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On *Kincaidiana* Altman, 1936 and *Guestphalinus* Michaelsen, 1933 (Annelida, Clitellata, Lumbriculidae), with the descriptions of three new species

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Abstract. Two formerly monotypic lumbriculid genera, *Guestphalinus* Michaelsen, 1933 and *Kincaidiana* Altman, 1936, are reviewed using morphological and molecular data, following the discovery of new northwestern, Nearctic species. Several populations of *Kincaidiana hexatheca* Altman, 1936 were examined, and both morphology and DNA data suggest a single, variable species in Pacific drainages extending from northern California through Washington, USA. Specimens of *Kincaidiana* from the Smith River drainage with a single, median atrium and differing genetically from *K. hexatheca* are assigned to *K. smithi* sp. nov. The chaetal morphology of North American *Guestphalinus* populations is variable, and two basic morphotypes are assigned to *G. elephantinus* sp. nov. and *G. exilis* sp. nov. This decision is supported by molecular data. The tree topology, based on the mitochondrial 16S rRNA and Cytochrome Oxidase I (COI), and the nuclear 28S rRNA gene sequences, confirmed the close phylogenetic relationships among the Nearctic *Guestphalinus*, *Kincaidiana* and *Uktena* Fend, Rodriguez & Lenat, 2015. Probable synapomorphies associating these genera include a filiform, ringed proboscis, a forward shift of reproductive organs relative to the usual position in the family, and spermathecae in the atrial segment.

Keywords. Taxonomy, new taxa, Clitellata, Lumbriculidae, *Kincaidiana*, *Guestphalinus*.

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Introduction

Recent worm collections from northwestern USA have yielded many specimens of large, proboscis-bearing lumbriculids with spermathecae in the atrial segment (a character uncommon within the family), and atria in a segment anterior to X (the most common position in the family). Thus, these distinctive worms show affinities with the genera *Guestphalinus* Michaelsen, 1933, *Kincaidiana* Altman, 1936 and *Uktena* Fend *et al.*, 2015.

The European *Dorydrilus* (*Guestphalinus*) *wiardi* Michaelsen, 1933, later transferred to the monotypic genus *Guestphalinus* by Hrabě (1936), was originally known from caves in Germany and had a unique combination of characters: semiprosoporous male ducts (atria with both anterior and posterior vasa deferentia), atria in IX, and paired spermathecae only in the atrial segment. Soon afterward, the genus *Kincaidiana* was designated for *K. hexatheca* Altman, 1936, a distinctive lumbriculid from western North America. *Kincaidiana hexatheca*, also with atria in IX, differed from *G. wiardi* in having prosoporous male ducts (each atrium with only posterior vas deferens) and spermathecae paired in three segments, IX–XI. Other defining characters of *K. hexatheca* included bifid chaetae in some anterior segments, and highly branched lateral blood vessels. More recently, Fend *et al.* (2015) described the monotypic *Uktena riparia* Fend, Rodriguez & Lenat, 2015 from southeastern USA. Like *K. hexatheca,* it had prosopore male ducts, and like *Guestphalinus,* spermathecae were restricted to a single pair in the atrial segment. However, the location of atria in VIII, and other diagnostic characters, such as large spermatophores and numerous copulatory chaetae, were unique to *Uktena* within the family Lumbriculidae.

In this paper, we describe and incorporate new collections of North American worms associated with the above taxa. Two new *Guestphalinus* species are described herein, based on both morphological and molecular data, and their morphology is compared with specimens of *G. wiardi* from two sites in southern Europe. *Kincaidiana hexatheca*, which has been reported in a variety of habitats in Pacific Coastal watersheds from northern California to southern British Columbia (Altman 1936; Cook 1971; Brinkhurst 1976; Fend 2009), shows considerable intra- and inter-population differences in size and other morphological details that will be examined here. The most unusual *Kincaidiana* population, collected from northern California, has a single, median male pore and atrium. This morphotype is here attributed to a new species, *Kincaidiana smithi* sp. nov., and is supported by molecular evidence. Phylogenetic relationships among these genera are also discussed on the basis of morphology and available molecular data.

Material and methods

Streams were sampled from both erosional and depositional habitats in northwestern U.S.A. (the states of Washington, Oregon, and California), using a 500 μm mesh dip net. In riffle habitats, worms were usually found by disturbing patches of finer gravel to a depth of about 20 cm. More depositional habitats in streams, as well as small seeps and pools, were generally sampled by sieving silt and organic detritus with a 500 μm screen. Some *Kincaidiana hexatheca* were collected by digging in saturated riparian mud. Most specimens were relaxed in dilute ethanol and fixed in formalin-aceto-alcohol for morphological study, or in 80–90% ethanol for DNA. Specific conductance was measured at a few sites in April, 2014, using a Cole Parmer model 1481-80 conductivity meter.

Most specimens used for morphological study were stained in either hematoxylin or borax carmine, partially destained in acidified alcohol, longitudinally dissected, dehydrated in an alcohol series, cleared in methyl salicylate, and mounted in Canada balsam. Sagittal sections were cut at 7 μm, transverse sections at 10 μm. All sections were stained in hematoxylin and eosin. The descriptions of reproductive structures are based on mature specimens, with sperm in the spermathecae, and usually with well-developed eggs. Characteristics of "nearly-mature" worms (e.g., atria and spermathecae appear incompletely developed, or without sperm in sperm funnels and spermathecae) and "post-reproductive" worms (e.g., some

resorption of reproductive organs, sperm sacs and egg sacs almost empty, spermathecae without darkly-staining sperm) are described in some cases. Measurements of chaetae are based on slide-mounted worms, but body lengths and widths were supplemented with measurements obtained from unmounted worms in alcohol; unless otherwise noted, measurements of somatic characters were based on specimens with at least partially-developed reproductive pores. Measurements are given as ranges, or as a range followed by the median value in parentheses. Segment numbers are indicated by Roman numerals; intersegments by Arabic numerals (as 9/10 to indicate the septum between IX and X). To simplify the descriptions, we use the general spatial terms "ental vs ectal" (internal vs body wall) for the orientation of internal organs having external pores. For description of chaetae we have used the terms "proximal/distal", which refer to the distance to the anchor point in the body wall.

Posterior parts of selected specimens (Table 1) were cut and transferred into 95% ethanol for the molecular analyses; anterior parts were treated following the procedures described above and identified to species level. DNA from tissue of posterior parts was extracted using the DNAeasy Tissue Kit (Qiagen) or the QuickExtract DNA Extraction Solution 1.0 from Epicentre (following instructions from the manufacturer). We used primers LCO1490 and HCO2198 (Folmer *et al.* 1994) or COI-E (Bely & Wray 2004) for COI, 16SAR-L and 16SBRH (Palumbi *et al.* 1991) for 16S and 28SC1 and 28SC2 (Dayrat *et al.* 2001) for the 28S rRNA. Each PCR reaction consisted of 21 µl ddH2O, 1 µl of each primer (10 µM), 2 µl template DNA and Illustra PuReTaq Ready-To-Go PCR Beads (0.2 ml tubes) (GE Healthcare). Conditions for PCR were 95ºC/300s, (95ºC/30–40s, 45–54ºC/30–45s, 72ºC/30–60s)* 35 cycles, 72ºC/480s. PCR products were purified using Exonuclease I (Fermentas) and FastAP Thermosensitive Alkaline Phosphatase (Fermentas) and sequenced at Macrogen Sequencing System, South Korea or at MWG Eurofins Operon in Edersberg, Germany. Genious 5.3 (Biomatters Ltd.) was used to obtain consensus sequences from assembled forward and reverse sequences, and to edit them. Alignments were made using Clustal X (Larkin *et al.* 2007) with default settings and then manually corrected in Geneious if necessary. Reading frame shifts were checked for the coding gene COI. Alignments resulted in 658 bp of COI, 496 bp of 16S and 331 bp of 28S data. Trees were built using Bayesian inference (BA) and maximum likelihood (ML). For Bayesian analysis (BA) we used MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003). The best-fit models were selected using the Akaike information criterion (AIC) in MrModeltest v. 2.3 (Nylander 2004) in conjunction with PAUP* v. 4.0b10 (Swofford 2002). For COI, a partitioned site specific rate model was used: we applied the Symmetrical model of sequence evolution (SYM) with a proportion of the sites invariable for the COI 1st codon position, the Felsenstein model (F81) for the COI 2nd codon position and the Hasegawa-Kishino-Yano model (HKY) with gamma distributed rates across sites for the COI 3rd codon position. For 16S, we applied the General Time Reversible model (GTR) with gamma distributed rates across sites. For 28S, the model selected was GTR with a proportion of the sites invariable. The number of generations was set to one million with four parallel chains (three hot and one cold), sample frequency to every 100th generation, and number of runs to two. The first 2500 samples were discarded as the 'burn-in' of the Markov chain. We checked for stationary and convergence of the chains with TRACER v. 1.6 (Rambaut *et al.* 2014). For ML analysis we used the online version of RaxML BlackBox (Stamakis *et al.* 2008), with 100 bootstrap replicates by using the GTR ϒ model. All model parameters were estimated by the program from its own maximum parsimony starting trees. The alignment was divided into the same 5 partitions as used for the Bayesian analysis. Phylogenetic relationships were considered strongly supported if posterior probabilities (pp) in Bayesian analysis were ≥ 0.95 and/or bootstrap values (bv) in maximum likelihood analysis were ≥ 70% (Hillis & Bull 1993; Alfaro *et al.* 2003). Uncorrected (p) sequence distances were generated using PAUP*.

Type specimens are deposited in the U.S. National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA (USNM), California Academy of Sciences, Invertebrate Zoology, San Francisco, California, USA (CASIZ) and Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN).

Table 1 (continued on next page)**.** List of taxa and specimens used in the molecular study, corresponding GenBank accession numbers, voucher data, localities and sampling data. "Specimen" numbers refer to the catalog number in the database of C. Erséus. New sequences are indicated by bold. Both COI sequences for *G. elephantinus* sp. nov. are identical.

Taxa	Locality and sampling date	Collectors	Specimen	GenBank Acc. No. COI	GenBank Acc. No. 16S	GenBank Acc. No. 28S	Voucher
Ingroup							
Guestphalinus elephantinus Fend & Rodriguez sp. nov.	Shale Creek at Clearwater Creek Road, Jefferson Co., Washington, USA, 4 Jun. 2003	Steven Fend	CE703_2	**KY884694**	**KY884700**	**KY884708**	CASIZ 220934 Paratype
Guestphalinus elephantinus Fend & Rodriguez sp. nov.	Clearwater River at Copper Mine Bottom Camp, Jefferson Co., Washington, USA, 25 Apr. 2004	Steven Fend	CE865_1	GU592303	–	–	SMNH105628
			CE865_2	–	GU592334	–	
Guestphalinus exilis Fend & Rodriguez sp. nov.	Squaw Creek, at Chirpchatter Campground, deep gravel-cobble riffle, Shasta Co., California, USA, 19 Apr. 2010	Steven Fend	CE10499	**KY884695**	**KY884701**	**KY884709**	CASIZ 220937 Paratype
Kincaidiana hexatheca Altman, 1936	Cow Creek, tributary to Umpqua River, Douglas Co., Oregon, USA, 28 Apr. 2004	Steven Fend	CE861	GU592304	GU592335	–	S. Fend collection
Kincaidiana hexatheca Altman, 1936	Inglenook Fen at McKerricher State Park, Mendocino Co., California, USA, 7 Jul. 2006	Steven Fend	CE2289	**KY884696**	**KY884702**	**KY884710**	CASIZ 220927
Kincaidiana smithi Fend & Rodriguez sp. nov.	Smith River below forks, Del Norte Co., California, USA, 8 Jun. 2003	Steven Fend	CE700	KY884697	KY884703	–	CASIZ 220929

Taxa	Locality and sampling date	Collectors	Specimen	GenBank Acc. No. COI	GenBank Acc. No. 16S	GenBank Acc. No. 28S	Voucher
Ingroup							
Uktena riparia Fend *et al.*, 2015	Flat Creek at Ft. Bragg, Hoke Co., North Carolina, USA, 17 Dec. 2013	David Lenat	CE20858	KY884698	KY884704	KY884711	CASIZ 220926
Outgroup							
Eclipidrilus frigidus Eisen, 1881	Big Spring at Bassets (near Downieville, Yuba River drainage), Sierra Co., California, USA, 4 Nov. 2002	Steven Fend	CE559	**KY884699**	**KY884705**	**KY884712**	S. Fend collection
Lumbriculus variegatus (Müller, 1774)			CE27	FJ639298	FJ639263	**KY884713**	no voucher
Stylodrilus heringianus Claparède, 1862	Dry stream on limestone at Drösarp, Mörbylånga, Öland, Sweden, grass-roots and sand, grass roots and sand; 56.5749° N, 016.6058° E, 13 Jun. 2007	Anna Ansebo, Lisa Matamoros and Christer Erséus	CE2993	JX993896	**KY884706**	**KY884714**	SMNH 126490
Trichodrilus strandi Hrabě, 1936	Spring fen in Jasénka (Kotrlé), Vsetín, Czech Republic, 3 May 2010	Jana Schenkova	CE8819	KR296745	**KY884707**	**KY884715**	SMNH 143121

Generated sequences are deposited in GenBank. Additional material is retained in the collections of S. Fend, U.S. Geological Survey, Menlo Park, California, USA or P. Rodriguez, University of the Basque Country, Spain. Detailed information on specimens used for molecular analysis, with museum voucher and GenBank numbers, are given in Table 1. Additional site locality data, with geographic coordinates, are given in Appendix 1.

Abbreviations

at = atrium
CASIZ = California Academy of Sciences, Invertebrate Zoology, San Francisco, California, USA
ff = female funnel
fp = female pore
gl = gland
mf = male funnel (mf1 anterior, mf2 posterior)
MNCN = Museo Nacional de Ciencias Naturales, Madrid, Spain
mp = male pore
np = nephridial pore
ov = ovary
pr = prostate
sa = spermathecal ampulla
SMNH = Swedish Museum of Natural History, Stockholm, Sweden
sp = spermathecal pore (sp1 first/anterior spermatheca, sp2 second spermatheca, ...)
st = spermatheca
te = testis
USNM = US National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA
UWBM = University of Washington Burke Museum of Natural History and Culture, Seattle, Washington, USA
vd = vas deferens

Results

Morphology

Class Clitellata Michaelsen, 1919
Order Lumbriculida Brinkhurst, 1971
Family Lumbriculidae Vejdovský, 1884

Kincaidiana Altman, 1936

Type species

Kincaidiana hexatheca Altman, 1936.

Included species

Kincaidiana hexatheca Altman, 1936
Kincaidiana smithi Fend & Rodriguez sp. nov.

Remarks

The diagnosis in Fend (2009) is modified to include the new species with a single, median male pore and atrium, and spermathecal pores in line with ventral chaetae. New observations of several populations of *K. hexatheca* (see below) indicate that chaetae in anterior segments may appear as simple bifids (as

in previous descriptions), but typically have more complex structure: a broad proximal tooth, a smaller dorsal tooth, and a thin dorsal keel. Anterior chaetae are rarely simple-pointed.

Altman (1936) described *K. hexatheca* and discussed the relationship of *Kincaidiana* to another proboscis-bearing (Holarctic) genus, *Rhynchelmis* Hoffmeister, 1843, but made no mention of the European *Guestphalinus wiardi* (Michaelsen, 1933). *Rhynchelmis* differs from *Kincaidiana*, *Guestphalinus* and *Uktena* in having spermathecae only in segments anterior to the atria, and the (usually) semiprosoporous atria are (almost always) located in segment X. *Kincaidiana freidris* Cook, 1966 (later transferred to *Altmanella* Fend, 2009) has prosoporous atria in VIII; however, it differs from *K. hexatheca* in most other respects: it is a small worm, without a proboscis, and spermathecae are present only in the post-atrial (not in the atrial) segment. The hexathecate condition, with three spermathecal segments beginning with the atrial segment, is only known in *Kincaidiana* and in *Cookidrilus* Rodriguez & Giani, 1987. In the latter genus, there is no proboscis, the atria and spermathecae begin in X, instead of IX, and the male ducts are paired and semiprosoporous.

Kincaidiana hexatheca Altman, 1936
Figs 1–3

Kincaidiana hexatheca Altman, 1936: 64–68, figs 53–59, 66.

Kincaidiana hexatheca – Brinkhurst & Cook 1966: 10, figs 2A, 5B, D, I. — Cook 1971: 237, figs 5.4 F–I, 5.5 D. — Fend 2009: 3–6, figs 1–2.

Material examined

Lectotype
UNITED STATES OF AMERICA: a sagittally sectioned worm on 4 slides, Series II, from the Altman collection, Washington, Pacific County, Loomis Lake, 11 Sep. 1929 (UWBM).

Other type material (Altman collection, UWBM)
UNITED STATES OF AMERICA: 1 dissected, same locality as lectotype, 14 Nov. 1931; 1 dissected, same locality as lectotype, 26 May 1932; 1 dissected, undetermined location (possibly Loomis Lake), 6 Apr. 1931; 1 dissected, undetermined location (possibly Loomis Lake), 23 Apr. 1931; 1 dissected, no date (#199); additional syntypes were examined by Fend (2009, figs 2D–E, G–H).

