Research Paper

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Synopsis of gastropod-associated nematodes of Ciscaucasia (Russian Federation) with the description of a new species of *Pellioditis* Dougherty, 1953 (syn. *Phasmarhabditis* Andrássy, 1976)

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Abstract

Caucasus is known as one of the few biodiversity hotspots in Europe and is characterised by rich gastropod fauna while the nematode fauna in association with gastropods has remained largely understudied. Surveys conducted in 2019 and 2021 in the North Caucasus of the Russian Federation (Stavropol Upland and western and central parts of Krasnodar Krai) has revealed the presence of three new species of *Pellioditis*, a facultative parasite of land gastropods, and two species of obligate parasites, the intestinal parasite *Angiostoma kimmeriense* and a new, still undescribed species of a larval ectoparasite *Alloionema* sp. The new associations of *Cruznema* sp. and *Rhabditophanes* sp. with land gastropods were recorded for the first time in the Russian Federation. The new species of *Pellioditis* Dougherty, 1953 described here is based on the analysis of morphology and molecular studies of two distant and morphologically distinct strains, *thermalis* and *sindicae*. *Pellioditis thermalis* n. sp. was characterised by females possessing a tail of about 95–100 μm long, broadly conical in shape in the *thermalis* and *sindicae* strains, with a rounded anterior part and a subulate terminal part as long as the former; prominent phasmids located at the mid-tail, equatorial vulva position, a lateral field of three ridges (four incisions), males with spicules featuring a hole at the distal tip, ensheathed infective juveniles with average length 717 μm in the *thermals* strain and 771 μm in the *sindicae* strain, and exsheathed ones 644 μm and 682 μm, respectively. ITS-based phylogenetic analyses revealed that all *Pellioditis* species found in Ciscaucasia and Transcaucasia probably belong to two separate clades, with independent evolutionary histories of colonisation of this area. The entire Caucasus range area appears to serve as a biodiversity hotspot for the genus *Pellioditis*, presumably due to its complicated geological history and repeated isolation events for its terrestrial mollusc hosts.

Introduction

The Caucasus is known as one of the few biodiversity hotspots in Europe (Williams *et al.* 2006), characterised by rich flora and fauna. The Caucasus Biodiversity Hotspot includes the territories of Armenia, Azerbaijan, Georgia (also known as Transcaucasia or the South Caucasus), and parts of Russia (represented by Ciscaucasia or the North Caucasus), Turkey, and Iran.

The land mollusc's fauna of the Caucasus (318 species of Gastropoda, according to Walther *et al.* 2014) has been intensively studied over the last decades (Hausdorf 2000, 2001; Neiber *et al.* 2015; 2017; 2022 etc.). At the same time, the nematode fauna associated with gastropods of this region has remained largely understudied.

Since 2017, several regions of the North Caucasus (a.k.a. Ciscaucasia) in the Russian Federation have been surveyed for the presence of gastropod-associated nematodes. Ciscaucasia is a vast territory with diverse landscapes (from plains to mountains) and climates (from semi-arid to warm and humid), and the gastropod and nematode faunas vary predictably from place to place. The areas chosen for the survey were those with the richest gastropod populations, including the Stavropol Upland and western and central parts of Krasnodar Krai, covering areas of different altitude, climate, and vegetation. Several similar surveys have been carried out in Europe over the last decade, and a number of new species have been described that originated from European land gastropods (Ivanova *et al.* 2013; Nermut' *et al.* 2016a, b, 2017; Singh *et al.* 2019; Ivanova *et al.* 2019; Andrus *et al.* 2022). The pattern of gastropod–nematode associations was recorded in each study, and the set of taxa generally replicated that described by H. Mengert in Germany almost 70 years ago, and later described by S. Morand in France (Mengert 1953; Morand 1988). To simplify, each survey recorded one to three species of opportunistic parasites of the genus *Pellioditis* Dougherty, 1953 (until the recent nomenclatural act by Tandigan de Ley *et al.* 2023 considered as *Phasmarhabditis* Andrássy, 1976); a common ectoparasite, *Alloionema*

appendiculatum Schneider, 1859, and a number of much rarer and less abundant internal parasites such as *Angiostoma* Dujardin, 1845/*Agfa* Chitwood, 1935/*Nemhelix* Morand & Petter, 1986.

As the Caucasus region is considered to be an area of significant endemism (Williams *et al.* 2006), we expected the local nematode fauna to be distinctive. For comparative analysis, gastropod-associated nematodes from the Republic of Georgia (belonging to the Transcaucasian geographical region) were included in the analysis.

Materials and methods

Areas surveyed and gastropod material examined

Adygea

The Republic of Adygea is an enclave within the boundaries of Krasnodar Krai, located in its central region, south of the city of Krasnodar. The gastropods were collected in the deciduous foothill forests surrounding the *Granite Gorge* formed by the Belaya River (Nickel settlement, Merkulaevka village, by the Sibirka stream, 44° 10'41" N, 40°09'21" E). In total, 533 individual gastropods identified as *Deroceras caucasicum* (Simroth, 1901), *Deroceras sp.*, *Helix albescens* Rossmässler, 1839, *Hygromiidae* gen. sp., *Fruticicola fruticum* (O.F. Müller, 1774), *Krynickillus melanocephalus* Kalenic-zenko, 1851, *Limacus flavus* (Linnaeus, 1758), *Metalimax varius* (O. Boettger, 1824), *Micropontica* sp., *Monacha cartusiana* (O.F. Müller, 1774), *Oxychilus* sp., *Oxychilus translucidus* (Mortillet, 1854), *Parmacella ibera* Eichwald, 1841, *Pomatias rivulare* (Eichwald, 1829), *Serrulina serrulata* L. Pfeiffer, 1847, and *Scrobi-fera* sp. were examined for the presence of parasites.

Krasnodar krai

In the *Guam Gorge* or Guamka (44°14'12"N 39°54'26" E), in the upper reaches of the Kurdzhips River, collections were made in the deciduous foothill forests. In total, 245 individuals (*Acrotoma* sp., *Caucasicola raddei* (Kobelt, 1880), *Caucasigena* sp., Clausiliidae spp., *Deroceras sp.*, *Helicopsis dejecta* (Rossmässler, 1838), *Helicopsis sp.*, *Helix lucorum* (Linnaeus, 1758), *Fruticicola fruticum*, *Pomatias rivulare*, *Krynickillus melanocephalus*, *Serrulina serrulata*, *Quadriduplicata* sp.) were collected.

In *Goryachiy Klyuch (Hot Spring)* (44º42'06" N 39º13'02" E), a settlement with a balneological resort with thermal springs in the rocky valley of the Psekups River, 147 gastropod specimens (*Acrotoma* sp., *Boetgerilla pallens* Simroth, 1912, Clausiliidae spp., *Metalimax varius, Oxychilus* sp., *O. translucidus*) were collected from forested areas outside the populated area.

In the *Bolshoi Utrish* nature reserve, near Anapa and the Black Sea coast at its eastern point 44º43'52" N 37º25'30"E, gastropods were collected in the foothill forest of oak, beech, pine, and juniper at the first level of the Navagir ridge (150 asl); in total, 17 specimens were collected (*Deroceras* sp., Clausiliidae spp., *Monacha cartusiana, Fruticicola fruticum*).

Stavropol krai

Collections were made in Georgievsk, Terskiy, and Pyatigorsk. In *Georgievsk* district (44°11'295" N, 43°30'500" E) collections were made on the banks of the Podkumok River at 239 m asl, in the ash/hornbeam forest, at Safonova dacha (44°11'02" N 43°30'13"E), and in city parks. In total, 192 gastropod specimens were collected and dissected (*Deroceras caucasicum, D. reticulatum* (O.F. Müller, 1774), *Chondrula tridens* (O.F. Müller, 1774), *Circassina frutis*

(L. Pfeiffer,1859), Clausiliidae spp., *Helix* sp., Hygromiidae gen. sp., *Monacha* sp., *P. rivulare*, *Quadriplicata* sp., *Xerolenta obvia* (Menke, 1828), Zonitidae gen. sp.).

At *Terskiy* (44°11'40" N 43°18'49" E) collections were made on roadsides and in wild vegetation near agricultural plots. In total, 36 specimens (*D. reticulatum, Caucasigena rengarteni* (Lindholm, 1913), *Circassina frutis, Cochlodina laminata* (Montagu, 1803), *H. albescens*, Hygromiidae gen. sp., *L. flavus, Serrulina serrulata*, Vitrinidae gen. sp., Zonitidae gen. sp.) were dissected. In *Pyati-gorsk* (44°02'56" N 43°04'14" E) collections were made on the wooded slopes of Mashuk Mountain and in city parks. In all, 56 specimens (*Cochlodina laminata*, Cochlicopidae gen. sp., *D. caucasicum, Helix lucorum, H. christophi* Boettger, 1881, *Limacus flavus, Succinella oblonga* (Draparnaud, 1801) and *Xerolenta obvia*) were dissected.

Nematode material

Live gastropods were brought to the lab and identified by dissection and morphological examination. Extracted parasites were preserved for morphological and molecular studies by preservation in hot 4–5% formalin and frozen for molecular work. Adult stages of *Pellioditis* were obtained by culturing juveniles on nutrient medium (pre-frozen gastropod flesh or pig kidney).

Nematodes preserved in formalin were processed with anhydrous glycerin for light microscopy as described by Seinhorst (1959). Light microscopic studies and drawings were done using a Nikon Eclipse 200 microscope (Nikon Corporation, Tokyo, Japan) equipped with a drawing attachment. Scale bars are given in micrometres (μm). Abbreviations: V% – distance from anterior extremity to vulva to body length in %; a, b, c – de Man indices. Illustrations were prepared using WACOM Intuos A4 USB drawing tablet (Wacom, Kazo, Japan) and Adobe Illustrator CS5. For the SEM studies, formalin-preserved material was dehydrated in a graded ethanol series, critical point dried using an HCP-2 HITACHI dryer (Hitachi Ltd, Tokyo, Japan), mounted on aluminum stubs, and coated with gold in a BIO-RAD SC502 sputter coater (Bio-Rad Laboratories Inc., Hercules, USA). Images were taken on a Mira 3 Tescan (Tescan Orsay Holding, a.s., Brno, Czech Republic).

Crossbreeding test

One male and 2–4 females from the Gorjachiy Klyuch (*thermalis*) and Utrish (*sindicae*) populations were placed in a Petri dish with a thin layer of water and a pinhead-sized piece of pig kidney. Nematode reproduction was observed for one week.

Molecular characterisation and phylogenetic analysis

DNA extraction from the discovered nematodes was done through digestion with proteinase K in the worm-lysis solution containing mercaptoethanol (Holterman *et al.* 2006). Single specimens of a female, male, and infective juvenile were used. The nematodes in the solution were incubated at 65 °C for 90 min, followed by deactivation of proteinase K at 99 °C for 5 min.

Two pairs of primers were used to amplify the small subunit ribosomal DNA (SSU rDNA) (Blaxter *et al.* 1998; Medlin *et al.* 1988): G18S4 (5'-GCT TGT CTC AAA GAT TAA GCC-3') and 26R (5'-CAT TCT TGG CAA ATG CTT TCG-3') for the 3' portion of this sequence, and 24F (5'-AGR GGT TGA AAT YCG TGG ACC-3') and Q39deg (5'-TAA TGA TCC WTC YGC AGG TTC ACC TAC-3') for the 5' flank of the SSU rDNA. The primer Q39deg was modified by V.V. Aleshin (personal communication). Polymerase chain reaction (PCR) cycling parameters for the first pair were: primary denaturation at 95 °C for 5 min followed by 35 cycles

of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and elongation at 72 °C for 60 s followed by final elongation at 72 °C for 5 min. PCR parameters for second pair were 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 60 s, annealing at 53 °C for 90 s, and elongation at 72 °C for 90 s, followed by final elongation at 72 °C for 5 min.

