

Morphological and molecular study of Californian species of the genus *Aporcelaimellus* Heyns, 1965 (Dorylaimida: Aporcelaimidae)

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Received: 16 May 2012; revised: 5 September 2012

Accepted for publication: 5 September 2012

Summary – Several species of *Aporcelaimellus* collected in natural areas in California, USA, are characterised on the basis of morphological, morphometric and molecular data. Two new species are identified and described here. *Aporcelaimellus californicus* sp. n. is characterised by its body length of 2.46–3.42 mm, lip region offset by constriction and 24–26 μm broad, odontostyle 23–24 μm long, neck 611–765 μm long, pharyngeal expansion occupying 50–52% of total neck length, a dorsal cellular mass present at level of cardia, uterus simple and 246–408 μm long, $V = 53\text{--}58$, short conoid tail (43–50 μm , $c = 56\text{--}71$, $c' = 0.8\text{--}1.0$) with a large hyaline portion occupying more than half of its total length, spicules 98 μm long, and 8–9 spaced ventromedian supplements. *Aporcelaimellus salicinus* sp. n. is distinguished by its body length of 1.45–1.94 mm, lip region offset by deep constriction and 16–18 μm broad, odontostyle 18–20 μm long, neck 393–521 μm long, pharyngeal expansion occupying 45–51% of total neck length, presence of a dorsal cellular mass at level of cardia, uterus simple and 21–45 μm long, $V = 51\text{--}57$, tail conical to conoid (31–38 μm , $c = 39\text{--}59$, $c' = 1.0\text{--}1.4$), and male unknown. Measurements, sequences and taxonomic comments are provided for the other three *Aporcelaimellus* species. Californian *Aporcelaimellus* display a notable morphological homogeneity but a remarkable molecular diversity, putting into question the monophyly of this group.

Keywords – *Aporcelaimellus californicus* sp. n., *Aporcelaimellus salicinus* sp. n., Bayesian inference, D2-D3 expansion segments, description, LSU ribosomal DNA, morphology, morphometrics, new species, phylogeny, RFLP-rDNA, taxonomy.

Aporcelaimellus Heyns, 1965 is a major dorylaimid taxon because of its high diversity, abundance and cosmopolitan distribution. Since its proposal, the taxonomy of *Aporcelaimellus* has been controversial and under continuous revision. The contributions by Tjepkema *et al.* (1971), Baqri & Khera (1975), De Ley *et al.* (1993), and Andrassy (2001, 2002a) are essential for understanding the changes proposed to its definition. More recently, and after studying Californian material, Álvarez-Ortega *et al.* (2013a, b) proposed new concepts for *Aporcella* Andrassy, 2002 and *Metaporcelaimus* Lordello, 1965, both being very close to *Aporcelaimellus*, and gave differential diagnosis for their separation.

This is the third paper in a series (Álvarez-Ortega *et al.*, 2013a, b) devoted to exploring the diversity of Californian

aporcelaims and it focuses on the characterisation of the representatives of *Aporcelaimellus*.

Materials and methods

The techniques and protocols used for extraction and mounting of nematodes, light microscopy, DNA extraction, PCR of the D2-D3 expansion segments of 28S rDNA gene and phylogenetic analysis were as described in the previous paper of this series (Álvarez-Ortega *et al.*, 2013b).

CLONING, SEQUENCING AND RFLP OF RDNA

PCR products were purified with QIAquick Gel Extraction Kit (Qiagen) and used for direct sequencing, cloning

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and RFLP. The PCR products for some samples were cloned and then sequenced as described by Tanha Maafi *et al.* (2003). The sequencing reactions were performed at 'Davis Sequencing', Davis, CA, USA. The newly obtained sequences were submitted to the GenBank database under accession numbers JX094336-JX094344.

Purified PCR product (4 μ l) of D2-D3 of 28S rDNA was digested by *MspI* or *RsaI* restriction enzymes in the buffer stipulated by the manufacturer. The digested DNA was run on a 1.5% TAE buffered agarose gel, stained with ethidium bromide, visualised with a UV transilluminator and photographed.

Species descriptions

*Aporcelaimellus californicus** sp. n. (Figs 1, 2)

MATERIAL EXAMINED

Six females and two males in a good state of preservation.

MEASUREMENTS

See Table 1.

DESCRIPTION

Adult

Moderately slender to slender nematodes of medium to large size, 2.46-3.42 mm long. Body cylindrical, tapering towards both extremities, but more so towards anterior end. Habitus curved ventrad after fixation, especially in posterior body region, C-shaped. Cuticle 3-3.5 μ m thick in anterior region, 5-10 μ m at mid-body and 6.5-10 μ m on tail, typical of genus, outer layer thin, inner layer thicker than outer. Cervical lacunae well visible in all specimens examined, but unusually short, at level of odontostyle and about as long as latter. Lateral chord 7-18 μ m wide or 8-19% of mid-body diam. Two ventral and two dorsal body pores usually present at level of odontophore. Lip region offset by constriction, 2.7-2.9 times as broad as high and one-fourth to one-third (24-34%) of body diam. at neck base; lips barely angular and moderately separated, labial papillae low. Amphid fovea cup-shaped, its aperture 12-14 μ m or *ca* one-half (50-56%) of lip region diam. Cheilostom nearly cylindrical,

lacking any differentiation. Odontostyle typical of genus, 3.9-4.3 times as long as wide, 0.9 times as long as lip region diam., and 0.67-0.93% of body length, aperture 17-18 μ m long or occupying three-fourths to four-fifths (76-82%) its length. Guiding ring plicate. Odontophore linear, rod-like, 1.9-2.2 times odontostyle. Anterior region of pharynx enlarging very gradually, basal expansion 6.5-8.5 times as long as wide, 3.7-4.4 times as long as body diam., and occupying 50-52% of total neck length, pharyngeal gland nuclei apparently located as follows: DN = 58-60, S₁N₁ = 69-70, S₁N₂ = 75 (n = 1), S₂N = 88 (n = 1). Nerve ring located at 209-251 μ m from anterior end or 32-35% of total neck length. Cardia conical to rounded, (19-25) \times (13-21) μ m, ring-like structure surrounding its junction with pharyngeal base, and dorsal cellular mass *ca* 60 μ m long present at level of or barely posterior to cardia. Tail short conoid with broadly rounded terminus; inner core very irregular and visibly notched so caudal region showing a large, hyaline portion occupying more than half of its total length. Two pairs of caudal pores quite close together, one lateral in middle of tail and other subdorsal in posterior half of tail.

Female

Genital system didelphic-amphidelphic, with both branches equally and well developed, anterior 392-596 μ m or 16-19% of body length (700 μ m or 21% of body length with uterine egg inside), and posterior 435-602 μ m or 16-19% of body length (673 μ m or 20% of body length with two uterine eggs inside). Ovaries relatively large, anterior 118-360 μ m and posterior 140-434 μ m long, oocytes arranged first in two or more rows, then in a single row. Oviduct 130-240 μ m long or 1.3-2.2 times corresponding body diam., consisting of slender part with prismatic cells and well developed *pars dilatata* with distinct lumen. Oviduct and uterus separated by a marked sphincter. Uterus a simple tube 246-408 μ m long or 2.6-4.2 times corresponding body diam., three females containing sperm cells. Uterine egg ovoid, 115 \times 50 μ m (n = 1), 2.3 (n = 1) times as long as wide. Vagina extending inwards 40-44 μ m or two-fifths to one-half (38-51%) of body diam., *pars proximalis* (28-32) \times (23-29) μ m, with somewhat sigmoid walls and surrounded by weak musculature, *pars refringens* with two triangular to drop-shaped pieces measuring 8-9 \times 6.5-7 μ m and with a combined width of 12-15 μ m, *pars distalis* 2.5-3 μ m long. Vulva a post-equatorial, transverse slit. Prerectum 1.7-2.7 and rectum 1.0-1.4 anal body diam. long.

* The specific epithet refers to geographical origin of the species.

