Molecular phylogeny of the family Cosmocercidae (Nematoda: Ascaridida), with description of a new species of Aplectana using an integrative approach

Hui-Xia Chen  
Hebei Normal University

Xiao-Hong Gu  
Hebei Normal University

Xue-Feng Ni  
Hebei Normal University

Liang Li (✉ liangliangex369@126.com)  
Hebei Normal University

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Abstract

Background

Nematodes of the family Cosmocercidae (Ascaridida: Cosmoceroidea) 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 of the Cosmocercidae and the other two families Atractidae and Kathlaniidae in the superfamily Cosmoceroidea, are still under debate. Moreover, the systematic position of some genera in Cosmocercidae remains unclear.

Methods

Nematodes collected from *Polypedates megacephalus* (Hallowell) (Anura: Rhacophoridae) were identified using morphological methods (light and scanning electron microscopy) and molecular approaches [sequencing and analyzing 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 Cosmoceroidea, and the systematic position of the genus *Aplectana* in Cosmocercidae.

Results

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

Conclusions

Our molecular phylogenetic results supported that the subfamily Cruziinae should be moved out from the hitherto-defined family Kathlaniidae and elevated to a separate family, and the genus Cosmocerca has closer relationship to the genus *Aplectana* in the family Cosmocercidae. Our present study provided the basic molecular phylogenetic framework for the superfamily Cosmoceroidea based on 18S + 28S sequence data for the first time. Moreover, a new species of *Aplectana, A. xishuangbannaensis* n. sp., was described using an integrative approach.
Background

The superfamily Cosmocercoidea is a group of zooparasitic nematodes and currently contains three families, namely Atractidae Railliet, 1917, Cosmocercidae Railliet, 1916 and Kathlaniidae Lane, 1914 [1–3]. Among them, the Cosmocercidae is the largest family, including approximately 200 nominal species, which are mainly parasitic in the alimentary 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 the Cosmocercoidea.

Our present knowledge of the molecular phylogeny of the Cosmocercoidea/ Cosmocercidae is still very limited. To date, several studies [8–11] provided molecular phylogenetic analyses to solve the systematic status of some genera using different genetic data. However, due to the paucity and inaccessibility of suitable material of Cosmocercoidea / Cosmocercidae for genetic analysis, all of these molecular phylogenetic studies have included only small numbers of representatives of the Cosmocercoidea / Cosmocercidae.

In order to clarify the phylogenetic relationships of the Cosmocercidae and the other families Atractidae and Kathlaniidae in the Cosmocercoidea, and the systematic position of the genus Aplectana in Cosmocercidae, phylogenetic analyses including the most comprehensive taxon sampling of Cosmocercoidea to date, were performed using maximum likelihood (ML) inference and Bayesian inference (BI) based on 18S + 28S sequence data, respectively. 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. Specimens were isolated from the intestine of this host, then fixed and stored in 80% ethanol until study.

Morphological observations

For light microscopical studies, nematodes were cleared in lactophenol. Drawings were made with the use of a Nikon microscope drawing attachment. For scanning electron microscopy (SEM), the anterior and posterior end 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).
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'-CGCGAATRGCTCATTACAACAGC-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'-AGCGGAGGAAAAGAAAACCTAA-3') and the reverse primer 28S-R (5'-ATCCGTGTGTTCAAGACGGG-3') [13]. The ITS-1 region of nuclear rDNA was amplified by PCR using the forward primer SS1 (5'-GTTTCCGTAGGGTAACCTGCG-3') and the reverse primer SS2R (5'-AGTGCTCAATGTGTCTGCAA-3') [14]. The partial cox1 region was amplified by PCR using the forward primer COIF (5′-TTTTTTTTCTCATCTGAGGTAT-3′) and the reverse primer COIR (5′-ACATAATGAAAATGACTAAAC-3′) [15]. The cycling conditions were as described previously [9]. PCR products were checked on GoldView-stained 1.5% agarose gels and purified with Column PCR Product Purification Kit (Shanghai Sangon, China). Sequencing was carried out using a Dye Deoxy Terminator Cycle Sequencing Kit (v.2, Applied Biosystems, California, USA) and an automated sequencer (ABI-PRISM 377). Sequencing for each sample was carried out for both strands. Sequences were aligned using ClustalW2. The DNA sequences obtained herein were compared (using the algorithm BLASTn) with those available in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov).

