

Three New Species of *Nothacrobeles* (Nemata: Cephalobidae) from the Mojave Desert, California

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Abstract: Three new species of *Nothacrobeles* are described from localities in the Mojave Desert, southern California. *Nothacrobeles triniglarus* n. sp. is characterized by the presence of a long post-vulval sac and three tubular adoral projections. Both *N. spatulatus* n. sp. and *N. nanocarpus* n. sp. are smaller than any other known species within the genus. *Nothacrobeles spatulatus* n. sp. has labial probolae that are short and spatulate without a basal ridge, whereas those of *N. nanocarpus* n. sp. are flattened and plate-like. Furthermore, *N. nanocarpus* n. sp. is unique by its extremely short esophageal corpus (less than 25 µm long in adult females) and the small size of its guard processes. An emended diagnosis of the genus is given to accommodate distinctive characteristics of these new species. A table comparing the 11 valid species of *Nothacrobeles* is presented.

Key words: Cephalobidae, morphology, new species, *Nothacrobeles nanocarpus* n. sp., *Nothacrobeles spatulatus* n. sp., *Nothacrobeles triniglarus* n. sp., SEM, southern California, taxonomy.

The Cephalobina (Rhabditida) is a suborder of mostly bacterial-feeding nematodes commonly found in desert soils. They are of particular interest because they have been depicted as part of a clade that includes certain parasites of vertebrates (*Strongyloides*), insects (*Steinernema*), and plants (Tylenchida) (Blaxter et al., 1998). The species described herein were collected during a long-term project to describe cephalobid diversity, including inventories of Cephalobina in the Mojave Desert of southern California.

The genus *Nothacrobeles* was proposed by Allen and Noffsinger (1971) within the family Cephalobidae Filipjev, 1934 to include four new species (*N. sheri*, *N. lepidus*, *N. maximus*, and *N. subtilis*) from Australia, India, and Israel, and one new combination for *Zeldia acrobeles* Andrassy, 1967, known only from a single male. Subsequently, one new species has been described and two known

species have been added or transferred to the genus: *N. lunensis* Shahina and De Ley, 1997 from Argentina; *N. prominens* (Andrassy, 1964) Andrassy, 1984 from Hungary, and *N. scaphovulva* (Rashid and Heyns, 1990) Shahina and De Ley, 1997 from South Africa.

Allen and Noffsinger (1971) characterized *Nothacrobeles* primarily by labial probolae that are “short to long,” always bifurcate, with a prominent basal ridge (swollen base, *sensu* Allen and Noffsinger, 1971) continuous with the bifurcations and projecting toward paired lips. They further described the genus as having two triangular guard processes (“two projecting, pointed structures” *sensu* Allen and Noffsinger, 1971) in each of the three deep primary axils. In this paper, we describe three new species that more or less differ from this characterization, and that complicate distinction of *Nothacrobeles* from putatively related genera. We therefore modify the diagnosis of the genus and briefly discuss its remaining differences from other Cephalobidae.

MATERIALS AND METHODS

Nematodes were extracted from soil samples with a combination of sieving and modified Baermann funnel techniques. Isolates of *N. triniglarus* n. sp. and *N. spatulatus* n. sp. were maintained on 1% water agar with 5 µl/ml cholesterol in petri dishes at 18–20 °C and fed with streaks of *Escherichia coli* OP50. In accordance with the recom-

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mendations of the *Caenorhabditis* Genetics Center, these strains, respectively, were designated with codes JB054 and JB060. In the case of *N. spatulatus* n. sp., all measured specimens are derived from JB060, since the fixed remainder of the original soil extract contained very few adults. We were unsuccessful in culturing *N. nanocorpus* n. sp., and collected relatively few specimens which restricted the extent of SEM studies compared to the other two species. For light microscopy, specimens were heat-killed (65 °C), fixed, and dehydrated as described by Seinhorst (1959) in a mixture of TAF (4 parts), FA (1 part), and distilled water (5 parts), and then processed to anhydrous glycerin.

The following morphometric characters are defined for the Cephalobidae to standardize measurements: esophageal corpus length (CL): measured along curvature of the lumen; labial probolae length (LPL): measured from tips of labial probolae to cheilorhabdia; neck length (NL): from tips of lips or labial probolae to the base of the basal bulb, measured along middle of the body; reproductive tract length (RTL): measured along body axis, from anteriormost tip to posteriormost tip, i.e. excluding all flexures; stoma length: from cheilorhabdia to base of the stoma. Stoma terminology (cheilostom, gymnostom, and stegostom) was adapted from De Ley et al. (1995), and terminology associated with the structures of the nematode's anterior is based on Rashid et al. (1988).

In many Cephalobidae, the corpus is visibly divided into two parts, and a distinctly swollen median bulb (= metacarpus) is almost never present. In such cases, the posterior part of the corpus is presumably homologous to the median bulb found in some Panagrolaimidae, many Rhabditina, and most Diplogasterida (De Ley, 1997). For descriptive purposes, we therefore refer to procorpus and metacarpus in Cephalobidae, even when the latter part is not bulbous.

Analogous to the ray formula commonly used in Rhabditidae, we herein introduce a papilla formula specifying the arrangement of paired genital papillae in male Cephalobidae. As it is usually possible in Cephalobi-

dae to distinguish phasmids from the papillae (unlike in most Rhabditidae), the phasmid pair is listed separately with a lower case "p". Pre- and postcloacal papillae are separated by "/", with precloacal papillae listed first, and the number of pairs in each cluster of papillae separated from the adjacent cluster with a "+".

For scanning electron microscopy, formalin-fixed (5% aqueous solution for > 24 hours) nematodes were rinsed in several changes of 0.1 M phosphate buffer (pH 7.0) and postfixed overnight in 4.0% aqueous osmium tetroxide solution. Postfixed specimens were rinsed in several changes of cold 0.1 M phosphate buffer within 15 minutes, dehydrated through a series of 20% to 100% absolute ethanol, critical-point dried in a Tousimis Autosamdri-810 critical-point drier, and mounted on double-sticking copper tape attached to aluminum stubs. Stubs with mounted nematodes were coated for 3 minutes with a 25-nm layer of gold palladium in a Hummer V sputter coater, and specimens were observed with a JEOL 35 Scanning Electron Microscope at 5 kV or a XL30-FEG Phillips 35 Scanning Electron Microscope at 10 kV.

SYSTEMATICS

Nothacrobeles triniglarus n. sp.
(Tables 1, 2; Figs. 1–3)

Description

Morphometrics of holotype and paratype females are given in Table 1. Morphometrics of paratype males are given in Table 2.

Females: Body almost straight or sigmoid to slightly curved ventrally in the vulval region (Fig. 1A). Cuticle 2–2.5 µm thick with prominent annules, 2.8–3.4 µm wide in widest part of body. Annules each with three to four irregular parallel rows of punctations (Fig. 1B). Lateral field, as observed with LM, with two alae separated by wide grooves resulting in 3–4 incisures depending on the specimen and body region (Figs. 1B,C,E,H). In SEM, two additional outer incisures parallel to the alae (Fig. 3F). Irregular breaks occasionally occurring in the dorso-

TABLE 1. Measurements of female paratypes of *Nothacrobeles trinigliarus* n. sp. ($n = 9$) and *N. subtilis*.

