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Chromatic and morphological anomalies in gymnophionans from India

Govindappa Venu[1,2], Govindaiah Venkatachalaiah[1], Halemane Ganesharao Seetharama[3], Gandlahalli Narasimaiah Balakrishna[1], Hmar Tlawmte Lalremsanga[4], Robert Kenneth Browne[5], Rajashekharaiah Nijagunaiah[6], Narayanappa Govinda Raju[7], Kulkarni Varadh[1], Sompalem Ramakrishna[1], Klaus Henle[8]

1 *Department of Zoology, Centre for Applied Genetics, Jnana Bharathi Campus, Bangalore University, Bengaluru, Karnataka, 560056, India*
2 *Department of Biosciences (UIBT), Chandigarh University, Mohali, Punjab, 140413, India*
3 *Division of Entomology, Central Coffee Research Institute, Coffee Research Station Post, 577 117, Chikmagalur District, Karnataka, India*
4 *Developmental Biology and Herpetology Laboratory, Department of Zoology, Mizoram University, Aizawl, Mizoram, 796004, India*
5 *Sustainability America, La Isla Road, Sarteneja, Corozal District, Belize*
6 *Biosystematics and Morphometry Laboratory, Department of Botany, Jnana Bharathi Campus, Bangalore University, Bengaluru, Karnataka, 560056, India*
7 *Department of Biotechnology, Karnataka State Open University, Mukthagangothri, Mysuru, Karnataka, 570006, India*
8 *UFZ – Helmholtz Centre for Environmental Research, Department of Conservation Biology and Social-Ecological Systems, Permoserstr. 15, 04318 Leipzig, Germany*

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Corresponding author: Govindappa Venu (venugcaecilian@gmail.com)

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Abstract

Caecilians (Gymnophiona) are commonly known as limbless amphibians and are the least understood vertebrate order. In this paper, we documented skin color, eye, jaw, snout, tentacular aperture and cloacal anomalies in 12 individuals of four species belonging to the three caecilian genera *Ichthyophis*, *Uraeotyphlus* and *Gegeneophis* collected from hotspots of caecilian diversity in India, the Western Ghats and Northeast India. As we found the majority of these individuals in coffee and tea plantations, we discuss the possibility that anomalies are the result of exposure to agrochemicals that are frequently used in plantations.

Key Words

agrochemicals, caecilian, coffee estate, hotspot, pesticides, tea estate, Western Ghats

Introduction

Caecilians (Gymnophiona) are the least speciose of the three orders of extant amphibians and are one of the least known taxa among vertebrates (Wilkinson 2012; Wake and Koo 2018; Mailho-Fontana et al. 2020). Currently, 214 extant caecilian species are recognized as inhabiting the wet tropics and subtropics. India is one of the hotspots of caecilian diversity with 41 species belonging to three endemic genera (*Gegeneophis* Peters, 1880, *Indotyphlus* Taylor, 1960 and *Uraeotyphlus* Peters, 1880), an endemic family the Chikilidae, and the more widely distributed genus *Ichthyophis* Fitzinger, 1826 (Frost 2022).

The Western Ghats of India is one of the eight recognized global biodiversity hotspots (Myers et al. 2000) and is inhabited by ~70% of the known Indian caecilian species (Venu et al. 2020). Northeast India, with the endemic family Chikilidae (four species) and nine species of *Ichthyophis*, accounts for more than 30% of Indian caecilians (Kamei et al. 2012; Gower et al. 2017; Lalremsanga et al. 2021). The Eastern Ghats of India with only one recognized species, *Gegeneophis orientalis* Agarwal, Wilkinson, Mohapatra, Dutta, Giri & Gower, 2013 is the least of the main Indian regions having caecilians (Agarwal et al. 2013). The paucity of information on various biological aspects of caecilians, an elusive, limbless, girdleless and snake-like amphibian order that comprises the sister group of the Batrachia (frogs and salamanders) results from both their restricted distribution and nocturnal and fossorial nature (Taylor 1968; Nussbaum and Wilkinson 1989; Gower and Wilkinson 2005).

Caecilians are known primarily from morphological (Wilkinson et al. 2007; Bhatta et al. 2011; Kamei and Biju 2016; Lalremsanga et al. 2021) and systematic studies (Gower et al. 2011, 2016; Torres-Sanchez et al. 2019) with only limited information on sporadic incidences of color and morphological anomalies. The color anomalies documented in caecilians to date include albinism in an adult male *Chthonerpeton indistinctum* (Reinhardt & Lutken, 1862) (Cacivio and Cespedez 1998) and in a juvenile *Gegeneophis mhadeiensis* Bhatta, Dinesh, Prashanth & Kulkarni, 2007 (Bhatta et al. 2007) and leucism in an adult female *Ichthyophis kodaguensis* Wilkinson, Gower, Govindappa & Venkatachalaiah, 2007 (Venu et al. 2021a). Morphological anomalies documented in caecilians are dicephalism in a larva of *Ichthyophis bannanicus* Taylor, 1960 (Bei et al. 2011) and burn-like wounds in *Ichthyophis glutinosus* (Linnaeus, 1758) (de Silva 2011).

The coffee, tea, and mixed crop plantations, that largely occupy the more fertile areas, support high caecilian populations due to their moist organically rich soils enriched by the application of organic matter (Bhatta 1997; Wilkinson et al. 2007; Venu et al. 2011; Kotharambath et al. 2012; Venu 2013; Venu et al. 2021a, b). However, many of these plantations are also subject to the unrestricted use of pesticides (such as alto-100, atrazine, Furadan, Lannate, Roundup, malathion) and fertilisers that may affect caecilian development (Hebrard et al. 1992; Daniels 2003; Venu 2008; de Silva 2011; Rathod and Rathod 2013; Hegde et al. 2019), and the potential of trauma through cultivation practices.

