Zoosymposia 23: 052–077 (2023)
https://www.mapress.com/j/zs

Article

ISSN 1178-9905 (print edition)
ZOOSYMPOSIA
ISSN 1178-9913 (online edition)

https://doi.org/10.11646/zoosymposia.23.1.9
http://zoobank.org/urn:lsid:zoobank.org:pub:B9D1269A-B646-4119-88E6-0C762E78CD19

New species of Lumbriculidae (Clitellata) from groundwater-dependent habitats in Texas

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Abstract

Recent hyporheic, crenal and phreatic groundwater collections suggest a rich oligochaete fauna associated with the Edwards and Edwards-Trinity Aquifers in central Texas, including many species in the family Lumbriculidae. Based on limited invertebrate collections from earlier sampling programs, four sites were selected for more intensive oligochaete sampling. The lumbriculid *Eclipidrilus palustris* (Smith, 1900) is widespread in cleaner, cooler waters of the southeastern USA, and was common in crenal habitats. However, several lumbriculid species appear to be new and endemic, four of which are described here. Two of these can be assigned to the genus *Eremidrilus* Fend & Rodriguez, 2003 based on semiprosoporous male ducts with male pores in X, spermathecae in XI, and a filiform proboscis. *Eremidrilus samacos* n. sp. has typical morphology for the genus; *E. parvitheca* n. sp. (provisionally assigned to the genus) has duct-like spermathecae that join the gut, and unusual dorsal glands in posterior segments. Two morphotypes are assigned to *E. parvitheca*, based on spermathecal location in XI (typical) or XII (variant). A third species (*Trichodrilus comalensis* n. sp.) lacks a proboscis and has unusually elongated ventral chaetae. A problematic fourth species (*Pararhynchelmis texana* n. sp.) has semiprosoporous atria in X and duct-like spermathecae joining the gut in VIII. As it lacks apomorphies previously proposed for *Rhynchelmis* Hoffmeister, 1843 and *Pseudorhynchelmis* Hrabě, 1982, it is provisionally assigned to *Pararhynchelmis* Fend & Lenat, 2010.

Key words: North America, oligochaetes, new species, biogeography, taxonomy, stygofauna, karst groundwater

1. INTRODUCTION

In contrast to Europe, groundwater-associated oligochaetes have received little attention in North America. The list of European species is extensive; a partial review of recent descriptions and summaries (Achurra *et al.* 2015, Artheau & Giani 2006, Creuzé des Chatelliers *et al.* 2009, Cernosvitov 1939, Dumnicka 2014, Giani *et al.* 2001, Giani *et al.* 2011, Martinez-Ansemil *et al.* 1997, Martinez-Ansemil *et al.* 2012, Martin *et al.* 2015, Rodriguez & Achurra 2010, Sambugar *et al.* 2005, Stoch *et al.* 2011) lists 39 in the family Lumbriculidae, over 40 in the Naididae, and over 30 in other families. In contrast, although North American lists of oligochaetes collected in crenal, hyporheic and phreatic habitats may be extensive (Wetzel & Taylor 2001, Strayer 2001), most collections have been dominated by species described from, or commonly associated with, epigean habitats. The North American faunal list includes less than 20 described oligochaete species known primarily from hyporheic and groundwater habitats (Cook 1971,

Submitted: 10 Jan. 2023; Accepted by Patrick Martin: 15 Mar. 2023; published: 30 Jun. 2023

1975, Erséus 1999, Kathman & Brinkhurst 1984, Rodriguez 1996, Rodriguez & Coates 1996, Strayer 2001, Strayer & Bannon-O'Donnell 1988, Wetzel & Taylor 2001). Nevertheless, these habitats (particularly hyporheic) may serve as important refugia for some species described from surficial sediments—e.g., the tubificine *Varichaetadrilus angustipenis* (Brinkhurst & Cook, 1966) (Wetzel & Taylor 2001) and the lumbriculids *Tenagodrilus musculus* Eckroth & Brinkhurst, 1996, *Uktena riparia* Fend, Rodriguez & Lenat, 2015 and some species of *Eremidrilus* Fend & Rodriguez, 2003.

Aside from a few widespread species (e.g., *Lumbriculus variegatus* (Mueller, 1774), *Stylodrilus heringianus* Claparède, 1862, *Eclipidrilus lacustris* (Verrill, 1871)), most species in the family Lumbriculidae are regionally or locally endemic (Timm 1999). Compared with the more widespread Naidinae and Tubificinae, the family seems associated with cooler, less organically enriched aquatic habitats (Milbrink 1983; Ohtaka 2014; Rodriguez & Reynoldson 2011; Timm 1999, 2012). Currently, about 80 described lumbriculid species appear to be endemic to North America, compared with about 165 Palearctic endemics—of which over 60 appear endemic to Lake Baikal (Semernoy 2004). Recent collections of aquatic oligochaetes in hyporheic, phreatic and crenal habitats in the region associated with the Edwards and Edwards-Trinity Aquifers, central Texas (Hutchins 2018, Hutchins *et al.* 2021, Gibson *et al.* 2008, Gibson *et al.* 2020) have produced as many as 12 potentially valid new species of Lumbriculidae, still undescribed. Initial collections were preserved in bulk, using ethanol, and it was difficult to describe some key characters. Subsequently, a more limited effort at some sites has yielded adequate type series of four of these species, described herein.

2. MATERIAL AND METHODS

Initial invertebrate collections covered extensive portions of the Edwards Plateau and Balcones Escarpment in central Texas, and lumbriculid worms were found at 23 of these sites from 2007 to 2020. The region is underlain by Cretaceous-age limestones that were uplifted to create broad, rolling plateaus (Edwards and Stockton Plateaus) bounded on the south and east by a series of en-echelon faults (Balcones Escarpment). Karstification, consisting of multiple stages of epigenic and hypogenic dissolution, contributes to permeability. Recharge occurs where limestones are exposed at the surface (Barker *et al.* 1994, Maclay 1995, Sharp & Banner 1997, Schindel & Gary 2017). The Edwards and hydrologically connected Edwards-Trinity Aquifers are a recognized hotspot of stygobiont biodiversity (Hutchins *et al.* 2021) and discharge at springs maintains flow in a number of surface streams originating on the Edwards Plateau or at the base of the Balcones Escarpment. Additional details on hydrogeology and stygobiont diversity are summarized in Hutchins *et al.* (2021). Sampled habitats included karst springs, caves, wells, and hyporheic habitats (surface streams with flow substantially or completely derived from groundwater inputs). Initial samples were collected using a Bou-Rouch pump (Bou and Rouch, 1967) or with drift nets placed at spring outflows for community-wide characterization. Samples were preserved in 95% ethanol in the field, and later sorted in the lab in 95% ethanol using a dissecting microscope at 10X magnification.

Based on the above information, additional specimens were obtained for improved morphological characterization during October 2021 from 4 priority sites: Comal Springs, San Marcos River at Scull Road, San Marcos Springs (Spring Lake), and Sessom Creek (Table 1). Near-surface collections were made by digging at least 20 cm deep in gravel streambeds and collecting the suspended material with a fine mesh (ca. 200 μm) net. Hyporheic collections were made with a Bou-Rouch pump: samples of approximately 9 L were collected from a depth of 30–50 cm using a 100 μm mesh net immediately after insertion of the Bou-Rouch spike. Spring drift samples were collected by placing drift nets (150–250 μm mesh) over spring openings for 24 hours and preserving captured invertebrates and debris in ethanol every 4 to 6 hours (Gibson *et al.* 2008, Gibson *et al.* 2020). Oligochaete worms were sorted in the field, and specimens for morphological study were fixed in either 5% formaldehyde or Kahle's fluid, and later preserved in 70% alcohol. Voucher specimens from an earlier study of Spring Lake (Worsham *et al.* 2016) were provided by McLean Worsham (Texas State University Masters research). Additional collections from Comal Spring run sites were provided by Mark J. Wetzel (Illinois Natural History Survey) and David Bowles (Texas Parks and Wildlife Department project).

TABLE 1. Collection data for Lumbriculidae observed in this study.

Species	No. specimens	Site	County	Latitude (WGS84)	Longitude (WGS84)	Sample type	Sampling date	Collector
Eremidrilus samacos n. sp	21	San Marcos River at Scull Rd.	Hays	29.8492	-97.8565	Bou-Rouch	11-Oct-21	Hutchins
Eremidrilus samacos n. sp.	4	Sessom Creek	Hays	29.8900	-97.9360	Bou-Rouch	11-Oct-21	Hutchins
Eremidrilus samacos n. sp.	4	Comal Spring Run 3	Comal	29.7143	-98.1362	drift net	12-Oct-21	Gibson
Eremidrilus cf. *samacos*	2	John Knox Ranch, Blue Hole Spring	Comal	29.9640	-98.1956	drift net	2-May-10	Gibson
Eremidrilus cf. *samacos*	1	Cold Springs, Austin	Travis	30.2797	-97.7807	drift net	3-Aug-18	Crow
Eremidrilus cf. *samacos*	2	Llano River SE of Mason	Mason	30.6610	-99.1096	Bou-Rouch	15-Apr-16	Hutchins
Eremidrilus cf. *samacos*	3	Independence Creek	Terrell	30.4598	-101.8246	Bou-Rouch	15Jul-13	Hutchins
Eremidrilus cf. *samacos*	6	McKittrick Canyon	Culberson	31.9859	-104.7697	Bou-Rouch	11-Sep-15	Hutchins
Eremidrilus parvitheca n. sp.	10	Sessom Creek	Hays	29.8900	-97.9360	benthic	11-Oct-21	Fend
Eremidrilus parvitheca var.	2	Comal Spring Run 3	Comal	29.7143	-98.1362	benthic	12-Oct-21	Fend
Eremidrilus parvitheca var.	2	Tahuaya Springs	Bell	31.0097	-97.5083	drift net	28-Aug-18	Diaz
Trichodrilus comalensis n. sp.	1	Comal Springs Run 3	Comal	29.7143	-98.1362	drift net	12-Oct-21	Gibson

...Continued on the next page

TABLE 1. (Continued)

Species	No. specimens	Site	County	Latitude (WGS84)	Longitude (WGS84)	Sample type	Sampling date	Collector
Trichodrilus comalensis n. sp.	1	Comal Springs Run 2	Comal	29.7143	-98.1362	drift net	29-Oct-20	Gibson
Trichodrilus comalensis n. sp.	1	Comal Springs Run 3	Comal	29.7143	-98.1362	drift net	19-Jun-20	Gibson
Trichodrilus comalensis n. sp.	1	Comal Springs Run 3	Comal	29.7143	-98.1362	drift net	2-May-19	Gibson
Trichodrilus comalensis n. sp.	1	Comal Springs Run 3	Comal	29.7143	-98.1362	drift net	27-Oct-17	Gibson
Trichodrilus comalensis n. sp.	1	Comal Springs Run 3	Comal	29.7143	-98.1362	drift net	12-May-17	Gibson
Trichodrilus comalensis n. sp.	1	Comal Springs Run 3	Comal	29.7143	-98.1362	drift net	16-Nov-14	Gibson
Trichodrilus cf. *comalensis*	1	Ezell's Cave	Hays	29.8738	-97.9594	other	29-Sep-06	Krejca
Pararhynchelmis texana n. sp.	6	Sessom Creek	Hays	29.8900	-97.9360	Bou-Rouch	11-Oct-21	Hutchins
Pararhynchelmis texana n. sp.	10	Sessom Creek	Hays	29.8900	-97.9360	benthic	11-Oct-21	Fend
Eclipidrilus palustris	2	Sessom Creek	Hays	29.8900	-97.9360	benthic	11-Oct-21	Fend
Eclipidrilus palustris	24	Comal Springs Run 7	Comal	29.7154	-98.1348	benthic	12-Oct-21	Fend
Lumbriculus sp.	5	Comal Springs	Comal	29.7111	-98.1319	benthic	23-May-97	Wetzel

Collection sites

The San Marcos River is a tributary to the Guadalupe River, which flows southeast to the Gulf of Mexico. Most collections were from the mainstem, where substrate is dominated by limestone gravels. In this area, the channel was approximately 18 m wide, with rapid, linear flow and an average depth of approximately 0.5 m. Sessom Creek is a small, heavily urbanized tributary of the San Marcos River. Except after rain, baseflow is maintained by springs discharging from the Edwards Aquifer. The streambed is dominated by limestone gravels over clay and marl. The creek averages about 2.5 m wide and 0.2 m deep. Comal Springs is the largest spring in Texas and the southwestern United States. The springs have flowed continuously except for 5 months in 1954 when surface flow ceased. These springs are the headwaters of the Comal River which flows for 2 km, and then joins the Guadalupe River. Spring Run 3 consists mostly of limestone cobble and gravel substrate with some fine sediments and relatively stable water quality. San Marcos Springs (Spring Lake) is the second largest spring in Texas and has flowed continuously throughout recorded history. It forms the headwaters of the San Marcos River, which flows for ca. 130 km before joining the Guadalupe River. The many spring sites consist of cobble, gravel, and sand substrates upwelling into the Spring Lake impoundment.

