

Morphology, genetic characterization and phylogeny of *Moniliformis tupaia* n. sp. (Acanthocephala: Moniliformidae) from the northern tree shrew *Tupaia belangeri chinensis* Anderson (Mammalia: Scandentia)

Hui-Xia Chen<sup>1, 3</sup>, Zhi-Jun Yu<sup>1, 2, 3</sup>, Jun Ma<sup>4</sup>, Cui-Hong Zhao<sup>1</sup>, Fu-Qiong Cao<sup>4</sup>, Liang Li<sup>1, 2</sup>

<sup>1</sup>Hebei Collaborative Innovation Center for Eco-Environment; Hebei Key Laboratory of Animal Physiology, Biochemistry and Molecular Biology; College of Life Sciences, Hebei Normal University, 050024 Shijiazhuang, Hebei Province, P. R. China

<sup>2</sup>Hebei Research Center of the Basic Discipline Cell Biology; Ministry of Education Key Laboratory of Molecular and Cellular Biology; 050024 Shijiazhuang, Hebei Province, P. R. China

<sup>3</sup>Ecological Postdoctoral Research Mobile Station, Hebei Normal University, 050024 Shijiazhuang, Hebei Province, P. R. China

<sup>4</sup>Key Laboratory of Veterinary Public Health of Yunnan Province, College of Veterinary Medicine, Yunnan Agricultural University, Kunming, Yunnan Province 650201, People's Republic of China

**Corresponding author:** Liang Li, E-mail: liangliangex369@126.com

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## Abstract

A new species of *Moniliformis*, *M. tupaia* n. sp. is described using integrated morphological methods (light and scanning electron microscopy) and molecular techniques (sequencing and analyzing the nuclear 18S, ITS, 28S regions and mitochondrial *cox1* and *cox2* genes), based on specimens collected from the intestine of the northern tree shrew *Tupaia belangeri chinensis* Anderson (Scandentia: Tupaiidae) in China. Phylogenetic analyses show that *M. tupaia* n. sp. is a sister to *M. moniliformis* in the genus *Moniliformis*, and also challenges the systematic status of *Nephridiacanthus major*. *Moniliformis tupaia* n. sp. represents the third *Moniliformis* species reported from China.

**Keywords:** Acanthocephala, Morphology, Phylogeny, *Moniliformis*, Tupaiidae

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## Introduction

The northern tree shrew *Tupaia belangeri chinensis* Anderson (Mammalia: Scandentia: Tupaiidae) is a novel ideal animal model for human disease, due to its small size, easy breeding, rapid reproduction and close genetic relationship to primates (Xu *et al.*, 2012; Xiao *et al.*, 2017; Tang *et al.*, 2018; Xu *et al.*, 2013; Wang *et al.*, 2021). *Tupaia belangeri chinensis* is omnivorous, eating fruits, seeds, insects and small vertebrates, which is mainly distributed in southwest China (including Yunnan and Sichuan Provinces) (Xiang & Yang, 2014) and can act as the intermediate and definitive host for some helminth parasites and protozoa (Brack *et al.*, 1987; Tian *et al.*, 1989; Xiang *et al.*, 2010; Xiang & Yang, 2014). However, our present knowledge of the species composition of the acanthocephalans of the northern tree shrew is very limited. To date, only *Prosthenorchis* sp. (Archiacanthocephala: Oligacanthorhynchidae) has been reported from *T. belangeri chinensis* (Tian *et al.*, 1989).

In the present study, some acanthocephalan specimens were collected from *T. belangeri chinensis* in China. In order to accurately identify these acanthocephalan specimens to species level, the detailed morphology of these specimens was studied using light and scanning electron microscopy. Moreover, the nuclear small subunit ribosomal DNA (18S), internal transcribed spacer (ITS) and large subunit ribosomal DNA (28S), and mitochondrial cytochrome c oxidase subunit 1 (*cox1*) and subunit 2 (*cox2*) genes were sequenced and analyzed. Phylogenetic analyses were also performed based on the 18S + *cox1* sequence data using maximum likelihood (ML) and Bayesian inference (BI) methods, in order to clarify the phylogenetic relationships between this species and its congeners.

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## Materials and methods

### *Morphological observation*

Acanthocephalans were isolated from the intestine of the northern tree shrew *T. belangeri chinensis* in Kunming, Yunnan Province, China. Specimens were washed and kept in cold water for several hours until the proboscis everted, and then stored in 80% ethanol until studied. For light microscopical studies, specimens were made in impermanent mount slide and cleared in lactophenol. Photomicrographs were recorded using a Nikon® digital camera coupled to a Nikon® optical microscopy. For scanning electron microscopy (SEM), specimens were post-fixed in 1% OsO<sub>4</sub>, dehydrated via an ethanol series and acetone, and then critical point dried. The specimens were coated with gold and examined using a Hitachi S-4800 scanning electron microscope at an accelerating voltage of 20 Kv. Measurements (range, followed by the mean in parentheses) are given in micrometres unless otherwise stated.

### *Molecular procedures*

Genomic DNA from the mid-body of one male and one female was extracted using a Column Genomic DNA Isolation Kit (Shanghai Sangon, China) according to the manufacturer's instructions. The partial 18S region was amplified by polymerase chain reaction (PCR) using the forward primer (5'-AGATTAAGCCATGCATGCGTAAG-3') and the reverse primer (5'-TGATCCTTCTGCAGGTTCACCTAC-3') (Garey *et al.*, 1996). The partial 28S region was amplified by PCR using four overlapping PCR fragments of 700–800 bp. Primers for

28S amplicon 1 were forward 5'-CAAGTACCGTGAGGGAAAGTTGC-3' and reverse 5'-CAGCTATCCTGAGGGAAAC-3'; amplicon 2, forward 5'-ACCCGAAAGATGGTGAACATG-3' and reverse 5'-CTTCTCCAAC(T/G)TCAGTCTTCAA-3'; amplicon 3, forward 5'-CTAAGGAGTGTGTAACAACCTACC-3' and reverse 5'-AATGACGAGGCATTTGGCTACCTT-3'; amplicon 4, forward 5'-GATCCGTAACCTCGGGAAAAGGAT-3' and reverse 5'-CTTCGCAATGATAGGAAGAGCC-3' (García-Varela & Nadler, 2005). The partial ITS region was amplified by PCR using the forward primer (5'-GTCGTAACAAGGTTTCCGTA-3') and the reverse primer (5'-TATGCTTAAATTCAGCGGGT-3') (Kráľová-Hromadová *et al.*, 2003). The partial *cox1* region was amplified by PCR using the forward primer (5'-GGTCAACAAATCATAAAGATATTGG-3') and the reverse primer (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Gómez *et al.*, 2002). The partial *cox2* region was amplified by PCR using the forward primer (5'-AATGTTTGATGGGTTAGAG-3') and the reverse primer (5'-AACACTGACCATATATAACC-3') (designed by the present study). The cycling conditions were as described previously (Li *et al.*, 2019). PCR products were checked on GoldView-stained 1.5% agarose gels and purified with Column PCR Product Purification Kit (Shanghai Sangon, China). Sequencing for each amplification product was carried out from both directions. Sequences were aligned using ClustalW2 and adjusted manually. The DNA sequences obtained herein were compared (using the algorithm BLASTn) with that available

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in the National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov>).