The current paper further extends the records of caecilian anomalies to instances associated with skin color, eye, jaw, snout, tentacular aperture and cloacal morphology in 12 individuals from four species in three genera, mainly collected from coffee and tea plantations in the Western Ghats and Northeast India.

Materials and methods

Extensive fieldwork was conducted in the Western Ghats of the southern states of India including Karnataka, Kerala, and Tamil Nadu, during the monsoon seasons from 2001 to 2020, to collect caecilian specimens for cytogenetic analyses (Venkatachalaiah and Venu 2002; Venu 2008; Venu et al. 2011; Venu and Venkatachalaiah 2012; Venu 2013). Caecilians were collected from varied habitats, such as backyard gardens, paddy fields, and mixed plantations of areca nut, banana, cardamom, coconut, coffee, and tea, by digging moist, dark soil using large hoes to a depth of 0.1– 0.4 m, by turning over logs, and by removing leaf litter and compost.

In total, 176 specimens of both sexes belonging to the genera *Ichthyophis* [*I. beddomei* Peters, 1880 (40), *I. kodaguensis* Wilkinson, Gower, Govindappa & Venkatachalaiah, 2007 (12), *I. longicephalus* Pillai, 1986 (5) and *I. tricolor* Annandale, 1909 (10)], *Uraeotyphlus* [*U. bombayensis* (Taylor, 1960) (10), *U. interruptus* Pillai & Ravichandran, 1999 (7), *U. menoni* Annandale, 1913 (9), *U. narayani* Seshachar, 1939 (36) and *U. oxyurus* (Dumeril & Bibron, 1841) (6)] and *Gegeneophis* [*G. carnosus* (Beddome, 1870) (2), *G. madhavaorum* Bhatta & Srinivasa, 2004 (6) and *G. ramaswamii* Taylor, 1964 (33)] were collected and examined for anomalies. In addition, we examined one *I. khumhzi* Kamei, Wilkinson, Gower & Biju, 2009 that was collected by Lalremsanga from Sateek, Aizawl District, Mizoram, in the very east of India.

The specimens were photographed in broad daylight after euthanization in 0.5% MS-222, with the total length and midbody width then measured using a piece of thread and ruler. Specimens were fixed in 10% formalin overnight, washed with tap water to remove all the traces of fixative, and then stored in 70% alcohol. Meristic data and metric data, to the nearest 0.01 mm using a Mitutoyo Digimatic calliper were recorded for the preserved specimens (Pillai and Ravichandran 1999; Gower and Wilkinson 2007; Wilkinson et al. 2007). Sex was determined by a midventral incision to expose the testes or ovaries. The numbers of vertebrae were determined from radiographs.

Animal procedures followed the ethical committee guidelines of the Department of Zoology, Bangalore University, Bengaluru (BUB), India and the Department of Zoology, Mizoram University, Mizoram, India. The vouchered specimens were deposited in the Cytogenetics Laboratory, Centre for Applied Genetics, Department of Zoology, Bangalore University, Bengaluru (BUB), India. The specimen of *I. khumhzi* (MZMU1460) was deposited in the Departmental Museum of Zoology, Mizoram University, Mizoram, India.

Specimens were compared with individuals described in Wilkinson et al. (2007) for *I. beddomei*, by Kamei et al. (2009) for *I. khumhzi*, by Pillai and Ravichandran (1999) and Gower and Wilkinson (2007) for *U. narayani*, and by Pillai and Ravichandran (1999) for *G. ramaswamii* to identify anomalies. We followed the terminology of Henle et al. (2017a) to identify the anomalies observed in the examined caecilians.

Results

In total, we detected anomalies in three individuals of *I. beddomei* (N = 40 individuals sampled), one *I. khumhzi* (N = 1), six *U. narayani* (N = 36) and two *G. ramaswamii*

(N = 33), (Table 1). Table 1 provides the collection localities and habitat types, the sex of the individuals and summarizes the body part affected in the abnormal individuals. Below, we describe the anomalies in detail.

Hypermelanism in *Ichthyophis beddomei* (BUB1180)

Ichthyophis beddomei is a striped ichthyophiid caecilian characterized by a relatively short and broad body, which is dark violet brown on the dorsum and pale brown on the venter. A bright yellow or cream lateral stripe of a uniform breadth of about 3 mm passes along the body from the snout to the tip of the tail. The yellow stripe is broader and more extensive on the mandible and widens a little towards the lower side on the first collar and bifurcates at the mouth angle with one branch getting thinner under the eye and terminating on the upper lip; the other branch reaches the tip of the chin along the lower jaw (Wilkinson et al. 2007) (Fig. 1A, B).

In the abnormal individual (BUB1180), the yellow lateral stripe is discontinued at the first collar on both sides. It is absent on the lower side of the jaw/collar region. On the left, the stripe again starts from the middle of the 2nd collar and is present up to the 9th annulus; on the right side, it is seen up to the 17th annulus. Yellow spots are evident on annuli 11, 12, 14, 17, 19, 20, 22, 24, 25, 31, 33, 37, 38, 50, 69, 71, 75, 99 and 101 on the left side and on annuli 24, 25, 42, 73, 78, 135, 163, 164, 165, 198, 227, 228 and 229 on the right side. The head is paler than the body and the dorsum darker than the venter. The vent disc is dark in comparison to the whitish brown in normal specimens (BUB1603) and the venter is darker than normal (Fig. 1C, D).

Albinism in *Ichthyophis beddomei* (BUB1648)

Dorsal and ventral surfaces are paler than normal: light grayish brown instead of dark violet brown (Fig. 2A, B). The annuli on the dorsal side are whitish and more distinct than in normal individuals (BUB1603). Bluish-white patches are present on the dorsum, lateral sides of the body and venter (Fig. 2A). The region between the

Table 1. Details of collection of the anomalous specimens and their anomalies.

