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DESCRIPTION OF *METAMPHIDELUS* GEN. N. AND *SCLERAMPHIDELUS* GEN. N. (ALAIMIDAE: NEMATODA)*

by
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Summary. Two new genera of Alaimidae (Nematoda), *Metamphidelus* gen. n. and *Scleramphidelus* gen. n., are described. The main diagnostic characters of *Metamphidelus* are: vagina with thick, infolded walls; vulva with open lips, didelphic female reproductive apparatus, amphids with small and shallow fovea and small, roundish, heavily sclerotized opening. Two new species of *Metamphidelus*, *M. brzeskii* gen. n. sp. n. and *M. winiszewskae*, gen. n. sp. n., both from Poland, are described. *Scleramphidelus* gen. n., erected to accommodate the species *Amphidelus lemani* (Hofmänner, 1913), is characterized by sclerotized vulva and vagina and by thick-walled, heavily sclerotized spicules, lacking a central piece. The status of *S. lemani* and of *Paramphidelus dolichurus* (de Man, 1876) are discussed.

Since its establishment (Micoletzky, 1922), the family Alaimidae has undergone many major changes in its taxonomic position and classification, while differing opinions have been expressed on its phylogenetical relationships. It presently comprises 12 genera, but the boundaries between some of them are uncertain and the family itself is not well defined.

To remove this state of uncertainty, we have undertaken the revision of the family, based on a study of the material kindly made available by scientists and Institutions from all over the world and on a review of all the relevant literature.

In this paper, which is the first of a series concerning the taxonomy of the family, two new genera and two new species of Alaimidae are described.

Materials and methods

The specimens described here came from the following collections: Institute of Zoology, Polish Academy of Sciences, Warsaw; Agricultural University, Wageningen (The Netherlands); Collection of G. Thorne, deposited at the Nematology Laboratory of USDA, ARS, Beltsville (Maryland, USA); collection of J. Juget, Université Claude Bernard, Lyon (France); collection of F. Schiemer, Institute of Zoology, University of Wien (Austria).

Specimens were already mounted in glycerin on slides for the observation by light microscopy. For SEM, some glycerin embedded specimens were first washed with gradually added distilled water and then dehydrated by a gradual series of ethanol concentrations increasing to 100%. They were then critical point dried with CO_2, mounted on stubs and coated with gold.

Terminology: "lip region" is from the anterior end to the outer labial papillae; "head width" is measured at the stoma base.

METAMPHIDELUS gen. n.

Body relatively slender ("a" value ranging from 43 to 97), tapering less at the anterior end than at the posterior one. Body ventrally curved

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(C shaped) after fixation. Cuticle smooth. Mouth cavity inconspicuous. Labial sensorial pits in two circles of six, those in the outer especially evident; a circle of four tiny cephalic sensorial pits is also present. Amphids 2.5-4.5 head diameters from anterior end; their opening relatively small, irregularly oval, with strongly sclerotized margin; amphidial fovea also small and shallow, triangular in outline; amphidial ductus 0.8-1.09% of body length. Pharynx rather short ("b" value ranging from 4.4 to 4.9), posteriorly enlarging at 80-88% of its length.

Female reproductive apparatus didelphic; genital tracts short (G' 6.3-8.4, corresponding to 3.7-6.6 body widths; G" 5.9-11.4, corresponding to 3.3-6.9 body widths). Ovaries reflexed, only partially covering oviducts. Vagina with thick walls, as in *Amphidelus*, but markedly infolded. Vulva transverse, with open lips. Vaginal glands well developed.

Male reproductive apparatus monorchic, straight. Spicules and sperms obscure. Two mid-ventral preanal papillae.

Differential diagnosis: Metamphidelus gen. n. is similar to the genus *Amphidelus* in being didelphic, in the thick-walled vagina, open vulval lips and well developed vaginal glands. It differs from it, as well as from any other genera of Alaimidae, in the characteristic infolding of the vaginal walls and in the structure of the amphids, which are the main diagnostic characters of the new genus.

Type species: Metamphidelus brzeskii gen. n., sp. n.

Other species: Metamphidelus winiszewskae sp. n.

METAMPHIDELUS BRZESKII gen. n. sp. n.

(Fig. 1, 4 and 5F)

Holotype (female): L = 1.54 mm; a = 61.5; b = 4.6; c = 12.3; c' = 8.9; V = 52.5; G' = 7.6; G" = 11.37. Pharynx 323 µm; tail 127 µm; V-anus 605 µm; amphids-anterior end 12-14 µm.

Paratypes (females; n = 6): L = 1.34-1.46 mm; a = 43-71; b = 4.4-4.95; c = 10.8-13; c' = 7-9; V = 49.5-53.2; G' = 6.3-8.4; G" = 5.9-11. Pharynx 299-312 µm; tail 108-133 µm; V-anus 520-610 µm; amphids-anterior end 12.5-15 µm.

Paratype (male): L = 1.37 mm; a = 68.7; b = 4.6; c = 13.4; c' = 6.8. Pharynx 296 µm; tail 102 µm; amphids-anterior end 12 µm.

