

13. Bradford, M., A rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein dye binding. *Anal. Biochem.*, 1976, **72**, 248–254.
14. Laemmli, U. K., Cleavage of structural protein during the assembly of the head of bacteriophage T<sub>4</sub>. *Nature*, 1970, **227**, 680–685.
15. Blum, H., Beier, H. and Gross, H. J., Improved silver staining of plant proteins, RNA, DNA in polyacrylamide gels. *Electrophoresis*, 1987, **8**, 93–99.
16. Towbin, H., Staehelin, T. and Gordon, J., Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets; procedure and some application. *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4350–4354.
17. Arif, A., Shanavas, A., Murthy, Ch. R. K. and Dutta-Gupta A., Juvenile hormone stimulated tyrosine kinase mediated protein phosphorylation in the CNS of the silkworm, *Bombyx mori*. *Arch. Insect Biochem. Physiol.*, 2002, **50**, 139–146.
18. Plantevin, G., Bosquet, G., Calvez, B. and Nardon, C., Relationships between juvenile hormone levels and the synthesis of major haemolymph proteins in *Bombyx mori* larvae. *Comp. Biochem. B. Physiol.*, 1987, **86**, 501–507.
19. Calvez, B., Him, M. and De Reggi, M., Ecdysone changes in the haemolymph of two silkworms (*Bombyx mori* and *Philosamia cynthia*) during larval and pupal development. *FEBS. Lett.*, 1976, **71**, 57–61.
20. Bonner, W. M. and Laskey, R. A., A film detection method for the tritium labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.*, 1974, **46**, 83–88.
21. Mogilner, A. and Oster, G., Cell motility driven by actin polymerization. *Biophys. J.*, 1996, **71**, 3030–3045.
22. Couderc, J. L., Hilal, L., Sobrier, M. L. and Datugue, B., 20-Hydroxyecdysone regulates cytoplasmic actin gene expression in *Drosophila* cultured cells. *Nucleic Acids Res.*, 1987, **15**, 2549–2561.
23. Marsh, L. and Letourneau, P. C., Growth of neurites without filopodial or lamellipodial activity in the presence of cytochalasin B. *J. Cell Biol.*, 1984, **99**, 2041–2047.
24. Hoffman, P. N., Distinct roles of neurofilament and tubulin gene expression in axonal growth. *Ciba Found. Symp.*, 1988, **138**, 192–204.
25. Kimble, M., Incardona, J. P. and Raff, E. C., A variant of  $\beta$ -tubulin isoform of *Drosophila melanogaster* ( $\beta$ 3) is expressed primarily in tissues of mesodermal origin in embryos and pupae and is utilized in populations of transient microtubules. *Dev. Biol.*, 1989, **131**, 415–429.
26. Chapel, S., Sobrier, M. L., Montpied, P., Micard, D., Bruhat, A., Couderc, J. L. and Dastugue, B., In *Drosophila* Kc cells 20-OHE induction of 60 C  $\beta$ 3 tubulin gene expression is a primary transcriptional event. *Insect Mol. Biol.*, 1993, **2**, 39–48.
27. Shanavas, A., Nayak, B. P. and Dutta-Gupta, A., Ecdysteroid mediated muscle actin synthesis during the larval development of rice moth, *Corcyra cephalonica*. *Biochem. Mol. Biol. Int.*, 1996, **40**, 955–963.
28. Vitek, M. P., Morganelli, C. M. and Berger, E. M., Stimulation of cytoplasmic actin gene transcription and translation in cultured *Drosophila* cells by ecdysterone. *J. Biol. Chem.*, 1984, **259**, 1738–1743.

ACKNOWLEDGEMENTS. Financial support from the Council of Scientific and Industrial Research, New Delhi is acknowledged. A.A. thanks University Grant Commission, New Delhi for financial support through a direct fellowship.

Received 12 November 2003; revised accepted 15 March 2004

## ***Gegeneophis nadkarnii* – a caecilian (Amphibia: Gymnophiona: Caeciliidae) from Bondla Wildlife Sanctuary, Western Ghats**

Gopalakrishna Bhatta<sup>1,\*</sup> and P. Prashanth<sup>2</sup>

<sup>1</sup>Department of Zoology, Bhandarkars' College, Kundapura 576 201, India

<sup>2</sup>Sri J.C.B.M. College, Sringeri 577 139, India

*Gegeneophis nadkarnii* is described as a species of Caeciliidae (Amphibia: Gymnophiona) based on two specimens collected from Bondla Wildlife Sanctuary, Goa, India. This species is differentiated from all *Gegeneophis* except *G. danieli*, in having many secondary annuli that begin on the anterior of the body. *G. nadkarnii* differs from *G. danieli* in having substantially fewer teeth.