Morphological methods

Whole and dissected specimens were usually stained with either borax carmine or hematoxylin and slide mounted in Canada balsam for examination with a compound microscope, in most cases using Nomarski differential interference contrast illumination. Measurements of distinct structures were made to about 5% precision, but indistinct or highly variable structures were measured to less. Measurements of reproductive organs were based on fully mature worms having sperm in the spermathecae; other measurements were made on worms with distinct genital pores (at least nearly mature). Figures were drawn with the aid of a camera lucida. Roman numbers are used to designate segments; septa are indicated by Arabic segment numbers, as “4/5”.

Museum abbreviations. USNM, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; CASIZ, California Academy of Sciences, Department of Invertebrate Zoology and Geology, San Francisco, California, USA.

Figure abbreviations: at, atrium; fp, female pore; gu, gut; mf, male funnel (1 = anterior, 2 = posterior); mp, male pore; mu, muscle; gl, gland; gu, gut; pr, prostate gland; sa, spermathecal ampulla (1 = anterior, 2 = posterior); sp, spermathecal pore; st, spermatheca; te, testis (1 = anterior, 2 = posterior); vd, vas deferens (1 = anterior, 2 = posterior).

3. RESULTS

New species included in this study:
3.1.1. *Eremidrilus samacos* n. sp. (Figs. 1, 2)
3.1.2. *Eremidrilus parvitheca* n. sp. (Figs. 3, 4)
3.1.3. *Eremidrilus parvitheca* variant (Figs. 3, 4)
3.2.1. *Trichodrilus comalensis* n. sp. (Figs. 5, 6)
3.3.1. *Pararhynchelmis texana* n. sp. (Figs. 7, 8)

3.1 *Eremidrilus* Fend & Rodriguez, 2003

Type species: *Eremidrilus elegans* Fend & Rodriguez, 2003.

Additional species: *Eremidrilus allegheniensis* (Cook, 1971), *Eremidrilus artzaini* Fend & Rodriguez, 2020, *Eremidrilus chalonensis* Rodriguez & Fend, 2022, *Eremidrilus coyote* Fend & Rodriguez, 2003, *Eremidrilus felini* Fend & Rodriguez, 2003, *Eremidrilus gilita* Fend & Rodriguez, 2020, *Eremidrilus humboldti* Fend & Rodriguez, 2020, *Eremidrilus montanensis* Fend & Rodriguez, 2020, *Eremidrilus owyhee* Rodriguez & Fend, 2022, *Eremidrilus pinedai* Rodriguez & Fend, 2022, *Eremidrilus ritocsi* Fend & Rodriguez, 2003.

Species described here: *Eremidrilus samacos* Fend n. sp., *Eremidrilus parvitheca* Fend n. sp.

Members of the genus *Eremidrilus* are small lumbriculids known only from the Nearctic Region. As in the mostly Palearctic genus *Trichodrilus* Claparède, 1862, male pores are in segment X and spermathecae are in one or two post-atrial segments; atria are semiprosoporous, with two functional male ducts serving testes in IX and X. All known species are differentiated from *Trichodrilus* by having a filiform proboscis, and male pores are usually on distinct porophores. Unlike in most *Trichodrilus* species (Rodriguez & Giani 1994), atria are elongate. The general diagnosis of the genus by Fend & Rodriguez (2020) can be applied to the new species described in the present contribution. A key to previously known species is given in Rodriguez & Fend 2022.

Up to 8 morphotypes having the *Eremidrilus* arrangement of reproductive organs and most other characteristics have been collected from Texas hyporheic and groundwater habitats. Most are represented by limited collections of damaged specimens, but there is sufficient material in good condition to describe two of these.

3.1.1. *Eremidrilus samacos* Fend n. sp.

(Figs. 1, 2)

Holotype: USNM 1683417. Whole worm, slide-mounted in Canada balsam.

Type Locality: Texas, Hays Co., San Marcos River at Scull Rd., N 29.8492, W 97.8565, 154 masl altitude, Bou-Rouch hyporheic sampler, 11 Oct 2021. Collected by B. Hutchins, S. Fend.

Paratypes: From the type locality, same collection data. USNM 1683418–1683420, 3 whole mounted worms. CASIZ 236635–236638, 4 whole mounted worms.

Etymology: From the type locality.

Other material: From the type locality, same collection data, 14 whole mounted worms. Texas, Hays Co., San Marcos, Sessom Creek near the Freeman Aquatic Building, N 29.8900, W 97.9360, 11 Oct 2021, 4 whole mounted worms. Comal Co., New Braunfels, Comal Springs Run 3, N 29.713506, W 98.136988, 12 Oct 2021; drift net at spring outflow, 2 whole mounted worms; benthic sample in upwelling area, 2 whole mounted worms.

Description: Segments 59–75. Length of fixed worms 11–22 mm; body diameter in X 0.41–0.79 mm. Proboscis 460–1030 µm long, about 60–100 µm diameter at middle (Fig. 1A, 2A). Secondary annulus in anterior 1/4−1/3 of preclitellar segments, distinct from V, may be narrower in IV. All chaetae simple-pointed, sigmoid (Figs. 1B, C; 2B); ventral pairs in anterior segments increasing in length from II to about VII (maximum length 155–224 µm), similar in posterior segments (140–210 µm); chaeta diameter 4–5 µm. Chaeta length in dorsal and ventral bundles similar, or dorsal pair slightly shorter. Outer chaeta in each pair usually shorter, with more distal nodulus (0.36–0.48 chaeta length from tip); nodulus of inner chaeta slightly distal to slightly proximal (0.43–0.55). Male pores on ventral chaetal lines, midway between chaetae and posterior septum (Fig. 1D–F); pores open on low (often indistinct) porophores about 20 µm high, 60–80 µm wide, formed of thickened, non-clitellar epidermis (Fig. 2C). One pair of simple spermathecal pores behind ventral chaetae in XI, on ventral chaetal line, at about 2/3 distance from ventral chaetae bundle to posterior septum (Fig. 1D–F).

Epidermis usually 10–16 µm thick in anterior segments, 14–24 µm in clitellum; clitellum weakly defined from X–XIII. Pharynx mostly in II–III (IV), without distinct dorsal pad; slightly thickened both dorsal and ventrally. Pharyngeal glands weakly developed in IV or V to V or VI (Fig. 1A). Nephridia paired in XIII, the next in about XVII, then in a few posterior segments; a short anteseptal funnel is followed by a thick, mostly postseptal granulated mass and a duct loop which may follow the ventral blood vessel into posterior segments, returning to originating segment; ectal end of duct slightly widens into a narrow vesicle (80–120 µm long by 20–35 µm in diameter); nephridiopore just anterior to ventral chaetae.

Testes to mid-segment in IX and X; ovaries to posterior part of segment in XI; female funnels to 160 µm high on 11/12. Sperm sacs extend anteriorly to VIII, posteriorly as far as XIV; egg sacs may extend 1–2 segments beyond. Both anterior and posterior male funnels (on 9/10 and 10/11, respectively) functional; both flat on septum, 70–150 µm high and directed anteriad (Figs. 1D–F). Vasa deferentia 14–24 µm wide; anterior pair 230–410 µm long, posterior usually slightly longer, 270–430 µm. Posterior vas forms distinct loop in XI; both anterior and posterior vasa join the muscle layer near middle of atrium and enter the lumen about 1/5 the atrial length from the apex. Atria club-shaped to somewhat petiolate with elongate ampulla, entirely in X (Figs. 1D–F, 2D), length 190–280 µm (0.3–0.5 times body diameter, 3–4 times porophore diameter), diameter in ampullar portion 50–90 µm, narrowing to 20–30 µm near the pore; atrial muscle layer thin (usually 2–3 µm), epithelium 10–25 µm thick (Fig. 2E), maximum lumen width 20–30 µm in most specimens. Prostate glands densely covering the ampulla, appearing diffuse in some specimens, but in others forming dense, petiolate clusters of cells, 25–50 µm high (Fig. 2D, E).

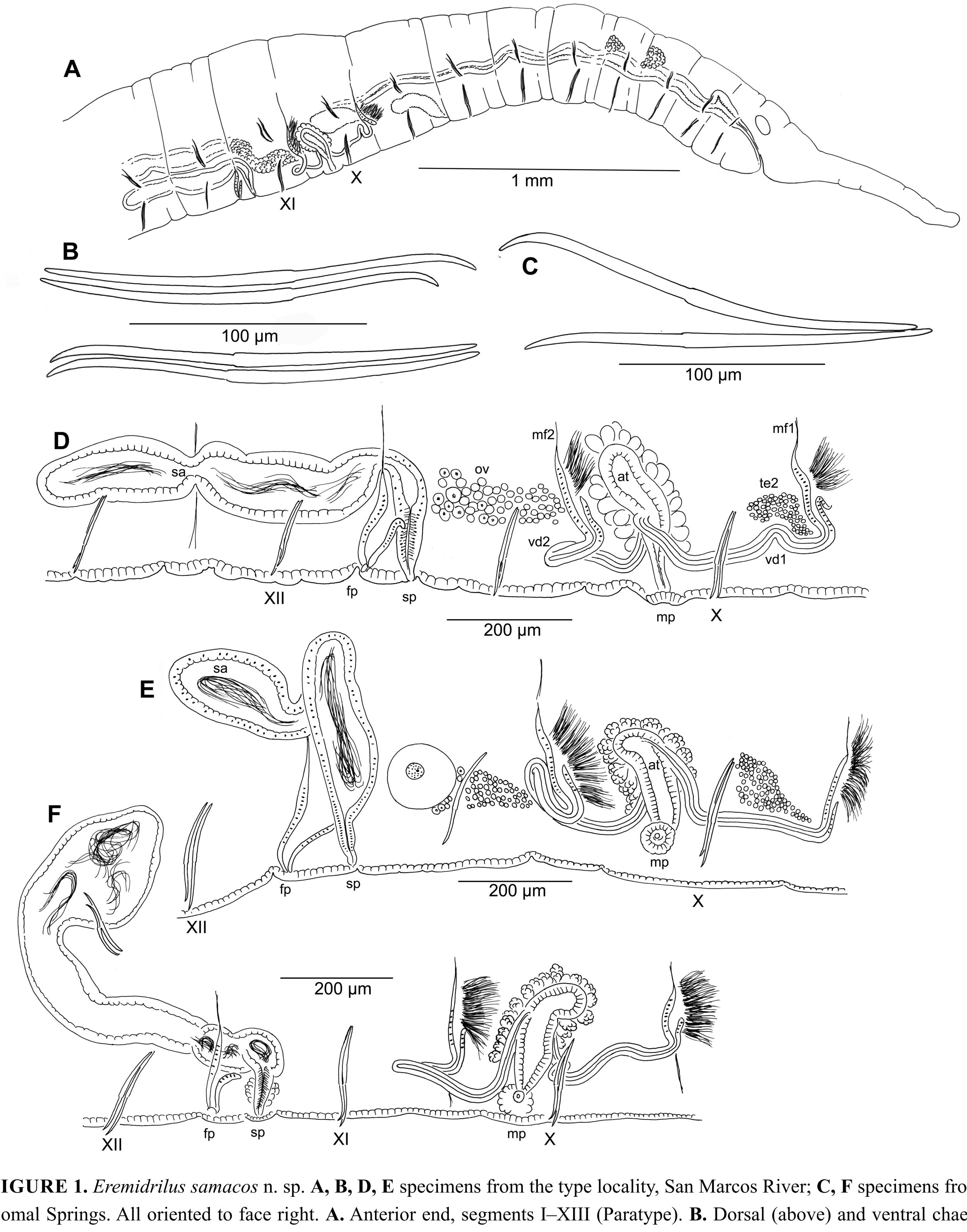

FIGURE 1. *Eremidrilus samacos* n. sp. **A, B, D, E** specimens from the type locality, San Marcos River; **C, F** specimens from Comal Springs. All oriented to face right. **A.** Anterior end, segments I–XIII (Paratype). **B.** Dorsal (above) and ventral chaeta pairs in segment IX (Paratype). **C.** Ventral chaeta pair in an anterior segment. **D–F**. Reproductive organs (D from Paratype, E from Holotype, F from non-type).

