SHORTER CONTRIBUTIONS

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The First Karyotypic Report of the Genus *Caudacaecilia* with Comments on its Generic Validity (Amphibia, Gymnophiona, Ichthyophiidae)

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The karyotype of the genus *Caudacaecilia* is first reported on the basis of *C. asplenia* from Sabah, Malaysia. The species has 2n = 42 chromosomes, consisting of two pairs of biarmed macrochromosomes, eight pairs of biarmed microchromosomes, and 11 pairs of uniarmed microchromosomes. These karyotypic characteristics do not differ from those of *Ichthyophis*, the other genus of Ichthyophiidae. We also provide the first documented account of the presence of splenial teeth in larval *Caudacaecilia*.

THE family Ichthyophiidae is distributed from South to Southeast Asia (Taylor, 1968) and includes 38–39 species belonging to two genera, *Caudacaecilia* and *Ichthyophis* (Frost, 2004; AmphibiaWeb, 2005). Although species of *Ichthyophis* have been well studied (Sarasin and Sarasin, 1885; Seshachar, 1936; Badenhorst, 1978) and have recently been examined in a phylogenetic context (Gower et al., 2002; Kupfer and Müller, 2004), comparatively little study has been done for *Caudacaecilia*.

The chromosomal study of caecilians was initiated long ago by Seshachar (1937) using an ichthyophiid, *Ichthyophis glutinosus*, which was reidentified as *I. beddomei* by Wake and Case (1975). Karyological knowledge of this family, however, is still very limited (Seto and Nussbaum, 1976; Nussbaum and Treisman, 1981). Four species of *Ichthyophis* (*I. beddomei*, *I. glutinosus*, *I. kohtaoensis*, and *I. orthoplicatus*) have been examined karyologically, however, no study has been undertaken for members of *Caudacaecilia*.

Caudacaecilia is a small genus established by Taylor (1968) and consists of five species: *C. asplenia* (southern Thailand, Sarawak, and doubtfully in Sri Lanka: see Taylor, 1965, 1968), *C. larutensis* (Malay Peninsula), *C. nigroflava* (Malay Peninsula, Sarawak, Kalimantan, and Sumatra), *C. paucidentula* (Sumatra), and *C. weberi* (Palawan). Taylor (1968) designated this genus on the basis of the absence of splenial teeth in adults, in contrast to species of *Ichthyophis* that have these teeth. No other characters are known to differentiate these two genera.

In this study, we describe a karyotype of *Caudacaecilia* based on specimens collected from Sabah, northern Borneo, Malaysia. The record of this genus is new for the region. We compare chromosomal and morphological characteristics of our specimens with other species of Ichthyophiidae and discuss the validity of the genus *Caudacaecilia*.

MATERIALS AND METHODS

We collected one immature individual and five larvae (with open gill slits and tail fin present) from a small tributary of Tawau River in Tawau Hills National Park, Sabah, Malaysia (4°27′N, 117°57′E; approximately 120 m) on 31 July and 1 August 2004 (Fig. 1). We kept the specimens for 12 h at room temperature (about 23 C) after an intraperitoneal injection of colchicine solution (0.2 mg/ml). After sufficiently anesthetizing animals with acetone chloroform saturated solution, we dissected the gonads to determine sex and maturity and removed liver tissues for genetic analyses and intestines for karyological analyses. Voucher specimens are stored at the Institute for Tropical Biology and Conservation, University Malaysia Sabah (BORNEENSIS 9226-9231).

Chromosomes were studied on squashed preparations of intestinal epithelium as described by Kezer and Sessions (1979) with a minor modification. Colchicine arrested metaphase chromosomes were stained with conventional 4% Giemsa solution. In the following karyological description, the nomenclature follows Green et al. (1980).

