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The invertebrate host of salmonid fish parasites *Ceratonova shasta* and *Parvicapsula minibicornis* (Cnidaria: Myxozoa), is a novel fabriciid annelid, *Manayunkia occidentalis* sp. nov. (Sabellida: Fabriciidae)

STEPHEN D. ATKINSON[1,3], JERRI L. BARTHOLOMEW[1] & GREG W. ROUSE[2]
[1]*Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA*
[2]*Scripps Institution of Oceanography, University of California, San Diego, CA 92093, USA*
[3]*Corresponding author. E-mail: atkinsos@oregonstate.edu*

Abstract

Myxosporea (Cnidaria: Myxozoa) are common fish parasites with complex life cycles that involve annelid hosts. Two economically important salmonid-infecting myxosporeans from rivers of the northwestern United States, *Ceratonova shasta* (Noble, 1950) and *Parvicapsula minibicornis* Kent *et al.*, 1997, have life cycles that require a freshwater annelid host, identified previously as *Manayunkia speciosa* Leidy, 1859. This species was described originally from Pennsylvania, with subsequent records from New Jersey, the Great Lakes and west coast river basins. Despite apparent widespread distributions of both suitable fish hosts and the nominal annelid host, both parasites are restricted to river basins in the northwestern US and have never been recorded from the Great Lakes or the eastern US. In this study, we sampled 94 infected and uninfected annelids from two northwestern US rivers to confirm the identity of the host. We found these new specimens had mitochondrial COI sequences with no more than 4.5% distance from each other, but with at least 11% divergence from *M. speciosa* sampled from near the type locality (New Jersey) and Lake Superior. We did not recover any *M. speciosa* from either west coast river. The annelid from the Klamath and Willamette rivers showed marked sexual dimorphism that has not been reported in any *Manayunkia* described to date, though it is apparent that this had been missed in *M. speciosa*. Accordingly, we describe a new taxon, *Manayunkia occidentalis* **sp. nov.**, and show that it can host both *C. shasta* and *P. minibicornis*. We suspect that previous records of *Manayunkia* from Pacific Northwest watersheds are likely to be *M. occidentalis* **sp. nov.** and not *M. speciosa*. Sampling of *Manayunkia* from additional localities is underway to test if the novel *Manayunkia* species is the only freshwater fabriciid annelid present across the Pacific Northwest.

Key words: Klamath River, Willamette River, myxozoan, Myxozoa, polychaete host, *Ceratomyxa shasta*

Introduction

Annelids are a diverse and speciose invertebrate group, particularly in marine systems. Apart from clitellate annelids, there are relatively few known freshwater species (Rouse 2004; Glasby & Timm 2008), and several of these belong to the genus *Manayunkia* Leidy, 1859. *Manayunkia* currently contains 11 accepted nominal species according to Read & Fauchald (2020), though this may need further assessment. The most recent taxa described are the marine *Manayunkia mizu* Rouse, 1996 and the freshwater *M. zenkewitschii* Sitnikova *et al*., 1997. Currently, there are freshwater *Manayunkia* described from Europe and North America, and brackish/marine species known from Australia, Europe, Papua New Guinea and Brazil (Rouse 1996). Two species of *Manayunkia* are recorded from North America: *M. aestuarina* Bourne, 1883 from brackish coastal environments (Bell 1982), though the type locality is in the UK. The type species of the genus, *M. speciosa* Leidy 1859, is recorded from freshwater habitats across North America. *Manayunkia speciosa* was first briefly described by Leidy (1859) from Schuylkill River (Pennsylvania, USA) and in much greater detail from specimens from New Jersey (Leidy 1883). Meehan (1929) and Krecker (1939) were the first to record *M. speciosa* from the Great Lakes, and this was later confirmed by Pettibone (1953) and others (e.g. Hiltunen 1965). It was also reported from the Klamath and Sacramento river basins of Oregon and California (Hazel 1966; Willson *et al*. 2010; Malakauskas *et al*. 2013; 2015) and lakes in North Carolina (Mackie & Qadri 1971) and Alaska (Holmquist 1967).

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Manayunkia speciosa was identified as the invertebrate alternate host of two myxosporean parasites of salmonid fishes: *Ceratonova shasta* (formerly *Ceratomyxa shasta*) and *Parvicapsula minibicornis* (Fig. 1; Bartholomew *et al*. 1997; 2006). Myxosporea (Cnidaria: Myxozoa) are common parasites with complex life cycles that require two hosts: a vertebrate (usually fish) in which parasite myxospores develop, and an invertebrate (usually an annelid) in which actinospores develop. Both *C. shasta* and *P. minibicornis* are widespread in the Pacific Northwest (Stinson *et al*. 2018; Atkinson *et al*. 2011), but have not been recorded from the Great Lakes, or east coast of North America, despite presence of both *M. speciosa* and susceptible salmon hosts in these regions. We hypothesized that these two myxozoans actually require a heretofore unrecognized annelid host, which is present only in western North American river basins, and which has been consistently mis-identified as *M. speciosa*.

