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Description of *Meloidogyne panyuensis* sp. n. (Nematoda: Meloidogynidae), parasitic on peanut (*Arachis hypogaea* L.) in China

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Summary. *Meloidogyne panyuensis* n.sp. is described and illustrated from peanut, *Arachis hypogaea* L., in Guangdong, P.R.China. It is characterized by: female stylet of 13 µm length, DGO of 10 µm; perineal pattern ovoid to oval shaped, smooth to moderately coarse striae, dorsal arch relatively low, lateral lines indistinct, tail terminus area with irregular striae; male stylet 24 µm long; labial disc oval shaped, slightly elevated and fused with crescent shaped medial lips; second-stage juveniles with small rounded labial disc, fused with medial lips; lateral field with four incisures, areolated; tail 55 µm long, gradually tapering towards a small pointed terminus, hyaline tail part distinct. A weak S1-F1 esterase pattern and a N1b malate dehydrogenase pattern were obtained from young single females. Additionally, distinguishing DNA information is presented.

Key words: *Arachis hypogaea*, *Meloidogyne panyuensis* sp. n., morphology.

Peanut (*Arachis hypogaea* L.) is an important economic crop in China as well as in other parts of the world. Originally, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 and *M. hapla* Chitwood, 1949 were considered as the major plant parasites of peanut in China. However, an undescribed root-knot nematode species was isolated recently and identified from the roots of peanut in the Guangdong province, Panyu county, China. It is described herein as *Meloidogyne panyuensis* sp. n.

MATERIAL AND METHODS

Cultures of *Meloidogyne panyuensis* n. sp. were established using single egg-masses from galled roots of peanut originally collected at the type locality and propagated on peanut in a glasshouse at 25°C. All stages used for morphological studies, isozyme and molecular detection were isolated from these purified cultures.

For morphological observations, adult females were obtained directly from galled peanut roots. Perineal patterns were prepared as described previously (Karssen *et al.*, 1998). Males were obtained after the galled roots were incubated in a moist chamber, and second-stage juveniles were hatched from egg-masses. Light microscopical observations from specimens in 2.5% formalin were performed using a Leica DMR research microscope. SEM observations for females, males and second-stage juveniles were made based on the method described previously with some modification (Karssen *et al.*, 1998).

For isozyme studies, single young egg-laying females were isolated from fresh galled roots. Isozyme patterns, including esterase (EST; EC 3.1.1.1) and malate dehydrogenase (MDH; EC 1.1.1.37) were obtained by the micro-gel method as described by Karssen *et al.* (1995).

The ITS region of rDNA including partial 18S, complete ITS and partial 26S was selected as a target and the Vrain primers 18S and 26S were used to amplify the fragment (Vrain *et al.*, 1992). PCR was performed using the following programme: 4 min at 94°C, then 30 cycles of 1 min at 94°C, 1 min at 53°C and 2 min at 72°C,

followed by 10 min at 72°C.

The generated ITS-fragments of *M. panyuensis* sp. n., *M. arenaria, M. javanica* (Treub, 1885) Chitwood, 1949, *M. incognita* (Kofoid & White, 1919) Chitwood, 1949, were purified from agarose gel and cloned into the pGEM-T vector (Promega). The cloned insert was sequenced and analysied on an ABI PRISM 377 DNA sequencer (Applied Biosystems). The sequences were deposited in GenBank (www.ncbi.nlm.nih.gov) under the accession numbers AY 394719. The alignment of consensus sequences were performed by Lasergene software (DNA star, Inc., Madison, WI, USA). Sequence pair distances were calculated with the (weighted) Jotun Hein Method.

DESCRIPTION

Meloidogyne panyuensis sp. n. (Figs. 1-6)

Measurements: Female, males and second stage juveniles in Table 1.

Eggs (n=20): Length: 90.0-105.0 (100.4±4.9, SE= 1.1) µm; width: 40.0-47.5 (45.8±2.2, SE= 0.5) µm; length/width: 1.9-2.5 (2.2±0.1, SE= 0.03). Tables 1-2.

Female. Body annulated, pearly white, globular to pear shaped, with slight posterior protuberance and distinct neck region. Cephalic framework weakly sclerotized. From the face view in SEM, head cap irregular and variable, labial disc fused with medial lips. Head region set off from the body. Fine annules in neck region, posterior body annules unclear. Excretory pore located between head end and metacorpus level. Stylet well developed, cone slightly curved dorsally, with large rounded knobs, set off from the shaft. Perineal pattern ovoid to oval shaped, striae smooth to moderately coarse, dorsal arch relatively low; lateral lines indistinct (with SEM, lateral lines appear as a weak indention); tail remnant area distinct with irregular striae, without punctuations; phasmids very small, difficult to observe.