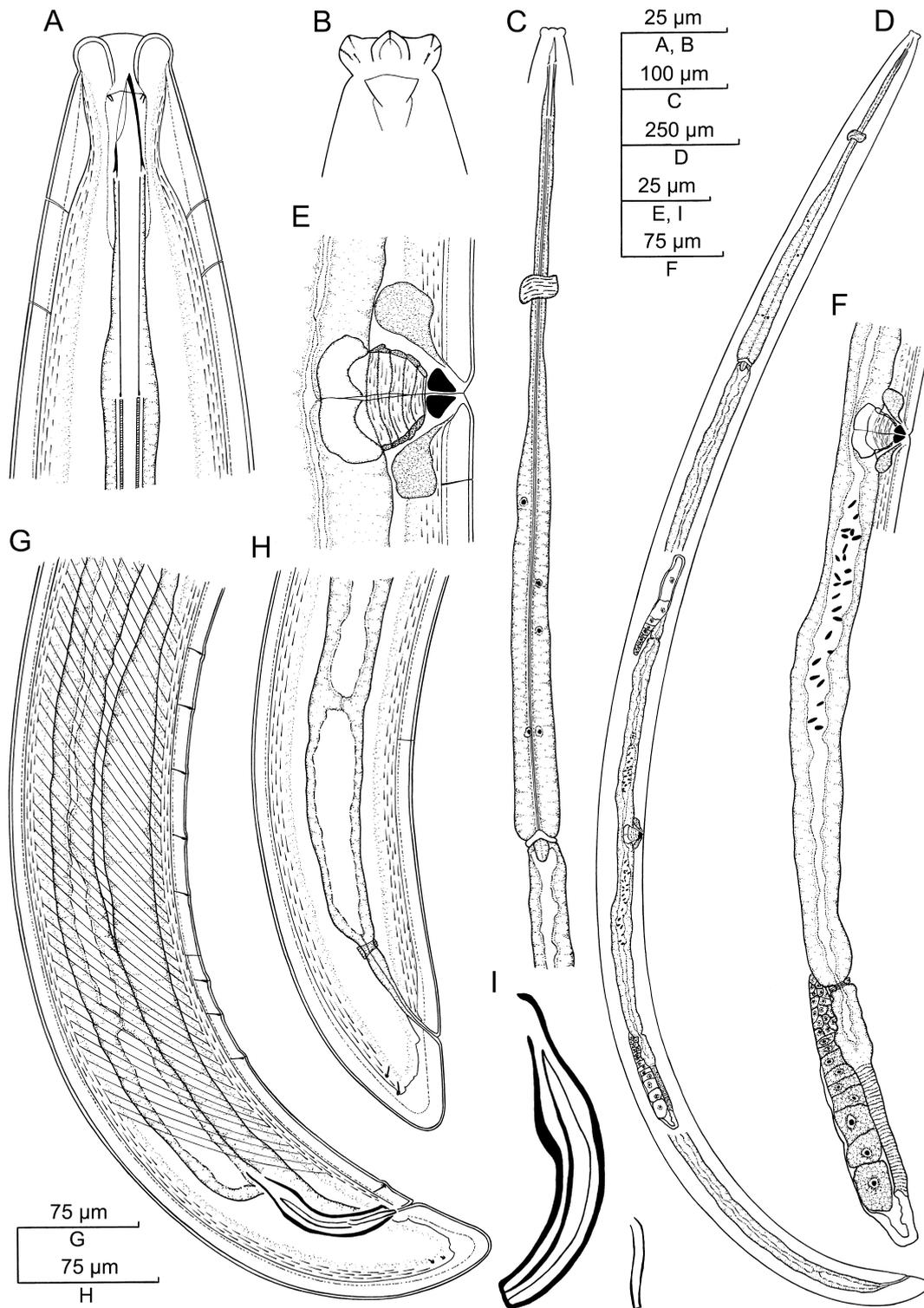


Fig. 1. *Aporcelaimellus californicus* sp. n. A: Anterior region in median view; B: Lip region in surface view; C: Neck; D: Entire body; E: Vagina; F: Posterior genital branch; G: Male, caudal region; H: Female, caudal region; I: Spicule and lateral guiding piece.

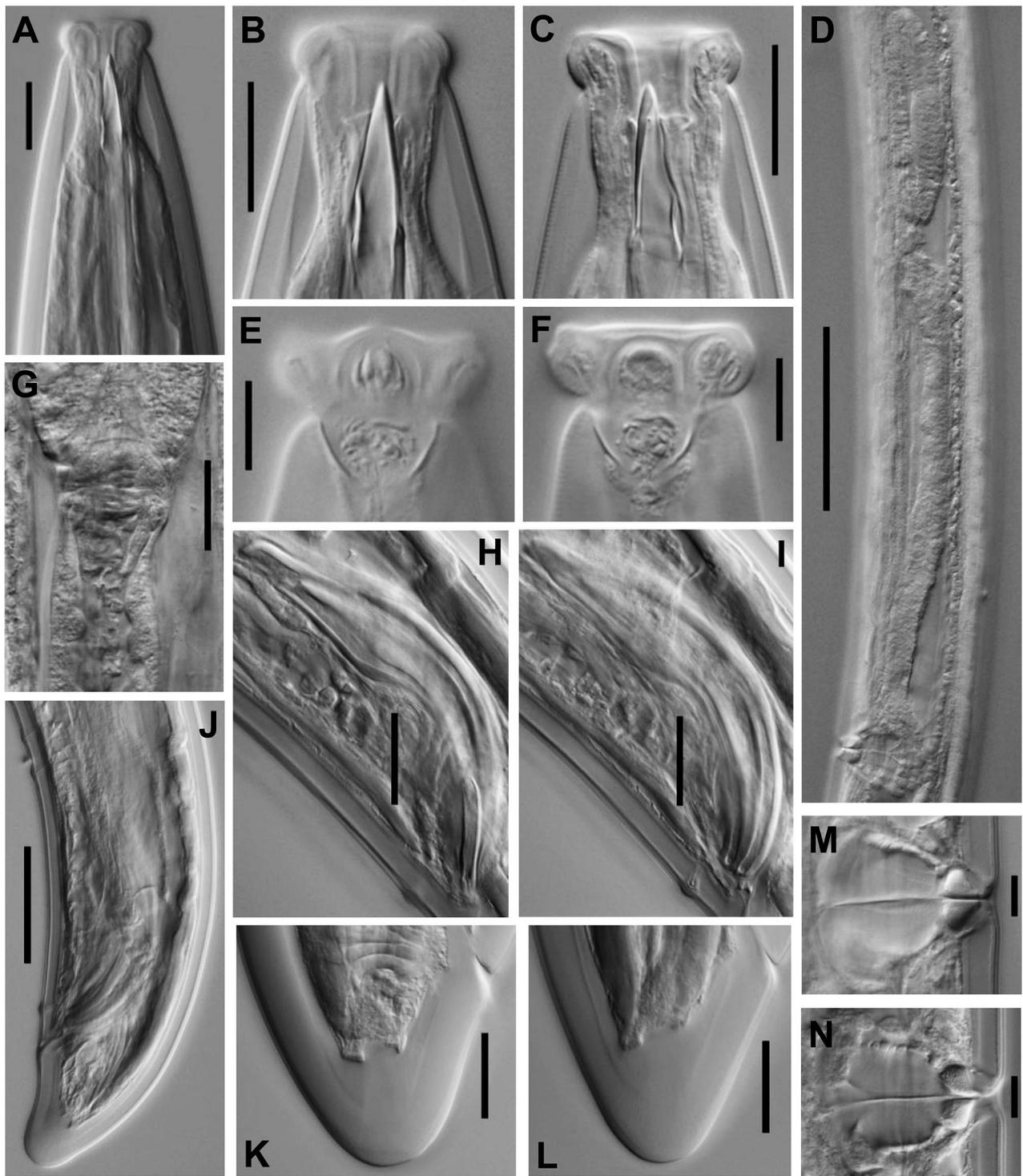


Fig. 2. *Aporcelaimellus californicus* sp. n. A-C: Anterior region in median view; D: Female anterior genital branch; E, F: Lip region in surface, lateral view; G: Pharyngo-intestinal junction; H, I: Spicules; J: Male posterior body region; K, L: Female, caudal region; M, N: Vagina. (Scale bars: A-C, G-I, K, L = 20 μ m; D = 100 μ m; E, F, M, N = 10 μ m; J = 50 μ m.)

Table 1. Morphometric data for *Aporcelaimellus californicus* sp. n. All measurements are in μm (except L, in mm), and in the form: mean \pm s.d. (range).

Character	Population						Total range
	Capell Valley Road, Napa Ct.		Lake Hennessey, Napa Ct.	Phoenix Lake Park, Marin Ct.		Female	
	Female		Male	Female			Female
	Holotype	Paratypes	Paratype	Male	Female	Female	
n	–	2	1	1	3	6	
L	3.13	3.10, 3.39	3.42	2.73	2.74 \pm 0.30 (2.46-3.07)	2.46-3.39	
a	32	27, 30	29	33	30.9 \pm 1.3 (30-32)	27-32	
b	4.1	4.3, ?	4.6	4.1	4.3 \pm 0.3 (4.0-4.6)	4.0-4.6	
c	68	68, 70	68	56	61.6 \pm 7.9 (56-71)	56-71	
c'	0.9	0.8, 0.8	0.8	0.9	0.8, 1.0	0.8-1.0	
V	58	57 (n = 2)	–	–	55.4 \pm 2.2 (53-57)	53-58	
Lip region diam.	25	24, 25	26	25	25.0 \pm 0.9 (24-26)	24-26	
Odontostyle length	24	23, 24	24	24	23.4 \pm 0.8 (23-24)	23-24	
Odontophore length	49	47 (n = 2)	47	45	45.6 \pm 1.7 (44-48)	44-49	
Guiding ring from ant. end	13	13, 14	14	14	13.6 \pm 0.5 (13-14)	13-14	
Neck length	765	719, ?	749	670	639 \pm 28 (611-668)	611-765	
Pharyngeal expansion length	385	371, ?	379	336	324 \pm 22 (308-349)	308-385	
Diam. at neck base	90	100, 99	101	76	82.2 \pm 4.4 (77-86)	77-100	
at mid-body	97	115, 112	116	82	88.5 \pm 7.2 (81-95)	81-115	
at anus/cloaca	54	59, 59	61	52	47, 52	47-59	
Prerectum length	125	114, 99	118	?	110 \pm 28 (89-142)	89-142	
Rectum length	68	58, 74	96	71	65.6 \pm 1.1 (65-66)	58-74	
Tail length	46	46, 48	50	49	44.5 \pm 1.2 (43-46)	43-48	
Spicule length	–	–	98	98	–	–	
Ventromedian supplements	–	–	8	9	–	–	

Male

Genital system diorchic, with opposed testes. In addition to ad-cloacal pair, situated at 17 or 21 μm from cloacal aperture, a series of 8-9 irregularly spaced (15-53 μm apart) ventromedian supplements, posteriormost of which is located outside range of spicules, at 100 or 117 μm from ad-cloacal pair. Spicules dorylaimoid, curved ventrad, 5.9 or 6.1 times as long as wide and 1.6 or 1.9 times as long as anal body diam. Lateral guiding pieces 28 or 31 μm long, 8.4 or 8.9 times as long as wide.

TYPE LOCALITY AND HABITAT

Napa County, CA, USA, crossroad between Capell Valley Road and Wragg Canyon Road, in association with willow trees (*Salix* sp.).

