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A New Species and New Combinations of *Brevitobrilus* Tsalolikhin, 1981 (Nematoda: Tobrilidae) from Spain

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Abstract: A new species of *Brevitobrilus* Tsalolikhin, *Brevitobrilus montanus* n. sp., found in high mountain lakes and rivers in Granada, Spain, is described. Additional data on *Brevitobrilus granatensis* (Ocaña & Zullini, 1988) n. comb. are provided. *Brevitobrilus montanus* n. sp. is characterized by moderate size (1.3–1.8 mm), amphid aperture width one-quarter the head width, subterminal seta distance from terminus four times the width of the terminal end of the tail, and supplements S5 and S6 separated. *Tobrilus granatensis* Ocaña & Zullini, 1988; *Tobrilus sardus* Vinciguerra & Zullini, 1991; and *Tobrilus siculus* Vinciguerra & Zullini, 1991 are all transferred to *Brevitobrilus*. Differences among the 13 species of *Brevitobrilus* are discussed.

Key words: *Brevitobrilus,* description, nematode, new species, taxonomy, *Tobrilus*

Tobrilus Andrássy, 1959 s.l. (=*Tobrilus* Bastian, 1865) (2), one of the larger nematode genera, has been studied by several taxonomists in attempts to clarify its taxonomy (3,5,7,9). Tsalolikhin (11,12) organized 72 species of *Tobrilus* into 13 different genera. Eleven species considered *species inquirendae* were not considered. The authors consider that this taxonomic grouping represents a positive step forward in clarifying affinities among *Tobrilus* s.l. Tsalolikhin (12) characterized *Brevitobrilus* spp. as having one tooth in each postbuccal chamber, a relatively small and slightly curved spicule, six supplements with a spherical internal portion, and supplements closest to the anus slightly smaller than the rest. This definition corresponded to Schiemer's group 3 (9). Since Tsalolikhin's revision (11), other *Tobrilus* spp. have described that fit the *Brevitobrilus* concept. The goals of this paper are to describe a new *Brevitobrilus* sp. from Spain, transfer additional *Tobrilus* spp. to *Brevitobrilus,* and provide additional data on *B. granatensis* n. comb.

Materials and Methods

Nematodes were extracted with modified Baermann funnels, fixed in 4% acetic formalin, and mounted in anhydrous glycerine by means of a modification of the Seinhorst method. Measurements are given as ranges, followed by means and standard deviations in parentheses.

Systematics

Brevitobrilus montanus n. sp.
(Figs. 1–2)

Description

Holotype (female): L = 1.7 mm; a = 23.2; b = 5.2; c = 9; c′ = 5.3; V = 44.2%.

Allotype (male): L = 1.3 mm; a = 32.3; b = 4.8; c = 10; c′ = 4.3; spicule length = 33 μm; supplements = 6.

Females (n = 17): L = 1.3–1.8 mm (1.6 ± 0.2); a = 23.2–33.5 (28.5 ± 2.9); b = 4.6–5.8 (5.2 ± 0.4); c = 7.9–10.4 (9.1 ± 0.7); c′ = 4.6–6.5 (5.4 ± 0.6); V = 39.3–48.3 (43.4 ± 2.3).

Males (n = 18): L = 1.3–1.9 mm (1.6 ± 0.2); a = 24.7–41.1 (33 ± 6.2); b = 4.8–6.6 (5.7 ± 0.5); c = 10–17.9 (13.5 ± 2.1); c′ = 3.2–4.5 (3.8 ± 0.5); spicule length = 28–38 (33.8 ± 3.2) μm; supplements = 6–7.

Cuticle thin, subcuticle sometimes faintly annulated, in total 1.2–1.5 μm thick at anterior end, 1.6–1.8 μm at base of esophagus, 1.9–2.3 μm at midbody, 1.2–1.6 μm at anus level. Body with scattered setae 3.5–6.2 μm long. Head outline rounded, not offset, with six distinct lips, each with a prominent papilla 2.5–4 μm long. Cephalic setae in outer circle 5.5–7.5 μm long and shorter setae in inner circle

