

Morphological and molecular characterisation of *Heliconema hainanensis* sp. nov. (Spirurina: Physalopteridae) from congers in the South China Sea, with a key to the species of *Heliconema*

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Heliconema hainanensis sp. nov. collected from *Uroconger lepturus* (Richardson) (Anguilliformes: Congridae), *Muraenesox cinereus* (Forsskål) and *Congresox talabonoides* (Bleeker) (Anguilliformes: Muraenesocidae) in the South China Sea was described using light and scanning electron microscopy. The new species differs from its congeners by the following morphology: pseudolabia, the number and arrangement of caudal papillae (4 pairs of pedunculate precloacal papillae arranged in 2 groups of 2 and 2 pairs and 6 pairs of pedunculate postcloacal papillae arranged in 4 groups of 1, 2, 1 and 2 pairs), the length of spicules [left spicule 0.51-0.69 mm, right spicule 0.20-0.27 mm, spicule (right:left) ratio 1:2.20-2.69] and the morphology of the female tail tip. In addition, specimens of the new species collected from the three different hosts and specimens of an unidentified species of *Heliconema* collected from *U. lepturus* were characterised using molecular methods by sequencing the internal transcribed spacer (ITS) of ribosomal DNA. Analyses and comparison of the ITS sequence of *H. hainanensis* sp. nov. with *Heliconema* sp. support the validity of the new species based on morphological observations. An identification key to the species of *Heliconema* is also provided.

Key words: Proleptinae - *Heliconema* - molecular taxonomy - morphology - new species

Adult *Heliconema* nematodes commonly occur in the digestive tract of fishes of the orders Anguilliformes and Synbranchiformes (Li 1934, Ogden 1969, Gupta & Duggal 1989, Crites & Overstreet 1991, Moravec et al. 2007), but some species can also parasitise snakes (Ortlepp 1923, Fusco & Palmieri 1980). Travassos (1919) erected the genus *Heliconema* and designated *Heliconema heliconema* Travassos 1919 from the chain moray *Echidna catenata* (Bloch) (Anguilliformes: Muraenidae) as the type species. Until 2012, there were only nine recognisable species in *Heliconema* worldwide, including *H. heliconema* Travassos 1919, *Heliconema longissimum* (Ortlepp 1923), *Heliconema brevispiculum* Baylis 1934, *Heliconema baylisi* Ogden 1969, *Heliconema serpens* Fusco and Palmieri 1980, *Heliconema psammobatidus* Threlfall and Carvajal 1984, *Heliconema kherai* Gupta and Duggal 1989, *Heliconema brooksi* Crites and Overstreet 1991 and *Heliconema savala* Akram 1996 (Travassos 1919, Ortlepp 1923, Baylis 1934, Li 1934, Ogden 1969, Fusco & Palmieri 1980, Threlfall & Carvajal 1984, Gupta & Duggal 1989, Crites & Overstreet 1991, Akram 1996, Moravec et

al. 2007). Of these, only three species have been reported in China: *H. longissimum* from *Anguilla japonica* Temminck & Schlegel (Anguilliformes: Anguillidae) and *H. heliconema* and *H. brevispiculum* from *Muraenesox cinereus* (Forsskål) (Anguilliformes: Muraenesocidae) (Li 1934, Wang et al. 1976, 1977, Pan et al. 1990). During a helminthological survey of marine fishes in the South China Sea from November 2009-December 2010, large numbers of spirurine nematodes identified morphologically as a new species, *Heliconema hainanensis* sp. nov. were collected from the intestine and stomach of the congers *Uroconger lepturus* (Richardson) (Anguilliformes: Congridae), *M. cinereus* (Forsskål) and *Congresox talabonoides* (Bleeker) (Anguilliformes: Muraenesocidae).

In recent years, morphological features combined with genetic information, such as sequences of the internal transcribed spacer (ITS) of nuclear ribosomal DNA (rDNA) and mitochondrial cytochrome C oxidase subunit I and II, have been successfully used for the accurate identification of nematodes at the species level for eggs, larvae and adults (Ivanova et al. 2007, Koubková et al. 2008, Mattiucci et al. 2008, Mašová et al. 2010, Guerrero et al. 2011, Li et al. 2012a, b, Testini et al. 2011). Consequently, specimens of this new species collected from three different hosts and specimens of *Heliconema* sp. collected from *U. lepturus* were characterised using molecular methods by sequencing and analysing the ITS region of rDNA to validate their taxonomic status. The present study is the first use of ITS-region sequencing for the purpose of species discrimination and identification in the genus *Heliconema* and represents a substantial step toward using sequence variation for species delimitation in this poorly characterised group. An identification key to the species of *Heliconema* is also provided.

