Description of a new species of *Aplectana* (Nematoda: Ascaridomorpha: Cosmocercidae) using an integrative approach and preliminary phylogenetic study of Cosmocercidae and related taxa

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**Abstract**

**Background:** Nematodes of the family Cosmocercidae (Ascaridomorpha: Cosmocercoidea) are mainly parasitic in the digestive tract of various amphibians and reptiles worldwide. However, our knowledge of the molecular phylogeny of the Cosmocercidae is still far from comprehensive. The phylogenetic relationships between Cosmocercidae and the other two families, Atractidae and Kathlaniidae, in the superfamily Cosmocercoidea are still under debate. Moreover, the systematic position of some genera within Cosmocercidae remains unclear.

**Methods:** Nematodes collected from *Polypedates megacephalus* (Hallowell) (Anura: Rhacophoridae) were identified using morphological (light and scanning electron microscopy) and molecular methods [sequencing the small ribosomal DNA (18S), internal transcribed spacer 1 (ITS-1), large ribosomal DNA (28S) and mitochondrial cytochrome c oxidase subunit 1 (cox1) target regions]. Phylogenetic analyses of cosmocercoid nematodes using 18S + 28S sequence data were performed to clarify the phylogenetic relationships of the Cosmocercidae, Atractidae and Kathlaniidae in the Cosmocercoidea and the systematic position of the genus *Aplectana* in Cosmocercidae.

**Results:** Morphological and genetic evidence supported the hypothesis that the nematode specimens collected from *P. megacephalus* represent a new species of *Aplectana* (Cosmocercoidea: Cosmocercidae). Our phylogenetic results revealed that the Cosmocercidae is a monophyletic group, but not the basal group in Cosmocercoidea as in the traditional classification. The Kathlaniidae is a paraphyletic group because the subfamily Cruzinae within Kathlaniidae (including only the genus *Cruzia*) formed a separate lineage. Phylogenetic analyses also showed that the genus *Aplectana* has a closer relationship to the genus *Cosmocerca* in Cosmocercidae.

**Conclusions:** Our phylogenetic results suggested that the subfamily Cruzinae should be moved from the hitherto-defined family Kathlaniidae and elevated as a separate family, and the genus *Cosmocerca* is closely related to the genus *Aplectana* in the family Cosmocercidae. The present study provided a basic molecular phylogenetic framework...
Background

The superfamily Cosmoceroidea is a group of zooparasitic nematodes and currently comprises three families, namely, Atractidae Railliet, 1917, Cosmocercidae Railliet, 1916, and Kathlaniidae Lane, 1914 [1–3]. Among them, Cosmocercidae is the largest family, including approximately 200 nominal species, which are mainly parasitic in the digestive tract of various amphibians and reptiles worldwide [4–6]. The evolutionary relationships of the Cosmocercidae and the other two families are not yet resolved. Based on morphological and ecological traits, some previous studies [1, 6, 7] considered that the Cosmocercidae represents the ancestral group in Cosmoceroidea.

The present knowledge of the molecular phylogeny of Cosmoceroidea/Cosmocercidae is still very limited. To date, several studies [8–11] have provided molecular phylogenetic analyses to solve the systematic status of some genera in Cosmoceroidea using different genetic data. However, due to the paucity and inaccessibility of some genera in Cosmocercidae using different genetic phylogenetic analyses to solve the systematic status of these taxa.

To clarify the phylogenetic relationships of the Cosmocercidae and the other families Atractidae and Kathlaniidae in Cosmoceroidea, and the systematic position of the genus *Aplectana* in Cosmocercidae, phylogenetic analyses including the most comprehensive taxon sampling of Cosmoceroidea to date were performed using maximum likelihood (ML) inference and Bayesian inference (BI) based on 18S+28S sequence data. Moreover, a new species of *Aplectana* was described using an integrative approach.

Methods

Parasite collection

A total of 91 *Polypedates megacephalus* (Hallowell) (Anura: Rhacophoridae) collected in the XiShuangBANNa Tropical Botanical Garden, Yunnan Province, China, were investigated for nematode parasites. Nematode specimens were isolated from the intestine of this host and then fixed and stored in 80% ethanol until study.

Morphological observations

For light microscopical studies, nematodes were cleared in lactophenol. Drawings were made using a Nikon microscope drawing attachment. For scanning electron microscopy (SEM), the anterior and posterior ends of nematodes were re-fixed in 4% formaldehyde solution, post-fixed in 1% OsO₄, dehydrated via an ethanol series and acetone, and then critical point dried. Samples were coated with gold and examined using a Hitachi S-4800 scanning electron microscope at an accelerating voltage of 20 kV. Measurements (the range, followed by the mean in parentheses) are given in micrometers (μm) unless otherwise stated. Type specimens were deposited in the College of Life Sciences, Hebei Normal University, Hebei Province, PR. China.