Character	<i>N. trinigliarus</i>				<i>N. subtilis</i>	
	Holotype	Paratypes ($n = 9$)			n	Range
		Mean	SD	Range		
Linear (μm)						
Body length	530	524.4	24.6	475–555	3	490–546
Vulva-anus distance	148	143.6	6.8	130.4–152	3	127–140
Neck length	136	132.4	4.7	123–149.5	3	126.5–131
Corpus length	59	58.6	2.5	55.5–64	3	53.5–62
Isthmus length	35	31.7	2.5	27–34.5	3	25–30
Basal bulb length	25	23.9	1.3	22–25.5	3	24.5–30
Nerve ring to anterior end	75	74.3	7.3	61–81	2	75–84
Excretory pore to anterior end	77.5	76.1	7.4	63.5–86.5	2	80.5–85
Deirid to anterior end	90	92.6	5.9	80–98.5	1	95
Maximum body width	33	32.3	1.7	28–33.5	1	37.5
Spermatheca length	45	43.5	4.2	40.5–46.5	3	31.2–40.8
Vagina length	7	12.2	3.6	7–19	2	8.8–13.5
Post-vulval sac length	44	55.2	12	44–83	3	23.2–30
Rectum length	20	22.3	2.8	16–25	3	12.8–15
Tail length	52	51.7	2.2	48–55	3	38.4–51.4
Anal body width	20	20.6	0.8	19.5–22	3	19.2–21.5
Phasmid-anus distance	16.5	17.4	2.6	15–22.5	3	12–16.8
Annule number						
R_{nr}^a	—	28.6	1.9	25–30	1	34
R_{ex}^b	—	29.4	2.2	25–33	1	34
R_{dei}^c	—	34.2	2.4	39–37	1	38
Ratios						
a	16	16.2	0.4	15.7–17	1	14.6
b	3.8	4.0	0.1	3.9–4.1	3	3.9–4.2
c	10.1	10.1	0.4	9.6–11	3	10.6–12.8
c'	2.6	2.5	0.1	2.4–2.7	3	2–2.4
C/I^d	1.6	1.86	0.15	1.7–2.1	3	1.8–2.5
Proportions						
Nerve ring ^e	55.1	56.0	4.0	49.4–60.0	2	59–64
Excretory pore ^e	56.9	57.4	4.0	49.4–62.0	2	64–65
Deirid ^e	66.1	69.9	3.5	65.0	1	72
V^f	62.2	65.1	6.5	61.6–82.1	3	65–66
G^g	31.8	35.1	2.7	31.1–39.2	3	30–32

^a R_{nr} : number of annules from the anterior end to the nerve ring.

^b R_{ep} : number of annules from the anterior end to the excretory pore.

^c R_{dei} : number of annules from the anterior end to the deirid.

^d C/I : the ratio between esophageal corpus length and isthmus length.

^e Nerve ring as percentage of neck length.

^f Vulva position as percentage of body length.

^g G : reproductive tract length as percentage of body length.

sublateral incisures in the area immediately posterior to the basal bulb (Fig 1B).

Lip region 13–15 μm wide, with four cephalic and six labial papillae; amphid openings small and oval, located slightly dorso-sublaterally on the base of the lateral lips (Fig. 2). Labial probolae 3–3.5 μm high, 0.2–0.3 times as long as lip region width, exterior surface shallow to moderately concave, slightly bifurcated into two very short prongs (i.e. branches of the bifurcation)

that have a plain inner margin lacking tines, and an outer (anterior) rim with widely spaced, low, triangular tines (Figs. 2;3A–E). Outer rim of each prong continuous on the side adjacent to the lips, with an anteriorly tilted and thickened prominent basal ridge. A narrow pedestal protruding adorally at the base of each labial probola just below the bifurcation. Lips peripheral to the probolae in pairs, with a finely fimbriate rim, tapering toward the tip (Figs. 2;3A,C,D). Combined

TABLE 2. Measurements of male paratypes of *Nothacrobeles trimiglarus* n. sp. ($n = 11$).

Character	Mean	SD	Range
Linear (μm)			
Body length (L)	526.0	42.6	450–590
Neck length	129.5	5.4	122–140.5
Corpus length	57.8	3.0	54–65
Isthmus length	32.4	4.1	28.5–44
Basal bulb length	23.0	1.3	21–25.5
Nerve ring to			
anterior end	76.8	6.2	64–84.5
Excretory pore to			
anterior end	79.0	8.5	57–89
Deirid to anterior			
end	91.5	9.3	68–107
Maximum body			
width	28.7	1.9	27–34
Testis flexure length	63.6	7.4	55.2–75.1
Tail length	52.0	4.1	46–60
Anal body width	20.8	1.6	19–23.5
Phasmid-anus			
distance	21.2	3.1	18–25.6
Spicule length	34.1	1.6	32–36.8
Gubernaculum			
length	21.1	1.4	19.2–24
Annule number			
R_{nr}^a	30.3	2.5	27–36
R_{ex}^b	31.0	2.0	29–34
R_{dei}^c	35.4	2.1	33–39
Ratios			
a	18.4	1.7	15.3–21.1
b	4.1	0.3	3.5–4.4
c	10.1	0.7	9.3–11.8
c'	2.5	0.2	2.2–2.8
C/I ^d	1.81	0.22	1.2–2.1
Proportions			
Nerve ring ^e	59.3	4.0	49.6–63.9
Excretory pore ^e	61.1	7.1	44.2–68
Deirid ^e	70.7	7.5	52.7–80.1
T ^f	54.9	3.4	50–61.3

^a R_{nr} : number of annules from the anterior end to the nerve ring.

^b R_{ex} : number of annules from the anterior end to the excretory pore.

^c R_{dei} : number of annules from the anterior end to the deirid.

^d C/I: the ratio between esophageal corpus length and isthmus length.

^e Nerve ring as percentage of neck length.

^f Reproductive tract length as percentage of body length.

lip pairs separated in the dorsal and ventrolateral positions by deep primary axils, with shallow secondary axils between the two members of each pair of lips. Lip margins also fimbriate in the primary axils, each of which two triangular guard processes emerging from a small annule surrounding the base of the lip region (Figs. 2;3A,C,D). Each primary axil flanked adorally by two hori-

zontal tooth-like processes directed toward the mouth opening but not reaching it (Fig. 3E). Tangential ridges absent. Rims of gymnostom extending anteriorly into three minute tubular projections pressed against the adoral surface of each labial probola and protruding through a central cleft in each adoral pedestal (Figs. 2;3A,D,E).

Mouth triangular with slightly convex sides (Fig. 3B,E). Stoma 9.5–12 μm long, 0.7–0.9 times lip region width, with distinct divisions into cheilostom, gymnostom, and stegostom (Fig. 1D). Cheilostom with conspicuous, transversely flattened cheilorhabdia. Gymnostom short, less than half length of stegostom. Stegostom with distinct subdivisions but lacking a clearly defined dorsal denticle.

Corpus cylindrical, 1.7–2.1 times as long as the isthmus (Fig. 1E). Isthmus clearly delineated from and narrower than corpus, with more conspicuous muscle fibers. Basal bulb pyriform with striated transverse valves. Excretory pore 0–3 annules posterior to the nerve ring, near the anterior end of the isthmus. Deirids small, located at 4–7 annules posterior to the excretory pore (Fig. 1E). Esophago-intestinal valve conoid, 3–5.5 μm long.