UNTIL 1999, the endemic Indian caeciliids were represented by five species belonging to two genera, viz. *Gegeneophis* Peters and *Indotyphlus* Taylor. These five species are *G. carnosus*, *G. fulleri*, *G. krishni*, *G. ramaswamii* and *I. battersbyi*<sup>1</sup>. Recently two more species from India were added to the genus *Gegeneophis*. Ravichandran *et al.*<sup>2</sup> reported *Gegeneophis seshachari* from Ratnagiri District, central western Maharashtra and Giri *et al.*<sup>3</sup> reported *Gegeneophis danieli* from Sindhudurg District, southern Maharashtra. In September 2001 we had collected two specimens resembling each other from Goa, India which fit into the generic diagnosis given by Taylor<sup>4</sup> and Ravichandran *et al.*<sup>2</sup> for *Gegeneophis*, but differ from all known species of the genus according to the recently developed key to the species of *Gegeneophis* by Giri *et al.*<sup>3</sup>. Here we describe this form (*G. nadkarnii* sp. nov.) that differs from all other species of the genus *Gegeneophis* except *G. danieli*, as having many secondary annuli that begin on anterior of the body. The species differs from *G. danieli* in having substantially fewer teeth.

*G. nadkarnii* sp. nov. is described as follows:

Holotype: Zoological Survey of India, Calicut, India (ZSI, CLT. No. V/A-573). A mature male, collected in Bondla Wildlife Sanctuary, Goa in September 2001, while conducting field-work.

Paratype: Bombay Natural History Society, Mumbai, India (BNHS) 4234. A mature male. Other data same as holotype.

This is a *Gegeneophis* differing from all other species of the genus except *G. danieli*, in having many secondary annuli (around 80, of which nearly 70 are complete middorsally) that appear on anterior part of the body. The anterior-most primary annuli (around 40) appear incomplete middorsally.

\*For correspondence. (e-mail: gkb@sancharnet.in)

The morphometric and meristic data are given in Table 1. The specimen is in good condition. The holotype measures 289 mm in length and 33 mm in circumference at mid-body. There are six artefactual horizontal ridges on the right side, beginning ca. 40 mm behind the snout. There is a 100 mm long midventral groove running from the first annulus to the region where the secondary annular grooves become complete. There is also a 70 mm long midventral incision in front of the vent.

In life, the body shape is subcylindrical and slightly dorsoventrally compressed (Figure 1). It is mostly of uniform width (10.9 mm at midbody), although slightly narrower anteriorly. Posteriorly, the body tapers strongly towards the terminus.

In dorsal view, the head tapers gently from the level of the occiput to the tentacular apertures (Figure 2). But anterior to this, the head tapers more strongly and ends in a bluntly rounded snout tip. In lateral view, the top of the head is straight and the margin of the upper lip slightly arched. The snout projects 1.4 mm beyond the mouth. The distance between the jaw angle and the top of the head (2.3 mm) is greater than the distance between the jaw angle and the ventral surface of the lower jaw (1.8 mm). In ventral view, the anterior margin of the lower jaw is much more rounded than the anterior margin of the snout.

The small, circular nostrils are 1.9 mm apart, visible dorsally and laterally but not ventrally. They are surrounded by a narrow whitish rim. In lateral view the nostrils

are slightly closer to the level of the snout tip than to the anteriormost margin of the mouth.

In life, the tentacles are globular. The tentacular apertures are lateral in position and 4 mm apart. They are situated at a distance of 2.4 mm from the tip of the snout and 1.3 mm from the nostrils. The slightly raised tentacular apertures are visible in both dorsal and ventral views. They are close to the margin of the upper lip than to the top of the head.

The eyes, which are scarcely visible under the bones in life, are invisible in the preserved specimen. In the live animal, the eyes occur at the posterior end of a light-pink-coloured stripe, the latter extends from behind the eye to immediately anterior to the tentacle. Laterally, the eyes lie approximately halfway between the margin of the upper lip and the top of the head. In dorsal view, the eyes are close to the lateral margins of the head.