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MATERIALS AND METHODS

Light and scanning electron microscopy (SEM) - Fishes collected from the South China Sea were examined for parasites. Nematodes recovered from the digestive tract of various fishes were washed in physiological saline and then fixed and stored in 70% ethanol until use. For light microscopy studies, nematodes were clarified in lactophenol. Drawings were rendered with the aid of a Nikon microscope drawing attachment. For SEM, a portion of the specimens was selected for fixation in 4% formaldehyde solution, post-fixed in 1% OsO₄, dehydrated via an ethanol series and acetone, then critical-point dried. The specimens were coated with gold and examined on a Hitachi S-570 scanning electron microscope at an accelerating voltage of 15 kV.

Measurements (the range, followed by the mean in parentheses) are given in micrometres unless otherwise stated. Type specimens are deposited at the College of Life Sciences, Hebei Normal University (HBNU), Hebei Province, China. The collection data for the specimens of *H. hainanensis* sp. nov. are presented in Table.

Molecular procedures - Twenty-one nematodes (i.e., 6 randomly selected paratypes of *H. hainanensis* sp. nov. from each of the host species, *U. lepturus*, *M. cinereus* and *C. talabonoides* and 3 specimens of *Heliconema* sp. from *U. lepturus*) were subjected to molecular analysis. Genomic DNA from individual worms was extracted with the Column Genomic DNA Isolation Kit (Shanghai Sangon, China) according to the manufacturer's instructions. DNA was eluted in elution buffer and stored at -20°C until use. The ITS region was amplified by polymerase chain reaction (PCR) using the primers NC5 (forward: 5'-GTAGGTGAACCTGCGGAAGGATCATT-3') and NC2 (reverse: 5'-TTAGTTTCTTCTCCGCT-3') (Zhu et al. 1998). PCR was performed in 50 µL of PCR reaction buffer containing 10 mM Tris HCl, pH 8.4, 50 mM KCl, 3.0 mM MgCl₂, 250 µM of each deoxynucleotide triphosphate, 50 pmol of each primer and 1.5 U of *Taq* polymerase (Takara) in a thermocycler (2720, Applied Biosystems) using the following conditions: 94°C for 5 min (initial denaturation), followed by 30 cycles of 94°C for 30 s (denaturation), 55°C for 30 s (annealing), 72°C for

70 s (extension) and a final extension at 72°C for 7 min. PCR products were verified on a GoldView-stained 1.5% agarose gel and purified with the Column PCR Product Purification Kit (Shanghai Sangon, China). Sequencing was performed using the DyeDeoxy Terminator Cycle Sequencing Kit (v.2, Applied Biosystems, California, USA) and an automated sequencer (ABI-PRISM 377). Sequencing for each sample was performed for both strands. Sequences were aligned using CLUSTALW2 (Thompson et al. 1994) and adjusted manually. The ITS sequences obtained were compared with those available in the National Center for Biotechnology Information (NCBI) database (ncbi.nlm.nih.gov) using the BLASTn algorithm.

RESULTS

H. hainanensis sp. nov. (Figs 1-4)

The morphometrics of specimens collected from the three different hosts, *U. lepturus*, *M. cinereus* and *C. talabonoides*, were strikingly similar (i.e., no notable morphological variations were detected between specimens from different hosts).

Diagnosis - Medium-sized, whitish nematodes. Cuticle with fine transverse striations. Cephalic end rounded. Cuticle in cephalic region inflated to form a cephalic vesicle extending posteriorly to the level of deirids (Fig. 1A). Oral aperture dorsoventrally elongated, surrounded by two lateral pseudolabia (Figs 1C, 2A, 3A); each pseudolabium bears two large submedian (dorsolateral and ventrolateral) cephalic papillae and one small amphid situated at the base of the pseudolabium (Figs 1C, 2A, 3A); inner surface of each pseudolabium with a large median conical tooth and ridged subdorsal and subventral teeth (Figs 3B, 4A); any series of denticles absent. Buccal cavity short. Oesophagus divided into a short, narrow anterior muscular portion and a significantly longer, wide glandular portion. Nerve ring encircles the muscular oesophagus posterior to its middle (Fig. 1A, B). Small simple deirids situated at the level of the nerve ring (Figs 1A, 3C). Excretory pore situated slightly posterior to the anterior end of the glandular oesophagus (Fig. 1B). Posterior end of the body sexual dimorphism (Figs 2C, 3D).