Reproductive system monodelphic, prodelphic (Fig. 1A,F), always dextral. Vulva shifted subventrally toward left of the body and often in a depression exaggerated in lateral view by the angle of view (Figs. 1H;3I). Length of vagina variable, extending obliquely over 32–59% of the vulval body width. Postvulval sac variable, 44–83 μm long, 1.5–2.6 times longer than vulval body width. Exact arrangement and number of uterus cells unclear; uterus apparently consisting of four rows of cells along most of its length, and extending anteriorly into an offset spermatheca. Oviduct emerging dorsally from the junction of uterus and spermatheca, leading to a reflexed, long ovary usually extending well posterior to the vulva (Fig. 1F). Rectum 0.8–1.2 times as long as anal body width. Anus an arcuate slit. Phasmid at 29–43% of tail length. Tail conoid with 13–18 ventral annules; tail tip hyaline, sub-acute to acute, occasionally slightly re-

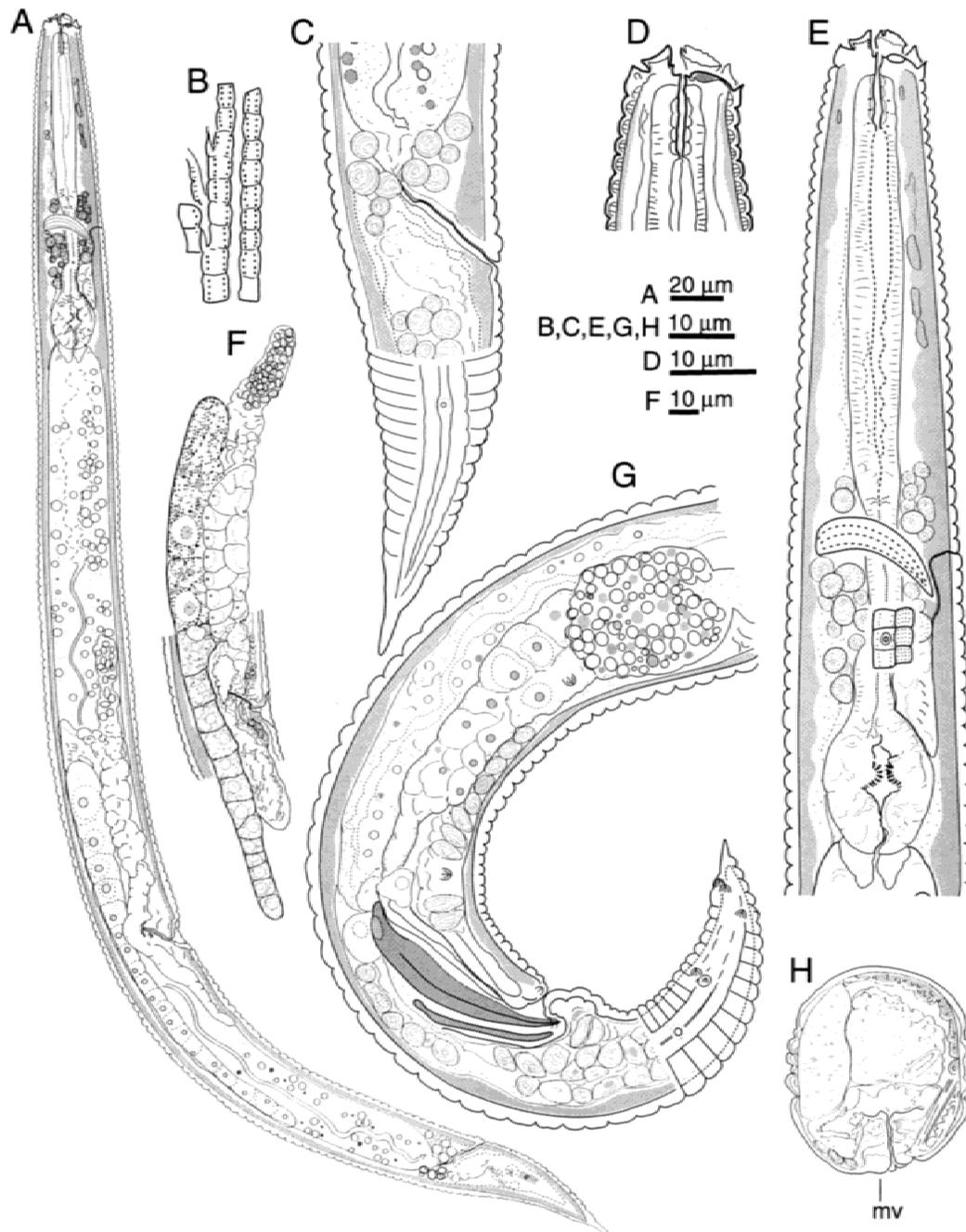


FIG. 1. Camera lucida drawings of *Nothacrobeles triniglarus* n. sp., female (A-F, H) and male (G). All are right lateral views unless otherwise indicated. A) Entire specimen. B) Irregular breaks in the dorso-sublateral wing at level posterior to basal bulb. C) Tail. D) Lip region. E) Anterior end. F) Reproductive tract. G) Tail region. H) Cross-section at vulval region showing subventral position of the vulva (mv: midventral).

curved dorsally and sometimes offset with a mucro of 5-7.5 μm (Figs. 1A,C,G).

Male: Body thinner than that of female, usually strongly curved posteriorly even

when relaxed. Lateral field with two separate alae and four incisures (Fig. 1G). Nerve ring and excretory pore in anterior part of isthmus, usually 1-2 annules apart, with deirids

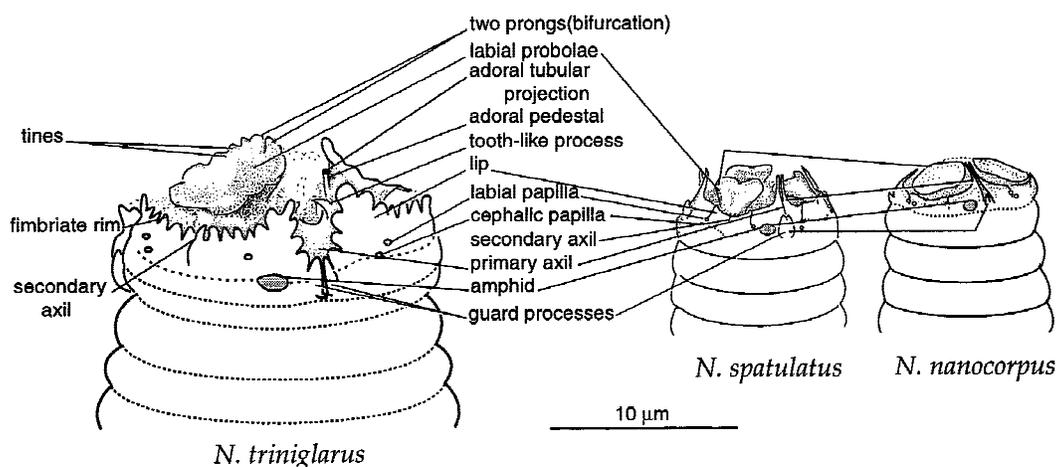


FIG. 2. Diagrammatic near-lateral views of the lip regions of *Nothacrobeles triniglarus* n. sp., *N. spatulatus* n. sp., and *N. nanocorpus* n. sp., redrawn from light and scanning electron microscope observations.

3-5 annules posterior to excretory pore. Reproductive tract occupying approximately half body length, monorchic, dextral, testis ventrally reflexed, extending posteriorly for 18-31% of the entire tract length. Spicule 32-36.8 μm , or 1.4-1.8 times as long as anal body width, ventrally arcuate, with a thin velum and a hemispherical, offset manubrium (Figs. 1G;3H,J). Gubernaculum canoe-shaped, more than half spicule length. A pair of distinct grooves extending 6-8 body annules anteriorly from edges of the cloaca (Fig. 3H). Two circular pads visible on the surface of everted cloaca, occurring on either side of the extruded spicules (Fig. 3J). Phasmids located at 30-45% of tail length (Fig. 1G). One medio-ventral papilla located on the anterior lip of the cloacal aperture, and eight pairs of genital papillae distributed as follows: three subventral preanal pairs and five postanal pairs: one subventral pair and one lateral pair posterior to the phasmids; one subdorsal pair, one lateral pair, and one subventral pair near tail terminus (Fig. 1G). Papilla formula 1+1+1/p+2+3. Tail conical, strongly curved ventrally (Fig. 1G).