### *Phylogenetic analyses*

Phylogenetic analyses were performed based on the 18S + *cox1* sequence data using maximum likelihood (ML) inference with IQ-TREE and Bayesian inference (BI) with Mrbayes 3.2 (Ronquist *et al.*, 2012; Nguyen *et al.*, 2015), respectively. *Polyacanthorhynchus caballeroi* Diaz-Ungria & Rodrigo, 1960 (Polyacanthocephala: Polyacanthorhynchida) was treated as the out-group. The in-group included 15 species of the class Archiacanthocephala representing six different genera belonging to three orders Gigantorhynchida, Moniliformida and Oligacanthorhynchida. The detailed information of acanthocephalan species included in the present phylogenetic analyses was provided in Table 1.

We used a built-in function in IQTREE to select a best-fitting substitution model for the sequences according to the Bayesian information criterion (Posada & Crandall, 2001). The GTR+F+I+G4 model was identified as optimal nucleotide substitution model. Reliabilities for ML tree were tested using 1000 bootstrap replications and BI tree was tested using 10 million generations. In the ML tree, bootstrap support (BS) values  $\geq 90$  were considered as fully supported; whereas BS values  $\geq 70$  and  $< 90$  were considered as generally supported. In the BI tree, Bayesian posterior probabilities (BPP)  $\geq 0.90$  were considered as fully supported, whereas BPP values  $\geq 0.70$  and  $< 0.90$  were considered as generally supported.

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## Results

### Description of *Moniliformis tupaia* n. sp. (Figs. 1–3)

*General.* Medium-sized worms with small proboscis (Figs. 1A; 3A, D). Trunk aspinose, nearly cylindrical, slender, showing pseudosegmentation characteristic of the genus *Moniliformis* (Fig. 3A). Anterior trunk tapering to gourd-shaped. Proboscis small compared to the trunk, cylindrical, with two apical sensory pores and 14 spiral longitudinal rows of 7–8 hooks each (Figs. 1A, B, E; 2A, C; 3B). Proboscis hooks small, with simple roots (Figs. 1E, F; 2A–C). Proboscis receptacle double-walled, cerebral ganglion at base of proboscis receptacle (Figs. 1A, B; 3D). Neck short. Lemnisci very long, unequal, distinctly longer than proboscis receptacle (Figs. 1A; 3D). Gonopore terminal in both sexes (Figs. 1C, D; 2D).

**Male** [Based on 5 mature specimens]. Trunk 34.0–47.5 (40.0) mm long, maximum width 1.83–2.07 (1.93) mm. Proboscis 366–439 (395) long, 146–171 (162) wide. Proboscis hooks similar in shape, 27–31 (30), 28–31 (29), 26–29 (28), 24–27 (26), 21–25 (24), 18–23 (21), 18–22 (20), 17–22 (19) in length from anteriorly to posteriorly. Neck 49–100 (68) long, 180–244 (204) wide. Proboscis receptacle 854–1195 (1000) long, 341–390 (368) wide. Shorter lemniscus 5.00–6.10 (5.72) mm long, longer lemniscus 7.68–9.32 (8.61) mm long. Testes two, oval, nearly equal in size; anterior testis 2.44–3.49 (3.05) mm long, 732–1024 (888) wide; posterior testis 2.44–3.54 (2.99) mm long, 585–1000 (849) wide (Fig. 1D). Cement glands eight, ovoid, clustered together; a short distance from posterior testis, 854–1829 (1256) long, 659–854 (761) wide (Figs. 1D, G; 3E). Saeftigen's pouch 927–1512 (1317) long, 293–463 (378) wide. Copulatory bursa evaginable or not everted, 780–1171

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(971) long, 366–854 (644) wide (Figs. 1G, 2E; 3F, G). Gonopore nearly terminal (Fig. 1D, G).

**Female** [Based on 1 mature specimen]. Trunk 41.0 mm long, maximum width 2.15 mm. Proboscis 390 long, 171 wide. Proboscis hooks similar in shape, 28–33 (31), 29–33 (32), 27–31 (29), 26–29 (27), 25–29 (27), 23–26 (24), 22–26 (24), 20–25 (22) in length from anteriorly to posteriorly. Neck 73 long, 195 wide. Proboscis receptacle 927 long, 439 wide. Shorter lemniscus 4.63 mm long, longer lemniscus 8.54 mm long. Uterine bell 350 long, 300 wide. Uterus 680 long, vagina 270 long (Fig. 1C). Eggs ellipsoid, 58–68 (65) × 24–32 (30) in size (n=20) (Figs. 1H; 3C). Gonopore nearly terminal (Figs. 1C, 2D).

**Type-host:** Northern tree shrew *Tupaia belangeri chinensis* Anderson (Scandentia: Tupaiidae).

**Type-locality:** Kunming, Yunnan Province, China.

**Site in host:** Intestine.

**Type specimens:** Holotype, male (HBNU-A-M20231201CL); allotype, female (HBNU-A-M20231202CL); paratypes: 4 males (HBNU-A-M20231203CL); deposited in the College of Life Sciences, Hebei Normal University, Hebei Province, China.

**Etymology:** The species name refers to the generic name of the type host.

### *Molecular characterization*

#### *Partial 18S region*



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Two 18S sequences of *M. tupaia* n. sp. obtained herein are both 1188 bp in length, with no nucleotide divergence detected. In the genus *Moniliformis*, there are six species with their 18S sequences available in GenBank, including *M. cryptosaudi* Amin, Heckmann, Sharifdini & Albayati, 2019 (MH401043), *M. ibunami* Lynggaard, García-Prieto, Guzmán-Cornejo & García-Varela, 2021 (MW136271, MW136272), *M. kalahariensis* Meyer, 1931 (MH401042), *M. moniliformis* (Bremser, 1811) (HQ536017, Z19562), *M. saudi* Amin, Heckmann, Mohammed & Evans, 2016 (KU206782) and *Moniliformis* sp. XH-2020 (OM388438). Pairwise comparison of the 18S sequences of *M. tupaia* n. sp. obtained herein with that of other *Moniliformis* species showed no nucleotide divergence (*Moniliformis* sp. XH-2020) to 0.66% (*M. ibunami*) nucleotide divergence. The 18S sequences of *M. tupaia* n. sp. obtained herein were deposited in GenBank database (<http://www.ncbi.nlm.nih.gov>) (under accession numbers PP002170, PP002171).

#### Partial 28S region

Two 28S sequences of *M. tupaia* n. sp. obtained herein are both 2692 bp in length, with no nucleotide divergence detected. In the genus *Moniliformis*, there are *M. ibunami* (MW136276, MW136277) and *M. moniliformis* (AY829086) with 28S sequences available in GenBank. Pairwise comparison of the 28S sequences of *M. tupaia* n. sp. obtained herein with that of other *Moniliformis* species showed 1.49% (*M. ibunami*) to 2.04% (*M. moniliformis*) nucleotide divergence. The 28S sequences of *M. tupaia* n. sp. obtained herein were deposited in GenBank database (<http://www.ncbi.nlm.nih.gov>) (under accession numbers PP002172, PP002173).

