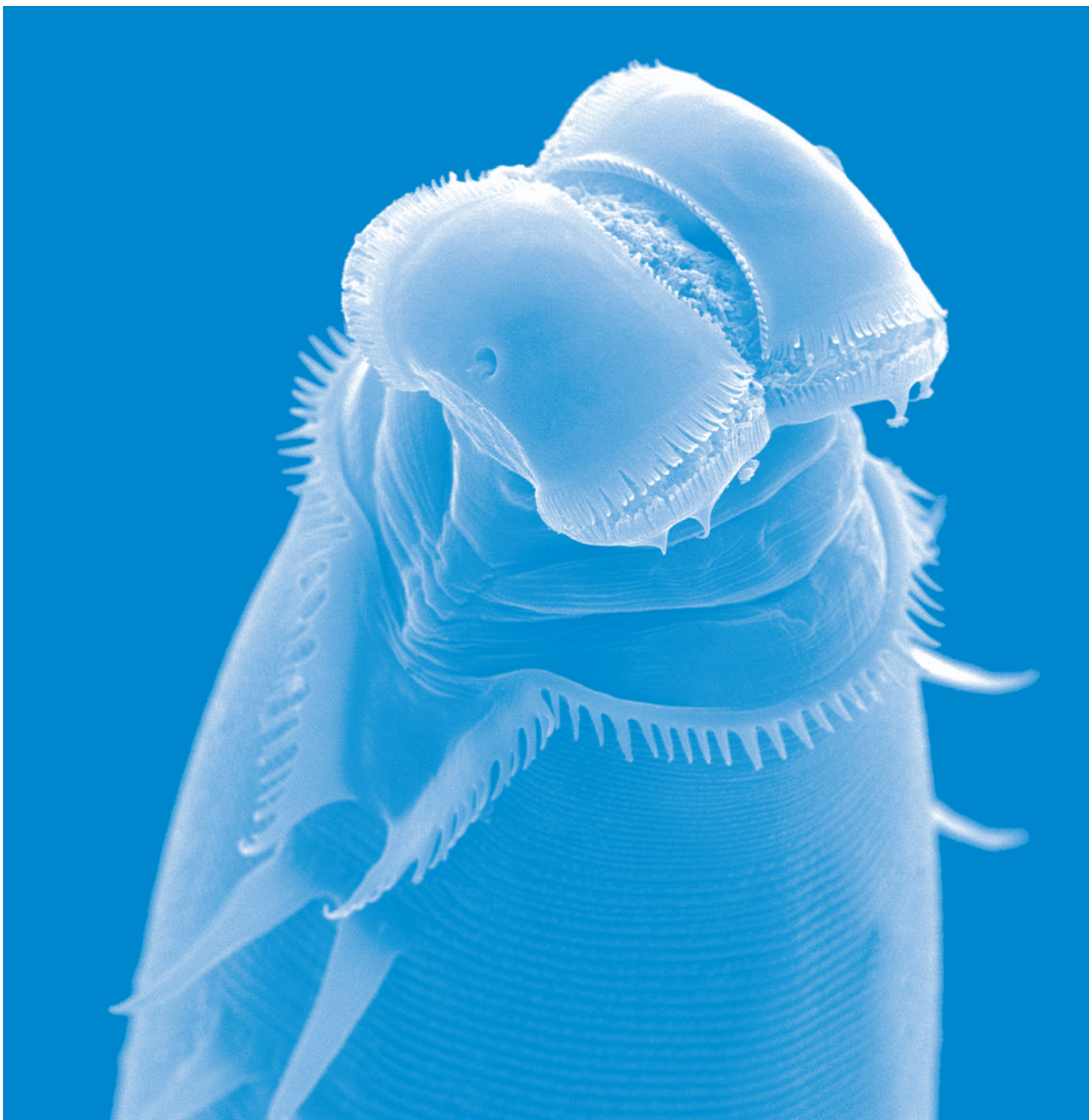


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Notes on morphological characteristics of 25 cyst nematodes and related Heteroderidae

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Summary. Based on the study of type specimens and other reliably identified nematode material, data supplementing or correcting the original descriptions of 18 *Heterodera* species (including four species mostly considered as *species inquirendae*), two *Meloidodera* and two *Globodera* species and one species each of the genera *Cactodera*, *Camelodera* and *Cryphodera* are presented. The main focus is on morphological characters of second-stage juveniles.

Key words: *Cactodera estonica*, *Camelodera eremophila*, *Cryphodera nothophagi*, diagnosis, *Globodera mali*, *Globodera millefolii*, *Heterodera* spp., identification, *Meloidodera* spp., morphology, taxonomy.

More than 160 species in the family Heteroderidae have been described so far, making identification of individual species increasingly difficult. Problems with correct identification are in part due to the fact that many species were poorly described and that a number of descriptions were based on insufficient material. Morphological details, which subsequently proved to be of diagnostic significance, were often not reported or not considered as ‘essential’ at the time of description. Among such characters were, in particular, data on second-stage juveniles. Lack of data may in part be due also to inadequate microscopical equipment. This situation resulted in a number of subsequent synonymisations, and a number of nominal species were considered as *species inquirendae*.

The objective of this paper is to provide supplementary information on morphological characters of a number of cyst nematodes and a few other heteroderid species based on the study of type specimens and other reliably identified nematode material in order to provide more data for reliable species identification. Most of the species included had been described from the former Soviet Union. Available for study were, in particular, permanent nematode microscope slides deposited in the nematode collection of the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia; nematode collection at the Institute of

Zoology, University of Tartu, Estonia, and the German Nematode Collection (DNST), Julius Kühn-Institut (formerly: Biologische Bundesanstalt für Land- und Forstwirtschaft), Münster, Germany. Some type or voucher specimens on permanent slides were used also from the Institute of Zoology, Uzbek Academy of Sciences, Tashkent, Uzbekistan; Rothamsted Research, Harpenden, UK; Nematode Department, Wageningen Agriculture University, Wageningen, The Netherlands; National Nematological Research Centre, University of Karachi, Pakistan; National Nematode Collection of New Zealand, Landcare Research, Auckland, New Zealand. In addition, nematode material collected by the author and deposited in the German Nematode Collection was used.

Some taxonomic problems within the family Heteroderidae and concerning particular species and their identity had been studied and discussed already earlier (Wouts & Sturhan, 1978; Sturhan & Wouts, 1995; Sturhan & Rumpfenhorst, 1996; Mor & Sturhan, 2000; Sturhan, 2002; Sturhan & Krall, 2002; Tanha Maafi *et al.*, 2007).

GENUS *HETERODERA*

Heterodera arenaria Cooper, 1955

According to the redescription of the species by Robinson *et al.* (1996) phasmids of the second-stage juveniles are “not prominent, found posterior to

anus and slightly anterior to hyaline tail". But in juveniles from the Gibraltar Point population, which was used for the redescription, as well as in second-stage juveniles from The Netherlands and Germany, phasmids are distinct with lens-like extension in the cuticle, in a position 2-4 annules posterior to the anus (voucher specimens in DNST Münster). All four incisures in the lateral field are only rarely fully developed.

***Heterodera cajani* Koshy, 1967**

The synonymisation of *H. vigni* Edward & Misra, 1968 with *H. cajani* by Kalha and Edward (1968) has been accepted in most subsequent publications, but curiously in several identification keys both species were still treated separately (Wouts, 1985; Golden, 1986; Shahina & Maqbool, 1995; Wouts & Baldwin, 1998), with four incisures in the lateral field of *H. cajani* and three in *H. vigni*, as originally reported for this species. Being a member of the *H. schachtii* group, four incisures in the lateral field are likely to be correct. The presence of four lateral incisures is confirmed for *H. cajani* second-stage juveniles, collected by G. Swarup and deposited in DNST Münster; the lateral fields in these specimens were irregularly areolated, the phasmids very faint.

***Heterodera cardiolata* Kirjanova & Ivanova, 1969**

Paratype second-stage juveniles from the St. Petersburg collection and from the Tashkent collection (the latter possibly used by Narbaev, 1987, for redescription of the species) had 20-20.5 µm long stylets with well separated slightly concave knobs of 4-4.5 µm diameter, three lip annules, lateral fields with three incisures (vs four according to the original description, three according to Narbaev, 1987) and a hyaline tail portion of 18-19 µm. The cyst wall showed a strong punctuation.

***Heterodera graduni* Kirjanova in Kirjanova & Krall, 1971**

The species has been considered as *species inquirenda* by Hesling (1978), Wouts (1985) and in subsequent publications. From two paratype slides examined (St. Petersburg collection and DNST Münster) it appears unlikely that the fenestra generally attains the peculiar shape which had been considered as a diagnostic character in the original description. In unhatched juveniles within eggs a stylet of 22.5-23.5 µm length (against 25 µm given in the original description for a single juvenile observed inside an egg), a hyaline tail portion of 21-24 µm, four equally developed lip annules and four

incisures in the lateral field were observed. Eggs measured 94 (92-98) x 41 (40-43) µm (n = 12), giving an average length/width ratio of 2.3, which is slightly less than that given in the original description (2.41).

***Heterodera koreana* (Vovlas, Lamberti & Choo, 1992) Mundo-Ocampo, Troccoli, Subbotin, Del Cid, Baldwin & Inserra, 2008**

Paratype second-stage juveniles deposited in the Wageningen nematode collection showed phasmids with large lens-like structure in a position 4-7 annules posterior to the anus.

Large phasmids were also seen in second-stage juveniles identified as *H. koreana* (stylet length 16-18 µm) isolated from a soil sample collected by the author from around bamboo in a natural forest in southern Thailand (voucher specimens in DNST Münster).

***Heterodera menthae* Kirjanova & Narbaev, 1977**

Poorly fixed second-stage juveniles on slides from the St. Petersburg collection and in DNST Münster had a hyaline tail portion of 24-26 µm, which are about two-thirds of the tail length given as 33.6-42 (39.4) µm in the original description. The measurements of 6.4-8 (7.6) µm originally mentioned for the hyaline tail length is incorrect. The lateral fields are irregularly areolated. Phasmids are punctiform. The lip region has three annules plus a labial disc. The stylet base is flat and the knobs are anteriorly projected.

***Heterodera mothi* Khan & Husain, 1965**

Controversial data exist with regards of the number of incisures in the lateral field of the second-stage juveniles. While Khan and Husain (1965), Sharma and Swarup (1984), Taya and Bajaj (1986) and Maqbool and Shahina (1986) report the presence of three incisures, Shahina and Maqbool (1991, 1995) and Tanha Maafi *et al.* (2004) give four incisures. In specimens collected by the present author in Iran and in second-stage juveniles obtained from Pakistan by M.A. Maqbool, three incisures were seen with the inner incisure occasionally diverging into two in a few juveniles.

***Heterodera oxiana* Kirjanova, 1962**

According to the original description this species is mainly characterised by the presence of two underbridges in the vulval cone. Examination of paratypes (St. Petersburg collection and DNST Münster) confirmed the presence of a long

underbridge deep below the fenestrae and irregular 'muscle strands' attached to the vagina mostly in right angle at a level between (lower) underbridge and fenestrae. The only data on juveniles given in the original description includes juveniles within eggs with a stylet length of 25 μm . Unhatched juveniles within eggs on a paratype slide revealed the following characters: stylet ($n=13$) = 25 (24-26) μm long, stylet base = 5-6 μm in diameter, stylet knobs with concave anterior faces, tail = 53 (48-57) μm long ($n=3$), hyaline tail portion = 29 (26-31) μm long ($n=8$), posterior lip annule wide, anterior lip annules indistinct, lateral field with four incisures, phasmids punctiform. *Heterodera oxiana* has been considered as *species inquirenda* by Hesling (1978) and Wouts (1985).

***Heterodera pakistanensis* Maqbool
& Shahina, 1986**

Second-stage juvenile paratype slides deposited at the University of Karachi were available for study. The morphological characters of the juveniles largely agreed with the original description of the species, but the lateral fields had mostly only three lines (instead of four according to the original description), with occasional splitting of the inner line into two lines (mostly less distinct) and presence of a distinct areolation. The head was weakly offset, the lip annules were indistinct. The stylet knobs were rounded (not anteriorly directed) and the stylet base was rather small (about 3 μm in diameter). The phasmids were small but distinct (not lens-like), located 24-28% of the tail length posterior to the anus. The tail measured 64-77 μm , the hyaline tail portion 28-34 μm (= 43-46% of the total tail length), ratio c' was 5.7-6.9 ($n=10$).

***Heterodera paratrifolii* Kirjanova, 1963**

Data on morphology of the second-stage juveniles were previously unknown. In juveniles within eggs on a paratype cyst slide deposited in DNST Münster, stylet lengths of 25-26 μm were measured; the stylet knobs were deeply concave, the stylet base was 5 μm in diameter. These juvenile characters support correctness for the synonymisation of *H. paratrifolii* with *H. trifolii* by Krall (1977).

***Heterodera phragmitidis* Kazachenko, 1986**

Paratype second-stage juveniles deposited in the Tartu nematode collection largely agreed with the original description: three distinct lip annules, stylet 18.7-20 μm long (vs 18-18.6 μm according to

original description) with flattened and only slightly concave knobs of 4.2-4.4 μm diameter, lateral field with three incisures (vs four according to original description) and irregular areolation, slender and pointed tail with hyaline part (26-34 μm) mostly longer than half total tail length, phasmids indistinct.

***Heterodera rosii* Duggan & Brennan, 1966**

In second-stage juveniles (specimens from Ireland in DNST Münster, collected by J. Duggan) lateral field only exceptionally areolated in mid-body region; phasmids indistinct, punctiform, 10-12 annules posterior to the anus; in general only the posterior one or two lip annules distinct; $c' = 4.6$ -5.2. The juvenile tail figured under Fig. 4D in the original description of the species probably does not belong to *H. rosii*.

***Heterodera rumicis* Poghossian, 1961**

For developed juveniles inside eggs a tail length of 55 μm and a hyaline tail portion of 30-32 μm were observed and measured, a stylet length of 28-30 μm , the lip region with only the posterior annule distinct and wide and the lateral field with four incisures; cyst cone characters resembled *H. trifolii* (slides from the St. Petersburg nematode collection and DNST Münster). Synonymisation of *H. rumicis* with *H. trifolii* by Wouts (1985) seems justified, although similarity to *H. rosii* may even be greater and both species have *Rumex* species as type hosts.

***Heterodera salixophila* Kirjanova, 1969**

Descriptions and data on morphology were presented by Kirjanova (1969), Kirjanova and Krall (1971), Mulvey (1972) and Krall (1977), but Brzeski (1998) already pointed out that the species "needs redescription as the original description lacks important morphological details". Paratype slides deposited in the St. Petersburg collection and additional material collected in Estonia and Germany from around *Salix* sp. were available for the present study.

Paratype cysts showed low semifenestrae and a vulva slit considerably extending beyond the fenestra width, distinct bullae in the vulva cone, most of them slender conoid but none were molar-shaped (similar to figs. 91 and 92 of Mulvey, 1972, but underbridge less developed). Cysts collected by E. Krall from *Salix* sp. at several sites in Estonia showed the same cyst cone characteristics, in particular the slender bullae; but bullae were absent in immature cysts.

From paratype second-stage juveniles ($n = 10$) the following measurements were taken: stylet = 28 (27.5-28.5) μm , width and height of stylet base = 6.3 (5.8-6.4) $\mu\text{m} \times 2.9$ (2.6-3.2) μm , width and height of lip region = 10 (9.6-10.7) $\mu\text{m} \times 4.8$ (4.6-4.9) μm , length of tail = 67 (64-71) μm , length of hyaline part of tail = 36 (32-42) μm . Some of these recent measurements differ from those given in the original description (stylet = 30 μm , height of lip region = 6.4-7.2 μm). In the paratype juveniles the anterior faces of the stylet knobs are flat to slightly concave; the lip region has three (occasionally four) distinct annules of almost the same diameter, the lateral fields show four incisures and no distinct areolation, the phasmids are small but distinct and situated 8-11 annules posterior to the anus (= approximately one anal body diameter), the tail terminus is slender and often slightly offset.

Specimens from Germany collected from *Salix* sp. agree closely with the type specimens. Cysts generally show the slender bullae, and the semifenestrae even of older cysts are often still covered by the cuticle. Second-stage juveniles have three (occasionally four) lip annules, the stylet knobs are concave anteriorly, the lateral fields are only irregularly areolated. The following measurements of second-stage juveniles ($n = 23$) are below those of the type specimens: $L = 453$ (410-495) μm , stylet = 25.5 (24.5-26) μm , tail = 58 (50-65) μm , hyaline part of tail = 32 (27-39) μm . The species appears to be widespread in Germany (Sturhan, 2006).

***Heterodera scleranthii* Kaktina, 1957**

Two slides from the St. Petersburg collection and one slide deposited in DNST Münster containing cyst cones and eggs with a few developed juveniles, not particularly designated as types, but determined by Kaktina and collected 10.09.1955, were available for study. Additions to the original description and the complementary description given by Kirjanova and Krall (1971): cyst cone with fenestra similar to *H. trifolii*, underbridge rather thick, bullae cone-shaped. Juveniles within eggs: stylet = 26-28 μm ($n=6$), stylet base = 6.3 μm in diameter, knobs robust, anterior faces cupped, three lip annules plus labial disc, lateral fields with four incisures, hyaline tail portion = 31-35 μm long ($n=5$), phasmids punctiform. Synonymisation of *H. scleranthii* with *H. trifolii* by Wouts (1985) appears justified.

***Heterodera tadshikistanica* Kirjanova & Ivanova, 1966**

The only information about second-stage juveniles given in the original description is that

juveniles within eggs had a stylet length of 23.4-26 μm . The study of a paratype slide (St. Petersburg collection) with cyst remnants and a few eggs with developed, unhatched juveniles revealed the following characters: stylet = 24-25 μm long, stylet base diameter = 4.5 μm , stylet knobs rounded with anterior faces flat to slightly cupped, lip region rather high, with 5-6 annules and posterior lip annule of almost the same diameter as other lip annules, lateral field with four incisures and irregular areolation, tail terminus more or less pointed, hyaline portion 21 μm long, phasmids punctiform.

***Heterodera turangae* Narbaev, 1988**

On two slides from the Tashkent nematode collection second-stage juveniles squashed from eggs had a 21 μm long stylet ($n=3$), which is slightly above the length given in the original description (mean = 19.4 μm).

Cyst characters such as the shape of the fenestra (semifenestrae almost rounded) and absence of bullae, short stylet in second-stage juveniles, four lines in lateral field and the type host being *Populus pruinosa* in Salicaceae, which are distantly related to Urticaceae, are indications that *H. turangae* appears to be a member of the *H. humuli* species group and does not belong to the *H. goettingiana* group as originally suggested (Narbaev, 1988).

***Heterodera uzbekistanica* Narbaev, 1980**

The study of cysts from three slides (in Tashkent nematode collection and DNST Münster) from *Salix olgae* in the Tashkent region confirmed the characters in addition to those mentioned in the original description (no bullae, very weak underbridge). Second-stage juveniles and eggs were not available for observation. Fenestration type with rather high semifenestrae, absence of bullae and hosts in Salicaceae indicate putative placement in the *H. humuli* group.

Other Heteroderidae genera

***Cactodera estonica* (Kirjanova & Krall, 1963) Krall & Krall, 1978**

From paratype slides deposited in the St. Petersburg collection the following characters of second-stage juveniles (most of them unhatched and some partly squashed from eggs) could be ascertained: lip region rounded, weakly set off, 9.0-9.5 μm in diameter and 4.0-4.8 μm high, with six (rarely five) annules; stylet 22.7 (22.2-23.6) μm long ($n=15$), with 4.0-4.8 μm wide knobs, which are

rounded and slightly inclined posteriad; tail 38.5 and 40 μm long ($n=2$), hyaline tail portion 19 (17-21.5) μm ($n=8$); $c' = 2.6$ and 3.0; tail terminus blunt; phasmids small, 8-10 annules posterior to the anus; lateral field with four incisures, areolation present, including central band. Stone (1986) and Sturhan (2002) already pointed out that the originally reported number of five incisures in the lateral field was incorrect. *Cactodera estonica* is an amphimictic species; males could be recovered from roots of the type host, but have not been described so far (Krall, 1977).

***Camelodera eremophila* Krall, Shagalina & Ivanova, 1988**

In a paratype second-stage juvenile (St. Petersburg nematode collection) the lateral field showed three incisures instead of four as reported by Krall *et al.* (1988). Other characters agreed with the original description. The phasmids are distinct but probably not lens-like, in a position nine annules posterior to the anus.

***Cryphodera nothophagi* (Wouts, 1973) Luc, Taylor & Cadet, 1978**

Re-examination of paratype second-stage juveniles (Auckland nematode collection) revealed three incisures in the lateral field, with the inner incisure occasionally diverging into two. Thus all known six *Cryphodera* species are characterised by the presence of basically three lateral incisures. Consequently, the key presented by Karssen and Van Aelst (1999) needs correction. In all described *Cryphodera* species the phasmids are lens-like.

***Globodera mali* (Kirjanova & Borisenko, 1975) Mulvey & Stone, 1976**

Sturhan (2002) refused synonymisation of *Heterodera chaubattia* Gupta & Edward, 1973 with *Heterodera mali* by Wouts (1985), a synonymy already suggested by Krall (1977), and proposed to consider *H. mali* as valid species in *Globodera*. The original description of the latter species had been based exclusively on cysts (see also Krall, 1977), measurements of eggs were given, but no data on females, males and juveniles.

On paratype slides deposited in the St. Petersburg collection and in DNST Münster, eggs with unhatched juveniles and juveniles partly squashed from eggs were found. The following characters could be ascertained: stylet ($n=20$) = 22 (21.5-22.5) μm , tail ($n=5$) = 41 (39-43) μm , hyaline tail portion ($n=20$) = 17 (13-20) μm , stylet knobs rounded and slightly inclined posteriad, 4 μm in diameter, lip region 8.2-8.5 μm wide and 3.6-3.8 μm

high with four annules, tail end rather blunt, annule width at mid-body about 1.5 μm .

***Globodera millefolii* (Kirjanova & Krall, 1965) Behrens, 1975**

The description of this species is based on a single immature cyst; males and juveniles were not described, eggs measured 132 x 49 μm . Krall (1977) considered *G. millefolii* as *species inquirenda*. A re-examination of the type slides deposited in the St. Petersburg collection did not reveal morphological characteristics in addition to those already published (Kirjanova & Krall, 1965; Krall, 1977).

A few cysts and juveniles squashed from eggs, collected by E. Krall in September 1972 at the type locality Tallinn-Pirita, Estonia (see Krall, 1977), around *Achillea millefolium* were used for the present study (slides deposited in DNST Münster). Cysts pale brown, in two of three cysts 6 and 9 irregularly rounded bullae of up to 25 μm in diameter at some distance below fenestra, one cyst without bullae-like structures. Two perineal areas had the following characteristics: fenestra 22 and 25 μm long, 30 and 22 μm wide, anus 25 and 35 μm apart from edge of fenestra, only in one cyst encircled with cuticular rings (thus, this is not a general characteristic as suggested from observations by Mulvey, 1972), 5 and 6 irregular cuticle ridges between anus and fenestra, Granek's ratio = 1.1 and 1.4. Second-stage juveniles ($n = 10$): L = 445 (420-470) μm , a = 28 (26-31), c = 9.8 (9.1-10.5), $c' = 3.9$ (3.6-4.4), stylet = 26 (25-27) μm , tail = 46 (42-52) μm , hyaline tail portion = 25.5 (21-29) μm . Lip region with four annules, 7.5-8 μm in diameter and 3-4 μm high; stylet knobs rounded, occasionally with slight anterior projection, 4.0-4.4 μm in diameter and 2.5-3.0 μm high; four incisures in the lateral field; phasmids punctiform, situated 8-9 annules posterior to the anus; tail conical with narrowly pointed terminus.

***Meloidodera alni* Turkina & Chizhov, 1986**

In second-stage juveniles from *Alnus* sp. and *Betula* sp. from the Moscow region and from *Alnus glutinosa* and *A. incana* from several sites in Germany the phasmids were large, lens-like, 4-9 annules posterior to the anus. In males from Russia and Germany indistinct phasmids with only slightly lens-like swelling in the cuticle were seen close to tail terminus; the lateral field was irregularly areolated. Re-measured paratype second-stage juveniles ($n=6$) had a body length of 400 (385-425) μm and a stylet length of 31 (30.5-31) μm ; the respective measurements of juveniles from Germany

were (n=44): L = 438 (405-490) µm and stylet = 31.5 (29.5-34) µm.

Meloidodera tianschanica Ivanova & Krall, 1985

In second-stage juveniles deposited in the Tartu nematode collection the phasmids are distinct with lens-like structure in the cuticle, not pore-like as given in the key presented by Cid del Prado (1991).

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D. Sturhan. Заметки о морфологических особенностях 25 видов цистообразующих нематод и близких к ним Heteroderidae.

Резюме. На основе переисследования типового материала, а также достоверно определенных до вида особей нематод, предлагаются дополняющие или корректирующие первоописания сведения по 18 видам *Heterodera* species (включая виды, обычно рассматриваемые как *species inquirendae*), по двум видам *Meloidodera* и двум видам *Globodera*, а также по одному виду из родов *Cactodera*, *Camelodera* и *Cryphodera*. Большая часть рассматриваемых морфологических особенностей относится к личинкам второй стадии.

Ecological characterisation of *Steinernema australe* (Panagrolaimomorpha: Steinernematidae) an entomopathogenic nematode from Chile

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Summary. This paper reports on studies of a recently described species of entomopathogenic nematode, *Steinernema australe*, from Chile. Under laboratory conditions *S. australe* had a fast life-cycle, with new infective juveniles (IJ) observed after 6 d at 20°C; however, adults and non-infective juveniles also emerged from cadavers and migrated away. The thermal and moisture profiles for infectivity were wide and significant infection occurred at cool, humid conditions, 7°C and 15.8% moisture content (MC). Five days at *ca* -1°C had no effect on IJ survival, but subsequent host infection was substantially reduced, a possible chilling-injury or phase-switching of the symbiotic bacteria. *Steinernema australe* infected a wide range of insect pests from Chile, including mobile and sedentary hosts, appearing most effective against Lepidoptera. No nictating or jumping behaviour of IJ was observed.

Key words: ecology, freeze, host, moisture, *Steinernema australe*, temperature.

Manipulation of entomopathogenic nematodes (EPN) in managed systems through inundation, inoculation and conservation can provide effective and environmentally benign methods for controlling insect pests (Koppenhöfer & Kaya, 1999; Grewal *et al.*, 2005; Stuart *et al.*, 2006). Key to the success of EPN as a control strategy is an understanding, based on both laboratory and field observations, of the biology, ecology and population dynamics of the EPN. The breadth of type-localities that 65+ valid EPN species, including recent collections in Tibet (Mráček *et al.*, 2009) the Sonoran Desert (Mexico) (Stock *et al.*, 2009) and Colombia (López-Núñez *et al.*, 2008), gives reason to suggest a rich biological diversity amongst these organisms, with characteristics of value for controlling insects. Koppenhöfer & Kaya (1999) suggest a protocol for ecological characterisation to complement a new EPN species description, providing basic information on host range, foraging strategy and abiotic profiles.

This paper describes a number of studies on the biology and ecology of *Steinernema australe* Edgington, Buddie, Tymo, Hunt, Nguyen, France, Merino, & Moore, 2009, a recently described species of nematode discovered during surveys in

Chile, and follows basic bionomic observations carried out during the original description (Edgington *et al.*, 2009). *Steinernema australe* was discovered on Isla Magdalena, an island in the south of Chile, approximately 2 km from the mainland (44° 35' 48.8" S, 72° 57' 35.7" W). Precipitation on the island is high (approximately 4000 mm per annum) with an average annual temperature of around 7°C. *Steinernema australe* was discovered within 200 m of a beach, below dense undergrowth, in a loamy-sand soil, shaded by trees.

We measured virulence of *S. australe* in the laboratory to a number of insect species of agricultural importance in Chile, the effect of temperature and moisture on infectivity and development, including exposure to temperatures below 0°C and behavioural aspects such as the ability to attach to an actively mobile host (i.e., the ability to ambush a host).

MATERIAL AND METHODS

Nematode culture. The nematodes used in the study were cultured in late instar waxmoth (*Galleria mellonella* L.) larvae (obtained from Live Foods Direct, Sheffield, UK). Emergent infective juveniles (IJ) were collected in modified White traps (White,

1927) and stored at $8 \pm 2^\circ\text{C}$ (a recognised storage temperature for temperate steinernematids) in tap water, prior to the trials. Only IJ collected within one week of first emergence were used in the trials and IJ in storage for > 20 d were discarded. All experiments were done in the laboratory.

Life cycle. Fifty late instar waxmoth larvae, in a Petri dish (15 cm diameter) lined with moistened filter paper, were exposed at a concentration of 50 IJ/ larva (in 5 ml sterilised tap water) at $20 \pm 2^\circ\text{C}$. There were five Petri dishes in total, i.e., 250 waxmoths. At 0, 1, 4, 12, 24 then every 24 h up to 240 h following inoculation, 10 waxmoth larvae were washed in tap water and dissected individually in 0.5% saline solution (NaCl). Penetration rates, sex ratios and *in vivo* EPN development were recorded. Fifty larvae were also checked for mortality every 24 h and cadavers transferred to modified White traps to monitor nematode emergence.

To test for self-fertility, one late instar waxmoth larva was placed into a chamber (1.5 cm^3) lined with moistened filter paper. One IJ in $10\text{ }\mu\text{l}$ sterilised tap water was topically applied to the dorsal region of each larva. Control waxmoths received $10\text{ }\mu\text{l}$ sterilised tap water without IJ. The larvae were maintained at $20 \pm 2^\circ\text{C}$ and monitored every 24 h for mortality. Cadavers were transferred to modified White traps to check for nematode emergence. There were 50 waxmoths for each treatment and the trial was carried out twice.

Temperature profile. Each chamber (1.5 cm^3) of a 25-chamber bioassay plate was partially filled with 0.5 g sterilised, air-dried sand (medium sized particles, mesh designation -30+50). The test temperatures were 7, 11, 16, 20, 23, 28 and 33°C , with IJ and waxmoth larvae equilibrated for 1 h at these temperatures prior to testing. Twenty five IJ in $50\text{ }\mu\text{l}$ sterilised tap water were transferred into each chamber, followed by one waxmoth larva. Control chambers received $50\text{ }\mu\text{l}$ sterilised tap water without IJ. Plates were placed in plastic bags to reduce desiccation and then maintained at one of the test temperatures. Larval mortality and time to first IJ emergence were recorded every 24 h for 20 d, then every 48 h thereafter for another 20 d. The trial was done twice.

Freeze tolerance. Plastic tubes (1.5 ml volume), containing 100 IJ in 1 ml sterilised tap water, were submerged in a water bath at $-1 \pm 1^\circ\text{C}$. The water bath contained approximately 30% antifreeze. Control treatment consisted of IJ in sterilised tap water maintained at $8 \pm 2^\circ\text{C}$. Five tubes containing IJ were taken out every 24 h for 120 h (destructive sampling), the IJ left to recover in distilled water for

4 h at room temperature and then the number of live and dead IJ counted. Nematodes that did not respond to gentle probing with a needle were counted as dead. Twenty live IJ were then picked out from pre-selected locations on a counting chamber and applied in $50\text{ }\mu\text{l}$ sterilised tap water to a chamber (1.5 cm^3), lined with filter paper, containing a single waxmoth larva. The chambers were covered and kept at $20 \pm 2^\circ\text{C}$. Mortality of waxmoth larvae was assessed after 144 h. The trial was set up as a randomised block design, with five blocks of five tubes, and was done three times.

Effect of soil moisture. Approximately 50 IJ in $50\text{ }\mu\text{l}$ sterilised tap water were placed at the bottom of a plastic tube (8 cm height, 1.5 cm diameter, 28 ml volume), which was then part-filled with pre-moistened sand (mesh designation -30+50) to a height of 4 cm, the column being gently compacted by tapping the tube on the bench. The moisture contents (MC) tested were (w/w) 0.2, 4.6, 8.3, 13.4 and 15.8% (saturation point of the sand was approximately 20.0% MC). Moisture contents were assessed using a HG53 Mettler Toledo Moisture Analyser. A disc of wire mesh was placed on the top surface of the sand onto which one late instar waxmoth larva was placed. The tubes were sealed and maintained at $20 \pm 2^\circ\text{C}$ for 72 h. Control treatment consisted of larvae maintained on top of the moistened sand column but without the addition of IJ. Mortality of waxmoth larvae was assessed after 72 h, with cadavers dissected in 0.5% NaCl to count the number of IJ that had penetrated. The MC of the sand was checked at 0 and 72 h, including measurements at the top and bottom of the column at 72 h. There were five tubes per treatment at each moisture level. The trial was done three times.

Ambush capacity. A Petri dish (9 cm diameter) was lined with filter paper with a thin layer of sand (10.0% MC w/w) on top. Approximately 500 IJ in $200\text{ }\mu\text{l}$ sterilised tap water were added to the dish and left at room temperature for 30 min. One late instar waxmoth larva was introduced into each dish and kept active for 20 min by gentle prodding with a pipette tip. After 20 min the larva was washed in 1 ml tap water and a count made of IJ in the wash. There were five waxmoth larvae per trial, i.e., five Petri dishes, and the trial was done three times.

Approximately 100 IJ on a circle of filter paper (0.5 cm diameter) were placed into the middle of a Petri dish (9 cm diameter) containing 2% tap water agar. The dish was left at room temperature for 30 min, following which IJ nictation and jumping was observed, for approximately 1 h, using a stereo microscope.

Laboratory host range. Fourteen insect species, either immature or adult stages, and representing five orders, were used to assess the laboratory virulence of *S. australe*. All insects were obtained within Chile and tested at the Instituto de Investigaciones Agropecuarias (INIA), Chillán, Chile (Region VII). Adult citrophilus mealybug *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae), leaf-footed bug *Leptoglossus chilensis* (Spinola) (Hemiptera: Coreidae), and late instar larvae of *G. mellonella* (Lepidoptera: Pyralidae), codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) and tussock moth *Orgyia antiqua* (Fitch) (Lepidoptera: Lymantriidae) were obtained from laboratory colonies INIA, Chillán, Chile; late instar blackmoth larvae *Dallaca pallens* (Blanchard) (Lepidoptera: Leporidae) and adult Argentine stem weevil *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) were obtained from natural pastures in Region X in the south of Chile (approx. latitude 41°S); late instar Mediterranean flour moth larvae *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) were obtained from a grain storage house in Region VII (approx. 35°S); late instar chafer larvae *Phytolema hermanni* (Germain) (Coleoptera: Scolytidae), *Brachysternus prasinus* (Guerin) (Coleoptera: Scarabaeidae), and adult burrito weevil *Aegorhinus nodipennis* (Hope) (Coleoptera: Curculionidae) were obtained from blueberry and raspberry crops in Regions VI to X (approx. 34 to 41°S); late instar eucalyptus weevil larvae *Gonipterus scutellatus* (Gyllenhal) (Coleoptera: Curculionidae) were obtained from a eucalyptus plantation in Region

VII; late instar pear slug larvae *Caliroa cerasi* (L.) (Hymenoptera: Tenthredinidae) and adult European earwig *Forficula auricularia* (L.) (Dermaptera: Forficulidae) were obtained from cherry trees and leaf litter respectively, on the INIA estate, Chillán. Each insect was placed into a chamber (1.5 cm high × 3 cm diameter) lined with moistened filter paper. Infective juveniles were applied at doses of 0, 10 and 100 IJ/ insect in 100 µl sterilised tap water, then left at 20 ± 2°C. Insect mortality was monitored every 24 h for 9 d; cadavers were monitored daily for a further 20 d to check for nematode emergence. The filter paper in each chamber was moistened occasionally. There were between 30 and 60 insects per treatment, depending on circumstances, arranged in three blocks. Adult *P. calceolariae* were exposed in groups of five per chamber.

Data analysis. In all experiments the results from the repeat trials were similar and were therefore combined. When appropriate, mortality data was corrected for control mortality using Abbott's formula (Abbott, 1925). 'Infectivity' was a function of host mortality and 'development' a function of progeny emergence. Any percentage data were arcsine transformed before significance testing (Dytham, 2003) (the data presented in the paper are pre-transformed data). Analysis of variance with appropriate factors was used to analyse treatment effects in all tests, with Tukey's test and linear regression used to analyse differences and relationships between treatments (Genstat 11th Edition, VSNi). Differences between treatment means (± SE) were considered significant at $P < 0.05$.

Table 1. Infectivity (%) and penetration of waxmoths by *Steinernema australe* in a 4 cm column of sand of MC 0.2, 4.6, 8.3, 13.4 and 15.8% (w/w). Waxmoths were exposed at a concentration of 50 IJ/ larva for 72 h, with IJ placed at the bottom of the sand column and waxmoths on the top. Included are MC readings taken at end of trial from top and bottom of sand column. Means followed by the same letter within a column are not significantly different ($P > 0.05$).

Moisture content % w/w (top to base range, 72 h)	Waxmoth infected % (± SE)	EPN penetrating (± SE)
0.2 (0.2-0.2)	0 ^a	0 ^a
4.6 (4.2-4.4)	100 ^b	20 (± 1.2) ^b
8.3 (7.5-8.7)	100 ^b	21 (± 1.1) ^b
13.4 (12.1-13.7)	100 ^b	17 (± 1.7) ^b
15.8 (14.9-16.0)	87 (± 9.1) ^c	9 (± 2.1) ^c

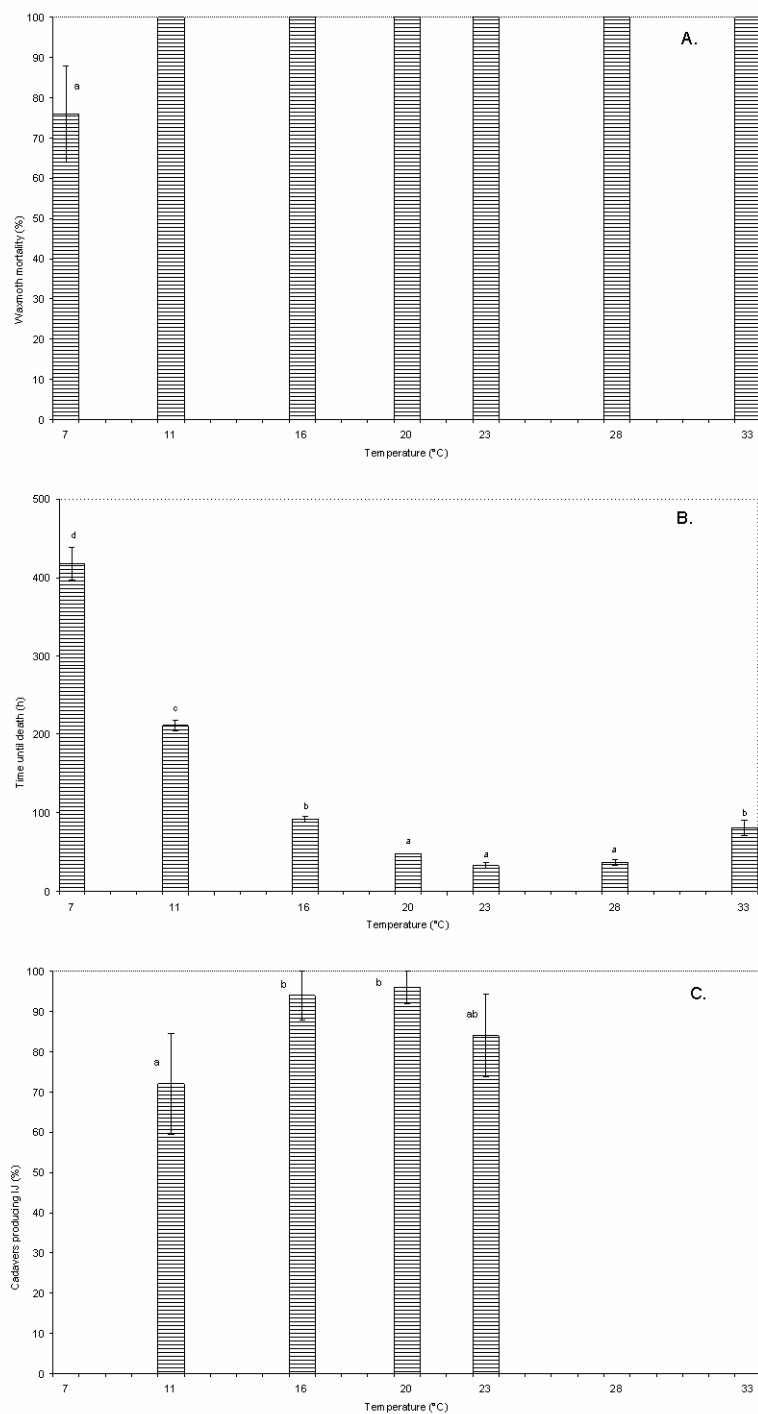


Fig. 1. Waxmoth larvae infected by *Steinernema australe* (%), time until waxmoth death (h) and development of *S. australe* *in vivo*, at test temperatures 7, 11, 16, 20, 23, 28 and 33°C. Waxmoth larvae were kept in chambers partially filled with sand and exposed at a concentration of 25 IJ/ larva, for a total of 40 d. Bars indicate means and vertical lines represent the 95% confidence intervals. Means sharing the same letter are not significantly different ($P > 0.05$).