Diagnosis

Nothacrobeles triniglarus n. sp. has a body length of about 0.5 mm and a long postvulval sac (see Table 3). Annules typically have four rows of punctations. The medium-sized

concave probolae have few irregularly spaced tines on the rim, a prominent basal ridge, and short prongs with plain inner margins and narrow space between them. The lips have fimbriate margins. Three tubular projections extend from the rims of the gymnostom to the adoral base of the labial probolae, a character that has not been observed in any previously published scanning micrographs of other *Nothacrobeles* spp. The corpus is longer than the isthmus.

Relationships

Nothacrobeles triniglarus n. sp. most closely resembles *N. subtilis* Allen & Noffsinger, 1971, *N. scaphovulva* (Rashid & Heyns, 1990) Shahina & De Ley, 1997 and *N. sheri* Allen & Noffsinger, 1971. The new species is similar to *N. subtilis* in morphology and measurements (Table 1) but differs in having a longer post-vulval sac (1.5-2.6 times the vulval body width vs. one vulval body width); mostly four horizontal rows of cuticular punctations on each annule, and labial probolae with shorter prongs ("arms," sensu Sauer et al., 1979) without inwardly inclined margins (cf Sauer et al., 1979). Males are present in *N. triniglarus* n. sp. but have not been collected for *N. subtilis*. Examination of paratypes of *N. subtilis* revealed that, as in *N. triniglarus* n. sp., the vulva is also positioned on the left subventral side. The shape of the labial probolae resembles

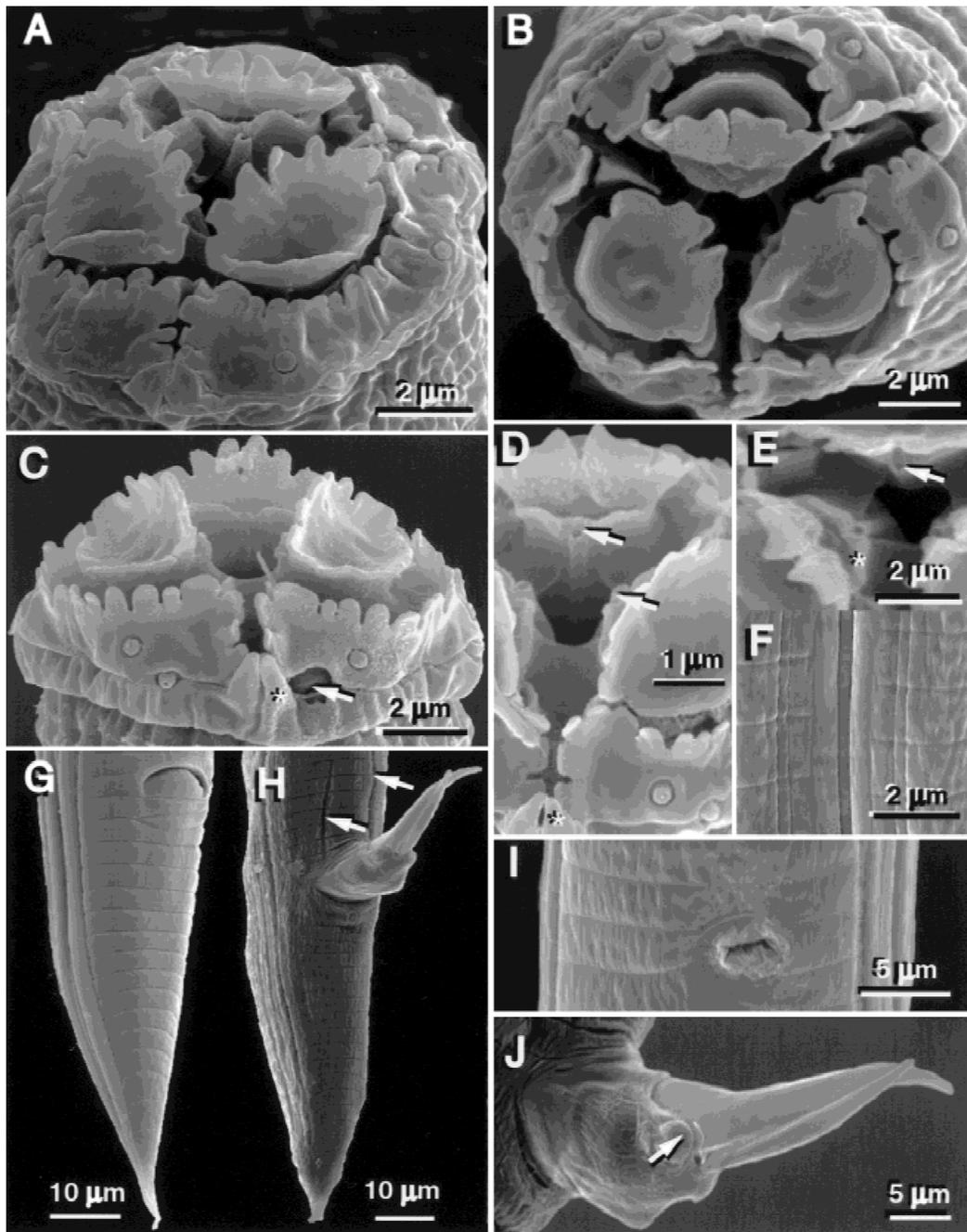


FIG. 3. Scanning electron micrographs of female (A–G, I) and male (H, J) *Nothacrobeles triniglarus* n. sp. A) Oblique ventral view of lip region. B) Nearly en face view. C) Lateral view of lip region. Arrowhead indicates amphid opening; asterisk indicates guard process. D) Oblique ventral view of mouth opening with the three tubular projections (arrowhead) and adoral pedestal. E) Nearly en face view of triangular stoma (sides slightly convex) with the three tubular projections (arrows) with tooth-like thickenings near their bases (asterisk), and adoral pedestal. F) Lateral field with two wings (alae) and six incisures (3 pairs; lines closely aligned within each pair). G) Female tail including anus. H) Posterior region of male showing spicules and subventral grooves (arrows). I) Vulval region, vulva subventral. J) Enlargement of protruding spicules and circular pads of the gubernaculum (arrow).

TABLE 3. Specific differential characters in the genus *Nothacrobeles* Allen & Noffsinger, 1971.