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### Partial ITS region

Two ITS sequences of *M. tupaia* n. sp. obtained herein are both 671 bp in length, with no nucleotide divergence detected. In the genus *Moniliformis*, only *M. moniliformis* (AF416415) has an ITS sequence available in GenBank. Pairwise comparison of the ITS sequences of *M. tupaia* n. sp. obtained herein with that of *M. moniliformis* showed 17.2% nucleotide divergence. The ITS sequences of *M. tupaia* n. sp. obtained herein were deposited in GenBank database (<http://www.ncbi.nlm.nih.gov>) (under accession numbers PP002174, PP002175).

### Partial *cox1* region

Two *cox1* sequences of *M. tupaia* n. sp. obtained herein are both 658 bp in length, with no nucleotide divergence detected. In the genus *Moniliformis*, seven species have their *cox1* sequences available in GenBank, namely *M. cryptosaudi* (MH401041), *M. ibunami* (MW115575, MW115576), *M. kalahariensis* (MH401040), *M. moniliformis* (AF416998), *M. necromysi* Gomes, Costa, Gentile, Vilela & Maldonado, 2020 (MT803593), *M. saudi* (KU206783, OQ078755) and *Moniliformis* sp. XH-2020 (OK415026). Pairwise comparison of the *cox1* sequences of *M. tupaia* n. sp. obtained herein with that of other *Moniliformis* species showed 24.9% (*M. ibunami*) to 27.3% (*M. moniliformis*) nucleotide divergence. The *cox1* sequences of *M. tupaia* n. sp. obtained herein were deposited in GenBank database (<http://www.ncbi.nlm.nih.gov>) (under accession numbers OR997666, OR997667).

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### Partial *cox2* region

Two *cox2* sequences of *M. tupaia* n. sp. obtained herein are both 514 bp in length, with no nucleotide divergence detected. In the genus *Moniliformis*, only *Moniliformis* sp. XH-2020 (OK415026) has a *cox2* sequence available in GenBank. Pairwise comparison of the *cox2* sequences of *M. tupaia* n. sp. obtained herein with that of *Moniliformis* sp. XH-2020 showed 23.6% nucleotide divergence. The *cox2* sequences of *M. tupaia* n. sp. obtained herein were deposited in GenBank database (<http://www.ncbi.nlm.nih.gov>) (under accession numbers PP002935, PP002936).

### Phylogenetic analyses

Phylogenetic trees of the class Archiacanthocephala constructed from the 18S + *cox1* sequence data using ML and BI methods have almost identical topology (Fig. 4). The representatives of Archiacanthocephala were divided into three major clades. Clade I included species of *Macracanthorhynchus*, *Nephridiacanthus*, *Oligacanthorhynchus* and *Oncicola*, representing the order Oligacanthorhynchida. Among them, the phylogenetic results showed *N. major* (Bremser, 1811) clustered together with *M. ingens* (Von Linstow, 1879). Clade II contained species of *Moniliformis*, representing the order Moniliformida. Clade III included species of *Mediorhynchus*, representing the order Gigantorhynchida. In the genus *Moniliformis*, *M. tupaia* n. sp. showed sister relationship with *M. moniliformis*.

### Discussion

The present specimens collected from the northern tree shrew *T. belangeri chinensis* belong

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to the genus *Moniliformis* (Moniliformida: Moniliformidae), due to the pseudosegmented trunk, the very small cylindrical proboscis, the double-walled proboscis receptacle, the very long lemnisci and the presence of eight spherical cement glands (Travassos, 1917; Van Cleave, 1923, 1953; Southwell & Macfie, 1925; Yamaguti, 1963; Schmidt, 1972; Amin, 1987). The genus *Moniliformis* currently comprises 19 species mainly parasitic in mammals (Amin, 2013; Amin *et al.*, 2016, 2019; Martins *et al.*, 2017; Gomes *et al.*, 2020; Lynggaard *et al.*, 2021). Among them, only *M. moniliformis* and *Moniliformis* sp. XH-2020 have been reported in China (Chen, 1933; Chandler, 1941; Dai *et al.*, 2022).

The proboscis of the new species has 14 spiral longitudinal rows of 7–8 simple rooted hooks each, which is similar to the proboscis of following species *M. acomysi* Ward & Nelson, 1967, *M. cryptosaudi*, *M. moniliformis*, *M. saudi* and *M. siciliensis* Meyer, 1932. *Moniliformis tupaia* n. sp. can be easily distinguished from *M. acomysi* by its much longer proboscis and lemnisci (proboscis 0.37–0.44 mm and lemnisci 5.00–9.32 mm long in the male of new species vs proboscis 0.19–0.36 mm and lemnisci 2.73–4.42 mm long in the male of *M. acomysi*). *Moniliformis tupaia* n. sp. differs from *M. cryptosaudi* and *M. saudi* by having larger cement glands (854–1829 long in the new species vs 312–811 long in the latter two species). Moreover, *M. cryptosaudi* and *M. saudi* are both parasitic in hedgehogs (Erinaceomorpha: Erinaceidae) in Saudi Arabia and Iraq, but the new species parasitizes the northern tree shrew *T. belangeri chinensis* in China. Furthermore, molecular analysis revealed strong genetic divergence (25.9–26.9% difference in nucleotides in the *cox1* region) between the new species and *M. cryptosaudi* and *M. saudi*. *Moniliformis siciliensis* is a poorly known acanthocephalan species only reported from the garden dormouse *Eliomys*

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*quercinus* Linnaeus (Mammalia: Rodentia) in the Italian island of Sicily (Meyer, 1932; Petrochenko, 1958). The new species differs from *M. siciliensis* in having shorter lemnisci (5.00–9.32 mm long in the former vs about 10.0 mm in the latter) and different localities and hosts.

*Moniliformis moniliformis* is an important zoonotic acanthocephan species, parasitizing rodents, canines and felines worldwide, including China (Meyer, 1932; Petrotschenko, 1958; Yamaguti, 1963; Ward & Nelson, 1967; Bhattacharya, 2007; Naidu, 2012). This species has a proboscis with 11–14 (usually 12) rows of 9–14 (usually 10–11) hooks each and much larger trunk (over 50.0 mm long in male), which is different from the new species (vs proboscis with 14 rows of 7–8 hooks each, and male 34.0–47.5 mm long in *M. tupaia* n. sp.). Additionally, molecular analysis displayed 27.3% and 17.2% nucleotide divergence in the *cox1* and ITS regions, between the new species and *M. moniliformis*, which strongly indicated that they represent two distinct species. Dai *et al.* (2022) reported *Moniliformis* sp. XH-2020 from the plateau zokor (*Eospalax fontanierii baileyi*) in China, but they only provided the mitochondrial genomic data of their specimens (they did not describe the morphology). Pairwise comparison between *M. tupaia* n. sp. and *Moniliformis* sp. XH-2020 showed 27.3% and 23.6% nucleotide divergence in the *cox1* and *cox2* regions. Consequently, they belong to different species.