RESULTS

Life cycle. Infectivity of waxmoth larvae at 20°C was observed after 24 h, with no larvae alive after 48 h. The level of penetration (mean $\pm$ SE) after 48 h was $62 \pm 4.6\%$ of the original IJ inoculum. First generation adults were observed 48 h following host death (female: male sex ratio of 1.8:1), second generation adults a further 48 h later. The mean time (range) from inoculation until IJ were first observed emerging from cadavers was 192 (144 – 216) h. Second generation adults and non-infective juveniles were observed outside the insect cadaver 144 h following inoculation. There were no indications of self-fertility for *S. australe* from the 1:1 trials; mortality of waxmoths was 38% vs 4% for IJ and control treatments respectively, but no progeny emerged.

Temperature profile. *Steinernema australe* infected 76% of the waxmoth larvae at 7°C and 100% at all other test temperatures (Fig. 1A). Temperature had a significant effect on mean time until waxmoth death ($F = 824.3$; $df = 6,331$; $P < 0.05$). Mortality of waxmoths was most rapid at 23°C and slowest at 7°C (33 ± 1.7 h and 418 ± 12.3 h

until death, respectively) (Fig. 1B). No progeny emerged at 7, 28 and 33°C, despite infectivity of $\geq 76\%$. The percentage of cadavers producing progeny was $> 90\%$ at 16 and 20°C, 84% at 23°C and 72% at 11°C (Fig. 1C). There was no mortality of waxmoth larvae in the control treatment at each test temperature. Temperature had a significant effect ($F = 333.6$; $df = 3,169$; $P < 0.05$) on the time until first nematode emergence after inoculation, with IJ observed outside the host $8.3 (\pm 0.4)$, $11.1 (\pm 0.7)$, $16.5 (\pm 0.6)$ and $44.8 (\pm 1.7)$ d after inoculation at 20, 23, 16 and 11 °C, respectively.

Freeze tolerance. At $-1 \pm 1^\circ\text{C}$ IJ survival was similar for all exposure times ($F = 1.82$, $df = 6,84$, $P > 0.05$); there was a significant effect of exposure time at $8 \pm 2^\circ\text{C}$ on IJ survival ($F = 5.81$, $df = 6,84$, $P < 0.05$) ($y = -0.04 + 95.9x$; $r^2 = 0.26$) although survival remained $> 90\%$ (Fig. 2). There was a significant effect of exposure time at $-1 \pm 1^\circ\text{C}$ on subsequent waxmoth infectivity ($F = 13.7$, $df = 6,84$, $P < 0.05$) ($y = -0.82 + 107.9x$; $r^2 = 0.77$), falling from 100% at 0 h exposure, to approximately 80% at 72 h and 0% after 120 h. Waxmoth infectivity by IJ stored at $8 \pm 2^\circ\text{C}$ was 100% throughout the study.

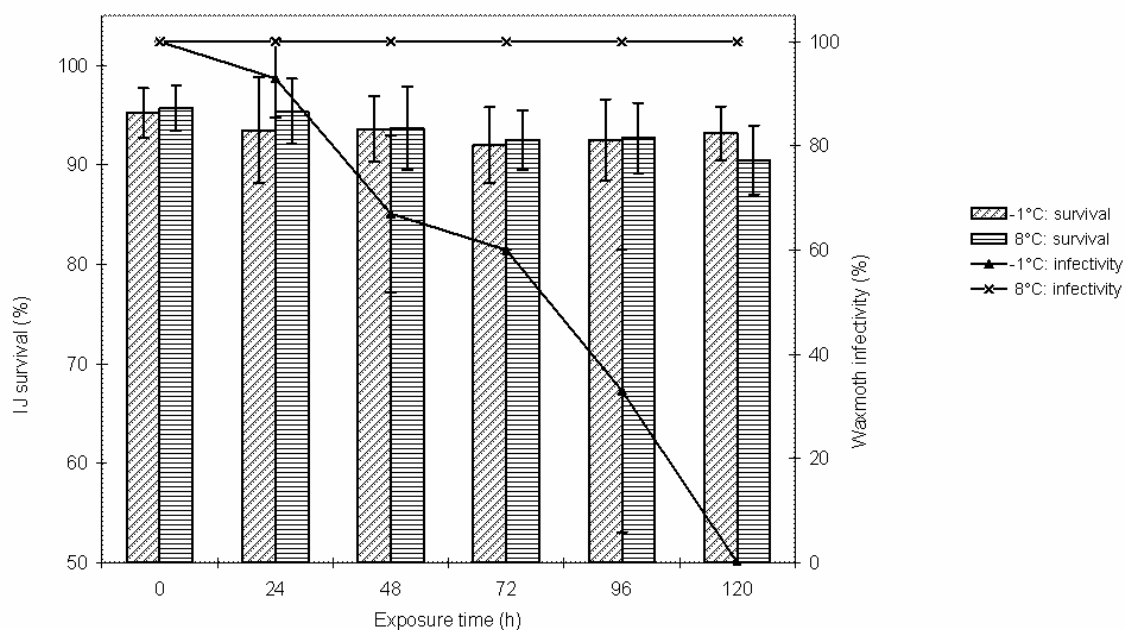


Fig. 2. Survival (%) of *Steinernema australe* IJ following exposure to $-1 \pm 1^\circ\text{C}$ and $8 \pm 2^\circ\text{C}$ for 0, 24, 48, 72, 96 and 120 h, and the subsequent infectivity of waxmoth larvae. Waxmoths were exposed at a concentration of 20 live IJ/larva, at $20 \pm 2^\circ\text{C}$ for 144 h. Bars and symbols ($\blacktriangle$ and $\times$) indicate means and vertical lines represent the 95% confidence intervals.

Table 2. Infectivity (%) of various insect species from Chile by *Steinernema australe* and subsequent EPN development. Insects were exposed at a concentration of 10 and 100 IJ/ insect for 9 d; IJ emergence from cadavers was monitored daily for 20 d. Stage: L = larva; A = adult. Means $\pm$ SE followed by the same letter within a column are not significantly different ($P > 0.05$).

Order	Family	Species	Stage	% Mortality		% Cadavers producing progeny	
				10 IJ	100 IJ	10 IJ	100 IJ
Lepidoptera	Pyrilidae	<i>Galleria mellonella</i>	L	83 $\pm$ 9.0 ^a	100 $\pm$ 0 ^a	39 $\pm$ 9.4 ^a	87 $\pm$ 1.7 ^{abc}
	Tortricidae	<i>Cydia pomonella</i>	L	55 $\pm$ 12.3 ^{abc}	79 $\pm$ 7.2 ^{ab}	59 $\pm$ 7.6 ^a	92 $\pm$ 1.6 ^{ab}
	Lymantriidae	<i>Orgyia antiqua</i>	L	49 $\pm$ 3.1 ^{abc}	95 $\pm$ 4.8 ^{ab}	46 $\pm$ 10.6 ^a	78 $\pm$ 2.8 ^{abcde}
	Leporidae	<i>Dallaca pallens</i>	L	-	19 $\pm$ 2.4 ^f	-	100 $\pm$ 0.0 ^a
	Pyrilidae	<i>Anagasta kuehniella</i>	L	51 $\pm$ 10.2 ^{abc}	91 $\pm$ 4.8 ^{ab}	42 $\pm$ 6.0 ^a	36 $\pm$ 2.9 ^{def}
Coleoptera	Scolytidae	<i>Phytolema hermanni</i>	L	13 $\pm$ 10.0 ^{cd}	40 $\pm$ 11.0 ^{cdef}	0 $\pm$ 0 ^b	28 $\pm$ 14.3 ^{ef}
	Scarabaeidae	<i>Brachysternus prasinus</i>	L	16 $\pm$ 1.0 ^{cd}	59 $\pm$ 4.8 ^{bcd}	32 $\pm$ 12.9 ^{ab}	49 $\pm$ 12.2 ^{bcd}
	Curculionidae	<i>Listronotus bonariensis</i>	A	4 $\pm$ 1.1 ^d	10 $\pm$ 6.3 ^f	0 $\pm$ 0 ^b	0 $\pm$ 0 ^f
	Curculionidae	<i>Aegorhinus nodipennis</i>	A	14 $\pm$ 7.2 ^{cd}	23 $\pm$ 8.4 ^{ef}	0 $\pm$ 0 ^b	33 $\pm$ 16.7 ^{def}
	Curculionidae	<i>Gonipterus scutellatus</i>	L	23 $\pm$ 9.4 ^{cd}	73 $\pm$ 6.8 ^{abc}	0 $\pm$ 0 ^b	14 $\pm$ 4.5 ^f
Hemiptera	Pseudococcidae	<i>Pseudococcus calceolariae</i>	A	37 $\pm$ 13.4 ^{bcd}	33 $\pm$ 6.4 ^{def}	0 $\pm$ 0 ^b	0 $\pm$ 0 ^f
	Coreidae	<i>Leptoglossus chilensis</i>	A	-	79 $\pm$ 11.5 ^{ab}	-	83 $\pm$ 11.8 ^{abcd}
Dermaptera	Forficulidae	<i>Forficula auricularia</i>	A	-	9 $\pm$ 5.9 ^f	-	17 $\pm$ 16.7 ^f
Hymenoptera	Tenthredinidae	<i>Caliroa cerasi</i>	L	72 $\pm$ 1.7 ^{ab}	73 $\pm$ 6.8 ^{abcd}	3 $\pm$ 2.8 ^b	42 $\pm$ 14.4 ^{cdef}

Effect of soil moisture. Results from the soil moisture study can be seen in Table 1. There were minor variations in MC in the tubes during the 72 h trial (Table 1). At 0.2% MC there was no infectivity of waxmoth larvae, for all other treatments infectivity was $\geq 87\%$ ($F = 115.2$, $df = 4,70$, $P < 0.05$). Control mortality was 0% for all treatments. There was a significant effect ($F = 41.3$, $df = 4,70$, $P < 0.05$) of MC on the number of IJ penetrating the host; highest penetration was 21 (± 1.1) IJ/ larva at 8.3% MC (approximately 42% of the original IJ inoculum level), falling to 9 (± 2.1) IJ/ larva (approximately 18% of original inoculum) at 15.8% MC.

Ambush capacity. There were no indications of an ability to attach to a mobile waxmoth larvae as no IJ were recovered from the insect cuticle after 20 min exposure. No IJ were seen nictating or jumping when on agar.

Laboratory host range. There were significant differences in corrected mortality among the target species at both 10 and 100 IJ/ target ($F = 8.92$, $df = 10,21$, $P < 0.05$ and $F = 19.53$, $df = 13,26$, $P < 0.05$ respectively) (Table 2). Infectivity $> 90\%$ was only observed in the Lepidoptera where *O. antiqua* and *A. kuehniella* suffered 95 and 91% mortality, respectively (interpretation excludes *G. mellonella*); infectivity $> 70\%$ was seen in all other orders apart from Dermaptera. A number of target species

showed relatively low levels of infection ($\leq 33\%$ at the highest IJ dose of 100), including four of the five species tested at the adult stage, viz., *F. auricularia*, *L. bonariensis*, *A. nodipennis* and *P. calceolariae*. Infection amongst the Coleoptera ranged from 10 – 73%, although emergence of new IJ never exceeded 50% of infected cadavers.

DISCUSSION

The results from this study provide baseline data on the biology and ecology of *S. australe*, thereby complementing the species description by Edgington *et al.* (2009). A new EPN species description, unless produced solely for taxonomic/systematic reasons, should be complemented by basic bionomic information as this will assist future researchers in selecting the most suitable EPN, optimising field efficacy and developing a suitable production process (see Grewal *et al.*, 2005).

The life-cycle of *S. australe* was relatively rapid, with IJ emergence observed after only 6 d post inoculation at 20°C. Other temperate *Steinernema* species emerged from waxmoth larvae after 8, 12 and 14 d at 20°C (Koppenhöfer & Kaya, 1999; Koppenhöfer *et al.*, 2000; Gungor *et al.*, 2006). There was no evidence of self-fertility, something that has only been recorded in *S. hermaphroditum*

(Stock *et al.*, 2004). It was not uncommon to see second generation males, females and non-infective juveniles of *S. australe* outside the cadaver, often moving into the water trap and surviving for several days. Adult emergence and migration away from the cadaver has been observed for *S. affine* (San-Blas pers. comm.), although it would appear to be a relatively rare characteristic for EPN. The IJ stage is morphologically and physiologically adapted to survive outside the host, possessing rich food reserves and being protected by the retained cuticle from the previous stage (O'Leary *et al.*, 1998; Griffin *et al.*, 2005), it is not clear whether adults and non-infective juvenile stages have suitable adaptations to survive for very long outside a host, although it would be surprising if they had.

The ability of *S. australe* to infect a host at 7°C may reflect an adaptation to its type-locality, a relatively cold island in southern Chile. Infectivity at cold temperatures can be a useful biological control feature. Steinernematid isolates sourced from cold localities in Canada and Scotland were infective at temperatures below 7°C (Mráček *et al.*, 1997; Long *et al.*, 2000), the Scottish isolate since becoming a successful commercial product for use against black vine weevil (*Otiorhynchus sulcatus* F.). The temperature profile of *S. australe* narrowed for development over infectivity, a feature not unusual for EPN (Molyneux, 1986; Koppenhöfer *et al.*, 2000; Gungor *et al.*, 2006). Infectivity has a wider window of opportunity than development and may also rely less on the proliferation of the symbiotic bacterium, itself restricted by lower temperatures (Wright, 1992). Indeed, EPN infectivity has been known to proceed in the absence of the symbiotic bacterium (see Ciche *et al.*, 2006). Steinernematids are found in some very cold localities including Arctic territories (see Haukeland *et al.*, 2006) where they are exposed to prolonged sub-zero temperatures. Infective juveniles of *S. australe* were able to survive 5 d at -1°C. Survival mechanisms of EPN to sub-zero conditions include the retained cuticle from the previous stage, the production of trehalose and cold-shock proteins (freeze-avoidance adaptations) or tolerance of a degree of tissue freezing (freeze-tolerance) (Glazer, 2002; Jagdale *et al.*, 2005). Ensheathed IJ of *S. australe* were used in the present study which may have enhanced their survival at -1°C. However, as exposure time to -1°C increased, the ability of *S. australe* to infect waxmoth larvae was substantially reduced, even at the near optimum temperature of 20°C. In similar studies, *Steinernema* and *Heterorhabditis* species both lost infectivity following exposure to sub-zero temperatures, the

reduction being attributed to sluggish movement and vacuolisation of the IJ (chilling-injuries) (Wharton & Surrey, 1994; Brown & Gaugler, 1998). Environmental factors are known to destabilise the symbiotic bacteria of EPN (Krasomil-Osterfeld, 1995), which in turn may reduce infectivity (Dowds & Peters, 2002); however, whether the bacteria of *S. australe* were sensitive to temperatures of -1°C is unknown.

Steinernema australe infected over a wide range of moisture levels. The high level of infectivity recorded at 15.8% MC may reflect the large body size of *S. australe* (IJ body length > 1300 µm, body diam. 38 µm), Koppenhöfer *et al.* (1995) suggesting that larger IJ are better adapted to move through thicker films of water as smaller nematodes may start floating. Establishment rates of *S. australe* at MC > 13% appeared slightly lower than *S. thermophilum* (now regarded as a junior synonym of *S. abbasi* (Hunt, 2007)) (Ganguly & Gavvas, 2004) and *S. monticolum* (Koppenhöfer *et al.*, 2000), both much smaller nematodes, although both these trials used shorter columns of substrate than the present trial and therefore their motility challenge would have been less. Infective juveniles active at a wide range of moisture levels could be of use in both badly drained (humid) soils and those experiencing considerable fluctuations in moisture levels, although desiccation tolerance of the IJ would be important as a soil dries out.

The natural host range of *S. australe* is unknown as this EPN was isolated from soil using waxmoth baiting. Reports on host ranges of EPN in their natural, non-agricultural habitats are relatively rare, most host range data generally originating from laboratory studies (and should be referred to as the 'laboratory host range') and/or insects collected from infested agricultural sites (see Peters, 1996). *Steinernema australe* infected a wide range of insect hosts in the laboratory, although there was clear host specificity. The laboratory host ranges of EPN are well documented. Temperate strains of *S. rarum*, *S. feltiae* and *S. monticolum* could be regarded as generalists with respect to insect order, infecting 11 insect orders between them, yet showing marked specificity at the insect family level (Koppenhöfer *et al.*, 2000; de Doucet *et al.*, 1999). Specificity is controlled by a combination of host finding, host acceptance and host suitability, which in turn relies on the host, EPN, bacterial symbiont and environmental conditions (Li *et al.*, 2007; Lewis *et al.*, 2006). During the ambush studies there were no indications that *S. australe* could attach to a mobile host and no nictation or jumping behaviour was observed. A similar result regarding attachment was

obtained for the cruise-forager *S. glaseri* (Koppenhöfer & Fuzy, 2003); Campbell & Kaya (2002) observed a lack of jumping behaviour for EPN species over 800 µm in length (*S. australe* body length > 1300 µm). An absence of nictation and/or jumping may preclude ambush foraging. During studies using inter-connected chambers filled with sand, *S. australe* was observed to travel at least 11 cm, in 48 h, in the absence of host cues (S. Edgington pers. obs.).

The results on the biology and ecology of *S. australe* presented here, whilst simple and to be treated with caution in making generalisations regarding behaviour in a field setting, can assist future researchers in assessing the use of this EPN as a control organism. The results complement both the formal description of *S. australe* and considerable EPN research being carried out by staff at INIA on other, locally sourced, EPN isolates as part of the national policy to find alternatives to chemical pesticides.

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S. Edgington, S. R. Gowen. Экологическая характеристика *Steinernema australe* (Panagrolaimomorpha: Steinernematidae) – энтомопатогенной нематоды из Чили.

Резюме. Приводятся результаты изучения недавно описанного вида энтомопатогенной нематоды *Steinernema australe* из Чили. В лабораторных условиях *S. australe* имеет довольно короткий жизненный цикл. Так, миграция инвазионных личинок (IJ) наблюдается при 20°C уже на 6-й день после заражения; при этом и взрослые нематоды, и питающиеся личинки также покидают труп насекомого. Пределы оптимальных температур и влажности для этих нематод оказываются достаточно широкими. Достаточно высокая степень заражения были получены при низкой температуре и высокой влажности (7°C и 15.8% содержания влаги). Инкубация при температуре около -1°C в течение 5 дней не влияла на выживаемость IJ, однако эффективность заражения хозяев такими личинками существенно снижалась. Предполагается, что причиной могло служить термическое повреждение симбиотических бактерий или воздействие низких температур на смену фаз у бактерий. *Steinernema australe* заражает широкий круг насекомых вредителей, отмеченных для Чили, включая высокоподвижные и малоподвижные формы, показывая при этом наивысшую эффективность против Lepidoptera. Для этого вида не отмечена способность IJ к прыжкам или никтации.

Assessment of genetic variability in population of *Ditylenchus destructor* (Thorne 1945) (Tylenchida: Anguinidae) from China

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Summary. *Ditylenchus destructor* is distributed widely in China and causes considerable yield losses of potatoes. Genetic diversity and variation of this species was analysed using Inter-Simple Sequence Repeats (ISSR) markers. Second-stage juveniles from 16 populations were analysed with three primer pairs and 32 fragments were considered for the analysis. Diversity levels within populations were relatively high. Cluster analysis and principle coordinate analysis grouped the majority of the nematode populations into three main clusters. At the regional level, the AMOVA indicated that about 91.4% variations in the data set were from genotypic variations within populations, 5.0% variations due to regional differences, and the remaining 3.6% due to differences among populations within regions. Low levels of genetic variation among populations suggested an extensive gene flow among them. Amphimictic mode in natural populations and passive dispersal of nematodes by anthropogenic activities and natural means would probably be responsible for the results observed. It was shown that the ISSR marker was an efficient method for detecting the genetic structure of *D. destructor* populations at a macro geographical level..

Key words: *Ditylenchus destructor*, genetic diversity, genetic variation, ISSR.

The nematode *Ditylenchus destructor* (Thorne, 1945) is an obligatory endoparasite species, able to grow in over 120 plant species and is the main pathogen affecting production of potato and sweet potato in most regions of China (Thorne, 1945; Yao & Cui, 2001). It belongs to the list of A2 pests regulated as quarantine pests in APPPC (Asia and Pacific Plant Protection Commission), COSAVE (Comite Regional de Sanidad Vegetal Parel Cono Sur) and China (Thorne, 1945; Hooper, 1973; OEPP/EPPO, 1978; Gubina, 1982; Esser, 1985). This species was first recorded in North America and has spread to about 52 countries of America, Europe, Asia, Africa and Oceania (Yao & Cui, 2001). *Ditylenchus destructor* was first detected in China infecting sweet potatoes in North China in 1937 (Ding & Lin, 1982). It was observed on other crops i.e. potato, pea, peanut (Liu et al. 2006), as well as on medicinal materials of the genera *Angelica* L. and *Mentha* L. etc. (Chen & Zhen, 1988; Zhang & Zhang, 2007). Now this nematode species is widely distributed in most sweet potato growing areas of China and causes considerable yield losses.

Despite their importance in ecosystems and for agriculture, the genetic structure of *D. destructor* populations is poorly known. Data on gene flow and the genetic structure of natural populations are scarce even for pests of the *Ditylenchus* genus. *Ditylenchus destructor* exhibits particular variability in many aspects. Populations from different hosts have shown different pathogenicity to the same or several other hosts (Ding & Lin, 1982; De Waele, 1989). The isolates of *D. destructor* from *Hyacinthus orientalis* could not infect sweet potato successfully. However, it cannot be classified as a different physiological race as originally was *D. dipsacii*. Variations in susceptibility of different populations to different types of nematicides have also been detected (Ding, 2007). Insecticide resistance level of Funing, Zuo Zhou and Lulong populations to aldicarb emulsifiable concentrate was twice to four times than that of the Changli population in Hebei province of China. Furthermore, genetic variability has been demonstrated using the D2D3 region of the ribosomal DNA (rDNA-D2D3); there are more than

10 bases that differ among 22 populations (Yu, 2008). High levels of variation were also detected by analysis of the internal transcribed spacer region of the ribosomal DNA (rDNA-ITS) (Wang *et al.*, 2007; Wan *et al.*, 2008; Zhang & Zhang, 2008). The sequence length of rDNA-ITS region was about 900 bp in A type populations but about 1100 bp in B type. Based on the variation of rDNA-ITS regions, several molecular detection methods were developed to identify two different types of this species (Liu *et al.*, 2007; Wan *et al.*, 2008).

The degree of genetic differentiation among local populations of a species largely depends on the magnitude of gene flow and other processes such as mutation, genetic drift, and locally differing selection pressure occurring independently in each subpopulation (Lax *et al.*, 2007). The use of population genetics to estimate dispersal provides additional information concerning the inbreeding, the genetic structure and the level of gene flow at various scales (Picard *et al.*, 2004). Thorough knowledge of the population structure of plant-parasitic nematodes is essential to develop efficient control strategies (Hyman, 1996). For pest species, measurement of dispersal ability is of crucial importance to formulating control methods to limit the economic losses in agriculture (Lenormand & Raymond, 1998; Carrière *et al.*, 2003). The Inter-Simple Sequence Repeats (ISSR) PCR technique has been developed to study genetic diversity in natural populations (Zietkiewicz *et al.*, 1994; Metge & Burgermeister, 2006; Lax *et al.*, 2007). This technique is rapid as well as sensitive, and capable of differentiating between closely related individuals. ISSRs take advantage of simple sequence repeats (SSR) or microsatellites, which are abundant in all eukaryotic genomes. Unlike SSR markers, the ISSRs do not require any prior knowledge of the genome sequence (Zietkiewicz *et al.*, 1994). This method is similar to RAPD-PCR and provides similar genomic information. However, ISSR markers may have certain advantages over RAPDs to assess genetic variation within and among population of the same and closely related species (Wolfe & Liston, 1998; Subbotin *et al.* 1999; Crawford *et al.*, 2001; Amiri *et al.* 2003; Ou *et al.*, 2008). ISSR primers are longer and have higher annealing temperatures, which results in greater band reproducibility than RAPD markers (Culley & Wolfe, 2001); they also reveal high genetic variability. ISSR markers have great potential for studies of natural populations and have been useful in the study of the population structure of many plant-parasitic nematodes, entomopathogenic nematodes and some crops with

resistance to nematodes (Zietkiewicz *et al.*, 1994; Berner & Schnetter, 2002; Metge & Burgermeister, 2006; Lax *et al.*, 2007; Dayteg *et al.*, 2008).

The objective of this work was to analyse the levels of genetic variability in Chinese populations of *D. destructor* from different geographic regions and to evaluate the degree of genetic difference among them using ISSR molecular markers.

MATERIAL AND METHODS

Nematode populations. Sixteen populations of *D. destructor* from different geographic regions were considered for this study (Table 1, Fig. 1). In autumn 2007, 10 soil samples of *ca* 3 kg each were random taken from an infested sweet potato field at each site and mixed together in a plastic case separately (except for the locality of KOSE, where 10 garlic samples were taken from the infested garlic intercepted by Shenzhen Entry-Exit Inspection and Quarantine Bureau of China). Second-stage juveniles (J2) were extracted from 200 g of soil by the Baermann's method. All populations were identified as *D. destructor*, according to the description of Thorne (1945) and morphological/morphometrical characterisations of other populations of the species (Thorne 1945; Ding & Lin, 1982). For nematode multiplication under laboratory conditions, 200 J2 of *D. destructor* were sterilized in 0.05% penicillin and 0.5% chloramphenicol solution for 30 min, and then the populations were cultured for 45 d on a colony of *Fusarium semitectum* Brek. & Rav. at 25°C in the dark. Then nematodes were transferred to another colony of *F. semitectum* to maintain populations until DNA extraction was conducted.

DNA Extraction. For each population, genomic DNA was isolated from individual nematode and fifteen individuals were used in total. Each specimen was crushed with a glass pestle inside the tube after it was immersed in liquid nitrogen for 30 s. Then 32 µl Worm Lysis Buffer (WLB, 500 mM KCl, 10 mM Tris-HCl, 15 mM MgCl₂, 1.0 mM DTT, 4.5% Tween20) and 8 µl Proteinase K (20 mg ml⁻¹) were added to each tube. The tubes were incubated at 65°C for 2 h, followed by 10 min at 94°C in a Master cycler 5332 PCR thermal sequencer (Eppendorf). DNA extracted from each nematode was kept at -20°C until use.

ISSR-PCR amplification. Several DNA and primer concentrations used in the reaction mix, number of cycles, annealing temperature and evaluation of multiple 2 µl aliquots from a single juvenile DNA extraction were tested to optimise PCR reaction conditions and guarantee the repeatability of the amplified products obtained. A

total number of 35 ISSR primers were tested (Zietkiewicz *et al.*, 1994; Tikunov *et al.*, 2003; Bornet & Branchard, 2001); those that produced clear, reproducible and polymorphic bands were selected for the analysis. Each ISSR reaction was carried out in a total volume of 25 μ l containing: 2 μ l of DNA, 2.5 μ l 10 $\times$ reaction buffer (500 mM KCl, 15 mM MgCl₂, 100 mM Tris-Cl, pH 9.0), 2.5 mM MgCl₂, 0.2 mM of each dNTP, 15 ng of primer and 1 U of Taq DNA Polymerase (TaKaRa Biosciences, China). PCR reactions were programmed for an initial denaturation at 94°C for 5 min, followed by 38 cycles of 30 s at 94°C, 45 s at 52°C, 1 min at 72°C, and a final extension of 10 min at 72°C. Negative controls were added in all assays to discount contamination. A positive control containing DNA at a concentration of 10 ng μ l⁻¹ of an individual of the plant species *Lycopersicon esculentum* Miller (Solanaceae) was used in each reaction. Its amplification products were known and gave high repeatability for the ISSR primers selected (Tikunov *et al.*, 2003). This control allowed us to verify the repeatability of the bands obtained and to check that the amplification experiment developed normally.

The PCR products were separated electrophoretically on 2% agarose gels in 1 $\times$ TAE buffer. DL2000 DNA Ladder (TaKaRa) was used as the molecular weight marker. Gels were stained with ethidium bromide and photographed with Gel Doc XR image analysis system (Bio-Rad).

Data analysis. Fifteen juveniles from each population were used for the analysis. Only bands that were clear and reproducible were included in the study. Amplified bands were scored as 1/0 (presence/absence) of homologous bands for all samples. The resulting presence/ absence data matrix was analyzed using POPGENE version 1.32 (Yeh *et al.* 1999) to estimate the level of genetic diversity by the percentage of polymorphic bands (PPB), average heterozygosity (H) and Shannon Information Index (I). Isolation by distance was investigated to estimate the correlation between geographical and genetic distance of the populations studied using a Mantel's test and a rank correlation coefficient (Mantel, 1967). In order to investigate the partition of genetic variation within and among populations, ARLEQUIN software (version 1.1) (Schneider *et al.*, 1997) was used to carry out analysis of molecular

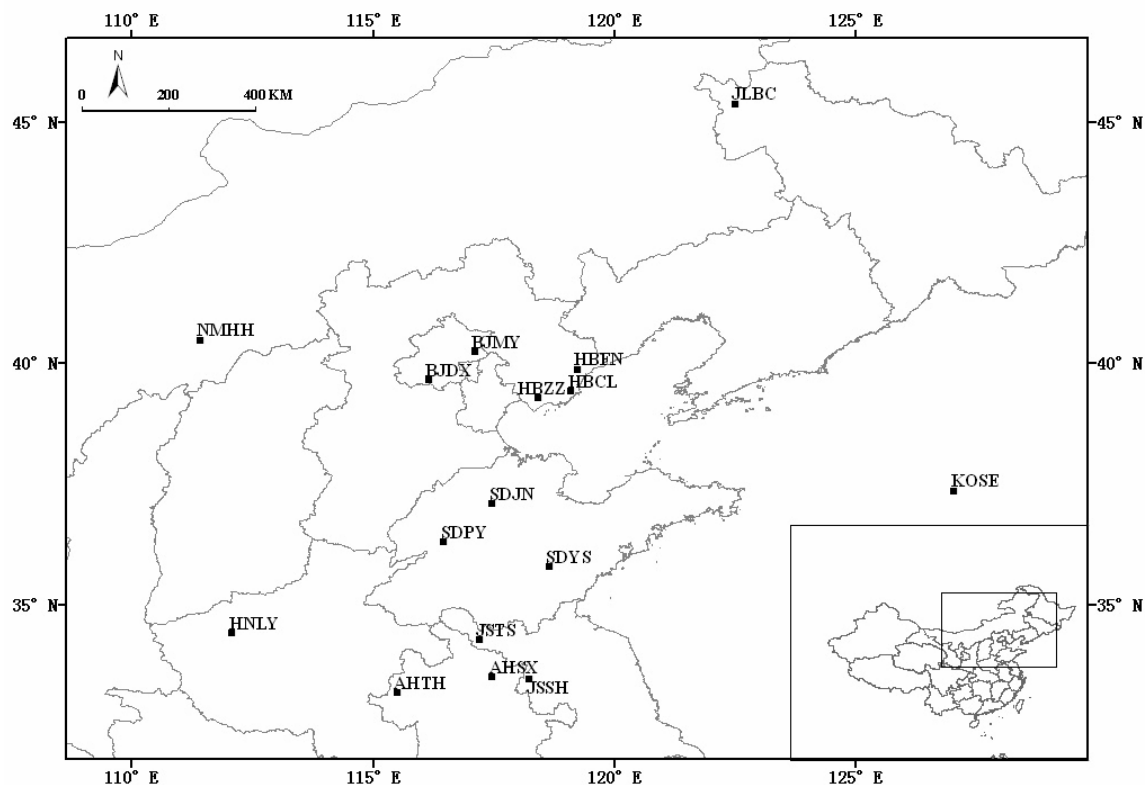


Fig. 1. Geographic location of the 16 *Ditylenchus destructor* populations from China obtained during 2007. For locality code information see Table 1. Altitude, latitude and longitude data of each location were recorded by a GPS locator.

Table 1. Location and genetic diversity of the 16 *Ditylenchus destructor* populations

Code	Province of origin	Locality	Altitude (m)	Longitude (E)	Latitude (N)	PPB (%)	Average heterozygosity (H)	Shannon Information Index (I)
HBZZ	Hebei	Zuozhou	17	118.41	39.28	81.3	0.2603	0.3975
HBCL	Hebei	Changli	32	119.10	39.42	68.8	0.1978	0.3042
HBFN	Hebei	Funing	76	119.22	39.88	68.8	0.2082	0.3130
BJDX	Beijing	Daxing	21	116.15	39.66	71.9	0.2169	0.3380
BJMY	Beijing	Miyun	68	117.10	40.24	75.0	0.2350	0.3620
SDYS	Shandong	Yishui	163	118.64	35.78	78.1	0.2344	0.3601
SDJN	Shandong	Jinan	54	117.46	37.09	75.0	0.2156	0.3384
SDPY	Shandong	Pingyin	212	116.46	36.29	78.1	0.2606	0.3964
JSSH	Jiangshu	Sihong	47	118.23	33.46	75.0	0.2504	0.3823
DDTS	Jiangshu	Tongshan	149	117.2	34.26	78.1	0.2650	0.4019
AHTH	Anhui	Taihe	26	115.49	33.17	78.1	0.2735	0.4123
AHSX	Anhui	Sixian	38	117.46	33.49	78.1	0.2532	0.3865
NMHH	Neimenggu	Huhot	1075	111.41	40.48	78.1	0.2523	0.3864
JLBC	Jilin	Baicheng	662	122.50	45.38	75.0	0.2335	0.3618
HNLY	Henan	Luoyang	329	112.07	34.40	71.9	0.2227	0.3439
KORE	Korea	Seoul	263	127.03	37.35	71.9	0.2301	0.3512
Average						75.2	0.2381	0.3647
Species						87.5	0.2769	0.4242

variance (AMOVA) with Euclidean distance matrices (ϕ_{ST}) from ISSR profiles. Pairwise F_{ST} estimates between different populations were also obtained with this program. Unweighted pair group arithmetic average (UPGMA) clustering was performed with the Phylip program, version 3.6, to describe the genetic relationship among the 16 *D. destructor* populations and dendrograms were created (Felsenstein, 2001; Rohlf, 2002). The robustness of the dendrogram was tested by bootstrapping with 1000 permutations. Results of all bands were also pooled into a principle coordinate analysis (PCO) with the Multi-Variate Statistical Package (MVSP) 3.0 (Kovach Computing Services 2005) in the 16 populations. Thus, the genetic variability of sampled populations was summarised into a few major components (e.g. PCO1, PCO2). The PCO on the data was considered as a check of the clusters formed by the cluster analysis.

RESULTS

Genetic diversity. Primers that could not amplify polymorphic bands in all populations or did not amplify polymorphic bands clearly enough were discarded. Sequences of the single non-anchored

and two anchored primers selected to amplify ISSR in all individuals are shown in Table 2. High polymorphism and genetic variability was observed using the selected primers (Fig. 2). The number of markers scored per primer ranged between 9 and 13. No population-specific bands were detected. At the species level, the average PPB was 87.5%, heterozygosity (H) was 0.2769 and Shannon information index (I) was 0.4242 (Table 1). Within populations, the PPB varied from 68.8% for population HBCL to 81.3% for population HBZZ, and the mean H was 0.2769, ranging from 0.1978 for population HBCL to 0.2735 for population AHTH. The I value showed similar trends, ranging from 0.3042 for HBCL to 0.4123 for AHTH.

Table 2. Sequence of ISSR primers used for the analysis of *Ditylenchus destructor* populations from China and number of polymorphic bands scored

Primer	Sequence(5'→3')	Number of loci scored
ISSR1	(AC) ₈ G	9
ISSR2	(GACA) ₄	13
ISSR3	(AC) ₁₀ AA	10

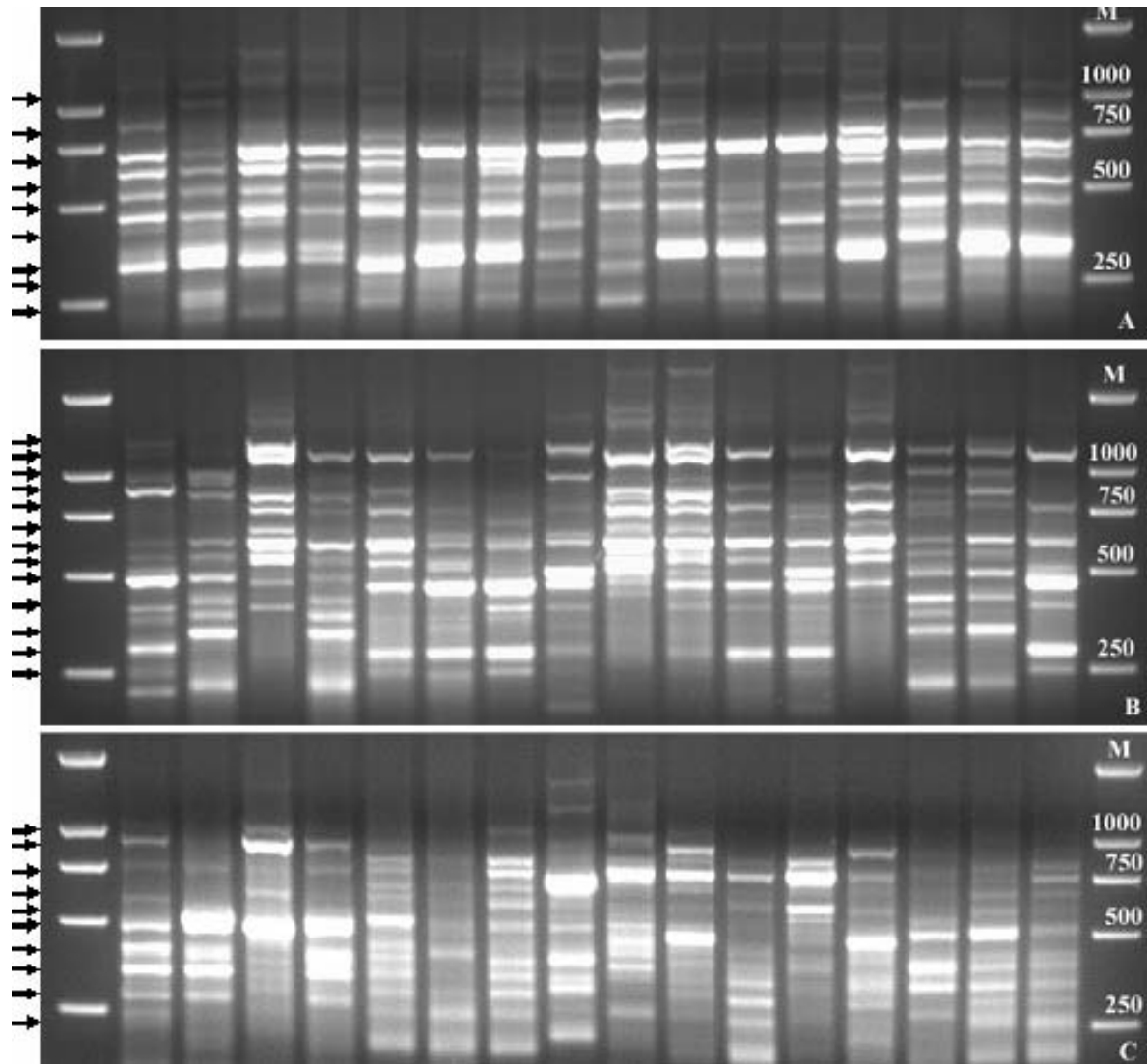


Fig. 2. ISSR bands in individual specimen of *Ditylenchus destructor* of different populations to indicate the selected bands (arrows) for the polymorphism analysis. A: Primer ISSR1; B: Primer ISSR2; C: Primer ISSR3. D: Primer could produce clear polymorphic bands in all populations.

Table 3. Hierarchical analysis of molecular variance (AMOVA) of the 16 *Ditylenchus destructor* populations in China.

Source of variation	d.f.	Sums of squares	Variance Component	Total variance (%)	P-value
Among populations	15	107.69	0.28	8.1	0.001
Within populations	224	703.40	3.14	91.9	0.001
Among regions	8	56.26	0.12	3.6	0.001
Among populations within regions	7	51.43	0.17	5.0	0.001
Within population	224	703.40	3.14	91.4	0.001

Genetic variation. Low levels of genetic variations were observed among *D. destructor* populations of different regions. F_{ST} values comparing pairs of populations ranged between 0.004–0.087, HBCL–NMHH ($F_{ST} = 0.087$), HBCL–JSTS ($F_{ST} = 0.082$) being the most different and HBFN–HBCL the most similar ($F_{ST} = 0.004$). Results of the cluster analysis among populations, based on their pairwise F_{ST} estimates, are shown in Fig. 3. Cluster analysis grouped the majority of the 16 *D. destructor* populations into three main clusters, which corresponded to their geographic distributions. Cluster I comprises the KORE, SDYS, JSSH and JSTS populations, which belong to the B type as classified by the rDNA-ITS region. Populations of A type classified by the rDNA-ITS region were divided to cluster II and cluster III. Cluster II comprises the JLBC, HBCL and HBFN populations, the remaining populations of the A type of rDNA-ITS region are more diverse and belongs to cluster III. However, the NMHH population

appeared clearly separated from the rest of the Chinese populations. Principal Coordinate Analysis (PCO) of the data set explained 27.9% and 16.8% of the total phenotypic variance along the first and second axes, respectively, implying a similar relationship among populations (Fig. 4). This confirmed the results obtained with the phenogram and the quantification of intra-population diversity. Mantel test showed a low positive correlation ($R^2 = 0.344$, $P = 0.009$, $t = 1.577$) between pairwise genetic and geographical distances (Fig. 5).