Male. Body vermiform, tapering, rounded at both extremities. Cuticle with distinct annulations. Lateral field with four incisures, areolated, incomplete incisure in middle of lateral field often present near mid-body. Head slightly set off, with a single post-labial annule. Cephalic framework moderately sclerotized. labial disc large and oval, elevated and fused with crescent shaped medial lips. Amphidial openings appear as elongated slits between labial disc and small lateral lips, Vestibule extension distinct. Stylet strong, straight and pointed at anterior end. Shaft cylindrical, knobs large and rounded, set off from the shaft. Pharynx with slender procorpus, metacorpus large and oval, ventrally overlapping pharyngeal gland lobe. Testis usually long with reflexed or outstretched germinal zone. Tail short, twisted ventrally with rounded terminus. Spicules arcuate and strong, curved ventrally.

Second-stage juveniles. Body vermiform, tapering slightly anteriorly and pronounced posteriorly. Body annules small and distinct. Lateral field with four incisures, aerolated. Head region slightly set off from body. Cephalic framework weakly sclerotized, labial disc rounded, small, and fused with relatively large medial lips. Medial lips with four distinct cephalic sensilla, lateral lips small. Vestibule extension distinct. Stylet slender and moderately long, cone straight; knobs distinct and rounded, set off from the shaft. Pharynx with slender procorpus and oval metacorpus. Pharyngeal gland lobe overlapping the intestine ventrally. Hemizonid anterior, adjacent to the excretory pore, 2 µm in length. Rectum slightly inflated. Tail tapering, terminus slightly pointed, sometimes irregular shaped and marked with cuticular constrictions.

Type host and locality. Described from the roots of peanut (*Arachis hypogaea* L.), originally isolated from infected roots of peanut from sandy soil of Panyu county, Guangzhou, Guangdong province, P.R.China.

Type material. Holotype female: slide collection of Wageningen University and Research Centre, Wageningen, The Netherlands. Paratypes: two female perineal patterns and heads, two males and five J2 deposited at each of the following collections: Wageningen University and Research Centre, Wageningen, The Netherlands; Rothamsted Research, Harpenden, UK; Plant Nematology Lab, South China Agricultural University, Guangzhou, China.

DIAGNOSIS AND RELATIONSHIPS

Meloidogyne panyuensis sp. n. is morphologically very different from the other known peanut *Meloidogyne* species *M. arenaria* and *M. hapla* and distinct from all other described species within the genus. It is characterized by a female stylet of 13 (12-15) µm in length and a DGO of 10.0 (9-12.5) µm. Perineal pattern ovoid to oval shaped, smooth to moderately coarse striae, indistinct lateral lines, tail terminus area with irregular striae. Male stylet 23.7 (22.5-26) µm long. Labial disc oval shaped, slight elevated and fused with crescent