New collections (mature specimens, unless otherwise noted)
UNITED STATES OF AMERICA: WASHINGTON: 2 sagittally sectioned, 2 dissected, several in alcohol, Jefferson County, small seep along Hoh River Road, 29 Apr. 1999, S. Fend leg.; 1 dissected, 2 in alcohol, same locality as preceding, 26 Apr. 2004, S. Fend leg.; 3 dissected, Hoh River drainage, roadside ditch, on Clearwater Road, 3 Jun. 2003, S. Fend leg.; 1 dissected, Pacific County, spring on Naselle River, 30 Apr. 1999, S. Fend leg.; 1 dissected, Clallam County, small pool along Bogachiel River on Undi Road, 26 Apr. 2004, S. Fend leg.; 1 mature, 3 post-mature, dissected, Clark County, Salmon Creek watershed, 10 Sep. 2001, R. Wisseman leg.; 2 partially-mature, dissected, Skamania County, Dog Creek near mouth, 26 Apr. 2014, P. Rodriguez and S. Fend leg. – OREGON: 2 dissected, 1 whole mount, Multnomah County, Oneonta Creek near mouth, downstream of Oneonta Gorge parking area, Columbia Gorge, 4 Jun. 2003, S. Fend leg.; 1 dissected (partially-mature), 1 whole mount, Tillamook County, small spring on Nestucca River 0.4 miles above Blaine, on Bible Creek Road, near Tillamook, 4 Jun. 2003, S. Fend leg.; 3 dissected, several in alcohol, Yamhill County, seep on east side of McGuire Reservoir near McMinnville, 4 Jun. 2003, S. Fend leg.; 7 dissected, several in alcohol, Lane County, muddy seep at Big Creek, FR5700, 11 May 2001, S. Fend leg.; 1 dissected, 1 in alcohol, same locality as preceding, 28 Apr. 2014, S. Fend leg.; 1 dissected, 4 immature, slide mounts, Lane County,

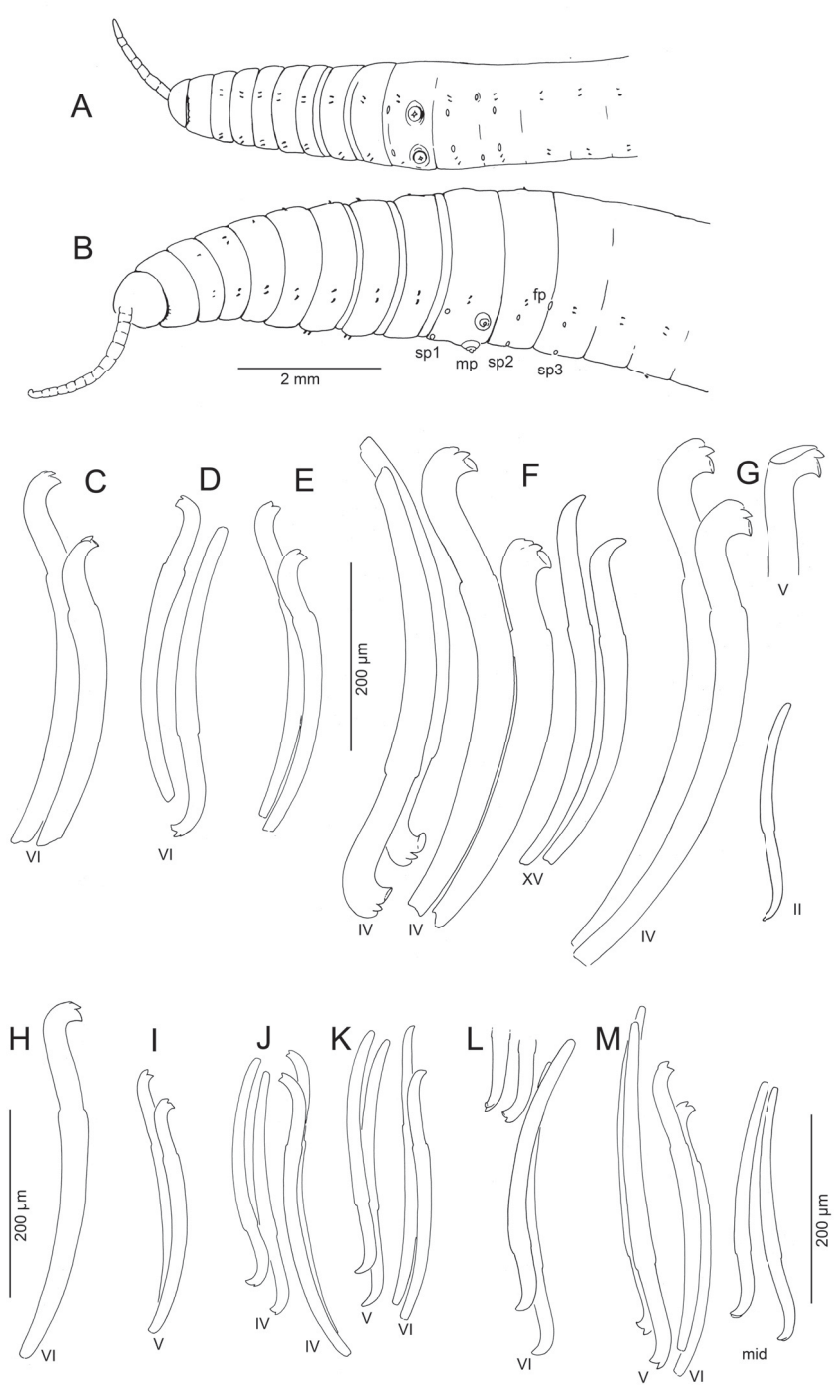

Fig. 1. *Kincaidiana hexatheca* Altman, 1936 from various sites. **A–B**. Complete worms, ventral view of anterior segments, from McKenzie River, OR (A), and from Big Creek, OR (B). **C–M**. Representative (mostly anterior) chaetae from different populations (ordered approximately north-south); dorsal chaetae with tips upward, ventrals with tips down. **C**. Hoh River, WA. **D**. Pool at Bogachiel River, WA. **E**. Salmon Creek watershed, WA, anterior dorsal pair. **F–G**. Two specimens from Big Creek, OR; tip of one dorsal chaeta with keel folded over. **H**. Rogue River, OR. **I**. From a *Darlingtonia* bog, O'Brien, OR. **J–K**. Two specimens from spring near Mule Creek, OR; K is from a smaller specimen, with simple-pointed chaetae. **L**. Illinois River, OR; older chaetae simple-pointed (worn?), replacements bifid. **M**. Inglenook Fen, CA. Scale bars: A–B = 2 mm; C–M = 200 µm.

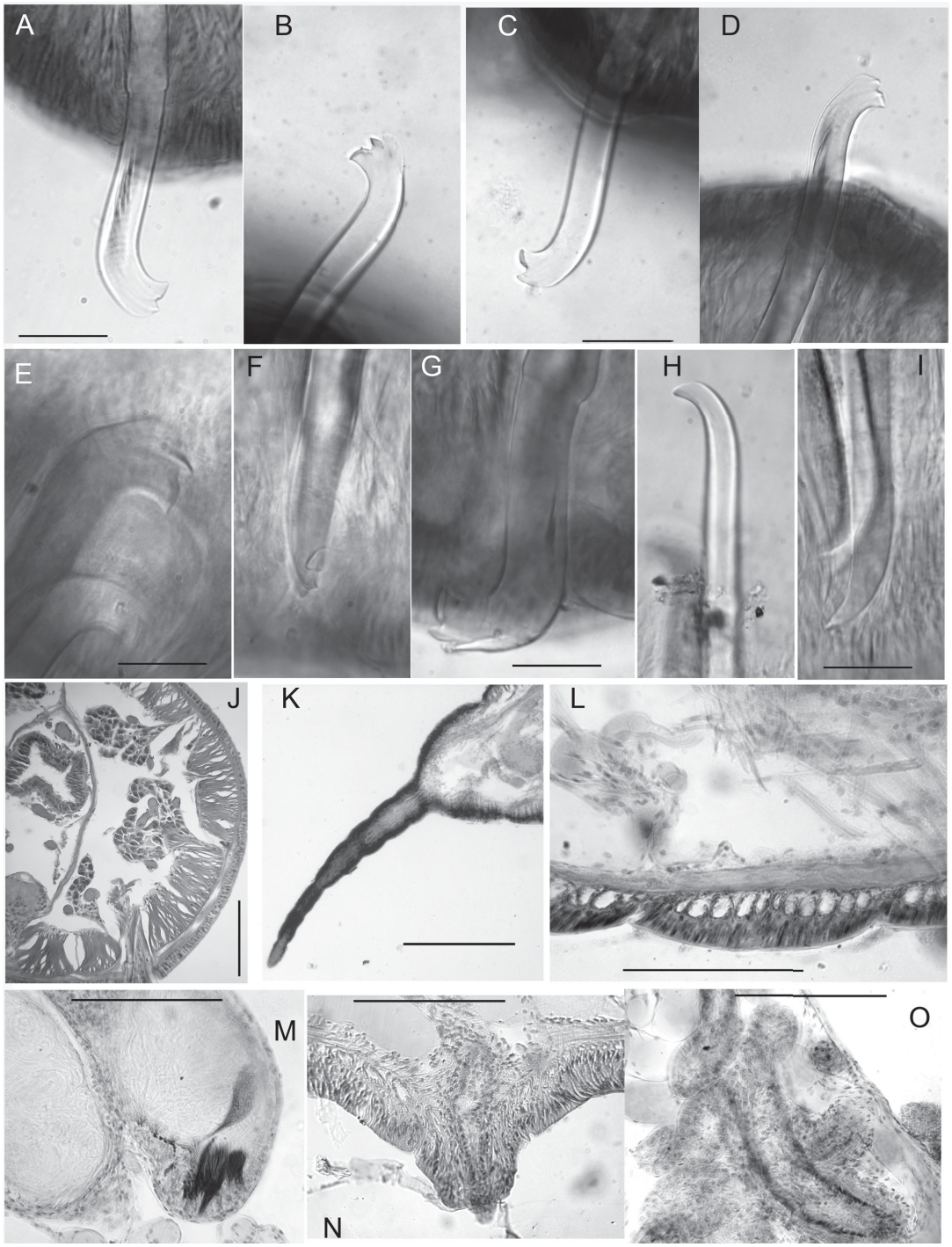

Fig. 2. *Kincaidiana hexatheca* Altman, 1936 from various sites. A, K–O from the sagittally sectioned lectotype, Loomis Lake, WA; B–C from Hoh River, WA; D from Inglenook Fen, CA; E–I from Big Creek, OR; J from O'Brien, OR. **A-I**. Tips of chaetae in anterior segments. **A**. IV ventral. **B**. VI dorsal. **C**. V ventral. **D**. V dorsal. **E**. IV dorsal. **F**. V ventral, frontal view. **G**. V ventral, chaeta with keel folded over. **H**. Dorsal, posterior segment. **I**. XV, ventral. **J**. Transverse section of anterior segment, showing 3-lobed pharyngeal gland. **K**. Ringed proboscis. **L**. Body wall in anterior segment, showing bands of circular muscle. **M**. Spermathecal ampulla, with concentrated sperm. **N**. Male porophore and penis. **O**. Atrium, prostate glands and vas deferens. Scale bars: A–I = 50 μm; J, L–O = 200 μm; K = 500 μm.

small spring at mouth of Tenmile Creek, 1 May 1999, S. Fend leg.; 1 whole mount (immature), Lane County, marsh at base of Mt Pisgah, near Eugene, 29 Jan. 2000, S. Fend leg.; 6 dissected, Lane County, outflow from Leaburg fish hatchery (McKenzie River), 19 May 2013, S. Fend leg.; 1 dissected, 2 in alcohol, Whittaker Creek at Siuslaw River, 12 Aug. 2016, S. Fend leg.; 3 immature whole mounts (1 is DNA voucher CE861), Douglas County, Cow Creek, tributary to Umpqua River, 28 Apr. 2004, S. Fend leg.; 6 dissected, 2 in alcohol, Douglas County, spring above Mule Creek, 7 Jun. 2003, S. Fend leg.; 1 transverse section, 4 dissected, 5 whole mounts, Josephine County, *Darlingtonia* bog near O'Brien, off Wimer-Lone Mt Road, 26 Oct. 1999, S. Fend leg.; 1 whole mount, Josephine County, Illinois River near Sixmile Creek, 25 Oct. 1999, S. Fend leg.; 1 whole mount, same locality as preceding, 7 Jun. 2003, S. Fend leg.; 1 dissected, 1 whole mount, same locality as preceding, 21 May 13, S. Fend leg.; 3 dissected, 1 whole mount, 3 immature dissected, Curry County, Rogue River at Quosatana Campground (NFS) ca 19 miles above Gold Beach, 8 Jun. 2003, S. Fend leg. – CALIFORNIA: 9 dissected (plus 3 immature dissected), Mendocino County, Inglenook Fen at McKerricher State Park, slow creek, roots of Apiaceae, 1 Jul. 2005, S. Fend leg.; 1 dissected DNA voucher, same locality as preceding, 7 Jul. 2006, S. Fend leg. (CE2289).

Molecular data

COI, 16S, and 28S sequences are from two specimens, collected at Inglenook Fen, California and Cow Creek, Oregon (details in Table 1).

Description of new material

Size variable (Table 2); specimens from swampy habitats usually larger than those from gravel-bed streams; largest specimens (length > 100 mm, diameter > 2 mm, segments > 200, Fig. 1B) from muddy seeps in Oregon. No obvious latitudinal difference in size (see length, width and segment numbers in Table 2). Largest specimens considerably larger than material collected by Altman (1936) (diameter 0.75–1.25 mm) from two sites in Washington and one in Oregon. Proboscis elongate, appearing ringed externally, but lacking internal septa (Figs 1A–B, 2K).

Chaetae in anterior segments (II to VI, VII or VIII) almost always modified, with distal ends oriented anteriad. Modified anterior chaetae may appear simply bifid in lateral view, with short upper tooth (Figs 1C, 2C), but structure usually more complex: lower tooth broad and flattened or concave, and thin dorsal keel may extend beyond upper tooth (Figs 1F–G, 2A–B, E–G). Keel visible as translucent outer edge in lateral view, or narrow point in frontal view (Fig. 2F); usually most prominent on replacement chaetae, but absent on many chaetae, possibly due to wear; keel often broken or folded over (Figs 1G, 2G) in mounted specimens, with chaeta appearing trifid. This structure occurs throughout the geographic range of the species, but not always visible in specimens from some populations. Chaetae in atrial and postatrial segments usually simple-pointed, moderately sigmoid and oriented posteriad; tips slightly keeled in posterior segments on some specimens (Fig. 1M).

Chaetae in segment II always smaller than those in next several segments; ventrals in II usually simple-pointed. Chaetae from III to about VII or VIII usually thicker than (but similar in length, see Table 2) to those in following segments, with more distal nodulus; distinctly longer in some individuals, and up to 50% longer in Big Creek (west-central Oregon) worms. Within a bundle, inner chaeta typically longer than outer, with more proximal nodulus (Fig. 1C, F). Greatest modification in size, position of nodulus, and development of teeth in anterior chaetae in specimens from Big Creek (Figs 1F–G, 2E–G).

Pharynx with high columnar cells from II to IV, with dorsal and lateral epithelium higher than ventral. Intestine begins in 6/7. Pharyngeal glands in V or VI to VII or VIII, on each side produced into 3 irregular, anteriorly directed lobes (Fig. 2J) joining at the base (posterior septum). Contrary to the description by Altman (1936), they were never observed in II or III, and are about equally distributed dorsally

Table 2. Descriptive measurements (mean, range, and n) for *Kincaidiana hexatheca* Altman, 1936 from different regions (from north to south: Washington, Oregon and California), and *K. smithi* Fend & Rodriguez sp. nov.

	Number of segments	Length, mm	Width in X, mm	Maximum width, mm	Anterior chaetae, max. length, µm	Mid-body chaetae, max. length, µm	Porophore height, µm	Atrium length, µm	Atrium length / body width	Spermathecal duct average length, µm	Spermatheca average length, µm	Spermatheca length/ body diameter
***K. hexatheca* Altman, 1936: Washington, USA**												
mean	**176**	**73**	**1.6**	**1.8**	**401**	**369**	**135**	**941**	**0.53**	**451**	**1563**	**0.83**
max	*220*	*95*	*2.1*	*2.2*	*446*	*396*	*170*	*1230*	*0.64*	*563*	*1913*	*0.92*
min	*146*	*62*	*1.1*	*1.2*	*335*	*314*	*80*	*690*	*0.47*	*246*	*1156*	*0.79*
n	11	11	19	18	8	8	10	11	8	11	5	4
***K. hexatheca* Altman, 1936: Oregon, USA**												
mean	**172**	**75**	**1.5**	**1.7**	**347**	**322**	**90**	**806**	**0.57**	**411**	**1292**	**0.91**
max	*289*	*183*	*2.6*	*2.8*	*628*	*555*	*140*	*1230*	*0.96*	*598*	*1770*	*1.18*
min	*92*	*40*	*0.7*	*1.0*	*199*	*214*	*60*	*209*	*0.24*	*120*	*545*	*0.70*
n	40	44	58	58	30	30	7	21	19	22	16	14
***K. hexatheca* Altman, 1936: California, USA**												
mean	**183**	**70**	**1.3**	**1.4**	**320**	**300**	**93**	**922**	**0.72**	**442**	**1498**	**1.15**
max	*235*	*104*	*1.9*	*2.1*	*385*	*372*	*160*	*1300*	*0.91*	*520*	*2165*	*1.42*
min	*130*	*48*	*1.1*	*1.2*	*259*	*244*	*44*	*725*	*0.59*	*355*	*985*	*0.90*
n	7	9	12	10	14	14	8	11	9	11	10	6
***K. smithi* Fend & Rodriguez sp. nov.: Smith River, California, USA**												
mean	**144**	**55**	**1.2**	**1.3**	**353**	**302**	**167**	**1169**	**1.02**	**472**	**1318**	**1.04**
max	*165*	*68*	*1.5*	*1.8*	*472*	*369*	*202*	*1620*	*1.18*	*717*	*1915*	*1.32*
min	*117*	*42*	*1.0*	*1.1*	*274*	*245*	*100*	*825*	*0.75*	*264*	*857*	*0.76*
n	9	9	13	13	10	10	6	9	8	12	13	7

and ventrally. Nephridia begin on 11/12, with narrow, dorsally-directed postseptale, as described by Fend (2009). Circular muscle layer of body wall forms distinct bands in anterior segments (Fig. 2L).

Lateral trunks of dorsal blood vessel join to form ventral vessel in III or IV, anterior to location in Altman's description (V). Unbranched, but highly convoluted commissural vessels in anterior segments to about XX. One or two pairs of lateral vessels in segments posterior to about XX; morphology of these vessels variable in middle segments; most commonly, the anterior is larger, branched and covered in chloragogen cells; alternatively, the posterior pair may be branched, and the anterior pair simple, as stated by Altman (1936). Posterior segments may have two pairs of branched vessels.

Male pores always paired, median and posterior to ventral chaetae on IX (Fig. 1A–B); conical porophores developed (Fig. 2N) or not (Fig. 3B–C). Spermathecae paired in IX, X, and XI; spermathecal pores slightly displaced towards ventral midline; pores in IX slightly anterior to chaetae, those in X–XI level with chaetae (Fig. 1A–B).

Atria generally more elongate-tubular than in the illustration by Altman (1936: fig. 59) (Fig. 3). Atrial ampulla and ectal duct weakly differentiated, ampulla distinguished only by slightly greater diameter and presence of prostates (Fig. 2O). Atrium length, including length relative to body width, shows overlap among regions (Table 2). Atria usually entirely in IX, but extend into X in four specimens from sites throughout the species distribution (Fig. 3C). Male funnels may be displaced back within sperm sacs as far as XI (Fig. 3A, C) or even XII.

Spermathecal duct about 300–600 µm long, tubular, histologically differentiated from ampulla, having thick, irregular epithelium of columnar cells and more well-developed muscular layer. Duct may be sharply constricted at ectal end, as it joins a narrow epidermal infolding. Spermathecal ampulla 800–1600 µm long; sharply expanded in basal part in some specimens (Fig. 3B), as in the original description (Altman 1936: fig. 57), but more typically elongate-tubular (Fig. 3A, C–D). Sperm usually lined up along epithelium near ectal end of ampulla (Figs 2M, 3D), absent in the duct.

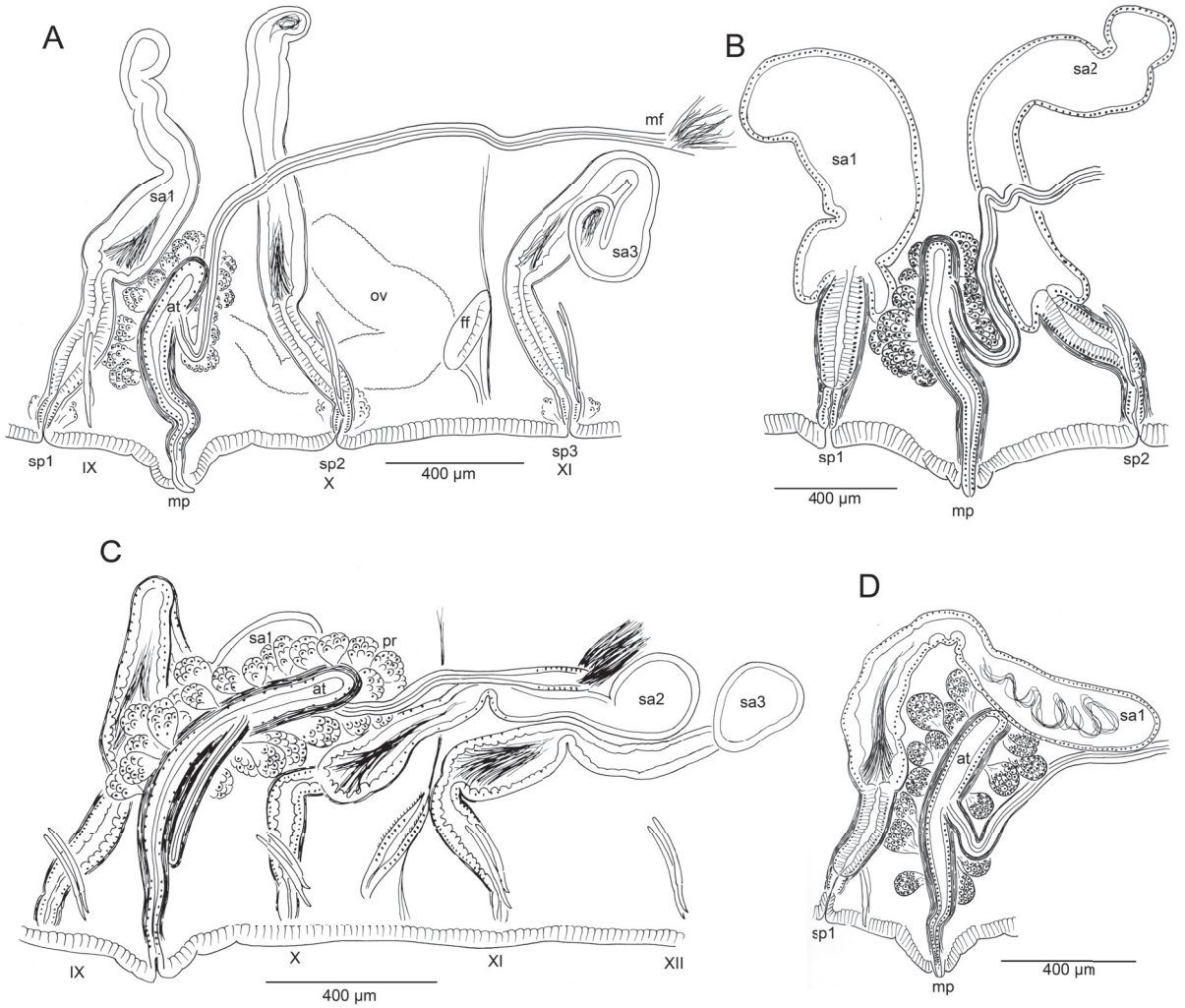

Fig. 3. *Kincaidiana hexatheca* Altman, 1936, reproductive organs. **A–B**. From Hoh River, WA; (A) segments IX-XI; (B) atrium and spermathecae with expanded ampullae in IX-X. **C–D**. From Rogue River, OR; (C) reproductive organs in IX-XI from an atypical worm with long atria; (D) atrium and spermatheca in IX, from a typical worm.