The Hierarchical AMOVA revealed small genetic divergence among populations from different locations. It further showed that the majority of genetic variation (91.9%) existed within populations, whereas small genetic variation (8.1%) occurred among populations. At the regional level, the AMOVA indicated that about 91.4% of the variations were genotypic variations within populations, 3.6% of the variations were due to regional differences, while the remaining 5.0% were due to differences among populations within re

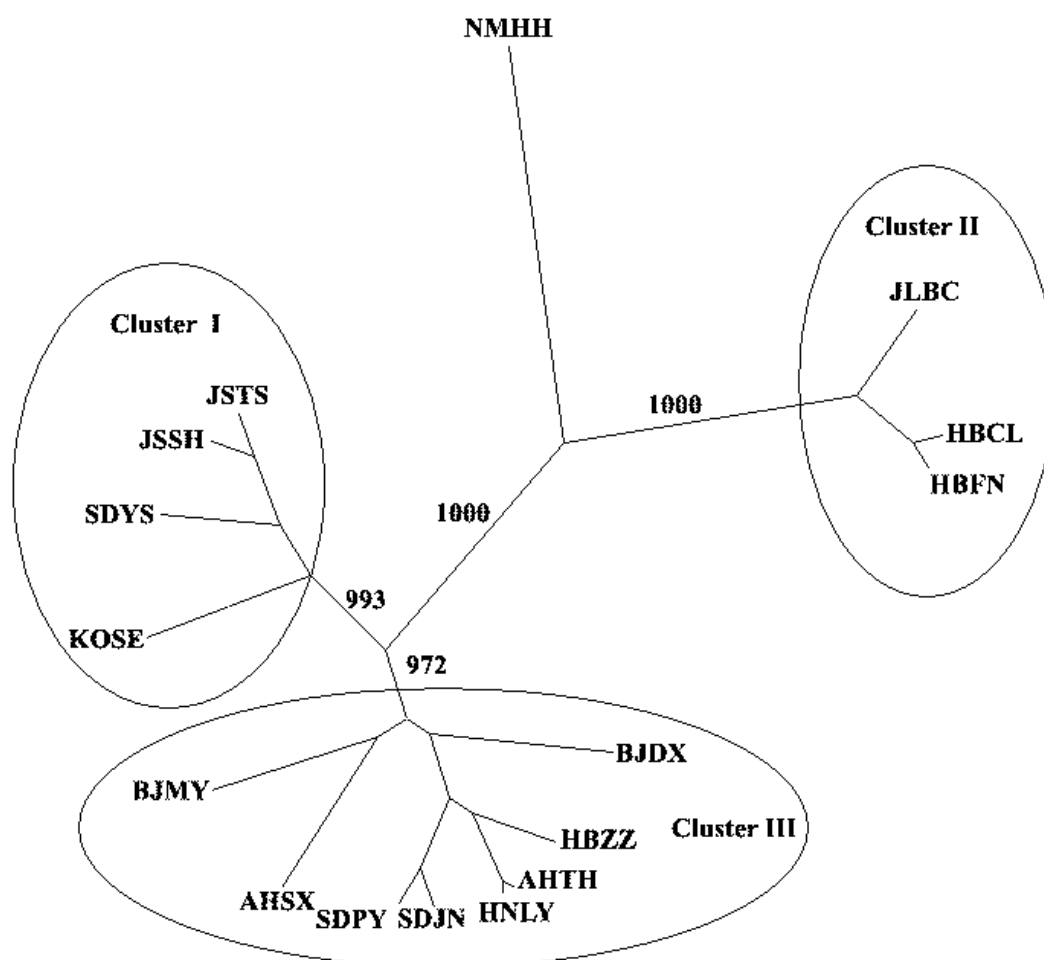


Fig. 3 Relationships among *Ditylenchus destructor* populations based on their pairwise F_{ST} values. UPGMA clustering was constructed using the Phylip program, version 3.6. Only bootstrap values higher than 950 are shown.

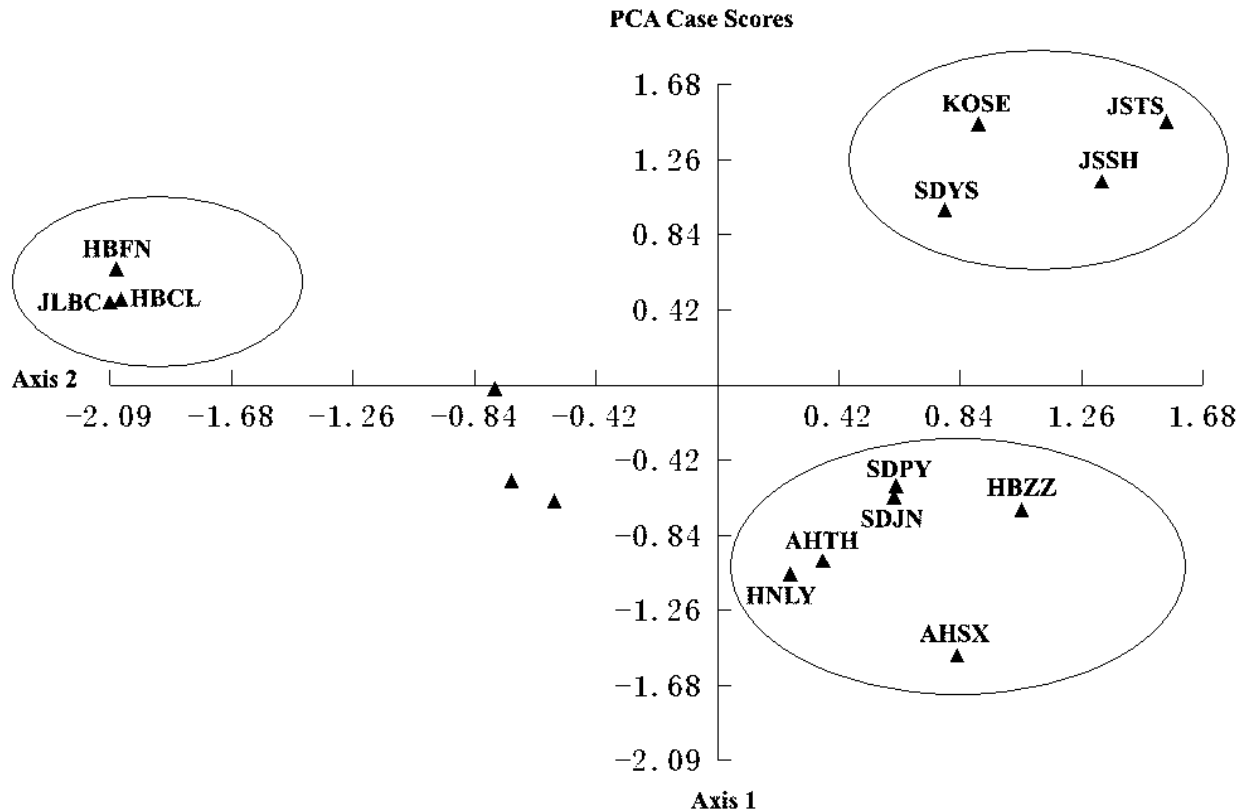


Fig. 4. Principal Coordinate Analysis (PCO) based on Euclidean distance among the 26 *Ditylenchus destructor* populations in China. PCO axis 1 and 2 accounted for 44.7% of the overall variation.

gions (Table 3). Despite the differences in proportion of the total variance, values for all three hierarchical levels were significantly different. This suggests that significant gene flow occurs among populations and even regions.

DISCUSSION

The application of the ISSR technique to detect intra-specific variation in nematodes and the usefulness of this technique in determining population genetic variation of some nematode species has been demonstrated (Berner & Schnetter, 2002; Metge & Burgermeister, 2006; Lax *et al.*, 2007). ISSR markers have been shown to provide useful information for resolving phylogenetic relationships among closely related species and relationships at or below the species level (Mort *et al.*, 2003). The present work is the first study of the genetic structure of *D. destructor* populations at a macrogeographical level using ISSR markers. At the species level, genetic variation within population of *D. destructor* is higher than that of *Heterodera glycines* and *Bursaphelenchus xylophilus* using

RAPD markers (Zhang *et al.*, 1998; Vieira *et al.*, 2007). However, higher genetic variation was detected within fields for *H. schachtii* (Plantard & porter, 2004; Madani *et al.* 2007), *Nacobbus aberrans* (Lax *et al.*, 2007) and *Globodera pallida* using different molecular markers.

Genetic similarity index is a reliable index that allows evaluation of genetic variation level among different individuals. In the present study, the Shannon genetic information index (I) was between 0.3042 and 0.4123, meaning that genetic diversity of *D. destructor* was very high. One of the possible sources of the high level of polymorphism found in *D. destructor* populations could be related to the mode of reproduction of the nematode. In the study based on the results obtained for four physiological races of *D. destructor*, Smart & Darling (1963) indicated that amphimixis is the only reproduction mode for this species. Anderson & Darling (1964) hypothesised that *D. destructor* secrete attractants that probably serve to bring nematodes of opposite sexes together prior to mating. They observed that a single male would mate with the same female if contact was made again and occasionally several males

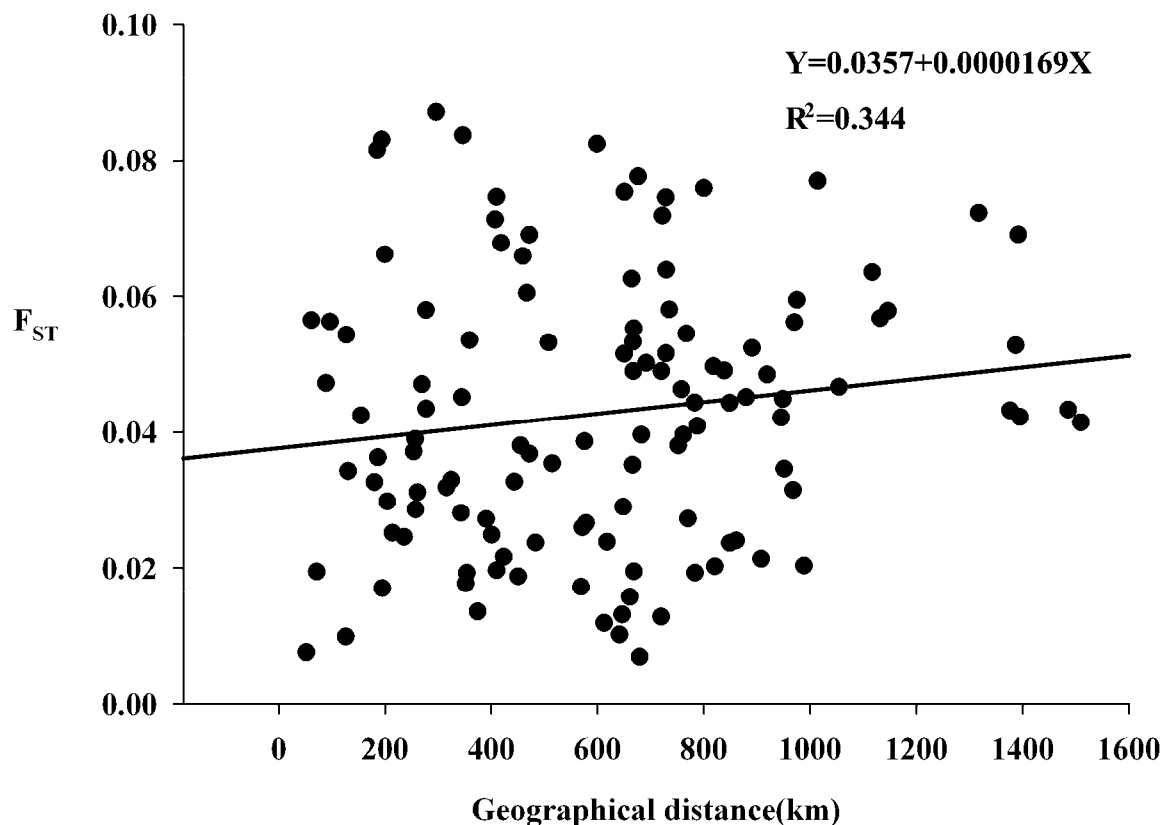


Fig. 5. Plot of pairwise F_{ST} values between populations of *Ditylenchus destructor* against geographical distance

would attempt to mate with the same female at the same time in the laboratory, which might be an indicator of multiple mating of *D. destructor*. In the same field, Wan *et al.* (2008) detected two different types of *D. destructor* rDNA-ITS region. This may be evidence of multiple mating in natural populations of this species. The multiple mating modes would favour genetic diversity among the offspring of *D. destructor*. In addition, the relatively high genetic diversity within populations may have resulted largely from multiple introductions of *D. destructor* from different origins, which might helped this species avoid the founder effect (Da Conceição *et al.*, 2003; Plantard *et al.*, 2008).

Ditylenchus destructor exhibits low genetic differentiation among populations and regions despite the great geographic distances (even 1500 km apart between HNLY and JLBC) that separate some of them, suggesting significant gene flow at these spatial scales. Because of their small size, active dispersal is probably limited to a few centimeters or decimeters. The nematodes can move only short distances in the soil and have no natural means of long-range movement. Juveniles from the same egg mass are, respectively, full or half-sibling following fertilisation of the female by one or

several males. Although males are slightly larger than J2, their active dispersal is also likely to be limited. This life cycle and behaviour should thus favour mating between siblings and enhances the production of homozygotes (Plantard & Porte, 2004). Even if the migration is very low, gene flow could occur and thus prevents genetic differentiation among populations. For *C. elegans*, which is also a soil nematode but a free-living one feeding on bacteria, Koch *et al.* (2000) suggest long-range dispersal to explain the weak genetic differentiation of this species at the global scale. Moreover, such gene flow could be due to passive transport of nematodes within and among fields by human activities (e.g. transport of soil by farm machinery, sewage farms around sweet tomato factories) or by water (flood, irrigation or drainage) (Plantard & Porte, 2004). In addition, infected root tubers and seedlings are important means of dissemination of this nematode. Third- (J3) and fourth-stage juveniles (J4) and immature females may be found on root tubers and seedlings of sweet potato (Anderson & Darling, 1964; Ding & Lin, 1982). They are sources of inoculum when infected seedlings are planted in sweet potato fields. These dispersal mechanisms would favour the maintenance of high effective

population sizes, which would contribute to increase the genetic diversity of natural populations of this species (Lax *et al.*, 2007).

The AMOVA performed with three variance components revealed that 3.6% of the total variation detected in *D. destructor* population using ISSR loci corresponded to significant differences among regions defined on the basis of their geographic origin. At the population level, the present results revealed that genetic variability within populations with ISSR loci (91.4%) in *D. destructor* was higher than that among different populations (5.0%). These results support the fact that there was some degree of genetic similarity among populations from the same region and would confirm the existence of different races or biological entities within the species (Plantard & Porte, 2004; Picard *et al.*, 2004). It has been reported that within particular nematode populations, some characteristics, such as host preference and aggressiveness, might be determined by alleles that vary in their frequencies (Triantaphyllou, 1987). Even the gene frequencies could change with time in response to host-induced selection (Kaplan *et al.*, 1999). This may have important consequences for the evolution of virulence in *D. destructor*. Indeed, in a classical gene-for-gene relationship with a dominant resistance gene, only homozygous virulent individuals are able to overcome the resistance gene. If a mutation in the virulence-avirulence gene changing the avirulence allele into a virulence allele occurs at a low frequency, mating between siblings will facilitate the association of two virulence alleles in the same individual. Thus, inbreeding induced by such a behaviour should be included in models of virulence evolution in phytoparasitic nematodes (Schouten, 1997; Plantard & Porte, 2004).

In this work, it was possible to define populations of *D. destructor* populations according to their genetic divergence using ISSR markers. Other molecular methods, such as analysis of the rDNA-ITS and rDNA-D2D3 regions, also showed affinities between populations of the species from the same country of origin (with some exception) (Liu *et al.* 2007; Wan *et al.* 2008; Yu, 2008). Sixteen populations of *D. destructor* were grouped to three main clusters using the ISSR marker, which corresponded to their characteristics from analysis of rDNA-ITS regions. The divergence of *D. destructor* also agrees with the results previously obtained by analysing the rDNA-D2D3 regions of 22 populations of *D. destructor* in China (Yu, 2008). These results would confirm the existence of two or three different races or biological entities within the species (Plantard & Porte, 2004; Picard *et*

al., 2004). However, the NMHH population appeared clearly separated from the other Chinese populations using ISSR markers. This population is located to the north of Yinshan mountains, the altitude of which is about 1500 m. Because of these geographic characteristics, the nematodes of this population are very unlikely to have a genetic exchange with the other populations. Given the clear genetic differentiation between NMHH and the other populations studies, it would be interesting to conduct a similar study to make a genetic comparison between that population and others.

The ISSR markers revealed high genetic diversity from different localities but low level genetic variation among different regions in *D. destructor* populations of China. It was shown that the ISSR marker was an efficient method for detecting genetic variation among the different geographic populations of this species. Further studies of *D. destructor* using the molecular markers would contribute to elucidate the taxonomic statuses and may assist in developing effective control measures for different biological entities of this species.

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Wen-Kun Huang, De-Liang Peng, Dong-Sheng Zhang, Hong-Yun Jiang, Zhong Ding, Huan Peng, Hai-Bo Long. Оценка генетического разнообразия в популяциях *Ditylenchus destructor* (Thorne 1945) (Tylenchida: Anguinidae) в Китае.

Резюме. Нематоды *Ditylenchus destructor* широко распространены в Китае и вызывают значительные потери урожая картофеля. Генетическое разнообразие и изменчивость этого вида исследовали с использованием метода ISSR-маркеров (Inter-Simple Sequence Repeats). Личинки 2-й стадии из 16 популяций исследовали с использованием трех пар праймеров, что позволяло проводить анализ 32 фрагментов ДНК. Уровень генетического разнообразия был довольно высоким. Кластерный анализ и метод основных координат подразделяли изученные популяции на три основные группы. На региональном уровне метод AMOVA показал, что около 91,4% наблюдаемой изменчивости связано с различиями генотипов в пределах популяций, 5,0% вариабельности определяется региональными различиями и остальные 3,6% определяются различиями между популяциями в пределах каждого из регионов. Невысокий уровень генетической изменчивости между популяциями предполагает значительный обмен генами между ними. Амфимиктическое размножение в природных популяциях и пассивный разнос за счет деятельности человека, а также естественные причины, вероятно, определяют наблюдаемую картину. Показано, что ISSR-маркеры представляют собой эффективный метод выявления генетической структуры популяций *D. destructor* на уровне географически удаленных популяций.

Response of roots of different plants to the presence of the false root-knot nematode *Nacobbus aberrans*

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Summary. *Nacobbus aberrans* is native to the American continent and produces severe damage to several crops. The response of roots of potato (*Solanum tuberosum*), tomato (*S. lycopersicum*) and weed quinoa (*Chenopodium album*) inoculated with nematodes from the localities Coronel Baigorria (CB) and Río Cuarto (RC) (province of Córdoba, Argentina) was evaluated. Quantitative parameters (number of galls and Gall Index) and qualitative parameters (histological studies that evaluate alterations in root tissues) were determined. The populations differed in their capacity to invade roots. Neither was able to infect potato; the most efficient hosts for CB and RC were quinoa and tomato, respectively. At the histological level, potato did not show symptoms of nematode attack. Hyperplastic tissue in the central cylinder with transformed cells forming syncytia along vascular cells was present in galls of tomato and quinoa. While each population showed preference for a single plant, the histological analyses did not reveal differences between the alterations induced by a single population on the two infested plants, but they did reveal between-population differences in the response of tissues of tomato and weed quinoa to the parasite attack.

Key words: Argentina, false root-knot nematode, histology, plant-parasite relationships, potato, tomato, weed quinoa.

The false root-knot nematode *Nacobbus aberrans* is native to the American continent (Sher, 1970); up to the present the species has been recorded in Argentina, Bolivia, Chile, Ecuador, Mexico, Peru and USA (Manzanilla-López *et al.*, 2002). It is a quarantine organism and is subject to strict pest regulations in various parts of the world (OEPP/EPPO, 1984). In Argentina, the species distribution has expanded significantly since it was first detected in 1977 (Costilla *et al.*, 1977), affecting several horticultural crops, both in the field and in glasshouse conditions (Doucet & Lax, 2005).

Nacobbus aberrans is an endoparasite of roots that induces galls in the tissues of infested plants. It has been cited as a parasite of about 84 plant species belonging to 18 families (Manzanilla-López *et al.*, 2002). Some of its populations exhibit a wide host range (Inserra *et al.*, 1985; Costilla, 1990; Doucet & Lax, 2005).

The nematode induces a number of cellular and histological alterations on infected roots, causing the formation of galls where the parasite feeding site (syncytium) develops. Several histopathological studies have been conducted in different crops, such as potato (*Solanum tuberosum*) (Finetti Sialer, 1990), sugarbeet (*Beta vulgaris*) (Inserra *et al.*, 1983, 1984), tomato (*S. lycopersicum*) (Doucet *et al.*, 1997; Lorenzo *et al.*, 2001; Vovlas *et al.*, 2007), pepper (*Capsicum annuum*) (Lorenzo *et al.*, 2001), eggplant (*S. melongena*) (Doucet *et al.*, 1997) and weeds (Doucet & Ponce de León, 1985; Ponce de León & Doucet, 1989; Tovar *et al.*, 1990; Doucet *et al.*, 1994, 1997, 2005). Evaluations to compare possible differences in the alterations induced by the same population among different plants (Moyetta *et al.*, 2007; Tordable *et al.*, 2007) or between populations on a single host (Tordable *et al.*, 2007) are scarce.

The aim of this work was to analyse the plant-nematode relationship in roots of three plant species inoculated with second-stage juveniles of two Argentine *N. aberrans* populations.

MATERIAL AND METHODS

Nematode populations and plant material.

Nacobbus aberrans populations were obtained from two localities of the department of Río Cuarto (province of Córdoba, Argentina) that are 33 km apart: Coronel Baigorria (CB) and Río Cuarto (RC). CB nematodes were obtained from the weed quinoa (*Chenopodium album*) where it occurs naturally; RC nematodes were extracted from infested pepper roots from a glasshouse in the locality of origin. To obtain the inocula, roots of infested plants were gently washed to remove adhering soil particles. Nematode egg masses present in the galls were extracted under a stereoscopic microscope and placed in Petri dishes with distilled water at room temperature to favour egg hatching. Mobile second-stage juveniles (J2) were recovered with a micropipette and concentrated in a test tube.

Seeds of tomato cv. Platense and weed quinoa were germinated in trays with sterile soil. Seedlings at four-leaf stage were placed individually in plastic containers with 50 g of soil and vermiculite (1:1). Roots were arranged horizontally on this substrate and 100 J2 1.5 ml⁻¹ of water were inoculated with a micropipette. Another 50 g of that substrate was then added to cover the roots. Potato cv. Spunta was obtained by *in vitro* multiplication of plants originated from meristem culture (Roca & Mroginski, 1993). Plants were planted in pots containing sterile soil and vermiculite (1:2) and maintained at 21°C for 15 days to favour development of the root system. After that period, they were transplanted to plastic pots containing 150

g soil and vermiculite (1:1) and the same number of J2 were inoculated following the procedure mentioned above. Six replications per plant were performed. The experiment was conducted at a mean temperature of 21 ± 3°C and a 14-h photoperiod. After 90 days of inoculation, plants were extracted and roots were washed free of adhering mineral particles.

Estimation of Gall Index and statistical analysis. The number of galls (NG) induced by the nematode was counted by observing the roots of each plant under a stereoscopic microscope. Gall Index (GI) was estimated based on a 0 to 5 scale proposed for *Meloidogyne* spp., where: 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, and 5 = more than 100 galls per root (Hartman & Sasser, 1985). Data of NG and GI were transformed into log₁₀ (x+1) and subjected to an analysis of variance ($P \leq 0.05$).

Histological studies. Healthy (without galls) and infected (with galls) roots were cut into small segments of about 5 mm in length and fixed in FAA. Then they were dehydrated in a series of ethyl alcohol and xylene baths and embedded in histowax. Serial transverse and longitudinal sections 8 to 10 µm thick were obtained with a rotary microtome. Sections were stained with triple staining (hematoxylin-safranin-fast green) and mounted in Depex (Johansen, 1940; O'Brien & McCully, 1981). Photographs of the exomorphological characteristics were taken with a Canon digital camera mounted on a stereoscopic microscope (SV6 Carl Zeiss). Micrographs were obtained with an AxioPhot Carl Zeiss microscope equipped with an AxioCam HRC camera and AxioVision 4.3 digital image analysis software.

Table 1. Mean value of the number of galls and Gall Index of two *Nacobbus aberrans* populations from Córdoba, Argentina, on three plant species (six replications).

Plant species	Common name	Number of Galls		Gall Index	
		CB	RC	CB	RC
<i>Chenopodium album</i>	Weed quinoa	7.7	0.7	2.2	0.5
<i>Solanum lycopersicum</i>	Tomato cv. Platense	3.0	6.5	1.2	2.0
<i>S. tuberosum</i>	Potato cv. Spunta	0.0	0.0	0.0	0.0

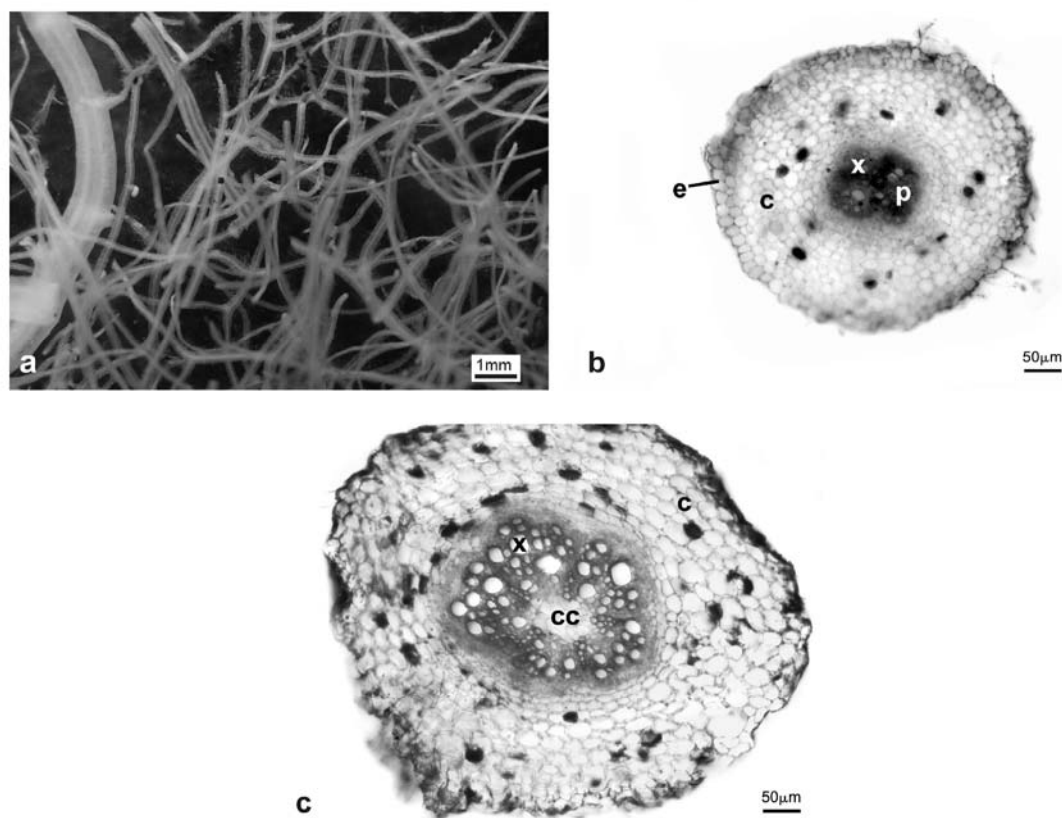


Fig. 1. Histopathology of potato (*Solanum tuberosum*) roots cv. Spunta inoculated with second-stage juveniles of *Nacobbus aberrans* from Coronel Baigorria and Río Cuarto (Córdoba, Argentina). a) External view of lateral roots; b, c) Transverse section of roots; b) Root with incipient secondary growth; c) Root with important development of secondary growth. Abbreviations. c: cortex; cc: central cylinder; e: epidermis; p: phloem; x: xylem.

RESULTS

The analysis of variance of the quantitative parameters (NG and GI) did not show significant differences between nematode populations for a single plant or between plant species for a single population ($P \leq 0.05$). Neither nematode population infested the potato cultivar (GI=0); weed quinoa was the most efficient host for CB population (GI=2.2), whereas tomato was the most efficient host for RC population (GI=2) (Table 1).

Potato. The potato plants did not show external symptoms of attack by either nematode population (Figure 1a). The two typical root zones, cortex and central cylinder, were assessed by means of the histological analysis performed in different root sectors both with primary and secondary growth. Both root zones were organised and composed of non-transformed tissues (Figure 1 b, c); no feeding sites or evidence of the nematode presence was observed.

Tomato. Tomato was infested by the two populations; gall formation was observed in main

and lateral roots (Figure 2, a). At gall level, the histological analysis showed the presence of hyperplastic tissue occupying part of the central cylinder. Cells of this tissue appeared transformed, forming syncytia, which were also composed of parenchymatic cells of vascular tissues. In the central root zone, *N. aberrans* females closely associated with feeding sites were also observed.

CB population. Galls containing between one and three mature females were observed. The hyperplastic tissue and syncytia were distributed in the central zone, producing tissue disorganisation, displacement, and fragmentation (Figure 2, b). Feeding sites developed adjacent to the vascular tissues, whose cells could be either incorporated into the sites or become crushed and broken (Figure 2 c). Feeding sites were composed of numerous cells (more than 30 as observed in a transverse section), of variable shape and different degrees of hypertrophy related to cell differentiation. Poorly-differentiated cells were approximately 26 µm, measured along the long axis and had dense, barely

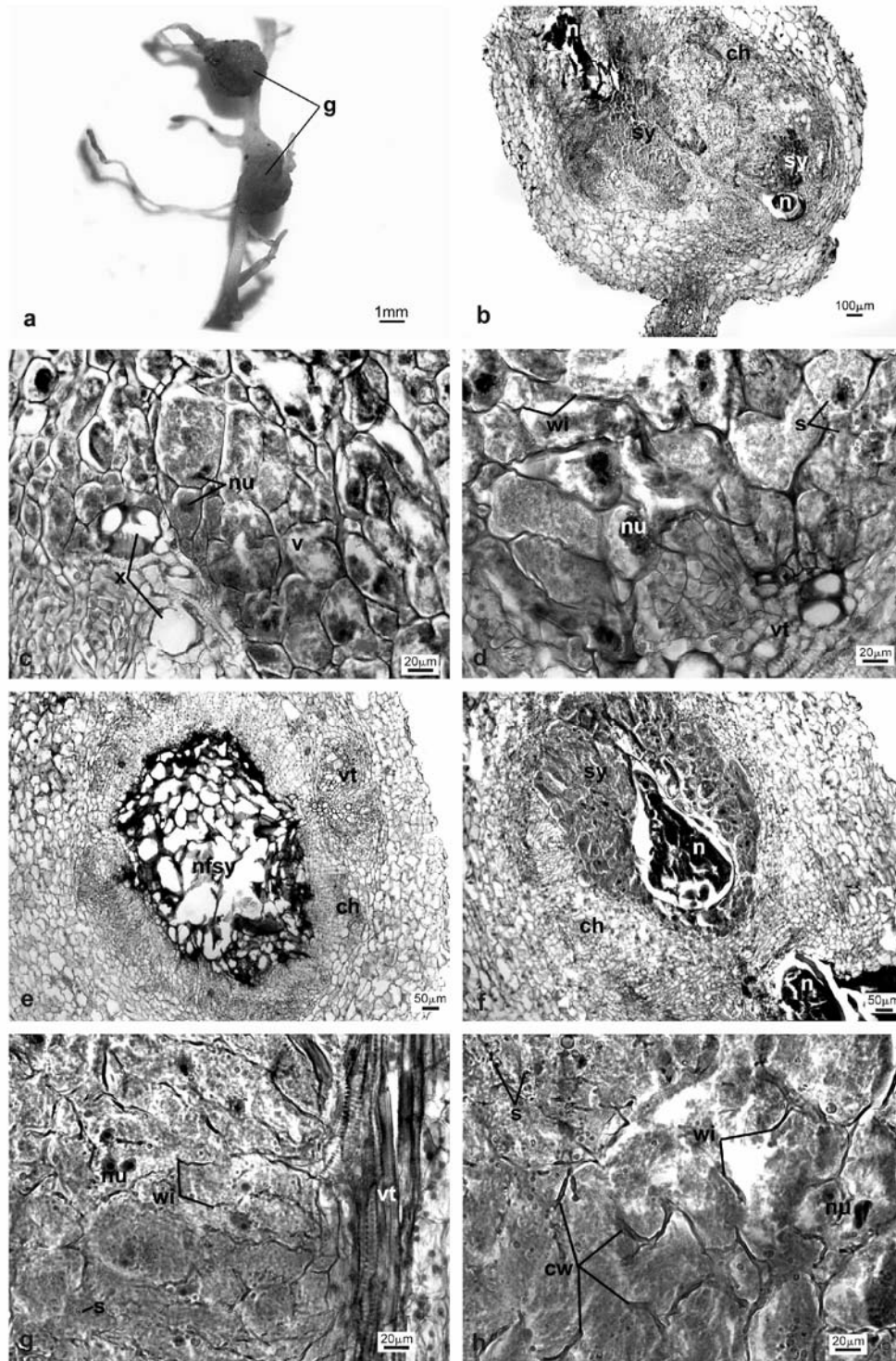


Fig. 2. Anatomical changes induced by *Nacobbus aberrans* populations from Coronel Baigorria (CB) and Río Cuarto (RC) (Córdoba, Argentina) on tomato (*Solanum lycopersicum*) roots cv. Platense. CB Population, a) External view of galls; b) Transverse section of gall with hyperplastic tissue, functional syncytia and nematodes; c) Close view of a sector with crushed and broken xylem cells; d) Close view of a sector showing syncytial features; e) Non-functional syncytium. RC Population, f) Gall with hyperplastic tissue, functional syncytium and nematodes; g, h) Detail of different sectors showing syncytial features. Abbreviations. ch: cellular hyperplasia; cw: cell wall; g: gall; n: nematode; nu: nucleous; nfsy: non-functional syncytium; s: starch; sy: syncytium; v: vacuole; vt: vascular tissues; wi: wall interruption; x: xylem.

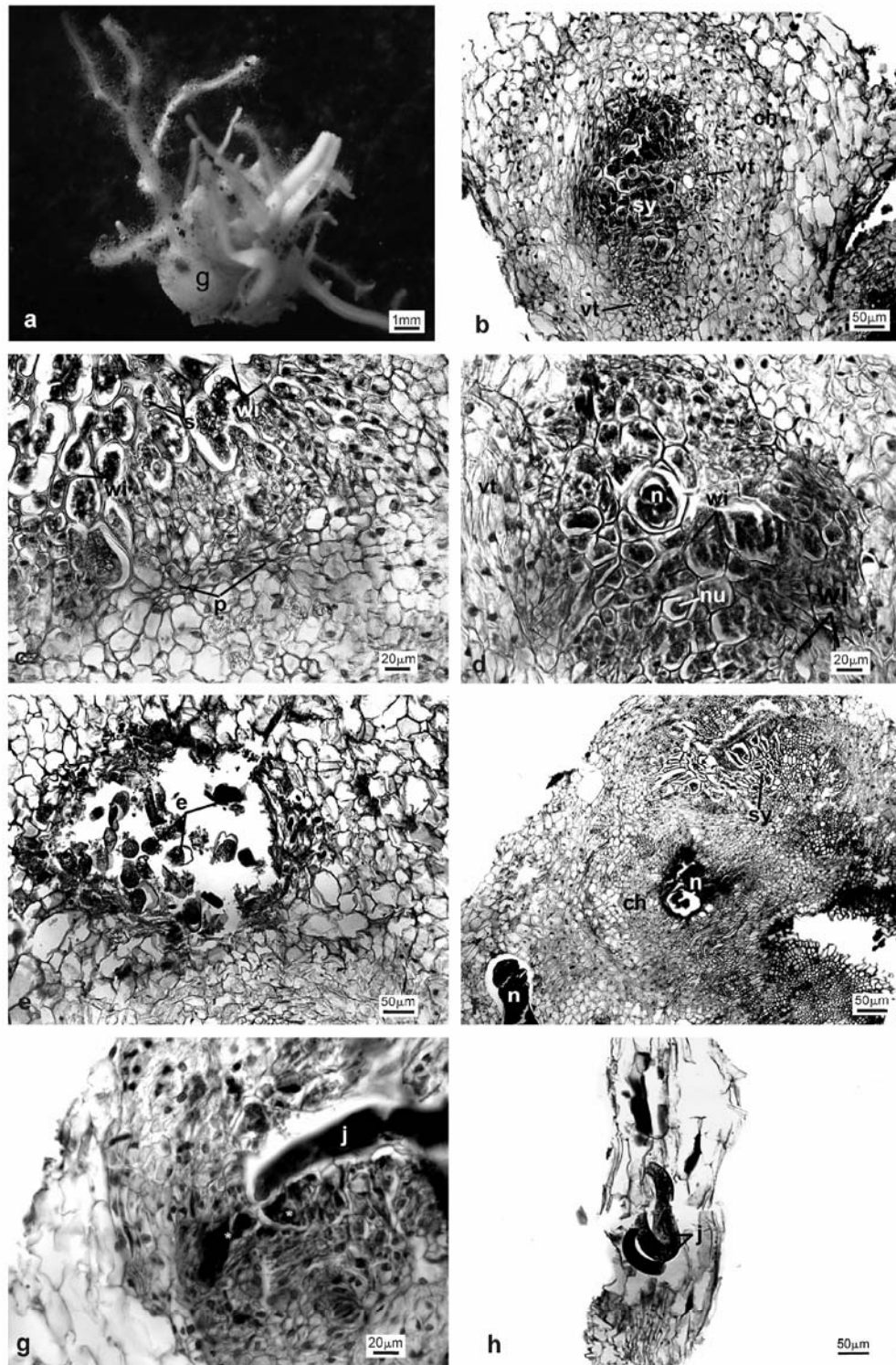


Fig. 3. Anatomical changes induced by *Nacobbus aberrans* populations from Coronel Baigorria (CB) and Río Cuarto (Córdoba, Argentina) on weed quinoa (*Chenopodium album*) roots. CB population, a) External view of galls; b) Transverse section of gall with hyperplastic tissue and functional syncytium; c, d) Close view of a sector showing syncytial features; e) Sector of gall with an egg mass. RC Population, f) Gall with hyperplastic tissue, functional syncytia and nematodes; g) Nematode juvenile stage in a gall and some syncytial cells in differentiation process (asterisk); h) Nematode juvenile stage in lateral root of gall. Abbreviations. ch: cellular hyperplasia; e: eggs; g: gall; j: juvenile; n: nematode; nu: nucleous; p: phloem; s: starch; sy: syncytium; vt: vascular tissues; wi: wall interruption.

vacuolated cytoplasm, some of them with starch grains. The largely-differentiated cells reached 45 μm along the long axis and exhibited a gradual regression of cytoplasm, which was separated from the cell walls and acquired a fibrillar texture (Figure 2 d). In all the cells observed, nuclei were hypertrophied, spherical or lobulated in shape, and contained prominent nucleoli. Cell walls were thin (approximately 2 to 3 μm), cellulosic, and partially fragmented, allowing neighbouring cytoplasms to join. Cells that originated the syncytium, which in some sectors were in contact with the body of females, were crushed and broken by the increasing nematode volume. Walls were thickened (approximately 6 μm) only in these cases. Non-functional syncytia composed of empty cells with somewhat thickened walls were also observed (Figure 2 e). Mature females with their egg masses were found associated with these syncytia.

RC population. Galls with two mature females on them were usually observed (Figure 2 f). During development, feeding sites also occupied the central zone of galls and syncytial characteristics were similar to those described in tomato attacked by the CB population. Cell walls in these syncytia, however, were thicker (approximately 6 μm), maintained their cellulosic nature, and were notably fragmented in wide sectors, which hindered individualisation of the most transformed cells (Figure 2 g, h).

Weed quinoa. Populations attacked quinoa, galls being detected both in main and lateral roots (Figure 3 a).

CB population. Galls with a female in the central zone associated with the feeding site were observed. The main effects of syncytia formation were displacement and separation of vascular tissues (Figure 3, b). As a consequence, some sections revealed that syncytium development caused a withdrawal of the phloem towards the periphery of the area, losing connection with the xylem in these sectors (Figure 3 c). During their formation, syncytia incorporated xylem cells, causing a reduction of this tissue enhanced by the interruption of the vascular cambium. Cells that originated the syncytium were slightly hypertrophied (15 μm in diameter) and maintained their shape and individuality because the walls, of cellulosic nature, exhibited partial interruptions or dissolutions in a few sectors. Syncytial cytoplasm was very dense, with scarce vacuolisation and starch, and had the particular feature of detaching from the cell walls in large sectors (Figure 3 c, d). Non-functional syncytia were composed of empty cells with thick walls, reaching approximately 9 μm in some sectors; mature females with their egg masses embedded in

the gall tissues were observed associated with those non-functional syncytia (Figure 3 e).