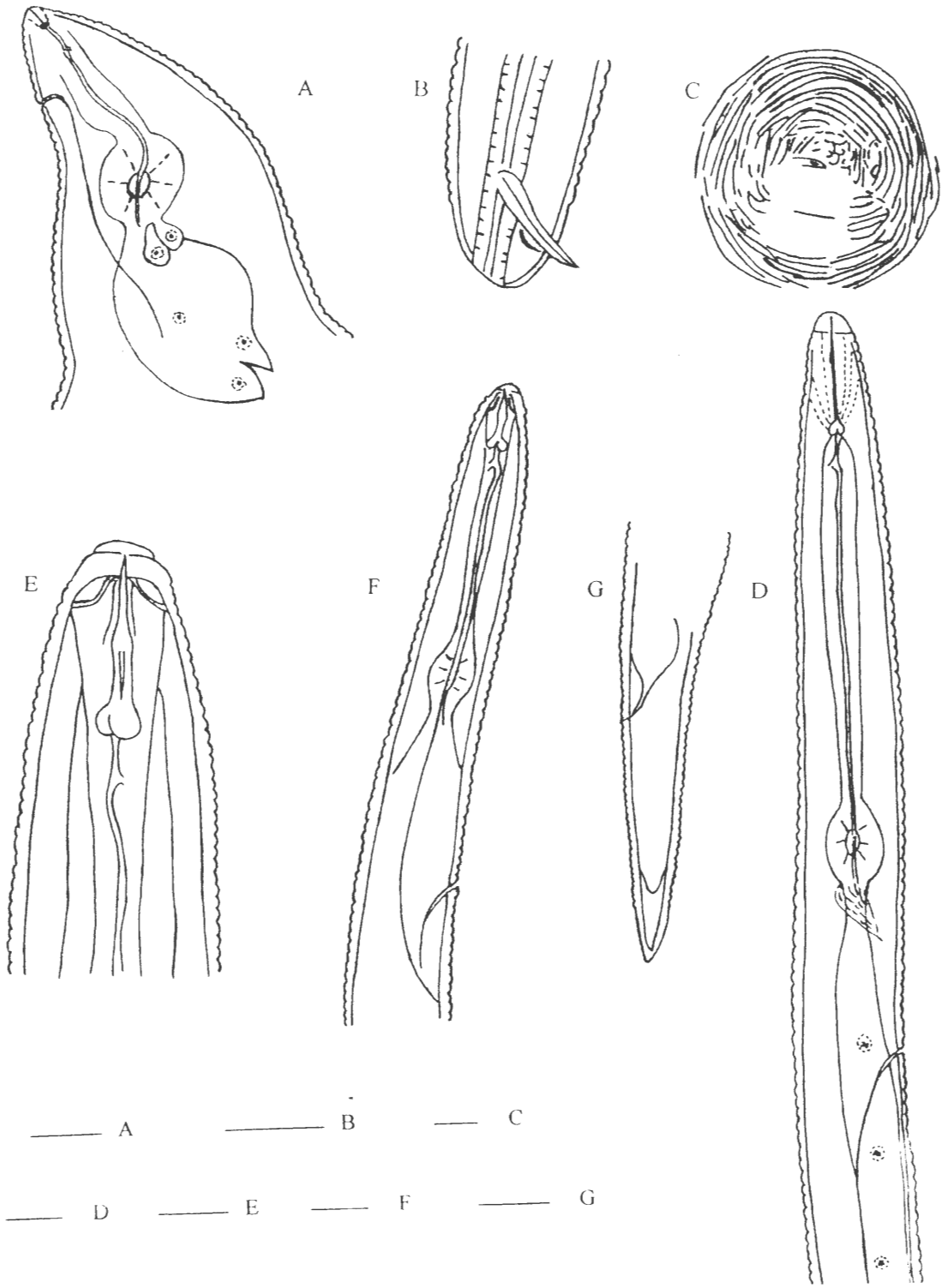

Fig. 1. *Meloidogyne panyuensis* sp. n. Female (A, C), Male (B, E, F), Second-stage juvenile (D, G). A: Anterior end (lateral); B: Posterior end (lateral); C: Perineal pattern; D-F: Anterior end (lateral); G: Tail (lateral). Scale bars: A-C – 20 μm; D-E – 10 μm.

Table1. Morphometrics of adults and second-stage juveniles (J2) of *Meloidogyne panyuensis*. sp. n., (mean ± SD and (range); n=20; all measurements in µm).

Character	Female	Male	J2
L	619 ± 77 (480-750)	1899 ± 89 (1710-2050)	409 ± 28 (353-455)
Greatest body diam	458 ± 75 (320-580)	47.4 ± 2.2 (43.8-52.5)	17.0 ± 1.2 (15.0-18.8)
Neck length	136 ± 33 (65-180)	–	–
Anterior end to metacorpus	81 ± 5 (73-88)	98 ± 6 (86-105)	61 ± 3 (55-68)
Excretory pore to anterior end	35.6 ± 2.1 (32.5-37.5)	161 ± 19 (135-200)	78 ± 6 (78-83)
Stylet	13.0 ± 0.9 (12.0-15.0)	23.7 ± 1.1 (22.5-26.3)	14.5 ± 0.6 (13.8-15.0)
Stylet knob height	2.8 ± 0.2 (2.5-3.0)	2.7 ± 0.3 (2.3-3.0)	1.6 ± 0.2 (1.3-2.0)
Stylet knob width	4.8 ± 0.3 (4.5-5.0)	5.1 ± 0.3 (4.5-5.8)	2.0 ± 0.2 (1.5-2.5)
DGO	10.0 ± 1.4 (8.8-12.5)	5.8 ± 0.7 (5.0-7.0)	4.1 ± 0.5 (3.0-4.5)
Metacorpus length	31.8 ± 2.6 (30.0-40.0)	19.5 ± 0.7 (18.8-20.0)	14.4 ± 0.9 (12.5-15.0)
Metacorpus diameter	31.7 ± 3.7 (30.0-37.5)	16.3 ± 1.0 (15.0-17.5)	10.2 ± 0.9 (8.8-12.5)
Vulva slit length	21.3 ± 1.1 (20.0-22.5)	–	–
Vulva-anus distance	15.9 ± 1.7 (15.0-17.5)	–	–
Spicule	–	31.7 ± 2.9 (25.0-35.0)	–
Tail length	–	–	55 ± 5 (48-63)
Hyaline length	–	–	9.1 ± 1.8 (7.5-10.0)
Esophageal base to anterior end	–	–	135 ± 16 (120-170)
a	1.3 ± 0.2(1.1-1.7)	40.2 ± 2.4 (34.2-43.6)	24.3 ± 2.4 (20.6-29.3)
c	–	–	7.5 ± 0.4 (6.7-8.1)