The pair of primers D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and D3B (5'-TCG AAA GGA ACC AGC TAC TA-3') was used to obtain the D2-D3 expansion segment of the large subunit ribosomal DNA (LSU rDNA) (Nunn 1992) with PCR cycling parameters: primary denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 1 min and elongation at 72 °C for 60 s. Primers CO1F1 (5'- CCT ACT ATG ATT GGT GGT TTT GGT AAT TG-3') and CO1R2 (5'- CGT GCT TAT TTT ACT GCT GCT AC-3') were used to obtain the sequence of the cytochrome oxidase subunit I (*Cox1* mtDNA) (Kanzaki & Futai 2002). The protocol for the reaction included primary denaturation at 95 °C for 5 min followed by 35 cycles at 95 °C for 45 s, 59 °C for 50 s, 72 °C for 60 s, and final elongation at 72 °C for 5 min. To amplify the ITS rDNA region of these two species, a pair of primers TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') was used. PCR cycling parameters were: primary denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 56 °C for 1 min, and elongation at 72 °C for 60 s, with final elongation at 72°C for 5 min. As direct sequencing of this ITS product was impossible because of multiple peaks in the middle of the ITS1 region, the product was cloned in pGEM-T vector and transformed into JM109 High Efficiency Competent Cells (Promega®, Leiden, The Netherlands) according to the manufacturer's instructions. PCR products from clones were sequenced with the same primers as used for primary amplification (TW81 and AB28). Five clones from each of two species were sequenced to confirm polymorphisms. Obtained sequences were deposited in NCBI Genbank. For *Pellioditis thermalis* n. sp. strain *sindicae* from Utrish, the obtained sequences were deposited as OR373882 for the partial SSU rDNA sequence; OQ102437 for the partial LSU rDNA sequence; OQ103047 for the partial sequence of *Cox1* mtDNA; OQ103124 and OQ103319 for the two haplotypes of ITS rDNA. For *Pellioditis thermalis* n. sp. strain *thermalis* from Gorjachiy Klyuch, the obtained sequences were deposited as OR373881 for the partial SSU rDNA sequence; OQ102435 for the partial LSU rDNA sequence; OQ103053 for the partial sequence of *Cox1* mtDNA; OQ103318 and OQ103320 for the two haplotypes of ITS rDNA. For comparative purposes and the phylogeny construction, relevant sequences from the NCBI GenBank database were used. Sequence alignments were generated using Clustal_X (Thompson *et al.* 1997) under default values for gap opening and gap extension penalties. All alignments were analysed using Mega X (Kumar *et al.* 2018) for maximum parsimony (MP), neighbour joining (NJ), and maximum likelihood (ML) methods. Bayesian Analysis (BA) was performed on an IQ-TREE web server (Trifinopoulos *et al.* 2016).

Results

We found two strains of the new species of the genus *Pellioditis* at two sites in Krasnodar krai (Gorjachiy Klyuch and Utrish, *thermalis* and *sindicae*, respectively), which revealed a number of morphological differences between them. The morphometric data for these strains are presented separately.

Description of Pellioditis thermalis n. sp.

Measurements
Morphometric data on adults and dauer juveniles of *P. thermalis* n. sp. are provided in Tables 1 & 2, respectively.

Description
Adults. Body robust, 0.9–1.7 mm long, straight when relaxed, slightly tapering to anterior end. Cuticle about 1 μm thick, bearing rows of transversal and longitudinal striations. Lateral field *c.* 10–12 μm wide at mid-body, bearing three ridges and four incisions (Figure 3C). Deirid located at level of excretory pore (Figure 4E); structure identified as postdeirid located at lateral field in one specimen *c.* 15 μm posterior to deirid (Figures 4A and 4D). Head rounded to truncate, lip region short, sometimes slightly offset (Figures 4B and 4C). Six distinct, slightly pointed lips, each lip bearing distinct labial papilla in apical position (Figures 3A, 3B, and 4B). Four less prominent cephalic papillae in sublateral position on dorsal and subventral lips, sometimes indistinct (Figures 3B and 4B). Amphids prominent with narrow convex rim situated in sublateral position on lateral lips (Figure 4C). Mouth aperture triangular (Figure 4B). Stoma tubular, wide and short (about as long as lip region diam.) (Figures 2D–F). Cheilostom not cuticularised. Gymnostom walls parallel, thickened. Stegostom with glottoid apparatus, isomorphic, isotropic, metarhabdions thickened, with three minute warts each (Figures 2D–F). Pharyngeal collar narrow, covering two thirds of stoma length. Cheilostom: gymnostom: stegostom ratio *c.* 1:1:2.2. Pharyngeal collar narrow, covering two thirds of stoma length (Figures 2D–F). Pharynx muscular, differentiated into corpus expanded posteriorly (median bulb), stout, well defined isthmus and pear-shaped terminal bulb as wide as metacorpal expansion (Figure 2I). Terminal bulb with valve and haustrulum. Nerve ring surrounding middle to posterior region of isthmus. Excretory pore position at mid-isthmus. Excretory duct short. Cardia prominent, projecting into intestine. Intestine well developed, forming proventriculus at anterior. Rectal glands present.

Female. Body 1.0–2.0 mm long, straight after fixation. Oviparous, viviparous (Figures 2A–B). Reproductive system typical for the genus: didelphic, amphidelphic, ovaries reflected on dorsal side. Anterior and posterior branches almost equal in length. Oocytes rounded, large, arranged in two or three rows. Oviducts short. Spermathecae and uteri with numerous large sperm cells *c.* 10 μm in diameter. Uteri spacious. Eggs with thin smooth shell. Vagina straight, muscular, more than half corresponding diameter long. Vulva median, a wide transverse slit with thickened lips (Figure 4F). Copulatory plug present in fertilised specimens. Rectum *c.* corresponding diam. long (Figures 2P and 2R). Anus an arcuate slit (Figures 4I and 4J). Tail elongated, initially widely conical with whip-like terminal part set by constriction to dome-shaped with spike slightly exceeding the rounded part in length (Figures 4I, 4J, and 4L). Phasmids rod-like, protruding, located at junction of tail parts or at the base of rounded part or on terminal spike in 2 μm from to its base (Figures 4G–L). Protruding part of phasmid *c.* 3 μm long.

Male. Slightly shorter and slimmer than females. Corpus to pharynx ratio slightly exceeds that of females (average 59.6 vs 57.6). Testis reflexion in *c.* three body diam. posterior to pharynx base. Spermatocytes in testis reflexion rounded, arranged in two rows; reflexion short (*c.* 150 μm). Immature sperm in *vas deferens c.* 8–10 μm x 10–12 μm and sperm cells in ejaculatory duct *c.* 5 μm in diam. Anal glands large. Tail short. Bursa open, peloderan

Table 1. Morphometric data of adults of *Pellioditis thermalis* n. sp. strains *thermalis* and *sindicae* from the North Caucasus. All measurements are in μm and in the form: mean ± s.d. (range)

Species	P. thermalis n. sp. strain thermalis			P. thermalis n. sp. strain sindicae	
	Female		Male	Female	Male
Character	Holotype	Paratypes	Paratypes	Paratypes	Paratypes
n	–	10	10	13	15
Body length	1755	1658 ± 199 (1228–1994)	1482 ± 136 (1228–1662)	1364 ± 182 (1074–1700)	892 ± 89 (769–1017)
a	17.9	15.7 ± 1.6 (13.1–18.1)	19.5 ± 2.4 (15.9–23.7)	18.1 ± 1.1 (15.9–20.2)	19.3 ± 1.5 (16.9–22.5)
b	8.4	8.1 ± 0.8 (7.1–9.6)	8.2 ± 0.9 (7.0–10.1)	6.2 ± 0.5 (5.4–6.9)	5.2 ± 0.4 (4.5–5.8)
c	15.5	17.7 ± 2.8 (15.1–22.5)	44.7 ± 6.2 (34.1–51.9)	13.7 ± 1 (11.4–15.4)	28.3 ± 3 (22.8–32.9)
c'	2.6	2.8 ± 0.7 (1.9–4.2)	1.1 ± 0.2 (0.9–1.5)	2.9 ± 0.3 (2.3–3.5)	1.2 ± 0.1 (0.9–1.5)
V%	50.7	51.5 ± 0 (48.4–56.7)	–	54 ± 0 (52.5–57)	–
Max. body diam	98	107 ± 16 (87–136)	77 ± 12 (57–98)	75 ± 10 (60–97)	47 ± 6 (37–56)
Anal body diam.	44	36 ± 11 (22–54)	31 ± 6 (21–40)	34 ± 4 (28–40)	27 ± 3 (21–32)
Lip region diam.	14	15 ± 2 (12–19)	12 ± 1 (11–14)	14 ± 1 (12–17)	13 ± 1 (10–15)
Stoma total length	18	19 ± 2 (15–22)	17 ± 2 (15–19)	22 ± 1 (20–23)	17 ± 1 (15–18)
Cheilostom length	4	5 ± 1 (3–7)	4 ± 1 (2–5)	4 ± 1 (3–5)	4 ± 1 (3–5)
Gymnostom length	5	4 ± 1 (3–5)	5 ± 1 (3–7)	6 ± 1 (5–7)	4 ± 1 (3–7)
Stegostom length	9	10 ± 1 (9–12)	9 ± 1 (6–10)	11 ± 1 (10–13)	9 ± 1 (7–10)
Stoma max. diam.	4	5 ± 1 (4–6)	5 ± 1 (3–6)	5 ± 1 (4–6)	4 ± 0.4 (4–5)
Corpus length	114	119 ± 11 (100–137)	108 ± 11 (97–132)	123 ± 10 (111–150)	102 ± 6 (93–112)
Mid–corpus diam.	16	14 ± 2 (11–16)	12 ± 2 (8–14)	18 ± 6 (11–28)	12 ± 2 (9–14)
Metacorpal expansion	27	24 ± 4 (18–30)	21 ± 4 (14–29)	22 ± 6 (14–32)	18 ± 3 (14–22)
Isthmus length	60	46 ± 10 (30–61)	42 ± 5 (37–50)	51 ± 4 (43–57)	37 ± 6 (24–48)
Isthmus diam.	15	13 ± 2 (9–15)	10 ± 1 (8–12)	11 ± 2 (8–14)	8 ± 1 (6–12)
Bulb length	38	37 ± 6 (26–45)	32 ± 4 (27–37)	44 ± 6 (32–55)	30 ± 3 (26–34)
Bulb diam.	30	29 ± 3 (24–35)	25 ± 3 (19–30)	29 ± 3 (25–35)	23 ± 3 (18–27)
Pharynx total length	209	207 ± 25 (168–245)	181 ± 22 (157–222)	219 ± 16 (198–255)	171 ± 12 (144–187)
Corpus to pharynx (%)	54.6	57.9 ± 0 (52.2–64.9)	59.7 ± 0 (53.3–67.5)	56.2 ± 0 (53.3–60.1)	59.6 ± 0 (56.3–64.6)
Apex to excretory pore	175	170 ± 20 (147–192)	177 ± 18 (154–209)	190 ± 21 (162–228)	154 ± 14 (125–179)
Apex to nerve ring	162	148 ± 22 (114–185)	137 ± 17 (115–172)	158 ± 17 (140–198)	129 ± 15 (109–164)
Apex to anterior ovary reflexion	469	377 ± 64 (265–447)	–	426 ± 94 (296–590)	–
Tail extremity to posterior ovary reflexion	389	397 ± 56 (335–484)	–	296 ± 63 (198–390)	–
Vagina length	54	62 ± 17 (45–80)	–	45 ± 10 (27–65)	–
Cardia length	20	16 ± 6 (11–28)	12 ± 3 (9–16)	15 ± 4 (10–24)	11 ± 3 (9–15)
Egg length	41	44 ± 4 (40–49)	–	46 ± 6 (37–58)	–
Egg diam.	26	31 ± 2 (29–34)	–	29 ± 4 (21–34)	–
Rectum length	30	39 ± 6 (32–43)	–	35 ± 6 (26–43)	–
Tail length	113	95 ± 11 (79–110)	33 ± 3 (29–38)	100 ± 11 (76–118)	32 ± 4 (25–38)
Terminus length	58	53 ± 11 (35–68)	–	64 ± 13 (44–84)	–
Anus to phasmid	55	42 ± 14 (24–60)	–	34 ± 8 (26–49)	–
Apex to testis reflexion	–	–	378 ± 48 (309–474)	–	306 ± 36 (255–388)
Testis reflexion length	–	–	150 ± 31 (110–193)	–	186 ± 43 (135–278)
Spicule length (chord)	–	–	63 ± 6 (47–66)	–	48 ± 6 (35–55)
Gubernaculum length	–	–	33 ± 3 (29–37)	–	23 ± 3 (18–30)

