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Morphological and molecular characterisation of some *Hemicriconemoides* species (Nematoda: Criconematidae) together with a phylogeny of the genus

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Summary – Sheathoid nematodes of the genus *Hemicriconemoides* are migratory root-ectoparasites of many plants including various agricultural crops and fruit trees. They are generally found inhabiting warm areas of the world and presently consist of 52 valid species. In this study we provide morphological and molecular characterisation of 12 species of this genus *viz.*: *H. alexis*, *H. brachyurus*, *H. californianus*, *H. chitwoodi*, *H. macrodorus*, *H. minutus*, *H. ortonwilliamsi*, *H. promissus*, *H. silvaticus*, *H. strictathecatus*, *H. wessoni* and *Hemicriconemoides* sp. originating from China, Greece, Japan, Myanmar, Spain, South Africa and the USA. Morphological descriptions, measurements, light and scanning electron microscopic observations and drawings are given for several species. Phylogenetic relationships within *Hemicriconemoides*, as inferred from the analyses of the D2-D3 of 28S rRNA and ITS-rRNA gene sequences, resulted in trees with three major clades that corresponded with species groupings based on morphology of the lip pattern and vulval flap. PCR with species-specific primers were developed for *H. californianus*, *H. chitwoodi* and *H. strictathecatus*.

Keywords – 28S rRNA gene, description, *Hemicriconemoides alexis*, *H. brachyurus*, *H. californianus*, *H. chitwoodi*, *H. macrodorus*, *H. minutus*, *H. ortonwilliamsi*, *H. promissus*, *H. silvaticus*, *H. strictathecatus*, *H. wessoni*, ITS-rRNA gene, PCR specific primers, phylogeny, plant-parasitic nematode, SEM.

The genus *Hemicriconemoides* was proposed by Chitwood & Birchfield (1957) to include those species which fitted neither the diagnosis of *Criconemoides* Taylor, 1936 nor that of *Hemicycliophora* de Man, 1921. These nematodes received the common name of 'sheathoid' nematodes, because the body cuticle of adult stages is covered by an outer accessory layer or sheath with smooth annuli. The sheath is missing in juveniles which have a single cuticle ornamented by rows of scales and spines. Presently, the genus has 52 valid species (Geraert, 2010), all of which are migratory root-ectoparasites of many plants, including various agricultural crops and fruit trees. Sheathoid nematodes are generally found inhabiting warmer areas of the world, particularly in Africa,

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the Americas, Australia, South and Southeast Asia and southern Europe. These nematodes have been reported associated with many crops, *viz.*: *H. cocophillus* (Loos, 1949) Chitwood & Birchfield, 1957 with small millets (Jain, 2009), rice (Sharma *et al.*, 1992) and root crops (Ray *et al.*, 1992), and *H. mangiferae* Siddiqi, 1961 with citrus (Crozzoli *et al.*, 1998), banana, date, pineapple (Siddiqi, 2000) and grapevine (Deimi & Mitkowski, 2010). However, damage is documented for only a few species and a few crops, which include the host-parasite combinations of litchi and mango with *H. litchi* Edward & Misra, 1964 (Liu & Feng, 1995; Nath *et al.*, 2008) and *H. mangiferae* (Milne, 1982; McSorley, 1992); sugarcane with *H. cocophillus* (Cadet & Albrecht, 1992) and tea with *H. kanayaensis* Nakasono & Ichinohe, 1961 (Nakasono & Ichinohe, 1961). In Florida, sheathoid nematodes, including *H. wessoni* Chitwood & Birchfield, 1957, have economic relevance because they suppress the growth and vigour of sod grasses at population levels ranging from 300-1000 specimens (100 cm^3 soil)$^{-1}$ (Crow, 2013). Disease symptoms induced by these parasites consist of stunting, premature wilting, leaf yellowing, root malformation, necrosis of cortical root tissues, and related signs characteristic of nutrient deficiencies (McSorley *et al.*, 1980).

Accurate and timely identification of sheathoid nematodes infesting crops is a prerequisite for the elucidation of host-parasite combinations of economic importance in agriculture and the implementation of effective management strategies and/or potential regulatory actions. Morphological identification of *Hemicriconemoides* is rather time consuming and difficult due to high intraspecific variability and the large number of described species in the genus. The most common morphological characters used for the delimitation of *Hemicriconemoides* species include female body and stylet lengths, number of body annuli (R), post-vulval body (VL/VB) and tail shape.

Hemicriconemoides was considered within Criconematinae Taylor, 1936 by Raski & Luc (1987), whereas Siddiqi (2000) and Geraert (2010) placed it within Hemicriconemoidinae, a subfamily proposed by Andrássy (1979). The last classification is supported by the analysis of sequences of sheathoid nematodes within Hemicriconemoidinae which cluster, in the phylogenetic trees, in a well supported clade separated from that containing members of Criconematinae *sensu* Siddiqi, 2000. The molecular analysis also confirmed the monophyly of the Hemicriconemoidinae and its distinct separation from the

sheath nematodes of the Hemicycliophoridae (Subbotin *et al.*, 2005, 2006).

In recent years, sequence data of the ribosomal rRNA genes have been increasingly used to provide a valuable tool in the identification of nematodes and reconstruction of phylogenetic relationships. Although several *Hemicriconemoides* species, *viz. H. alexis* Vovlas, 1980, *H. californianus* Pinochet & Raski, 1975, *H. chitwoodi* Esser, 1960, *H. cocophillus*, *H. gaddi* (Loos, 1949) Chitwood & Birchfield, 1957, *H. kanayaensis*, *H. ortonwilliamsi* Ye & Siddiqi, 1979, *H. parasinensis* Chen & Liu, 2003, *H. pseudobrachyurus* De Grisse, 1964, *H. strictathecatus* Esser, 1960 and *H. wessoni* have been molecularly characterised with the D2-D3 of 28S rRNA, ITS-rRNA or 18S rRNA genes, the relationship between species within the genus remains uncertain (Subbotin *et al.*, 2005; Chen *et al.*, 2007, 2008, 2011; van Megen *et al.*, 2009; Powers *et al.*, 2011). In addition, topotype populations for most species were not included in these studies.

The objectives of this work were: *i*) to carry out a detailed morphological and morphometric characterisation of topotype specimens and populations of some species belonging to *Hemicriconemoides* from several countries; *ii*) to provide molecular characterisation of the topotype specimens and populations of *Hemicriconemoides* species using sequences of the D2-D3 expansion segments of the 28S nuclear ribosomal RNA and the ITS of rRNA gene; *iii*) to analyse phylogenetic relationships within *Hemicriconemoides* using rRNA genes sequences and their congruence with morphological characters; and *iv*) to develop PCR with species-specific primers for identification of some *Hemicriconemoides* species.

Materials and methods

Nematode populations

Nematode populations studied in this research were obtained from soil samples from different locations in China, Greece, Japan, Myanmar, Spain, South Africa and USA (Table 1). The populations of *H. alexis*, *H. chitwoodi*, *H. macrodorus* Vovlas, Troccoli & Castillo, 2000 and *H. minutus* Esser, 1960 were obtained from the type localities. Samples were collected from the rhizosphere of cultivated and natural environments listed in Table 1. Morphological and molecular characters of topotype specimens were compared with those of populations of the same species collected from other localities. Specimens of *H. ortonwilliamsi* from Italy and *H. strictathecatus* from

Table 1. *Hemicriconemoides* species and populations used in the present study.

Species	Locality	Host	Sample code	Molecular study		Collector or identifier
				D2-D3 of 28S rRNA	ITS-rRNA	
H. alexis	Epirus, Greece (type locality)	*Zea mays* L.	849a	AY780959	KF856561	N. Vovlas, Subbotin *et al.* (2005)
H. alexis	Brooklyn Park, Minnesota, USA	Unknown	CD1163	KF856530	KF856562	D. Mollov, S.A. Subbotin
H. brachyurus	Aoshima, Japan	*Ischaemum anthephoroides* (Steud.) Miq., *Carex kobomugi* Ohwi.	P1	KF856515	KF856553	J.E. Palomares Rius
H. californianus	Folsom, Sacramento County, California, USA	Unknown	CD847	KF856536, KF856537	KF856559, KF856560	S.A. Subbotin
H. californianus	San Jose, Santa Clara County, California, USA	*Platanus* sp.	CD907	KF856538	KF856557, KF856558	S.A. Subbotin
H. californianus	Yolo County, California, USA	*Salix* sp.	CD1021	KF856539	–	S.A. Subbotin
H. chitwoodi	Monticello, Florida, USA (type locality)	*Liquidambar styraciflua* L.	CD1185	KF856533, KF856535	KF856543, KF856544	R.N. Inserra, J.D. Stanley
H. chitwoodi	Imported from Japan to Tuscany, Italy	*Camellia sasanqua* Thunb.	Tus	KF856534	–	P. Castillo, N. Vovlas
H. chitwoodi	Miyazaki, Japan	*Prunus* sp., *Rhododendron* sp.	Miy	KF856532	–	J.E. Palomares Rius
H. macrodorus	Santa Elena, Jaén, Spain (type locality)	*Quercus suber* L.	topotype, CD339	KF856522, KF856523	–	P. Castillo, N. Vovlas
H. macrodorus	Alcalá de los Gazules, Spain	*Olea europaea* L.	Alcala	KF856525, KF856526	KF856556	P. Castillo
H. macrodorus	Tarifa, Spain	*Olea europaea* L.	Tarifa	KF856524	–	P. Castillo
H. minutus	University of Florida Campus, DPI, Gainesville, Florida, USA (type locality)	*Carya* sp.	CD1181	KF856516, KF856517	KF856545, KF856546	R.N. Inserra, J.D. Stanley

Table 1. (Continued.)

Species	Locality	Host	Sample code	Molecular study		Collector or identifier
				D2-D3 of 28S rRNA	ITS-rRNA	
H. minutus	Haines City, Florida, USA	*Quercus* sp.	CD1306	KF856518	–	R.N. Inserra, J.D. Stanley
H. ortonwilliamsi	Rociana, Huelva, Spain	*Vitis vinifera* L.	Rociana	KF856514	KF856552	P. Castillo
H. ortonwilliamsi	Sanlúcar de Barrameda, Spain	*Vitis vinifera* L.	J215	KF856513	KF856551	P. Castillo
H. promissus	Bolonia, Cádiz, Spain	*Ammophila arenaria* L.	Bolonia	KF856529	KF856554	P. Castillo
H. promissus	Monteagudo Island, Spain	*Pinus pinaster* Aiton	Monteagudo	KF856527, KF856528	KF856555	P. Castillo
H. silvaticus	Aoshima, Japan	*Ischaemum anthephoroides* (Steud.) Miq., *Carex kobomugi* Ohwi.	097	KF856531	KF856542	J.E. Palomares Rius
H. strictathecatus	Cuyagua, Venezuela	*Theobroma cacao* L.	839	AY780957	KF856566	R. Crozzoli
H. strictathecatus	Dela Rey farm, near Tzaneen, Limpopo Province, South Africa	*Persea americana* Mill.	Tvl1948	–	–	H. Swart, E. Van den Berg
H. strictathecatus	Outlook Farm, near South Broom, Kwa-Zulu Natal Province, South Africa	*Litchi chinensis* Sonn.	N826, CD896	KF856541	KF856564, KF856565	P. Burger, E. Van den Berg
H. strictathecatus	Imported from China to Ruvo, Bari, Italy	*Largestroemia indica* (L.) Pers.	Ruvo	KF856540	KF856563	N. Vovlas
H. wessoni	Bent Oak Farm, Ocala, Florida, USA	*Bermuda* sp., *Ulmus* sp.	CD1107	KF856521	KF856549	R.N. Inserra, J.D. Stanley
H. wessoni	DPI, Gainesville, Florida, USA	*Bermuda* sp., *Stenotaphrum secundatum* (Walt.) Kuntze	CD1154	KF856519	KF856547, KF856548	R.N. Inserra, J.D. Stanley
H. wessoni	Riverside County, California, USA	Turf grasses	CD1262	KF856520	–	S.A. Subbotin
Hemicriconemoides sp.	Myanmar	Unknown	712	–	KF856550	D. Sturhan, J. Hallmann

Venezuela (Subbotin *et al.*, 2005) were also used for morphological and molecular comparisons, respectively.

Samples were collected from the upper 10-40 cm of soil of 4-5 plants arbitrarily chosen in each locality. Nematodes were extracted from 500 cm^3 of soil by centrifugal-flotation (Coolen, 1979) or rapid centrifugal-flotation methods (Jenkins, 1964).

LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDY

Specimens for light microscopy (LM) were killed by gentle heat, fixed in a solution of 4% formaldehyde + 1% propionic acid or FPG (Netscher & Seinhorst, 1969) and temporarily mounted in 4% formalin (American specimens) or processed to pure glycerin using Seinhorst's (1962) or De Grisse's (1969) methods and mounted on permanent slides. Light micrographs were taken with an automatic Infinity 2 camera attached to a compound Olympus BX51 microscope equipped with Nomarski differential interference contrast. Measurements were made with a research microscope (Nikon Labophot-2) equipped with a drawing tube.

For scanning electron microscopy (SEM), samples were fixed in 70% ethanol for at least 12 h, and then dehydrated in an ethanol series of 80, 90 and 100% for 15 min each. The samples were critical point dried using liquid carbon dioxide in a critical point dryer. The dried samples were mounted on SEM stubs with double sided carbon tape and sputter coated with 15 nm gold/palladium (66/33%). The coated samples were viewed under a FEI Quanta FEG 250 SEM under high vacuum mode at 5-10 kV.

DNA EXTRACTION, PCR AND SEQUENCING

For molecular analyses, nematode DNA from *Hemicriconemoides* samples was extracted from single or several individuals using proteinase K as described by Castillo *et al.* (2003). PCR and sequencing was completed in two laboratories: IAS-CSIC, Spain and CDFA, USA. All detailed protocols, were described by Castillo *et al.* (2003) and Tanha Maafi *et al.* (2003), respectively. The primer sets used for amplification of the D2-D3 expansion segments of 28S rRNA and ITS-rRNA genes are given in Table 2. Two μl of the PCR product were run on a 1% TAE buffered agarose gel.

PCR products were purified after amplification using ExoSAP-IT (Affmetrix, USB products) or QIAquick (Qiagen) gel extraction kits and used for direct sequencing in both directions with the primers referred above or for cloning. The PCR products were cloned into the pGEM-T vector and transformed into JM109 High Efficiency Competent Cells (Promega). Several clones of each sample were isolated using blue/white selection and submitted to PCR with same primers. PCR products from each clone were sequenced in both directions at the Stab Vida sequencing facilities (Caparica, Portugal) and Davis Sequencing (Davis, CA, USA). The newly obtained sequences were submitted to the GenBank database under the accession numbers KF856513-KF856566 as indicated in Table 1.

PHYLOGENETIC ANALYSIS

The D2-D3 expansion segments of 28S rRNA and ITS-rRNA gene sequences of several *Hemicriconemoides* from GenBank were used for phylogenetic reconstruc-

Table 2. Primer sets used in the present study.

Primer code	Sequence (5′ → 3′)	Amplified gene	Amplicon length (bp)	References
TW81	GTT TCC GTA GGT GAA CCT GC	ITS-rRNA	850-880	Curran *et al.* (1994)
AB28	ATA TGC TTA AGT TCA GCG GGT			
D2A	ACA AGT ACC GT GAG GGA AAG TTG	D2-D3 of 28S rRNA	*ca* 780	Subbotin *et al.* (2006)
D3B	TCG AAA GGA ACC AGC TAC TA			
TW81	GTT TCC GTA GGT GAA CCT GC	ITS-rRNA	*ca* 186	This study
H_califor	CTA TTC CGA AAG GGG TGT TC			
TW81	GTT TCC GTA GGT GAA CCT GC	ITS-rRNA	*ca* 730	This study
H_strict	CAG TCG TCA GTG AAC AAG TCA			
TW81	GTT TCC GTA GGT GAA CCT GC	ITS-rRNA	*ca* 333	This study
H_chitw	CGC ACC GCG TAT CAG TGC			

tion. Outgroup taxa for each dataset were chosen according to previous published data (Subbotin *et al.*, 2005). The newly obtained and published sequences for each gene were aligned using ClustalX (Thompson *et al.*, 1997) with default parameters. The alignments were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). The general time reversible substitution model with estimation of invariant sites and assuming a gamma distribution with four categories (GTR + I + G) was selected as the optimal nucleotide substitution model for the analyses. BI analysis for each gene was initiated with a random starting tree and was run with four chains for 1.0×10^6 generations. Two runs were performed for each analysis. The Markov chains were sampled at intervals of 100 generations. After discarding burn-in samples other trees were used to generate a 50% majority rule consensus tree.

The combined D2-D3 and ITS-rRNA alignment with one sequence from each species was also generated. This alignment was analysed with BI and maximum parsimony (MP) using PAUP* 4.0b 10 (Swofford, 2003) with 1000 bootstrap replicates. Sequence analyses of alignments were also performed with PAUP. Pairwise divergences between taxa were computed as absolute distance values and as percentage mean distance values based on whole alignment, with adjustment for missing data.

PCR WITH SPECIES-SPECIFIC PRIMERS

Species-specific primers for three *Hemicriconemoides* species (Table 2) were designed using the sequence alignment of ITS-rRNA gene. The PCR mixture was prepared as described by Tanha Maafi *et al.* (2003). The PCR amplification profile consisted of 4 min at 94°C; 30 cycles of 1 min at 94°C, 45 s at 57°C and 45 s at 72°C, followed by a final step of 10 min at 72°C. Then 2-3 μl of the PCR products were run on a 1.4% TAE buffered agarose gel, stained and photographed. Several *Hemicriconemoides* samples were used to test the specificity of PCR with newly designed species specific primers.

Results

We distinguished 11 valid species within the studied samples: *viz.*, *H. alexis*, *H. brachyurus* (Loos, 1949) Chitwood & Birchfield, 1957, *H. californianus*, *H. chitwoodi*, *H. macrodorus*, *H. minutus*, *H. ortonwilliamsi*, *H. promissus* Vovlas, 1980, *H. silvaticus* Eroshenko & Volkova, 1985, *H. strictathecatus* and *H. wessoni*. A sample of

Hemicriconemoides sp. from Myanmar was not identified to species level because of the absence of adult females. Morphological and morphometric characterisation of some species are given below (Figs 1-13; Tables 3-6).

Hemicriconemoides alexis Vovlas, 1980
(Figs 1A, B; 3A, B)

Females of this species were found in the rhizosphere of maize, Epirus, Greece (Vovlas, 1980), and males and juveniles were described later (Vovlas *et al.*, 2000). A single female of this species was found in a sample collected from Brooklyn Park, MN, USA (Table 1). Its morphological characteristics matched those of the type population. This is the first record of *H. alexis* in the USA and outside the type locality.