TABLE
Specimens of *Heliconema hainanensis* sp. nov. collected from the South China Sea

Hosts	Date of collection	Locality of collection	Prevalence and intensity (mean)
<i>Uroconger lepturus</i>	24-30 November 2010	Off Sanya, Hainan Island	2/11, 42-126 (84.0)
	24-25 November 2010	Off Sanya, Hainan Island	1/2, 30 (15.0)
<i>Muraenesox cinereus</i>	25-26 November 2009	Off Beihai, Guangxi Province	6/6, 25-100 (66.2)
	1 December 2010	Off Sanya, Hainan Island	1/2, 90
<i>Congresox talabonoides</i>	26 November 2009	Off Beihai, Guangxi Province	2/2, 3-84 (43.5)
	29 April 2010	Off Zhanjiang, Guangdong Province	1/1, 54

Male (24 specimens) - Body 17.3-23.8 (20.5) mm long; maximum width 274-392 (337). Pseudolabia 29-39 (35.0) long, 118-127 (122) wide. Cephalic vesicle 127-343 (204) long, 20-29 (22.9) wide. Entire oesophagus 1.96-2.74 (2.37) mm long, representing 10.4-13.4 (11.7)% of body length; muscular oesophagus 245-363 (312) long, 78-108 (90.9) maximum width; glandular oesophagus 1.72-2.38 (2.06) mm long, 147-196 (162) maximum width; length ratio of two parts of the oesophagus 1:5.71-8.00 (1:6.66). Oesophageal-intestinal valve 49-98 (75.6) long, 118-157 (141) wide. Nerve ring, deirids and excretory pore 167-343 (253), 186-372 (289) and 235-441 (376), respectively, from the anterior extremity. Spicules dissimilar in shape, unequal in length; left spicule relatively long, 510-686 (574)

in length, arcuate, narrow, tapering to fine point distally, representing 2.26-3.25 (2.83)% of body length; right spicule short, 196-265 (238) long, thicker than left spicule, with distal end recurved, representing 1.01-1.40 (1.17)% of body length; spicule (right:left) ratio 1:2.20-2.69 (1:2.42) (Fig. 1E, G). Gubernaculum absent. Posterior end of body spirally coiled ventrally (Fig. 2C). Caudal alae slightly expanded, supported by four twin pairs of subventral pedunculate precloacal papillae and six pairs of subventral pedunculate postcloacal papillae arranged in four groups of one, two, one and two pairs (Figs 1E, F, 2D). Each caudal papilla surrounded by a ring consisting of numerous small cuticular, papilla-like protuberances (in young males, caudal papilla as knob-like projection without sur-