The class Archiacanthocephala currently includes four orders, namely Gigantorhynchida, Moniliformida, Oligacanthorhynchida and Apororhynchida (Amin, 2013). However, the phylogenetic relationships of the four orders remain unclear, due to a lack of genetic data of some taxa, especially the order Apororhynchida. The previous phylogenetic study using 18S

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or 18S + *cox1* genetic data suggested a close affinity between Moniliformida and Gigantorhynchida (Amin *et al.*, 2013, 2020). However, our phylogenetic results based on the 18S + *cox1* data suggested Moniliformida is a sister to Oligacanthorhynchida, rather than Gigantorhynchida, which are consistent with some previous studies based on *cox1* or 18S data (Gomes *et al.*, 2020; Rodríguez *et al.*, 2021; Amin *et al.*, 2021, 2022). In the order Oligacanthorhynchida, the present phylogeny displayed *Nephridiacanthus major* nested in representatives of *Macracanthorhynchus* (clustered together with *M. ingens*), which challenged the current systematic position of this species. The present results agreed well with the previous phylogenetic study based on *cox1* data (Rodríguez *et al.*, 2021). In the genus *Moniliformis*, our molecular phylogenetic analyses indicate that *M. tupaia* n. sp. is a sister to *M. moniliformis*.

**Data availability.** The nuclear and mitochondrial DNA sequences of *M. tupaia* n. sp. obtained herein were deposited in the GenBank database [<http://www.ncbi.nlm.nih.gov>, accession numbers: PP002170, PP002171 (18S); PP002172, PP002173 (28S); PP002174, PP002175 (ITS); OR997666, OR997667 (*cox1*); PP002935, PP002936 (*cox2*)]. Type specimens of *M. tupaia* n. sp. (HBNU-A-M20231201-3CL) were deposited in the College of Life Sciences, Hebei Normal University, Hebei Province, China.

**Authors' contributions.** HXC and LL contributed to the study design and identification of the acanthocephalan specimens. HXC, CHZ and LL sequenced and analyzed genetic data. HXC, ZJY and LL conducted the phylogenetic analyses. JM and FQC provided

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acanthocephalan specimens. HXC and LL wrote the manuscript. All authors read and approved the final manuscript.

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**Competing interests.** The authors declare that they have no conflict of interest.

**Ethical standards.** This study was conducted under the protocol of Hebei Normal University. All applicable national and international guidelines for the protection and use of animals were followed.

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## References

- Amin OM** (1987) Key to the families and subfamilies of Acanthocephala with the erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida). *Journal of Parasitology* **73**, 1216–1219.
- Amin OM** (2013) Classification of the Acanthocephala. *Folia Parasitologica* **60**, 273–305.
- Amin OM, Evans P, Heckmann RA and El-Naggar AM** (2013) The description of *Mediorhynchus africanus* n. sp. (Acanthocephala: Gigantorhynchidae) from galliform birds in Africa. *Parasitology Research* **112**, 2897–2906.
- Amin OM, Heckmann RA, Mohammed O and Evans RP** (2016) Morphological and molecular descriptions of *Moniliformis saudi* sp. n. (Acanthocephala: Moniliformidae) from the desert hedgehog, *Paraechinus aethiopicus* (Ehrenberg) in Saudi Arabia, with a key to species and notes on histopathology. *Folia Parasitologica* **63**, e014.
- Amin OM, Heckmann RA, Sharifdini M and Albayati NY** (2019) *Moniliformis cryptosaudi* n. sp. (Acanthocephala: Moniliformidae) from the long-eared hedgehog *Hemiechinus auritus* (Gmelin) (Erinaceidae) in Iraq; a case of incipient cryptic speciation related to *M. saudi* in Saudi Arabia. *Acta Parasitologica* **64**, 195–204.
- Amin OM, Sharifdini M, Heckmann RA and Zarean M** (2020) New perspectives on *Nephridiacanthus major* (Acanthocephala: Oligacanthorhynchidae) collected from hedgehogs in Iran. *Journal of Helminthology* **94**, e133.
- Amin OM, Heckmann RA, Sist B and Basso WU** (2021) A review of the parasite fauna of the black-bellied pangolin, *Phataginus tetradactyla* LIN. (Manidae), from central Africa with the description of *Intraproboscis sanghae* n. gen., n. sp. (Acanthocephala:



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Gigantorhynchidae). *Journal of Parasitology* **107**, 222–238.

**Amin OM, Chaudhary A, Heckmann RA, Swenson J and Singh HS** (2022) Redescription and molecular characterization of *Pachysentis canicola* Meyer, 1931 (Acanthocephala: Oligacanthorhynchidae) from the maned wolf, *Chrysocyon brachyurus* (Illiger, 1815) in Texas. *Acta Parasitologica* **67**, 275–287.

**Bhattacharya SB** (2007) Handbook on Indian Acanthocephala. *Zoological Survey of India*, 1–225.

**Brack M, Naberhaus F and Heymann E** (1987) *Tupaia taenia quentini* (Schmidt & File, 1977) in *Tupaia belangeri* (Wagner, 1841): transmission experiments and Praziquantel treatment. *Laboratory Animals* **21**, 18–19.

**Chandler AC** (1941) The specific status of *Moniliformis* (Acanthocephala) of Texas rats, and a review of the species of this genus in the western hemisphere. *Journal of Parasitology* **27**, 241–244.

**Chen HT** (1933) A preliminary report on a survey of animal parasites of Canton, China, rats. *Lingnan Science Journal* **12**, 65–74.

**Dai GD, Yan HB, Li L, Zhang LS, Liu ZL, Gao SZ, Ohiolei JA, Wu YD, Guo AM, Fu BQ and Jia WZ** (2022) Molecular characterization of a new *Moniliformis* sp. from a plateau zokor (*Eospalax fontanierii baileyi*) in China. *Frontiers in Microbiology* **13**, e806882.

**Dessi G, Cabras P, Mehmood N, Ahmed F, Porcu F, Veneziano V, Burrai GP, Tamponi C, Scala A and Varcasia A** (2022) First molecular description of *Macracanthorhynchus hirudinaceus* in wild boars from Italy with pathomorphological

- 
- and epidemiological insights. *Parasitology Research* **121**, 197–204.
- García-Varela M and Nadler SA** (2005) Phylogenetic relationships of Palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences. *Journal of Parasitology* **91**, 1401–1409.
- García-Varela M and Nadler SA** (2006) Phylogenetic relationships among Syndermata inferred from nuclear and mitochondrial gene sequences. *Molecular Phylogenetics and Evolution* **40**, 61–72.
- García-Varela M, Pérez-Ponce de León G, de la Torre P, Cummings MP, Sarma SS and Laclette JP** (2000) Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. *Journal of Molecular Evolution* **50**, 532–540.
- García-Varela M, Cummings MP, Pérez-Ponce de León G, Gardner SL and Laclette JP** (2002) Phylogenetic analysis based on 18S ribosomal RNA gene sequences supports the existence of class polyacanthocephala (acanthocephala). *Molecular Phylogenetics and Evolution* **23**, 288–292.
- Garey JR, Near TJ, Nonnemacher MR and Nadler SA** (1996) Molecular evidence for acanthocephala as a subtaxon of Rotifera. *Journal of Molecular Evolution* **43**, 287–292.
- Gomes APN, Costa NA, Gentile R, Vilela RV and Maldonado A** (2020) Morphological and genetic description of *Moniliformis necromysi* sp. n. (Archiacanthocephala) from the wild rodent *Necromys lasiurus* (Cricetidae: Sigmondontinae) in Brazil. *Journal of Helminthology* **94**, e138.
- Gómez A, Serra M, Carvalho GR and Lunt DH** (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis*

---

(Rotifera). *Evolution* **56**, 1431–1444.

