significantly different from the published sequences of *P. abditovesiculata* (Williams and Burreson, 2006). The primary purpose of this paper is to describe the specimens of *Pterobdella* from the Pacific Coast as *Pterobdella occidentalis* n. sp. This study documents the genetic identity of *P. occidentalis*, examines and emends the morphological description of this form by Burreson (1976), and identifies distinguishing features that occur between *Pterobdella* from the Pacific Coast and new live specimens collected from the ʻoʻopu ʻakupa in Hawaii, the type locality of *P. abditovesiculata*.

MATERIALS AND METHODS

Specimens of *P. occidentalis* were taken from fishes collected by minnow trap or beach seine in estuaries and wetlands along the Pacific Coast over 5 yr (Table I). Specimens of *P. abditovesiculata* from Hawaii were collected on 2 February 2022 and 21 June 2022 from the ʻoʻopu ʻakupa, caught by hook and line in Wailoa River Estuary, Hilo, Hawaii (19°43′512″N, −155°4′36.3″W). Specimens of *Pterobdella* from Hawaii were removed from ʻoʻopu ʻakupa at the site of collection and placed in aerated water at 3 parts per thousand (ppt) salinity. All fish were released, and leeches from Hawaii were shipped live to California on the day of collection. *Pterobdella* specimens from the Pacific Coast hosts were removed from their host in the field or the lab. Living hosts and leeches were anesthetized or euthanized using Tricane (MS222) before removal of leeches, which were subsequently placed in seawater. Some specimens were relaxed by slowly adding 95% molecular grade ethanol and preserving in either 95% ethanol or 10% formalin for molecular analysis or histology, respectively. Specimens of *Pterobdella* from Hawaii were gradually acclimated over several hours to an increasing salinity of 30 ppt, either in small plastic cups or on a fish host. *Pterobdella* specimens from Hawaii were placed in containers with 1 individual of several fish including longjaw mudsuckers, arrow gobies, *Clevelandia ios* (Jordon and Gilbert, 1882), California killifish, *Fundulus parvipinnis* Girard, 1854, diamond turbot, *Hypsopsetta guttulata* (Girard, 1856), and staghorn sculpin. The morphology of living specimens from both Hawaii and the Pacific Coast was examined. For histology, specimens were embedded in paraffin, and 6 μm transverse and sagittal sections were placed on slides and stained with hematoxylin and eosin. Whole mounts were prepared by lightly compressing relaxed specimens with a slide before adding 10% formalin. Whole mount specimens were then stained in carmine or celestine blue b, dehydrated in an ethanol series, cleared in pure methyl salicylate, and mounted on slides in Canada balsam. For comparison, preserved specimens were borrowed from and/or deposited in the following institutions: Bernice P. Bishop Museum (BM) in Hawaii and United States National Museum of Natural History (Smithsonian Institution, Washington, D.C. [USNM] and Cabrillo Marine Aquarium [CMA]). Measurements are in millimeters, unless stated otherwise, and are of specimens relaxed in ethanol and preserved in 95% ethanol.

For molecular analysis, DNA was extracted from specimens of *P. occidentalis* from the U.S. Pacific Coast and *P. abditovesiculata* from Hawaii, initially preserved in 95% EtOH, using the Qiagen DNeasy kit, following the manufacturer's recommendation (Qiagen, Valencia, California). Phylogenetic relationships were determined via sequencing of 2 mitochondrial markers, cytochrome c oxidase subunit I (*COI*) and NADH dehydrogenase subunit I (*ND1*), and the nuclear *18S* rRNA gene. The partial *COI* gene was amplified using the primers LCO1490/HCO2198 (Folmer et al., 1994). The partial *ND1* gene was amplified using the primers LND300/HND1932 (Light and Siddall, 1999). The partial *18S* rRNA gene was amplified via primers *18S*-3F/*18S*-5R (Giribet et al., 1996). Amplification products were sequenced directly using Sanger sequencing, via Laragen Inc. (Culver City, California), and are available at GenBank (Fig. 2). Attempts to extract and sequence genetic material from voucher specimens of *A. abditovesiculata* in the Smithsonian were not successful.

Statistical analysis was carried out using XLSTAT and included the Mann–Whitney U-Test (U) and linear regression. Infection quantification follows Bush et al. (1997).

DESCRIPTION

Pterobdella occidentalis

(Figs. 1, 2; Table I)

Aestabdella abditovesiculata (Moore, 1952) Burreson, 1976 (in part)

Diagnosis: Small slender leeches, up to 16.0 mm total length. Oral sucker very eccentrically attached, 2 pairs of poorly developed eyes. First nuchal annuli constricted. Caudal sucker well developed, as wide as greatest body width. Smaller individuals lack significant pigmentation but have eyes on oral sucker, 1 pair of punctiform ocelli on first and last trachelosome annuli, both dorsally and ventrally, and on 13 urosome annuli. Caudal sucker without ocelli. Eyes and ocelli may be obscured by pigmentation on mature individuals. Pigmentation variable, sparse and light brown in smaller individuals, more dense and darker brown/olive in larger individuals. Oral sucker with 2 pigment bands, separated by an unpigmented band, the darker, posterior band obscuring eyes. Trachelosome and urosome metameric in appearance due to lateral unpigmented areas. Mid-lateral stripe coalesces with outermost dorso-lateral stripe segmentally with unpigmented areas between, giving the leech a transverse banded appearance, especially on the margins. Pigmentation on caudal sucker diffuse, not forming any discernable pattern. Internal morphology includes a spacious coelom, segmental nephridia, 5 pairs of testisacs, and 2 pairs of mycetomes.