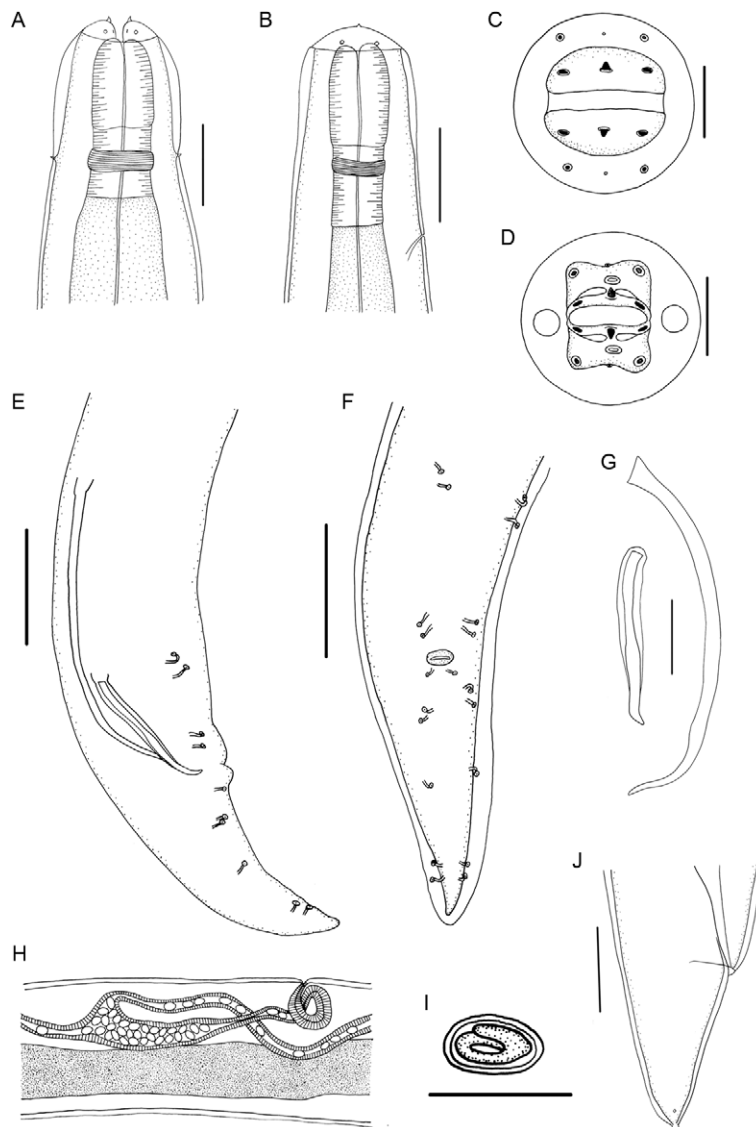


Fig. 1: *Heliconema hainanensis* sp. nov. from *Uroconger lepturus* (Richardson) in the South China Sea (A-C, E-J). A: anterior part of female, lateral view; B: anterior part of female, ventral view; C: cephalic end of male (reconstructed from scanning electron microscopy, apical view); D: cephalic end of *Heliconema longissimum* (Ortlepp), apical view [(redrawn after Moravec et al. (2007))]; E: posterior end of male (showing spicules and caudal papillae), lateral view; F: posterior end of male (showing caudal papillae and caudal alae), ventral view; G: spicules; H: region of vulva; I: larvated egg; J: posterior end of female, lateral view. Bars: A, B, E, F, H, J: 200 μ m; C: 40 μ m; D: 30 μ m; G: 100 μ m; I: 50 μ m.

rounding cuticular, papilla-like protuberances) (Figs 2E, F, 4B-D). Ventral precloacal surface forming 20 or more rows of tessellated longitudinal ridges (Fig. 2H) (in young males, tessellated longitudinal ridges weak, ridges lack clear demarcations) (Fig. 2G). Tail 294-412 (364) long with rounded tip (Figs 2B, 4C). Phasmids not observed.

Female (24 specimens) - Body 23.6-42.1 (35.3) mm long; maximum width 490-735 (622). Pseudolabia 29-49 (44.8) long, 127-147 (140) wide. Cephalic vesicle 196-294 (216) long, 29-39 (31.4) wide. Entire oesophagus 2.89-3.92 (3.38) mm long, representing 8.27-12.2 (10.4)% of body length; muscular oesophagus 392-461 (425) long, 118-176 (147) maximum width; glandular oesophagus 2.50-3.47 (2.96) mm long, 176-265 (234) maximum width; length ratio of two parts of the oesophagus 1:6.23-7.76 (1:6.98). Oesophageal-intestinal valve 78-137 (103) long, 157-274

(213) wide. Nerve ring, deirids and excretory pore 294-363 (317), 343-372 (353) and 392-441 (415), respectively, from the anterior extremity. Rectum a hyaline tube. Vulva slit-like without a protruding flap (Figs 1H, 3E) situated 11.2-16.9 (14.2) mm from anterior extremity at 35.4-47.4 (40.9)% of the body length. Vagina narrow, muscular, initially directed anteriorly from the vulva and then oriented posteriorly (Fig. 1H). Uteri didelphic, amphidelphic, containing numerous oval, thick-shelled, embryonated (larvated) eggs. Eggs suboval to rounded, 39-49 (45.7) long, 20-39 (30.5) wide (Fig. 1I). Posterior end of body nearly straight, not spirally coiled ventrally (Fig. 3D). Tail 294-412 (363) long with nipple-like tip. A pair of small lateral phasmids is present near tail tip (Figs 1J, 3F).

Type host - *U. lepturus* (Richardson) (Anguilliformes: Congridae).

Site of infection - Intestine and stomach.

Type locality - South China Sea, Off Sanya (109°30'E 18°12'N), Hainan Island, China.

Type data and depository - Holotype: male from *U. lepturus*, South China Sea, Off Sanya (HBNU-F1135); allotype: female from *U. lepturus*, South China Sea, Off Sanya (HBNU-F1136); paratypes: 75 males, 91 females from *U. lepturus* (HBNU-F1137), 29 males, 61 females from *C. talabonoides* (HBNU-F1138), six males, 24 females from *M. cinereus* (HBNU-F1139), South China Sea, Off Sanya; 22 males, 65 females from *C. talabonoides* (HBNU-F1140), 172 males, 225 females from *M. cinereus* (HBNU-F1141), South China Sea, Off Beihai; 23 males, 31 females from *C. talabonoides* (HBNU-F1142), South China Sea, Off Zhanjiang.

Other material examined - *M. cinereus* (Forsskål) (Anguilliformes: Muraenesocidae) and *C. talabonoides* (Bleeker) (Anguilliformes: Muraenesocidae).