**Kamimura K, Yonemitsu K, Maeda K, Sakaguchi S, Setsuda A, Varcasia A and Sato H**

(2018) An unexpected case of a Japanese wild boar (*Sus scrofa leucomystax*) infected with the giant thorny-headed worm (*Macracanthorhynchus hirudinaceus*) on the mainland of Japan (Honshu). *Parasitology Research* **117**, 2315–2322.

**Král'ová-Hromadová I, Tietz DF, Shinn AP and Spakulová M** (2003) ITS rDNA

sequences of *Pomphorhynchus laevis* (Zoega in Müller, 1776) and *P. lueyi* Williams and Rogers, 1984 (acanthocephala: Palaeacanthocephala). *Systematic Parasitology* **56**, 141–145.

**Li L, Wayland MT, Chen HX and Yang Y** (2019) Remarkable morphological variation in

the proboscis of *Neorhadiorhynchus nudus* (Harada, 1938) (acanthocephala: Echinorhynchida). *Parasitology* **146**, 348–355.

**López-Caballero J, Mata-López R, García-Varela M and Pérez-Ponce de León G** (2015)

Genetic variation of *Oligacanthorhynchus microcephalus* (Acanthocephala: Archiacanthocephala: Oligacanthorhynchidae), parasite of three species of opossums (Mammalia: Didelphidae) across central and southeastern Mexico. *Comparative Parasitology* **82**, 175–186.

**Lynggaard C, García-Prieto L, Guzmán-Cornejo C and García-Varela M** (2021)

Description of a new species of *Moniliformis* (Acanthocephala: Moniliformidae) from *Peromyscus hylocetes* (Rodentia: Cricetidae) in Mexico. *Parasitology International* **83**, e102315.

**Martins NB, Del Rosario Robles M and Navone GT** (2017) A new species of *Moniliformis*

- 
- from a Sigmodontinae rodent in Patagonia (Argentina). *Parasitology Research* **116**, 2091–2099.
- Meyer A** (1932) Acanthocephala. *Bronn's Klassen und Ordnungen des Tierreichs* **4**, 1–332.
- Naidu KV** (2012) Fauna of India and the adjacent countries. Acanthocephala. *Zoological Survey of India, Kolkata*, 1–638.
- Near TJ, Garey JR and Nadler SA** (1998) Phylogenetic relationships of the Acanthocephala inferred from 18S ribosomal DNA sequences. *Molecular Phylogenetics and Evolution* **10**, 287–298.
- Nguyen LT, Schmidt HA, Haeseler A and Minh BQ** (2015) IQ-tree: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32**, 268–274.
- Petrochenko VI** (1958) Acanthocephala of domestic and wild animals, vol. 2. *Izdatel'stvo Akademy Nauk SSSR, Moscow*, 1–478.
- Posada D and Crandall KA** (2001) Selecting the best-fit model of nucleotide substitution. *Systematic Biology* **50**, 580–601.
- Richardson DJ, Hammond CI and Richardson JE** (2016) The florida ivory millipede, *Chicobolus spinigerus* (Diplopoda: Spirobolidae): a natural intermediate host of *Macracanthorhynchus ingens* (Acanthocephala: Oligacanthorhynchidae). *Southeastern Naturalist* **15**, 7–11.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP** (2012) Mrbayes 3.2: efficient Bayesian phylogenetic inference and model choice across across a large model space. *Systematic*

---

*Biology* **61**, 539–542.

**Rodríguez SM, Amin OM, Heckmann RA, Sharifdini M and D'Elia G** (2021) Phylogeny and life cycles of the Archiacanthocephala with a note on the validity of *Mediorhynchus gallinarum*. *Acta Parasitologica* **67**, 369–379.

**Schmidt GD** (1972) Revision of the class Archiacanthocephala Meyer, 1931 (Phylum Acanthocephala), with emphasis on Oliganthorhynchidae Southwell et Macfie, 1925. *Journal of Parasitology* **58**, 290–297.

**Southwell T and Macfie JWS** (1925) On a collection of Acanthocephala in the Liverpool School of Tropical Medicine. *Annals Tropical Medicine Parasitology* **19**, 141–284.

**Tang B, Wu T, Xiao SF, Ge JY, Wei D, Li CM, Wang QH, Zeng W, Fu BM and Zhang J** (2018) Using tree shrews (*Tupaia belangeri*) as a novel animal model of liver transplantation. *Current Medical Science* **38**, 1069–1074.

**Telford MJ and Holland PW** (1993) The phylogenetic affinities of the chaetognaths: a molecular analysis. *Molecular Biology and Evolution* **10**, 660–676.

**Tian BP, Pen YZ and Hou YD** (1989) Investigation of parasites on Chinese tree shrews. *Zoological Research* **10**, 90, 110.

**Travassos L** (1917) Contribuição para o conhecimento da fauna helmintológica brasileira, XVII. Revisão dos acantocéfalos brasileiros. Parte I. Fam. Gigantorhynchidae Hamann, 1982. *Memórias do Instituto Oswaldo Cruz* **9**, 5–62.

**Van Cleave HJ** (1923) A key to the genera of Acanthocephala. *Transactions of the American Microscopical Society* **12**, 184–191.

**Van Cleave HJ** (1953) Acanthocephala of North American mammals. *Illinois Biological*

---

*Monographs* **23**, 1–79.

**Ward HL and Nelson DR** (1967) Acanthocephala of the genus *Moniliformis* from rodents of Egypt with the description of a new species from the Egyptian spiny mouse (*Acomys cahirinus*), *Journal of Parasitology* **53**, 150–156.

**Xiang Z, Rosenthal BM, He YS, Wang WL, Wang H, Song JL, Shen PQ, Li ML and Yang ZQ** (2010) *Sarcocystis tupaia*, sp. nov., a new parasite species employing treeshrews (Tupaiaidae, *Tupaia belangeri chinensis*) as natural intermediate hosts. *Parasitology International* **59**, 128–132.

**Xiang Z and Yang ZQ** (2014) Advances in tree shrews parasite. *Chinese Journal of Zoonoses* **30**, 955–959.

**Xiao J, Liu R and Chen CS** (2017) Tree shrew (*Tupaia belangeri*) as a novel laboratory disease animal model. *Zoological Research* **38**, 127–137.

**Wang X, Ruan LY, Song QK, Wang WG, Tong PF, Kuang DX, Lu CX, Li N, Han YY, Dai JJ and Sun XM** (2021) First report of *Schistosoma sinensium* infecting *Tupaia belangeri* and *Tricola* sp. LF. *International Journal for Parasitology: Parasites and Wildlife* **14**, 84–90.