Table 2. Morphometric data of dauer juveniles of *Pellioditis thermalis* n. sp. strains *thermalis* and *sindicae* from the North Caucasus. All measurements are in μm and in the form: mean ± s.d. (range)

Character	*P. thermalis* n. sp.			
	Strain *thermalis*		Strain *sindicae*	
	DL ensheatded (n=20)	DL exsheatded (n=10)	DL ensheatded (n=20)	DL exsheatded (n=10)
Body length	717 ± 72 (596–822)	644 ± 71 (531–773)	771 ± 57 (699–937)	682 ± 84 (564–862)
Maximal width	36 ± 1 (29–42)	28 ± 5 (21–38)	33 ± 6 (27–50)	31 ± 6 (27–44)
Anal width	19 ± 3 (15–25)	15 ± 2 (13–18)	18 ± 2 (13–23)	15 ± 1 (15–16)
a	20.3 ± 2.1 (22.2–27.3)	23.7 ± 2.7 (20.3–26.8)	23.4 ± 2.2 (18.7–26.9)	22.6 ± 3 (17.2–25.9)
b	5.5 ± 0.5 (4.5–6.9)	4.7 ± 0.4 (4.1–5.6)	5.2 ± 0.3 (4.8–6)	4.4 ± 0.5 (3.8–5.5)
c	7.6 ± 1.3 (6.1–11.4)	11.8 ± 0.8 (10.5–13.3)	7.6 ± 0.7 (6.4–8.9)	11.3 ± 1.6 (8.8–15.1)
c'	5.1 ± 0.7 (3–5.9)	3.8 ± 0.3 (3.2–4.4)	5.6 ± 0.8 (4–8.1)	4.1 ± 0.3 (3.6–4.6)
Lip region width	7 ± 1 (6–9)	7 ± 1 (6–8)	8 ± 1 (6–9)	8 ± 1 (7–9)
Stoma total length	18 ± 2 (13–22)	20 ± 1 (18–22)	20 ± 1 (17–21)	20 ± 1 (19–22)
Cheilostom length	4 ± 1 (3–5)	4 ± 1 (4–5)	4 ± 1 (3–5)	5 ± 1 (4–6)
Gymnostom length	4 ± 1 (2–6)	5 ± 1 (4–6)	5 ± 1 (4–7)	5 ± 1 (4–6)
Stegostom length	10 ± 1 (8–13)	11 ± 1 (9–12)	11 ± 1 (8–13)	10 ± 1 (8–12)
Stoma max. width	2 ± 1 (1–3)	2	2 ± 0 (1–2)	2 ± 0 (2–3)
Corpus length	81 ± 8 (64–95)	81 ± 5 (75–88)	89 ± 5 (82–98)	96 ± 4 (87–100)
Mid–corpus width	6 ± 1 (4–8)	8 ± 1 (6–10)	7 ± 1 (6–8)	8 ± 1 (6–10)
Metacorpal expansion	10 ± 2 (8–12)	11 ± 1 (9–13)	10 ± 1 (7–12)	13 ± 1 (11–14)
Isthmus length	30 ± 6 (21–42)	32 ± 4 (22–37)	34 ± 7 (27–47)	35 ± 5 (27–45)
Isthmus width	5 ± 1 (4–8)	5 ± 1 (4–7)	6 ± 1 (4–7)	6 ± 1 (5–8)
Bulb length	20 ± 3 (15–26)	22 ± 2 (18–26)	20 ± 3 (14–27)	24 ± 2 (22–27)
Bulb width	12 ± 2 (8–17)	15 ± 2 (12–18)	13 ± 2 (11–17)	16 ± 2 (14–18)
Pharynx total length	131 ± 10 (114–145)	136 ± 8 (124–149)	148 ± 8 (132–166)	157 ± 8 (138–166)
Corpus to pharynx ratio	61.6 ± 0 (55.2–70.4)	59.4 ± 0 (57.6–61)	60.2 ± 0 (55.4–64.8)	62.1 ± 0 (59.2–65.3)
Apex to excretory pore	110 ± 13 (94–125)	112 ± 8 (102–126)	129 ± 13 (112–154)	123 ± 6 (116–132)
Apex to nerve ring	99 ± 10 (83–115)	98 ± 8 (85–109)	110 ± 8 (99–134)	108 ± 5 (100–114)
Cardia length	8 ± 2 (5–11)	7 ± 2 (5–11)	8 ± 2 (6–11)	8 ± 2 (5–10)
Tail length	96 ± 13 (68–113)	55 ± 5 (47–60)	102 ± 11 (82–129)	61 ± 3 (56–65)
Anus to phasmid	28 ± 3 (24–33)	23 ± 3 (20–29)	40 ± 6 (29–49)	25 ± 3 (21–30)
Genital primordium length	49 ± 12 (37–67)	83 ± 29 (48–142)	82 ± 29 (29–145)	122 ± 45 (85–220)

(Figures 2S–U, 3E, and 3J). Arrangement of circumcloacal papillae typical for the genus: unpaired ventral median papilla in front of anus. Two prominent circumcloacal papillae located at lateral margins of anus (Figures 3D, 3F, and 3K). Nine pairs of bursal papillae: Genital papilla (GP) formula 1 + 1 + 1 + 2/1 + 3 + phasmids (Figures 2S–U; 3E, and 3J). GP1 slightly shorter than the rest of papillae, situated latero-ventrally, distanced from following GPs. GP 2 and 3 opened on lateral edge of bursa, GP 4 and 5 grouped, and GP5 opens dorsally, GP 5 and 6 not reaching edge of bursa; GP 7–9 grouped with GP 7 and 9 not reaching edge of bursa, GP9 opening dorsally (Figures 2S–U, 3J, and 3L). Phasmids papilliform, situated closely to tail tip (Figures 2S and 2U). Tail tip not reaching the edge of bursa (Figures 2S, 2U, and 3E). Spicules nearly straight (Figures 2V, 2W, and 3H). Velum nearly indiscernible. Distal tips with holes (Figures 3G, 3I, 3K, and 3L). Manubrium not prominent, not distinctly separated from blade. Gubernaculum without processes, about half as long as spicules (Figure 2W).

Infective juvenile (ensheathed). Body 717 μm long on average in *thermalis* strain and 771 μm in *sindicae* strain, slender, tapering gently towards head end, straight when heat killed (Figure 2C). Lateral field *c.* 10 μm wide at mid-body; composed of narrow central band flanked with three closely situated ridges by each side (Figure 5E) but a lateral field pattern of 10 ridges also present. Head rounded, lip region flat, not offset from body contour, cephalic cap absent, though slightly risen flaps around mouth aperture formed by lips present (Figures 2H and 5A–C). Six cephalic papillae slightly distanced from oral aperture; amphidial apertures situated at margin of second annulus (Figures 5A–C); stoma long and thin (or seven times longer than wide); cheilostom not cuticularised, gymnostom strongly cuticularised, stegostom about four times longer than
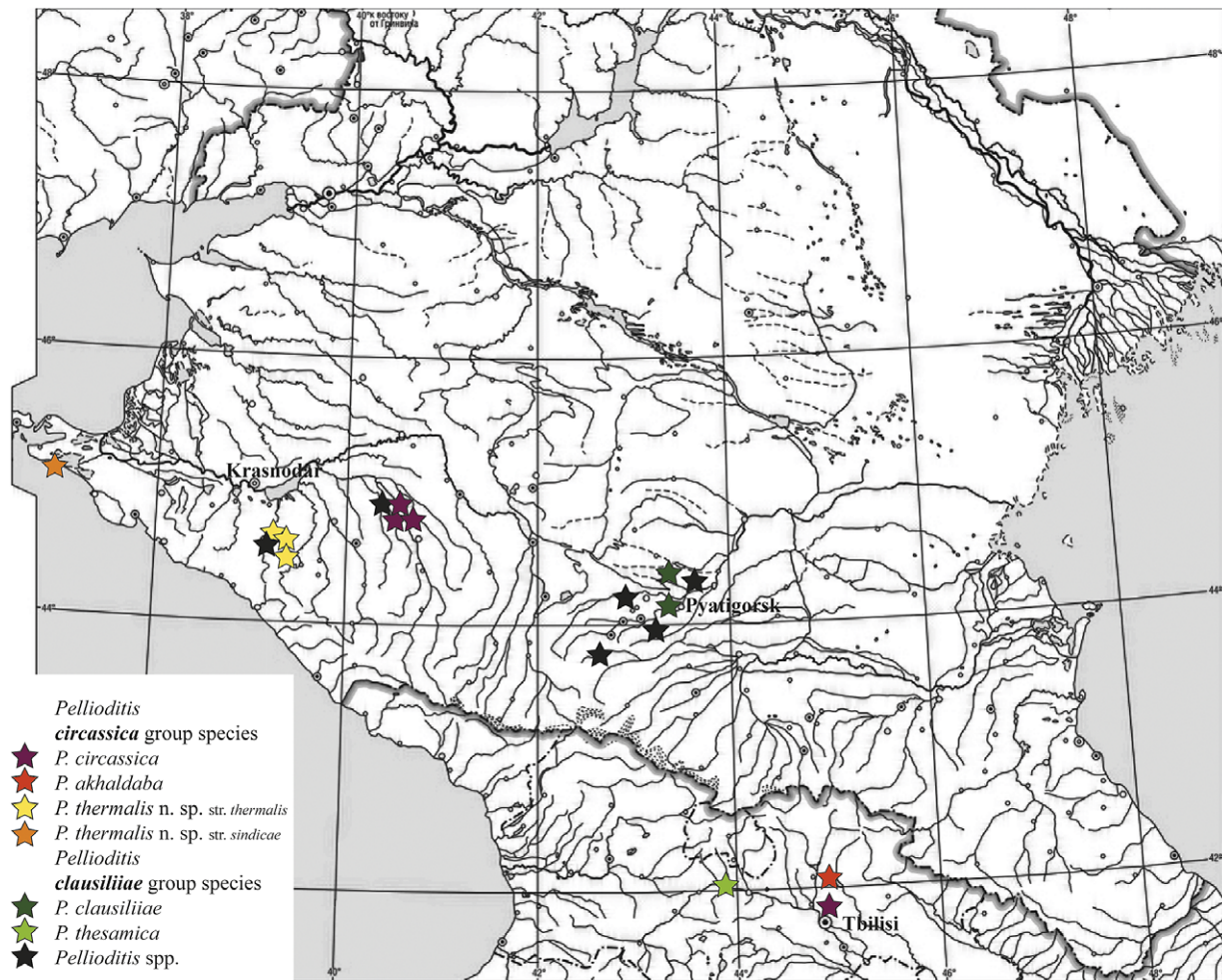
Figure 1. Distribution map of *Pellioditis* species in the Caucasus region.

gymnostom (Figure 2H). Pharynx structure typical for the genus. Nerve ring surrounding isthmus (Figure 2J). Cardia elongated, protruding into intestine (Figure 2J). Excretory pore located at nerve ring level or slightly posterior to it (Figure 5D). Deirids barely observable (Figure 5D). Hemizonid not observed. Intestine expanded at anterior and containing a mass of bacteria and collapsed posterior to expansion, filled with fat globules. Average length of genital primordium located at mid-body 49–82 µm (*thermalis* and *sindicae* strains, respectively) (Figure 2C). Tail conical, attenuated (Figures 2K–M and 5G). Rectum collapsed. Phasmids pore-like, situated in 20–29 µm posterior to anus (Figures 5F and H).

Infective juvenile (exsheathed). Similar to ensheathed dauer juvenile in internal morphology. Mean body length 644–682 µm (*thermalis* and *sindicae* strains, respectively). Cuticle without longitudinal striations (Figure 5O). Lateral fields with slightly sunken central band *c.* 9 µm wide at mid-body flanked by protruding ridges (Figures 5I–O). Lip region continuous with body contour, divided into six lip sectors, prominent amphid openings at base of lateral lip sectors, six papillae on top of lips (Figures 5J and 5K). Excretory pore situated anterior to basal bulb. Tail narrowly conical, half as long as in ensheathed juvenile, tip rounded (Figures 2N and 5M). Genital primordium well developed, nearly twice longer than in ensheathed juvenile. Deirids indistinct. Phasmids pore-like, distinct, located in short distance from anus to nearly mid-tail (Figure 5N).