Distribution - South China Sea, Off Sanya (109°30'E 18°12'N), Hainan Island, Off Beihai (109°12'E 21°48'N), Guangxi Province, Off Zhanjiang (110°39'E 21°26'N), Guangdong Province, China.

Host-parasite data - For details, see Table.

Etymology - The species name refers to the name of the type locality, Hainan Island.

DNA characterisation - Ten sequence types were obtained for the ITS region of the partial rDNA in *H. hainanensis* sp. nov. based on the specimens collected from the three different hosts examined here. The length of the ITS sequence of *H. hainanensis* sp. nov. ranged from 1,191-1,203 bp and showed low intraspecific nucleotide variability among the different sequence types (nucleotide differences from 0.16-1%) (Supplementary data). In *Heliconema* sp., two sequence types were obtained for the ITS region and the length of the ITS varied between 1,349-1,354 bp. A pairwise comparison between the two sequence types of *Heliconema* sp. demonstrated 0.96% nucleotide differences (Supplementary data). No ITS sequence from *Heliconema* is registered in GenBank; therefore, a pairwise comparison could only be performed between *H. hainanensis* sp. nov. and *Helicone-*

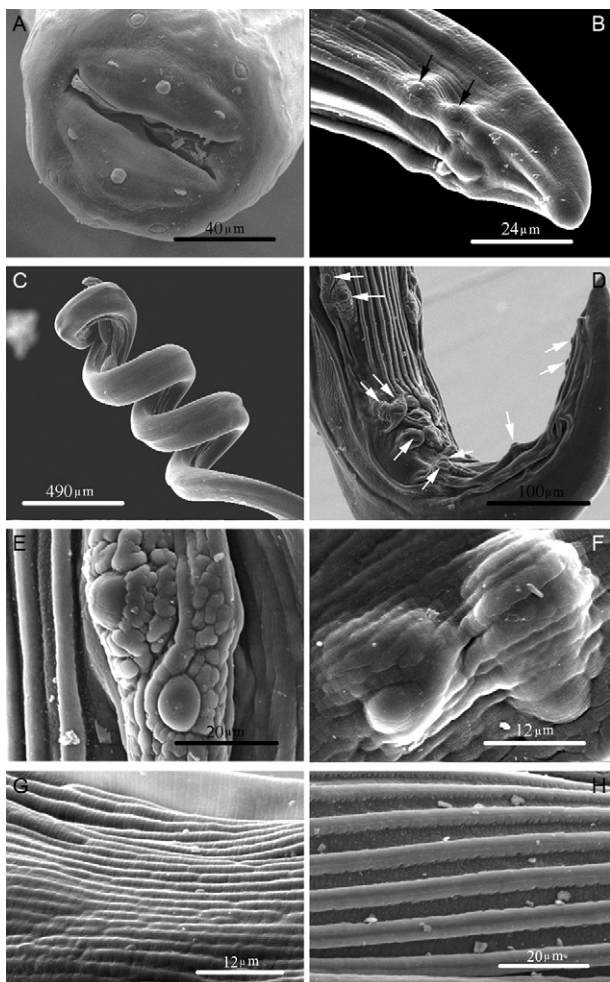


Fig. 2: scanning electron micrographs of *Heliconema hainanensis* sp. nov. from *Uroconger lepturus* (Richardson) in the South China Sea. A: cephalic extremity of mature male, apical view; B: apical portion of young male tail (last 2 postcloacal papillae arrowed), ventral view; C: posterior end of mature male, dorsal view; D: posterior end of mature male (caudal papillae arrowed), ventrolateral view; E: postcloacal papillae of mature male; F: postcloacal papillae of young male; G: precloacal tessellated longitudinal ridges of young male; H: precloacal tessellated longitudinal ridges of mature male.

ma sp. A high level of sequence variation was detected between the two species (greater than 50% nucleotide differences) because of the large number of nucleotide insertions, deletions and substitutions present (Supplementary data). The ITS sequences of *H. hainanensis* sp. nov. and *Heliconema* sp. were deposited in the GenBank database (ncbi.nlm.nih.gov) under the following accessions: *H. hainanensis* sp. nov., JQ750474-JQ750483 and *Heliconema* sp., JQ750472 and JQ750473.