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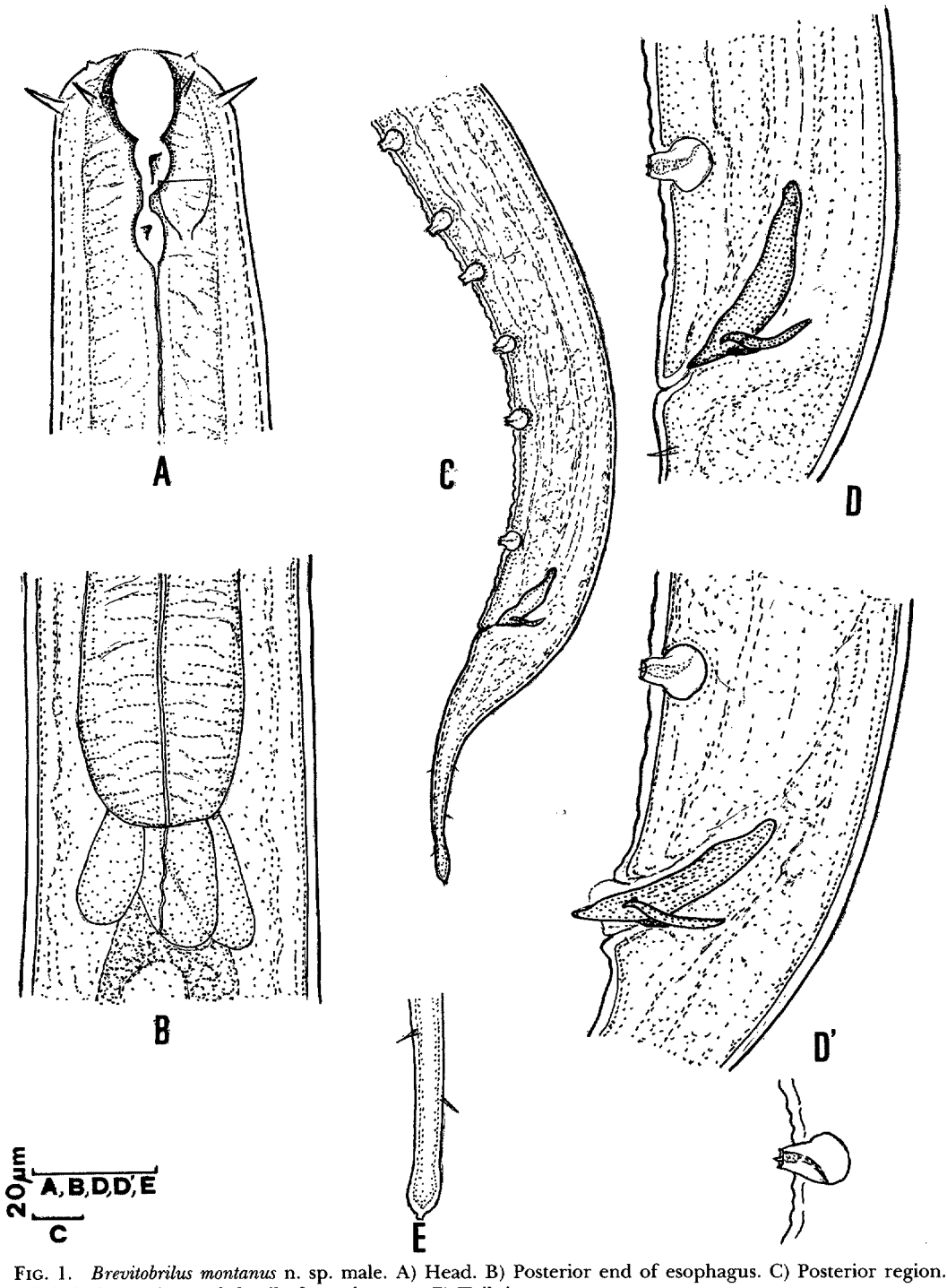

FIG. 1. *Brevitobrilus montanus* n. sp. male. A) Head. B) Posterior end of esophagus. C) Posterior region. D-D') Spicular region and detail of supplements. E) Tail tip.