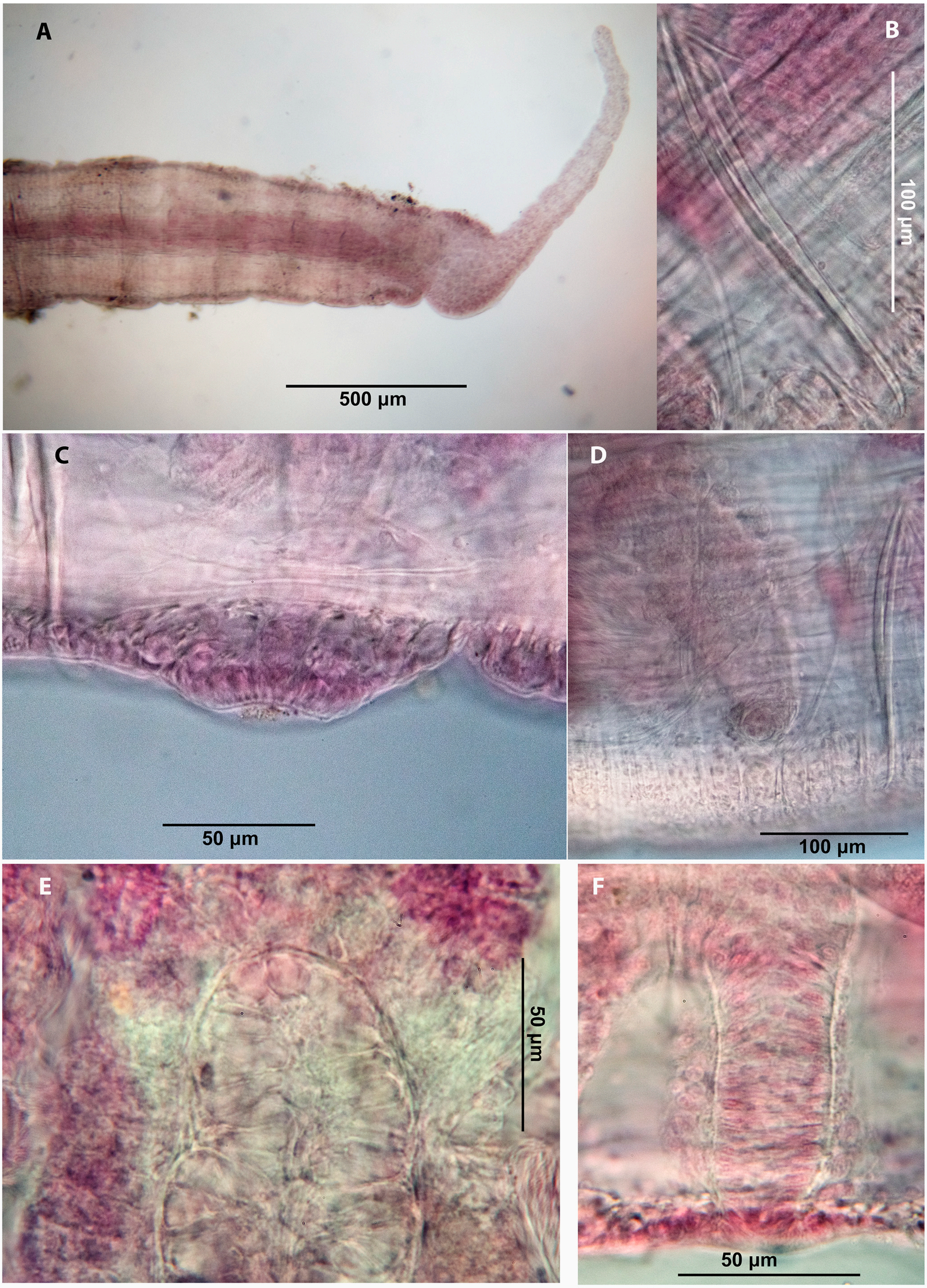

FIGURE 2. *Eremidrilus samacos* from San Marcos River. **A.** Anterior end of whole mount, showing long proboscis. **B.** Ventral chaeta pair, mid-body. **C.** Male pore, lateral view. **D.** Atrium, ventrolateral view. **E.** Ental end of atrium, showing thick epithelium (Paratype). **F.** Spermathecal duct (Paratype).

One pair of spermathecae in XI, distinctly petiolate with elongate, sac-like ampulla, length more than 3 times width in mated worms, usually extending into XII or XIII (Fig. 1D–F); ampullar epithelium about 10–20 µm thick, not obviously glandular. Sperm in the ampulla in loose, irregular bundles, not associated with epithelium. Short spermathecal ducts 50–100 µm long, 20–40 µm wide entally, tapered near the pore, with a thin muscle layer and columnar lining cells (Fig. 2F); ratio of duct length to body diameter 0.08–0.20. In nearly-mature, unmated specimens, spermathecal ducts are similar to those in mature worms, but ampullae are smaller.

Additional material examined (see Table 1)

A few specimens from additional sites, collected on earlier sampling dates, generally resembled typical *E. samacos* with respect to morphology of chaetae and proportions of reproductive organs. However, material was in poor condition for detailed observations, and these specimens are provisionally assigned to *E. samacos*, pending future collections.

Remarks: *Eremidrilus samacos* n. sp. resembles California *Eremidrilus* species (*E. elegans*, *E. chalonensis*, *E. coyote*, *E. felini*, *E. pinedai*, and *E. ritocsi*) in having a club-shaped atrium and a single pair of spermathecae in the ovarian segment. In contrast to most of those species (Fend & Rodriguez 2003, Rodriguez & Fend 2022), the male porophores in *E. samacos* are not prominent; they are basically patches of thickened epidermis, without well-developed circular musculature. The spermathecal pores are in line with the ventral chaetae, instead of the slightly to strongly lateral position in most described California species. The structure of spermathecae, with an elongate (instead of nearly spherical) ampulla, and a very short duct also distinguishes *E. samacos* from California species, other than *E. chalonensis*. In contrast to *E. chalonensis*, the spermathecal pores are simple openings, without conspicuous musculature or accessory glands. Guts of most specimens contained large amounts of fine organic matter and small amounts of mineral particles, indicating that these are selective deposit feeders.

In addition to *E. samacos*, *Pararhynchelmis texana* n. sp. was found in both hyporheic and near-surface sediment at the type locality, and *Eremidrilus parvitheca* n. sp. was collected at the Sessom Creek site. At Comal Spring, *Trichodrilus comalensis* n. sp. was found in drift samples, and a possible variant form of *Eremidrilus parvitheca* occurred in near-surface sediment. Among other aquatic oligochaetes, near-surface benthic collections at these sites included the widespread lumbriculid *Eclipidrilus palustris* (Smith, 1900), the tubificine *Varichaetadrilus angustipenis* (Brinkhurst & Cook, 1966), and an undescribed species of Haplotaxidae that may be regionally endemic (unpublished).

3.1.2. *Eremidrilus parvitheca* Fend n. sp.: typical specimens

(Figs. 3A–E, 4A, C, D, F–I)

Holotype: USNM 1683421. Whole worm, slide-mounted in Canada balsam.

Type Locality: Texas, Hays Co., San Marcos, Sessom Creek near the Freeman Aquatic Building, N 29.8900, W 97.9360, 175 masl altitude, benthic sample, 11 Oct 2021, collected by S. Fend and B. Hutchins.

Paratypes: USNM 1683421–1683422. From the type locality, same collection data, 2 whole mounted worms. CASIZ 236639–236641, 3 whole mounted worms.

Etymology: For the reduced spermathecae, which lack the large ampullae typical for the genus.

Other material: From the type locality, same collection data, 8 whole mounted worms, some partially mature. Hyporheic (Bou-Rouch) samples, collected by B. Hutchins: 27 Mar 2015, 2 whole mounted; 21 Jul 2015, 2 whole mounted.

Description: Segments (5 complete worms) 50–57, length of fixed worms 6–11 mm; body diameter in X 0.2–0.3 mm. Proboscis short, 70–160 µm long, about 30–46 µm diameter at middle (Figs. 3A, 4A). Secondary annulus in anterior 1/4−1/3 of preclitellar segments, from segment III or IV. All chaetae simple-pointed, sigmoid; in anterior segments increasing in length from II to VI or VII, 67–87 µm long in mid-body; shorter in posterior segments; chaeta length in dorsal and ventral bundles similar (Fig. 3B). Outer chaeta in each pair usually shorter than inner, with a more distal nodulus (0.36–0.41 chaeta length from tip; 0.40–0.46 in inner chaeta). Male pores on the ventral chaetal line, about 2/5–1/2 the distance between chaetae and posterior septum (Fig. 3C, D); pores open on small papillae within indistinct porophores formed of thickened, non-clitellar epidermis (Fig. 4F); porophores 30–50 µm wide. One pair of simple spermathecal pores behind ventral chaetae in XI, on the ventral chaetal line, usually near the posterior septum (Fig. 3C–E), but sometimes more anteriorly placed.

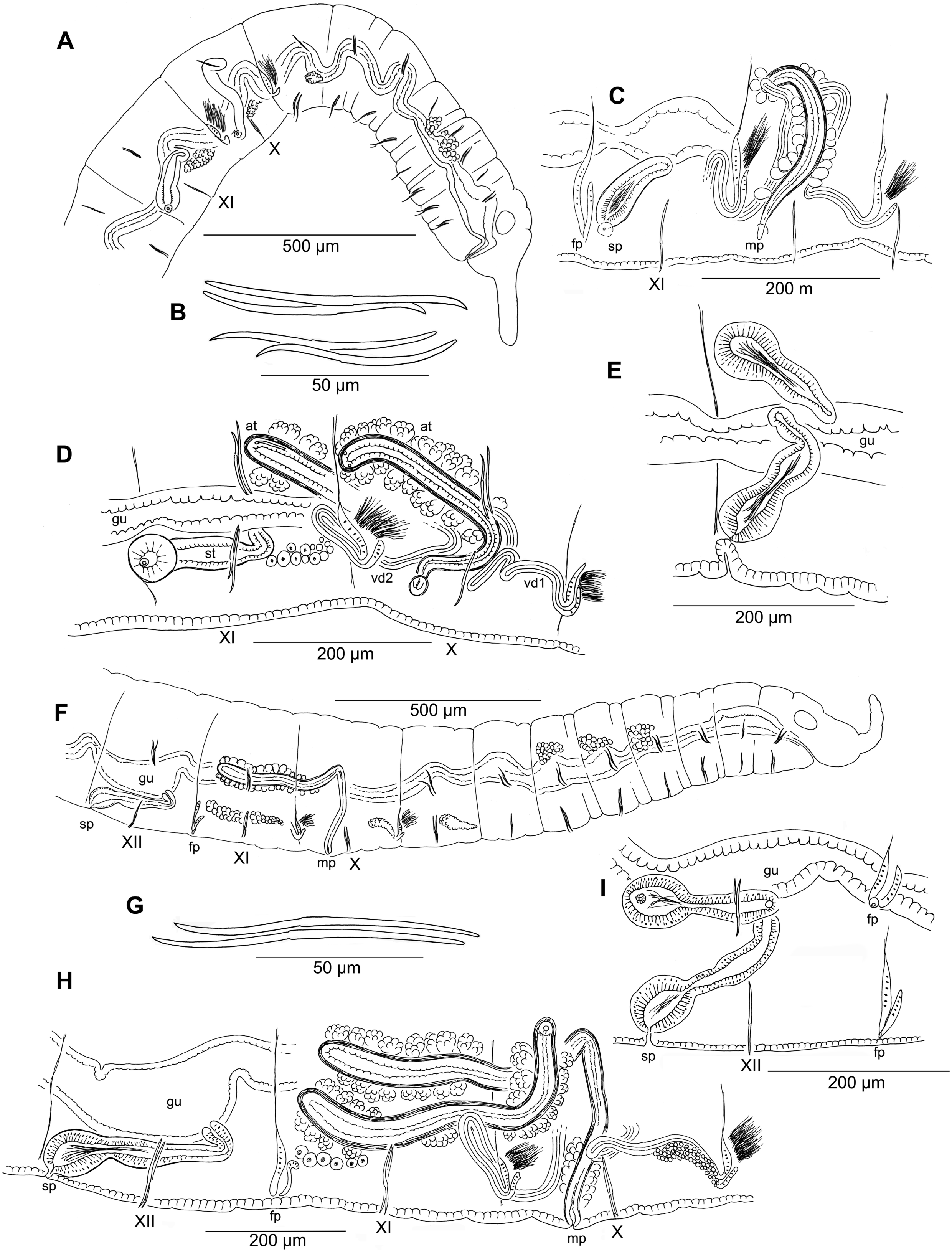

FIGURE 3. A–E. *Eremidrilus parvitheca* n. sp. (typical) from Sessom Creek. **A.** Anterior end (segments I–XII) (Paratype). **B.** Chaetal bundles in XI, upper pair dorsal, lower pair ventral. **C, D.** Reproductive organs in X–XI (D is from Holotype). **E.** Detail of spermathecae in XI, dorsal view showing ental ends approaching gut. **F–I.** *Eremidrilus parvitheca* variant from Comal Springs. **F.** Anterior segments I–XII. **G.** Chaetal bundle, XI ventral. **H.** Reproductive organs, with atria in X and spermathecae in XII. **I.** Spermathecae in XII, ventrolateral view, with ental ends at gut.

