Levipalatum texanum n. gen., n. sp. (Nematoda: Diplogastridae), an androdioecious species from the south-eastern USA

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Summary – A new species of diplogastrid nematode, *Levipalatum texanum* n. gen., n. sp., was isolated from scarab beetles (Coleoptera: Scarabaeidae) in Texas and baited from soil in Virginia, USA. *Levipalatum* n. gen. is circumscribed by stomatal and pharyngeal morphology, namely a long, hooked dorsal tooth connected to a 'palate' projecting anteriad and mediad, subventral telostegostomatal ridges of denticles, and the dorsal radius of the pharynx bulging anteriad. The males of the new species are distinguished from most other Diplogastridae by the frequent presence of ten pairs of genital papillae. Phylogenetic relationships inferred from 11 ribosomal protein-coding genes and a fragment of the small subunit rRNA gene strongly support *L. texanum* n. gen., n. sp. to be a sister group to *Rhabditolaimus*, which lacks all the stegostomatal and pharyngeal characters diagnosing the new genus. The new species expands comparative studies of the radiation of feeding morphology that are anchored on the model organism *Pristionchus pacificus*. The phylogenetic position of *L. texanum* n. gen., n. sp. indicates a new case of convergent evolution of hermaphroditism in Diplogastridae.

Keywords – *Cyclocephala*, description, hermaphrodite, molecular, morphology, morphometrics, new genus, new species, phylogeny, Scarabaeidae, stoma, taxonomy.

The nematode family Diplogastridae Micoletzky, 1922 consists of 33 valid genera, following the revision by Sudhaus & Fürst von Lieven (2003) and the inclusion of new or re-erected genera since then (Kanzaki *et al.*, 2009, 2012b, 2014; Fürst von Lieven *et al.*, 2011; Susoy & Herrmann, 2012; Herrmann *et al.*, 2013). Diplogastrid nematodes have a widespread association with insects, a feature which has allowed the discovery of diverse new taxa by sampling the potentially large number of possible hosts. Particularly striking among new diplogastrid species is the diversity of their stomatal morphology, including the presence of moveable teeth, an evolutionary novelty of the family. Consequently, stomatal morphology comprises the most important characters for circumscribing diplogastrid taxa (Sudhaus & Fürst von Lieven, 2003). The freedom afforded by complex mouthparts may facilitate the colonisation of new feeding niches, such as those encountered by associations with new hosts. The description of new forms in Diplogastridae thus highlights the family as an

exemplar for radiation of feeding morphology and function.

The potential of Diplogastridae for integrative studies of morphological evolution has been realised by the utility of *Pristionchus pacificus* Sommer, Carta, Kim & Sternberg, 1996 as a model system with advanced tools for genetic analysis (Sommer & McGaughran, 2013). For example, research anchored on *P. pacificus* has yielded mechanistic insight into the developmental regulation of morphological novelties, particularly by developmental plasticity (Ragsdale *et al.*, 2013a). In *P. pacificus* and several other diplogastrid taxa, moveable teeth are associated with dimorphism (Fürst von Lieven & Sudhaus, 2000), whereas other diplogastrids and outgroups are monomorphic with respect to their mouthparts, which are often, but not always, simpler. This partial correlation between teeth and dimorphism raises the questions of whether and how developmental plasticity and the addition of morphological complexity are related. Here, we expand the comparative framework for Diplogastridae, a model for

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these emergent questions, by describing a new species and genus of this family. The new genus is circumscribed by its monomorphic but complex stomatal morphology and is placed into an independent phylogenetic context inferred from 11 ribosomal protein-coding genes and a partial sequence of the small subunit (SSU) rRNA gene. In addition to providing a new reference point for comparative studies of feeding morphology, the androdioecious new species represents a distinct lineage of Diplogastridae in which hermaphroditic sex has evolved.

Materials and methods

NEMATODE ISOLATION AND CULTIVATION

The new species was isolated from an adult scarab beetle (Coleoptera: Scarabaeidae) as described for the type strain below. Host beetles were placed alive on to a 2.0% agar plate seeded with *Escherichia coli* OP50. After 2 h, a few nematode juveniles were visible on the plate, after which this plate was kept at room temperature for several weeks. Single individuals were thereafter transferred to nematode growth medium (NGM) agar plates seeded with *E. coli* OP50 and have since been kept in laboratory culture on this medium.

MORPHOLOGICAL OBSERVATION AND PREPARATION OF TYPE MATERIAL

One- to two-week-old cultures provided material for morphological observation. Observations by light microscopy (LM) and differential interference contrast (DIC) microscopy were conducted using live nematodes, which were hand-picked from culture plates. Because of the clarity and integrity of its morphology, live material was used for morphometrics. For line drawings, specimens were mounted into water or M9 buffer (3.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 0.5 g NaCl, 1.0 g NH_4Cl, brought to a volume of 1 l in water) on slides with silicon grease and then relaxed by applying gentle heat (Kanzaki, 2013). Stoma morphology was additionally analysed by pressing the head region to evert and separate stomatal structures (Kanzaki, 2013). For morphometrics, specimens were mounted on slides with pads of 5% noble agar and 0.15% sodium azide and were additionally relaxed by heat when necessary. To prepare type material, nematodes were isolated from type strain cultures, rinsed in water to remove bacteria, heat-killed at 65°C, fixed in 5% formalin, and processed through a glycerin and ethanol se-

ries using Seinhorst's method (see Hooper, 1986). Alternatively, some paratypes were fixed in 5% formalin after heat killing at 65°C. Nomarski micrographs were taken using a Zeiss Axio Imager Z.1 microscope and a Spot RT-SE camera supported by the program MetaMorph v.7.1.3 (Molecular Devices).

SCANNING ELECTRON MICROSCOPY

Nematodes were prepared for scanning electron microscopy (SEM) by fixation in 2.5% glutaraldehyde in PBS buffer and then post-fixed with 1% osmium tetroxide. After several rinses with water, samples were dehydrated through a graded ethanol series, followed by critical point drying using carbon dioxide. Specimens were mounted on polylysine-coated cover slips, sputter-coated with 20 nm gold/palladium, and then imaged with a Hitachi S-800 field emission scanning electron microscope operating at 20 kV.

MOLECULAR CHARACTERISATION AND PHYLOGENY

For species diagnosis and phylogenetic analysis, we amplified and sequenced a *ca* 500 bp fragment of the small subunit (SSU) rRNA gene of the new species using the primers SSU18A (5′-AAA GAT TAA GCC ATG CAT G-3′) and SSU9R (5′-AGC TGG AAT TAC CGC GGC TG-3′) (Floyd *et al.*, 2002). A partial SSU rRNA sequence of the new species was deposited in the GenBank database under accession number KJ395504. Additionally, we amplified and sequenced 11 ribosomal protein-coding genes for the two new species: *rpl-2*, *rpl-6*, *rpl-9*, *rpl-10*, *rpl-14*, *rpl-23*, *rpl-35*, *rps-7*, *rps-14*, *rps-27* and *rps-28*. All information regarding genes, primers and PCR conditions is given in Mayer *et al.* (2009). Except for *rps-28*, sequences of which were less than 200 bp, all ribosomal protein gene sequences for the new species have been deposited in GenBank under accession numbers KJ395553-KJ395562. Phylogenetic analysis included species from all diplogastrid genera for which homologous sequences have been published (Mayer *et al.*, 2009; Kanzaki *et al.*, 2012a; Herrmann *et al.*, 2013).