RC population. Galls with marked proliferation of lateral roots were observed. Although these galls held established mature females and syncytia similar to those observed associated with the CB population (Figure 3 f), the presence of juvenile stages in different gall sectors was remarkable. Some syncytial cells with thick (approximately 6 to 7 μm) and lignified walls were observed closely associated with the anterior portion of nematode juvenile stages (Figure 3 g). Juveniles inside the lateral roots were also observed (Figure 3 h).

DISCUSSION

Although the two *N. aberrans* populations considered were at a relatively short distance, they showed different behaviour towards the same plant and a clear preference for a given host. Furthermore, the invading capacity of each population also differed between the three plant species. This can be considered as an indicator of the physiological variability of different populations of the nematode, suggesting the existence of races/groups within the species (Inserra *et al.*, 1985; Costilla, 1990; Manzanilla-López *et al.*, 2002).

The histological and cytological features of syncytia in infested tomato and weed quinoa are, in general, consistent with features already mentioned for other Argentine *N. aberrans* populations parasitising the same plants (Doucet & Ponce de León, 1985; Doucet *et al.*, 1997; Lorenzo *et al.*, 2001; Vovlas *et al.*, 2007). Similar situations were reported for other hosts (Inserra *et al.*, 1983, 1984; Moyetta *et al.*, 2007). In the present analysis, the galls of both hosts exhibited an important amount of hyperplastic tissue in the central cylinder zone, which may be a defence feature (Suárez, 2007). This feature was not recorded in tomato infected by an *N. aberrans* population from the locality of Oliva (province of Córdoba, Argentina) (Lorenzo *et al.*, 2001). Regarding the location of syncytia, both in tomato and quinoa they occupied only the central zone of galls, whereas in previous research conducted on the same hosts, syncytia were detected in cortex and central cylinder (Doucet & Ponce de León, 1985; Doucet *et al.*, 1997; Lorenzo *et al.*, 2001).

In syncytia induced in weed quinoa no cell specialisation features ('wall ingrowths') were observed in the walls adjacent to the xylem, as previously observed in the same host (Doucet *et al.*, 1997). In this work, the scarce amount of starch in syncytial cells was also noticeable, a feature that does not agree with previous observations in this host, in which cells full of starch were found (Doucet *et al.*,

2005). The presence of this carbohydrate is one of the characteristics that distinguish *N. aberrans*-induced feeding sites and might indicate an intense metabolic activity of syncytial cells (Sousa, 2001). The presence of starch-rich amyloplasts in syncytia is among the earliest events in feeding-site formation; these nutrient reserves would be used by the nematode during the reproductive stage (Schuster *et al.*, 1964). Therefore, while syncytia observed in this work remain functional, they may be already composed of fully differentiated and more mature cells.

Although each population showed preference for a single host (tomato in RC and quinoa in CB), at the histological level no differences between alterations induced by a single population on both host plants were observed. However, each host reacted differently to the attack of each population. Tomato roots inoculated with CB juveniles were characterised by the presence of: galls harbouring a larger number of females, central cylinders with greater reduction of vascular tissues, accompanied by conductive elements of the xylem that were broken or had an atypical arrangement, and hypertrophied cells of the vascular cambium with dense cytoplasm and secondary vacuoles being part of the syncytia. This group of features would indicate that the CB population would be more aggressive on tomato than the RC population. This assumption is not consistent with GI values, since the attack of the CB population on tomato was less aggressive. Although these nematodes would be less capable of invading tomato roots, the few individuals that would succeed in penetrating the roots would produce more damage in the tissues, at the histological level, than the other population.

Quinoa roots parasitised by RC individuals were characterised by the presence of juvenile stages in different gall sectors, as well as a pronounced proliferation of lateral roots with the presence of nematodes. This would indicate that the plant is not an efficient host for that nematode population. Once inside the roots, some juveniles might not have continued to develop their life cycle, which accounts for the low GI values recorded. These differences in aggressiveness to a plant observed between populations agree with results recently obtained for CB and RC on pepper cv. California Wonder and sugarbeet cv. Detroit (Tordable *et al.*, 2007). In that work, while both plants were susceptible, RC nematodes were more aggressive in tissues of both hosts.

None of the *N. aberrans* populations was able to invade tissues of the potato cultivar Spunta. However, *S. tuberosum* is an efficient host of other

N. aberrans populations in Argentina, mainly in the provinces of Catamarca and Tucumán (Costilla *et al.*, 1977; Costilla, 1985) and in the northwest of the country it is closely associated with numerous varieties of Andean potato (*S. tuberosum* subsp. *andigenum*) (Lax *et al.*, 2006, 2008). Within the species *N. aberrans*, some groups of populations have been proposed, depending on the plants they are able to infest: *i*) bean (populations that attack beans and pepper but not potato or sugarbeet); *ii*) sugarbeet (populations infecting sugarbeet, pepper, and tomato but not potato); and *iii*) potato (populations that damage potato, sugarbeet, and tomato but not pepper) (Manzanilla-López *et al.*, 2002). The fact that the populations evaluated are capable of infesting pepper and sugarbeet (Tordable *et al.*, 2007), along with the present results, suggests that RC and CB populations would belong to the so called 'sugarbeet group'.

Intraspecific variation in *N. aberrans* affects crop rotation planning, such as the selection of sources of resistance for breeding (Manzanilla-López *et al.*, 2002). Knowing the response of crops to different nematode populations is very important for selecting suitable management strategies. In Argentina, quinoa is a widely distributed weed of great importance for crops both in the field and in the glasshouse. Therefore, in studies of this type it is important to include weeds, since many of them may be excellent reservoirs for the nematode in the absence of a crop (Doucet & Lax, 2005).

The evaluation of the response of 41 species of cultivated and non-cultivated plants to nine populations of *N. aberrans* from different geographical origin showed that roots of some plants that were efficient hosts had an asymptomatic reaction (Castiblanco *et al.*, 1999). However, different stages of the nematode life cycle (females, males and eggs), which are indicators of the normal development of the parasite in the plant, were found inside the tissues. Histological studies are useful to analyse situations like the present one, in which no external symptoms are apparent in the root tissues attacked by the nematode. At the same time, possible differences in the reaction of a single host to different nematode populations can be detected with such studies.

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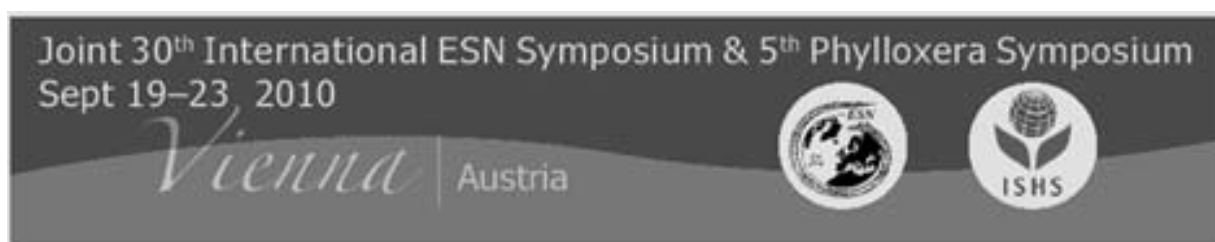
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M. del Carmen Tordable, P. Lax, M. Edmundo Doucet, P. Bima, D. Ramos, L. Vargas. Реакция корней различных растений на поражение нематодами *Nacobbus aberrans*.

Резюме. Нематоды *Nacobbus aberrans* происходят из Южной Америки и причиняют существенный вред различным культурам. Изучена реакция корней картофеля (*Solanum tuberosum*), томатов (*S. lycopersicum*) и чилийской мари (*Chenopodium album*) на инокуляцию нематодами, выделенными в Coronel Baigorria (CB) и Río Cuarto (RC) (обе точки в провинции Córdoba в Аргентине). Определяли количественные (число галлов и индекс галлообразования) и качественные параметры (результаты гистологического исследования тканей корней растений). Две изученные популяции различались по способности поражать корни растений. Ни одна из популяций нематод не была способна поражать картофель, так что наиболее подходящим растением-хозяином для них были томаты и мари. На гистологическом уровне не были выявлены признаки поражения на картофеле. Разрастание ткани в центральном цилиндре корня с трансформацией нормальных клеток в синцитий была выявлена в галлах на томатах и мари. Каждая из изученных популяций наковбусов показала предпочтение одного из видов растений. Гистологическое исследование не выявило различий в строении измененной ткани у двух восприимчивых растений, однако были отмечены различия в характере реакции растений на поражение нематодами из двух разных популяций паразитических нематод.



The European Society of Nematologists will hold its 30th International Symposium in Vienna, Austria from September 19th - 23rd 2010.

The Symposium will be hosted by the University of Natural Resources and Applied Life Sciences in Vienna. This venue provides excellent facilities on a campus in a charming green environment and with a blend of traditional and modern architecture.

Contact:

<http://esn-online.org/esn-2010-vienna>

Keynote Speakers

ESN is delighted to announce Prof. Jonathan Hodgkin and Prof Heribert Hirt as the Keynote Speakers for the meeting.

Prof Hodgkin is a Professorial Fellow of Keble College Oxford and holder of the Genetics Chair in the Department of Biochemistry. His work has covered a wide range of aspects of developmental biology of *C. elegans*, including sex determination, developmental morphogenesis, germline immortality and telomere function, nematode-bacteria interactions and innate immunity.

Prof Hirt is Director of the URGV Plant Genomics Research Unit, Paris, France and until last year was Head of the Department of Plant Molecular Biology at the University of Vienna. His research is focused on perception and signaling of stress responses in plants.

Invited speakers at the meeting will include Mark Blaxter, Thomas Baum and Ralf Sommer.

CCN workshop

The ESN 2010 meeting will include a workshop on Cereal Cyst Nematodes, designed to follow on from the highly successful first workshop held in Turkey in 2009.

Local Organizers: Florian Grundler, Monika Pribil, Julia Hofmann, Krzysztof Wieczorek, Holger Bohlmann, Shahid Siddique, Nasser El Nashry

Scientific Board: Silvia Bulgheresi, Keith Davies, Ralph Udo Ehlers, Carolina Escobar, Johannes Hallmann, John Jones, Marie-Noelle Rosso, Patricia Timper, Miroslav Sobczak, Loes de Nijs.



Nematodes of the order Dorylaimida from Romania: two interesting species of the subfamily Qudsianematinae Jairajpuri, 1965

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Summary. Two known species of the nematode family Qudsianematidae were studied on the basis of material collected from natural habitats in Romania. Two populations of *Labronema carusoi* are compared to the original description, with new observations that allow a better characterisation of this taxon, especially those referring the morphology of lip region and female genital system. The female of *Labronemella labiata* is reported and described for the first time. Descriptions, measurements, illustrations, including LM pictures for both species and SEM pictures for *L. carusoi*, are provided. Data concerning the occurrence of the species in Romania are also given.

Key words: Carpathians, Danube Delta, distribution, *Labronema carusoi*, *Labronemella labiata*, morphology, morphometrics, SEM, survey, taxonomy.

As a result of an extensive ecological survey on natural ecosystems aiming to evaluate the diversity of nematode communities throughout Romania, material including several genera belonging to the family Qudsianematidae was deposited in the nematode collection of the Institute of Biological Research in Cluj-Napoca.

According to Popovici *et al.* (2008), Romanian nematode fauna belonging to the genera *Labronema* Thorne, 1939 and *Labronemella* Andrassy, 1985 is poorly known: *Labronema plica* Ciobanu, Popovici & Decraemer, 2004, probably a Romanian endemic species, was reported from a salt-affected area at Cojocna/Cluj (Ciobanu *et al.*, 2004) and *Labronemella czernowitziensis* (Micoletzky, 1922) Andrassy, 2002 was originally described from Northern Moldova.

This paper provides information on the occurrence of two rare species, *Labronema carusoi* Vinciguerra & Orselli, 1998 and *Labronemella labiata* Andrassy, 1985 as new records in the Romanian fauna, and new data about them is provided for their characterisation.

MATERIAL AND METHODS

Nematodes were collected by the second author (I.P.) during two field sampling trips carried out in 1993 and 1995 in natural ecosystems located in the Eastern Romanian Carpathians and within the Danube Delta

Biosphere Reserve (see Table 1). Nematodes were extracted using the centrifugation method of De Gisse (1969), killed and preserved in a 4% formaldehyde solution, heated at 65°C and mounted in anhydrous glycerol according to Seinhorst (1959). Microphotographs were taken with a Nikon Eclipse 80i light microscope provided with differential interference contrast optics (DIC) and Nikon Digital Sight DS-U1 camera. For scanning electron microscopy (SEM), glycerol embedded nematodes in the permanent slides were first transferred, after measuring, into a drop of glycerol. Distilled water was then gradually added until nematodes were in almost pure distilled water and they were left so for 24 h. The nematodes were then initially dehydrated by passing through a gradual ethanol concentration series of 25, 30, 50, 70, 95 and 100% at intervals of 2 h, followed by an overnight dehydration in 100% ethanol, and subsequently putting them into 100% acetone for about 1h. After critical point drying with CO₂, dried specimens coated with gold were examined with a JEOL (JSM-5800) microscope operating at 13 kV.

Data on the presence and distribution of the species were included in the Romanian nematode fauna database. The paper is also a contribution towards an inventory of the species belonging to the family Qudsianematidae in Romania.

Table 1. Site locations, vegetation and soil types of a nematological survey in Romania.

Site no.	Locality	Altitude (m)	Geographical position	Plant association*	Soil type**
1	Danube Delta	0.3	45°08'N-29°39'E	<i>Plantaginietum coronopi</i>	salt-affected sand dune
2	Danube Delta	1.5	44°50'N-29°37'E	<i>Acorelletum pannonici</i>	salt-affected sand dune
3	Gurghiu Mts.	830	46°45'N-25°01'E	<i>Symphyto cordati-Fagetum</i>	acid brown

*according to Coldea (1991) and Popescu *et al.* (1980)**according to the Romanian System of Soil Classification (Conea *et al.*, 1980).**Table 2.** Measurements for *Labronema carusoi* Vinciguerra & Orselli, 1998 and *Labronemella labiata* Andr ssy, 1985 from Romania. Measurements in μm (except L, in mm), and in the form: mean $\pm$ standard deviation (range).

Species	<i>Labronema carusoi</i>			<i>Labronemella labiata</i>
Population	Sulina Sand dunes	Sacalin Island Sand dunes		Gurghiu Valley Beech forest
Character				
n	7 ♀♀	15 ♀♀	8 ♂♂	1 ♀
L	1.52 $\pm$ 0.0 (1.38-1.65)	1.57 $\pm$ 0.1 (1.30-1.98)	1.66 $\pm$ 0.1 (1.46-1.85)	2.56
a	29.2 $\pm$ 0.9 (28.3-30.8)	30.8 $\pm$ 3.0 (27.4-36.2)	32.8 $\pm$ 4.7 (27.6-39.0)	33.2
b	5.0 $\pm$ 0.6 (4.3-6.0)	4.7 $\pm$ 0.4 (4.1-5.4)	4.9 $\pm$ 0.6 (3.9-5.6)	4.1
c	71.9 $\pm$ 9.3 (61.4-77.0)	70.6 $\pm$ 9.0 (56.1-83.3)	62.3 $\pm$ 7.5 (53.3-74.1)	94.8
c'	0.7 $\pm$ 0.0	0.7 $\pm$ 0.1 (0.6-0.8)	0.8 $\pm$ 0.1 (0.7-0.9)	0.6
V	54.0 $\pm$ 3.5 (52.1-55.8)	54.3 $\pm$ 1.5 (51.0-56.7)	-	50.6
Lip region diam.	20.0 $\pm$ 0.0 (20.0-20.0)	19.9 $\pm$ 0.8 (18-22)	19.8 $\pm$ 0.4 (19-20)	25
Odontostyle	23.4 $\pm$ 1.8 (20-25)	24.1 $\pm$ 0.9 (22.5-25)	24.2 $\pm$ 1.6 (21-25)	30
Odontophore	46.5 $\pm$ 4.2 (38-50)	45.1 $\pm$ 5.5 (38-58)	47.1 $\pm$ 4.0 (40-50)	53
Guiding ring from ant. end	15.0 $\pm$ 1.4 (12.5-17.5)	16.0 $\pm$ 1.5 (12.5-18)	15.6 $\pm$ 1.0 (15-17.5)	20
Neck length	307.1 $\pm$ 43.4 (245-373)	332.5 $\pm$ 25.0 (283-370)	339.6 $\pm$ 29.6 (288-373)	619
Pharyngeal expansion length	161.1 $\pm$ 22.5 (125-195)	159.2 $\pm$ 11.6 (137-175)	164.2 $\pm$ 15.3 (138-180)	300
Diam. at neck base	47.0 $\pm$ 1.5 (47-50)	47.0 $\pm$ 1.5 (47-50)	47.0 $\pm$ 1.5 (47-50)	78
at mid-body	52.1 $\pm$ 2.6 (48-56)	51.3 $\pm$ 4.5 (45-60)	51.2 $\pm$ 2.8 (47-55)	77
at anus	30.4 $\pm$ 1.5 (29-33)	33.3 $\pm$ 2.9 (30-40)	34.4 $\pm$ 2.6 (33-39)	45
Prerectum length	99.6 $\pm$ 18.8 (68-125)	79.8 $\pm$ 18.8 (60-110)	118.0 $\pm$ 45.0 (75-193)	95
Rectum length	33.3 $\pm$ 3.4 (28-38)	34.3 $\pm$ 4.8 (25-43)	33.8 $\pm$ 6.3 (30-45)	50
Tail length	21.3 $\pm$ 1.9 (20-23)	22.6 $\pm$ 2.5 (19-28)	26.9 $\pm$ 1.6 (25-29)	27
Spicule length	-	-	52.9 $\pm$ 4.3 (45-58)	-
Ventromedian supplements	-	-	19-23	-

DESCRIPTIONS

Labronema carusoi Vinciguerra & Orselli, 1998 (Figs. 1, 2)

Material examined: Twenty-two females and eight males from two localities, in general in good condition.

Measurements: See Table 2.

New observations (based on Romanian specimens):

Adult: Cuticle two-layered: outer layer thin and with very fine transverse striations, easier to distinguish under SEM; inner layer about as twice as thick as the outer one; cuticle thickness 2.0-3.5 μm

at anterior region, 2.5-3.5 μm in mid-body and 4.0-7.0 μm on tail. Cervical pores present, two or three dorsal and two or three ventral ones at level of odontostyle plus odontophore; lateral pores present along the whole body, but more developed in the caudal part. Lateral chord occupying about one-third of the corresponding body diameter at midbody, containing gland bodies which are very distinct in some specimens. Lip region 2.7-3.3 times as wide as high and about half of body diameter at neck base. SEM observations show that lips are mostly fused, each lip consisting of a few concentric striations with one papilla at the centre; lips are separated by radial striations extending across the oral field; outer and inner labial papillae appear close together at the



Fig. 1. *Labronema carusoi* Vinciguerra & Orselli, 1998. A, B: Anterior region in median view; C: Lip region in surface view; D: Female, posterior genital branch; E: Female, posterior body region; F: Vagina; G: Male, posterior body region; H: Female tail; I, J: Male, caudal region and spicules. (Scale bar: A-C, F, H-J = 10 μ m; D, E, G = 20 μ m).

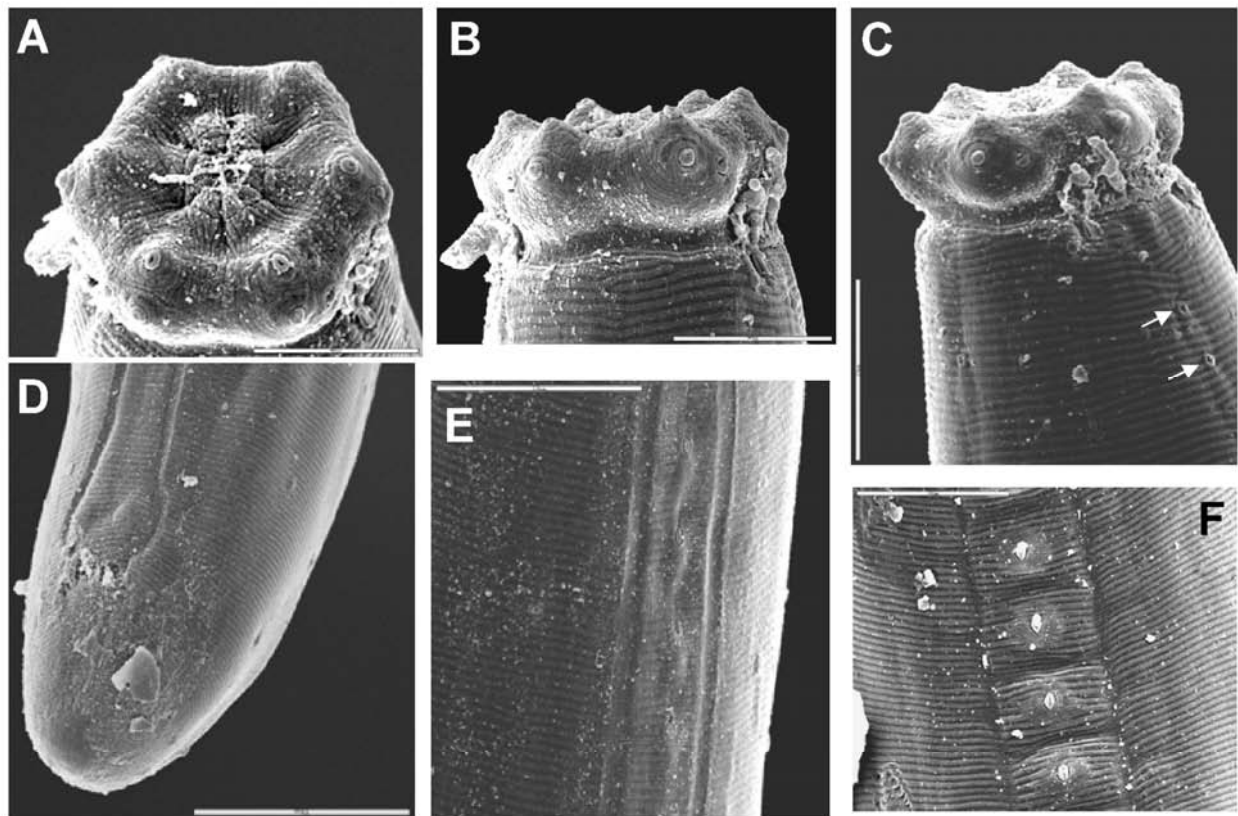


Fig. 2. *Labronema carusoi* Vinciguerra & Orselli, 1998 (SEM). A: Lip region, in frontal view; B, C: Same in dorsal or ventral view (arrow heads pointing to lateral body pores); D: Male caudal region in ventral view; E: Male posterior region showing the most anterior ventromedian supplements; F: Same showing contiguous ventromedian supplements. (Scale bar: 10 μ m).

margin of lip region; and perioral area divided into six sectors differentiated as small, low liplets. Amphid opening cup-like, occupying 8.0-12.5 μ m or about half of lip region width. Odontostyle 7-8 times as long as wide, 1.0-1.3 times longer than lip region width, and equal to or thicker than body cuticle at its level. Guiding ring double, but this condition is difficult to observe in some specimens due to fixation process. Basal pharyngeal expansion 4.1-5.3 times as long as wide or 3.0-3.4 times longer than body diameter at neck base, and occupying about half (48-52%) of total neck length. Pharyngeal gland nuclei difficult to observe in the material examined. Nerve ring located at 38-41% of the total neck length. Junction between pharyngeal base and cardia surrounded by a ring-like structure.

Female: Genital system didelphic-amphidelphic, with both genital branches equally and well developed, the anterior 182-415 μ m, the posterior 190-390 μ m long. Ovaries with variable development, reflexed but not always surpassing the sphincter level; oocytes first in two rows and then in a single row. Oviduct joining subterminally the ovary and consisting of a tubular part and a poorly

developed *pars dilatata*. Oviduct and uterus separated by an indistinct sphincter. Uterus 145-175 μ m or about 2.6-2.8 times the corresponding body diameter, and tripartite, *i.e.* consisting of three regions: proximal section, close to the vagina, a tube 80-90 μ m long with clearly visible lumen; an intermediate, shorter (50-55 μ m long), muscular section, with narrow lumen; and distal section, close to sphincter, a tube 90-100 μ m long, comparable in texture to proximal section, although slightly narrower. Spindle-shaped spermatozoa, 3.0-5.0 μ m long, often observed within the uterus. Uterine egg 92 x 35 μ m. Vagina cylindrical, extending inwards about half of the corresponding body diameter: *pars proximalis* 20-21 x 9-10 μ m with slightly sigmoid walls (*i.e.* proximally divergent and distally convergent) and surrounded by weak musculature; *pars refringens* with small, drop-shaped pieces separated by a less refringent area, with a combined width of 8.5-9 μ m; *pars distalis* short, 2.0-2.5 μ m long. Vulva a small longitudinal slit, usually preceded by a depression of body surface. Prerectum 2.0-4.3 times as long as anal body diameter. Rectum from slightly shorter to slightly

longer than anal body diameter. Tail broadly rounded to hemispheroid; two pairs of caudal pores, one subdorsal, another lateral, both at middle of tail.

Male: Prerectum 1.9-5.5 times the cloacal body diameter. Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair situated close to cloacal opening, there is a series of 19-23 almost contiguous ventromedian supplements, starting out the range of spicules, with the posteriormost supplement situated at 65-80 μm from ad-cloacal pair. Spicules curved ventrad, 4-5 times as long as wide and 1.2-1.8 times the anal body diameter long. Lateral guiding pieces relatively short, 7.5-10.0 μm , 3.5-4.5 times as long as wide. Tail bluntly conoid, ventrally almost straight, dorsally more convex; six pairs of caudal pores, two subdorsal, two subventral and two subterminal.

Distribution: Salt-affected sand dunes on the beach at Sulina and on Sacalin Island, both locations in the Danube Delta Biosphere Reserve - sites no. 1 and 2 in Table 1.

Remarks: This species was originally described from sand dunes in Italy on the basis of specimens collected in six different localities. The Romanian specimens perfectly fit the type material, with only minor morphometric differences. These differences include thicker cuticle (2.5-3.5 vs 1.0-2.0 μm in midbody), slightly more slender body (vs $a = 19-30$), and somewhat shorter odontostyle (vs 23-30 μm), although overlapping in the range of these measurements and ratios can be noted. Nevertheless, the above description provides many new details, especially those derived from SEM study of lip region and others concerning the morphology of female genital system, which allow a better characterisation of the taxon.

This is the first record of *L. carusoi* in Romania, extending its geographical distribution. By reporting the species from the same type of habitat as it was originally described, its preference for sand dunes is confirmed.

***Labronemella labiata* Andr ssy, 1985 (Fig. 3)**

Material examined: One female collected from the Gurghiului Valley (Gurghiului Mountains), in excellent condition, making suitable a detailed study of its morphological features.

Measurements: See Table 1.

Female: Slender nematode of medium size, 2.56 mm long. Habitus strongly curved, G-shaped upon fixation. Cuticle with two layers: outer layer smooth and thin, the inner layer is very thick (3-4 times the outer one) and bears distinct radial striations along entire body; cuticle 3 μm thick at anterior region, 5

μm in mid-body and 7.5 μm on tail. Lateral chord occupying about one-fourth of the corresponding body diameter at midbody. Lip region offset by constriction, sucker-like, and 3.5 times as wide as high or about one-third of body diameter at neck base; lips amalgamated in their most part; oral field deeply sunk in head contour, with six small rounded liplets surrounding the oral aperture. Amphidial fovea funnel-like, its aperture 9 μm or about two-fifths (41%) of lip region width. Odontostyle straight, slender (about 12 times as long as wide), with slender walls and distinct lumen; it is 1.2 times longer than lip region width and 1.17% of body length; aperture 37% of the total length. Odontophore rod-like, 1.8 times the odontostyle. Guiding ring double. Pharynx consisting of a slender but distinctly muscular anterior part, enlarging gradually; basal expansion 8.7 times as long as wide or 4.1 times longer than body diameter at neck base, and occupying 49% of total neck length. Pharyngeal gland nuclei and outlets situated as follows: DN = 58, DO = 56, S₁N₁ = 69, S₁N₂ = 78, S₂N = 88. Nerve ring located at 38% of neck length. Cardia conoid, 23 μm long, 1.5 times as long as wide, surrounded by intestinal tissue that forms a conical extension measuring 40 μm including the cardia. Genital system didelphic-amphidelphic, with both genital branches equally and well developed, the anterior 437 μm , the posterior 525 μm long. Ovaries very large, extending beyond the sphincter level; oocytes numerous, arranged first in two or more rows and then in one single row. Oviduct joining the ovary subterminally and consisting of a tubular part and a well developed, elongate *pars dilatata* containing spindle-shaped spermatozoa, 4.0-5.0 μm long. Sphincter separating oviduct from uterus, but not very marked. Posterior uterus 549 μm long, the anterior one convoluted; it is tripartite, that is consisting of three specialized regions: proximal portion, close to the vagina, a tube 110-154 μm long with wide lumen; an intermediate dilated portion, 46-56 μm long, containing spermatozoa; and distal portion, close to the sphincter, a narrower tube 73-110 μm long with practically no distinct lumen. Vagina extending inwards to more than half (55%) of the corresponding body diameter: *pars proximalis* 23 x 6.5 μm , with slightly sigmoid walls (*i.e.* proximally divergent and distally convergent) and surrounded by moderately developed musculature; *pars refringens* with two trapezoidal, adjacent pieces measuring 9 x 6-6.5 μm and with a combined width of 12.5 μm , almost reaching the body surface; *pars distalis* very short, measuring about 1.5 μm . Vulva a transverse slit. Prerectum relatively short, about twice the anal body diameter long. Rectum slightly longer than

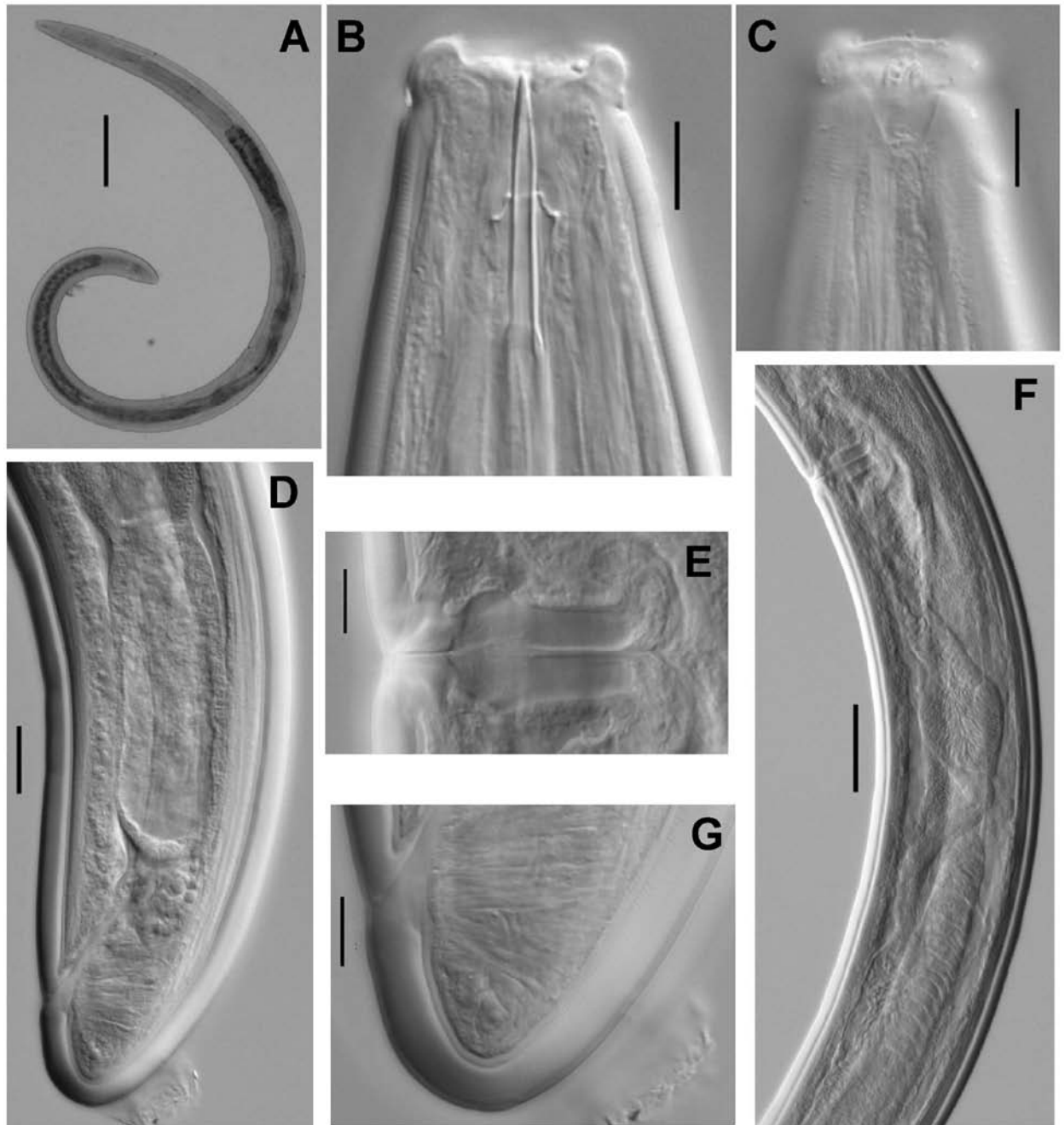


Fig. 3. *Labronemella labiata* Andr ssy, 1985 (female). A: Entire female; B: Anterior region in lateral median view; C: Same in lateral surface view; D: Posterior body region; E: Vagina; F: Posterior genital branch; G: Tail. (Scale bar: A = 200 μ m; B, C, E, G = 10 μ m; D = 20 μ m; F = 50 μ m).

anal body diameter. Tail broadly rounded; three pairs of caudal pores at the posterior half of tail, two subdorsal and the other lateral.

Male: Not found.

Distribution: Natural beech forest (*Fagus sylvatica*) on acid soil located in the Gurghiului Valley (Gurghiului Mountains, Eastern Romanian Carpathians), site no. 3 (Table 1.)

Remarks: Although Andr ssy (1985, see also 2009) described this species on the basis of only one male from Hungary, the Romanian female specimen herein studied is considered to be conspecific with the Hungarian male because there are many morphometric similarities between them, namely body length (2.56 vs 2.54 mm in the Hungarian male), inner cuticle layer with radial striation (vs

“cuticle not annulated but finely radially striated”), lip region width (25 vs 23 µm), odontostyle length (30 vs 31 µm or 1.2 vs 1.3 times the lip region width), odontostyle aperture (37% vs one-third of total length), etc.; moreover, both specimens were collected from the same biogeographical area. There are, nevertheless, some morphometric differences, such as body slenderness ($a = 33$ vs $a = 48$) or neck length (619 vs 410 µm) but, taking into consideration that only two specimens of different sex are compared, they are provisionally interpreted as intraspecific variations. Then, with due caution, the female of this species is reported and described for the first time.

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M. Ciobanu, I. Popovici, R. Peña-Santiago. Нематоды отряда Dorylaimida из Румынии: два вида Qudsianematinae Jairajpuri, 1965.

Резюме. По материалу, собранному в естественных экосистемах Румынии, исследованы два известных вида семейства Qudsianematidae. Проведено сравнение двух обнаруженных популяций *Labronema carusoi* с первоначальным описанием. Новые морфологические наблюдения позволяют дополнить первоописание деталями строения губ и женской половой системы. Впервые приводится описание самки *Labronemella labiata*. Даны описания, измерения и иллюстрации для обоих видов, включая фотографии, сделанные в световом и сканирующем электронном микроскопе (для *L. carusoi*). Приводятся сведения о встречаемости этих видов в Румынии.

***Devibursaphelenchus wangi* sp. n. (Nematoda: Ektaphelenchinae) feeding on *Aphelenchoides* sp.**

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Summary. *Devibursaphelenchus wangi* sp. n. is described and figured. The new species was isolated from pine packaging wood from the USA and inspected in Ningbo harbour, China in 2009. The new species is characterised by relatively slender body ($a = 36.5$ and 37.1 for males and females, respectively); three lines in the lateral field; stylet with relatively wide lumen but lacking basal knobs; vulval flap absent, very short post-uterine sac; non-functional and indistinct female rectum and anus; spicules relatively small (14.2 – 15.6 μm) with a flattened cucullus; two pairs of caudal papillae. The new species is morphologically close to *D. eproctatus* and *D. hunanensis* and can be distinguished by shape and size of spicules and smaller stylet. The separate species status is supported by ITS-RFLP patterns and molecular phylogenetic analysis based on ITS1/2 and partial LSU sequences, which revealed that the new species is close to *D. hunanensis*. The feeding habit of the new species is also observed and discussed.

Key words: Ektaphelenchidae, morphology, molecular taxonomy, new species.

In March 2009, during a routine inspection of imported packaging wood, a new species of *Devibursaphelenchus* was isolated together with an *Aphelenchoides* species from pine packaging wood coming from USA. It is described and figured herein as *Devibursaphelenchus wangi* sp. n.

MATERIAL AND METHODS

Sawn samples taken from packaging wood were cut into small pieces no more than 1 cm wide. Nematodes were extracted by the modified Baermann funnel technique for 24 h. The feeding habit of the new species was observed on a slide in water and recorded. Multiplication on agar-fungi plates (*Botryotinia fuckeliana*) failed. Measurements were made on permanent slides fixed in TAF and processed to glycerol following the method of Seinhorst (1959). The light micrographs were made using a Zeiss Imager Z1 microscope equipped with a Zeiss AxioCam MRm CCD camera.

DNA samples of *Devibursaphelenchus wangi* sp. n. were prepared according to Li *et al.* (2008). Two sets of primers (synthesised by Invitrogen, Shanghai, China) were used in the PCR analyses to amplify the ITS1/2 region and the D2D3 LSU region of rDNA, respectively. Primers for amplification of ITS1/2 were forward primer F194 (5'- CGT AAC AAG GTA GCT

GTA G -3') (Ferris *et al.*, 1993) and reverse primer 5368r (5'- TTT CAC TCG CCG TTA CTA AGG -3') (Vrain, 1993). Primers for amplification of D2/D3 LSU were forward primer D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and reverse primer D3Br (5'-TCG GAA GGA ACC AGC TAC TA-3') (De Ley *et al.*, 1999). PCR conditions were as described by Li *et al.* (2008). PCR products were separated on 1% agarose gels and visualised by staining with ethidium bromide. PCR products of sufficiently high quality were purified for cloning and sequencing by Invitrogen, Shanghai, China.

For ITS-RFLP profiles, suitable aliquots of the amplified ITS rDNA were digested for at least 3 h at 37° using 10 U of each of the five restriction endonucleases (*RsaI*, *HaeIII*, *MspI*, *HinfI* and *AluI*) (Takara, Japan) following the manufacturer's instructions. Fragments were resolved by electrophoresis in a 2.5% agarose gel and stained with ethidium bromide.

The ITS1/2 and partial LSU sequences were analysed and aligned using the program ClustalW implemented in MEGA version 4.0 (Tamura *et al.*, 2007). Phylogenetic trees were generated with the Neighbor Joining (NJ) method using the Tajima-Nei distance option. Bootstrapping analysis was performed with 1000 replicates.