Hemicriconemoides brachyurus (**Loos, 1949**) **Chitwood & Birchfield, 1957**
(Figs 1C; 3C)

This species was originally described from a tea nursery soil from Sri Lanka (Loos, 1949) and was characterised in the original description by females with large vulval flaps, a short postvulval body part, and a bluntly rounded tail. The morphological and molecular characters of a *H. brachyurus* population collected in Japan (Table 1) are provided in this study.

MEASUREMENTS

See Table 3.

DESCRIPTION

Female

Body shape almost straight to slightly arcuate ventrad. Cuticle sheath attached to body at labial disc, but clearly separated on tail. Lip region flattened anteriorly, slightly set off, labial disc elevated. Stylet knobs anchor-shaped. Excretory pore situated from 6-8 annuli posterior to base of pharynx. Hemizonid and hemizonion not seen. Vulval flaps present and vulval opening a distinct slit with prominent lips. Vagina straight. Spermatheca not seen. Tail conoid, ending in a rounded tip. Anus situated 6-8 annuli posterior to vulva.
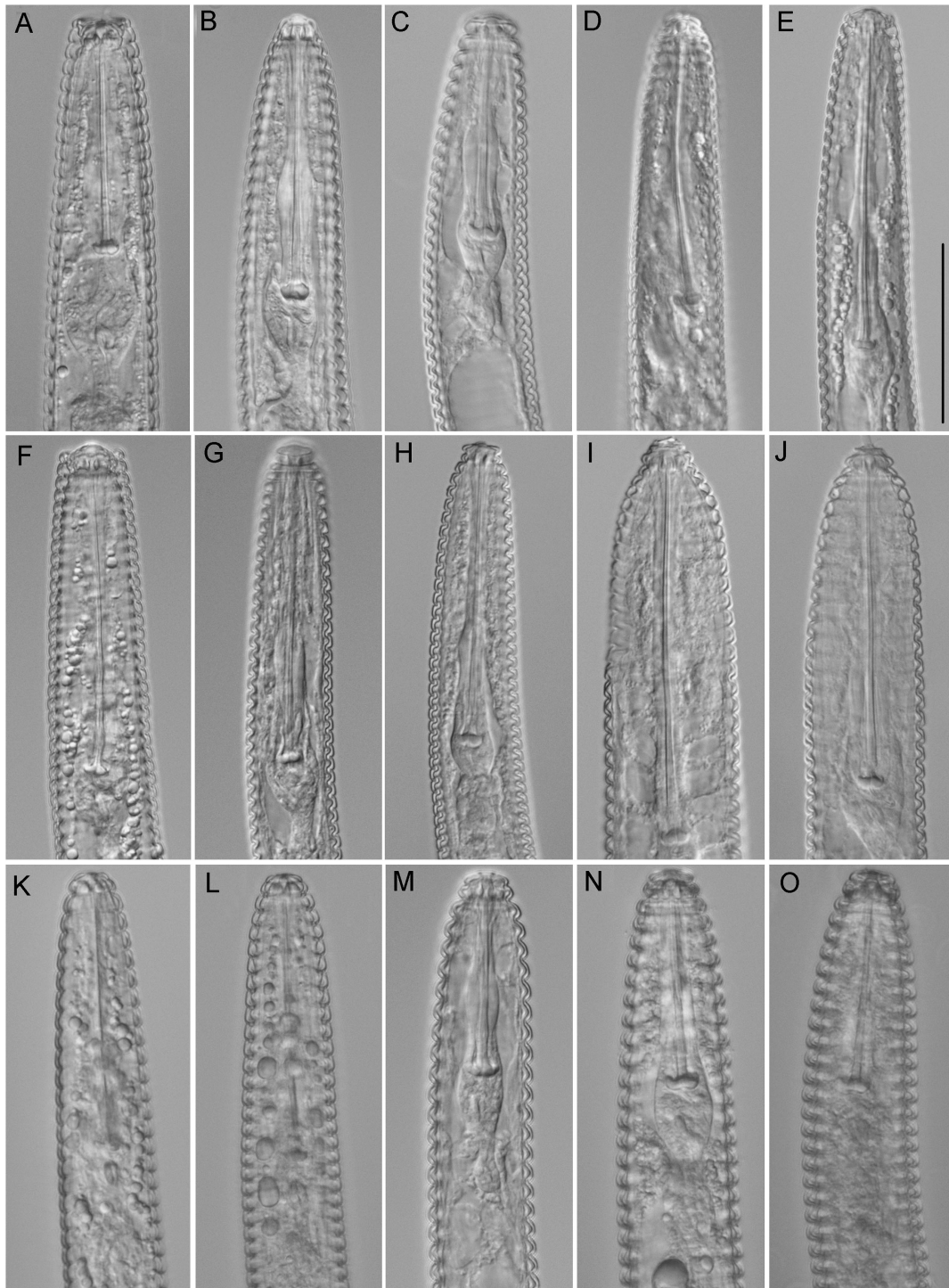
Male

Not found.

Fig. 1. Anterior regions of *Hemicriconemoides* species. A: *H. alexis*, MN, USA (CD1163); B: *H. alexis*, topotype Greece; C: *H. brachyurus*, Japan; D: *H. californianus*, CA, USA (CD1021); E, F: *H. chitwoodi*, topotype FL, USA (CD1185); G: *H. chitwoodi*, Japan (intercepted in Italy); H: *H. chitwoodi*, Japan; I, J: *H. macrodorus*, topotype, Spain; K, L: *H. minutus*, topotype FL, USA; M: *H. ortonwilliamsi*, Spain; N, O: *H. ortonwilliamsi*, Italy. (Scale bar = 50 μm.)
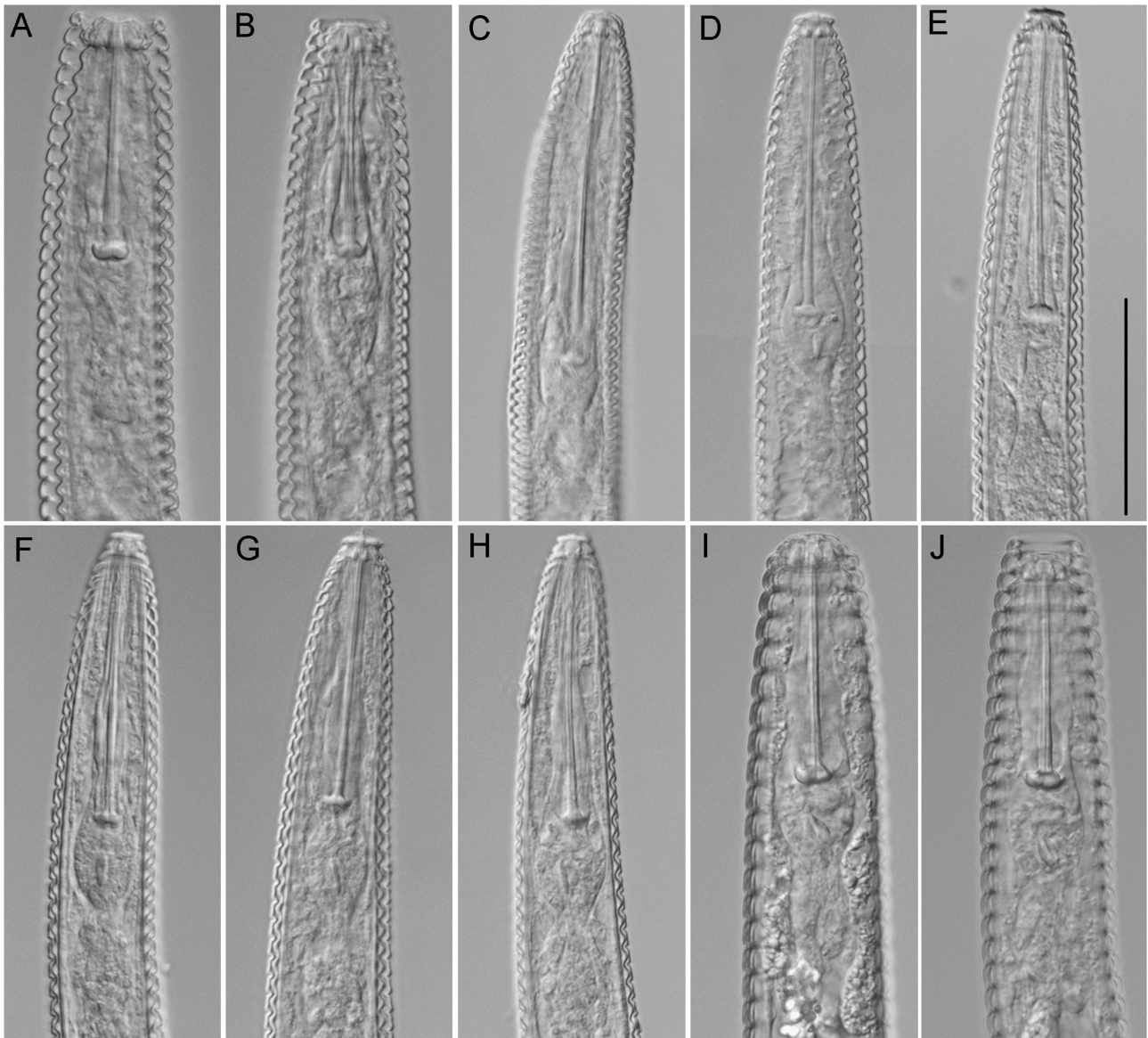
Fig. 2. Anterior regions of *Hemicriconemoides* species. A, B: *H. promissus*, Spain; C: *H. silvaticus*, Japan; D: *H. strictathecatus*, China (intercepted in Italy); E-H: *H. strictathecatus*, South Africa; I-J: *H. wessoni*, FL, USA. (Scale bar = 50 μm.)

REMARKS

Morphometrics of the Japanese population from Aoshima (Table 3) were coincident with the original description, as well as those by Van den Berg & Heyns (1977) from sugarcane and grass in South Africa and Dasgupta *et al.* (1969) from sugarcane in Sri Lanka and Taiwan. This species has also been cited from Japan (Yaegashi, 1977), as well as in Indonesia (Rashid *et al.*, 1988), India (Ye & Siddiqi, 1994), Korea (Choi & Jeong, 1995), Vietnam

(Germani & Anderson, 1991) and California, USA (Ye & Robbins, 2000).

Hemicriconemoides californianus
Pinochet & Raski, 1975
(Figs 1D; 3D, E; 5; 6)

This species was originally described from specimens collected from the rhizosphere of *Vitis vinifera* L. at the
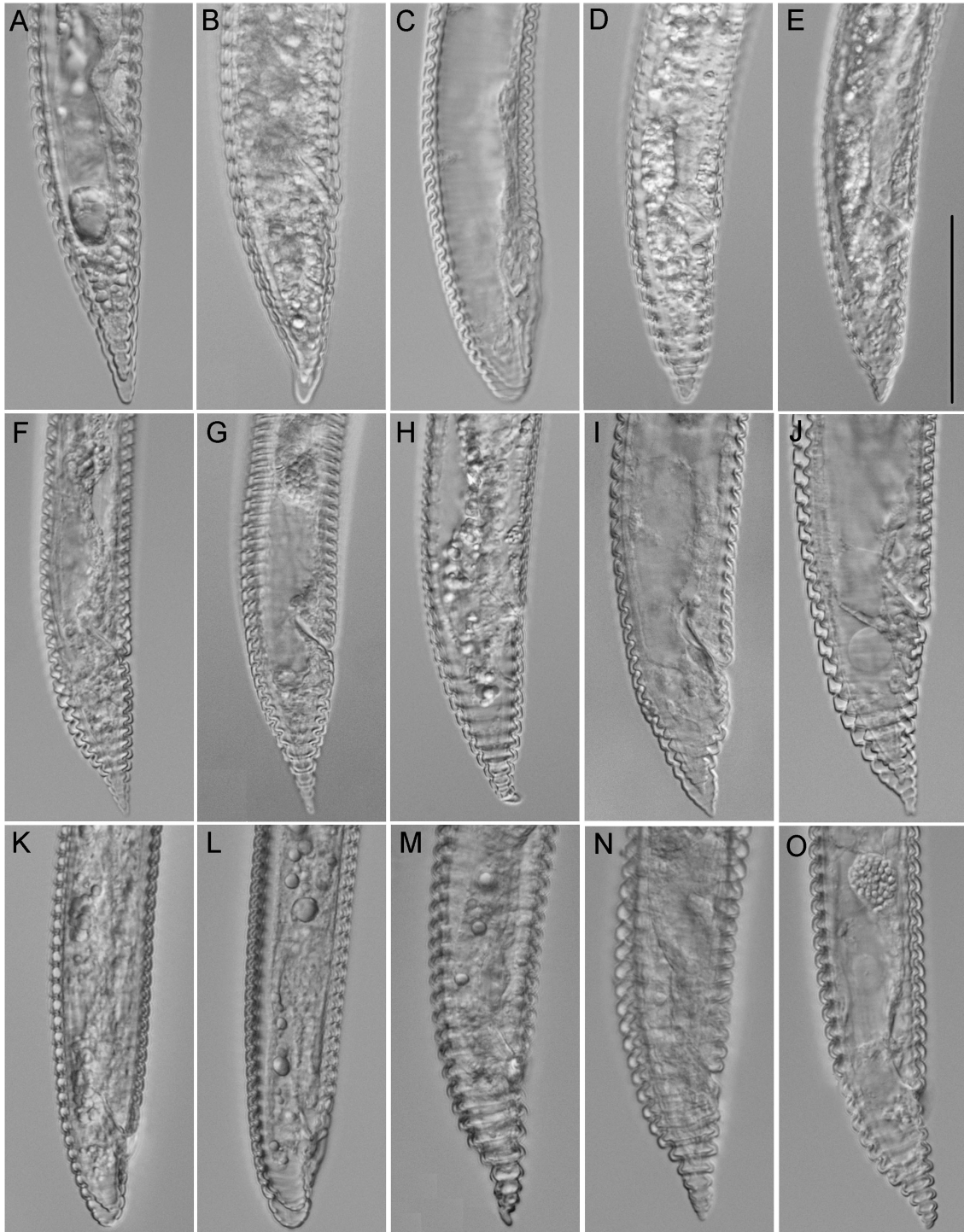
Fig. 3. Posterior regions of *Hemicriconemoides* species. A: *H. alexis*, topotype Greece; B: *H. alexis*, MN, USA (CD1163); C: *H. brachyurus*, Japan; D, E: *H. californianus*, CA, USA (CD1021); F: *H. chitwoodi*, Japan (intercepted in Italy); G: *H. chitwoodi*, Japan; H: *H. chitwoodi*, topotype FL, USA (CD1185); I, J: *H. macrodorus*, topotype, Spain; K, L: *H. minutus*, topotype FL, USA; M: *H. ortonwilliamsi*, Italy; N, O: *H. ortonwilliamsi*, Spain. (Scale bar = 50 μm.)

Table 3. Morphometrics of females of *Hemicriconemoides brachyurus*, *H. californianus* and *H. chitwoodi*. All measurements are in μm and in the form: mean ± s.d. (range).