Description: (Based on examination of numerous living and preserved specimens, several adult and juvenile specimens stained and mounted on slides, and a set of transverse and sagittal sections. Observations were compared with the description by Burreson [1976] of *P. abditovesiculata* from the staghorn sculpin from Yaquina Bay, Oregon, and mostly repeated here with some modifications primarily related to the digestive tract. Morphometrics of the type specimens from the longjaw mudsucker include measurements of 1 holotype and 5 paratypes, relaxed in ethanol and preserved in 95% ethanol. Measurements include the mean, the holotype [in square brackets], and the range [in parentheses].)

Body smooth, lacking gills, tubercles, papillae, and pulsatile vesicles. Body 12.2 [11.9] (10.8–13.8) long; trachelosome 2.6 [2.4] (2.2–3.4) long by 0.9 [0.8] (0.7–1.0) wide; urosome 8.0 [7.9] (7.3–8.9) long by 1.2 [1.2] (1.1–1.3) wide. Two eyes on cephalic sucker, and 1 pair of punctiform ocelli on first and last trachelosome annuli, both dorsally and ventrally on 13 urosome segments. Caudal sucker without ocelli. Dark stellate chromatophores scattered over most of body, which give it a brownish coloration except at lateral margins where there are metameric unpigmented areas. Pigment density variable, some individuals solid brown with scattered large chromatophores. Cephalic sucker with 2 pigment bands, a light anterior one, which may be broad and cover the anterior third of the sucker, and a darker posterior one, which has 2 large, darkly pigmented areas, which obscure the eyes. Caudal sucker sparsely pigmented, light brown with scattered white "corpuscles" visible in some living specimens. Cephalic sucker shallow, attached to neck at posterior edge, 0.7 [0.8] (0.6–0.8) long by 0.7 [0.8] (0.7–0.8) wide. First few nuchal annuli constricted and can be telescoped into other neck annuli, which gradually widen and become continuous with clitellar region. At segment XIII body widens to form slight shoulders, which mark beginning of the subcylindrical urosome. From segments XIII through XX sides of body almost parallel. Mid-body segments 7(14) annulate. Urosome gradually narrows and becomes rounded before joining caudal sucker slightly anterior to its center. Caudal sucker 1.5 [1.7] (1.1–1.7) long by 1.5 [1.5] (1.3–1.6) wide and slightly wider than greatest body width.

Mouth pore centrally located in oral sucker. Proboscis extending to ganglion in IX; salivary glands located between ganglia in VII and IX. Paired mycetomes emerge laterally from esophagus in posterior portion of XI and curve posteriorly on the outer dorsal margin of the ejaculatory bulbs. A second pair of mycetomes emerge from the esophagus in the anterior portion of XIII just posterior to the female gonopore and project anteriorly to the level of the male gonopore. The crop lumen expands between the testisacs in the form of paired blunt diverticula. The intestine and postceca originate immediately posterior to the ganglion in XIX. The intestine has 3 pairs of diverticula and a series of smaller compartments including a glandular-like widening prior to a tubular rectum. The postceca are fused with a fenestra at each ganglion.

Five pairs of large testisacs located intersegmentally in XIV/XV in lateral sinuses. Vasa deferentia enlarge in XIII and enter loosely coiled epididymis in anterior portion of XIII, continue anteriorly, and become confluent with thick-walled ejaculatory bulbs. At ganglion in XI, ejaculatory bulbs bend ventrad and enter atrial cornua. Terminal portions of ejaculatory bulbs and dorsal portions of atrial cornua covered with accessory gland (prostate) cells. Atrial cornua merge into common atrium that opens to moderately large bursa that terminates as the male gonopore in the anterior portion of XII. Female reproductive system with paired, convoluted ovisacs that merge into common oviduct and open through the female gonopore in the posterior portion of XII. Cocoons measure 0.61 (0.56–0.68) long by 0.49 (0.48–0.52) wide and are slightly thicker at 1 pole when viewed in lateral view.

Coelomic system expansive, consists of dorsal, ventral, and lateral sinuses. No testicular sinuses. Ramifications of the connecting sinuses extend laterally to the position typically occupied by lateral sinuses, suggesting that lateral sinuses are present. Intersegmentally, the dorsal sinus ramifies ventrolaterally but does not connect with the ventral sinus.

Large segmental nephridia in urosome with first pair beginning in segment XIII and opening to the outside in segment XIV. The single large trunk of each nephridium begins near ventral body wall in posterior portion of segment. Nephridium then passes posteriorly until it is posterior to a testisac, if one is present in the segment, and then proceeds dorsally and then laterally to occupy a mid-lateral position along body wall. It then proceeds posteriorly again and eventually enters a bladder that opens to outside at a mid-lateral position immediately posterior to ganglion in next segment posteriorly. Nephridia open to outside in segments XIV through XXIII.

