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Description of *Acromoldavicus xerophilus* n. sp. (Nematoda, Rhabditida, Elaphonematidae) from the southern Iberian Peninsula, including a key to species of the genus

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Research Paper

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Abstract

A new species of the genus *Acromoldavicus* is described from coastal sand dunes and sandy soil in the southeast of the Iberian Peninsula. *Acromoldavicus xerophilus* n. sp. is characterized by its 557–700 µm body length, cuticle tessellated, lip region with three pairs of expanded lips bearing a large labial expansion, primary axils bearing guard processes with two different morphology, secondary axils lacking guard processes, stoma short and tubular with prostegostom bearing prominent rhabdia directed towards the stoma lumen, female reproductive system monodelphic-prodelphic, post-vulval sac 0.6–0.9 times body diameter, rectum very large, female tail short with biacute terminus and males unknown. The description, light micrographs, scanning electron microscope images, illustrations, and molecular analyses are provided. Molecular analyses (based on 18S and 28S rDNA) revealed its relationship with some species of the genera *Cephalobus* (18S tree), *Nothacrobeles*, *Paracrobeles*, and *Spinocephalus* (28S tree). Keys to species identification of this genus are also included.

Introduction

Nesterov (1970) proposed the genus *Acromoldavicus* Nesterov, 1970 to accommodate the species previously described as *Acrobeloides skrjabini* by Nesterov and Lisetskaya (1965). Also, this author placed this genus in the subfamily Acrobelinae Thorne, 1937 within the family Cephalobidae Filipjev, 1934. Thereafter, Andrássy (1976) proposed the new subfamily Kirjanoviinae Andrássy, 1976 within the family Cephalobidae and transferred the genus *Acromoldavicus* to this new subfamily. Later, Karegar *et al.* (1997) reported several morphological similarities between *Acromoldavicus* and *Elaphonema* Heyns, 1962 and therefore transferred the subfamily Kirjanoviinae to the family Elaphonematidae, which was previously suggested by Nesterov (1979).

Currently, the genus *Acromoldavicus* contains two valid species: *A. mojavicus* Baldwin, De Ley, Mundo-Ocampo, De Ley, Nadler and Gebre, 2001, which was only described in sandy soil from the Mohave Desert by Baldwin *et al.* (2001), and *A. skrjabini* (Nesterov and Lisetskaya, 1965) Nesterov, 1970, which has been found in agricultural soils from several countries: Nesterov and Lisetskaya (1965) and Nesterov (1970, 1979) in Moldavia, Boström (1989, 1992) in Greece, Karegar *et al.* (1997) in Iran and Spain, Susulovsky *et al.* (2001) in Ukraine and Israel, Iliev *et al.* (2003) in Bulgaria, and recently in Spain, Abolafia *et al.* (2021). The genus *Acromoldavicus* Nesterov, 1970 is an infrequent taxon belonging to the infraorder Cephalobomorpha De Ley and Blaxter, 2002, superfamily Cephaloboidea Filipjev, 1934, family Elaphonematidae Heyns, 1962.

Acromoldavicus is characterized by having cuticle tessellated, lip region with modified lips, flattened labial probolae, primary axils broad with triangular guard processes, secondary axils narrow lacking guard processes, stoma short and tubular with reduced rhabdia, pharynx with basal bulb bearing well developed and striated transverse valves, female system monodelphic-prodelphic with vulva not prominent and rectum very long, and males infrequent.

In this study, a new species of the genus *Acromoldavicus* is described from two localities of the southeast Iberian Peninsula found in sand dunes and sandy soil using morphological, morphometric, and molecular characterization. Additionally, a key for the species identification of the genus *Acromoldavicus* is provided.

Material and methods

Nematode extraction and processing

Soil samples were collected from natural areas (Figure 1) and processed following several nematological techniques, which were described in detail by Abolafia (2022). The nematodes

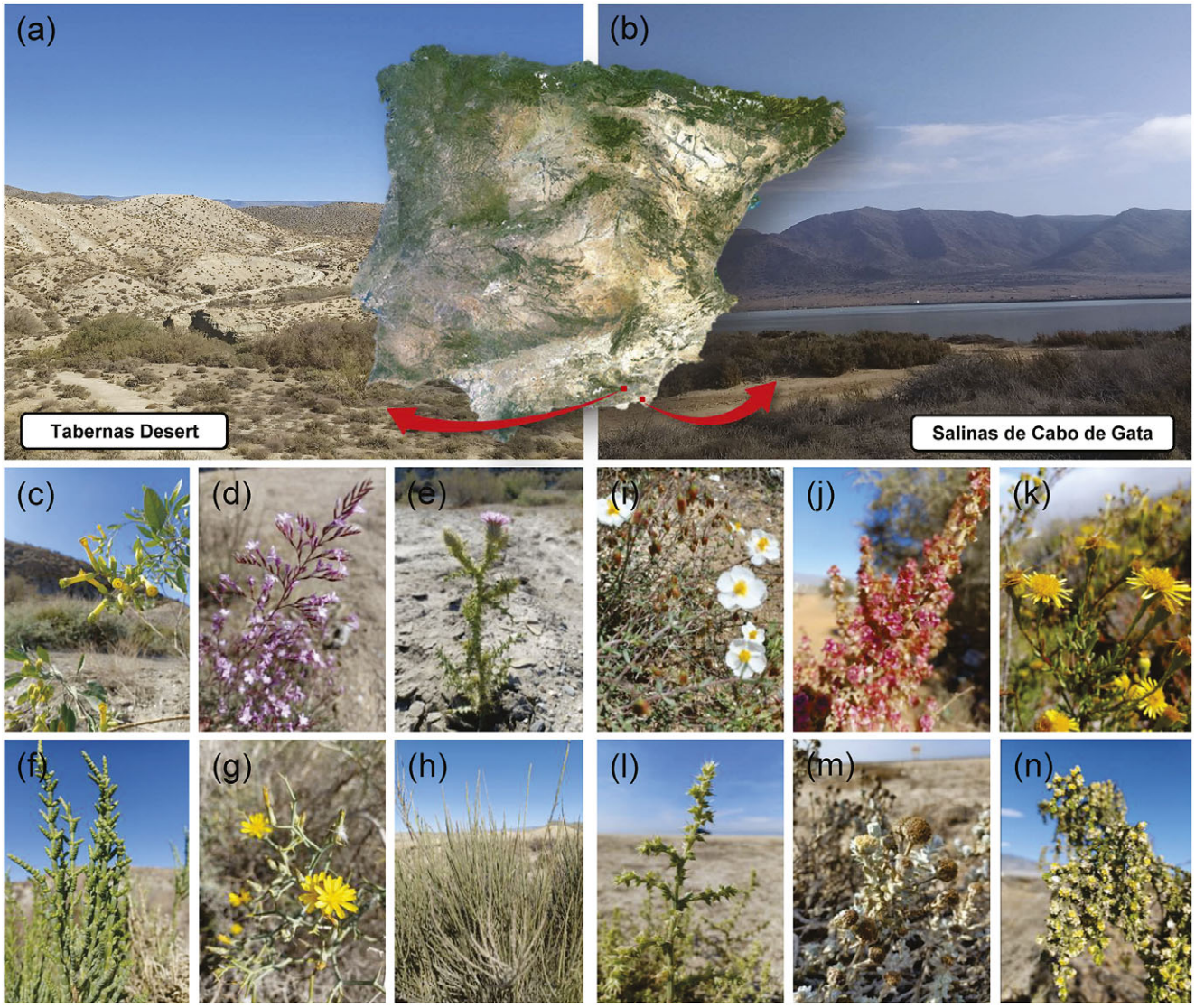

Figure 1. Map of Spain showing landscape views of the study area: (a) Tabernas Desert and (b) Salinas de Cabo de Gata; and xerophilic vegetation associated with *A. xerophilus* n. sp.: (c) *Nicotiana glauca* Graham; (d) *Limonium insigne* (Coss.) Kuntze; (e) *Carduus tenuiflorus* Curtis; (f) *Arthrocnemum macrostachyum* (Moric.) C. Koch.; (g) *Launaea arborescens* (Batt.) Murb; (h) *Ephedra fragilis* Desf; (i) *Helianthemum almeriense* Pau; (j) *Caroxylon vermiculatum* (L.) Akhani and Roalson; (k) *Limbarda crithmoides* (L.) Dumort; (l): *Salsola kali* L.; (m) *Onthatus maritimus* Hoffmanns and Link; (n) *Thymelaea hirsuta* (L.) Endl.

were extracted from sandy soil using the modified Baermann's (1917) funnel technique, killed by heat, and fixed in a 4% formaldehyde solution (except for specimens used for molecular analyses, which were not fixed). The nematodes were processed to anhydrous glycerine according to Siddiqi's (1964) method, using lactophenol-glycerine solutions, and were permanently mounted on glass microscope slides with the glycerine-paraffin method (de Maeseneer and d'Herde 1963) somewhat modified using hot liquid paraffin.