OTHER LOCALITIES AND HABITATS

Napa County, CA, USA, 1770 Sage Canyon Road, Lake Hennessey, in association with willow trees (*Salix*

sp.); and Marin County, Phoenix Lake Park, CA, USA, in association with laurel trees.

TYPE MATERIAL

Female holotype, one female and one male paratypes deposited in the nematode collection of the University of Jaén, Spain. One female paratype deposited with USDA Nematode Collection, Beltsville, MD, USA.

DIAGNOSIS AND RELATIONSHIPS

The new species is characterised by its body length of 2.46-3.42 mm, presence of short cervical lacunae at level of odontostyle, lip region offset by constriction and 24-26 μm broad, odontostyle 23-24 μm long with its aperture occupying 76-82% of its length, neck 611-765 μm long, pharyngeal expansion 308-386 μm long or 50-52% of total neck length, dorsal cellular mass at level of cardia, uterus simple and 246-408 μm long or 2.6-4.2 times the corresponding body diam., *pars refringens vaginae* with

two well developed sclerotised pieces, vulva transverse ($V = 53-58$), tail short conoid ($43-50 \mu\text{m}$, $c = 56-71$, $c' = 0.8-1.0$) and with a large hyaline portion occupying more than half of its total length, spicules $98 \mu\text{m}$ long, and 8-9 spaced ventromedian supplements. Four features are especially characteristic of this species and hence useful for its identification: short cervical lacunae, dorsal cellular mass at level of cardia, long but simple uterus, and large hyaline portion of caudal region.

The new species resembles *A. kazirangus* Khan, Ahmad & Jairajpuri, 1995, *A. micropunctatus* Botha & Heyns, 1990, *A. obtusicaudatus* (Bastian, 1865) Altherr, 1968 and *A. porcus* Thorne, 1974. It differs from *A. kazirangus* by its larger general size ($L = 2.46-3.42$ vs $1.9-2.3$; neck length $611-765$ vs $506-581 \mu\text{m}$), broader lip region ($24-26$ vs $19-21 \mu\text{m}$), comparatively longer uterus ($2.6-4.2$ vs up to one body diam. according to original Fig. 3F), longer spicules (98 vs $72 \mu\text{m}$) and fewer ventromedian supplements ($8-9$ vs 11); from *A. micropunctatus* by its larger general size ($L = 2.46-3.42$ vs $1.72-2.58$; neck length $611-765$ vs $440-625 \mu\text{m}$), absence of conspicuous ventral pores (vs $37-47$, distributed throughout entire body), comparatively longer uterus ($2.6-4.2$ vs one body diam.), caudal region with irregular and notched inner core (vs regular) and male present (vs absent); from *A. obtusicaudatus*, a cosmopolitan and highly variable species (see De Ley *et al.*, 1993), in its short lacunae (vs well developed, often as long as odontophore or more), longer uterus ($246-408$ vs $48-147 \mu\text{m}$, $2.6-4.2$ vs up to one body diam.), and caudal region with irregular (vs regular) inner core and large (vs no) hyaline portion; and from *A. porcus*, a poorly characterised species, by its shorter lacunae (vs well developed, at level of odontophore and as long as the latter, see original Fig. 6A), longer neck ($611-765$ vs $581-595 \mu\text{m}$), more posterior vulva ($V = 53-58$ vs 46), inner core of tail very irregular and with large hyaline portion (vs regular and no hyaline portion).

MOLECULAR CHARACTERISATION

Two sequences of the D2-D3 of 28S rDNA (Fig. 3) gene were obtained from two females, one from soil collected in Capell Valley Road, the other from the Phoenix Lake Park population. These two sequences are very similar (99%) and differ by only two nucleotides. The phylogenetic position of this species within the dorylaims is presented in Figure 4. PCR-D23 of 28S rDNA-RFLP diagnostic profiles with two enzymes are given in Figure 5.

REMARKS

Although the material studied was collected from several localities and shows wide morphometric variability in body and neck length, it is quite homogenous in relevant features such as lip region diam., odontostyle length, uterus length, tail morphology, spicule length, *etc.* Accordingly, this material is assumed to belong to the same species.

*Aporcelaimellus salicinus** sp. n. (Figs 6, 7)

MATERIAL EXAMINED

Fifteen females from three locations and in a good state of preservation.

MEASUREMENTS

See Table 2.

DESCRIPTION

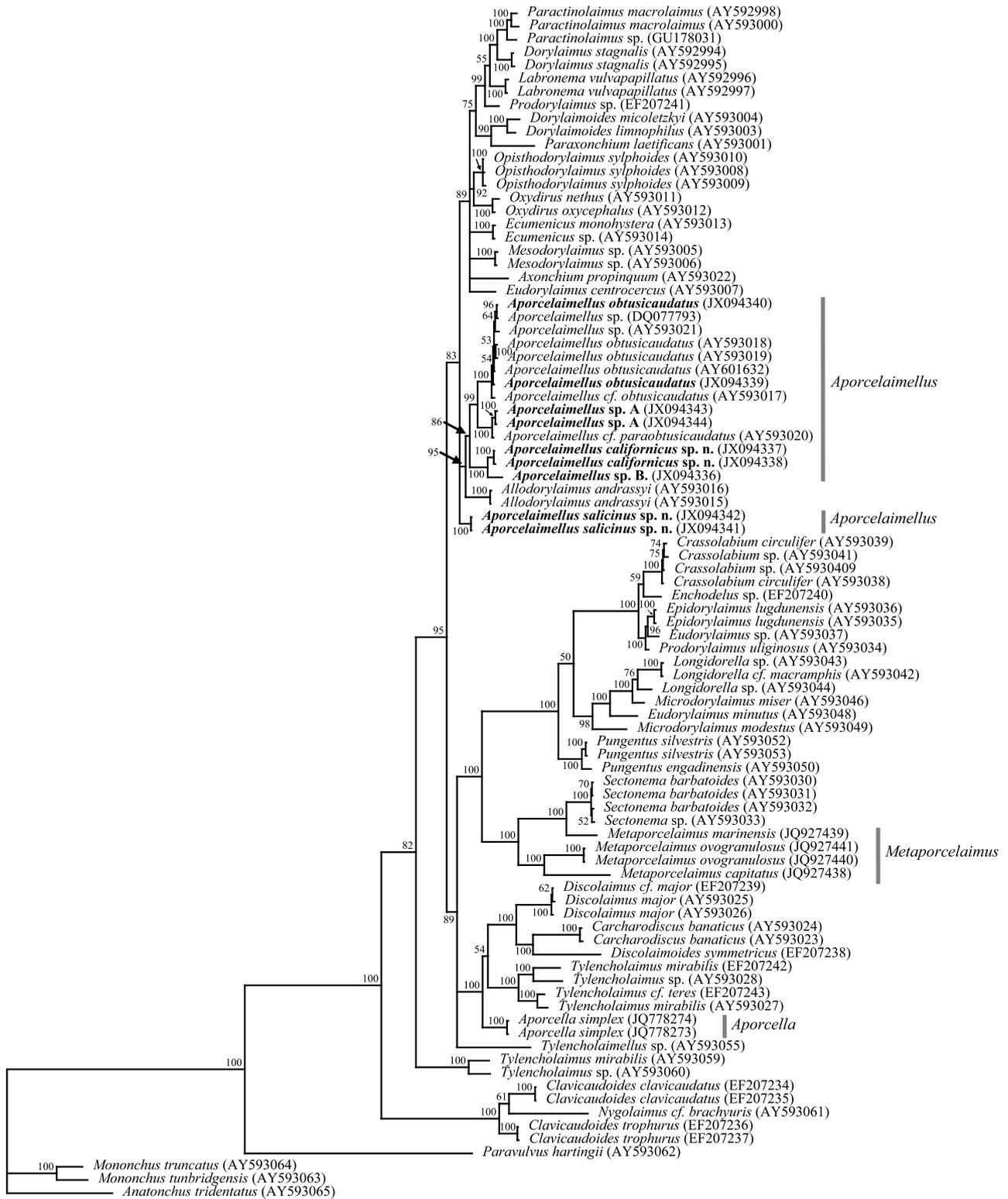
Female

Moderately slender nematodes of medium size, 1.45-1.94 mm long. Body cylindrical, tapering towards both extremities, but more so towards posterior end. Habitus curved ventrad after fixation, C-shaped. Cuticle 1.5-2 μm in anterior region, 2-3 μm at mid-body and 3-6 μm on tail, outer cuticle layer thin and smooth, inner layer thicker than the outer. Cervical lacunae small, nearly at level of odontostyle, absent in specimens from American River Parkway. Lateral chord 8-14 μm wide or 15-22% of mid-body diam. Lip region offset by deep constriction, 2.7-3.0 times as broad as high and about one-third (30-34%) of body diam. at neck base, lips barely angular and moderately separated, labial papillae weakly protruding. Amphid fovea funnel-shaped, its aperture 9-9.5 μm or somewhat more than one-half (53-58%) of lip region diam. Cheilostom nearly cylindrical, lacking any differentiation. Odontostyle relatively slender, 5.8-6.3 times as long as wide, 1.1-1.2 (0.9 in only one specimen with odontostyle 16 μm long) times as long as lip region diam., and 1.01-1.28% (0.89 in only one specimen with odontostyle 16 μm long) of body length,

* The specific epithet refers to willow (*Salix* sp.), the plant species with which the new species was associated.