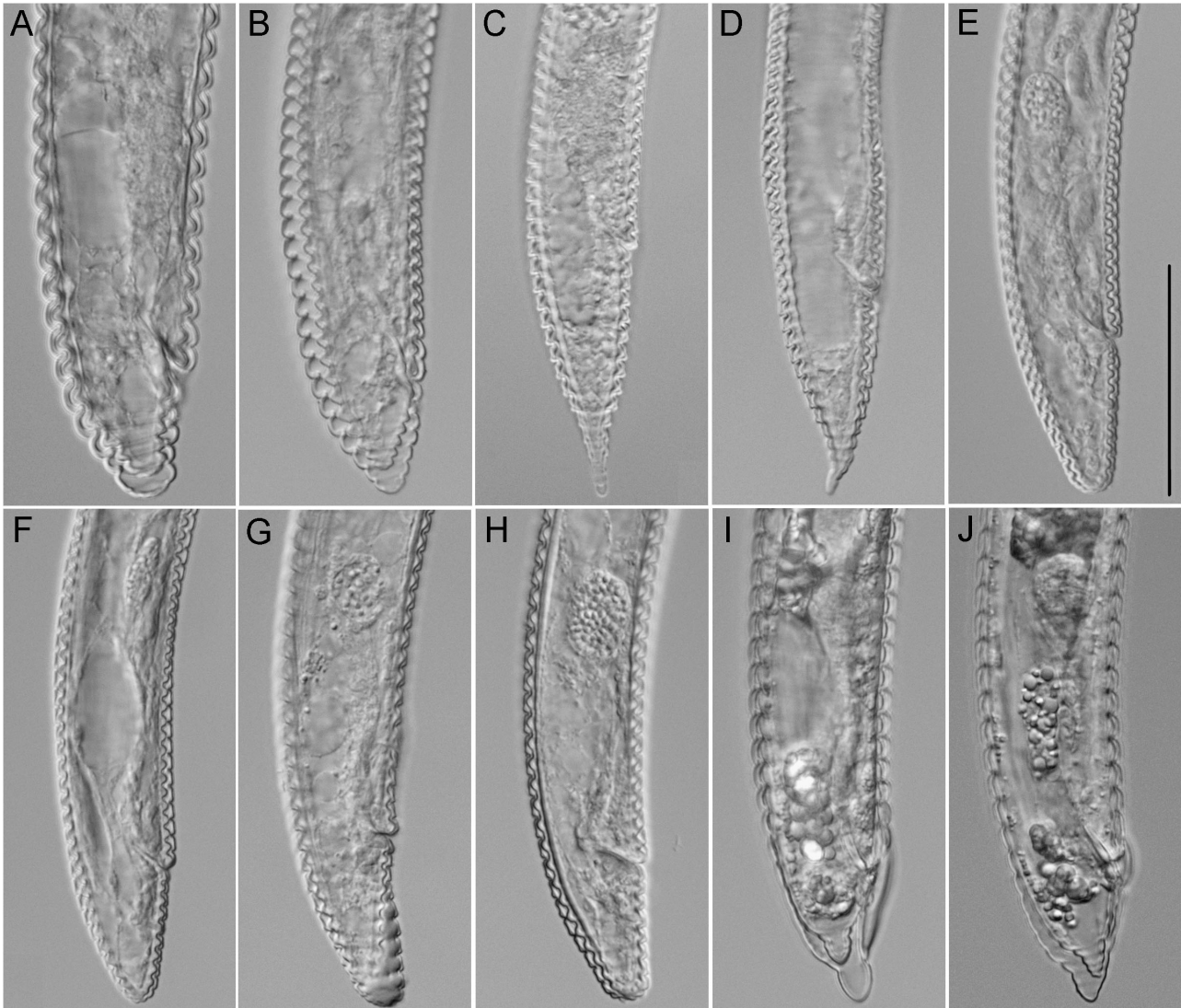| Character	*H. brachyurus* Aoshima, Japan	*H. californianus* CA, USA (CD847)	*H. californianus* CA, USA (CD1021)	*H. chitwoodi* Topotype FL, USA (CD1185)	*H. chitwoodi* Miyazaki, Japan (Miy)	*H. chitwoodi* Japan (intercepted in Italy) (Tus)
n	12	11	7	7	9	8
L	443 ± 59 (344-584)	429 ± 22.2 (387-457)	439 ± 19 (416-460)	496 ± 3.8 (435-537)	471 ± 53.8 (364-544)	477 ± 51.3 (413-556)
a	13.1 ± 2.6 (10.5-18.8)	15.9 ± 0.9 (14.4-17.3)	14.4 ± 1 (13.1-15.9)	13.6 ± 1.7 (11.9-16.2)	16.9 ± 2.1 (13.5-19)	17.8 ± 1.0 (15.9-18.8)
b	4.5 ± 0.4 (4.0-5.2)	3.6 ± 0.4 (3.2-4.5)	3.7 ± 0.3 (3.5-4.4)	3.7 ± 0.1 (3.5-3.8)	3.8 ± 0.4 (3.4-4.4)	3.9 ± 0.5 (3.5-4.8)
c	26.5 ± 3.6 (20-33.2)	23.1 ± 3.1 (19.3-29.7)	15.7 ± 1.6 (13-17.9)	17.2 ± 1.2 (15.2-19.1)	15.5 ± 2.1 (11-17.7)	16.5 ± 1.0 (14.4-17.7)
c'	1.0 ± 0.1 (0.9-1.2)	1.2 ± 0.1 (1.0-1.5)	1.6 ± 0.2 (1.4-1.8)	1.3 ± 0.2 (1.1-1.4)	1.8 ± 0.3 (1.4-2.3)	1.7 ± 0.1 (1.6-1.8)
o	6.2 ± 0.6 (5.7-6.6)	7.8 ± 0.5 (6.8-8.5)	7.2 ± 2 (5.3-9.3)	8.4 ± 0.6 (8.2-9.1)	7.6 ± 0.4 (7.0-7.8)	7.7 ± 0.5 (7.0-8.5)
DGO	3.3 ± 0.4 (3.0-3.5)	6.5 ± 0.5 (6.0-7.5)	5.5 ± 2 (5.3-9.3)	7 ± 0.5 (6.5-7.5)	6.3 ± 0.3 (6.0-6.5)	6.4 ± 0.3 (6-7)
V	92.8 ± 1.1 (91-95)	91.5 ± 0.5 (91-92.5)	89 ± 0.8 (87.5-90)	90 ± 2.8 (84-92)	89.9 ± 1.0 (88-91)	90.5 ± 0.5 (90-91)
G₁	59.5 ± 8.0 (50.3-64.9)	32 ± 4.8 (27.5-40.5)	37.5 ± 2.6 (35.5-43)	38.5 ± 8.9 (31.5-40.5)	51.2 ± 19.7 (36-73)	43.5 ± 2.3 (40-45)
Ovary length	249 ± 66 (173-289)	138 ± 18.5 (121.5-170.5)	163 ± 10.6 (148.5-178)	187 ± 49.7 (142-281)	218 ± 97.8 (131-324)	201 ± 29.5 (173-250)
Stylet length	56.5 ± 3.7 (52-63)	86 ± 2.8 (78.5-89.5)	79.5 ± 2.3 (76.5-82)	88 ± 5.2 (81-96.5)	84.3 ± 3.9 (77-89)	83 ± 2.6 (80-88)
Metenchium length	45.7 ± 3.0 (41.5-51)	76 ± 2.8 (69-79.5)	69.5 ± 2.1 (65.5-71.5)	79 ± 4.7 (72-85.5)	75.9 ± 3.8 (69-82)	75.5 ± 3.0 (72-81)
Telenchium length	10.8 ± 1.9 (7.0-13.5)	10 ± 0.5 (9-11)	10 ± 0.7 (9-11)	9.5 ± 1.1 (8-11)	8.4 ± 1.2 (7.0-10)	7.6 ± 0.5 (7.0-8.0)
m	81.0 ± 2.9 (76.7-86.8)	88.5 ± 0.7 (87.5-89)	87.3 ± 0.9 (86-88.4)	89.6 ± 1.0 (88.3-91)	90.0 ± 1.4 (88-92)	90.8 ± 0.8 (90-92)
Stylet knob height	4.0 ± 0.8 (2.5-5.0)	3 ± 0.3 (3-3.5)	3.5 ± 0.3 (3.0-3.5)	2.5 ± 0.6 (2.0-3.5)	2.9 ± 0.2 (2.5-3.0)	2.8 ± 0.3 (2.5-3.0)
Stylet knob width	9.2 ± 0.6 (8-10)	6 ± 0.5 (6.0-7.5)	6.5 ± 0.5 (5.5-7.0)	7.5 ± 0.4 (6.5-8.0)	6.9 ± 0.7 (6-8)	6.4 ± 0.3 (6-7)
Excretory pore from anterior end	118 ± 8.4 (108-131)	127 ± 9.3 (113-142)	121 ± 18.8 (104.5-154.5)	140 ± 6.4 (129.5-150)	134 ± 2.2 (132-137)	128 ± 6.0 (120-133)
Diam. at mid-body	34.2 ± 3.7 (31-42)	26.5 ± 1.1 (24-28)	30.5 ± 1.1 (28.5-32.5)	37 ± 4.7 (33-42.5)	28.0 ± 2.8 (24-32)	26.9 ± 3.3 (24-32)
Diam. at anus	–	–	–	24.5 ± 5.6 (18.5-35)	–	–
Diam. at vulva	–	–	–	30.5 ± 4.2 (25.5-36)	–	–
Annulus width	4.3 ± 0.4 (4-5)	4 ± 0.4 (3.5-5.0)	4.5 ± 0.4 (4-5)	4 ± 0.3 (3.5-4.5)	3.7 ± 0.3 (3.5-4.0)	3.6 ± 0.2 (3.5-4.0)
Tail length	17.0 ± 2.1 (13-20)	18.5 ± 2.3 (14.5-22)	28 ± 3.3 (25.5-35.5)	29 ± 2.6 (25-31.5)	30.6 ± 3.6 (26-35)	29.1 ± 3.6 (25-35)
Pharynx length	96 ± 7.9 (83-112)	119 ± 8.7 (101.5-130.5)	119 ± 7.4 (103.5-125)	134 ± 7.9 (122.5-144)	125 ± 8.9 (106-135)	124 ± 5.7 (116-130)
First lip diam.	18.4 ± 0.9 (17-20)	10.5 ± 0.9 (9.5-12)	11.5 ± 0.4 (11-12)	12.5 ± 0.7 (11.5-13)	12.2 ± 0.5 (11.5-13)	12.5 ± 0.4 (12-13)
Second lip diam.	–	11.5 ± 0.8 (10.5-12.5)	11.5 ± 1.0 (10.5-13)	12 ± 0.7 (11.5-13)	11.3 ± 0.7 (10-12)	–
First body annulus diam.	–	13.5 ± 1.0 (12-15)	14.5 ± 1.0 (12.5-16.5)	17 ± 0.8 (15.5-17.5)	14.7 ± 1.2 (13-16)	–
Second body annulus diam.	–	15.5 ± 0.8 (14.5-17)	16.5 ± 1.4 (15.5-18.5)	19 ± 1.4 (17.5-21.5)	12.2 ± 0.5 (11.5-13)	–
Third body annulus diam.	–	17 ± 1.1 (16-18.5)	17.5 ± 0.7 (16-18)	20.5 ± 2.1 (17.5-23.5)	–	–
R	98 ± 9.8 (86-118)	115 ± 4.7 (104-121)	116 ± 3.9 (108-120)	122 ± 2.2 (118-125)	131 ± 7.3 (117-141)	131 ± 6.7 (119-139)
RSt	13.3 ± 1.5 (11-16)	27 ± 2.4 (23-31)	28 ± 1.5 (26-29)	23 ± 1.5 (22-26)	22.1 ± 2.5 (19-25)	21.9 ± 3.0 (17-25)
ROes	19.8 ± 1.9 (17-23)	37 ± 4.6 (31-46)	37 ± 2.6 (32-40)	33 ± 2.3 (30-36)	32.1 ± 1.8 (30-35)	32.4 ± 2.3 (28-35)
Rex	28.7 ± 4.7 (25-34)	36 ± 2.1 (34-40)	36 ± 3.1 (33-42)	35 ± 1.7 (33-37)	33.8 ± 1.7 (32-36)	33.5 ± 2.1 (30-36)
RV	8.9 ± 1.2 (8-11)	12 ± 0.8 (11-13)	14.5 ± 0.8 (13-15)	14 ± 1.3 (13-16)	13.6 ± 0.7 (13-15)	13.6 ± 0.7 (13-15)
RVan	2.2 ± 0.8 (1-3)	4 ± 0.9 (3-6)	4	3 ± 0.5 (3-4)	3.9 ± 0.8 (3-5)	3.8 ± 0.7 (3-5)
Ran	6.7 ± 0.9 (6-8)	7 ± 0.6 (6-8)	9 ± 0.7 (8-10)	10 ± 0.8 (9-11)	9.6 ± 0.7 (9-11)	9.8 ± 0.9 (9-11)
VL/VB	1.1 ± 0.1 (1.0-1.5)	1.6 ± 0.1 (1.5-1.8)	2 ± 0.2 (1.7-2.3)	1.6 ± 0.2 (1.3-1.9)	1.9 ± 0.1 (1.7-2.1)	1.8 ± 0.1 (1.7-2.0)
St%L	13.0 ± 1.4 (10.1-15.4)	20 ± 0.8 (18.3-20.9)	18.4 ± 0.7 (17.2-19.1)	17.8 ± 1.4 (16.5-20.1)	18.1 ± 2.0 (15.3-21.2)	17.7 ± 2.2 (14.7-19.9)
Spermatheca length	–	–	18.5 ± 2.1 (16-22.5)	15.5 ± 5.1 (10.5-22)	–	–
Spermatheca diam.	–	–	12 ± 2.3 (9.5-15)	12.5 ± 6.9 (9.5-18.5)	–	–

Fig. 4. Posterior regions of *Hemicriconemoides* species. A, B: *H. promissus*, Spain; C, D; *H. silvaticus*, Japan; E: *H. strictathecatus*, China (intercepted in Italy); F-H: *H. strictathecatus*, South Africa; I, J: *H. wessoni*, FL, USA. (Scale bar = 50 μm.)

University of California campus, Davis, Yolo County, California, USA. It was also found from soil around various trees such as sycamore, willow, alder, black walnut, macadamia and grapevine in California (Pinochet & Raski, 1975). Ye & Robbins (2000) found it on rose, loquat, turf, cabbage, grapevine, willow, citrus and *Philodendron* sp. in California. Konorbis & Dobosz (1996) reported it from North Korea, and Chen & Liu (2002) from China. Three new populations of this species from California (Table 1) were characterised morphologically and molecularly in this study. A population from *Salix* sp. was collected close to the type locality.

MEASUREMENTS

See Table 3.

DESCRIPTION

Female

Body shape slightly arcuate ventrad. Sheath closely fitting. Lip region flattened anteriorly, slightly set off with two annuli with almost same diam., first annulus sometimes appearing to be slightly wider than second. However, this is not a consistent feature as is shown in Figure 6A. Stylet very long, slender frequently curved dor-
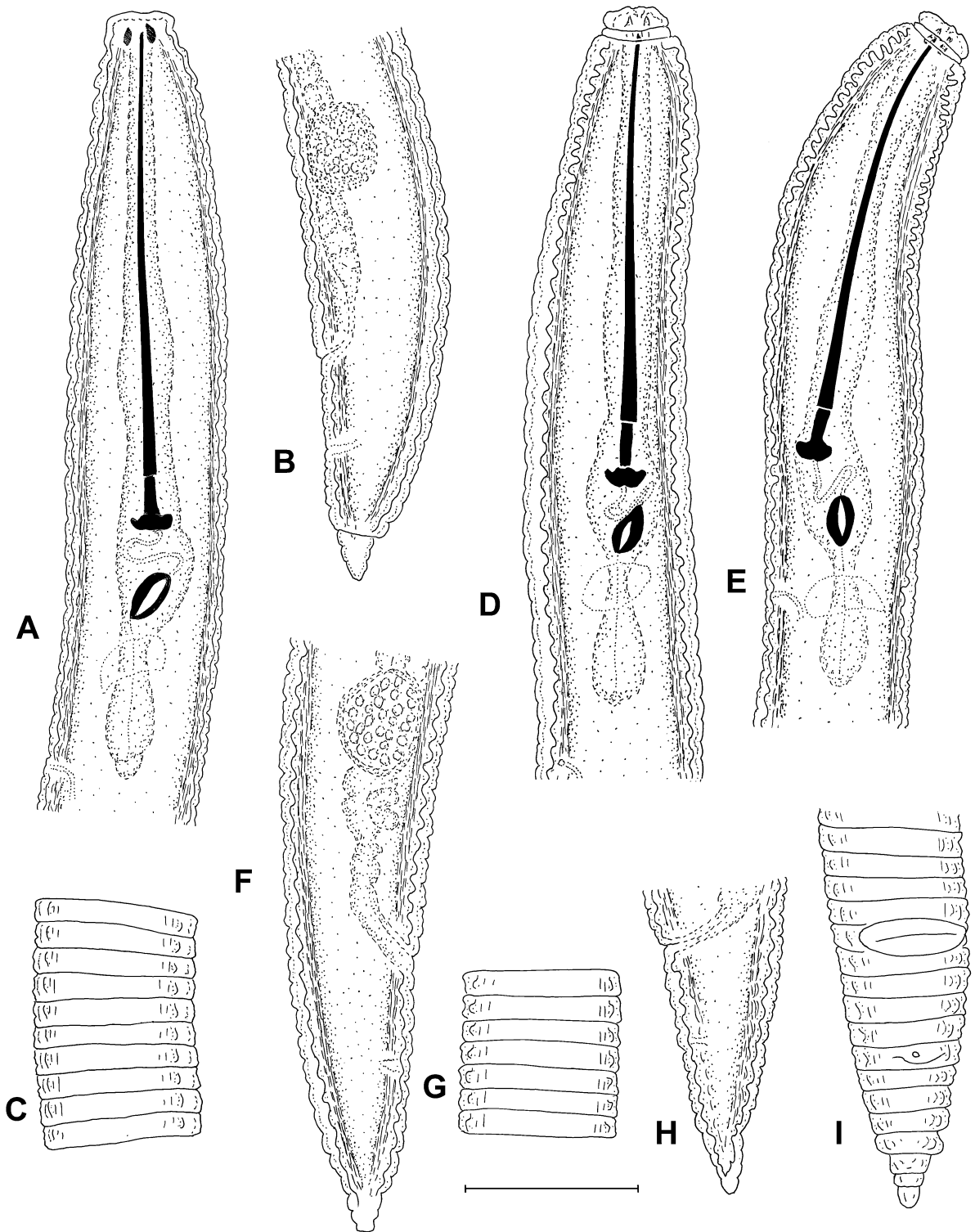
Fig. 5. *Hemicriconemoides californianus* female CA, USA (CD847). A: Anterior region; B: Posterior region; C: Annuli at mid-body. CD1021. D, E: Anterior region; F: Posterior region; G: Annuli at mid-body; H, I: Posterior regions. (Scale bar = 30 μm.)
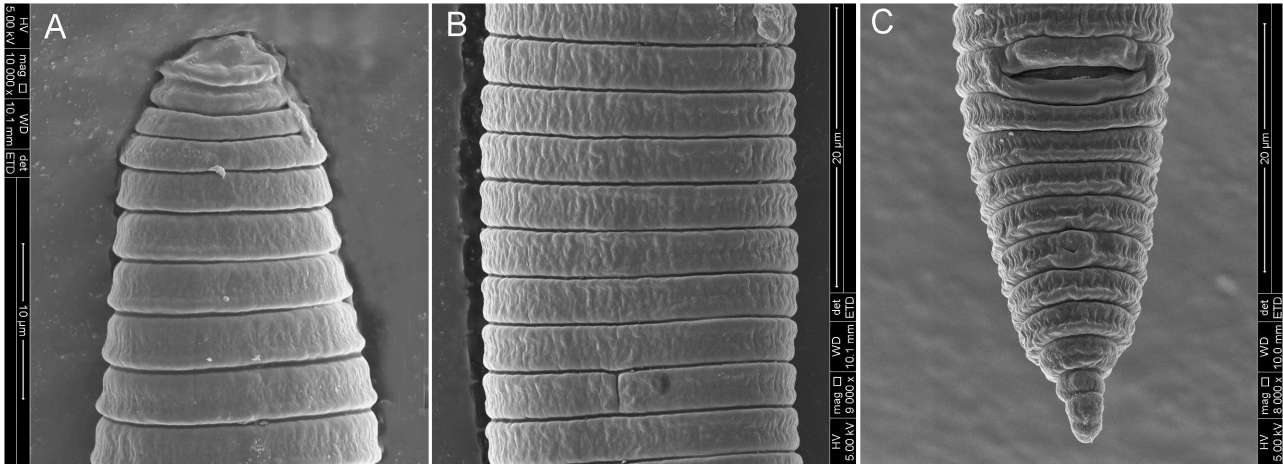
Fig. 6. *Hemicriconemoides californianus* female CA, USA (CD847). A: Anterior region; B: Annuli at mid-body; C: Posterior region, ventral view.

sad. Stylet knobs small, indented anteriorly and rounded posteriorly. Dorsal pharyngeal gland opening situated quite near to base of stylet. Excretory pore situated from five annuli anterior to nine annuli posterior to base of pharynx. Hemizonid and hemizonion not seen. Sheath annuli flattened to mostly indented over whole length of body except last two or three annuli on tail tip, no anastomosis present. Vulval flaps absent, vulval opening a distinct slit with slightly prominent lips. Vagina straight. Spermatheca not seen in some specimens but otherwise large, rounded to oblong and filled with rounded sperm cells. Tail tapering to a finely rounded tip; sheath appearing to end *ca* three annuli before tail tip giving the last three annuli a set off appearance. Anus situated 3-6 annuli posterior to vulva.

Male

Not found.

REMARKS

The Californian populations of this species fit well with *H. californianus* described by Pinochet & Raski (1975) and Ye & Robbins (2000), except for minor intraspecific differences (Table 3). Our observations indicated that the new Californian populations show variability in the diam. of the lip annuli, mean of stylet length (79.5-86 μm), and shape of the spermatheca. Their sheath annuli are ornamented by slight indentations, a feature missing in the original description. *Hemicriconemoides californianus* is close to *H. chitwoodi* and *H. gaddi*. These Californian populations differ from *H. chitwoodi* in having the first

and second lip annuli of the same diam. *vs* a first lip annulus larger than the second in *H. chitwoodi*, a slightly larger c ratio (13-29.7 *vs* 11-22) and a more broadly rounded tail terminus compared with a more slender, conoid tail with a finely rounded tip for *H. chitwoodi*. Pinochet & Raski (1975) reported shorter male spicules (22-25 μm) compared with 29.1 μm (Esser, 1960) and 27-31 μm (Dasgupta *et al.*, 1969) for *H. chitwoodi*. The *H. californianus* females reported from Taiwan (Chen *et al.*, 2007) seem to have a tail more similar to that of *H. chitwoodi*.