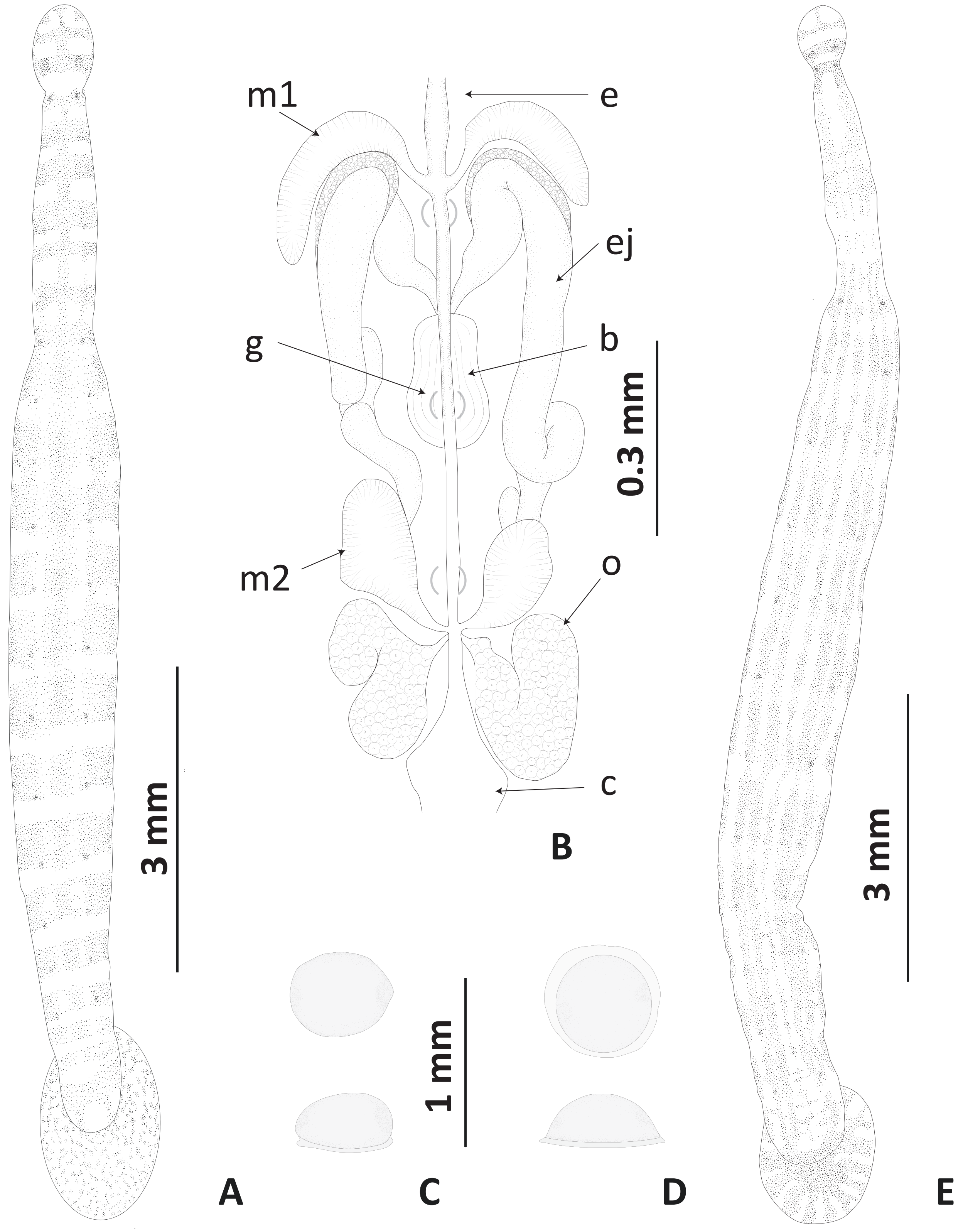
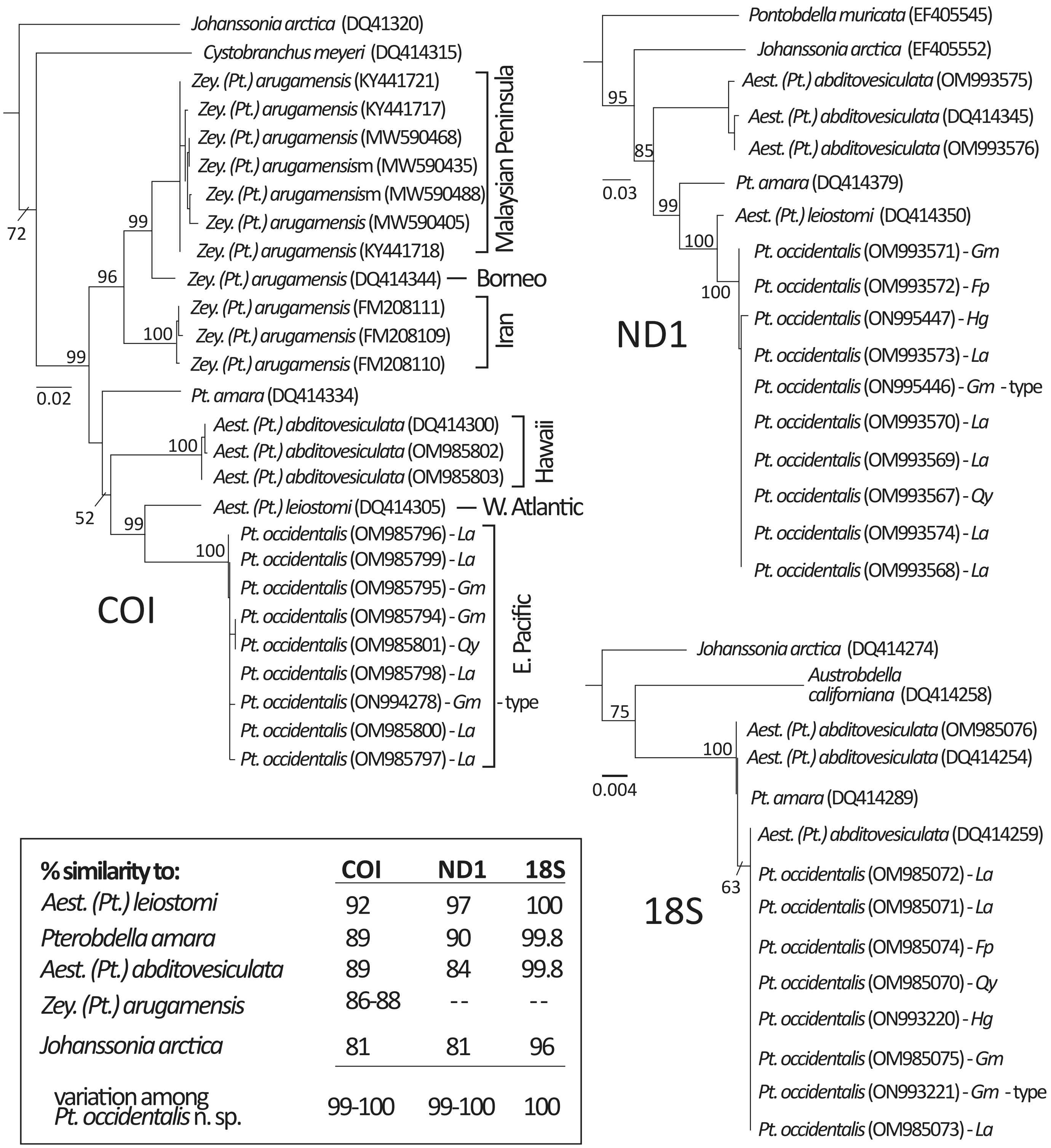