Accordingly, we sampled both infected and uninfected annelids from the two localities where the parasites were recorded originally from their annelid hosts: the Klamath River, California, and the Willamette River, near Corvallis, Oregon. Using morphology and mitochondrial Cytochrome oxidase subunit I (COI) DNA sequencing, we compared these specimens with *M. speciosa* samples from New Jersey and the Great Lakes. We discovered that all Klamath and Willamette *Manayunkia*, both infected and uninfected, were a novel species, that we describe here as *Manayunkia occidentalis* **sp. nov.** We sequenced annelid samples from our collections from 2006–2018 and identified only *Manayunkia occidentalis* **sp. nov.** We did not detect any *M. speciosa* from either river basin.

Materials and methods

Specimens. Annelids were collected by hand at depths of 10–50 cm from rocks and periphyton from the Klamath River, California, from below Iron Gate Dam (41°52’02.6”N 122°48’37.4”W). Additional specimens were collected from the Willamette River, Oregon (44°34’57.7”N 123°13’41.5”W). Under a dissection microscope, living annelids were separated from substrate, then examined by light microscopy either alive or fixed in 10% neutral-buffered formalin, frozen for DNA analysis, or fixed in 2% glutaraldehyde in cacodylate buffer for electron microscopy. Type and voucher specimens were deposited in the Benthic Invertebrate Collection at Scripps Institution of Oceanography (SIO-BIC), La Jolla, California (USA). To confirm identity and infection status, we examined additional archived annelid samples collected opportunistically 2006–2018 by Oregon State University as part of our long term, on-going ecological and disease studies of parasites *C. shasta* and *P. minibicornis*. These samples were collected from localities in the Klamath and Willamette Rivers (Stocking & Bartholomew 2007; Bjork & Bartholomew 2009; Atkinson *et al*. 2011; Hurst *et al.* 2012; Alexander *et al.* 2016). We also obtained *M. speciosa* from Lake Superior (St. Marys River, Ontario, Canada) and South River, New Jersey, in 2009. A marine fabriciid, *Echinofabricia goodhartzorum* Huang *et al*., 2011, was used as the outgroup, since *Echinofabricia* has been shown to be the sister group to *Manayunkia* (Huang *et al*. 2011).

Microscopy. Myxozoan parasite infections were determined visually by observing developing and mature actinospores in either the tegument (*C. shasta*; Fig. 1A–C) or coelom (*P. minibicornis*; Fig. 1D–E). Images of living *Manayunkia* in tubes (Fig. 2A–B) were taken using a stereomicroscope. Images of both fresh and formalin-fixed *Manayunkia* were taken from wet-mount specimens compressed gently under a coverslip, using bright field or Nomarski interference contrast illumination (Figs 2C–E; 3A–E). For scanning electron microscopy, fixed annelids were dehydrated in a graded ethanol series for 15 min each step, then critical point-dried, before being mounted on stubs and sputter-coated with gold:palladium (40:60). Annelids were examined using a FEI Quanta 600 FEG Scanning Electron Microscope (FEI, USA) at Oregon State University’s Electron Microscopy facility (Figs 3F–H; 4A–H).

DNA sequencing. Whole specimens were used for DNA extraction due to the small size of the annelid. Total DNA was extracted using a DNeasy Blood & Tissue Kit per manufacturer’s instructions (Qiagen Inc., Valencia, California). General PCR primers LCO1490 and HCO2198 (Folmer *et al*. 1994) were used initially to amplify the *COI* gene fragment. For reference annelids *M. speciosa* and *E. goodhartzorum,* sequencing methods followed Tilic *et al*. (2019). For additional sequences from Klamath and Willamette River annelids we designed taxon-specific primers MANSPEC2F (GCAGAACTAGGTCAACCTGG; which binds ~90 bases downstream of LCO1490) and MANSPEC2R (CTCCTCCACCAGCTGG; which binds ~40 bases upstream of HCO2198) to improve amplification and sequence quality, with a ~560 bp amplicon. PCRs were in 10 µL volumes and comprised: 2.0 µL DNA (~100 ng), 0.25 µM each primer, 1.25 U GoTaq Flexi polymerase, 200 µM each dNTP, 5 µg bovine serum albumin,

1.0 µL Rediload dye, 1.5 mM $MgCl_2$, 2.0 µL 5× GoTaq Flexi clear buffer and water. PCRs were run on an MJ Research PTC-200 thermocycler, using a touch-down reaction profile: primary denaturation of 120 sec at 95°C; followed by 6 cycles of 94°C for 30 sec, 45°C for 40 sec (dropping 1 degree per cycle to 40°C) and 72°C for 60 sec; then 35 cycles of 94°C for 30 sec, 51°C for 40 sec and 72°C for 60 sec; with a final extension at 72°C for 10 min. Samples were sequenced using the PCR primers, at the OSU Center for Genome Research and Biocomputing, using an ABI BigDye Terminator Cycle Sequencing Kit v3.1 and ABI3730 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Sequences of a paratype annelid from the Klamath River, and one from the Willamette River were deposited in GenBank under accession numbers (MN991228 & MN991229, respectively). Reference *M. speciosa* sequences from New Jersey and Lake Superior specimens were deposited under accession numbers (MT002969 & MT002970, respectively).

Myxozoan infections. Presence of parasite infections was determined either by visual microscopic examination (see previous section), or by parasite-specific PCR assay: *C. shasta* (Hallett & Bartholomew 2006) or *P. minibicornis* (Hallett & Bartholomew 2009).