From *H. gaddi*, the present specimens differ in having a flattened lip region, with a set off first annulus of the same or larger diam. than that of second annulus *vs* rounded lip region with first annulus always smaller than second lip annulus, and a slightly larger c value (13-29.7 *vs* 11.2-20). The opening of the dorsal pharyngeal gland is further from the stylet base (6-9.3 *vs* 3-5 μm).

Hemicriconemoides chitwoodi **Esser, 1960**
(Figs 1E-H; 3F-H; 7; 8)

This species was described from the roots of *Camellia* sp., Monticello, FL, USA, by Esser (1960). Records from other localities in the USA include Lake Alfred, FL, California, New Jersey and North Carolina. Outside the USA it has been reported from China and Japan (Dasgupta *et al.*, 1969; Ye & Robbins, 2000). Topotype specimens were collected in Florida and characterised morphologically and molecularly in this study. Two Japanese populations (one of which was detected in a plant shipment in
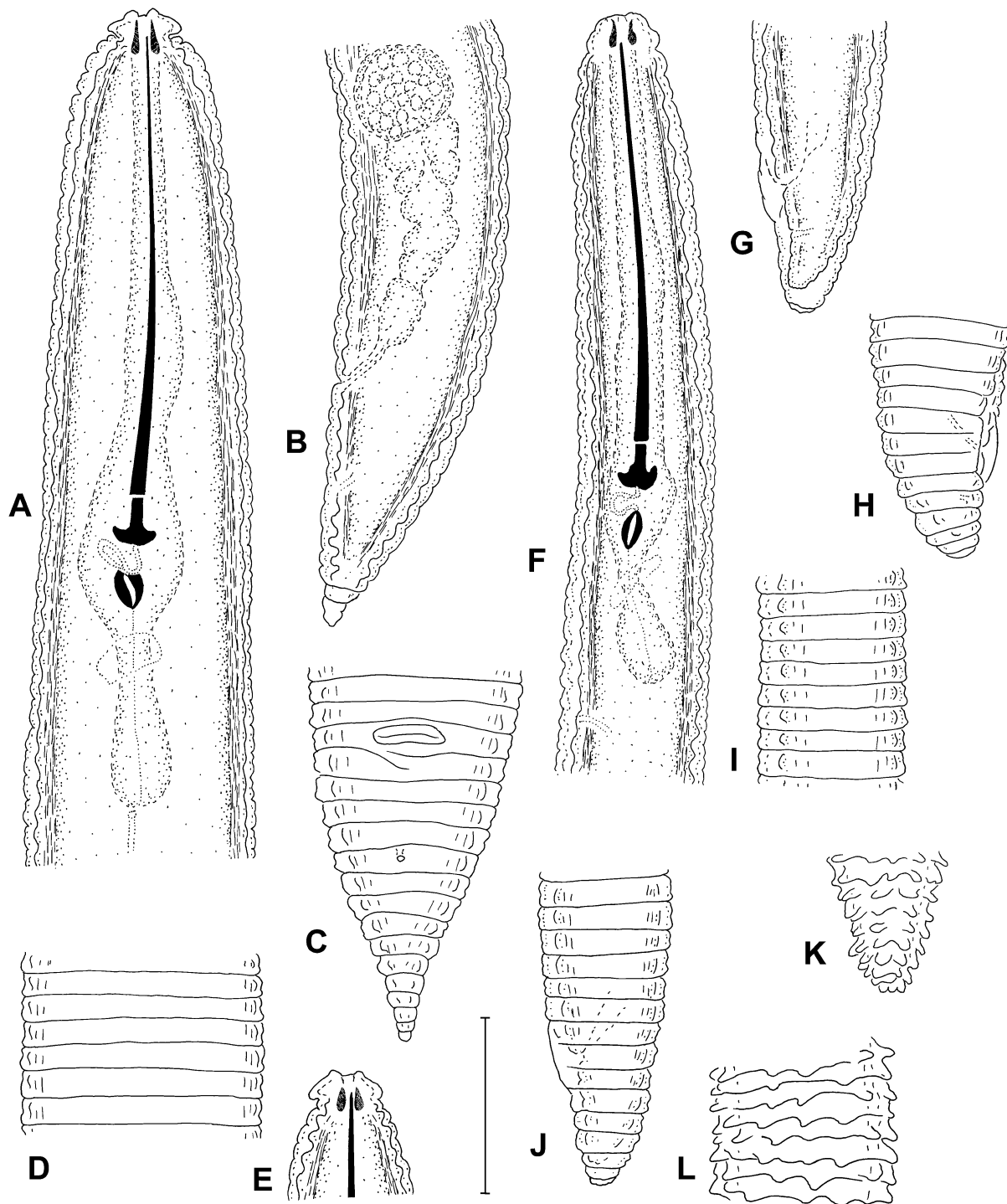
Fig. 7. *Hemicriconemoides chitwoodi* topotype female FL, USA (CD1185). A: Anterior region; B, C: Posterior regions; D: Annuli at mid-body; E: Lip region of another female. *Hemicriconemoides minutus* topotype female FL, USA (CD1181). F: Anterior region; G, H: Posterior regions; I: Annuli at mid-body; J: Tail of another female. J4 Juvenile (CD1181). K: Tail region; L: Annuli in posterior part of body. (Scale bar = 30 μm.)
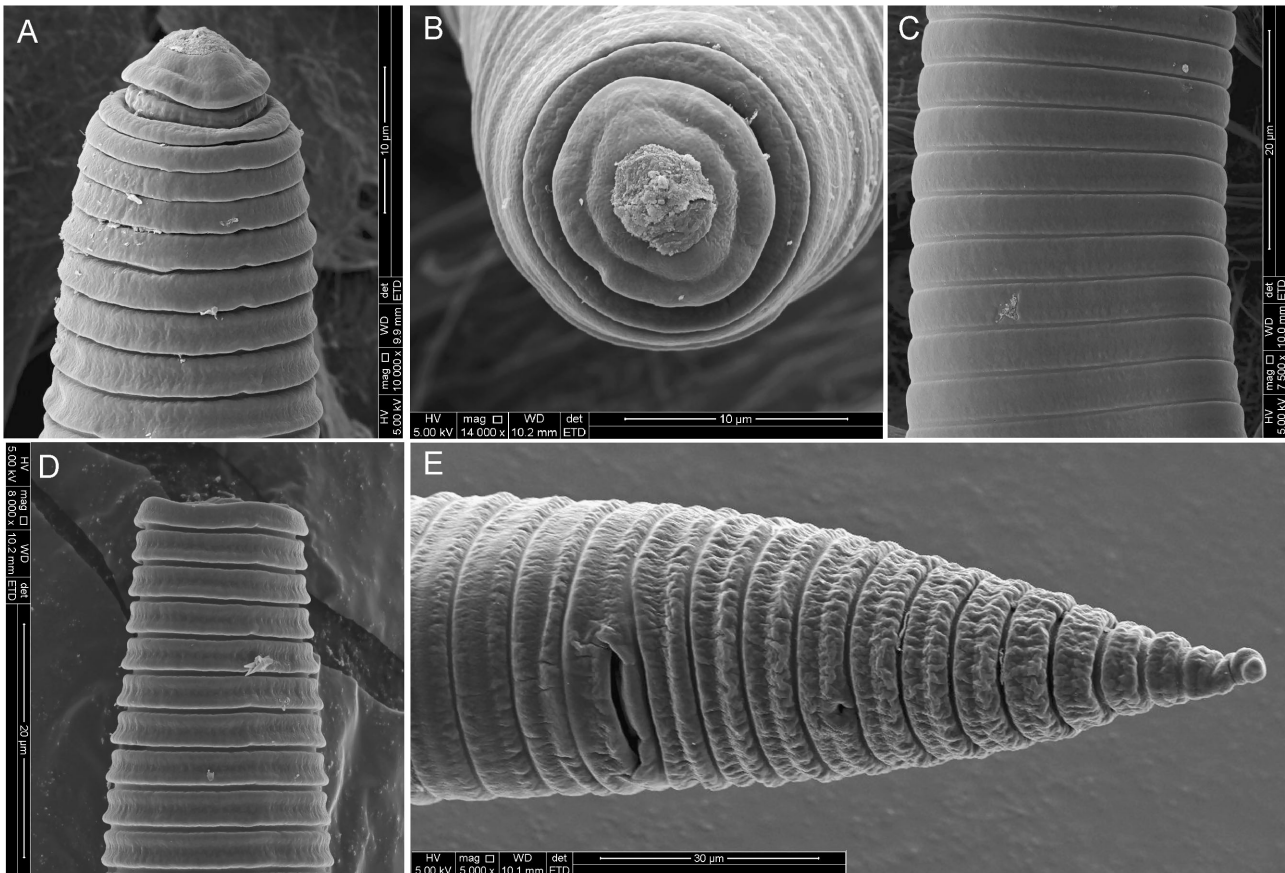
Fig. 8. *Hemicriconemoides chitwoodi* topotype female FL, USA (CD1185). A: Anterior region with lateral view of lip region; B: *En face* view of lip region; C: Annuli at mid-body; D: Anterior region with sheath pushed anteriorly over lip region; E: Posterior region, ventral view.

Italy) were also analysed morphologically and compared with the topotype population (Table 1).

MEASUREMENTS

See Table 3.

DESCRIPTION

Female

Body shape slightly arcuate ventrad. Sheath closely fitting except sometimes in lip region. Lip region angular, with two annuli, set off from body, first annulus of larger diam. than second, first annulus higher with two distinct rounded lobes on labial disc. In SEM photographs, labial disc appearing rounded, but morphological details obscured by soil particles attached to cuticle. Stylet long and very slender, frequently curved dorsad. Stylet knobs small, indented anteriorly and rounded posteriorly. Dorsal pharyngeal gland opening situated quite near to base of stylet. Excretory pore situated from six annuli anterior to three annuli posterior to base of pharynx. Hemizonid and hemizonion not seen. Sheath annuli flattened to mostly indented over whole length of body, no anastomoses present. Vulval flaps absent, vulval opening a distinct slit without prominently raised lips. Vagina straight. Spermatheca small to large, rounded to oblong and in a few specimens filled or half-filled with rounded sperm cells. Tail tapering to a narrow rounded tip. Anus situated from 3-4 annuli posterior to vulva.

Male

Not found.

REMARKS

The measurements of the Florida topotype population were more similar to those of the original description than those of the two Japanese populations of this species. The Japanese populations had smaller range values of stylet lengths than those of the topotype population and the original description (89 *vs* 96.5 μm). The mid-body diam. in the Floridian specimens was greater (33-42 μm) than that of the Japanese specimens (24-32 μm), but was similar to the 38-43 μm was reported by Ye & Robbins (2000). Other diam. values reported in the literature, such as 24-32 μm (Crozzoli & Lamberti, 2003), 26.5-36 μm (Decraemer & Geraert, 1992) and 30-41 μm (Van den Berg *et al.*, 1999) indicate that this character is very variable.

Chen *et al.* (2007) provided photos, measurements and descriptions of three populations of *Hemicriconemoides* which they identified as *H. californianus* from Taiwan. However, although the morphometric values reported by Chen *et al.* (2007) for this Taiwanese population of '*H. californianus*' overlap with both those of *H. californianus* and *H. chitwoodi*, the morphology of the female anterior body of specimens of this population, illustrated in Figure 3 in Chen *et al.* (2007), shows the lip region with the first lip annulus larger that the second, as found in *H. chitwoodi* and in contrast to that of *H. californianus*, which has both lip annuli with the same diam. or the first with a smaller diam. than the second. Furthermore, in this illustration the stylet is 92 μm long, the same as that of the topotype population of *H. chitwoodi* (86-95 μm) and much longer than that of *H. californianus* paratypes (74-86 μm). The shape of the tail in this figure also is more similar to that of *H. chitwoodi* than *H. californianus*. Our analysis of the ITS-rRNA gene sequence under accession number EU180057 from a sample that Chen *et al.* (2007) deposited in GenBank, showed that this sequence is very similar to those of the topotype and populations of *H. chitwoodi* in our study and clustered with them in the phylogenetic tree, confirming the results of our morphological observations. On the basis of these morphological and molecular findings, we concluded that sequences ascribed to *H. californianus* from Taiwan and nematode species under this name in the work by Chen *et al.* (2007) should be considered as representative of *H. chitwoodi*.

Hemicriconemoides macrodorus Vovlas, Troccoli & Castillo, 2000
(Figs 1I, J; 3I, J)

This species was described from southern Spain and is characterised by the truncate lip region, the very long stylet of 90-110 μm, the lack of lateral cuticular vulval flaps, and the conical tail, curved dorsally and with a rounded tip (Vovlas *et al.*, 2000). Two populations of this species were collected in Spain (Table 1) and characterised morphologically and molecularly in this study.

MEASUREMENTS

See Table 4.

DESCRIPTION

Female

Body shape slightly arcuate ventrad, almost cylindrical, narrowing slightly at anterior and post-vulval portion. Cuticle sheath fitting closely on anterior part of body, but well separated on tail. Lip region with two distinct annuli and cephalic framework strongly sclerotised. Stylet knobs with small anteriorly directed projections. Excretory pore situated slightly posterior to pharyngo-intestinal junction. Hemizonid and hemizonion not seen. Vulval flaps absent. Body narrowing posteriorly to vulva. Post-vulval body region 1.4 (1.1-1.7) of corresponding vulval body diam. Tail conoid, tapering uniformly, ending in a rounded tip. Anus situated 3-5 annuli posterior to vulva.

Male

Not found.

REMARKS

Since the morphology of the two new Spanish populations of *H. macrodorus* is almost identical to that published for this species in the original description, no morphological drawings of these new populations are provided here. Discovery of the two populations constitutes new records of this species for Spain. Minor morphometric differences of the two populations from those of the original description include a shorter body (522-661 and 569-678 *vs* 548-761 μm), slightly lower V ratio (90-94 and 93-96 *vs* 94-96), smaller annulus width (4.0-4.5 and 4.0-4.5 *vs* 4.5-6.2 μm), and longer tail (18-22 and 18-23 *vs* 13-22 μm). These differences may be a result of geographical intraspecific variability (Table 4).

Table 4. Morphometrics of females of *Hemicriconemoides macrodorus, H. minutus* and *H. ortonwilliamsi.* All measurements are in μm and in the form: mean ± s.d. (range).

Character	*H. macrodorus* Alcalá de los Gazules, Spain	*H. macrodorus* Tarifa, Spain	*H. minutus* Topotype, FL, USA (CD1181)	*H. ortonwilliamsi* Rociana, Spain (Rociana)	*H. ortonwilliamsi* S. Barrameda, Spain (J215)
n	6	6	19	12	7
L	580 ± 48 (522-661)	622 ± 46 (569-678)	404 ± 29.3 (357-445)	461 ± 18 (433-484)	468 ± 36 (431-540)
a	14.5 ± 1.5 (13.1-17.4)	17.6 ± 1.1 (16.3-18.8)	13.2 ± 2.0 (10.3-16.4)	14.7 ± 0.9 (13.5-16.3)	14.0 ± 0.5 (13.5-14.9)
b	4.1 ± 0.5 (3.6-5.0)	4.4 ± 0.3 (4.1-4.9)	3.5 ± 0.2 (3.0-3.8)	5.2 ± 0.3 (4.5-5.5)	5.5 ± 0.4 (5.0-6.1)
c	28.9 ± 3.0 (26-33.1)	32.2 ± 4.2 (24.7-37.4)	22.2 ± 2.5 (17.7-25.9)	17.4 ± 2.5 (15.2-22.4)	16.7 ± 1.7 (14.8-19.6)
c'	1.1 ± 0.03 (1.0-1.1)	0.9 ± 0.1 (0.9-1.1)	1.1 ± 0.1 (0.8-1.2)	1.5 ± 0.1 (1.4-1.6)	1.5 ± 0.1 (1.4-1.6)
o	4.8 ± 0.4 (4.5-5.5)	4.5 ± 0.7 (3.7-5.2)	6.2 ± 1.0 (4.6-7.6)	7.0 ± 0.9 (5.5-8.0)	7.0 ± 0.6 (6.4-8.0)
DGO	4.8 ± 0.5 (4.0-5.5)	4.7 ± 0.6 (4.0-5.5)	5 ± 0.8 (3.5-6.0)	3.6 ± 0.4 (3-4)	3.7 ± 0.3 (3.5-4.0)
V	92 ± 1.3 (90-94)	94.4 ± 1.2 (93-96)	94 ± 0.5 (93-95)	91.8 ± 0.9 (91-93)	91.1 ± 0.7 (90-92)
G_1	45.2 ± 11.6 (35.5-62.2)	38.6 ± 4.1 (35.4-44.1)	40 ± 5.2 (30-44)	44.4 ± 11.4 (29.7-59.5)	48.2 ± 11.0 (34.3-59.5)
Ovary length	266 ± 90.5 (192-411)	238 ± 12.1 (222-251)	161 ± 32.6 (110.5-195)	205 ± 53.8 (140-278)	225 ± 50.6 (153-275)
Stylet length	98 ± 6.6 (89.5-106)	103 ± 4.3 (98-109)	76 ± 3.2 (70.5-80.5)	52 ± 2.4 (49-55)	53 ± 2.9 (49-57)
Metenchium length	89.1 ± 6.2 (81-96.5)	89.9 ± 2.8 (86-93)	67.5 ± 3.2 (62-73)	43.6 ± 2.5 (41.0-46.5)	44.4 ± 3.6 (41-51)
Telenchium length	9.0 ± 1.0 (7.5-10)	13.1 ± 1.8 (11-16)	8.5 ± 0.7 (7.5-9.5)	8.3 ± 1.0 (6-9)	8.3 ± 1.0 (6-9)
m	90.8 ± 1.0 (89-91.9)	87.3 ± 1.3 (85.3-88.9)	88.9 ± 1.0 (86.8-89.9)	84.1 ± 2.1 (82-88.6)	84.2 ± 2.5 (82-89.5)
Stylet knob height	3.3 ± 0.3 (3.0-3.5)	3.3 ± 0.4 (3.0-3.5)	2.5 ± 0.3 (2-3)	3.5 ± 0.4 (3-4)	3.5 ± 0.4 (3-4)
Stylet knob width	8.4 ± 0.4 (8-9)	8.3 ± 0.3 (8.0-8.5)	6.5 ± 0.4 (6.0-7.5)	8.1 ± 0.4 (7.5-8.5)	8.1 ± 0.4 (7.5-8.5)
Excretory pore from anterior end	156 ± 14.1 (138-170)	152 ± 3.0 (149-156)	132 ± 9.2 (118.5-145.5)	121 ± 10.7 (102-131)	113 ± 12.6 (95-129)
Diam. at mid-body	40.1 ± 1.6 (38-42.5)	35.4 ± 0.5 (35-36)	30.5 ± 4.7 (24.5-37.5)	31.5 ± 1.5 (28-33)	33.3 ± 2.2 (32-38)
Diam. at anus	–	–	17 ± 1.8 (14-20)	–	–
Diam. at vulva	–	–	21.5 ± 2.4 (18.5-25.5)	–	–
Annulus width	4.1 ± 0.2 (4.0-4.5)	4.2 ± 0.3 (4.0-4.5)	5 ± 0.6 (3.5-6.0)	4.4 ± 0.2 (4.0-4.5)	4.5 ± 0.4 (4-5)
Tail length	20.2 ± 1.8 (18-22)	19.5 ± 1.9 (14-23)	18.5 ± 2.5 (14-25)	26.8 ± 3.2 (21-30)	28.1 ± 2.3 (25-31)
Pharynx length	142 ± 11.6 (128-161)	142 ± 4.8 (136-149)	114 ± 5.4 (105-122)	88 ± 8.1 (81-107)	85 ± 6.3 (75-94)
First lip diam.	9.3 ± 0.6 (8.5-10)	7.8 ± 0.3 (7.5-8.0)	13 ± 0.8 (12-14)	12.3 ± 0.6 (11.5-13)	12.2 ± 0.4 (11.5-12.5)
Second lip diam.	–	–	17.5 ± 0.7 (17-18.5)	–	–
First body annulus diam.	–	–	22 ± 1.4 (20-24)	–	–
Second body annulus diam.	–	–	25 ± 1.7 (23.5-28)	–	–
Third body annulus diam.	–	–	26 ± 1.9 (24.5-29.5)	–	–
R	142 ± 7.4 (131-151)	143 ± 4.5 (133-150)	92 ± 2.7 (87-97)	105 ± 4.0 (99-110)	105 ± 4.6 (98-111)
RSt	25.0 ± 2.8 (20-28)	24.3 ± 2.4 (21-27)	17 ± 1.3 (15-19)	12.1 ± 1.0 (11-14)	12.3 ± 1.0 (11-14)
ROes	34.2 ± 2.6 (30-38)	31.8 ± 2.2 (29-35)	25 ± 1.9 (21-29)	19.8 ± 1.8 (17-22)	20.1 ± 1.7 (17-22)
Rex	37.3 ± 2.7 (32-39)	36.7 ± 1.9 (35-39)	29 ± 2.3 (25-35)	25.8 ± 2.1 (23-28)	26.0 ± 2.2 (22-28)
RV	13.8 ± 0.8 (13-15)	13.2 ± 1.0 (12-14)	8 ± 0.7 (7-9)	10.6 ± 1.1 (9-12)	10.7 ± 1.0 (9-12)
RVan	3.8 ± 0.4 (3-4)	4.0 ± 0.9 (3-5)	0	1.3 ± 0.5 (1-2)	1.3 ± 0.5 (1-2)
Ran	10.0 ± 0.6 (9-11)	9.2 ± 1.2 (8-11)	7 ± 0.7 (6-8)	9.4 ± 1.5 (7-11)	9.4 ± 1.1 (7-10)
VL/VB	1.5 ± 0.2 (1.3-1.7)	1.3 ± 0.2 (1.1-1.5)	1.1 ± 0.1 (1.0-1.4)	1.5 ± 0.1 (1.4-1.6)	1.6 ± 0.1 (1.4-1.7)
St%L	17.0 ± 1.9 (13.5-19)	16.6 ± 0.7 (15.8-17.5)	19.1 ± 1.7 (17.1-22)	11.2 ± 0.4 (10.7-11.7)	11.3 ± 0.4 (10.6-11.7)
Spermatheca length	–	–	12.5, 19 (n = 2)	–	–
Spermatheca diam.	–	–	11, 17.5 (n = 2)	–	–