Phylogenetic analyses

Phylogenetic trees were constructed based on the 18S + 28S sequence data using maximum likelihood (ML) inference with IQ-TREE and Bayesian inference (BI) with Mrbayes 3.2 [16, 17]. *Ascaris lumbricoides* Linnaeus, 1758 (Ascaridida: Ascaridoidea) was treated as the outgroup. The ingroup included 16 cosmocercoid species belonging to 8 genera in three 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 optimal nucleotide substitution model. Reliabilities for ML tree was tested using 1000 bootstrap replications and BI tree was tested using 50 million generations, and bootstrap values exceeding 70% were showed in the phylogenetic tree.
Table 1
Cosmocercoidea species with their detailed information of genetic data included in the phylogenetic analyses

| Species                                      | Host                                | Locality     | GenBank ID       | Reference                            |
|----------------------------------------------|-------------------------------------|--------------|-----------------|---------------------------------------|
| Aplectana xishuangbannaensis n. sp.          | Polypedates megacephalus (Hallowell) | China        | MW329041        | Present study                         |
| Aplectana sp.                                | Hylarana spinulosa (Smith)          | China        | MW329991        | Present study                         |
| Cosmocerca omata (Dujardin, 1845)            | Hylarana spinulosa (Smith)          | China        | MW326676        | Present study                         |
| Cosmocerca simile Chen, Zhang, Feng & Li, 2020| Bufo gargarizans Cantor             | China        | MN839758        | Chen et al. (2020a) [10]              |
| Cosmocerca sp. 1                             | Hoplobatrachus chinensis (Osbeck)   | China        | MW329987        | Present study                         |
| Cosmocerca sp. 2                             | Bufo melanostictus Schneider        | China        | MW329990        | Present study                         |
| Cosmocercoides pulcher Wilkie, 1930          | Bufo japonicus formosus             | Japan        | LC018444        | Tran et al. (2015) [46]              |
| Cosmocercoides qingtianensis Chen, Zhang, Nakao & Li, 2018 | Bufo gargarizans Cantor | China        | MH178321        | Chen et al. (2018) [47]; Present study |
| Cosmocercoides tonkinensis Tran, Sato & Luc, 2015 | Acanthosaura lepidogaster (Cuvier) | Vietnam      | AB908160        | Tran et al. (2015) [46]              |
| Cruzia americana Maplestone, 1930           | Didelphis virginiana Kerr           | USA          | U94371          | Nadler & Hudspeth (1998) [13]         |
| Falcaustra sp.                               | Lithobates catesbeianus (Shaw); Indotestudo elongate (Blyth) | Japan, China | AB818380        | Hasegawa et al. (2013) [48]; Li et al. (2018) [49] |
| Species | Host | Locality | GenBank ID | Reference |
|---------|------|----------|------------|-----------|
| Megalobatrachonema hainanensis Chen, Zhang & Li, 2019 | *Amolops hainanensis* (Boulenger) | China | – | MH545569 | Chen et al. (2019) [9] |
| Megalobatrachonema terdentatum (Linstow, 1898) | *Lissotriton vulgaris* (Linnaeus) | Germany | – | MN444705 | Sinsch et al. (2019) [50] |
| Megalobatrachonema wangi Chen, Zhang, Sinsch, Scheid, Balczun & Li, 2020 | *Quasipaa exilispinosa* (Liu & Hu) | China | MW325957 | MN245660 | Present study; Chen et al. (2020b) [11] |
| Orientattractis moravecii Cavalcante, Silva, Santos, Chagas-Moutinho & Santos, 2016 | *Pimelodus blochii* Valenciennes | Brazil | KX524513 | KX524514 | Cavalcante et al. (2016) [51] |
| Rondonia rondoni (Travassos, 1920) | *Pterodoras granulosus* (Doradidae); *Pimelodus blochii* Valenciennes | Peru; Brazil | DQ442679 | KX524512 | Wijova et al. (2006) [52]; Cavalcante et al. (2016) [51] |
| Ascaris lumbricoides Linnaeus, 1758 | *Homo sapiens* Linnaeus | USA | M74585 | U94751 | Müller et al. (1992) [53]; Nadler & Hudspeth (1998) [13] |

**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 out of 91 *P. megacephalus*) 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-C3AF-42E6-80E6-B734D6B084B8. The LSID for the new name *Aplectana xishuangbannaensis* is urn:lsid:zoobank.org:act:5E4C6C18-7B72-4C28-BD28-6964C6D8F0A3.

Etymology

The specific epithet refers to the type location XiShuangBanNa Tropical Botanical Garden, Yunnan Province, China.