Phylogenetic analyses. In addition to the nominated *M. occidentalis* **sp. nov.** type sequence from the Klamath River, we composed a matrix of our novel sequences from *M. occidentalis* **sp. nov.** from the Willamette River, our *M. speciosa* reference sequences from Lake Superior and New Jersey, plus representative sequences from each of the available *Manayunkia* taxa in GenBank that were linked with published collection records, plus the outgroup sequence of *Echinofabricia goodhartzorum* (Fig. 5). Sequences were aligned using Muscle (Edgar 2004) within Mesquite (Maddison & Maddison 2018). Maximum Likelihood (ML) and Bayesian inference (BI) phylogenetic analyses using RaXML (Stamatakis, 2014) and MrBayes (Ronquist *et al*. 2012), respectively, were conducted with all positions using the GTR+I+G model of nucleotide substitution based on the Akaike Information Criterion score, as calculated with jModeltest (Darriba *et al.* 2012). Support for the ML result was assessed using a bootstrap analysis with 1000 pseudoreplicates. The BI analysis was done via a run of four Markov Monte Carlo chains with 5 million generations, saving a tree every 1,000 generations. The log-likelihoods and other parameters were assessed for stationarity using Tracer 1.6 (Rambaut *et al.* 2018). Following this, the first 10% of the trees were discarded as burn-in; the posterior probabilities were estimated by combining the remaining trees from each run in a majority-rule consensus tree.

Distance analysis. Pairwise distances were estimated for an expanded sequence alignment that included sequences from the archival Klamath River annelid samples (94 sequences of sufficient length/quality). Distances were calculated using the online Automatic Barcode Gap Discovery tool (Puillandre *et al.* 2012) using the simple distance method.

Results

Live annelids were found in clusters of tubes, most often attached to rocks or intertwined with periphyton. Females were observed frequently with broods of larvae in their tubes. Males and females with overt parasite infections often lacked gametes. Five individuals were sequenced from the type collection locality/date, with an additional 79 from archival material from the Klamath River; 10 were sequenced from the Willamette River. The five paratype specimens were up to 1.6% divergent with each other (0–6 nucleotides (nt) differed over the total alignment of 439 nt), compared with a divergence of up to 2.8% among all 84 Klamath River annelids sequenced. The 10 Willamette River specimens were up to 0.4% divergent with each other, and pairwise distances between Willamette and Klamath River specimens were 3.1–4.1%. These west coast samples were 10.5%–12.2% divergent from our Great Lakes and East Coast *M. speciosa* reference sequences. The parasite *C. shasta* was detected in 16 annelids from the Klamath River, whereas *P. minibicornis* was detected in four annelids from the Klamath River and one from the Willamette River; these parasite abundances were typical (Stocking & Bartholomew 2007; Atkinson *et al*. 2011; Alexander *et al*. 2014).

The Maximum Likelihood and Bayesian analyses of the COI data for *Manayunkia* were congruent (Fig. 5). All sequences from freshwater *Manayunkia* species were distinct from the only marine exemplar available, *M. athalassia*. Each showed a distinct and well supported clade of *M. occidentalis* **sp. nov.** that was sister to a clade of *M. speciosa* sequences, which included both our Great Lakes and New Jersey reference sequences and three published

sequences from GenBank (nominally from the Great Lakes, Klamath River & Sacramento River). This sister group relationship was poorly supported, however. Also, both analyses showed only weak support for the relationships between these two groups of North American *Manayunkia* sequences and sequences from five Russia freshwater *Manayunkia* spp., mainly from Lake Baikal. One of these, *Manayunkia* sp. '*kolymaensis*' refer to an undescribed species from rivers in far eastern Russia rather than Lake Baikal (Pudnikova *et al.* 2014) and this appeared to be closer to the North America *Manayunkia* than to the well supported Lake Baikal clade of species. This also was fairly poorly supported and further loci will be needed to better resolve the relationships within *Manayunkia* (Rouse in prep.).

Systematics

Fabriciidae Rioja, 1923

Manayunkia Leidy, 1859

Manayunkia occidentalis sp. nov.

Figures 2–4

Manayunkia speciosa: Hazel, 1966; Bartholomew *et al*. 1997; 2006; 2007; Alexander *et al.* 2014; Stocking and Bartholomew, 2007; Bjork & Bartholomew 2009; Willson *et al*. 2010; Atkinson *et al*., 2011; Malakauskas *et al*., 2013; 2015.

Holotype (in 70% ethanol, initially fixed in 10% formalin)**.** Adult male from Klamath River mainstem near confluence with Beaver Creek, California, USA. 41.867240°N, -122.809763°E, collected by Julie Alexander (OSU), 25 April 2014 (SIO-BIC A12115)