Hemicriconemoides minutus Esser, 1960
(Figs 1K, L; 3K, L; 9)

This species was described from roots of *Carya glabra* var. *megacarpa* Sarg. from Gainesville, FL, USA (Esser, 1960). The *H. minutus* from Florida is a component of the nematofauna of mixed hardwood forests in north and central Florida where many trees such as hickory, oaks, sweet gum and other plants coexist. Topotype specimens and a new population of this species were collected in Florida and characterised morphologically and molecularly (Table 1).

MEASUREMENTS

See Table 4.

DESCRIPTION

Female

Body shape slightly curved ventrad. Sheath closely fitting to body except posteriorly to vulva. Occasionally sheath protruding anteriorly over lip region or sheath slightly pushed anteriad so that first body annulus is slightly wider than second lip annulus and appearing almost as a third lip annulus. Lip region rounded, not set off with two annuli, first smaller than second. SEM photographs show labial disc to be slightly rectangular, not raised much above first lip annulus with four small rounded lobes around mouth opening. Stylet long, slender, frequently curved slightly dorsad. Stylet knobs distinctly anchor-shaped and rounded posteriorly. Dorsal pharyngeal gland opening situated near to base of stylet knobs. Excretory pore situated 2-6 annuli posterior to base of
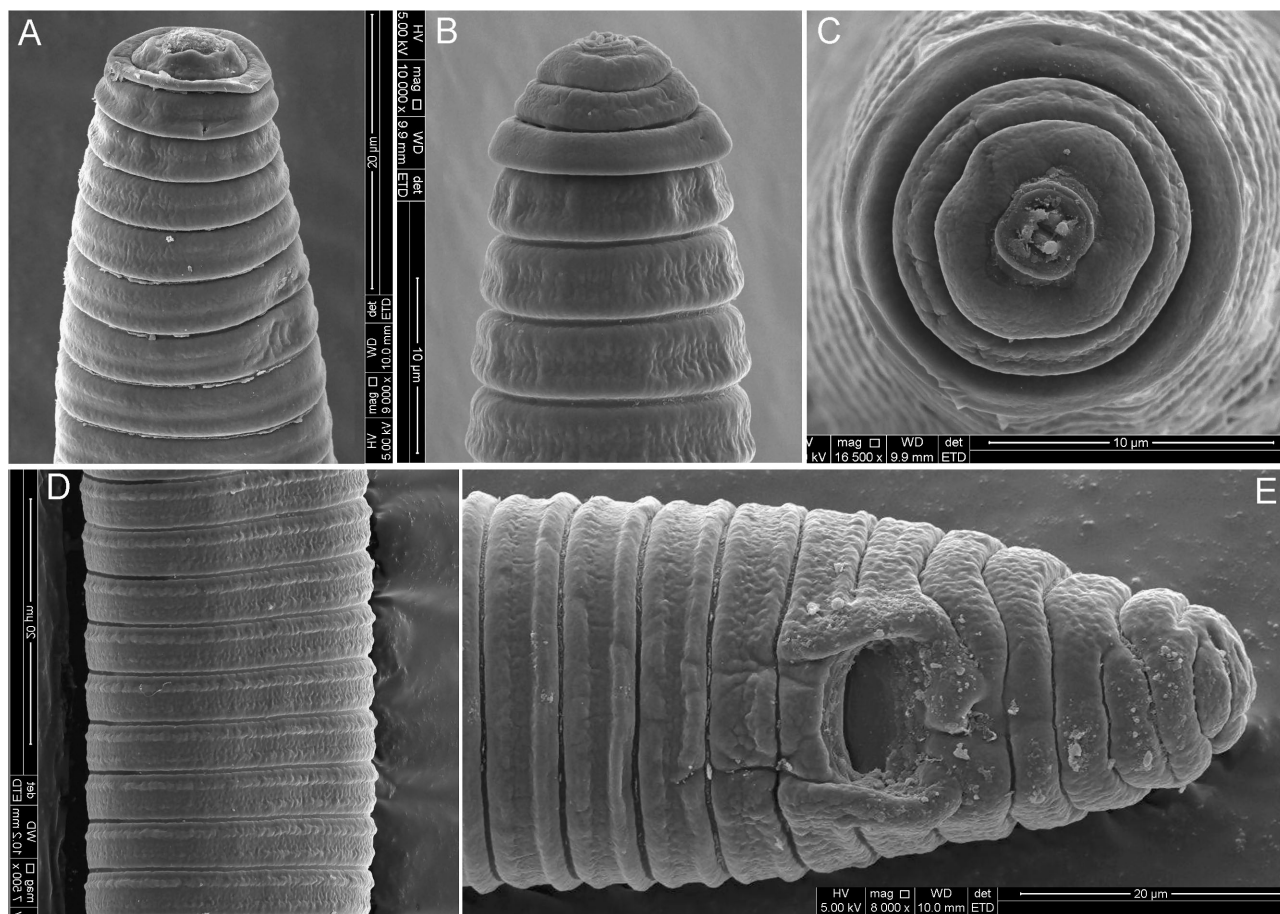

Fig. 9. *Hemicriconemoides minutus* topotype female FL, USA (CD1181). A, B: Anterior region; C: *En face* view of lip region; D: Annuli at mid-body; E: Posterior region with vulva and two small vulval flaps laterally with rounded tail.

pharynx. Hemizonid seen in one specimen only, one annulus long, situated opposite to excretory pore. Hemizonion not seen. Sheath annuli smooth, mostly flattened or slightly indented over whole length of body except sometimes on last few annuli where more rounded, no anastomoses present. Vulval flaps small, 2-3 annuli long with smooth or slightly crenate margins. Vulva a distinct slit. Vagina straight. Spermatheca poorly visible, but in a few specimens appearing small, round and empty, or with a few rounded sperm cells. Body tapering slightly posterior to vulva to a rounded tail tip. Anus situated on first annulus posterior to vulva.

Male

Not found.

Juvenile

Posterior end of one female enclosed in a fourth-stage juvenile cuticle. Margins of posterior annuli with *ca* 12 rounded lobes apparently not forming longitudinal rows.

REMARKS

The present specimens compare very well with those described by Esser (1960). Esser (1960) noted that the one stylet knob appeared to protrude more strongly anterior than the others. This was also seen in the present specimens. Although no males were found in the original specimens, Esser (1960) noted that the form of the spermatheca and sperm cells suggested evidence for the presence of males. In one of studied females there appeared to be a few sperm cells in the spermatheca.

Hemicriconemoides ortonwilliamsi **Ye & Siddiqi, 1994**
(Figs 1M-O; 3M-O)

This species was described from the Samoa Islands (Ye & Siddiqi, 1994) and is characterised by an elongated postvulval body part and a conical tail with a smooth rounded terminus. The morphological and molecular features of two populations of this species, found in Spain (Table 1), are reported in this study.

MEASUREMENTS

See Table 4.

DESCRIPTION

Female

Body shape slightly arcuate ventrad, narrowing at post-vulval portion. Cuticle sheath fitting closely on whole body. Lip region with two annuli. Stylet knobs distinctly anchor-shaped, rounded posteriorly. Excretory pore situated 5-9 annuli posterior to pharyngo-intestinal junction. Hemizonid and hemizonion not seen. Vulval flaps present. Spermatheca oval. Body narrowing posteriorly to vulva. Tail conoid, with annuli clearly separated from each other. Anus situated 1-2 annuli posterior to vulva.

Male

Not found.

REMARKS

The two Spanish populations were recovered from sandy soil collected in southern Spain. The morphology of both populations agrees well with that of the original type material from the Samoa Islands (Ye & Siddiqi, 1994), although small differences can be detected in greater number of body annuli (98-111 *vs* 86-98), RSt (11-14 *vs* 15-19), and a slightly smaller stylet (49-57 *vs* 52-64 μm). These new records of this species from Spain expand the geographical distribution of this species in the Mediterranean region, since a population of this species was reported previously in Italy (Vovlas *et al.*, 2000). Measurements of the present specimens compare well with the paratypes of Ye & Siddiqi (1994) and the specimens reported by Vovlas *et al.* (2000) (Table 4).

Hemicriconemoides promissus **Vovlas, 1980**
(Figs 2A, B; 4A, B)

Germani & Anderson (1991), based on light microscope observations of female paratypes, studied the morphology of *H. promissus* and *H. intermedius* Dasgupta, Raski & Van Gundy, 1969. They proposed both species as junior synonyms of *H. brachyurus*. However, SEM studies by Vovlas *et al.* (2006) from Italian paratype material and female specimens of a population from Spain showed that both *H. promissus* populations lacked lateral vulval flaps, which are present in *H. brachyurus*, justifying the rejection of the synonymy proposed by Germani & Anderson (1991), and the re-establishment of *H. promissus* as a species distinct from *H. brachyurus*. The morphological

and molecular data of two additional populations found in Spain (Table 1) are reported in this study.

MEASUREMENTS

See Table 5.

DESCRIPTION

Female

Body shape slightly curved ventrally, cylindrical. Lip region truncate. Cuticle sheath closely fitting body from anterior end to vulva. Body annuli without ornamentations or anastomoses. Stylet short and robust, with anchor-shaped knobs. Excretory pore situated 2-7 annuli posterior to pharyngo-intestinal junction. Hemizonid and hemizonion not seen. Vulval flaps absent. Post-vulval body region 0.9-1.1 vulval body diam. Tail conoid-rounded, ending with a small rounded lobe.

Male

Not found.

REMARKS

The two Spanish populations were recovered from sandy soil collected in southern and north-western Spain. Both populations were characterised by a short and robust stylet with anchor-shaped knobs, absence of a vulval flap, and tail ending in a small rounded lobe. The morphology and morphometric data agree very well with the original type description of specimens from halophytic plants in southern Italy (Vovlas, 1980). Some morphometric differences were detected (Table 5), including variable Ran values, 1-2 and 5-9 for the new Spanish populations *vs* 6-7 reported in the original description, which confirms some intraspecific variation of this character depending on the geographical origin of the population. Other minor differences included a smaller c value (20-28.6 *vs* 27-35), slightly higher RSt value (8-14 *vs* 8-9), and slightly higher RV value (6-10 *vs* 7-8).

Hemicriconemoides silvaticus Eroshenko & Volkova, 1986 (Figs 2C; 4C, D)

This species was originally described from *Pinus* species in the Amur district, Russian Far East (Eroshenko & Volkova, 1986). A population of this species was found in Japan (Table 1) and it is described morphologically and molecularly in this study.

MEASUREMENTS

See Table 5.

DESCRIPTION

Female

Body shape slightly arcuate ventrad. Sheath closely fitting. Lip region flattened anteriorly, rounded. Oral disc not prominent. Stylet long and thin, slightly curved, knobs anchor-shaped. Excretory pore situated from 4-6 annuli posterior to base of pharynx. Hemizonid and hemizonion not seen. Vulva without vulval flaps. Spermatheca undifferentiated. Postvulval body part elongated. Tail conical with an annulated obtusely rounded terminus. Anus situated 3-5 annuli posterior to vulva.

Male

Not found.

REMARKS

The morphometric data of the specimens (Table 5) from Japan fit well those of the original description (Eroshenko & Volkova, 1986). This is the first report of this species outside the type locality of Eastern Russia. The morphological and molecular results obtained in the present study also confirmed the close relationship of *H. silvaticus* with *H. gaddi*.

Hemicriconemoides strictathecatus Esser, 1960 (Figs 2D-H; 4D-H; 10; 11)

Esser (1960) described this species from specimens collected from coconut, *Cocos nucifera* L., in Florida, USA. In our study, we characterised morphologically and molecularly three *H. strictathecatus* populations from China (intercepted in Italy), South Africa and Venezuela (Table 1).

MEASUREMENTS

See Table 5.

Table 5. Morphometrics of *Hemicriconemoides promissus*, *H. silvaticus* and *H. strictathecatus*. All measurements are in μm and in the form: mean ± s.d. (range).