Morphological variation between the strains

Adult nematodes of the strain *thermalis* differ from those of the strain *sindicae* by generally greater average body length (1658 µm vs 1364 µm, females and 1482 µm vs 892 µm, males). Females of the former strain differ from the latter ones by the less rounded anterior part of a tail and the slightly more posterior position of phasmids (displaced to the beginning of a tail spike vs the spike base (Figures 2O–R, 4H, and 4K), and remarkably more frequent occurrence of *endotokia matricida*. Males of the *thermalis* strain are common and only slightly slimmer and shorter than females of the same strain, whereas males of *sindicae* are rare and remarkably shorter and slimmer than their female counterparts. There is also a significant difference in the spicule length (average 63 µm vs 48 µm, *thermalis* vs *sindicae*, respectively). The average lengths of both ensheathed and exsheathed juveniles differ less significantly (717 µm vs 771 µm and 644 µm vs 682 µm; *thermalis* vs *sindicae*).

Taxonomic summary

Type material. Strain *thermalis*. Holotype female on slide accession number 1380, the paratype female, male and infective juveniles on slide accession number 1381, the paratype male and female on slide
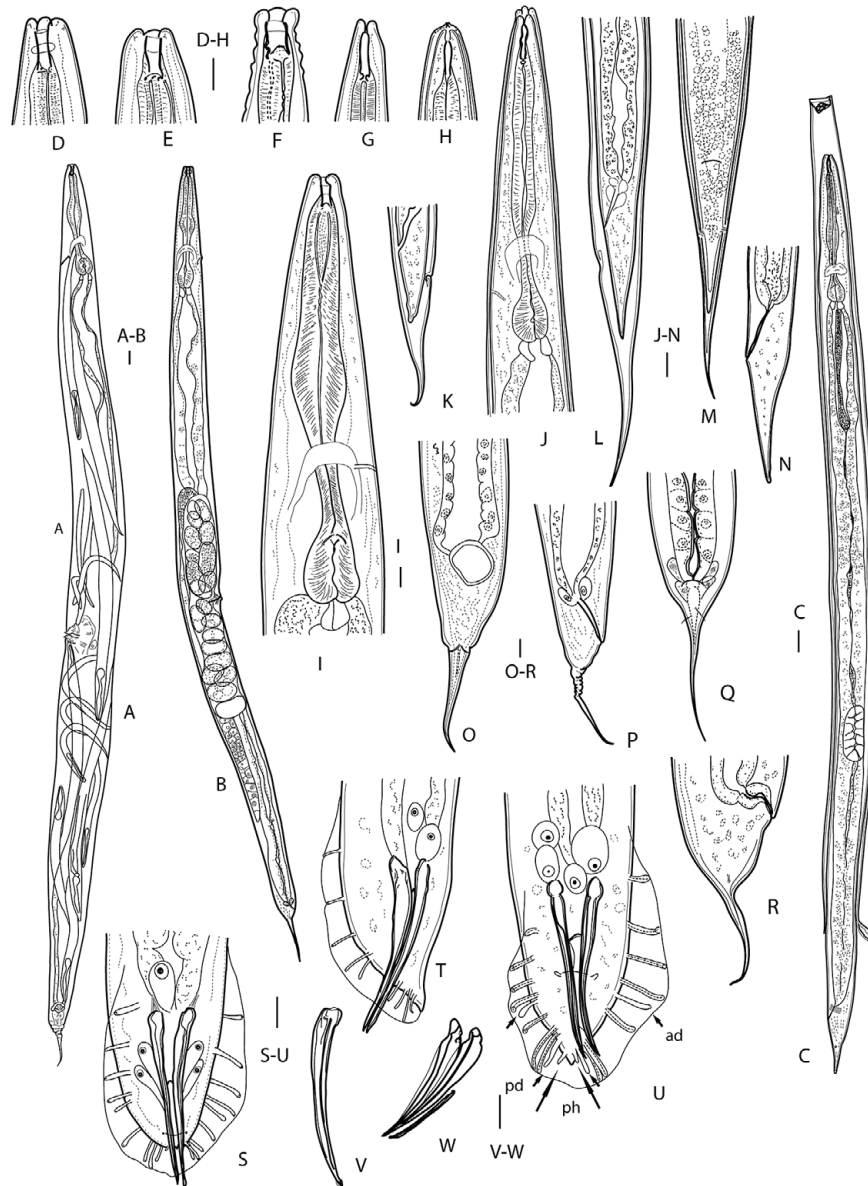
Figure 2. *Pellioditis thermalis* n. sp. (A) Entire female (*endotokia matricida*), lateral view; (B) entire female with eggs, lateral view; (C) Infective juvenile shedding extra cuticle, lateral view; (D–H) Head (D, female, sublateral view; E, Male, subdorsal view; F, Female, matricidal hatching specimen, subdorsal view; G, Exsheathed infective juvenile, lateral view; H, ensheathed juvenile, lateral view); (I and J) Pharyngeal region, lateral view (I, female; J, exsheathed juvenile); (K–M) Ensheathed infective juvenile, tail, lateral (K–L) and dorsal (M) view; (N) exsheathed infective juvenile, tail, lateral view; (O–R) Female, tail (O, K, ventral view, P, R, lateral view); (S–U): Male, tail (S, U, ventral view and T, lateral view); (V) spicule, lateral view; (W) spicules and gubernaculum, lateral view. A, C, E–G, I–L, O, P, S–V *thermalis* strain; B, D, H, Q, R, W *sindicae* strain. Abbreviations: ad, anterior dorsal genital papilla; pd, posterior dorsal genital papilla; ph, phasmid. (Scale bars 10 μm.)

accession number 1382, two paratype males on slide accession number 1383, and two paratype males and infective juveniles on slide accession number 1384. Strain *sindicae*. Paratype female on slide accession number 1385, the paratype male on slide accession number 1386, the paratype male, two paratype females, and juveniles on slide accession number 1387, four paratype females and juveniles on slide accession number 1388, and the paratype male, female, and juveniles on slide accession number 1389. Slides were deposited in the Museum of the Helminthological Collections of the Centre of Parasitology at the Severtsov Institute of Ecology and Evolution, Moscow, Russian Federation.

Type host. Type host *Tandonia* sp.
　Other hosts. *Acrotoma* sp., *Oxychillus* (*translucidus*?), and *Deroceras* sp.

Prevalence. Fourteen of 63 gastropod specimens infected (22.2%).

Type locality. Psekups River valley between Kotkh and Pshaf ridges 44º42'06" N 39º13'02"E.

Other locality. Bolshoi Utrish nature reserve by Anapa near the Black Sea coast at its east point 44º43'52" N 37º 25'30"E. Foothill forest of oak, beech, pine, and juniper at the first level of Navagir ridge (150 asl).

Etymology. The specific epithet reflects the type locality, an area rich in hot mineral springs. The strain *thermalis* comes from the type locality and is named after the species name. The strain *sindicae*, from the other locality, was named after the ancient district of Sindica on the Taman peninsula inhabited by the Circassian people.
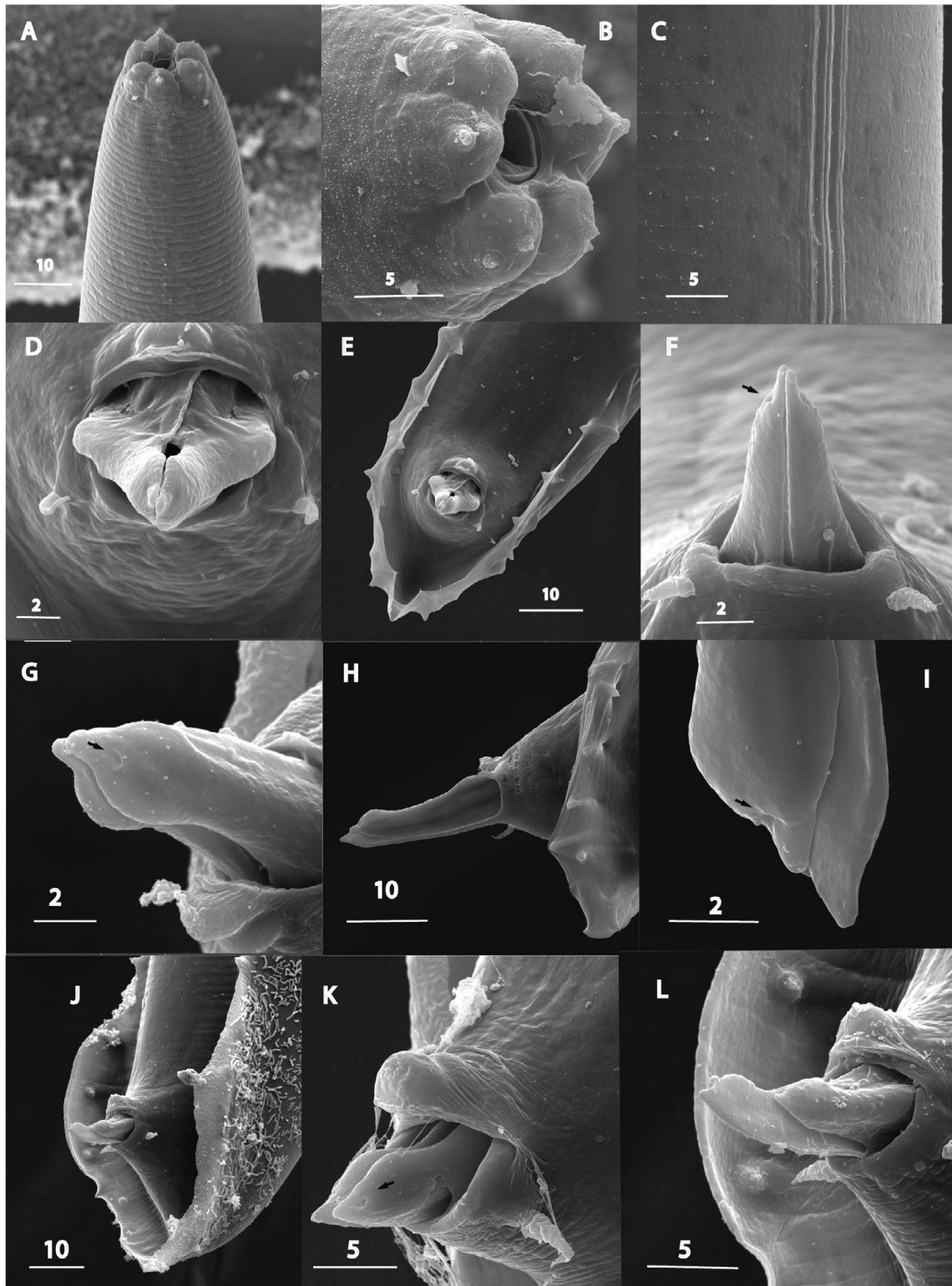
Figure 3. *Pellioditis thermalis* n. sp. SEM images. Male. (A) Sublateral view of anterior body region; (B) Head, subapical view; (C) Lateral field; (D) Cloacal region, ventral view; (E, J) Tail, ventral and sublateral view; (F, G, I) Spicule tips, dorsal and lateral view; (H, K, L) Cloacal region, lateral view. Arrow indicating hole at spicule tip. A–C and J–L *sindicae* strain; D–I *thermalis* strain. (Scale bars in μm.)