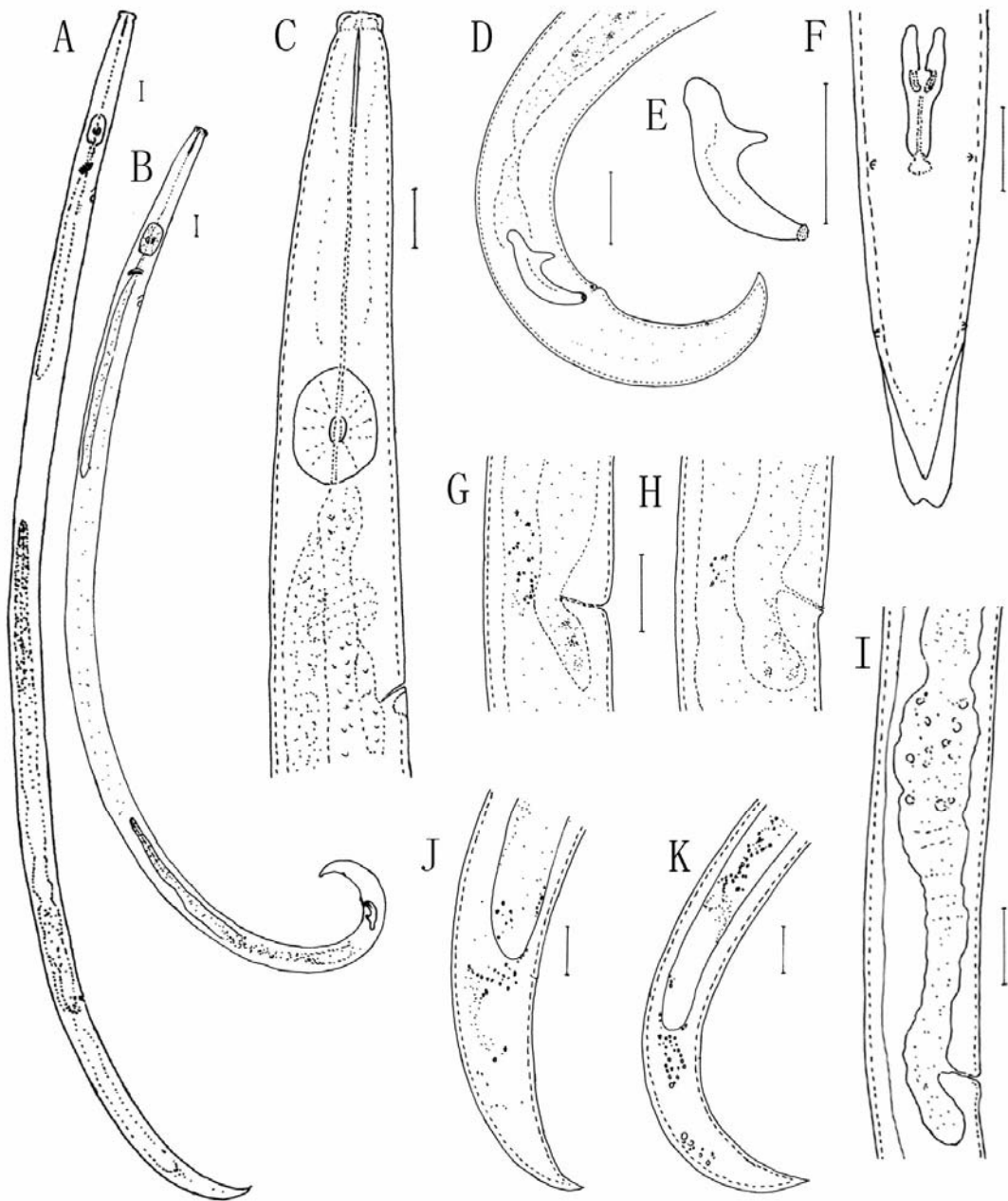


Fig. 1. *Devibursaphelenchus wangi* sp. n. A: Female; B: Male; C: Anterior body; D: Lateral view of male tail; E: Spicules; F: Ventral view of male tail; G-I: Vulva region; J, K: Variation of female tail. (Scale bars=10 µm).

DESCRIPTIONS

Devibursaphelenchus wangi sp. n. (Figs. 1-3)

Measurements (Table 1).

Male. Body slender, cylindrical, posterior region sharply curved ventrally when heat-killed. Cuticle weakly annulated, lateral field with three incisures (*i.e.*, two ridges). Lip region offset, about 3.5 µm in

height and 7.1 µm wide. Stylet short and relatively broad lumened, lacking basal swellings, conus forming *ca.* 38-40% of total length. Procorpus cylindrical. Median bulb strongly developed, elongate-oval, 15.7 ± 0.8 µm long, 9.7 ± 0.8 µm wide, with valve plates situated slightly posteriorly. Pharyngeal gland lobe slender and well developed, about six body diameters long, overlapping intestine dorsally. Nerve ring located at *ca.* 12-15 µm posterior to median bulb. Excretory pore located at *ca.*

Table 1. Measurements of *Devibursaphelenchus wangi* sp. n., all measurements in μm ; mean $\pm$ s.d. (range).

	Female		Male
	Holotype	Paratypes	Paratypes
n	-	15	6
L	655.0	704.3 $\pm$ 62.0 (606.0-803.3)	614.3 $\pm$ 22.9 (578.0-644.0)
a	36.5	37.1 $\pm$ 4.3 (26.4-45.1)	36.5 $\pm$ 2.4 (31.3-38.2)
b	7.7	8.5 $\pm$ 0.8 (7.6-10.6)	7.2 $\pm$ 0.4 (6.8-8.1)
b'	3.2	3.5 $\pm$ 0.3 (3.0-4.0)	3.5 $\pm$ 0.3 (3.3-4.0)
c	-	-	17.5 $\pm$ 0.9 (16.2-18.8)
c'	-	-	2.8 $\pm$ 0.1 (2.7-2.9)
V or T	78.6	78.2 $\pm$ 1.7 (73.7-80.0)	29.1 $\pm$ 0.6 (28.0-29.7)
Max body diam.	17.9	19.1 $\pm$ 3.3 (15.0-29.5)	16.9 $\pm$ 1.7 (15.2-20.6)
Lip diam.	7.0	7.7 $\pm$ 0.8 (6.9-9.0)	7.1 $\pm$ 0.7 (6.9-8.8)
Lip height	3.1	3.7 $\pm$ 0.5 (3.1-4.5)	3.5 $\pm$ 0.4 (3.1-4.0)
Stylet length	16.8	17.1 $\pm$ 0.3 (16.7-17.4)	14.8 $\pm$ 1.4 (12.4-16.6)
Median bulb length	16.3	17.7 $\pm$ 1.2 (16.3-19.8)	15.7 $\pm$ 0.8 (15.0-17.0)
Median bulb diam.	10.3	11.0 $\pm$ 1.8 (8.3-14.5)	9.7 $\pm$ 0.8 (8.3-11.1)
Median bulb length/diam.	1.6	1.6 $\pm$ 0.2 (1.3-2.0)	1.6 $\pm$ 0.2 (1.5-2.0)
Excretory pore position	95	105.4 $\pm$ 8.2 (94.0-119.1)	98.2 $\pm$ 3.2 (93.0-104.0)
Spicule (dorsal limb)	-	-	18.3 $\pm$ 0.6 (17.3-19.1)
Spicule (chord)	-	-	15.0 $\pm$ 0.5 (14.2-15.6)
Spicule (curved median line from the middle of condylus to the end)	-	-	15.9 $\pm$ 0.3 (15.4-16.4)
Ovary or testis length	350	298.6 $\pm$ 34.6 (256.0-355.0)	180.7 $\pm$ 4.7 (160.0-199.0)
Post-uterine sac length	11.7	10.2 $\pm$ 1.8 (7.6-13.2)	-
Blind sac	85.0	107.0 $\pm$ 14.8 (79.0-128.0)	-
Tail length	-	-	35.2 $\pm$ 2.2 (33.0-39.8)

30-35 μm posterior to median bulb. Hemizonid located just posterior to excretory pore, but sometimes anterior to excretory pore. Testis single, about 180.7 $\pm$ 4.7 μm long, spermatocytes arranged in two rows. Cloacal opening lips slightly protruding. Spicules arcuate, condylus rounded, elongated, lamina smoothly and symmetrically curved, rostrum conical with bluntly pointed tip. Distal ends of spicules forming a flattened cucullus. Tail strongly recurved, terminus finely pointed, spade-shaped terminal bursa clearly visible

under light microscope. Two pairs of caudal papillae present: one pair located slightly precloacal and the second subventral pair located just anterior to the beginning of bursal flap.

Females. Body slightly ventrally arcuate when heat-relaxed. Cuticle and lip region similar to male. Ovary outstretched, developing oocytes in two rows. Vulva slightly inclined anteriorly, vulva lips not protruding, anterior vulva lip does not form a vulval flap. Vagina not sclerotized. Spermatheca elongate-

oval, sometimes containing round sperms. Post-uterine sac short, less than one body diameter long, rectum and anus indistinct. Intestine terminating as a blind sac. Tail conical, tapering to ventrally bent terminus, tail terminus finely rounded or sharply pointed.

Diagnosis and relationships. *Devibursaphelenchus wangi* sp. n. is characterised by relatively slender body ($a = 36.5$ and 37.1 for males and females, respectively); three lines in the lateral field; stylet with relatively wide lumen and lacking basal knobs; relatively high vulva position (average 78%); vulval flap absent, very short postuterine sac; non-functional and indistinct female rectum and anus; spicules relatively small (14.2 – 15.6 μm) with a flattened cucullus; two pairs of caudal papillae; presence of a distinct bursal flap.

Braasch (2009) re-established the genus *Devibursaphelenchus* Kakuliya, 1967 belonging to Ektaphelenchinae, which contains five species: *D. typographi* Kakuliya, 1967; *D. eproctatus* (Sriwati, Kanzaki, Phan & Futai, 2008) Braasch, 2009; *D. hunanensis* (Yin, Fang & Tarjan, 1988) Braasch, 2009; *D. lini* (Braasch, 2004) Braasch, 2009 and *D. teratospicularis* (Kakuliya & Devdariani, 1965) Braasch, 2009.

Devibursaphelenchus wangi sp. n. is particularly close to *D. eproctatus* and *D. hunanensis* in the shape of spicules and female tail. *Devibursaphelenchus wangi* sp. n. is distinguished from *D. eproctatus* by the shape and size of spicules (15.4 – 16.4 μm vs 18.8 – 20.8 μm long measured along curved median line, condylus not recurved dorsally vs sometimes recurved dorsally); different size of stylet (12.4 – 16.6 μm and 16.7 – 17.4 μm for males and females, respectively vs 15 – 20 μm and 19 – 22 μm); different ovary length (256 – 355 μm vs 152 – 243 μm) and testis length (160 – 199 μm vs 129 – 143 μm); different c ratio of males ($c = 16.2$ – 18.8 vs $c = 13.3$ – 15.0).

Devibursaphelenchus wangi sp. n. is distinguished from *D. hunanensis* by the presence of three vs four lateral lines, absence vs presence of a functional rectum and anus, shorter stylet (12.4 – 16.6 μm and 16.7 – 17.4 μm for males and females, respectively, vs 19 – 21 μm and 20 – 26 μm); the shape of spicules (distal end of spicules with a distinct cucullus vs distal end of spicules obtuse, without cucullus).

Devibursaphelenchus wangi sp. n. is distinguished from *D. typographi* by the body shape ($a = 31.3$ – 38.2 and 26.4 – 45.1 for males and females, respectively vs $a = 21.2$ – 22.5 and 20.2 – 20.9); shorter stylet (averaging 17 and 15 μm for females and males, respectively, vs 21 – 22 μm); the shape of

female tail terminus (finely rounded or sharply pointed vs broadly rounded), the V value ($V = 73.7$ – 80.0 vs $V = 85.3$ – 85.8), and the size of spicules (14.2 – 15.6 μm vs 12 μm long measured in chord).

Devibursaphelenchus wangi sp. n. is distinguished from *D. lini* by the shape and size of spicules (14.2 – 15.6 μm long vs 16 – 21 μm long measured in chord, rostrum bluntly pointed vs sharply pointed); different length of stylet (12.4 – 16.6 μm and 16.7 – 17.4 μm for males and females, respectively vs 17 – 21 μm and 18 – 23 μm); and the vulva structure (no sclerotization in the vulva region vs a strong half ring-like sclerotization in the anterior vulva part).

Devibursaphelenchus wangi sp. n. is distinguished from *D. teratospicularis* by stylet length (averaging 17 and 15 μm for females and males, respectively, vs 18 – 22 μm); lack of basal swellings at the stylet vs presence of minute swellings in *D. teratospicularis*; finely rounded or pointed vs blunt tip of female tail; and by shape of spicules (rostrum position in the anterior part of spicules vs rostrum in the middle part of spicules due to very high condylus of *D. teratospicularis*, smoothly ventrally curved dorsal limb vs sunken distal part of dorsal limb).

Molecular profiles and phylogenetic status.

The rDNA based sequences of ITS1/2 and D2D3 LSU are deposited in the GenBank database with the accession numbers GQ894739 and GQ903770, respectively. The molecular phylogenetic status of the new species is shown in Figures 4 and 5, and the ITS-RFLP profiles of rDNA are shown in Figure 6 and Table 2. The ITS-RFLP pattern of *D. wangi* sp. n. is different from the patterns of *D. hunanensis* and *D. lini* (Burgermeister *et al.*, 2009).

Table 2. Sizes of PCR products and DNA restriction fragments obtained in ITS-RFLP analysis and calculated on sequencing results of the ITS1/2 regions

Species	PCR product (bp)	Restriction fragments (bp) ¹				
		<i>Rsa</i> I	<i>Hae</i> III	<i>Msp</i> I	<i>Hinf</i> I	<i>Alu</i> I
<i>D. wangi</i> sp. n.	966	403	717	769	488	798
		335	249	197	433	73
		155			45	65
		73				15
<i>D. hunanensis</i> ²	947	374	580	765	497	641
		305	367	182	181	293
		196			164	13
		72			63	
					42	

¹ Fragment sizes (bp) were calculated with a computer program DNASTAR MapDraw 5.01.

² According to Burgermeister *et al.*, 2009.

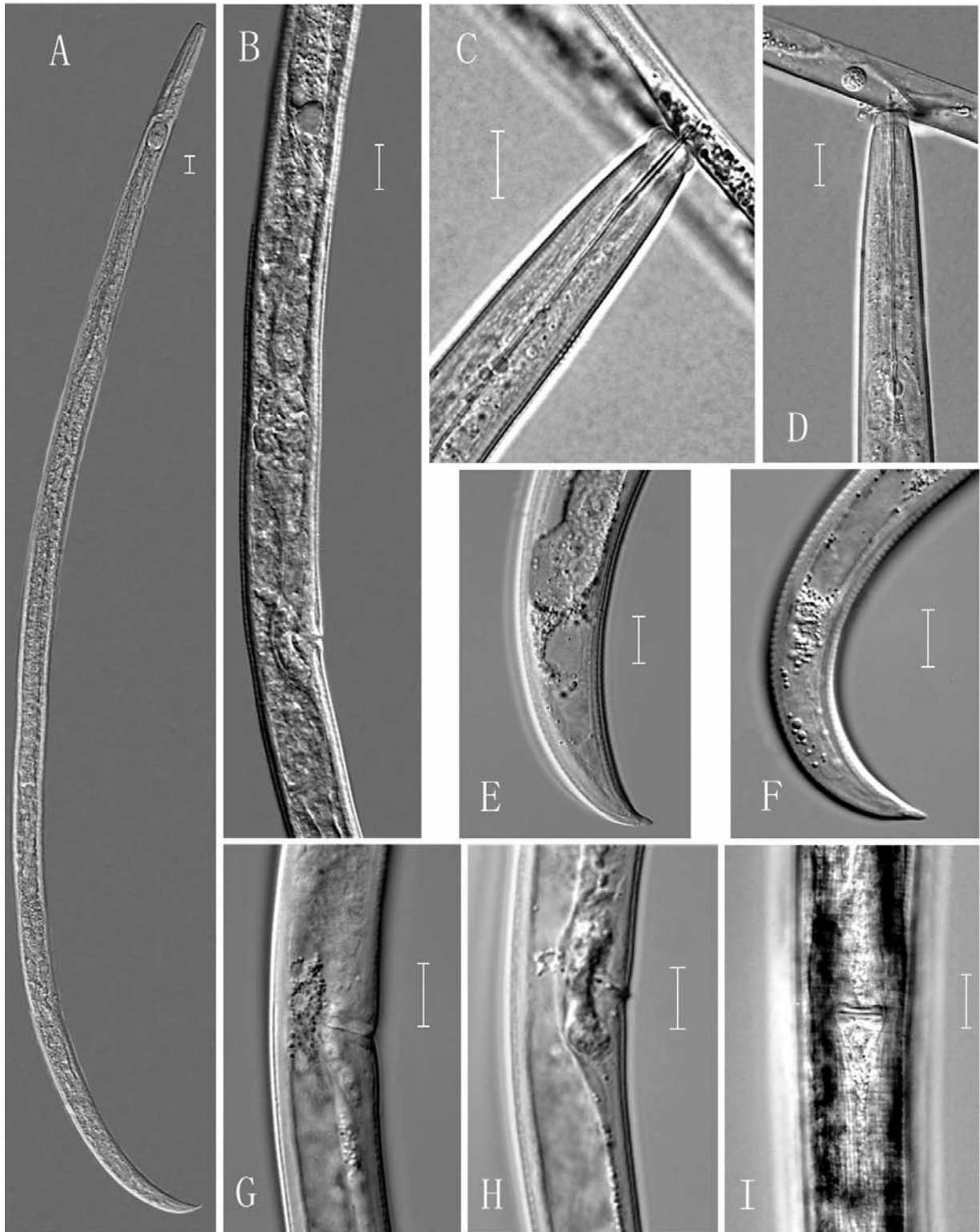


Fig. 2. Light photomicrographs of *Devibursaphelenchus wangi* sp. n. (female) A: Whole body; B: Vulva region; C, D: Feeding on *Aphelenchoides* sp.; E, F: Tail; G, H: Vulva region (lateral view); I: Vulva region (ventral view). (Scale bars=10 μ m).

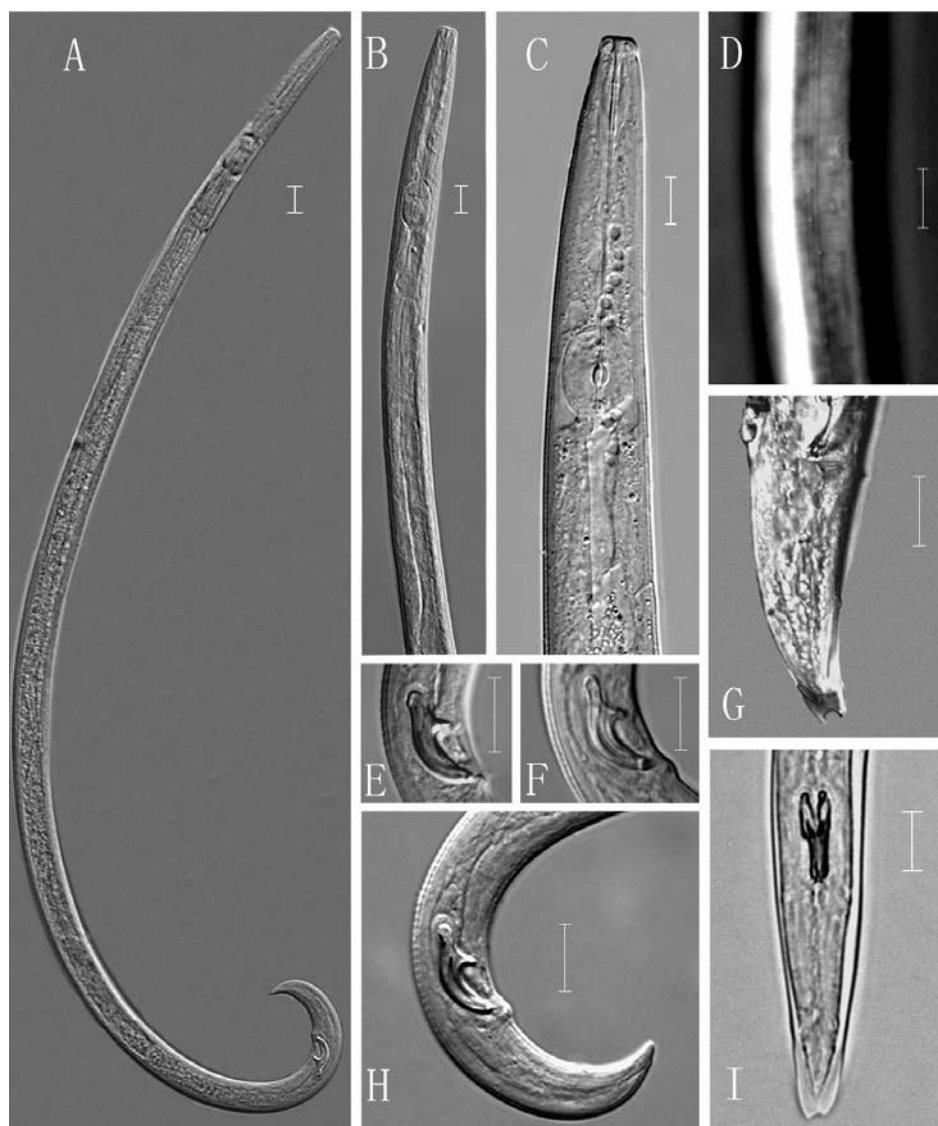


Fig. 3. Light photomicrographs of *Devibursaphelenchus wangi* sp. n. (male) A: Whole body; B, C: Anterior body; D: Lateral field; E, F: Spicules; G & I: Tail (ventral view, showing papillae and bursa); H: Tail. (Scale bars=10 μ m).

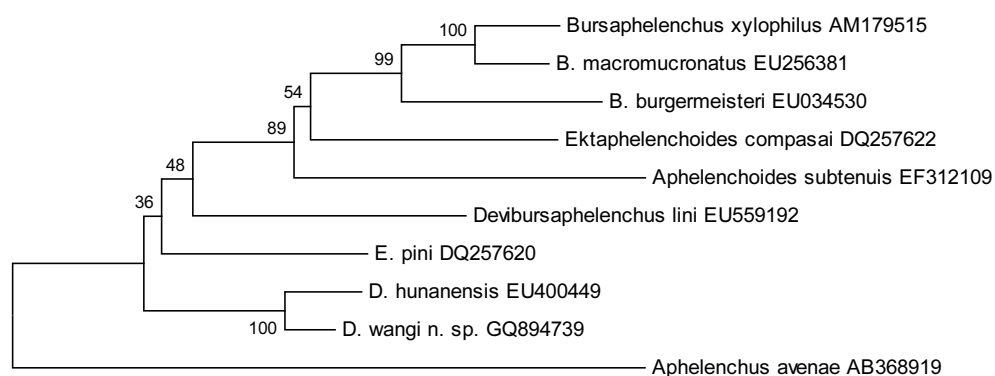


Fig. 4. Molecular phylogenetic status of *Devibursaphelenchus wangi* sp. n. based on ITS1/2 sequences. *Aphelenchus avenae* served as the outgroup species. Numbers at branching points are bootstrap values obtained using 1000 repetitions. Scale bar: substitutions/site.

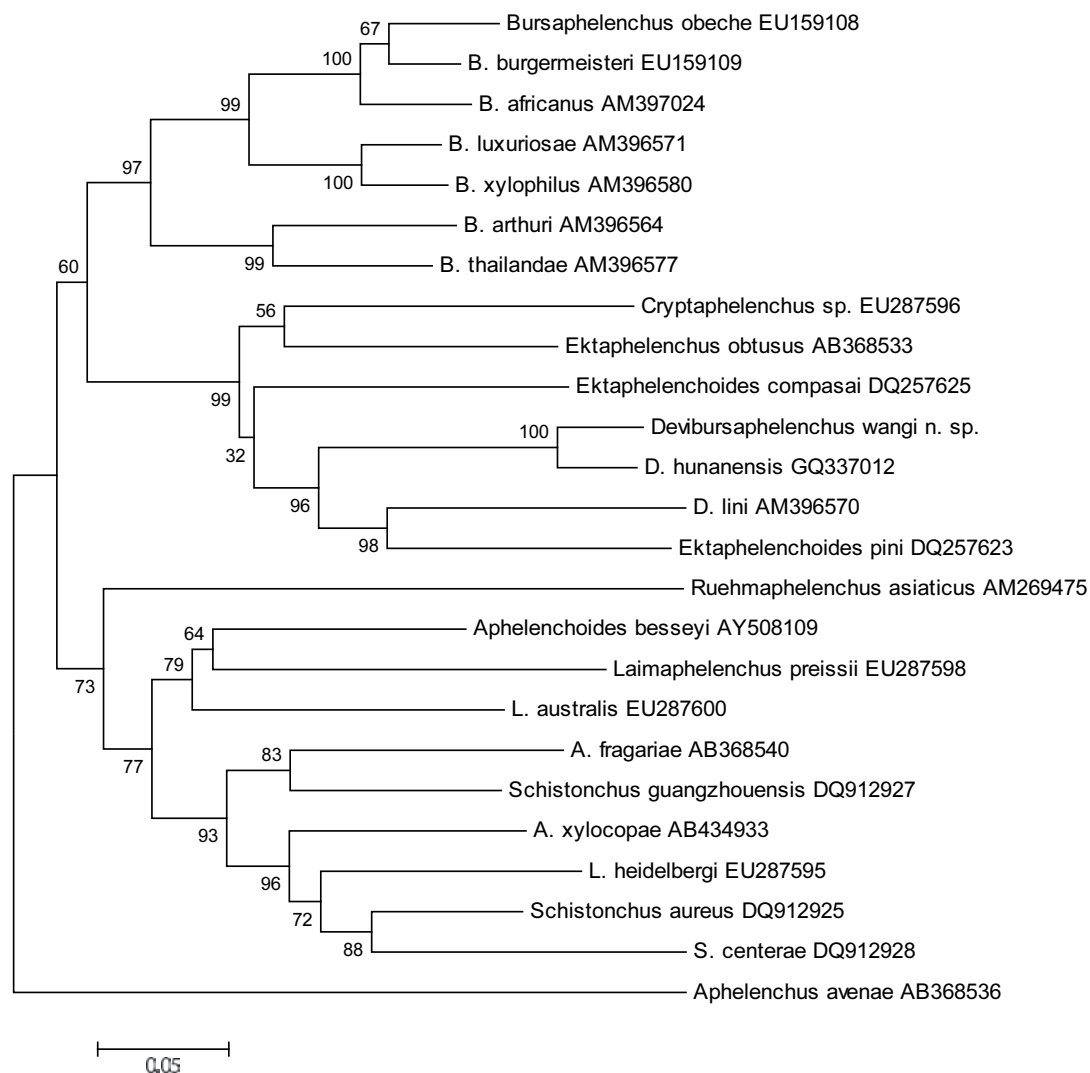


Fig. 5. Molecular phylogenetic status of *Devibursaphelenchus wangi* sp. n. based on partial LSU sequences. *Aphelenchus avenae* served as the outgroup species. Numbers at branching points are bootstrap values obtained using 1000 repetitions. Scale bar: substitutions/site.

The two molecular trees show that the new species is close to *D. hunanensis* (Figs 4 and 5), and that the genus *Devibursaphelenchus* is close to other genera of Ektaphelenchinae such as *Ektaphelenchoides*, *Ektaphelenchus* and *Cryptaphelenchus* (Fig. 5).

Type habitat and locality. Packaging wood of *Pinus* sp. from USA, inspected in Ningbo Entry-exit Inspection and Quarantine Bureau, China, in 2009.

Feeding habitat. Seven specimens of *Devibursaphelenchus wangi* sp. n., including males, females and juveniles were found feeding on *Aphelenchoides* sp. The bodies of *Aphelenchoides* sp. were penetrated by the stylets of *D. wangi* sp. n., which could not easily be separated from their food (Fig. 2).

Types. Holotype male, ten female and five male paratypes (slide numbers 848-1 to 848-11) deposited in the nematode collection of Ningbo Entry-exit Inspection and Quarantine Bureau, China. One paratype male and four paratype females (slide numbers 9319 and 9320) deposited in the Canadian National Collection of Nematodes, Ottawa, Ontario, Canada. One paratype male and four paratype females (slide numbers 848-12 and 848-13) deposited in the Institute of Biotechnology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China.

Etymology. *D. wangi* sp. n. is named after Wang Songqing, the deputy director of Ningbo Entry-exit Inspection and Quarantine Bureau, who had supported the authors' research.

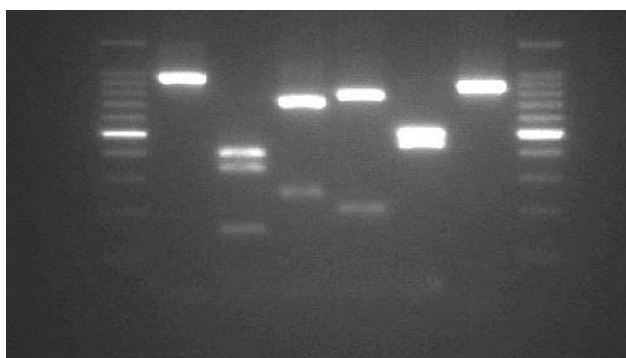


Fig. 6. ITS-RFLP pattern of *Devibursaphelenchus wangi* sp. n. M = Molecular size marker (100 bp ladder); Lane 1: rDNA amplification product; Lanes 2-6: Digestion products obtained with *RsaI*, *HaeIII*, *MspI*, *HinfI* and *AluI*. Sizes of PCR product and its restriction fragments are shown in Table 2.

DISCUSSION

The genus *Devibursaphelenchus* clearly belongs to Ektaphelenchinae with the following typical characters: stylet with relatively wide lumen, rectum and anus obscure in females, intestine ending in a blind sac. The molecular phylogenetic analysis also showed that *Devibursaphelenchus* species cluster together with those of the genera of Ektaphelenchinae (Fig. 5). Additionally, the predatory behaviour of *Devibursaphelenchus* species supports their placement within Ektaphelenchinae. Among the genera of Ektaphelenchinae, only *Devibursaphelenchus* has a bursa, which is otherwise typical for Parasitaphelenchinae.

The genus *Devibursaphelenchus* differs from *Ektaphelenchus* by having a male tail with a terminal bursa, but if the male is absent, the females of these two genera are similar especially for those *Ektaphelenchus* species without distinct basal swellings (all the stylets of *Devibursaphelenchus* species are without basal swellings).

According to Braasch (2009), the genus *Devibursaphelenchus* is characterised by a slender body; strong stylet (20–26 µm long) with relatively wide lumen and usually without basal thickenings; metacarpus elongate-oval, with valve plates located posterior to the middle of bulb; excretory pore posterior to metacarpus; vulva relatively posterior ($V = 76\text{--}80$), not protruding and without flap; vagina sclerotized; post-uterine branch short (up to 1.5 times the corresponding body diameter long); rectum and anus obscure in females; male tail strongly recurved, with distinct terminal bursa seen in dorso-ventral position; two pairs of male caudal papillae; spicules strong, almost straight and arcuate

in their distal part, with prominent rostrum, relatively high condylus, without cucullus, and in some species with a hook-like appendix at the distal end, with appearance of a cucullus.

The re-establishment of *Devibursaphelenchus* is supported by this new species description. However, the new species lacks a highly sclerotized vagina shown in other species of the genus (Braasch, 2009) and has a cucullus. Therefore, these features may not be used for genus characterisation.

Devibursaphelenchus hunanensis has several features in common with the other *Devibursaphelenchus* species (Braasch, 2009), but it was described as having four lateral lines (two refractive inner lines), and distinct rectum and anus of females (Yin *et al.*, 1988). Gu *et al.* (2006) reported that *D. hunanensis* was detected in Ningbo, China, but the rectum and anus of the reported species were not clearly seen. The identity of this species with the originally described *D. (B.) hunanensis* remains questionable.

Braasch (2004) observed that a specimen of *D. lini* was feeding on a *Bursaphelenchus xylophilus* juvenile. *D. hunanensis* has been observed feeding on *B. mucronatus* and *B. rainulfi* (unpubl. observations). *Devibursaphelenchus wangi* sp. n. has been observed feeding on *Aphelenchoides* sp. Therefore, we assume that *Devibursaphelenchus* species are predatory on other nematodes and cannot be multiplied on fungi. It will be very interesting and of practical interest to investigate the potential efficiency of *Devibursaphelenchus* species for a possible future control strategy of *B. xylophilus*.

Because packaging wood is a circulating product and there is no phytosanitary treatment mark, the exact geographic origin of the new species remains unclear. The vector of the new species is also unknown.

ACKNOWLEDGEMENT

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Jianfeng Gu, Jiangling Wang, Jingwu Zheng. *Devibursaphelenchus wangi* sp. n. (Nematoda: Ektaphelenchinae), питающийся на *Aphelenchoides* sp.

Резюме. Приводится описание и иллюстрации для *Devibursaphelenchus wangi* sp. n. Новый вид выделен из прибывшей из США упаковки, изготовленной из древесины сосны. Древесина упаковки была обследована в гавани Нингбо в Китае в 2009 году. Новый вид характеризуется сравнительно тонким телом ($a = 36.5$ и 37.1 для самцов и самок, соответственно); тремя линиями латерального поля, стилетом со сравнительно широким просветом и без базальных утолщений, отсутствием вульварной складки, не функционирующим и не различимым ректумом и анальным отверстием у самок; сравнительно небольшими (14.2 - 15.6 μm) спикулами с уплощенным кукулюсом, двумя парами хвостовых папилл. Новый вид морфологически близок к *D. eproctatus* и *D. hunanensis*, но может быть дифференцирован от них по размеру и форме спикул и меньшей длине стилета. Независимый статус этого нового вида подтвержден спектрами ITS-RFLP, а также результатами молекулярно-филогенетического анализа, основанного на сравнении полной ITS последовательности и частичной LSU последовательности. Показано, что новый вид близок к *D. hunanensis*. Исследован характер питания нематод нового вида.

Description of *Bursaphelenchus braaschae* sp. n. (Nematoda: Aphelenchoididae) found in dunnage from Thailand

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Summary. *Bursaphelenchus braaschae* sp. n. is described and figured. The new species was isolated from deciduous dunnage from Thailand. The new species belongs to the *fungivorus* group of the genus. It is characterised by relatively stout body ($a = 23.5$ and 24.0 for males and females, respectively); four lines in the lateral field; spicules relatively small and delicate (14.2 - 16.3 μm) with blunt rostrum and rounded distal end without cucullus, condylus thin and high, not dorsally bent, dorsal edge of spicules along condylus with characteristic darker section; and by females with strongly protruding vulva lips and a slim, attenuated tail with rounded terminus. The new species is morphologically closest to *B. willibaldi* and can be distinguished by the shape and size of spicules, post-uterine sac length and vulva position. The new species status is supported by ITS-RFLP patterns and molecular phylogenetic analysis based on partial SSU, ITS1/2 and partial LSU sequences, which revealed that *B. braaschae* sp. n. is closest to *B. willibaldi*.

Key words: *Bursaphelenchus braaschae* sp. n., DNA sequencing, morphology, molecular taxonomy, PCR-RFLP, SEM.

To prevent the spread of the pine wood nematode *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) Nickle, 1970 and other pests through packaging wood, almost all packaging wood imported through Ningbo Harbour, Zhejiang, China has been inspected and sampled since 1997 (Gu *et al.*, 2006).

In June 2009, big dunnage pieces made from deciduous wood without a phytosanitary mark were sampled and inspected during inspection of medium density fibreboard from Thailand. In addition to *Cryptaphelenchus* sp., *Ruehmaphelenchus* sp. and *B. fraudulentus*, a new species of *Bursaphelenchus* was detected. It is described and figured herein as *Bursaphelenchus braaschae* sp. n.

MATERIAL AND METHODS

Sawn samples taken from packaging wood were cut into small pieces no more than 1 cm wide. Nematodes were extracted by the modified Baermann funnel technique for 24 h and reared successfully on *Botryotinia fuckeliana*. Measurements were made on permanent slides fixed in TAF and processed to glycerol following the method of Seinhorst (1959). The light micrographs

were made using a Zeiss Imager Z1 microscope equipped with a Zeiss AxioCam MRm CCD camera. The SEM micrographs were taken with a scanning electron microscope Hitachi TM1000.

DNA samples of *Bursaphelenchus braaschae* sp. n. were prepared according to Li *et al.* (2008). Three sets of primers (synthesised by Invitrogen, Shanghai, China) were used in the PCR analyses to amplify the partial SSU region, the ITS1/2 region and the D2/D3 LSU region of rDNA, respectively. Primers for amplification of SSU were forward primer K4f (5'-ATG CAT GTC TAA GTG GAG TAT TA -3') and reverse primer K1r (5'- TTC ACC TAC GGC TAC CTT GTT ACG ACT -3') (Penas *et al.*, 2006). Primers for amplification of ITS1/2 were forward primer F194 (5'- CGT AAC AAG GTA GCT GTA G -3') (Ferris *et al.*, 1993) and reverse primer 5368r (5'- TTT CAC TCG CCG TTA CTA AGG -3') (Vrain, 1993). Primers for amplification of D2/D3 LSU were forward primer D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and reverse primer D3Br (5'-TCG GAA GGA ACC AGC TAC TA-3') (De Ley *et al.*, 1999). PCR conditions were as described by Li *et al.* (2008). PCR products were separated on 1% agarose gels and visualised by staining with ethidium bromide.

PCR products of sufficiently high quality were purified for cloning and sequencing by Invitrogen, Shanghai, China.

For ITS-RFLP profiles, suitable aliquots of the amplified ITS rDNA were digested for at least 3 h at 37°C using 10 U of each of the five restriction endonucleases (*Rsa*I, *Hae*III, *Msp*I, *Hinf*I and *Alu*I) (Takara, Japan) following the manufacturer's instructions. Fragments were resolved by electrophoresis in a 2.5% agarose gel and stained with ethidium bromide.

Partial SSU, ITS1/2 and partial LSU sequences of many other *Bursaphelenchus* species were available as GenBank accessions which had been contributed by various research teams (Ye *et al.*, 2007). Sequences were analysed and aligned using the program Clustal W implemented in MEGA version 4.0 (Tamura *et al.*, 2007). Phylogenetic trees were generated with the Neighbor Joining (NJ) method using the Tajima-Nei distance option. Bootstrapping analysis was performed with 1000 replicates.

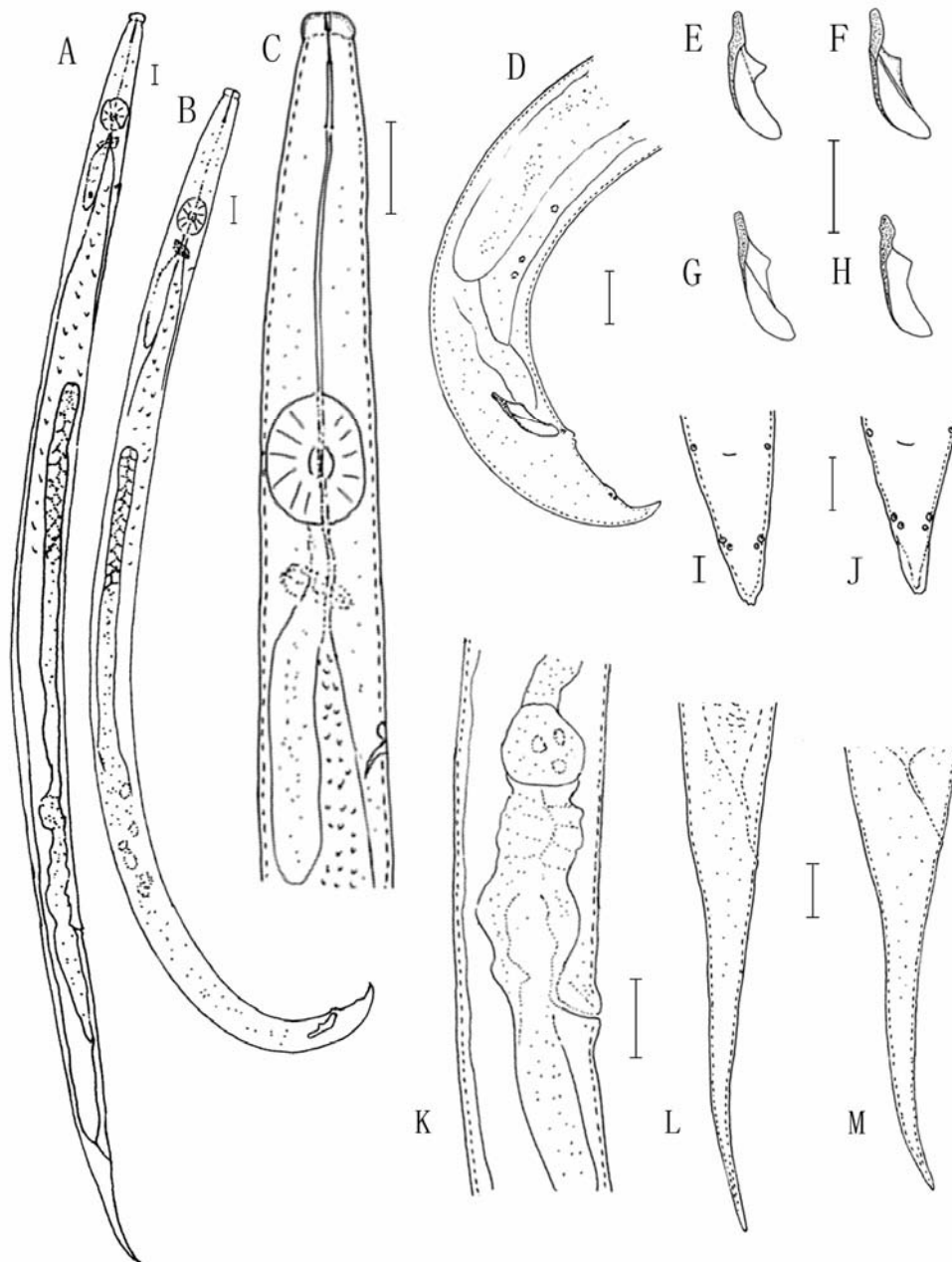


Fig. 1. *Bursaphelenchus braaschae* sp. n. A: Female; B: Male; C: Anterior body; D: Lateral view of male tail; E-H: Variation in shape of spicules; I, J: Ventral view of male tail; K: Vulva region; L, M: Variation of female tail. (Scale bars=10 µm).

DESCRIPTIONS

Bursaphelenchus braaschae sp. n. (Figs. 1, 2, 3)

Measurements. Table 1.