Description: body C-shaped after fixation, slender, tapering more markedly at the posterior end; head width 27-29.5% as wide as body width at cardia. Cuticle smooth, about 1 µm thick. Mouth cavity 1.7-2 µm long. Lip region cap-like, rounded, continuous with body contour. Anterior sensilla consisting of a circle of six tiny inner labial pits at the level of stoma base, a circle of six large outer labial pits 3.5-4 µm from anterior end and of 4 tiny cephalic pits 6-8 µm from anterior end. Amphids 12-15 µm from anterior end (2.5-3.5 head widths); in holotype their position is slightly asymmetrical. Amphidial opening small, 22-26% as wide as corresponding body diameter, drop-like, obliquely directed, with strongly sclerotized margin. Amphidial fovea shallow, small, triangular in outline. Fusus 17-20 µm far from amphidial opening. Amphidial ductus 12-15 µm long, 0.8-1% of body length. Pharynx slightly posteriorly expanded at 82-88% of its length. Excretory pore opening 55-64 µm from anterior end (18-20% of pharynx length), markedly anterior to nerve ring, which is located 139-165 µm from anterior end. Cardia conoid. Rectum 11.5-12.5 µm long, slightly longer than anal body width. Distance between pharynx base and vulva corresponding to 1.2-1.4 pharynx lengths. Vulva-anus distance corresponding to 4-6 tail lengths. Female reproductive apparatus didelphic, with symmetrical gonads; both anterior and posterior genital tracts 5-7 body widths long. The reflexed ovaries contain 4-6 oocytes in a single row; proximal part of oviducts, not covered by ovary tips, expanded, sac-like, with numerous nuclei in their walls; between ovary and oviduct a rather long ovarial sac is

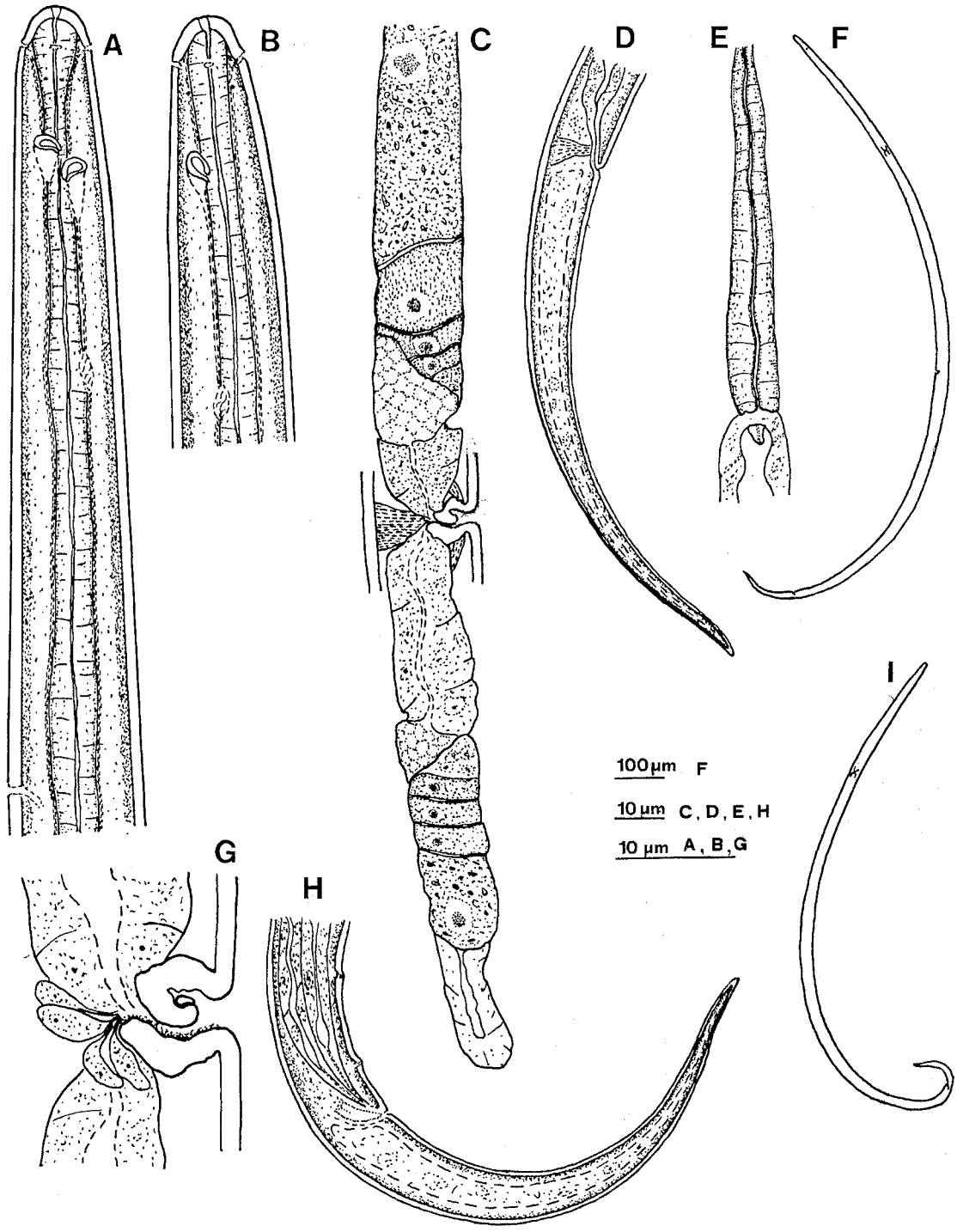

Fig. 1 - *Metamphidelus brzeskii* gen. n., sp. n.: A and B, anterior end; C, female reproductive apparatus; D, female tail; E, pharynx end; F, female body; G, vulval region; H, male tail; I, male body.

present. Vagina with thick walls, variously infolded; vulva transverse, with open lips. From the ventral area of the vaginal walls two small muscular bundles direct towards uterus; from the dorsal walls of vagina various muscular bundles direct towards the dorsal body wall. Two pairs of glands are located dorsally to vagina and open into it.