Remarks

The type series was not clearly designated by Altman (1936), and material used in the original description appears to have been collected from 3 sites in southwestern Washington and northwestern Oregon. The sagittally sectioned specimen here designated as the lectotype (Fig. 2A, K–O) shows most of the diagnostic characters: annulated proboscis, bifid/keeled anterior chaetae, spermathecae paired in atrial and 2 postatrial segments, conical male porophores, short-tubular atria with prosopore male ducts paired in IX. Other worms from the type series (mostly from undetermined localities) also show these characters (see fig. 2D–E, G in Fend 2009).

In addition to examining 18 apparent syntypes, Fend (2009) verified diagnostic characters in specimens from additional sites in Washington, Oregon, and California, but did not discuss regional or population differences. Here we examine variation in morphological characters in specimens from throughout the known geographic distribution of the species and molecular differences between specimens from two sites in the southern part of the range, near the type locality of *Kincaidiana smithi* sp. nov. (see below).

The bifid, anteriorly-directed chaetae in preclitellar segments are one of the most distinctive characters for *K. hexatheca*. Despite some population differences in size of these chaetae, the general pattern was consistent in most populations. However, the occurrence of worms from a few sites in southwestern Oregon, having typical *K. hexatheca* reproductive organs, but with simple-pointed or only slightly bifid anterior chaetae, cautions against reliance on this character alone for identifying immature specimens. All chaetae (including replacements) were simple-pointed in a partially-mature specimen from Cow Creek (Umpqua River drainage), and immature worms from the same site were similar; chaetae in the first few anterior segments were not enlarged, although they were anteriorly-directed. This condition was variable in mature and immature specimens from two sites in the Rogue River drainage: a spring near Mule Creek (Fig. 1J–K) and the Illinois River (Fig. 1L); in one such specimen the replacement chaetae are bifid (Fig. 1L), but in other individuals all chaetae (including replacements) were simple-pointed. This suggests that simple-pointed chaetae do not simply reflect wear in worms from gravel-bed streams.

There was no obvious latitudinal difference in size and exent of modification of anterior chaetae (Table 2). The maximum chaeta length in anterior segments of several populations was considerably larger than the 0.218 mm reported by Altman (1936).

Morphology of reproductive organs varied within populations (Fig. 3), possibly masking regional differences. Our observations differ from prior descriptions in minor details. Cook (1971) stated that spermathecal pores are behind the ventral chaetae in IX, but Altman (1936) placed them “between, and just ventral” (median?) to the ventral chaetae. All of the new material has spermathecal pores distinctly displaced towards the ventral midline, and the first pair is clearly anterior to the ventral chaetae (Fig. 1A–B). Atrium length varied by about a factor of 2 within each region, although this was less when normalized by body diameter (Table 2). The limited data suggest that atria of specimens in the California population were larger relative to body size. Spermatheca size varied similarly due to the elongate, irregular ampullae; however, the duct length was less variable.

The species appears to be endemic to the Pacific northwestern USA and British Columbia (Kathman & Brinkhurst 1998); reported records from other regions cannot be verified, as material was mostly unavailable for study. Some confusion may be based on ambiguous somatic characters regarded as distinctive in published keys (e.g., Kathman & Brinkhurst 1998). In particular, wrinkling due to fixation of other proboscis-bearing species may be interpreted as a “pseudo-segmented” proboscis, and minutely bifid chaetae (possibly a result of wear) on some specimens of *Rhynchelmis* may also cause confusion. For example, Spencer & Denton (2003) tentatively attributed immature specimens from

Utah to *K. hexatheca*, but recent examination by one of the authors (S. Fend) of some of this material deposited in the Bean Life Science Museum (Brigham Young University, Provo, Utah) suggested that they were more likely to be a species of *Rhynchelmis*. Therefore, morphology-based identification of immature specimens of *K. hexatheca* should ideally be based not only on the proboscis and presence/absence of modified anterior chaetae, but also on other morphological characters. For example, two pairs of lateral blood vessels in segments X–XX of the Nearctic *Rhynchelmis* species (if present) are short, usually branched, and do not join the ventral vessel (Fend & Brinkhurst 2000).

Habitat

Kincaidiana hexatheca has been collected in many coldwater habitats, ranging from cobble riffles in large streams to small, muddy seeps, typically associated with aquatic plants (e.g., skunk cabbage, *Lysichiton americanus* Hultén & St. John and water parsley, *Oenanthe sarmentosa* Presl).

Kincaidiana smithi Fend & Rodriguez sp. nov.
urn:lsid:zoobank.org:act:9485884B-2496-42B0-8488-5A1A89514F9C
Figs 4–5

Etymology

Named for Prof. Jerry Smith, in recognition of his contributions to the ecology and management of Pacific coastal streams.

Material examined

Holotype

UNITED STATES OF AMERICA: a dissected worm, stained in hematoxylin and mounted in Canada balsam, California, Del Norte County, Smith River below forks, 8 Jun. 2003, S. Fend leg. (USNM 1422281).

Paratypes

UNITED STATES OF AMERICA: 1 dissected, same data as for holotype (USNM 1422282); 1 dissected, same data as for holotype (CASIZ 220930); 1 sagittally sectioned, same locality as for holotype, 10 May 2009, S. Fend and P. Rodriguez leg. (CASIZ 220928); 1 dissected, seep by South Fork Smith River, 10 May 2009, S. Fend and P. Rodriguez leg. (CASIZ 220931); 1 dissected, same data as preceding (MNCN 16.03/3102).

Additional material (all partially-mature)

UNITED STATES OF AMERICA: 1 sagittally sectioned, 2 dissected, 1 whole mount, 3 in alcohol, from type locality, 8 June 2003; 2 dissected, same locality as preceding, 10 May 2009.

Molecular data

COI and 16S sequences correspond to topotypic voucher CASIZ 220929 (details in Table 1).

Description

Medium-sized to large worms (Table 2); prostomium short, length about ½ width; filiform proboscis 1–2 mm long, 0.1–0.16 mm diameter, not widened at base, externally ringed with multiple shallow constrictions (Fig. 4A). Body segmentation externally distinct in anterior segments, weak in clitellum and posteriorly; secondary annulation may appear as a narrow posterior ring in V–VII or VIII.

Chaetae two per bundle; those in II to (VII)VIII (IX) directed anteriad, others directed posteriad. Chaetae in II or III to VI or VIII appear bifid with large, flat ventral tooth and thinner dorsal tooth (Fig. 5D–E);

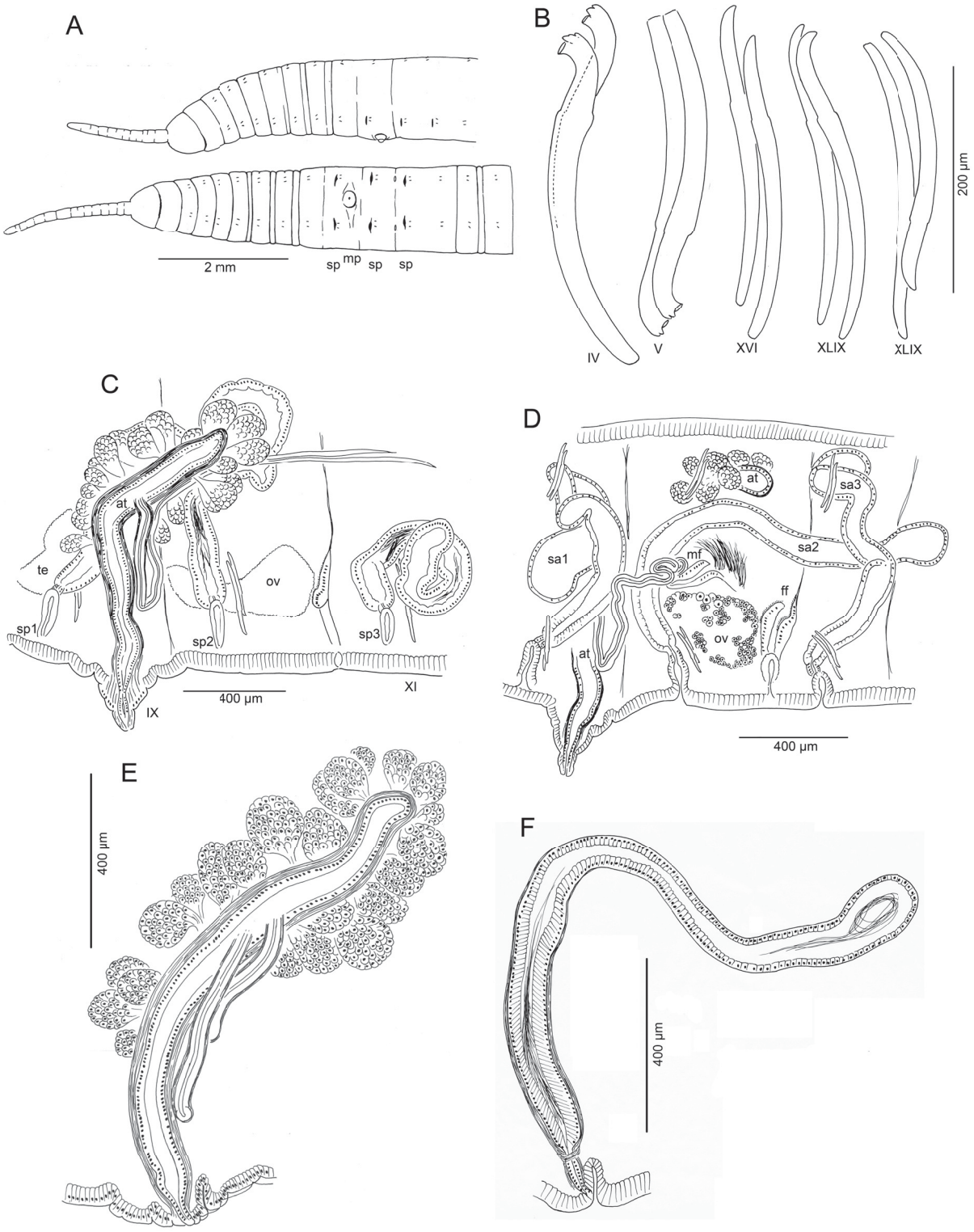

Fig. 4. *Kincaidiana smithi* Fend & Rodriguez sp. nov., from the type locality, except for C**,** from seep by South Fork Smith River, CA. **A**. Complete worm, lateral and ventral views, showing location of reproductive pores. **B**. Representative chaetae: Roman numerals indicate segment number; dorsal chaetae with tips upward, ventrals with tips down. **C**. Reproductive organs in IX-XI. **D**. Reproductive organs in IX-XI, from a sagittal section (middle portion of atrium missing). **E**. An atrium, joined by both vasa deferentia. **F**. A spermatheca.

some chaetae with a thin dorsal keel above the upper tooth (Fig. 4B). Modified anterior chaetae strongly arcuate distal to the nodulus; shortest in II, gradually increasing in size from III to VI, maximum anterior chaeta length 270–470 μm. Within each pair, median (inner) chaeta has nodulus 0.3–0.4 the distance from the tip; lateral (outer) chaeta may be slightly thicker, with more distal nodulus, 0.25–0.3 distance from tip. Chaetae in IV–VII may be longer and thicker (width 15–22 μm) than more posterior chaetae (width 11–15 μm). Posterior to VI–VIII, chaetae usually simple-pointed (Fig. 4B), moderately sigmoid, and directed posteriad; dorsals about the same length as ventrals. Within each posterior pair, lateral chaeta has slightly more distal nodulus. Chaetae in segments posterior to about LXXX may be strongly arcuate; dorsal pairs may appear slightly bifid or with an upper keel (Fig. 5F).

Epidermis in anterior segments 25–32 μm thick; in clitellum 50–70 μm, thinner in posterior segments. Clitellum from about mid-VII to mid-XIII. In anterior segments, circular muscle layer of body wall arranged in a series of transverse bands with gaps between (Fig. 5B); this layer is 25–50 μm thick in pre-clitellar segments, gradually narrowing posteriorly down to 5–7 μm and appearing homogeneous. Longitudinal muscle layer of body wall 70–100 μm thick in anterior and middle segments. Pharynx from I–III or IV, with dorsal and lateral wall moderately thickened; transition to esophagus indistinct. Pharyngeal glands V–VII, with dorsolateral, median and ventrolateral lobes joining at posterior septa; lobes are joined between segments by thin extensions. No abrupt division between esophagus and intestine. Chloragogen cells cover gut beginning in VII. Brain in the peristomium, not deeply lobed.

Dorsal blood vessel separate from gut to about VIII, then closely appressed posteriorly. One pair of commissural blood vessels join dorsal blood vessel near posterior septum between II and about XX; these vessels lack a dense chloragogen layer; in pre-clitellar segments they are long and sinuous; those originating in II–VI join the ventral vessel(s) in the next segment; posteriorly, they are shorter, and join both dorsal and ventral vessels in the same segment. A pair of lateral, blind blood vessels, covered with chloragogen cells, joins the dorsal vessel in the anterior part of segments beginning in about XVI; at first, these are short and unbranched, but by XXV they reach the ventral part of the body, and have up to 10 long branches; by XL they may have more than 20 branches and fill much of the coelom. A second pair of blind, branched lateral blood vessels is located in the posterior part of each segment, posterior to about segment L. In more posterior segments (by about C), both pairs of lateral vessels have many short dorsal branches.

Nephridia usually paired on 11/12; occurring irregularly in posterior segments, absent from many segments. A small anteseptal funnel is followed by narrow, dorsally-directed, granular postseptal mass (Fig. 5C). Efferent duct forms a closely-appressed loop, extending to dorsal part of body cavity, forming a convoluted mass; duct ends in a narrow vesicle in front of ventral chaetae in the originating segment; nephridiopore inconspicuous.

Male pore single, median and posterior to ventral chaetae on IX; ectal tip of the atrial duct protrudes as a short penis. When everted, penis is subtended by a conical, tiered porophore (Fig. 5M–N), the entire structure up to about 200 μm high by 250–300 μm wide at base; porophore circular, usually consisting of two concentric epidermal folds. Spermathecae paired in IX, X, and XI; spermathecal pores on or very slightly median to ventral chaetal lines, all slightly anterior to respective chaetae (Figs 4A, 5A). One pair of testes in IX, reaching to mid-segment; one pair of ovaries in X, extending to back of segment or into XI; female funnels intersegmental in 10/11, up to 280 μm high. Sperm sacs extend posteriad to XVII–XXIV; no anterior sperm sacs; egg sacs with large eggs extend 1–2 segments behind sperm sacs.

Spermathecal ducts terminate within narrow vestibules, 100–130 μm deep; junction usually constricted by a muscular ring, forming a short sphincter (Fig. 5K). Spermathecae nearly tubular, about 1000–2000 μm long, weakly divided into two sections, both of which may contain sperm (Figs 4F, 5H–J). Ectal

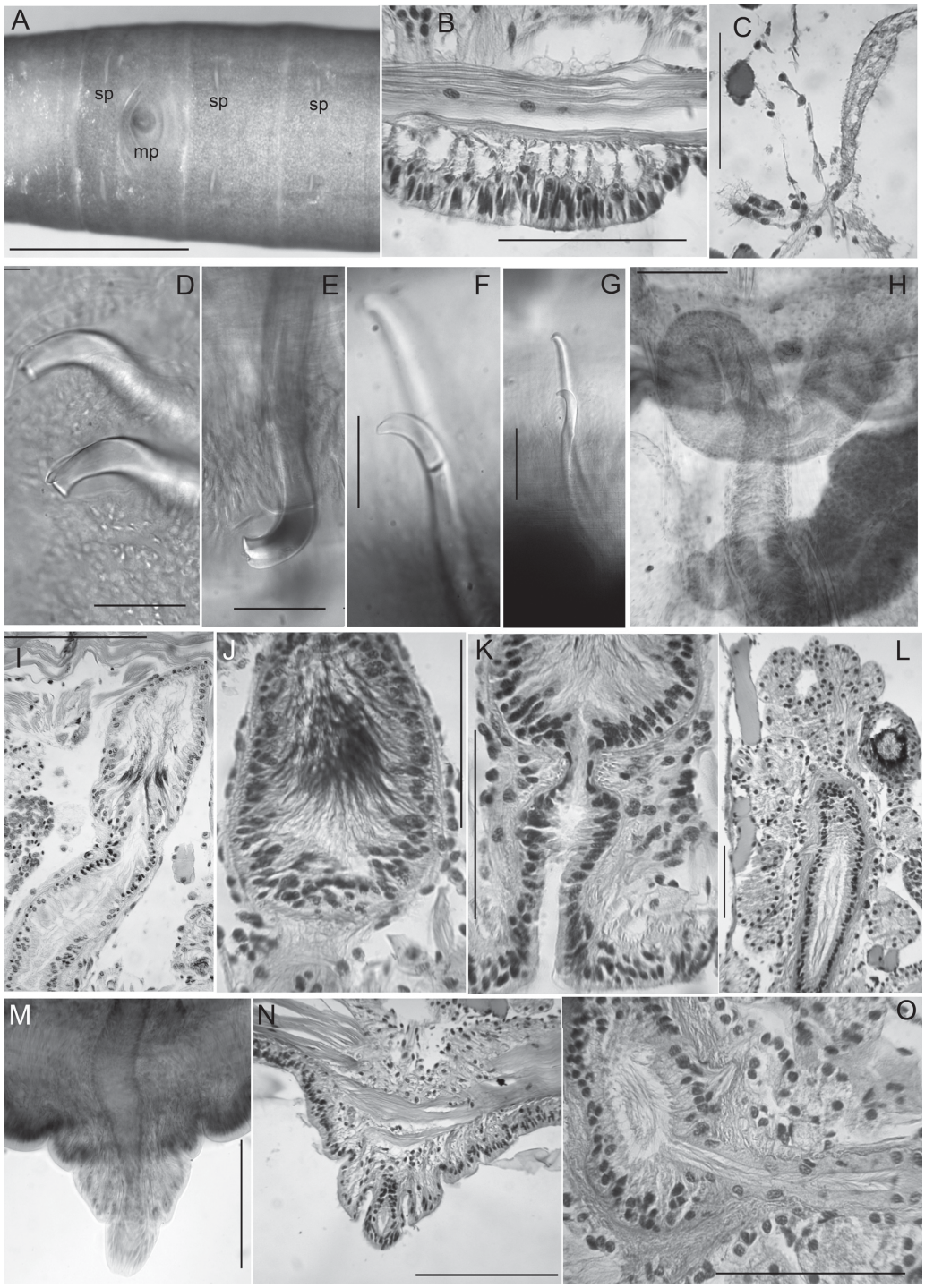

Fig. 5. *Kincaidiana smithi* Fend & Rodriguez sp. nov., from the type locality. B–C, I–L, N–O from sagittal sections; D–H, M from dissections. **A**. Ventral view of an entire worm, stained with hematoxylin, showing positions of genital pores. **B**. Body wall in segment III, showing longitudinal and transverse muscle layers. **C**. Nephridial funnel and postseptal expansion. **D**. Chaetae in V dorsal. **E**. IV ventral. **F–G**. Chaetae in posterior segments; (F) is detail of tip, showing slight keel. **H**. Spermatheca. **I**. Junction of spermathecal "duct" and "ampulla"; sperm are in the ampullar part. **J**. Ectal end of spermathecal duct, with sperm lined up along epithelium. **K**. Spermathecal vestibule and ectal end of duct, with muscular sphincter. **L**. Atrium with prostate glands. **M–N**. Penis and male porophore. **O**. Vas deferens joining atrium near middle. Scale bars: A = 1 mm; B–C, G, J–L, O = 100 μm; D–F = 50 μm; H–I, M–N = 200 μm.

third is duct-like, 100–130 μm wide, with uneven, columnar epithelium 25–50 μm thick, and distinct muscle layer 5–8 μm thick. Ental, ampullar section mostly tubular (about 75–85 μm wide), but may expand to 100–200 μm wide near ental end. Epithelium of ectal, duct-like section of spermatheca may be penetrated by heads of darkly-staining sperm cells which densely fill the lumen (Fig. 5J). Epithelium thinner (about 10 μm) in ental part of ampulla, and lumen wider, containing small amount of unordered, poorly staining sperm. Spermathecae in IX usually largest; those in XI smallest and may lack sperm. Spermathecae may extend posteriad through 1–2 segments (Fig. 4D).