Table 2. Sequence pair distances (% identity and divergence) of *Meloidogyne panyuensis* sp. n., *M. fallax, M. arenaria, M. javanica* and *M. incognita* calculated using the J. Hein alignment method. Over the diagonal - %identity, below the diagonal – divergence.

Species	*M. panyuensis*	*M. fallax*	*M. arenaria*	*M. javanica*	*M. incognita*
M. panyuensis	–	73.7	73.6	73.3	73.4
M. fallax	33.0	–	81.6	81.6	81.8
M. arenaria	32.7	21.1	–	99.1	99.6
M. javanica	33.2	21.1	0.9	–	99.3
M. incognita	33.0	20.9	0.4	0.7	–

shaped medial lips. Second-stage juveniles with small rounded labial disc, disc fused relatively large medial lips. The lateral field has four incisures, areolated. The tail is 55.3 (47.5-62.5) µm long and tapering towards a slight pointed terminus with distinct hyaline tail part.

The closest related species is *M. fallax* Karssen, 1996. The new species differs morphologically from it by a longer DGO (10.0 ± 1.4 µm *vs* 4.3 ± 0.5 µm in females; 5.8 ± 0.7 µm vs 4.4 ± 0.7 µm in males; 4.1 ± 0.5 µm vs 3.5 ± 0.3 µm in second-stage juveniles); a longer distance between excretory pore to anterior end (35.6 ± 2.1 µm *vs* 22.5 ± 5.3 µm in females; 161.4 ± 19.2 µm *vs* 121±11.4 µm in males; 77.8±6.2 µm *vs* 69.0± 3.4 µm in second-stage juveniles); different labial disc shape (oval *vs* rounded) in males; longer male stylet length (23.7±1.1 µm *vs* 19.6± 0.8 µm) and a longer tail (55.3±4.5 µm *vs* 49.3±2.2 µm) in second-stage juveniles.

In addition, a N1b malate dehydrogenase pattern was also found in *M. fallax* and *M. artielli* Franklin, 1961 (see Karssen, 2002), the weak 2 banded esterase pattern, S1-F1, is considered as unique for *M. panyuensis* n. sp.

The ITS amplification fragment from the primer 18S and 26S, as described previously, produced a band of 870 bp for *M. panyuensis* sp. n. ITS