Light microscopy (LM)

Photomicrographs were taken with a Nikon Eclipse 80i (Nikon, Tokyo, Japan) microscope provided with differential interference contrast (DIC) optics and a Euromex sCEMX-6 camera (Euromex Microscopen BV, Arnhem, The Netherlands). The micrographs were edited using Adobe® Photoshop® CS (Adobe Inc., San José, California, USA), and figures were mounted using Microsoft® PowerPoint® (Microsoft Corporation, Redmond, Washington, USA). Demanian indices (de Man 1881) and other ratios were calculated. The terminology used for the morphology of stoma and spicules/gubernaculum follows the proposals by De Ley *et al.* (1995) and Abolafia and Peña-Santiago (2017), respectively.

Scanning electron microscopy (SEM)

Specimens preserved in glycerin were selected for observation under SEM according to Abolafia (2015). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol-acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope (5 kV) (Zeiss, Oberkochen, Germany).

DNA extraction, PCR, and sequencing

Specimens were processed according the Abolafia and Ruiz-Cuenca (2021) methodology. Nematode DNA was extracted from single fresh specimens using the proteinase K protocol and PCR assays as

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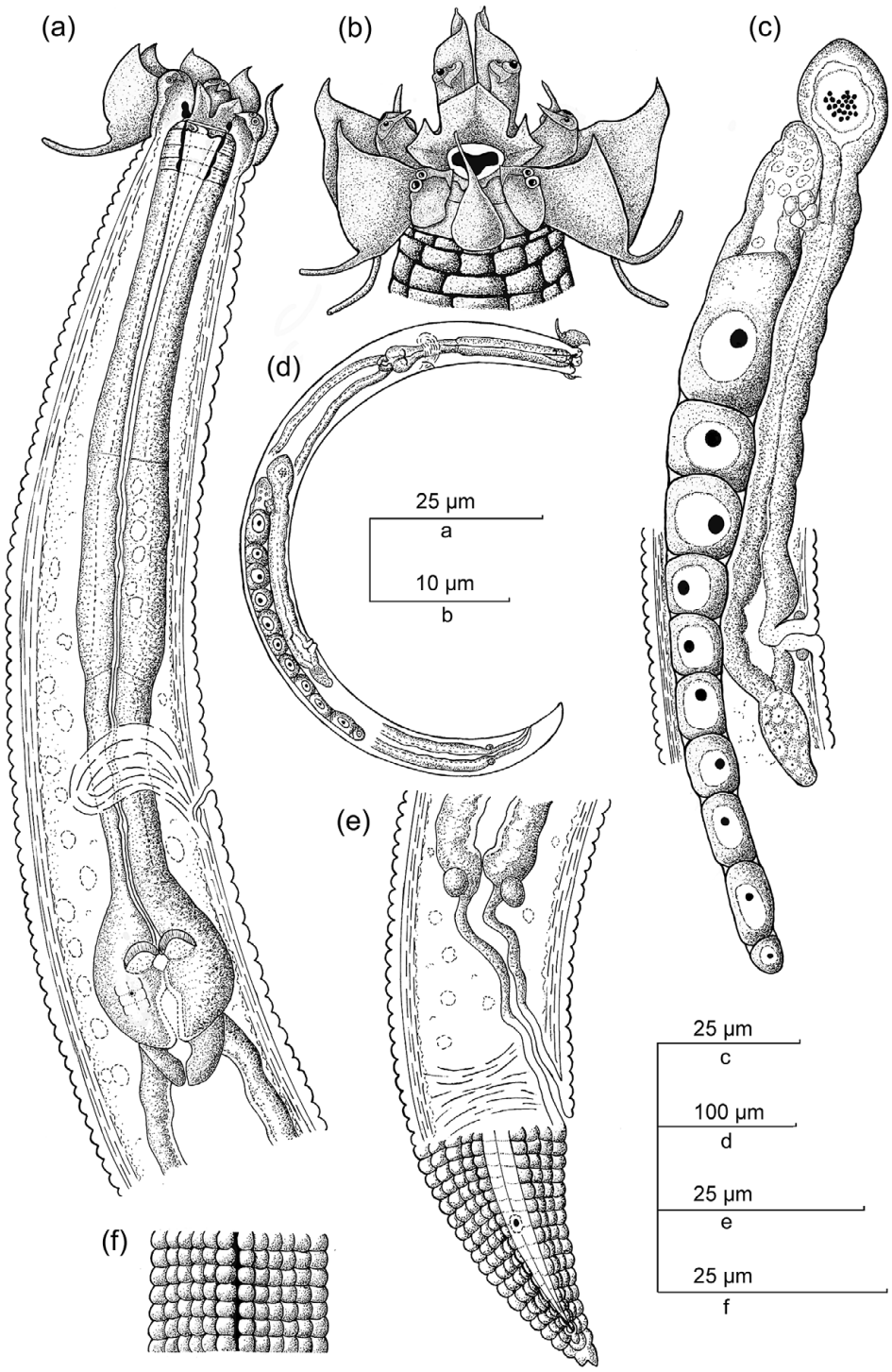

Figure 2. *Acromoldavicus xerophilus* n. sp. (female). (a) neck; (b) lip region in ventral view; (c) reproductive system; (d) entire body; (e) tail; (f) lateral field.

described Castillo *et al.* (2003), albeit somewhat modified (Archidona-Yuste *et al.* 2016). The specimens were cut into small pieces using a sterilized dental needle on a clean slide with 18 ml of TE (Tris-EDTA) buffer [10 mM Tris-Cl (tris hydrochloride) + 0.5 mM EDTA (ethylene-diamine-tetra acetic acid); pH=9.0], transferred to a microtube, adding 2 µl proteinase K (700 µg/ml) (Roche, Basel, Switzerland), and stored to -80ºC within 15 min (for several days). The microtubes were incubated at 65°C (1 h), then at 95°C (15 min). For DNA amplification, 3 µl of the extracted DNA was transferred to a microtube containing: 0.6 µl of each primer (10 mM), 3 µl Master Mix Taq DNA Polymerase (5x Hot FirePol Blend Master Mix, Solis BioDyne, Tartu, Estonia), and double distilled water (ddH2O) to a final volume of 20 µl. The primers used for amplification of the region of 18S rRNA gene were the forward primer 988F (5′-CTCAAAGATTAAGCCATGC-3′) and the reverse primer 1912R (5′-TTTACGGTCAGAACTAGGG-3′)

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Figure 3. *Acromoldavicus xerophilus* n. sp. (light microscopy, female). (a) lip region; (b) reproductive system; (c) lateral field at deirid level (arrow); (d) uterine egg; (e) spermatheca with spheroid structure containing small round corpuscles (arrow); (f) entire body (black arrow pointing to the vulva, white arrow pointing to the anus); (g) lateral field.

(Holterman *et al.* 2006). The primers used for amplification of the D2-D3 region of 28S rRNA gene were the D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and the D3B (5'-TCGGAAGGAACCAGCTACTA-3') primers (Nunn 1992; De Ley *et al.* 1999). PCR cycle conditions were as follows: one cycle of 94°C for 15 min., followed by 35 cycles of 94°C for 45 s + annealing temperature of 55°C for 45 s + 72°C for 45 s, and finally one cycle of 72°C for 5 min. After DNA amplification, 5µl of product was loaded on a 1% agarose gel in 0.5% Tris-acetate-EDTA (40 mM Tris, 20 mM glacial acetic acid and 2 mM EDTA; pH=8) to verify the amplification using an electrophoresis system (Labnet Gel XL Ultra V-2, Progen Scientific, London, UK). The bands with DNA products were stained with SYBR Green I (10,000x concentrate in DMSO; Invitrogen, Waltham, USA) and