DISCUSSION

In the genus *Heliconema*, the new species described here is similar to the congeners *H. longissimum*, *H. baylisi*, *H. serpens* and *H. kherai* in that they exhibit a vulva that lacks a prominent protruding flap. *H. hainanensis* sp. nov. can be readily distinguished from *H. serpens* and *H. kherai* by having six pairs of pedunculate postcloacal papillae (vs. 4 pairs in the latter 2 species). The new species differs from *H. baylisi* by having distinctively unequal spicules (right:left spicule ratio of 1:2.20-2.69 in the former vs. 1:1.11-1.36 in the latter), a different arrangement of postcloacal papillae and a different morphology of the female tail tip. *H. hainanensis* sp. nov. differs from

H. longissimum by having a pseudolabium without depressions or grooves (Fig. 1C, D), a female tail with a nipple-like tip and a different number, morphology and arrangement of postcloacal papillae; all of the six pairs of postcloacal papillae are pedunculate in the former, whereas three of the seven pairs of postcloacal papillae are sessile in the latter. The *Heliconema* sp. material in the present study was also collected from the same host and locality (*U. lepturus*, Off Sanya, Hainan Island) as *H. hainanensis* sp. nov. We were unable to precisely identify the present material to the species level morphologically because only four female specimens were found in the digestive tract of *U. lepturus*. However, from *H. hainanensis* sp. nov., *Heliconema* sp. differs by having a significantly larger body size (body length 43.3-56.7 mm in *Heliconema* sp. vs. 23.6-42.1 mm in the new species in the female) and the female tail lacks a nipple-like tip.

According to the traditional diagnostic characters and keys to the *Heliconema* species provided by Fusco and Palmieri (1980), Gupta and Duggal (1989), Crites and Overstreet (1991) and Akram (1996), the number of ventral precloacal tessellated longitudinal ridges (area rugosa) is critical to discriminating species of *Heliconema*. However, based on observations in some spirurine nematodes, such as *Rhabdochona* spp, Moravec et al. (2007) determined that this feature might be unreliable and variable within a species of *Heliconema* because the number of such ridges depends in part on the body size and age of the worm. In our study, the precloacal tessellated longitudinal ridges in the young males of *H. hainanensis* sp. nov. were weak (ridges without clear demarcation) and difficult to observe by light microscopy.

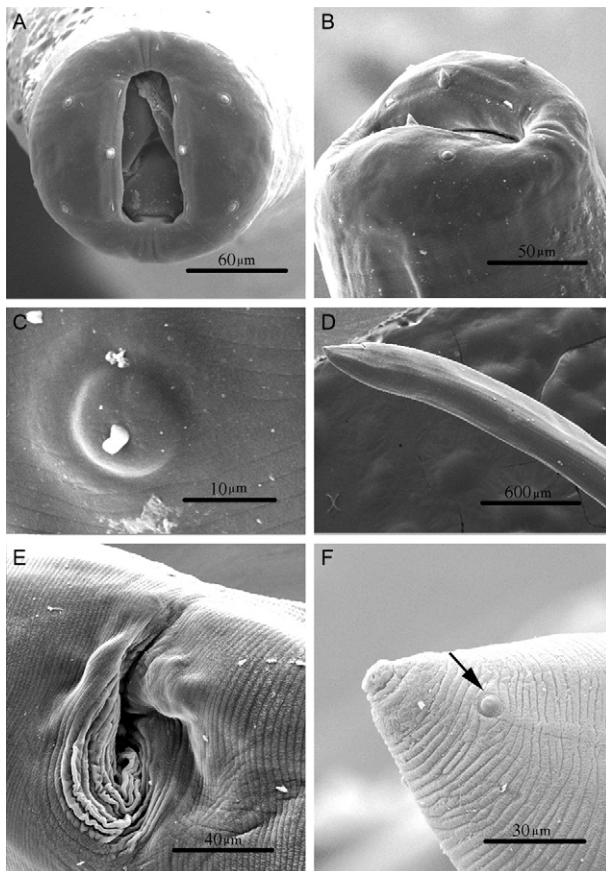


Fig. 3: scanning electron micrographs of *Heliconema hainanensis* sp. nov. from *Uroconger lepturus* (Richardson) in the South China Sea, female. A: cephalic extremity, apical view; B: cephalic extremity, lateral view; C: deirid; D: posterior end of body, lateral view; E: vulva; F: apical portion of tail (phasmid arrowed), lateral view.