Male pore without obvious glands, but surrounded by diffuse mass of muscle tissue. Single, prosoporous atrium is joined by both (posterior) vasa deferentia near the midpoint; they enter the atrial lumen directly at the junction (Fig. 5O). Atrium narrows ectally within the porophore; the remainder tubular, not divided into distinct regions, extending at least into mid-X (Fig. 4C–D); length 825–1620 μm, width 110–130 μm, including thick (12–25 μm) muscle layer. Atrial muscle layer thickest in ectal part, with fibers unevenly transverse to slightly diagonal, but not lined up (parallel) or in distinct layers; epithelium cuboidal, 10–15 μm thick. Ental ½–⅔ of the atrium covered by thick layer of multicellular prostate glands, 120–190 μm high; glands petiolate, broadly pyriform, with many cells (Figs 4E, 5L). Vasa deferentia 1200–1400 μm long, 34–40 μm wide; widest near the atrium (up to 40–48 μm), where they are covered by a thin muscle layer. Vasa extend posteriorly one or more segments, terminating in elongate, narrowly conical sperm funnels, up to 400 μm long.

Remarks

Both morphology and molecular results (see below) support *K. smithi* sp. nov. as a distinct species, closely related to *K. hexatheca*. Despite the morphological variability of *K. hexatheca* throughout its broad geographic range, *K. smithi* sp. nov. is distinctive in that all specimens from the type locality and a nearby site have a single, median male pore and atrium joined by both of the posterior vasa deferentia. The single, median atrium of *K. smithi* sp. nov. is unusual within the family, but this character appears in both species of *Tatriella* Hrabě, 1939, as well as in some species of *Eclipidrilus* Eisen, 1881 (see Fend & Lenat 2012). As in *K. smithi* sp. nov., this arrangement does not usually represent a simple loss of one of the male ducts from the normal paired condition. In *Eclipidrilus pacificus* Fend, 2005, for example, all four vasa deferentia join the median atrium (Fend 2005: fig. 9B). Aside from rare and presumably teratological variation within populations, only *Eclipidrilus ithys* Brinkhurst, 1998 was described as having either one or two atria (Brinkhurst 1998); but when only one is present, the entire male duct is missing from one side.

Compared with most specimens of *K. hexatheca*, the atrium in *K. smithi* sp. nov. is more elongate, usually entering the post-atrial segment; nevertheless, this occasionally occurs within the range of variation in populations of *K. hexatheca* (Table 2, Fig. 3C). The spermathecal pores are clearly displaced from the ventral chaetae line towards the mid-body line in *K. hexatheca*, while in *K. smithi* sp. nov. the pores are on the chaetal line, anterior to the chaetal bundles (Fig. 1A–B vs 4A). The morphology of the spermathecae is similar to that of *K. hexatheca*; however, they are typically more narrow-elongate, with faint distinction between ampullar and duct portions.

Other characters of *K. smithi* sp. nov. conform closely to those of its congener, *K. hexatheca*. Both have a large, rather cylindrical body, with a ringed proboscis. The chaetal morphology resembles that of typical *K. hexatheca*, and the distribution and orientation of modified anterior chaetae are also similar. Less conspicuous, but nevertheless unusual characters are also shared with *K. hexatheca* (see Fend 2009): the circular musculature of the body wall, the narrow, dorsally-directed nephridia, and the muscle layer extending along the ectal end of the vasa deferentia.

Habitat

The Smith River site is a large (average discharge >100 m^3/s), free-flowing stream with riffle-pool structure and gravel-boulder substrate. Specific conductance was 90 µS cm^{-1} in April 2014 (62–150 µS cm^{-1} in 1978–1981, NWIS 2016a). *Kincaidiana smithi* sp. nov. was only found in a backwater area with some silt deposition. The other collection site was a small roadcut seep, with slow current and some rooted aquatic vegetation. Guts were filled with undetermined organic matter and very fine mineral particles, with diatoms.

Guestphalinus Michaelsen, 1933

Type species

Dorydrilus (*Guestphalinus*) *wiardi* Michaelsen, 1933.

Included species

Guestphalinus wiardi (Michaelsen, 1933)
Guestphalinus exilis Fend & Rodriguez sp. nov.
Guestphalinus elephantinus Fend & Rodriguez sp. nov.

Emended diagnosis (modified from Michaelsen 1933; Cook 1971)

Medium-sized to large worms with a filiform proboscis. Chaetae two per bundle. Paired testes in VIII and IX, one pair of ovaries in X. Male pores paired in IX, near 9/10. Spermathecal pores paired in IX, anterior to the male pores. Petiolate copulatory glands (= Pubertätsdrüsen in Michaelsen 1933) associated with male and/or spermathecal pores. Male duct semiprosoporous. Penes absent. Atria elongate, cylindrical to club-shaped, ental part loosely covered with multicellular, pyriform prostate glands. Anterior vasa deferentia form a loop in the pre-atrial segment before entering the atrial segment. Paired spermathecae in the atrial segment, anterior to the atria.

Distribution

Europe and northwestern USA (present study). *Guestphalinus wiardi* is rarely reported, but is known from subterranean or spring habitats in Germany (Michaelsen 1933; Griepenburg 1941), Slovenia (Hrabě 1973) and Italy (new material used for the present description, see below), with other records from mountain streams in Crimea (Dembitsky 1987).

Remarks

Among the lumbriculids with a filiform proboscis, *Guestphalinus* is distinguished from the semiprosoporous species of *Rhynchelmis*, *Eclipidrilus* (*Premnodrilus*) Smith, 1900 and *Eremidrilus* Fend & Rodriguez, 2003 by the location of the male pores in IX (instead of X), the spermathecae in the atrial segment, the anterior vasa deferentia entering the pre-atrial segment, and the morphology of the elongate spermathecae. In addition to being prosoporous, the two *Kincaidiana* species are easily distinguished from *Guestphalinus* by the presence of spermathecae also in the first two post-atrial segments, and by a different type of modified chaetae in several pre-clitellar segments. *Uktena* is distinguished from other proboscis-bearing lumbriculids by having atria and spermathecae in VIII (rather than IX), in addition to characters unique within the family: spermatophores, a spermathecal copulatory organ, and multiple genital chaetae (Fend *et al.* 2015).

Guestphalinus was originally described as a subgenus of *Dorydrilus* Piguet, 1913 (Michaelsen 1933), but was later elevated to generic status (Hrabě 1936, although spelled *Questphalinus* in that paper). This decision, although considered provisional in the 1936 paper, has been maintained in subsequent literature (Cook 1971; Hrabě 1973; Dembitsky 1987).

Guestphalinus wiardi (Michaelsen, 1933)
Fig. 6

Dorydrilus (*Guestphalinus*) *wiardi* Michaelsen, 1933: 7, figs 1–2.

Questphalinus wiardi – Hrabě 1936: 10; 1973: 45, figs 4–6.
Guestphalinus wiardi – Cook 1968: 281, fig. 2e; 1971: 237. — Dembitsky 1987: figs I–III.

Material examined

SLOVENIA: a single specimen, sagittally sectioned on 3 slides, near Dornberk (Czech National Museum (Prague), Hrabě collection, 1970–25 I1–3).

ITALY: a sagittally dissected anterior end, Grotta Sulfurea, Frassasi Cave system, Genga/Ancona, 43.401° N, 12.966° E, Marche, Sharmishtha Dattagupta and Jennifer L. Macalady leg., Jun. 2007.

Description of new material and remarks

Hrabě (1973) described a mature, unmated specimen from Slovenia, with sperm on the male funnels. This specimen from Hrabě's collection is reexamined here, but only the anterior body section was available. The figures in Hrabě (1973: figs 6–7) correspond to photographs 6B and 6C, respectively, in the present paper (note that labels for spermathecal and male pores should be reversed in Hrabě: fig. 6). The Italian specimen is mature, with clitellum from VIII–XIII and sperm in the spermathecae; the anterior 100 segments are represented. Descriptions by Michaelsen (1933) and Hrabě were quite detailed, and were reviewed by Dembitsky (1987); nevertheless, we can add the following remarks based on both specimens.

A pair of lateral blood vessels is visible in posterior segments of the Italian specimen, and some of these have a few blind branches (Fig. 6I). Chaetae in anterior segments are simple-pointed, but tips of most chaetae in posterior segments bear a distinct dorsal groove, which may appear as a small dorsal tooth in lateral view (Fig. 6F).

The anterior vasa deferentia form a loop in the pre-atrial segment (VIII) before penetrating 8/9 and entering IX to join the atrium (Fig. 6A, E, G) in both of our specimens; this unique character was described and illustrated by Michaelsen (1933), but not mentioned in later descriptions. Posterior vasa deferentia do not penetrate septum 9/10. Michaelsen (1933) described and illustrated a greatly expanded posterior sperm funnel extending well into the posterior sperm sacs. Both posterior and anterior male funnels are small in the Slovenian worm (Fig. 6A); they are much larger in the Italian specimen, and the posterior extends back into X (Fig. 6G). Vasa deferentia are very thick (to 50 μm) in the Italian worm, joining the atrium subapically; they join the atrium before the apex in the Slovenian worm, running a short distance under the muscle layer to enter the lumen apically, as described by Dembitsky (1987) for Crimean specimens. Compared with other descriptions, the atrial ampulla appears rather short and ovate in the Slovenian and Italian worms, although the total atrium length (320 and 335 μm, respectively) and width are similar to those of the Crimean specimens (Dembitsky 1987). Male pores are on a rounded protrusion (referred to as a porophore by Michaelsen 1933), closely behind a groove containing the spermathecal and copulatory gland pores in the Slovenian worm (Fig. 6A–B). Although this structure was also illustrated by Dembitsky (1987: fig. III) it was not seen in our Italian specimen (Fig. 6G). Prostate glands are large (to about 100 μm high), petiolate clusters of cells, more similar to fig. 2 in Michaelsen (1933) than to fig. III in Dembitsky (1987).

The large copulatory glands (Fig. 6C, H) are quite similar in structure to the copulatory glands described below in the new Nearctic species, although a distinct muscle layer was not seen at the duct.

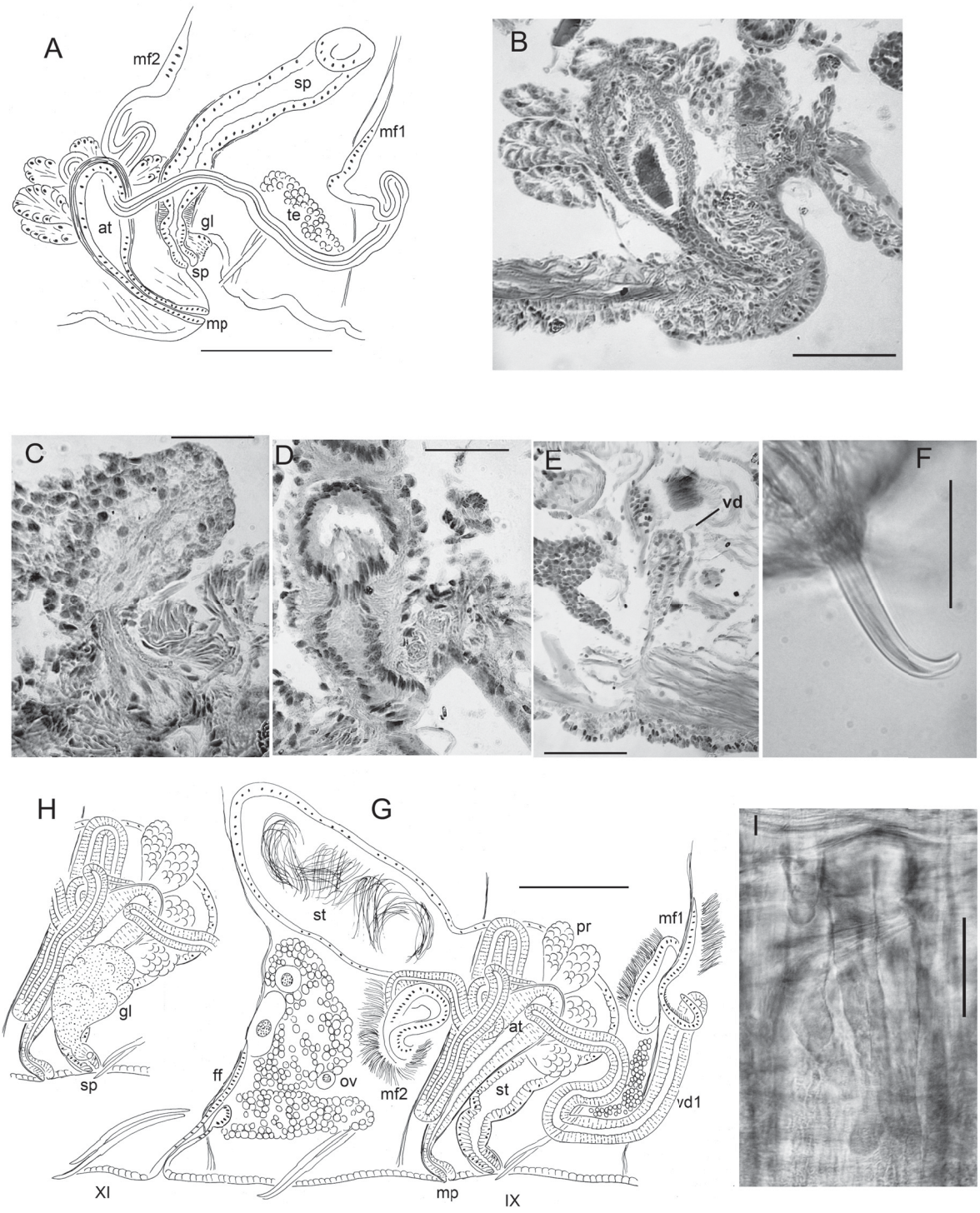

Fig. 6. *Guestphalinus wiardi* (Michaelsen, 1933); A–E a sectioned worm from Slovenia, Hrabě's collection; F–I, a dissected Italian specimen. **A**. Male duct and spermatheca in IX, reconstructed from sections; pore from a copulatory gland (not shown) opens next to spermathecal pore. **B**. Atrium and male porophore. **C**. Copulatory gland. **D**. Spermathecal pore and duct. **E**. Anterior male funnel with vas deferens entering pre-atrial segment. **F**. Posterior ventral chaeta, showing grooved dorsal tooth. **G**. Reproductive organs in IX-XI, figure rotated to face to right. **H**. Detail of G, showing position of copulatory gland, from median view. **I**. Branched lateral blood vessel in posterior segment. Scale bars: A, G–H = 200 μm; B, E, I = 100 μm; C–D, F = 50 μm.

The illustration in Hrabě (1973: fig. 7) is similar to ours. The slight difference in placement (adjacent to the spermathecae in both of our specimens), compared with Michaelsen's material (at the ventral chaetae), suggests that the position of these glands may be variable, as in *Guestphalinus exilis* Fend & Rodriguez sp. nov. (see below). Their occurrence may also vary with stage of reproductive development, as Dembitsky (1967) was unable to find these glands in the Crimean specimens, attributing their absence to possible resorption.

The spermatheca is not clearly differentiated into duct vs ampullar sections, although there is a sphincter-like constriction, with thickened circular muscles near the pore of the Slovenian worm (Fig. 6D). The female funnel is clearly visible only in the Italian worm (Fig. 6G), where it appears to penetrate the posterior septum 10/11 (the plesiopore condition), as in the illustration in Michaelsen (1933). *Guestphalinus* was the only lumbriculid genus considered by Brinkhurst (1989: character 16) to have plesiopore female ducts, in common with *Haplotaxis*, one of the outgroup taxa in that analysis. However, this character has not been observed in *G. elephantinus* sp. nov. nor in *G. exilis* sp. nov., where female pores appear to be intersegmental (see below, Fig. 12Q). Plesiopore female ducts appear to be unusual in the Lumbriculidae (Brinkhurst 1989; Fend & Ohtaka 2004), but they may simply have escaped notice in other species descriptions. The exact position of the septum may be difficult to define, as muscle fibers from septa may join the body wall on either side of the female funnel.

Guestphalinus exilis Fend & Rodriguez sp. nov.
urn:lsid:zoobank.org:act:37A5A3E0-3DC2-4D24-84AC-62E21131E70E
Figs 7–9

Etymology

Derived fom the Latin word '*exilis*' (small), with reference to *G. elephantinus* sp. nov. (see below) and to the name of the extinct Californian pygmy mammoth *Mammuthus exilis* (Stock & Furlong, 1928).

Material examined

Holotype

UNITED STATES OF AMERICA: a dissected specimen, slide-mounted in Canada balsam, California, Shasta County, Squaw Creek at Chirpchatter Camp, deep riffle, gravel patches in cobble bottom, 6 May 2012, S. Fend leg. (USNM 1422283).

Paratypes (all collected by S. Fend at the type locality)

UNITED STATES OF AMERICA: 1 dissected on 2 slides, 19 Apr. 2010 (USNM 1422284); 1 dissected, 17 May 2010 (USNM 1422285); 1 dissected, 6 May 2012 (CASIZ 220935); 1 whole mount, 17 May 2010 (CASIZ 220936); 1 sagittally sectioned, 17 May 2010 (CASIZ 220938); a whole mount, immature but with gonads (DNA voucher), 19 Apr. 2010 (CASIZ 220937); 2 dissected on slides, 17 May 2010 (MNCN 16.03/3100–16.03/3101).

Additional material

UNITED STATES OF AMERICA: CALIFORNIA: 2 sagittal sections, 3 dissected, 4 whole mounts (all immature or partially mature), Shasta County, Squaw Creek at Chirpchatter Camp, 17 Jun. 1996, S. Fend leg.; 1 dissected (partially mature), same locality as preceding, 19 Apr. 2010, S. Fend leg.; 4 dissected (1 mature), 8 whole mounts (immature), several in alcohol, same locality as preceding, 17 May 2010, S. Fend leg.; 1 dissected (mature), several immature in alcohol, same locality as preceding, 6 May 2012, S. Fend leg.; 2 whole mounts (partially mature), Humboldt County, South Fork Eel River at Elk Creek, 24 Jun. 2001, S. Fend leg.; 1 whole mount (partially mature), Mendocino County, Garcia

River, 23 Mar. 1996, W. Fields leg.; 1 whole mount (immature, with small gonads), Colusa County, Stony Creek at Stonyford, 4 Nov. 2002, S. Fend leg.