The concatenated dataset of ribosomal protein genes and the partial SSU rRNA gene was aligned using MUSCLE (Edgar, 2004), followed by manual alignment in MEGA5.10 (Tamura *et al.*, 2011), which included deletion of ambiguous alignment positions. The final alignment comprised 5777 sites, 5328 of which were in ribosomal protein genes. For analysis by maximum likelihood

(ML), the alignment was partitioned into four subsets – one for the partial SSU rRNA gene and three according to codon position for the concatenated set of ribosomal protein genes. The analysis was implemented in RAxML v.7.2.8 (Stamatakis, 2006). Fifty independent runs were performed for the ML analysis, in which trees were inferred under a general time reversible (GTR) model with a gamma-shaped distribution of rates across sites (Γ). Model parameters were unlinked across character partitions. Bootstrap support was calculated by 1000 pseudoreplicates on the most likely tree among all runs.

Bayesian analyses, as implemented in MrBayes 3.2 (Ronquist *et al.*, 2012), were also used to infer the phylogeny. In one analysis, the alignment partitioned four ways as described above for the ML analysis, and invoked a GTR + Γ model. This analysis was initiated with random starting trees and was run with four chains for 4×10^6 generations, sampling Markov chains at intervals of 100 generations, and was performed twice. After confirming convergence of runs and discarding the first 2×10^6 generations as burn-in, the remaining topologies were used to generate a 50% majority-rule consensus tree with clade credibility values given as posterior probabilities (PP). In a second analysis, the dataset was partitioned into a subset including the SSU rRNA fragment, which was analysed under a GTR + Γ model, and a subset including ribosomal protein genes and that invoked a codon model of substitution + Γ. This second analysis was performed four times, in each case for 10×10^6 generations. The first 4×10^6 generations were discarded as burn-in, after which trees were summarised as above. Model parameters were unlinked across character partitions in both Bayesian analyses.

Results

Levipalatum[*] n. gen.

Description

Diplogastridae. Body cylindrical, stout. Cuticle with fine annulation, fine striations observed by LM but not appearing on surface. Labial region with six lips and bearing six short, bristle-like, labial sensilla present in both male and morphological female (only hermaphrodites are known) and four bristle-like cephalic papillae present in male, as typical for diplogastrid nematodes. Stomatal opening surrounded by six rounded per- and interradial flaps, although flaps were not always confirmed by SEM (*e.g.*, Fig. 5B); apparently variable presence may be due to procedural artefact or true dimorphism, although there was no correlation with either other stomatal morphology or sex. Labial and cephalic papillae visible by LM. Lumina of cheilostom and gymnostom together forming a tube. Cheilostom a thick toroid, curving away from stomatal axis mid-way along its length and thickest at its mid-point or slightly posterior. Lumen of cheilostom as wide as long. Gymnostom barrel-shaped, slightly longer than wide, thicker at mid-point but at most half as thick as cheilostom, gymnostomatal lumen about as wide as cheilostomatal lumen. Gymnostom shorter dorsally than ventrally. Combined pro- and mesostegostom forming a sclerotised ring that is longest dorsally and shortest ventrally, dorsal part directed anteriad, 30° or less from stomatal wall, ventral part directed distad. Associated with ring is pronounced anisomorphy of anterior pharynx, dorsal radius of which bulging far anterior, often to level of cheilostom. Dorsally, stegostom (presumptive pro- and mesostegostom) has anterior projection (= 'palate') extending to level of cheilostom, wide at its posterior base and pointed anteriorly such that it is triangular in ventral view, and is close to gymnostomatal wall (it is also possible that this structure is gymnostomatal). Posterior to palate is a long, hooked, moveable metastegostomatal tooth extending to more than two-thirds of stoma diam. Dorsal pharyngeal gland orifice penetrating tooth subapically. Dorsal tooth musculature inserted in part on pro- and mesostegostomatal ring. On both subventral sectors are symmetrical ridges of denticles, hypothesised to be metastegostom. These ridges are surrounded distally by a ring, hypothesised to be telostegostom that is grooved circumferentially. Posterior to telostegostomatal ring, where stoma makes a transition to lumen of rest of pharynx, are two subventral thickenings of telostegostom. Articulated apodemes absent. Procorpus and metacorpus muscular. Metacorpus wider than procorpus in diam. Isthmus narrow, not muscular. Basal bulb glandular. Pharyngo-intestinal junction clearly observed, well developed. Morphological females amphidelphic, ovotestes reflexed. Male testis reflexed. Bursa or bursal flap absent.

Type and only species

Levipalatum texanum n. gen., n. sp.

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[*] Derived from the Latin words levo (= lift) and palatum (= roof of the mouth), referring to the projecting dorsal wall of the stoma.

RELATIONSHIPS

Levipalatum n. gen. is distinguished from all other Diplogastridae by a stoma with an anteriorly pointed 'palate' (presumptive stegostom) projecting or 'raised' into the stomatal lumen, a long, hooked dorsal tooth connected to the projecting palate, and the dorsal radius of the pharynx bulging anteriad such that the dorsal part of a pro- and mesostegostomatal ring is bent anteriad. The new genus is further diagnosed by a combination of a tube-shaped stoma with complex stegostomatal morphology that includes ridges of subventral denticles and a grooved stegostomatal ring.

Morphologically close to *Levipalatum* n. gen. is *Diplogastrellus* Paramonov, 1952, which also has a bipartite, tube-shaped stoma with a barrel-shaped cheilostom, *i.e.*, which curves distad mid-way along its length and which has a pronounced anisomorphy in the anterior pharynx. Distinguishing *Levipalatum* n. gen. from *Diplogastrellus* are: a strongly thickened *vs* thin cheilostom; dorsal gymnostom of about same length as ventral *vs* dorsal gymnostom much shorter than ventral; a raised stomatal palate *vs* no such dorsal projection; dorsal radius of pharynx bulging anteriad from the lumen and with a conspicuous, sclerotised pro- and mesostegostomatal ring *vs* anterior margin of pharynx perpendicular to lumen and lacking a pro- and mesostegostomatal ring; a long, hooked *vs* short, triangular dorsal tooth; subventral sectors each with a ridge of denticles *vs* no ridges and one or no small denticle; didelphy *vs* monodelphy. Also close to *Levipalatum* n. gen. is *Demaniella* Steiner, 1914 (Fürst von Lieven, 2000) and *Pseudodiplogasteroides* Körner, 1954 in that the stoma of the latter two genera has a dorsal longitudinal ridge. Distinguishing the new genus from *Demaniella* are: cheilostomatal processes and pouches absent *vs* present; subventral stegostomatal flap-like process absent *vs* present; a long, hooked *vs* short, blunt dorsal tooth; dorsal radius of pharynx bulging anteriad from lumen *vs* perpendicular to lumen; subventral ridges of denticles present *vs* absent. *Levipalatum* n. gen. is diagnosed from *Pseudodiplogasteroides* by: a cheilostom and gymnostom that are thick and curved *vs* a stoma with thin, straight walls; a cheilostom that is almost as long as *vs* much shorter than gymnostom; the dorsal radius of pharynx bulging anteriad from lumen *vs* perpendicular to lumen; a long, hooked *vs* short, blunt dorsal tooth; the basal bulb with a haustrulum and associated musculature absent *vs* present.