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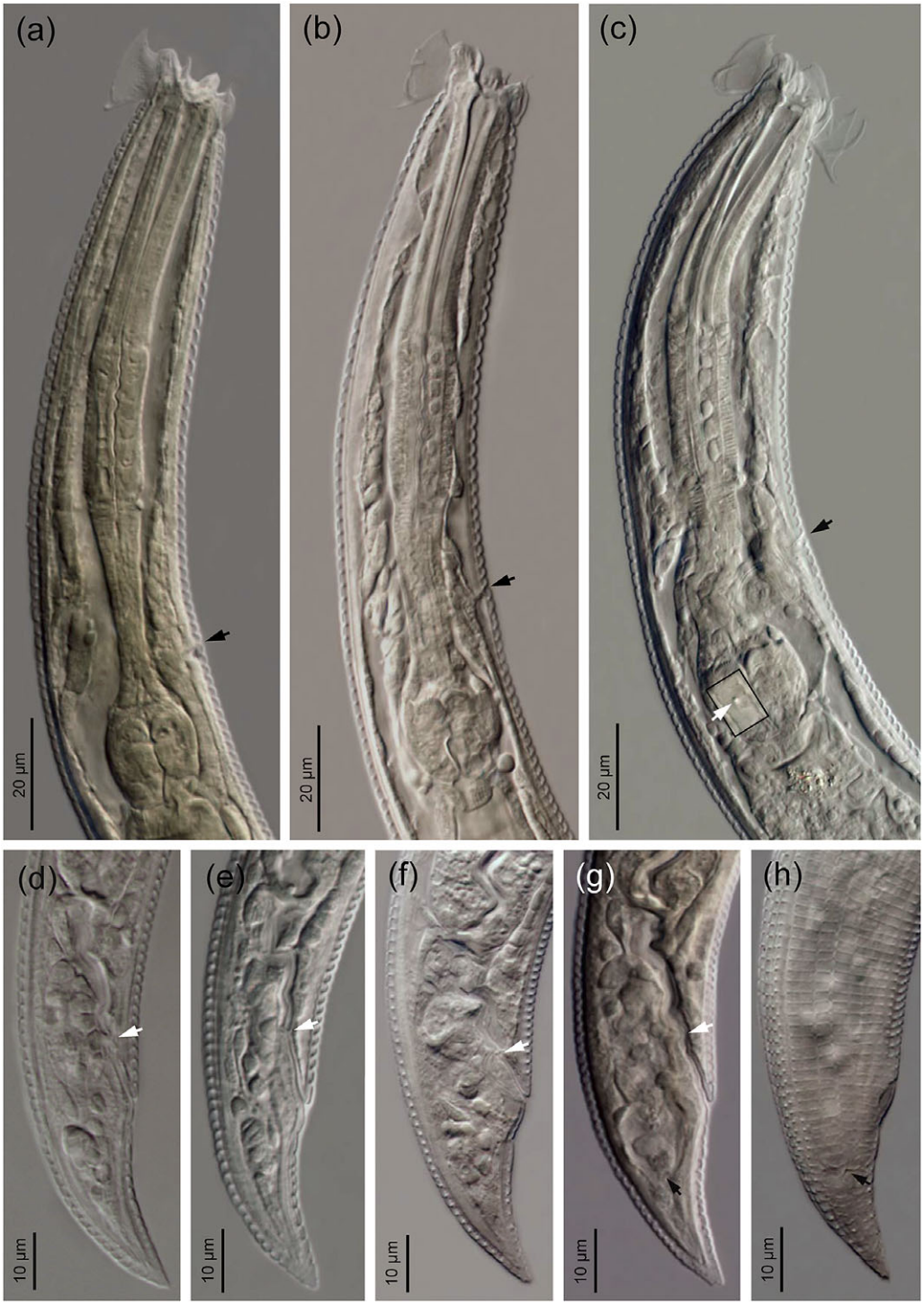

Figure 4. *Acromoldavicus xerophilus* n. sp. (light microscopy, female). (a–c) neck region showing the morphological variation of the isthmus and position of the excretory pore (black arrow pointing to the excretory pore, white arrow pointing to the deirid); (d–h) posterior region showing the morphological variation of the tail (arrow pointing to the cellular-cuticular limit at the rectum, black arrow pointing to the phasmid).

the DNA-loading buffer 6x (GeneON, Ludwigshafen, Germany). The sequencing reactions of the PCR products were performed at Sistemas Genómicos (Paterna, Valencia, Spain) according to the Sanger *et al.* (1977) method. The sequences obtained were submitted to the GenBank database. The obtained sequences were deposited in NCBI GenBank under accession numbers PP069740 and PP069741 (18S rDNA) and PP069742 (28S rDNA).

Phylogenetic analyses

For phylogenetic relationships, analyses were based on 18S and 28S ribosomal DNA (rDNA) fragments. The newly obtained sequences were manually edited using Chromas 2.6.6 (Technelysium, Queensland, Australia) and aligned with another 18S or 28S rDNA sequences available in GenBank using the ClustalW (Thompson

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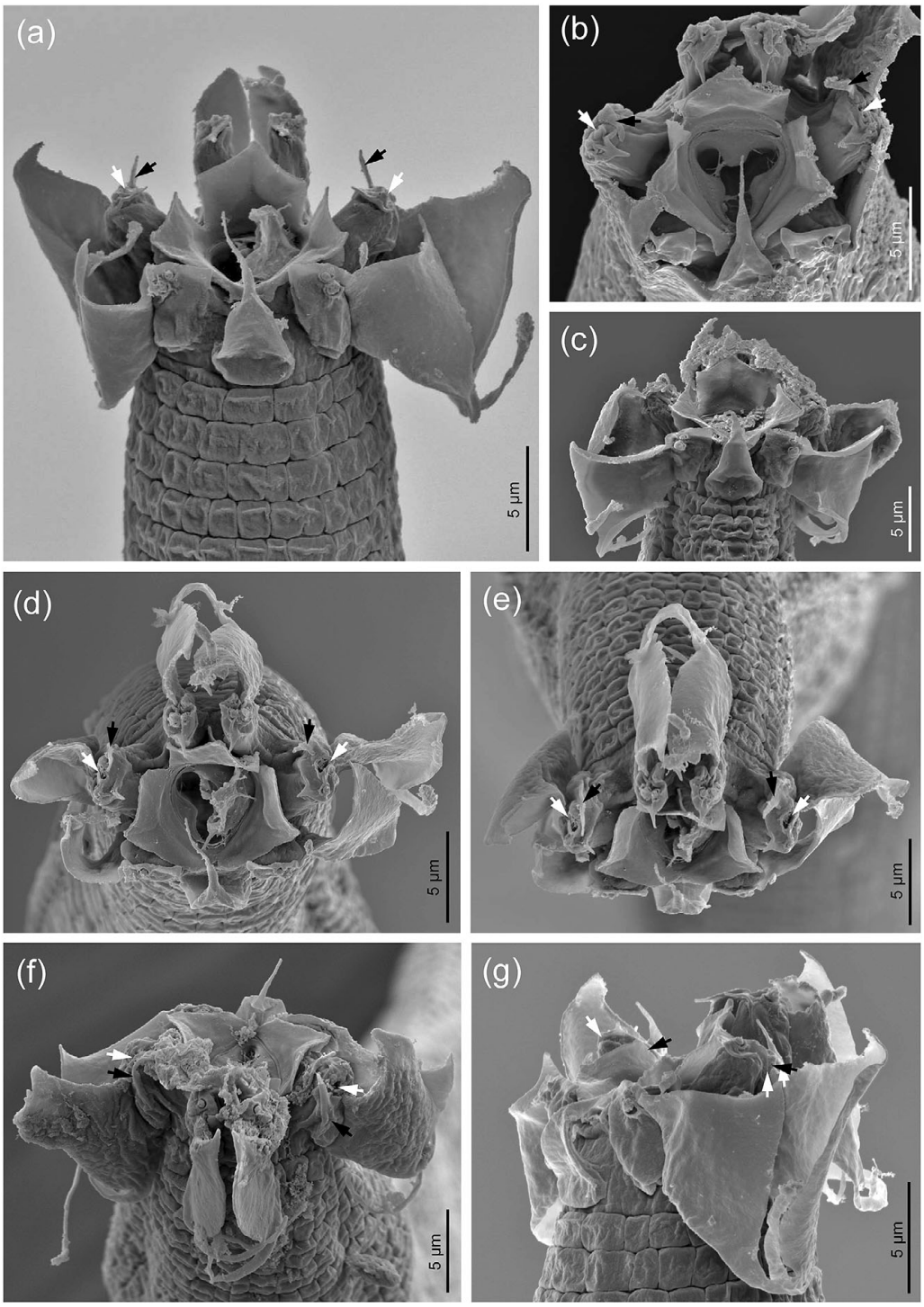

Figure 5. *Acromoldavicus xerophilus* n. sp. (scanning electron microscopy, female). (a, c) lip region in ventral view; (b) oral opening; (d–g) lip region in frontal, subdorsal, dorsal, and left lateral views, respectively. Black arrows pointing to the lateral axillar guard process; white arrows pointing to the amphids.

et al. 1994) alignment tool implemented in MEGA7 (Kumar *et al.* 2016). Poorly aligned regions at extremes were removed from the alignments using MEGA7. The best-fit model of nucleotide substitution used for the phylogenetic analysis was statistically selected using jModelTest 2.1.10 (Darriba *et al.* 2012). Phylogenetic trees were generated with the Bayesian inference method using MrBayes 3.2.6 (Ronquist *et al.* 2012). *Aphelenchus avenae* (JQ348399) for 18S rDNA and *Teratolobus* sp. (KJ652552) for 28S rDNA were chosen as outgroups. The analysis under a General Time Reversible Plus Invariant sites plus Gamma distribution (GTR+I+G) model was selected with a random starting tree and run with the Markov Chain Monte Carlo (MCMC) method (Larget and Simon 1999) for 1 x 10^6 generations. The resulting trees were visualised and saved with FigTree 1.4.4 (Rambaut 2018).