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 present (Fig. 1c, e, i). Lateral alae extending from some distance 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 lip with inner flanges (Figs. 1a, b, d, 2b). Dorsal lip with one pair of large double cephalic papillae; subventral lips each with single large double cephalic papilla and amphid (Figs. 1a, 2b). Oesophagus divided into anterior short pharynx, cylindrical corpus, slightly narrow isthmus and terminating posterior bulb with valves (Fig. 2a). Nerve ring located at about 1/2 of oesophageal length. Excretory pore suited at level of anterior of 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) in total length, representing 12.6–16.1 (15.0) % of body length; pharynx + corpus + isthmus 248–356 (307) long, size of bulb 59–69 (67) × 50–59 (54) (Fig. 3a). 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. 1e, 2f). Spicules, small, more or less equal in length, 139–178 (161) long, distal end pointed, representing 5.98–7.09 (6.47) % of body length (Fig. 2g). Gubernaculum absent. Caudal papillae distributed as: 6 pairs of pre-cloacal papillae, 3 pairs of paracloacal papillae (distinguishable from somatic papillae) and 4 pairs of cloacal papillae. Single median, ventral 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) in total length, representing 11.0–12.6 (11.8) % of body length; pharynx + corpus + isthmus 347–366 (356) long, size of 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, representing 44.8–54.5 (51.8) % of body length. Vagina muscular, each uterus full of eggs in different stages of development; egg oval, large, with smooth surface, 149–297 (205) ÷ 99–238 (146) (n = 20) (Fig. 2d, e). Tail 347–406 (384) long, representing 9.78–11.1 (10.5) % of body length (Figs. 1i, 2c).

**Genetic characterization**

**Partial 18S region**

Three 18S sequences of *Aplectana xishuangbannaensis* n. sp. obtained herein are all 1539 bp in length and represent only one genotype. There is no species of *Aplectana* with 18S sequence registered in GenBank. Pairwise comparison between *A. xishuangbannaensis* n. sp. and the other species of Cosmocercidae with 18S sequence data available in GenBank, including *Cosmocerca simile* (MN839758–MN839760), *Cosmocercoides dukae* (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. The 18S sequences of *A. xishuangbannaensis* n. sp. are deposited in the GenBank database (http://www.ncbi.nlm.nih.gov) (accession numbers MW329041–MW329043).

**Partial ITS-1 region**

Three ITS-1 sequences of *A. xishuangbannaensis* n. sp. obtained herein are all 554 bp in length and represent only one genotype. There are two species of *Aplectana* with ITS sequence data available in GenBank, including *A. chamaeleonis* (MN907375–MN907378) and *Aplectana* sp. 'Neyraplectana' PNLS-530 (MH836325). Pairwise comparison between *A. xishuangbannaensis* n. sp and *A. chamaeleonis* and *Aplectana* sp. 'Neyraplectana' PNLS-530 showed 46.67% and 45.47% of nucleotide divergence, respectively. Pairwise comparison between *A. xishuangbannaensis* n. sp. and the other species of Cosmocercidae with ITS sequence data 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% nucleotide divergence. The ITS-1 sequences of *A. xishuangbannaensis* n. sp. are deposited in the GenBank database (http://www.ncbi.nlm.nih.gov) (accession numbers MW329035‒MW329037).

**Partial 28S region**

Three 28S sequences of *A. xishuangbannaensis* n. sp. obtained herein are all 740 bp in length and represent 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 *Aplectana* sp. 'Neyraplectana' PNLS-530 showed 20.67% of nucleotide divergence. Pairwise comparison between *A. xishuangbannaensis* n. sp. and the other species of Cosmocercidae with 28S sequence data available in GenBank, including *Cosmocerca simile* (MN839755‒MN839757), *Cosmocercoides pulcher* (LC018444) and *C. tonkinensis* (AB908160), displayed 16.78‒17.94% nucleotide divergence. The 28S sequences of *A. xishuangbannaensis* n. sp. are deposited in the GenBank database (http://www.ncbi.nlm.nih.gov) (accession numbers MW329038‒MW329040).