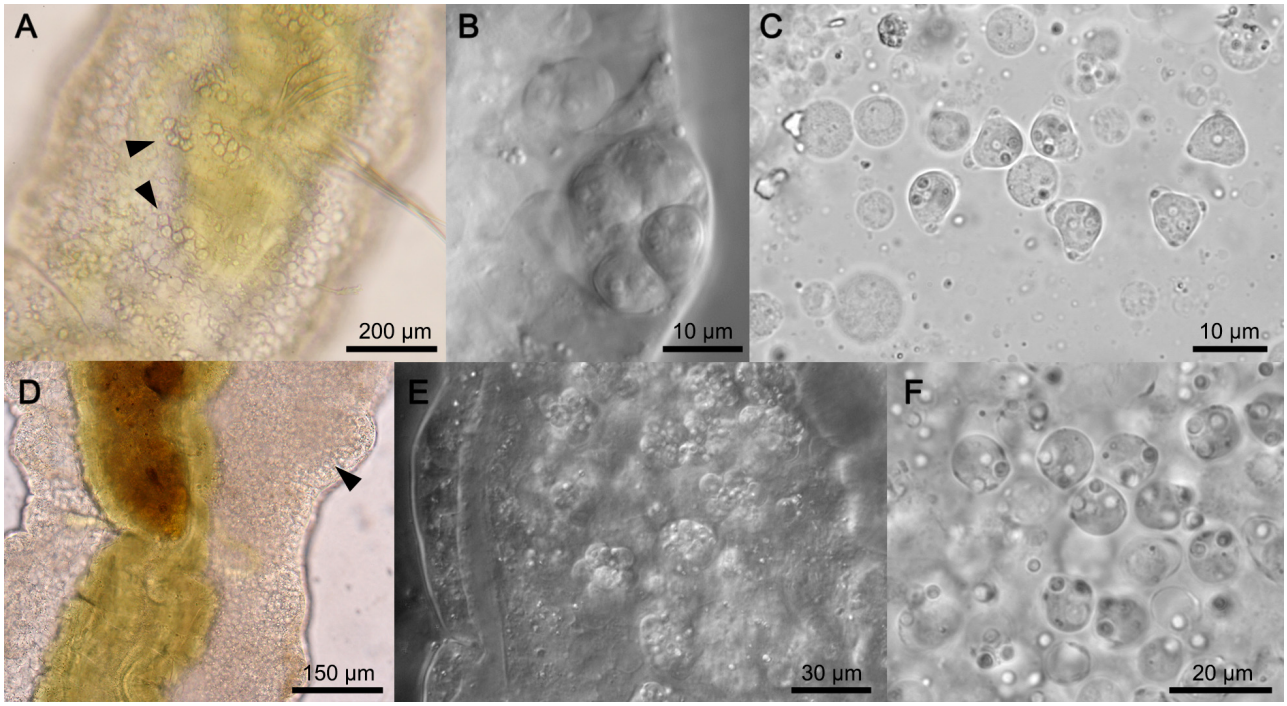

FIGURE 1. *Manayunkia occidentalis* **sp. nov.** from the Klamath River, CA, infected with myxozoan parasites *Ceratonova shasta* (A–C) and *Parvicapsula minibicornis* (D–F). A, bright field image of *C. shasta* actinospores (arrowheads) in tegument; B, Nomarski interference contrast image of *C. shasta* pansporocyst in tegument; C, Individual tetractinomyxon type actinospores of *C. shasta*; D, bright field image of P. *minibicornis* actinospores (arrowhead) in coelom of infected annelid; E, mass of developing *P. minibicornis* spores in coelom; F, Individual saccimyxon type actinospores of *P. minibicornis*; morphologies after Atkinson *et al.* (2019).

Paratypes (in 70% ethanol, fixed in 10% formalin); One specimen, adult female, same locality, collector and date as holotype (SIO A12116); One specimen, adult male, same locality, collector and date as holotype (SIO A12117).

Etymology. The specific epithet is derived from the Latin word for "west", to contrast with *M. speciosa*, which occurs in eastern and central North American drainages.

Description. Holotype (SIO-BIC A12115) a male with eight thoracic segments and three abdominal segments. Total length (unfixed) 3.0 mm, crown 0.3 mm. Body cylindrical with tapering, dorso-ventrally flattened abdomen. Body wall translucent grey with minor brown pigmentation on peristomium and pygidium, and green pigmentation on chaetiger 6. Branchial crown ~10% of total body length. Two pairs of radioles, each with six pinnules. One ventral pinnule on radiole with 4–5 yellow-white spots in life (Fig. 2B–C). One pair of vascularised, unbranched ventral filamentous appendages present, extending for about two-thirds length of radiolar crown, approximately same width as pinnules (Fig. 2B–C). Anterior peristomial ring with membranous collar, followed by posterior peristomial ring (Fig. 4A–B,D). Collar margin smooth, higher ventrally, separated by a narrow dorsal gap that corresponds with ciliated faecal groove (Fig. 4D). Peristomial eyes, black rounded spots (Fig. 2E). Pygidial eyes absent. Superior thoracic notochaetae elongate, narrowly hooded; 5–7 per fascicle (chaetigers 1–5), 4–5 per fascicle (chaetigers 6–8) (Figs 3A; 4A–C). Inferior thoracic notochaetae on chaetigers 1–8 short; 3–4 per fascicle except chaetiger 2, which has 6 per fascicle. Thoracic neuropodia each with 4–7 uncini in chaetigers 2–8 (Fig. 3B). Thoracic uncini with rows of evenly small teeth gradually decreasing in size away from main fang (Figs 3B; 4E). Position of chaetae and uncini reversed in abdominal segments; number of abdominal uncini per fascicle decreasing posteriorly from 22 to 9. Abdominal uncini each with multiple rows of teeth that are uniform in size (Figs 3C; 4H); manubrium at least five times longer than dentate region, with base about two thirds the width of the dentate region. Abdominal neuropodia are elongate, narrowly hooded, decreasing posteriorly from 4–6 to 2 per fascicle (Fig. 4G).