Diagnosis and relationships

Pellioditis thermalis n. sp. is characterised by females possessing a tail about 95–100 μm long with a broadly conical to a rounded anterior part and a subulate terminal part as long as the former, prominent phasmids located at the mid-tail, equatorial vulva position, a lateral field of three ridges (four incisions), males with spicules featuring a
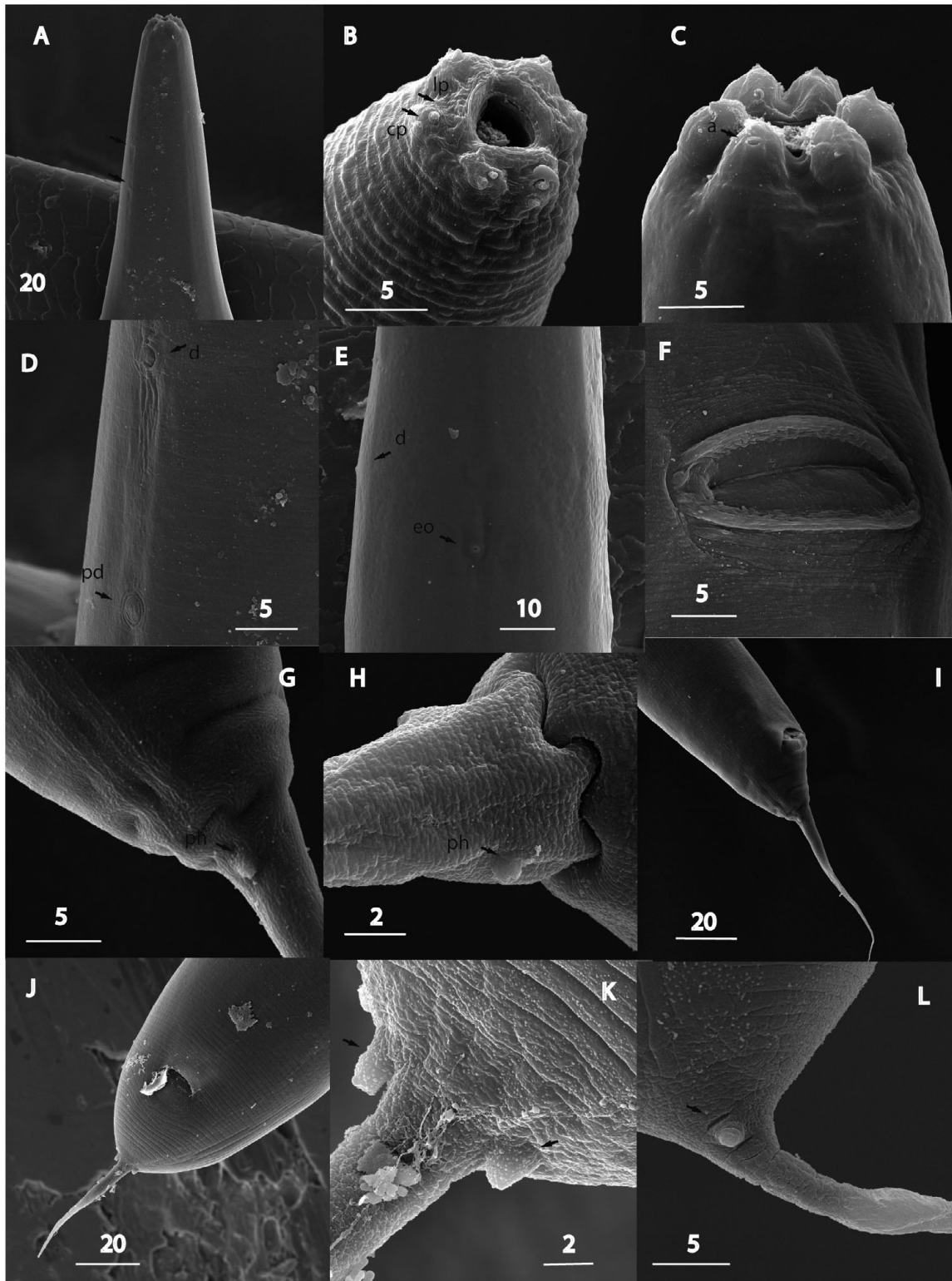
Figure 4. *Pellioditis thermalis* n. sp. SEM images. Female. (A) Sublateral view of anterior body region, arrows indicating deirid and posdeirid; (B) Head, subapical view; (C) Head, sublateral view; (D) Anterior body region showing deirid (d) and postdeirid (pd), sublateral view; (E) Anterior body region showing deirid (d) and excretory pore (eo), ventral view; (F) Vulva, subventral view; (G, H, K, L) Mid-tail region showing phasmids; (I, J) Tail. Abbreviations: a, amphid; cp, cephalic papilla; lp, labial papilla; ph, phasmid; A, D, F and J–L *sindicae* strain; B, C and G–I *thermalis* strain. (Scale bars in µm.)

hole at the pointed distal tip (Figures 3G, 3I, 3K, and 3L); ensheathed infective juveniles of average length 717–771 µm with the pointed tail terminus and exsheathed ones 644–682 µm with the rounded tail tip and the distinct molecular characteristics of the new species. The unusual feature of recognisable and specifically structured deirids and postdeirids was also discovered.
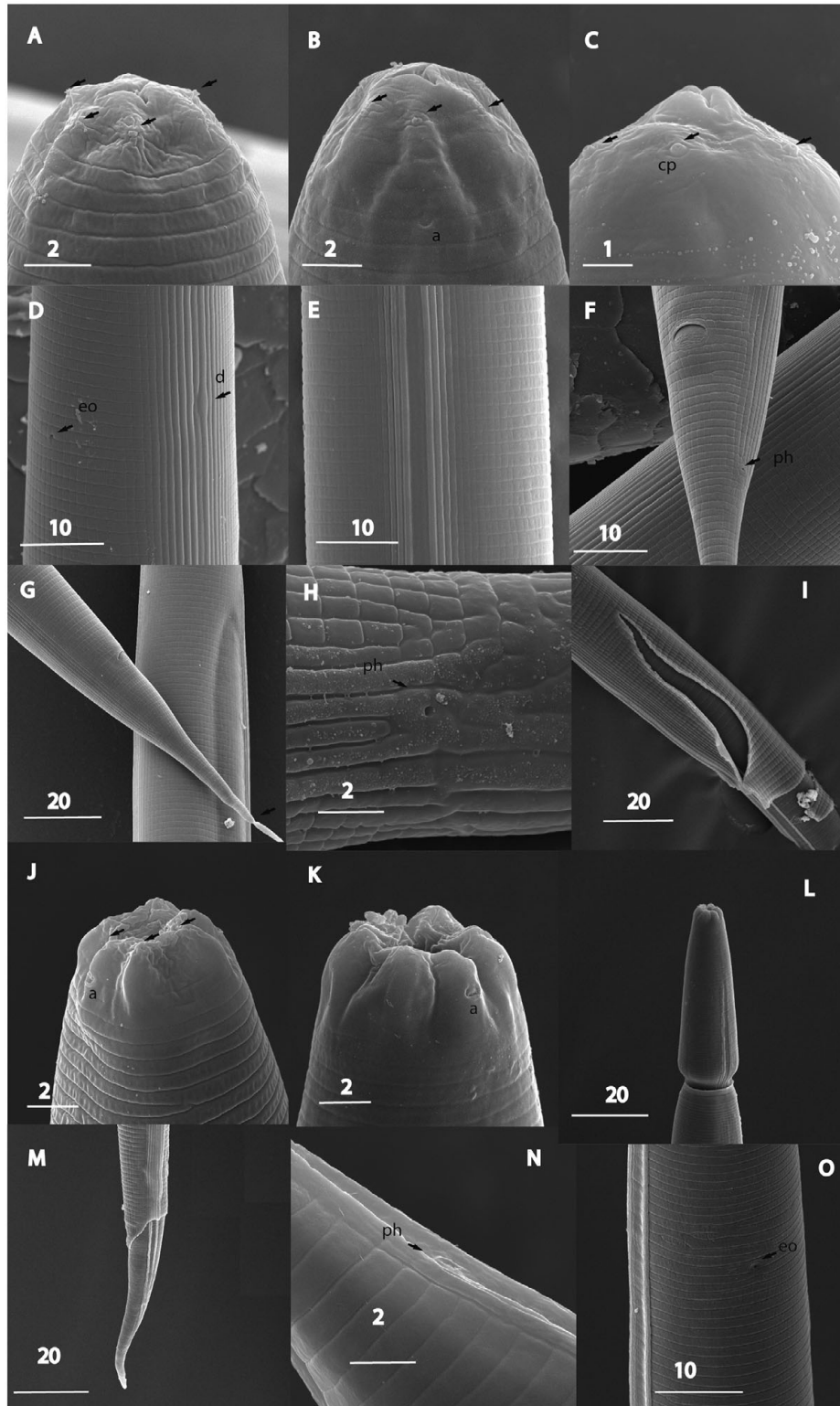
Figure 5. *Pellioditis thermalis* n. sp. SEM images. Infective juvenile. (A–C) Head, ensheathed infective juvenile, sublateral view; (D) Body region at mid-pharynx level, ensheathed juvenile, subventral view; (E) Lateral field, ensheathed juvenile; (F) Tail region, ensheathed juvenile, subventral view; (G) Tail region, ensheathed juvenile with broken tail (arrow); (H) Phasmid region, ensheathed juvenile, lateral view; (I, L, M) Infective juvenile shedding cuticle; (J, K) Head, exsheathed juvenile, sublateral view; (N) Phasmid region, exsheathed juvenile, subventral view; (O) Body region at mid-pharynx level, exsheathed juvenile, subventral view. Abbreviations: a, amphid; cp, cephalic papilla; eo, excretory opening; ph, phasmid; A, D–F, I and M *thermalis* strain; B, C, G, H, J–L and N-O *sindicae* strain. (Scale bars in µm.)
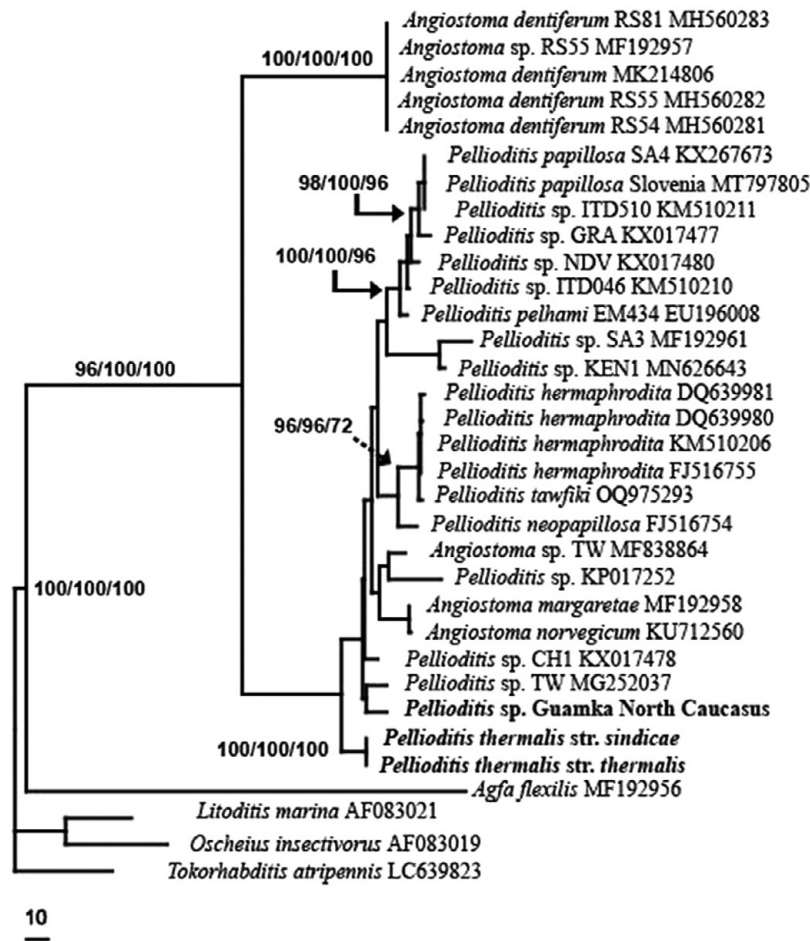
Figure 6. Phylogenetic relationships of *Pellioditis* species from the Caucasus region and other related *Pellioditis* species using the SSU 18S rDNA gene as inferred from Bayesian Inference. *Litoditis marina* AF083021, *Oscheius insectivorus* AF083019 and *Tokorhabditis atripennis* LC639823 were used as outgroup taxa. Bootstrap values and posterior probability (as a percentage) are assigned next to the relevant nodes as MP/NJ/ML.

Adult nematodes of both sexes of the new species differ from *P. quinamensis* Ivanova & Spiridonov, 2022 in having six separate lips vs three pairs of fused lips and ensheathed infective juveniles in having a rounded vs forked tail tip.