In order to identify species, we measured the following characters: total length (TL); tail length (TAL); head width at jaw angle (HW); body width at middle (BWM); interorbital distance (IOD); internarial distance (IND);

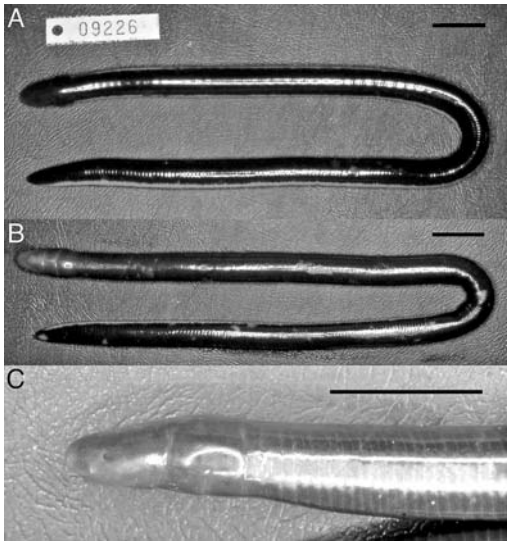

Fig. 1. A metamorph of caecilian collected from Tawau Hills National Park, Sabah, Malaysia. (A) Dorsal view, (B) ventral view, (C) lateral view of the head. Scale bar = 10 mm.

eye–nostril distance (END). Eye–tentacle distance (ETD) and tentacle–nostril distance (TND) were measured only for the metamorph as the larvae lacked them. We also counted the following characters: total annuli (TA); premaxillary–maxillary teeth (PMM); vomeropalatine teeth (VP); dentary teeth (DE); splenial teeth (SP); vertebrae (VER). These measurements and counts were made based on Kupfer and Müller (2004) with minor modifications. Measurements were taken to the nearest 0.1 mm with a dial caliper. The number of vertebrae was counted from soft X-ray photographs using Fuji Medical X-Ray Film.

Results

Karyotype.—We obtained a total of 12 well-spread metaphase plates for all six individuals examined. The diploid chromosome number was 2n = 42 (Fig. 2). No marked variation between individuals was observed. The 21 homologous pairs could be divided into four groups by size and shape (Table 1). The first group contained large chromosomes (Nos. 1 and 2), both of which were metacentric. Pairs 3–6 composed the second group of medium chromosomes of nearly equal size. This group contained one subtelocentric (No. 3) and three metacentric chromosomes. The remaining two groups included microchromosomes; four pairs of the third group (Nos. 7–10) were metacentric, and

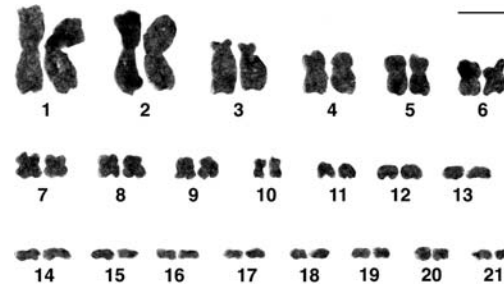

Fig. 2. The Giemsa stained karyotype of a caecilian specimen from Sabah, Malaysia (BORNEENSIS 9226). Scale bar = 10 μm.

11 of the fourth group (Nos. 11–21) were uniarmed.

Morphology.—Measurements and counts of morphological characters of six specimens are shown in Table 2. Eye–tentacle distance and TND, measured only for the metamorph, were 0.8 mm and 1.9 mm, respectively. All specimens had distinct yellow stripes on lateral sides of the body from below eye on upper jaw to upper center of cloacal disc. The metamorph proved to be an immature female and lacked splenial teeth. Although the larvae did have splenial teeth, they had identical mitochondrial DNA sequences (cytochrome *b*, 12sRNA, and 16sRNA) to the metamorph (Matsui et al., unpubl. data). The metamorph had 282 body folds that were incomplete dosally at the anterior two-ninths of the body. The number of vertebrae varied from 105 to 109 (Table 2) and was uncorrelated with the total length.