We counted 20 premaxillary-maxillary, 18 vomeropalatine, 10 dentary and 5 splenial teeth (the tooth count includes the empty sockets also). With the exception of splenials, all the other types of teeth are found in equal numbers on each side of the jaw. Of the five splenials, three are on the right and two on the left side of the jaw. The teeth in all four series are generally recurved. They are smaller posteriorly than anteriorly. Both in the upper jaw and lower jaw, the teeth of the outer rows are markedly larger than those of the inner rows. The premaxillary-maxillary and vomeropalatine teeth rows, which run parallel to each other anteriorly, clearly extend posterior to the choanae and occur closer together posteriorly.

The small, oval choanae are obliquely placed and slightly longer than wide. They are separated by a distance that is approximately 2.5 times the width of each choana anteriorly and 3 times posteriorly.

The tongue is rounded, unattached anteriorly and is separated by a groove from the gingivae. The tongue has a pair of laterally positioned globular narial plugs. The

**Table 1.** Morphometric and meristic data (in mm) for the holotype and paratype of *Gegeneophis nadkarnii*. Measurements were taken to the nearest 0.1 mm with vernier callipers. For measuring the length and circumference, a calibrated 0.3 m scale and a piece of thread were used

	Holotype	Paratype
Total length	289	225
Head length	6.7	5.6
Distance between external nostrils	1.9	1.5
Distance between tentacles	4.0	3.0
Distance from nostril to tentacle	1.3	1.0
Distance between snout tip and tentacle	2.4	2.3
Distance between snout tip and jaw angle	7.4	5.1
Distance between jaw angle and tip of lower jaw	5.7	4.7
Distance between jaw angle and tentacle	4.5	3.9
Distance between snout tip and 1st nuchal groove	8.9	6.9
Distance between snout tip and 2nd nuchal groove	11.1	9.1
Distance between snout tip and 3rd nuchal groove	14.3	11.5
Width of head at jaw angle	6.5	5.2
Width of head at 1st nuchal groove	7.3	5.8
Width of head at 2nd nuchal groove	7.9	6.4
Width of body (at the broadest region)	10.9	9.5
Length by width	26.5	23.7
Width of body in front of vent	7.0	5.0
Width of body at the level of vent	4.9	4.1
Circumference at mid-body	33	28
Number of primary annuli	114	114
Number of secondary annuli	86	86
Premaxillary-maxillary teeth	20	20
Vomeropalatine	18	20
Dentary	10	10
Splenial	5	4



**Figure 1.** Holotype of *Gegeneophis nadkarnii* in life (ZSI, CLT. No. V/A-573).



upper surfaces of the narial plugs are on level with the surface of the tongue.



**Figure 2.** *a*, Dorsolateral view of the head; *b*, Ventral view of the head; *c*, Ventral view of the body terminus.

The nuchal region is broader and higher than the adjacent parts of the body. It has two nuchal collars that are marked clearly by three white-coloured nuchal grooves. The nuchal region at first nuchal groove (7.3 mm) is slightly expanded than the back of the head at jaw angle (6.5 mm). However, it is less expanded than the body at first annular groove (8.3 mm). The first collar (2.2 mm) is narrower than the second (3.2 mm), laterally. The first and second nuchal grooves are complete all around the nuchal region. The third nuchal groove, though complete dorsally and laterally, is incomplete midventrally. The first collar bears two short, equidistantly placed transverse grooves (folds) middorsally. The second collar bears a middorsal, whitish transverse groove that extends fully on the dorsum. This transverse groove is slightly nearer to the second nuchal groove. On the ventral surface of the nuchal region a longitudinal, whitish groove extends from about half way between the tip of the lower jaw and the first nuchal groove, to about two-thirds the length of the second collar. Further, there is a small transverse groove (may be artefactual) just in front of the first nuchal groove. These two grooves, i.e. longitudinal and transverse, together give the appearance of '+' mark on the chin. The nuchal and annular grooves are perpendicular to the long axis of the body.