In having a gonochoristic way of reproduction, *P. thermalis* n. sp. differs from hermaphroditic *P. hermaphrodita* (Schneider, 1859) Andrássy, 1983 and *P. californica* Tandingan De Ley, Holovachov, McDonnell, Bert, Paine & De Ley, 2016.

By the shape of a female tail, the new species differs from all species of *Pellioditis* with a cupola-shaped tail joining a slender terminus, i.e., *P. bonaquensis* [1] Nermuť, Půža, Mekete & Mráček, 2016b, *P. huizhouensis* Huang *et al.* 2015, *P. meridionalis* Ivanova & Spiridonov, 2017, *P. papillosa* (Schneider, 1866) Andrássy, 1976, *P. safricana* Ross, Pieterse, Malan & Ivanova, 2018, and *P. zhejiangensis* Zhang & Liu, 2020. The female tail shape of *P. thermalis* n. sp. differentiates it from the group of species with a conical or conoid female tail gradually tapered to a pointed terminus, i.e., *P. pellio* (Schneider, 1866), *P. pelhami* Tandigan de Ley, Kiontke, Bert, Sudhaus, Fitch, 2023, *P. hermaphrodita*, *P. californica, P. neopapillosa* (Mengert in Osche, 1952) Andrássy

1983, *P. tawfiki* Azzam, 2003, *P. apuliae* Nermuť, Půža & Mráček, 2016a, *P. kenyaensis* Pieterse, Rowson, Tiedt, Malan, Haukeland & Ross, 2020, *P. quinamensis*, and *P. villasmundi* Ivanova, Clausi, Leone & Spiridonov, 2023. From the following members of this group, the new species can be also distinguished by shorter female tails (vs 168 μm in *P. kenyaensis;* 157 μm in. *P. neopapillosa*; vs 152 μm in *P. apuliae*; vs 128 μm in *P. tawfiki*; vs 122 μm in *P. villasmundi*; vs 114 μm in *P. pellio*; and 108 μm in *P. pelhami*).

Pellioditis bohemica Nermuť, Půža, Mekete & Mráček, 2017 was considered a junior synonym of *P. pellio* by Tandigan de Ley *et al.* (2023). The authors also expressed the opinion that *P. tawfiki* (still molecularly characterised only by the SSU sequence) represents the species of another genus, possibly *Pelodera*.

By the shape of the female tail, the present species is most similar to four other species from the Caucasus region, i.e., *P. circassica* Ivanova, Geraskina & Spiridonov, 2020, *P. clausiliiae* Ivanova, Geraskina & Spiridonov, 2020, *P. akhaldaba* Ivanova, Gorgadze, Lorthipanidze & Spiridonov, 2021, and *P. thesamica* Gorgadze, Troccoli, Fanelli, Tarasco & De Luca, 2022. While the female tails of *P. circassica*, *P. clausiliiae* and *P. thesamica* show little variation in shape, two discovered populations (strains 'quick' and 'slow') of *P. akhaldaba* show variation in female tail shape similar to *P. thermalis* sp. n. strains. From females of two known species from Georgia (*P. akhaldaba* and *P. thesamica*), the females of the present

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[1] Here and further on the specific name is used in the form 'bonaquensis' instead of original 'bonaquense' as was proposed by Nematology's Editor – see a correction in Ivanova & Spiridonov, 2022.
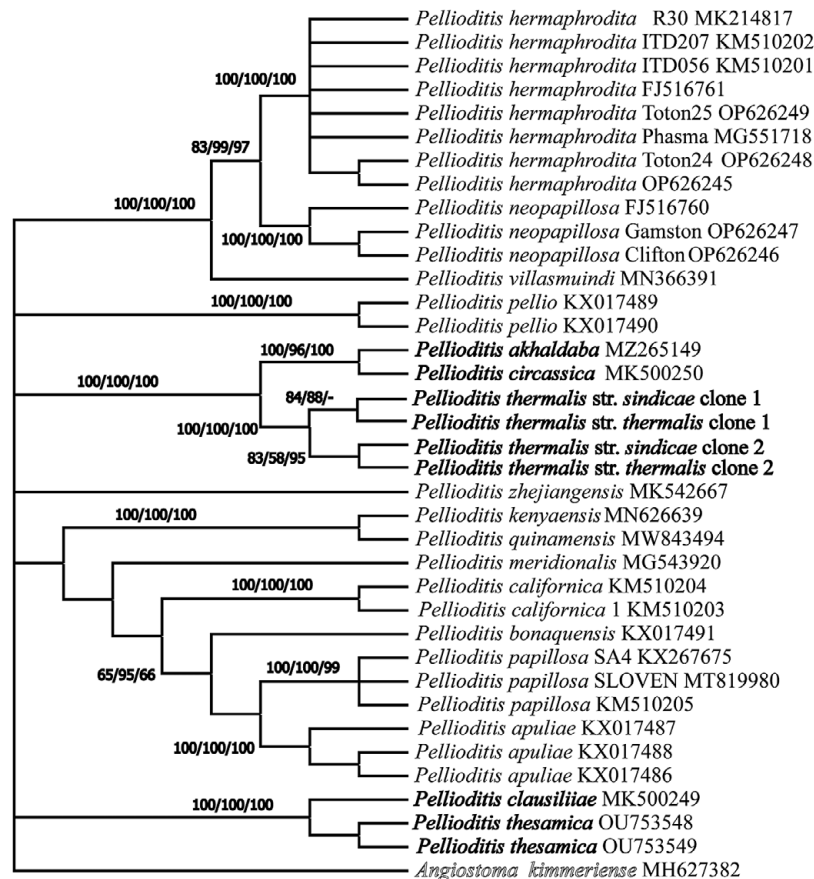
Figure 7. Phylogenetic relationships of *Pellioditis* species from the Caucasus region and other related *Pellioditis* species using the ITS rDNA gene as inferred from the Maximum parsimony (MP, GTR+G model), Neighbour joining and Maximum likelihood (ML, T92 +G model) analyses. *Angiostoma kimmeriense* MH627382 was used as outgroup taxa. Bootstrap values and posterior probability (as a percentage) are assigned next to the relevant nodes as MP/NJ/ML.

species can be differentiated by significantly shorter tails (95–100 µm vs 125–134 µm in *P. akhaldaba* and 138 µm in *P. thesamica*). Average female tail length of the present species is close to that of *P. circassica* (82 µm) and *P. clausiliiae* (94 µm). Females of *P. thermalis* sp. n. differ from the latter two species in having generally wider bodies (a = 15.7–18.1 in *thermalis* and *sindicae* vs 21.7–22 in *P. circassica* and *P. clausiliiae*). In addition, the unusually structured deirids and postdeirids that were observed in the *sindicae* strain have never been recorded elsewhere in the species of the genus.

Females of the present species differ from *P. mairei* (Maupas, 1919) in having a much smaller body length (vs 3400 µm). The latter species was also isolated from earthworms vs land gastropods.

Males of *P. thermalis* n. sp. are characterised by spicules with a mean length of 63 µm in the strain *thermalis* and 42 µm in *sindicae* and holes at their tips. In having holes in spicules, males of the new species can be compared with *P. circassica*, *P. clausiliiae*, *P. akhaldaba*, and *P. villasmundi*. From *P. villasmundi*, the present species differs in having significantly shorter spicules (vs 82 µm) and a different shape of the female tail. The males of the *thermalis* strain are comparable to the rest of species from Caucasus in the length of the spicules (vs 58 µm in *P. circassica*, vs 54 µm in *P. clausiliiae*, vs 60 µm in *P. thesamica*, and vs 65–67 µm in *P. akhaldaba*). The males of the *sindicae* strain differ from all mentioned species in having the shortest spicules (48 µm vs 58, 54, 65–67, 63, and 82 µm in *P. circassica*, *P. clausiliiae*, *P. thesamica*, *P. thermalis* n. sp., and *P. villasmundi*, respectively).

In having the same spicule length, males of *P. thermalis* n. sp. of the strain *thermalis* can be compared with *P. tawfiki*, but both species can be distinguished in having different female tail shape (see above) and much shorter infective juveniles (717–771 µm vs 966 µm). Spicule lengths of a similar range were recorded for *P. safricana* (61 µm), *P. pellhami* (60.4 µm), *P. pellio* (58.5 µm), *P. papillosa* (62 µm), *P. neopapillosa* (68 µm), and *P. zhejiangensis* (58 µm).

P. thermalis n. sp. differs from *P. pellio* and *P. pelhami* by having a non-furcated vs a furcated tip of the spicules and by a different shape of the female tail. Also, copulation in the present species follows a spiral rather than parallel type as was reported by Tandigan de Ley *et al.* (2023) for the former species. From *P. safricana* and *P. zhejiangensis* the present species can be distinguished by longer IJs (717 µm vs 543 µm and 610 µm) and from *P. papillosa* and *P. neopapillosa* by shorter IJs (vs 900 µm and 1010 µm). Additionally, the new species differs from *P. zhejiangensis* in the more anterior location of an excretory pore (shortly behind a nerve ring vs at the pharynx base). From *P. huizhouensis*, the present species differs in having the non-cuticularised vs cuticularised cheilostom, remarkably more anterior location of an excretory pore in females, and not-forked vs forked spicule tips.

From the recently described (under the name *Phasmarhabditis*) *P. eagyptiaca* Azzam, 2023 the present species can be distinguished by much shorter male tail (32–33 µm vs 63 µm) not extending vs extending beyond bursa margin and infective juveniles (717–771 µm vs 1112 µm).
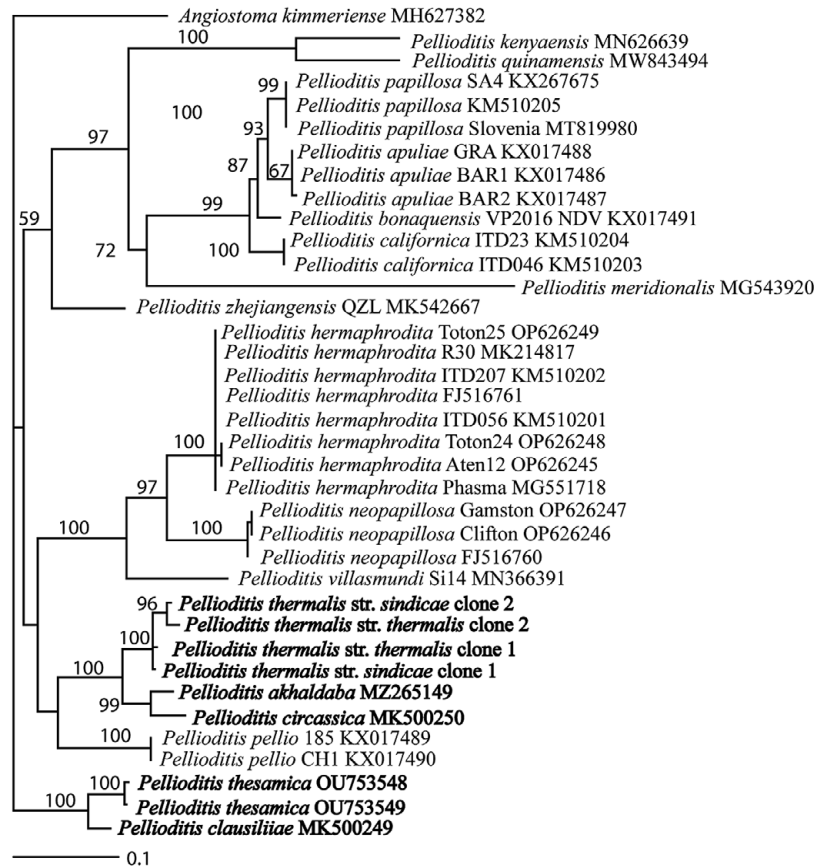
Figure 8. Phylogenetic relationships of *Pellioditis* species from the Caucasus region and other related *Pellioditis* species using the ITS rDNA gene as inferred from Bayesian Inference. *Angiostoma kimmeriense* MH627382 was used as outgroup taxa. Bootstrap values and posterior probability (as a percentage) are assigned next to the relevant nodes as MP/NJ/ML.
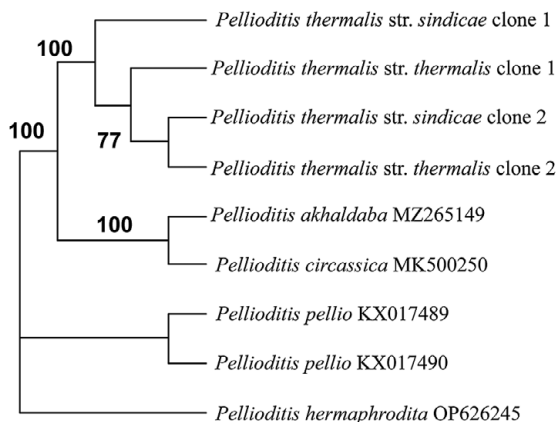

Figure 9. Phylogenetic relationships between the strains of *Pellioditis thermalis* n. sp. and the closest Caucasian species of the genus. MP analysis of ITS rDNA.