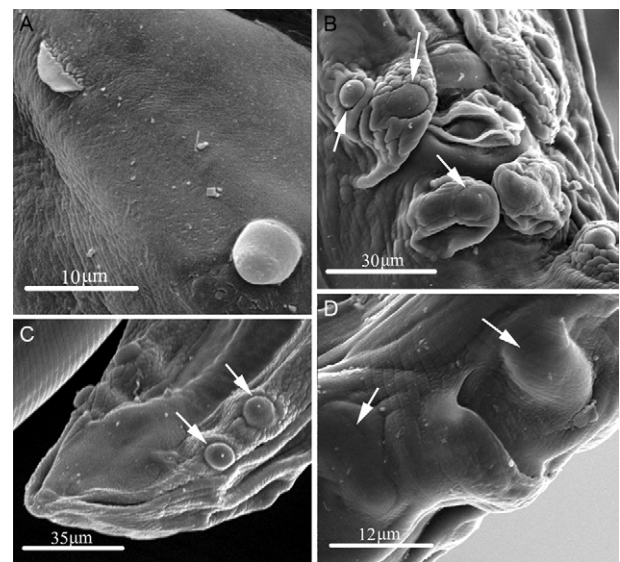


Fig. 4: scanning electron micrographs of *Heliconema hainanensis* sp. nov. from *Uroconger lepturus* (Richardson) in the South China Sea. A: large median conical tooth and ridged subdorsal tooth, apical view; B: cloacal region of mature male (precloacal and postcloacal papillae arrowed), ventral view; C: apical portion of mature male tail (last 2 postcloacal papillae arrowed), ventral view; D: cloacal region of young male (precloacal and postcloacal papillae arrowed), ventral view.

This result agrees well with the opinion of Moravec et al. (2007) and this feature was discontinued from use in our identification key. In addition, the morphology of the caudal papillae varies markedly, depending mainly on the age of the worm. In mature males, the caudal papillae are mammiform and surrounded by a ring consisting of numerous small cuticular, papilla-like protuberances. However, in young males, caudal papillae only form as knob-like projections, without surrounding cuticular, papilla-like protuberances. This observation indicates that care must be taken in designating the age of specimens when differentiating species according to the morphology of caudal papillae.

The molecular analyses of the ITS sequence data obtained for *H. hainanensis* sp. nov. revealed 10 different sequence types based on the limited number of specimens collected from the three different hosts and exhibited a low level of sequence variation (nucleotide differences from 0.16-1%), which is far lower than the interspecific sequence differences (greater than 50% nucleotide difference between *H. hainanensis* sp. nov. and *Heliconema* sp.). Therefore, it is reasonable to consider the sequence differences in *H. hainanensis* sp. nov. as intraspecific nucleotide variations, similar to *Heliconema* sp. Moreover, the level of the intraspecific sequence differences in *H. hainanensis* sp. nov. do not appear to relate to any particular group of specimens, such as from different hosts or geographical locations. This result agrees with the morphological observations (i.e., no notable morphological variations were detected between specimens from different hosts or geographical locations) and confirms that all of the nematode material of the morphospecies *H. hainanensis* sp. nov. collected from the three different hosts represents a single species. The results from the present study demonstrate that utilising the ITS sequence of rDNA as a genetic marker is a powerful approach for the accurate identification and distinction of species of *Heliconema*.

Key to species of the genus *Heliconema*

1. Vulva with prominent protruding flap 2
 - Vulva without protruding flap 6
2. Spicule (right:left) ratio of 1:9.1-20.0 3
 - Spicule (right:left) ratio of 1:2.4-4.3 5
3. Paracloacal papillae absent, left spicule of 2.09-3.50 mm *H. brooksi*
 - Paracloacal papillae one-two pairs, left spicule of 4.00-7.30 mm 4
4. Four pairs precloacal papillae, one pair paracloacal papillae; left spicule of 5.40-7.30 mm, right spicule of 0.30-0.35 mm; vulva at approximately the second quarter of the body *H. heliconema*
 - Three pairs precloacal papillae, two pairs paracloacal papillae; left spicule of 4.00 mm, right spicule of 0.27 mm; vulva at approximately the first third of the body *H. savala*
5. Spicule (right:left) ratio averaging 1:2.4
 - *H. brevispiculum*
 - Spicule (right:left) ratio of 1:4.1-4.3
 - *H. psammobatidus*
6. Postcloacal papillae five-seven pairs 7
 - Postcloacal papillae four pairs 9

7. Pseudolabium with oval lateroterminal depression (Fig. 1D), postcloacal papillae seven pairs
 - *H. longissimum*
 - Pseudolabium without oval lateroterminal depression (Fig. 1C), postcloacal papillae five-six pairs 8
8. Spicule distinctively unequal (right:left) ratio of 1:2.20-2.69 (averaging 1:2.42); tail of female with a nipple-like tip *H. hainanensis* sp. nov.
 - Spicule subequal (right:left) ratio of 1:1.11-1.36 (averaging 1:1.21); tail of female is rounded, without a nipple-like tip *H. baylisi*
9. Spicule (right:left) ratio averaging 1:1.7 collected from water snake *H. serpens*
 - Spicule (right:left) ratio averaging 1:2.1 collected from freshwater fish *H. kherai*