**Xu L, Chen SY, Nie WH, Jiang XL and Yao YG** (2012) Evaluating the phylogenetic position of Chinese tree shrew (*Tupaia belangeri chinensis*) based on complete mitochondrial genome: implication for using tree shrew as an alternative experimental animal to primates in biomedical research. *Journal of Genetics and Genomics* **39**, 131–137. (in Chinese)

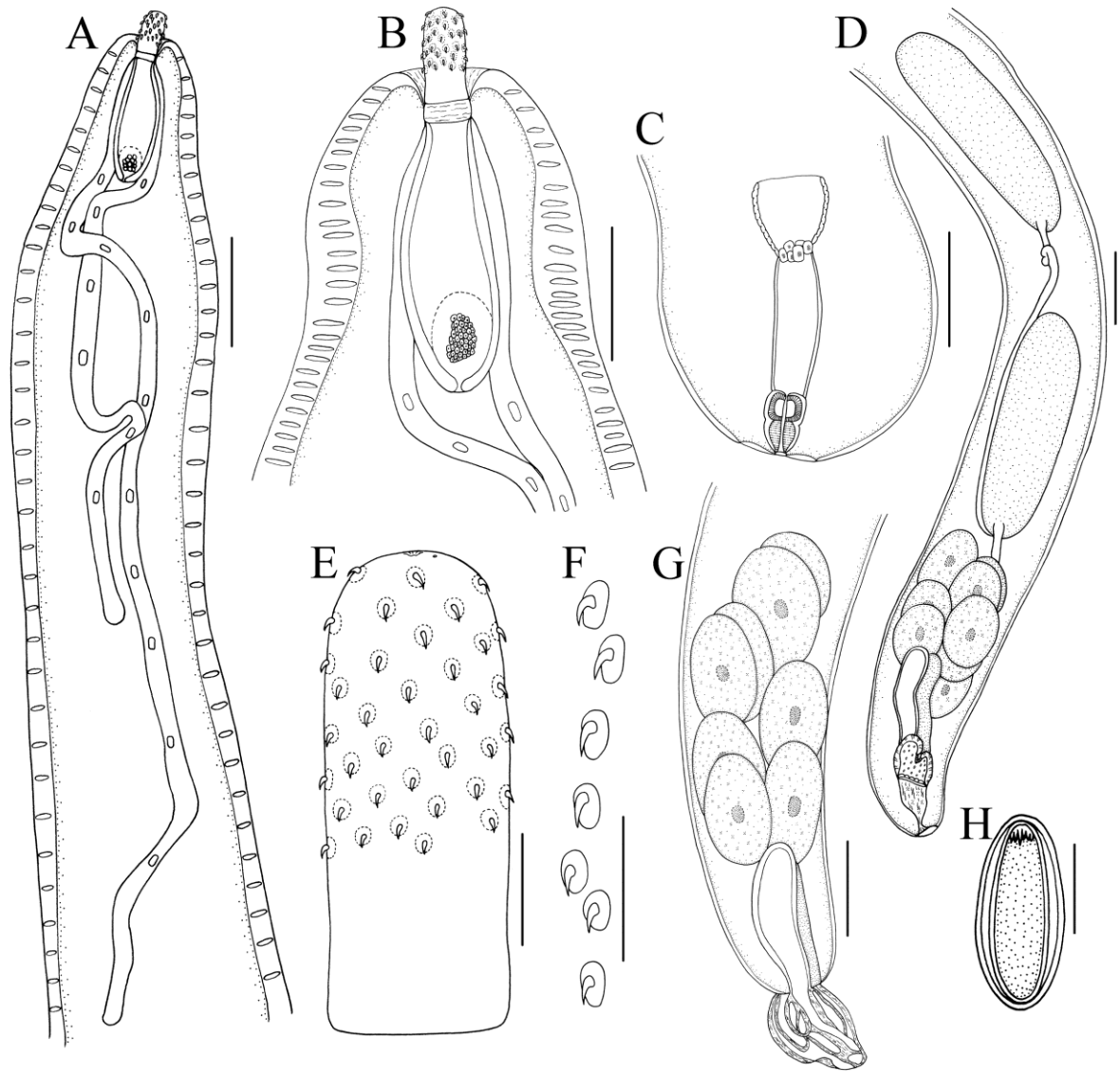
**Xu L, Zhang Y, Liang B, Lü LB, Chen CS, Chen YB, Zhou JM and Yao YG** (2013) Tree

---

shrews under the spot light: emerging model of human diseases. *Zoological Research* **34**, 59–69. (in Chinese)

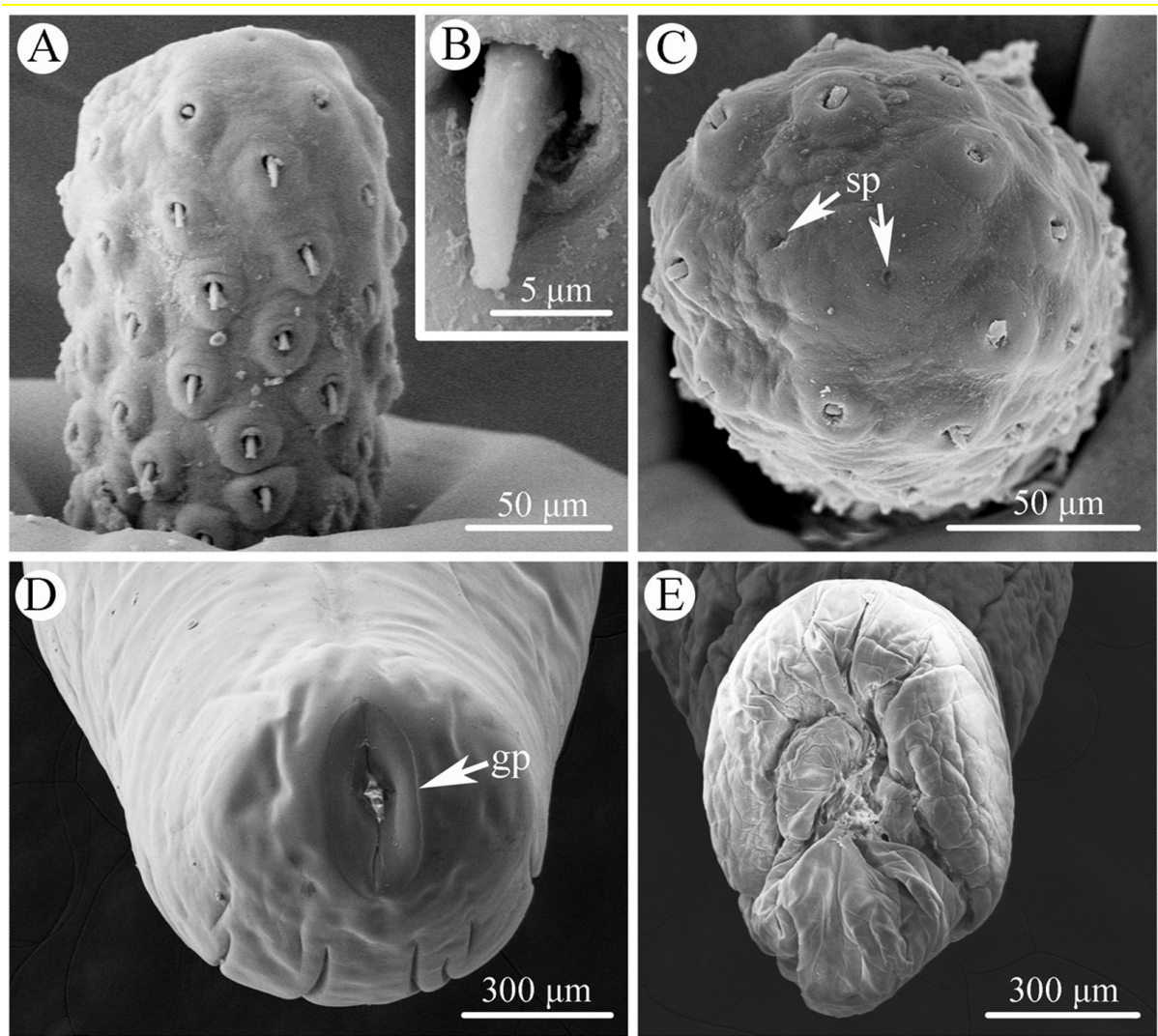
**Yamaguti S** (1963) *Systema Helminthum*. Vol. V. Acanthocephala. New York, John Wiley and Sons Interscience Publishers, 1–423.