Molecular procedures

Genomic DNA from each sample 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 18S-F (5′-CGC GAATRGCTCATTACAACAGC-3′) and the reverse primer 18S-R (5′-GGGCGGTATCTGATCGCC-3′) [12]. The partial 28S region of nuclear rDNA was amplified by PCR using the forward primer 28S-F (5′-AGCCGA GGAAAGAAAACTAA-3′) and the reverse primer 28S-R (5′-ATCCGTGT TTCAAGACGGG-3′) [13]. The ITS-1 region of nuclear rDNA was amplified by PCR using the forward primer SS1 (5′-TTTCCGTAGGTAACCT GCG-3′) and the reverse primer SS2R (5′-AGTGCT CAATGTGTCGCA-3′) [14]. The partial cox1 region was amplified by PCR using the forward primer COIF (5′-TTTGGTCCATCTTCGTTTAT-3′) and the reverse primer COIR (5′-ACATAATGAAAATGACTA ACAAC-3′) [15]. The cycling conditions were described by the previous study [9]. PCR products were checked on GoldView-stained 1.5% agarose gels and purified with the Column PCR Product Purification Kit (Shanghai Sangon, China). Sequencing was carried out using a DyeDeoxy Terminator Cycle Sequencing Kit (v.2, Applied Biosystems, Foster City, CA, USA) and an automated sequencer (ABI-PRISM 377). Sequencing of each sample was carried out on both strands. Sequences were aligned using ClustalW2. The DNA sequences obtained herein were deposited in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov) and compared (using the BLASTn algorithm) with those available in the GenBank database.

for the superfamily Cosmoceroidea based on 18S+28S sequence data for the first time to our knowledge. Moreover, a new species, *A. xishuangbannaensis* n. sp., was described using integrative approach.

Keywords: Nematoda, Ascaridomorpha, Systematics, Genetic data, Molecular phylogeny, New species
Phylogenetic analyses

Phylogenetic trees were constructed based on the 18S+28S sequence data using maximum likelihood (ML) in IQ-TREE and Bayesian inference (BI) in MrBayes 3.2 [16, 17]. *Ascaris lumbricoides* Linnaeus, 1758 (Ascaridida: Ascaridoidea) was used as the outgroup. The ingroup included 16 cosmocercoid species belonging to 8 genera in 3 different families: Cosmocercidae, Atractidae and Kathlaniidae. The detailed information of nematode species included in the phylogenetic analyses, is provided in Table 1. We used a built-in function in IQ-TREE to select a best-fitting substitution model for the sequences according to the Bayesian information criterion [18]. The TIM3e+G4 model for 18S+28S sequence data were identified as the optimal nucleotide substitution model. Reliabilities for the ML tree were tested using 1000 bootstrap replications, and the BI tree was tested using 50 million generations, and bootstrap values exceeding 70% were shown in the phylogenetic tree.

Results

Family Cosmocercidae (Railliet, 1916)

Genus *Aplectana* Railliet & Henry, 1916

*Aplectana xishuangbannaensis* n. sp.

*Type host:* White-spotted thigh tree-frog *Polypedates megacephalus* (Hallowell) (Anura: Rhacophoridae).

*Type-locality:* XiShuangBanNa Tropical Botanical Garden (21°41′ N, 101°25′ E), Yunnan Province, China.

*Type specimens:* Holotype: male (HBNU–N-2020A009L); allotype: female (HBNU–N-2020A010L); paratypes: 41 males, 122 females (HBNU–N-2020A011L).

*Site of infection:* Intestine.

*Prevalence and intensity of infection:* 12.1% (11 *P. megacephalus* infected out of 91 examined) were infected with intensity of 1–88 (mean 15.0) nematodes.

*ZooBank registration:* To comply with the regulations set out in Article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN) [19], details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:09F4B1EF-C3AF42E6-80E6-B734D6B848B. The LSID for the new name *Aplectana xishuangbannaensis* is urn:lsid:zoobank.org:act:5E4C6C18-7B72-4C28-BD28-6964C6D8F0A3.

*Description*

**General**

Small-sized, whitish nematodes. Body cylindrical, maximum width at about region of middle body. Cuticle with fine transverse striations and longitudinal stockade-like ornamentation (Fig. 1a–c). Somatic papillae small, distributed irregularly over body surface (Figs. 1a–c, e, i, 2b). Lateral alae extending from 60–70 posterior to base of lips as far as about middle of tail in both sexes (Fig. 1b, f, i). Oral aperture simple, triangular, surrounded by 3 small lips, each with inner flanges (Figs. 1a, b, d, 2b). Dorsal lip with pair of large double cephalic papillae; subventral lips with single large double cephalic papilla and amphid each (Figs. 1a, 2b). Oesophagus divided into anterior short pharynx, cylindrical corpus, slightly narrower isthmus and terminal posterior bulb with valves (Fig. 2a). Nerve ring located at about 1/2 of oesophageal length. Excretory pore slightly anterior to oesophageal bulb (Fig. 2a). Tail of both sexes conical, with long filamentous tip (Figs. 1e–g, i, 2c, f, h).