Although both *Levipalatum* n. gen. and *Acrostichus* Rahm, 1928 have a bipartite but relatively narrow stoma,

a prominent dorsal tooth and symmetry of the subventral sectors, *Levipalatum* n. gen. is distinguished from *Acrostichus* by: the cheilostom being undivided and forming a thick toroid *vs* divided into adradial plates; duplicature of gymnostom absent *vs* present; the dorsal radius of pharynx bulging anteriad from lumen *vs* perpendicular to lumen; a long dorsal tooth that is hooked at its apex *vs* a dagger-like dorsal tooth that is strongly sclerotised at its base; a dorsal *receptaculum seminis* absent *vs* present. *Levipalatum* n. gen. is diagnosed from the phylogenetically close genus *Rhabditolaimus* Fuchs, 1914 by a suite of stomatal and pharyngeal characters including: the labial region divided into six *vs* four lips; cheilostomatal toroid transversely curved *vs* not curved; gymnostom < 1.5 *vs* >5 times as long as wide; gymnostom thickened and curved *vs* thin and straight; conspicuous palate present *vs* absent; anterior pharynx strongly *vs* not anisomorphic; dorsal tooth present *vs* absent; subventral stomatal armature present *vs* absent; a two-part pharyngeal corpus (*i.e.*, procorpus narrower than metacorpus) *vs* cylindriform corpus.

NOMENCLATURAL REGISTRATION

The new generic name has been registered in ZooBank (zoobank.org) under the identifier 4C04012E-D9FB-451F-81DF-FF1A77BDE9C0.

MOLECULAR CHARACTERISATION AND PHYLOGENY

The concatenated alignment consisted of 2992 variable and 2307 parsimony-informative sites. Tree topologies inferred by ML and Bayesian analyses under the GTR + Γ model of molecular evolution were largely congruent, differing in the presence of a single node, and so only the Bayesian tree is shown (Fig. 1A). In both trees, *Levipalatum* n. gen., n. sp. was highly supported (100% BS, PP) as a sister group to *Rhabditolaimus* sp. RS5442. The tree inferred in the alternative Bayesian analysis, namely under a codon model of evolution, differed from the former two analyses and is presented separately (Fig. 1B).

In all analyses, *Levipalatum* n. gen. and *Rhabditolaimus* sp. were fully supported (100% BS, PP) as sister taxa. The relationships of this clade to other taxa in Diplogastridae was incongruent among analyses. In the ML and Bayesian analyses under a GTR + Γ model, this clade formed a poorly to moderately supported (53% BS, 99% PP) sister group of a moderately to well supported (83% BS, 100% PP) clade including *Diplogas-*
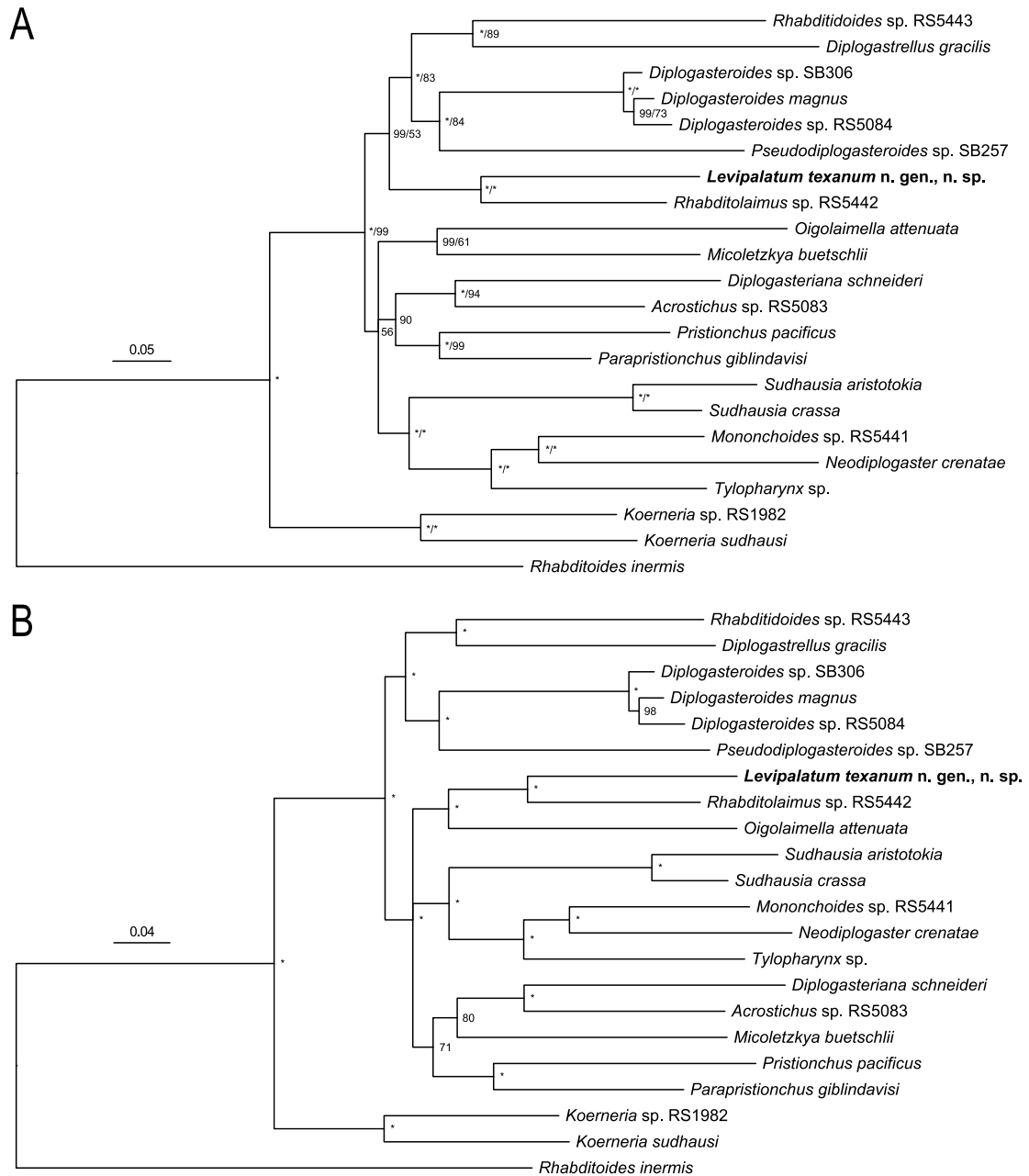
Fig. 1. Phylogenetic relationships of Diplogastridae, including *Levipalatum texanum* n. gen., n. sp. Relationships were inferred from a *ca* 450 bp fragment of the small subunit (SSU) rRNA gene and 11 ribosomal protein-coding genes. Trees are drawn to scale, with branch lengths measured in the number of substitutions per site. Support values above 50% are shown next to nodes. Asterisks indicate 100% support. A: Bayesian inference from SSU rRNA and partitions of protein-coding genes by codon position and invoking a GTR + Γ model. The topology is nearly identical with the most likely tree inferred by maximum likelihood (ML) from the same partitioned dataset, with the exception of one separate, poorly resolved node indicating the position of *Micoletzkya buetschlii* + *Oigolaimella attenuata* in the ML tree. Left support values are the posterior probability (PP) of nodes in Bayesian analyses; right values, the proportion of trees in which the associated taxa clustered together in 1000 bootstrap pseudoreplicates in the ML analysis. Dashes indicate <50% PP (left value) or the absence of the node in the ML tree (right value); B: Bayesian inference from SSU rRNA under a GTR + Γ model and protein-coding genes under a codon model of evolution. Posteror probabilities (PP) are shown next to nodes.