Male. Body stout, cylindrical, posterior region sharply curved ventrally when heat-killed. Cuticle weakly annulated, lateral field with four incisures (*i.e.*, three ridges). Lip region rounded, offset, about 3.5 μm high and 6.8 μm wide. Stylet with small basal swellings, conus *ca.* 39% of total length. Procorpus cylindrical. Median bulb strongly developed, almost oval, with valve plates placed slightly posteriorly. Pharyngeal gland lobe slender and well developed, about two to three body diameters long, overlapping intestine dorsally. Nerve ring located at *ca.* 6 μm posterior to median bulb. Excretory pore located at *ca.* 15 μm posterior to nerve ring. Hemizonid just anterior to excretory pore. Testis single, spermatocytes arranged in multiple rows. Cloaca opening lips slightly protruding. Spicules small (14.2–16.3 μm long) and separate, with blunt rostrum and rounded distal end without cucullus. Condylus thin and high, not dorsally bent. Rostrum small and low, bluntly conical. Junction of rostrum and calomus smoothly curved. Dorsal edge of spicules along condylus with characteristic darker section in light microscopy. Tail strongly recurved, terminus sharply pointed. Three pairs of caudal papillae present: the first lateral pair located slightly anterior to cloacal slit, the second and the third pairs about 1–2 μm apart from each other at the base of bursa, the third pair more ventral than the second. A single P1 papilla in front of cloaca has not been detected. Bursa 6–8 μm long, often truncated with two to three points.

Female. Body slightly arcuate ventrally when heat-relaxed. Cuticle and lip region similar to male. Ovary outstretched, developing oocytes in multiple rows. Vulva slightly inclined anteriad, vulva lips often strongly protruding, but sometimes only slightly thickened. Spermatheca oval, sometimes containing round sperms. Post-uterine sac well developed, about half vulva to anus distance, sometimes containing round sperms. Tail slim, attenuated with finely rounded terminus.

Diagnosis and relationships. *Bursaphelenchus braaschae* sp. n. is characterised by relatively stout body ($a = 23.5$ and 24.0 for males and females, respectively); four lines in the lateral field; spicules relatively small and delicate (14.2–16.3 μm) with blunt rostrum and rounded distal end without cucullus, condylus thin and high, not dorsally bent, dorsal edge of spicules along condylus with

characteristic darker section; and by females with strongly protruding vulva lips and a slim, attenuated tail with rounded terminus. Based on number of lateral lines, spicule shape (broad spicules with highly rounded apex, conspicuous ventral and dorsal limb, rounded distal end without cucullus), number and arrangement of caudal papillae of males and the other characters mentioned above (Figs. 1–3), *B. braaschae* sp. n. is affiliated to the *fungivorus* group of the genus (Braasch *et al.*, 2009).

According to Braasch *et al.*, (2009), the *fungivorus* group contains nine species: *B. huntii* (Steiner, 1935) Giblin & Kaya, 1983, *B. sychnus* Rühm, 1956 (J.B. Goodey, 1960), *B. steineri* Rühm, 1956 (J.B. Goodey, 1960), *B. fungivorus* Franklin & Hooper, 1962, *B. gonzalezi* Loof, 1964, *B. seani* Giblin & Kaya, 1983, *B. thailandae* Braasch & Braasch-Bidasak, 2002, *B. arthuri* Burgermeister, Gu & Braasch, 2005 and *B. willibaldi* Schönfeld, Braasch & Burgermeister, 2006.

Bursaphelenchus braaschae sp. n. is most similar to *B. willibaldi*. It differs from *B. willibaldi* by the size and shape of spicules (spicules average 15.3 μm vs 17 μm ; condylus not dorsally bent vs condylus slightly dorsally bent; rostrum in obtuse angle and indistinct vs rostrum in sharp angle and conspicuous), by the post-uterine sac length (average 50.4 μm , which occupies 55.3% of the vulva-to-anus distance vs average 95 μm , which occupies 68% of the vulva-to-anus distance), by slightly stouter body ($a = 23.5$ and 24.0 for males and females, respectively vs $a = 32$ and 29), and also the vulva lips protrude more in the new species.

Bursaphelenchus braaschae sp. n. is distinguished from: *B. huntii* by the shape of spicules (blunt rostrum vs sharp rostrum); *B. sychnus* by the shape and size of spicules (broadly rounded distal end vs pointed distal end; 14.2–16.3 μm vs 19–23 μm); *B. steineri* by the shape and size of spicules (blunt rostrum vs sharp rostrum; 14.2–16.3 μm vs 17 μm), and by different shape of female tail terminus (rounded vs pointed with a cuticular mucron); *B. fungivorus* by body size (average 535 μm and 554 μm for males and females vs 850 μm and 980 μm , respectively) and shape of spicules (blunt rostrum vs sharp and long rostrum), and by the shape of female tail (slightly ventrally curved vs ventrally hooked); *B. gonzalezi* by the shape of spicules (condylus high vs condylus low); and by the shape of female tail terminus (rounded vs pointed); *B. seani* by the shape and size of spicules (blunt rostrum vs sharp rostrum; 14.2–16.3 μm vs 18–27 μm) and the ratio c' in females (5.7 vs 3.4 on average); *B. thailandae* by different body shape ($a=23.5$ and 24.0 for males and females vs $a=39$ and 38 , respectively), ratio c' (5.7

Table 1. Measurements of *Bursaphelenchus braaschae* sp. n. Measurements in μm and in format: mean $\pm$ s.d. (range).

	Female		Male
	Holotype	Paratypes	Paratypes
n	-	15	15
L	629.0	554.1 $\pm$ 46.9 (466.5-623.6)	534.5 $\pm$ 42.8 (467.0-615.0)
a	25.6	24.0 $\pm$ 2.3 (18.6-28.0)	23.5 $\pm$ 2.4 (19.3-26.9)
b	8.2	7.4 $\pm$ 1.0 (5.6-8.9)	7.0 $\pm$ 0.7 (5.5-8.6)
b'	6.0	5.4 $\pm$ 0.5 (4.4-6.1)	4.7 $\pm$ 0.6 (3.8-6.2)
c	11.1	9.3 $\pm$ 0.7 (8.2-11.0)	22.2 $\pm$ 1.8 (19.5-25.8)
c'	4.9	5.7 $\pm$ 0.4 (5.1-6.4)	1.8 $\pm$ 0.1 (1.5-2.0)
V or T	70.7	70.5 $\pm$ 0.8 (69.3-71.9)	67.5 $\pm$ 7.2 (50.4-79.5)
Max. body diam.	24.6	23.8 $\pm$ 3.7 (17.8-30.7)	23.2 $\pm$ 2.8 (19.1-27.9)
Lip diam.	6.9	6.9 $\pm$ 0.5 (6.1-7.5)	6.8 $\pm$ 0.5 (5.9-7.5)
Lip height	3.1	3.5 $\pm$ 0.2 (3.1-3.8)	3.5 $\pm$ 0.3 (3.1-3.9)
Stylet length	14.8	14.6 $\pm$ 0.7 (13.4-15.7)	13.7 $\pm$ 0.8 (12.1-15.0)
Median bulb length	16.7	16.8 $\pm$ 1.0 (14.8-18.1)	16.8 $\pm$ 0.9 (14.6-18.0)
Median bulb diam.	13.7	13.7 $\pm$ 1.1 (12.1-15.8)	14.5 $\pm$ 1.4 (12.7-16.4)
Median bulb length/diam.	1.2	1.2 $\pm$ 0.1 (1.1-1.4)	1.2 $\pm$ 0.1 (1.0-1.4)
Excretory pore position	92.5	86.9 $\pm$ 5.0 (80.7-99.0)	88.0 $\pm$ 5.1 (82.0-99.0)
Spicule (chord)	—	—	15.3 $\pm$ 0.6 (14.2-16.3)
Spicule (dorsal limb)	—	—	17.4 $\pm$ 0.8 (16.1-18.6)
Ovary or testis length	191.3	248.7 $\pm$ 46.8 (156.0-318.0)	345.3 $\pm$ 66.6 (205.0-455.0)
Post-uterine sac length	76.8	50.4 $\pm$ 16.4 (29.0-78.3)	—
Post-uterine sac length/ Vulva to anus (%)	—	55.3 $\pm$ 14.8 (34.4-78.3)	—
Tail length	56.6	60.1 $\pm$ 4.9 (50.1-69.9)	24.2 $\pm$ 1.8 (21.5-27.1)
Anal body diam.	11.5	10.6 $\pm$ 1.3 (8.9-13.6)	13.3 $\pm$ 1.1 (11.7-16.2)

vs 4.2 on average), shape of female tail terminus vs (rounded pointed), and the length of bursa (only 2-4 μm in *B. thailandae*); *B. arthuri* by different shape and size of spicules (blunt rostrum vs sharp rostrum; 14.2-16.3 μm vs 16.0-20.9 μm), body length (average 535 μm and 554 μm for males and females vs 923 μm and 961 μm , respectively), and ratio c' of females (5.7 vs 4.8 on average).

Key to the *fungivorus* group

1. Six male papillae, rostrum low.....2

Seven male papillae, rostrum high and sharply pointed.....7

2. Rostrum sharply conical, spicules tip pointed or narrow.....3

Rostrum bluntly conical, spicule tip broad and blunt.....4

3. Spicules broad, female tail terminus sharply pointed.....*B. sychnus*

Spicules slim, female tail terminus mucronate.....*B. sternerii*

4. Female tale terminus pointed.....5

Female tale terminus finely rounded.....6

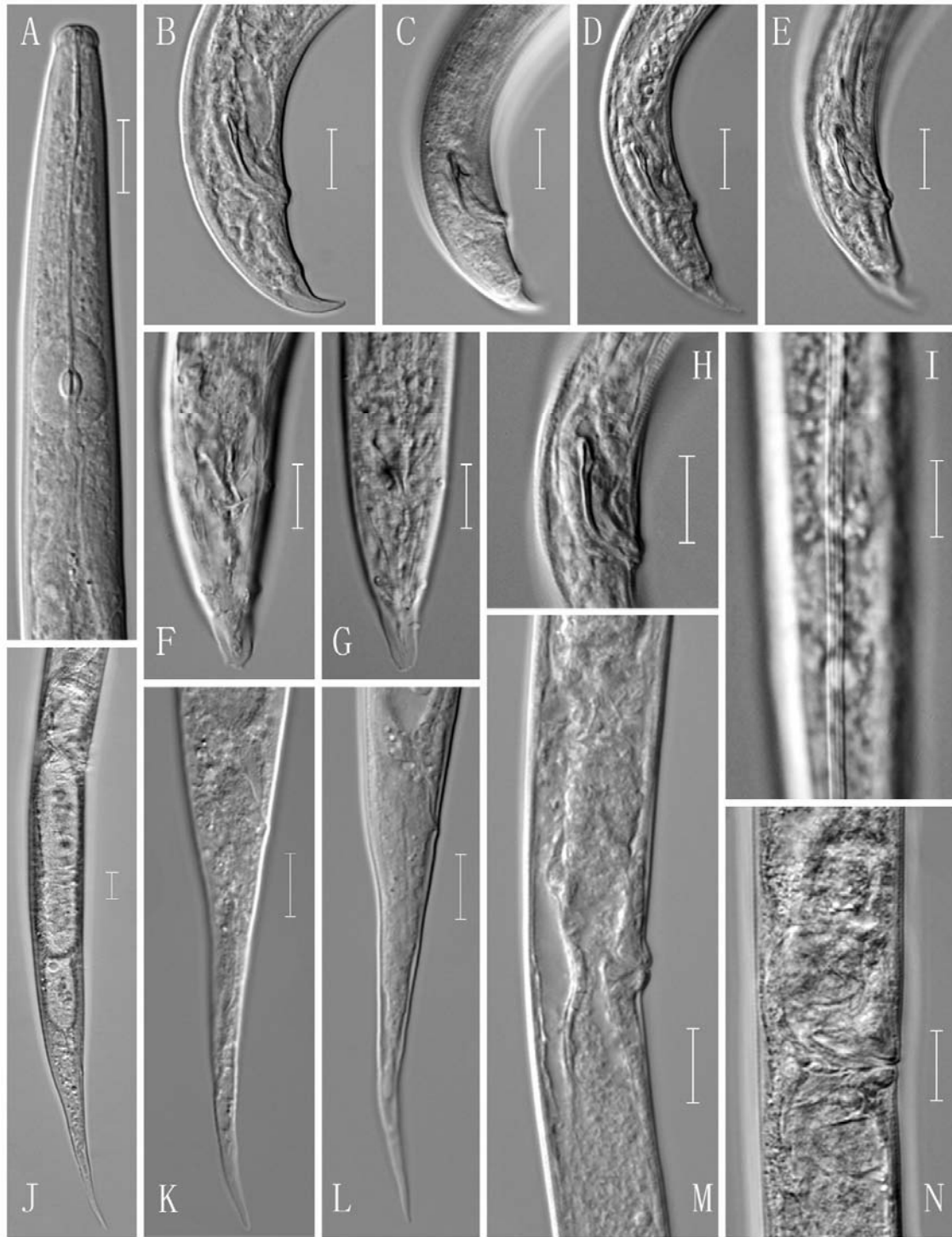


Fig. 2. Light photomicrographs of *Bursaphelenchus braaschae* sp. n. A: Female anterior region; B-E & H: Lateral view of male tail; F, G: Ventral view of male tail with variations of terminal bursa; I: Lateral lines; J-L: Female tail; M, N: Vulva region. (Scale bars=10 μ m).

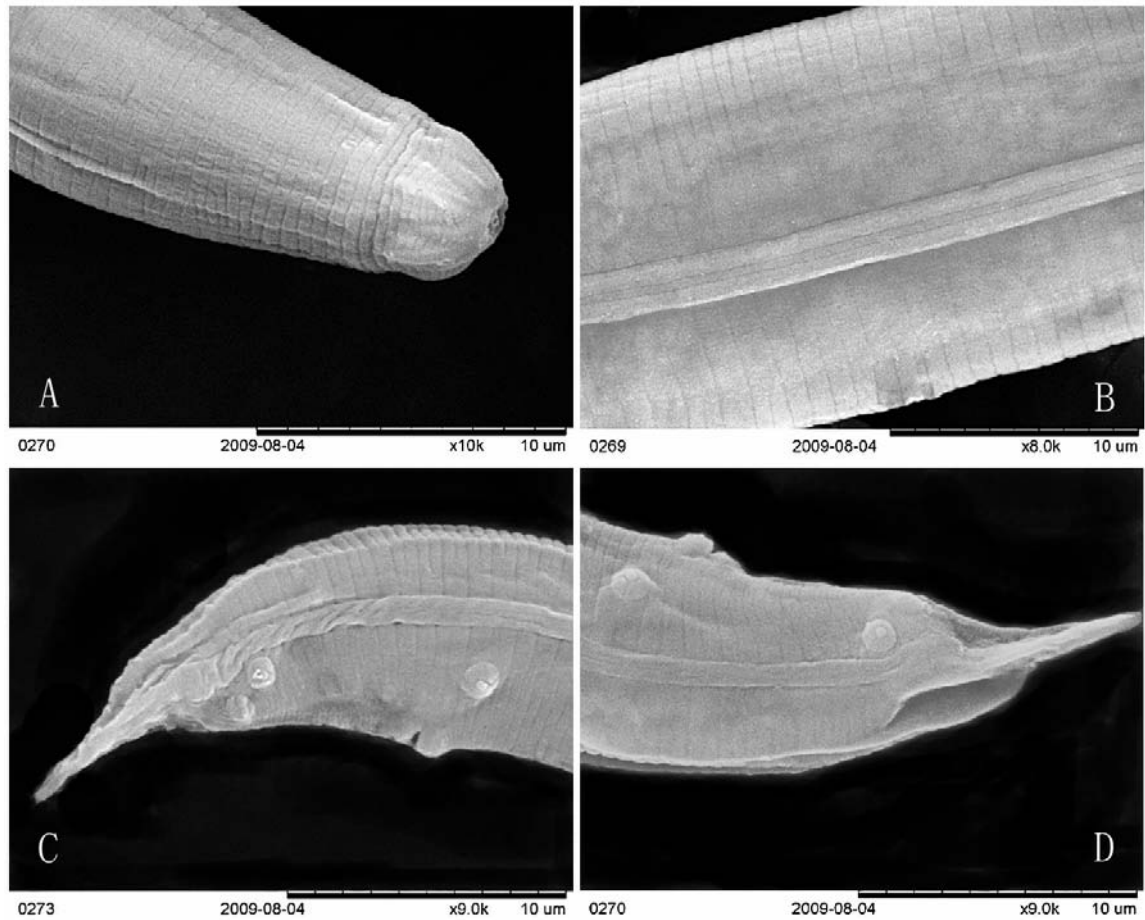


Fig. 3. Scanning electron microscope (SEM) observations of *Bursaphelenchus braaschae* sp. n. A: Head region; B: Lateral view; C, D: Male tail.

Table 2. Sizes of PCR products and DNA restriction fragments obtained in ITS-RFLP analysis and calculated on sequencing results of the ITS1/2 regions.

<i>Bursaphelenchus</i> species	PCR product (bp)	Restriction fragments (bp) ¹				
		<i>Rsa</i> I	<i>Hae</i> III	<i>Msp</i> I	<i>Hinf</i> I	<i>Alu</i> I
<i>B. braaschae</i> sp. n.	1060	552	852	1060	358	622
		508	208		325	332
					212	92
					94	14
					47	
					24	
<i>B. willibaldi</i>	1132	543	1132	731	488	534
		301		401	359	379
		288			215	136
					46	93
					24	
<i>B. thailandae</i>	880	482	880	880	382	555
		333			226	273
		65			202	52
					46	
					24	

¹Fragment sizes (bp) were calculated with the computer program DNASTAR MapDraw 5.01.

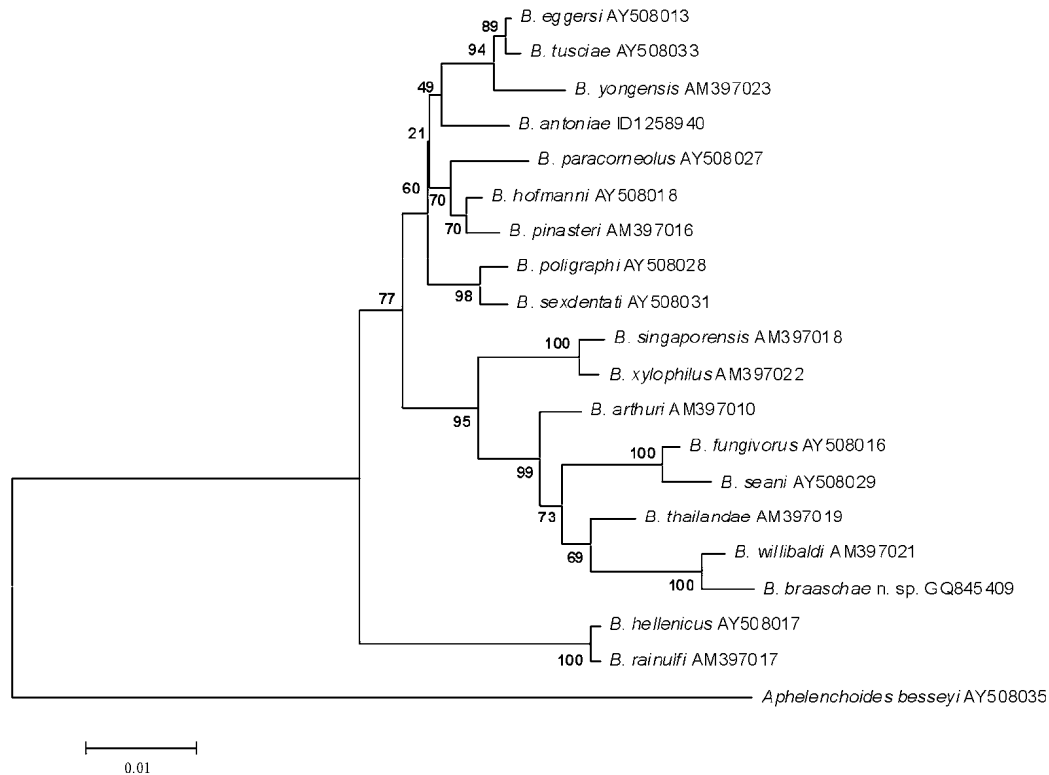


Fig. 4. Molecular phylogenetic status of *Bursaphelenchus braaschae* sp. n. based on partial SSU sequences. *Aphelenchus besseyi* served as outgroup species. Numbers at branching points are bootstrap values obtained using 1000 repetitions. Scale bar: substitutions/site.

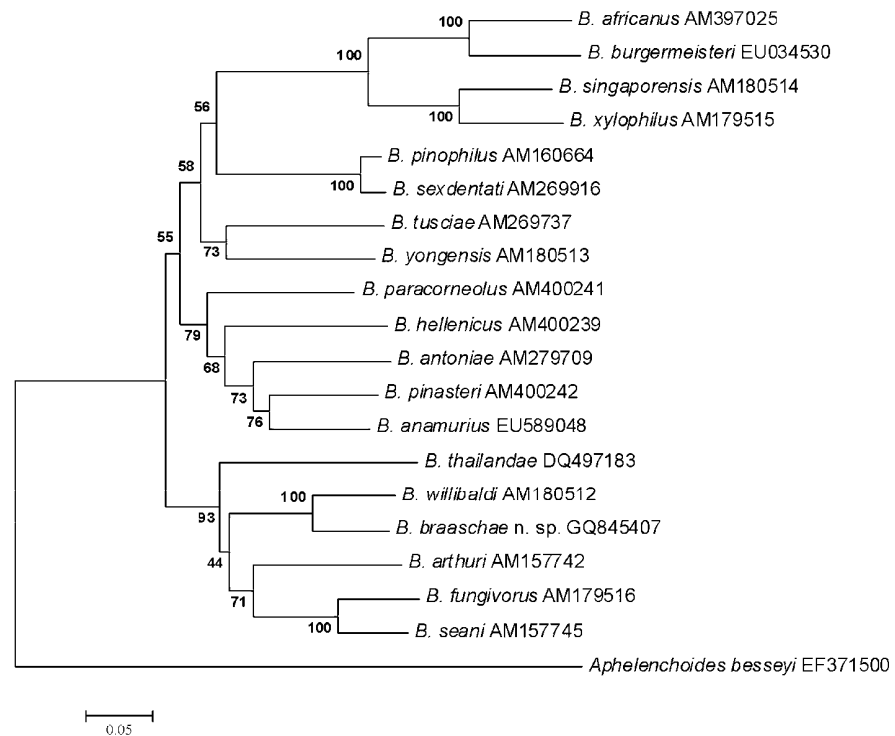


Fig. 5. Molecular phylogenetic status of *Bursaphelenchus braaschae* sp. n. based on ITS1/2 sequences. *Aphelenchoides besseyi* served as outgroup species. Numbers at branching points are bootstrap values obtained using 1000 repetitions. Scale bar: substitutions/site.

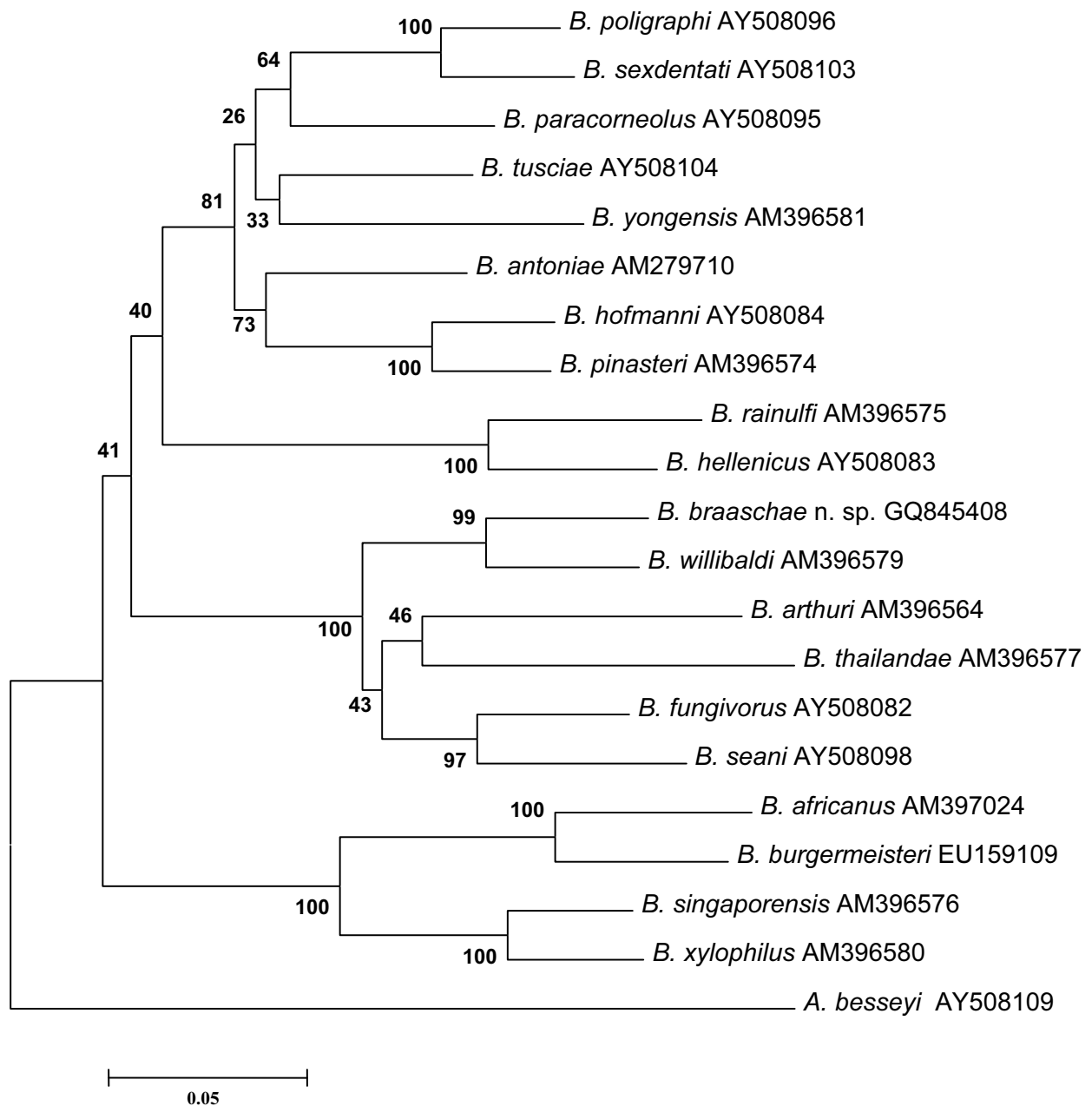


Fig. 6. Molecular phylogenetic status of *Bursaphelenchus braaschae* sp. n. based on partial LSU sequences. *Aphelenchus besseyi* served as outgroup species. Numbers at branching points are bootstrap values obtained using 1000 repetitions. Scale bar: substitutions/site.

5. Condylus low, female tail ventrally curved.....*B. gonzalezi*
 Condylus high, female tail straight.....*B. thailandae*
 6. Mean spicules length 15 μ m, vulva lips protruding.....*B. braaschae* sp. n.
 Mean spicules length 17 μ m, vulva lips not protruding.....*B. willibaldi*
 7. Female tale terminus pointed.....*B. seani*
 Female tale terminus finely rounded.....8

8. Condylus low.....*B. hunti*
 Condylus high.....9
 9. Female tail relatively short ($c'=4-5$) and slightly ventrally bent, post-uterine branch length occupies 75% of the vulva-to-anus distance.....*B. arthuri*
 Female tail relatively long ($c'=6-7$) and ventrally hooked, post-uterine branch length occupies 50% of the vulva-to-anus distance.....*B. fungivorus*

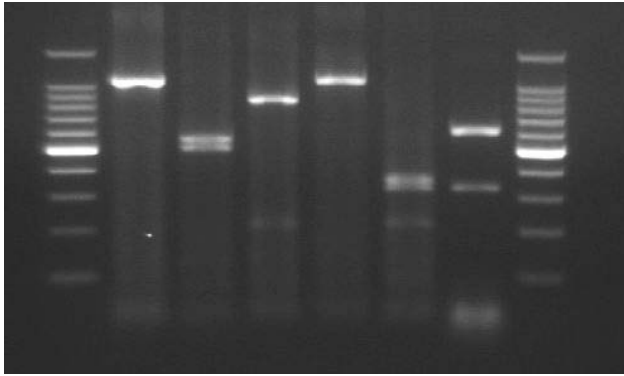


Fig. 7. ITS-RFLP pattern of *Bursaphelenchus braaschae* sp. n. M = Molecular size marker (100 bp ladder); Lane 1: rDNA amplification product; Lanes 2-6: Digestion products obtained with *RsaI*, *HaeIII*, *MspI*, *HinfI* and *AluI*. Sizes of PCR product and its restriction fragments are shown in Table 2.

Molecular profiles and phylogenetic status.

The rDNA base sequences of partial SSU, ITS1/2 and D2D3 LSU are deposited in the GenBank database with the accession numbers GQ845409, GQ845407 and GQ845408, respectively. The molecular phylogenetic status of the new species is shown in Figures 4, 5 and 6, and the ITS-RFLP profiles of rDNA are shown in Figure 7 and Table 2. The ITS-RFLP pattern of *B. braaschae* sp. n. is different from the patterns of known *fungivorus* group species, such as *B. fungivorus*, *B. seani*, *B. thailandae*, *B. arthuri* and *B. willibaldi* as shown in Burgermeister *et al.* (2009).

In all the molecular trees, the new species is close to *B. willibaldi* and the species of the *fungivorus* group distinctly grouped in one clade and separated from other species of the genus. The paired sequence similarities of *B. braaschae* sp. n. compared to *B. willibaldi* are 0.988, 0.751 and 0.924 for partial SSU, ITS1/2 and D2D3 LSU sequences.

Type locality and habitat. Deciduous dunnage from Thailand, inspected in Ningbo Entry-exit Inspection and Quarantine Bureau, China, in 2009.

Type specimens. Holotype male, eighty-three female and ninety male paratypes (slide numbers 14190-1 to 14190-30) deposited in the nematode collection of Ningbo Entry-Exit Inspection and Quarantine Bureau, China. Eight paratype males and eleven paratype females (slide numbers 14190-31 and 14190-32) deposited in the Canadian National Collection of Nematodes, Ottawa, Ontario, Canada. Nine paratype males and ten paratype females (slide numbers 14190-33 and 14190-34) deposited in the Institute of Biotechnology, College

of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China.

Etymology. *Bursaphelenchus braaschae* sp. n. was named after Dr Helen Braasch from the former Federal Biological Research Center for Agriculture and Forestry of Germany for her outstanding contributions to the understanding of the genus *Bursaphelenchus* and her great help to the first author in many nematological investigations.

DISCUSSION

According to Braasch *et al.* (2009), position and number of male caudal papillae are important in grouping of *Bursaphelenchus*, apart from other characters. Males of the *fungivorus* group appear to have seven preanal and postanal papillae as observed in *B. seani*, *B. fungivorus*, *B. huntii* and *B. arthuri*, with the single P1 papilla occurring relatively far above the cloacal slit, and the two postanal pairs close to each other (1-2 μ m distance). However, the single P1 papilla has not been observed in *B. sychmus*, *B. steineri*, *B. gonzalezi*, *B. thailandae*, *B. willibaldi* and *B. braaschae* sp. n. Possibly, these species represent two subgroups of the *fungivorus* group as supported by the phylogenetic trees based on rDNA sequences (Figs. 4 and 5), which show that *B. fungivorus*, *B. seani* and *B. arthuri* group together (seven papillae, rostrum high and sharply pointed), whereas *B. thailandae*, *B. willibaldi* and *B. braaschae* sp. n. cluster together in another branch (six papillae, rostrum low and bluntly conical). However, it is also possible that the P1 papilla is relatively indistinct or does not open through the cuticle in the species in question.

Without further molecular evidence, the grouping status of *B. sychmus*, *B. steineri*, *B. gonzalezi* and *B. huntii* is still questionable.

The vector of the new species is unknown.

ACKNOWLEDGEMENT

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Jianfeng Gu, Jiangling Wang. Описание *Bursaphelenchus braaschae* sp. n. (Nematoda: Aphelenchoididae) из материала упаковочных подстилок, изготовленных в Таиланде.

Резюме. Дано описание и иллюстрации для *Bursaphelenchus braaschae* sp. n. Новый вид был выделен из подстилок под морские грузы, изготовленных из древесины лиственных пород в Таиланде. Новый вид относится к группе видов *fungivorus* и характеризуется сравнительно мощным телом ($a = 23.5$ и 24.0 для самцов и самок, соответственно); четырьмя линиями в латеральном поле, сравнительно короткими и тонкими спикулами ($14.2-16.3 \mu m$) с притупленным роостромом, округлой дистальной частью и без кукулуса, тонким и высоким кондилусом без загиба на спинную сторону, дорсальным краем спикул вдоль кондилуса с характерной затемненной частью, самками с сильно выступающими губам вульвы и тонким вытянутым хвостом с закругленным терминусом. Новый вид морфологически близок к *B. willibaldi*, но отличается от него формой и размером спикул, длиной заднего маточного мешка и положением вульвы. Статус отдельного вида для него подтвержден спектром ITS-RFLP, а также результатами молекулярно-филогенетического анализа, основанного на частичной последовательности SSU, полной ITS и частичной последовательности LSU рибосомальной ДНК. Этот же анализ подтвердил близость *B. braaschae* sp. n. к *B. willibaldi*.

Studies on the genus *Aporcelaimellus* Heyns, 1965 (Dorylaimida: Aporcelaimidae) - material studied by Thorne and Swanger in 1936 but not named

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Summary. The identity of four species of *Aporcelaimellus sensu lato* studied, but not originally described, by Thorne and Swanger in 1936 are analysed and discussed on the basis of studies on the material deposited in Thorne's collection. *Dorylaimus papillatus* is described and illustrated, and provisionally considered to be a valid species since it is distinguishable from *A. obtusicaudatus* by the vagina, which is without *pars refringens*. The material labelled as *Dorylaimus perfectus*, often regarded as a synonym of *A. obtusicaudatus*, consists of two males very similar to those described for *Labronema goodeyi*, and seven females certainly belonging to *A. obtusicaudatus*; measurements and illustrations of these specimens are provided. One male labelled as *Dorylaimus paraobtusicaudatus* is *Metaporcelaimus romanicus*; it is described in detail and illustrated. Part of the material originally described as *Dorylaimus propinquus* is actually *Aporcelaimellus waenga*, of which new data, including detailed description, measurements and illustrations, are provided; in addition, *Aporcelaimellus laevis* is regarded as a junior synonym of *A. waenga*.

Key words: *Aporcelaimellus*, *Aporcelaimellus laevis*, *Aporcelaimellus papillatus*, *Aporcelaimellus waenga*, description, *Dorylaimus perfectus*, identity, *Metaporcelaimus romanicus*, morphology, new synonym, taxonomy.

This is the second in a series of papers devoted to studying species of the genus *Aporcelaimellus sensu lato*. For more detailed information about the rationale and the objectives of this work the reader is referred to the first contribution on the matter (Álvarez-Ortega & Peña-Santiago, 2010).

In their classical monograph on dorylaimid nematodes, Thorne and Swanger (1936) described several new as well other known species currently classified under *Aporcelaimellus*. The taxa originally described by the American authors were treated in our first paper; those not formally named in 1936 are discussed here.

MATERIAL AND METHODS

The material studied, deposited at the USDA Nematode Collection, was available to the authors by courtesy of Dr. Z. Handoo. The specimens are preserved in five permanent glycerin slides (the technique used for preparing

and mounting nematodes is described in Thorne and Swanger, 1936), and placed on 76 × 26 mm aluminium slides to allow handling. The information included on the labels of each slide and the specimens they contain is summarised in Table 1. Additionally, Fig. 1 displays the original labels and disposition of nematodes in every slide.

All the specimens supposedly belonging to the genus *Aporcelaimellus* in acceptable condition were studied using a light microscope. Morphometrics included De Man's indices and most of the usual measurements. The location of the pharyngeal gland nuclei is expressed according to Loof and Coomans (1970). Some of the best preserved specimens were photographed with a Nikon Eclipse 80i microscope and a Nikon DS digital camera. Raw photographs were edited using Adobe® Photoshop® CS. Drawings were made using a *camera lucida*.

The species are presented as originally labelled on the corresponding slide.

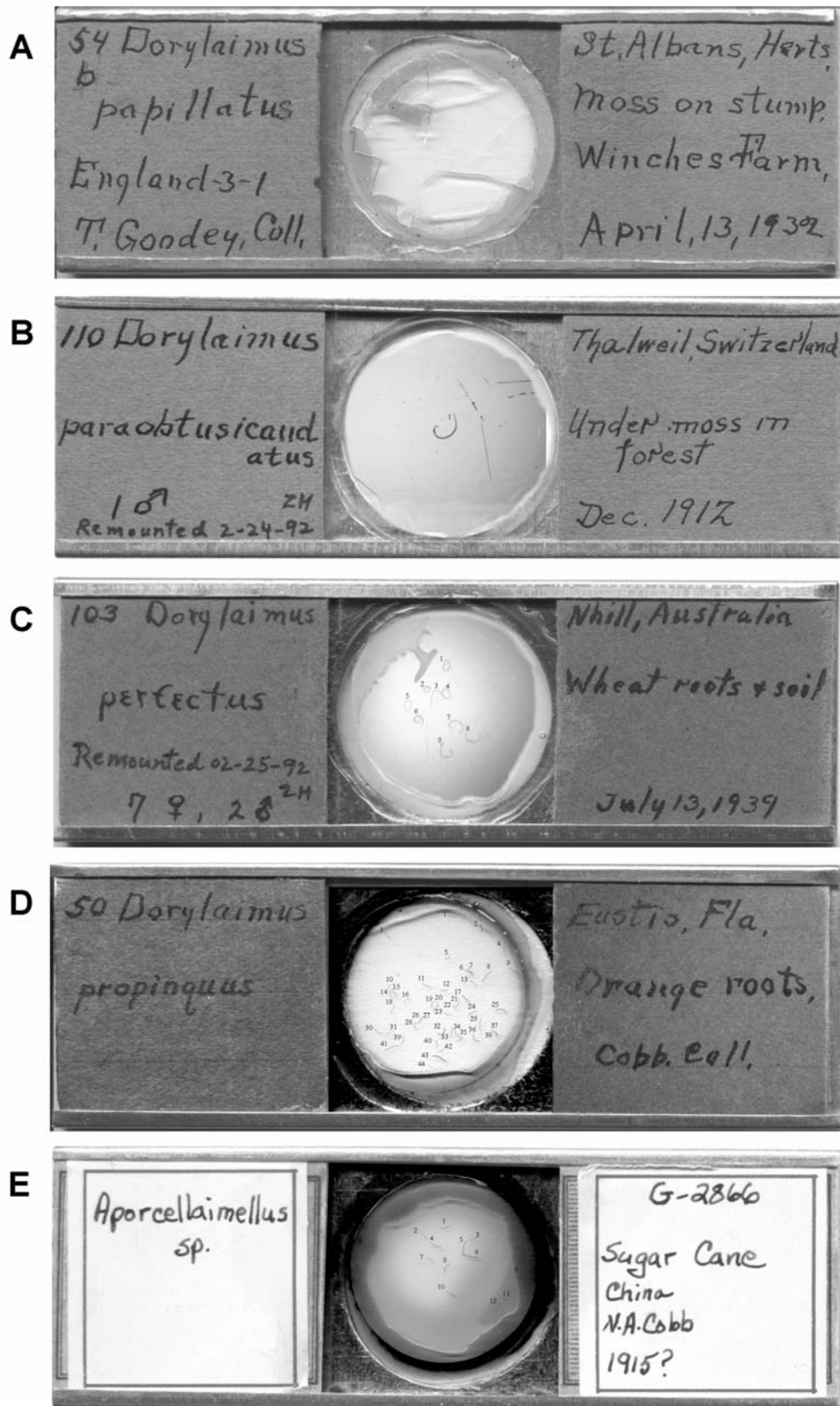


Fig. 1. Slides studied with their labels. A: *Dorylaimus papillatus*; B: *D. paraobtusicaudatus*; C: *D. perfectus*; D: *D. propinquus*; E: *Aporcellaimellus* sp.

Table 1. Origin and content of the slides studied.

Slide label		Locality and habitat	Date	Specimens contained
54b	<i>Dorylaimus papillatus</i> 12 spec.	St. Albans, Herts. (U.K.) Moss on stump, Winches farm.	April 13, 1932	<i>Aporcelaimellus papillatus</i> : 11 females and 1 male.
110	<i>Dorylaimus paraobtusicaudatus</i> 1 spec.	Thalweil, Switzerland Under moss in forest	December 1912	<i>Metaporcelaimus romanicus</i> : 1 male.
103	<i>Dorylaimus perfectus</i> 9 spec.	Nhill, Australia Wheat roots and soil	July 13, 1939	<i>Aporcelaimellus obtusicaudatus</i> : 7 females <i>Labronema</i> sp.: 2 males.
50	<i>Dorylaimus propinquus</i> 44 spec.	Eustis, Florida (USA) Orange roots		<i>Aporcelaimellus waenga</i> : 3 females and 11 juv. <i>Aporcelaimellus propinquus</i> : 2 females, 5 males, and 17 juv. Non <i>Aporcelaimellus</i> : 1 male and 5 juv.
G-2866	<i>Aporcelaimellus</i> sp. 12 spec.	China Sugar Cane	1915	<i>Aporcelaimellus waenga</i> : 1 female and 11 juv.