**Male**

[Based on 10 mature specimens; Figs. 1b, d–h, 2a, f–h]: Body 2.32–2.72 (2.49) mm long, maximum width 139–178 (158). Oesophagus 317–426 (374) long, representing 12.6–16.1 (15.0) % of body length; pharynx + corpus + isthmus 248–356 (307) long, bulb 59–69 (67) × 50–59 (54) (Fig. 2a). Nerve ring 158–198 (176) and excretory pore 257–376 (334) from anterior extremity, respectively (Fig. 2a). Posterior end of body distinctly curved ventrally (Figs. 1f, 2f). Spicules small, similar in shape and length, 139–178 (161) long, distal end pointed, representing 5.98–7.09 (6.47) % of body length (Fig. 2g). Gubernaculum absent. Caudal papillae: 6 pairs of precloacal, 3 pairs paracloacal (distinguishable from somatic papillae) and 4 pairs postcloacal papillae. Single median, ventral precloacal papilla present (Figs. 1g, h, 2h). Tail 198–248 (230) long, representing 8.26–9.84 (9.26) % of body length (Figs. 1e–g, 2f, h).

**Female**

[Based on 10 mature specimens; Figs. 1a, c, i, 2b–e]: Body 3.54–3.86 (3.65) mm long, maximum width 248–297 (272). Oesophagus 416–446 (431) long, representing 11.0–12.6 (11.8) % of body length; pharynx + corpus + isthmus 347–366 (356) long, bulb 69–79 (74) × 50–69 (62). Nerve ring 208–228 (215) and excretory pore 347–386 (366) from anterior extremity, respectively. Vulva transverse slit, 1.60–2.10 (1.89)
mm from anterior extremity, at 44.8–54.5 (51.8) % of body length. Ovaries two, located anterior to vulva. Vagina muscular (Fig. 2d). Uteri amphidelphic, full of eggs in different stages of development; egg oval, large, with smooth surface, 149–297 (205) × 99–238 (146) (n = 20) (Fig. 2e). Tail 347–406 (384) long, representing 9.78–11.1 (10.5) % of body length (Figs. 1 i, 2c).

Genetic characterization

Partial 18S region

Three 18S sequences of Aplectana xishuangbannaensis n. sp. (accession numbers MW329041–MW329043) obtained were all 1539 bp long, representing only one genotype. There is no species of Aplectana with 18S sequenced registered in GenBank. Pairwise comparison between A. xishuangbannaensis n. sp. and the other species of Cosmocercidae regarding the 18S sequences available in GenBank, including Cosmocerca simile (MN839758–MN839760), Cosmocercoides dukaee (FJ516753), C. pulcher (LC018444, MH178322–MH178326), C. qingtianensis (MH032769–MH032771, MH178319–MH178321), C. tonkinensis (AB908160), C. wuyiensis (MK110872), Nemhelix bakeri (DQ118537) and Raillietnema sp. (DQ503461), displayed 1.88–3.77% nucleotide divergence.

Partial ITS-1 region

Three ITS-1 sequences of A. xishuangbannaensis n. sp. (accession numbers MW329035–MW329037) obtained were all 554 bp long, representing only one genotype. There are two species of Aplectana with ITS sequences available in GenBank, including A. chamaeleonis (MN907375–MN907378) and Neyraplectana PNLS-530 (MH836325). Pairwise comparison between A. xishuangbannaensis n. sp. and the previously mentioned taxa showed 46.67 and 45.47% nucleotide divergence, respectively.