2.7–4 µm. Cephalic diameter 24.2–30.3 µm. Anterior part of buccal cavity 12–14 µm long, 8.2–10.3 µm wide. Teeth in two pockets, apices 9–11.5 µm apart. Amphid aperture 6.3–7.5 µm wide, 19–22.5 µm from anterior end. Pharynx 262–341 µm

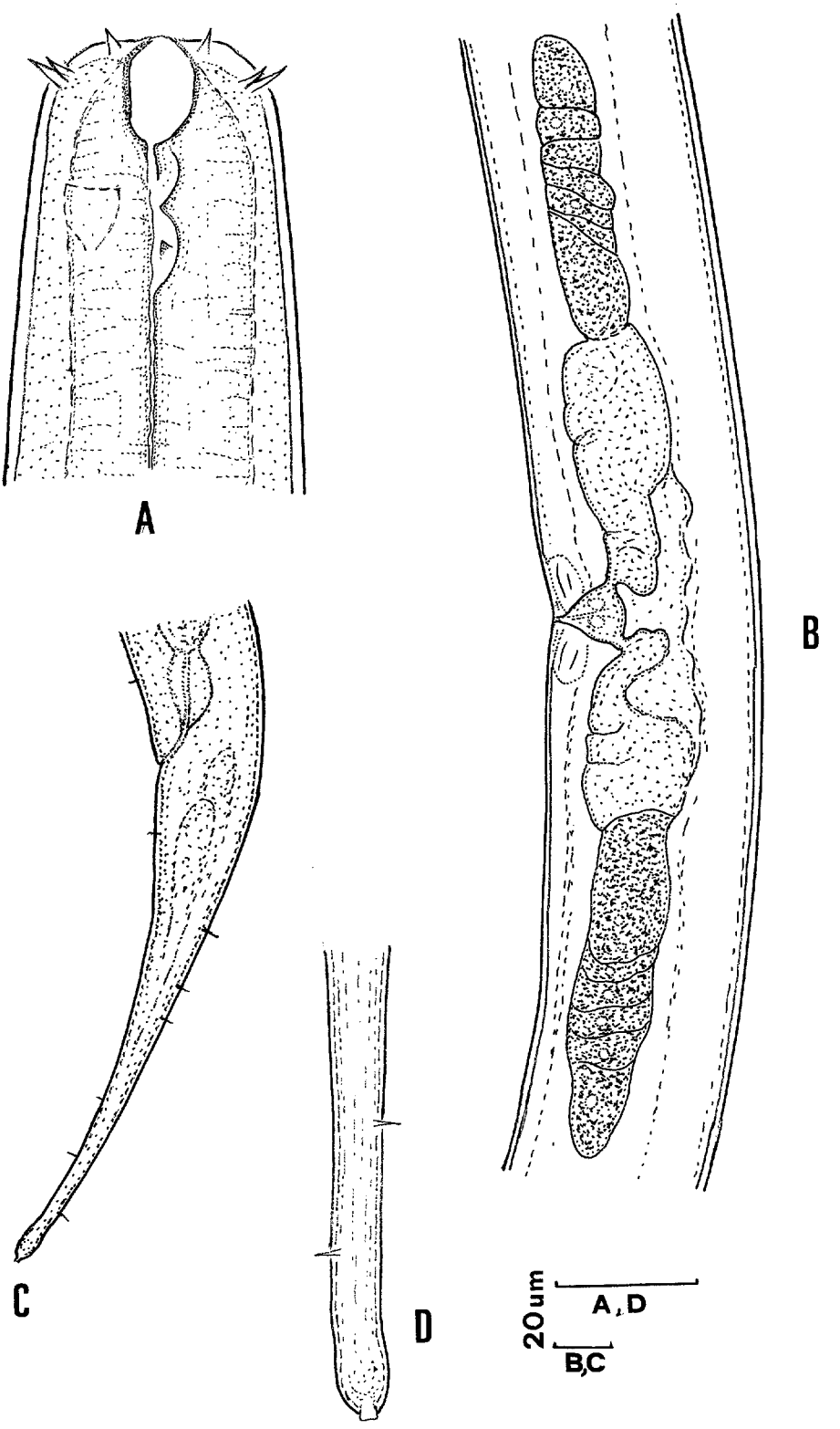

FIG. 2. *Brevitobrilus montanus* n. sp. female. A) Head. B) Vulva region. C) Tail. D) Tail tip.

(290 ± 29) long, cardia 19.5–26 μm long (6–8% of esophagus length), cardia with three glandular cells, nerve ring about one-third esophagus length posterior to stoma. Small, finely granulated, crystalloid bodies (1.5–3.2 μm in diam.) in pseudocoel, more frequent in posterior half of body.

Female gonads paired, lengths equal. Uterus length 180–237 μm. Vulva transverse, vaginal length about 1/3–1/2 body diameter, musculature developed but not clearly striated. One egg seen in uterus (59 × 41 mm). Vulva-anus distance 4–4.5 times tail length. Female tail length 148–205 μm (180 ± 19), rectum 32–38 μm long.