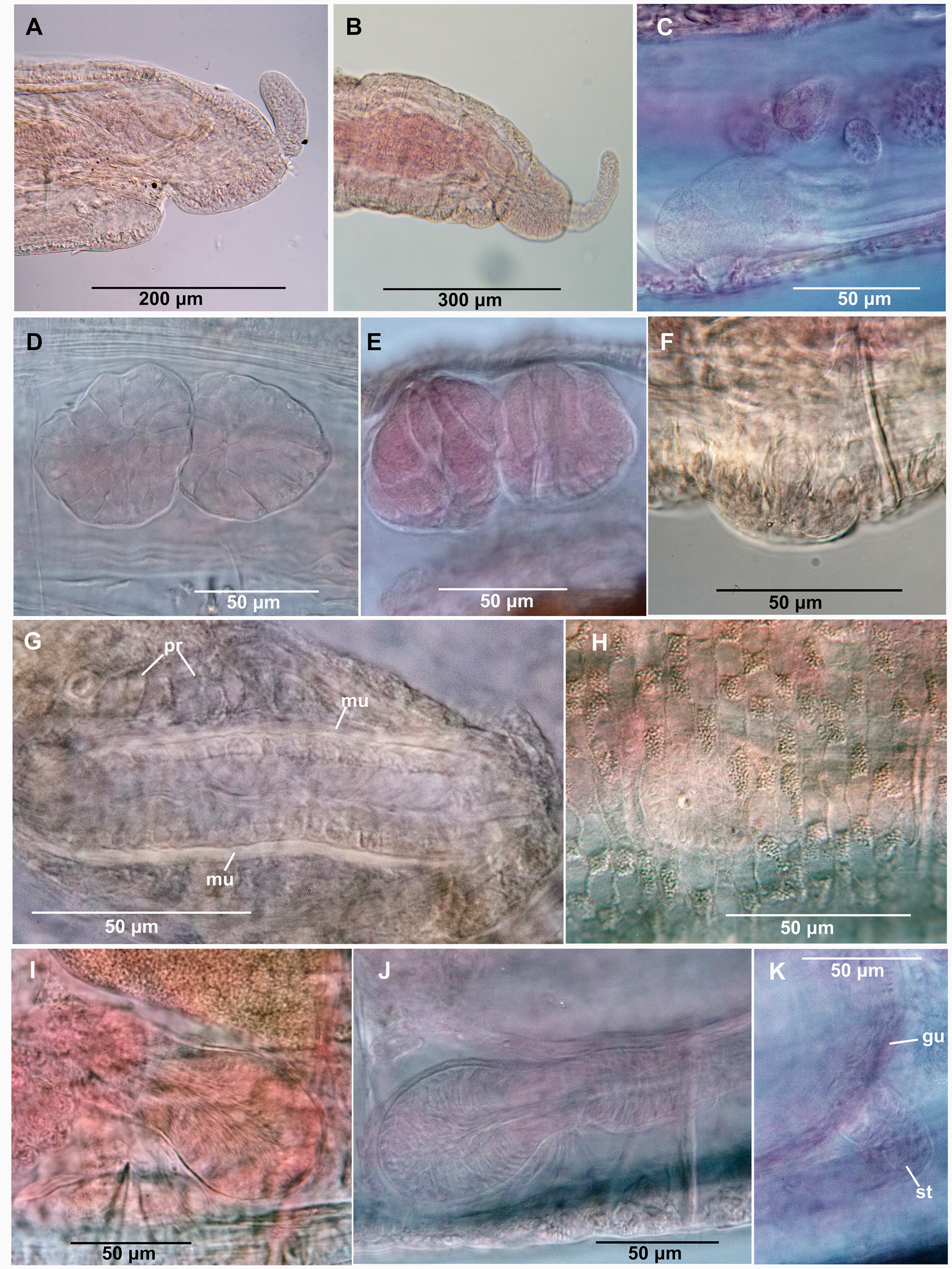

FIGURE 4. A, C, D, F–I. *Eremidrilus parvitheca* from Sessom Creek. **A.** Head with proboscis, lateral view. **C.** Nephridial vesicle, lateral view. **D.** Paired dorsal glands in tail segment, dorsal view. **F**. Male porophore, lateral view (Paratype). **G.** Portion of atrium, showing epithelium, muscle, and prostate layers (Paratype). **H.** Spermathecal pore and clitellum, ventrolateral view. **I.** Ectal part of spermatheca in XI, lateral view. **B, E, J, K.** *Eremidrilus parvitheca* variant, from Comal Springs. **B.** Head with proboscis, lateral view. **E.** Paired dorsal glands in tail segment, lateral view. **J.** Ectal part of spermatheca in XII, lateral view. **K.** Spermatheca joining gut in XII.

Clitellum from X–XIII, appearing granular, with strongly developed epidermal glands (Fig. 4H). Pharynx mostly in II–III, slightly thickened both dorsally and ventrally. Pharyngeal glands weakly developed in about V–VII. First nephridia paired in XIII, nephridial duct widens into a small, rounded vesicle just anterior to ventral chaetae (Fig. 4C). Brain appears deeply lobed.

In both mature and immature worms, all segments from about XXV with a prominent pair of mid-dorsal glands; glands are near mid-segment, and arranged longitudinally (Fig. 4D). Glands are multicellular, nearly spherical, diameter 30–60 µm.

Testes in IX and X; ovaries in XI; female funnels on 11/12. Sperm sacs extend posteriorly to as far as XII; egg sacs to XIII. Sperm funnels about 50–70 µm high, flat on septa 9/10 and 10/11 and directed anteriad. Vasa deferentia 10–12 µm wide, partially obscure in all available specimens. Posterior vas forms a distinct loop in XI; both vasa free for most of their length, joining atrium near apex (Fig. 3C, D). Atria elongate-tubular, about 260–500 µm long (Fig. 3C, D); both atria commonly extending posteriad through XI, or as far as XII; atrium length 0.9–1.6 times body diameter. Diameter in ampullar portion 32–37 µm, gradually narrowing to 10–16 µm near male pore. Atrial muscle layer 3–5 µm thick, uniform epithelium 5–10 µm thick (Fig. 4G). Prostate glands densely covering most of atrium, forming small, dense, petiolate clusters 15–30 µm high (Fig. 4G).

One pair of spermathecae in XI, usually near posterior margin of XI. Pores simple, inconspicuous (Fig. 4H). Spermathecae 170–210 µm long, without a distinct duct or ampulla; ectal part distinctly expanded (usually ovate, 40–65 µm wide), with thin muscle layer and columnar lining cells 7–10 µm thick (Figs. 3C–E; 4I). Ectal expansion tapers to 20–30 µm wide; ental 2/3 may be nearly cylindrical, or may be expanded again (giving a "dumbbell" shape to the spermatheca); ental end joins the gut anywhere between mid-segment and the posterior septum. Sperm heads associated with epithelium (Fig. 4I).

Remarks: This species is difficult to classify, and attribution to the genus *Eremidrilus* is provisional. Like most *Eremidrilus*, these are small worms with a proboscis; they have semiprosoporous atria, with male pores in X; the posterior vasa deferentia form a loop in XI, and spermathecae are paired in XI. Nephridia begin in segment XIII, and there are no lateral blood vessels in posterior segments.

Eremidrilus parvitheca differs from all other *Eremidrilus* species in that the spermathecae lack a distinct ampulla, and join the gut. Within the family Lumbriculidae, this character has only been described for most species of *Rhynchelmis* and for *Pararhynchelmis murdocki* Fend & Lenat, 2010. Although this unusual character would appear to be diagnostic at the genus level, it should be noted that within the subgenus *Rhynchelmis* (*Rhynchelmoides*), two species (*Rhynchelmis elrodi* Smith & Dickey, 1918 and *Rhynchelmis glandula* Altman, 1936) have spermathecae (without ampullae) joining the gut, and the remaining five species have normal spermathecae with large ampullae (Fend & Lenat 2010).

No other *Eremidrilus* has been described with dorsal glands in posterior segments, although similar glands were described for the European *Trichodrilus angelieri* Giani & Rodriguez, 1994. As in other Texas species, male porophores are weakly developed compared with most previously described *Eremidrilus* species (cf. Fig. 9 in Fend & Rodriguez 2003). The relatively short proboscis (cf. Figs. 4A, B vs. 2A) also seems atypical for the genus.

The atria are elongate-tubular, with vasa deferentia joining at the ental end—thus somewhat resembling male ducts of *E. allegheniensis* (Cook, 1975). *Eremidrilus allegheniensis* is a larger, southeastern species that differs in having 2 spermathecal segments, spermathecae with narrow ducts and sacciform ampullae, and prominent male porophores (Cook 1975, Fend & Rodriguez 2020).

3.1.3. *Eremidrilus parvitheca* variant

(Figs. 3F–I; 4B, E, J, K)

Material examined: Texas, Comal Co., New Braunfels, Comal Springs Run 3 at Landa Park. N 29.713506 , W 98.136988, 196 masl altitude, benthic sample, 11 Oct 2021, collected by S. Fend and R. Gibson, 2 whole mounts. Texas, Bell Co., Tahuaya Springs, N 31.009653, W 97.508281, 28 Aug 2018, collected by P. Diaz, 1 whole mount.

Length of 1 complete worm 6 mm; 56 segments. Body diameter in X 0.27–0.32 mm. Proboscis 155–193 µm long, about 35 µm diameter at middle (Figs. 3F, 4B). All chaetae simple-pointed, sigmoid; in anterior segments 72–94 µm long; nodulus 0.39 to 0.43 chaeta length from the tip (Fig. 3G). Male pores as in typical specimens (cf. Fig. 3F, H). One pair of simple spermathecal pores behind ventral chaetae in XII, on ventral chaetal line, just anterior to septum 12/13 (Fig. 3F, H).

Pharynx, pharyngeal glands and nephridia as described for typical specimens. All segments from about XXV with a prominent pair of mid-dorsal glands; glands are near mid-segment, and arranged longitudinally. Glands are multicellular, nearly spherical, diameter 30–65 µm (Fig. 4E).

Testes, ovaries, vasa deferentia, and male and female funnels as described for typical specimens (Fig. 3H). Atria elongate-tubular, about 290–580 µm long; both atria extending posteriad through XI (Fig. 3H). Diameter in ampullar portion about 40 µm, gradually narrowing to 16–17 µm near male pore. Atrial muscle layer well developed (about 5 µm thick). Prostate glands densely covering the ampulla, forming small, dense, petiolate clusters 20–30 µm high.

One pair of spermathecae near posterior margin of XII, total length 160–260 µm. Pores small and inconspicuous, not associated with ducts; ectal part of spermatheca distinctly expanded to about 60 µm, with thin muscle layer and columnar lining cells, tapering entally to 24–26 µm; ental portion slightly expanded (35 µm) before narrowing at junction with gut, either anterior or posterior to the chaetae (Figs. 3H, I; 4J, K).