### Table 1

| Species                        | Host                        | Locality       | GenBank ID        | References                  |
|--------------------------------|-----------------------------|----------------|-------------------|-----------------------------|
| Aplectana xishuangbannaensis n. sp. | Polypledes megacephalus (Hallowell) | China            | MW329041 MW329038 | Present study               |
| Aplectana sp.                  | Hylarana spinulosa (Smith)  | China           | MW329991 MW364062 | Present study               |
| Cosmocerca ornata (Dujardin, 1845) | Hylarana spinulosa (Smith)  | China           | MW326676 MW326675 | Present study               |
| Cosmocerca simile Chen, Zhang, Feng & Li, 2020 | Bufo garガりーズ Cantor | China            | MN839758 MN833301 | Chen et al. [10]            |
| Cosmocerca sp. 1               | Hoplobatrachus chinesis (Oisebeck) | China           | MW329987 MW329989 | Present study               |
| Cosmocerca sp. 2               | Bufo melanostictus Schneider | China           | MW329990 MW329988 | Present study               |
| Cosmocercoides pulcher Wilkie, 1930 | Bufo japonicus formosus    | Japan           | LC018444 LC018444 | Tran et al. [46]            |
| Cosmocercoides qingtianensis Chen, Zhang, Nakao & Li, 2018 | Bufo garガりーズ Cantor | China            | MH178321 MW325956 | Chen et al. [47]; Present study |
| Cosmocercoides tonkinensis Tran, Sato & Luc, 2015 | Acanthosaura lepidogaster (Cuvier) | Vietnam         | AB908160 AB908160 | Tran et al. [46]            |
| Cruzia americana Maplestone, 1930 | Didelphis virginiana Kerr | USA             | U94371 U94757     | Nadler and Hudspeth [13]    |
| Falcaustra sp. _T               | Lithobates catesbeianus (Shaw); Indotestudo elongate (Blyth) | Japan; China   | AB818380 MF094270 | Hasegawa et al. [48]; Li et al. [49] |
| Megalobatrachonema hainanensis Chen, Zhang & Li, 2019 | Aranolps hainanensis (Boulenger) | China            | – MH545569       | Chen et al. [9]             |
| Megalobatrachonema terdentatum (Linstow, 1898) | Lissomiton vulgaris (Linnaeus) | Germany         | – MN444705       | Sinsch et al. [50]          |
| Megalobatrachonema wangi Chen, Zhang, Sinsch, Scheid, Balczaun & Li, 2020 | Quasisipa exilipinnosa (Liu & Hu) | China           | MW325957 MN245660 | Present study; Chen et al. [11] |
| Orientattractis moravec Cavalcante, Silva, Santos, Chagas-Moutinho & Santos, 2016 | Pimełodus blochii Valenciennes | Brazil           | KXS24513 KXS24514 | Cavalcante et al. [51]      |
| Rondonia rondoni (Travassos, 1920) | Pterodoras granulosus (Doradidæ); Pimełodus blochii Valenciennes | Peru, Brazil | DQ442679 KXS24512 | Wijova et al. [52]; Cavalcante et al. [51] |
| Ascaris lumbricoides Linnaeus, 1758 | Homo sapiens Linnaeus | USA             | M74585 U94751     | Müller et al. [53]; Nadler and Hudspeth [13] |
Pairwise comparison between *A. xishuangbannaensis* n. sp. and the other species of Cosmocercidae regarding the ITS sequences available in GenBank, including *Cosmocerca japonica* (LC052772–LC052782), *C. longicauda* (MG594349–MG594351), *C. ornata* (MT108302), *Cosmocerca* sp. LL-2020 (MT108303), *C.*
simile (MN839761–MN839768), Cosmocercoides pulcher (MH178314–MH178318, LC018444), C. qingtianensis (MH178311–MH178313, MH032772–MH032774), C. tonkinensis (AB908160, AB908161) and C. wuyiensis (MK110871), displayed 28.53–47.52% of nucleotide divergence.
Partial 28S region
Three 28S sequences of *A. xishuangbannaensis* n. sp. (accession numbers MW329038–MW329040) obtained were all 740 bp long, representing only one genotype. There is only one species of *Aplectana, Aplectana* sp. ‘Neyraplectana’ PNLS-530, with 28S sequence data (MH909070) available in GenBank. Pairwise comparison between *A. xishuangbannaensis* n. sp. and the previously mentioned taxon showed 20.67% of nucleotide divergence. Pairwise comparison between *A. xishuangbannaensis* n. sp. and the other species of Cosmocercidae with 28S sequences available in GenBank, including *Cosmocerca similis* (MN839755–MN839757), *Cosmocercoides pulcher* (LC018444) and *C. tonkinesis* (AB908160), displayed 16.78–17.94% of nucleotide divergence.

Partial cox1 region
Three cox1 sequences of *A. xishuangbannaensis* n. sp. (accession numbers MW327586–MW327588) obtained were all 384 bp long, representing only one genotype. There is no species of *Aplectana* with cox1 sequence registered in GenBank. Pairwise comparison between *A. xishuangbannaensis* n. sp. and the other species of Cosmocercidae regarding the cox1 sequences available in GenBank, including *C. japonica* (LC052756–LC052770), *C. ornata* (MT108304), *C. similis* (MN833301–MN833303), *C. pulcher* (MH178306–MH178310, LC052771) and *C. qingtianensis* (MH178303–MH178305, MH032775–MH032777), displayed 10.23–21.09% of nucleotide divergence.

Phylogenetic analyses
Phylogenetic trees inferred from maximum likelihood (ML) and Bayesian inference (BI) showed that representatives of Cosmocercoida were divided into four major clades (Fig. 3). Clade I included the species of three genera *Cosmocerca, Cosmocercoides* and *Aplectana*, representing the family Cosmocercidae. Among the three genera, *Cosmocerca* displayed a closer relationship to *Aplectana* rather than *Cosmocercoides*. Clade II included only *Crucia americana* (a common nematode parasite in the digestive tract of opossums), which belongs to the subfamily Crucinae in the family Kathlaniidae according to the current classification [1]. Clade III included species of *Falcaustra* and *Megalobatrachonema*, which represent the family Kathlaniidae. The representatives of *Orientattractis* and *Rondonia* formed Clade IV, representing the family Atractidae.

