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Title Morphological variations in *Ichthyophis garoensis* with comparative reference to *Ichthyophis moustakius* (Amphibia: Gymnophiona: Ichthyophiidae)

Authors Sejuti Biswas[1], Kaushik Deuti[1*]

[1]Amphibia & Protochordata Section, Zoological Survey of India, FPS Building, Indian Museum Complex, Kolkata 700016, West Bengal, India

* Corresponding author email: kaushikdeuti@gmail.com

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Ichthyophis garoensis Pillai and Ravichandran,1999 is a striped Asiatic caecilian species belonging to the genus *Ichthyophis* Fitzinger, 1826 under the family Ichthyophiidae and the order Gymnophiona. The genus *Ichthyophis* is distributed across India, Sri Lanka, eastern Nepal, southern China, and throughout Southeast Asia, extending as far south as the Wallace's Line (Kamei, 2018). Globally, 50 species of *Ichthyophis* are currently recognized (Frost, 2024), of which 15 species are known from India (Dinesh et al., 2024). Notably, the Northeastern part of India houses more than 50% (8 out of 15 species) of India's *Ichthyophis* diversity, most of which are endemic to the region (Kamei, 2018). The type locality of *I. garoensis* Pillai & Ravichandran, 1999 is Anogiri Lake, Garo Hills, Meghalaya, India and the holotype was collected by Akhlaq Husain (Kamei & Biju, 2016). Morphologically, the species is characterized by its long subtriangular head and approximately 315 annular grooves and a prominent lateral yellow stripe on both side of the body extending from the head to the tail tip, including the vent (Pillai and Ravichandran, 1999).

It is relevant to mention here that, *Ichthyophis husaini* Pillai & Ravichandran, 1999 was described based on a single specimen from Rongram in the Garo Hills, Meghalaya. In the original description, it was documented as an unstriped species of *Ichthyophis*. But, subsequent herpetological surveys conducted between 2008 and 2014 in and around the type locality failed to locate any unstriped caecilians. In a comprehensive taxonomic revision, Kamei and Biju (2016) re-examined the holotype of *I. husaini* and found a faint yet detectable lateral stripe, contradicting the original description. Furthermore, it was found to be morphologically indistinguishable from *I. garoensis*, the type locality of which is only 20 km away from that of *I. husaini*. Given the lack of diagnostic morphological differences and the close geographic proximity of the two type localities, Kamei and Biju (2016) synonymized it under *I. garoensis* as a junior subjective synonym (Kamei & Biju, 2016).

Morphological variation within *I. garoensis* has been understudied across its range. To better understand the intraspecific variation in *I. garoensis*, we examined a specimen collected by Prof. Saibal Sengupta from Basista, Garbhanga Reserve Forest, Kamrup District, Assam, in June 1999. This specimen (ZSIA 16280), deposited in the National Zoological Collection, Zoological Survey of India, Kolkata, was identified to species level based on the original description by Pillai & Ravichandran (1999) and compared with three *I. garoensis* specimens (ZSIA 12494, ZSIA 12495, ZSIA 12496) collected by R. G. Kamei from Tebronggre, West Garo Hills, Meghalaya, as well as another *Ichthyophis* species from Northeast India, *Ichthyophis moustakius* Kamei et al., 2009 holotype (BNHS 5213) and three paratypes (BNHS 5214, BNHS 5215, BNHS 5216). Notable morphological variations were observed in the nuchal grooves, the nostril-tentacular aperture line (N-TA line), and the yellow band on the mandible.

We highlight the comparative variations in more detail here.

Nostril-Tentacular Aperture line (N-TA Line): Pillai & Ravichandran (1999) mentioned "a faint lavender stripe originating from below the nostril runs towards the tentacular aperture" in the original description of *I. garoensis*. This narrow lavender area is above the upper lip and is stripe - like in the holotype of *I. garoensis* and a clear pale - yellow line is present above it, not mentioned by Pillai & Ravichandran (1999) but examined by Kamei & Biju (2016). This N-TA Line in the

holotype of *I. garoensis* resembles partly the 'moustache' of *I. moustakius* Kamei et al., 2009 and *I. sendenyu* Kamei et al., 2009 and is one of the significant characters used by Kamei et al. (2009) to identify those species. In the holotype of *I. garoensis*, the pale - yellow area beneath the lavender stripe appears relatively broad, likely due to the fading of less pigmented areas in the preserved specimen. The N-TA Line which is also very prominent in the specimen (ZSIA 16280), originates near nostril and runs halfway towards the Tentacular aperture.

Nuchal Grooves: Mathew and Sen (2009) provided morphometric and meristic data based on two specimens (Reg. Nos. V/A/ERS/ZSI/858 and V/A/ERS/ZSI/859), reporting that the collars in these specimens are well-defined. The length of the first collar ranges from 1.76 to 2.89 mm, the second collar from 1.70 to 2.55 mm; and the distance from the snout to the third nuchal groove measures between 11.51 and 14.40 mm.

In these specimens, the first nuchal groove (NG1) is distinctly visible laterally and ventrally, and nearly complete dorsally; in comparison, NG1 is moderately distinct in the holotype; whereas in specimen ZSIA 16280, it is clearly visible across both dorsal and ventral sides.

The second nuchal groove (NG2) is clearly observed laterally in both V/A/ERS/ZSI/858 and V/A/ERS/ZSI/859; in the holotype**,** NG2 is clear on ventral and lateral sides but it becomes indistinguishable on the dorsal side; in specimen ZSIA 16280, NG2 is prominently visible both laterally and ventrally.