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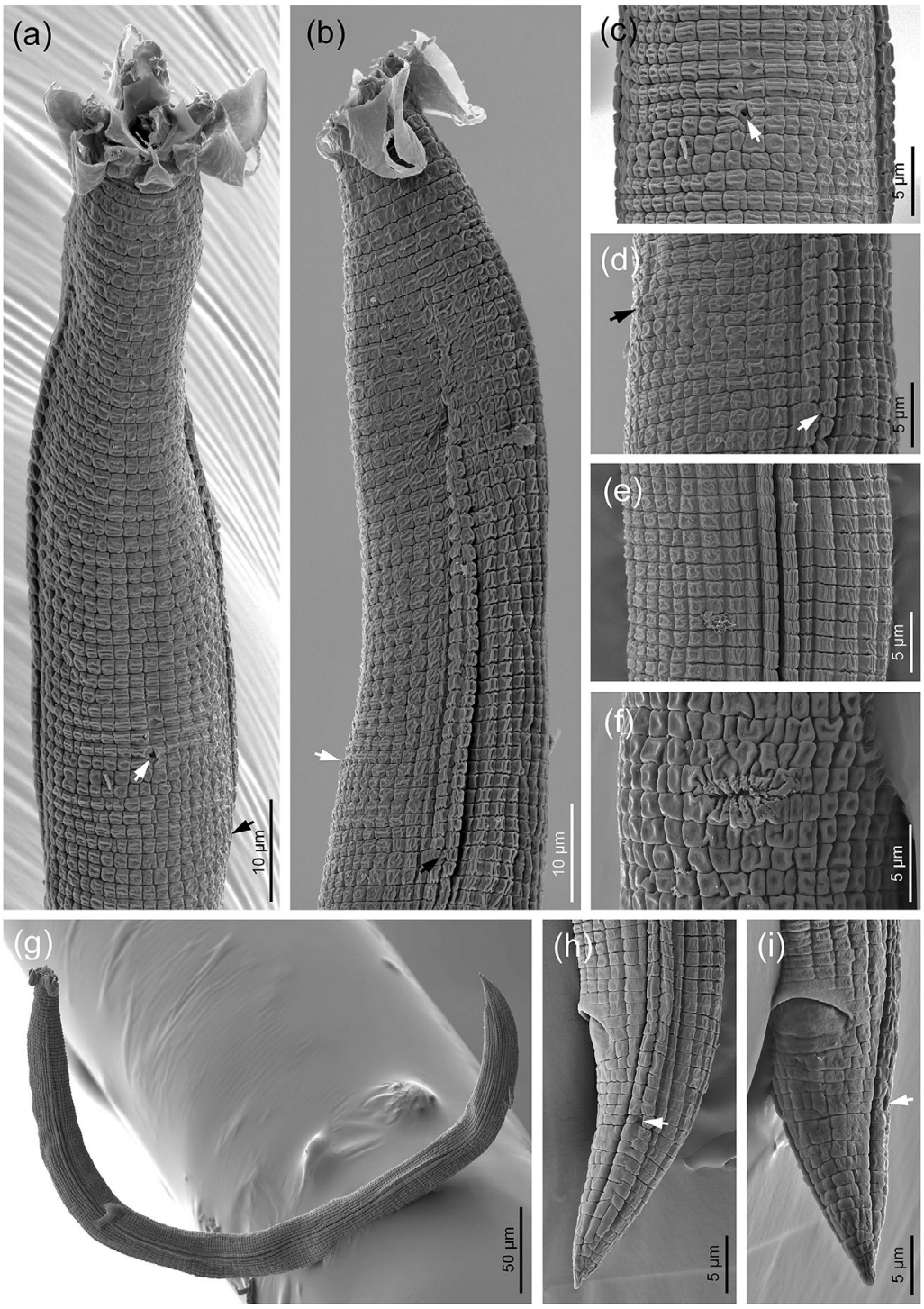

Figure 6. *Acromoldavicus xerophilus* n. sp. (scanning electron microscopy, female). (a, b) Neck region in ventral and left lateral views, respectively (white arrow pointing to the excretory pore, black arrow pointing to the deirid); (c) excretory pore in ventral view (arrow); (d) excretory pore in lateral view (black arrow) and deirid (white arrow); (e) lateral field; (f) vulva in ventral view; (g) entire body; (h, i) tail in left lateral and ventral views, respectively (arrow pointing to the left phasmid).

Results

Acromoldavicus xerophilus *n. sp*

Zoobank: urn:lsid:zoobank.org:act:93E003AF-1F8F-42FF-AD42--A5F0F9432294

Material examined

Twenty-one females (holotype and paratypes) from Salinas de Cabo de Gata and fifteen females from Tabernas Desert (province of Almería, Spain) were examined.

Table 1. Morphometrics of *Acromoldavicus xerophilus* n. sp. from Spain. Measurements in μm and in the form: mean ± standard deviation (range) where appropriate

Province	Almería		
Locality	Salinas de Cabo de Gata		Tabernas Desert
Habitat	Sand dune		Sandy soil
n	1 ♀ Holotype	20 ♀♀ Paratypes	15 ♀♀
Body length	670	639.5 ± 34.2 (566–700)	606.6 ± 32.4 (557–656)
a	20.9	20.1 ± 1.1 (18.2–22.1)	19.1 ± 2.0 (14.6–21.6)
b	5.2	4.9 ± 0.3 (4.1–5.4)	4.7 ± 0.3 (3.8–5.2)
c	19.1	18.2 ± 1.4 (16.1–22.4)	17.9 ± 1.3 (15.8–21.2)
c’	1.8	1.8 ± 0.1 (1.6–2.0)	1.7 ± 0.1 (1.5–2.0)
V	61	59.8 ± 2.2 (51–62)	61.3 ± 1.1 (60–63)
Labial probolae length	8	7.9 ± 0.3 (7–8)	7.5 ± 0.8 (6–9)
Lip region width	23	22.9 ± 0.5 (21–24)	19.3 ± 3.3 (15–23)
Stoma length	9	8.9 ± 0.2 (8–9)	8.6 ± 0.7 (7–10)
Pharyngeal corpus length	72	69.2 ± 1.5 (67–72)	69.1 ± 5.1 (64–86)
Isthmus length	22	26.0 ± 2.8 (23–34)	25.3 ± 2.6 (22–30)
Bulbus length	27	24.5 ± 1.8 (21–27)	24.4 ± 2.5 (21–29)
Pharynx length	121	119.8 ± 4.3 (111–128)	118.8 ± 5.5 (112–137)
Nerve ring-anterior end distance	85	87.4 ± 3.7 (81–95)	85.4 ± 4.2 (81–96)
Excretory pore-anterior end distance	97	98.8 ± 4.5 (90–109)	96.2 ± 4.6 (90–106)
Deirid-anterior end distance	103	111.7 ± 4.9 (100–121)	108.3 ± 7.8 (95–121)
Neck length (stoma + pharynx)	130	128.7 ± 4.3 (120–137)	127.5 ± 5.7 (120–146)
Body diam. at neck base	30	29.5 ± 1.1 (28–32)	30.2 ± 2.4 (27–35)
Body diam. at midbody	32	31.7 ± 1.0 (30–35)	32.0 ± 2.8 (29–38)
Ovary length	156	156.8 ±15.7 (135–184)	155.8 ± 18.8 (107–184)
Oviduct length	11	14.8 ± 4.0 (11–29)	9.7 ± 0.6 (9–11)
Spermatheca length	23	37.4 ± 6.6 (26–50)	26.8 ± 5.1 (16–34)
Uterus length	83	77.0 ± 10.6 (58–93)	76.3 ± 8.7 (52–87)
Post-vulval uterine sac length	28	27.5 ± 1.3 (25–29)	24.7 ± 1.6 (21–27)
Vagina length	14	12.3 ± 0.9 (11–14)	10.9 ± 1.2 (9–13)
Vulva-anterior end distance	408	382.6 ± 23.1 (338–427)	372.2 ± 20.7 (341–402)
Rectum length	37	38.0 ± 1.8 (34–40)	37.4 ± 2.1 (33–40)
Anal body diameter	20	18.8 ± 0.8 (18–20)	19.0 ± 1.3 (17–22)
Tail length	35	35.1 ± 1.8 (30–37)	34.0 ± 2.2 (30–38)
Phasmid-anus distance	12	14.3 ± 1.5 (12–16)	13.1 ± 1.1 (11–15)

Demanian indices (de Man 1881): *a* = body length/body diameter; *b* = body length/pharynx length; *c* = body length/tail length; *c’* = tail length/anal body diameter; *V* = (distance from anterior region to vulva/body length) x 100.