Two male gonads, length 220–313 μm (276 ± 32). Ductus ejaculatorius weakly muscled. Junction of ductus ejaculatorius-vas deferens with narrow constriction. Six precloacal supplements approximately same diameter as globular internal part (the smallest 7.5 μm [S5 and especially S6]; the largest 11 μm [S1–S4]). Each supplement externally terminating in crown of four projections and central spine. Ventral cuticle in supplement region with papilliform ripples: 8–10 between S1–S2, 4–5 (S2–S3), 5–6 (S3–S4), 6 (S4–S5), 7–8 (S5–S6), and 4 (S6-anus). Distances between supplements variable (n = 16 males): S1–S2, 40 ± 4.9; S2–S3, 28 ± 4.6; S3–S4, 34 ± 5.2; S4–S5, 33 ± 4, S5–S6, 47.6 ± 5.6; S6-anus, 33.3 ± 4. Two spicules, length 28–38 μm (33.8 ± 3.2); bifurcate gubernaculum, length 12–19 μm (14 ± 2). Male tail 119 ± 10 μm long. Distance of subterminal seta to tail terminus four times width of tail tip.

Type locality

Holotype, allotype, and 22 paratypes in 0.5–2 m-deep high mountain lakes in Granada, Spain (Virgen Inferior, Aquas Verdes, Río Seco Inferior, Laguna de las Yeguas), at altitudes between 2,850–3,050 meters above sea level, pH 6.2–8.7, temperature 5–20.9 °C, oxygen concentrations 5.2–8.2 mg/liter. Thirteen additional paratypes from Tunca and Genil riverbeds, Granada, Spain, exposed to organic pollution, altitudes 450–840 meters above sea level, pH values 6.8–8, temperature 12.5–19.6 °C, oxygen concentrations 5.7–10 mg/liter.

Type specimens

Holotype, allotype, and paratypes from lakes in Granada, Spain, slide numbers 75, 78–81, 83, 84, 86, and 87, deposited in the Departamento de Biología Animal y Ecología, Facultad de Ciencias, Universidad de Granada, Granada, Spain. Other paratypes, slide numbers 76, 77, 82, and 85, deposited in the Dipartimento di Biologia, Sezione di Ecologia, Universitá di Milano, Italy. Paratypes from Granada rivers, slide numbers 88–90 and 92, deposited in the Departamento de Biología Animal y Ecología, Facultad de Ciencias, Universidad de Granada, Granada (Spain), and one slide deposited in the U.S. Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland

Diagnosis

Brevitobrilus montanus n. sp. is characterized by body length (1.3–1.8 mm), cephalic diameter (24–30 μm), length of longer cephalic setae (5.5–7.5 μm), and distance between buccal denticles (9–11.5 μm). Amphid aperture width one-fourth the corresponding head width. Subterminal seta distance from tail tip four times width of terminal tail portion. Supplements S5–S6 widely separated.

Relationships

Brevitobrilus montanus n. sp. is closely related to six species currently placed in *Brevitobrilus* or *Tobrilus* (Table 1). It differs from *Brevitobrilus graciloides* (Daday, 1908) Tsalolikhin, 1981 (15) in having shorter spicules (28–38 μm vs. 35–49 μm) and S6 is not found in the spicular area, *Brevitobrilus africanus* (Zullini, 1988) Tsalolikhin, 1981 syn *Brevitobrilus graciloides* (Daday, 1908) Tsalolikhin, 1981 (see 13). It differs from *Tobrilus granatensis* Ocaña & Zullini, 1988 in that S5 and S6 are not smaller than the other supplements and because there are no subterminal setae next to tail end,

TABLE 1. Morphometrics of *Brevitobrilus* spp.