Discussion
The genus *Aplectana* (Cosmocercoida: Cosmocercidae) is a group of zooparasitic nematodes, with approximately 50 nominal species mainly parasitic in various amphibians, and rarely occurring in reptiles worldwide [4, 5, 20–22]. The absence of rosette papillae or plectanes in males and presence of somatic papillae, lateral alae and two prodelphic ovaries, uteri containing numerous eggs of normal size in females, allocate the present specimen to the genus *Aplectana*. To date, only four species of *Aplectana* have been reported in China, namely *A. hainanensis* Bursey, Goldberg & Grismer, 2018, *A. hylae* Wang, 1980, *A. macintoshii* (Stewart, 1914) and *A. paucipapillosa* Wang, 1980 [22–24]. Lacking a gubernaculum, the new species can be easily distinguished from the four above-mentioned species (the four species all possessing a gubernaculum) [20, 22, 23].

In the genus *Aplectana*, *A. akhrami* (Islam, Farooq & Khanum, 1979), *A. artigasi* Puga & Torres, 1997, *A. chilensis* Lent & Freitas, 1948, *A. crossodactyli* Baker, 1980, *A. crucifer* Travassos, 1925, *A. delirae* (Fabio, 1971), *A. dubrajipuri* Sou & Nandi, 2015, *A. hoplobatrachusia* Sou, Sou & Nandi, 2018, *A. meridionalis* Lent & Freitas, 1948, *A. papillifera* (Araujo, 1977), *A. praeputialis* (Skrjabin, 1916), *A. tarija* Ramallo, Bursey & Goldberg, 2007, and *A. vercammeni* Le Van Hoa, 1962, have no gubernaculum [20, 22, 25–35], similar to the new species.

*Aplectana xishuangbannaensis* n. sp. differs from *A. dubrajipuri* and *A. meridionalis* in the different position of the excretory pore (situated at anterior end of oesophageal bulb vs at 1/2 between nerve ring and oesophageal bulb in the latter two species). With only one pair of precloacal papillae, *A. tarija*, which has six pairs of precloacal papillae, can be easily differentiated from the new species. *Aplectana artigasi, A. chilensis, A. crucifer, A. praeputialis, A. vercammeni* and *A. hoplobatrachusia* differ from *A. xishuangbannaensis* n. sp. by having relatively longer spicules (spicules representing 9.10–15.2% of body length in the new species) and *A. papillifera* vs *A. xishuangbannaensis* n. sp. by having relatively longer spicules (9.10–15.2% of body length in the new species) and a different arrangement and number of caudal papillae (precloacal: 10 pairs; paracloacal: 1–2 pairs; postcloacal: 8 pairs in the former vs precloacal: 6 pairs; paracloacal: 3 pairs; postcloacal: 4 pairs in *A. xishuangbannaensis* n. sp.).

The new species differs from *A. crossodactyli* by having relatively longer spicules (vs representing 3.78–4.64%...
of body length in *A. crosodactyli* and fewer precloacal papillae (6 pairs in the new species vs 20 pairs in the latter). *Aplectana xishuangbannaensis* n. sp. can be easily distinguished from *A. akhrami* by having a different position of the vulva (vulva from anterior extremity at 44.8–54.5% of body length in the new species vs vulva from anterior extremity at 29.0–30.6% of body length in *A. akhrami*) and a much longer female tail (vs 0.16 mm, representing 4.44–5.33% of body length in *A. akhrami*).

Currently, the specific diagnosis of *Aplectana* spp. remains based on morphology, and the genetic data of these parasites are severely limited. Based on the genetic analysis of *A. xishuangbannaensis* n. sp., no intraspecific nucleotide differences in 18S, ITS-1, 28S and cox1 regions among different individuals were noted, but a high level of interspecific genetic variation in these regions among species of the other genera in the Cosmocercidae was clear.

Our phylogenetic results are largely congruent with the traditional classifications of the Cosmocercoidea, which have been proposed based on morphological characters and ecological traits, including the structure of the oesophagus, the presence or absence of a precloacal sucker, the morphology of caudal papillae, the morphology of female reproductive organs and the reproductive strategies [1, 2, 36].

The systematic position of the subfamily Cruziinae has long been under debate. Our molecular phylogenetic results conflicted with the traditional classification [1, 5, 40–42], which suggested that the subfamily Cruziinae should be moved out from the hitherto-defined family Kathlaniidae and elevated to a separate family. The highly specialized structure of the pharynx (the presence of unique pharyngeal lamellae) and the unique digestive system (the presence of an intestinal caecum) of this group support its full family status [43]. However, a more rigorous molecular phylogenetic study with broader representatives of the Cruziinae using different nuclear and/or mitochondrial genetic markers is required to further ascertain its systematic position.