Remarks: Only two specimens from Comal Spring and one from Tahuaya Springs were available, so differences from the *Eremidrilus parvitheca* type series at Sessom Creek need to be confirmed with more material. This morphotype is very similar to typical specimens, but the spermathecae are in XII, behind the female segment. No other *Eremidrilus* species has an intervening post-atrial segment without spermathecae. Nevertheless, although segmental position of reproductive organs is an important character for generic diagnoses in the Lumbriculidae, variation has been accepted within some genera, and even intraspecifically in some species of *Lumbriculus* Grube, 1844. Species with 1 vs. 2 spermathecal segment(s) appear within several genera, including *Eremidrilus*, *Trichodrilus* and *Rhynchelmis*; the entire sequence of reproductive segments may be shifted forward in *Styloscolex* Michaelsen, 1901, some species of *Stylodrilus* Claparède, 1862 (e.g., *Stylodrilus wahkeenensis* Rodriguez & Coates 1996), and *Trichodrilus* (*Trichodrilus strandi* Hrabě, 1936); spermathecae may even insert before or after the atrial segment in different species of *Styloscolex*.

3.2. *Trichodrilus* Claparède, 1862

Over 35 lumbriculid species have been assigned to the genus *Trichodrilus*, although most are known from the Palearctic Region, particularly Europe. North American records are limited to *Trichodrilus culveri* Cook, 1971 from groundwater in southeastern USA, an unconfirmed record of the European *Trichodrilus allobrogum* Claparède, 1862, from a well in Illinois, and an unidentified species from Washington, D.C. (Cook 1971). As in the Nearctic *Eremidrilus*, male pores are in segment X and spermathecae are in one or two post-atrial segments; atria are semiprosoporous, with two functional male ducts serving testes in IX and X. All species are differentiated from *Eremidrilus* by lacking a filiform proboscis, but other characters are highly variable within the genus (Rodriguez & Giani 1994).

3.2.1. *Trichodrilus comalensis* Fend n. sp.

(Figs. 5, 6)

Holotype: USNM 1683423. Whole worm, slide-mounted in Canada balsam.

Type Locality: Texas, Comal Co., New Braunfels, Comal Springs, Run 3, N 29.7143, W 98.1362, drift net, 12 Oct 2021, collected by R. Gibson.

Paratypes: USNM 1683424–1683425. The type locality, 29 Oct 2020, 16 Nov 2014, 1 whole mount on each date. CASIZ 236643–236644. 2 May 2019, 19 Jun 2020, 1 whole mount on each date. All drift net samples, collected by R. Gibson.

Other material: The type locality, 27 Oct 2017 and 12 May 2017, drift net, collected by R. Gibson. 1 whole mount on each date. Texas, Hays Co., Ezell's Cave, N 29.87382, W 97.95939, 29 Sep 2006, collected by J. Krejca. 1 whole mount.

Etymology: From the type locality.

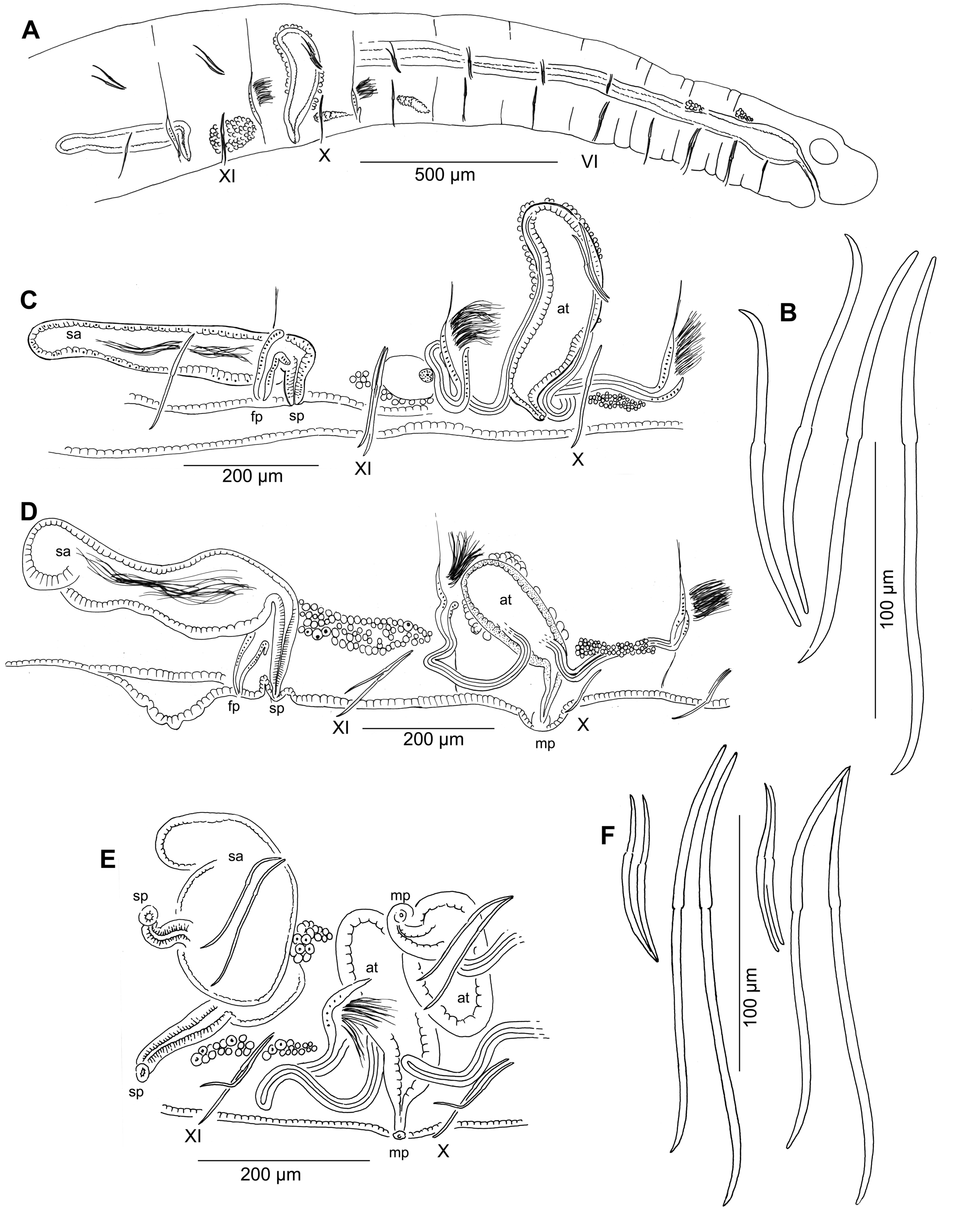

FIGURE 5. A–D. *Trichodrilus comalensis* n. sp. from the type locality, Comal Springs; drawings of whole mounted worms (A, B, C from Holotype, D from Paratype). **A.** Anterior segments I–XII. **B.** Dorsal (left) and ventral (right) chaeta pairs, showing proximal nodulus and elongated distal part in ventral chaetae. **C–D.** Reproductive organs in X–XI; C is a detail of worm shown in 2A. **E–F.** *Trichodrilus cf. comalensis* from Ezell's Cave. **E**. Ventrolateral view of reproductive segments X–XI. **F.** Dorsal (left) and ventral (right) chaetal pairs from two mid-body segments. All oriented to face right.

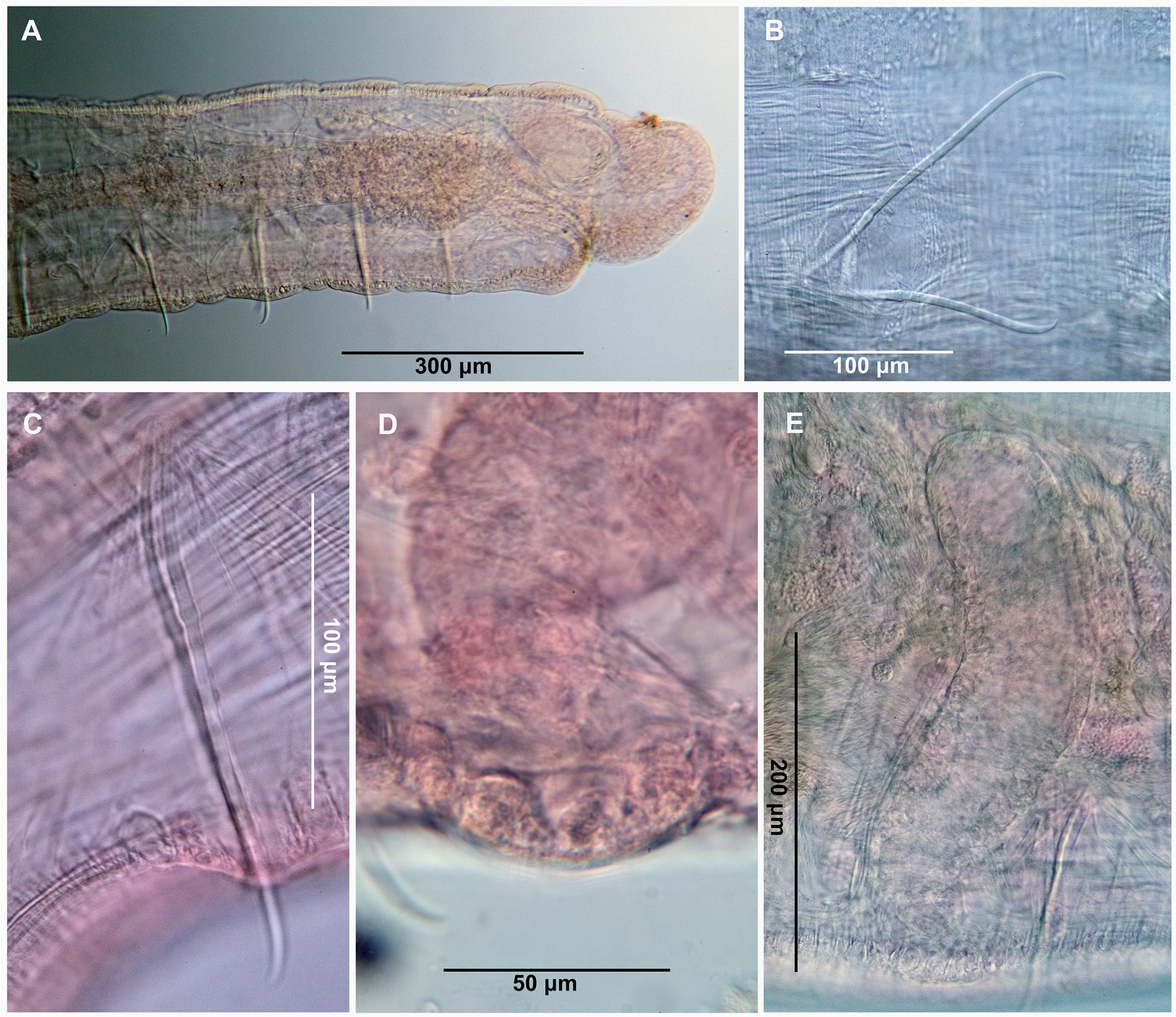

FIGURE 6. *Trichodrilus comalensis* from the type locality, Comal Springs. **A.** Anterior end, showing rounded prostomium; large ventral chaetae in II–VI, small dorsal chaeta in VI. **B, C.** Ventral chaeta pairs with elongate distal part, mid-body (B ventral and C lateral views). **D.** Male pore, lateral view. **E.** Atrium, lateral view.