Discussion

Identification.—Because the metamorph and larvae were collected simultaneously from a restricted portion of a small stream, and because our preliminary genetic analyses demonstrated that DNA sequences were identical, we believe they are conspecific. Although all larvae had splenial teeth, the single metamorph collected lacked them. Thus, presence in larval *Caudacaecilia* of splenial teeth that are lost after metamorphosis, as suggested by Taylor (1968), is confirmed for the first time by these data.

Among *Caudacaecilia*, *C. asplenia*, *C. nigroflava*, and *C. paucidentula* have yellowish stripes on the lateral body surfaces (Taylor, 1968) like those specimens collected in the present study. Of these, *C. paucidentula* has inconspicuous and partly broken stripes and is only known from Sumatra. The number of vertebrae in our

TABLE 1. MEDIANS AND RANGES (IN PARENTHESES) OF QUANTITATIVE CHROMOSOMAL CHARACTERS IN *Caudacaecilia asplenia* EXAMINED IN THIS STUDY. *Arm ratio = length of long arm/length of short arm. **Centromere position is based on the definitions of Levan et al. (1964). m = metacentric, st = subtelocentric, t = telocentric.

Chromosome pair no.	Relative length	Arm ratio*	Centromere position**	Classified group
1	16.2 (14.6–17.8)	1.0 (0.7–1.4)	m	1
2	15.5 (13.7–17.3)	1.2 (1.0–1.3)	m	1
3	9.7 (9.1–10.2)	3.9 (3.6–4.1)	st	2
4	7.5 (7.2–7.8)	1.3 (1.1–1.4)	m	2
5	6.8 (6.5–7.2)	1.2 (1.0–1.5)	m	2
6	6.3 (5.8–6.7)	1.0 (0.9–1.2)	m	2
7	4.9 (4.6–5.3)	1.1 (0.7–1.4)	m	3
8	4.5 (4.0–4.9)	1.1 (1.0–1.3)	m	3
9	3.8 (2.9–4.8)	1.4 (1.2–1.7)	m	3
10	3.6 (2.7–4.6)	1.1 (1.0–1.3)	m	3
11	2.8 (2.5–3.1)	–	t	4
12	2.7 (2.4–2.9)	–	t	4
13	2.3 (2.1–2.4)	–	t	4
14	2.1 (1.8–2.3)	–	t	4
15	1.8 (1.5–2.3)	–	t	4
16	1.8 (1.5–2.0)	–	t	4
17	1.7 (1.6–1.8)	–	t	4
18	1.6 (1.5–1.7)	–	t	4
19	1.6 (1.4–1.7)	–	t	4
20	1.6 (1.3–1.6)	–	t	4
21	1.4 (1.3–1.5)	–	t	4

TABLE 2. MEASUREMENTS AND COUNTS OF CAECILIANS EXAMINED IN THIS STUDY. See text for abbreviations. The number of teeth are shown for the right and left side following Taylor (1960). *Specimen number of the BORNEENSIS collection at the Institute for Tropical Biology and Conservation, University Malaysia Sabah; **TA of three larvae could not be counted.

Specimen no.*	Stage	TL	TAL	HW	BWM	IOD	IND	END	TA**	PMM	VP	DE	SP	VER
9226	metamorph	182.5	3.1	6.4	7.5	4.6	2.5	3.2	282	25/23	24/21	20/20	0/0	107
9227	larva	131.0	3.2	5.5	6.3	3.3	3.3	2.4	281	13/13	15/14	14/15	5/5	105
9228	larva	128.5	2.9	5.7	6.4	3.4	2.0	2.1	281	11/12	16/15	15/15	4/3	105
9229	larva	119.1	2.5	4.8	4.8	3.3	1.9	1.9	–	11/13	15/13	13/15	5/5	109
9230	larva	94.1	2.1	3.8	4.3	2.8	1.5	1.5	–	13/13	14/14	15/14	4/4	107
9231	larva	85.2	2.8	3.8	4.2	2.5	1.2	1.6	–	13/13	15/16	13/13	5/5	106

samples (105 to 109) did not overlap with that of *C. nigroflava* (118–123) or *C. paucidentula* (122). Unfortunately, we cannot compare our sample with *C. asplenia*, because the number of vertebrae is presently unknown for the latter species.