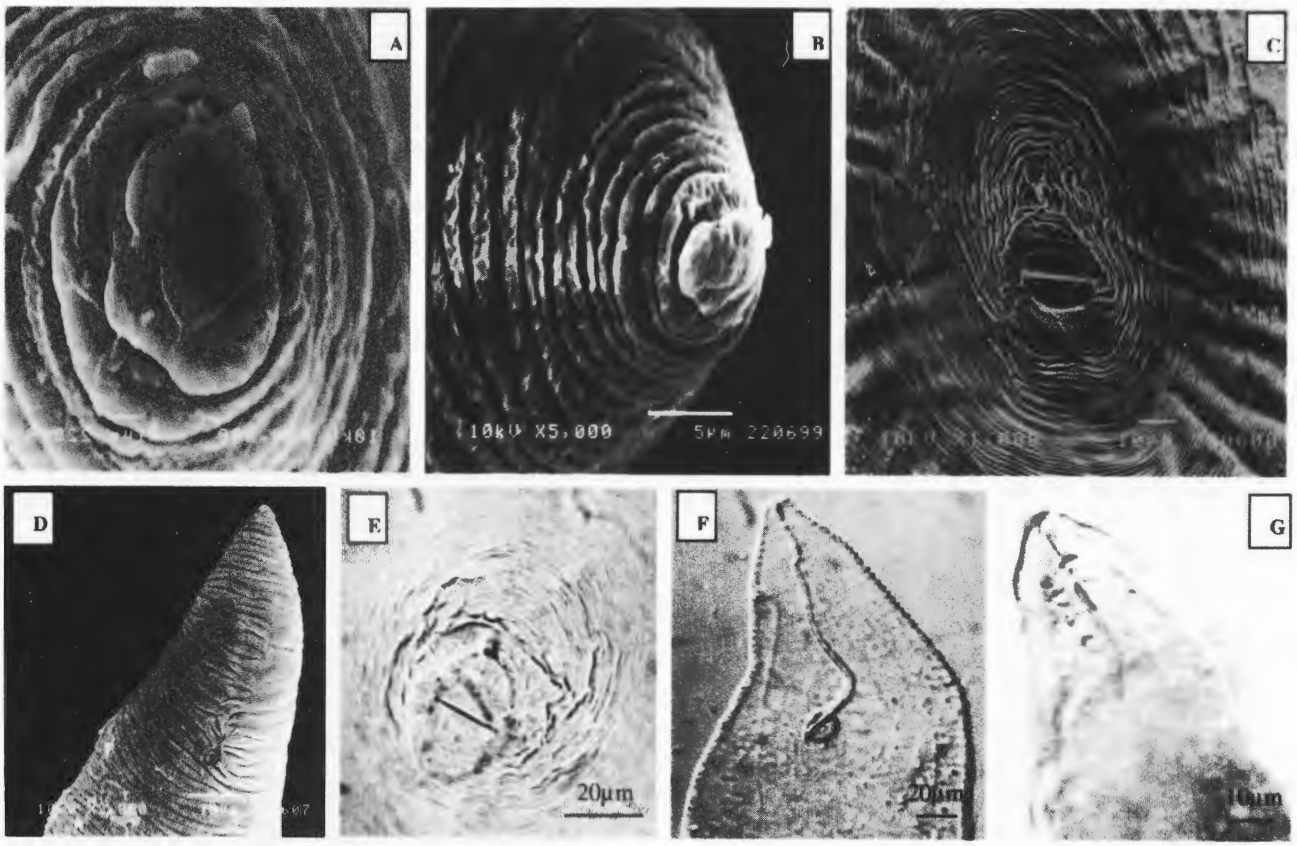

Fig. 2. SEM (A-D) and LM (E-G) photographs of *Meloidogyne panyuensis* sp. n., Female. A, B: Lip region; C, E: Perineal pattern; D: Neck region with excretory pore; F, G: Anterior end (lateral). (Scale bars: A = 1 μm; B = 5 μm; C, D, G = 10 μm; E, F = 20 μm).