Male reproductive apparatus monorchic, with straight test. Since the single male specimen was in poor condition, sperm shape and spicule structure were obscure. Spicule length 7.5 µm, 0.6 anal body widths. Two midventral preanal papillae present, respectively 1.2 and 2.9 µm from cloacal opening.

Tail in both sexes elongate conoid, with pointed terminus, ventrally curved, especially in male.

Diagnosis: M. brzeskii sp. n. is characterized by a rather stout body, a cap-like lip region with rounded terminus, drop-like amphids, elongate conoid tail 7-9 anal body widths long, didelphic gonads.

Type locality and habitat: Bialowieza (Poland): forest, division 664, *Querco-carpinetum medioeuropaeum* Tuxen, 1937; soil. FD-4-3b (collected in September 1988 by M. W. Brzeski).

Type material: holotype female deposited in the Muzeum i Instytut Zoologii PAN (Warsaw, Poland); 2 females and 1 male paratypes deposited in the Dipartimento di Biologia Animale of the Catania University (Italy).

Derivatio nominis: the new species is named in honour of the Prof. M. W. Brzeski, who kindly provided the specimens.

METAMPHIDELUS WINISZEWSKAE gen. n. sp. n. (Fig. 2)

Holotype (female): L = 1.937 mm; a = 97; b = 4.7; c = 8; c' = 24.1; V = 46.6; G' = 6.55; G" = 10.2. Pharynx 410 µm; tail 241 µm; V-anus 793 µm; amphids-anterior-end 16 µm.

Description: body slightly ventrally curved after fixation, very slender, tapering at both extremities, more markedly at the posterior one. Head width 26.5% as wide as body width at cardia. Cuticle smooth, about 1 µm thick. Mouth cavity 1.4 µm long. Lip region truncate - conoid, continuous with body contour. Anterior sensilla consisting of a circle of 6 small inner labial pits, located at the level of the stoma base, a circle of 6 large outer labial pits, 4 µm far from anterior end, and a circle of 4 tiny cephalic pits, 8 µm far from anterior end. Amphids 16 µm from anterior end (4.5 head widths). Laterally observed, they show a small opening, strongly sclerotized, and a short and shallow fovea. Fusus 29 µm from amphidial opening; amphidial ductus 21 µm long, 1.1% of body length. Pharynx gradually enlarging at 80% of its length. Cardia truncate-conoid. Excretory pore ventrally opening 52 µm from anterior end (at 13% of pharynx length), considerably anterior to nerve ring, 198 µm far from anterior end. Rectum length 15 µm, 1.5 anal body widths. Distance between pharynx base and vulva 1.2 pharynx lengths. Vulva-anus distance 3.3 tail lengths. Female reproductive apparatus didelphic, with symmetric gonads. Anterior and posterior genital tracts respectively 6 and 7 body widths long. Ovaries reflexed, short, with a few oocytes in a single row and numerous oogonies in several rows at the tip. Oviduct only partially covered by the reflexed ovary. An ovarial sac is present between oviduct and ovary, longer in the anterior genital tract. Uterus long, sac-like; vagina with very thick walls, markedly infolded; vulva transverse with open lips. A strong muscular bundle joins the dorsal vaginal wall to the dorsal body wall. Tail long and slender, regularly tapering to the finely rounded terminus, never becoming filiform.

Diagnosis: M. winiszewskae sp. n. is characterized by its long and very slender body, small amphids with strongly sclerotized opening, female reproductive apparatus didelphic and long tail. It differs from *M. brzeskii* sp. n. in being