Pellioditis incilaria (Yokoo & Shinohara in Shinohara & Yokoo, 1958) has been accepted as a valid species by Tandigan de Ley *et al.* (2023) in their recent paper. However, the presence of 10 pairs of genital papillae distinguishes the males of this species from the rest of the genus.

Based on the length of infective juveniles, the new species can be compared with *P. clausiliiae* (742 μm), *P. akhaldaba* strain 'slow' (702 μm), *P. pellio* (= *P. bohemica*) strain 185 (691 μm), *P. thesamica* (670 μm), and *P. villasmundi* (773 μm), but it can

be distinguished by the combination of features and molecular characteristics.

In addition, the new species can be distinguished by the different average length of the IJs (717–771 μm vs 839 μm in *P. meridionalis*; vs 900 μm in *P. papillosa*; vs 543 μm in *P. safricana*; vs 610 μm in *P. zhejiangensis*; vs 1232 μm in *P. kenyaensis*; vs 812 μm *P. apuliae*; vs 553 μm in *P. pellio* (as *P. bohemica* CH1); vs 902 μm in *P. bonaquensis*; vs 1010 μm in *P. neopapillosa*; vs 839 μm in *P. meridionalis*; vs 966 μm in *P. tawfiki*; vs 1098 μm in *P. quinamensis*.

No data on morphology and morphometrics for infective juveniles of *P. pellio* and *P. pelhami* were provided by Tandigan de Ley *et al.* (2023).

Crossbreeding test

The test has shown that the offspring have been obtained in all variants of crossbreeding.

Molecular characterisation and phylogeny of P. thermalis n. sp.

We have obtained the sequences for three loci of the *P. thermalis* n. sp. nuclear genome, i.e., the partial SSU rDNA, the LSU rDNA, and the ITS-region of the rDNA, and a mitochondrial taxonomic marker, the partial *Cox*1 mtDNA. An analysis of partial SSU rDNA revealed that *P. thermalis* n. sp. clustered with a weakly supported clade consisting of all known *Pellioditis* sequences (including undescribed species from the North Caucasus) and several *Angiostoma* sequences (*A. margaretae*, *A. norvegicum*, and *Angiostoma*

sp. MF838864). Only the 'megaclade' containing all known *Pellioditis* and *Angiostoma* spp. SSU rDNA sequences was strongly supported (Figure 6). Phylogenetic analysis of LSU rDNA and *Cox*1 mtDNA was not informative for assessing the position of the new species in the genus *Pellioditis* due to the low number of informative positions for the former marker and scarcity of NCBI deposited data for the latter. In the LSU rDNA sequence, *P. thermalis* n. sp. was close to *P. circassica* (7 bp difference), but it showed nucleotide differences of 21–40 bp (in a 707 bp long alignment) from other species in the genus with known LSU rDNA sequences. The analysis of the *Cox*1 mtDNA showed that the difference between the strains of the new species was 41 bp. The partial sequence of *P. thermalis* n. sp. *Cox*1 mtDNA differed from *Pellioditis* spp., for which this locus is known, by 57–71 bp. Direct sequencing of the ITS rDNA amplicon was not possible as double peaks were observed after approximately 210 nucleotides in several repeated attempts. The primary PCR product was then cloned and analysed. For the strain *thermalis*, the analysis of five clones of primary PCR-product revealed the presence of two haplotypes, with or without the insert of 3 bp in the ITS1 rDNA. Similar to *thermalis* strain, two haplotypes were detected between clones (in total, four clones were examined) of *sindicae* strain due to the insert of 3 bp in the ITS1 rDNA.

Clone 1 of the *P. thermalis* n. sp. strain *sindicae* ITS rDNA was almost identical to clone 1 of *thermalis*. These two clones differed in five positions of the alignment (data not shown): one deletion and four insertions. All these indels were located at one site in the ITS rDNA region. Clones were identical throughout the rest of the ITS region. Two other clones (clone 2 of *P. thermalis* n. sp. *sindicae* and clone 2 of the *P. thermalis* n. sp. *thermalis*) showed more pronounced nucleotide differences, e.g., with the exclusion of positions in this variable site of the ITS1 rDNA, clone 2 of *P. thermalis* n. sp. *thermalis* differed by 10 bp from clone 1 of the same strain and by 12 bp from clone 1 of strain *sindicae*. A comparison of the sequences obtained from both haplotypes revealed the phyletic relationship of this species with two other Caucasian species: *P. akhaldaba* and *P. circassica*. Depending on the haplotype, *P. thermalis* n. sp. differed from *P. circassica* by 42 or 51 bp and from *P. akhalbada* by 38 or 48 bp.

As the ITS rDNA bp differences between the *thermalis* and *sindicae* strains were much lower, we concluded that they represent only two geographically distinct populations with some morphological variability, rather than independent species. The differences between *thermalis* and *sindicae* in the ITS rDNA, currently considered the most reliable marker, were slightly above the reported intraspecific level (8 bp). At the same time, the difference in *Cox*1 mtDNA between these strains was very pronounced, accounting for 41 bp or 7.5% of the sequences compared. The LSU rDNA sequences obtained from both strains were found to be identical. However, the LSU rDNA gene proved to be of little value for the taxonomy of this genus, being unable to resolve relationships between *Pellioditis* and certain *Angiostoma* species (Nermut' *et al.* 2016; Pieterse *et al.* 2020; Ivanova *et al.* 2020). Nematodes from both strains were shown to be able to reproduce in the interfertility test.

Other species of Pellioditis discovered in Caucasus region, and their interrelationships

We have isolated several strains of *Pellioditis* in land gastropods in Caucasus region (see Table 3) and described three new species from the territory of Ciscaucasia and one from Transcaucasia. No known

Pellioditis species occurring in Europe were discovered. The species distribution is shown in Figure 1.

Morphological and molecular-phylogenetic analyses of *Pellioditis* were carried out and included yet another recently described species of the genus, *P. thesamica* (Figures 6–9). The ITS-based analyses carried out have placed the new species close to *P. circassica* and *P. akhaldaba*. The former species was originally described from Adygea and later discovered in the Mineralnye Vody-Pyatigorsk zone (Russia) and Tbilisi (Georgia) and the latter in the Borjomi Valley (Georgia). One of the strains of *P. akhaldaba* was isolated from the surface of *Galleria melonella* cadavers, which were used as bait for entomopathogenic nematodes. All other strains of *P. circassica* and *P. akhaldaba* were isolated from gastropods collected at lower elevations of the mountains, similar to the newly described species. Two strains of *P. thermalis* n. sp. were discovered from gastropods collected on different sides of the Caucasian range (westernmost spurs for the *sindicae* strain and northwestern ones for *thermalis*), although separated by no great geographical distance. Phylogenetic analysis of *P. thermalis* n. sp. revealed the presence of two haplotypes in each strain, with crossing affinity between them. Notable biological differences were observed between the strains: in *thermalis* there were almost as many males as females, with habitual matricidal hatching observed, whereas both traits were rare in *sindicae*.

In the present study, ITS-based analyses placed *P. thermalis* n. sp. sp. in a subclade that forms the sister group with *P. circassica* and *P. akhaldaba* (Figures 6 and 7). The analysis also grouped two other Caucasian species, *P. clausiliiae* and *P. thesamica*, which show close relationships with the exotic *P. zhejiangensis*, although with weak support. Our analysis is consistent with the data of Gorgadze *et al.* 2022 who recently described *P. thesamica* from eastern Georgia. Thus, the present five *Pellioditis* species from the Caucasus form two subclades that are closest to the European *P. pellio* (previously described as *P. bohemica*), *P. neopapillosa*, *P. hermaphrodita*, and *P. villasmundi* and at the same time, to Chinese *P. zhejiangensis*. The latter group of species represents one of the clades of *Pellioditis* in the evolutionary reconstruction carried out by Tandigan de Ley *et al.* (2023).

To date, the species of *Pellioditis* originating from the Caucasus, i.e., *P. circassica*, *P. clausiliiae*, *P. akhaldaba*, and *P. thesamica*, form a morphologically comparable group characterised by an average body length of ensheathed infective juveniles of around 700 μm, females with a broadly conical to a rounded anterior part and a subulate terminal part of the tail, and males of all but the latter species with the holed spicule tips.

All species of Caucasian *Pellioditis* were associated with more than one gastropod species, both slugs and snails, both native and alien. Snail hosts were represented by smaller species, mainly from Clausiliidae and Oxychilidae families. Infection of larger helicids was not detected.

Angiostoma

The only species of *Angiostoma* discovered in the Caucasus (Adygea) was *A. kimmeriense* Korol & Spiridonov, 1991, for which contradictory phylogenetic relationships were described earlier (Ivanova & Spiridonov 2018). In short, the D2–D3 and ITS-based phylogenetic analyses placed the species among the members of the *Pellioditis* clade. *Angiostoma kimmeriense* is one of the few members of the genus that retains a rhabditoid stoma as an adult. Such a rhabditoid stoma structure is apparently an ontogenetic trait for this genus, and Morand (1988) had shown for four species of

Table 3. Comparison of morphological characters of five Caucasian species of *Pellioditis* Dougherty, 1953

Species and locality/character	Mean body length (male/female) (in mm)	Mean stoma size (male/female) (in μm)	Mean DL length (ensheathed/exsheathed) (in μm)	Cephalic cap	Lateral field pattern (DL)	Lateral field pattern (adults)	Female tail shape	Mean c (male/female)	Female phasmids' location	Mean spicule length	Hole at spicule tip
P. circassica Russia, Adygea, Nikel, Sibirka Creek, Merkulaevka, Stavropol Krai, Pyatigorsk, Georgia: Tbilisi	1.2/1.8	20 × 4/23 × 5	896/773	Present	9 ridges	4 ridges	Widely conoid with spike	37.2/22.7	At spike base or shortly anteriad	58	Present
P. clausiliiae Russia, Stavropol Krai, Georgievsk, Terskyi	1.2/1.3	17 × 3/19 × 4	742/810	Present	9 ridges	4 ridges	Widely conoid with spike	45.1/13.7	At spike base or shortly anteriad	54	Present
P. thermalis n. sp. strain *thermalis* Russia, Krasnodar Krai, Goryachiy Klyuch, Guam Gorge	1.5/1.7	17 × 4/19 × 5	717/644	Present but small	6 ridges	3 double ridges	Widely conoid with spike	44.7/17.7	On spike	63	Present
P. thermalis n. sp. strain *sindicae* Russia, Krasnodar krai, Bolshoi Utrish by Anapa	0.9/1.4	17 × 4/22 × 5	771/682	Absent	10 ridges	3 ridges	Dome-shaped with spike	28.3/13.7	At spike base	48	Present
P. akhaldaba strain 'quick' Georgia: Borjomi valley	1.3/2	17 × 7/21 × 7	862/667	Present	6 ridges	3 ridges	Widely conoid with spike	17/38.9	At spike base or shortly anteriad	65	Present
P. akhaldaba strain 'slow' Georgia: Borjomi valley	2.2/2.7	21 × 6/23 × 8	702/669	Present	6 ridges	3 ridges	Dome-shaped with spike	20.6/57.1	On spike	67	Present
P. thesamica Georgia: Mtskheta-Mtianeti region	2/1.2	18.8 × 5.7/21 × 6.9	670	Absent	10 incisures	3 ridges	Spike-like, distal part = 2/3 tail length	33/14.8	At spike base	60	No info

Angiostoma that at least two first juvenile stages of these species had similar stoma structures. The position of the male phasmids of *A. kimmeriense* (posterior to the posteriormost genital papillae) also places it with *Pellioditis* sp.

