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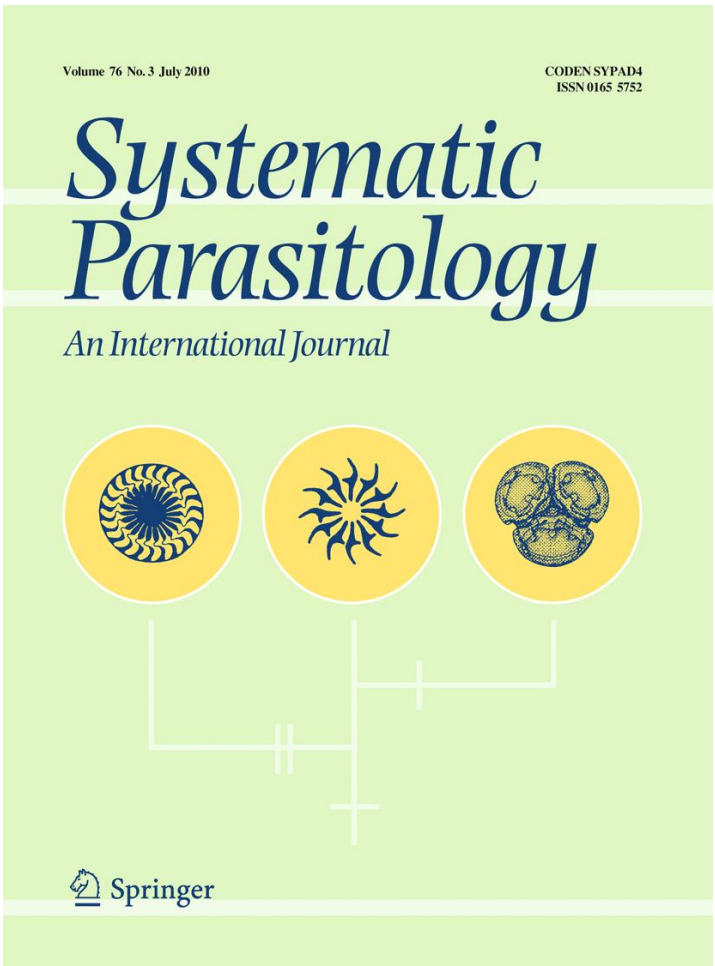

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Serpentoanisocladium sinense n. g., n. sp. (Digenea: Cryptogonimidae) from the eastern water snake *Sinonatrix percarinata* (Boulenger) (Serpentes: Colubridae) in Guizhou Province, China

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Abstract *Serpentoanisocladium sinense* n. g., n. sp. (Digenea: Cryptogonimidae) is described from the intestine of the eastern water snake *Sinonatrix percarinata* (Boulenger) (Serpentes: Colubridae) from Guizhou Province, China. This digenean is morphologically most similar to members of *Anisocladium* Looss, 1902 and, to a lesser extent, *Anisocoelium* Lühe, 1900, which are parasites of marine teleost fish in the Mediterranean and Black Seas. The new genus and species can be differentiated from the two known species of *Anisocladium* by the position of the vitellarium in relation to the gonads and seminal vesicle, a much longer oesophagus, a shorter caecum only reaching the anterior margin of the anterior testis, the presence of a muscular sucker-like gonotyl, the lack of circumoral spines and the peculiar position of the uterus, which is confined to the same half of the body as the longer caecum. The new genus and species can be differentiated from the only known species of *Anisocoelium* by the substantially higher body length to width ratio, a much longer oesophagus, the arrangement of the vitelline follicles, the postovarian versus pre-ovarian seminal receptacle, the presence of a well-defined muscular gonotyl and the length of the shorter caecum. A diagnosis of the new genus and a description of the new species are provided. This is the first cryptogonimid found in snakes from China and the first cryptogonimid reported from *S. percarinata*.