Species	Length of prongs of labial probolae	Lateral lines	Post-vulval sac length ^d (µm)	Body length of female (µm)	Spicule length ^e (µm)	Gubernaculum length ^e (µm)	Corpus length (C/I ratio) ^f	Lip region width (µm)	Other features
<i>N. nanocorpus</i>	— ^a	3	8–15	240–295	NA	NA	16.4–24.5 (0.5–0.9)	5.5–8.5	Short corpus; labial probolae plate-like, without basal ridge
<i>N. spatulatus</i>	— ^a	3	10–13	320–355	NA	NA	28–34 (1.2–1.5)	8–9	Labial probolae spatulate, without basal ridge
<i>N. tringularis</i>	3	4–6	44–83	475–555	32–37	19–24	55.5–64 (1.7–2.1)	13–15	3 tubular adoral projections, annules with 4 rows of punctations
<i>N. lunensis</i>	1–2	2–3	43–67	357–519	21–24	9–13	40–52 (1.3–1.6)	11–13	Cuticle appearing double
<i>N. scaphovulva</i>	1–2 ^b	3	48–53 ^c	420–620	20–34	11–19	?	12–14 ^b	Sunken vulva, annules with 3 rows of punctations
<i>N. subtilis</i>	3.5	4	11–30	480–700	NA	NA	53.5–62 (1.8–2.5)	15 ^b	Annules with 2 rows of punctations
<i>N. acrobeles</i>		4	?	?	36	26	?	13 ^b	Annules with 2 rows of punctations
<i>N. maximus</i>	4–5 ^b	4	13–35	640–870	?	?	?	17 ^b	Cuticle tessellate
<i>N. lepidus</i>	5–6 ^b	4	13–21	520–640	22–25	12–14	?	13 ^b	Cuticle tessellate
<i>N. sheri</i>	7–10 ^b	4	54–78	670–900	30–38	15–19	103 ^b (2.3)	18 ^b	Cuticle tessellate, annules with 3 rows of punctations
<i>N. prominens</i>	7 ^b	3	?	600–780	35	16	?	17 ^b	

^a Prongs absent.
^b Derived from figures and descriptions in Allen and Noffsinger (1971).
^c Based on redescription of holotype by Allen and Noffsinger (1971).
^d VBW: Length of post-vulval sac/vulval body width.
^e NA: Not applicable (males unknown).
^f C/I ratio: Length of esophageal corpus/length of isthmus.

N. scaphovulva; however, in the latter species, prongs are longer and triangular (Rashid and Heyns, 1990). Tines that are present in the primary axils of *N. subtilis* are absent in *N. scaphovulva*. Although the vulva

of both species is sunken and perhaps shifted subventrally, *N. scaphovulva* has a vulval opening surrounded by a prominent cuticular “flap” (rim as shown in SEM). The presence of annular punctations is a charac-

ter shared with *N. sheri* and *N. scaphovulva*, both of which have three rows of punctations per annule. Postvulval sac length (mean = 55.2 μm) is close to that of *N. sheri* (mean = 66 μm ; 54–78 μm), although *N. triniglarus* has a broader range (44–83 μm). *Nothacrobeles sheri* is generally longer than *N. triniglarus* n. sp. and has longer, more elaborately fringed labial probolae.

Type locality

Desert sand collected 25 October 1997 around roots of a small Joshua tree (*Yucca brevifolia*) on the south side of Cactus Flats Road, 100 m east of California State Highway 395 and Olancho Fire Station, Olancho, California. Vegetation in the area is dominated by *Artemisia tridentata* and includes a few scattered creosote bushes (*Larrea tridentata*).

Type designations

Holotype slide 30040 deposited in the University of California Riverside Nematode Collection (UCRNC). Four female, three male, and one juvenile paratypes also deposited at UCRNC. Five females and three male paratypes deposited at the University of California Davis Nematode Collection (UCDNC). One female, two male, and one juvenile paratypes deposited at the Swedish Museum of Natural History, Stockholm, Sweden. Additional topotypes and specimens from laboratory culture are deposited in the UCRNC.

Etymology

The specific epithet is derived from the Greek $\nu\gamma\lambda\alpha\rho\sigma$ (small pipe, whistle) and refers to the three minute tubular structures emerging from the mouth.

Nothacrobeles spatulatus n. sp.
(Table 4, Figs. 2,4,5)

Description

Morphometrics of holotype and paratype females are given in Table 4. Males were not collected.

Females: Body almost straight to slightly curved ventrally, 320–355 μm long (Fig. 4A). Cuticle thin ($\leq 2 \mu\text{m}$), with fine annules 1.3–

2.5 μm wide in widest part of the body, with 3 lines in the lateral field occupying 2.5–4.5 μm (Fig. 4B). Lip region 8–9 μm wide, with four cephalic and six labial papillae (Figs. 2;5A–C). Labial probolae 1.5–2.5 μm high, 0.2–0.3 times as long as lip region width, resembling a spatula, the shallow concavity directed aborally (Figs. 2;5A–C). Lips in pairs, smooth-edged, almost rectangular, demarcating primary axils; primary axils each associated with two triangular guard processes emerging from a small annule around the base of the lip region (Figs. 2C,5C). Tangential ridges absent. Amphid openings small, oval, located slightly dorsal to the bases of lateral lips (Figs. 2;5C).

Mouth triangular, with slightly convex sides (Fig. 5A,B). Stoma 4–5.5 μm long, 0.4–0.6 times LRW, distinctly divided into cheilostom, gymnostom, and stegostom. Gymnostom short, less than half as long as stegostom. Stegostom often with distinct subdivisions, but never with distinct dorsal denticle (Fig. 4B). Corpus muscular, cylindrical, clearly delineated into two parts, the procorpus longer than the metacarpus. Metacarpus leading to a narrower but longer and more muscular isthmus. Basal bulb pyriform with transverse valves located anteriorly (Fig. 4B). Excretory pore very small, 0–2 annules anterior to the nerve ring, at level of the anterior half of the isthmus. Deirids minute, 1–8 annules posterior to the excretory pore. Esophago-intestinal valve short, conoid.

Reproductive system typically cephalobid, monodelphic, prodelfic, consistently dextral, occupying almost one-third of the body length. Ovary long, extending far posterior to the vulva (Fig. 4A), containing 13–20 oocytes and cap cell, few cells in double rows, a single row of cells leading to the oviduct. Oviduct attached dorsally to the junction of the offset spermatheca and uterus (Fig. 4A,C). Uterus opening into a short vagina; vulval slit located about two-thirds of the body length from the anterior end. Vulva midventral, vagina straight, 0.2–0.4 times vulval body width (Fig. 4C). Post-vulval sac about one-half as long as vulval body width. Rectum 1–1.6 times as long as anal body

TABLE 4. Measurements of females of *Nothacrobeles spatulatus* n. sp. ($n = 15$) and *Nothacrobeles nanocorpus* n. sp. ($n = 15$).

Character	<i>N. spatulatus</i>				<i>N. nanocorpus</i>			
	Holotype	Paratypes ($n = 14$)			Holotype	Paratypes ($n = 14$)		
		Mean	SD	Range		Mean	SD	Range
Linear (μm)								
Body length (L)	345	340	9.5	320–355	248	266.8	18.4	240–295
Vulva-anus distance	114	103	6.4	91–114	70	78.0	10.6	66–105
Neck length	85	80.3	2.1	77–85	72	70.0	2.9	64.5–76.5
Corpus length	33	31.9	1.3	28–34	21	20.7	2.2	16.5–24.5
Procorpus	20	19.4	1.4	16–22	12	12.6	0.3	12.5–13
Metacorpus	13	12.5	0.8	11–14	9	9.5	0.7	8.5–10
Isthmus length	25	24.2	1.2	22–26	27	29.5	2.1	26–33.5
Basal bulb length	18	16.2	1.3	15–19	15	14.6	0.6	13.5–15.5
Nerve ring to anterior end	56	55.3	2.6	49–59	48.5	44.8	6.6	30–52.5
Excretory pore to anterior end	51	53.3	2.8	46–57	48	45.6	6.0	31.75–51
Deirid to anterior end	62	66.2	4.0	58–73	— ^e	56.3	5.3	52.5–60
Maximum body width	21	20.8	1.5	19–24	19	17.5	3.2	8.5–20.5
Spermatheca length	14	12.3	1.7	10–15	16	12.9	2.6	9–16.5
Post-vulval sac length	13	10.7	0.8	10–13	8	11.2	1.9	8–15
Rectum length	17	14.8	1.6	13–17	10	12.8	1.5	11–15.5
Tail length	21	21.6	1.2	20–23	19	19.9	1.4	18–23
Anal body width	12	11.8	1.1	10–14	11	10.0	0.9	8.5–12
Phasmid-anus distance	12	11.2	1.1	9–13	8	7.4	0.8	6–8.5
Ratios								
a	16.4	16.4	1.0	14–18	13.2	16.1	4.1	13.4–28.8
b	4.05	4.24	0.2	4–4.6	3.5	3.8	0.2	3.5–4.2
c	16.4	15.8	0.9	14–17	12.9	13.4	0.8	12.3–15
c'	1.8	1.84	0.2	1.6–2.3	1.7	2.0	0.1	1.8–2.2
C/I ^a	1.3	1.32	0.1	1.2–1.5	0.7	0.7	0.1	0.5–0.9
Proportions								
Nerve ring ^b	66	68.9	3.1	62–72	67	64.0	8.8	43–70
Excretory pore ^b	60	66.3	3.8	59–72	66	65.2	8.6	45–74
Deirid ^b	73	71	29.0	58–91	— ^e	83.8	10.1	77–91
V ^c	61	63.5	1.5	61–66	64	63.3	3.0	55–67
G ^d	35	33.3	2.6	27–37	22	32.2	4.9	23–37