	Ml	Ssd/Ted	Gds	S6-A	Sl	Lcs	A/Hd	c′
B. confusus (Khera, 1975) Tsalolikhin, 1991 (6)	1.1	?	S1–S2	47	22.5	3	?	4.2
B. consimiloides (Altherr, 1965) Tsalolikhin, 1991 (1)	1.7	3	S5–S6	30	30	6–7	1/5–1/6	4
B. findeneggi (Schiemer, 1971) Tsalolikhin, 1991 (9)	1.2	1	S3–S4	15	26	2.9	?	2.4
B. graciloides (Daday, 1908) Tsalolikhin, 1991 (11)	1.9	3	S5–S6	35–48	49–51	4–5	1/6	5.3–6.5
B. granatensis (Ocaña & Zullini, 1988) n. comb. (8)	1.5–2.3	1	S3–S4	13–21	32–46	6–7	1/5	3.4–4.9
B. kenyensis (Tsalolikhin, 1992) (13)	1.8	4–5	S5–S6	20	48	6	1/6–1/7	6.2
B. malayanus (Schneider, 1938) Tsalolikhin, 1991 (11)	1–1.3	?	S1–S2	33	28–32	6.5	1/5	5
B. sardus (Vinciguerra & Zullini, 1991) n. comb. (14)	1–1.4	1.3	S3–S4;S1–S2	9–15	23–31	5–6	1/6	2.5–4.3
B. sexsetiferus (Khera, 1975) Tsalolikhin, 1991 (6)	0.9–1.4	?	S5–S6	29	20–22	5–6	1/8(?)	4
B. siculus (Vinciguerra & Zullini, 1991) n. comb. (14)	1.8–2.1	1.3	S3–S4	15–20	40–46	6–7	1/5	2.5–3.3
B. stefanskii (Micoletzky, 1925) Tsalolikhin, 1991 (4)	1.3–1.6	1.5	S5–S6	42–48	35	5–6	1/5	3.4–3.6
B. vibratus (Sukul, 1967) Tsalolikhin, 1991 (10)	0.9–1	?	Sd	14.5	15	4.5	?	4
B. montanus n. sp.	1.3–1.8	4	S5–S6	28–36	28–38	6.5–7.5	1/4	3.5–4.5

Ml = Male length (mm); Ssd/Ted = Subterminal setae distance/tail end diameter (terminal seta is less than one tail end diameter from the tail tip, and subterminal seta is equal to or more than one tail end diameter from the tail tip); Gds = Greatest distance between supplements; S6-A = Supplement 6-anus distance (µm); Sl = Spicule length (µm); Lcs = Longest cephalic setae (µm); A/Hd = Amphid/head diameter; Sd = all to similar distance; c′ = tail length/anal body width; (number) = number of the reference in the literature cited.

and because S6 is not in the spicular region (8). It differs from *Tobrilus sardus* Vinciguerra & Zullini, 1991 in that *Brevitobrilus montanus* is larger (1.3–1.8 mm vs. 1.0–1.4 mm), the amphid has a wider diameter (1/4 vs. 1/6 the head diameter) and because S6 is not distinctly smaller than S1–S5 (14). It differs from *Tobrilus siculus* Vinciguerra & Zullini, 1991 in that S6 is not located at anus level and the subterminal setae are farther away from the tail end (14). It differs from *Brevitobrilus stefanskii* (Micoletzky, 1925) Tsalolikhin, 1981 in that the cephalic setae of the outer circle are longer (6.5–7.5 µm vs. 5–6 µm), subterminal setae are far removed from the tail end, and there is no muscular constriction between the vas deferens and ductus ejaculator at or slightly anterior to the level of S1 (4). Finally, it differs from *Brevitobrilus malayanus* (Schneider, 1937) Tsalolikhin, 1981 in larger amphids, different buccal cavity, and larger S6 (11).

Discussion

Currently, eight species are placed in *Brevitobrilus* Tsalolikhin (12). An additional three species described since 1981 as *Tobrilus* spp. fit the diagnosis of *Brevitobrilus* and therefore are transferred as follows: *Brevitobrilus granatensis* (Ocaña & Zullini, 1988) n. comb. (=*Tobrilus granatensis* Ocaña & Zullini, 1988); *Brevitobrilus sardus* (Vinciguerra & Zullini, 1991) n. comb. (=*Tobrilus sardus* Vinciguerra & Zullini, 1991; and *Brevitobrilus siculus* (Vinciguerra & Zullini, 1991) n. comb. (=*Tobrilus siculus* Vinciguerra & Zullini, 1991).

Brevitobrilus granatensis (Ocaña & Zullini, 1988) was found in the Genil and Fardes Rivers, Granada, Spain, in riverbed sediment exposed to organic pollution. Morphological and anatomical features coincide entirely with those described to date (8), except that the vulva is more anterior (V = 41–46%).

This species appears to be a characteristic inhabitant of subterranean waters that outcrop at the surface.

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