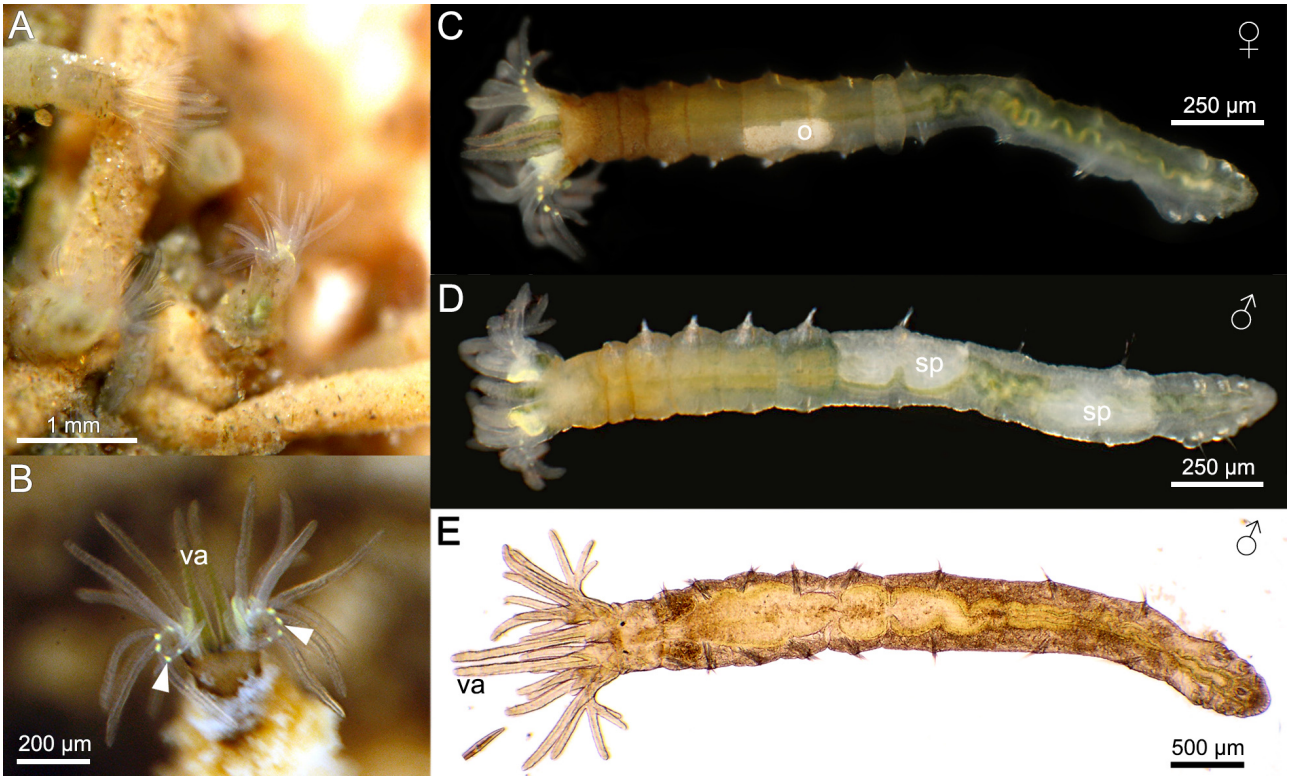

FIGURE 2. Gross morphological features of *Manayunkia occidentalis* **sp. nov.** resolved by light microscopy: A, multiple individuals emerged and feeding from their tubes; B, Individual annelid with prominent vascularized appendages (va) and showing refractile spots on one feeding palp on each side (arrowheads); C, female in reflected light, showing white oocytes (o) in segments 4–6; D, male in reflected light showing bright spermatogonic tissue (sp) in multiple posterior segments; E, Male in transmitted light showing parallel vascularized appendages (va) and fanned-out feeding palps anteriorly with two dark eyespots in peristomium, and granular coelom posteriorly due to presence of developing sperm.

Variation. Paratypes are similar to the holotype. However, females have oocytes in thoracic chaetigers 4–5 (Fig. 2C) and a pair of pigmented spermathecae in the base of the radiolar crown. Females differ from males in having thoracic neuropodia with uncini in chaetigers 2–5 followed by 3–6 elongate hooded chaetae in neuropodia of chaetigers 6–8 (Fig. 3E,G).