Species	Locality	Habitat	Voucher details	Sex	Associated organ	Type of anomaly
Ichthyophis beddomei	Kuchikunnel tea plantations, Gudalur, Nilgiris, Tamil Nadu, (11°30.78'N, 76°29.21'E)	Mixed plantations of tea, banana, pepper, orange, coffee, silver oak	BUB1180	M	Skin	Hypermelanism
	Central Coffee Research Institute, Balehonnur, Chikkamagalur, Karnataka (13°34.90'N, 75°46.51'E)	Mixed plantations of coffee, pepper, cardamom	BUB1396	F	Eye	Macrophthalmy (left eye)
	Regional Agricultural Research Station, Ambalavayal, Wayanad, Kerala, (11°36.84'N, 76°12.64'E)	Mixed plantations of coffee, pepper, cardamom, arecanut, banana, jackfruit, chikoo	BUB1648	F	Skin	Albinism
Ichthyophis khumhzi	Sateek, Aizawl, Mizoram, (23°54.77'N, 92°70.32'E)	Secondary forest dominated by a mixture of *Bambusa tuda*, *Melocanna baccifera*, *Macaranga indica*, *Michelia champaca*, *Schima wallichi*, *Trema orientalis*	MZMU1460	M	Cloaca	Abnormal shaped cloacal opening
Uraeotyphlus narayani	Regional Agricultural Research Station, Ambalavayal, Wayanad, Kerala, (11°36.84'N, 76°12.64'E)	Mixed plantations of coffee, pepper, cardamom, arecanut, banana, jackfruit, chikoo	BUB1171	M	Skin	Partial melanism Annuli groves wider than normal
			BUB1502	F		
			BUB1336	F	Eye	Unilateral anophthalmy
					Jaw	Abnormal jaws
					Snout	Left snout absent
					Tentacular aperture	Absent on left side
	Kuchikunnel tea plantations, Gudalur, Nilgiris, Tamil Nadu, (11°30.78'N, 76°29.21'E)	Mixed plantations of tea, banana, pepper, orange, coffee, silver oak	BUB1149	M	Eye	Microphthalmy (right eye)
	Regional Coffee Research Station, Chundale, Wayanad, Kerala, (11°57.21'N, 76°05.80'E)	Mixed plantations of coffee, silver oak, orange, pepper, coconut, banana	BUB1467	F	Skin	Blue
			BUB1147	M		
Gegeneophis ramaswamii	Chuldmanur, Thembakalu, Thiruvananthapuram, Kerala, (8°52.41'N, 76°93.66'E)	Plantations of banana, arecanut, coconut	BUB1169	F	Skin	Blue
			BUB1516	M		

Figure 1. Lateral views of normal (**A, B**) and melanistic (**C, D**) *Ichthyophis beddomei*.

tail tips to the posterior edge of the vent is whitish; the lateral stripe touches the outer margin of the vent and is paler than normal; the snout tip is paler than normal; and the vent and its disc are whitish (Fig. 2B).

Macrophthalmy (left eye) in *Ichthyophis beddomei* (BUB1396)

The diameter of the macrophthalmic left eye (Fig. 3B) is 1.96 mm (normal 0.60 mm – Fig. 3A), its distance to the upper lip 0.08 mm (normal 0.86 mm in BUB1603), to the nostril 3.32 mm (same as normal), to the jaw angle 0.95 mm (normal 1.82 mm), to the tentacle 0.96 mm (normal 1.49 mm), and to the snout tip 3.88 mm (normal 3.82 mm). The ratio of the distances between the tentacle and the nostril and that of the tentacle to the eye is 2.15 (Fig. 3B); which is more than that in *I. longicephalus* (1.75–2.0) (Venu et al. 2020).

Abnormal shaped cloacal opening in *Ichthyophis khumhzi* (MZMU1460)

The abnormal individual (MZMU1460) was identified following Kamei et al. (2009) and has a blunt tail, unlike the tapering pointed tail in normal individuals (Fig. 4A, B: MZMU1005). The number of vertebrae, determined from radiographs, was 119, which is fewer than the 127 vertebrae in the normal individual MZMU1005 (Kamei et al. 2009). The posterior vertebrae start to bend strongly downwards from the 118th vertebra onward (Fig. 4C).

Partial melanism in *Uraeotyphlus narayani* (BUB1171 and BUB1502)

Uraeotyphlus narayani is an ichthyophiid caecilian of the *oxyurus* group with a grayish dorsum and pale, flesh-colored belly. A median greenish line runs between the chin and the tail. Its eyes are distinct with a white patch around them. The nostrils are placed dorsally on the snout and visible from above. The tentacles are placed below the nostrils, not visible from above. The tip of the tail is whitish, and the snout tip and lower jaw are cream-colored (Gower and Wilkinson 2007).