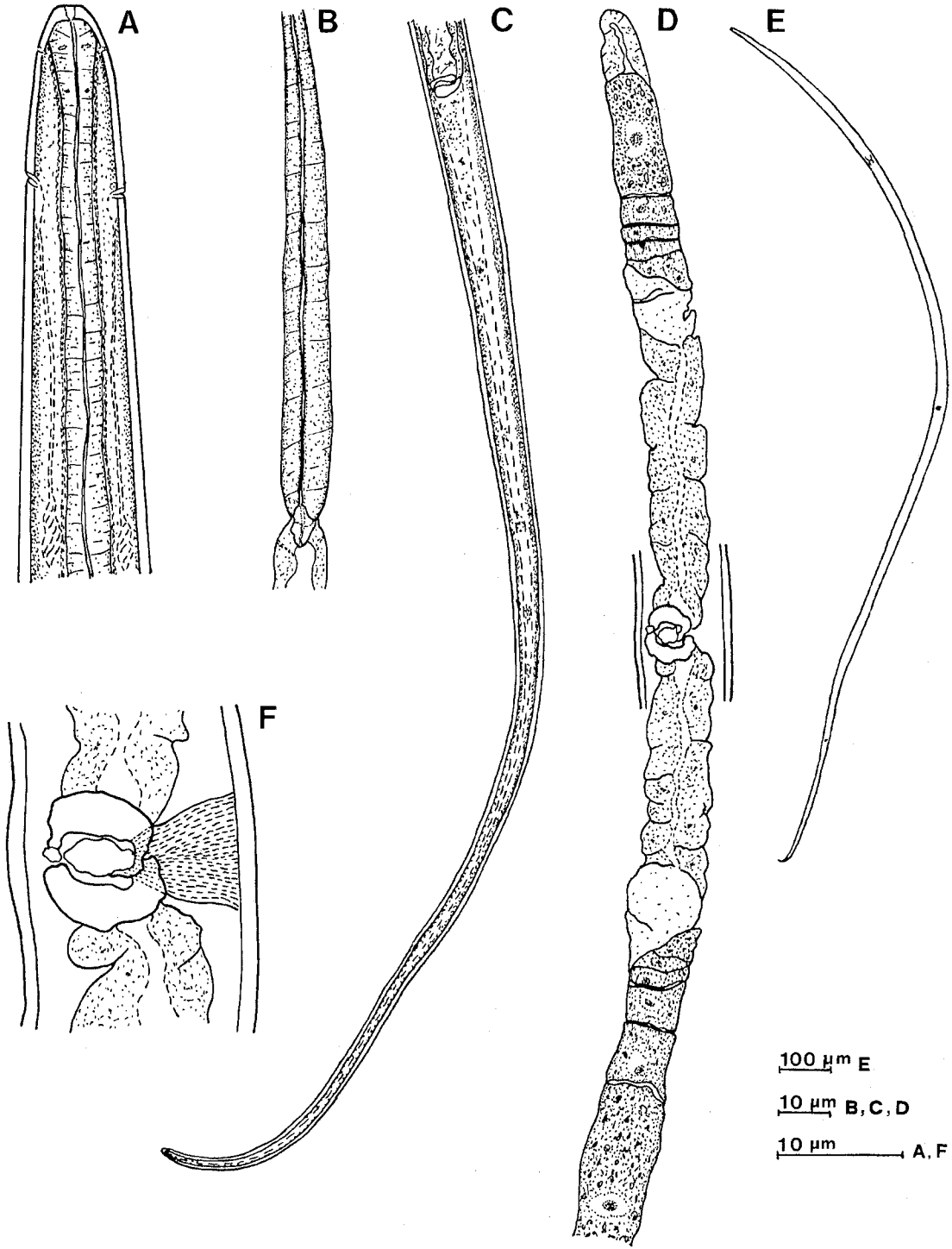

Fig. 2 - *Metamphidelus winiszewskae* gen. n., sp. n.: A, anterior end; B, pharynx end; C, female tail; D, female reproductive apparatus; E, entire body; F, particular of the vulvo-vaginal area.

longer and thinner, in the more truncate head, the posterior amphids, the anterior vulva, the longer and differently shaped tail.

Type locality and habitat: Rekowo, prov. Gdansk (Poland), meadow on muck soil. CF-1-3d (collected on May 5, 1980 by A. Skwiercz).

Type material: the holotype is deposited in the Muzeum i Instytut Zoologii PAN (Warsaw, Poland).

Derivatio nominis: the new species is named in honour of Dr Grazyna Winiszewska - Slipinska, who kindly provided the material.

KEY TO THE SPECIES OF METAMPHIDELUS *gen. n.*

– a < 70; c > 10; c' < 9; lip region rounded; amphids less than 4 head widths from anterior end . . . . . *M. brzeskii* sp. n.

– a > 90; c < 9; c' > 20; lip region truncate; amphids more than 4 head widths from anterior end . . . . . *M. winiszewskae* sp. n.

SCLERAMPHIDELUS gen. n.

Body almost straight after fixation, rather stout ("a" ranges between 44.8 and 63.5), tapering at both extremities, particularly at the posterior one. Lip region more or less continuous with body contour. Anterior sensilla consisting of three circles of sensorial pits, corresponding respectively to 6 inner and 6 outer labial and to 4 cephalic pits. Amphids located at 2.6-4.6 head widths from anterior end; they are large, stirrup-shaped; amphidial opening slit-like to oval, with sclerotized margin; fovea wide and deep, with evident innervation. Fusus joined to fovea by an amphidial ductus long 1.1-1.5% of body length. Pharynx rather short ("b" ranges between 4.1 and 5.7), gradually expanding at 85-90% of its length.

Female reproductive apparatus mono-opisthodelphic, with prevulval sac in the single species known; the genital tract is 7.4-8.5 body widths long. Distal part of uterus forming a spermathaeca, reached by the tip of the reflexed ovary. Vagina very short, with walls thin and sclerotized; vulva with strongly sclerotized lips.

Male reproductive apparatus monorchic. Sperms spindle-shaped in the seminal vesicle. Spicules large, tubular, with thick, strongly sclerotized walls, slightly S-shaped, without central piece. Midventral preanal papillae 4-5 in number.

Tail long and slender in both sexes.

Diagnosis and discussion: Scleramphidelus gen. n. resembles the genus *Paramphidelus* Andrássy, 1977 in the amphid structure, the thin-walled vagina and the opisthodelphic gonad, but differs from it and from any other genus of Alaimidae in the sclerotized vagina and vulva and in the peculiar shape and structure of the spicules, which - uniquely among Alaimidae - lack a central piece.

The single species so far described with such characteristics, *Alaimus lemani* Stefanski, 1914, had been transferred to the genus *Amphidelus* Thorne, 1939 by Juget (1969) and, when this genus was revised and split by Andrássy (1977), to *Paramphidelus*. The latter author, however, had observed only a single male; therefore he could not observe the peculiar structure of the vulvo-vaginal area, nor could he compare the structure of those spicules to that of the spicules of other males of *Paramphidelus*, since all the specimens of this genus studied by him were females. Moreover, the spicule structure of the few males of *Paramphidelus* known at that time had been rather vaguely, or even erroneously, described. The present, detailed study of the morphology of spicules in all the described species of Alaimidae allowed us to recognize the absolute peculiarity of the structure of the spicules of *Alaimus lemani* and hence the high diagnostic value of this character in defining a taxon of supraspecific rank.