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Introduction

The Cryptogonimidae Ward, 1917 is a large, globally distributed digenean family comprising at least 64 genera with more than 200 species found in marine and freshwater fishes, amphibians and reptiles (Miller & Cribb, 2008). Most cryptogonimids are parasitic in fish; the relatively few species that do infect reptiles belong to five genera (*Proctocaecum* Baugh, 1957; *Timoniella* Rebecq, 1960; *Caimanicola* Freitas & Lent, 1938; *Acanthostomum* Looss, 1899; and, rarely, *Neochasmus* Van Cleave & Mueller, 1932; for references, see Brooks, 1980; Miller & Cribb, 2008). Almost all cryptogonimids known from reptiles belong to the former subfamily Acanthostominae Poche, 1926; however, in the most recent treatment of the Cryptogonimidae in the "Keys to the Trematoda" (Miller & Cribb, 2008) cryptogonimids are no longer separated into subfamilies due to insufficient knowledge of the phylogenetic relationships between lineages within the family. Most cryptogonimids parasitic in reptiles have been reported from crocodilians and turtles, but a few species are known from

snakes in Mexico, Burma, India, Bangladesh, Malaysia and Venezuela (Bennett, 1935; Brooks, 1980; Brooks & Holcman, 1993; Miller & Cribb, 2008). The eight or nine species of cryptogonimids from snakes are known from three genera, namely *Acanthostomum* (*A. megacetabulum* Thatcher, 1963; *A. burminis* Bhalerao, 1926; *A. simhai* Khalil, 1963; *A. pakistanensis* Coil & Kuntz, 1960; *A. asymmetricum* Simha, 1958; *A. proctophorum* Dwivedi, 1966; plus one undescribed species reported by Brooks (1980) from the collection of John Holmes), *Timoniella* (*T. incognita* Brooks, 1980) and *Neochasmus* (*N. labeosus* Bennett, 1935, considered a *nomen nudum* by Hughes et al. (1942), but still a record of a cryptogonimid in snakes).

As part of a survey of biodiversity of terrestrial vertebrates and their parasites in south-eastern China, we examined a snake, *Sinonatrix percarinata* (Boulenger), collected in Guizhou Province. It harboured, among other parasites, several digenean specimens that represented a new species and new genus within the family Cryptogonimidae. To the best of our knowledge, the new species described herein, is the fourth cryptogonimid genus reported from snakes and the first cryptogonimid reported from *Sinonatrix*.

Materials and methods

During April, 2007, one individual of the eastern water snake *Sinonatrix percarinata* (Boulenger) was collected in the vicinity of the Shuipu Village, Libo County, Guizhou Province, China (25°29′4.74″N, 107°49′4.80″E). The reptile was collected as a part of a biodiversity survey and inventory project along the southern borderlands of China. Upon capture, the snake was killed with nembutal. Its intestine was removed, flash-frozen in liquid nitrogen, shipped to the US and dissected in the laboratory upon thawing. Seven specimens of a new digenean species belonging to the Cryptogonimidae were recovered from the intestine. Worms were fixed in AFA and subsequently stained with aqueous alum carmine or Mayer's haematoxylin, dehydrated in a graded ethanol series, cleared in methyl salicylate (after haematoxylin) or clove oil (after carmine) and mounted permanently in Damar gum. Type-specimens were deposited in the US National Parasite Collection (USNPC). One specimen of the new species was used for DNA extraction and sequencing; the results of a phylogenetic analysis will be published separately.

Measurements were taken from Olympus BX51 compound microscope with DIC optics using digital imaging and Rincon measurement software (v. 7.1.2, Imaging Planet, Goleta, California). All measurements are in micrometres unless otherwise stated.

Serpentoanisocladium n. g.