Molecular data

COI, 28S and 16S sequences correspond to a topotypic paratype (see Table 1 for details).

Description

Specimens from the type locality: body measurements in Table 3. Prostomium rounded to nearly conical; filiform proboscis 0.9 to 2.1 mm long, diameter at midpoint 0.1–0.15 mm, appearing ringed with multiple shallow constrictions (Figs 7A–C, 9A). Body segmentation not strong in external view; secondary annulation a narrow anterior ring in IV–IX, weak in post-clitellar segments. Clitellum from VIII to XII or mid-XIII, absent ventrally in IX in the area surrounding male and spermathecal pores (Figs 7B, 9A).

Chaetae paired, in 4 bundles in each segment, beginning in II. Chaetal measurements given in Table 3. Chaetae in II bluntly simple-pointed to shallowly notched, directed anteriorly (Figs 7E–G, 9B–C, E); these chaetae slightly sigmoid, with distal nodulus; within each bundle, lateral chaeta slightly longer than the median, with more distal nodulus. Posterior to II, chaetae sigmoid, mostly simple pointed, with nodulus about ⅓ of distance from tip; perpendicular to body axis or posteriorly directed; chaetae in III slightly shorter, but those in more posterior segments similar in length to those in II. Chaetae in posterior segments may have a slight dorsal keel (Fig. 9D). Ventral chaetae absent in IX in mature and post-reproductive worms.

Epidermis in anterior segments 12–24 μm thick, in clitellum 30–50 μm, posteriorly 5–10 μm. In pre-clitellar segments, circular muscle layer of body wall arranged in a series of transverse bands (cf. Fig. 12K), 10–15 μm thick; posteriorly a simple layer about 5 μm thick. Longitudinal muscle layer 50–60 μm thick. Brain in the peristomium, lateral lobes rounded. Pharynx begins dorsally and laterally in II, ventrally in III, extending through IV. Pharyngeal glands in IV to VI or VII; on each side, three lobes (dorsal, lateral and median) (Fig. 9G) broadly connected at posterior septum of each segment and extending anteriorly, joining corresponding lobe in previous segment by a thin extension. No abrupt division between esophagus and intestine. Chloragogen cells cover the gut usually beginning in VII; in the most posterior segments many free eleocytes present in the coelomic cavity (Fig. 9H).

First nephridia usually on 6/7, absent in VIII–XI, usually paired on 11/12, occuring irregularly in posterior segments. Each nephridium with small anteseptal funnel; granular postseptal expansion elongate to ovate (length 130–250 μm, diameter 40–80 μm), directed posteriad or somewhat dorsad (Fig. 9I); convoluted efferent duct may pass through one or more adjacent (anterior or posterior) segments, ventral or ventrolateral to the gut, usually near the ventral blood vessel, terminating in a short ectal branch to a simple nephridiopore anterior to the ventral chaetae; indistinct vesicle at the pore in some specimens.

Dorsal blood vessel passes under brain: two forks pass around the pharynx and join in IV, forming the ventral vessel. Dorsal vessel free anteriorly, closely appressed to top of gut posterior to VI or VII. Ventral vessel separate from gut, but 2–3 short vessels join it to the perivisceral sinus in each segment posterior to about VII. One pair of commissural vessels in anterior segments; those in II–VI (or VII) long and sinuous, extending through most of originating segment; those in VIII to XII (or XIV) restricted to posterior part of the segment, but a posterior loop from those in IX and X may enter sperm and egg sacs. No lateral blood vessels observed behind about segment XIV. Perivisceral sinus begins in about VII.

Genital field covers ventral side of IX, flattened or slightly concave in preserved, mature worms. Male and spermathecal pores paired in IX. Male pores near posterior intersegmental groove (Fig. 7A–B) and inside lines of ventral chaetae; spermathecal pores in line with, and in front of male pores, about level

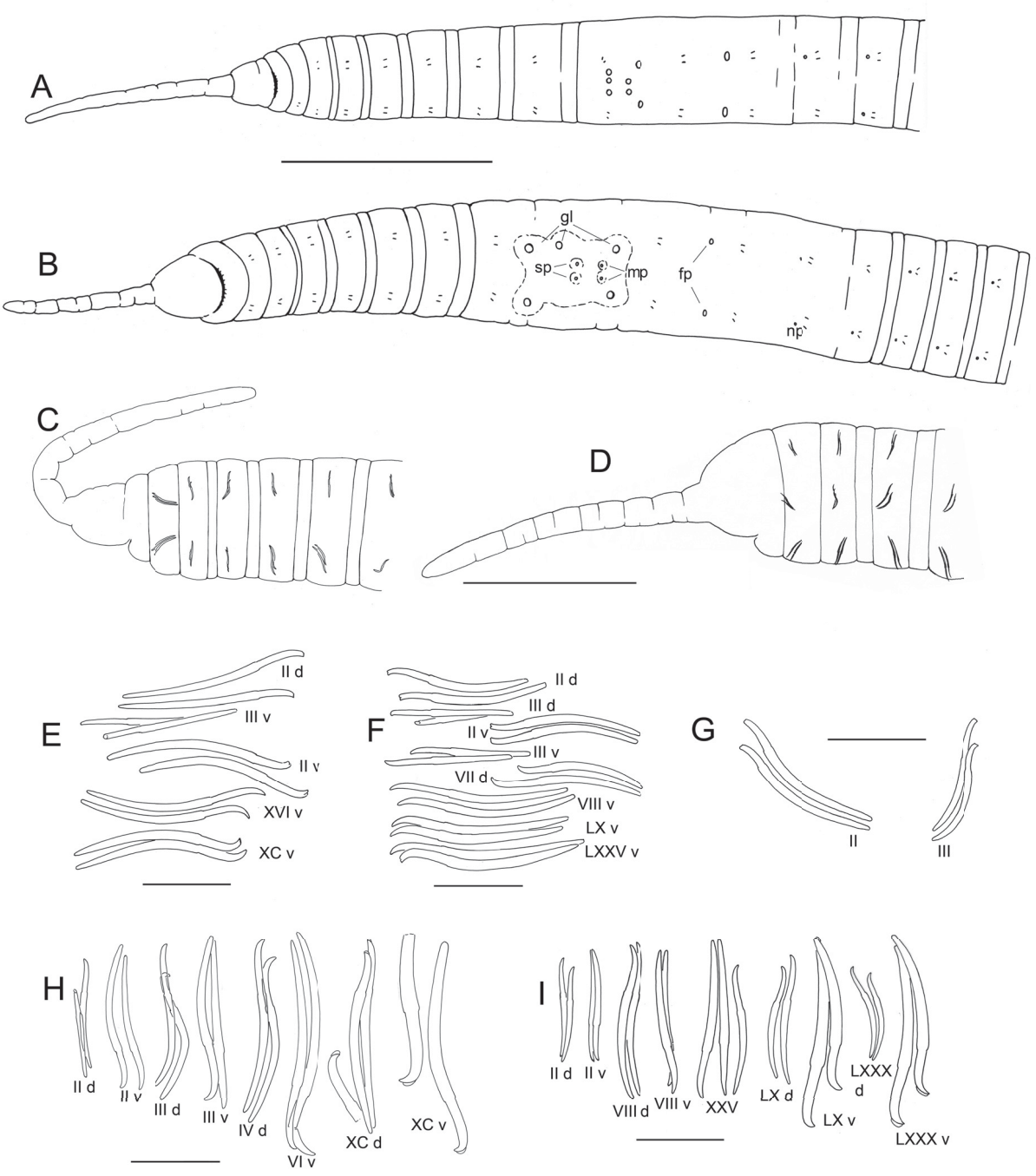

Fig. 7. *Guestphalinus exilis* Fend & Rodriguez sp. nov., external characters. A–C, E–G from Squaw Creek, CA (the type locality); D, H from South Fork Eel River, CA; I from Stony Creek, CA. **A–B**. Ventral views of two worms, showing positions of genital pores: A is partially-mature, with 3 glands opening around male and spermathecal pores; B is from a reproductively-mature worm, dashed line encloses the non-clitellar genital field with 5 glands opening around spermathecal and male pores. **C–D**. Anterior end, showing orientation of chaetae. **E–G**. Selected chaetae from typical specimens; G shows relative position of dorsal chaetae in II and III. **H–I**. Selected chaetae from partially-mature worms. Chaetae are numbered by segment (Roman numerals), followed by "d" (dorsal) or "v" (ventral). Scale bars: A–B = 2 mm; C–D = 1 mm; E–I = 100 μm.

with dorsal chaetal bundles (ventral chaetae absent in IX). Female pores paired, on chaetal line at intersegmental groove 10/11. Secretory openings of copulatory glands in IX, small and circular areas, lateral to genital pores, and either in front of or behind them. Paired testes on anterior septa in VIII and IX may be large, extending through anterior half of segment; ovaries in X, reaching to posterior septum, or into XI. Sperm sacs extending back to XIV–XVII in mature worms, egg sacs as far as XV–XVII.

Spermathecae to over 2000 μm long in mated worms, nearly tubular, with two, weakly differentiated sections, both containing sperm. Ectal, duct-like section gradually expanding to form ental ampulla; ampulla may be folded within IX (both in unmated or mated specimens) or extending into X–XII. Ectally, spermatheca passes through a short (12 μm), narrow constriction within ring of muscle fibers (Figs 8A–B, 9J) and terminates in a shallow epidermal infolding, about 70–110 μm deep. Duct-like section (80–100 μm diameter) with irregular, columnar epithelium (up to 40–45 μm high); dense sperm may be lined up along epithelium (Fig. 9K); very thin outer muscle layer about 2 μm thick. Ampulla-like section nearly tubular, diameter about 100 μm for much of its length, but may be entally expanded to over 200 μm (Figs 8A, 9L), with thinner epithelium and wider lumen containing sparse spermatozoa.

Typically, 3–6 stalked copulatory glands are associated with genital pores in mature and nearly-mature worms (Fig. 8A–C); they are absent at early stages of reproductive development. Glands are a pyriform cluster of granular, petiolate cells 160–300 μm long (Fig. 9M–N), sometimes appearing as a group of smaller clusters. Conjoined cell extensions of the gland are constricted by a ring of circular muscle fibers and then surrounded by thin epithelium before opening in a round secretory surface (to 50 μm diameter) on the body wall.

Anterior and posterior male funnels about equal in size, to 240 μm high; anterior pair rather flat on septum 8/9; posterior pair on 9/10, but usually directed back into X; both pairs functional, with sperm when fully mature. Anterior vas deferens extends into VIII (Figs 8A–C, 9O), forming a convoluted mass, then penetrates 8/9, running along ventral body wall to near the male pore, then follows atrium to near the ental end, joining the atrium apically. Length of anterior vasa deferentia to 2600 μm, width 36–50 μm. Posterior vas deferens forms a compact, convoluted mass in posterior IX, then follows atrium within the sperm sac, joining it at or near the apical end. Length of posterior vasa over 2000 μm, width 38–46 μm. Vasa deferentia histologically similar throughout, with ciliated, non-glandular epithelium.

Atria of mature worms usually extend back into X or XI; in nearly-mature worms they may be entirely in IX. Atria petiolate in mature worms (Fig. 8A–B); a short ectal duct (150 μm long) has thick, columnar epithelium and a thin (to 2 μm) muscle coat; no distinct penis, although duct may be somewhat expanded at male pore, with thickened epithelium (Figs 8B, 9Q). An abrupt transition from the atrial duct to the tubular or sacciform atrial ampulla. Ampulla length to 1060–1105 μm, width 130–150 μm, thin-walled (5–8 μm) with cuboidal epithelium and wide lumen (Figs 8A, 9P); clusters of prostatic cells, 70–140 μm long, sparsely cover the atrial ampulla. In nearly-mature, unmated worms, atria tubular (Fig. 8C); ampulla and duct not greatly differentiated; prostates small but appear more densely packed than in mated worms.

Eel River, Stony Creek and Garcia River, northern California: The few, partially-mature specimens from these localities are tentatively assigned to *Guestphalinus exilis* sp. nov. based on the ringed proboscis, the chaetae, and gonads in VIII, IX and X. They differ from the type diagnosis in that chaetae in II are smaller than other anterior chaetae, and simple-pointed (but oriented forward, as in the type locality population). Ventral chaetae in posterior segments distinctly keeled and larger than corresponding dorsals (Fig. 7H–I). As in typical *G. exilis* sp. nov., specimens from Eel River have semiprosoporous male ducts, with vasa deferentia joining atrium apically and the anterior vas deferens penetrating 8/9 (Fig. 8D). Spermathecal and male pores are behind ventral chaetae of IX and 5 large copulatory glands surround the genital pores. One specimen has an additional, developing spermatheca on one side, in X.

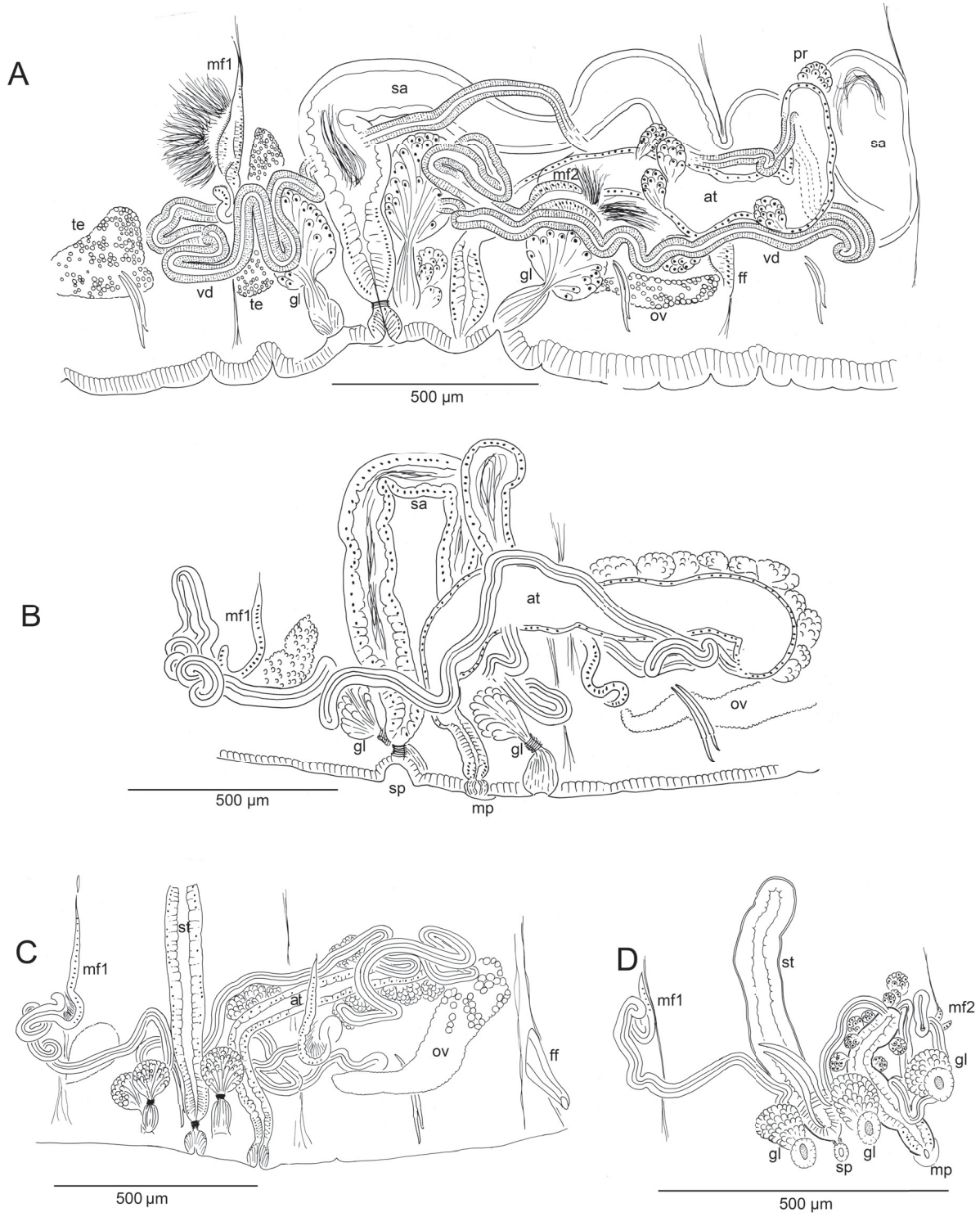

Fig. 8. *Guestphalinus exilis* Fend & Rodriguez sp. nov., reproductive structures, all oriented with anterior end to left. A–C from Squaw Creek (the type locality), D from the South Fork Eel River **A**. Segments VIII–XI of a mature worm, showing reproductive organs; anterior vas deferens broken at spermatheca. **B**. Segments IX–X of a recently-mated worm; spermathecal ampulla not yet developed; atrium expanded. **C**. Segments IX–X of a partially-mature worm; atrium and spermathecae tubular, but copulatory glands well-developed; ental end of spermatheca missing. **D**. Segment IX of a partially-mature worm; glands well developed; atrium and spermatheca small, tubular.

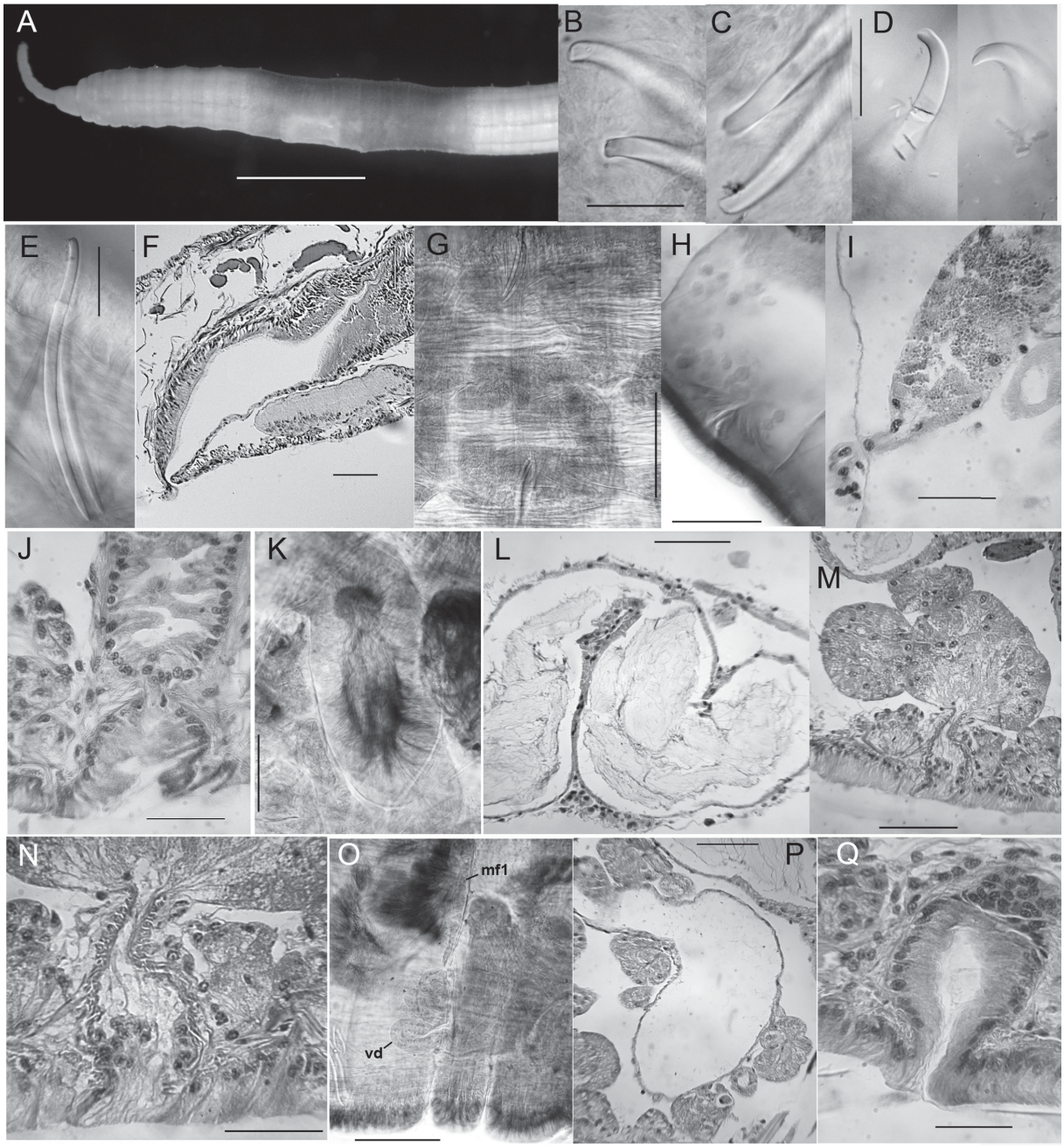

Fig. 9. *Guestphalinus exilis* Fend & Rodriguez sp. nov., from Squaw Creek (the type locality); F, I–N, P–Q from sagittal sections. **A**. A complete worm, anterior end, lightly stained with hematoxylin, showing clitellum and genital field. **B**. Tips of chaetae in II dorsal. **C**. Chaetae in II ventral. **D**. Tips of chaetae in two posterior segments, showing dorsal keel. **E**. Chaetae in II dorsal. **F**. Pharynx. **G**. Pharyngeal glands from a whole mount, 3 lobes facing anteriad (to left). **H**. Eleocytes, in a posterior segment. **I**. Nephridial funnel (left) and postseptal expansion. **J**. Spermathecal pore and constricted ectal end of duct. **K**. Spermathecal ampulla, ectal part, with sperm penetrating epithelium. **L**. Spermathecal ampulla, ental part with weakly-staining sperm. **M**. Ventral copulatory gland. **N**. Pore of copulatory gland. **O**. Anterior vas deferens protruding into VII (to left), with male funnel on 8/9. **P**. Expanded atrial ampulla with prostates. **Q**. Male pore. Scale bars: A = 2 mm; B–E, H–J, N, Q = 50 μm; F–G, K–M, O–P = 100 μm.