	*	20	*	40	*	60	*	80	*	100													
A. obtusicaudatus1	:	AAAAGA	ACTTTGAAGAGAGAG	TTCAA	AAGGGCGTGA	AACCCTTAGA	AGAAAACGGACGGAC	CGCGTTCGGGAGCCTTCGGG	AATCAGTCTCGGATAGGCG	:	100												
A. obtusicaudatus2	:										:	100											
A. sp.B1	:										:	100											
A. sp.B2	:										:	100											
A. salicinus1	:										:	100											
A. salicinus2	:										:	100											
A. californicus1	:										:	100											
A. californicus2	:										:	100											
A. sp.A	:										:	100											
	*	120	*	140	*	160	*	180	*	200													
A. obtusicaudatus1	:	AACGTG	-CGCGATT	TCGGAAC	CAAGTCCGAT	CGTTG	-TCCGG	-CTAGTAC	CTTCGCTT	GGCGCAT	TTTCGAAGGTAAGCACCGC	GACGATACGAA	: 196										
A. obtusicaudatus2	:											: 196											
A. sp.B1	:	CT	.A				GG	.A	.A			TC	.T	: 199									
A. sp.B2	:	CT	.A				C	GG	.A	.A		TC	.T	: 199									
A. salicinus1	:	AT	.CG	.T		G		C	CG	--	AT	.A		TG	.T	C	: 197						
A. salicinus2	:	AT	.CG	.T		G		C	CG	--	AT	.A		TG	.T	C	: 197						
A. californicus1	:	C	.T	.CGAT				CG	--	A	.T	--	T		G	.T	: 195						
A. californicus2	:	C	.T	.CGAT				CG	--	A	.T	--	T		G	.T	: 195						
A. sp.A	:	C	.T	.CAAT				CG	--	A	.T	--	TT		G	.T	: 197						
	*	220	*	240	*	260	*	280	*	300													
A. obtusicaudatus1	:	AGCCCG	CAGACGGT	TTGGATT	CGGGCGAG	ACTTCTC	ACGGG	-ACTCGT	AACAGTCT	TTACGTCG	T--AGTCG	TCCGACCGCAGTAT	GTATCGATAG	: 293									
A. obtusicaudatus2	:											: 295											
A. sp.B1	:											: 298											
A. sp.B2	:											: 298											
A. salicinus1	:	T																: 294					
A. salicinus2	:	T																: 294					
A. californicus1	:	T																: 292					
A. californicus2	:	T																: 292					
A. sp.A	:	T																: 296					
	*	320	*	340	*	360	*	380	*	400													
A. obtusicaudatus1	:	CC--AA	GTATAT	TATATAC	GGCCG	CGTAA	AGCTCG	CTCGAG	TACCGGAT	AGTTGGT	CAAT-AG	TGGTACT	TGCTCG	CAGGTC	CTAAT	TCG	: 390						
A. obtusicaudatus2	:											: 392											
A. sp.B1	:	A	.CG		AT		C											: 395					
A. sp.B2	:	A	.CG		AT		C											: 395					
A. salicinus1	:	AG	T-AA															: 388					
A. salicinus2	:	AG	T-AA															: 388					
A. californicus1	:	A	.CG	AA														: 388					
A. californicus2	:	A	.CG	AA														: 388					
A. sp.A	:	GA	.A	ATATA														: 396					
	*	420	*	440	*	460	*	480	*	500													
A. obtusicaudatus1	:	ATT	CGCTTT	TGAAGT	TATCG	CGGAC	GTT-TC	GGCGT	ATAGG	CGTCAG	CGGTT	CAGTGT	CGGCC	ACCCAT	CCGAC	CCGCT	TGAAAC	CGGAC	CAAGGA	: 489			
A. obtusicaudatus2	:											: 490											
A. sp.B1	:											: 494											
A. sp.B2	:											: 494											
A. salicinus1	:	A																		: 486			
A. salicinus2	:	A																		: 486			
A. californicus1	:	A																		: 486			
A. californicus2	:	A																		: 486			
A. sp.A	:	A																		: 495			
	*	520	*	540	*	560	*	580	*	600													
A. obtusicaudatus1	:	GTCTA	ACATAT	CGCGAG	CTAAT	GGGTG	GAAAAC	CCAAAG	CGGTA	ATAAA	AGTAA	ACCGC	GTTTC	CGGAC	GGCTA	AGGTAC	GATCCT	AGTGT	CCTTT	GCG-	: 588		
A. obtusicaudatus2	:											: 589											
A. sp.B1	:											: 592											
A. sp.B2	:											: 592											
A. salicinus1	:											: 584											
A. salicinus2	:											: 584											
A. californicus1	:											: 585											
A. californicus2	:											: 585											
A. sp.A	:											: 594											
	*	620	*	640	*	660	*	680	*	700													
A. obtusicaudatus1	:	ACATT	AGCGC	GACTACT	GTCCC	GTC	CCGAC	CGCAT	GTCCG	TAGG	CGGAG	ATAG	AGTGC	ATAC	GTTGG	GACCC	GAAAG	TGGT	GAAC	TATG	CCTG	GACG	: 688
A. obtusicaudatus2	:											: 689											
A. sp.B1	:	TT																					: 692
A. sp.B2	:	TT																					: 692
A. salicinus1	:	TA																					: 684
A. salicinus2	:	TA																					: 684
A. californicus1	:	TTC																					: 685
A. californicus2	:	TTC																					: 685
A. sp.A	:	TTC																					: 694
	*	720	*	740	*	760																	
A. obtusicaudatus1	:	AGCGA	AGCC	GAGGAA	AACTCT	GGTGG	AGGTT	CGTAG	CGGTT	CTGAC	GTGCA	AAATCG	ATCG	TGAC	: 754								
A. obtusicaudatus2	:											: 755											
A. sp.B1	:											: 758											
A. sp.B2	:											: 758											
A. salicinus1	:											: 750											
A. salicinus2	:											: 750											
A. californicus1	:											: 751											
A. californicus2	:											: 751											
A. sp.A	:											: 760											

Fig. 3. 28S rDNA gene sequences of *Aporcelainellus* spp. showing the differences between the sequences.



aperture 9-10.5 μm long or occupying *ca* one-half (50-53%, 57 in only one specimen with odontostyle 16 μm long) its length. Guiding ring plicate, at 8.0-9.5 μm from anterior end. Odontophore linear, rod-like, 1.6-2.0 (2.5 in only one specimen with odontostyle 16 μm long) times odontostyle. Anterior region of pharynx enlarging very gradually, basal expansion 6.0-7.9 times as long as broad, 3.3-4.6 times as long as body diam., occupying 44-51% of total neck length, pharyngeal gland nuclei

apparently located as follows: DN = 63-68, DO-DN = 17, 20 μm ($n = 2$), S₁N₁ = 75-76 ($n = 3$), S₁N₂ = 80-82 ($n = 4$), S₂N = 90-92. Nerve ring located at 134-167 μm or 32-38% of total neck length from anterior end. Cardia conical, (12-17) \times (10-14) μm , ring-like structure present surrounding its junction with pharyngeal base. Dorsal cellular mass *ca* 30 μm long present at level of cardia. Genital system didelphic-amphidelphic, both branches equally developed and moderately large, anterior 111-220 μm or 6-12% of body length (201-259 μm or 12-17% of body length with uterine egg), and posterior 98-183 μm or 7-13% of body length (217-259 μm or 13-16% of body length with uterine egg). Ovaries relatively small, anterior 76-197 μm and posterior 87-195 μm long, oocytes arranged first in two or more rows, then in a single row. Oviduct 44-91 μm long or 0.9-1.6 times corresponding body diam., consisting of slender part with prismatic cells and poorly developed *pars dilatata*. Oviduct and uterus are separated by a weak sphincter. Uterus a short simple tube, 21-45 μm long or 0.4-0.8 times corresponding body diam., not containing sperm cells. Vagina extending inwards for 19-25 μm or one-third to two-fifths (33-43%) of body diam., *pars proximalis* (12-19) \times (13-21) μm , with somewhat sigmoid walls and surrounded by weak musculature, *pars refringens* with two triangular to rounded pieces measuring (5-7) \times (3-4.5) μm and with a combined width of 8-10 μm , *pars distalis* 1.5-2 μm long. Two glandular cells, one anterior and other posterior present close to vagina. Vulva a post-equatorial, transverse slit. Prerectum 1.5-2.9 and rectum 1.2-1.8 times anal body diam. long. Tail conical with rounded terminus to convex conoid, sometimes with slight dorsal concavity. In specimens from American River Parkway, the inner cuticle layer shows a distinct refringent band at both (dorsal and ventral) sides, ending at *ca* two-thirds of tail length, such differentiation being less perceptible in females from other locations. Hyaline portion of tail 10-11 μm long or *ca* one-third of tail length. Two pairs of caudal pores, one dorsal, other subdorsal.

Male

Unknown.

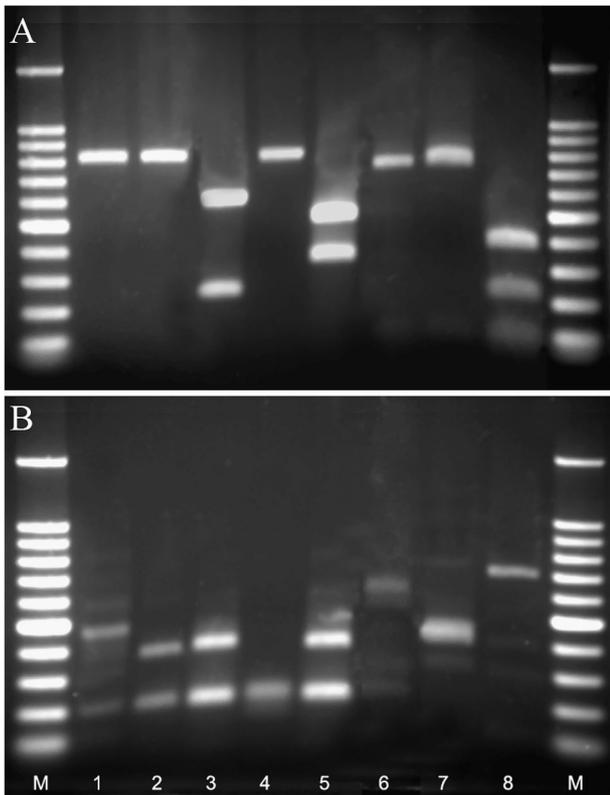


Fig. 5. PCR-RFLP of D2-D3 of 28S rDNA for aporcelaim species. A: *MspI* restriction enzyme; B: *RsaI* restriction enzyme. Lane codes: M = 100 bp DNA ladder (Promega); 1: *Aporcelaimellus salicinus* sp. n.; 2: *A. californicus* sp. n.; 3: *Aporcelaimellus* sp. B; 4: *Aporcelaimellus* sp. A; 5: *A. obtusicaudatus*; 6: *Metaporcelaimus ovogranulosus* Álvarez-Ortega, Subbotin & Peña-Santiago, 2013; 7: *M. marinensis* Álvarez-Ortega, Subbotin & Peña-Santiago, 2013; 8: *Aporcella simplex* (Thorne & Swanger, 1936) Álvarez-Ortega, Subbotin & Peña-Santiago, 2013.