Diagnosis

Body very elongate; length/width ratio 10.1–14.9:1. Oral sucker subterminal to terminal, unarmed, larger than ventral sucker. Forebody 18–27% of body-length. Prepharynx very short; oesophagus very long; intestinal bifurcation immediately anterior to ventral sucker; caeca blind, unequal; shorter caecum not extending posteriorly beyond anterior margin of anterior testis; longer caecum ends posterior to posterior testis, but does not reach posterior end of body. Testes two, tandem, entire, separate, in hindbody. Seminal vesicle free in parenchyma, bipartite, with its base at level of anterior margin of vitellarium. Genital pore median, just anterior to ventral sucker. Muscular gonotyl sucker-like, median, immediately anterior to ventral sucker. Ovary spherical, entire, midway between anterior testis and seminal vesicle, in middle third of body. Seminal receptacle median, just posterior to ovary. Vitellarium consists of 4 lateral groups of medium-sized follicles, 2 on either side of body; vitelline follicle groups extend from base of seminal vesicle to end of shorter caecum, separated on each side of body by gap at level of ovary. Uterus in hindbody, reaches posterior extremity of body; both descending and ascending parts of uterus extracaecal on one side of body only, always on same side as longer caecum. Eggs numerous. In snakes (Colubridae); China. Type-species *S. sinense* n. sp.

Serpentoanisocladium sinense n. sp.

Type-host: Eastern water snake *Sinonatrix percarinata* (Boulenger, 1899) (Reptilia: Serpentes: Colubridae).

Type-locality: Shuipu Village, Libo County, Guizhou Province, China (25°29′4.74″N, 107°49′4.80″E).
Prevalence and intensity of infection: One snake was infected with 7 digeneans.
Site: Intestine.
Type-material: Holotype: USNPC no. 102783; paratypes: USNPC no. 102784 (3 slides). All specimens labeled ex *Sinonatrix percarinata*, Shuipu Village, Libo County, Guizhou Province, China, Coll. S. Bush, V. Tkach.
Etymology: The generic name refers to morphological similarity between the new genus and *Anisocladium* and its parasitism in snakes (as opposed to fish in case of *Anisocladium*). The specific epithet refers to the country where the new species was found (China).

Description (Fig. 1)

[Measurements based on 5 adult specimens; measurements of holotype given first, followed by entire series in parentheses (minimum–maximum followed by mean values). Not all measurements could be taken from 2 specimens due to distortion.]

Body very elongate, with almost parallel lateral margins and rounded at both ends; body length 2,804 (2,622–2,865; 2,754); maximum body width 279 (187–279; 239). Body length/width ratio 10.5:1 (10.1–14.9:1; 11.8:1). Oral sucker and tegument unarmed; there are no signs of lost spination on oral sucker, although tegumental spines might have been lost. Oral sucker terminal or subterminal, 111 × 115 (98–116 × 89–115; 106 × 102), substantially larger than ventral sucker. Ventral sucker round, 68 × 70 (68–74 × 64–76; 71 × 68), in anterior third of body.

Prepharynx very short, but distinct, 15.6 (15–17; 16) long, wider than oesophagus. Pharynx small, 46 × 49 (40–54 × 45.5–50; 45 × 48). Oesophagus very thin and long, 374 (295–495; 388). Intestinal bifurcation just anterior to ventral sucker. Caeca blind, unequal; shorter caecum not extending posteriorly beyond anterior margin of anterior testis; longer caecum extends posteriorly to posterior testis, but does not reach posterior end of body, terminating 313 (280–470; 354) from it.

Testes 2, tandem, spherical to subspherical, postovarian, midway between seminal vesicle and posterior end of body; anterior testis 181 × 139 (168–204 × 113–139; 181 × 126); posterior testis 178 × 131 (157–225 × 125–146; 184 × 133). Seminal vesicle free in parenchyma, bipartite, with its base at level of anterior margin of vitellarium and its distal part tortuous. Pars prostatica not observed. Genital pore median, just anterior to ventral sucker. Muscular gonotyl sucker-like, median, immediately anterior to ventral sucker.

Ovary spherical, entire, 103 × 103 (103–141 × 97–110; 118 × 102), midway between anterior testis and seminal vesicle, in middle third of body. Seminal receptacle 113 × 71(88–125 × 64–94; 109 × 77), median, just posterior to ovary and usually partly overlapping it. Vitellarium consists of 4 lateral groups of medium-sized follicles, 2 on either side of body, in middle third of body; vitelline follicle groups extend from base of seminal vesicle to end of shorter caecum or anterior margin of anterior testis, separated on each side of body by gap at level of ovary. Uterus in hindbody, reaches posterior extremity of body; both descending and ascending parts of uterus mostly extracaecal on one side of body only, always on same side as longer caecum. Metraterm indistinct. Eggs numerous, operculate, 22 × 13 (21–24 × 13–14.5; 22.5 × 13.5). Excretory vesicle not observed.