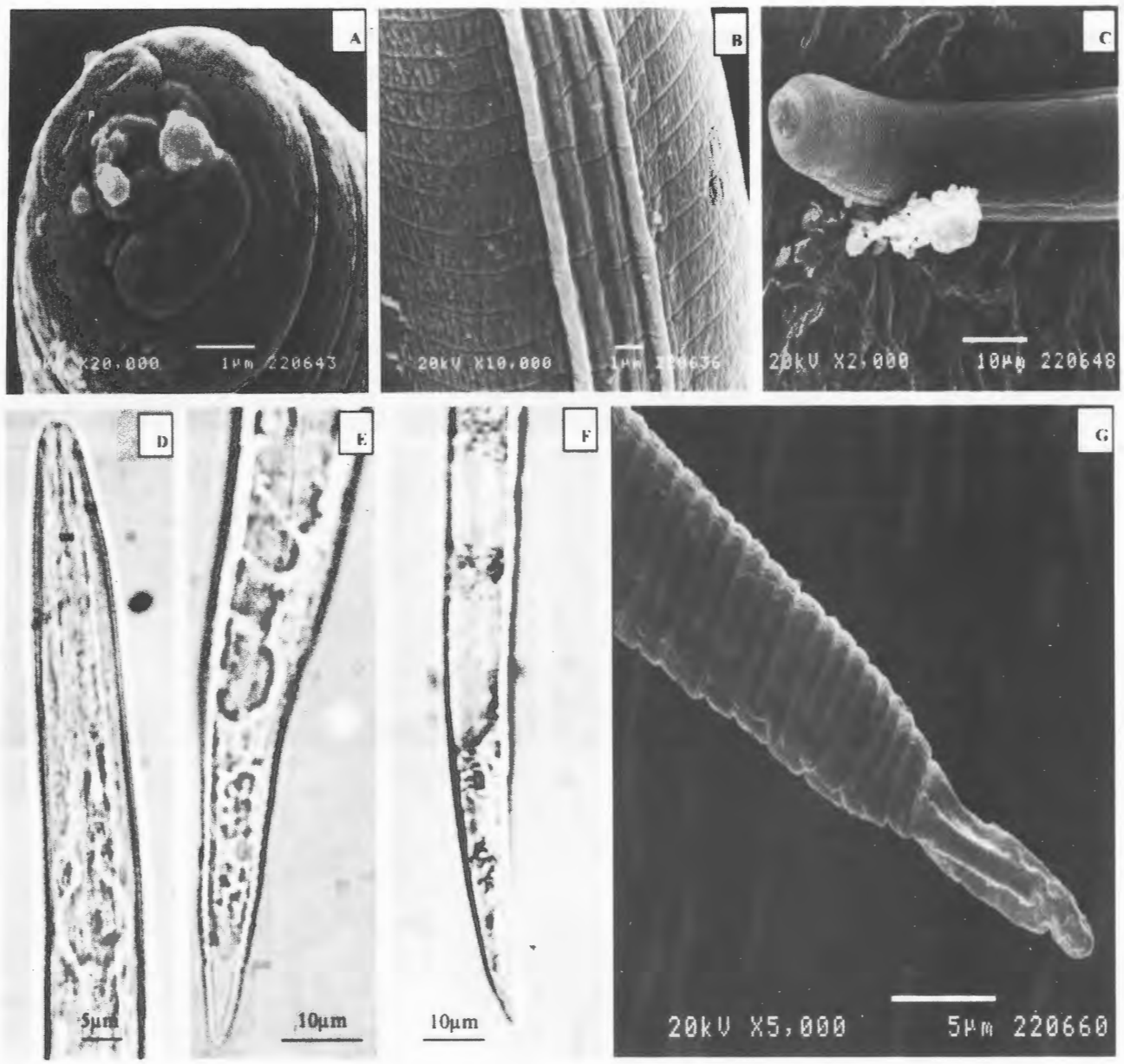

Fig. 3. SEM (A-C, G) and LM (D-F) photographs of *Meloidogyne panyuensis* sp. n. Second-stage juvenile. A: Lip region; B: Lateral field; C: Anterior end with excretory pore; D: Anterior end (lateral); E, F: Tail (lateral); G: Tail tip. (Scale bars: A, B = 1 μm; D, G = 5 μm; C, E = 10 μm).