DESCRIPTIONS

Dorylaimus papillatus Bastian, 1865 (Fig. 2; as *Aporcelaimellus papillatus*)

Nomenclature. The original species described by Bastian was transferred to *Eudorylaimus* Andr ssy, 1959 by Andr ssy (1959) and later to *Aporcelaimellus* by Baqri and Khera (1975). The material of *D. papillatus* studied by Thorne and Swanger was considered to belong to *Aporcelaimellus obtusicaudatus* by De Ley *et al.* (1993), but it is herein separated from this and provisionally regarded as conspecific with Bastian's population (see remarks).

Material examined. Eleven females, one male and four juveniles, mounted on slide "54b *Dorylaimus papillatus*"; in bad condition.

Measurements. See Table 2; as *Aporcelaimellus papillatus*.

Adult. Moderately slender to slender nematodes of medium size, 2.43-3.08 mm long. Body cylindrical, tapering towards both extremities, but more so towards the anterior end. Habitus curved ventrad after fixation, especially in posterior body region, J-shaped. Cuticle not well observed due to bad condition of specimens. Lateral chord 6-14 μ m wide at mid-body, occupying 8-15% of the corresponding body diameter. Body pores inconspicuous. Lip region offset by constriction, but its precise morphology difficult to observe because the cuticle is often lost; lips apparently rather separated. Amphids not observed. Cheilostom nearly cylindrical, lacking any differentiation. Odontostyle typical of the genus, 4.1-4.8 times as long as wide, and 0.62-0.77% of body length;

aperture 14.5-17.0 μ m long or occupying about four-fifths (77-85%) its length. Guiding ring plicate. Odontophore linear, rod-like, 2.0-2.3 times as long as odontostyle. Anterior region of pharynx enlarging very gradually; basal expansion 6.1-7.6 times as long as wide, 3.2-4.6 times as long as body diameter, and occupying 51-55% of total neck length; pharyngeal gland nuclei located as follows: DN = 58-62, S₁N₁ = 68-72, S₁N₂ = 79-81, S₂N = 90-92. Nerve ring located at 174-206 μ m from anterior end or 31-34% of total neck length. Cardia conical, 17.5 x 13.0 μ m (n=1); its junction to pharyngeal base surrounded by a ring-like structure. Prerectum 3.4-4.9, rectum 1.0-1.4 anal body widths long.

Female. Genital system didelphic-amphidelphic; both branches equally and well developed, the anterior 310-446 μ m and the posterior 303-450 μ m long. Ovaries large, reflexed, usually reaching and surpassing the sphincter level, even reaching vulva; the anterior 313-321 μ m, the posterior 290-343 μ m long; oocytes arranged first in two or more rows, then in a single row. Morphology of genital tract not well observed. Oviduct 117-188 μ m long or 1.2-2.2 times the corresponding body diameter; it consists of slender part with prismatic cells and a poorly to moderately developed *pars dilatata*. Oviduct-uterus junction surrounded by a sphincter. Uterus a simple tube, 111-131 μ m or 1.3-1.5 times the corresponding body diameter. Uterine egg ovoid, 100-117 x 50-68 μ m, 1.5-2.2 times as long as wide. Vagina extending inwards about 24 μ m (n = 2) or one-fourth of body diameter; *pars proximalis* 16 x 11 μ m (n = 1); *pars refringens* apparently absent; *pars distalis* 8 μ m long. Vulva a pre-equatorial, transverse slit. Tail rounded conoid, slightly more straight in its ventral side, but other details obscure (see remarks).

Male. Genital system diorchic, with opposite testes. Ventromedian supplements not observed due

to bad condition of the only available specimen. Spicules somewhat curved ventrad, about 4.8 times as long as wide. Lateral guiding pieces about 26 µm long, 8.8 times as long as wide. Tail similar to that of female, but more straight ventrally.

Diagnosis. *Aporcelaimellus papillatus* is characterised by its body 2.43–3.08 mm long, lip region offset by constriction, odontostyle 19–24 µm long with aperture occupying 77–85% of total length, neck 555–649 µm long, pharyngeal expansion 288–349 µm long or 51–55% of total neck length, female genital system amphidelphic, uterus 111–131 µm or 1.3–1.5 times the corresponding body diameter, *pars refringens vaginae* (?) absent, $V = 45$ –50, female tail convex conoid to rounded (31–43 µm, $c = 60$ –87, $c' = 0.8$ –1.1), spicules 73 µm long, and eight widely spaced ventromedian supplements, the posteriormost at level of anterior end of spicules.

Relationships. Assuming that *pars refringens vaginae* is absent (see remarks) and in having body more than 2.40 mm long, *A. papillatus* resembles *A. maximus* Rahman, Jairajpuri, Ahmad & Ahmad, 1986, *Metaporcelaimus oceanicus* Andrassy, 2001 and *A. shamini* Ahmad, 1995. It differs from *A. maximus* in its smaller size (*vs* $L = 3.37$ –3.96), larger odontostyle aperture (*vs* about half its length), shorter pharyngeal expansion (*vs* 63–66% of total neck length), more anterior vulva (*vs* $V = 51$ –54), and male present (*vs* absent). From *M. oceanicus* in its longer odontostyle (*vs* 16–18.5 µm), shorter pharyngeal expansion (*vs* about three-fifths of total neck length), more anterior vulva (*vs* $V = 51$ –55), shorter and more rounded tail (*vs* 61–74 µm, $c' = 1.4$ –1.8, conical), and males known (*vs* unknown). And from *A. shamini* in its shorter uterus (*vs* 202–215 µm long), more anterior vulva (*vs* $V = 59$ –61), and shorter tail (*vs* $c' = 1.3$ –1.6).

Distribution. According with the label on Thorne's slide, the material herein studied was collected from moss on stump, in Winches farm, St. Albans, Herts, UK, by T. Goodey on April 13, 1932.

Remarks. The material studied is not in good condition, and this is the reason why some morphological features are not described in detail. The cuticle is lacking in many specimens, especially at level of lip region and tail. Descriptions of vagina and vulva should be taken with caution because, lacking the cuticle, the interpretation of these structures is problematic. The *pars refringens vaginae* is apparently absent, since no refractive piece has been observed. Thorne and Swanger (1936) illustrate the tail being convex-conoid to rounded, but, lacking the cuticle, it looks more conical in the material herein studied. There is no doubt that the specimens examined are the same that were studied by the American authors, and the

above description agrees perfectly with that provided by them.

A different question is whether this material is conspecific with that described by Bastian (1865) from UK, Berks, Broadmoor, although no significant difference is found with his simple, original description: $L = 2.54$, $b = 4.0$, stylet (certainly odontostyle plus odontophore) 51 µm long, tail 38 µm long. De Ley *et al.* (1993) considered that the material described by Thorne and Swanger (*op. cit.*) belongs to *A. obtusicaudatus*. The re-examination of the material studied by Thorne and Swanger has revealed that *pars refringens vaginae* is certainly absent, and this is a major difference with *A. obtusicaudatus*; thus, this material is, with the due caution, provisionally regarded as belonging to *A. papillatus*.

Bütschli (1873) reported *Dorylaimus papillatus*, but Thorne and Swanger (1936) considered this record did not belong to the true *papillatus*. Figure 1a of Bütschli apparently shows a female with distinct *pars refringens vaginae*, which might be better placed in *A. obtusicaudatus*.

De Man (1876) also reported *Dorylaimus papillatus* from The Netherlands, but, later, the same author (1880, 1884) considered this material to be *A. obtusicaudatus*.

De Bruin & Heyns (1992) identified as *A. papillatus* two females and two males from South Africa, but some doubts exist on the identity of this material due to significant differences with the material herein examined, some of which have already been mentioned by the South African authors, including longer (*vs* $L = 2.12$ –2.23) and more stout (*vs* $a = 40$ –43) body, longer odontostyle (*vs* 14–16 µm) with larger aperture (*vs* 53–57% of total length), longer neck (*vs* 460–510), shorter uterus (*vs* ca. twice the body diameter, according with original illustration), longer spicules (*vs* 57–61 µm), and higher number (*vs* five) of ventromedian supplements.

Cobb (1893) described *Dorylaimus domus Glauci* from Pompeii (Italy), but available information on this species is extremely poor. Thorne and Swanger (1936) synonymised it with *A. papillatus*, but De Ley *et al.* (1993) regarded it as *species inquirendae*. Taking into account that nothing is known about odontostyle nature (no illustration was provided in the original description of the species), it should be better considered as a *species incertae sedis*.

Dorylaimus perfectus Cobb, 1893

(Figs. 3 & 4; as *Aporcelaimellus obtusicaudatus* and *Labronema* sp., see remarks)

Nomenclature. The original species described by Cobb was regarded as a junior synonym of

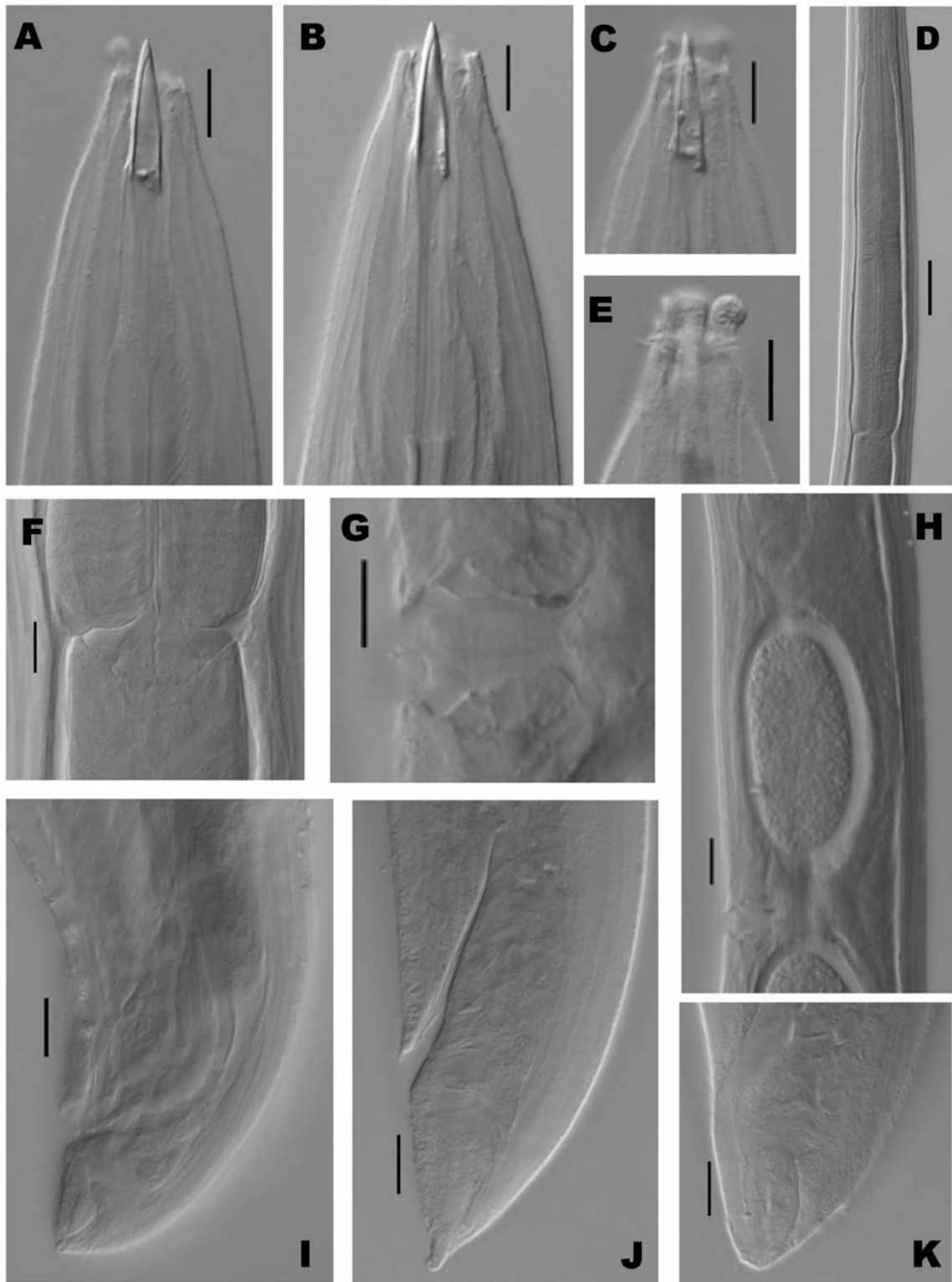


Fig. 2. *Aporcelaimellus papillatus* (Bastian, 1865) Baqri & Khera, 1975. A, B: Anterior region; C: Lip region in median view; D: Pharyngeal expansion; E: Lip region in surface view; F: Pharyngo-intestinal junction; G: Vagina; H: Uterine egg; I: Male tail and spicules; J, K: Female tail. (Scale bars: A-C, E-G, I-K = 10 µm; D = 50 µm; H = 20 µm).

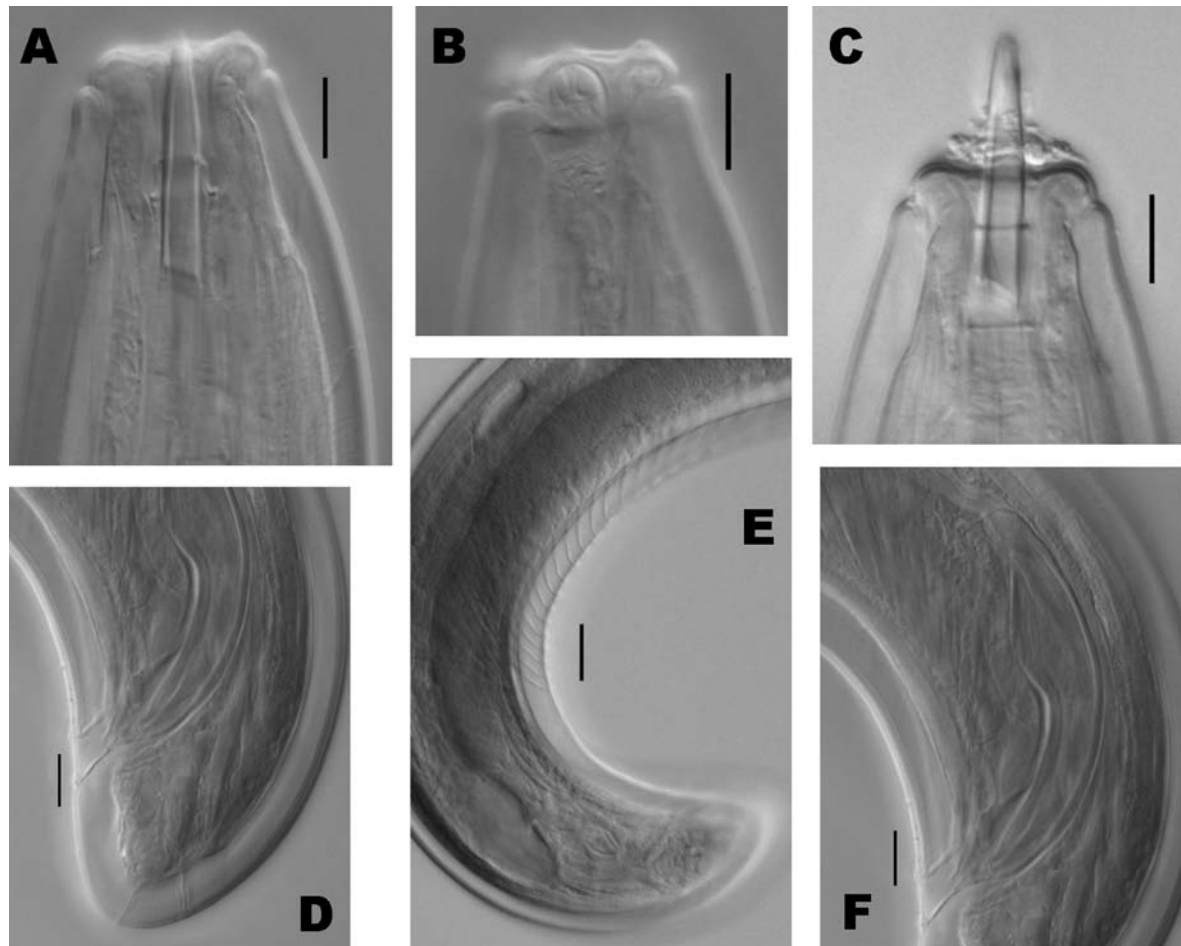


Fig. 3. *Labronema* sp. (male). A, C: Anterior region in median view; B: Lip region in surface view; D: Tail; E: Posterior body region; F: Spicules. (Scale bars: A-D, F = 10 μ m; E = 20 μ m).

Dorylaimus obtusicaudatus by Micoletzky (1922), and as a subspecies of the same species by Schneider (1937). In discussing its identity, De Ley *et al.* (1993) concluded that both taxa are “distinguishable ... on the basis of original description, but re-examination desirable”. The material herein studied of *D. perfectus* belongs to (see remarks) two different species, namely *Aporcelaimellus obtusicaudatus* and *Labronema* sp.; meanwhile the true identity of the original *D. perfectus* remains obscure.

Material examined. Seven females and two males, mounted on slide “103 *Dorylaimus perfectus*”, collected from wheat roots and soil, in Nhill, Victoria, Australia, on (according to the slide label) July 13, 1939.

Measurements. See Table 2, as *Aporcelaimellus obtusicaudatus* and *Labronema* sp.

Remarks. Cobb (1893) described this species on the basis of one female and several males

collected from soil about the roots of banana plants in Fiji Islands on July, 1891, but the same author stated that he was “not perfectly certain that the male and female here described together really belong to one and the same species”. The males in question were described as having “about twenty-three innervated closely approximated low papillae”, an unusual feature within the genus *Aporcelaimellus*.

In their monograph, Thorne and Swanger (1936) included the original description by Cobb, but, apparently, they did not examine Cobb’s original material or any new material. As mentioned above, the slide herein studied, belonging to Thorne’s collection, dates from 1939, but the American authors either never studied this slide or did not publish their observations. The re-examination of the specimens mounted on Thorne’s slide has revealed new relevant data.

Table 2. Morphometric data of *Aporcelaimellus papillatus* (Bastian, 1865) Baqri & Khera, 1975, *A. obtusicaudatus* (Bastian, 1865) Altherr, 1968 and *Labronema* sp. Measurements in μm (except L, in mm), and in the form: mean $\pm$ standard deviation (range).

Species Population		<i>A. papillatus</i> St. Albans, UK		<i>A. obtusicaudatus</i> Nhill, Australia	<i>Labronema</i> sp. Nhill, Australia
Character	n	11 ♀♀	♂	7 ♀♀	2 ♂♂
L		2.79 $\pm$ 0.20 (2.43-3.08)	2.96	2.48 $\pm$ 0.13 (2.26-2.64)	2.53, 2.40
a		31.0 $\pm$ 2.8 (27.2-34.3)	?	28.6 $\pm$ 2.3 (26.5-32.2)	30.3, 31.3
b		4.6 $\pm$ 0.2 (4.2-4.9)	?	4.5 $\pm$ 0.4 (4.0-5.0)	4.9, 5.3
c		73.6 $\pm$ 8.0 (60.2-87.4)	?	66.4 (n=1)	94.3, 91.5
c'		1.0 $\pm$ 0.1 (0.8-1.1)	?	0.8 (n=1)	0.6, 0.7
V		48 $\pm$ 2 (45-50)	-	49.3 $\pm$ 2.0 (46.7-51.6)	-
Lip region diam.		?	?	18.2 $\pm$ 0.6 (17.5-19.0)	21.5, 20.5
Odontostyle length		19.7 $\pm$ 0.5 (19.0-20.5)	24	20.2 $\pm$ 1.0 (19.5-22.0)	29.0, 28.5
Odontophore length		40.6 $\pm$ 1.6 (38-43)	45	23.9 $\pm$ 2.3 (20.5-25.5)	27.5, 26.5
Guiding ring from ant. end		?	?	10.2 $\pm$ 1.1 (9.5-12.0)	17.5, ?
Neck length		604 $\pm$ 40.2 (555-649)	?	566.9 $\pm$ 35.4 (530-619)	519, 451
Pharyngeal expansion length		323 $\pm$ 25.6 (288-349)	?	293.4 $\pm$ 28.3 (268-346)	261, 232
Diam. at neck base		79.3 $\pm$ 6.4 (73-90)	?	77.0 $\pm$ 5.0 (71-84)	73, 69
at midbody		91.0 $\pm$ 8.4 (79-100)	?	86.9 $\pm$ 7.9 (77-97)	84, 77
at anus		38.4 $\pm$ 2.6 (35.5-44.0)	?	46.0 $\pm$ 0.7 (45-47)	42, 39
Prerectum length		156 $\pm$ 17.3 (130-183)	?	106.3 $\pm$ 23.2 (84-139)	90, 119
Rectum length		45.8 $\pm$ 4.0 (40-50)	?	50.5 $\pm$ 1.5 (49-51)	58, 60
Tail length		37.3 $\pm$ 3.4 (31.5-42.5)	?	39 (n=1)	27.0, 26.5
Spicule length		-	73	-	73, 62
Ventromedian supplements		-	?	-	22, 19

The seven females and the two males are not con-specific, since the two males (Fig. 3) belong to the genus *Labronema*: cuticle at level of odontostyle very thick, with very distinct dorsal and ventral body pores, and on tail it is typically two-layered, with very thin outer layer and very thick inner layer; odontostyle strong, with comparatively thicker walls, about 1.4 times than lip region width, and aperture hardly reaching one-half its length; and there are 19-22 contiguous ventromedian supplements. On the other hand, the females (Fig. 4) fit the typical *Aporcelaimellus* pattern.

Lacking their corresponding females, the precise identity of the males is problematic, but there is evidence that allow a first approximation. They are near identical to those described for *Labronema goodeyi* by Altherr & Delamare-Deboutteville (1972) from Massachusetts, USA (but also reported from Russia and Ethiopia, according to Andr  ssy, 1991), from which they can be separated by minor differences as shorter odontostyle (28-29 vs 30 μm) and neck ($b = 4.9$ -5.3 vs $b = 3.9$ -4.1). Concerning the identity of the male material described by Cobb as belonging to *Dorylaimus perfectus*, it is very similar to the two males of Thorne's collection since

its ratios and morphometrics, calculated from Cobb's original description, are: $L = 2.35$, $a = 26.4$, $b = 4.9$, $c = 71$, $c' = 0.7$; lip region 19 μm wide, neck 477 μm long, tail 33 μm long, spicules about 70 μm long, and 23 contiguous ventromedian supplements out the range of spicules; then, only minor differences are noted between Cobb's material and the specimens herein studied. Nevertheless, the two males belonging to Thorne's collection do not show any indication of the existence of large unicellular glands in neck region, a remarkable feature of *Dorylaimus perfectus*, but totally unknown in dorylaims, which was mentioned and illustrated by Cobb (see also comments by De Ley *et al.*, 1993). In consequence, it is impossible to clarify definitively the identity of the male of *Dorylaimus perfectus* described by Cobb, but, it certainly belongs to *Labronema* and probably is con-specific with Thorne's specimens, very close to *L. goodeyi*.

Concerning the females (Fig. 4), both general morphology and morphometry perfectly fit the re-description of *Aporcelaimellus obtusicaudatus* (Bastian, 1865) Altherr, 1968 provided by De Ley *et al.* (1993). The only female of *Dorylaimus perfectus*

measured by Cobb does not differ significantly from *A. obtusicaudatus* in its ratios and morphometrics: $L = 2.58$, $a = 20.8$, $b = 3.9$, $c = 66$, $V = 54$, $c' = 0.7$, lip region 26 μm wide, neck 660 μm long, and tail 39 μm long. Nevertheless, the question of the existence of large unicellular glands in the neck region remains unsolved.

***Dorylaimus paraobtusicaudatus* Micoletzky, 1922**
(Fig. 5; as *Metaporcelaimus romanicus*)

Nomenclature. The original species described by Micoletzky was transferred to *Eudorylaimus* by Andr ssy (1959), later to *Aporcelaimellus* by Andr ssy (1986) and, more recently, retained under *Eudorylaimus* also by Andr ssy (2002). The only male specimen labelled by Thorne and Swanger as *D. paraobtusicaudatus* is herein identified (see remarks) as *Metaporcelaimus romanicus* (Popovici, 1978) Andr ssy, 2001.

Material examined. One male, mounted on slide “110 *Dorylaimus paraobtusicaudatus*”; in acceptable condition.

Measurements. See Table 3; as *Metaporcelaimus romanicus*.

Male. Slender nematode of medium size, 3.07 mm long. Body cylindrical, tapering towards both extremities, but more so towards the anterior end. Habitus curved ventrad after fixation, especially in posterior body region, G-shaped. Cuticle 2 μm at anterior region, 3 μm in mid-body and 5.5 μm on tail. Lateral chord width about one-seventh of body diameter at neck base. Body pores inconspicuous. Lip region offset by constriction, 2.6 times as wide as high and about one-fifth (18%) of body diameter at neck base; lips apparently separated. Amphid fovea funnel-shaped, its aperture 8 μm or hardly more than half (55%) of lip region diameter.

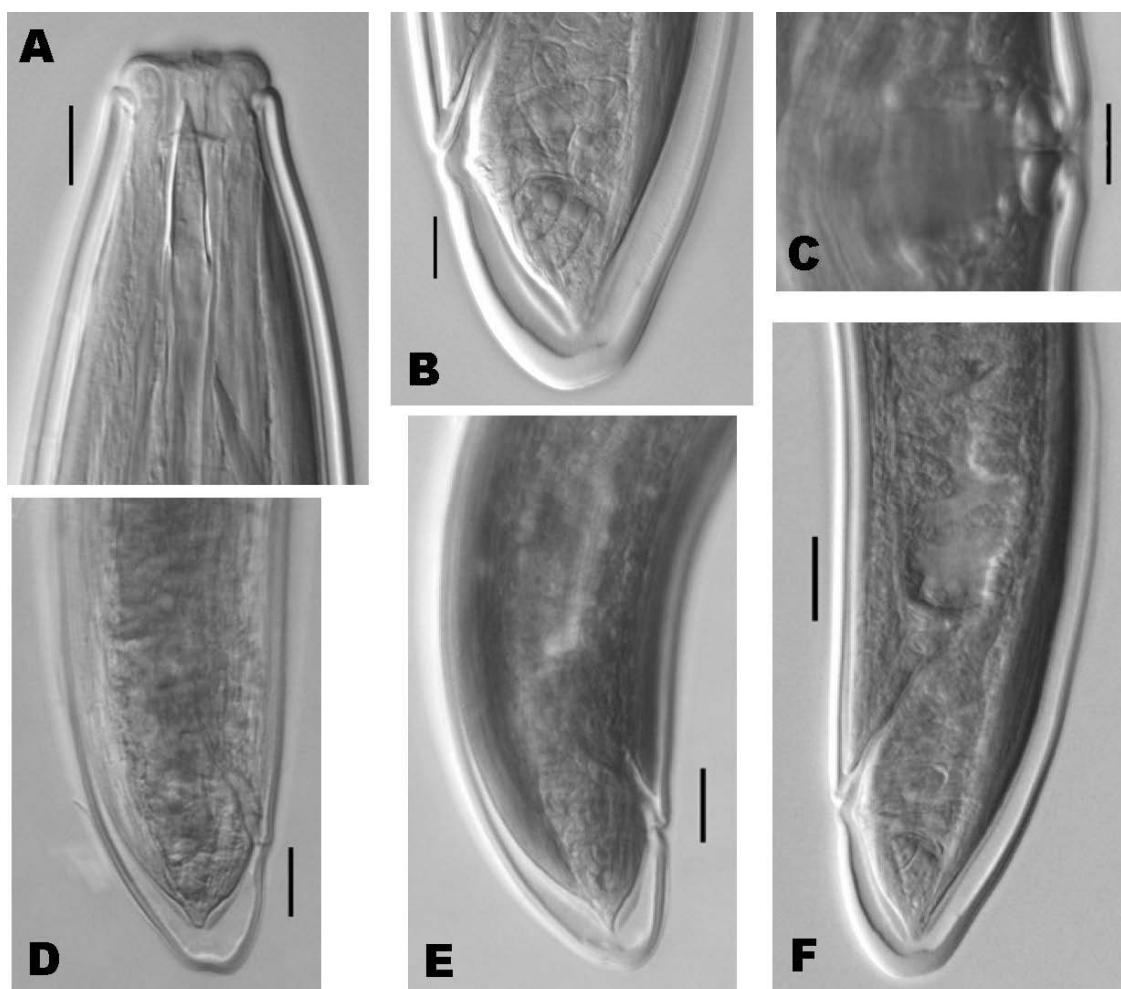


Fig. 4. *Aporcelaimellus obtusicaudatus* (Bastian, 1865) Altherr, 1968 (female). A: Anterior region; B: Tail; C: Vagina; D-F: Posterior body region. (Scale bars: A-C = 10 μm ; D-F = 20 μm).

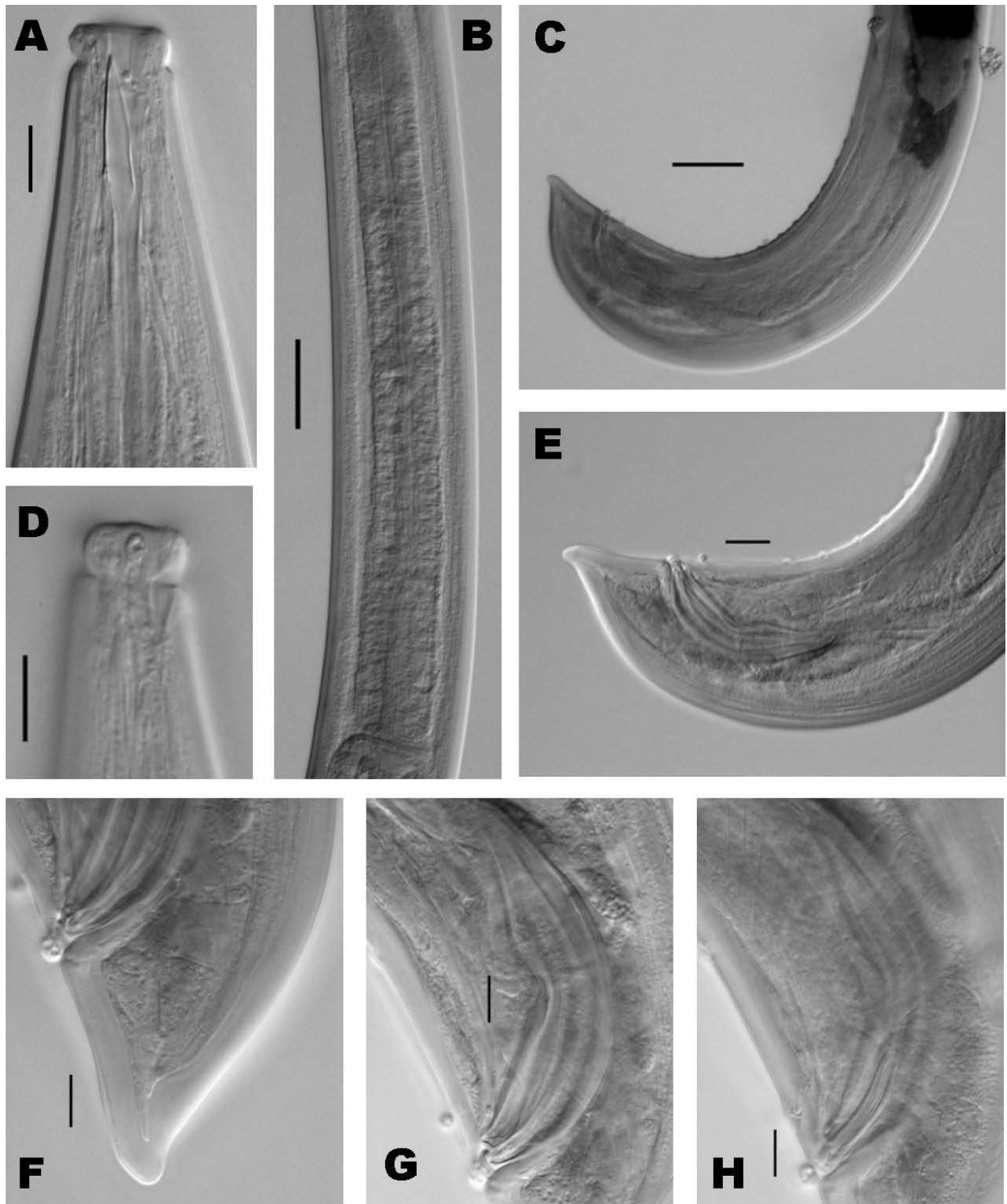


Fig. 5. *Metaporcelaimus romanicus* (Popovici, 1978) Andr ssy, 2001 (male). A: Anterior region; B: Pharyngeal expansion; C, E: Posterior body region; D: Lip region in surface view; F: Tail; G, H: Spicules. (Scale bars: A, D, F-H: 10 μ m; B, C, E = 20 μ m).

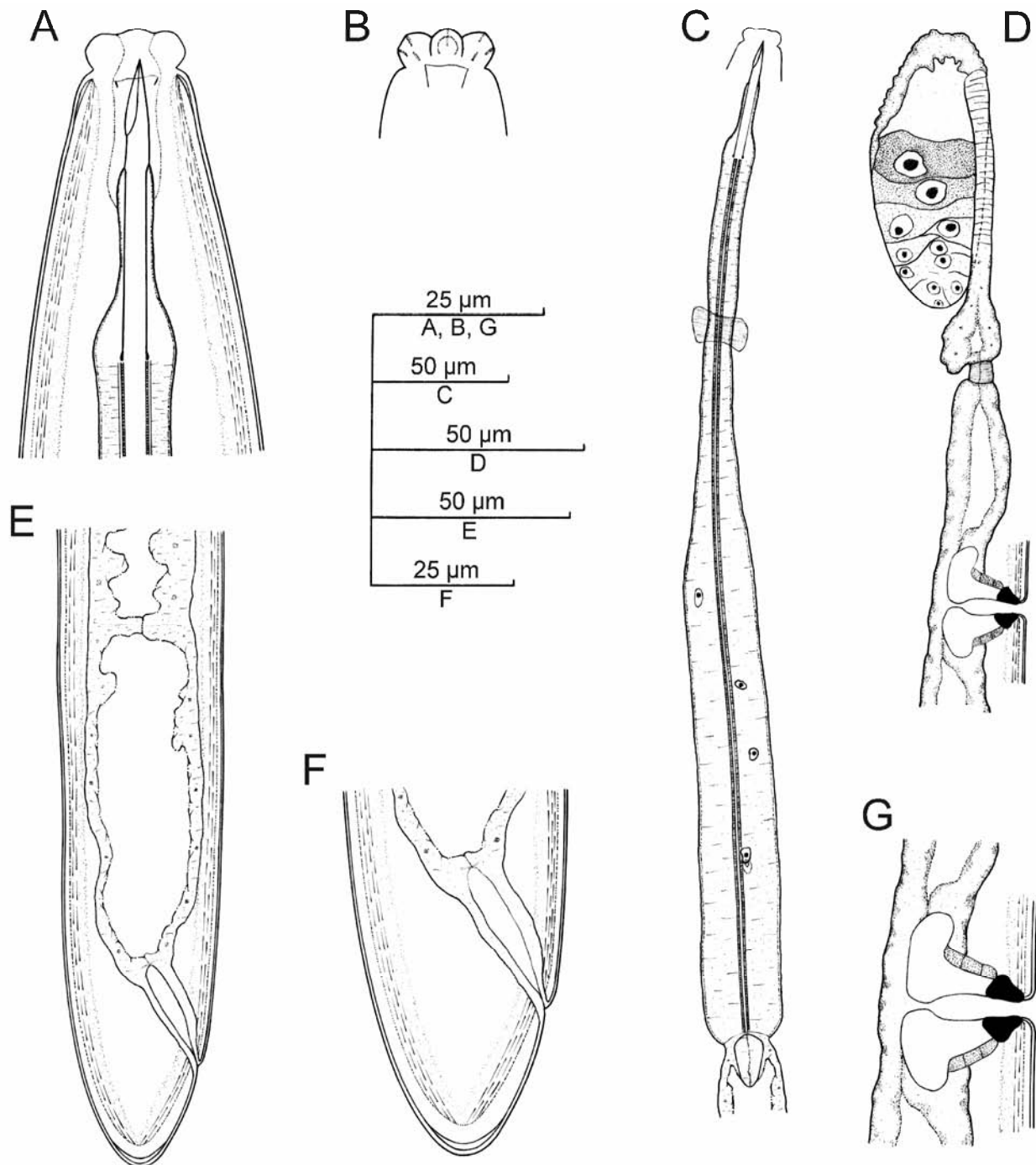


Fig. 6. *Aporcelaimellus waenga* (Yeates, 1967) Peña-Santiago & Ciobanu, 2008 (female). A: Anterior region; B: Lip region in surface view; C: Pharyngeal region; D: Anterior genital branch; E: Posterior region; F: Tail; G: Vagina.

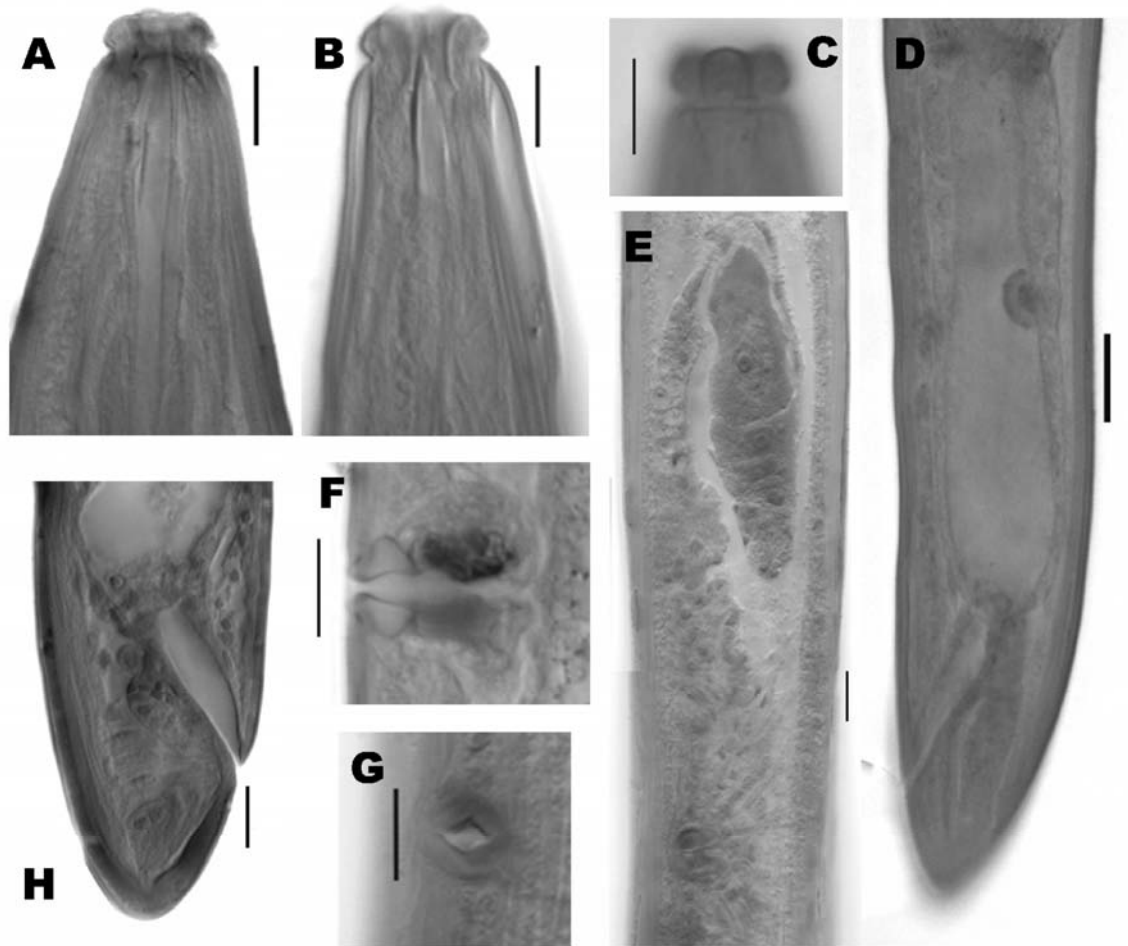


Fig. 7. *Aporcelaimellus waenga* (Yeates, 1967) Peña-Santiago & Ciobanu, 2008 (female). A, B: Anterior region; C: Lip region in surface view; D: Posterior body region; E: Anterior genital branch; F: Vagina; G: Vulva in frontal view; H: Tail. (Scale bars: A-C, F-H = 10 μ m ; D, E =20 μ m).