Remarks

Based on its general morphology, the new species clearly corresponds to the diagnosis of the Cryptogonimidae according to Miller & Cribb (2008). At the same time, it differs substantially from members of all known cryptogonimid genera in several significant morphological features. The new species is morphologically closest to *Anisocladium* Looss, 1902 and, to a lesser extent, *Anisocoelium* Lühe, 1900.

Despite the fact that digeneans were obtained from a flash-frozen snake intestine, which may result in loss of circumoral and tegumental spines, we are confident that this species lacks circumoral spines. Upon very careful examination with DIC optics at high magnification, no signs of spine loss were found. Normally, when the larger circumoral/collar spines are lost in members of the Cryptogonimidae and Echinostomatidae, the spine placeholders are still visible. Besides, in the same intestine, we found several other digeneans that have circumoral spines and they did not lose them. In contrast, tegumental spines are more easily lost during freezing and, if the

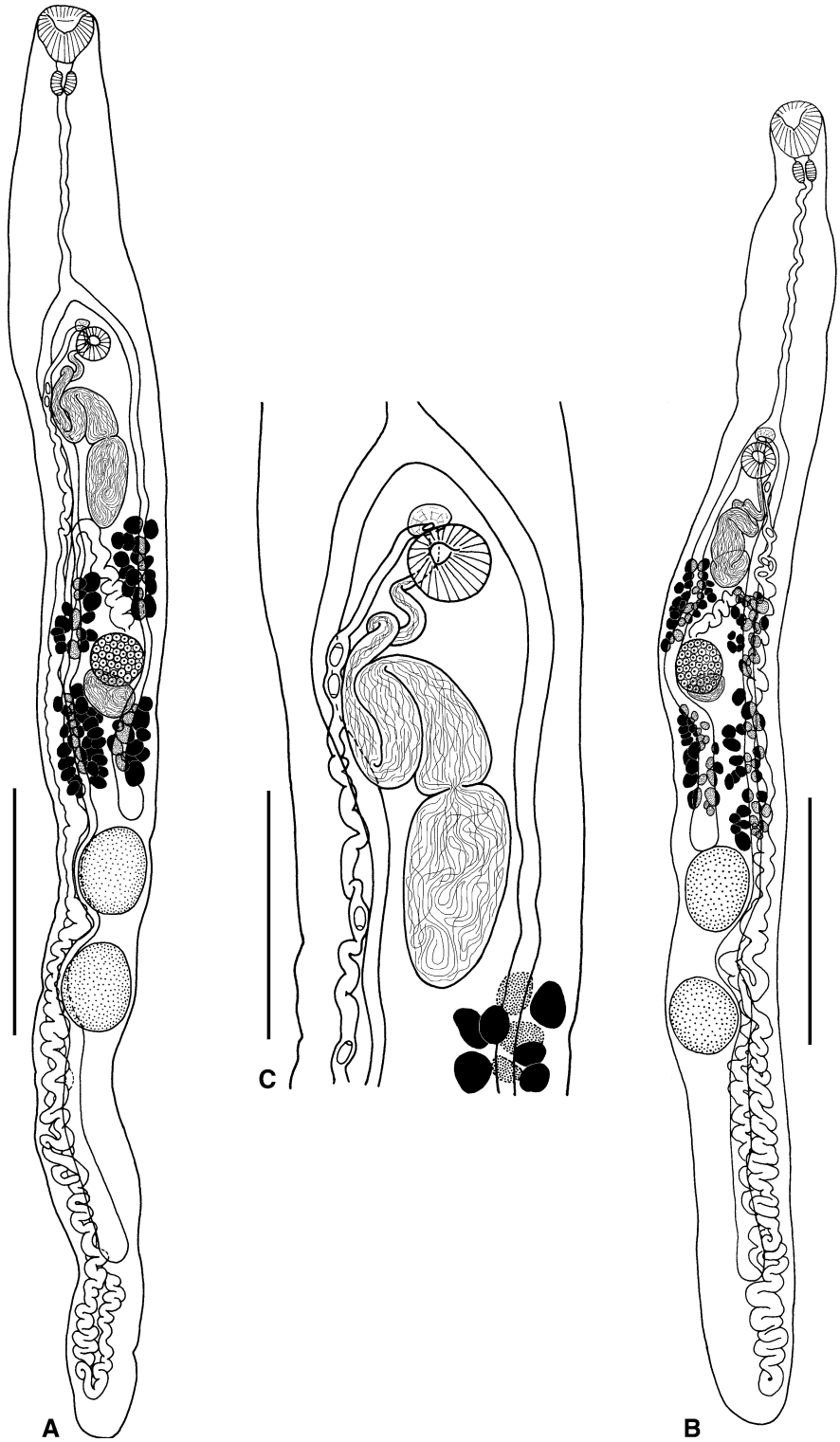

Fig. 1 *Serpentoanisocladium sinense* n. g., n. sp. A. Ventral view of holotype; B. Ventral view of a paratype; note the 'mirror' arrangement of caeca and reproductive system organs in the two specimens; C. Region of ventral sucker and distal parts of reproductive system in the holotype. *Scale-bars*: A,B, 500 μm; C, 200 μm

new species had tegumental spines, they might have been lost. Freshly collected specimens would be necessary to verify that this species lacks tegumental spines. We would like to emphasize, however, that the differentiation between the new species and members of known genera presented below, is not based on presence or absence of circumoral/tegumental spines; other morphological characters provide sufficient basis for our taxonomic conclusions.

The new species is most similar to *Anisocladium* and shares with this genus several features, such as a very elongated body, unequal caeca, the extent and position of the uterus and the vitelline follicles grouped in grape-like clusters. Moreover, one of the two known *Anisocladium* species, namely *A. gracile* (Looss, 1901), lacks enlarged circumoral spines, which