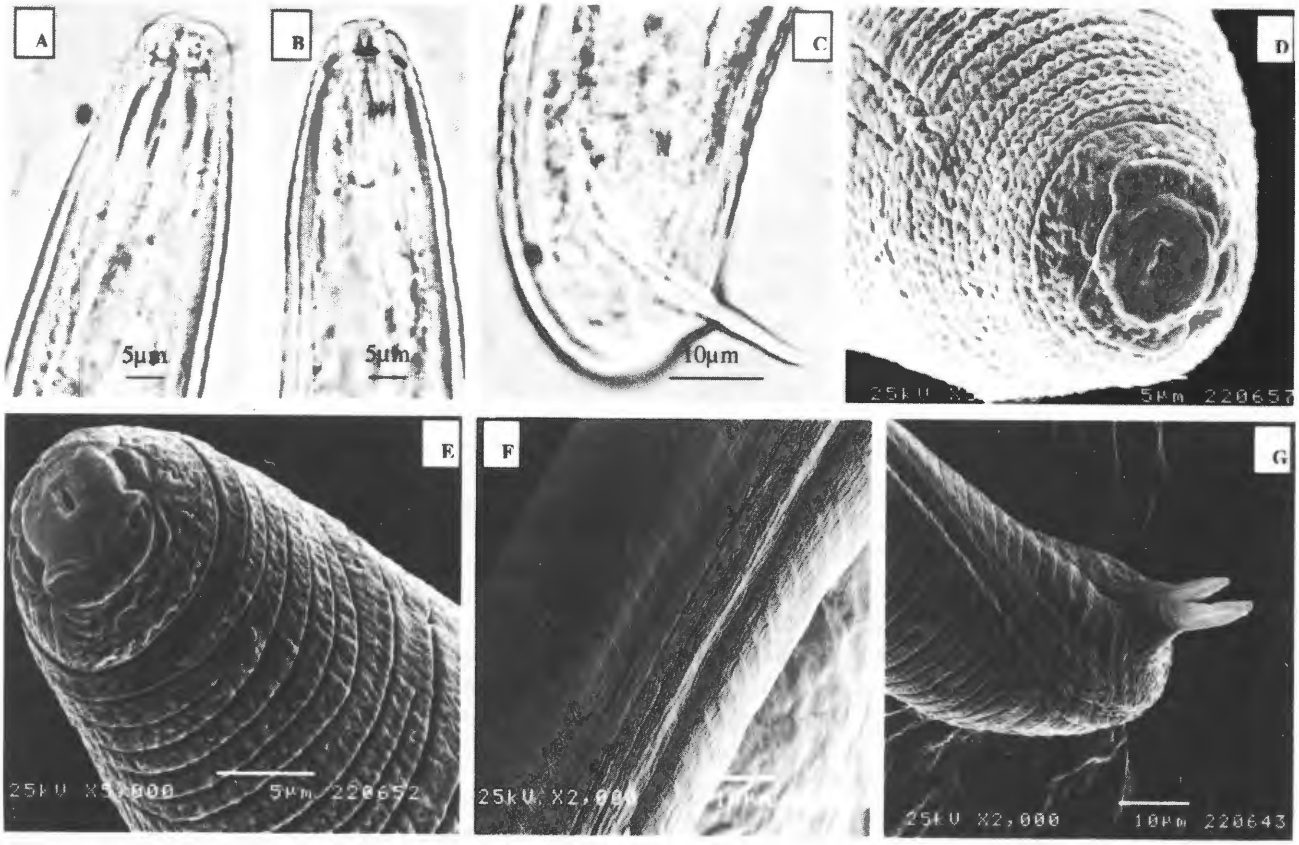

Fig. 4. LM (A-C) and SEM (D-G) photographs of *Meloidogyne panyuensis* sp. n. Male. A,B: Anterior end (lateral, dorsal); C: Posterior end (lateral); D, E: Head region; F: Lateral field; G: Tail region. (Scale bars: A, B, D, E = 5 μm; C, F, G = 10 μm).

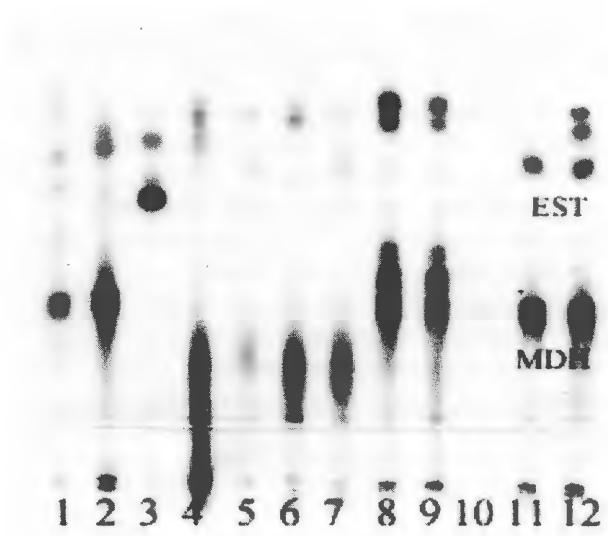

Fig.5. Esterase (upper part) and malate dehydrogenase (lower part) isozymes of *Meloidogyne javanica* (lane 1, 12); *M. incognita* (lane 2, 10, 11); *M. panyuensis* sp. n. (lane 4-7) and *M. arenaria* (lane 8, 9).