trellus gracilis Bütschli, 1876 + *Rhabditidoides* sp. and *Pseudodiplogasteroides* sp. + *Diplogasteroides* spp. Exclusive of this clade was a negligibly supported clade (<50% BS, 56% PP) that included all other sampled diplogastrids except for *Koerneria* spp. In this clade, *Micoletzkya buetschlii* (Fuchs, 1915) Baker, 1962 + *Oigolaimella attenuata* Fürst von Lieven & Sudhaus, 2008 were poorly to moderately supported (61% BS, 99% PP) as sister taxa; the relationship of these two taxa to others in that clade was the only incongruence between the ML and Bayesian analyses under the same model, and in the ML tree those species were sister to a group including nine other species, although the node indicating that relationship was only negligibly supported (<50% BS). In contrast to these analyses, the Bayesian analyses under the codon model supported *O. attenuata* as a fully supported (100% PP) sister group to *Levipalatum* n. gen. + *Rhabditolaimus* sp., and all three of these species were part of a fully supported (100% PP) clade that included *M. buetschlii* and the other nine species mentioned above. Therefore, the genus phylogenetically closest to *Levipalatum* n. gen. was in all analyses highly supported as *Rhabditolaimus*, although the relationship of these two taxa to other genera in Diplogastridae, especially *Oigolaimella*, remains unresolved.

Levipalatum texanum[*] n. gen., n. sp.
(Figs 2-5)

MEASUREMENTS

See Table 1.

DESCRIPTION

Adult

Androdioecious, *i.e.*, with male and self-fertile hermaphrodite. No lateral lines observed. Amphidial apertures elliptical, located subdorsally at level of posterior cheilostom. Dorsal pharyngeal gland clearly observed, penetrating dorsal tooth to gland opening. Procorpus occupying half to two-thirds of corresponding body diam. Corpus as long, or up to 1.3 times as long, as postcorpus. Nerve ring surrounding anterior part to middle of isthmus. Excretory pore at level of middle of isthmus to anterior part of basal bulb. Hemizonid not observed. Deirid at level of anterior or middle part of basal bulb.

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[*] The species epithet refers to Texas, the state in the USA where the type locality is located.

Male

Spontaneous (*i.e.*, fatherless) males relatively rare in culture (<5%). Testis single, located along ventral side. Spermatogonia arranged in *ca* three rows in reflexed part, in two to three rows in anterior half of main branch, then mature amoeboid spermatids filling most of remaining, proximal part of gonad. *Vas deferens* not clearly separated from other parts of gonad. Spicules paired, separate, adjacent to each other for distal fifth of their length. Spicule in lateral view ventrally arcuate, giving spicule total curvature of *ca* 135°; this curvature is slightly warped, such that its radius is shorter in anterior half of spicule. Manubrium ovoid, short, 1.0-1.5 times as wide as long, wider than spicule shaft and blade (calomus and lamina) in lateral view. Spicule shaft and blade smoothly curved along ventral surface, such that there is no expansion (*i.e.*, at anterior margin of lamina). Gubernaculum two fifths of spicule length, shaped as a stout hook in lateral view, hook anterior, pointed ventrally, *ca* 25% or less of spicule length, ventral wall of spicule straight and spicule thickness constant from anterior hook to posterior groove, posterior groove not offset from rest of spicule in lateral view but medially flattened laterad to enclose spicules. Cloacal opening U-shaped in ventral view. Three cloacal glands, two subventral and one dorsal, present but not obvious. Ten, sometimes nine, pairs of genital papillae and one small, unpaired, ventral genital papilla on anterior cloacal lip. The most anterior two pairs (v1a and v1p), when both are present, are of uncertain homology as they include a pair additional to typical nine of most diplogastrids, and are named herein as presumptive duplicates of v1 (following nomenclature of Sudhaus & Fürst von Lieven, 2003). However, because ten pairs were not found in all specimens examined, the duplication is tentatively considered diagnostic only of the new species. Papillae and phasmid arranged as ⟨v1a, v1p, (v2d, v3), v4, ad, (Ph, v5, v6, v7, pd)⟩ (= ⟨P1a, P1p, (P2d, P3), P4, P5d, (Ph, P6, P7, P8, P9d)⟩ in homology-agnostic nomenclature of Kanzaki *et al.*, 2012a), whereby v1a and v1p are within one-third cloacal body diam. of each other, v1p is *ca* one cloacal body diam. from v2d, v4 is closer to ad than to v3, Ph is immediately anterior to v5, and pd overlaps v5-v7, often as anterior as level of v6. Tail conical, tail tip thinly conical, *ca* two-thirds of tail length. Bursa or bursal flap absent.