Cheilostom nearly cylindrical, lacking any differentiation. Odontostyle typical of the genus, 4.7 times as long as wide, 1.3 times the lip region width and 0.57% of body length; aperture 12.5 μ m long or occupying about 71% its length. Guiding ring plicate, situated at 8 μ m or about one-half of lip region diameter from anterior end. Odontophore linear, rod-like, 1.8 times as long as odontostyle. Anterior region of pharynx enlarging very gradually; basal expansion 9.0 times as long as wide, 4.8 times as long as body diameter, and occupying 57% of total neck length. Pharyngeal gland nuclei located as follows: DN = 48, S₁N₁ = 59, S₁N₂ = 71, S₂N = 88. Nerve ring situated at 197 μ m from anterior end or 28% of total neck length. Cardia conical, as long as wide; its junction to pharyngeal base apparently surrounded by a ring-like structure. Prerectum 3.9, rectum 1.7 anal body widths long. Genital system diorchic, with opposite

testes. In addition to the adcloacal pair, situated at 14 μ m from cloacal aperture, there is a series of 12 ventromedian supplements regularly and widely spaced, the posteriormost one lying within the range of spicules and at 40 μ m from adcloacal pair; ventromedian supplements 16-21 μ m apart. Spicules somewhat curved ventrad, about 4.9 times as long as wide. Lateral guiding pieces about 24.5 μ m long, 7.1 times as long as wide. Tail conical with rounded terminus, ventrally almost straight, dorsally convex but with a slight dorsal concavity at the end; inner core of tail extending along its terminal part, so that the hyaline portion is very short, about 9 μ m long. Caudal pores two pairs, one nearly dorsal, at the middle of tail; another lateral, at the posterior half of tail.

Distribution. According to the label on Thorne's slide, the material herein studied was collected

“under moss in forest”, in Thalweil, Switzerland, in December, 1912.

Remarks. The label of the slide 110 of Thorne’s collection indicates it contains one male of *Dorylaimus paraobtusicaudatus*. Available information of this species (for instance, see Thorne & Swanger, 1936; Kirjanova, 1951; Altherr, 1952; Andrásy, 1952; Bongers, 1988) is of poor quality, sometimes contradictory, and does not allow its characterisation. However, it is obvious that the male specimen herein examined does not belong to *D. paraobtusicaudatus* since the body is larger (*vs* *L* up to 2.00 mm in all the records of *paraobtusicaudatus*), and the tail is longer (*vs* less than 40 µm long) and with different appearance (*vs* more conoid, lacking a terminal projection of the inner core). Nevertheless, this male is identical to that reported for *M. romanicus* (see Popovici, 1978; Andrásy, 2001), a species described from Carpathian Mountains, and an indication that the species might be spread in Central Europe.

Thorne and Swanger (1936) either did not study this male or did not publish their results, because

they apparently incorporated the original data by Micoletzky in their monograph.

***Dorylaimus propinquus* Thorne &
Swanger, 1936**
(Figs. 6 & 7; as *Aporcelaimellus waenga*)

Nomenclature. See first contribution of the series and remarks below.

Material examined. Three females, mounted on slide “50 *Dorylaimus propinquus*”, and one female mounted on slide relabelled “G-2866 *Aporcelaimellus* sp.”. Both slides certainly belong to N.A. Cobb’s collection, and are deposited with Thorne’s collection. The specimens are not in good condition, apparently were stained and now appear pink coloured.

Measurements. See Table 3; as *Aporcelaimellus waenga*.

Female. Moderately slender to slender nematodes of medium size, 1.31-1.71 mm long. Body cylindrical, tapering towards both extremities,

Table 3. Morphometric data of *Metaporcelaimus romanicus* (Popovici, 1978) Andrásy, 2001 and *Aporcelaimellus waenga* (Yeates, 1967) Peña-Santiago & Ciobanu, 2008. Measurements in µm (except *L*, in mm), and in the form: mean ± standard deviation (range).

Species	<i>M. romanicus</i>	<i>A. waenga</i>	
		Eustis, Florida	China
Population	Thalweil, Switzerland		
Character	n	3 ♀♀	♀
L	3.07	1.46 ± 0.22 (1.31-1.71)	1.62
a	32.7	28.8 ± 2.2 (26.3-30.4)	37.5
b	4.4	3.4 ± 0.2 (3.2-3.6)	4.0
c	68.3	54.4, 60.7 (n=2)	68.0
c'	1.0	0.8, 1.0 (n=2)	0.9
V	-	56.1 ± 1.2 (54.9-57.4)	55.3
Lip region diam.	14.5	14.8 ± 1.3 (13.5-16.0)	13
Odontostyle length	18.5	17, 18 (n=2)	17.5
Odontophore length	31.5	25, 31 (n=2)	?
Guiding ring from ant. end	8	7.0, 9.5 (n=2)	?
Neck length	706	430.2 ± 45.9 (390-480)	407
Pharyngeal expansion length	401	223.5 ± 32.5 (195-259)	197
Diam. at neck base	83	48.3 ± 4.5 (43-51)	42
at midbody	94	50.7 ± 7.3 (43-58)	43
at anus	47	29.9 ± 1.5 (29.0-31.5)	26
Prerectum length	182	95.9 ± 25.2 (79-125)	110
Rectum length	79	38.3 ± 7.0 (31-44)	32.5
Tail length	45	24-28 (n=2)	24
Spicules length	87	—	—
Ventromedian supplements	12	—	—

but more so towards the anterior end. Habitus curved ventrad after fixation, especially in posterior body region, C-shaped. Cuticle 1.0, 1.5 (n=2) μm at anterior region, 1.0-1.5 μm at midbody and 1.5-2.0 μm on tail. Lateral chord 5.5, 9.5 μm (n=2) wide at midbody, occupying 13, 16% of body diameter. Body pores inconspicuous. Lip region offset by very deep constriction, 2.4-3.1 times as wide as high and one-fourth to one-third (26-35%) of body diameter at neck base; lips separated, with scarcely protruding papillae. Amphid fovea funnel-shaped, its aperture 6.5-7.0 μm or two-fifths to one-half (41-48%) of lip region diameter. Cheilostom nearly cylindrical, lacking any differentiation. Odontostyle typical of the genus, 4.4-4.9 times as long as wide, and 1.1-1.3% of body length; aperture 11-12 μm long or occupying about two-thirds (67-69%) its length. Guiding ring plicate. Odontophore linear, rod-like, 1.5, 1.8 (n=2) times as long as odontostyle. Anterior region of pharynx enlarging very gradually; basal expansion 6.2-7.0 times as long as wide, 4.3-5.0 times as long as body diameter, and occupying 48-54% of total neck length. Pharyngeal gland nuclei located as follows: DN = 57-61, S₁N₁ = 67-69, S₁N₂ = 74-77, S₂N = 83-86; DN quite posterior and S₂N comparatively anterior. Nerve ring situated at 130-150 μm from anterior end or 31-34% of total neck length. Cardia conical, 19-22 x 12.5-15.5 μm . Genital system didelphic-amphidelphic; both branches equally and poorly developed, the anterior 93-164 μm and the posterior 90-170 μm long. Ovaries of variable length, usually not surpassing the sphincter level; the anterior 53-153 μm , the posterior 55-170 μm long; oocytes arranged first in two or more rows, then in a single row. Oviduct 47-72 μm long or 1.1-1.7 times the corresponding body diameter; it consists of slender part with prismatic cells and a poorly developed *pars dilatata*. Oviduct-uterus junction marked by a sphincter. Uterus a simple, short tube, 29-51 μm or 0.6-1.0 times the corresponding body diameter. Uterine egg not observed. Vagina extending inwards 12.5-19.0 μm or one-third (29-36%) of body diameter, in the four specimens examined appearing dilated; *pars proximalis* 11, 12 (n=2) x 9.5-12 μm ; *pars refringens* 2, 3.5 x 5, 6.5 μm (n=2) and with a combined width of 7.0, 10.5 μm ; *pars distalis* short, about 1.5, 2 μm long. Vulva a post-equatorial, oval, transverse slit. Prerectum 2.7-4.3, rectum 1.1-1.5 anal body widths long. Tail convex conoid. Caudal pores two pairs, one lateral, at the middle of tail, another subdorsal, more posterior.

Diagnosis (based on present specimens). This species is characterised by its body 1.31-1.71 mm

long, lip region offset by very deep constriction and 13-16 μm wide, odontostyle 17-18 μm long with aperture occupying 67-69% of its length, neck 390-480 μm long, pharyngeal expansion 195-259 μm long or 48-54% of total neck length, uterus a simple tube 29-51 μm or 0.6-1.0 times the corresponding body diameter long, *pars refringens vaginae* present, $V = 55-57$, tail convex conoid (24-28 μm , $c = 54-68$, $c' = 0.8-1.0$), and male unknown.

Distribution. Three females collected around orange roots, Eustis, Florida, USA; and one female found in sugar cane field, China in 1915.

Remarks. In our previous paper on *Aporcelaimellus* species (Álvarez-Ortega & Peña-Santiago, 2010), we mentioned that three females of the material originally identified as *Dorylaimus propinquus* by Thorne and Swanger (1936), and later re-described by Tjepkema *et al.* (1971) as *Aporcelaimellus propinquus*, were not conspecific with this taxon. Their detailed study revealed that they belong to *A. waenga*, which was originally described as type species of *Takamangai* Yeates, 1967, a genus with an intricate taxonomic history (for details see Peña-Santiago & Ciobanu, 2007, 2008) that is currently regarded as a junior synonym of *Aporcelaimellus*. Type material of *A. waenga* was recently re-examined by Peña-Santiago and Ciobanu (*op. cit.*), although its bad condition did not enable a detailed description to be made, but it was confirmed as belonging to the genus *Aporcelaimellus*. Orselli and Vinciguerra (2000) reported it from coastal dunes in Italy.

Aporcelaimellus laevis Tjepkema, Ferris & Ferris, 1971 is very similar to *A. waenga*, but the two species were never compared, certainly because the later was classified in that time under other genus. The above description fits very well the original one of *A. laevis*, with the exception of minor differences, such as narrower lip region (13-16 vs 17.6 ± 0.5 μm in *A. laevis*), longer odontostyle aperture (about two-thirds vs $54 \pm 1\%$ its length), and slightly shorter female tail ($c' = 0.7-0.9$ vs $c' = 0.9-1.1$). *Aporcelaimellus laevis* was originally described from several localities in Indiana, and later reported from India (Ahmad & Jairajpuri, 1982; Ahmad, 1995; Rahaman & Ahmad, 1995) and Italy (Orselli & Vinciguerra, 2000, 2005), but only Ahmad and Jairajpuri (*op. cit.*) and Ahmad (*op. cit.*) provided additional measurements that do not differ significantly from those given in this contribution. As conclusion, *A. laevis* should be regarded as a junior synonym of *A. waenga*, this becoming a widely, probably worldwide, distributed species.

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S. Álvarez-Ortega and R. Peña-Santiago. Изучение рода *Aporcelaimellus* Heyns, 1965 (Dorylaimida: Aporcelaimidae) по материалу, исследованному, но не идентифицированному Торном и Свангером в 1936.

Резюме. На основании переисследования материала из коллекции Торна проведено определение материала по четырем видам *Aporcelaimellus sensu lato*, изученного, но не описанного Торном и Свангером в 1936 году. Приведено описание и иллюстративный материал для *Dorylaimus papillatus*, который рассматривается как валидный вид, поскольку легко отличим от *A. obtusicaudatus* по отсутствию *pars refringens* в вагине. Материал с этикеткой «*Dorylaimus perfectus*», часто рассматриваемый как синоним *A. obtusicaudatus*, состоит из двух особей самцов, напоминающих по строению самцов, описанных для *Labronema goodeyi*, и семи самок, относящихся, несомненно, к *A. obtusicaudatus*. Даны измерения и иллюстративный материал для этих экземпляров. Один самец, этикетированный как *Dorylaimus paraobtusicaudatus*, представляет собой *Metaporcelaimus romanicus*. Приводится детальное описание и иллюстрации для этого вида. Часть материала, описанного изначально как *Dorylaimus propinquus*, в действительности представляет собой *Aporcelaimellus waenga*, для которого предложено детальное описание, измерения и иллюстративный материал. Кроме того, *Aporcelaimellus laevis* рассматривается как младший синоним *A. waenga*.

Book review

R. N. Perry, M. Moens and J. L. Starr (Editors) 2009. Root-knot nematodes. CABI, Wallingford, United Kingdom, 488 pp. ISBN – 13: 978 1 84593 492 7.

Three aims of this book were defined by the authors in the Preface: 1) to 'focus on the main findings'; 2) 'reflect recent advances in the molecular genetics' and 3) 'to highlight the control options'. This book, bound in an attractive cover, meets all these targets and provides the reader with an 'in-depth' vision of this very interesting group of parasites. The book is organised into separate chapters, with each chapter accompanied by its own list of relevant references. The colour plates are excellent and deserve special compliments; the printing of these was supported by Syngenta.

In the first chapter '*Meloidogyne* species – a diverse group of novel and important plant parasites', authored by all three editors of the book, a condensed synopsis of the overall book content is presented. The main aspects of root-knot nematodes are briefly reviewed in this chapter and are considered in detail in subsequent chapters, but the reader of Chapter 1 will surely be satisfied with such an introduction into the complex information in the specialist chapters. Such primary data will be especially helpful for nematologists not dealing directly with plant-parasitic nematodes, as it explains such phenomena as the initiation and development of giant cells and the host reaction to nematode invasion, or the concept of host races. Several emerging species of *Meloidogyne* are also considered.

Chapter 2 'General morphology' contains descriptions of the morphology of several stages of *Meloidogyne* development: adult nematodes and second-stage juveniles (J2). *Meloidogyne* nematodes have rather unusual morphogenesis. The feeding J2 has a swollen body, which can increase to give a nearly spherical female body or slim into a vermiform male. Peculiarities of the root-knot nematode structures are illustrated with numerous photographs (both light microscopy and SEM) and traditional art-line diagrams. Some figures are very visual, e.g. Fig. 2.1 providing an immediate image of the comparative sizes of adults and J2. This chapter has self-contained value for any nematologist, not only those interested in plant nematodes. *Meloidogyne* are among the most intensively studied groups of the Phylum Nematoda. Intensive TEM studies of the anterior end in general and amphids in particular have resulted in rich information about the morphology of this genus of plant-parasitic nematodes. One can only wonder how adaptation of parasitism in plants can change the quite stable archetype of secernentean (=Rhabditida) nematodes. The depth of coverage is different for separate systems and organs: digestive and reproductive systems are described in more detail than, for example, the nervous system of the sensory structures. What makes this chapter quite unusual is the template for the description of new species of *Meloidogyne*. All the required information is presented, and authors of future first descriptions are invited to provide molecular and isozyme characterisation, and ensure the inclusion of drawings for all taxonomically-sensitive features. It is a really interesting example of the self-organization of science. We have to propose regulations in order not to be swamped by numerous descriptions of low diagnostic value.

Chapter 3 'Taxonomy, identification and principal species' will bring very special satisfaction to anyone who, like the author of this review, likes the history of science and considers as a proper act any homage to the previous generations of biologists. The first part of this chapter is illustrated with portraits of scientists who initiated the study of root-knot nematodes: the Rev. Miles Joseph Berkeley and Emilio Augusto Goeldi. The citation of Rev. Berkeley's description of the nematode-induced galls is an amazing example of how precise were the observation of biologists in the XIX century, despite still primitive microscope optics. In this chapter, the authors (Drs D. Hunt and Z. Handoo) reveal several surprising stories, like the discovery of *Meloidogyne* eggs in the stool of US soldiers in Texas which led to the description of *M. incognita* Kofoid and White, 1919. Narration on the history of *Meloidogyne* research continues through the times of N.A. Cobb and B.G. Chitwood and ends in the contributions of recent years. As is common with nematode taxonomy, an understanding of the taxonomic importance of separate characters was changing with time. For example, the authors indicate that the value of perineal patterns was considered as highly important until pronounced intraspecific variation was recorded. Still the preparation of slides with perineal cuticle is described in full and perineal patterns for 12 *Meloidogyne* species of major importance are presented.

Chapter 4 'Biochemical and molecular identification' is authored by two leading specialists in molecular taxonomy and biology of plant-parasitic nematodes, Drs V. C. Block and T. O. Powers. *Meloidogyne* is

probably quite a rare example of a group in which the taxonomy was based for more than a decade on the intensive use of isozyme patterns. Characteristic esterase and malate dehydrogenase phenotypes were described for nearly three dozen *Meloidogyne* species. After a short explanation about the use of antibodies in root-knot nematode identification, the authors move to probably the most intriguing part of the chapter – the impact of molecular techniques on *Meloidogyne* taxonomy. This part of the chapter contains general descriptions of the principal molecular techniques, the composition of the most popular primers and a map of the *Meloidogyne* mitochondrial genome. On page 109 one can find a list of ‘species-specific’ primers for *Meloidogyne* identification.

The problems of molecular taxonomy and phylogeny of root-knot nematodes are presented separately in Chapter 5. In addition to the quite well known phylogenetic analyses inferred from different domains of ribosomal RNA genes, the authors (Drs B.J. Adams, A.R. Dillman and C. Finlinson) are proposing several, still not complete, surveys based on single-copy orthologous nuclear genes. The problem of misrepresentation of the taxon phylogeny because of the presence of different copies of rDNA cistrons can be avoided through the use of such nuclear genes. Not all such genes are helpful for construction of *Meloidogyne* phylogeny – some of these are too conservative to distinguish between species. The part of the chapter devoted to the construction of a *Meloidogyne* ‘supertree’ is especially interesting. The authors present arguments in favour of construction of the tree, which will unite the phylogenetic signals of several trees based on different DNA domains. Two different methods of supertree construction – ‘matrix representation of parsimony’ and ‘distance fit’ produced similar topologies (e.g. *Meloidogyne ichinohei* is in basal position in all the supertrees).

Several of the following chapters describe specific events in the *Meloidogyne* life cycle (like Chapters 6, ‘Hatch and host location’ and 7, ‘Invasion, feeding and development’), or special functions and systems of organs (Chapter 8, ‘Reproduction, physiology and biochemistry’), or adaptations (Chapter 9, ‘Survival mechanisms’).

The subsequent part of the book deals with ecological aspects of root-knot nematode existence in soils. In Chapter 10, ‘Interaction with other pathogens’, several examples of synergistic effects are given, when the cumulative effect of penetration of root-knot nematodes and another (e.g. fungal) pathogen is greater than the simple additive effect. Chapter 11, ‘Population dynamics and damage levels’, is a classical analysis of factors influencing the population densities in root-knot nematodes. Such an analysis is accompanied by a short overview of models describing root-knot nematode dynamics and damage levels. Nematologists working in the field will find as very helpful that part of the chapter describing the experimental schemes (glasshouse and field experiments, microplots, fitting the models to data etc). Chapter 12, ‘Sampling root-knot nematodes’, presented by Drs L.W. Duncan and M.S. Philips can be considered as a logical outcome of the ecological data presented in previous chapters, as it stipulates the main principles of representative sample collection. Nematode spatial patterns dictate sampling schemes. The flotation method in solutions of higher gravity is considered by the authors of this chapter as the most effective of the various sample processing methods. It is mentioned in the chapter that the importance of root-knot nematode sampling and precise estimation of nematode densities became more important with the exclusion of chemical compounds from agricultural practice. Growers are interested in the detailed picture of root-knot nematode distribution in the field, as this can help to focus use of control measures on focal points of nematode densities.

The next three chapters cover the problems of resistance against *Meloidogyne* in plants and the possibility of manipulating resistance mechanisms for control. In Chapter 13, ‘Mechanisms and genetics of resistance’, the most completely described resistance genes of different plants preventing the parasitism by root-knot nematodes are summarised. The short introduction into the mechanisms of plant resistance to pathogens in plants is very helpful, at least for a beginner in this field. The action and properties of the most studied root-knot nematode resistance gene, *Mi-1* of tomato, are presented in detail. The fine details of the mechanisms that trigger the action of this gene are still not understood, although the gene is known to be suppressed by the addition of cytokinin and enhanced by salicylic acid. Rich genetic resources of natural host plant resistance traits described in this chapter are critical for the development of control measures based on resistance genes. However, the next chapter, Chapter 14, ‘Development of resistant varieties’, brings more sober information on this topic. Five successive crops of tomato plants with the *Mi* gene were sufficient to see the rise of *M. incognita* population virulent for this cultivar. Still, the resistance approach is possible, and the authors (Drs J.L. Starr and C.F. Mercer) propose a compendium of steps and actions for the development of a resistance-breeding programme, including search for sources of resistance, methods of screening etc. Chapter 15, ‘Plant biotechnology and control’, by H.W. Atkinson, P. E. Urwin and R. S. Hussey covers the problems very

similar to those in focus in the previous chapter, but with a more pronounced bias toward protein engineering. Novel for nematologists, is the information about *Cry* proteins as biopesticides, which will be especially interesting in Russia with our long lasting Bt-research programmes. RNAi is now in the arsenal of tools for suppression of root-knot nematodes. Such an approach is especially interesting as no new proteins are expressed by transgenic plants, producing only these double-stranded RNA molecules.

Towards the end of the book the reader will find Chapter 16, 'The complete sequence of the genomes of *Meloidogyne incognita* and *Meloidogyne hapla*'. The subject area of this chapter is closer to Chapters 4 and 5 (related to molecular phylogeny), and not to chapters about the problems of root-knot nematode control that comprise the final part of the book. The chapter is extremely helpful for the readers inexperienced in molecular biology, as it explains the rationale of the EST approach for genomic studies, the problems of the search for parasitism-specific genes etc. Several fascinating facts about the genome of the two species of *Meloidogyne* are presented in the text. The structure of the genome is quite different in *Meloidogyne incognita* and *M. hapla*. The repetitive or transposable elements comprise 36% of *M. incognita* genome. Special attention is devoted to plant parasitism genes. Table 16.3 demonstrates the remarkable richness of the genes encoding cell-wall-degrading enzymes in *M. incognita* in comparison with *Caenorhabditis* and *Drosophila*. Many of these genes are thought to have been acquired by horizontal gene transfer from bacteria.

Three chapters in the final part of the book, Chapter 17 'Biological control using microbial pathogens, endophytes and antagonists', Chapter 18 'Current and future management strategies in intensive crop production systems' and Chapter 19 'Current and future management in resource-poor farming' are all oriented toward practical application. Dr J. Hallmann and his co-authors of Chapter 17 admit that biological preparations against root-knot nematodes are an 'extremely elusive management tool'. This is the case for all 'biologicals' but for root-knot nematode management the use of biological control has to be accompanied by modern crop production technology.

The book contains three separate indices, 'Gene Index', 'Nematode genus and species index' and 'General Index', which is very convenient for the reader.

Several editorial patterns of the book deserve special praise. Thus, 'conclusions and future directions' are presented at the end of each chapter and represent a very valuable part of this book. What could be more helpful for the beginner in this field than the opinion of experienced colleagues, who can define still unresolved key problems in all this complicated 'megaproblem' of root-knot nematode research? Numerous new facts and concepts are directly included in the book. The reader thus obtains a set of carefully selected published papers and completely new scientific facts about *Meloidogyne*. It is very important to have such book in any laboratory dealing with root-knot nematodes, both as source of basic facts and as an invigoration for further studies.

Sergei E. Spiridonov

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COOMANS, A. 1996. Phylogeny of the Longidoridae. *Russian Journal of Nematology* 4: 51-60.

TCHESUNOV, A.V., MALAKHOV, V.V. & YUSHIN, V.V. 1996. Comparative morphology and evolution of the cuticle in marine nematodes: *Russian Journal of Nematology* 4: 43-50.

Article in Russian

ROMANENKO, N.D. 1976. [Longidorids of fruit and berry crops]. *Zashchita Rastenii* 9: 52-53.

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Book

DECRAEMER, W. 1995. *The Family Trichodoridae: Stubby Root and Virus Vector Nematodes*. The Netherlands, Kluwer Academic Publishers. 360 pp.

Article in book

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In memoriam

Professor Günther Osche (1926 – 2009) and his scientific legacy

Günther Osche died on February 2, 2009, in Freiburg (Southern Germany) at the age of 82 after a period of illness. He leaves behind his wife, three children and two grandchildren. He was born on the 7th of August, 1926, in Haardt near Neustadt (Weinstraße) in Southwest Germany as the only child of a bank officer and his wife. A short time later, his family moved to Nürnberg (Bavaria). There, he spent his childhood and developed his early interest in natural history as a bird watcher, which continued throughout his life. The world war took its toll when he became a soldier in 1944; he was deployed in France where he was seriously injured the same year. He came back from war captivity in 1946 with a stiff knee and entered Erlangen University to study Natural Sciences with zoology as principal subject. There, under the supervision of the distinguished zoologist Hans-Jürgen Stammer he obtained his Dr. rer. nat. in 1951 with his famous treatise on the systematics, phylogeny and ecology of *Rhabditis* [1, 2]. As a scientific assistant he was able to perform research in the nematology group of Stammer, who initiated a large-scale investigation of parasites of small mammals in Germany. Osche's task was to identify the parasitic nematodes. However, in addition he used these specimens, which could be studied alive, to elaborate his own topics of more general significance. In 1963 he received the *venia legendi* in zoology with a *habilitation* thesis on the embryology and comparative morphology of Pentastomida and became a *private* lecturer at the University in Erlangen. From 1967 until his retirement in 1988 at age 62 he was *ordinary* professor at the University of Freiburg in Breisgau and in this capacity he was responsible for teaching systematic zoology, comparative morphology, ecology, and evolutionary biology. Over the years there, he led a successful research group in evolutionary biology, systematics and ecology, from which five professors can trace their academic lineage. He authored many research papers and text books in evolutionary biology, parasitology and ecology.

In his original work and as an author of reviews and textbooks, Günther Osche was a highly successful scientist in five very different fields: systematics of nematodes, parasitology, evolutionary biology, human biology and flower biology, thus demonstrating his wide-ranging interest in natural sciences. Throughout his scientific work he sought for historical-narrative explanations [3]. Here, "only" his contributions on morphology, phylogeny, systematics, ecology, preparasitic associations, life cycles and the evolution of free-living and parasitic nematodes including coevolution with vertebrate hosts will be recognised. He authored about 20 outstanding articles with about 650 pages on nematodes, starting with Rhabditidae, of which more than half dealt with parasitic groups (Acaridae, Ascaridae, Rhigonematidae, Strongylidae), always focusing on a subject of general interest to biology. Topics of Osche's contributions included: *i*) evolutionary change by paedomorphosis (which he called fetalisation), which he proposed could be used to understand the origin of sexual dimorphism; *ii*) cryptotypic characters – which are genetically maintained but not phenotypically expressed – as one source of character evolution (something he termed "latent potential"); *iii*) the evolution of complex and synorganised structures; and *iv*) parasite-host coevolution. In different groups, he observed recapitulations in the morphogenesis of structures and used them as Ariadne's thread for a rough outline of the phylogeny of that particular group. Taxonomy played only a small part in his



publications insofar as he had to clarify the objects of his investigations, although he discovered and described some new species and created some genera names like *Caenorhabditis*, *Matthesonema* and *Stammerinema*.

Günther Osche was a scientist with an eye for biological detail and a view of the broader context. By recognising a diversity of minute structures on the glottoid apparatus of the stoma of 65 investigated species of *Rhabditis sensu lato*, which he used for systematisation in his doctoral thesis, he set the cornerstone for understanding the phylogeny of the “Rhabditidae” [1]. His comprehensive revision of this group was the foundation for 25 years. In this publication, he gave new names for six taxa of the genus group, from which *Caenorhabditis* is the most famous, and he described three new species. It has been little recognised that, in this paper, he debated about possible modes of speciation without separation (sympatric speciation) in rhabditids, assuming that all the species were cosmopolitan (today we know that this is not the case). He took up this subject again and dealt with it in greater detail in his trailblazing paper on sibling species and “complementary species” [4], the latter differing in their mode of reproduction (gonochoristic *versus* autogamous hermaphroditic). For groups of three such species he developed the idea that a new gonochoristic nematode species could evolve *via* a detour through an intermediate hermaphroditic offshoot. After a certain period of evolution and ecological differentiation from the gonochoristic complementary species, the hermaphroditic one would revert to gonochorism, likely to be ecologically and genetically incompatible to its sibling species. The occurrence of such a possible mode of speciation with both events occurring sympatrically is still an open question. His discussions were based on crossing experiments between the sibling species and comprehensive studies on the variability of morphological structures and aberrations to bridge the gap between the species. In this context, he redefined the term “cryptotype” (K. Saller) as complexes of the genotype maintained for features that usually are not realised phenotypically. The most impressive example in nematodes of such a latent character harboured for a very long time were the three tips (one dorsal, two subventral; comparable to the caudal glands of “Adenophorea”) on the tail observed in a rhabditid (*Pellioiditis papillosa*), which he interpreted as atavistic and found in the same arrangement as “reanimated” characters of species disjunctively distributed in mostly parasitic groups. Thus, it was a cryptic part of the bauplan, at least of the Secernentea (for him all nematodes), that could reemerge independently [5, 6]. In an evolutionary scenario, Osche connected this plesiomorphic “triply pointed” tail to the behaviour of aquatic nematodes that adhere to the substratum by secretions of the caudal glands; specifically, such nematodes display an oscillating body motion, from which he derived the “waving” of dauer and infective larvae of the terrestrial Secernentea by “change of function” [7].

Günther Osche [8] first observed waving “in a tube” in unrelated rhabditid species. His preliminary results on the genetics of waving behaviour of dauer larvae from crossing experiments between a waving and a non-waving strain of *Rhabditoides inermis* [2] were cited in many textbooks. He also discovered some interesting associations, like *Rhabditella typhae* (as we now call it) living in the frass of the caterpillar of *Nonagria typhae* [1], or *Matthesonema tylosum* existing entoecic in the gill chamber between the pleopods of the isopod *Tylos latreillei* [9], a phenomenon which should be reinvestigated. He discovered that third-stage juveniles of a saprobiontic nematode (*Rhabditis “strongyloides”*) could invade the skin or the conjunctival sac of small rodents and were morphologically distinguishable according to the source. This phenomenon could be resolved 30 years later by his scientific “grandson” Franz Schulte. Schulte showed that instead of one species with different behaviours, a complex of ecologically different species exists, namely *Pelodera strongyloides*, *P. cutanea* and *P. orbitalis*, the last two living in nests of rodents and using the hosts for transport and nourishment [10]. The premature statement by Osche [11, 12] that the juveniles in a rodent possibly were waiting to develop on the decaying cadaver turned out to be wrong, but was unfortunately perpetuated in recent literature [13].

As mentioned before, in his morphological investigations of different parasitic nematode groups ontogenetic changes that could be interpreted as recapitulations played an important part and suggested ideas about evolutionary transformation. For example, the cuticular cordons at the anterior end of *Stammerinema soricis* (Acuariidae) pass through different stages, which are known from the adults of related taxa (stages like the terminal characters in *Paracuaria* → *Acuaria* → *Dispharynx* → *Synhimantus*) [14]. The various cordon types were organismically explained as a result of heterochrony of different growth processes. In Ascaridoidea [15] the morphogenesis of the lip complex of *Porrocaecum ensicaudatum* passes through stages that are characteristic for adults of five different ascarid taxa (*Acanthocheilus* → *Contracaecum* → *Stomachus* → *Amplicaeum* → *Ophidascaris*). Hypothesising this as a recapitulatory development, Osche proposed a guideline for ordering the groups in a phylogenetic sequence, which was supported by the host-range (“*Wirtskreis*”)

and the life cycles of the nematodes (primarily heteroxenous, secondarily monoxenous). For him, a strong argument for this sequence and his phylogenetic hypothesis was the coevolution of the ascarids with the phylogeny of jawed vertebrates nearly from their beginning (cartilaginous fishes → bony fishes → amphibians/reptiles → birds/mammals). The main exception of this parallel evolution (not cospeciation!) of parasitic groups and host groups are the Stomachinae, which could be explained as multiple transfers from teleosts as primarily definitive hosts to fish-eating marine mammals (whales and seals), birds (gulls, petrels, penguins etc.), and turtles with the ancestral hosts becoming intermediate hosts [15, 16]. This “host-range expansion” (“*Wirtskreiserweiterung*”: Osche) of primarily “fish parasites” was facilitated because the ancestral (autochthonous) ascarids in these taxa (e.g. whales) were lost when entering the marine environment in the course of evolution.

The conclusions of this article on ascarids, which Osche regarded as his best publication on nematodes [3], are hard to accept from an eco-evolutionary point of view. As parasitic lineages within Secernentea originated terrestrially, “it is difficult to accept any scheme that jumps from terrestrial free-living bacterial feeders to the marine environment and sharks” [17: p. 250]. To resolve this problem, a new approach combining data from morphology, life-cycles and host-range with a phylogenetic tree generated from molecular data is required.

On a broad scale of parasitic taxa, Osche investigated the different modes of host shifting [18]. With good arguments, he assumed earthworms as primary intermediate hosts of the ascarids *Porrocaecum*, whereas predators of earthworms like shrews and moles were added later as second intermediate hosts, which made possible a host switch to birds of prey as definitive hosts. By contrast, a host switch to herbivores was possible by abandoning the intermediate host, so that *Parascaris* and others became secondarily monoxenous, with a complex migration in the host that recapitulates the behaviour in an earlier intermediate host before (ontogenetically) the same host becomes the definitive host. In the heteroxenous Acuariidae (arthropods as intermediate hosts and birds feeding on arthropods as definitive hosts) a host switch to birds of prey or owls and herons was achieved *via* small vertebrates (shrews, frogs, fishes) as paratenic hosts, whereupon in the further course of evolution *Stammerinema* succeeded in capturing former paratenic hosts (shrews) as definitive hosts.

Other comparative morphological studies helped in understanding the origin of complex, synorganised structures like the lip complex of *Paraspidodera uncinata* (Heterakidae) by interdigitation of three lips, each with different processes, while individual preconditions for this closing device were found in related groups and in aberrations [19]. The study of aberrations, a theme for Osche from the beginning of his scientific career, to demonstrate the evolutionary potentialities for transformations within a group, was exemplified with the bursal rays of Strongylida by investigating thousands of unfixed specimens and considering a vast literature [6]. Since that time, we are clear that strongylids are closely related to rhabditids. Not knowing about ray-like phasmids in males of rhabditids he wrongly assumed ten pairs of rays as typical and (based on some aberrations) suggested the loss of one ray by fusion of rays nos. 7 and 8, thus forming the externodorsal ray in Strongylida. This fusion of two rays cannot be accepted. The bursa of Strongylida is formed by nine pairs of rays with nos. 4 and 7 pointing dorsally (d), the others ventrally (v), and terminal phasmids (ph). In our formula it reads: v1(v2,v3)/[ad(v4,v5)]pd(v6,v7,ph).

In the only article resulting from his profound research on parasito-phylety and biogeography of Rhigonematidae (parasites of the hind gut of subtropical and tropical diplopods), Osche concentrated on sexual dimorphic structures of the buccal cavity and the pharynx [20]. The pronounced sexual dimorphism in these organs – as different as in two genera – results by prolongation of development in females or an arrest in males at an earlier ontogenetic and evolutionary stage (paedomorphosis). Osche wondered about the selective value for structures concerned with nutrition, but at that time did not yet think of different partial econiches of the sexes [21]. The phylogenetic significance of his analysis of the terminal bulb that exhibits five levels of valves has still to be established. In *Brumptaemilius* he discovered four rows of special sense organs in the vulva region and suggested a stimulatory effect of two rows of cuticularised male papillae precloacal and a spiny area postcloacal (*area rugosa*) during courtship behaviour. His attempt to use the ancient Rhigonematidae as an auxiliary means of research on the historical biogeography of the hosts (Diplopoda) got stuck in its infancy. This was not only due to the onset of his extensive teaching as Professor in Freiburg, but because dozens of new species would first have to have been described, and Osche was not thrilled to do so extensive a taxonomic study. In addition, anticipated biological observations on special rhigonematids like spermatophores [pers. comm. 1970; 22: p. 54] or giant sperms remained unpublished. Osche only casually documented observations about *Rhigonema* feeding on filiform fungi (Ecclinidae) attached on the wall of the hindgut of the host [23: p. 101]. He also documented an undescribed species of rhigonematids with a voluminous buccal cavity carnivorous on other endozooic nematodes [23: p. 115], which he thought

emerged from an ancestor grazing on such fungi that also settled on the cuticle of nematodes. The evolution of nematophagous nematodes in the alimentary tract of a host would not have been possible if the ancestors were real parasites and certain substances of the host were indispensable for them. Not until decades later were all these facts discovered and published by other authors. In contrast to most scientists, Osche was not very much interested to claim credit because of priority of observation or thinking; rather, he was delighted if he was confirmed by others coming to the same conclusions. In the same vein, he generously bestowed a cornucopia of new ideas to those close to him.

Günther Osche developed a convincing scenario for the transition of nematodes from free-living to zooparasitic ones. His “preadaptation concept” states that adaptations, successively evolved in saprobiontic nematodes (like rhabditids) living as bacteria-feeders in ephemeral biochores of decaying organic matter and using other organisms initially for transport (phoresy), at least served as a complex of preadaptations for a transition to parasitism in animals [24, 11, 16]. Parasites originated in multiple lineages of Secernentea and in different geological periods, so that very old and relatively young parasitic groups exist side by side. Phoretic and pre-parasitic species are “models” demonstrating, respectively, the successive steps in which parasitism evolved in the past. The “rule of the infective third stage” in parasitic Secernentea, due to the infective larva being a transformation of the phoretic dauer larva, is just as much part of this concept as is the “void in the sea”, i.e., the situation that marine invertebrates are nearly free from parasitic “Adenophorea” because the pre-adaptive plateau was not achieved in this paraphyletic group [12]. (Benthimermithidae and Marimermithida are rare exceptions.) This vacancy demonstrates that there exist no “empty niches” to be “occupied”; moreover the animals offer “ecological licences” (redefined by Osche) that cannot be exploited until the organisms first evolved necessary preadaptations (“organismic licences”, coined by Osche) to establish a new econiche.

One reason that Osche was so successful in discovering new and phylogenetically relevant structures and aberrations was that both in free-living and in parasitic nematodes he could always study ample numbers of live specimens, where many structures are more clearly recognisable. Besides A. G. Chabaud and W. G. Inglis, he was certainly *the* authority in understanding the phylogeny of parasitic nematodes at that time. His articles are an endless source of ideas for scientists even today. Asking a wide range of questions, he approached a subject from every possible angle. By considering the variety of organisms and structures, his aim was to develop a coherent image of evolution of a group, synthesising facts from very different branches of biology to allow predictions. He was a master of such syntheses, resulting in a rounded overall picture. Although he had read the publications of Willi Hennig from the beginning (cited already in his 1952 article [1]) he never in his studies on phylogeny of nematodes employed cladistic methods to reconstruct cladograms or to find apomorphies to establish monophyletic groups, which is the benchmark for all such discussions today. His main interest was anagenesis, not cladogenesis, for which he pursued a strictly gradualistic approach. Among colleagues giving talks or plenary discussions that involve gradualistic transformation series and require continuous functionality to cope with daily life, Osche's most-cited statement is that, in contrast to the possibilities that can occur during ontogeny, “evolving organisms cannot close in order to rebuild”.

As a professor, Osche had a huge impact on the thinking of students and prospective schoolteachers by his comprehensive and stimulating lectures. He was an exceptionally gifted speaker, who could “explain complex subjects in such a clear way that even non-biologists could follow and could understand the issues” [25]. His excellent lecture on the special zoology of invertebrates, refreshed every year over two decades, nearly attained cult status. At one time he intended to publish a textbook on this topic, but he gave up the idea, among other reasons possibly because he was afraid to satisfy the growing expectations of cladistics in the early seventies. Also brilliant were his many lectures at various societies and symposia, which covered all the domains mentioned at the beginning of this article. He was an enthusiastic participant in scientific debates, where he often clarified discussions with convincing arguments based on his excellent memory and enormous background knowledge in all fields of biology. Whether it was in a greater audience or in a small circle, he enjoyed discussing all aspects of natural history, and during such discussions and dialogues he developed novel ideas by establishing new connections between facts. We owe to him many hypotheses on evolution that accompanied us during our research. Many scientists from different disciplines consulted him, as they appreciated his stimulating enthusiasm and open-minded discussion on their questions and results. He was a supervisor of many student theses and served as editor of several high-class German scientific journals [25], where he altruistically put much work into improving submitted papers. He was beloved, as he was a person of highest integrity, fighting for a matter or a friend, not for his personal

gain. His profound knowledge in very different fields of his wide interests and pleasing colourful explanations were admired, and therefore his dominant part in discussion circles was respected.

Prof. Osche was a great scientist and a major figure in German evolutionary biology and zoology. He was a member of many visiting groups and national committees and served as president of the Deutsche Zoologische Gesellschaft (1973–74). His reputation from his nematological work was international, although with two exceptions he published only in German and participated only on two international symposia: 1957 in Neuchatel (Switzerland) on host specificity among parasites of vertebrates and 1960 in Asilomar (California) on comparative biology and phylogeny [16]. Since 1979, he was made a fellow of the Deutsche Akademie der Naturforscher Leopoldina (Halle). In recognition of his outstanding contributions to zoology and evolutionary biology he was awarded the Dr. *honoris causa* at the University of Bonn in 2001. He is honoured in some species names of protists, gastropods and arthropods and the nematode names *Diplolaimelloides oschei* Meyl, 1954 (Monhysteridae), *Parasitylenchus* (*Metaparasitylenchus*) *oschei* Rühm, 1956 (Allantonematidae), *Macdonaldius oschei* Chabaud & Frank, 1961 (Onchocercidae), *Brumptaemilius oschei* Dollfus, 1964 (Rhigonematidae), and the “Rhabditidae” *Oscheius* Andrassy, 1976, *Rhabditis* (*Mesorhabditis*) *oschei* Körner, 1954 and *Rhabditis* (*Oscheius*) *guentheri* Sudhaus & Hooper, 1994. Günther Osche maintains a significant presence in his pioneering publications and concepts pointing to the routes for prolific research, and he lives in the memories and hearts of his friends and his alumni.

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