Type species: Scleramphidelus lemani (Stefanski, 1914) n. comb.

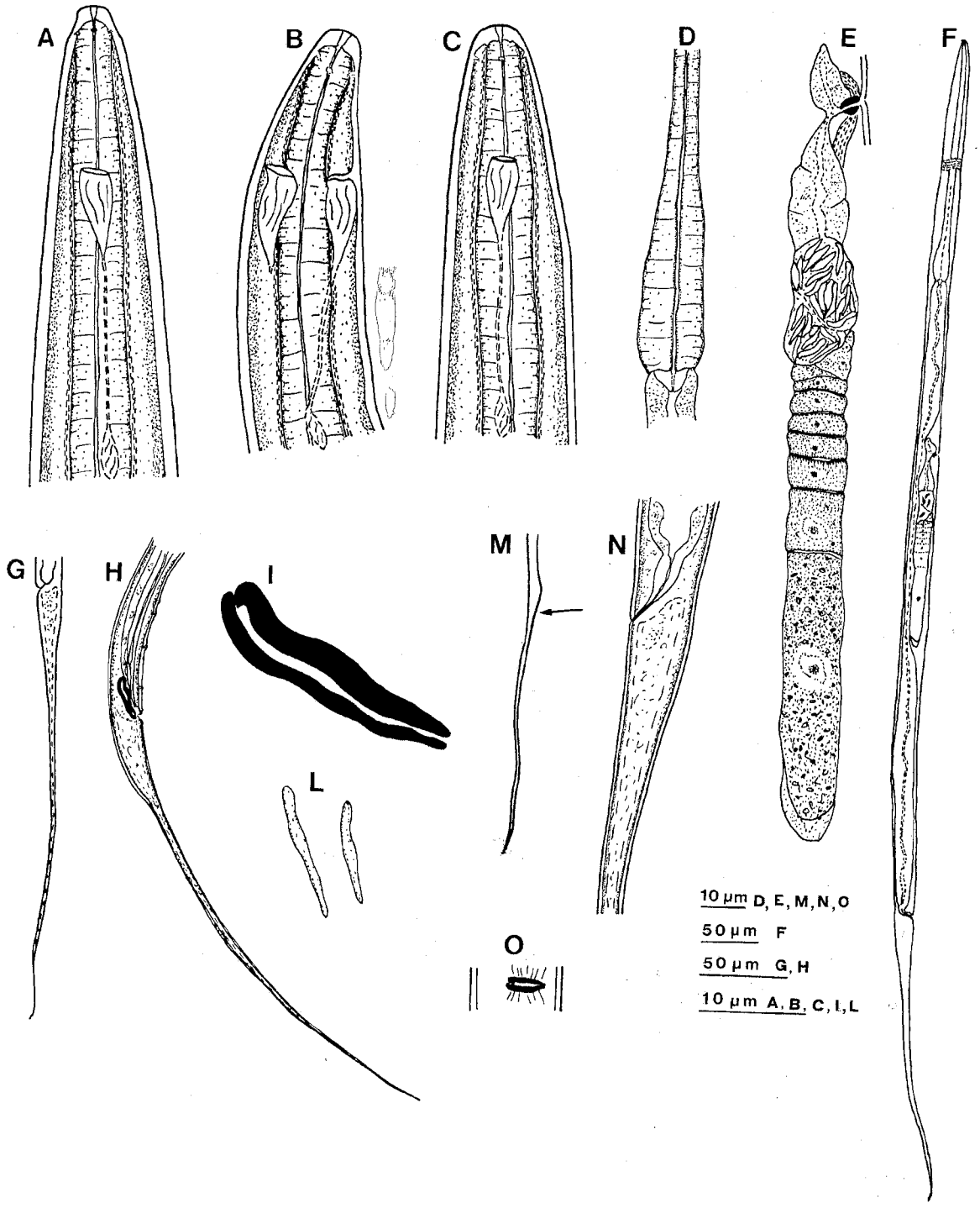

Fig. 3 - *Scleramphidelus lemani*: A, B and C, anterior end; D, pharynx end; E, female gonad; F, entire female body; G, female tail; H, male tail; I, spicule; L, sperms; M, female tail end; N, anal region; O, vulva.

Syn: *Alaimus lemani* Stefanski, 1914, partim.
Amphidelus lemani (Stefanski, 1914) Juget, 1969.
Paramphidelus lemani (Stefanski, 1914) Andrássy, 1977.
Amphidelus lemani longicaudatus Schiemer, 1978.
Alaimus dolichurus apud Hofmänner *et* Menzel, 1915 nec de Man, 1876 n. syn.
Amphidelus dolichurus apud Thorne, 1939 nec de Man, 1876 n. syn.
Other species: not known.

SCLERAMPHIDELUS LEMANI (Stefanski, 1914) N. Comb.

(Fig. 3 and 5 A-E; Table I)

Alaimus lemani
Stefanski, 1914; These: p. 11-12; fig. 1 A-C.
Amphidelus lemani
Thorne, 1939; *Capita Zool.*, 8: p. 174.
Andrássy, 1961; *Acta Zool. Hungar.*, 7: p. 7-8; fig. 3 A-C.
Meyl, 1961; Die Tierwelt Mitteleuropas: p. 115; fig. 608 a-b.
Juget, 1969; *Bull. Soc. Vaud. Sci. Nat.*, 70: p. 171-172; fig. 14 A-G.
Amphidelus lemani longicaudatus
Schiemer, 1978; *Hydrobiologia*, 28: p. 190-191; fig. 12 a-c.
Alaimus dolichurus
Hofmänner, 1913; *Rev. Suisse Zool.*, 21: p. 41-42.
Hofmänner e Menzel, 1915; *Rev. Suisse Zool.*, 23: p. 119.
Amphidelus dolichurus
Thorne, 1939; *Capita Zool.*, 8: p. 175; fig. 239-239a.