Figure 1. Morphology of *Pterobdella occidentalis* n. sp. From the longjaw mudsucker, *Gillichthys mirabilis* Cooper, 1864, from California and *Pterobdella abditovesiculata* (Moore, 1952) from the ʻoʻopu ʻakupa, *Eleotris sandwicensis* Vaillant and Sauvage, 1875, from Hawaii. (**A**) Body of *P. occidentalis*; (**B**) internal morphology of *P. occidentalis*; (**C**) dorsal and lateral views of *P. occidentalis* cocoons; (**D**) dorsal and lateral views of *P. abditovesiculata* cocoons; I body of *P. abditovesiculata*. Abbreviations: crop, c; esophagus, e; male ejaculatory duct with prostate cells, ej; ganglion, g; first and second pairs of mycetomes, m1, m2; ovary, o.



% similarity to:	**COI**	**ND1**	**18S**
Aest. (Pt.) leiostomi	92	97	100
Pterobdella amara	89	90	99.8
Aest. (Pt.) abditovesiculata	89	84	99.8
Zey. (Pt.) arugamensis	86-88	--	--
Johanssonia arctica	81	81	96
variation among *Pt. occidentalis* n. sp.	99-100	99-100	100

Figure 2. Phylogenetic relationships between *Pterobdella occidentalis* n. sp. and related species, based on the partial *18S* rRNA gene (570 bp), cytochrome oxidase subunit 1 (*COI*) gene (700 bp), and the NADH dehydrogenase subunit 1 (*ND1*) gene (530 bp), as neighbor-joining trees with a Jukes-Cantor genetic distance model. All nodes shown are supported by bootstrap values > 50% for both parsimony and neighbor-joining distance methods, obtained from 1000 replicate samplings. The outgroup was *Branchellion lobata* Moore, 1952 (not shown). The inset table shows similarity values for comparisons between species and within *Pterobdella occidentalis*. Abbreviations: *Zeylanicobella*, *Zey.*; *Pterobdella*, *Pt.*; *Aestabdella*, *Aest.*; *Gillichthys mirabilis*, *Gm*; *Leptocottus armatus*, *La*; *Hypsopsetta guttulata*, *Hg*; *Fundulus parvipinnis*, *Fp*; *Quietula y-cauda*, *Qy*.