Description

See Figures 2–6 and Table 1.

Female. Body stout, 0.55–0.70 mm long. Habitus sigmoid, C-shaped o slightly curved ventrally after fixation. Cuticle tessellated, 1–2 μm thickness, having transversal incisures forming annuli with 2–3 μm of thickness at mid-body and longitudinal incisures dividing the cuticle in small and rectangular blocks. Lateral field 6–7 μm wide, occupying 17–24% of mid-body diameter, with two alae limited by three longitudinal incisures, beginning at the anterior third of the neck and continuing to near tail terminus. Lip region continuous with body contour having three pairs of expanded lips, one dorsal and two subventral. Lips bearing an acute process at tip bent to the oral opening and a large expansion outwards or vexillum (pl. vexilla), acute at anterior side and with a filiform process at posterior side. Primary axils V-shaped, having a triangular guard process, larger at ventral primary axil and smaller and fused to the adjacent lateral lip at subdorsal primary axils. Secondary axils U-shaped, all of them lacking guard processes. Amphids oval, located almost apical at each lateral lip. Sensilla papilliform, appearing both labial and cephalic papillae almost apical at each lip, except the lateral ones lacking the cephalic papilla. Oral opening almost triangular, surrounded by three pentagonal labial probolae, connected at their base to each other. Stoma short and tubular: cheilostom

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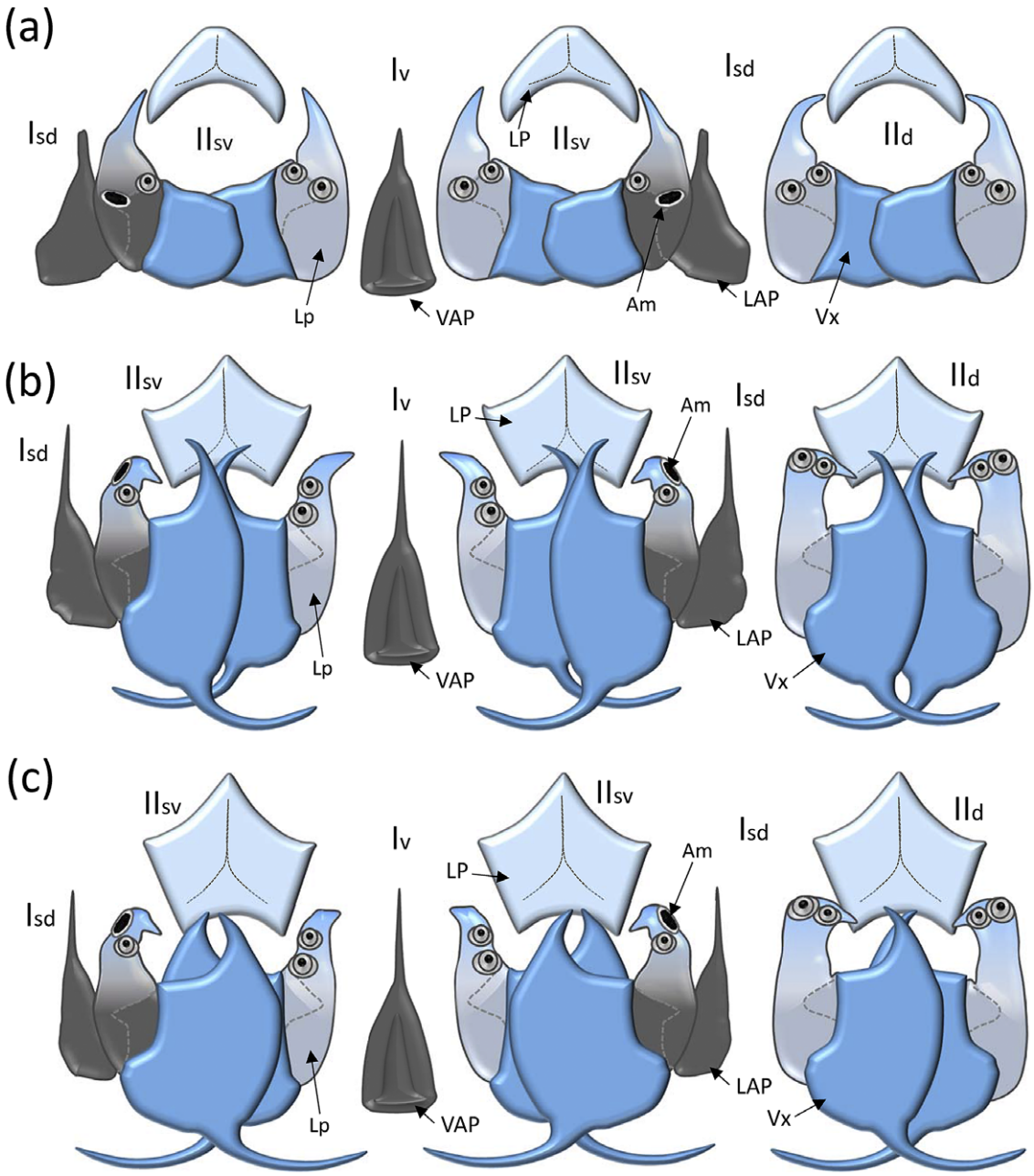

Figure 7. Schematic view of the lip region of the *Acromoldavicus* species. (a) *A. skrjabini*; (b) *A. mojavicus*; (c) *A. xerophilus* n. sp. Iv: ventral primary axil; Isd: subdorsal primary axil; IId: dorsal secondary axil; IIsv: subventral secondary axil; Am: amphid; LAP: lateral axillar process; Lp: lip; LP: labial probola; VAP: ventral axillar process; Vx: vexillum.

short with small and rounded to elongated rhabdia; gymnostom reduced with very small rhabdia; stegostom robust, muscular, with prostegostom having rhabdia directed toward the stoma lumen, meso-, meta- and telostegostom with small rhabdia, scarcely visible. Pharynx cephaloboid: pharyngeal corpus subcylindrical, 2.2–3.9 times isthmus length; isthmus more slender, slightly narrower than metacorpus; basal bulb pyriform, bearing well-developed and striated transverse valves. Cardia more or less conoid. Nerve ring at 61–76% of neck length, surrounding isthmus. Excretory pore at 63–85% of neck length, at isthmus level. Deirids at 83–96% of neck length, at level of basal bulb or at posterior part of isthmus. Reproductive system cephaloboid, monodelphic-prodelphic, in dextral position to intestine: ovary long posteriorly directed, with or without flexures posterior to vulva; ovary differentiated at its junction with the junction in a diverticulum having ovoid small cells at its lumen; oviduct short; spermatheca well developed, 0.7–1.6 times body diameter, divided in two sections, a proximal tubular part with narrow lumen and a distal part swollen with a spheroid structure containing very small rounded corpuscles; uterus tubular, 1.6–3.1 times body diameter long, differentiated in a long distal tubular part with a scarce lumen and thick walls, and a short proximal swollen part with thinner walls and distinct lumen; post-vulval uterine sac reduced, 0.6–0.9 times the corresponding body diameter long, with two sections, the proximal one similar to the swollen part of the uterus, and the distal one more swollen lacking lumen; vagina slightly sigmoid, 34–45% of body width; uterine eggs elongate, about three times longer than wide; vulva a ventral slit, not prominent. Rectum very long, 1.7–2.2 times anal body diameter; three small gland-like cells are distinguishable around the intestine-rectum