The following description is based on the specimens observed by us.

Material examined: 2 females and 1 male from Switzerland (collection of Juget); 1 female from Austria (collection of Schiemer); 1 female from Utah (collection of Thorne); 5 females and 1 male from The Netherlands (collection of Loof).

Description: body not very slender, generally straight after fixation, tapering at both extremities, particularly at the posterior one. Head width 14.4-22% as wide as body width at cardia. Cuticle smooth, about 1.5 µm thick. Mouth cavity 1.5-1.9 µm long. Lip region truncate conoid, more or less high in specimens of different populations, slightly set off from body. Anterior sensilla consisting of a circle of 6 minute inner labial pits, a circle of 6 well evident outer labial pits, 2.3-3.4 µm far from anterior end, and a circle of 4 tiny cephalic pits, 7.5-10 µm far from anterior end. Amphids 12-22 µm from anterior end (2.6-4.6 head widths). Amphidial opening slit-like to oval, with sclerotized margin, 2.5-3 µm wide, about 1/3 as wide as corresponding body width. Amphidial fovea large, goblet-shaped, rather deep and swollen, with conspicuous innervation. Fusus 25-30 µm from amphidial opening; amphidial ductus 14-20 µm long, 1.1-1.5% of body length. Pharynx rather slender, enlarging at 85-90% of its length. Cardia truncate conoid. Excretory pore generally obscure, in a single specimen 45 µm from anterior end (20% of pharynx length). Nerve ring 117-132 µm from anterior end. Rectum 10-19 µm long (about half anal body width), with thick walls and a sphincter at the intestinal junction. Distance between pharynx base and vulva 0.6-0.9 pharynx lengths. Vulva-anus distance 1.5-7.8 tail lengths.

Female reproductive apparatus mono-opisthodelphic with prevulval sac. Genital tract 7.4-8.5 body widths long. Pre-vulval sac 12.5-40 µm long (0.6-1.5 body widths). Ovary reflexed, its tip reaching the spermathaeca located in the distal part of uterus. Spermathaeca generally full of spindle-shaped sperms, about 8 µm long. Vagina very short, with thin walls, internally sclerotized. Vulva lips strongly sclerotized. In some specimens an egg is present in the uterus, cylindroid, 77-80 µm x 16 µm in size. From the ventral body wall encircling the vulval lips numerous muscles radiate ventro-laterally.