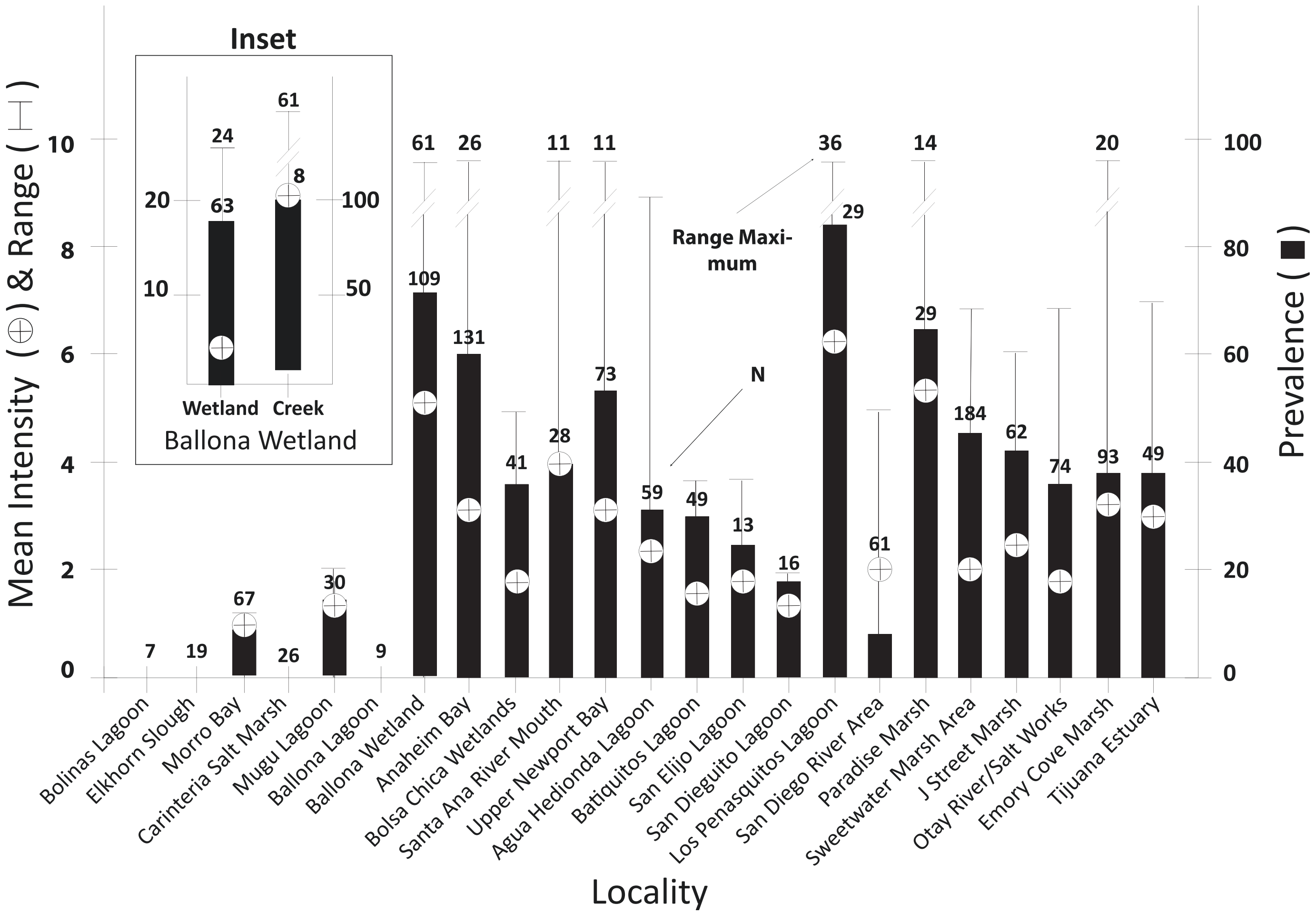


Figure 3. Prevalence and intensity of *Pterobdella occidentalis* n. sp. collected from longjaw mudsucker, *Gillichthys mirabilis* Cooper, 1864, in California wetlands in various months between 2016 and 2021 and comparison of the intensity of infection at wetland and stream collection sites in Ballona Wetland, California.

Taxonomic summary

Type host: Longjaw mudsucker, *Gillichthys mirabilis* (Gobiidae).

Other hosts: See Table I.

Type locality: Seal Beach National Wildlife Refuge, Seal Beach, California, U.S.A. (33°44′7.047″N, 118°4′58.0722″W; 18 May 2022).

Other localities: See Table I.

Sites of infection: Predominantly on pectoral, ventral, and caudal fins; occasionally on head and body.

Prevalence and intensity: See Figure 3.

Specimens deposited: USNM 1522355 (holotype); USNM 1522356 (paratypes), CMA 2022.06.0003 (paratypes); USNM 1522357 (paragenophores); USNM (color digital photo); USNM 1522358 (syngenophores, *Leptocottus armatus*, Willapa Bay, Washington); USNM 1522359 (vouchers) *Hypsopsetta guttulata*, San Diego Bay, California).

Specimens examined: USNM 53254, 53255, 532560 (*Leptocottus armatus*, Yaquina Bay, Oregon; Burreson, 1976).

GenBank accession numbers: See Figure 2.

Remarks

Specimens collected in southern California were nearly identical to specimens described by Burreson (1976) as *Aestabdella abditovesiculata* from Yaguina Bay, Oregon. The presence of 2 pairs of mycetomes was not mentioned by Burreson (1976), but this feature was visible in sectioned material and some whole mounts of *P. occidentalis* and similar in position to those described by Burreson and Thoney (1991) for *Pterobdella leiostomi*. While specimens of *Pterobdella* from Yaquina Bay, Oregon, where *A. abditovesiculata* was originally described, were not available for genetic sequencing, specimens from the staghorn sculpin collected in Willapa Bay, Washington, which is north of Yaquina Bay, were genetically identical to *P. occidentalis* specimens from the longjaw mudsucker and other hosts in southern California (Fig. 2).

Genetic sequencing of leeches from the longjaw mudsucker, staghorn sculpin, and other hosts from the Pacific Coast, as well as the presumptive *P. abditovesiculata* from the ʻoʻopu ʻakupa from Hawaii (Williams and Burreson, 2006; present study), indicate that *P. occidentalis* is genetically distinct (Fig. 3). The closest relatives based on the *COI* gene are also the most similar morphologically: *Aestabdella leiostomi* (= *P. leiostomi*) from the East Coast of the United States at 92% similarity and *A. abditovesiculata* (= *P. abditovesiculata*) from Hawaii at 89% similarity (Fig. 2). Interspecific differences in the *COI* gene from the next closest relatives, including *P. abditovesiculata*, are far greater than intraspecific differences (0 to 1%) for *P. occidentalis*. This level of dissimilarity, also observed in the *ND1* gene, indicates a new species designation is warranted (Fig. 2).

Living *P. occidentalis* can be readily distinguished from *Pterobdella* specimens collected from Hawaii in lacking dark "n"-shaped nuchal pigmentation, having a metameric pigmented urosome (versus striped in other species), and having no discernable chromatophore pattern on the caudal sucker (Fig. 1). Sectioned material of *P. occidentalis* and *P. abditovesiculata* showed no obvious differences in internal anatomy between the 2 species and are consistent with the descriptions of Moore (1952) and Burreson (1976) with the exception that both species have 2 pairs of mycetomes.