**Partial cox1 region**

Three cox1 sequences of *A. xishuangbannaensis* n. sp. obtained herein are all 384 bp in length and represent 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 with cox1 sequence data available in GenBank, including *Cosmocerca japonica* (LC052756‒LC052770), *C. ornata* (MT108304), *Cosmocerca* sp. LL-2020 (MT108305), *C. simile* (MN833301‒MN833303), *Cosmocercoides pulcher* (MH178306‒MH178310, LC052771) and *C. qingtianensis* (MH178303‒MH178305, MH032775‒MH032777), displayed 10.23‒21.09% nucleotide divergence. The cox1 sequences of *A. xishuangbannaensis* n. sp. are deposited in the GenBank database (http://www.ncbi.nlm.nih.gov) (accession numbers MW327586‒MW327588).

**Phylogenetic analyses** (Fig. 3)

Our phylogenetic trees using Maximum likelihood (ML) and Bayesian inference (BI) analyses both showed that the representative of Cosmocercoidea were divided into four large clades (Fig. 3). The clade I included the species of three genera *Cosmocerca*, *Cosmocercoides* and *Aplectana*, which represented the family Cosmocercidae. Among the three genera, *Cosmocerca* displayed closer relationship to *Aplectana* rather than *Cosmocercoides*. The clade II only included *Cruzia americana* (a common nematode parasite in the digestive tract of opossums), which belongs to the subfamily Cruzinae in the family Kathlaniidae according to the current classification. The clade III contained the species of *Falcaustra* and
Megabatrachonema, which represented the family Kathlaniidae. The representatives of Orientatractis and Rondonia formed Clade IV, representing the family Atractidae.

**Discussion**

The genus *Aplectana* (Cosmoceroidea: Cosmocercidae) is a group of zooparasitic nematodes, with approximately 50 nominal species mainly parasitic in various amphibians, and unfrequently occurring in reptiles worldwide [4, 5, 20–22]. To date, only four species of *Aplectana* have been reported in China, namely *A. hylae* Wang, 1980, *A. macintoshii* (Stewart, 1914), *A. hainanensis* Bursey, Goldberg & Grismer, 2018 and *A. paucipapillosa* Wang, 1980 [22–24].

Among the four species of *Aplectana* recorded in China, the new species can be easily distinguished from *A. hylae* and *A. macintoshii* by the position of the excretory pore (located at anterior edge of oesophageal bulb in *A. xishuangbannaensis* vs slightly posterior to nerve ring in *A. hylae* and at the middle position between nerve ring and oesophageal bulb in *A. macintoshii*). Without somatic papillae, *A. hainanensis* and *A. paucipapillosa* differs from *A. xishuangbannaensis* n. sp. (with somatic papillae in both sexes). Moreover, *A. xishuangbannaensis* n. sp., lacking of gubernaculum, is different from all the above-mentioned Chinese species of *Aplectana* (all possessing gubernaculum) [20, 22, 23].

In the genus *Aplectana*, the following species have no gubernaculum, which are similar to the new species, including *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. dubrajpuri* Sou & Nandi, 2015, *A. meridionalis* Lent & Freitas, 1948, *A. papillifera* (Araujo, 1977), *A. praeputialis* (Skrjabin, 1916), *A. tarija* Ramallo, Bursey & Goldberg, 2007, *A. vercammeni* Le Van Hoa, 1962 and *A. hoplobatrachusia* Sou, Sow & Nandi, 2018 [20, 22, 25–35].