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JQ750473 CGGGTCGCTGATCCTCCGCGCTCGCACAGCAGAACAAAAAAGAGGGACGAAAC 1209

JQ750482 TCACTCATGCAATG-TCCGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG-T 1095
JQ750475 TCACTCATGCAATG-TCCGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG-T 1098
JQ750477 TCACTCATGCAATG-TCCGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG-T 1105
JQ750476 TCACTCATGCAATG-TCCGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG-T 1096
JQ750474 TCACTCATGCAATG-TCCGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG-T 1096
JQ750479 TCACTCATGCAATG-TCCGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG-T 1093
JQ750481 TCACTCATGCAATG-TCCGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG-T 1099
JQ750480 TCACTCATGCAATG-TCCGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG-T 1100
JQ750483 TCACTCATGCAATG-TCCGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG-T 1095
JQ750478 TCACTCATGCAATG-TCCGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG-T 1098
JQ750472 CAGTAAAAAAGAAATCAGAGGATAGAG-GAGCACAAGAGG--ACTGCCAAGAAAGT 1261
JQ750473 CAGTAAAAAAGAAATCAGAGGATAGAG-GAGCACAAGAGG--ACTGCCAAGAAAGT 1266

JQ750482 TGCTACTGCGCAGTACTTACCAGTGGAAAGCAGCAGCACTGTGTT-TGA-GACTCA 1153
JQ750475 TGCTACTGCGCAGTACTTACCAGTGGAAAGCAGCAGCACTGTGTT-TGA-GACTCA 1156
JQ750477 TGCTACTGCGCAGTACTTACCAGTGGAAAGCAGCAGCACTGTGTT-TGA-GACTCA 1163
JQ750476 TGCTACTGCGCAGTACTTACCAGTGGAAAGCAGCAGCACTGTGTT-TGA-GACTCA 1154
JQ750474 TGCTACTGCGCAGTACTTACCAGTGGAAAGCAGCAGCACTGTGTT-TGA-GACTCA 1154
JQ750479 TGCTACTGCGCAGTACTTACCAGTGGAAAGCAGCAGCACTGTGTT-TGA-GACTCA 1151
JQ750481 TGCTACTGCGCAGTACTTACCAGTGGAAAGCAGCAGCACTGTGTT-TGA-GACTCA 1157
JQ750480 TGCTACTGCGCAGTACTTACCAGTGGAAAGCAGCAGCACTGTGTT-TGA-GACTCA 1158
JQ750483 TGCTACTGCGCAGTACTTACCAGTGGAAAGCAGCAGCACTGTGTT-TGA-GACTCA 1153
JQ750478 TGCTACTGCGCAGTACTTACCAGTGGAAAGCAGCAGCACTGTGTT-TGA-GACTCA 1156
JQ750472 TGCTAAAG--ATTGTG--CCGCGCTC-----CCGCTCTTCTTCTATGCTGACTCG 1309
JQ750473 TGCTAAAG--ATTGTG--CCGCGCTC-----CCGCTCTTCTTCTATGCTGACTCG 1314

JQ750482 GCTCAGTGTGAGCACCCTGAAATTAAGCATAATACTA 1193
JQ750475 GCTCAGTGTGAGCACCCTGAAATTAAGCATAATACTA 1196
JQ750477 GCTCAGTGTGAGCACCCTGAAATTAAGCATAATACTA 1203
JQ750476 GCTCAGTGTGAGCACCCTGAAATTAAGCATAATACTA 1194
JQ750474 GCTCAGTGTGAGCACCCTGAAATTAAGCATAATACTA 1194
JQ750479 GCTCAGTGTGAGCACCCTGAAATTAAGCATAATACTA 1191
JQ750481 GCTCAGTGTGAGCACCCTGAAATTAAGCATAATACTA 1197
JQ750480 GCTCAGTGTGAGCACCCTGAAATTAAGCATAATACTA 1198
JQ750483 GCTCAGTGTGAGCACCCTGAAATTAAGCATAATACTA 1193
JQ750478 GCTCAGTGTGAGCACCCTGAAATTAAGCATAATACTA 1196
JQ750472 TGTTAGGATAGATACCCGCTAAATTAAGCATAATACTA 1349
JQ750473 TGTTAGGATAGATACCCGCTAAATTAAGCATAATACTA 1354

Alignment of internal transcribed spacer sequences of Heliconia hainanensis sp. nov. (JQ750474-JQ750483) and Heliconia sp. (JQ750472, JQ750473). Numbers to the right of alignment indicate the alignment position and the same nucleotide sites were designated using the asterisks.