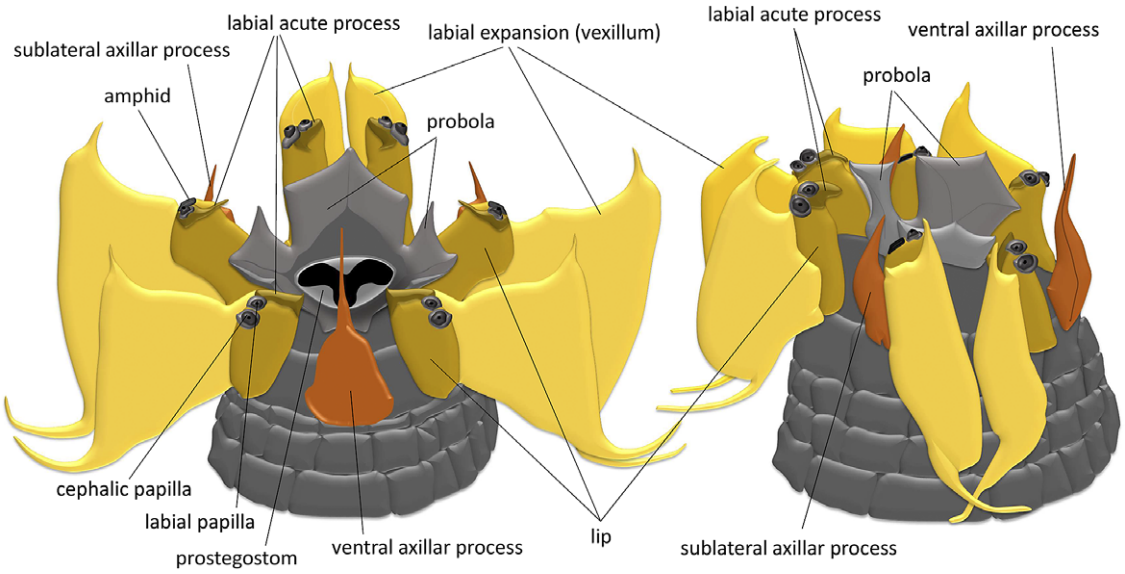

Figure 8. Schematic view of the lip region in ventral view of *Acromoldavicus xerophilus* n. sp. in ventral (left) and lateral (right) views.

junction. Tail conoid, slightly curved ventrally, with terminus scarcely biacute, being the dorsal tip smaller. Phasmids located at 33–50% of tail length.

Male. Unknown.

Etymology

The specific name refers to the presence of this species in xeric or water-lacking environments [from ancient greek ξηρός (xērós, "dry") and φίλος (philos, "love/friendship")].

Diagnosis

Acromoldavicus xerophilus n. sp. is characterised by its body length (557–700 µm in females), cuticle tessellated, lateral fields with three longitudinal incisures, lips with a large labial expansion, primary axils with a triangular guard process with the ventral one larger, secondary axils lacking guard processes, amphids oval, labial probolae pentagonal, stoma short with prostegostom bearing prominent rhabdia directed towards the stoma lumen, pharynx cephaloboid, nerve ring surrounding the isthmus, excretory pore at isthmus level, female reproductive system monodelphic-prodelphic, spermatheca 0.7–1.6 times the corresponding body diameter, post-vulval sac reduced 0.6–0.9 times body diameter long, rectum 1.7–2.2 times anal body diameter, female tail conoid (30–38 µm long, c=15.8–22.4, c'=1.5–2.0) with biacute terminus and males unknown.

Relationships

Acromoldavicus xerophilus n. sp. is similar to other species of the genus, especially *A. mojavicus,* by having a lip region with very expanded lips. However, the new species differs in having cuticle with quadrangular block (vs. rectangular in general), lips with larger lobular expansion (7–9 vs. 6–8 µm long), with filiform posterior process shorter (similar in length as expanded lips part vs. visibly longer), primary axils with smaller guard process, seta-like, and fused to adjacent lateral lip (vs. large, triangular, and scarcely fused to adjacent lateral lip), labial probolae with lateral lips angular (vs. slightly conoid), amphids almost apical (vs. at lip base according the original description, although it could be more apical), longer pharynx (111–137 vs. 62–74 µm long), vulva located slightly more anterior (V=51–63 vs. V=60–63), post-vulval sac lacking lumen along most of its length (vs. with lumen occupying *ca.* half its length), rectum with cuticular part one third of its length (vs. more than 50%), tail terminus with smaller hyaline part (as long as wide vs. about 1.5 times longer than wide), tail tip biacute (vs. finely rounded), and males absent (vs. frequent).

On the other hand, with respect to *A. skrjabini*, the new species is very different and clearly distinguished by the morphology of their lip region, with lips and probolae visibly more reduced in *A. skrjabini.*

Type locality and habitat

Acromoldavicus xerophilus n. sp. was found in two localities from the province of Almería, Spain: i) Salinas de Cabo de Gata (GPS coordinates: latitude 36º45'53.73"N, longitude 2º13'8.10"O), in sand dunes; ii) Tabernas Desert (GPS coordinates: latitude 37º0'4.34"N, longitude 2º27'1.43"O), in sandy soil, both associated with xerophilic vegetation: *Arthrocnemum macrostachyum* (Moric.) C. Koch, *Carduus tenuiflorus* Curtis, *Caroxylon vermiculatum* (L.) Akhani and Roalson, *Ephedra fragilis* Desf, *Helianthemum almeriense* Pau, *Launaea arborescens* (Batt.) Murb, *Limbarda crithmoides* (L.) Dumort, *Limonium insigne* (Coss.) Kuntze, *Nicotiana glauca* Graham, *Onthatus maritimus* Hoffmanns and Link, *Salsola kali* L., and *Thymelaea hirsuta* (L.) Endl.

Type material

Thirty-four females (holotype and paratypes) are deposited in the Nematode Collection of the Department of Animal Biology, Plant Biology and Ecology of the University of Jaén. Two females (paratype) are deposited in the nematode collection of the Swedish Museum of Natural History (Stockholm, Sweden).

Molecular characterization

Three sequences of *Acromoldavicus xerophilus* n. sp. were obtained: two 18S rDNA fragments, both with 925 bp (PP069740, PP069741), and one 28S rDNA fragment with 1057 bp (PP069742). For 18S

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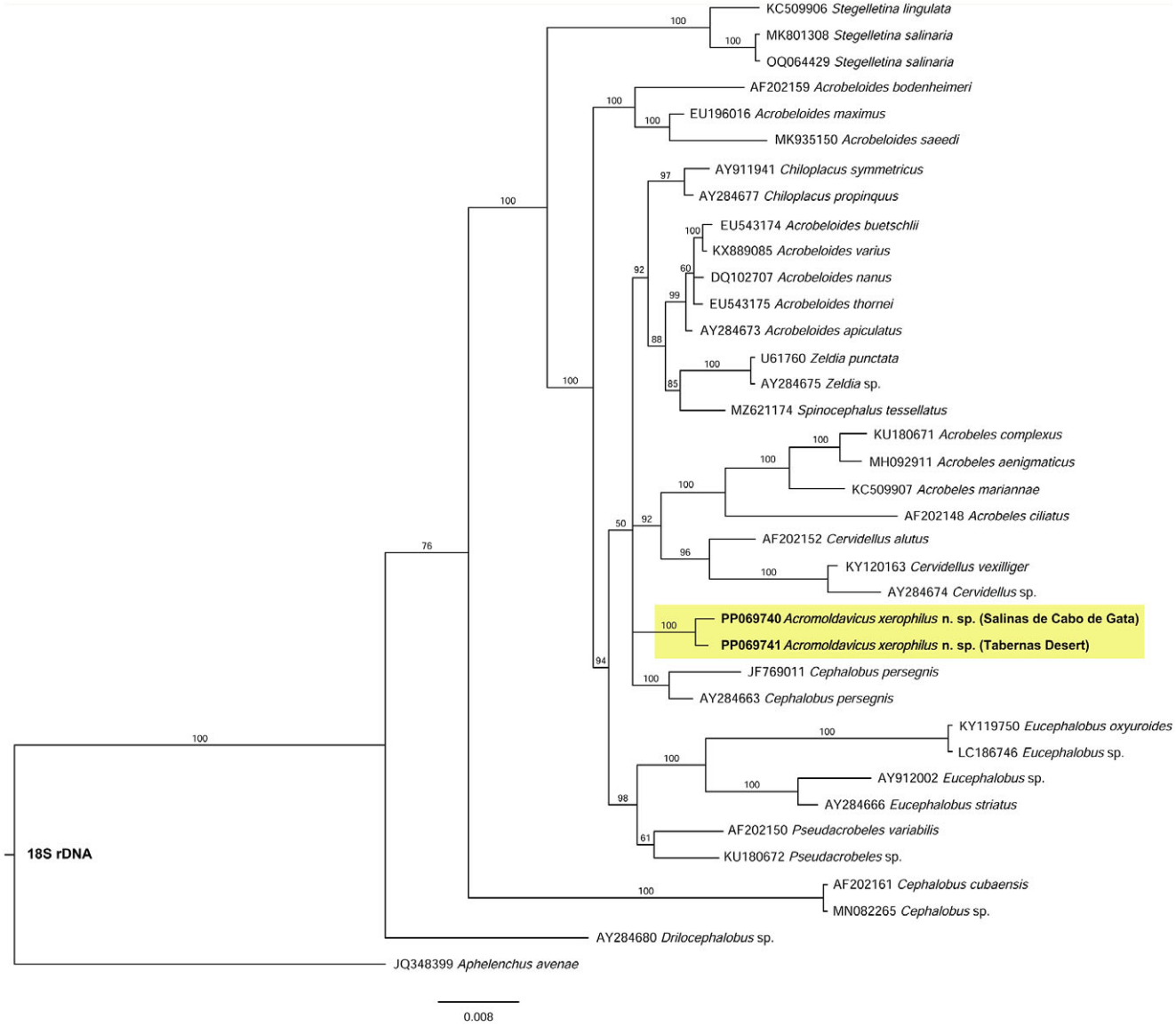

Figure 9. Bayesian inference tree from the newly sequenced *Acromoldavicus xerophilus* n. sp. based on sequences of the 18S rDNA region. Bayesian posterior probabilities (%) are given for each clade. Scale bar shows the number of substitutions per site.

rDNA, the two sequences of *A. xerophilus* n. sp. show 100% similarity, in a fragment in common with 625 bp. With respect to the 28S rDNA, in a fragment in common with 519 bp, the sequence of *A. xerophilus* n. sp. shows 97.5% similarity (13 bp differences) compared with the sequences of *A. mojavicus* (DQ145626, AY027536), and 97.3% similarity (14 bp differences) with respect to the sequence of *A. skrjabini* (AY027535). On the other hand, the two sequences of *A. mojavicus* and the sequence of *Acromoldavicus* aff. *mojavicus* have 100% similarity, which shows that these taxa are probably conspecific.