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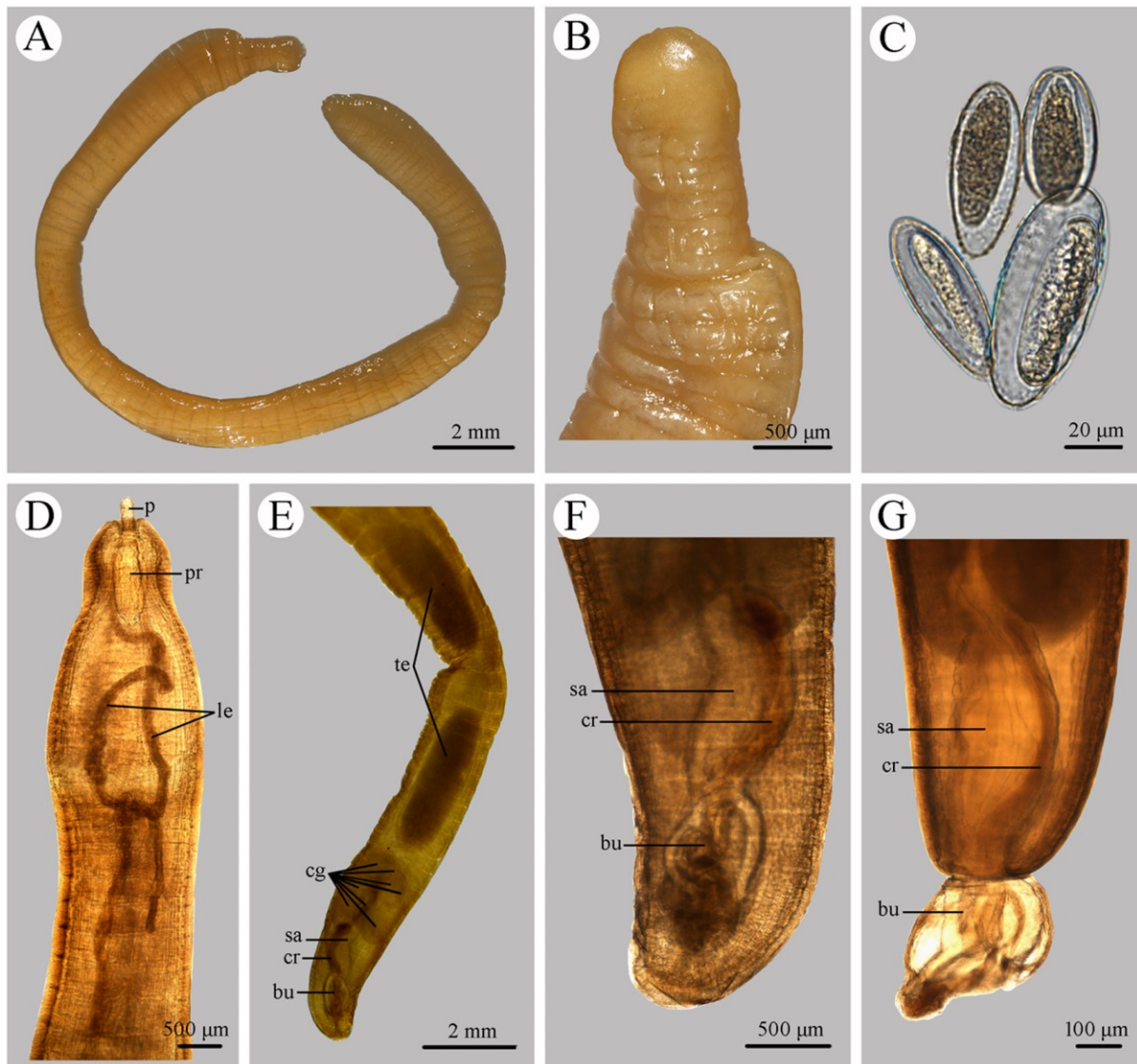


**Figure 1.** *Moniliformis tupaia* n. sp. collected from *Tupaia belangeri chinensis* (Scandentia: Tupaiidae) in China. (A) anterior part of male. (B) anterior end of male. (C) posterior end of female. (D) posterior part of male. (E) proboscis. (F) longitudinal row of hooks. (G) posterior end of male. (H) egg. *Scale bars:* A, D, G = 1000  $\mu\text{m}$ ; B, C = 500  $\mu\text{m}$ ; E, F = 100  $\mu\text{m}$ ; H = 30  $\mu\text{m}$ .