In BUB1171 and BUB1502, the dorsal and ventral surfaces and the tip of the tail are darker than normal (Fig. 5A) and the annular grooves are wider than normal (BUB1499). Whereas in BUB1171 the distinct white borders of the annuli are retained, they are absent in BUB1502 (Fig. 5B). In the latter specimen, the annuli borders are darker than normal. Likewise, the first, second and third nuchal grooves, the throat, chins, tail tip and the area around the eye and the

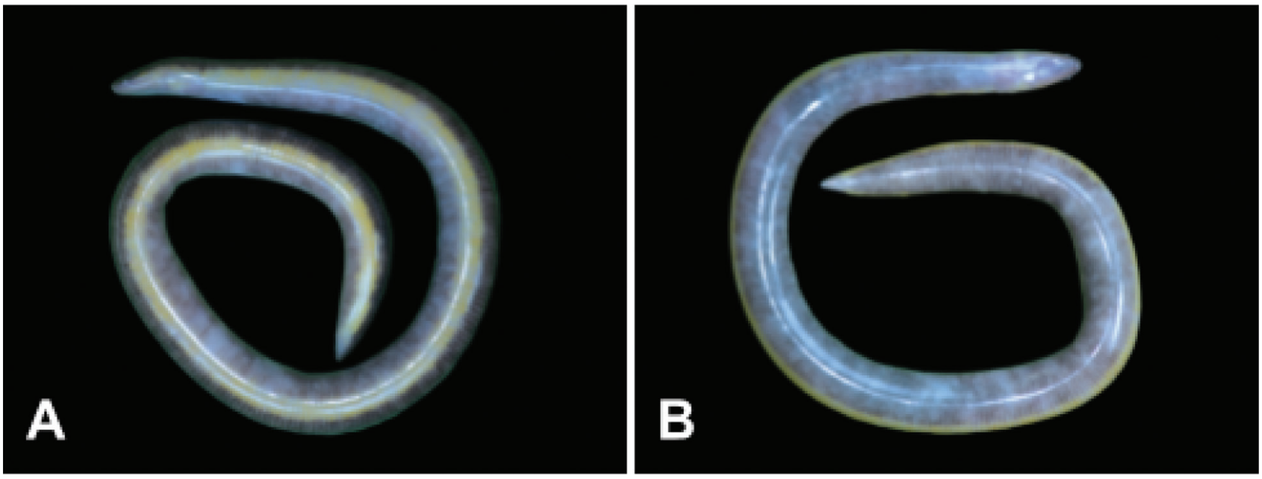

Figure 2. Dorsolateral (**A**) and ventral (**B**) views of albinistic *Ichthyophis beddomei*.

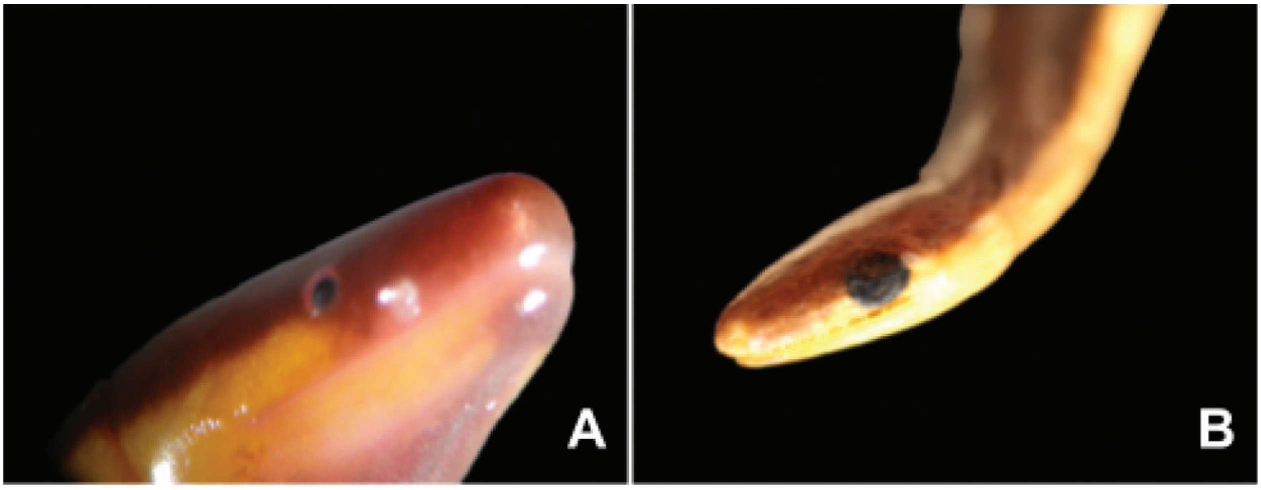

Figure 3. Normal (**A**), macrophthalmic (**B**) eyes of *Ichthyophis beddomei*.

surrounding areola are darker than normal. In contrast, the snout tip is paler than normal. The venter is brownish rather than whitish in normal individuals. The annuli have distinct white borders on the ventral and lateral sides; they are less distinct on the dorsal side.

Blue colored *Uraeotyphlus narayani* (BUB1467 and BUB1147)

In BUB1467, the dorsal surface, annuli borders, snout tip, the areola surrounding the eye, the jaw borders, the area surrounding the throat, the chin, the tail tip, and the first, second and third nuchal grooves are bluish. The venter turned yellowish upon preservation. Annuli borders are bluish on the dorsal surface and yellowish on the lateral and ventral surfaces (Fig. 6C). The normal dorsal color, in contrast, consists of narrow darker and lighter pinkish-gray cross bands (Fig. 6A) and the ventral one is pale gray with darker gray posteriorly (BUB1499) (Fig. 6B).

In BUB1147, the dorsum is pale blue and darker than normal (BUB1499). The borders of the jaws and the vent disc are also darker than normal. The eye is also darker than normal and its surrounding area is bluish. The snout tip is bluish and the tail tip is bluish to black (Fig. 6D).

Unilateral anophthalmia in *Uraeotyphlus narayani* (BUB1336)

The eye, tentacular aperture and nostril are absent on the left side of the head in the abnormal individual (Fig. 7B). Fig. 7A shows a normal individual (BUB1499) for comparison.

Microphthalmia in the right eye of *Uraeotyphlus narayani* (BUB1149)

The diameter of the abnormal eye (0.22 mm) is smaller than that of the normal (BUB1499) eye (Fig. 8A) on the left side of the head (0.42 mm). The white areola surrounding the abnormal eye is incomplete and its maximum distance from the tip of the eye is 0.34 mm, whereas the white areola is completely surrounding the left eye and its distance from the outer curvature of the eye is 0.06 mm (Fig. 8B, C).