Description: Segments (2 complete worms) 61–66, length of fixed worms 10–13 mm; body diameter in X (5 slide-mounted worms) 0.35–0.58, maximum diameter 0.40–0.62 mm. Proboscis absent (Figs. 5A, 6A), prostomium rounded or conical, slightly shorter than width at base. Secondary annulus in anterior 1/4−1/3 of preclitellar segments, from IV. All chaetae simple-pointed, sigmoid; dorsal chaetae begin in VI (Fig. 5A), ventral chaetae in all segments from II. Nodulus proximal in ventral chaetae (Figs. 5B; 6B, C); within each chaetal pair, the outer one is usually shorter (152–168 µm) with slightly proximal nodulus (0.50–0.56 x chaeta length from tip); nodulus distinctly proximal (0.60–0.68 x chaeta length from tip) in longer (172–203 µm) inner chaeta. Dorsal chaetae about 0.7–0.8 times as long as corresponding ventrals in anterior and middle segments, and with more distal nodulus; difference is less in posterior segments. Male pores on ventral chaetal line, about 1/3–1/2 the distance between chaetae and posterior septum (Fig. 5C, D); pores open on indistinct porophores, to 20 µm high, 50–75 µm wide, formed of thickened epidermis (Fig. 6D). One pair of simple spermathecal pores behind ventral chaetae in XI, on ventral chaetal line, at about 3/4 distance from chaetae bundle to posterior septum (Fig. 5C, D).

Epidermis thin, 5–6 µm in anterior segments, 8–10 µm in clitellum. Clitellum from X–XII, with weakly developed epidermal glands. Pharynx mostly in II–III, slightly thickened both dorsal and ventrally. Pharyngeal glands inconspicuous, may be weakly developed in III–IV (Fig. 5A). Nephridia difficult to see in available material.

Testes paired in IX and X, ovaries in XI; female funnels on 11/12, pores intersegmental on chaetal line. Sperm sacs extend anteriorly to VIII, posteriorly to XII; egg sacs to XIII or XIV. Anterior and posterior male funnels (on 9/10 and 10/11, respectively) functional; both flat on septum and directed anteriad; anterior funnel slightly smaller than posterior, usually with less sperm. Vasa deferentia 14–20 µm wide; posterior vas deferens penetrates septum 10/11, forming a variable posterior loop in XI (Fig. 5C, D); both vasa join muscle layer near ectal end of atrial ampulla, running under muscle layer and entering the lumen subapically. Atria club-shaped to somewhat petiolate with elongate ampulla, entirely in X (Figs. 5C, D; 6E), length 300–390 µm (0.6–1 times body diameter, about 5–6 times porophore diameter), diameter in ampullar portion 82–96 µm, narrowing to 20–30 µm near pore; atrial muscle layer thin (less than 2 µm) and inconspicuous; epithelium 10–20 µm. Prostate glands relatively sparse and small (to 20 µm), in indistinct bundles.

One pair of spermathecae in XI, distinctly petiolate with elongate, sac-like ampulla, length (300–400 µm) about 3 times width (95–130 µm), usually extending through XII (Fig. 5C, D); ampullar epithelium about 5–15 µm thick, not obviously glandular, or thickened entally. Sperm in the ampulla in loose, irregular bundles, not associated with epithelium. Spermathecal ducts 70–110 µm long; 30–35 µm wide entally, tapered (narrowest near the pore), with a thin muscle layer and columnar lining cells.

A single specimen from Ezell's Cave differs slightly from specimens from the type locality (Fig 5E–F) and may represent another species. Dorsal chaetae appear to begin in X; they are smaller than in typical specimens (58–76 µm long), and the nodulus is more basal (0.39–0.51 chaeta length from the tip). Ventral chaetae are similar in length to those in typical specimens (the inner chaeta 149–228 µm), and appear more highly modified: nodulus of inner chaeta 0.68–0.70 chaeta length from tip, nodulus of outer chaeta 0.62–0.64 (Fig. 5F).

Remarks: *Trichodrilus comalensis* n. sp. is provisionally assigned to the genus, as arrangement and morphology of reproductive structures could also associate it with *Eremidrilus* (all Nearctic), rather than the heterogeneous (and mostly Palearctic) *Trichodrilus*. However, the presence of a prostomial proboscis in *Eremidrilus* has been the primary character separating these two genera, along with a distinct male porophore in most *Eremidrilus* species. Nevertheless, it should be noted that within the typically proboscis-bearing lumbriculid genus *Rhynchelmis* Hoffmeister, 1843, some species living in ancient lakes appear to have lost the proboscis (Michaelsen 1901, Hrabě 1931, Fend & Carter 2014).

Position and morphology of reproductive organs resembles those of sympatric *Eremidrilus samacos* except that (1) the atrium is longer, (2) vasa deferentia join the atrium more basally, and (3) prostatic glands are smaller and less dense. Although both occur at Comal Springs, *T. comalensis* was only collected in low numbers from groundwater outflow ("spring drift" samples), whereas *E. samacos* was also collected in hyporheic samples.

Absence of dorsal chaetae in some anterior segments is unusual in the family (exceptions *Stylodrilus wahkeenensis* Rodriguez & Coates, 1996 and some *Lamprodrilus* Michaelsen, 1901); dorsal chaetae in remaining segments are distinctly smaller than corresponding ventrals. Morphology of ventral chaetae is also unusual for the family: the nodulus is distinctly proximal, as opposed to slightly distal (or sometimes median) in other species, and the distal portion of the inner chaeta in each pair appeared remarkably long and thin.

3.3 ***Pararhynchelmis*** **Fend & Lenat, 2010**

The single previously described species in this genus, *Pararhynchelmis murdocki* Fend & Lenat, 2010, is a small lumbriculid without a filiform proboscis. The first nephridia are in VII, and lateral blood vessels are present in middle and posterior segments. Testes are in IX and X, ovaries in XI. Male ducts are semiprosoporous, with the posterior vasa deferentia not penetrating the posterior septum; atria are elongate- petiolate; male pores have short, simple penes in segment X. Paired spermathecae in VIII and IX are simple and nearly tubular, without distinct ampullae, and join the gut in their originating segments. *Pararhynchelmis murdocki* is known from small springs and seeps in a small area in the Blue Ridge segment of the Appalachian Mountains, Virginia, USA (Fend & Lenat 2010).

3.3.1. *Pararhynchelmis texana* Fend n. sp.

(Figs. 7, 8)

Holotype: USNM 1683426. Whole worm, carmine stain, slide-mounted in Canada balsam.

Type Locality: Texas, Hays Co., San Marcos, Sessom Creek near the Freeman Aquatic Building, N 29.8900, W 97.9360, 175 masl altitude, Bou-Rouch and near-surface benthic samples, 11 Oct 2021, collected by B. Hutchins and S. Fend.

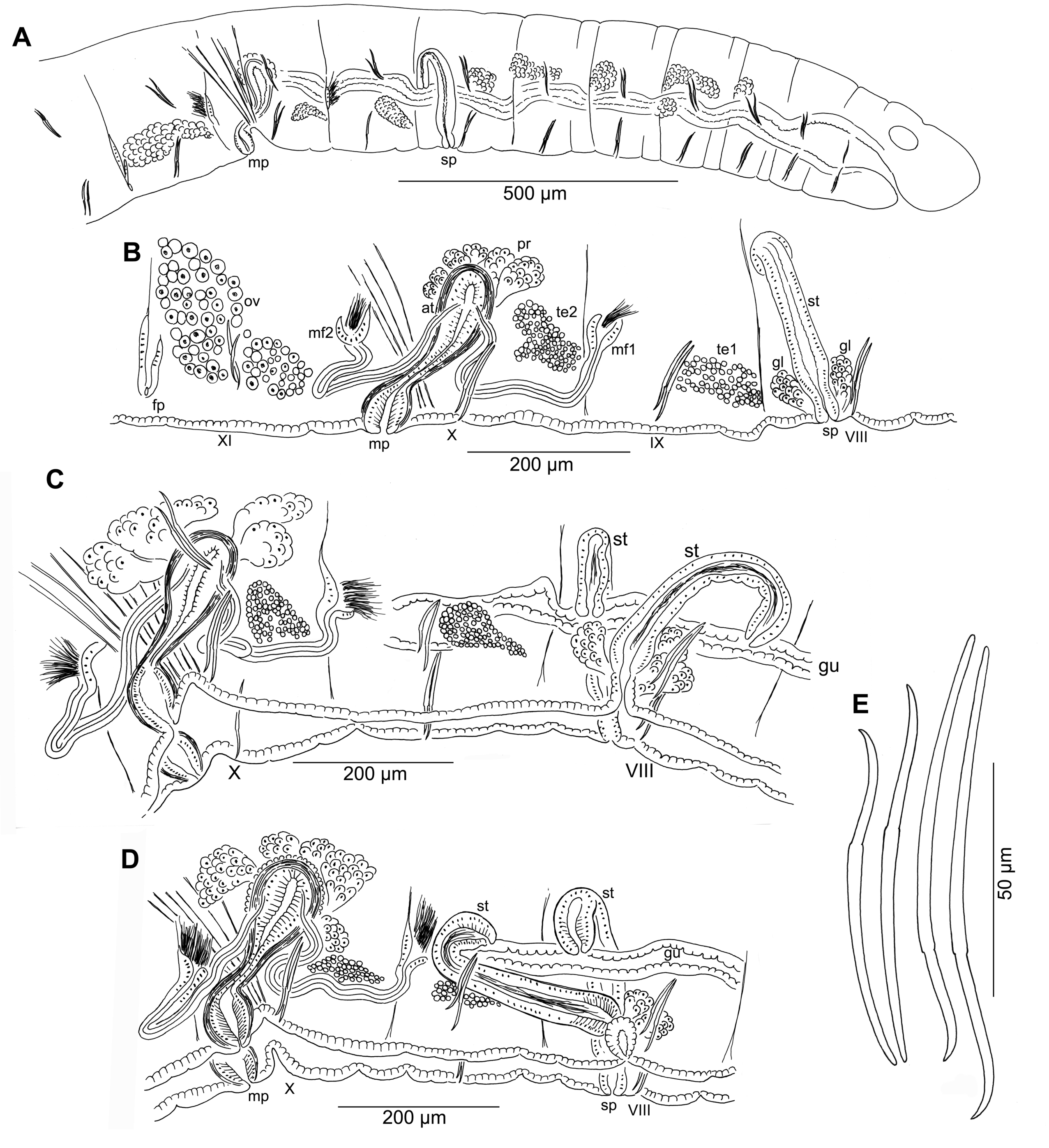

FIGURE 7. *Pararhynchelmis texana* n. sp. from the type locality, Sessom Creek. **A.** Anterior segments (Paratype). **B.** Reproductive system in segments VIII–XI, male pore without sulcus (Paratype). **C, D.** Reproductive system in VIII–X, showing variation in spermathecal orientation relative to gut; male pore at posterior side of ventral sulcus (C from Paratype, D from Holotype). **E.** Dorsal (left) and ventral (right) chaeta pairs, from segment XI (from a Paratype).