Remarks

Guestphalinus exilis sp. nov. from western North America resembles the Palearctic *G. wiardi* in characters considered diagnostic for the genus (see above). Compared with *G. wiardi*, *G. exilis* sp. nov. has modified chaetae in II, a much larger, sacciform atrium, and more copulatory glands; the male pore was never strongly protruding (Fig. 6B vs Fig. 8A–B). The reproductive organs of *G. exilis* sp. nov. are morphologically similar to those of *G. elephantinus* sp. nov. (see below); both lack a distinct male porophore or penis, although the atrial duct may be somewhat expanded at the male pore, with thickened epithelium, suggesting that lining cells may be protrusible (Figs 9Q, 12M, O) as "type-2 penes" (temporary structures formed by extruded lining cells of the male duct, see Rodriguez & Giani 1994: fig. 17D–F).

Molecular results strongly suggest more than one Nearctic species (see below). Both new *Guestphalinus* species present distinctive chaetal morphotypes, and these chaetal characters are therefore used here as main diagnostic characters. Nevertheless, there is some variation among populations assigned to both basic morphotypes. In segment II chaetae of typical (from Squaw Creek) *G. exilis* sp. nov. are slightly modified relative to those in other segments. In most Squaw Creek specimens the chaetae appear slightly notched, slightly larger than those in III, and a little shorter than those in middle segments (Table 3). In specimens from two other California sites, chaetae in II are simple-pointed and smaller than those in III – and thus similar to most other lumbriculids. These are provisionally assigned to *G. exilis* sp. nov., based on the limited and only partially-mature available material. Partially developed reproductive organs (Fig. 8D) clearly indicate that the Eel River specimens belong to *Guestphalinus* as defined here. The single specimens from Stony Creek and the Garcia River, although immature, have gonads in VIII–X, distinguishing them from other proboscis-bearing lumbriculid genera in the region (e.g., *Kincaidiana*, *Rhynchelmis* and *Eremidrilus*).

Habitat

The type locality is Squaw Creek, a tributary to the Sacramento River, in northern California; the collection site is a 5th order riffle-pool stream with gravel-cobble sediment (U.S.D.A. 1999). The type series was found only at the lower end of a single riffle, and no additional material has been found at other sites within the stream. Other oligochaetes were collected throughout the stream; dominant species were *Mesenchytraeus pedatus* Eisen, 1904 and *Rhyacodrilus clio* Rodriguez & Fend, 2013 (also the type locality for *R. clio*). The four streams where *G. exilis* sp. nov. was collected were similar in size and sediment characteristics, and are coldwater streams, supporting salmonid fishes. The species was usually found in patches of finer gravel within cobble-boulder riffles. Specific conductance at the Squaw Creek and Eel River sites (April 2014) was 265 and 250 µS cm^{-1}, respectively. Earlier measurements at a downstream site on Squaw Creek (Rettig & Bortleson 1983) gave temperatures from 13.5 to 22.5 °C and a specific conductance of 109–261 µS cm^{-1}. Visits to the type locality from July to September did not produce any specimens of *G. exilis* sp. nov. The sporadic occurrence and patchy distribution suggest that, like *G. wiardi*, they may also be primarily groundwater or hyporheic worms that only occasionally show up in surface collections.

Guestphalinus elephantinus Fend & Rodriguez sp. nov.
urn:lsid:zoobank.org:act:A30D385C-06C9-438E-AAFC-1F145905B74F
Figs 10–12

"*Guestphalinus* sp. nov." – Zhou *et al.* 2010: 381, figs 1–6 (sequenced, but not morphologically described; voucher SMNH 105628).

Table 3. Descriptive measurements (mean, range, and n) for different populations of *G. exilis* Fend & Rodriguez sp. nov. and *G. elephantinus* Fend & Rodriguez sp. nov. Measurements of "nodulus" refer to the proportional distance from the tip of the chaeta (i.e., "0.5" means the nodulus is at the midpoint of the chaeta).

	Number of segments	Length, mm	Width in IX, mm	Chaeta length in II, outer dorsal, µm	Chaeta length in III, µm	Maximum chaeta length in mid-body, µm	Dorsal chaeta length in II / mid-body chaeta length	Chaetal nodulus in II, dorsal outer	Chaetal nodulus in II, dorsal inner	Chaetal nodulus in III	Chaetal nodulus in mid-body
***G. exilis* Fend & Rodriguez sp. nov.: Squaw Creek, California, USA (type locality)**											
Mean	**101**	**35.5**	**0.89**	**182**	**157**	**204**	**0.91**	**0.23**	**0.23**	**0.32**	**0.31**
max	*123*	*54*	*1.17*	*218*	*182*	*226*	*1.20*	*0.27*	*0.28*	*0.38*	*0.34*
min	*78*	*19*	*0.67*	*134*	*113*	*170*	*0.76*	*0.19*	*0.19*	*0.24*	*0.25*
n	16	22	24	21	18	17	16	16	16	14	12
***G. elephantinus* Fend & Rodriguez sp. nov.: Olympic Peninsula, Washington, USA (including type locality)**											
Mean	**117**	**46.3**	**0.76**	**355**	**142**	**207**	**1.73**	**0.15**	**0.23**	**0.32**	**0.35**
max	*131*	*61*	*1.11*	*510*	*180*	*256*	*2.42*	*0.18*	*0.26*	*0.44*	*0.41*
min	*92*	*26*	*0.62*	*252*	*106*	*165*	*1.20*	*0.1*	*0.2*	*0.23*	*0.28*
n	36	52	58	52	44	43	42	52	50	43	43
***G. elephantinus* Fend & Rodriguez sp. nov.: other northwestern sites, USA (Satsop River, Washington; Euchre Creek, Oregon)**											
Mean	**131**	**47.1**	**0.93**	**701**	**280**	**230**	**3.07**	**0.13**	**0.21**	**0.24**	**0.36**
max	*145*	*54*	*1.3*	*916*	*372*	*276*	*3.32*	*0.14*	*0.25*	*0.3*	*0.4*
min	*116*	*42*	*0.7*	*554*	*154*	*182*	*2.95*	*0.12*	*0.19*	*0.2*	*0.29*
n	2	3	5	6	6	5	5	6	6	6	5
***G. elephantinus* Fend & Rodriguez sp. nov.: Peavine Ridge spring, Oregon, USA**											
Mean	**114**	**42.6**	**0.67**	**423**	**162**	**236**	**1.79**	**0.18**	**0.25**	**0.37**	**0.37**
max	*128*	*54*	*0.8*	*458*	*187*	*253*	*1.91*	*0.19*	*0.27*	*0.39*	*0.4*
min	*84*	*33*	*0.53*	*360*	*154*	*223*	*1.58*	*0.16*	*0.22*	*0.35*	*0.35*
n	8	10	10	9	9	9	9	9	9	8	9
***G. elephantinus* Fend & Rodriguez sp. nov.: Guadalupe and Alamitos Creeks, California, USA**											
Mean	**119**	**40.4**	**0.78**	**489**	**251**	**213**	**2.38**	**0.12**	**0.22**	**0.21**	**0.37**
max	*135*	*74*	*1.17*	*794*	*437*	*316*	*3.28*	*0.14*	*0.29*	*0.28*	*0.41*
min	*88*	*18*	*0.49*	*372*	*187*	*114*	*1.58*	*0.11*	*0.2*	*0.15*	*0.33*
n	6	7	9	11	11	11	11	11	11	11	11

Etymology

Based on the fanciful and homoplasious similarity to an elephant (proboscis and anteriorly-directed "tusks").

Material examined

Holotype

UNITED STATES OF AMERICA: a dissected specimen, slide-mounted in Canada balsam, Washington, Jefferson County, Shale Creek at Clearwater Creek Road, 4 Jun. 2003, S. Fend leg. (USNM 1422286).

Paratypes (all from the Clearwater River drainage, on Olympic Peninsula, Washington)
UNITED STATES OF AMERICA: 1 dissected (post-reproductive), Jefferson County, Clearwater River at Upper Clearwater Camp, 27 Apr. 2004 (USNM 1422287); 1 dissected (post-reproductive), same data as preceding (CASIZ 220932); 1 dissected (post-reproductive), Hurst Creek near Clearwater River, 25 Apr. 2004 CASIZ 220933); 1 whole mount, immature but with gonads (DNA voucher), from type locality, 4 Jun. 2003 (CASIZ 220934); 1 dissected (post-reproductive), Clearwater River at Upper Clearwater Camp, 27 Apr. 2004 (MNCN 16.03/3098); 2 partially-mature whole mounts on 1 slide, Shale Creek at Clearwater Creek Road, 4 Jun. 2003 (MNCN 16.03/3099).

Additional material (typical specimens)
UNITED STATES OF AMERICA: WASHINGTON, Jefferson County (all from the Clearwater River drainage, on Olympic Peninsula): 2 dissected (mature, reproductive organs slightly resorbed), Bull Creek near Clearwater River, 2 Apr. 2016; 2 whole mounts (immature, with small gonads), Clearwater River at Upper Clearwater Camp, 4 Jun. 2003; 4 dissected, (immature or post-reproductive), same locality as preceding, 27 Apr. 2004; 1 dissected and 16 whole mounts, several in alcohol (all immature or partially mature), Clearwater River at Copper Mine Bottom Camp, 25 Apr. 2004; 8 whole mounts (immature or partially mature), Shale Creek at Clearwater Creek Road, 4 Jun. 2003; 4 whole mounts (immature), same locality as preceding, 14 Apr. 2010; 5 whole mounts (immature), Hurst Creek, 25 Apr. 2004.

Other sites

UNITED STATES OF AMERICA: WASHINGTON: 1 dissected (slightly post-reproductive), 2 whole mounts (post-reproductive), Grays Harbor County, Middle Fork Satsop River, 27 Apr. 2004, S. Fend leg.; 2 whole mounts (1 nearly mature), Columbia County, Tucannon River below Turner Road, 29 Jul. 2011, Uttam Rai leg. – OREGON: 1 sagittal section, 1 transverse section, 3 dissected, 6 whole mounts (dissected and sectioned nearly mature, others with gonads),Yamhill County, spring at Peavine Ridge near McMinnville, 5 Dec. 1999, S. Fend leg.; 1 whole mount (immature, with gonads), Curry County, Mule Creek at upstream bridge, near Rogue River, 7 Jun. 2003, S. Fend leg.; 1 dissected (immature, with gonads), Curry County, Euchre Creek near mouth, 20 May 2013, S. Fend leg. – CALIFORNIA: 1 whole mount (immature), Santa Clara County, Alamitos Creek above Almaden Reservoir, 1 May 1997, S. Fend leg.; 1 whole mount (immature), Santa Clara County, Guadalupe Creek above Guadalupe Reservoir, below Rincon Creek, 3 Apr. 2001, S. Fend leg.; 1 dissected (mature) and 2 whole mounts (partially mature), same locality as preceding, 25 Mar. 2007, S. Fend leg.; 1 whole mount (immature), same locality as preceding, 10 Feb. 2008, S. Fend leg.; 1 whole mount (immature), same locality as preceding, 5 Dec. 2009, S. Fend leg.; 1 dissected (post-mature), same locality as preceding, 20 Jun. 2011, S. Fend leg.

Molecular data

COI, 28S and 16S sequences are based on specimens from Shale Creek and Clearwater River, Washington (details in Table 1). An additional ITS sequence (GenBank acc. no. GU592364) corresponds to the Clearwater River voucher SMNH105628. A topotypic voucher is included in the type series (see above). Both COI sequences for *G. elephantinus* sp. nov. are identical.

Description

Specimens from the Clearwater River drainage, Olympic Peninsula, Washington
Description of somatic characters is based on mature or partially-mature (with gonads) worms from Clearwater River, Hurst Creek, Shale Creek and Bull Creek. Reproductive organs are described from the only fully-mature specimen (the holotype), from Shale Creek, and supplemented with 9 post-reproductive specimens with partially-resorbed organs from nearby sites

Body form and segmentation as described for *G. exilis* sp. nov. (see above), measurements given in Table 3. Length of proboscis 0.9–2.6 mm (Figs 11A, 12A), clitellum from VIII to XII in the mature worm.

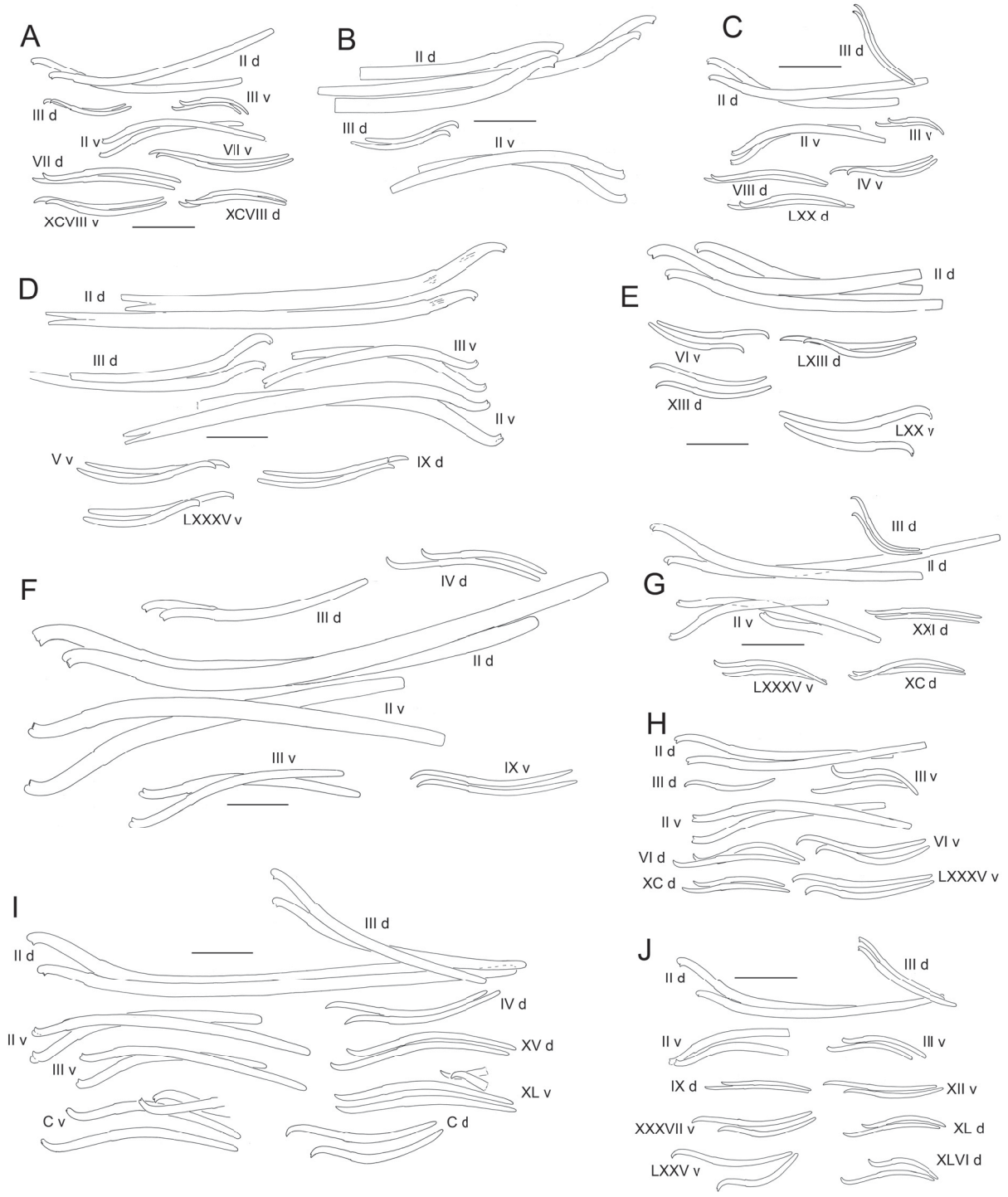

Fig. 10. *Guestphalinus elephantinus* Fend & Rodriguez sp. nov., various sites, arranged approximately north-south; paired chaetae; d = dorsal bundle, v = ventral. **A**. From Shale Creek, WA (type locality). **B–C**. From Clearwater River, WA; C shows II and III in relative positions at top. **D**. West Fork Satsop River, WA. **E**. Tucannon River, WA; II dorsal bundle with 1 replacement chaeta. **F**. Euchre Creek, OR. **G**. Mule Creek at Rogue River, OR; II and III dorsal are shown in relative positions. **H**. Spring at Peavine Ridge, OR. **I–J**. A mature and immature worm from Guadalupe Creek, CA; II and III shown in relative positions; XL ventral and C ventral in I have partially-developed replacements; II ventral chaetae partially-developed in J. Scale bars: 100 µm.