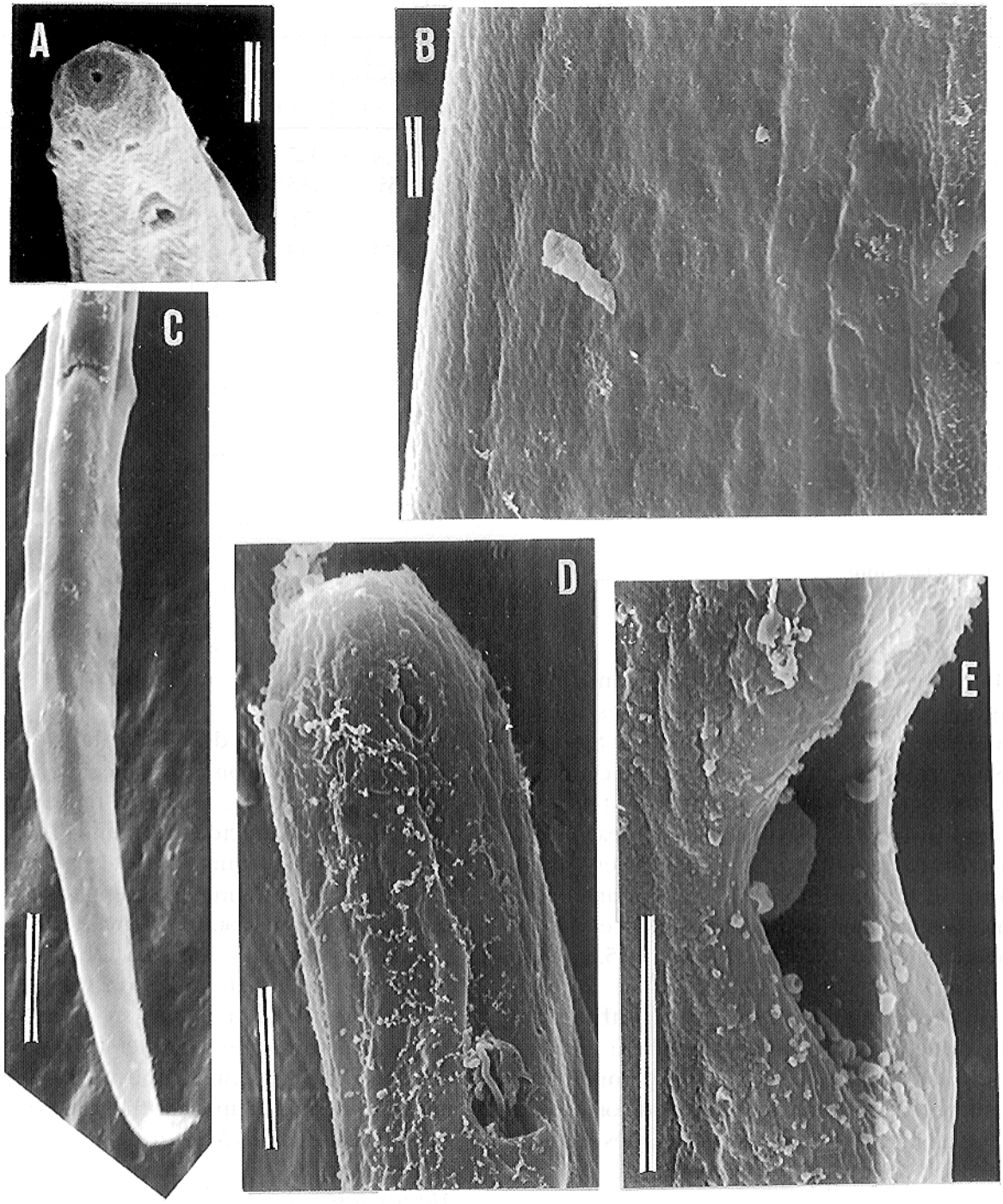

Fig. 4 - *M. brzeskii* gen. n., sp. n.: A and D, anterior end; B, vulval region; C, tail; E, particular of vulva. Scale bars 2.5 µm for A, B, D, E and 10 µm for C.

TABLE I - *Measurements of* Scleramphidelus lemani *(Stefanski, 1914).*

	Holotype (Stef.)*	Allotype (Juget)*	Switzerland (Juget)*		(Hofm.)*	Hungary (Andr.)*	Austria (Schiemer)*		Geldermalsen		Leiderdorp	Utah
	♂	♀	♀ (n = 4)	♂ (n = 4)	♀ (n = 3)	♂ (n = 1)	♀ (n = 1)	♂ (n = 1)	♀ (n = 3)	♂ (n = 1)	♀ (n = 2)	♀ (n = 1)
L (mm)	1.32	1.39	1.23-1.39	1.21-1.29	1.25-2.15	1.36	1.34	1.27	1.12-1.22	1.28	1.02-1.07	1.21a
a	58	50	48-57	48-63	50-65	53	55	63.5	44.8-48.8	55.6	51-53.5	40.3
b	4.2	4.5	4.5-5	4.1-5	5-5.7	5.1	5.6	5.1	4.8-5.7	5.1	4.75-4.8	5.5
c	6	5.8	5.7-5.9	5.5-6.2	6-7	4.7	3.75	3.65	4-4.3	4.4	4-4.28	4
c'									17.3-20	14.5	16.7-17.3	15
V		38	35-38				34.5		35-36.6		34.8-36.5	34.7
G"									13-23		16-17.4	
Phar (µm)									211-225	250	215-230	220
Tail (µm)									260-295	280	247-256	297
V-anus (µm)									454-484		380-410	490
Amph.-Ant. end (µm)									12.2-12.5	14	12.2-12.7	14.3

* Data from literature.

Male reproductive apparatus monorchic, straight; seminal vesicle containing slender, spindle-shaped sperms 11-13 µm long. Spicules massive, tubular, lacking central piece, with thick, strongly sclerotized walls, 24-25 µm long (1.2 anal body widths). Midventral preanal papillae 4-5 in number; in two specimens their distance in µm from cloacal opening and from each other in postero-anterior sequence was as follows: 1), 4, 15.2, 21.6, 18.4, 8; 2) 5.8, 16.7, 17.5, 9.

Tail very long and slender in both sexes, where three parts can be distinguished: convex-conoid proximally, after an abrupt constriction, the tail becomes elongate-cylindroid for a long tract, until it tapers again and becomes filiform in the distal tract.

Geonemy and ecology: Leman Lake (Switzerland): lake sediments; Neusiedlersee (Austria): lake bottom; Gerdelmassen and Leiderdorf (The Netherlands): soil; Lake Mary and Maple Canyon (Utah, USA): lake shore and soil. Baradla cave (Hungary): river mud.

Discussion

Stefanski (1914) described the new species *Alaimus lemani* from two specimens, one female and one male, found in the Leman lake. Thorne (1939) erected the genus *Amphidelus* and transferred Stefanski's species to this genus. Juget (1969), studying the nematode fauna of the Leman basin, found 5 females and 4 males belonging to *A. lemani* and, checking Stefanski's nematode collection from that locality, realized that this two specimens belonged to two different species; therefore, he erected for the female specimen the new species *Amphidelus stefanskii* and left the male in the species *Paramphidelus lemani.*

De Man (1876) described the new species *Monhystera? dolichura* from The Netherlands, which he later (1880) transferred to the new genus *Alaimus.* Since then many authors have reported this species from various localities. Thorne (1939) transferred this species to the new genus *Amphidelus.* He compared his pop-