*Aplectana xishuangbannaensis* n. sp. is different from *A. dubrajpuri* by the different position of excretory pore (situated at anterior edge of oesophageal bulb in *A. xishuangbannaensis* vs at the middle position between nerve ring and oesophageal bulb in *A. dubrajpuri*) and the presence of somatic papillae in female (vs absence of somatic papillae in female in *A. dubrajpuri*). With only one pair of precloacal papillae, *A. tarija* can be easily differentiated from the new species (*A. xishuangbannaensis* n. sp. with 7 pairs of precloacal papillae). *Aplectana artigasi*, *A. chilensis*, *A. crucifer*, *A. praeputialis*, *A. vercammeni* and *A. hoplobatrachusia* differs from *A. xishuangbannaensis* n. sp. by having relatively longer spicules (spicules representing 9.10–15.2% of body length in the former species vs spicules representing 5.98–7.09% of body length in *A. xishuangbannaensis* n. sp.). *Aplectana papillifera* can be easily distinguished from the new species by having larger female body (5.90–8.50 mm in *A. papillifera* vs 3.54–3.86 mm in *A. xishuangbannaensis* n. sp.), relatively shorter female tail (representing 4.47–5.59% of body length in *A. papillifera* vs representing 9.78–11.1% of body length in the new species) and different arrangement and number of caudal papillae (precloacal 10 pairs, paracloacal 1–2 pairs and postcloacal 8 pairs in the former vs precloacal 6 pairs, paracloacal 3 pairs and postcloacal 4 pairs in *A. xishuangbannaensis* n. sp.).
The new species differs from *A. crossodactyli* by having relatively longer spicules (representing 5.98–7.09% of body length in *A. xishuangbannaensis* n. sp. vs representing 3.78–4.64% of body length in *A. crossodactyli*), less precloacal papillae (precloacal 6 pairs in the new species vs precloacal 20 pairs in the latter) and different position of excretory pore (situated at anterior edge of oesophageal bulb in *A. xishuangbannaensis* vs at the middle position between nerve ring and oesophageal bulb in *A. crossodactyli*). *Aplectana xishuangbannaensis* n. sp. can be easily distinguished from *A. akhrami* by having different position of vulva (vulva from anterior extremity representing 44.8–54.5% of body length in the new species vs vulva from anterior extremity representing 29.0–30.6% of body length in *A. akhrami*) and much longer female tail (0.35–0.41 mm, representing 9.78–11.1% of body length in *A. xishuangbannaensis* n. sp. vs 0.16 mm, representing 4.44–5.33% of body length in *A. akhrami*). The new species can be differentiated from *A. meridionalis* by the morphology of male tail (tail tapering abruptly at about proximal second to a long caudal filament in *A. xishuangbannaensis* n. sp. vs tail tapering abruptly at about proximal seventh to a long caudal filament in *A. meridionalis*) and the different position of excretory pore (situated at anterior edge of oesophageal bulb in *A. xishuangbannaensis* vs at about the middle position between nerve ring and oesophageal bulb in *A. meridionalis*). Moreover, *A. meridionalis* was reported from *Ceratophrys americana* and *Pleurodema borelli* (Anura: Leptodactylidae) in the Neotropical region (Uruguay and Argentina) [20, 26]. However, our specimens were collected from *Polypedates megacephalus* (Hallowell) (Anura: Rhacophoridae) in the Oriental region (Yunan Province, China).

The current species identification of *Aplectana* remains mainly based on the morphological methods. The genetic data of species of *Aplectana* are severely insufficiency. The 18S, ITS1, 28S and cox1 regions of *A. xishuangbannaensis* n. sp. were sequenced and analyzed in the present study. The results displayed no intraspecific nucleotide differences in 18S, ITS-1, 28S and cox1 regions among different individuals, but a high level of interspecific genetic variation in these regions among species of the other genera in the Cosmocercidae. Our genetic data of *A. xishuangbannaensis* n. sp. obtained herein can be used for molecular identification of *Aplectana* species and phylogeny of the Cosmocercidae.

Our phylogenetic results are largely congruent with the traditional classifications of the Cosmoceroidea, which have been proposed based mainly on morphological characters and ecological traits, including the structure of the oesophagus, the presence or absence of precloacal sucker, the morphology of caudal papillae, the host-type, the form of female reproductive organs and the mode of reproduction [1, 2, 36]. The results supported the Cosmocercidae and Atractidae to be monophyletic groups with strong support from both Maximum likelihood (ML) and Bayesian inference (BI) analyses. However, the Kathlaniidae was not monophyly, and the representatives of the sampled Kathlaniidae were divided into two distinct clades (clade II including the genus *Cruzia* and clade III including the genera *Falcaustra* and *Megalobatrachonema*).

According to Chabaud (1978) [1], the family Kathlaniidae currently includes three subfamilies, namely Kathlaniinae, Cruziinae and Oxyascaridinae. Baker & Vaucher (1985) [37] considered that the Oxyascaridinae (only including the genus *Oxyascaris*) should be transferred into the Cosmocercidae, and
treated as a synonym of the subfamily Cosmocercinae, based on the morphological characters. This proposal was accepted by Bursey & Goldberg (2007) [38] and González & Hamann (2008) [39]. Unfortunately, we could not test Baker & Vaucher's (1985) [37] proposal in the present study, because of suitable material of Oxyascariidae unavailable.