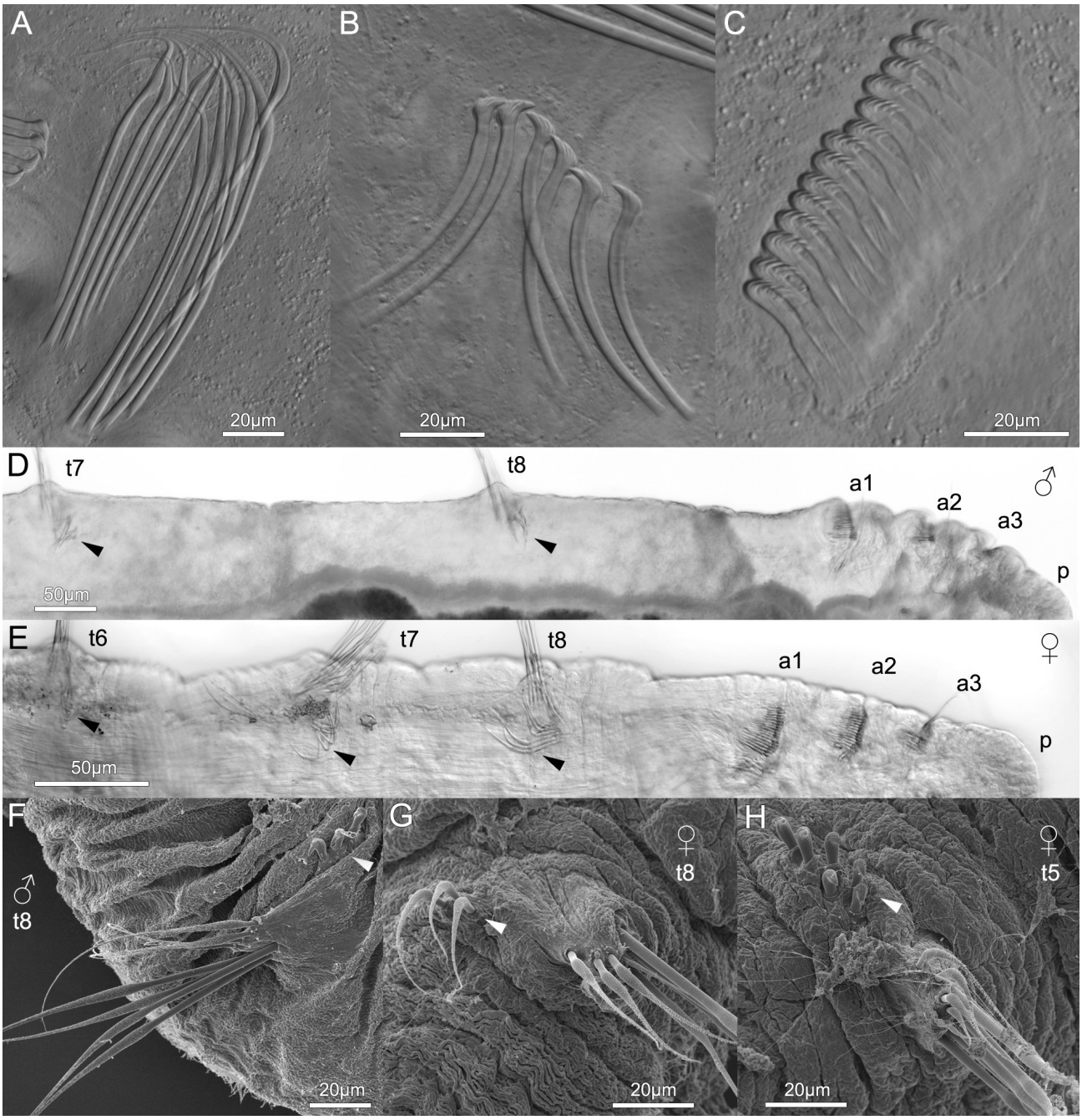

FIGURE 3. Images showing chaetal characters of *Manayunkia occidentalis* **sp. nov.**: A, inferior and superior notochaete typical of thoracic segments 2–8; B, thoracic uncini; C, abdominal uncini; D, dorsal view of posterior thoracic segments and abdomen of male showing typical uncini in chaetigers t7 and t8 (arrowheads) and in scanning electron micrograph shown in F; E, dorsal view of posterior thoracic segments and abdomen of female showing neuropodia of thoracic chaetigers 6–8 with elongate neuropodia instead of uncini, seen also in scanning electron micrograph in G (arrowhead). H, anterior thoracic segment of female showing notochaetae and uncini (arrowhead), characteristic of thoracic segments 2–5 in both sexes.

Remarks. *Manayunkia occidentalis* **sp. nov.** is the sister group to *M. speciosa* based on COI sequence data with a minimum pairwise distance (simple distance) to our *M. speciosa* samples of 11.0%, clearly supporting that they can be regarded as a separate taxon, although phylogenetic support for this relationship was weak (bootstrap

28%). *Manayunkia occidentalis* **sp. nov.** and *M. speciosa* are the only species of *Manayunkia,* and in fact of any Fabriciidae (see Huang *et al*. 2011), to show sexual dimorphism with regards to thoracic chaetal complement. Males of *M. occidentalis* **sp. nov.**, and *M. speciosa* (Rouse pers. obs.) on specimens from near the type locality (SIO-BIC A12106), show the typical arrangement of thoracic uncini in each of chaetigers 2–8, while females have thoracic uncini in chaetigers 2–5 only (Fig. 3H). The neuropodia of chaetigers 6–8 instead have elongate hooded chaetae (Fig. 3G). These elongate hooded chaetae were seen in all females examined. Larval development would be worth studying to see if there is a loss of thoracic uncini or if they never develop in chaetigers 6–8. It is somewhat surprising that this major difference in chaetal complement was never noted previous in observations on *M. speciosa. Manayunkia occidentalis* **sp. nov.** differs markedly from descriptions of *M. speciosa* by Leidy (1883) and Pettibone (1953) in that it has far fewer pinnules on the radioles. In this regard *Manayunkia occidentalis* **sp. nov.** is similar to other *Manayunkia* species that have relatively few pinnules (see Rouse 1996).