^a C/I: the ratio between esophageal corpus length and isthmus length.

^b Nerve ring as percentage of neck length.

^c Vulva position as percentage of body length.

^d Reproductive tract length as percentage of body length.

^e Unobserved character.

width. Anus an arcuate slit. Phasmid at 29–43% of the tail length. Tail 20–23 μm long, with 7–11 ventral annules, subcylindrical, ending in a bluntly conoid tip (Fig. 4D).

Diagnosis

Nothacrobeles spatulatus n. sp. has a short body and post-vulval sac (see Table 3). The annules are not punctated. The labial probolae are short and spatulate without any basal ridge, prongs, or tines. The lip margins are smooth, and the corpus is longer than the isthmus.

Relationships

Nothacrobeles spatulatus n. sp. is very similar to *N. nanocorpus* n. sp. Both species apparently are parthenogenetic, and they also are the two smallest species within the genus. However, *N. spatulatus* n. sp. is slightly longer, and the esophagus has a longer corpus and shorter isthmus relative to that of *N. nanocorpus* n. sp. The labial probolae of both species are similar in not having bifurcations; however, they differ in that those of *N. spatulatus* n. sp. are spatulate, while those of *N. nanocorpus* n. sp. are plate-like. *N. spatu-*

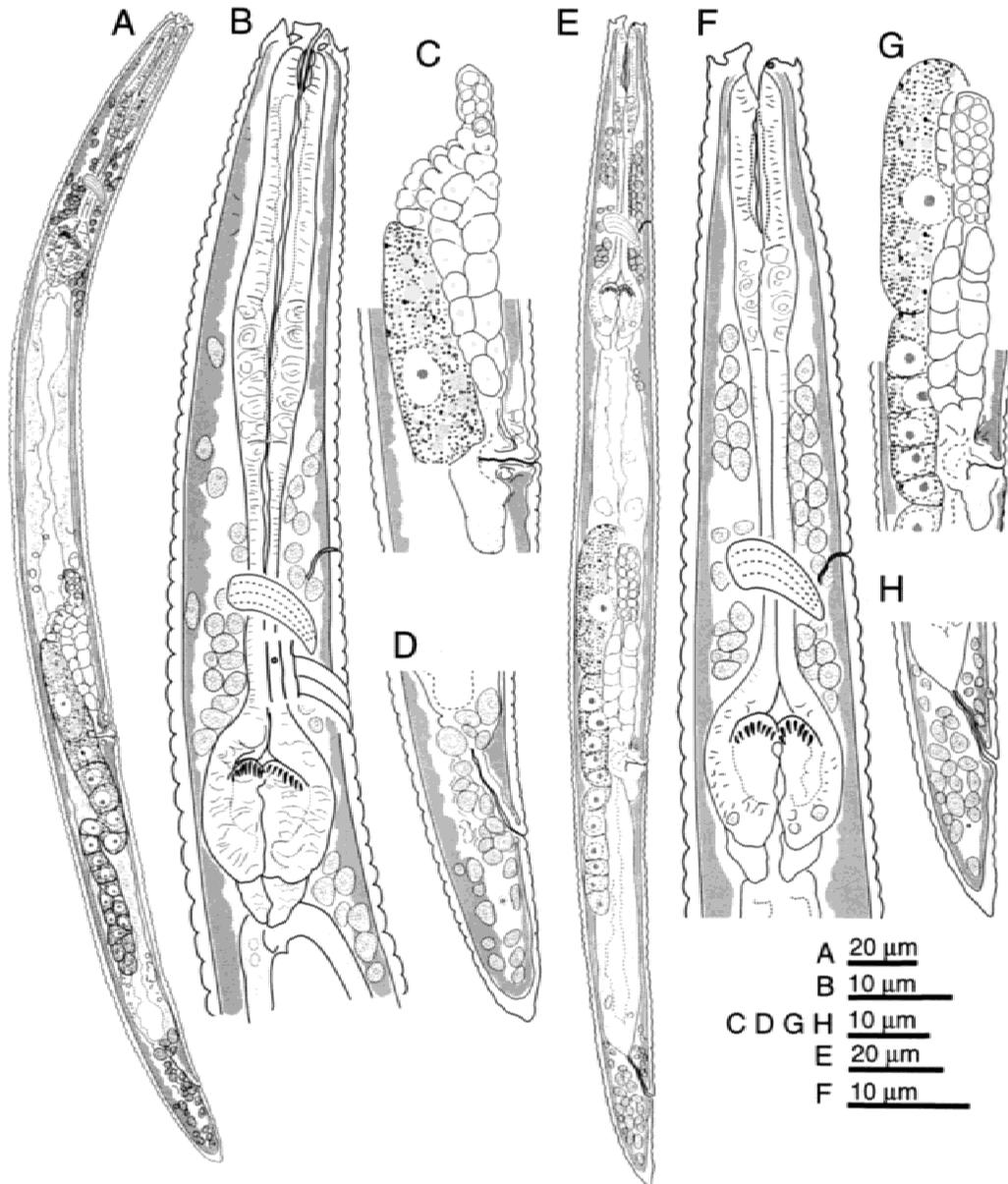


FIG. 4. Camera lucida drawings of females of *Nothacrobeles spatulatus* n. sp. (A–D) and *Nothacrobeles nanocarpus* n. sp. (E–H). A) Entire specimen. B) Anterior end. C) Reproductive tract. D) Tail. E) Entire specimen. F) Anterior end. G) Reproductive tract. H) Tail.

latus n. sp. also has guard processes that are larger than those of *N. nanocarpus* n. sp.