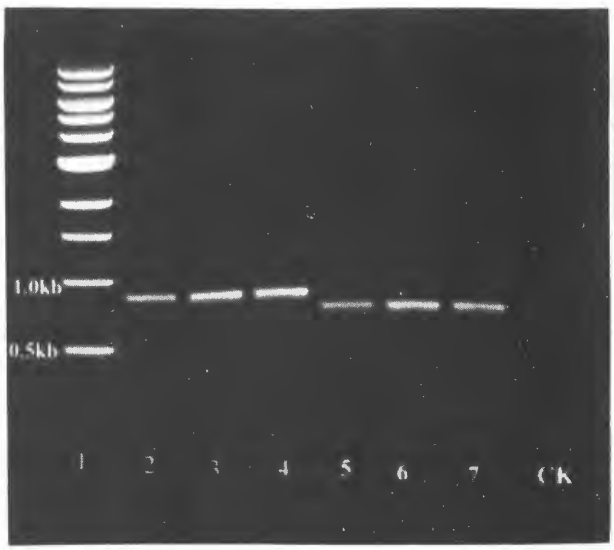

Fig.6. rDNA-ITS fragments of *Meloidogyne panyuensis* sp. n. (lane 2-4); *M. arenaria* (lane 5, 6); *M. incognita* (lane 7) and negative controle (lane 8).

sequence homology analysis shows no match with ITS sequences for *M. arenaria, M. fallax, M. incognita, M. javanica* or other *Meloidogyne* species deposited in GenBank (Table 2).

DISCUSSION

Historically, there are two root-knot nematodes recorded as infecting peanuts in China, i.e. *M. hapla* and *M. arenaria*. *M. arenaria* was found to be the main pathogenic nematode on peanut in the Guangdong province so far. Whether *M. panyuensis* sp. n. is also distributed in other parts of China is unknown and needs to be determined. According to the observation in glasshouse and field tests, the symptoms of *M. panyuensis* sp. n. infested peanuts are stunting plants, yellow and small leaves with slightly swollen roots.

Female isozyme patterns are very important for *Meloidogyne* identification (Esbenshade & Triantaphyllou, 1985; Karssen, 2002). The malate dehydrogenase pattern for *M. panyuensis* sp. n., clearly belongs to the N1b type, i.e. the same rare type as so far only found in *M. fallax* and *M. artiellia* Franklin,1961. This is also the first time that this Mdh type is reported outside Europe. Importantly, the weak 2 banded esterase pattern (S1-F1) in *M. panyuensis* sp. n. is unique, and has not been described so far.

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REFERENCES

Esbenshade, P.R. & Triantaphyllou, A.C. 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species. *Journal of Nematology* 17: 6-20.

Karssen, G., Van Hoenselaar, T., Verkerk, B. & Janssen, R. 1995. Species identification of cyst and root-knot nematodes from potato by electrophoresis of individual females. *Electrophoresis* 16: 105-109.

Karssen, G., Van Aelst, A. & Van Der Putten, W.H. 1998. *Meloidogyne duytsi* n.sp. (Nematoda: Heteroderidae), a root-knot nematode from Dutch coastal foredunes. *Fundamental and Applied Nematology* 21: 299-306

Karssen, G. 2002. The plant-parasitic nematode genus *Meloidogyne* Göldi, 1892 (Tylenchida) in Europe. Leiden, The Netherlands,Brill Academic Publisher, 160 pp.

Vrain, T.C., Wakarchuk, D.A., Lévesque, A.C. & Hamilton, R.I. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* 15: 563-573.

Liao J. , Yang W., Feng Z. и Karssen G. Описание *Meloidogyne panyuensis*sp. n. (Nematoda, Meloidogynidae), паразитирующих на арахисе (Arachis hypogaea L.) в Китае.

Резюме. Приводится описание *Meloidogyne panyuensis* n.sp. – нового вида, выделенного из арахиса *Arachis hypogaea* L. в провинции Гуандун Китая. Новый вид характеризуется стилетом самок длиной 13 μm, DGO 10 μm; перинеальными структурами от овоидных до овальных, почти гладкой или умеренно выраженной исчерченностью, сравнительно слабо выраженной дорсальной дугой, плохо различимыми латеральными линиями, терминусом хвоста с нерегулярной исчерченностью, стилетом самца длиной 24 μm; овальным лабиальным диском, слегка возвышающимися и слитыми средними губами, личинками второй стадии с небольшим округлым губным диском слитым с губами, латеральным полем с 4 инцизурами и ареолами, хвостовым отделом длиной 55 μm, постепенно сужающимся к небольшому заостренному терминусу, и четко различимой гиалиновой частью хвоста. От отдельных особей молодых самок были получены слабые профили S1-F1 эстераз и N1b малат-дегидрогеназ. Представлена молекулярно-таксономическая информация для дифференциации нового вида.