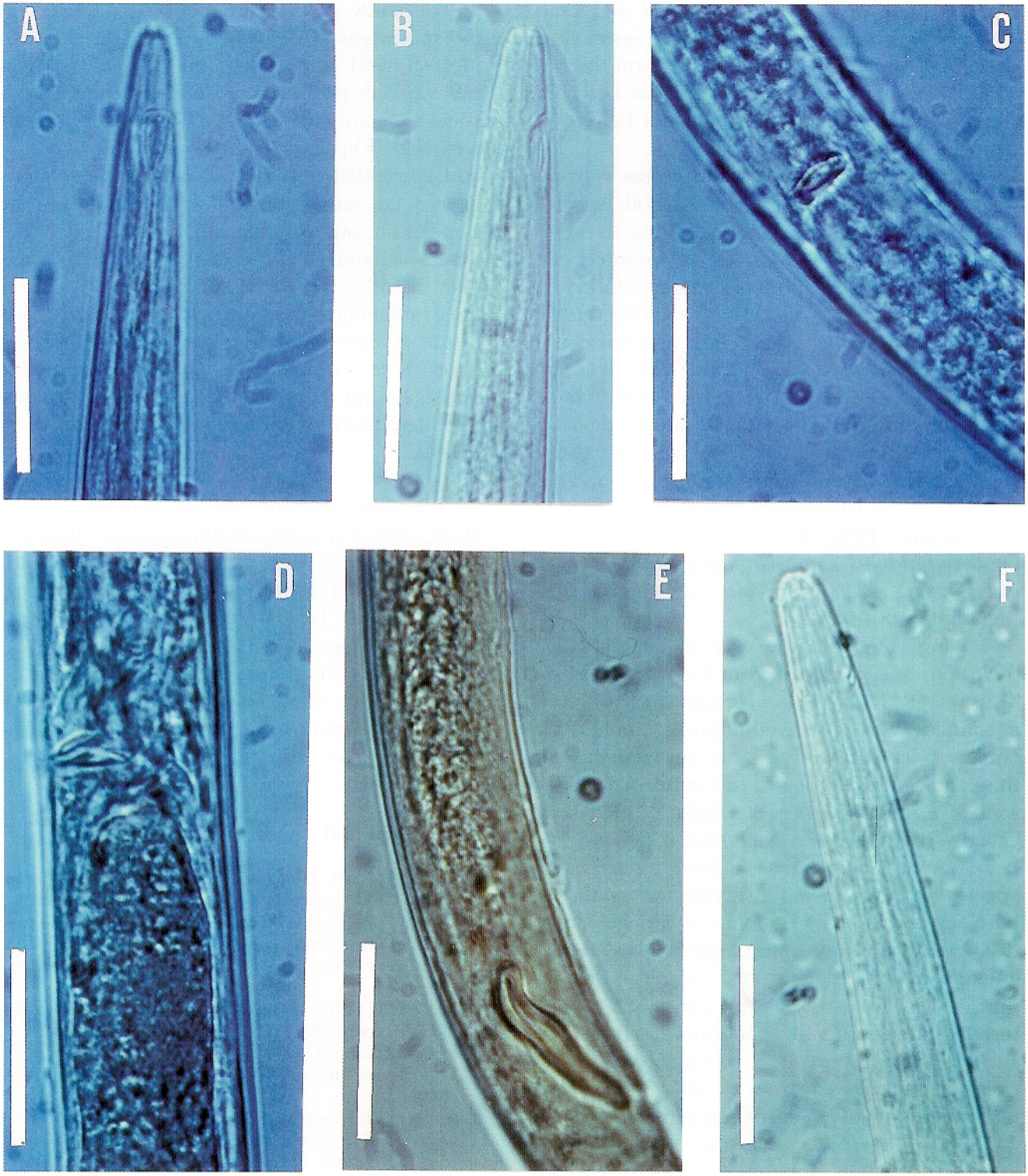

Fig. 5 - A-E *S. lemani*: A and B, anterior end; C and D, vulval region; E, spicular region. F, *M. brzeskii* gen. n., sp. n.: anterior end. Scale bars 25 µm.

ulations of *A. dolichurus* from Brazil and Utah to the type population and to some specimens described by Hofmänner (1913) and on the basis of some morphometric differences between them, concluded that they might not belong to a single species.

Andrássy (1977) erected the genus *Paramphidelus*, to which he transferred both species *A. dolichurus* and *A. lemani*. Referring to previous reports of those species, he pointed out that the specimens reported as *A. dolichurus* by Hofmänner and Menzel (1915) should be attributed to *A. lemani*; further, he considered the specimens reported by Thorne (1939) and by Hofmänner (1913) as being true *dolichurus*. The latter attribution is certainly due to an oversight, since Hofmänner and Menzel (1915) simply reported measurements and description already given by Hofmänner (1913).

Schiemer (1978) described the new subspecies *Amphidelus lemani longicaudatus*, which differs from *P. lemani* in the longer tail and in the amphid position.

Having compared the type specimens of *P. lemani* to one specimen from Utah, reported by Thorne (1939) as *A. dolichurus*, and to some specimens from The Netherlands, labelled by Loof also as *P. dolichurus*, we realized that all these specimens belong to the same species described as *A. lemani* by Stefanski, 1914, which actually resembles *P. dolichurus* in general appearance and has similar habitats as the latter species, thus somewhat justifying the confusion between the two. *P. dolichurus* can be easily distinguished from de Man's species by the peculiar structures of its vulva, vagina and spicules, which also support the attribution to different genera, and in other minor characters such as the presence of a prevulval sac. However, the mistakes in the identification of the two species made so far suggest that many reports of *P. dolichurus*, a species very widespread and rather common, might actually refer to *S. lemani*, which might thus have a wider geographic distribution than that reported here.

The study of the variability of characters in the specimens of *S. lemani* from different localities showed some slight differences between the various populations. Namely, the Dutch specimens are characterized by a lower and not well set off lip region and shorter prevulval sac and amphidial ductus; the Austrian specimens have a tail rather longer than that of the type population, but as long as that of the Dutch; the type specimens have more posterior amphids than the others; the characters of the Utah specimen are intermediate. However, the existence of these slight differences between the populations, does not justify the establishment of distinct taxa and therefore the subspecies *A. lemani longicaudatus* is considered synonym of *S. lemani*.

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