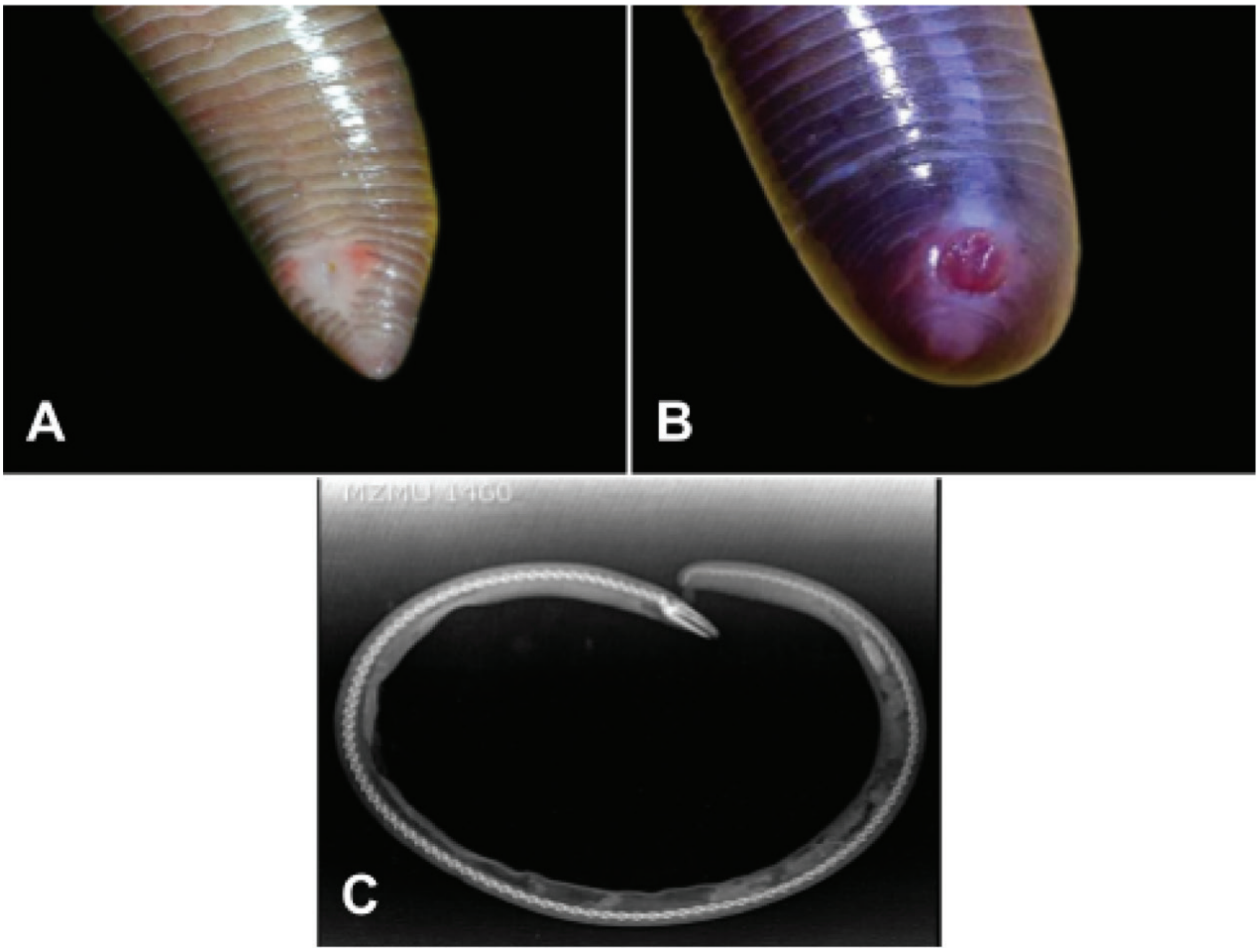

Figure 4. Ventral views of the tail tip of *Ichthyophis khumhzi*: normal (**A**), abnormal (**B**) and radiograph of the abnormal individual highlighting the curved posteriormost vertebrae (**C**).

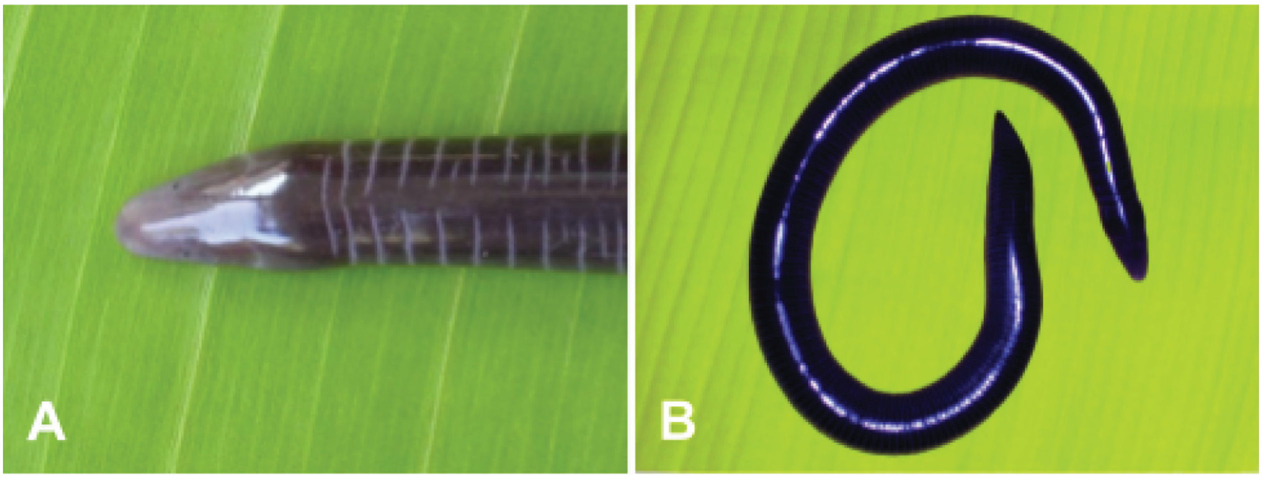

Figure 5. Enlarged view of the head of normal (**A**) and dorsal view of *Uraeotyphlus narayani* (BUB1502) with partial melanism (**B**).