Type locality

Nothacrobeles spatulatus n. sp. was collected from a sample of dark soil with abundant organic matter including needles and cones at the foot of a large pinyon pine, *Pinus edu-*

lis, growing between rocks along a creek near Kissing Rocks, southwestern tip of the Sweeney Granite Mountain Desert Research Facility, California, 18 March 1998. Cultures were given the strain code designation JB060 and kept at the J. G. Baldwin laboratory, UC Riverside, and in the culture collection of P. De Ley, Department of Biology,

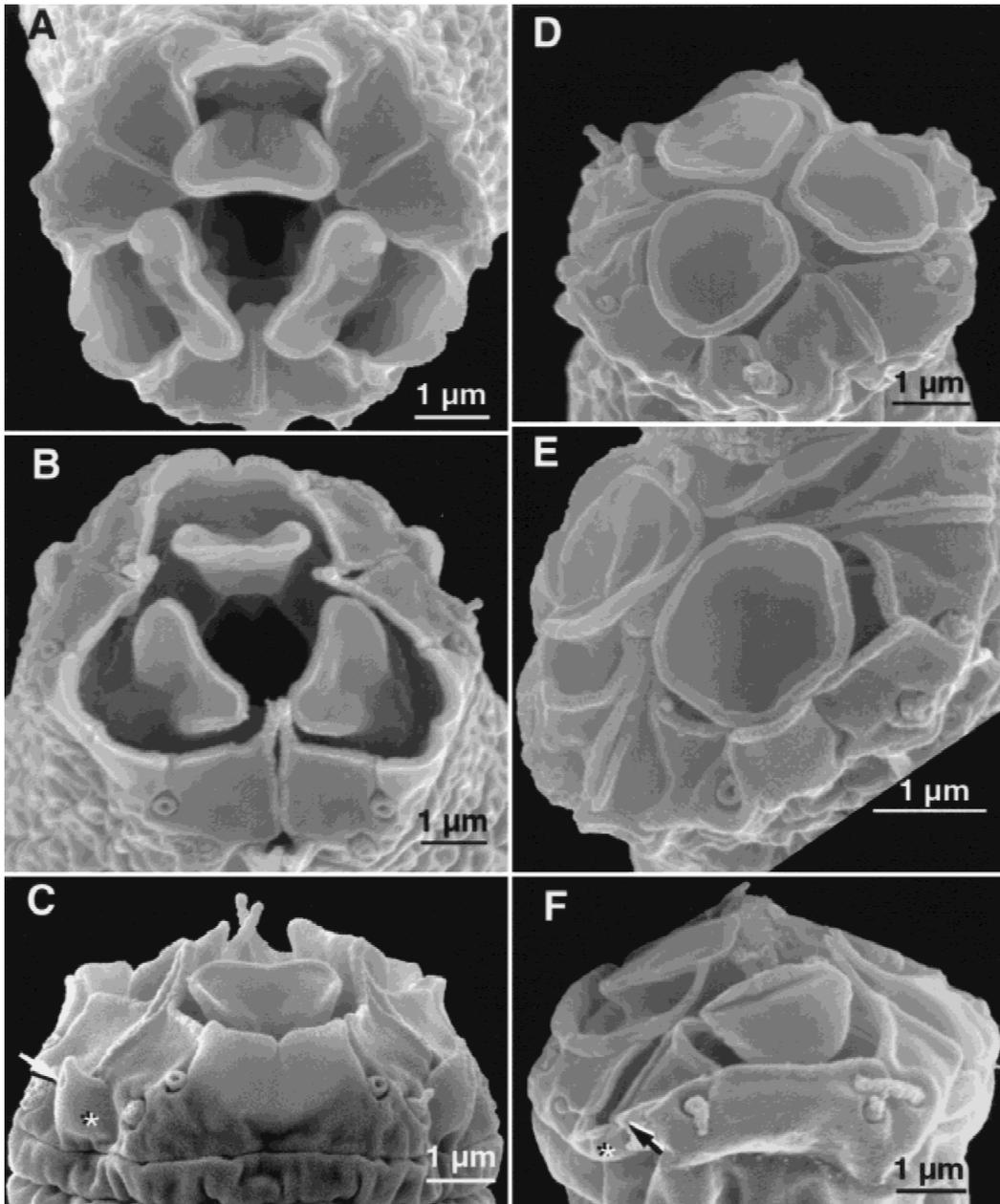


FIG. 5. Scanning electron micrographs of lip region of females of *Nothacrobeles spatulatus* n. sp. (A–C) and *Nothacrobeles nanocorpus* n. sp. (D–F). A) Nearly en face view. B) Nearly en face view tilted slightly ventrally. C) Ventro-lateral view showing amphid (arrowhead) and guard process (asterisk). D) Nearly en face view tilted slightly dorsolaterally. E) Enlargement showing dorsal plate-like probola. F) Dorso-lateral view showing amphid (arrow) and guard process (asterisk).

University of Gent. All measured specimens and type specimens are derived from this culture, since the fixed remainder of the original soil extract contained very few adults.

Type designations

Holotype slide 30045 deposited at UCRNC. Ten female paratypes are deposited at UCRNC, 11 at UCDNC, and 4 at the Swedish Museum of Natural History.

Etymology

The specific epithet is derived from the Latin word *spatula* (spoon) and refers to the three short ladle-like labial probolae in this new species.

Nothacrobeles nanocorpus n. sp.
(Table 4, Figs. 2,4E–H,5D–F)

Description

Morphometrics of holotype and paratype females are given in Table 4. Males not collected.

Females ($n = 15$): Body very short, 240–295 μm , straight to slightly curved when heat-relaxed (Fig. 4E). Cuticle about 1.3 μm thick and annules 1.7–2.2 μm wide at level of maximum body width. Lateral field with 3 faint lines rarely visible in LM. Lip region 5.5–8.5 μm wide, with four cephalic and six labial papillae. Amphid openings located slightly dorsal to bases of lateral lips (Figs. 2;5F). Labial probolae three, very short (1–1.5 μm), 0.1–0.3 lip region width, without prongs or tines, but with a basal ridge encircling each entire probola. Each labial probola thus resembles a plate or bowl, being slightly concave aborally, and mounted on a pedestal (Figs. 2;6D–F). Lips six, peripheral to probolae and partially separated into pairs by a fold or indentation (Fig. 2E). Primary axils often folded toward the oral opening; two very small triangular guard processes occur at lip bases (Figs. 2E,F). Stoma 3–5 μm long; 0.4–0.8 lip region width, conspicuously divided into cheilostom, gymnostom, and stegostom, the latter more than two times as long as the gymnostom (Fig. 4F). No distinct dorsal denticle on the stegostom. Corpus cylindrical, muscular, distinctly delineated into the procorpus and metacarpus which both are extremely short, together only 0.5–0.9 times as long as the isthmus (Fig. 4E,F). Isthmus long, narrow, muscular. Basal bulb pyriform with striated transverse valves located anteriorly. Excretory pore usually opening at the level of the nerve ring. Deirid rarely visible with LM (noted in only two of 15 specimens), 3 to 5 annules posterior to excretory pore. Esophago-intestinal valve conoid, short. Rectum 11–15.5 μm long, 1–1.5 anal

body width, terminating as arcuate slit anus. Tail 18–23 μm long, subcylindrical, with 6–10 ventral annules and conoid-rounded tip (Fig. 4E,H). Phasmid located at 31–42.5% of the tail.

Reproductive system monodelphic, prodelphic, and dextral (Fig 4E,4G). Vulva one-half to two-thirds of the body length from the anterior end, extent of protrusion apparently depending on fixation and thus confounding measurement of vagina length. In three specimens, vagina 3–5 μm long, 0.2–0.3 vulval body width. Postvulval sac 8–15 μm long, 0.4–0.8 vulval body width. Spermatheca and oviduct each consisting of two rows of four cells. Uterus cells arranged in complex pattern: anteriorly with two pairs of cells, centrally with two dorsal rows of 7–8 cells and two ventral rows of 5–6 cells, posteriorly with one pair of cells adjacent to vagina and post-vulval sac. Ovary containing 8–18 oocytes at different developmental stages. Oviduct with two rows of cells, emerging dorsally between the junction of uterus and spermatheca, continuing into a single row and toward the distal end, single to double rows of 8–18 oocytes at different developmental stages.

Diagnosis

Nothacrobeles nanocorpus n. sp. is the shortest known species within the genus and has a short post-vulval sac (see Table 3). The annules are not punctated. The labial probolae are short and plate-like without prongs or tines, but with a circular rim that can be considered to include the basal ridge. The lip margins are smooth, and the guard processes are extremely small compared to those of all other *Nothacrobeles* species. The corpus is distinctly shorter than the isthmus.