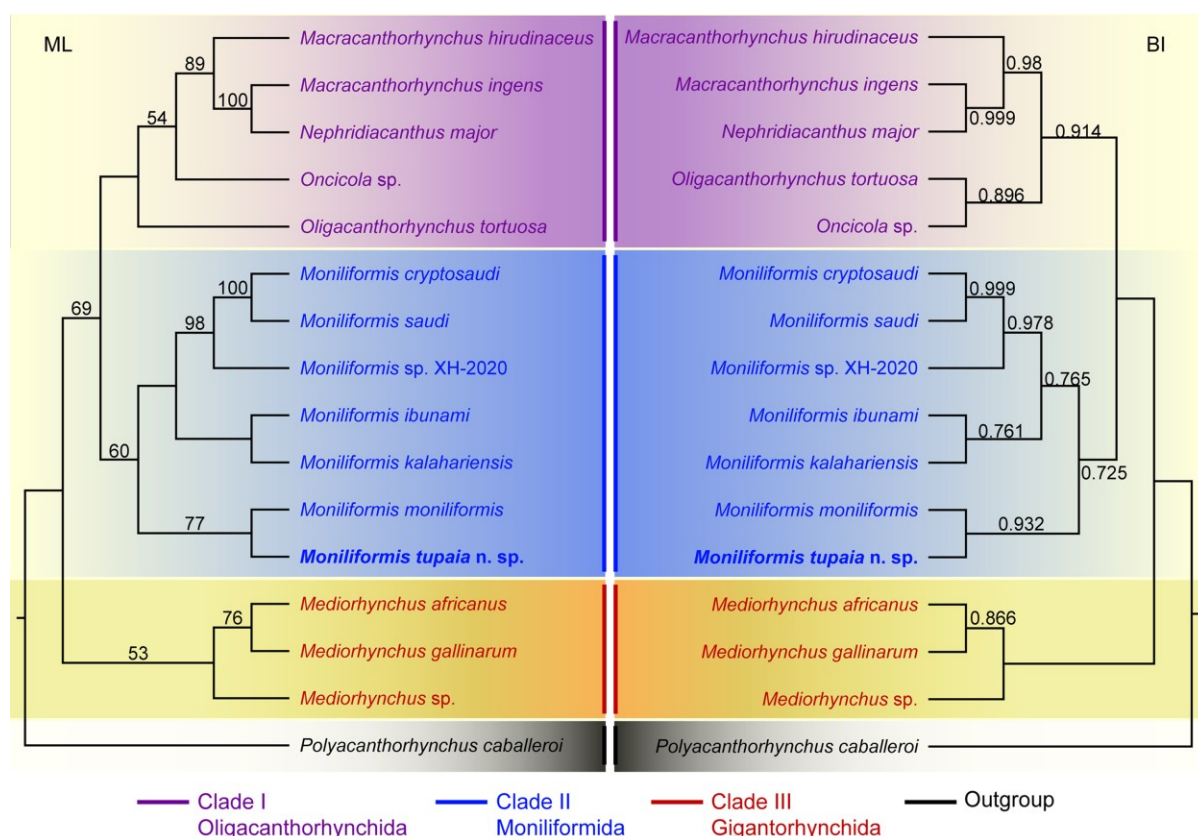




**Figure 2.** Scanning electron micrographs of *Moniliformis tupaia* n. sp. collected from *Tupaia belangeri chinensis* (Scandentia: Tupaiidae) in China. (A) proboscis of male, lateral view. (B) magnified image of proboscis hook. (C) proboscis of male (sensory pores arrowed), apical view. (D) posterior end of female (gonopore arrowed). (E) copulatory bursa. *Abbreviations:* sp, sensory pores; gp, gonopore.



**Figure 3.** Photomicrographs of *Moniliformis tupaia* n. sp. collected from *Tupaia belangeri chinensis* (Scandentia: Tupaiidae) in China. (A) mature female. (B) proboscis of female. (C) eggs. (D) anterior part of male. (E) posterior part of male. (F) posterior part of male (copulatory bursa not everted). (G) posterior part of male (copulatory bursa evaginable).  
*Abbreviations:* bu, bursa; cg, cement glands; cr, cement reservoir; le, lemniscs; p, proboscis; pr, proboscis receptacle; sa, saeftigen's pouch; te, testis.



**Figure 4.** Maximum likelihood (ML) inference and Bayesian inference (BI) based on the 18S + *cox1* sequence data showing the phylogenetic relationships of representatives of Archiacanthocephala. *Polyacanthorhynchus caballeroi* (Polyacanthocephala: Polyacanthorhynchidae) was chosen as the outgroup. Bootstrap support (BS) values  $\geq 50$  in ML tree and Bayesian posterior probabilities (BPP)  $\geq 0.70$  in BI tree are shown.

**Table 1.** Species of Archiacanthocephala with their detailed information of genetic data included in the phylogenetic analyses

Species	Host	Locality	GenBank ID for 18S region	GenBank ID for <i>cox1</i> region	References
<b>Ingroup</b>					
<b>Order Gigantorhynchida</b>					
<b>Family Gigantorhynchidae</b>					
<i>Mediorhynchus africanus</i>	<i>Numida meliagriss</i> (Aves: Galliformes)	Africa	KC261353	KC261351	Amin <i>et al.</i> , 2013
<i>Mediorhynchus gallinarum</i>	Chickens (Aves: Galliformes)	Indonesia	KC261354	KC261352	Amin <i>et al.</i> , 2013
<i>Mediorhynchus</i> sp.	<i>Cassidix mexicanus</i> (Aves: Passeriformes)	N/A	AF064816	AF416996	García-Varela <i>et al.</i> , 2000, 2002
<b>Order Moniliformida</b>					
<b>Family Moniliformidae</b>					
<i>Moniliformis cryptosaudi</i>	<i>Hemiechinus auratus</i> (Mammalia: Erinaceomorpha)	Iraq	MH401043	MH401041	Amin <i>et al.</i> , 2019
<i>Moniliformis ibunami</i>	<i>Peromyscus hylocetes</i> (Mammalia: Rodentia)	Mexico	MW136271	MW115576	Lynggaard <i>et al.</i> , 2021
<i>Moniliformis kalahariensis</i>	<i>Atelerix frontalis</i> (Mammalia: Erinaceomorpha)	South Africa	MH401042	MH401040	Amin <i>et al.</i> , 2019
<i>Moniliformis moniliformis</i>	<i>Rattus norvegicus</i> (Mammalia: Rodentia); N/A	England; N/A	Z19562	AF416998	Telford & Holland, 1993; García-Varela <i>et al.</i> , 2002
<i>Moniliformis saudi</i>	<i>Paraechinus aethiopicus</i> (Mammalia: Erinaceomorpha)	Saudi Arabia	KU206782	KU206783	Amin <i>et al.</i> , 2016
<i>Moniliformis</i> sp. XH-2020	<i>Eospalax fontanierii baileyi</i> (Mammalia: Rodentia)	China	OM388438	OK415026	Dai <i>et al.</i> , 2022
<i>Moniliformis tupaia</i> n. sp.	<i>Tupaia belangeri</i> (Mammalia: Scandentia)	China	PP002170	OR997666	This study
<b>Order Oligacanthorhynchida</b>					

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**Family Oligacanthorhynchidae**

<i>Macracanthorhynchus hirudinaceus</i>	<i>Sus scrofa leucomystax</i> (Mammalia: Artiodactyla); <i>S. scrofa meridionalis</i> (Mammalia: Artiodactyla)	Japan; Italy	LC350000	MZ683370	Kamimura <i>et al.</i> , 2018; Dessì <i>et al.</i> , 2022
<i>Macracanthorhynchus ingens</i>	N/A; <i>Procyon lotor</i> (Mammalia: Carnivora)	N/A; USA	AF001844	KT881244	Near <i>et al.</i> , 1998; Richardson <i>et al.</i> , 2016
<i>Nephridiacanthus major</i>	<i>Hemiechinus auratus</i> (Mammalia: Erinaceomorpha)	Iran	MN612079	MN612080	Amin <i>et al.</i> , 2020
<i>Oligacanthorhynchus tortuosa</i>	<i>Didelphis virginiana</i> (Mammalia: Didelphimorphia)	N/A; Mexico	AF064817	KM659378	García-Varela <i>et al.</i> , 2000; López-Caballero <i>et al.</i> , 2015
<i>Oncicola</i> sp.	<i>Nasua narica</i> (Mammalia: Carnivora); N/A	N/A	AF064818	AF417000	García-Varela <i>et al.</i> , 2000, 2002

**Outgroup**
**Class Polyacanthocephala**
**Order Polyacanthorhynchida**
**Family Polyacanthorhynchidae**

<i>Polyacanthorhynchus caballeroi</i>	<i>Caiman yacare</i> (Reptilia: Crocodylia)	Bolivia	AF388660	DQ089724	García-Varela <i>et al.</i> , 2002; García-Varela & Nadler, 2006
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