According to Taylor (1968), the number of body folds is diagnostic in separating *C. asplenia* (247–270), *C. nigroflava* (389–433), and *C. paucidentula* (384–390). The metamorph had 282 body folds and was closest to *C. asplenia*. Further, Taylor (1965) reported that the body folds are incomplete, at least on the anterior half of the dorsal surface in *C. asplenia*, but are complete in *C. nigroflava* and sometimes in *C. paucidentula*. Our metamorphic sample had body folds that were incomplete, but the degree to which folds failed to meet along the anterior surface was much lower than described in *C. asplenia* (Taylor, 1965). However, this condition may change ontogenetically.

Thus, our samples are morphologically most similar to *C. asplenia* and could be tentatively identified as that species. Regardless of species identification, this is the first record of *Caudacaecilia* from Sabah, Malaysia. Although Sabah is one of the most intensively studied regions in Southeast Asia in terms of batrachian inventories (Inger, 1966; Inger and Tan, 1996; Inger et al., 2000; Malkmus et al., 2002), caecilian diversity is clearly underestimated in this region at present.

Karyotype.—Among ichthyophid karyotypes reported thus far, those of *Ichthyophis glutinosus*, *I. kohtaoensis*, and *I. orthoplicatus* (Seto and Nussbaum, 1976; Nussbaum and Treisman, 1981) are comparable to our data. These species are reported to have 2n = 42 chromosomes just like our sample. Pair three is reported to be submetacentric in *I. kohtaoensis* and *I. orthoplicatus*, but telocentric in *I. glutinosus*. In this regard, our sample differs from all of these species in having a subtelocentric pair three. *Ichthyophis glutinosus* and *I. kohtaoensis* have nine and 11 biarmed chromosomes, respectively, differing from our sample with ten pairs. Although *I. orthoplicatus* has ten biarmed chromosomes (Nos. 1–10) as in our sample, pair nine is submetacentric and pair 10 is subtelocentric. It differs from our sample in which corresponding pairs are all metacentric.

Karyologically, our sample of *C. asplenia* is very similar to *Ichthyophis*, although not completely identical to any species hitherto reported, and no distinct karyological differences are found to separate *Caudacaecilia* from *Ichthyophis*. As noted above, Taylor's (1968) designation of *Caudacaecilia* was made solely on the basis of the absence of splenial teeth in the postmetamorphic stage. Actually, there are several species of *Ichthyophis* that have only two splenial teeth in adults (Taylor, 1968), and morphological differentiation of the two genera by only a single character is highly problematic. Nussbaum and Wilkinson (1989) already discussed this issue and suggested the possible polyphyletic nature of the two genera. In order to evaluate the validity of *Caudacaecilia* more definitively, additional morphological and molecular comparisons among Ichthyophiidae are necessary.

Ichthyophiidae, including *Caudacaecilia*, has the largest number of both total chromosomes and microchromosomes among Gymnophiona (Seto and Nussbaum, 1976). Morescalchi (1973) proposed that primitive species in amphibians have many total chromosomes and microchromosomes and, among Gymnophiona, Ichthyophiidae is estimated to represent the second most primitive lineage (San Mauro et al., 2004). Therefore, the karyological feature of this family does not contradict the evolutionary trend generally observed in amphibians. However, our understanding of the pattern of karyological evolution in caecilians is far from complete because no chromosomal studies have ever been done for the most primitive family, Rhinatrematidae (San Mauro et al., 2004).

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