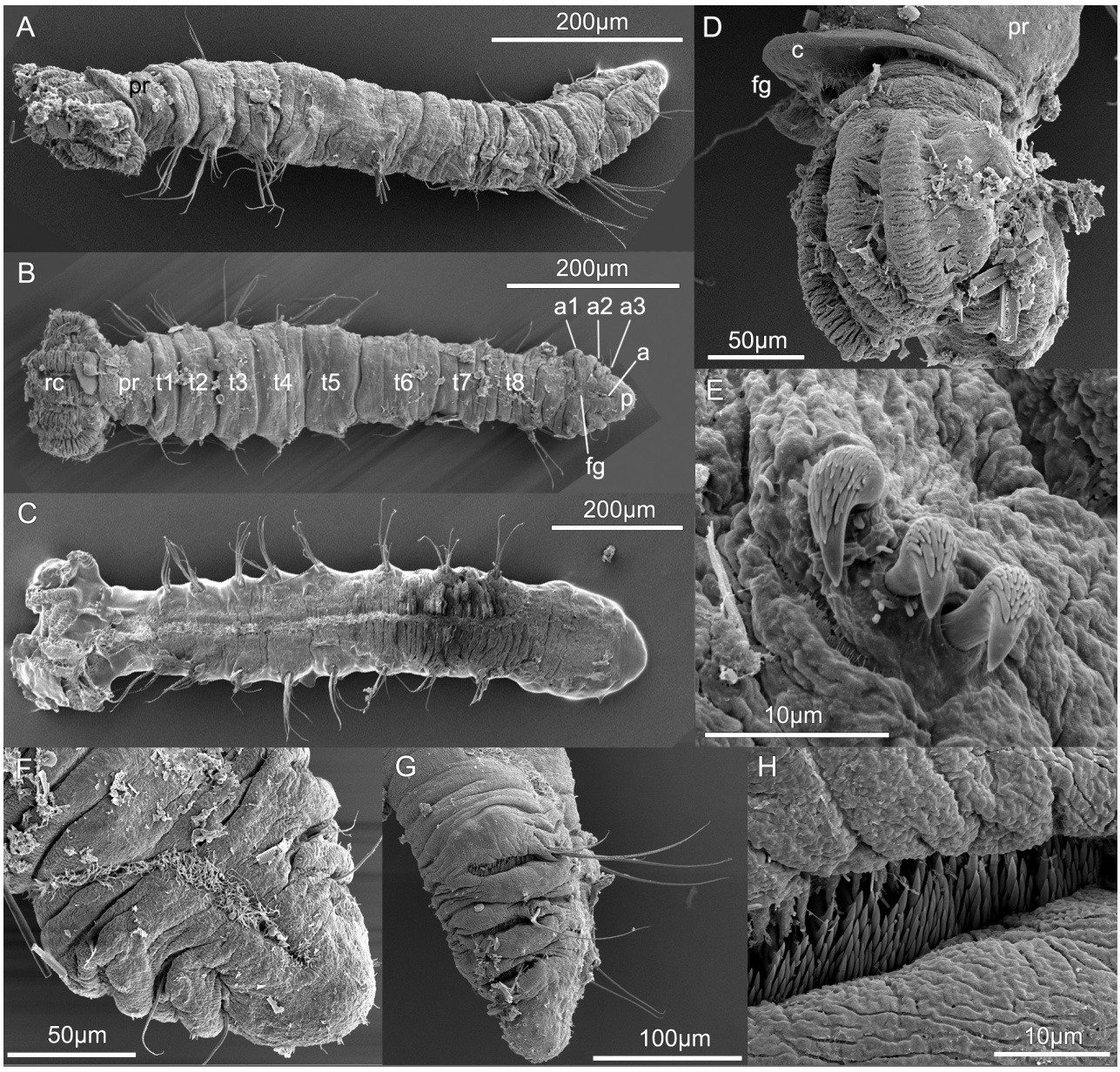

FIGURE 4. Morphological features of *Manayunkia occidentalis* **sp. nov.** resolved by scanning electron microscopy: A, lateral view; B, dorsal view; C, ventral view; D, anterior showing membranous collar (c) of the peristomal ring (pr), margin with fecal groove (fg); mucus covers the pinnules and adheres diatoms; E, detail of typical thoracic uncini; F, abdominal segments and anus, showing ciliated fecal groove extending anteriorly and wrapping around to the ventral surface at boundary between abdominal and thoracic segments; G, lateral view of thoracic segments showing contracted uncini and prominent neuropodia; H, higher magnification view of retracted abdominal uncini.

Discussion

Manayunkia speciosa was described originally from Philadelphia, USA with subsequent reports from the Great Lakes and several west coast river basins. This annelid is reported to be the obligate alternate host of two myxozoan parasites *Ceratonova shasta* and *Parvicapsula minibicornis* (Bartholomew *et al*. 1997; 2006). Unambiguous identification of the invertebrate hosts of myxozoans, which produce the infectious stage to fish, is essential for risk assessment and stock management as these parasites cause disease and death within populations of salmon and trout. Our present findings support the hypothesis that the annelid host of the parasites has likely been mis-identified as *M. speciosa*, and is actually a novel taxon *Manayunkia occidentalis* **sp. nov.**

The 94 west coast *M. occidentalis* **sp. nov.** specimens that we characterized had *COI* sequences at least 11% different from our reference *M. speciosa* sequences from Lake Superior and New Jersey. This east-west genetic divergence underpinned our rationale for describing the west coast annelids as a distinct, novel taxon. Among the *M. occidentalis* **sp. nov.** specimens, the greatest genetic difference (4.1%) was between isolates from the two rivers, which are more than 500 km apart. These genetic differences are not surprising given the relative immobility of the annelids, particularly from above- and below-dam localities, and between river basins separated by saltwater. We hypothesize that similar degrees of genetic differences, with corresponding novel haplotypes, will be observed among populations in other unconnected river basins on the West Coast (Fraser, Rogue, Sacramento), and probably within large basins like the Columbia.