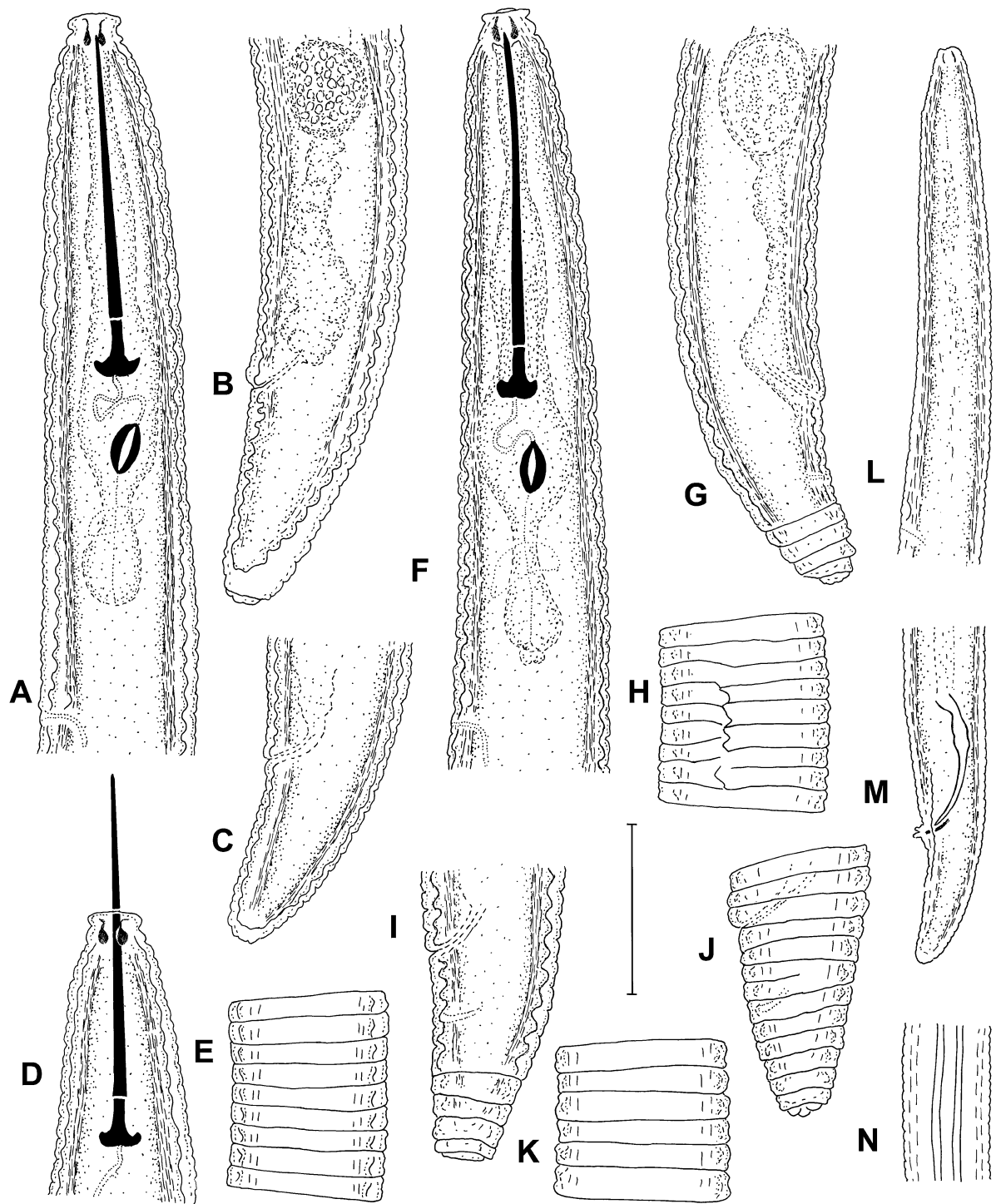| Character	*H. promissus* Bolonia, Spain	*H. promissus* Monteagudo Island, Spain (Monteagudo)	*H. silvaticus* Aoshima, Japan (O97)	*H. strictathecatus* China (intercepted in Italy) (Ruvo)	*H. strictathecatus* South Africa (Tvl1948)	*H. strictathecatus* South Africa (N826)	*H. strictathecatus* South Africa (N826)
n	16 females	10 females	10 females	12 females	7 females	15 females	2 males
L	491 ± 51 (438-560)	447 ± 43 (400-527)	438 ± 56 (351-518)	460 ± 31 (429-517)	504 ± 44.9 (429-563)	520 ± 37.5 (475-607)	392, 379
a	12.2 ± 0.8 (11.2-13)	13.9 ± 1.2 (11.8-15.8)	14.4 ± 0.6 (13.9-15.2)	16.4 ± 0.8 (14.8-17.3)	17.6 ± 1.2 (15.5-19.2)	16.4 ± 1.8 (12.8-19.1)	26.7, 24.6
b	4.6 ± 0.5 (4.1-5.3)	4.6 ± 0.5 (4.0-5.4)	4.2 ± 0.6 (3.6-5.1)	4.5 ± 0.4 (3.9-5.1)	4.7 ± 0.5 (3.8-5.4)	4.5 ± 0.3 (4.2-5.3)	–
c	22.8 ± 2.1 (20.8-25.4)	25.7 ± 3.0 (20.0-28.6)	11.9 ± 1.0 (10.2-12.7)	22.9 ± 2.3 (20.4-28.3)	19.9 ± 3.1 (14.6-23.4)	23 ± 2.9 (17.2-27.1)	14, 15.6
c'	0.99 ± 0.04 (0.95-1.05)	0.8 ± 0.1 (0.7-1.0)	1.8 ± 0.1 (1.8-1.9)	1.2 ± 0.1 (1.1-1.3)	–	–	2.8, 2.2
o	9.1 ± 0.9 (8.3-10.4)	8.3 ± 0.8 (7.3-9.2)	8.3 ± 0.5 (7.5-8.9)	8.6 ± 0.9 (7.7-9.4)	9.5 ± 1.2 (8.0-11.8)	9.4 ± 1.5 (7.6-12.6)	–
DGO	4.4 ± 0.5 (4-5)	4.4 ± 0.4 (4-5)	6.4 ± 0.5 (6-7)	5.5 ± 0.6 (5-6)	6 ± 0.7 (5.0-7.5)	6.5 ± 0.9 (5-8)	–
V	95.0 ± 1.0 (94-96)	93.2 ± 0.8 (92-94)	88.4 ± 0.5 (88-89.5)	91.8 ± 0.8 (91-93)	92.5 ± 0.9 (90.5-93)	93 ± 0.6 (91.5-94)	–
G$_1$	55.8 ± 1.4 (54.5-57.9)	50.8 ± 5.9 (40.5-58.2)	40.2 ± 6.3 (33-45)	36.8 ± 6.0 (28.2-42.1)	39.5 ± 4.0 (31.5-43.5)	40 ± 4.8 (31.5-45.5)	–
Ovary length	274 ± 35.5 (242-324)	225 ± 35.5 (162-260)	197 ± 53.9 (116-254)	167 ± 16.1 (146-185)	198 ± 23.1 (166-222)	203 ± 26.8 (156.5-248)	–
Stylet length	48.0 ± 0.8 (47-49)	52.9 ± 1.4 (51-55)	80.7 ± 4.4 (72-86)	67.3 ± 2.8 (64-72)	65 ± 2.0 (62.5-68)	67 ± 1.7 (64.5-69.5)	14.5, 15.6
Metenchium length	37.3 ± 1.0 (36-38)	44.7 ± 1.8 (43-47)	70.9 ± 4.1 (64-77)	56.8 ± 2.2 (53-60)	56 ± 1.9 (53-58)	58.5 ± 1.5 (55-60.5)	–
Telenchium length	10.7 ± 1.3 (9-12)	8.6 ± 0.9 (8-10)	9.5 ± 2.1 (6.5-11)	10.5 ± 1.6 (7-13)	9 ± 0.5 (8.5-10)	9 ± 0.7 (7.5-10.5)	2.8, 2.2
m	77.6 ± 2.4 (75-80.8)	83.9 ± 1.6 (81.7-85.5)	87.8 ± 2.5 (85.3-91)	84.4 ± 1.2 (81.9-89.6)	86.1 ± 0.9 (84.6-86.9)	86.2 ± 0.8 (85-87.5)	–
Stylet knob height	–	2.9 ± 0.3 (2.5-3.0)	2.3 ± 0.3 (2-3)	2.5	3 ± 0.3 (3.0-3.5)	3 ± 0.4 (2.0-3.5)	–
Stylet knob width	8.5 ± 0.6 (8-9)	9.3 ± 0.3 (9.0-9.5)	6.6 ± 0.4 (6-7)	6.8 ± 0.3 (6.5-7.0)	6.5 ± 0.3 (6.0-7.5)	7 ± 0.8 (5.5-8.5)	–
Excretory pore from anterior end	130 ± 10.0 (124-145)	98 ± 6.1 (90-104)	118 ± 6.0 (112-124)	126 ± 10.6 (109-135)	128 ± 7.9 (118-137.5)	126 ± 7.8 (116-142)	85, 86
Diam. at mid-body	40.3 ± 1.9 (39-43)	32.2 ± 2.4 (28-35)	28.9 ± 2.2 (25-32)	28.0 ± 1.8 (26-30.5)	28.5 ± 1.0 (27-29.5)	32 ± 3.5 (27-37)	14.5, 15.5
Annulus width	5.5 ± 0.6 (5-6)	4.3 ± 0.3 (4.0-4.5)	3.6 ± 0.4 (3-4)	3.7 ± 0.3 (3.5-4.0)	4 ± 0.6 (3.5-5.0)	4.5 ± 0.4 (4-5)	1.5
Tail length	21.5 ± 0.6 (21-22)	17.6 ± 2.2 (14-21)	35.3 ± 4.3 (28-43)	20.3 ± 2.9 (16-25)	26 ± 3.7 (20.5-30)	23 ± 3.0 (18.5-28.5)	28, 24.5
Pharynx length	106 ± 2.6 (104-110)	97 ± 6.7 (89-102)	108 ± 12.6 (86-121)	103 ± 4.2 (99-111)	107 ± 3.2 (104.5-112)	114 ± 3.0 (109.5-122.5)	–
First lip diam.	–	–	9.6 ± 0.6 (9.0-10.5)	–	9 ± 0.7 (7.5-9.5)	9 ± 0.6 (8.5-10.5)	–
Second lip diam.	–	–	–	–	10 ± 0.8 (9.0-11)	10.5 ± 0.4 (10-10.5)	–
First body annulus diam.	–	–	–	–	13 ± 0.9 (12-14)	13.5 ± 0.8 (12-15.5)	–
Second body annulus diam.	–	–	–	–	15 ± 1.2 (13.5-16)	15 ± 0.8 (14.5-17.5)	–
Lip region diam.	–	12.5 ± 0.5 (12-13)	9.6 ± 0.6 (9.0-10.5)	–	–	–	–
R	95.5 ± 4.1 (92-100)	105 ± 5.2 (97-117)	118.1 ± 10.0 (106-138)	132 ± 5.9 (123-141)	129 ± 5.1 (122-136)	125 ± 5.1 (112-131)	–
RSt	8.8 ± 0.9 (8-10)	12.2 ± 0.9 (11-14)	31.5 ± 2.1 (29-36)	18.8 ± 1.5 (17-21)	20 ± 1.0 (19-21)	20 ± 1.2 (18-22)	–
ROes	15.8 ± 1.0 (15-17)	20.9 ± 1.8 (19-25)	31.5 ± 2.1 (29-36)	27.3 ± 2.2 (25-30)	30 ± 2.4 (26-33)	31 ± 1.9 (28-35)	–
Rex	24.5 ± 1.3 (23-26)	22.3 ± 1.9 (20-26)	36.0 ± 3.5 (33-42)	34.5 ± 1.0 (33-36)	34.5 ± 2.0 (31-37)	34 ± 2.1 (31-38)	–
Rhem	–	–	–	–	33 ± 2.0 (30-35)	33 ± 1.9 (31-36)	–
RV	7.3 ± 0.5 (7-8)	7.3 ± 1.1 (6-10)	15.1 ± 1.0 (13-16)	11.5 ± 1.0 (10-13)	11 ± 0.9 (10-12)	11 ± 1 (9-12)	–
RVan	1.0	1.0	3.9 ± 0.6 (3-5)	3.7 ± 0.5 (3-4)	2.5 ± 1.0 (1-4)	3 ± 0.7 (1-4)	–
Ran	1.3 ± 0.5 (1-2)	6.3 ± 1.1 (5-9)	11.0 ± 1.3 (9-13)	7.8 ± 1.0 (6-9)	7.5 ± 1.1 (6-9)	6.5 ± 0.7 (6-8)	–
VL/VB	0.9 ± 0.05 (0.9-1.0)	1.1 ± 0.1 (0.9-1.2)	2.2 ± 0.2 (1.8-2.5)	1.5 ± 0.1 (1.4-1.6)	1.7 ± 0.2 (1.5-1.9)	1.5 ± 0.2 (1.3-1.8)	–
St%L	9.8 ± 0.8 (8.8-10.7)	11.9 ± 1.1 (10.4-13.6)	19.2 ± 1.0 (18.2-20.5)	14.6 ± 1.2 (12.6-15.9)	13 ± 1.0 (12.1-15)	12.9 ± 0.9 (11.5-14.1)	–
Lip region diam.	–	–	–	–	–	–	6.5, 6
Lip region height	–	–	–	–	–	–	3.5, 4
Lateral field width	–	–	–	–	–	–	5
Spiculum length	–	–	–	–	–	–	24, 25
Gubernaculum length	–	–	–	–	–	–	6

Fig. 10. *Hemicriconemoides strictathecatus* female South Africa (Tvl1948). A: Anterior region; B, C: Posterior regions; D: Lip region of another female; E: Annuli at mid-body. Female (N826). F: Anterior region; G: Posterior region; H: Anastomosing annuli at mid-body; I, J: Tail regions; K: Annuli at mid-body. Male (N826). L: Anterior region; M: Tail region; N: Lateral field at mid-body. (Scale bar = 30 μm.)
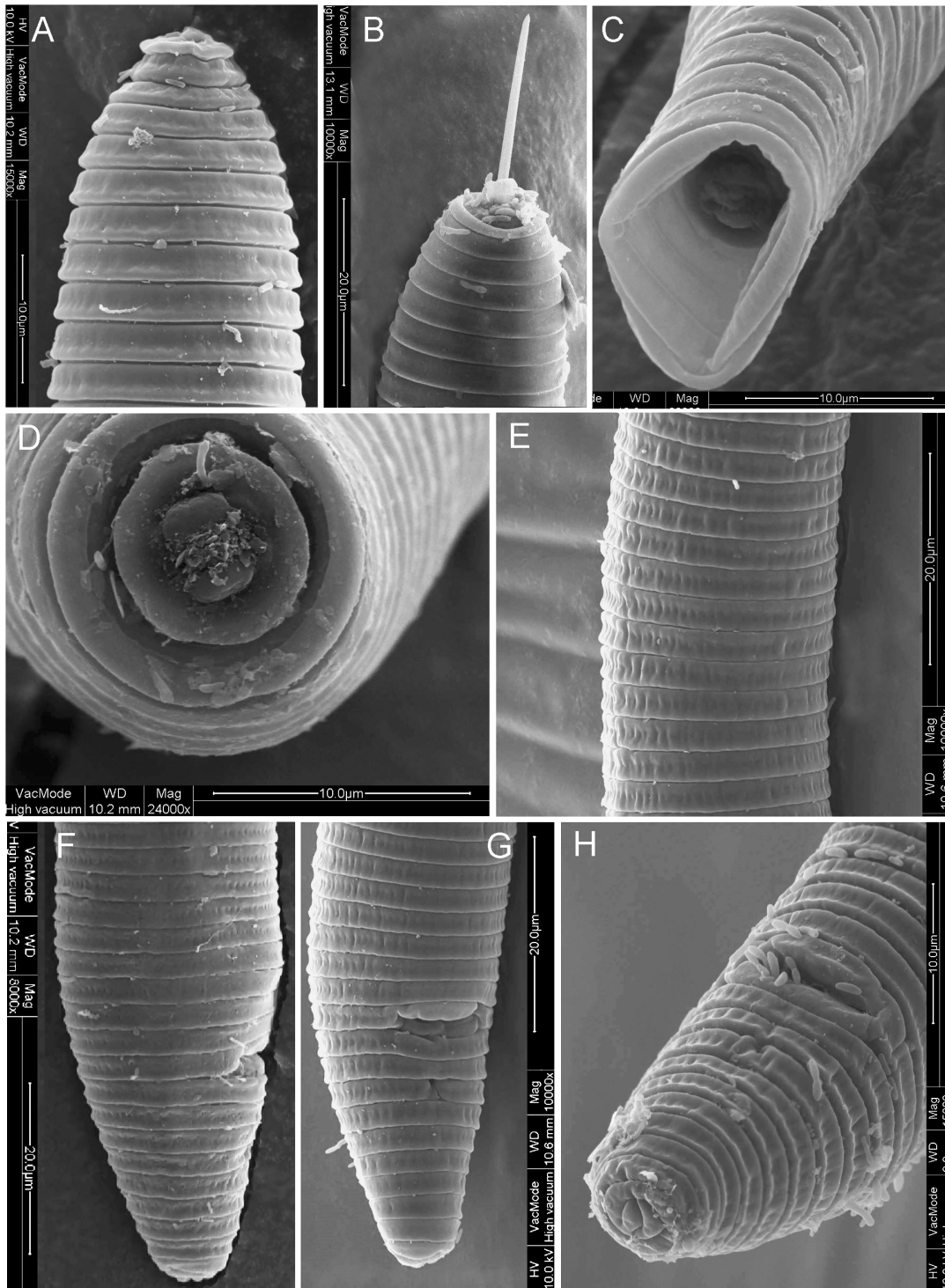
Fig. 11. *Hemicriconemoides strictathecatus* female South Africa (N826). A: Anterior region with lateral view of lip region; B: Another view of lip region; C: Lip region with sheath pushed forward; D: *En face* view of lip region; E: Annuli at mid-body; F-H: Various tail regions.

DESCRIPTION

Female

Body shape slightly arcuate ventrad. Sheath mostly closely fitting except on tail, sheath rarely protruding anteriorly over lip region. Lip region with two annuli, not set off, first lip annulus protruding outward while second annulus sloping more posteriad, first lip annulus mostly with smaller diam. than second. SEM photographs showing labial disc to be more rectangular, slightly set off from lip annulus; mouth opening not clear due to dirt covering opening. Stylet long and slender, frequently slightly curved dorsad. Stylet knobs indented anteriorly, rounded posteriorly. Dorsal pharyngeal gland opening situated near to base of stylet knobs. Excretory pore situated from 1-8 annuli posterior to base of pharynx. Hemizonid one annulus long situated from opposite to three annuli anterior to excretory pore. Hemizonion not seen. Sheath annuli flattened to mostly indented over whole length of body, smooth with a few anastomoses seen in N826 population. Vulval slit without prominent lips. Vagina straight. Spermatheca large, round to oblong, filled with rounded sperm cells. Body tapering slightly behind vulva to a mostly bluntly rounded tip. Anus situated 2-4 annuli posterior to vulva.

Male

Body slightly arcuate ventrally. Lip region rounded with slight indent anteriorly, not distinctly set off, annuli not distinct. Stylet absent and pharynx reduced. Lateral field with four distinct lines. Excretory pore distinct. Hemizonid two annuli long, situated directly anterior or one annulus anterior to excretory pore. Hemizonion seen in one specimen, one annulus long, situated eight annuli posterior to hemizonid. Body tapering slightly posterior to cloaca, ending in a slightly narrower, rounded tail tip. Cloacal lips slightly elongated with posterior lip slightly longer.

REMARKS

The morphological features of the populations from China (intercepted in Italy), South Africa and Venezuela agree well with those of the original and other descriptions of *H. strictathecatus* (Siddiqi, 1961; Edward & Misra, 1964; Edward *et al.*, 1965; Dasgupta *et al.*, 1969; Germani & Luc, 1970; Choi & Geraert, 1975; Decraemer & Geraert, 1992; Van den Berg & Quénéhervé, 1999; Crozzoli & Lamberti, 2003).

When the ITS-rRNA gene sequences of *H. strictathecatus* populations from China, South Africa and Venezuela were analysed with those of *H. litchi* reported by Chen *et al.* (2011), all of these sequences clustered together in the phylogenetic tree indicating that these populations were conspecific. As a consequence of these findings the two ITS-rRNA gene sequences under accession numbers GQ354786 and GQ354787 identified as *H. litchi* in Chen *et al.* (2011) should be considered as representatives of *H. strictathecatus*. The comparison of the measurements of *H. strictathecatus* populations from Taiwan and South Africa with those reported by Chen *et al.* (2011) for populations of *H. litchi* from Taiwan also indicated similarity among many measurements. Furthermore, the morphology of the Taiwanese females of putative *H. litchi*, illustrated in Figure 3 in Chen *et al.* (2011), shows a close similarity with that of the South African population of *H. strictathecatus*. The two populations share a tapered tail with a bluntly rounded terminus, a stylet of the same length and the first lip annulus with a slightly smaller diam. than the second. These morphological similarities support the conspecificity of the two populations as suggested by the results of the molecular analysis. The fact that the stylet knobs of the South African populations of *H. strictathecatus* are anchor-shaped indicates that rounded stylet knobs, which are considered to be a character of diagnostic value for the identification of *H. strictathecatus*, are not a reliable diagnostic feature since populations with both rounded and anchor-shaped knobs occur in this species. These findings also complicate the morphological separation of *H. strictathecatus* from the closely related species *H. mangiferae*, since these two species differ mainly by the shape of stylet knobs, which are supposed to be round in *H. strictathecatus* and anchor-shaped in *H. mangiferae*. The work conducted by Chen *et al.* (2011) and by us indicates that the populations of *H. strictathecatus* used in these studies are very close morphologically and molecularly to a putative Taiwanese population of *H. mangiferae* (Chen *et al.*, 2011). Since no topotype specimens of *H. mangiferae* are available for molecular studies, we accept this Taiwanese population as a representative of *H. mangiferae*. However, the morphological separation of this Taiwanese *H. mangiferae* from *H. strictathecatus* is not reliable without the support of the molecular analysis because is based on a few characters with overlapping ranges such as a longer stylet (69.3-82.0 μm) and larger R value (125-152) for *H. mangiferae* *vs* shorter stylet (58.7-74.7 μm) and smaller R values (112-136) for *H. strictathecatus*.