Fig. 4. Bayesian 50% majority rule consensus trees as inferred from D2-D3 expansion segments of 28S rDNA gene sequence alignments under the GTR + I + G model. Posterior probabilities are given for appropriate clades. Newly obtained sequences are indicated by bold letters.

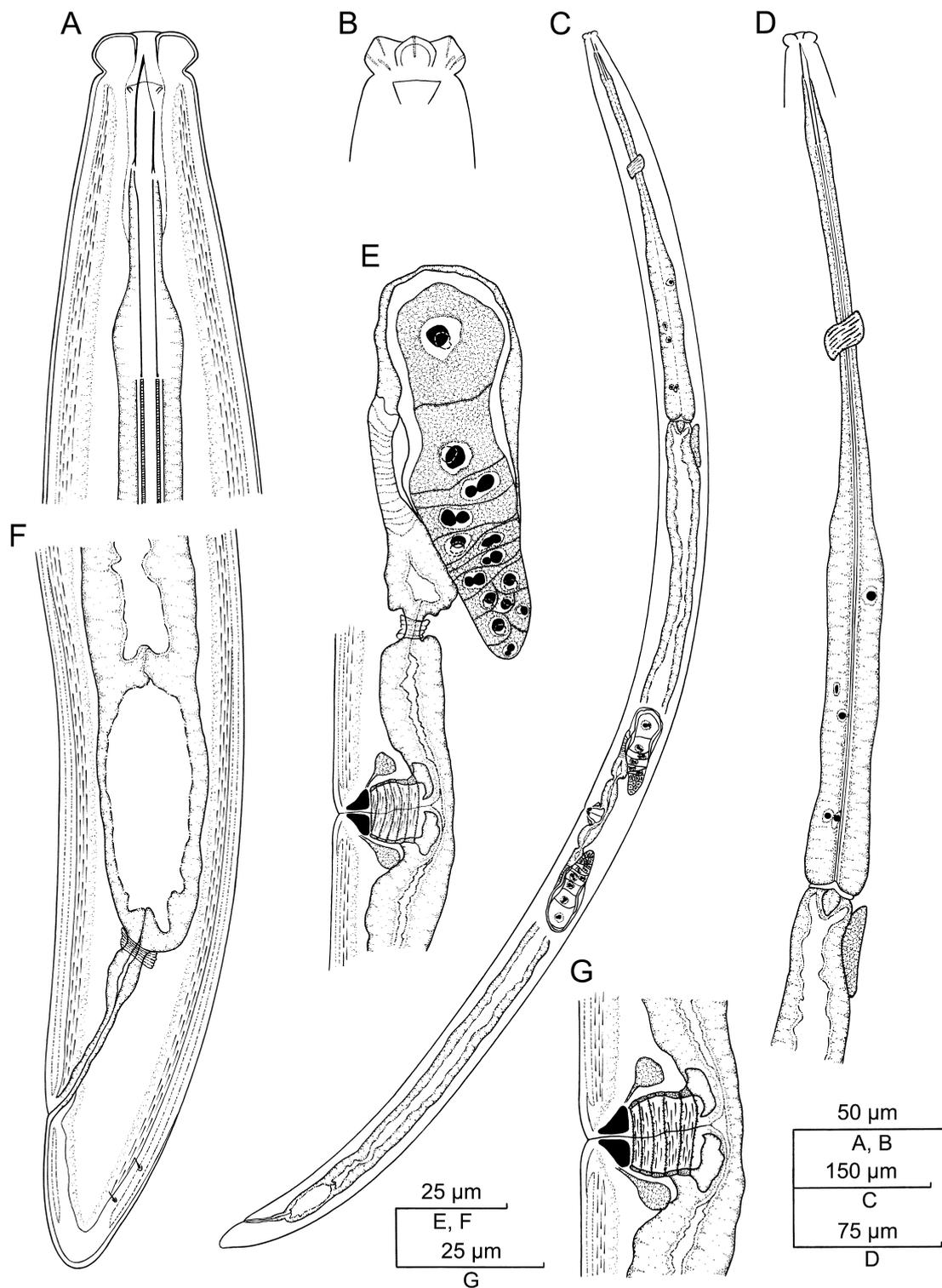


Fig. 6. *Aporcelaimellus salicinus* sp. n. (female). A: Anterior region in median view; B: Lip region in surface view; C: Entire body; D: Neck; E: Anterior genital branch; F: Caudal region; G: Vagina.

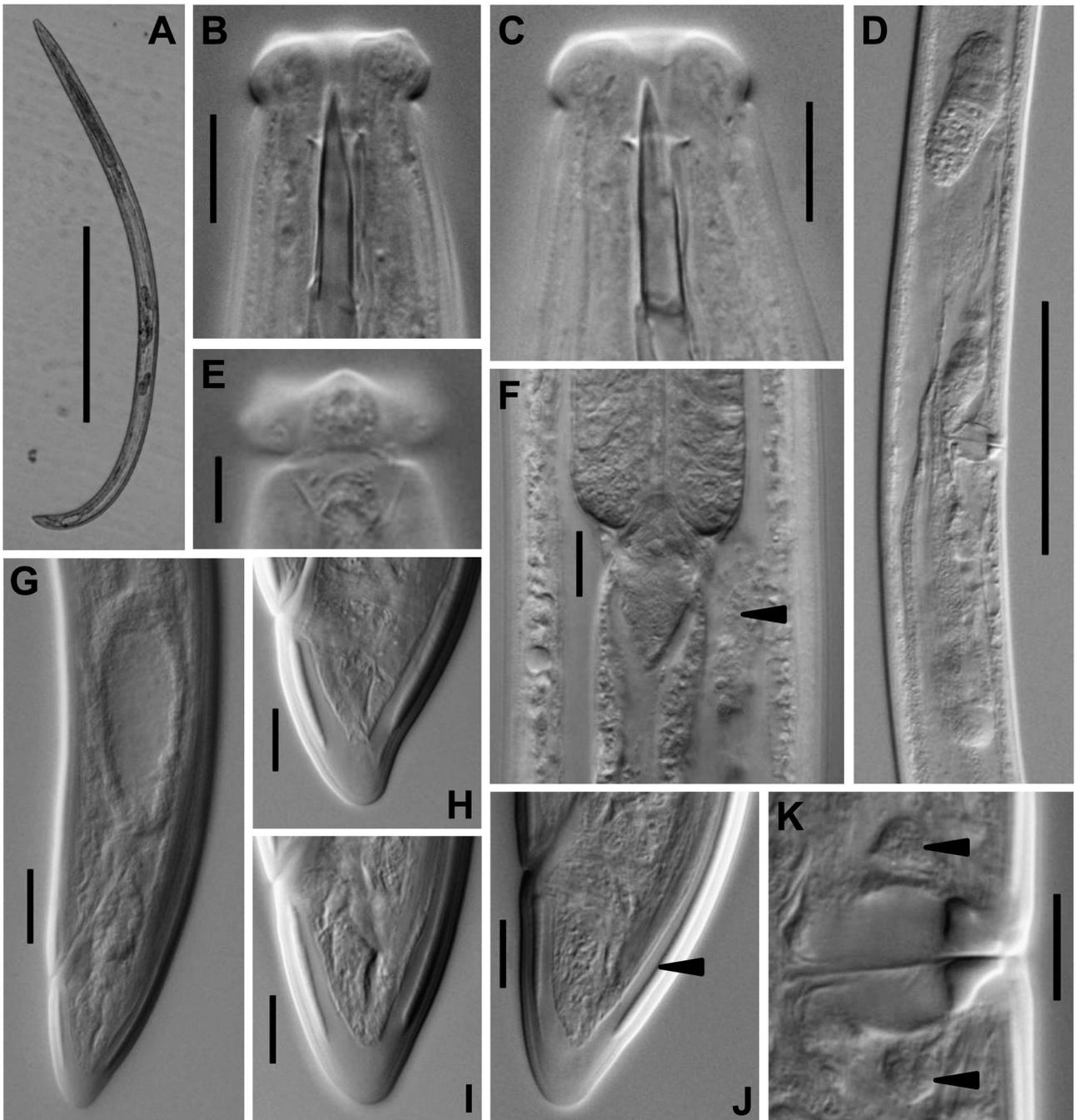


Fig. 7. *Aporcelaimellus salicinus* sp. n. (female). A: Entire body; B, C: Anterior region in median view; D: Genital system; E: Lip region in lateral, surface view; F: Pharyngo-intestinal junction, with arrowhead pointing at dorsal cellular mass; G: Posterior body region; H-J: Caudal region, with arrowhead pointing at refringent layer of cuticle; K: Vagina, with arrows pointing at adjacent cells. (Scale bars: A = 500 μ m; B, C, F, H-K = 10 μ m; D = 50 μ m; E = 5 μ m; G = 20 μ m.)