Discussion

Morphological differences between Acromoldavicus species

According to the morphology of the lip region, the species of the genus *Acromoldavicus* (Figure 7) can be divided into two groups: a first group with less developed lip region (*A. skrjabini*) and a second group with more developed lip region (*A. mojavicus* and *A. xerophilus* n. sp.). Thus, the *skrjabini* group is characterized by having lips with acute apical process, slightly laterally curved, smaller vexilla lacking processes, axillar guard process wider at base, triangular, with short elongate tip, and more reduced probolae, almost triangular. On the other hand, the *mojavicus* group is characterized by having lips with apical process bent toward the oral opening (claw-like) and larger vexilla bearing an elongate process, axillar guard process narrower, triangular, with filiform tip, and larger probolae, pentagonal. In addition, the prostegostom is not expanded toward the stoma lumen in the *skrjabini*-group (vs. expanded in three tongue-like processes in the *mojavicus*-group). Comparing both species of the *mojavicus* group, *A. xerophilus* n. sp. (Figure 8) shows labial characters slightly more developed than *A. mojavicus*, female tail more acute (vs. finely rounded in *A. mojavicus*), and males absent (vs. as frequent as females). With respect to the sexual condition, *A. mojavicus* and *A. skrjabini* are amphimictic species, appearing as males frequently, while *A. xerophilus* n. sp. is, apparently, a parthenogenetic species where males are absent.

https://doi.org/10.1017/S0022149X24000051

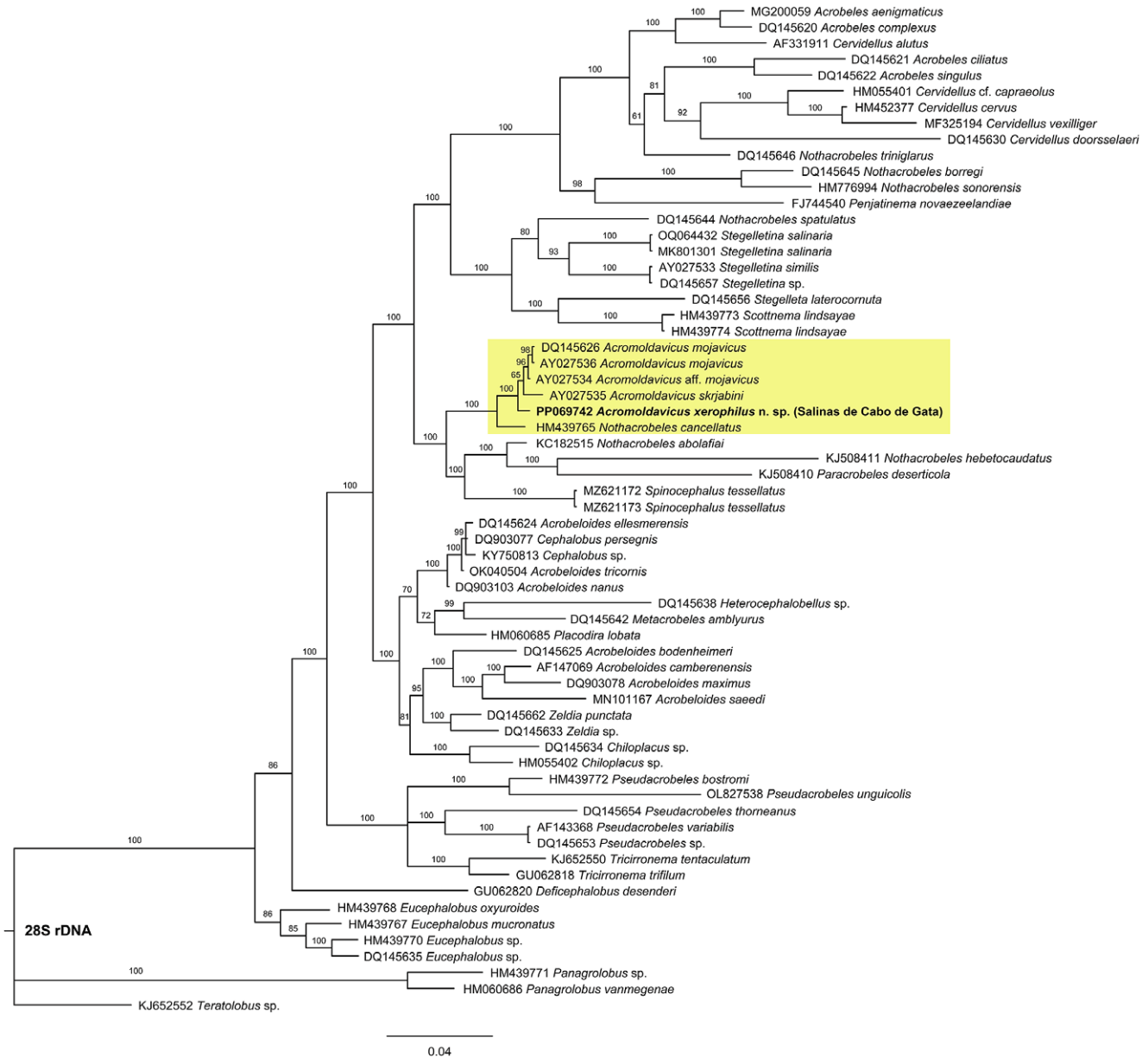

Figure 10. Bayesian Inference tree from the newly sequenced *Acromoldavicus xerophilus* n. sp. based on sequences of the 28S rDNA region. Bayesian posterior probabilities (%) are given for each clade. Scale bar shows the number of substitutions per site.

With respect to other genera, *Acromoldavicus* is morphologically related to *Scottnema* (as reported by Boström 1985), showing less modified lips, primary axils with two axillar guard processes (only the sublateral and one ventral process are maintained in *Acromoldavicus*), having similar pentagonal labial probolae (plesiomorphic condition), and *Elaphonema*, having lips lacking vexillum but having similar claw-like labial apical processes, axillar guard processes absent, and developing three large oral processes, probably a very modified labial probolae (apomorphic condition).

Phylogenetic position of the genus Acromoldavicus

The phylogenetic analysis based on 18S (Figure 9) and 28S rDNA (Figure 10) fragments clearly shows that the genus *Acromoldavicus* is monophyletic. However, its relationship with other genera is not clear, appearing in both trees as many clades with low consistency. Thus, the 18S rDNA tree, with an arrangement of genera not well distributed according to morphological relationships, placed the genus *Cephalobus* Bastian, 1865 as a sister group of *Acromoldavicus* Nesterov, 1970, showing 96.24% similarity (25 bp differences) in a fragment in common with 665 bp, although they do not maintain close morphological similarities.