Hermaphrodite

Each genital system arranged form vulva and vagina as uterus, oviduct, and ovotestis. Uterus and oviduct extend-
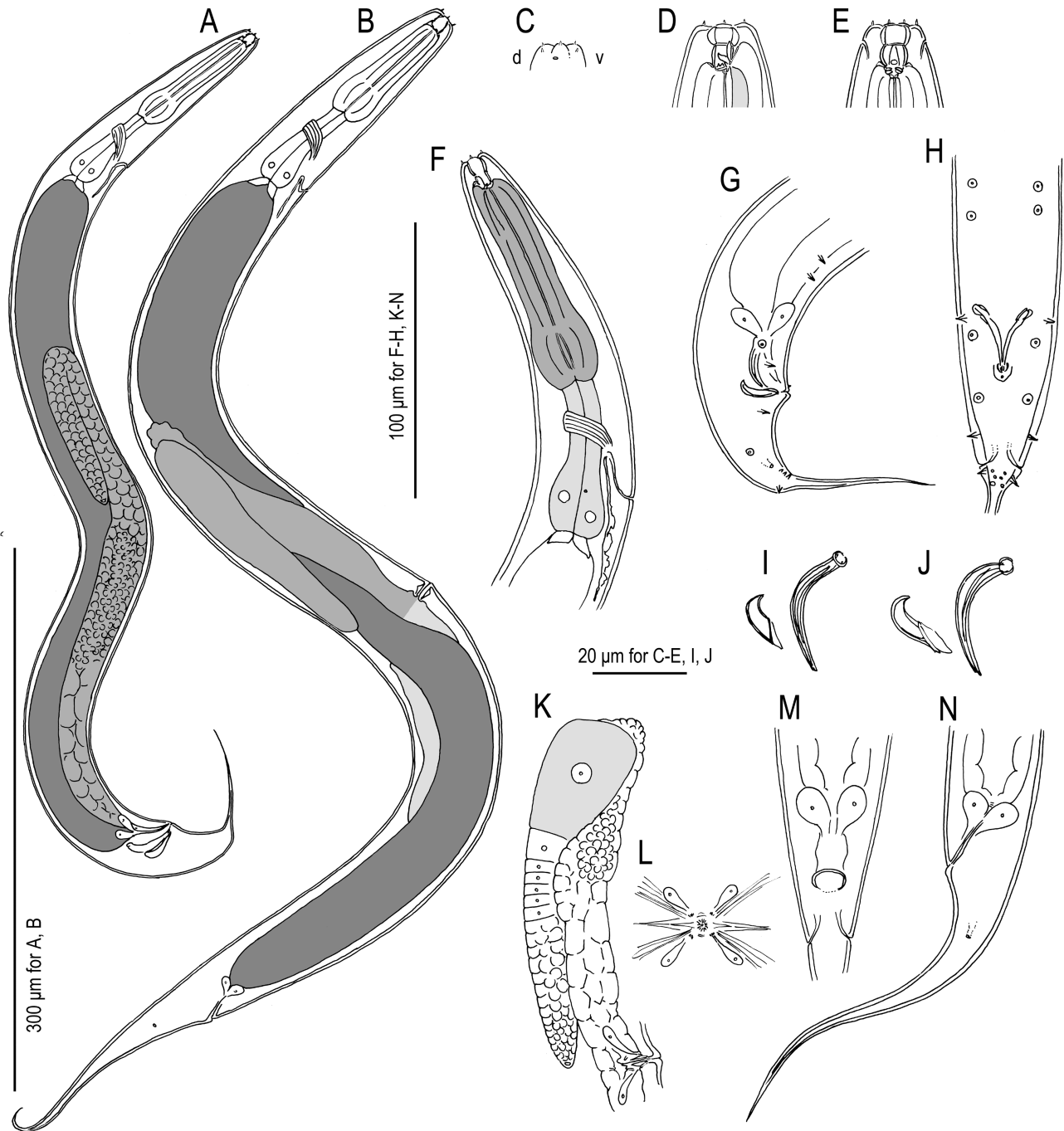
Fig. 2. *Levipalatum texanum* n. gen., n. sp. A: Entire male, right lateral view; B: Entire hermaphrodite, right lateral view; C: Lip region of male, right lateral view; D: Stomatal region of hermaphrodite, left lateral view; E: Stomatal region of hermaphrodite, ventral view; F: Neck region of hermaphrodite, right lateral view; G: Male tail, right lateral view; H: Male tail, ventral view; I: Gubernaculum and spicule, right lateral view; J: Variation of gubernaculum and spicule, right lateral view; K: Anterior gonad branch of female, right lateral view; L: vulva, ventral view; M: Anus, ventral view; N: Female tail, left lateral view.
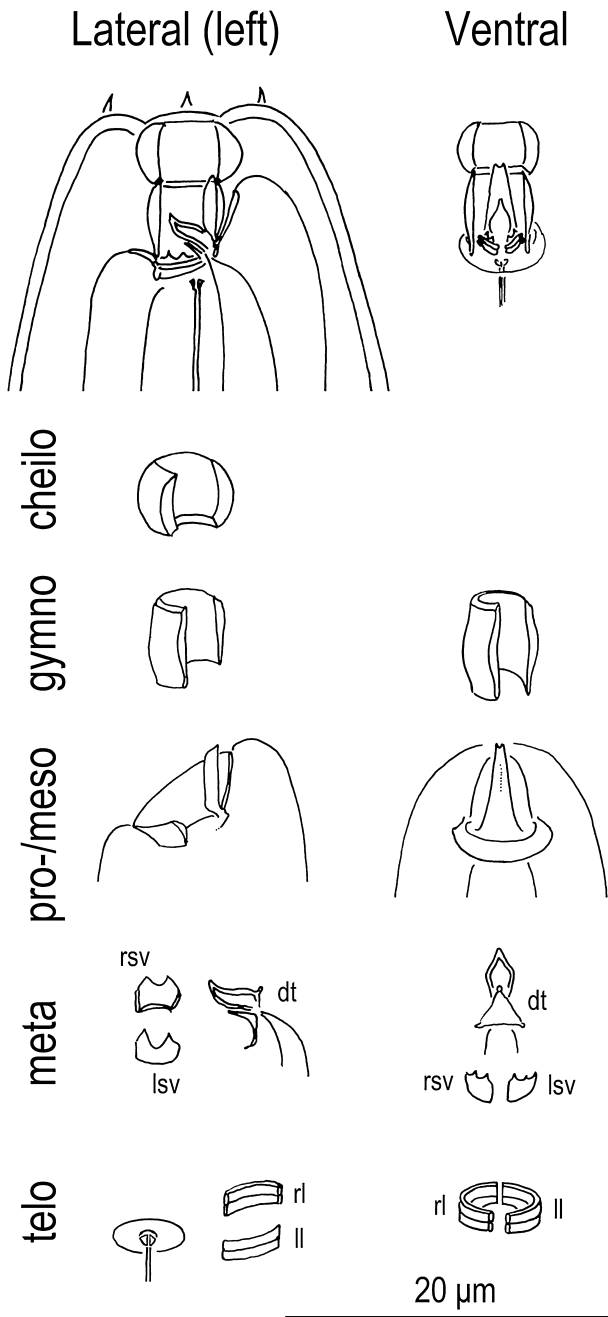
Lateral (left) Ventral

Fig. 3. Schematic representation of the stoma of *Levipalatum* n. gen., n. sp. with aspect of morphology given above each column. Metastegostom (meta) and telostegostom (telo) are separated into: dorsal tooth (dt); left and right subventral denticles (lsv and rsv, respectively); left and right lateral rings (ll and rl, respectively); and posterior cuticularised swellings. (Abbreviations: cheilo = cheilostom; gymno = gymnostom; pro-/meso = pro- and mesostegostom.)

ing ventrally and anteriorly on right of intestine, as typical for diplogastrids. Gonad totally reflexed, extending dorsally, with flexure occuring at boundary of ovotestis and oviduct. Dorsal flexure of gonad variable, shorter to longer than proximal part of gonadal branch. In distal half of ovotestis, oocytes arranged in multiple (five or more) rows, in proximal half, ooocytes in young adults arranged in a single row and culminating in one large oocyte. At distal end of main gonad branch, oviduct serving as spermatheca and filled with sperm. Proximal to spermatheca-like region, oviduct of young hermaphrodites composed of flattened, rectangular cells. Proximal, remaining part of oviduct (= uterus) also composed of flattened cells. Vaginal glands present but obscure, observed in ventral and lateral view. Vagina perpendicular to body surface. Vulva slightly protuberant in lateral view, pore-like in ventral view. Rectum *ca* one anal body diam. long, intestinal-rectal junction surrounded by well developed sphincter muscle. Three rectal glands, two subventral and one dorsal, present. Anus in form of crescent-shaped slit, posterior anal lip slightly protuberant. Phasmid conspicuous, located an anal body diam. posterior to anus. Tail conical, *ca* 4-5 anal body diam. in length, with conical terminus.