Table 2. Morphometric data for *Aporcelaimellus salicinus* sp. n. All measurements are in μm (except L, in mm), and in the form: mean \pm s.d. (range).

Character	Population				Total range
	American River Parkway, El Dorado Ct.		Stebbins Cold Canyon Reserve, Napa Ct.	Capell Valley Road, Napa Ct.	
	Holotype	Paratypes			
n	–	3	7	4	14
L	1.49	1.46 \pm 0.01 (1.46-1.47)	1.56 \pm 0.08 (1.45-1.69)	1.83 \pm 0.12 (1.73-1.94)	1.45-1.94
a	28	26.6 \pm 1.2 (26-28)	28.5 \pm 1.1 (26-29)	30.5 \pm 0.9 (30-32)	26-32
b	3.5	3.7	3.8 \pm 0.3 (3.4-4.0)	3.6 \pm 0.1 (3.5-3.7)	3.4-4.0
c	45	43.7 \pm 2.6 (42-47)	46.2 \pm 3.8 (39-52)	53.3 \pm 4.1 (50-59)	39-59
c'	1.1	1.2 \pm 0.1 (1.0-1.2)	1.2 \pm 0.1 (1.1-1.4)	1.2 \pm 0.1 (1.1-1.2)	1.0-1.4
V	56	56.9 \pm 0.6 (56-57)	54.6 \pm 1.6 (52-57)	52.1 \pm 1.1 (51-53)	51-57
Lip region diam.	17	16.5 \pm 0.4 (16-17)	16.4 \pm 0.5 (16-18)	17.0 \pm 0.5 (17-18)	16-18
Odontostyle length	18	18.4 \pm 0.5 (18-19)	19.1 \pm 0.3 (19-19)	20.1 \pm 0.2 (20-20)	18-20
Odontophore length	33	31.3 \pm 1.1 (30-32)	32.0 \pm 0.7 (31-33)	38.1 \pm 0.5 (37-39)	30-39
Guiding ring from ant. end	9	9.3 \pm 0.2 (9-9)	8.3 \pm 0.3 (8-9)	8.4 \pm 0.3 (8-9)	8-9
Neck length	422	393	412 \pm 18 (392-430)	502 \pm 17 (487-521)	392-521
Pharyngeal expansion length	195	175	192 \pm 16 (174-211)	247 \pm 13 (229-256)	174-256
Diam. at neck base	51	52	51.0 \pm 2.0 (47-53)	55.7 \pm 1.0 (54-57)	47-57
at mid-body	53	55.0 \pm 2.8 (52-58)	55.3 \pm 3.3 (50-59)	59.8 \pm 2.1 (58-62)	50-62
at anus	29	29.2 \pm 1.3 (28-31)	28.4 \pm 2.1 (27-32)	29.8 \pm 1.8 (28-32)	27-32
Prerectum length	57	67.3 \pm 20.4 (44-81)	58.3 \pm 8.1 (47-65)	67.3 \pm 5.3 (63-74)	44-81
Rectum length	47	40.1 \pm 3.2 (38-44)	40.4 \pm 4.6 (35-47)	43.3 \pm 2.5 (42-46)	35-47
Tail length	33	33.6 \pm 1.7 (32-35)	34.0 \pm 2.6 (31-38)	34.4 \pm 2.6 (32-38)	31-38

* One specimen with odontostyle 16 μm long, not included in the range.

TYPE LOCALITY AND HABITAT

American River Parkway, River Bend Park, El Dorado County, CA, USA, in association with willow tree (*Salix* sp.).

OTHER HABITATS AND LOCALITIES

Putah Creek, Wildlife Area, Stebbins Cold Canyon Reserve, in association with maple (*Acer* sp.); and Capell Valley Road, crossroad with Wragg Canyon Road, in association with grass, both localities being in Napa County, CA, USA.

TYPE MATERIAL

Female holotype and two female paratypes deposited in the nematode collection of the University of Jaén, Spain. One female paratype deposited with USDA Nematode Collection, Beltsville, MD, USA.

DIAGNOSIS AND RELATIONSHIPS

This species is characterised by its body length of 1.45-1.94 mm, cervical lacuna present, lip region offset by deep constriction and 16-18 μm broad, odontostyle 18-20 μm (16 μm in one specimen) long with its aperture occupying 50-53% (57% in one specimen) of its length and, neck 392-521 μm long, pharyngeal expansion 174-256 μm long or 45-51% of total neck length, presence of a dorsal cellular mass at level of cardia, uterus simple and 21-45 μm long or 0.4-0.8 times the corresponding body diam., *pars refringens vaginae* with two well developed sclerotised pieces, two gland cells at both sides of the vagina, vulva transverse (V = 51-57), tail conical to conoid (31-38 μm , c = 39-59, c' = 1.0-1.4), and male unknown.

This species is very close to *A. waenga* (Yeates, 1967) Peña-Santiago & Ciobanu, 2008 (= *A. laevis* Tjepkema, Ferris & Ferris, 1971) (see recent description by Álvarez-Ortega & Peña-Santiago, 2010), from which it differs in its comparatively more slender odontostyle (5.8-6.3 vs 4.4-4.9 times as long as wide), presence vs absence of

a dorsal cellular mass close to the cardia, presence *vs* absence of glandular cells close to the vagina, and female tail longer (31-38 *vs* 23-30 μm) and more conical. See also Remarks.

Some features of this species, mainly the relatively slender odontostyle with a short aperture (*ca* one-half of total length) and female tail conical to conoid, resemble those found in members of *Crassolabium* Yeates, 1967 and *Eudorylaimus* Andr ssy, 1959, but it is not possible to find comparable species in both genera.

MOLECULAR CHARACTERISATION

Two identical sequences (750 bp) were obtained (Fig. 3) from two females, one collected in the American River Parkway, another in the Stebbins Cold Canyon Reserve. The phylogenetic position of this species within dorylaims is presented in Figure 4, and their PCR-D23 of 28S rDNA-RFLP diagnostic profiles are given in Figure 5.

REMARKS

The material examined shows wide variability in its morphometrics. The four females from Capell Valley Road can be distinguished from the others by several measurements and ratios, *viz.*, larger general size (body, neck and pharyngeal expansion lengths), longer odontostyle and odontophore, somewhat more anterior vulva, comparatively shorter tail, *etc.* Nevertheless, and taking into account that only a few specimens from this location were available for study, we adopt a conservative position and provisionally consider them as belonging to the same species as the females from other locations.

OTHER MATERIAL STUDIED

In addition to the two new taxa above described, a few specimens belonging to other *Aporcelaimellus* species were collected and characterised by morphological (see corresponding LM illustrations), morphometric and molecular data. Unfortunately, their precise identification was not always possible because of the limited number of specimens available. The information concerning this material is presented below in an abbreviated form as it provides evidence of the complex diversity of the group.

Aporcelaimellus obtusicaudatus (Bastian, 1865) Altherr, 1968 (Fig. 8; Table 3)

REMARKS

Six females of two populations (three specimens from Yolo County, Davis, near to a river; and three specimens from the Karas line, Sonoma County, Guerneville Road, in association with *Rubus* sp.) were collected and studied. The specimens of two populations can be separated based on their general body size. The three specimens from Davis were larger in several relevant morphometric features, features that did not overlap with those of the females from the Karas line: body diam. (including ratio a), lip region diam., odontostyle and odontophore lengths, neck and pharyngeal expansion lengths, and vulva position. In addition, the *pars distalis vaginae* was smaller and the inner layer of the tail cuticle lacks any differentiation in the three females from the Karas line (*vs* small, more refringent areas present in Yolo County specimens, probably due to fixation process). Nevertheless, the morphological and morphometric variability observed was within the known ranges of the species (for instance, see re-description by De Ley *et al.*, 1993). Two sequences, each obtained from single females from two locations, were 754 and 755 bp, respectively (Fig. 3), and highly similar (98%), differing in eight nucleotides. This variation is hence considered as intraspecific. The phylogenetic position of this species is given in Figure 4. Californian *A. obtusicaudatus* clustered with other representatives of this species available from GenBank. The PCR-D23 of 28S rDNA-RFLP diagnostic profile for this species is given in Figure 5.