Of the 5 species of *Pterobdella*, *P. occidentalis* is the only species presently known from the Pacific Coast and can be distinguished from other congeners by its metamerically pigmented urosome compared to the striped urosome of *P. arugmensis* and *P. platycephalus* (Burreson, 2020) and the uniform light pigmentation of *P. leiostoma* (Burreson and Thoney, 1991). *Pterobdella occidentalis* can be distinguished from *Pterobdella amara*, by lacking significant differentiation of the trachelosome and urosome, being pigmented, having separate male and female gonopore, and being a parasite of bony fishes, as opposed to cartilaginous fishes (Burreson, 2006, 2020). There is some variability in the amount of pigmentation on *P. occidentalis*. As noted by Burreson (1976) small *Pterobdella* specimens on staghorn sculpins lack significant pigmentation relative to larger specimens. The same is true of specimens on longjaw mudsuckers, although some larger specimens raised on light-colored longjaw mudsuckers in the lab had very little pigmentation. In contrast, specimens collected from diamond turbot and California halibut were uniformly heavily pigmented, which obscured the metameric pattern, and the pigment on the oral sucker formed 2 very dark/distinct bands. When turbot *Pterobdella* leeches were transferred to mudsuckers, their dark pigmentation did not change over 3 days. Similarly, *P. occidentalis* from longjaw mudsuckers placed on turbot remained lighter in color. While longer trials are warranted, it may be that definition of pigmentation is established at an early stage of leech development and does not change thereafter.

Pterobdella occidentalis has been found in bays and estuaries and wetlands on staghorn sculpin, longjaw mudsucker, and other fishes (Table I) from Willapa Bay, Washington, to the Tijuana Estuary, California, U.S.A. Prevalence and intensity on longjaw mudsuckers in California wetlands are highly variable but diminish in the northern collection localities (Fig. 3). While *P. occidentalis* is rare on mudsuckers north of Mugu wetland, it is common on staghorn sculpins even where both species are abundant. In Morro Bay tidal channels, *P. occidentallis* is rarely found on longjaw mudsuckers but was present on 17 of 27 staghorn sculpins captured in shallow bay marina habitat (R. G. Appy, unpubl. data). In southern California *P. occidentallis* is predominantly a parasite of the longjaw mudsucker, although large numbers have been collected from the non-native yellowfin goby, *Acanthogobius flavimanus* (Temminck and Schlegel, 1845), and staghorn sculpin in the Santa Margarita River mouth (R. G. Appy, unpubl. data). In addition, 6 of 9 juvenile diamond turbot, *Hypsopsetta guttulata* Girard, 1856, and 1 juvenile California halibut, *Paralichthys californicus* (Ayres, 1859) caught in south San Diego Bay in the spring and summer of 2022 were infected. *Pterobdella occidentalis* collected from staghorn sculpin and diamond turbot readily attached and fed on longjaw mudsuckers in captivity and subsequently deposited cocoons. Conversely, specimens from longjaw also readily attached to and fed on staghorn sculpins, diamond turbots, California killifish, and arrow gobies.

Pterobdella occidentalis appears to be common in wetlands with significant freshwater input (e.g., Ballona Wetlands and Los Penasquitos Lagoon), but is also common in wetlands with little freshwater input (e.g., Anaheim Bay), areas that are slightly hypersaline (south San Diego Bay), or in small intertidal pools with salinities as high as 37 ppt (R. G. Appy, unpubl. data) (Fig. 3). In Ballona Wetland, where hourly salinity data have been collected, *P. occidentalis* was significantly more abundant in the tidal portion of the freshwater stream/inlet ($\bar{x} = 18.6$ [1.2–20] ppt) than the more saline tidal marsh ($\bar{x} = 28.3$ [13.3–33.9] ppt) ($U = 72$, $n_1 = 56$, $n_2 = 8$, $P = 0.022$; Fig. 3, inset). Additionally, as expected, *Pterobdella occidentallis* from Ballona Wetland, Anaheim Bay, and Paradise Marsh combined were slightly more abundant on larger mudsuckers ($R^2 = 0.063$, $F[1,177]$, $f = 12.756$, $P = 0.000$). On 2 occasions, cocoons of this leech have been found on the shells of the lined shore crab, *Pachygrapsus crassipes* Randall, 1839, which cohabitates salt marshes with the longjaw mudsucker.

Pterobdella occidentalis is easily raised in the lab on longjaw mudsuckers and deposits cocoons on the wall of aquaria which hatch in approximately 30 days at 21C. This leech readily swims and will curl in a ball and drop to the bottom before reattaching.

***Pterobdella abditovesiculata* (Moore, 1952)**

(Figs. 1, 2)

Amended diagnosis: Small slender leeches, up to 15.0 mm total length. Oral sucker very eccentrically attached, with 1 pair of poorly developed eyes. Caudal sucker well developed, as wide as greatest body width. Newly hatched individuals lack pigmentation, but have 1 pair of eyes on oral sucker, 1 pair of punctiform ocelli on first and last trachelosome annuli, both dorsally and ventrally, and on 13 urosome annuli. Caudal sucker without ocelli. Eyes and ocelli may be obscured by pigmentation on mature individuals. Pigmentation variable from brown in smaller individuals to black in larger individuals. Oral sucker with 2 pigment bands, posterior band obscuring eyes and interrupted mid-dorsally by unpigmented band. First nuchal segments heavily pigmented in the shape of an "n." Trachelosome with 2 pairs of dorso-lateral longitudinal stripes interrupted segmentally by unpigmented bands. Urosome with 5 longitudinal stripes; mid-dorsal stripe narrow, paired dorso-lateral stripes wider. Mid-lateral stripe coalesces with outermost dorso-lateral stripe segmentally with unpigmented areas between, giving the leech a transverse banded appearance, especially on margins. Caudal sucker with 14 diffuse pigment bands radiating from center of sucker to outer margins. Internal morphology includes spacious coelom, segmental nephridia, 5 pairs of testisacs, and 2 pairs of mycetomes. Postcaeca fused with fenestra at each ganglion.