TYPE HOST (CARRIER) AND LOCALITY

The type strain was established from juvenile nematodes isolated from the body of an adult of a species of *Cyclocephala* Dejean collected by Andre Pires-da Silva on May 5, 2006 from a compost pile close to the University of Texas at Arlington, on Summit Avenue, Arlington, TX, USA. Two of four beetles examined were positive for the nematode.

OTHER HABITAT AND LOCALITY

In addition to the type carrier and locality, *L. texanum* n. gen., n. sp. was collected by P. Cole Eskridge and Theresa Grana on May 29, 2011 along the shore of the Rappahannock River close to Fair Hill Drive (Virginia Secondary 639), Fredericksburg, VA, USA. The nematode was trapped with an apple set as bait on sandy soil. After letting pieces of apple sit on the soil for 1 h, they were transferred to 10 cm NGM plates seeded with OP50, 1 day after which individuals of *L. texanum* n. gen., n. sp. emerged onto plates. No potential insect hosts or carriers were observed.
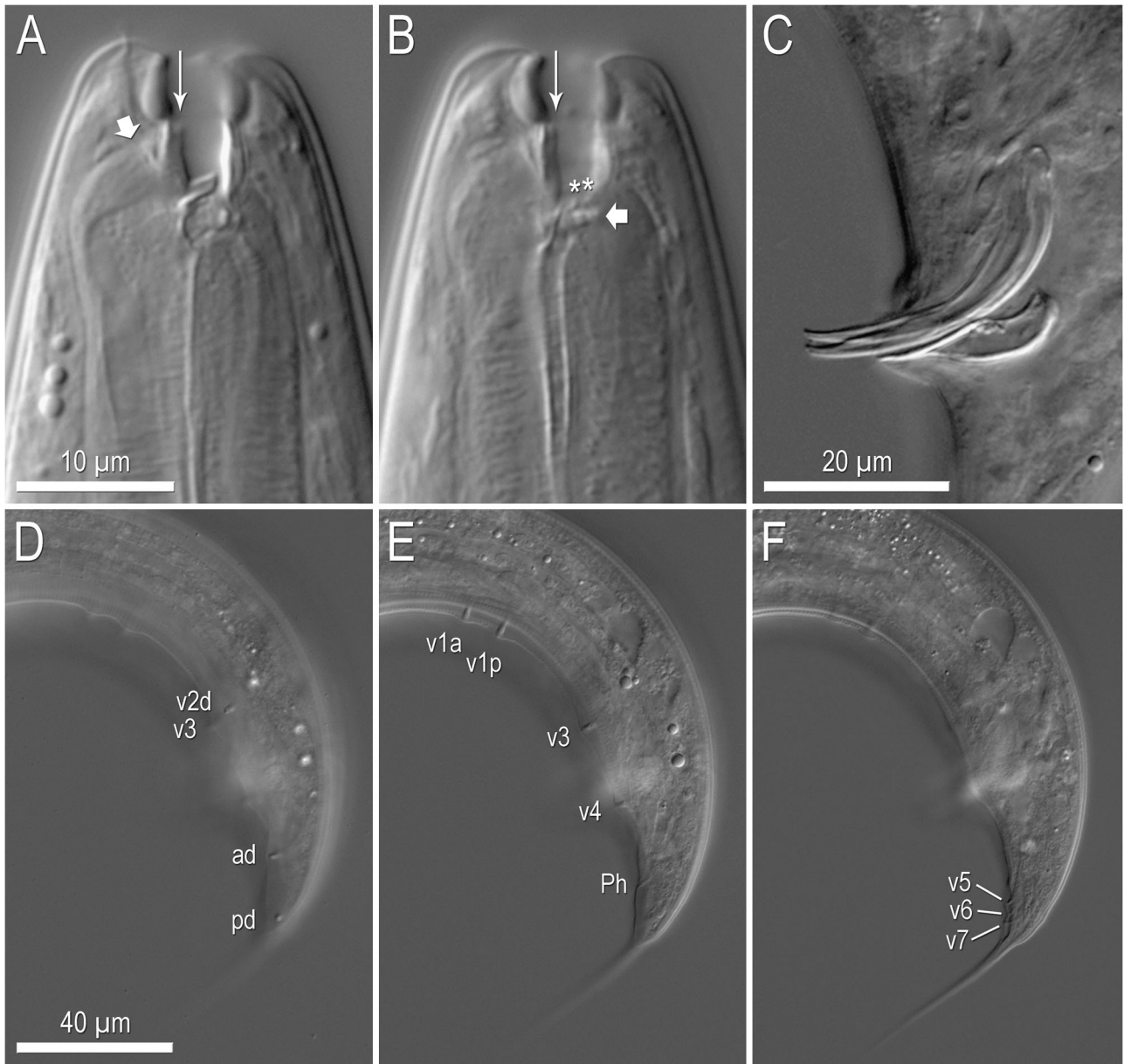
Fig. 4. Nomarski micrographs of *Levipalatum texanum* n. gen., n. sp. A, B. Stoma of a single hermaphrodite in two focal planes and at same scale. A: Sagittal plane. Dorsal radius of pharynx extends anteriad (short arrow), such that ring of pro- and mesostegostom forms an acute angle with stomatal wall. A dorsal 'palate' of putative stegostom (long arrow) extends anteriad from tooth and medially into stomatal lumen. Long, hooked dorsal tooth is also shown; B: Putative telostegostom in each subventral sector forms a thick, grooved ring (short arrow) supporting two denticles (asterisks). Long arrow indicates projecting dorsal palate; C: Spicules and gubernaculum. D-F. Genital papillae of a single male in different focal planes and at same scale. D: Dorsal papillae; E: Phasmid and anterior ventral papillae, including two pairs far anterior (v1a, v1p); F: Posterior ventral papillae.
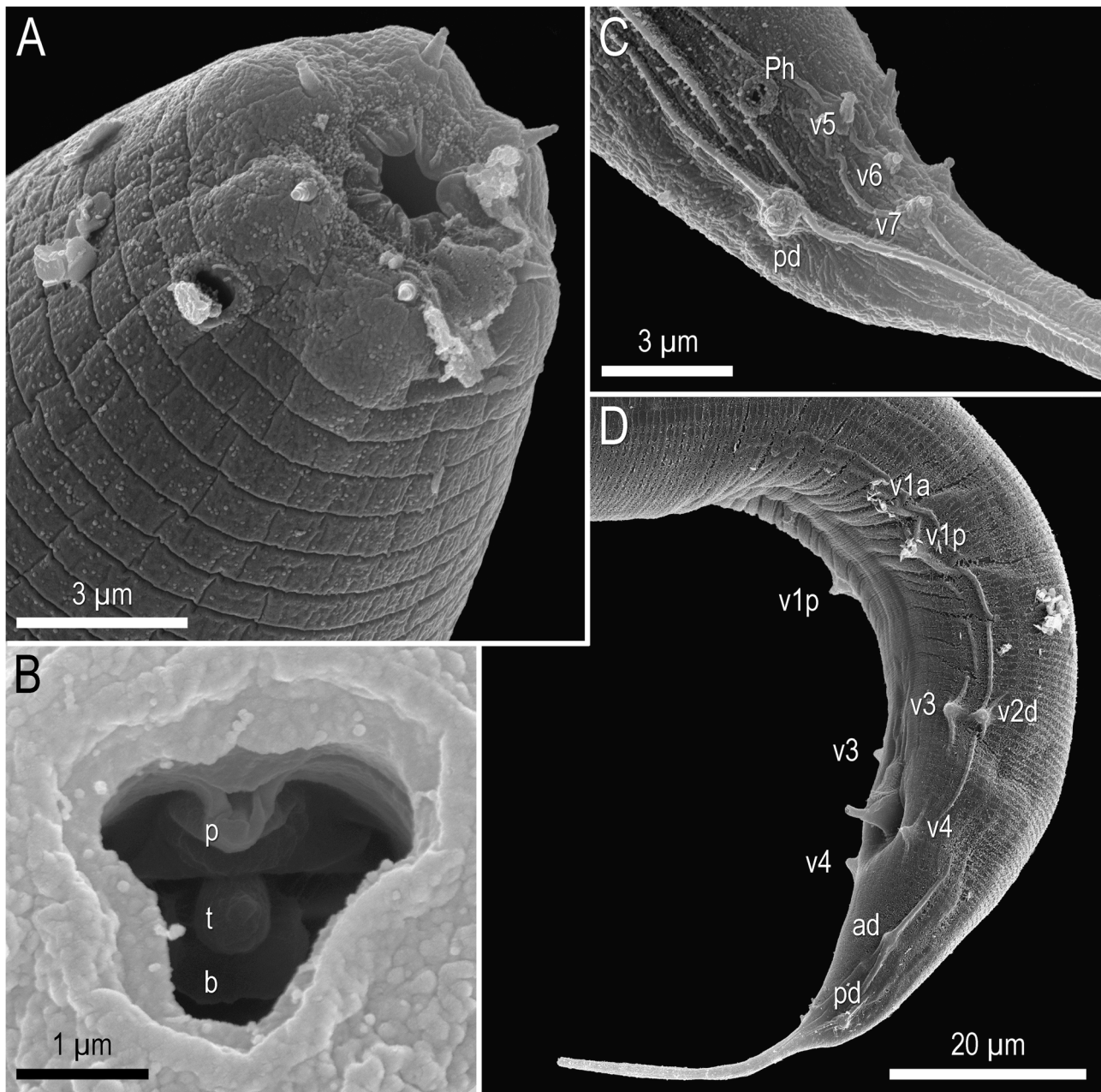
Fig. 5. Scanning electron micrographs of *Levipalatum texanum* n. gen., n. sp. A: Lip region of hermaphrodite, oblique left lateral view; B: Stoma, *en face* view. 'Palate' (p) has a wide base, narrow apex, and projects mediad from dorsal stomatal wall. Also shown is apex of tooth (t) and the posterior base (b), *i.e.*, part posterior to dorsal gland orifice, of tooth; C: Male tail, right lateral view; D: Male tail, left ventrolateral view. Refer back to p. 700 for definitions or state (abbreviations after Sudhaus & Fürst von Lieven, 2003).