Aporcelaimellus sp. A (Fig. 9; Table 3)

REMARKS

The identification of this material (only one female from Cazadero, 116 HWY, Austin Creek Road, in association with grass; and one female and one male from Napa County, Stebbins Cold Canyon Reserve, Putah Creek, Wildlife Area, in association with *Quercus* sp.) is problematic. The three specimens are very close to the representatives of *A. obtusicaudatus* in general morphology and morphometrics, but the tail is longer (50 μm , $c' = 1.1$ *vs* 23-43 μm , $c' = 0.5-0.9$, $n = 58$, after De Ley *et al.*,

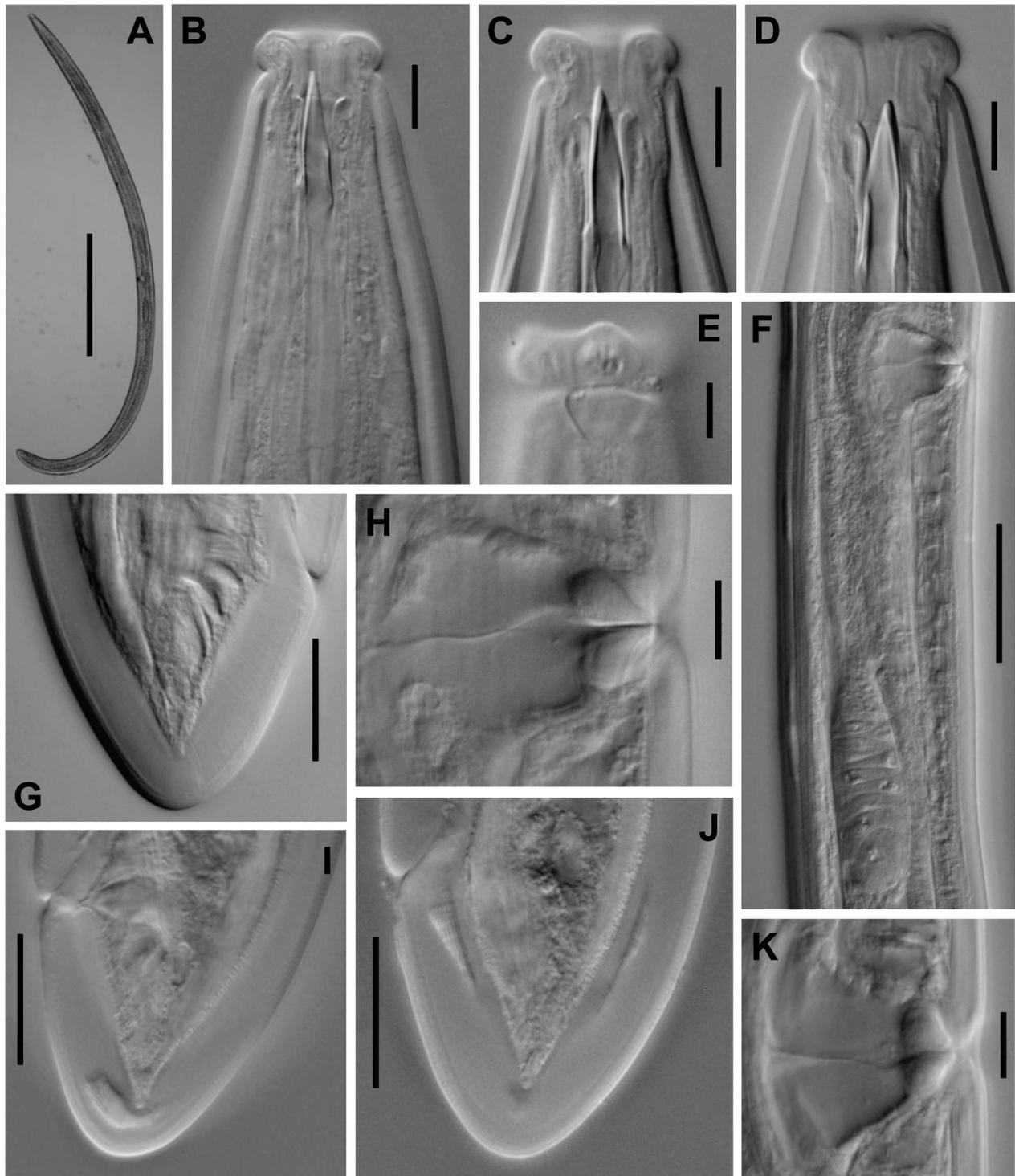


Fig. 8. *Aporcelaimellus obtusicaudatus* (female). A: Entire body; B-D: Anterior region in median view; E: Lip region in lateral, surface view; F: Vagina and posterior genital branch; G, I, J: Caudal region; H, K: Vagina. (Scale bars: A = 500 μm ; B-D, H, K = 10 μm ; E = 5 μm ; F = 50 μm ; G, I, J = 20 μm .)

Table 3. Morphometric data for *Aporcelaimellus obtusicaudatus* (Bastian, 1865) Altherr, 1968, and *Aporcelaimellus* spp. All measurements are in μm (except L, in mm), and in the form: mean \pm s.d. (range).

Character	<i>A. obtusicaudatus</i>			<i>Aporcelaimellus</i> sp. A			<i>A. sp. B</i>
	Davis, Yolo Ct.	Karas line, Sonoma Ct.	Total range	Austin Creek Road, Cazadero	Stebbins Cold Canyon Reserve, Napa Ct.	Stebbins Cold Canyon Reserve, Napa Ct.	
	Females	Females	Females	Female	Male	Female	Female
n	3	3	6	1	1	1	1
L	2.42 \pm 0.12 (2.35-2.56)	2.81 \pm 0.42 (2.33-3.12)	2.33-3.12	2.73	2.80	2.41	1.62
a	33.1 \pm 0.1 (33-33)	27.1 \pm 1.1 (26-28)	26-33	31	31	31	27
b	4.3 \pm 0.1 (4.2-4.4)	4.1 \pm 0.3 (3.7-4.3)	3.7-4.4	4.5	4.2	3.6	3.9
c	67.2 \pm 2.0 (66-70)	71.0 \pm 10.7 (59-79)	59-79	54	82	61	43
c'	0.9 \pm 0.0 (0.8-0.9)	0.7 \pm 0.1 (0.7-0.8)	0.7-0.9	1.1	0.7	0.9	1.2
V	51.4 \pm 1.5 (50-52)	48.2 \pm 1.4 (47-50)	47-52	45	-	51	53
Lip region diam.	19.0 \pm 1.0 (18-20)	22.6 \pm 0.8 (22-23)	18-23	19	21	21	21
Odontostyle length	19.0 \pm 0.2 (19-19)	22.4 \pm 0.2 (22-23)	19-23	19	20	21	18
Odontophore length	39.0 \pm 0.4 (39-39)	46.0 \pm 0.7 (45-47)	39-47	37	40	41	34
Guiding ring from ant. end	10.7 \pm 0.5 (10-11)	13.1 \pm 0.6 (12-14)	10-14	13	11	10	9
Neck length	569 \pm 16 (553-585)	690 \pm 53 (635-740)	553-740	602	667	665	419
Pharyngeal expansion length	296 \pm 4 (291-300)	356 \pm 40 (314-392)	291-392	292	340	335	208
Diam. at neck base	69.5 \pm 1.6 (68-71)	91.4 \pm 9.8 (81-99)	68-99	74	85	73	60
at mid-body	73.2 \pm 3.6 (71-77)	103.2 \pm 12.1 (90-114)	71-114	87	92	77	61
at anus/cloaca	41.3 \pm 0.5 (41-42)	53.2 \pm 3.6 (49-55)	41-55	44	47	43	32
Prerectum length	108 \pm 16 (98-126)	137 \pm 20 (118-158)	98-158	87	115	85	70
Rectum length	54.2 \pm 4.0 (50-59)	65.3 \pm 4.6 (60-68)	50-68	52	73	59	41
Tail length	36.1 \pm 2.4 (34-39)	39.5 \pm 0.0 (39-40)	34-40	50	34	40	38
Spicule length	-	-	-	-	73	-	-
Ventromedian supplements	-	-	-	-	8	-	-

1993) in the female from Cazadero, and the presence of a male in the population from Napa County is a relevant novelty, as well as the presence of a dorsal cellular mass at the level of the anterior end of the intestine. On the other hand, the female from Cazadero is morphometrically very similar to *A. porcus*, itself a poorly characterised species, in having a comparable tail length (50 μm) and quite anterior vulva ($V = 45$ vs 46 in *A. porcus*), although the odontostyle is shorter (19 vs 22 μm , respectively). The couple of specimens from Napa County are also morphometrically similar to *A. porcus* in having the male with eight irregularly spaced ventromedian supplements, although the tail length is shorter (34-39 μm , $c = 61$ -82 vs 50 μm , $c = 50$ -51 in *A. porcus*). PCR-D23 of 28S rDNA-RFLP diagnostic profile for this species is given in Figure 5. The two sequences obtained (from one female in each loca-

tion, 758 bp, Fig. 3) are very similar (99%), differing in three nucleotides, and cluster (Fig. 4) with those available from GenBank for *Aporcelaimellus cf. paraobtusicaudatus* (Micoletzky, 1922) Andr ssy, 1986 and *A. obtusicaudatus*.

***Aporcelaimellus* sp. B**
(Fig. 10; Table 3)

REMARKS

The identity of the only female available, collected from Napa County, Stebbins Cold Canyon Reserve, Putah Creek, Wildlife Area, in association with holm oak *Quercus* sp., also remains obscure. Its general, the morphometric data fit well with those of *A. amylovorus* (Thorne &