Hemicriconemoides wessoni
Chitwood & Birchfield, 1957
(Figs 2I, J; 4I, J; 12; 13)

This species was originally described from *Myrica cerifera* L. from Alturas, FL, USA. It has since been reported from various habitats and localities in Florida, Georgia, Alabama and South and North Carolina (Chitwood & Birchfield, 1957; Dasgupta *et al.*, 1969; Ye & Robbins, 2000; Zeng *et al.*, 2012). Two populations from Florida and one from California were characterised morphologically and molecularly in our study.

MEASUREMENTS

See Table 6.

DESCRIPTION

Female

Body shape straight to slightly arcuate ventrad. Sheath mostly closely fitting except on tail, sheath frequently protruding forward over lip region. In all specimens, sheath over vulval area is not or very slightly annulated. In many specimens, sheath forming a distinct fold over vulva and ending abruptly. Lip region rounded but flattened anteriorly, not set off with two annuli, first smaller than second. Labial disc not distinctly seen under light microscope but SEM photographs showing disc to be slightly rectangular with six small rounded lobes around mouth opening, well separated from first lip annulus. Stylet short and sturdy with stylet knobs distinctly anchor-shaped anteriorly and rounded posteriorly. Dorsal pharyngeal gland opening situated near to base of stylet knobs. Excretory pore situated from 4-9 annuli posterior to base of pharynx. Hemizonid seen in one specimen only, one annulus long, situated opposite excretory pore. Hemizonion not seen. Sheath annuli smooth, flattened to slightly indented over whole length of body except in vulval region where they are more rounded to almost non-existent. No anastomoses present. Vulval flaps small to large. Spermatheca round to oblong, mostly filled with large rounded sperm cells. Vagina straight. Body mostly tapering abruptly posterior to anus, especially ventrally. Tail tip varying from slightly knob-like, rounded, irregular to very slightly curved ventrad with distinct annuli to rarely not annulated. Anus a distinct opening just posterior to vulva.

Male

Not found.

REMARKS

The present specimens compare well with those described by various authors. Ye & Robbins (2000) gave a good description of the great variation in tail form and structure.

Sequence and phylogenetic analysis

The D2-D3 of 28S rRNA gene alignment included 37 sequences of *Hemicriconemoides* and two sequences selected as outgroup taxa and was 695 bp in length. Twenty-nine new sequences were obtained in the present study. Intraspecific sequence diversity (uncorrected p-distance) for species were: *H. chitwoodi*, 0-0.3% (0-2 bp); *H. strictathecatus*, 0-0.3% (0-2 bp); *H. californianus*, 0-1.3% (0-7 bp); *H. ortonwilliamsi*, 0-0.3% (0-2 bp); *H. wessoni*, 0-0.4% (0-3 bp); *H. minutus*, 0.2-2.4% (1-16 bp) and *H. macrodorus*, 0% (0 bp). Difference between *H. alexis* type A and type B was 4.7% (22 bp); and *H. cocophillus* type A and type B, 5.3% (29 bp) and thus deserve further study to clarify this high variation. Phylogenetic analysis resulted in majority consensus BI tree with three major weakly to moderate supported clades (Fig. 14).

The ITS-rRNA gene alignment included 35 sequences of *Hemicriconemoides* and three sequences selected as outgroups from the genera *Paratylenchus* and *Gracilacus* and was 938 bp in length. Twenty-five new sequences were obtained in the present study. Intraspecific sequence diversities were for: *H. chitwoodi*, 0.3-0.7% (1-3 bp); *H. strictathecatus*, 0.1-0.9% (1-7 bp); *H. californianus*, 0.7-1.5% (4-12 bp); *H. ortonwilliamsi*, 0.8% (4 bp); and *H. wessoni*, 0.1-1.1% (1-8 bp). Majority consensus phylogenetic tree generated by the BI under the GTR + G + I model contained three major moderately to highly supported clades (Fig. 15).

The combined D2-D3 and ITS-rRNA alignment was 1429 bp in length and included 16 sequences of *Hemicriconemoides* and three outgroup sequences. The BI and MP analyses resulted in trees with similar topology. Three major clades can be distinguished in the tree (Fig. 16).

Morphological and molecular groupings

LIP REGION PATTERNS

After analysis of original and other published *en face* view illustrations made with SEM and LM, Decraemer
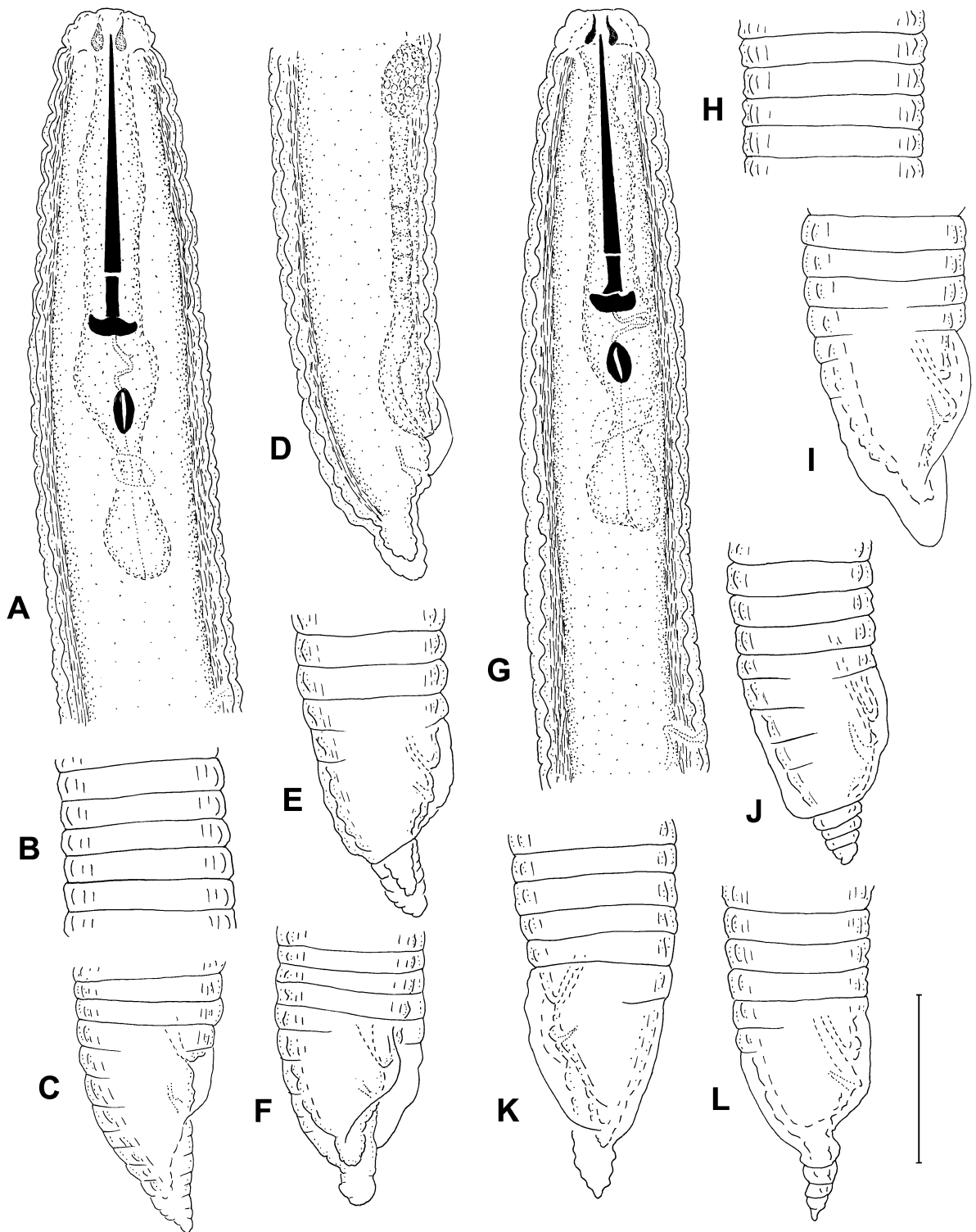
Fig. 12. *Hemicriconemoides wessoni* female FL, USA (CD1107). A: Anterior region; B: Annuli at mid-body; C-F: Various tail regions. Female (CD1054). G: Anterior region; H: Annuli at mid-body; I-L: Various tail regions. (Scale bar = 30 μm.)
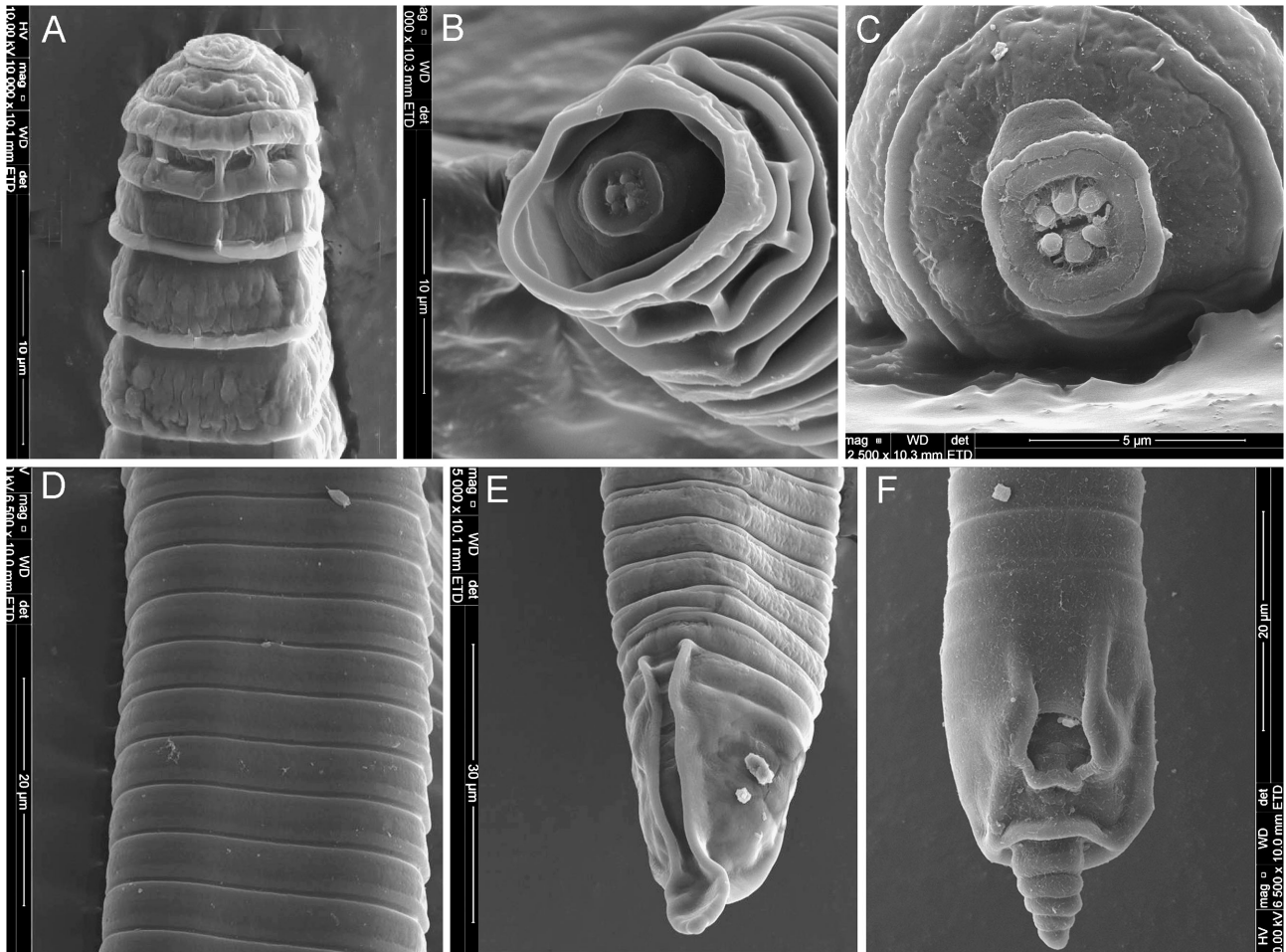
Fig. 13. *Hemicriconemoides wessoni* female FL, USA (CD1107). A: Lateral view of lip region; B: *En face* view of lip region with sheath pushed anteriorly; D: Annuli at mid-body; E: Tail region with distinct vulval flaps. Female FL, USA (CD1154). C: *En face* view of lip region; F: tail region, ventral view with vulval opening and vulval flaps.

& Geraert (1992) revealed four different types of lip regions for *Hemicriconemoides*, of which the first two are common whilst the third and fourth type are each represented by a single Australasian species. The first type of lip pattern (*H. mangiferae* type) consists of a narrow, oval, dorsoventrally orientated oral disc (with slit-like oral opening), flanked by wide amphidial apertures that are almost always covered by a plug (Fig. 16; Lip pattern – A1). This type was identified by Decraemer & Geraert (1992) in several *Hemicriconemoides* species including *H. mangiferae*, *H. gaddi*, *H. californianus*, *H. alexis* and *H. kanayaensis*. Illustrations published by Chen & Liu (2002) and Chen *et al.* (2008) allowed us to consider the lip region of *H. parasinensis* as belonging to this type. Our present study showed that the lip regions of *H. chitwoodi*

and *H. strictathecatus* likely represent variations of the first type.

The second type of lip pattern (*H. cocophillus* type) consists of a large, round to oval, raised oral disc with a slit-like oral opening surrounded by six sectors and an outer rim of one or two fine annuli. Laterally from the oral disc are two slit-like amphidial apertures with narrow to wider protruding *corpus gelatum*, amphids sometimes indistinct (Fig. 16; Lip pattern A2). The lip patterns of *H. cocophillus* and *H. wessoni* belong to this type according to Decraemer & Geraert (1992). SEM photos of lip regions of *H. brachyurus* (Esser & Vovlas, 1990), *H. ortonwilliamsi* (Ye & Siddiqi, 1994; Vovlas *et al.*, 2000) and *H. minutus* (present study) revealed that they can also be classified as belonging to the second type.

Table 6. Morphometrics of female *Hemicriconemoides wessoni*. All measurements are in μm and in the form: mean ± s.d. (range).

Character	FL, USA (CD1107)	FL, USA (CD1154)
n	8	17
L	442 ± 27.8 (399-491)	454 ± 27 (421-505)
a	13.7 ± 0.7 (12.3-14.8)	13.9 ± 1.3 (12-15.7)
b	4.6 ± 0.5 (4.2-5.6)	4.6 ± 0.3 (4.1-5.1)
c	19.8 ± 2.7 (16.2-22.9)	20.6 ± 3.3 (16.4-28.2)
c'	1.2 ± 0.2 (0.9-1.4)	1.2 ± 0.2 (0.9-1.7)
o	10.0 ± 0.7 (8.6-10.7)	8.8 ± 1.5 (5.6-10.3)
DGO	5 ± 0.5 (4.5-5.5)	4.5 ± 0.7 (3-5)
V	93 ± 0.7 (92-94)	93.5 ± 0.8 (92-95)
G_1	40 ± 4.3 (35-45)	42.5 ± 7.5 (25-53.5)
Ovary length	181 ± 28.8 (147-222)	189 ± 36.5 (107.5-252)
Stylet length	52 ± 1.9 (48.5-55.5)	53 ± 2.5 (50-58)
Metenchium length	41 ± 1.9 (39.5-45)	42.5 ± 2.0 (39.5-47)
Telenchium length	11 ± 1.0 (9.5-12.5)	10 ± 0.8 (8.0-11)
m	79.1 ± 1.9 (76.4-80.7)	80.8 ± 1.2 (78.9-84.3)
Stylet knob height	4 ± 0.8 (2.0-4.5)	3.5 ± 0.4 (3.0-4.5)
Stylet knob width	9 ± 1.0 (7.5-10.5)	8.5 ± 0.4 (8-9)
Excretory pore from anterior end	131 ± 10.3 (114-145)	128 ± 6.4 (116-140.5)
Diam. at mid-body	32 ± 1.1 (30-33)	33 ± 3.1 (29.5-39.5)
Diam. at anus	19 ± 1.1 (17-20)	19 ± 2.7 (15-24.5)
Diam. at vulva	24 ± 1.3 (22.5-25.5)	23 ± 1.8 (20-27)
Annulus width	5.5 ± 0.5 (5.0-6.5)	5.5 ± 0.6 (5.0-7.5)
Tail length	22 ± 3.4 (17.5-27)	23 ± 3.9 (17.5-28.5)
Pharynx length	100 ± 12.2 (87.5-125)	99 ± 8.5 (86-111)
First lip diam.	12.5	12.5 ± 1.2 (11-15.5)
Second lip diam.	16.0	16 ± 1.0 (15-18.5)
First body annulus diam.	20.0	20 ± 1.0 (18.5-21.5)
Second body annulus diam.	22.5	22 ± 1.8 (20.5-25.5)
Third body annulus diam.	23.5	24 ± 1.7 (22-27)
R	84 ± 3.4 (78-87)	87 ± 3.4 (82-94)
RSt	10 ± 0.7 (9-11)	11 ± 0.7 (10-12)
ROes	17 ± 1.4 (16-20)	19 ± 1.7 (16-22)
Rex	24 ± 1.4 (22-26)	24 ± 1.5 (23-28)
Rhem	24	–
RV	9 ± 1.0 (8-10)	9 ± 1.3 (7-12)
RVan	0-1	1-2
Ran	7.5 ± 0.8 (6-8)	7 ± 1.1 (6-10)
VL/VB	1.2 ± 0.1 (1.0-1.4)	1.3 ± 0.2 (0.9-1.8)
ST%L	11.8 ± 0.6 (11.3-12.7)	11.7 ± 0.5 (10.8-12.5)
Spermatheca length	16 ± 1.8 (13-17)	14.5 ± 2.0 (12.5-18.5)
Spermatheca diam.	12 ± 2.2 (9-14)	12.5 ± 1.5 (11-14.5)

Mapping of types of lip pattern on the combined dataset tree (Fig. 16) showed that the first type is associated with species from the Clade I (the lip pattern for *H. silvaticus* is unknown) and the second type with species from Clade II. The lip patterns for species from Clade III are still unknown.