TYPE MATERIAL AND STRAIN CULTURE

Holotype hermaphrodite (accession 31381), eight paratype hermaphrodites and three paratype males (31382-31384) deposited in the University of California Riverside Nematode Collection (UCRNC), CA, USA. Five paratype hermaphrodites and three paratype males deposited in the Swedish Museum of Natural History, Stockholm,

Table 1. Morphometrics of hermaphrodite holotype (in glycerin) and hermaphrodite and male specimens (temporary water mounts) of *Levipalatum texanum* n. gen., n. sp. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Hermaphrodite		Male
	Holotype	Temporary water mounts	Temporary water mounts
n	–	20	20
L	1244	1108 ± 303 (784-1622)	761 ± 87 (626-929)
L′	1093	963 ± 284 (665-1436)	667 ± 85 (541-827)
a	14	16 ± 1.6 (13-18)	16 ± 1.4 (14-19)
b	9.0	7.4 ± 1.8 (5.5-11)	5.7 ± 0.6 (4.9-6.6)
c	8.2	7.6 ± 1.1 (6.6-10.2)	8.1 ± 0.8 (6.5-9.4)
c′	4.2	4.6 ± 0.6 (3.4-6.3)	3.3 ± 0.4 (2.9-4.4)
V or T	46	50 ± 1.5 (46-52)	55 ± 4.1 (48-62)
Max. body diam. (not detached cuticle)	91	74 ± 27 (43-121)	47 ± 4.9 (33-52)
Stoma diam.	4.3	4.6 ± 0.5 (3.6-5.3)	3.0 ± 0.4 (2.2-3.8)
Stoma length (gymno- + cheilostom), ventral	8.9	8.5 ± 0.6 (7.2-10.1)	8.1 ± 0.5 (7.2-8.9)
Stoma length (gymno- + cheilostom), dorsal	6.8	6.5 ± 0.6 (5.7-8.2)	6.2 ± 0.5 (5.1-7.0)
Total stoma length	12.5	12.9 ± 0.9 (10.9-14.8)	11.4 ± 0.6 (10.3-12.5)
Anterior end to amphidial aperture	4.6	4.8 ± 0.6 (3.7-5.7)	4.8 ± 0.4 (3.7-5.5)
Corpus length	66	78 ± 3.3 (73-86)	65 ± 2.6 (60-70)
Pharynx length	131	142 ± 5.4 (131-151)	126 ± 3.8 (120-135)
Corpus as % pharynx length	50	82 ± 12 (62-101)	51 ± 2.7 (48-58)
Neck length	138	149 ± 5.8 (139-158)	134 ± 3.8 (127-143)
Median bulb diam.	23	25 ± 2.3 (21-29)	18 ± 1.1 (17-21)
Basal bulb diam.	25	23 ± 4.2 (16-31)	19 ± 1.6 (15-21)
Excretory pore from anterior end	101	134 ± 15 (120-175)	119 ± 14 (93-142)
Nerve ring from anterior end	86	104 ± 5.8 (96-123)	92 ± 3.9 (86-98)
Vulva from anterior end	570	549 ± 138 (406-779)	–
Vulva to anus distance	523	415 ± 147 (259-670)	–

Table 1. (Continued.)

Character	Hermaphrodite		Male
	Holotype	Temporary water mounts	Temporary water mounts
Length of anterior gonad branch	285	234 ± 97 (136-404)	–
Length of anterior flexure	172	236 ± 146 (77-482)	–
Length of posterior gonad branch	230	137 ± 67 (85-303)	–
Length of posterior flexure	338	228 ± 175 (84-520)	–
Testis length	–	–	418 ± 79 (302-571)
Testis flexure	–	–	76 ± 14 (52-104)
Cloacal or anal body diam.	36	33 ± 8.1 (19-48)	29 ± 3.1 (21-33)
Tail length	151	145 ± 21 (119-195)	94 ± 5.0 (85-102)
Spicule length (curve)	–	–	40 ± 2.6 (35-44)
Spicule length (chord)	–	–	33 ± 2.1 (28-36)
Gubernaculum length	–	–	21 ± 1.4 (17-23)

Sweden. Four paratype hermaphrodites and two paratype males deposited in the Harvard Museum of Comparative Zoology, Cambridge, MA, USA. The type strain is available in living culture and frozen stocks under strain code RS5280 from the Department of Evolutionary Biology, Max Planck Institute (MPI) for Developmental Biology, Tübingen, Germany, and can be provided to other researchers upon request.