is similar to our new species. Bartoli & Gibson (2000) provided detailed morphological re-descriptions of both *Anisocladium* species (*A. gracile* (Looss, 1901) and the type-species, *A. fallax* (Rudolphi, 1819)) and discussed their morphology and taxonomy. Despite the above-mentioned similarities, the new species differs substantially from both members of *Anisocladium* in having a much lower body length to width ratio (10.1–14.9:1 in the new species *vs* 34:1 on average in *A. fallax* and 24:1 on average in *A. gracile*), a much longer oesophagus (it is very short in both *Anisocladium* species), a distinct arrangement of the vitelline follicles in four lateral groups extending between the seminal vesicle and the anterior testis (the vitelline fields in *Anisocladium* are entirely pre-ovarian) and a postovarian seminal receptacle (it is anterior to the ovary in *Anisocladium*). The shorter caecum only reaches the anterior testis in the new species, but extends to the posterior testis in *Anisocladium*.

The new species differs substantially from the only known species of *Anisocoelium*, *A. capitellatum* (Rudolphi, 1819), in having a substantially greater body length to width ratio (10.1–14.9:1 in the new species *vs* 6.7:1 on average in *A. capitellatum*), a much longer oesophagus (it is very short in *A. capitellatum*), a distinct arrangement of the vitelline follicles in four lateral groups extending between the seminal vesicle and the anterior testis (the vitelline fields in *Anisocoelium* are entirely pre-ovarian) and a postovarian seminal receptacle (it is anterior to the ovary in *Anisocoelium*). The shorter caecum only reaches the anterior testis in the new species, but extends posteriorly well beyond the posterior testis in *Anisocladium*.

The new species is somewhat similar to some members of the former genus *Atrophecaecum* Bhalerao, 1940 (now synonymised with *Acanthostomum* Looss, 1899) in possessing unequal caeca and having the seminal receptacle posterior to the ovary, but differs dramatically from the latter genus in most other features, such as the position of the gonads and uterus, the distribution and shape of the vitellarium, body proportions, the lack of oral sucker armament and other characters.