We did not detect any *M. speciosa* among the 94 annelids sampled from multiple localities in two river basins. This was surprising given historical records of that taxon in West Coast basins, particularly associated with myxozoan parasites (see citations listed under Systematics). Our collection localities in the Klamath River included one where "*M. speciosa*" was collected and sequenced previously (Malakauskas *et al*. 2015; GenBank accession number KX026668). This published sequence is only minimally divergent (0–1%) from our own *M. speciosa* reference sequences collected from Lake Superior and the East Coast (Fig. 5). We cannot explain how these trans-continental *M. speciosa* sequences could be so similar, given that we observed genetic distances up to 2.8% among *M. occidentalis* **sp. nov.** samples from the Klamath Basin, and up to 4.1% between specimens from different West Coast river basins. Based on our survey data, we postulate that *M. speciosa* may not be present in the Klamath or Willamette rivers, and we suspect that the earlier assignations of Klamath River annelids as *M. speciosa* are incorrect. Almost all previous identifications relied on morphology, and we have now shown that there are few morphological characters to distinguish the two taxa, though the markedly smaller number of pinnules in *M. occidentalis* **sp. nov.** is an obvious difference from *M. speciosa*. This and the *COI* sequence divergence of at least 10.5% makes future discrimination between *M. speciosa* and *M. occidentalis* **sp. nov.** relatively straightforward.

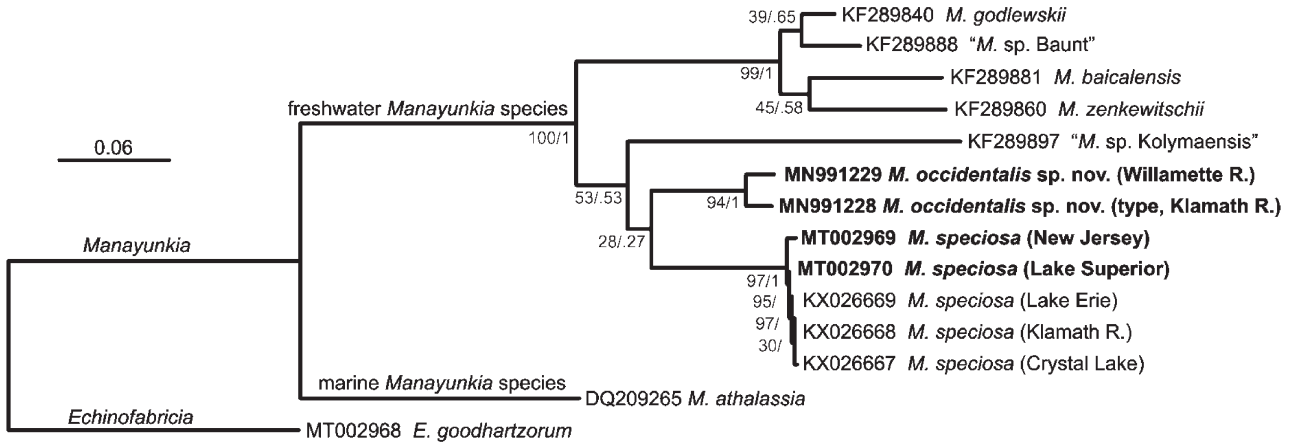

FIGURE 5. Phylogram from Maximum Likelihood (ML) analysis of COI data for representative *Manayunkia* species, with bootstrap values given at nodes and GenBank accession numbers given in parentheses; novel sequences indicated by boldface. Bayesian analysis gave equivalent tree topology (posterior probabilities shown after ML bootstrap values at nodes).

Twenty-one of the 94 examined annelids were infected with either or both *C. shasta* and *P. minibicornis*, and all were identified by sequence as *M. occidentalis* **sp. nov.**, and thus we could not confirm that *M. speciosa* is a host for these myxozoans. If *M. speciosa* is not a permissive host, then this would explain why *C. shasta* and *P. minibicornis*

have not become established in the Great Lakes or in East Coast river systems where *M. speciosa*, only, is present. It is likely, however, that *M. speciosa* hosts other myxozoan species, as evidenced by detection of *Ceratonova*-like DNA in annelids from the Great Lakes (Malakauskas *et al.* 2016). We predict that we will find *M. occidentalis* **sp. nov.** in all river basins where the parasite is found on the west coast, and none in the Great Lakes. Accordingly, we are sampling and sequencing additional annelid populations from river basins where *C. shasta* and *P. minibicornis* occur, to determine the phylogeography of the host, any correlation between infection and host haplotype, and if any *M. speciosa sensu stricto* exists on the west coast of North America.

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