Similar to the nuchal grooves, the annuli are also marked by white-coloured grooves. They are increasingly more conspicuous posteriorly. There are 114 complete primary annuli. Starting from the nuchal region, on the first 45 annular grooves middorsally, and on the first 104 annular grooves midventrally, the white colouration of the grooves is indistinct, as a result of which these grooves appear incomplete. The secondary annular grooves appear for the first time dorsolaterally on the 30th primary annulus at the right side and on the 31st primary annulus at the left side. But they are absent on the left side of the 30th primary annulus and on the right side of the 31st primary annulus. On 32nd and 33rd primary annuli, the secondary grooves are seen on both sides dorsolaterally. On the 34th primary annulus the secondary groove is seen only on the left side. From the 35th primary annulus, the secondary annular grooves are regular in their occurrence till the end of the body. But from the 35th primary annulus to the 45th primary annulus, the secondary annular grooves do not meet middorsally. From the 46th primary annulus onwards, the secondary annular grooves are complete middorsally. The secondary annular grooves extend across the midline on the ventral surface from the 105th primary annulus up to the subcircular disc surrounding the vent. The subcircular disc is 2.8 mm wide, 2.6 mm long and is interrupted by the 114th primary annulus and the 85th and 86th secondary annuli. The rounded terminus ends in a small cap, which is incompletely demarcated by the last secondary annular groove. The transverse vent (1.5 mm wide) lies just 1.4 mm from the body terminus. The vent is surrounded by five anterior and six poste-

rior denticles. Ten secondary annular grooves in front of the vent are complete. There are 86 secondary annuli.

The scales were searched at three different points along the body. No scales are found on the 20th primary annulus on both the dorsal and ventral surfaces. On the 75th primary annulus oval scales are found only on the dorsal surface in a single row in each of the primary and secondary annular grooves. On the 105th primary annulus, the scales are found on the dorsal surface in three rows and on the ventral surface in a single row. The oval scales of the 105th primary annulus (each 1.4 mm wide) are larger than the scales of the 75th annulus.

In life, the body is dark, slaty-grey coloured on the dorsal surface and light grey on the ventral surface. The head is of body colour with a light pink bar between the tentacle and the position of the eye. The tip of the snout, the lower jaw and the ventral surface of the first collar are pinkish in colour. The dorsal and ventral surfaces of the hind end of the body are darker than the rest of the body. The skin is with profuse white glandules all over the body.

In the preserved specimen, the body is dark grey in colour on the dorsal surface and light grey on the ventral surface. The annular and nuchal grooves are white in colour, being more prominent laterally. Dorsally, the tip of the snout bears a thin arc of cream colour. The stripes, which extend from just behind the position of the eyes to just in front of the tentacular apertures and the ventral surface of the rostrum, are pale cream in colour.

The under side of the lower jaw and the part of the body behind it are light grey in colour and are indistinguishable from each other. The rim of the lower jaw is pale cream in colour as also those of the tip of the snout and ventral surface of the rostrum. The disc surrounding the vent is white, both in live and preserved specimens.

The paratype is in good condition. It measures 225 mm in length and 28 mm in circumference at mid-body. There are four artefactual horizontal ridges on the dorsal side, beginning ca. 27 mm behind the snout. There is a 90 mm long midventral groove running from the first annulus to the region where the secondary folds become complete. There is also a 60 mm long midventral incision in front of the vent.

The paratype is light grey in colour when compared to the holotype. The paratype resembles the holotype in the pattern of the nuchal grooves with the exception that in the paratype, the second collar bears three equidistantly placed, middorsal, white transverse grooves among which the first one is shorter than the other two. The second and third transverse grooves are of equal length. The third transverse groove, which is incomplete on the middorsal line, meets the third nuchal groove on the right side. On the chin there are two small transverse grooves just in front of the first nuchal groove. In the paratype, on the first 42 annular groove, middorsally and on the first 106 annular groove midventrally, the white colouration of the

grooves is indistinct. In the paratype, secondary annular grooves appear for the first time dorsolaterally on the 29th primary annulus at the left side and on the 31st primary annulus at the right side. The secondary annular grooves are absent on the 30th, 32nd and 33rd primary annuli. From the 34th primary annulus onwards till the end of the body, the secondary annular grooves are regular in their occurrence. But from the 34th primary annulus to the 42nd primary annulus they are positioned only dorsolaterally. From the 43rd primary annulus onwards, they become complete middorsally. They extend across the midline on the ventral surface from 106th primary annulus up to the disc surrounding the vent. The subcircular disc surrounding the vent is 1.8 mm wide, 2.3 mm long and is interrupted by three annuli of which two are primaries and one secondary. The transverse vent (1.1 mm wide) is surrounded by four anterior and five posterior denticles. Eight secondary annular grooves in front of the vent are complete. There are 86 secondary annuli. The paratype has nine premaxillary-maxillary teeth on right side and eleven on the left side. Further, it has two vomeropalatine teeth more and 1 splenial tooth less when compared to the holotype.

Both the holotype and paratype were collected from a heap of rotting elephant grass at a distance ca. 5 m from a cement-walled stream inside a mini zoo. The locality is situated in the Western Ghats, at approximately 200 m asl. The soil where the specimens were spotted was brown in colour with pH 6.07, soil temperature 26°C (at 30 cm depth) and 0% canopy cover.