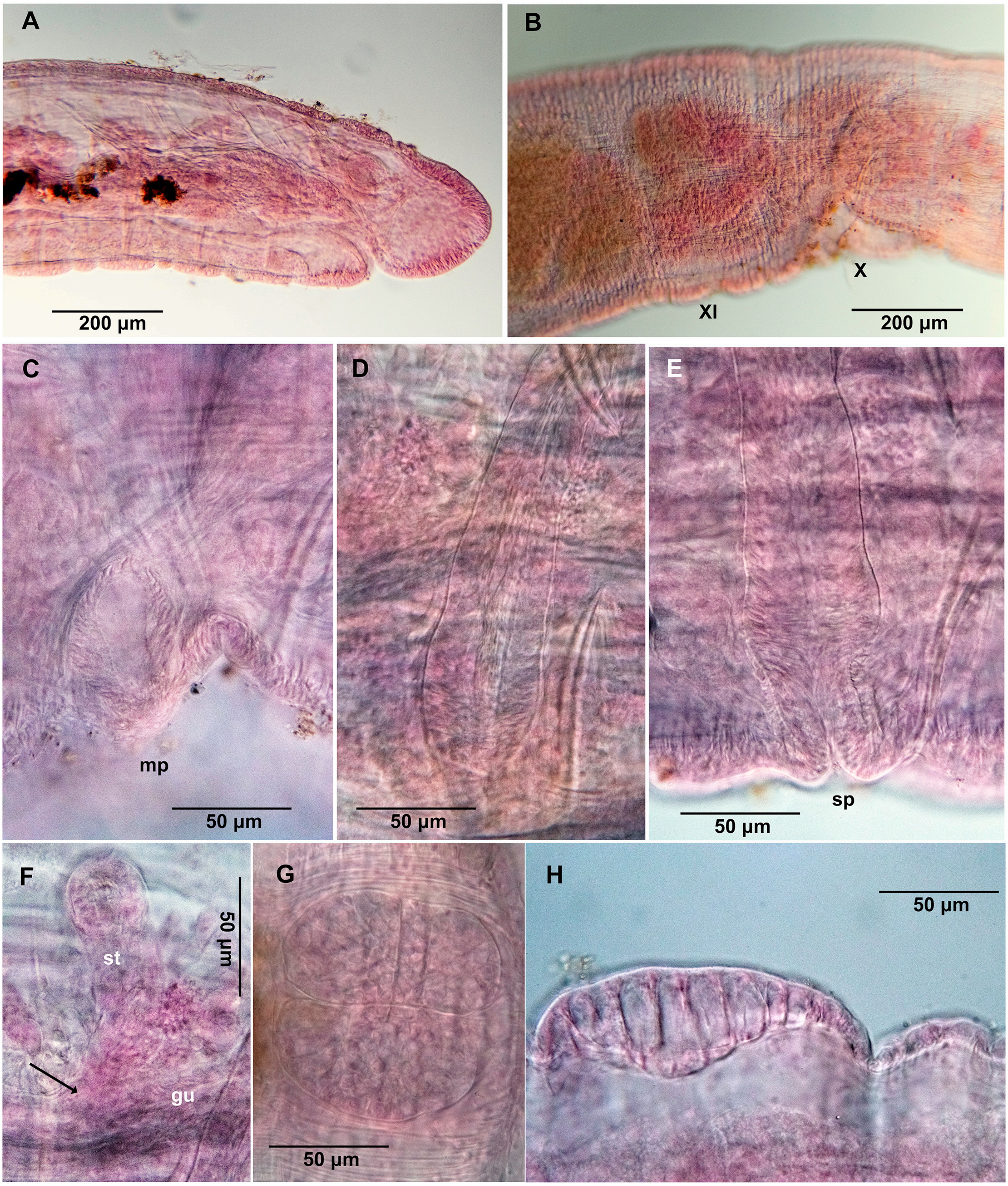

FIGURE 8. *Pararhynchelmis texana* n. sp. from the type locality, Sessom Creek (all from Paratypes). **A.** Anterior segments. **B.** Clitellum, with male pore in sulcus in segment X. **C.** Detail of male pore at posterior side of sulcus. **D, E.** Ectal parts of 2 spermathecae. **F.** Ental part of spermatheca, curving over gut and joining it (arrow indicates junction). **G, H.** Dorsal glands in posterior segments; (G) dorsal view, (H) lateral view.

Paratypes: From the type locality, same collection data, 11 Oct 2021. USNM 1683427–1683429, 3 whole mounts. CASIZ 236646–236648, 3 whole mounts.

Other Material: From the type locality, same collection data, 11 Oct 2021, 9 whole mounts.

Etymology: This species is known only from Texas.

Description: Segments 62–67. Length of fixed worms 8.5–15 mm; body diameter in X 0.27–0.43 mm. Prostomium rounded-conical, 145–200 µm long, 160–260 µm wide at base (Figs. 7A; 8A). Secondary annulus in anterior 1/4−1/3 of preclitellar segments, from IV, usually weak posteriorly. All chaetae simple-pointed, sigmoid; increasing in length from II to VI or VII (to 90–120 µm long, 3–3.5 µm diameter); similar in middle to posterior segments (80–115 µm); nodulus 0.35 to 0.41 x chaeta length from the tip; chaeta length in dorsal and ventral bundles similar or dorsals slightly shorter (Fig. 7E). Within each chaetal pair, the outer one usually shorter. Ventral face of segment X may form a deep, transverse sulcus (groove) which varies with contraction (Figs. 7A–D; 8B, C). Male pores on ventral chaetal line in X, about midway between chaetae and posterior septum, usually on posterior side of sulcus (Figs. 7C, D; 8C). One pair of simple spermathecal pores just behind ventral chaetae in VIII, on chaetal line (Figs. 7A–D; 8E).

Epidermis usually 10–15 µm thick in anterior segments, 15–25 µm in clitellum. Clitellum may be well developed, from X–XII, with prominent epidermal glands in transverse bands (Fig. 8B). Posterior segments (from about XLV–L) with mid-dorsal glands, consisting of a rounded cluster of elongate epidermal cells; gland mass 60–120 µm wide, 40–90 µm high (Fig. 8G, H).

Pharynx mostly in II–III, moderately thickened dorsally, but without a distinct dorsal pad. Pharyngeal glands well developed in V–VII (Fig. 7A). Chloragogen layer on gut weak from VIII, stronger in post-clitellar segments. First nephridia in VII, next in XIII; with small anteseptal funnel, followed by a thick, mostly postseptal granulated mass; short ectal duct widens into a terminal vesicle (50–60 µm long by about 30 µm diameter) at nephridiopore, just anterior to ventral chaetae; posteriorly, a duct follows the ventral blood vessel into posterior segments. No obvious lateral blood vessels in posterior segments.

Testes extend to mid-segment in IX and X; ovaries large (to near posterior septum) in XI (Fig. 7B). Sperm sacs rarely extend anteriorly to VIII, posteriorly to about XI or XII; egg sacs to XII or XIII (XIV). Female funnel simple, about 100 µm high, on septum 11/12; inconspicuous female pore intersegmental, on chaetal line.

Anterior and posterior sperm funnels about 60–80 µm high, flat on septa 9/10 and 10/11 respectively and directed anteriad. Vasa deferentia 66–22 µm wide, both about the same length, 250–350 µm long; posterior vas forms a short loop in XI; both vasa join the muscle layer near middle of atrial ampulla and enter lumen subapically (Fig. 7B–D). Atria petiolate, with elongate-ovate ampulla (110–170 µm long, 65–105 µm wide), gradually narrowing to form an ectal duct; duct abruptly terminates in an ectal "bulb" (Fig. 8C). Atria entirely in X, total length 190–330 µm (0.6–0.95 x body diameter). Atrial ampulla muscle layer well-developed (9–14 µm thick); epithelium thick (16–24 µm), appearing granular; lumen narrow in most specimens. Prostate glands large, petiolate clusters of cells, 60–120 µm high, sparsely covering the ampulla. Ectal bulb 62–84 µm long, 50–72 µm wide, muscle layer distinct (3–5 µm); epithelium with elongate cells. Male pores in most preserved specimens on posterior side of transverse, ventral sulcus formed by contraction of prominent muscle bands, arranged fanwise, extending to dorsolateral body wall in posterior X through anterior XI (Figs. 7B–D; 8C).

One pair of spermathecae in VIII, behind ventral chaetae; pores usually closer to chaetae than to posterior septum (Fig. 7B–D). Epidermis slightly thickened around pore; actual pore a prominent, transverse slit. Short spermathecal ducts (about 40–50 µm long, 25–40 µm wide), surrounded by multicellular glands (Figs. 7B–D; 8E). Main part of spermatheca slightly expanded near duct (50–70 µm wide, Fig. 8D), narrowing entally to form an elongate tube (30–40 µm wide) which extends dorsally, then bends ventrally to join the gut (Figs. 7C, D; 8F); total spermatheca 200–400 µm long. Location of junction with gut varies from mid-segment to near posterior septum, but may extend into an adjacent segment (Fig. 7C–D). Spermatheca with thin/inconspicuous muscle layer and columnar epithelium 12–22 µm thick in ectal expansion (Fig. 8D, E), cuboidal and 8–18 µm thick in remainder. Sperm in the spermathecae longitudinally arranged in a loose bundle, not oriented towards epithelium.

Remarks: Most lumbriculids with spermathecae in VIII, semiprosoporous male ducts, and atria in X have been assigned to *Rhynchelmis* Hoffmeister, 1843, in which most species also have spermathecae joining the gut. However, *Rhynchelmis* species are generally much larger, and usually have a distinct proboscis. Other proposed *Rhynchelmis* apomorphies not seen in either *Pararhynchelmis* species include branched lateral blood vessels, elongate-tubular atria and anterior (GI) testes and male ducts reduced or absent. Posterior vasa deferentia form a postseptal loop in *P. texana* (a character considered plesiomorphic by Hrabě [1984]), but not in *Rhynchelmis* or *P. murdocki* (Fend & Brinkhurst 2010).

A few other lumbriculid genera have atria in X and spermathecae in VII, and as in the new species, members of these genera are relatively small worms and lack a proboscis. However, all differ in having normal spermathecae with ampullae that do not connect to the gut. *Pseudorhynchelmis* Hrabě, 1982 is a Palearctic group of small lumbriculids, largely confined to Lake Baikal and the surrounding region. Apomorphies proposed by Martin & Kaygorodova (2008), which were largely intended to distinguish the genus from *Rhynchelmis*, include (1) lack of proboscis, (2) single pair of spermathecal pores in VIII, (3) no connection of spermathecal ampullae with gut, (4) two pairs of testes, in IX and X, (5) one pair of male pores in X, (6) absence of non-functional atria, (7) large fascicles of prostate glands. The monotypic *Secubelmis limpida* Fend & Gustafson, 2001, from western North America, appears to be parthenogenetic; it has non-functional testes in IX–X, and is semiprosoporous, but with non-functional male funnels on 9/10 and 10/11. Atria are petiolate, with a single large prostate gland, and the posterior vasa deferentia do not penetrate the posterior septum (10/11). Two Palearctic species of *Tatriella* Hrabě, 1936 have similar segmental arrangement of male ducts and spermathecae, except that both spermatheca and atrium are single and median.

At this point, the most appropriate genus seems to be the monotypic *Pararhynchelmis* Fend & Lenat, 2010. The single species, *Pararhynchelmis murdocki* Fend & Lenat, 2010, is a small worm, without a proboscis, known from a small area in North Carolina, USA. *Pararhynchelmis murdocki* also has petiolate atria in X, and the spermathecae lack an ampulla and join the gut. *Pararhynchelmis murdocki* differs from the new species in having a more elongate atrium without a distinct terminal "bulb", and the posterior vasa deferentia do not penetrate the posterior septum (10/11). Additionally, spermathecae are paired in both VIII and IX, have a simple tubular form, and always join the gut in the originating segment. *Pararhynchelmis murdocki* also has conspicuous lateral blood vessels in middle to posterior segments.

3.4. Other groundwater/hyporheic oligochaetes

3.4.1 Lumbriculidae

Limited numbers of poorly-preserved specimens, apparently representing additional undescribed lumbriculid species, were also collected at several sites during the initial sampling efforts. There appears to be at least one undescribed species that could be attributed to *Stylodrilus* Claparède, 1862, based on paired atria in X and spermathecae in IX. Several morphotypes (all with a proboscis, male pores in X, and spermathecae in XI or XI–XII) can be attributed to *Eremidrilus*, differing from the newly described species in atrial morphology, number of spermathecal segments or chaetal morphology. Developmental stage may account for some of this variability, but it is likely that some of these represent additional new species. Some other morphotypes resemble *P. texana* in general morphology of the atria, but differ in location (in VII, VI, or IV) or morphology of spermathecae (with or without gut connection). Formal description of these taxa awaits adequate material from future collections.

3.4.2 Other families

Based on morphology of chaetae, gizzard, pharyngeal musculature, and glands, there also appear to be multiple species attributable to the Haplotaxidae, a family usually associated with groundwater. These will be discussed in a future contribution. A single specimen of an unknown species of Phallodrilinae (Naididae) was collected at Tahuaya Springs, Bell Co., Texas, on 28 March 2018. Although diverse members of Phallodrilinae are a major component of European cave fauna (e.g., Artheau & Giani 2006), they are rarely reported in North America, most likely due to their small size, which requires specialized collecting methods. These small worms are associated with marine lineages (Giani *et al.* 2001).

3.5. Epigean oligochaetes

3.5.1. Lumbriculidae

Material examined: see Table 1

Eclipidrilus (*Premnodrilus*) *palustris* (Smith, 1900) was found at several sites, including springs and in both hyporheic and benthic stream samples (see also Worsham *et al.* 2016; as Lumbriculidae sp1). A short proboscis, petiolate spermathecae in IX, elongate atria with spiral muscles and long, thin, cuticular penes conform to Wassell's (1984) diagnosis of *Eclipidrilus* (*Premnodrilus*), and although distant from the type locality in central Florida, USA, the thick atrial musculature distinguishes them from the more widespread *Eclipidrilus daneus* Cook, 1966. This appears to be a primarily epigean species in southeastern North America, with records from lowland streams, springs and swamps in Florida, Alabama (Fend 2005), South Carolina (Wassell 1984), and North Carolina (D.R. Lenat & S. Fend, unpublished).