Abnormal jaw (left side) in *Uraeotyphlus narayani* (BUB1336)

The upper jaw is discontinuous: premaxillary teeth at the position of the anomaly are absent. There is a break in the jaw between the position of the eye and the snout tip (Fig. 9B) and a perforation above the margin of the upper jaw, opposite to the anomaly in the lower jaw. The lower jaw is projected upward between the position of the eye and the position of the nostril. The nostril and tentacular aperture are absent. Fig. 9A shows a normal individual (BUB1499) for comparison.

Blue colored *Gegeneophis ramaswamii* (BUB1169 and BUB1516)

The body of *Gegeneophis ramaswamii* is grayish dorsally and pale gray ventrally (Pillai and Ravichandran 1999) (Fig. 10A, B). In the abnormal individuals the dorsal surface, vent disc and lower jaw are bluish and darker than in normal individuals (Fig. 10C, D), but its nostril and tentacular aperture are white like that of normal individuals (BUB1013).

Discussion

Our observations almost double the number of caecilian species with anomalies reported in natural populations. Anophthalmy, microphthalmy and macrophthalmy, jaw, snout, tentacular aperture and cloacal anomalies, as well as abnormal blue color and melanism in caecilians, are reported for the first time in our study. This list of species includes microphthalmia in *Uraeotyphlus narayani*, although the unilateral anophthalmia and abnormal jaw could possibly be a consequence of physical trauma.

Albinism and leucism have been previously recorded in caecilians (Cacivio and Cespedez 1998; Bhatta et al. 2007; Venu et al. 2021a) and in many other amphibians (Henle et al. 2017a). Anophthalmy and melanism also have been observed in many amphibian species (Henle et al. 2017a). Abnormal blue coloration has often been reported in European anurans (genera *Hyla* and *Pelophylax*), but rarely for other amphibian species (Bagnara et al. 2007; Henle et al. 2017a; Marushchak et al. 2021). The blue color in amphibians usually is a structural color caused by the scattering of blue wavelengths when xanthophores or their pigments are absent (Bagnara et al. 2007). Usually, it is assumed that abnormal blue coloration has a genetic basis but this has been rarely tested experimentally (Henle et al. 2017a; Marushchak et al. 2021).

The other anomalies we currently report, such as tentacular aperture – a unique feature of caecilians and cloacal forms, have never been documented in the literature for amphibians (Henle et al. 2017a; Marushchak et al. 2021).

Due to the soil-dwelling habits of most (at least adult) caecilians, it can be difficult to get a large number of samples. However, the frequency of anomalies (6–17%) in the three species, *G. ramaswamii*, *I. beddomei* and in *U. narayani*, even when considering the possibility of trauma, with the largest sample sizes (N = 33–40) were well above

Figure 6. Dorsal (**A**), ventral (**B**) views of normal and dorsolateral (**C**) and ventral (**D**) views of blue *Uraeotyphlus narayani*.

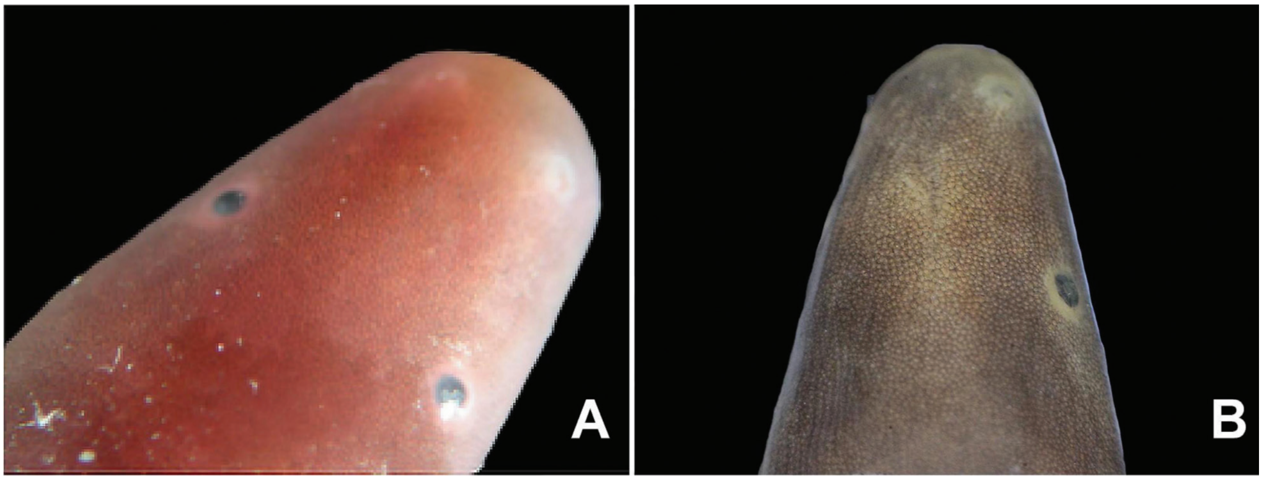

Figure 7. Normal eyes (**A**) and unilateral anophthalmia (**B**) in *Uraeotyphlus narayani*.