NOMENCLATURAL REGISTRATION

The new species binomial has been registered in ZooBank under the identifier DDE51B2A-B769-42AE-9062-0C1D3B4145BD.

Discussion

The description of *L. texanum* n. gen., n. sp. provides a new reference point for comparative studies of feeding morphology in Diplogastridae. The new genus is characterised by greater stomatal complexity than in other genera with tube-like stomata, including *Demaniella*, *Diplo-* *gasteroides* de Man, 1912a, *Diplogastrellus*, *Pseudodiplogasteroides*, *Rhabditidoides* and *Rhabditolaimus*. Species of those six genera lack many or all of the stomatal structures present in species with multiple teeth and a dimorphism and, as a consequence, were previously considered to be intermediates between rhabditid outgroups and diplogastrids with more complex stomata (Fürst von Lieven & Sudhaus, 2000). Characters that are divergent between the new genus and closely related genera are the form or presence of the 'palate', the stegostomatal ridges of denticles, the shape of the dorsal tooth, and the bulge of the dorsal pharyngeal radius. Because typological distinctness and relationships of *L. texanum* n. gen., n. sp. preclude its inclusion in any nominal taxon within Diplogastridae, we describe *Levipalatum* n. gen. as a monotypic new genus.

Phylogenetic analysis of partial SSU rRNA and 11 ribosomal protein gene sequences robustly supported *L. texanum* n. gen., n. sp. to be a sister group to *Rhabditolaimus* sp. *Rhabditolaimus* comprises species with highly derived stomatal and pharyngeal morphology, warranting its earlier status as a separate family, Cylindrocorporidae (Goodey, 1939; Andrássy, 1984). Morphological and

molecular characters have since supported the genus to be a monophyletic group nested within Diplogastridae (Susoy & Herrmann, 2012; Kanzaki & Giblin-Davis, 2014). Characterising *Rhabditolaimus* in part is a deep, narrow stoma devoid of observable complexity, in contrast to the multiple projecting structures in the stegostom of *L. texanum* n. gen. n. sp. The relationship between *Levipalatum* n. gen. and *Rhabditolaimus* is thus puzzling to reconcile, although a putative synapomorphy of the two genera is a thickened toroid in the cheilostom, in contrast to the thin cheilostomatal cuticle of other diplogastrid genera with tube-like stomata (Sudhaus & Fürst von Lieven, 2003).

Several aspects of stomatal and pharyngeal morphology show a greater similarity to outgroups than to *Rhabditolaimus*. First, a bipartite pharyngeal corpus characterises *L. texanum* n. gen., n. sp. and all other Diplogastridae except for most species of *Rhabditolaimus* (but see Susoy & Herrmann, 2012) and *Odontopharynx* de Man, 1912b, indicating a bipartite pharynx to be symplesiomorphic. Second, the lips of *L. texanum* n. gen., n. sp. and outgroups show hexaradiate symmetry rather than the peculiar tetraradiate divisions of *Rhabditolaimus* spp. Third, the bipartite structure of the stoma, *i.e.*, with a division of the cheilostom and gymnostom into distinct regions of comparable length, is reminiscent of the stoma of *Diplogastrellus*. However, the ambiguous relationships among *L. texanum* n. gen., n. sp., *Diplogastrellus gracilis* and more distant outgroups confound ancestral state reconstructions of this character. A fourth possible symplesiomorphy of *L. texanum* n. sp. with respect to *Demaniella* spp., *Diplogasteroides* spp., *Pseudodiplogasteroides* spp. and *Rhabditidoides* spp. is an axially extended dorsal tooth (Fürst von Lieven, 2000). In species of the four latter genera, the tooth is laterally short and extended anteriad from the dorsal gland orifice; in *L. texanum* n. gen., n. sp., the putatively homologous dorsal armature is also long, although in this species it comprises a transversely long tooth and an anterior palate. Finally, the raised palate of *L. texanum* n. gen., n. sp. appears similar to the gymnostomatal fold in species of *Demaniella* and *Pseudodiplogasteroides*, but whether the fold or palate derives from a homologous ancestral structure or is convergent in the three genera is still ambiguous given the unclear relationships among those taxa.

In addition to stomatal structures, distinctive characters of *L. texanum* n. gen., n. sp. include male sexual morphology, specifically the arrangement of genital papillae. Examined males of *L. texanum* n. gen., n. sp. usually had ten pairs of genital papilla, *i.e.*, one pair more than the typ-

ical nine of most other Diplogastridae. The presence of two pairs far anterior to the cloacal opening suggests that the additional pair may have arisen due to a duplication of v1. The presence of an extra anterior papilla is not unknown for diplogastrid nematodes, and has been reported for *Pristionchus maupasi* Potts, 1910, *P. boliviae* Kanzaki, Ragsdale, Herrmann, Susoy & Sommer, 2013, and *Micoletzkya masseyi* Susoy, Kanzaki & Herrmann, 2013. The presence of an extra papilla in these cases, however, was much less consistent and almost always confined to a single side of the body. In *Pristionchus*, extra papillae were reported for androdioecious species, soliciting the hypothesis that such characters were degenerate: specifically, the result of "selfing syndrome" (Thomas *et al.*, 2012), a condition whereby stabilising selection on male-specific morphology is relaxed by a mostly self-fertilising mode of reproduction (Kanzaki *et al.*, 2013). A correlation between the putative duplication of v1 in *L. texanum* n. gen., n. sp. with androdioecy might also be speculated. However, because androdioecy is not a prerequisite for aberrant papillae patterns (Susoy *et al.*, 2013), tests of this correlation require denser sampling of closely related gonochoristic species.

Finally, the new species described here further demonstrates the labile nature of the reproductive mode in Diplogastridae. Specifically, hermaphroditic sex has evolved independently in *Diplogasteroides magnus* (Völk, 1950) Weingärtner, 1955 (Kiontke *et al.*, 2001), *Koerneria sudhausi* Fürst von Lieven, 2008, and *Sudhausia* spp. (Herrmann *et al.*, 2013), as well as at least six times in *Pristionchus* Kreis, 1932 alone (Mayer *et al.*, 2007; Kanzaki *et al.*, 2013c; Ragsdale *et al.*, 2013b), representing a stunning example of repeated convergent evolution. Based on the inferred phylogenetic position of *L. texanum* n. gen., n. sp., the new species represents yet another independent acquisition of this trait in Diplogastridae. With increasing genetic resources available for *Pristionchus* and other genera of the family, it may ultimately be possible to unravel the mechanism underlying the evolution of sex (*e.g.*, Haag, 2009). Our report of androdioecy in *L. texanum* n. gen., n. sp. further supports the utility of Diplogastridae as a model for this evolutionary question.

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