Lumbriculus cf. *variegatus* may be the most commonly encountered lumbriculid in North America. Only a few immature specimens were seen in this study, from Comal Springs. Mature specimens are needed for species identification, but as members of this eurytopic species complex typically reproduce asexually, the diagnostic reproductive structures are rarely seen.

3.5.2 Other epigean taxa

Varichaetadrilus angustipenis (Brinkhurst & Cook,1966) (Naididae, Tubificinae) was common at Comal Springs in 2021 samples, as well as in earlier samples collected by Mark J. Wetzel and David Bowles; it also occurred at Sessom Creek. This species is widespread in North America, although rarely common in habitats dominated by other tubificines. It appears to be common in cave and spring habitats (Wetzel & Taylor 2001), although it is occasionally abundant in different conditions (e.g., San Francisco Bay [Wayne Fields, pers. comm.] and Lake Michigan [Stimpson *et al.* 1975]).

Other species of Tubificinae were occasionally collected. A few mature *Potamothrix bavaricus* (Öschmann, 1913) were collected at Comal Springs. Other, less frequently encountered tubificine species included *Limnodrilus cf. hoffmeisteri* Claparède, 1862 and *Branchiura sowerbyi* Beddard, 1892. Several species of Naidinae (Naididae) were reported by Worsham *et al.* 2016, and some of these were also found in the present collections; the most common were *Nais pseudobtusa* Piguet, 1906, *Pristina aequiseta* Bourne, 1891, and *Pristina breviseta* Bourne, 1891.

Fragmented and mostly immature megadriles (Crassiclitellata) were common in initial sampling efforts. The most frequent appeared to be species of *Sparganophilus* Benham, 1892 (Sparganophilidae), based on absence of a gizzard or obvious gut diverticula; some of these may correspond to "Lumbriculidae sp2" in an earlier report (see Fig. 10 in Worsham *et al.* 2016). Specimens with gut diverticula are attributed to family Ocnerodrilidae, while a few perichaetine specimens are in the family Megascolecidae.

Low numbers of Enchytraeidae were collected in initial samples. Although these specimens were poorly preserved for observation of internal organs, chaetal morphology suggested several genera, including *Fridericia* Michaelsen, 1889; *Achaeta* Vejdovsky, 1878 and *Cernosvitoviella* Nielsen & Christensen, 1959.

4. DISCUSSION

All of the sites from which the new species were collected are groundwater-influenced, mostly associated with the Edwards Aquifer and Balcones Fault Zone. Three of these sites (Sessom Creek Springs, Comal Springs, and Ezell's Cave) have previously been identified as particularly species-rich with respect to stygobionts (Hutchins *et al.*, 2021), but it should be noted that large portions of the Edwards and Edwards-Trinity Aquifers, including biodiverse areas such as the San Antonio and Del Rio areas, remain largely unsampled for oligochaetes. The Edwards and Edwards-Trinity Aquifers are hydrologically and geologically complex, with deep and shallow sections, confined and unconfined components, and distinct food sources (e.g., Hutchins *et al.*, 2016). Additionally, the hyporheic zone of surface streams fed by Edwards springs may ecologically function as an extension of the aquifer habitat for stygobionts: cobble substrate is dominated by limestone and water chemistry is thermally stable and ionically similar to Edwards groundwater (dominated by Ca^{2+} and HCO_3^-). With the exception of Ezell's Cave, the new species were all collected from hyporheic habitats or sites that represent shallow portions of the Edwards Aquifer, where food webs are supported by photosynthetically derived organic matter from the surface. Whether oligochaetes preferentially occur in these shallower groundwater areas, or in deeper portions of the aquifer that have not been adequately sampled for groundwater-obligate oligochaetes, is unknown.

The list of stygophilic invertebrates from the Edwards Aquifer region is extensive (Bowles & Arsuffi 1993, Hutchins *et al.*, 2021), yet aside from a study of San Marcos River headsprings by Worsham *et al.* (2016), freshwater oligochaetes have rarely been mentioned. This is likely due to difficulties in adequate preservation of oligochaetes in groundwater and hyporheic invertebrate collections. In North America, collecting efforts in these habitats have focused on arthropods, which can be adequately preserved in alcohol. Although oligochaetes common in surface-water habitats can usually be identified using external or hard characters and readily available keys (e.g., Kathman & Brinkhurst 1999), taxa more common in hyporheic and groundwater habitats require detailed observation of fragile, soft, internal organs, requiring more careful preservation and curation.

Most other North American studies of hyporheic/groundwater annelids have been geographically limited, but have uncovered a few unusual lumbriculids (Cook 1971, 1975; Rodriguez 1996, Rodriguez & Coates 1996). Although some of these species are known only from their type localities, they may represent more widespread lineages. *Eremidrilus allegheniensis* (Cook, 1975), from a cave in West Virginia, was the first species described in the genus, and subsequent collections in surface, hyporheic and cave habitats have shown that the genus is widespread in North America (Fend & Rodriguez 2003, 2020; Rodriguez & Fend 2022). Lumbriculids resembling *Stylodrilus wahkeenensis* Rodriguez & Coates, 1996, which has chaetal morphology otherwise unknown in the family, appear to have a wide distribution. Although *S. wahkeenensis* was described from hyporheic habitat in Oregon, USA, similar worms have been collected in Tennessee and Alabama, USA (Strayer 2001). Other subterranean genera may be regionally endemic. For example, the monotypic *Spelaedrilus multiporus* Cook, 1975 has a uniquely modified male duct, and is known from a single locality: Smith Cave, Russell Co., Virginia, USA.

The study by Strayer (2001) is the most extensive regional survey of hyporheic annelids, encompassing much of Eastern North America; lumbriculids were not common in that sampling program, although several other unusual annelid taxa were found. Low numbers of Lumbriculidae in Illinois caves (Wetzel & Taylor 2001) and in New York hyporheic samples (Strayer & Bannon-O'Donnell 1988) may reflect prior glaciation. Extensive Pleistocene glaciation has limited the distributions of native terrestrial oligochaetes in northern North America (Gates 1967), although this was not identified as a major factor in hyporheic annelid distributions in the survey by Strayer (2001). Caves may actually provide refugia for some lumbriculid taxa in changing climates. Although the genus *Rhynchelmis* is common in unglaciated northern Alaska (Holmquist 1976), it appears mostly absent from regions with Pleistocene glacial history. Two isolated northern records for *Rhynchelmis* species are from caves: *Rhynchelmis saxosa* Fend & Brinkhurst, 2000 in coastal Alaska (Carlson 1997), and an undetermined *Rhynchelmis* species in Walkin Cave, Alberta (unpublished, see below). As many lumbriculids appear to be cool-stenotherms (Timm 1999), taxa experiencing a warming climate at southern latitudes (e.g., central Texas) may be restricted to groundwater-associated habitats.

The discovery of four new species may seem noteworthy, but these results represent only a fraction of the undescribed annelid diversity in Texas groundwater-associated habitats. Several additional, as-yet unidentified lumbriculid species, as well as undescribed species in other oligochaete families, were collected during earlier surveys, but there was insufficient material in good condition for clear morphological descriptions. Although the most common lumbriculids could be attributed to the widespread genus *Eremidrilus*, other undescribed lumbriculids are difficult to classify and may represent unknown groundwater-associated lineages.

It is difficult to identify morphological traits that can be considered stygomorphic for aquatic oligochaetes. Their elongated, flexible body form is a clear adaptation to interstitial life (regardless of depth) (Creuzé des Châtelliers *et al.*, 2009, Glasby *et al.*, 2021) and other common subterranean adaptations (absence of pigmentation and eyes) are the general rule in these animals. The unusually elongated ventral chaetae of *T. comalensis* (absent from shallow benthic samples in this study) have rarely been described in lumbriculid oligochaetes. Similar chaetae were described in a population attributed to *Rhynchelmis rostrata* (Eisen, 1888) from profundal Lake Tahoe (Fig. 53d in Fend & Brinkhurst 2000). The deep lake habitat has many similarities to subterranean aquatic habitats, and other stygobionts have been recorded from depth in Lake Tahoe (e.g., *Stygobromus tahoensis* Holsinger, 1974). Ventral chaetae with elongated distal ends also occur in an undescribed *Eremidrilus* species from a cave in Illinois (Steven Taylor, Illinois Natural History Survey, unpublished), and another cave-dwelling *Rhynchelmis* with similar chaetae (represented only by immature specimens) has been collected from Walkin Cave, Alberta (David Critchley, Northern Alberta Institute of Technology, unpublished). Dorsal chaetae have particularly elongated, hairlike distal ends in the hyporheic *Stylodrilus wahkeenensis* Rodriguez & Coates 1996. Elongation of sensory appendages has been associated with other invertebrates in subterranean habitats, including some annelids (Glasby *et al.* 2021). Rodriguez & Giani (1994) provide additional examples of unusual chaetal modifications in lumbriculids in interstitial/hyporheic habitats; this includes dorsal chaetae smaller than ventral ones in some *Trichodrilus* species.

The occurrence of two seemingly unrelated, hyporheic species (*E. parvitheca* and *P. texana*) with similar, but unusual dorsal glands and modified spermathecae seems noteworthy. Within the family, reduced spermathecae, which lack an ampulla and appear to join the gut, have been reported only in *Rhynchelmis* (several species), and in *Pararhynchelmis murdocki* Fend & Lenat 2010. Sambugar *et al.* (1999; 2008) suggested that elongation or asymmetrical placement of spermathecae in interstitial Phallodrilinae and in the lumbriculid *Trichodrilus cernosvitovi* Hrabě, 1937 may be related to a reduction in body diameter in the gonadal body region. The function of the mid-dorsal glands in the tail segments is unknown, although similar glands in the hyporheic *Trichodrilus angelieri* Giani & Rodriguez, 1994, as well as in the marine interstitial *Marionina glandulifera* (Jansson, 1960) were suggested to be adhesive glands (Coates 1983, Rodriguez & Giani 1994), although adhesive glands are more commonly associated with annelids in unstable habitats (Glasby *et al.* 2021).

Texas *Eremidrilus* morphotypes mostly have one spermathecal segment, but one undescribed form has two. Up until now, most described *Eremidrilus* species having one spermathecal segment (always in XI, the post-atrial segment) have been found in Pacific Coastal drainages (Fend & Rodriguez 2003, Rodriguez & Fend 2022); one Snake River species (*E. owyhee*) is from the intermountain region, but the other 6 species are from coastal ranges. All these species are from near-surface sediments, in streams with extensive alluvium, and thus may be primarily hyporheic. Described species having two spermathecal segments (XI–XII) are from more eastern localities, including the Rocky Mountains, intermountain areas, and *E. allegheniensis* from a single cave in the Appalachians (Cook 1971, Fend & Rodriguez 2020). The new Texas *Eremidrilus* species also differ from some West Coast species in the basic form of the spermathecae: duct short, and ampulla elongate-sacciform (vs. nearly spherical), with sperm unordered (vs. in a characteristic pattern) (see figs. 5, 10, 11 in Fend & Rodriguez 2003).

Acknowledgements

Collections of Texas material were provided by Amelia Hunter, Ashley Seagroves, and Alex Klingele, San Marcos Aquatic Resources Center, U.S. Fish and Wildlife Service. Voucher specimens from a San Marcos Springs study were provided by McLean Worsham, Texas State University, San Marcos. David Bowles (retired, National Park Service), Mark J. Wetzel (Illinois Natural History Survey), and Jean Krejka (Zara Environmental) provided additional specimens from prior surveys of Texas springs. David Critchley (Northern Alberta Institute of Technology) provided specimens of an undetermined *Rhynchelmis* species from Walkin Cave, Alberta, Kent Carlson (Karst Biosciences) provided specimens of *Rhynchelmis saxosa* from a cave in southeastern Alaska, and Steven J. Taylor (Illinois Natural History Survey) provided specimens of an undetermined *Eremidrilus* species from a cave in Illinois. Wayne Fields (Hydrozoology) provided information and examples of worms from San Francisco Bay. The final version of this paper greatly benefitted from constructive reviews and discussion with Pilar Rodriguez (University of the Basque Country, Spain), Mark Wetzel, and Patrick Martin (Royal Belgian Institute of Natural Sciences). The views expressed herein are those of the authors and do not necessarily represent those of the United States Fish and Wildlife Service.

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