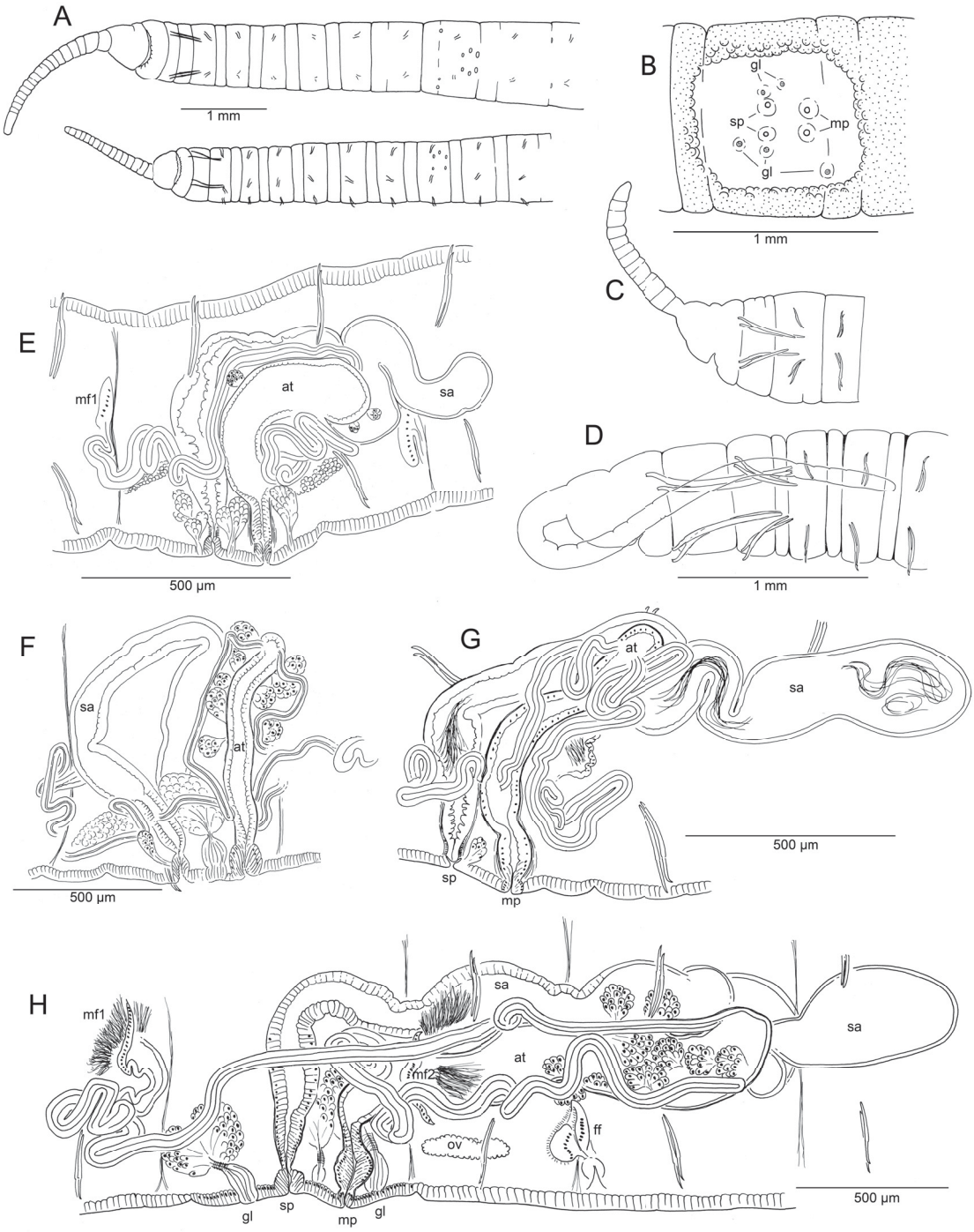

Fig. 11. *Guestphalinus elephantinus* Fend & Rodriguez sp. nov. **A**. Ventral view of two worms from Shale Creek, WA (type locality); upper drawing is the holotype, lower is a post-reproductive worm. **B**. Ventral view of segment IX, showing genital field of a mature worm from Guadalupe Creek, CA; secretory openings of 5 glands are visible, in addition to paired male and spermathecal pores. **C**. Anterior end, from Peavine Ridge spring, OR, showing position of chaetae in II-IV. **D**. Anterior end of worm from Middle Fork Satsop River, WA. **E**. Reproductive organs of a mated worm from Shale Creek (holotype). **F**. Segment IX of an unmated worm, Peavine Ridge. **G**. Atrium and spermatheca in IX of a mated, but slightly post-mature worm from Clearwater River, WA; sperm present in spermatheca, but atrium is reduced in size. **H**. Reproductive organs in segments VIII–XII of a mated, mature worm from Guadalupe Creek, CA.

Chaetae in II much larger and thicker than those in posterior segments (Table 3), bifid, with a short, thick lower tooth and a larger, rounded and laterally compressed upper tooth (Figs10A–C, 12C), directed anteriorly, with proximal ends protruding into posterior segments (cf. Figs 11C–D, 12B). Dorsal chaetae in II about 30–40% longer than those in ventral bundles (Table 3); within each bundle in II, the lateral (i.e., outer) chaeta is slightly longer, with a more distal nodulus (0.15 from tip, vs 0.24 in the median (inner) chaeta), and usually has a distal thickening that appears as a "secondary nodulus" between nodulus and tip (Fig. 12C–D). Chaetae in III simple-pointed or slightly bifid, and oriented more perpendicularly to body, but still facing anteriad (Figs 10C, 12B); much shorter than chaetae in II and slightly shorter than those in other segments (Table 3). Chaetae in IV and posterior segments usually oriented posteriorly, simple-pointed, sigmoid (Fig. 12H). From III to about XX, nodulus about 0.3–0.4 from the tip, but the median (inner) chaeta in each bundle is slightly longer. In posterior segments, chaetae of similar length, but slightly thicker, and nodulus 0.25–0.35 from the tip; simple-pointed or with a small notch or dorsal keel in tail segments. Ventral chaetae absent in IX in mature or post-mature worms.

Epidermis in anterior segments 15–20 μm thick; in clitellum to 48 μm in the holotype; narrower, densely packed cells up to 30 μm on ventral side of IX (in area surrounding genital pores) in the holotype and in the post-reproductive worms; posteriorly 10–12 μm. Body-wall musculature, brain, pharynx, pharyngeal glands, nephridia, blood vessels and chloragogen as described above for *G. exilis* sp. nov., except that pharyngeal glands in *G. elephantinus* sp. nov. extend to VII in all specimens observed.

Male and spermathecal pores paired in IX. Male pores just anterior to the posterior intersegmental groove (Fig. 11A), slightly inside the line of ventral chaetae; spermathecal pores aligned with and in front of male pores, between or slightly posterior to chaetal bundles. Female pores paired, on chaetal line at 10/11; female funnel up to 130 μm high (Fig. 12Q). A variable number of copulatory glands with internal structure as described for *G. exilis* sp. nov.; with round secretory openings located close to spermathecal and male pores. The mature worm (holotype) has 3 pairs of petiolate copulatory glands in IX, 100–140 μm high, lateral to, in front of, and behind genital pores (Fig. 11E), although not all of these were visible externally (Fig. 11A). Copulatory glands smaller and indistinct or absent in the post-reproductive worms (male duct and spermathecae expanded; large but nearly empty sperm and egg sacs extending to XV), but faint external secretory openings visible in one specimen.

Spermathecae up to 1700 μm long in the holotype; ectal ¼ is duct-like, nearly tubular (about 90 μm in diameter); ental ampulla-like portion irregular, up to about 100 μm in diameter, folded within X or extending into XI. Ectal part with irregularly columnar cells up to 35 μm high, epithelium gradually becoming thinner in ental ampulla. Spermathecae end in a short, narrow constriction surrounded by a ring of muscle fibers (Fig. 11E, cf. Fig. 12L) which opens in a shallow epidermal infolding, about 40 μm deep. Spermathecae of a paratype similar (Fig. 11G), 1200–2600 μm long, with loose sperm; ampulla thin-walled, ental diameter up to 230 μm, extending into X or as far as XII.

Anterior pair of male funnels on 8/9; posterior male funnels on 9/10, directed back into X; male funnels of post-reproductive worms indistinct, without or with small amount of sperm. Anterior vas deferens extends into VIII (Fig. 11E), forming a compact, convoluted mass, then penetrates 8/9, running along ventral body wall to near the male pore, then follows the atrium before joining the atrial wall and entering the lumen near the apex (junction not clear on holotype). Length of anterior vas deferens up to about 1500 μm, width of both anterior and posterior vasa deferentia 30–36 μm in the holotype. Posterior vas deferens forms a compact, convoluted mass in posterior part of IX, then follows the atrium externally before joining it near the ental end. Atria of holotype extend back into X; ectal duct (length 160 μm, width near midpoint 30–40 μm) has a thick, columnar epithelium and thin muscle coat; ectal part of duct expanded to 50 μm, with epithelial cells directed outward. Entally, there is an abrupt transition to the sacciform atrial ampulla; ampulla length up to 550 μm, width up to 130 μm, with thin-walled

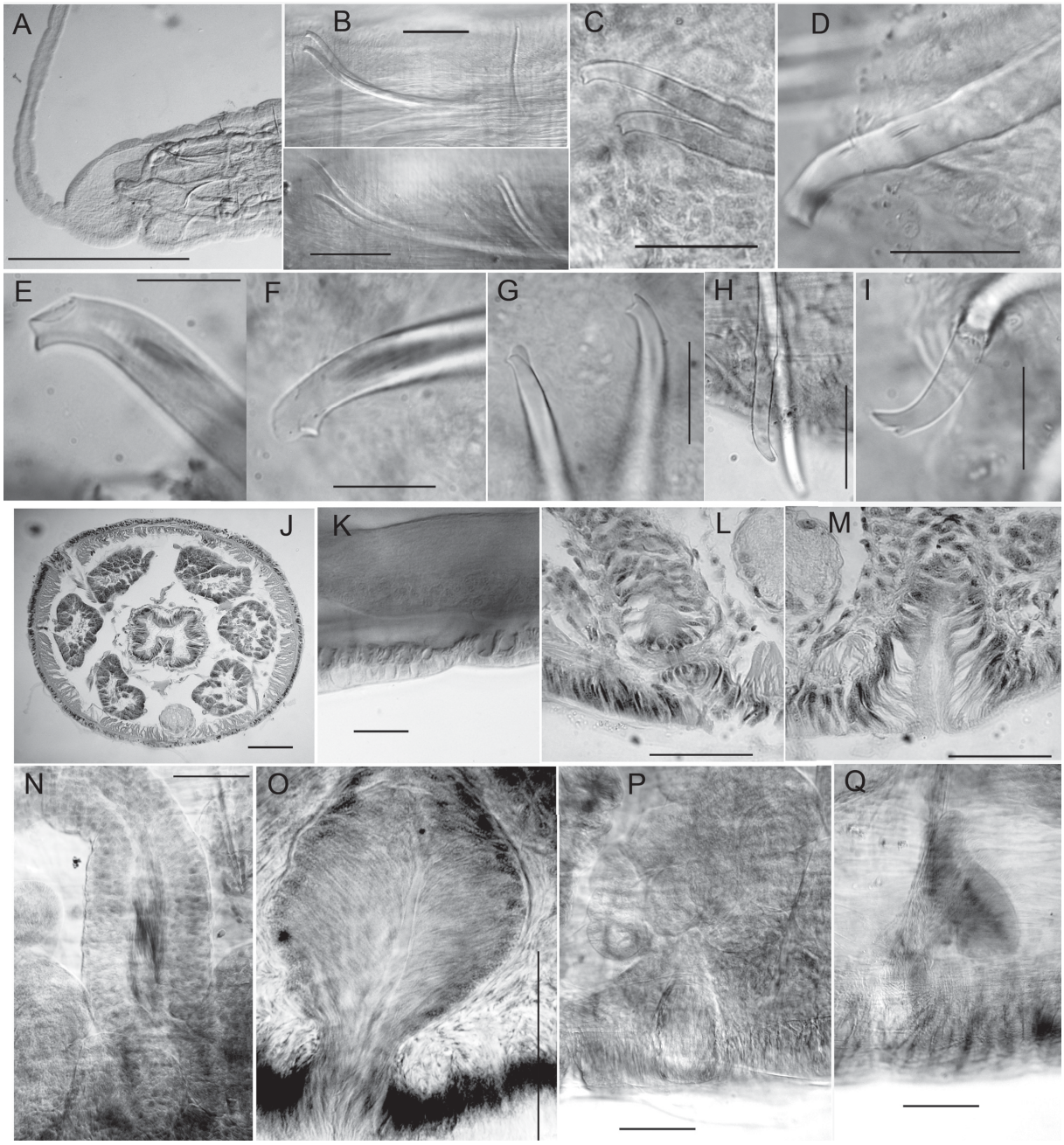

Fig. 12. *Guestphalinus elephantinus* Fend & Rodriguez sp. nov. A–B (upper) from Clearwater River, WA; C, H, K from Shale Creek, WA (type locality); D–E from Satsop River, WA; B (lower), F–G, N–Q from Guadalupe Creek, CA; I–J, L–M from Peavine Ridge, OR. **A**. Anterior end of cleared worm. **B**. Chaetae in II-III, comparing worms from northern (upper) and southern (lower) populations. **C**. Dorsal chaetae in II. **D**. The outer chaeta in II, showing the "secondary nodulus". **E–F**. Inner dorsal chaeta in II; E shows folded keel. **G**. Modified dorsal chaetae in II. **H**. Posterior ventral chaeta. **I**. Posterior ventral chaeta with bifid tip. **J**. Transverse section of anterior segment, showing lobes of pharyngeal glands. **K**. Body wall in anterior segment. **L**. Spermathecal pore. **M**. Male pore. **N**. Ectal part of spermatheca from a dissected, mated worm. **O**. Expanded atrial duct (type 2 penis?) at male pore. **P**. Copulatory gland. **Q**. Female funnel and pore on intersegment 10/11. Scale bars: A = 1 mm; B–I, K–M = 50 μm; J, N–Q = 100 μm.

epithelium and a wide lumen (Fig. 11E). Small clusters of prostatic cells, 20–40 μm high, very sparse. Atria tubular, with ampulla not expanded in a paratype (apparently at a more advanced stage of maturity, after copulation, Fig. 11G); length 400–800 μm, diameter 60–80 μm; prostates very small or absent; atrial duct and male pore as in the holotype.

Specimens from other localities

Satsop River, western Washington: Modified chaetae in both II and III; chaetae in II very long (Table 3); lateral (outer) chaeta in the largest specimen with secondary nodulus, as in topotypic specimens (Fig. 12D). Chaetae in III smaller, but bifid and larger than chaetae in the next segments, and directed anteriad (Fig. 11D).

Tucannon River, eastern Washington: Typically modified chaetae in II, but without secondary nodulus; small, simple-pointed chaetae in III; ventral chaetae in posterior segments enlarged, longer and thicker than corresponding dorsals or ventrals in anterior segments posterior to III; some posterior chaetae are slightly keeled (Fig. 10E). In the unmated, nearly-mature worm, tubular spermatheca, vasa deferentia, atrium and prostates similar to those of the Peavine Ridge material (cf. Fig. 11F). Copulatory glands large, up to about 150 μm high, anterolateral and posterolateral to the male pores.

Peavine Ridge, northern Oregon: Chaetae in II enlarged and modified as above, without secondary nodulus (Figs 10H, 11C); chaetae in III simple-pointed, smaller than other anterior chaetae. Posterior segments with ventral chaetae larger than dorsals; both dorsal and ventral chaetae slightly bifid or keeled (Fig. 12I). In unmated, nearly-mature worms, there is a variable number (2–4) of well-developed, petiolate copulatory glands with distinct external secretory openings, total height up to 155–290 μm (Fig.11F). Copulatory glands usually present between spermathecal and male pores (but lateral to both), other glands may be present anterior, lateral, or posterior to genital pores. Much smaller glands may insert at male and spermathecal pores. Atria and spermathecae both tubular, without sperm; male and spermathecal pores (Fig. 12L–M) as described above.

Coastal ranges, central California and southern Oregon: Highly modified chaetae in II as in typical specimens, but without secondary nodulus; the two specimens with the largest chaetae are from this region (Table 3, Fig. 10F, I). Chaetae in III also modified, shorter than those in II but similar in general form (bifid, with rounded, laterally flattened upper tooth; nodulus distal); longer than other chaetae in anterior segments (Fig. 12B, F–H). Posterior chaetae usually simple-pointed, ventral chaetae keeled in tail segments (posterior to XC) of some specimens; ventrals in posterior segments larger than those in anterior segments, and larger than corresponding dorsals in some specimens.

Reproductive organs in the mature worm from Guadalupe Creek include the openings of 5 copulatory glands visible externally in IX, in ventral field, but lateral to male and spermathecal pores (Fig. 11B, H). Copulatory glands up to 250 μm high; extensions of granular, petiolate cells constricted by a muscular ring, terminating in a small, external secretory opening (Fig. 12P). Clitellum from ¼ VIII through XIII. Ectal, duct-like portion of spermatheca (Figs 11H, 12N) weakly differentiated from ampulla; extending to XII, thin-walled and expanded entally. Vasa deferentia as described above (Fig. 11H), 50–60 μm wide, joining atrium subapically; histologically similar throughout, with thick, ciliated epithelium. Atria petiolate; well-defined ectal duct; ectal part of atrial duct expanded, bulbous (up to 130 μm wide), with epithelial cells directed outward, possibly indicating a “type 2” penis (Fig. 12O). Sacciform atrial ampulla extending to 11/12, with thin-walled epithelium and a wide lumen (Fig. 11H); petiolate prostate glands sparsely covering ampulla.

Remarks

All populations here attributed to *G. elephantinus* sp. nov. have highly modified bifid chaetae in II (or II and III), and their morphology differs from chaetae in all other lumbriculids. These anterior chaetae are distinctly enlarged, very weakly sigmoid, and anteriorly directed with a characteristic short, broad proximal tooth and a laterally-flattened distal tooth. In comparison, typical *G. exilis* sp. nov. have similar but only slightly modified (blunt-tipped or slightly notched) chaetae in II. Additional inter-population variation in chaetal morphology suggests the existence of a species complex, but the rarity of mature specimens from most localities makes it difficult to compare populations with the usual morphological criteria, which focus on reproductive structures. Although our limited DNA sampling strongly supports species status for *G. elephantinus* sp. nov. and *G. exilis* sp. nov. morphotypes (see below), further molecular studies will be necessary to resolve the diversity of this genus in a region that appears to have extensive radiation within other lumbriculid genera (McKey-Fender & Fender 2001; Fend & Rodriguez 2003).

The morphology of modified chaetae in segment II varies among collection sites, but appears consistent within large series of immature worms collected at some of these sites. Increased chaetal size has been associated with high water conductivity in the Naididae (Loden & Harman 1980, for *Pristina aequiseta* Bourne, 1891, or for *Tubifex tubifex* var. *grandiseta* Rodriguez, 1986). This does not appear to be the case for *G. elephantinus* sp. nov., since populations with very enlarged chaetae inhabit streams with a wide range of conductivity (<100 to >400 μS cm^{-1}). The other western Nearctic species, *G. exilis* sp. nov., with much less-enlarged anterior chaetae, was collected from streams with intermediate conductivity values (ca 200–300 μS cm^{-1}, see *G. exilis* sp. nov. habitat notes, above).

The reproductive organs of *G. exilis* sp. nov. and *G. elephantinus* sp. nov. appear similar, although the morphology of these structures is difficult to define, as it varies with stage of development. Although atrial morphology is commonly used in defining lumbriculid species, it has been shown that it can vary considerably over the reproductive period in *Stylodrilus mollis* Timm, 1998 and *Trichodrilus seirei* Timm, 1979. The thin-walled, sacciform atrial ampulla in some mated specimens of both Nearctic species of *Guestphalinus* appears to be an unusual character for the Lumbriculidae. Unmated, mature worms (Fig. 11F) have tubular atria with a thick epithelium, whereas sac-like atria with thin walls lacking a glandular epithelium or an obvious muscle layer were observed in worms at a more advanced stage of maturity (Fig. 11E, H), including post-reproductive specimens with partially-resorbed reproductive organs.

Habitat

All sites except the spring on Peavine Ridge were alluviated streams with gravel to cobble substrate. All appear to have permanent flow, except for Guadalupe and Alamitos Creeks, where surface flow may disappear during summer months. Worms were typically collected by digging at least 20 cm deep in patches of finer gravel.

The Clearwater drainage sites, on the Olympic Peninsula, northwestern Washington, are larger streams in a watershed dominated by commercial forest, but with riparian buffers. These streams support populations of several salmonid species (Harrington 2005). Limited available water quality data for the Clearwater River near Clearwater (NWIS 2016b) indicate low specific conductance (measured in 1972–1974: 13–94 μS cm^{-1}). Guadalupe, Alamitos and Euchre Creeks are small, coastal drainages ranging from southern Oregon to central California. The Peavine Ridge collection was from an isolated spring-fed, seasonally inundated pool with fine sediment, and the species was found on only one of several visits. Values for specific conductance in both the Peavine Ridge spring and in Euchre Creek (measured in April 2014: 95 and 63 μS cm^{-1}, respectively) were low. However, values tend to be much higher (at

summer base flow, 340–490 μS cm^{-1}) in Guadalupe and Alamitos Creeks (unpublished field data, J.L. Carter, US Geological Survey, 3 sampling dates in May, June, September 1997–1998).