Description: (Measurements of 5 specimens from ʻoʻopu ʻakupa include the mean and the range [in parentheses].) Body 12.8 (11.3–14.4) long; trachelosome 2.1 (2.0–2.5) long by 0.56 (0.5–0.7) wide; urosome 8.9 (8.0–9.9) long by 1.0 (0.9–1.2) wide. Cephalic sucker shallow, attached to neck at its posterior edge (Fig. 1), 0.6 (0.5–0.7) long by 0.6 (0.6–0.7) mm wide. Caudal sucker 1.5 (1.3–1.7) long by 1.4 (1.2–1.6) wide and slightly wider than greatest body width. Cocoons measure 0.61 (0.56–0.68) long by 0.49 (0.48–0.52) wide and uniformly concave in lateral view (Fig. 1).

Taxonomic summary

Type host: Whitespotted puffer, *Arothron hispidus* (Tetradontidae).

Type locality: Honolulu Aquarium (Moore 1952).

Other hosts and localities: ʼoʻopu ʻakupa, *Eleotris sandwicensis* (Eleotridae) Wailoa River Estuary, Hilo Hawaii, present study.

Sites of infection: On pectoral fins and body surface.

Prevalence and intensity: One hundred percent of 19 *E. sandwicensis* collected in the Wailoa River Estuary infected with 4 to 16 leeches.

Specimens deposited: Eleotris sandwicensis, USNM 1522360 (voucher); USNM 1522361 (syngenophore); USNM (color digital photo); CMA 2022.06.0004 (voucher).

Specimens examined: Types *Arothron hispidus*, BM R139 (holotype photo), R155 (paratypes).

GenBank accession numbers: See Figure 2.

Remarks

Living *Pterobdella abditovesiculata* can be readily distinguished from *P. occidentalis* in having a radiating chromatophore pattern on the caudal sucker. In larger specimens, there is also a well-developed dark "n"-shaped pigmentation on the first nuchal segment and a striped pigmented urosome (vs. metmeric in *P. occidentalis*). Large *P. abditovesiculata* macroscopically appear black, while even the darkest specimens of *P. occidentalis* were more brownish. While no pigmentation pattern was present in paratypes or a photo of the holotype examined from the Bishop Museum, the description by Moore (1952, p. 30) from the white-spotted puffer fish clearly describes the presence of a striped urosome and "dorsal face (of caudal sucker) darkened by a dense chromatophore reticulum, divided by 14 paler rays." The sectioned material of *P. abditovesiculata* and *P. occidentalis* showed no obvious differences between the 2 species and is consistent with the descriptions of Moore (1952), except for 2 pairs of mycetomes in both species, noted in the generic diagnosis by Burreson (2020). The cocoons of *P. abditovesiculata* are more oval than those of *P. occidentalis* and are uniformly concave in lateral view (Fig. 1). In light of the ability of *P. abditovesiculata* to adapt to salinity ranging from 3 to 30 ppt and an ability to attach to a range of fish hosts (present study), it would be plausible for this species to be present on white-spotted puffers in a marine aquarium exhibit. Additionally, the white-spotted pufferfish is commonly found in estuarine environments (Randall et al., 2012) in the same areas occupied by ʻoʻopu ʻakupa (Peyton et al., 2015) where it could have become infected with *P. abditovesiculata*.

Pterobdella abditovesiculata can be distinguished from 2 of the 4 congeners. *Pterobdella leiostomi* lacks any definitive pigment pattern and is present in the western Atlantic (Burreson and Thoney, 1991), and *P. amara* has a well-differentiated trachelosome, lacks pigmentation, has a common genital opening, and is a parasite of sharks (Burreson, 2006, 2020).

There is little morphological evidence supporting the separation of *P. abditovesiculata* from *Pterobdella arugamensis* and *Pterobdella platycephali*, although the last is generally larger (Burreson, 2020). All 3 species have similar pigmentation patterns (see Burreson, 2020), and *P. abditovesiculata* and *P. arugamensis* appear to favor estuarine environments. However, genetic evidence based on 3 genes (Fig. 2) indicates that the specimens from Hawaii are distinct from specimens identified as *P.* (*Zeylanicobdella*) *arugamensis* (Fig. 2).

All ʻoʻopu ʻakupa collected in the Wailoa River Estuary on 2 February 2022 (n = 12) were infected with leeches (4–50 each). In a sample of 7 fish collected on 21 June 2022, larger ʻoʻopu ʻakupa (133–147 mm total length) were more heavily infected (13–16 leeches) than smaller fish (78–97 mm total length), which were infected with 4–7 leeches. Salinity at time of capture was 3 ppt, but the estuary is subject to daily tidal fluctuations to 20 ppt, and salinity reaches 30 ppt during spring tides (T. S. Sakihara, unpubl. data). All specimens placed in a salinity of 30 ppt in captivity survived for at least 14 days. Specimens readily attached to and fed on eastern Pacific longjaw mudsuckers and arrow gobies held at 15 ppt, raised to 30 ppt; some leeches detached within 2 to 3 days and re-attached to the container, while others remained on the host for at least 14 days. Leeches deposited cocoons, which subsequently hatched and juveniles attached to and fed on mudsuckers. Leeches move inch-worm-like when disturbed, are capable of swimming, and will curl in a ball and drop to the bottom before reattaching.