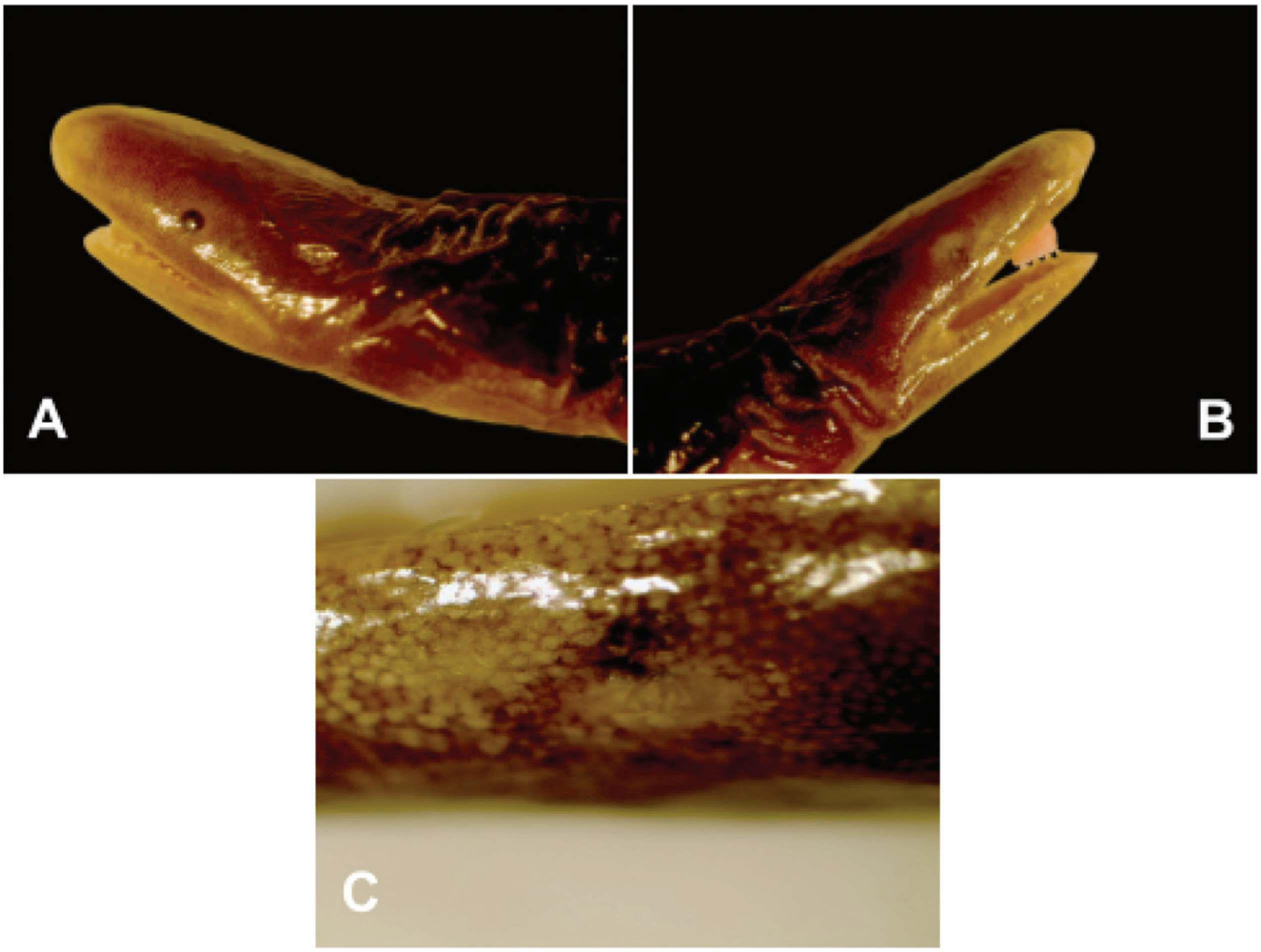

Figure 8. *Uraeotyphlus narayani* with normal left (**A**) and, microphthalmic right eyes (**B**). Enlarged view of the microphthalmic eye (**C**).

the 5% level of concern suggested in various publications (e.g. Henle et al. 2017a; Haas et al. 2018). Furthermore, due to decreased relative survival rates the absolute rates of anomalies at birth or hatch would be expected to be higher than those found in adults. Because all types of anomalies can have a range of different causes (Henle et al. 2017b), it is difficult to specify individual sources, especially when sample sizes and therefore associated habitat variables are limited. However, the high prevalence of anomalies in these three species indicates that environmental factors and possibly contaminants may be implicated. Moreover, high frequencies of color anomalies are considered signals of environmental distress (Henle et al. 2017a; Dubois and Ohler 2018). The human footprint was significantly higher at locations with abnormal blue coloration in *Pelophylax* species across Ukraine (Marushchak et al. 2021).