Molecular phylogeny and genetic distances

Two species of *Kincaidiana*, the two Nearctic species of *Guestphalinus* and *Uktena riparia* form a clade with high support: BA posterior probability (BA pp) = 0.99, bootstrap value under ML (ML bs) = 87 in the consensus tree based on concatenated sequence data (COI, 16S rRNA and 28S rRNA) (Fig. 13). *Uktena riparia* is the sister species to the clade formed by *Kincaidiana* and *Guestphalinus* species in the ML tree (not shown); however, in the BA tree (Fig. 13), *U. riparia* is the sister species to the *Kincaidiana* species (BA pp = 0.88), with *Guestphalinus* as the sister group to *Kincaidiana* + *Uktena*. Resolving the phylogenetic relationships among these 3 genera needs further molecular work; however, their relatedness is well supported by morphological evidence (see Discussion).

Together, *K. hexatheca* and *K. smithi* sp. nov. form a strongly supported clade (BA pp = 1.00, ML bs = 100). The two specimens of *K. hexatheca* group together (despite morphological differences, already mentioned above), and they are separated from *K. smithi* sp. nov. The uncorrected (p) distance between the two specimens of *K. hexatheca* is 3% for COI, and 7.9–8.5% between *K. hexatheca* and *K. smithi* sp. nov.; these values are concordant with intra- and interspecific values for COI found in other lumbriculids (Achurra & Erséus 2013; Achurra *et al.* 2015). Note that the type locality of *K. smithi* sp. nov. (on the Smith River) is geographically located between the localities of the two sequenced specimens of *K. hexatheca*, and the closest of the latter to the Smith River (Cow Creek, about 150 km to the north) corresponds to the lowest genetic distance (7.9%). For Clitellata, Erséus & Gustafsson (2009) proposed distances of 10% or more for congeneric species; distances of about 5% were interpreted as

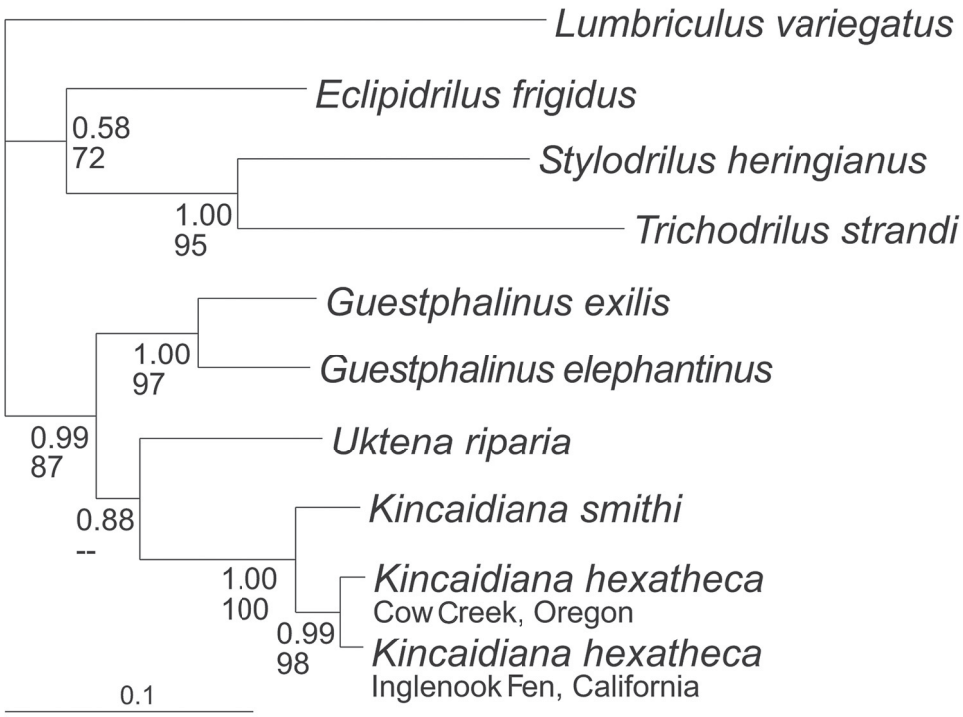

Fig. 13. BA consensus tree derived from the concatenated sequences of COI, 16S rRNA and 28S rRNA genes. Numbers beside internal branches indicate BA posterior probabilities (≥ 0.90) and ML bootstrap values (≥ 70%). The geographical location for two specimens of *K. hexatheca* Altman, 1936 is given under the taxon name.

due to putative ongoing speciation (Zhou *et al.* 2010), which could be the case for the *Kincaidiana* species under study.

Guestphalinus exilis sp. nov. and *G. elephantinus* sp. nov. group together with high support (BA pp = 1.00, ML bs = 97), having an uncorrected (p) distance of 13.2% for COI; this value is high and together with the morphological differences (mainly based on chaetae, see above) supports the recognition of two distinct species.

Discussion

Both morphology and DNA suggest that *Kincaidiana*, *Guestphalinus* and *Uktena* are phylogenetically related. With respect to morphology, the three genera share several distinctive, and probably synapomorphic characters: an elongate-filiform proboscis, spermathecae in the atrial segment, male ducts and pores in a segment anterior to X, and elongate or tubular atria and spermathecae.

The filiform proboscis appears superficially ringed in preserved specimens of all three genera, and was referred to as "pseudo-segmented" by Cook (1971) for *K. hexatheca*, as rings are not associated with septa. Other lumbriculid taxa, *Rhynchelmis*, *Eclipidrilus* (*Premnodrilus*) and *Eremidrilus*, are also characterized by an elongate, although usually shorter proboscis, and surface wrinkling (which may appear as rings) varies with fixation.

Most other lumbriculid genera have the spermathecae in either pre- or post-atrial segments, and only the three *Cookidrilus* species have paired spermathecae in the atrial segment. Two species of *Dorydrilus* (*D. michaelseni* Piguet, 1913 and *D. tetrathecus* Hrabě, 1960) also have spermathecae in the atrial segment. *Dorydrilus* is generally placed in the Dorydrilidae, although sometimes assigned to the Lumbriculidae (e.g., Hrabě 1983). *Cookidrilus* and *Dorydrilus* are small worms, without a proboscis, and also differ from *Kincaidiana, Guestphalinus* or *Uktena* in most other respects.

The position of the male pores in VIII (*Uktena*) or IX (*Kincaidiana* and *Guestphalinus*) likely represents a forward shift from an ancestral position in X (Brinkhurst 1989). A forward shift in the position of reproductive organs is known in both semiprosoporous (particularly the *Trichodrilus* group having bifid chaetae: *T. strandi* Hrabě, 1936, *T. diversisetosus* Rodriguez, 1986, *T. campoyi* Rodriguez, 1988 and *T. isabellae* Popchenko, 1988) and prosoporous lumbriculids (e.g., *Altmanella*).

The general form of the elongate spermathecae and atria is similar in *Kincaidiana*, *Guestphalinus* and *Uktena*. In most cases, the ectal, duct-like section of the spermatheca widens gradually to form an elongate, sacciform (irregular) ampulla, and in both *Kincaidiana* and *Guestphalinus* the spermathecae terminate in a muscular sphincter just before the pore. As in some other lumbriculids having elongate spermathecae (e.g., some *Rhynchelmis* (*Rhynchelmoides*) and *Pilaridrilus* Fend & Lenat, 2007), dense accumulations of sperm are also lined up along an ectal region with more columnar epithelium, and more diffuse sperm is present in the ampulla (Fend & Lenat 2007, 2010).

Chaetae

Apart from the occurrence of modified genital chaetae in a few species (e.g., *U. riparia* and four species of *Pseudorhynchelmis* Hrabě, 1982; Martin & Kaygorodova 2008), most lumbriculids have rather uniform, sigmoid chaetae with a nodulus somewhat distal to the midpoint. Simple-pointed chaetae are most common in the family, although bifid somatic chaetae occur as intra-generic variation in several lumbriculid genera (e.g., *Stylodrilus*, *Rhynchelmis*, *Lumbriculus*) as well as in the monotypic *Wsewolodus* Semernoy, 2004. Bifid chaetae in those taxa have a typical lumbriculid form, with a small, thin upper tooth; furthermore, bifids occur throughout the worm, or if variable, teeth are usually more developed

in posterior segments. Chaetal differences among body regions are usually minor, and limited to greater size of ventrals vs dorsals, or a gradual decrease in size along the body axis. Ventral chaetae may be larger than dorsals in some species of several lumbriculid genera, e.g., *Stylodrilus mirus* (Cekanovskaya, 1956), *Lamprodrilus mrazeki* Hrabě, 1928, *Sylphella puccoon* Rodriguez *et al.*, 2014 and *Trichodrilus diversisetosus* Rodriguez, 1986. The strongly hooked dorsal chaetae of the predaceous *Phagodrilus* McKey-Fender, 1988 are developed only in posterior segments. Other unusual chaetal modifications, such as elongate distal ends of dorsal chaetae in *Trichodrilus capilliformis* Giani & Rodriguez, 1994 and *Stylodrilus wahkeenensis* Rodriguez & Coates, 1996, the elongated ventral chaetae of *Lamprodrilus bythius* Michaelsen, 1905 and some *Rhynchelmis rostrata* (Eisen, 1888) (Fend & Brinkhurst 2000), or the partial or complete loss of chaetae in some species of *Lamprodrilus* (e.g., *L. achaetus* Isossimov, 1962) could also be interpreted as derived characters related to a particular lifestyle.

Some anterior chaetae are distinctly modified in both *Kincaidiana* and the Nearctic *Guestphalinus*; they may appear larger and bifid or even trifid in lateral view, and where most developed, modified chaetae in both taxa show a flattened, dorsal keel. The wide range in size or degree of modification among populations in these taxa suggest local speciation and perhaps adaptation to different external conditions (e.g., sediment particle size distribution). In both *Kincaidiana* and *G. elephantinus* sp. nov., the forward orientation of the enlarged and modified anterior chaetae suggests a particular mechanical adaptation to locomotion or feeding (including the possibility of predatory behavior).

The inconspicuous dorsal keel at the tip of posterior chaetae in some specimens of *Kincaidiana* and the Nearctic species of *Guestphalinus* has not been reported in other lumbriculids, and the grooved tips on posterior chaetae of one specimen of *G. wiardi* also appear unusual. Nevertheless, similar chaetae with keeled tips have been described in unrelated taxa, e.g., two species in the family Haplotaxidae: *Delaya leruthi* (Hrabě, 1958) (Delay 1972: figs 1–2) and *D. navarrensis* (Delay, 1973) (Delay 1973: fig. 1).

Male ducts

Guestphalinus has a semiprosoporous male duct (with 2 male ducts per atrium), in contrast to the prosoporous ducts of the related *Kincaidiana* and *Uktena*. Although this has been considered a phylogenetically important character, it is likely that a reduction of the anterior vas deferens has occurred more than once in lumbriculids (see Brinkhurst 1989).

Both of our specimens of *G. wiardi* and the description by Michaelsen (1933: fig. 2) indicate that the anterior vasa deferentia form a loop in the pre-atrial segment, as in *G. exilis* sp. nov. and *G. elephantinus* sp. nov. This character has not been reported in other Lumbriculidae so far. Hrabě (1984) considered the penetration of the posterior septum by the posterior vas deferens in semiprosoporous lumbriculids to be an atavistic character, associated with the loss of the posterior pair of atria from a double-pair of prosoporous atria; following that logic, this new structure could suggest the loss of an anterior atrium. The male funnels extend several segments back within posterior sperm sacs in *Kincaidiana*, *Uktena*, and possibly in some *G. wiardi* (Michaelsen 1933: fig. 2; but not confirmed in our material); this character is also somewhat unusual in the family, even in genera having very elongate atria (e.g., *Rhynchelmis* and *Eclipidrilus*).

Copulatory glands

Large, stalked glands (Pubertätsdrüsen in Michaelsen 1933), are associated with the male and/or spermathecal pores in *Guestphalinus*. The glands have a similar structure in all species, with cell extensions passing through a narrow muscular constriction and terminating in small, circular secretory openings in the epidermis. These glands are here regarded as probable copulatory glands, as they are well developed in both nearly-mature (unmated) and mature worms, often smaller in post-copulatory worms with well-developed eggs, and disappear in post-reproductive worms. Species of *Kincaidiana* have well-defined penes, the most common condition in Lumbriculidae, but the absence of this efficient

means of sperm transfer in *Guestphalinus* further suggests that the glands in the ventral region (close to the genital pores) are associated with mating. Large glands of this form also occur in *Uktena riparia* Fend *et al.*, 2015, where they probably have some function in the transfer of spermatophores, but, instead of opening externally, these open within the complex spermathecal and male bursae.

Glands of this form are uncommon in the Lumbriculidae, but similar structures, with external openings in (or just behind) the male segment, have been described in *Rhynchelmis brooksi* Holmquist, 1976 and some species of *Lamprodrilus* (e.g., Isossimov 1962: figs 14, 28), where they have been termed "accessory copulatory glands" (Cook 1971; Michaelsen 1901). Midventral glands with a similar form have also been described in anterior segments of sexually mature specimens of some species of *Rhynchelmis* (*Rhynchelmoides*) (Holmquist 1976; Fend & Brinkhurst 2000, 2010). All of the above glands lack a distinct duct, and the conjoined ends of the gland cells open via a small secretory surface. Members of other oligochaete families (e.g., Naididae, Enchytraeidae, Haplotaxidae) have similar glands opening externally near the genital pores at maturity, in some cases associated with genital chaetae (e.g., Rodriguez & Fend 2013); their sporadic occurrence in different lineages suggests that they are convergent.

Glands associated with reproductive structures in some other lumbriculid genera differ in structure. More elaborate, glandular "Kopulationsdrüsen" (e.g., Michaelsen 1901), or "accessory organs" (Fend & Brinkhurst 2010) occur in some *Rhynchemis* (*Rhynchelmis*) species. These organs have an internal lumen, opening through a duct into a simple pore, and have been considered to be rudimentary atria. Michaelsen considered the glands in *G. wiardi* as possible "Geschlechtsborstendrüsen", similar to glands associated with copulatory chaetae in other microdriles, but he was unable to associate them with the ventral chaetae. Although common in other microdrile families, glands associated with genital chaetae are rare within the Lumbriculidae, and have only been described in detail for some species of *Pseudorhynchelmis* Hrabě, 1982 (Martin & Kaygorodova 2008).

Conclusions

Morphology (proboscis, spermathecae in the atrial segment, and general morphology of reproductive organs) suggests that *Kincaidiana*, *Guestphalinus* and *Uktena* are phylogenetically related. This relationship is confirmed by the molecular data available at this time: the three genera form a strongly supported clade in the tree topology (Fig. 13), based on a limited set of lumbriculid outgroup taxa. At this point, it should be noted that no molecular phylogeny of the family Lumbriculidae as a whole has been published. Further molecular analyses, including more genera, are clearly a priority for any understanding of relationships within the family.

Populations of both *Kincaidiana* and *Guestphalinus* are quite variable across their distributions in western North America. Our DNA results provide good confirmation that the two most distinctive populations, *K. smithi* sp. nov. (with a single median atrium) and *G. exilis* sp. nov. (with only slightly modified anterior chaetae), are closely related to their more widespread congeners, *K. hexatheca* (with paired atria) and *G. elephantinus* sp. nov. (with highly modified anterior chaetae), yet differ by interspecific COI distances of 8% or more. Still, given the geographic variation in morphological characters in Nearctic populations of *Kincaidiana* and *Guestphalinus*, further molecular analyses among and within populations would also be of interest, and could provide evidence of speciation processes in the region. Finally, behavioral observations, including the feeding behavior of *Kincaidiana* and *Guestphalinus*, may eventually reveal the function of modified chaetae.

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Appendix 1 (continued on next page)**.** Locality data for collection sites (WGS84 datum). Abbreviations: Rd = road; Cr = creek; R = River; Rv = reservoir; Pk = park; Cg = campground.

State	County	River Drainage	Site	Longitude (° W)	Latitude (° N)
			***K. hexatheca* Altman, 1936**		
WA	Jefferson	Hoh	small seep along Hoh River Rd	124.1128	47.8167
WA	Jefferson	Hoh	Hoh River drainage, ditch by Clearwater Rd	124.1532	47.7923
WA	Pacific	Naselle	spring on Naselle R	123.7283	46.4000
WA	Clallam	Bogachiel	small pool along Bogachiel R on Undi Rd	124.3499	47.8910
WA	Skamania	Columbia	Dog Cr near mouth	121.672	45.712
OR	Multnomah	Columbia	Oneonta Cr near mouth	122.0759	45.5905
OR	Tillamook	Nestucca	spring on Nestucca R, Bible Creek Rd	123.7053	45.2709
OR	Yamhill	Nestucca	seep on east side of McGuire Rv	123.3950	45.2910
OR	Lane	Big Creek	muddy seep at Big Cr, FR5700	124.1133	44.1736
OR	Lane	Willamette	Small spring at mouth of Tenmile Cr	124.1083	44.2250
OR	Lane	Willamette	Marsh at base of Mt. Pisgah, near Eugene	122.9494	43.9492
OR	Lane	McKenzie	Outflow from Leaburg fish hatchery	122.6758	44.1075
OR	Lane	Siuslaw	Whittaker Cr at Siuslaw R	123.6650	49.9861
OR	Douglas	Umpqua	Cow Cr, tributary to Umpqua R	123.4832	42.9122
OR	Douglas	Rogue	spring near Mule Cr	123.7549	42.7846
OR	Josephine	Rogue	*Darlingtonia* bog near O'Brien	123.7611	42.0333
OR	Josephine	Rogue	Illinois R near Sixmile Cr	123.7681	42.3044
OR	Curry	Rogue	Rogue R at Quosatana Cg	124.2339	42.4995
CA	Mendocino	(coastal)	Inglenook Fen at McKerricher State Pk	123.78	39.52
			***K. smithi* Fend & Rodriguez sp. nov.**		
CA	Del Norte	Smith	Smith R below forks	124.08973	41.81543
CA	Del Norte	Smith	Seep by South Fork Smith R	–	–
			***G. exilis* Fend & Rodriguez sp. nov.**		
CA	Shasta	Sacramento	Squaw Cr at Chirpchatter Cg	122.1169	40.8597
CA	Humboldt	Eel	South Fork Eel R at Elk Cr	123.8472	40.2647
CA	Colusa	Sacramento	Stony Cr at Stonyford	122.5514	39.3815
			***G. elephantinus* Fend & Rodriguez sp. nov.**		
WA	Jefferson	Queets	Shale Cr at Clearwater Creek Road	124.2533	47.6370
WA	Jefferson	Queets	Clearwater R at Upper Clearwater Cg	124.1170	47.6785
WA	Jefferson	Queets	Hurst Cr near Clearwater R	124.2917	47.5734
WA	Jefferson	Queets	Bull Cr near Clearwater R	124.1662	47.6670
WA	Jefferson	Queets	Clearwater R at Copper Mine Bottom Cg	124.1996	47.6559
WA	Grays Harbor	Satsop	Middle Fork Satsop R	123.5103	47.1778
WA	Columbia	Snake	Tucannon R below Turner Rd	117.7524	46.4402

OR	Yamhill	Willamette	spring at Peavine Ridge near McMinnville	123.3807	45.2386
OR	Curry	Rogue	Mule Cr at upstream bridge, near Rogue R	123.8829	42.7220
OR	Curry	Euchre Creek	Euchre Cr near mouth	124.376	42.56
CA	Santa Clara	Guadalupe	Guadalupe Cr above Guadalupe Rv	121.8719	37.1827
CA	Santa Clara	Alamitos	Alamitos Cr above Almaden Rv	121.8389	37.1506