The presence or absence of secondary annuli and grooves, the number of primary and secondary annuli, the position of secondary annuli and the external visibility of eyes are the important characters used for differentiating the species of *Gegeneophis*<sup>3,4</sup>.

*G. nadkarnii* shares certain common characters with *G. danieli*. The overall colouration of the body, including the white grooves, the absence of white colouration approximately in the anterior one-third of the body, the contour of the head with snout and jaws, the structure of the nostrils, tentacles, choanae and tongue, the relative position of the nostrils and tentacles, the stripe between the position of the eye and the tentacle, the position of the occurrence of scales and their shape, structure of the terminal cap and the number of denticles surrounding the vent do exhibit similarity between *G. nadkarnii* and *G. danieli*. But *G. nadkarnii* differs from *G. danieli* mainly in the position and pattern of the secondary annular grooves. In *G. nadkarnii*, the secondary annular grooves commence from around the 30th primary annulus and they are positioned dorsolaterally, while in *G. danieli* the secondary annular grooves appear from the first primary annulus itself and are middorsal in position. In *G. nadkarnii*, from the 46th primary annulus onwards in the holotype and from the 43rd primary annulus onwards in the paratype, secondary grooves are complete middorsally, while



in *G. danieli* posterior to the 35th primary annulus there are few primary annuli on which the secondary annular grooves are incomplete middorsally. In addition to the above, *G. nadkarnii* differs from *G. danieli* also in having (a) first nuchal groove complete all around, (b) two transverse grooves on the 1st nuchal collar, (c) lesser number of secondary annuli, their position and arrangement (d) invisible eyes and (e) lesser number of premaxillary-maxillary, vomeropalatine and dentary teeth.

*G. nadkarnii* differs from *G. seshachari* in having secondary annuli, scales, invisible eyes and segmented terminal shield. *G. nadkarnii* differs from *G. krishni*, *G. ramaswamii*, *G. fulleri* and *G. carnosus* mainly in the number, position and pattern of the secondary annuli. In all these species, the number of secondary annuli is less than fifteen and these are restricted to the posterior half or less of the body.

*G. nadkarnii* is named in honour of Vinayaka Baburao Nadkarni, Department of Zoology, Karnatak University, Dharwad, India in recognition of his contributions to the field of comparative vertebrate endocrinology and biology of reproduction.

Bhatta<sup>5</sup> had stated that there is good chance of discovering new species of caecilians with additional sampling, and using Chao's estimate he expected this number to rise up to 27. The recent discoveries of two species by Ravichandran *et al.*<sup>2</sup> and Giri *et al.*<sup>3</sup> and the present

description of another species thereby expanding the Indian caecilian diversity number from 20 to 23, support the expectation of Bhatta<sup>5</sup>. They also substantiate the observations of Ravichandran *et al.*<sup>2</sup> and Bhatta<sup>5</sup> that the northern part of the Western Ghats has been less explored than the southern part and further exploration of the northern part might enrich the caecilian diversity.

1. Pillai, R. S. and Ravichandran, M. S., *Rec. Zool. Surv. India, Occas. Pap.*, 1999, **172**, 1–117.
2. Ravichandran, M. S., Gower, D. J. and Wilkinson, M., A new species of *Gegeneophis* Peters (Amphibia: Gymnophiona: Caeciliidae) from Maharashtra, India. *Zootaxa*, 2003, **350**, 1–8.
3. Giri, V., Wilkinson, M. and Gower, D. J., A new species of *Gegeneophis* Peters (Amphibia: Gymnophiona: Caeciliidae) from Maharashtra, India, with a key to the species of the genus. *Zootaxa*, 2003, **351**, 1–10.
4. Taylor, E. H., *The Caecilians of the World*, Univ. Kansas Press, Lawrence, 1968, p. 848.
5. Bhatta, G., Caecilian diversity of the Western Ghats: In search of the rare animals. *Curr. Sci.*, 1997, **73**, 183–187.

**ACKNOWLEDGEMENTS.** We thank the Department of Science and Technology, New Delhi for a research grant and the Director, Wildlife and Eco Tourism, Forest Department, Goa for permission to conduct the field work in the forests of Goa. We thank the Academy of General Education, Manipal for facilities and encouragement, and Vivek Broome for help with photography.

Received 7 August 2003; revised accepted 12 April 2004