The combination of the above-mentioned differentiating characters, especially the shape and position of the vitellarium, the postovarian seminal receptacle and the presence of a gonotyl clearly separates the new species from members of all previously known cryptogonimid genera. Furthermore, the two morphologically most similar genera, *Anisocladium* and *Anisocoelium*, are known exclusively from marine fish in the Mediterranean and Black Seas. The new species was found in a semi-aquatic snake in China, which makes a close relationship with these two genera highly unlikely. Therefore, we establish a new genus, *Serpentoanisocladium* n. g., with *S. sinense* n. sp. as the type-, and currently only, species.

Discussion

Serpentoanisocladium sinense n. g., n. sp. is the first cryptogonimid found in snakes from China and the first cryptogonimid reported from *Sinonatrix percarinata*.

The most unusual morphological feature of the new species is that the vitelline follicles are arranged in four lateral groups, which, to the best of our knowledge, has not been reported in any other cryptogonimid. Although members of *Anisocladium* also have relatively compact groups of vitelline follicles, their position in the body is very different. The morphological similarity of *Serpentoanisocladium sinense*, a parasite of freshwater semi-aquatic snakes to the two genera from marine fishes, appears to be a case of phenotypic convergence rather than due to a close evolutionary relationship. The postovarian position of the seminal receptacle in the new species (*vs* pre-ovarian in both *Anisocladium* and *Anisocoelium*), the presence of a muscular gonotyl and the arrangement of

the vitellarium suggest that these taxa most probably belong to different lineages.

We suggest that, most probably, the new species is phylogenetically close to some other cryptogonimids from snakes, such as former members of *Atrophecaecum* (now merged with *Acanthostomum*) found in several Asian countries. We have obtained DNA sequences from the new species, but, in the absence of sequences or fresh material of *Anisocladium* and *Anisocoelium* as well as from other potentially related genera, our sequences are not able to resolve the relationships between these genera at the present time. Patterns of host associations and the geographical distribution of cryptogonimids in reptiles suggest that colonisation of reptiles (and snakes in particular) by cryptogonimids occurred on multiple occasions in the evolutionary history. However, it would be premature to speculate on this subject before a robust phylogeny of the group is available. Miller & Cribb (2008), in their recent treatment of the Cryptogonimidae, have abandoned all subfamilies previously recognised in this family due to obvious difficulties in their differentiation and the uncertain relative 'weight' of various morphological characters used in the systematics of this group. We support this decision and anticipate that a combined molecular and morphological approach will allow clarification of numerous systematic problems in the Cryptogonimidae and its constituent genera, and will illuminate many questions of their evolution and host associations.

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References

Bartoli, P., & Gibson, D. I. (2000). Three little known acanthostomine digeneans from *Uranoscopus scaber* L. in the western Mediterranean. *Systematic Parasitology, 46*, 123–141.

Bennett, H. J. (1935). Four new trematodes from reptiles. *Journal of Parasitology, 21*, 83–90.

Brooks, D. R. (1980). Revision of the Acanthostominae Poche, 1926 (Digenea: Cryptogonimidae). *Zoological Journal of the Linnean Society, 70*, 313–382.

Brooks, D. R., & Holcman, B. (1993). Revised classification and phylogenetic hypothesis for the Acanthostominae Looss, 1899 (Digenea: Cryptogonimidae). *Proceedings of the Biological Society of Washington*, 106, 207–220.

Hughes, R. C., Higginbotham, J. W., & Clary, J. W. (1942). The trematodes of reptiles. Part I. Systematic section. *American Midland Naturalist, 27*, 109–134.

Miller, T. I., & Cribb, T. H. (2008). Family Cryptogonimidae Ward, 1917. In: Bray, R. A., Gibson, D. I., & Jones, A. (Eds) *Keys to the Trematoda* Vol. 3. Wallingford: CAB International, pp. 51–112.