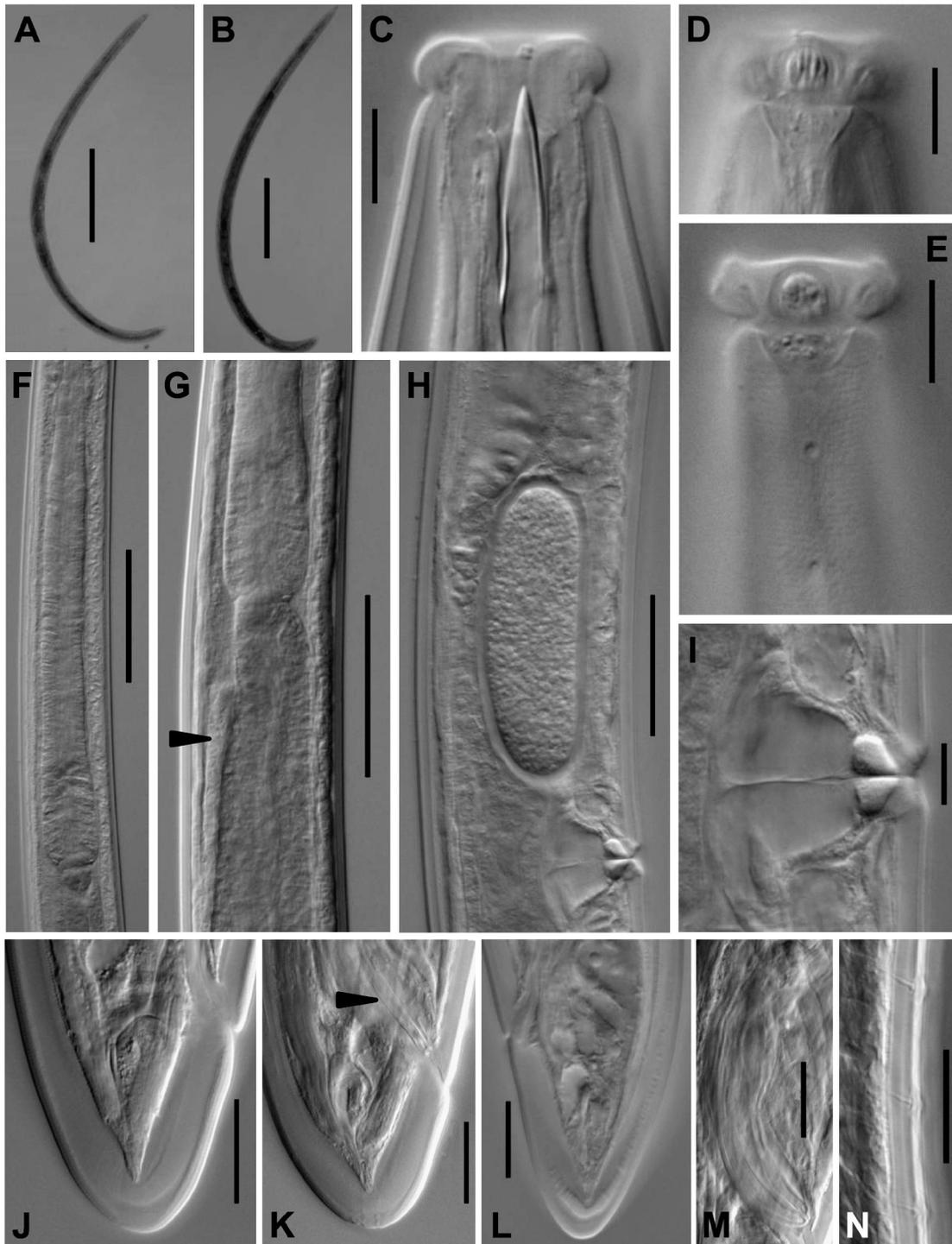


Fig. 9. *Aporcelaimellus* sp. A: A: Female, entire body; B: Male, entire body; C: Lip region in median view; D, E: Lip region in lateral, surface view; F: Pharyngeal expansion; G: Pharyngo-intestinal junction showing dorsal cellular mass (arrowhead); H: Female, anterior genital branch in part; I: Vagina; J, L: Female, caudal region; K: Male, caudal region, showing lateral guiding piece (arrowhead); M: Spicule; N: Ventromedian supplements. (Scale bars: A, B = 500 μ m; C-E, I = 10 μ m; F, G = 100 μ m; H = 50 μ m; J-N = 20 μ m.)

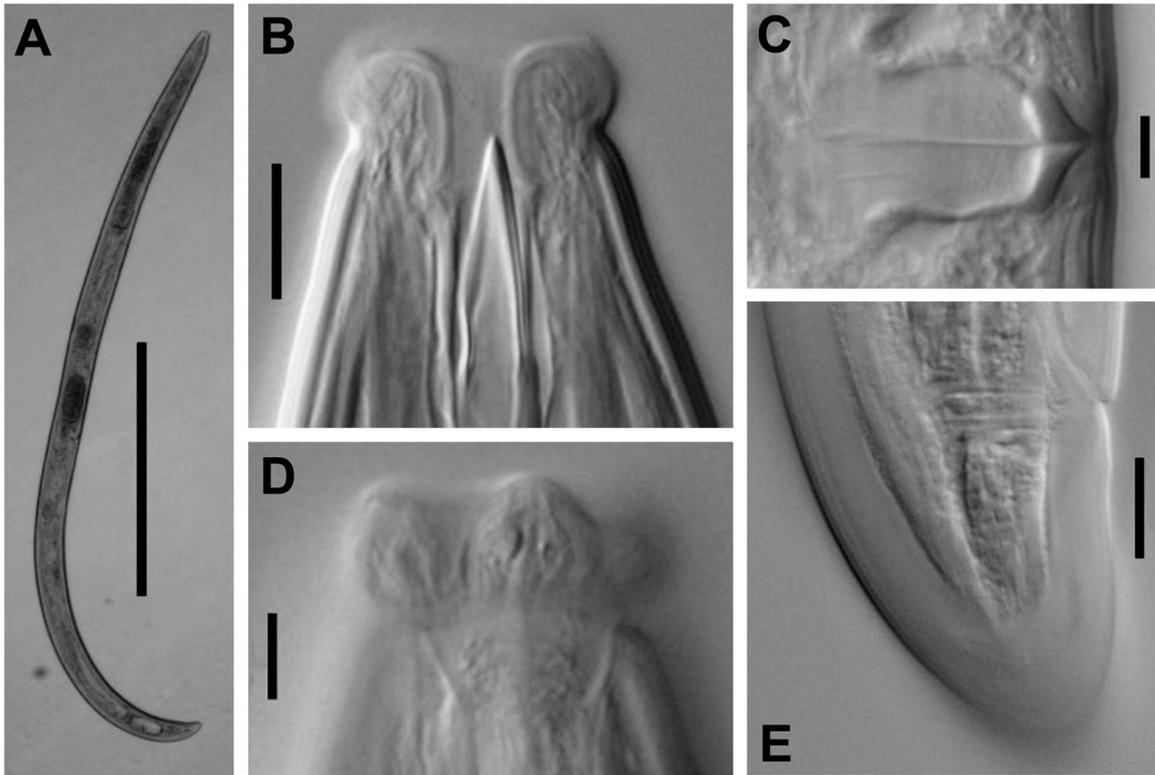


Fig. 10. *Aporcelaimellus* sp. B (female). A: Entire body; B: Anterior region in median view; C: Vagina; D: Lip region in lateral, surface view; E: Caudal region. (Scale bars: A = 500 μm ; B, E = 10 μm ; C, D = 5 μm .)

Swanger, 1936) Heyns, 1965 and *A. waenga*, but there are also some differences which might be significant. It can be distinguished from both of them by the broader lip region (21 vs 15-18 μm in *A. amylovorus*, and 13-18 μm in *A. waenga*), and the odontostyle being distinctly shorter than the lip region diam. (vs as long as or barely longer than lip region diam.). In addition, it differs from *A. amylovorus* by its shorter pharyngeal expansion (208 μm or 50% of total neck length vs 231-239 μm or ca 61% of total neck length, see data by De Ley *et al.*, 1993), and from *A. waenga* by its longer female tail (38 vs 23-30 μm , $c = 49-68$, $c' = 0.7-1.1$). PCR-D23 of 28S rDNA-RFLP diagnostic profile for this species is given in Figure 5. The sequence obtained (760 bp, Fig. 3) is similar (94%) to those obtained for *A. californicus* sp. n., but there are major morphological differences with this species. For instance the uterus is about one body diam. (vs very long, 2.6-4.2 body diam. in the new species). This female might therefore belong to a non-described taxon.

Discussion

The study of Californian material has confirmed that, from a morphological point of view, *Aporcelaimellus* is (apparently) a rather homogeneous taxon. It can be distinguished from *Aporcella* Andr ssy, 2002 and *Metaporcelaimus* Lordello, 1965 (see the new concepts of these taxa by  lvarez-Ortega *et al.*, 2013a, b) by the presence of *pars refringens vaginae* (vs absent in *Aporcella*) and tail shape and size (vs conical, two-layered, and with a discontinuity in its inner layer in *Metaporcelaimus*). Phylogenetic analysis of the D2-D3 of 28S rDNA gene sequences also confirmed that these three genera represent three separate lineages in the tree (Fig. 4).

Regarding the interspecific diversity of *Aporcelaimellus*, the five Californian species show a remarkable morphological homogeneity, differing in more or less distinct morphometric features, namely body length, odontostyle length, uterus length, tail length, *etc.* However, the analysis of the D2-D3 of 28S rDNA gene sequences revealed distinct differences between all species, since in the phy-

logenetic tree, *Aporcelaimellus* species formed two major subclades. The analysis of additional genes is needed to test and confirm monophyly of this genus. PCR-RFLP technique provides an attractive solution for molecular diagnostics of dorylaimids, showing enough discriminative power for separation of *Aporcelaimellus* species. Restriction of D2-D3 amplicons by *MspI* provides distinct differences for RFLP diagnostics. It is likely that molecular diversity is more prominent than morphological diversity and should therefore be well studied and understood.

As currently defined, and in spite of the recent advances to elucidate its identity and clarify its separation from *Aporcella* and *Metaporcelaimus* (see above), *Aporcelaimellus* is still a difficult and intriguing dorylaimid genus. Further studies should be undertaken to explore and analyse its morphological and molecular diversity, not only for theoretical objectives but also for applied purposes, since it should not be forgotten that it is very rich in number of species, some being of worldwide distribution, and that its type species, *A. obtusicaudatus*, “might be the most common soil nematode species” (cf., Andrásy, 2009).

Acknowledgements

The authors are especially grateful for the financial support received from the project entitled *Fauna Ibérica: Nematoda, Dorylaimoidea (excepto Longidoridae)* (Spanish Ministry of Science and Innovation, ref. CGL2007-66786-C08-08; co-financed FEDER). The first author thanks the ‘*Estancias Breves*’ Programme, also of the Spanish Ministry of Science and Innovation, for financing a research stay at the Plant Pest Diagnostics Center (California Department of Food and Agriculture, Sacramento, CA, USA), the Director of which is also thanked for the facilities given.

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