The Cosmocercidae currently includes about 200 nominal species allocated in more than 20 genera, representing the largest family within Cosmocercoidea [1, 3, 21, 44]. However, the phylogenetic relationships among genera within Cosmocercidae is poorly understood because of the lack of genetic data. According to Chabaud (1978) [1] and Gibbons (2010) [44], the morphology of caudal papillae in males is one of the most important characters for generic diagnosis in the Cosmocercidae. Species of the genus *Aplectana* have no modified papillae (plectanes and/or rosette papillae), but those of *Cosmocerca* and *Cosmocercoides* have this character. Wilkie (1930) [45], Skrjabin et al. (1961) [5] and Chabaud (1978) [1] considered these genera with modified papillae more closely related to each other than *Aplectana*. However, our results indicated that *Cosmocerca* is closer to *Aplectana* rather than *Cosmocercoides*, conflicting with the traditional systematics based on morphology.

**Fig. 3** Maximum likelihood (ML) inference and Bayesian inference (BI) based on the 18S + 28S sequences of the rDNA showing the phylogenetic relationships of representatives of Cosmocercoida. *Ascaris lumbricoides* Linnaeus, 1758 (Ascaridida: Ascaridoidea) was chosen as the outgroup. Bootstrap values exceeding 70% are shown.
Conclusions

The present study provided a preliminary molecular phylogenetic framework for the superfamily Cosmoceroidea based on 18S + 28S sequence data for the first time to our knowledge. The family Kathlianaeidae is not a monophyletic group. Cruziidae is probably valid as a family for Cosmoceroidea. The genus Aplectana is closer to Cosmocerca than to Cosmoceroidea, which is basal within Cosmoceroidea. Moreover, morphological and genetic evidence both supported the hypothesis that our nematode specimens collected from P. megacephalus represent a new species of Aplectana, which is the fifth species in the genus reported from China. However, the phylogenetic aspects of Cosmoceroidea are far from being well understood.

Abbreviations

SEM: Scanning electron microscopy; PCR: Polymerase chain reaction; ML: Maximum likelihood; BI: Bayesian inference; 18S: Small subunit ribosomal DNA; 28S: Large subunit ribosomal DNA; ITS: Internal transcribed spacer; cox1: Cytochrome c oxidase subunit 1; dl: Dorsal lip; vl: Ventrolateral lip; pp: Large double cephalic papillae; sp: Single median precloacal papilla.

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Authors’ contributions

All authors contributed to the study design. HXC and LL carried out sample collection and identified the nematode specimens. HXC, XHG, XFN and LL analyzed morphological and genetic data. HXC and LL conducted the phylogenetic analyses and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The nuclear and mitochondrial DNA sequences of Aplectana xishuangbanaensis n. sp. obtained in this study were deposited in GenBank database under the accession numbers MW5329041–MW5329043 (18S sequences), MW329035–MW329037 (ITS-1 sequences), MW329038–MW329040 (28S sequences) and MW327586–MW327588 (cox1 sequences). Type specimens of the new species were deposited in the College of Life Sciences, Hebei Normal University, Hebei Province, under the accession numbers HBNU–N-2020A009–11L, China.

Declarations

Ethics approval and consent to participate

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.

Consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Chabaud AG. Keys to genera of the superfamilies Cosmoceroidea, Seuratoidae, Heterakoidea, and Subuluroidea. In Anderson RC, Chabaud AG, Willmott S, editors. CIH keys to the nematode parasites of vertebrates. Farnham Royal: Commonwealth Agricultural Bureaux; 1978. p. 71.
2. Anderson RC. Nematode parasites of vertebrates. Their development and transmission. 2nd edition. Wallingford: CAB Publishing; 2000:650 pp.
3. Zhang Z-Q. Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness. Zootaxa. 2011;3148:63–95.
4. Travassos L. Pesquisas helminthicologicas realizadas em Hamburgo. IX. Ensaio monographica da familia Cosmoceroidea Trav., 1925 (Nematoda). Mem I Oswaldo Cruz. 1931;19(25):237–98.
5. Skrjabin KI, Shikhobalova NP, Lagodovskaya EA. Oxyurata of animals and man. Part 2. Translated from Russian. Israel Program for Scientific Translations, Jerusalem, Israel, 1974. p. 460.
6. Baker MR. Nematode parasitism in amphibians and reptiles. Can J Zool. 1984;62:747–57.
7. Baker MR. Systematic relationships of the Atractidae and Cosmoceroidea (Nematoda: Cosmoceroidea): two new atractids parasitic in amphibians and fish. Can J Zool. 1982;60:2395–402.
8. Pereira FB, Tavares LER, Paiva F, Scholz T, Luque JL. A morphological and molecular study of Spectatus spectatus (Kthlianaeidae), including redescriptions of the species and amendment of genus diagnosis. J Parasitol. 2015;101:468–75.
9. Chen H-X, Zhang L-P, Li L. Morphological and molecular characterization of Megaboltrachonema hainanensis sp. n. (Nematoda: Ascaridida), with phylogenetic position of Megaboltrachonema in Cosmoceroidea. J Helminthol. 2019;94:1–7.
10. Chen H-X, Zhang L-P, Feng YY, Li L. Integrated evidence reveals a new species of Cosmocerca (Ascaridomorpha: Cosmoceroidea) from the Asiatic toad Bufo gargarizans Cantor (Anphibia: Anura). Parasitol Res. 2020;119:1795–802.
11. Chen H-X, Zhang L-P, Sinsch U, Scheid P, Balczun C, Li L. Molecular phylogeny of Megaboltrachonema (Nematoda: Ascaridida), with description of a new species based on morphological and molecular evidence. Infect Genet Evol. 2020;80:104172.
12. Floyd RM, Rogers AD, Lambshead PJD, Smith CR. Nematode-specific PCR primers for the 18S small subunit rRNA gene. Mol Ecol Notes. 2005;5:611–2.
13. Nadler SA, Hudspeth DSS. Ribosomal DNA and phylogeny of the Ascaridoidea (Nematoda: Secernentes): implications for morphological evolution and classification. Mol Phylogenet Evol. 1998;10:221–36.
14. Zhu X, D’Amelio S, Paggi L, Gasser RB. Assessing sequence variation in the internal transcribed spacers of ribosomal DNA within and among members of the Contracaecum-osculatum complex (Nematoda: Ascaridoidea: Anisakidae). Parasitol Res. 2000;86:677–83.
15. Lazarova SS, Malloch G, Oliveira CMG, Hübschen J, Neilson R. Ribosomal and mitochondrial DNA analyses of Xiphiphium americanum-group populations. J Nematol. 2006;38:404–10.
16. Nguyen LT, Schmidt HA, Haeseler A, Minh BQ. IQ-tree: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32:268–74.
17. Ronquist F, Teslenko M, Mark PVD, Ayres DL, Darling A, Hühn S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012;61:539–42.
18. Posada D, Crandall KA. Selecting the best-fit model of nucleotide substitution. Syst Biol. 2000;50:580–601.
19. ICZN. International Commission on Zoological Nomenclature: Amendment of articles 8, 9, 10, 21 and 78 of the International Code of Zoological
Nomenclature to expand and refine methods of publication. Bull Zool Nomencl. 2012;69:161–9.

20. Baker MR. Revision of old world species of the genus Aplectana Railliet & Henry, 1916 (Nematoda: Cosmocercidae). Bull Mus Natn Hist Nat. 1980;4:955–98.

21. Baker MR. Synopsis of the nematode parasitic in amphibians and reptiles. St. John's, NFld., Canada, Memorial University of Newfoundland, Occasional papers in Biology. 1987. p. 325.

22. Bursey CR, Goldberg SR, Grismer LL. A new species of Aplectana (Nematoda, Cosmocercidae) in Goniurosaurus bawanglingensis (Squamata, Eublepharidae), from Hainan Province. China Acta Parasitol. 2018;63:190–7.

23. Wang P-Q. Studies on some new nematodes of the suborder Oxyurata.

24. Wang P-Q, Wang Y-Y. Synopsis of trematodes and nematodes of amphibians and reptiles. St. John's, NFld., Canada, Memorial University of Newfoundland, Occasional papers in Biology. 1987. p. 325.

25. Traversos L. Contribuições para o conhecimento da fauna helminthológica dos batarROs do Brasil. Sci Med. 1925;9:673–87.

26. Lent H, Freitas JFT. Uma coleção de nematódeos, parasitos de vertebrados do Museu de História Natural de Montevideo. Mem Inst Oswaldo Cruz. 1948;46:1–71.

27. Le Van HL. Nematodes parasites de mammifères, reptiles et amphibiens du Congo. Brussels. 1962:653–85.

28. Vicente JJ, Santos E. Nova espécie do gênero Neysaplectana Ballastero‑Marques, 1945 (Nematoda, Subuluroidea). Atas Soc Biol Rio de Janeiro. 1970;13:21–3.

29. Fabio SP Sóbre uma nova espécie do gênero Neysaplectana Ballastero‑Marques, 1945 (Nematoda, Cosmocercidae). Atas Soc Biol Rio de Janeiro. 1971;15:11–3.

30. Araujo P. Uma nova espécie do gênero Neysaplectana (Nematoda, Subuluroidea, Cosmocercidae) encontrada em ovidios. Mem Inst Butantan. 1977;40/41(1):259–64.

31. Islam A, Farooq M, Khanum Z. Two new genera of nematode parasitidae (Oxyuridae: Oxysoomatinae) from toad, Bufo andersoni. Pak J Zool. 1979;11:69–73.

32. Puga S, Torres P. Aplectana artigasi n. sp. (Nematoda: Cosmocercidae) from the frog Euphryctus calcarius (Anura: Leptodactylidae) in Southern Chile. Mem I Oswaldo Cruz. 1997;92:767–70.