DISCUSSION

A description of *P. occidentalis* and a redescription of *P. abditovesiculata* confirms the generic synapomorphies identified by Burreson (2020): spacious coelomic sinuses, 2 pairs of mycetomes, and large nephridia. Species within the genus *Pterobdella* form a morphologically homogeneous group, except for *P. amara*, the only species described to date that has a markedly distinct trachelosome and urosome, a common genital pore, and is a parasite reported from stingrays (Burreson, 2006, 2020).

The genus *Pterobdella* forms a well-supported genetic clade, based on both mitochondrial and nuclear genes (Fig. 2; Williams and Burreson, 2006), with close relationships between *Pterobdella occidentalis*, *P. leiostomi*, and *P. abditovesiculata* supported, and less similarity to *P. arugamensis* (de Silva, 1963). Notably, species attributed to *P. arugamensis* are polyphyletic with at least 2, and possibly 3, clades from different geographic regions that may represent different species (Fig. 2). Given that similar genetic distances were observed among leeches identified as *P. arugamensis* from Malaysia (Ravi and Yahaga, 2017; Azemy, 2022), Iran (Polgar et al., 2009), and Borneo (Williams and Burreson, 2006), as observed between *P. occidentalis* and *P. abditovesiculata*, *P. arugamensis* likely represents a species complex (Polgar et al., 2009; Burreson, 2020; Azemy, 2022) and requires further examination. Recent interest in *P. arugamensis* has resulted from its apparent dispersal in fish culture and pathogenic effects on its hosts (Murwantoko et al., 2018; Azemy et al., 2020, 2022).

Font (2007) identified *P. abditovesiculata* as 1 of only 3 endemic adult parasites of fish in streams and estuaries in Hawaii. While this endemicity is supported genetically with the specimens examined to date (Fig. 2), *P. abditovesiculata* may be an invasive species introduced with non-native host species, as documented for other parasites (Font, 2007). While *P. abditovesiculata* was described in 1952, 60 species of fish had been reported as introduced into the Hawaii ecosystem by 1984, including 13 established species (Maciolek, 1984; Eldredge, 1994), and it could be that this leech arrived on one of these introduced fish. It is unlikely that *P. abditovesiculata* arrived naturally with its usual host, ʻoʻopu ʻakupa, since this species has an amphidromous life cycle, i.e., this estuarine benthic fish disperses by pelagic eggs/larvae. Additional genetic studies in the Indo-Pacific, Australia, Micronesia, and Polynesia are needed to clarify the relationship of *P. abditovesiculata* to worms identified as *P. arugamensis* and if *P. abditovesiculata* represents an endemic Hawaiian species or a more recent introduction.

Ecologically, *Pterobdella occidentalis*, *P. arugmensis*, and *P. abditovesiculata* do not appear to be specific in host preference and can withstand significant ranges in salinity of their preferred estuarine environments (Polgar et al., 2009; Burreson, 2020; present study). Even *P. amara*, the close relative to *P. occidentalis* found on stingrays (*Pastinaca*), is found on this host in brackish waters (Sanjeeva Raj et al., 1977), and this host has been found in brackish regions of rivers (Compagno et al., 1989). Except for specimens from San Diego Bay, where salinity rarely falls below 32 ppt (Delgadillo-Hernandez et al., 2008), all *P. occidentalis* collected in the present study were from wetland habitats subject to annual changes in salinity and temperature. Both *P. occidentalis* and *P. abditovesiculata* were able to survive at least 7 days in salinities ranging from 5 ppt to 30 ppt.

While *P. occidentalis* is capable of infecting at least 10 fish host species, north of Pt. Conception they are predominantly parasites of the staghorn sculpin, and south of Pt. Conception they are predominantly parasites of the longjaw mudsucker, even where the fish species overlap in distribution. Polgar et al. (2009) similarly found that while *P. arugamensis* was found predominantly on 1 host in the wild, it readily attached to other hosts in aquaria. The plasticity of some *Pterobdella* species appears to mirror those of their host. Both the longjaw mudsuckers and staghorn sculpins are ecologically and morphologically similar in their ability to acclimate to a wide variety of temperatures and salinities. In particular, the longjaw mudsucker, which burrows in the mud when the tide recedes, can withstand extreme ranges in salinity and temperature and can even travel short distances out of the water (Love, 2011). Similarly, mudskippers *Scartelaos tenuis* (Day, 1876), host to *P. arugamensis* in Iran, live a quasi-amphibious existence in intertidal habitats with extreme fluctuations in temperature, salinity, and oxygen (Polgar et al., 2009), and *P. abditovesiculata* infects the ʻoʻopu ʻakupa from Hawaii, which can withstand a large range of salinities. The spacious coelom and well-developed nephridia present in *Pterobdella* species may be adaptations allowing cohabitation with hosts living in extreme and dynamic habitats. The physiological plasticity of *P. occidentalis* and the longjaw mudsucker host, and the ease of raising *P. occidentalis* in the lab, make it an excellent candidate for the study of leech physiology, behavior, and bacterial symbionts associated with a sanguivorous lifestyle.

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