The 28S rDNA tree, with genera well distributed according the morphological relationships, shows that the genus *Acromoldavicus* is related to species of the genera *Nothacrobeles*, *Paracrobeles*, and *Spinocephalus*, all of them containing species with tessellated cuticles. Thus, in the 28S tree, the closer species are *Nothacrobeles abolafiai* Mehdizadeh and Shokoohi, 2013 with 94.21% similarity (58 bp differences), *N. cancellatus* (Thorne, 1925) Ruiz-Cuenca and Abolafia, 2020 with 96.21% similarity (20 bp differences), *N. hebetocaudatus* Abolafia, Divsalar, Panahi and Shokoohi, 2014 with 86.72% similarity (137 bp differences), *Paracrobeles deserticola*

https://doi.org/10.1017/S0022149X24000051

Table 2. Morphometrics of *Acromoldavicus* species. Measurements in μm and in the form: mean ± standard deviation (range) where appropriate

Species	Acromoldavicus mojavicus		Acromoldavicus skrjabini													*Acromoldavicus xerophilus* n. sp	
Reference	Baldwin *et al.* (2001)		Nesterov and Lisetskaya (1965)		Nesterov (1970)		Boström (1992)	Karegar *et al.* (1997)			Susulovsky *et al.* (2001)			Iliev *et al.* (2003)		Present study	
Country	USA		Moldavia		Moldavia		Greece	Iran		Spain	Israel		Ukraine	Bulgaria		Spain	
Province/State	California		Kishinev		Kinishev		Réthymno	Tehran		Granada	Haifa		Mykolaiv Oblast	Blagoevgrad		Almería	
Locality	Mojave Desert		?		?		Réthymno Beach	Tajrish		Sierra de la Sagra	Mount Carmel		Elanets District	Tisata Reserve		Salinas de Cabo de Gata	Tabernas Desert
Habitat	Sandy soil		Agricultural soil		Crops		Pine forest	Wild plants		Dry soil	Dry soil		Steppe cereals	Wild plants		Sand dune	Sandy soil
n	13♀♀	12♂♂	4♀♀	2♂♂	20♀♀	15♂♂	1♀	7♀♀	5♂♂	3♀♀	20♀♀	11♂♂	2♀♀	10♀♀	10♂♂	20♀♀	15♀♀
Body length	500–605	500–630	720–740	724–735	599–740	615–735	512	557–677	602–647	611–671	526–617	537–618	509–570	590–679	600–683	566–700	557–656
a	12.0–17.0	15.0–18.0	15.0–16.0	15.0	15.0–18.3	15.0–25.0	17.0	17.3–19.7	17.2–22.3	16.5–18.1	15.8–19.3	16.9–21.7	13.7–16.7	14.5–18.7	17.0–24.0	18.2–22.1	14.6–21.6
b	3.4–4.4	3.8–4.8	4.6	4.5	4.0–4.6	4.2–4.8	4.4	3.9–4.9	4.0–4.7	4.5–4.9	3.8–4.9	4.2–4.8	3.8–3.9	4.2–4.7	4.3–5.0	4.1–5.4	3.8–5.2
c	15.0–18.0	14.0–19.0	16.0	16.0	16.0–17.0	14.2–16.0	20.0	14.1–18.9	13.4–21.6	16.8–19.7	14.5–18.3	12.8–15.5	13.6–16.7	15.3–21.1	13.9–16.1	16.1–22.4	15.8–21.2
c’	1.9–2.3	1.4–3.0	?	?	?	?	1.8	1.9–2.8	1.2–1.9	2.0–2.4	2.1–3.1	1.6–2.0	2.5–2.2	1.5–2.3	1.1–1.6	1.6–2.0	1.5–2.0
V	56–64	–	62	–	58–62	–	65	60–64	–	52–62	59–63	–	62–63	56–64	–	51–62	60–63
Labial probolae length	6–9	6–10	?	?	?	?	?	?	?	?	7–10	6–9	9–10	?	?	7–8	6–9
Lip region width	22–26	22–26	?	?	?	?	?	16–19	?	?	18–21	18–20	21–22	14–16	14–16	21–24	15–23
Stoma length	6–8	6–8	?	?	?	?	7	?	?	?	7–9	7–9	7–8	7–8	7–8	8–9	7–10
Pharyngeal corpus length	37–46	35–45	?	?	?	?	?	48–89	55–83	70–79	57–84	66–83	85–88	?	?	67–72	64–86
Isthmus length	22–34	23–44	?	?	?	?	?	32–45	31–45	25–30	18–37	17–40	19–27	?	?	23–34	22–30
Bulbus length	24–26	21–25	?	?	?	?	?	22–28	23–27	24–25	22–28	21–28	26–27	?	?	21–27	21–29
Pharynx length	62–74	60–73	?	?	?	?	116	102–162	109–155	?	97–149	104–151	130–142	133–144	133–144	111–128	112–137
Nerve ring-anterior end distance	74–99	77–98	?	?	?	?	?	81–112	85–106	103–108	69–95	69–89	87–82	?	?	81–95	81–96
Excretory pore-anterior end distance	69–103	70–105	?	?	?	?	?	85–103	88–103	94–103	74–98	76–86	93–90	89–101	89–101	90–109	90–106
Deirid-anterior end distance	82–126	84–126	?	?	?	?	?	109–135	117–130	130	99–115	99–111	110–113	?	?	100–121	95–121

(*Continued*)

Table 2. (*Continued*)

Species	Acromoldavicus mojavicus		Acromoldavicus skrjabini													*Acromoldavicus xerophilus* n. sp	
Reference	Baldwin *et al.* (2001)		Nesterov and Lisetskaya (1965)		Nesterov (1970)		Boström (1992)	Karegar *et al.* (1997)			Susulovsky *et al.* (2001)			Iliev *et al.* (2003)		Present study	
Country	USA		Moldavia		Moldavia		Greece	Iran		Spain	Israel		Ukraine	Bulgaria		Spain	
Province/State	California		Kishinev		Kinishev		Réthymno	Tehran		Granada	Haifa		Mykolaiv Oblast	Blagoevgrad		Almería	
Locality	Mojave Desert		?		?		Réthymno Beach	Tajrish		Sierra de la Sagra	Mount Carmel		Elanets District	Tisata Reserve		Salinas de Cabo de Gata	Tabernas Desert
Habitat	Sandy soil		Agricultural soil		Crops		Pine forest	Wild plants		Dry soil	Dry soil		Steppe cereals	Wild plants		Sand dune	Sandy soil
n	13♀♀	12♂♂	4♀♀	2♂♂	20♀♀	15♂♂	1♀	7♀♀	5♂♂	3♀♀	20♀♀	11♂♂	2♀♀	10♀♀	10♂♂	20♀♀	15♀♀
Neck length (stoma + pharynx)	126–150	129–140	?	?	?	?	123	123–167	131–156	130–137	120–147	120–137	134–145	?	?	120–137	120–146
Body diam. at midbody	32–45	31–39	?	?	?	?	30	29–37	29–35	37	31–37	27–32	37–34	?	?	30–35	29–38
Spermatheca or spicula length	30–54	27–29	?	31	?	28–33	42	29–40	25–33	29–40	33–69	23–34	52	?	31–35	26–50	16–34
Post-vulval uterine sac or gubernaculum length	20–38	13–16	?	20–21	?	?	27	20–37	14–18	36–45	20–41	13–18	22–27	33–39	20–22	25–29	21–27
Vulva-anterior end distance	305–375	–	?	–	?	–	?	?	–	?	?	–	?	?	–	338–427	341–402
Rectum length	22–40	–	?	–	?	–	21	23–37	–	28–31	32–41	–	28–30	24–31	–	34–40	33–40
Anal body diameter	15–19	12–28	?	?	?	?	?	15–19	24–26	15–16	12–15	20–24	15–16	?	?	18–20	17–22
Tail length	30–37	34–41	?	?	?	?	26	31–46	28–48	32–37	30–40	37–42	34–38	32–39	?	30–37	30–38
Phasmid-anus distance	11–15	16–27	?	?	?	?	?	10–16	18–21	15	9–14	12–19	11–12	?	?	12–16	11–15

Demanian indices (de Man 1881): *a* = body length/body diameter; *b* = body length/pharynx length; *c*=body length/tail length; *c'* = tail length/anal body diameter; *V* = (distance from anterior region to vulva/body length) x 100.

Abolafia, Divsalar, Panahi and Shokoohi, 2014 with 89.53% similarity (108 bp differences), and *Spinocephalus tessellatus* Abolafia, Hosseinvand and Eskandari, 2021 with 88.76% similarity (72 bp differences). However, *Scottnema lindsayae* Timm, 1971, its more related genus with respect to the morphology of the lip region, appears further in the tree with respect to *Acromoldavicus*, showing 89.98% similarity (101 bp differences).

List of species of the genus *Acromoldavicus* Nesterov, 1970

The genus *Acromoldavicus* includes three species (Table 2).

Type species

Acromoldavicus skrjabini (Nesterov and Lisetskaya, 1965) Nesterov, 1970

= *Acrobeloides skrjabini* Nesterov and Lisetskaya, 1965

Other species

Acromoldavicus mojavicus Baldwin, De Ley, Mundo-Ocampo, De Ley, Nadler and Gebre, 2001

Acromoldavicus xerophilus n. sp.

Keys to species identification

1a – Lips with small labial expansion .... *skrjabini*

1b – Lips with large labial expansion 2

2a – Subdorsal axillar guard processes longer; female tail acute ..... *mojavicus*

2b – Subdorsal axillar guard processes shorter; female tail slightly biacute .... *xerophilus* n. sp.

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Competing interest. The authors declare no conflict of interest.

Ethical standard. All procedures contributing to this study comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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