Relationships

Nothacrobeles nanocorpus n. sp. is very similar to *N. spatulatus* n. sp., but *N. nanocorpus* n. sp. is even shorter and is distinguished from all known Cephalobidae by a corpus that is extremely short, both in absolute length and in relation to the isthmus. The labial probolae of *N. nanocorpus* n. sp. are concave and plate-like, while those of *N.*

spatulatus n. sp. are spatula-shaped. It is tempting to conclude that these two new species are each other's closest relatives within the genus, but this would be premature in the absence of other data, since it cannot be excluded that the resemblances between the two species resulted from parallel evolution toward small size or, conversely, that these similarities represent plesiomorphic characters.

Type locality

Nothacrobeles nanocarpus n. sp. was extracted from the same sample from which *N. spatulatus* n. sp. was isolated. This sample consisted of dark soil with abundant organic matter including needles and cones at the foot of a large pinyon pine, *Pinus edulis*, growing between rocks along a creek near Kissing Rocks, southwestern tip of the Sweeney Granite Mountain Desert Research Facility, California, 18 March 1998.

Type designations

Holotype slide 30046 is deposited in UCRNC. Seven female paratypes are deposited at UCRNC and eight at UCDNC.

Etymology

The species epithet is a compound of the Latin nouns *nanus* (dwarf) and *corpus* (body), since this new species has both a very short esophageal corpus and a very short body.

DISCUSSION

Nothacrobeles is a diverse genus with 11 clearly differentiated species (Table 3). The genus appears to be well adapted to desert habitats. For example, it has been recovered from sand near scattered vegetation on the edge of an ephemeral river (Shahina and De Ley, 1997), soil around *Acacia albida* on a riverbank (Rashid and Heyns, 1990), and sand around *Euphorbia* sp. in a stream bed (Allen and Noffsinger, 1971). However, it also may occur in agricultural (wheat) or garden (*Heterodendron* sp.) soil (Allen and Noffsinger, 1971). Our surveys found representatives in desert dominated with *Artemisia*

tridentata, Joshua trees (*Yucca brevifolia*), creosote, and also from dark organic soil of decomposing *P. edulis* needles and cones accumulating at the base of trees growing among granite rock outcrops.

Nothacrobeles spp. share a number of unusual features of the lip region, but many of these are further modified at the species level. The three tubular projections in *N. triniglarus* n. sp. seem to be unique to this species, but may be homologous to the adoral "triangular prorhabdion plates" on the inner base of each labial probola of some previously studied Cephalobidae (e. g., *Acrobeles complexus*, *Zeldia punctata*, and *N. subtilis* [Sauer et al., 1979]). None of these species, however, show the tiny pores at the end of the projections characteristic of *N. triniglarus* n. sp. Morphologically and morphometrically, *N. spatulatus* n. sp. and *N. nanocarpus* n. sp. are similar and share a similar habitat; it is therefore tempting to speculate that their distinctive lip regions may be specialized for different food preferences. In both species, light microscope observations suggest the cephalic probolae may be capable of folding or closing over the oral opening, and the shape and position of probolae, as seen in SEM, suggest such closure might be most complete in *N. nanocarpus* n. sp. However, a possible relationship between lip structure and feeding biology needs careful evaluation and testing in living specimens. Although a basal ridge has been considered a character shared among *Nothacrobeles* spp., the discovery of *N. spatulatus* n. sp., which lacks this character, necessitates a redefinition of *Nothacrobeles* to accommodate the presence or absence of a basal ridge. Unlike many other Cephalobidae, *N. subtilis*, *N. maximus* Allen and Noffsinger, 1971, *N. triniglarus* n. sp., *N. spatulatus* n. sp., and *N. nanocarpus* n. sp. do not have tangential ridges connecting the labial probolae. In all these species, there are two narrow radial ridges inserted between the lip (secondary) axils and labial probolae, and these terminate anteriorly in horizontal tooth-like processes.

Nothacrobeles spatulatus n. sp. and *N. nanocarpus* n. sp. do not fit the most recent

genus diagnosis (Shahina and De Ley, 1997) since neither species has bifurcated labial probolae and, as noted, *N. spatulatus* n. sp. lacks a basal ridge. However, these two species fit even more poorly into other genera. *Cervidellus* Thorne, 1937 currently contains species with bifurcate and relatively elongate labial probolae, distinct tines on the lip margins, and much less pronounced, flattened lip margins. *Zeldia* Thorne, 1937, on the other hand, contains species with a single guard process per primary axil and typically with a long, slender esophageal corpus. Therefore, *N. spatulatus* n. sp. and *N. nanocorpus* n. sp. cannot be placed in *Cervidellus* or *Zeldia*. It is not justified to propose a new genus for *N. spatulatus* n. sp. and *N. nanocorpus* n. sp. defined by lack of bifurcation of the labial probolae because the expression of bifurcation is ambiguous in additional species. For example, the prongs of *N. triniglarus* n. sp. are no larger or thicker than tines, and the same probably is true for *N. lunensis* Shahina & De Ley, 1997.

The genus diagnosis of *Nothacrobeles* is emended as follows:

Nothacrobeles Allen & Noffsinger, 1971

Emended diagnosis

Cephalobidae. Body small to relatively large (L = 0.2–0.9 mm). Cuticle appearing single or double, with small to large annules, with or without longitudinal striae and (or) punctations. Lateral field with two to six incisures, often with undulating internal pseudolines if cuticle appears double. Labial probolae short to long, bifurcated except in *N. spatulatus* n. sp. and *N. nanocorpus* n. sp.; probolae with or without tines. Outer rim of probolae usually continuing into a prominent basal ridge and swollen toward the lips. Lips in pairs, low, demarcating deep primary axils and shallow secondary axils. Primary axils with two triangular guard processes larger than any neighboring tines. Radial ridges each with one tooth-like process. Stoma cephalobid with transversely flattened cheilorhabdia. Esophageal corpus cylindrical to fusiform, not distinctly swollen posteriorly, lumen sometimes expanded af-

ter fixation. Excretory pore at or near level of isthmus. Female reproductive system cephalobid, spermatheca and postvulval sac small to large. Vulva not protruding, rarely sunken or shifted subventrally. Males with three pairs of preanal papillae, five pairs of caudal papillae, and one median papilla on the precloacal lip. Tails in both sexes conical, subcylindrical, usually with acute tip. Male phasmids level with or anterior to the anteriormost caudal papillae.

Type species

Nothacrobeles sheri Allen & Noffsinger, 1971

Other species

N. acrobeles (Andrássy, 1967) Allen & Noffsinger, 1971 = *Zeldia acrobeles* Andrásy, 1967

N. lepidus Allen & Noffsinger, 1971

N. lunensis Shahina & De Ley, 1997

N. maximus Allen & Noffsinger, 1971

N. nanocorpus n. sp.

N. prominens (Andrássy, 1964) Andrásy, 1984 = *Acrobeles prominens* Andrásy, 1964

N. scaphovulva (Rashid & Heyns, 1990) Shahina & De Ley, 1997 = *Namibinema scaphovulva* (Rashid & Heyns, 1990)

N. spatulatus n. sp.

N. subtilis (Allen & Noffsinger, 1971) = *Nothacrobeles subtilis* Allen & Noffsinger, 1971

N. triniglarus n. sp.

Species inquirendae

N. distinctus (Kirjanova, 1951) Shahina & De Ley, 1997 = *Acrobeles distinctus* Kirjanova, 1951 = *Acrobeles innoxius* Kirjanova, 1951

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