33. Ramallo G, Bursey CR, Goldberg SR. Two new species of cosmocercids (Ascaridida) in the toad Chaunus arenarum (Anura: Bufonidae) from Argentina. J Parasitol. 2007;93:910–6.

34. Sou SK, Nandi AP. Aplectana dubrajpuri sp. nov. (Nematoda: Cosmocercidae) in Hoplobatrachus tigerinus (Anura: Dicroglossidae) from Dubaijpur, Birbhum, West Bengal, India. Acta Parasitol. 2015;60(3):430–4.

35. Sou SK, Sow KK, Nandi AP. Cosmocerca bengalensis sp. nov. (Nematoda: Cosmocercidae) in Hoplobatrachus tigerinus (Daudin, 1803) (Amphibia, Anura, Dicroglossidae) from West Bengal, India. Acta Parasitol. 2018;63(3):715–20.

36. Chabaud AG. Ordre des Ascaridida. In: Traité de zoolOgie: anatomie, systématique, biologie. IV. Paris: Masson; 1965. p. 732–1497.

37. Baker MR, Vaucher C. Parasitic helminths from Paraguay. VII. Systematic position of Oxyascaris Traversos, 1920 (Nematoda: Cosmocercidae). Rev Suisse Zool. 1985;92:303–10.

38. Bursey CR, Goldberg SR. A new species of Oxyascaris (Nematoda, Cosmocercidae) in the Costa Rica brook frog, Duellmanohyla uranochroa (Anura, Hylidae). Acta Parasitol. 2007;52:58–61.

39. González CE, Harann M. Nematode parasites of two anuran species Rhinella schneideri (Bunifonidae) and Scinax acuminatus (Hylidae) from Corrientes. Argentina Rev Biol Trop. 2008;56:147–61.

40. Travassos L. Alguns helmintos da coleção do Instituto Bacteriológico de São Paulo. Bras Med. 1917;31:99–100.

41. Ortepp RJ. On a collection of helmintos from Durch Guiana. J Hel‑minthol. 1924;2:15–40.

42. Khalil M. Cosmocerca mexicana n. sp. parasite d'un lizard mexicain. Ann Parasitol Hum Comp. 1927;5:41–5.

43. Li L. Redescription of Cruzia americana Mapleston, 1930 (Nematoda: Kathanidae), a parasite of Didelphis virginiana (Kerr) (Mammalia: Didel‑phidae) in USA. Syst Parasitol. 2019;96:433–40.

44. Gibbons LM. Keys to the nematode parasites of vertebrates. Wallingford: CABI Publishing. 2010. p. 416.

45. Wilke JS. Some parasitic nematodes from Japanese Amphibia. Ann Mag Nat Hist Series. 1930;106:606–14.

46. Tran BT, Sato H, Luc PV. A new Cosmocercoides species (Nematoda: Cosmocercidae), C. tonkinessis n. sp., in the scalebellied tree lizard (Acanthosaura lepidogaster) from Vietnam. Acta Parasitol. 2015;60:407–16.

47. Chen H-X, Zhang L-P, Nakao M, Li L. Morphologicaland molecular evidence for a new species of the genus Cosmocercodes Wilkie, 1930 (Ascaridida: Cosmocercidae) from the Asiatic toad Bufo gargarizans Cantor (Amphibia: Anura). Parasitol Res. 2018;117:1857–64.

48. Hasegawa H, Sato A, Kai M, Uchida A. Helminth parasites of bullfrogs, Lithobates catesbeianus (Shaw, 1802), in Kanto District, Japan, with special reference to those introduced from North America. Japan J Vet Parasitol. 2013;12:1–10.

49. Li L, Lu L, Nadler SA, Gibson DI, Zhang L-P, Chen H-X, et al. A terrestrial origin for ascaridoid nematodes in the Early Carboniferous. Syst Biol. 2018;67:888–900.

50. Sinsch U, Heneberg P, Těšínský M, Balczun C, Scheid P. Helminth endo‑parasites of the smooth newt Triturus vulgaris: linking morphological identification and molecular data. J Helminthol. 2019;93:332–41.

51. Cavalcante PHO, Silva MT, Santos EGN, Chagas‑Moutinho VA, Santos CP. Oentactis maraviezae n. sp. and Rondonia rondoni Traversos, 1920 (Nematoda: Atractidae), parasites of Pimelodus blochii (Osteichthyes, Pimelodidae) from the Acre and Xapuri Rivers, Western Amazon, Brazil. Parasitol. 2016;144(2):226–36.

52. Wijová M, Moravec F, Horák A, Lukes J. Evolutionary relationships of Spirurina (Nematoda: Chromadorea: Rhabditida) with special emphasis on dracunculoid nematodes inferred from SSU rRNA gene sequences. Int J Parasitol. 2006;36:1067–75.

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