The systematic position of the subfamily Cruziinae has long been under debate. Travassos (1917) [40] erected the family Cruziidae for the reception of the genus *Cruzia* and considered this family has close relationship with the Kathlaniidae. Later, Ortlepp (1924) [41] treated it as a subfamily of Kathlaniidae. Subsequently, Khalil (1927) [42] rejected the validity of the family Cruziidae or subfamily Cruziinae, but he recognized the genus *Cruzia* to be a member of the Kathlaniidae. However, Skrjabin et al. (1961) [5] considered the Cruziidae/Cruziinae should be placed in the Atractoidea/Atractidae. Chabaud (1978) [1] questioned Skrjabin's et al. (1961) [5] treatment and accepted Cruziinae as a separate subfamily in the Kathlaniidae. Our molecular phylogenetic results conflicted with these traditional opinions, which supported 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 pharynx (the presence of unique pharyngeal lamellae) and the unique digestive system (the presence of intestinal caecum) of this group supported its full family-status [43]. However, only one species of the Cruziinae was included in our phylogenetic analyses, thus we do not make any immediate systematic changes in the Cosmoceroidea, because a more rigorous molecular phylogenetic study with broader representatives of the Cruziinae using different nuclear and/or mitochondrial genetic markers (including mitochondrial genomic data) is required to further ascertain its systematic position.

The Cosmocercidae currently includes approximately 200 nominal species placed in more than 20 genera, which is the largest family in the superfamily Cosmoceroidea [1, 3, 21, 44]. However, the phylogenetic analyses at the generic level are generally lacking in the Cosmocercidae, due to the paucity and inaccessibility of suitable material. According to Chabaud (1978) [1] and Gibbons (2010) [44], the morphology of caudal papillae in male is one of the most important genetic diagnosis characters in the Cosmocercidae. Species of the genus *Aplectana* have no modified papillae (plectanes and/or rosette papillae), but species of the genera *Cosmocerca* and *Cosmocercoides* have modified papillae (plectanes and/or rosette papillae). Wilkie (1930) [45], Skrjabin et al. (1961) [5] and Chabaud (1978) [1] all considered these genera with modified papillae (plectanes and/or rosette papillae) seem to have close relationships in the Cosmocercidae. However, our molecular phylogenetic results supported the genus *Cosmocerca* has closer relationship to the genus *Aplectana*, rather than the genus *Cosmocercoides* in the Cosmocercidae, which are conflicted with the traditional views on the systematics of these groups.

**Conclusions**

Our knowledge of the phylogeny of the Cosmoceroidea and its included families/genera is still far from comprehensive. The present molecular phylogenetic results supported that the subfamily Cruziinae should be moved out from the hitherto-defined family Kathlaniidae and elevated to a separate family, and the genus Cosmocerca has closer relationship to the genus *Aplectana* in the family Cosmocercidae.
present study provided the basic molecular phylogenetic framework for the superfamily Cosmoceroidea based on 18S + 28S sequence data for the first time. Moreover, a new species of *Aplectana, A. xishuangbannaensis* n. sp., was described using an integrative approach.

**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; *cox*1: cytochrome *c* oxidase subunit 1; dl, dorsal lip; vl, ventrolateral lip; cp, large double cephalic papillae; pp, paracloacal papilla.

**Declarations**

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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 xishuangbannaensis* n. sp. obtained in this study were deposited in the GenBank database under the accession numbers MW329041–MW329043 (*18S* sequences), MW329035–MW329037 (*ITS*-1 sequences), MW329038–MW329040 (*28S* sequences), MW327586–MW327588 (*cox*1 sequences). Type specimens of the new species were deposited in College of Life Sciences, Hebei Normal University, Hebei Province under the accession numbers HBNU–N-2020A009–11L, China.
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.

Author details

1Key Laboratory of Animal Physiology, Biochemistry and Molecular Biology of Hebei Province, College of Life Sciences, Hebei Normal University, 050024 Shijiazhuang, Hebei Province, People’s Republic of China.

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**Figures**
Figure 1

Scanning electron micrographs of Aplectana xishuangbannaensis n. sp. collected from Polypedates megacephalus (Hallowell) (Anura: Rhacophoridae) in Yunnan Province, China. a Cephalic end of female (amphids arrowed), subapical view. b Anterior part of male (lateral ala arrowed), lateral view. c Magnified image of somatic papilla and longitudinal stockade-like ornamentation of cuticle of female. d Cephalic end of male (inner flanges arrowed), subapical view. e Posterior end of male (precloacal papillae
arrowed), lateral view. f Tail of male (lateral ala arrowed), lateral view. g Tail of male (four pairs of postcloacal papillae arrowed), ventral view. h Magnified image of single, median precloacal papilla. i Tail of female (somatic papillae arrowed).

Figure 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 Cosmocercoidea. Ascaris lumbricoides Linnaeus, 1758 (Ascaridida: Ascaridoidea) was chosen as the outgroup. Bootstrap values exceeding 70% were showed.