VULVAL REGION

More than a third of the presently known *Hemicriconemoides* species have females bearing lateral vulval flaps or sheath, which can vary from poorly to fully developed in various species. Mapping of presence or absence
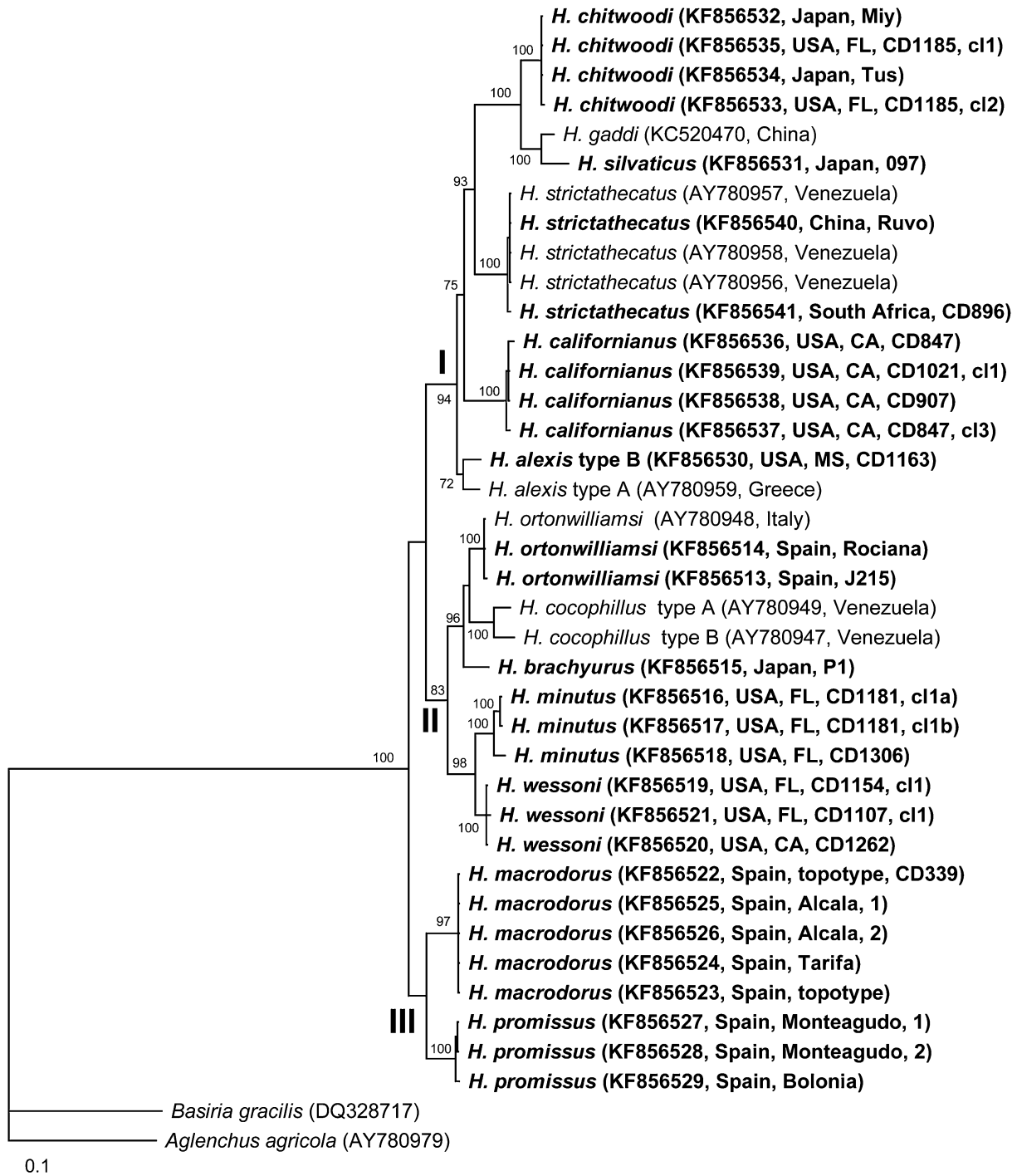
Fig. 14. Phylogenetic relationships within populations and species of *Hemicriconemoides* as inferred from Bayesian analysis using the D2-D3 of 28S rRNA gene sequence dataset with the GTR + I + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.
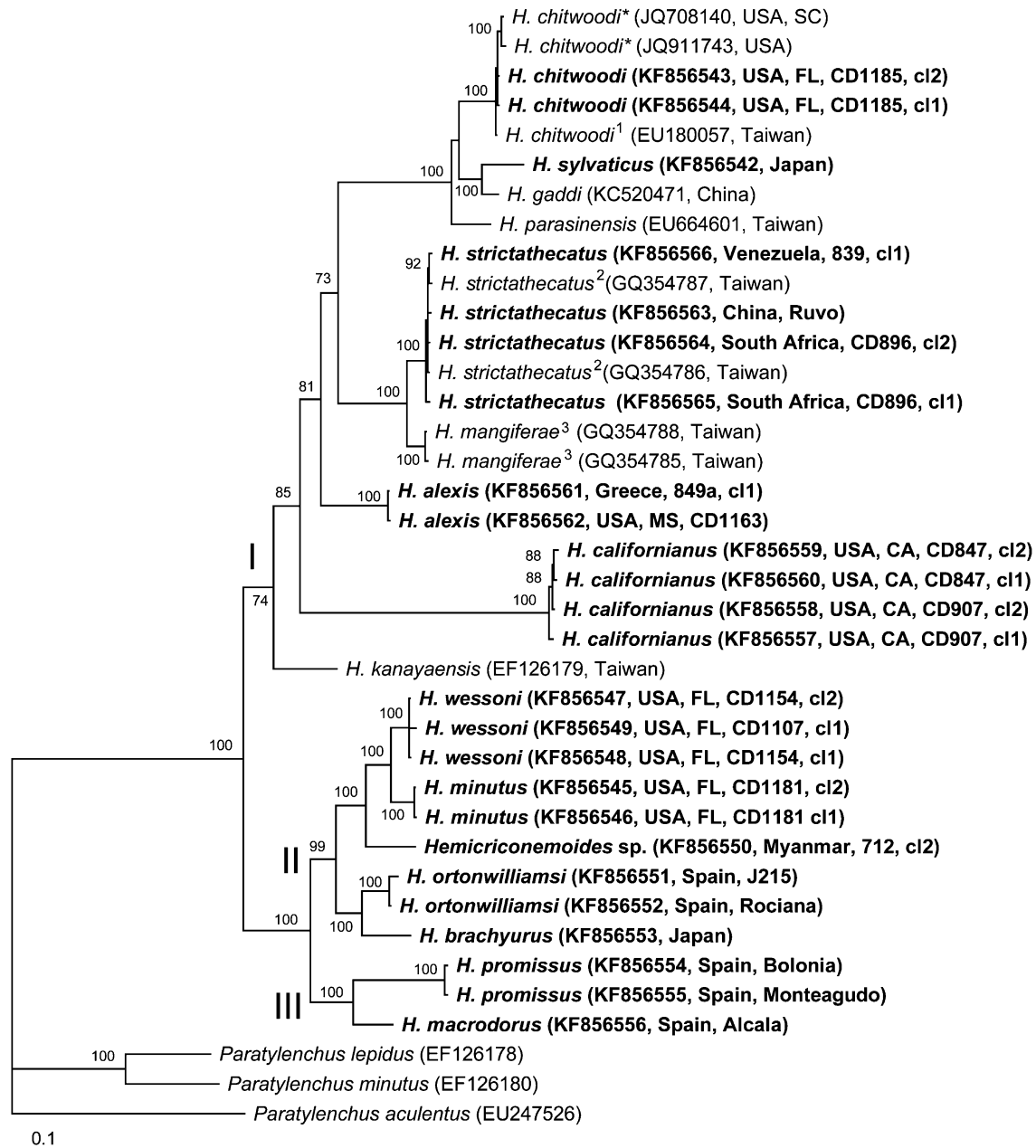
Fig. 15. Phylogenetic relationships within populations and species of *Hemicriconemoides* as inferred from Bayesian analysis using the ITS-rRNA gene sequence dataset with the GTR + I + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold. * – several nucleotides from 3′ end were excluded from the original sequence due to possible mistakes in sequence reading; [1] – identified as *H. californianus* in GenBank and the article by Chen *et al.* (2007); [2] – identified as *H. strictathecatus* in GenBank and as *H. litchi* in the article by Chen *et al.* (2011); [3] – identified as *H. strictathecatus* in GenBank and as *H. mangiferae* in the article by Chen *et al.* (2011).

of this character on the combined dataset tree (Fig. 16) revealed that lateral vulval flaps are associated with all species from Clade II only. Females of the species belonging to Clades I and III do not have vulval flaps.
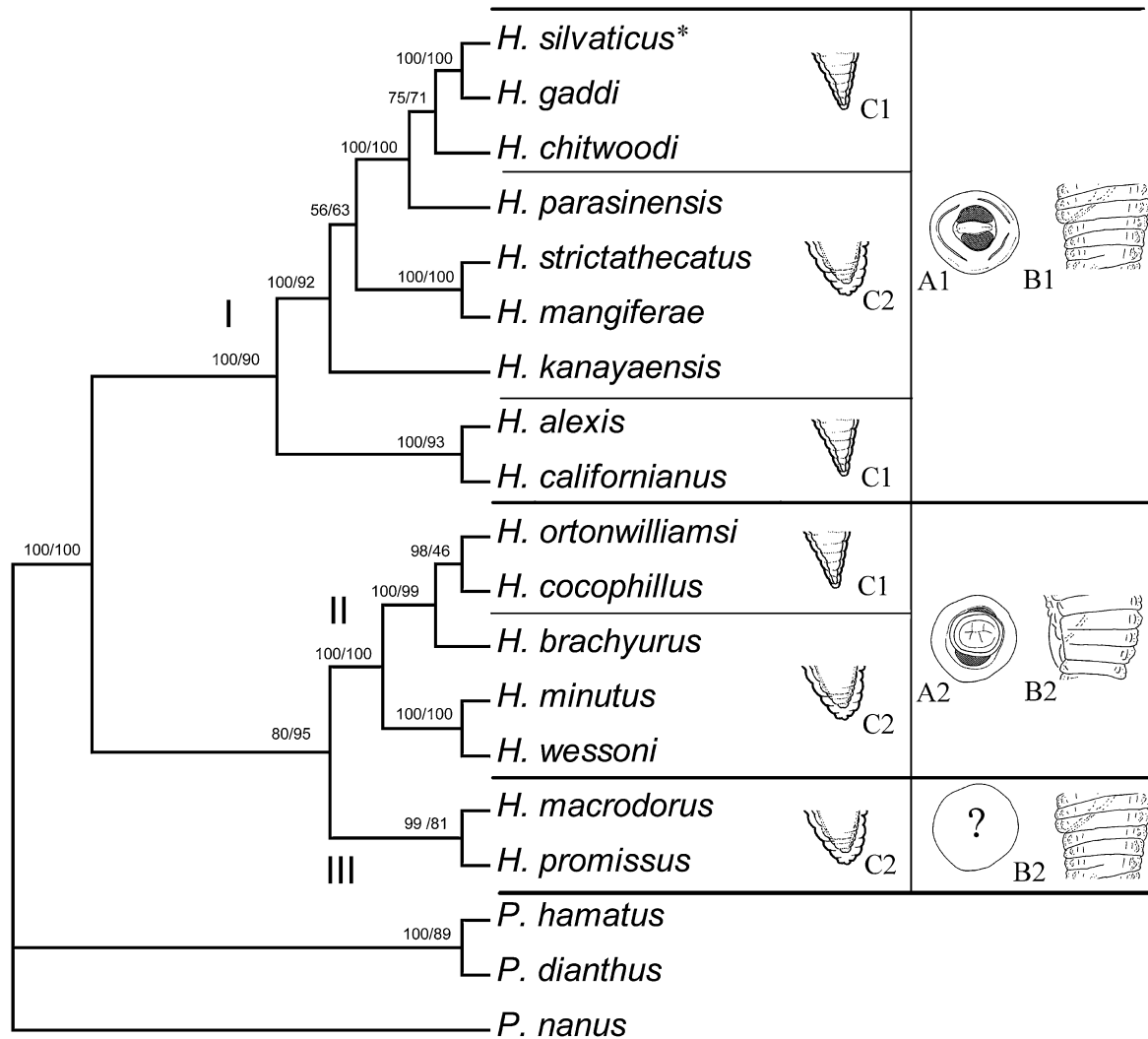
Fig. 16. Phylogenetic relationships within populations and species of *Hemicriconemoides* as inferred from Bayesian analysis using the combined dataset (the D2-D3 of 28S rRNA and the ITS-rRNA gene sequences) with mapping of lip region structure, vulval region and tail terminus shapes. Posterior probabilities for BI (GTR + I + G model) and bootstrap values for MP are given for appropriate clades. Lip pattern: A1: first type; A2: second type. Vulval flaps: B1: absent; B2: present. Tail terminus shape: C1: pointed; C2: rounded. * Lip region structure of *H. silvaticus* is unknown. *Paratylenchus* species were used as outgroups.

TAIL TERMINUS SHAPE

Tail shape in females of *Hemicriconemoides* varies from a pointed to a bluntly rounded terminus. Nearly half of the known species have a rounded terminus and other half have a pointed terminus. Species with both terminus shapes occur in Clade I and as well as in Clade II. Species with a rounded terminus are present only in Clade III (Fig. 16).

Molecular diagnostics of *Hemicriconemoides* species

Species-specific primers were developed for three *Hemicriconemoides* species based on differences in the ITS-rRNA gene sequences (Table 2). Results of PCR with the species-specific primers developed in this study for the three most common *Hemicriconemoides* species are given in Figure 17. The combination of the universal primer TW81 with the corresponding species-specific primers
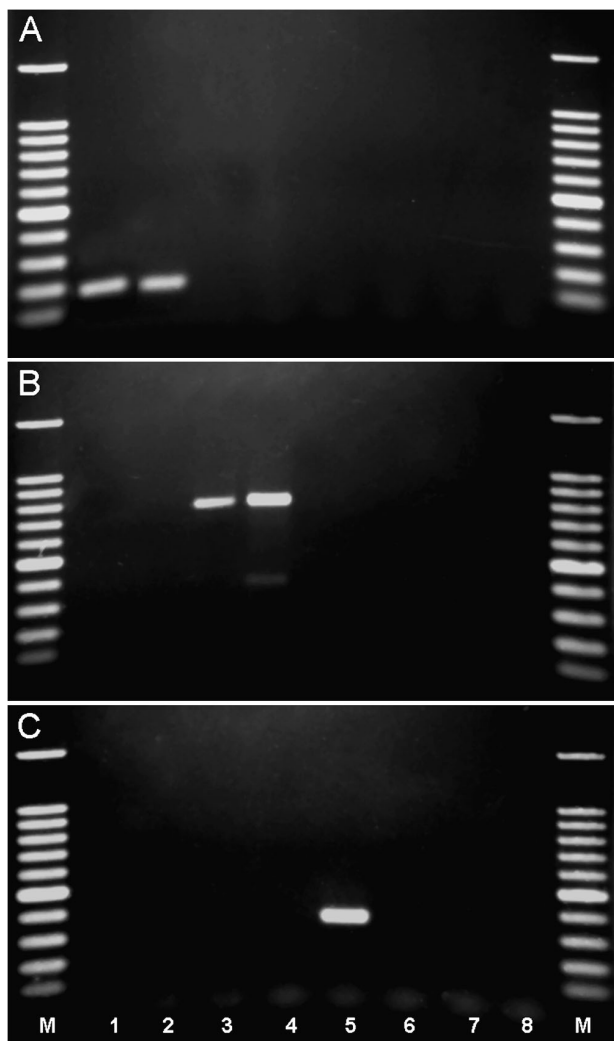
Fig. 17. Gel with specific amplicons obtained in the results of PCR with species-specific primers. A: PCR with the *Hemicriconemoides californianus*-specific primer (TW81 + H_califor); B: PCR with the *H. strictathecatus*-specific primer (TW81 + H_strict); C: PCR with the *H. chitwoodi*-specific primer (TW81 + H_chitw). Lanes: M = 100 bp DNA ladder (Promega, USA); 1 = *H. californianus* CA, USA (CD847); 2 = *H. californianus* CA, USA (CD907); 3 = *H. strictathecatus* Venezuela (839); 4 = *H. strictathecatus* South Africa (CD896); 5 = *H. chitwoodi* topotype FL, USA (CD1185); 6 = *H. minutus* topotype FL, USA (CD1181); 7 = *H. wessoni* FL, USA (CD1107); 8 = control without DNA.

yielded a PCR product of *ca* 186 bp for *H. californianus*, 730 bp for *H. strictathecatus* and 333 bp for *H. chitwoodi*. PCR with the specific primers were tested with several *Hemicriconemoides* samples.

The results of our study clarified some controversial aspects of the classification of sheathoid nematodes. The morphological analysis confirmed the small differences between *H. strictathecatus* and *H. mangiferae*. These two species share anchor-shaped style knobs, but *H. strictathecatus* has a shorter stylet than *H. mangiferae* and also has both rounded or anchor-shaped knobs. These close morphological affinities were also reflected in the close grouping of *H. strictathecatus* populations with populations of *H. mangiferae* from Taiwan in the phylogenetic tree generated with the ITS sequences. The variability in the morphological characters of *Hemicriconemoides* species observed in this study showed the great importance of the integration of molecular and morphological features for the separation of these nematodes and the need for morphological and molecular standards for each species, especially from topotype populations. The data reported here strengthen the idea that *Hemicriconemoides* species delimitation should be the result of integrated studies based on morphological, morphometric and molecular analyses. Using this procedure, this study demonstrated misidentifications in deposited sequences in GenBank. PCR-RFLP and sequencing of ribosomal DNA (D2-D3 region, ITS1) markers appear to be a useful and appropriate method for characterisation and accurate identification of sheathoid nematodes. However, future phylogenetic studies should include additional genetic markers such as mitochondrial and nuclear protein-coding genes (*e.g.*, cytochrome c oxidase subunit I or heat shock protein 90 genes) in order to resolve the relationships within *Hemicriconemoides* and to confirm the findings made in this work.

Our research provided evidence of the wide distribution of *Hemicriconemoides* species. However, some species seem to be more localised than others. Amongst the species described in Florida, *H. chitwoodi* and *H. strictathecatus* are more widespread outside the USA than *H. minutus* and *H. wessoni*. The presence of *H. wessoni* in Florida, California and other states in the USA may be due to the trade of sod grasses among the states. Phylogeographical studies are required to determine if the pathways followed by these *Hemicriconemoides* species are spread throughout many continents.

As a final comment, we would like to emphasise the fact that many of the species characterised in this study are a component of the nematofauna of mixed hardwood forests and natural areas, including *H. chitwoodi* and *H. minutus* in north Florida. Only three species were reported from fruit trees and a corn field. However, their economic importance in agriculture and the environment

is undetermined. In this selection of studied sheathoid nematodes, *H. wessoni* is the only species having economic relevance because this nematode parasitises and damages many sod grasses, including Bermuda grass (*Cynodon dactylon*), seashore paspalum (*Paspalum vaginatum*), St Augustine grass (*Stenotaphrum secundatum*), and *Zoysia* sp. in Florida and North and South Carolina (Zeng *et al.*, 2012; Crow, 2013).

Acknowledgements

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