Third Nuchal groove (NG3) appears distinct laterally and dorsally, but is not visible ventrally in the specimens (V/A/ERS/ZSI/858 and V/A/ERS/ZSI/859); in the holotype**,** NG3 is faint but crosses the dorsum and extends laterally to the ventral region (Pillai and Ravichandran, 1999; Mathew and Sen, 2009); in specimen ZSIA 16280, NG3 is prominent dorsally and laterally, but absent ventrally.

Mandibular pigmentation: In the holotype of *I. garoensis*, the yellow band on each side of the mandible broadens and joins at the front, making the anterior ventral tip of lower jaw appear yellowish (Pillai and Ravichandran, 1999). In contrast, in case of specimen ZSIA 16280, the yellow bands on either side do not meet, leaving a distinct unpigmented gap at the anterior-most tip of the mandible, which is clearly visible upon examination.

The morphological variations observed in *I. garoensis* specimen ZSIA 16280, particularly in the N-TA Line, nuchal grooves, and the mandibular yellow band demonstrate the significant intraspecific variation within the species. Despite these variations, these features align with the established diagnostic criteria for *I. garoensis* Pillai & Ravichandran, 1999, highlighting the risk of relying on single morphological characters for species identification. This study emphasizes the necessity of assessing multiple specimens from diverse populations and using both morphological characteristics and genetic data to better define species boundaries within the genus *Ichthyophis*.

Fig 1: Dorsal view of *I. garoensis* (ZSIA 16280)

Fig 2: Ventral view of *I. garoensis* (ZSIA 16280)

Comparative morphological and meristic characters:

Characters	Holotype of *I. garoensis* (ZSIS 18458)	ZSIA 12494 - ZSIA 12496	ZSIA 16280	Holotype of *I. moustakius* (BNHS 5213) and Paratypes (BNHS 5214 - 5216)
Total Length	225 mm	198 - 281 mm	268 mm	196 - 287 mm
Tail Length	4 mm	4.18 - 5.19 mm	4.7 mm	-
Annular Grooves (AG)	315	282 - 295	268	238 - 292
AGs in tail	Tail short with 9 AGs	10 - 11	9	-
AGs broken by vent	3	3 - 4	2	3 - 5
AGs behind vent	6 complete folds behind vent	7 complete folds behind vent	7 complete folds behind vent	4 - 6 complete folds behind vent
Head Structure	Subtriangular	Subtriangular	U- shaped	More U-shaped than V-shaped
Head Length	12.2 mm	9.78 - 11.40 mm	11.93 mm	10.4 - 11.7 mm
Head Width	8 mm	5.78 - 7.23 mm	7.71 mm	6.7 - 9.9 mm
Head Depth	4.5 mm	3.22 - 5.59 mm	4.9 mm	3.9 - 6.3 mm
Width at collar	9.5 mm	6.56 - 7.37 mm	8.67 mm	-
Maximum body width	10.5 mm	7.57 - 7.64 mm	10.45 mm	9.0 - 11.8 mm
Tentacular Aperture (TA)	Close to upper lip, nearer to eye (2.0 mm) than to nostril (3.5 mm).	Very close to upper lip, nearer to eye (1.38 - 2.15 mm) than to nostril (2.78 - 3.43 mm).	Very close to upper lip, nearer to eye (2.2 mm) than to nostril (3.35 mm).	Very close to upper lip, nearer to eye (1.2 - 1.5 mm) than to nostril (2.7 - 3.1 mm)
Distance between eye and maxilla	1.3 mm	0.94 - 1.08 mm	1.22 mm	0.8 - 1.2 mm
Distance between nostril and maxilla	1 mm	0.63 - 0.70 mm	0.89 mm	-
Distance between eye and nostril	5.0 mm	4.11 - 4.24 mm	4.67 mm	3.7 - 4.6 mm

Snout Length	5.6 mm	4.69 - 5.07 mm	5.85 mm	4.8 - 6.0 mm
Inter-ocular distance	6.5 mm	4.87 - 5.12 mm	6.11 mm	4.2 - 6.1 mm
Ring around Eye	Eye with a pale circular ring around it.	Eye with a pale circular ring around it.	Eye with a pale circular ring around it.	Eye with a narrow whitish ring.
First Nuchal Groove (NG1)	NG1 crosses dorsum dimly, arches forward on either side and meet medially on the ventrum.	NG1 does not cross dorsum but arches forward and meets ventrally.	NG1 crosses dorsum and ventrum prominently.	NG1 crossed dorsum and ventrum prominently.
Second Nuchal Groove (NG2)	NG2 is distinct both laterally and ventrally.	NG2 is present laterally and faintly to prominently ventrally.	NG2 is seen prominently both laterally and ventrally.	NG2 is seen prominently both laterally and ventrally, but not dorsally.
Third Nuchal Groove (NG3)	NG3 is faint, crosses dorsum and runs down to side of ventrum.	NG3 is prominent laterally but faint ventrally and dorsally.	NG3 is prominent dorsally and laterally but absent ventrally.	NG3 is complete dorsally with gentle anteromedial curvature.
Nostril-TA Line	A faint lavender stripe originates from below the nostril and runs towards the TA.	Not present at all.	A prominent yellow stripe originates from below the nostril and runs halfway towards the TA.	An arched yellow stripe extends between nostril and TA, broader towards the nostril.
Yellow band on the mandible	The yellow band on lower jaw widens and joins the corresponding band on other side rendering the anterior ventral tip of the mandible yellowish.	Does not join the other band leaving a distinct gap at the anterior tip.	Does not join the other band on other side leaving a distinct gap at the tip of the mandible.	Does not join the other band leaving a prominent gap at the anterior most tip of the mandible.

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