The new host and locality for *A. kimmeriense* have been recorded. However, angiostomatids as internal parasites and large-sized nematodes unable to live in the dead host represent a further step towards parasitism compared with *Pellioditis*. The new host in the Caucasus was the snail species of the same genus *Oxychillus* as found on the Crimean peninsula and also the local endemic.

Alloionema

The genus *Alloionema* Schneider, 1859 belongs to the family Alloionematidae of the Panagrolaimorpha order, which, like the "Rhabditidae", contains mainly free-living forms but also has a number of species switched to parasitism, mostly in invertebrates. The undescribed *Alloionema* sp. was found by the authors in Adygea in the foot of a glass snail *Oxychilus* sp. in 2019. Sequences for the LSU and ITS rDNA and *COX1* mtDNA regions of this nematode isolate (AlloS) were obtained, and comparison with the molecular characteristics of other representatives of the *Alloionema* (*A. appendiculatum* Schneider, 1859, *A. similis* Holovachov et al. 2016, and *A. luofuensis* Huang et al. 2016) revealed sufficient differences between them. In the phylogenetic analyses based on ITS rDNA and *COX1* mtDNA regions, the North Caucasus isolate of *Alloionema* was in a separate position and clustered with the basal node of the *A. appendiculatum* + *A. similis* clade (Ivanova et al. 2022). All other *Alloionema* isolates found in RF were shown to be represented by *Alloionema appendiculatum* of Central and Western European origin, co-introduced with the invasive slug *Arion vulgaris* Moquin-Tandon, 1855 (Ivanova et al. 2022; Nermuť et al. 2015). Unfortunately, we were not able to collect complete morphological material from Adygea because parasitic larvae from a snail foot did not develop into free-living adults, but died. Repeated attempts to collect infected gastropods from the original site also failed, and this new species is still awaiting description. It is the only record of *Alloionema* sp. in the Caucasus region.

Cruznema

Cruznema Artigas, 1927 are free-living rhabditid nematodes sometimes found in association with insects and rarely with land gastropods. The species was recovered from two specimens of small dead Hygromiidae gen. sp. in the steppe vegetation adjacent to agricultural fields on the slopes of the Stavropol Upland. Once recovered, it was easily maintained on pig kidney agar. In small-scale laboratory experiments, it has shown the ability to kill *Deroceras reticulatum*. This is the first report of *Cruznema* sp. associated with land gastropods in the RF and the Caucasus region.

Rhabditophanes

Rhabditophanes sp. (Rhabditophanes Fuchs, 1930, Alloionematidae) was found in *Xerolenta obvia* collected in the Podkumok River valley, with its relatively dry and hot climate. The *Rhabditophanes* are usual associates of beetles while *Alloionema* and *Neoalloionema* are always associated with snails and slugs. The new finding supports the affinity of the genera that make up the family Alloionematidae.

Discussion

The name *Phasmarhabditis* was confirmed as the junior synonym of *Pellioditis* Dougherty, 1953 after 40 years of work (Tandigan de Ley et al. 2023). To prove this, the authors (Tandigan de Ley et al. 2023) presented the redescription of the re-isolated type species of the *Pellioditis*, *Pellioditis pellio* (Schneider, 1866), and performed molecular reconstruction of the genus. They listed 22 species in the *Pellioditis*, combining all valid species of *Phasmarhabditis* except one (they synonymised *P. bohemica* Nermuť, Půža, Mekete & Mráček, 2017 with *P. pellio*), the newly described *P. pelhami* and *P. pellio*, *P. mairei*, and *P. incilaria*, which were listed in the *Pellioditis* by Sudhaus (2011) before to the study. Of all species in the genus, *P. mairei* and *P. incilaria* have not been molecularly characterized, and their brief descriptions provide little morphological information. For the validity of *P. mairei*, see the opinion of W. Sudhaus (2023), who also suggested that *P. tawfiki* may belong to a different genus, possibly *Pelodera*. While most members of the genus are known from terrestrial molluscs, the type species and *P. mairei* and *P. pelhami* were originally isolated from earthworms. At least two species (*P. pellio* and *P. hermaphrodita*) were later found in association with both types of host (Tandigan de Ley et al. 2023). The Caucasian *P. akhaldaba* was isolated from a gastropod as well as the cadaver of the baited *Galleria melonella*. Another species of the genus was found in rotten leaves (*P. huizhouensis*). This shows the ecological plasticity of the members of *Pellioditis*. The ability of *Pellioditis* spp. to grow on various organic materials has also been demonstrated experimentally (Wilson et al. 1995; Nermuť et al. 2014). While some gastropod-associated species can be pathogenic to their hosts (McDonnel et al. 2020; Laznik et al. 2020), no such effect was observed in the remainder of the genus.

For the phylogenetic reconstruction of *Pellioditis*, Tandigan de Ley et al. (2023) carried out the concatenated analysis based on nuclear and mitochondrial genes and constructed a supermatrix. This revealed the presence of three or four clades containing members of *Pellioditis* (= *Phasmarhabditis*) as well as *Angiostoma norvegicum* Ross et al. 2016 as a representative of the Angiostomatidae. All species originated from the Caucasus were shown to belong to the same clade as *P. pellio*, *P. neopapillosa*, *P. hermaphrodita*, *P. villasmundi* known from Europe, and *P. zhejiangensis* from China. In our ITS-based analysis, five Caucasian *Pellioditis* sp. formed two subclades: one related to *P. circassica* and another related to *P. clausiliiae* (Figures 6 and 7). The *circassica* group included *P. circassica*, *P. akhaldaba*, and *P. thermalis* n. sp., while the *clausiliiae* group included *P. clausiliiae* and *P. thesamica*, i.e., each group (subclade) included species isolated on both sides of the Caucasian range.

In terms of nucleotide number, the difference between species from different subclades of Caucasian *Pellioditis* ranged from 66 to 104 and between species within a subclade, from 22 to 51.

We detected the presence of two ITS rDNA haplotypes in both strains. All haplotypes grouped together in all analysis methods. At the same time, the haplotypes from different populations showed closer relationships to each other than to the haplotype from the same population (Figure 9). Hypothetically, the observed pattern could be the result of two separate evolutionary events. A population of initially non-polymorphic species was split by successive nucleotide changes in the ITS1 rDNA. After the reunion of the two separated populations and their hybridisation, a polymorphic species emerged. This polymorphic species passed through the area fragmentation, and being separated on both slopes of the Caucasian range, the populations underwent independent mutations in the

ITS rDNA region. The Western Caucasus region has undergone the deep geological changes. Primarily, in the Jurassic-Eocene there was the rift basin of significant depth, but in the Middle-Late Miocene, the inversion occurred there (Nikishin *et al.* 2010), accompanied by the immediate submergence of both slopes of the Greater Caucasus. Such events must have affected the populations of hosts such as snails and slugs on both slopes of the range, increasing the number of isolation events for terrestrial molluscs and accelerating intense speciation in the genus *Pellioditis*.

Species radiation has been investigated for several elements of the biota, including terrestrial gastropods, within the Caucasus hotspots (Mumladze *et al.* 2014; Neiber *et al.* 2015, 2017), but no data were available for the gastropod-associated nematodes prior to our study. However, the gastropod fauna of the Caucasus region has been extensively studied, and its phylogenetic relationships with gastropods of neighbouring regions and the evolutionary history of many taxa have been discussed (Hausdorf, 2000, 2001; Neiber *et al.* 2022). The richness of the gastropod fauna in the Caucasus (318 species, 66% of which are endemic; Walther *et al.* 2014) suggests the same for the nematode fauna. Considering the number of *Pellioditis* species found in the Caucasus region alone, it can be assumed that the diversity of gastropods and the high percentage of the endemic species definitely contributed to the nematode diversity of the region.

Apart from the aforementioned species from Caucasus, world fauna of *Pellioditis* includes species from Europe (*P. pellio*, *P. hermaphrodita*, *P. papillosa*, *P. neopapillosa*, *P. bonaquaensis*, *P. apuliae*, and *P. villasmundi*), North America (*P. californica* and *P. pelhami*), Africa (*P. tawfiki, P. safricana, P. kenyaensis, P. eagyptiaca*, and likely *P. mairei*), and East Asia (*P. incilaria, P. huizhouensis, P. meridionalis, P. zhejiangensis*, and *P. quinamensis*). Unidentified species of *Pellioditis* (as *Phasmarhabditis*) have been recorded in Japan (Waki & Sawahata 2019) and Egypt (Azzam & El-Abd 2021). According to recent research, *P. californica* is thought to have a cosmopolitan distribution, being isolated in the USA, Canada, UK, Ireland, Germany, and New Zealand (Tandingan De Ley *et al.* 2016; Wilson *et al.* 2016; Carnaghi *et al.* 2017; Andrus & Rae 2019, Mc Donnell *et al.* 2020; Brophy *et al.* 2020; Keyte *et al.* 2022). *Pellioditis papillosa* described from Europe has been reported in South Africa and the USA (Tandigan De Ley *et al.* 2016; Pieterse *et al.* 2017), and *P. hermaphrodita* in the USA (Tandigan de Ley *et al.* 2014), Chile (France & Gerding 2000), Egypt (Genena *et al.* 2011), Iran (Karimi *et al.* 2003), and New Zealand (Wilson *et al.* 2012). The distribution of *P. hermaphrodita* is thought to be the result of the introduction of its commercialised strain, which is on sale in Europe (Howe *et al.* 2020; Schurkman *et al.* 2022).

However, no common European species of *Pellioditis* has been found in the Caucasian region. Phylogenetically, the Caucasian species show more affinity in phylogenetic analysis to species from Europe than to the species of more distant origin, with the exception of *P. zhejiangensis* from China. Yet two Old World species from Egypt, both associated with a Mediterranean snail *Eobania vermiculata*, were found to group with European *P. hermaphrodita* (*P. tawfiki*) and *P. neopapiilosa* (*P. eagyptiaca*) in the ITS rDNA-based analysis (Azzam 2023).

The distribution of Caucasian species of *Pellioditis* reflects the distribution of the gastropod hosts on which the distribution of the parasites is based. The distribution/dispersal pattern of Caucasian *Pellioditis* shows overlapping ranges of species of the *circassica* and *clausiliiae* groups. The presence of different haplotypes could be explained in part by the lack of strict host specificity within the

genus *Pellioditis* and the presence of two (or more) species of *Pellioditis* infecting a single host. The co-infection of multiple parasitic taxa in a gastropod is a common event (Morand 1988; Singh *et al.* 2019; Ivanova *et al.* 2020, etc.) wherein nematode parasites occupy different ecological niches within a host. However, a co-infection of two *Pellioditis* species has also been observed (Keyte *et al.* 2022, own unpublished data).

According to the ITS-based analyses performed, five *Pellioditis* species discovered in the Caucasus region are united in two related subclades. The subclades include species from both Ciscaucasia and Transcaucasia, so the geographical barrier of the Caucasian range may or may not serve to delimit the faunas. From the other side, ancient geographical events probably formed the separate nematode faunas of Western Europe and the Caucasus region, which is reflected in the absence of species typical for Western Europe. All analyses in this study confirmed the monophyletic status of *Pellioditis* as a genus containing gastropod-associated species. Comparison of the morphology of species within the Caucasus group allowed us to identify certain common characters (Table 3), of which we consider the presence of holes at the tips of male spicules, the mean length of spicules, the mean length of dauer juveniles, and their lateral field pattern to be of main diagnostic value. The GP formula, the length of the bursal rays, and their position in relation to the ventral/dorsal side of the bursa are common to all these species. In general, it applies to the majority of *Pellioditis* species, where little variation is observed, except for *P. quinamensis*, which is characterised by the position of the anteriormost pair of papillae outside the bursa.

Broadly speaking, our results support the general distribution pattern of gastropod-associated nematodes described in previous studies. Compared to the diversity in the areas closest to the study area, i.e., in southern Europe, namely Bulgaria, Crimea, and Sicily, the Caucasus region shows a much higher diversity of *Pellioditis* species, which outweighs all other taxa found. At the same time, the numerous dissections of gastropods made in the study have shown the complete absence of *Agfa* Chitwood, 1935 in the Caucasian gastropods. Since *Agfa* species parasitise mainly in the salivary glands of larger limacid slugs, we tend to attribute this to the fact that this gastropod group is rare in the region.

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