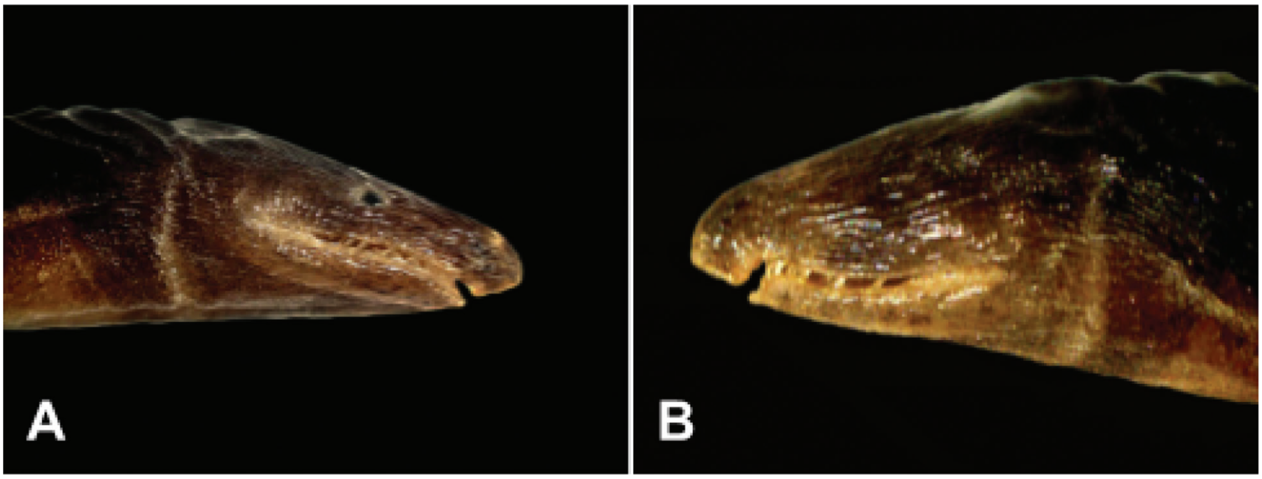

Figure 9. *Uraeotyphlus narayani* with normal- right (**A**) and abnormal- left (**B**) jaws.

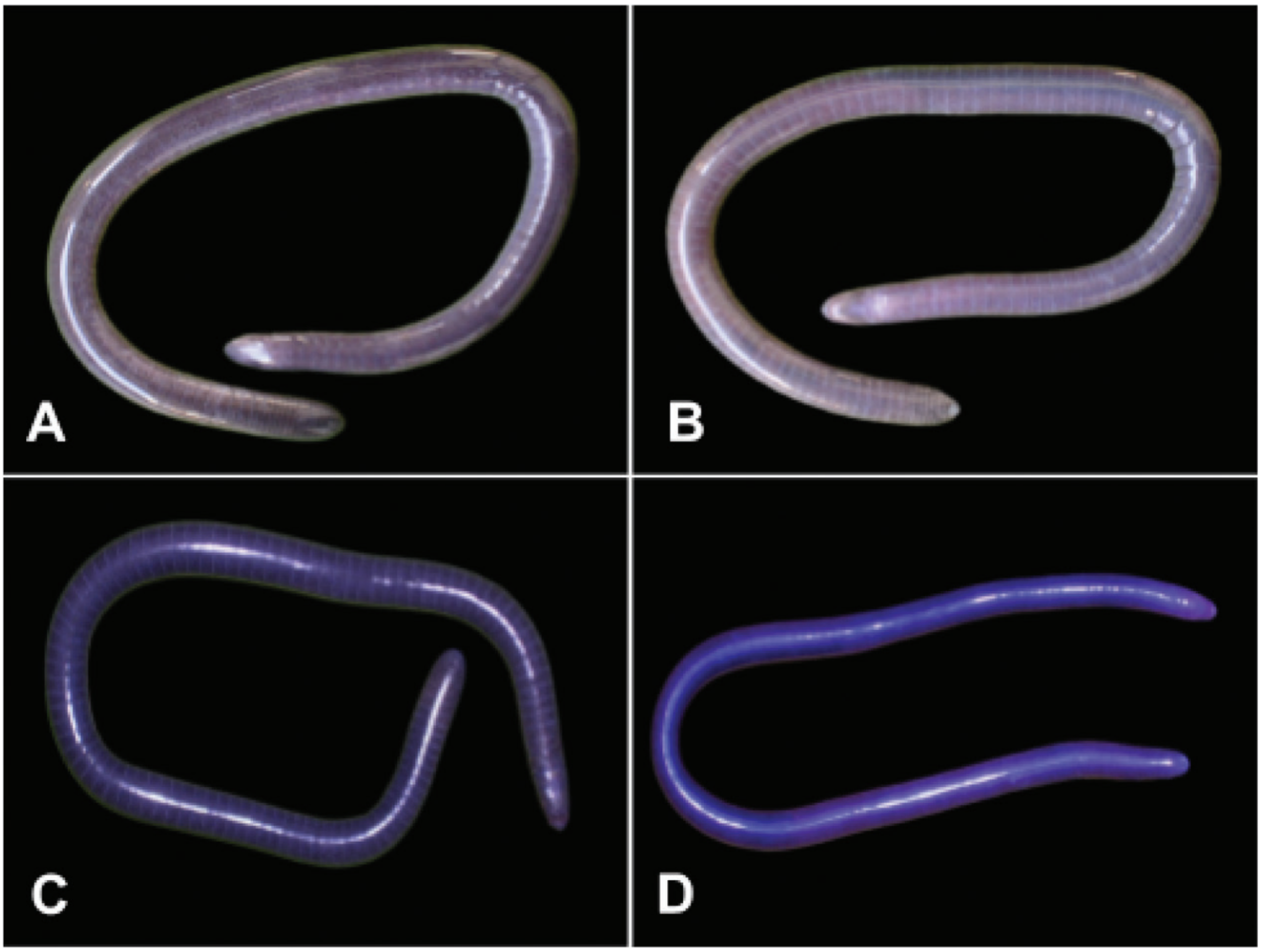

Figure 10. *Gegeneophis ramaswamii*: normal dorsal (**A**) and ventral (**B**) colouration, and abnormal bluish colouration in ventrolateral (**C**) and ventral views (**D**).

There has been only one publication on anomalies in caecilians making a strong association with pollutants; three individuals of *I. glutinosus* with fresh burn-like lesions were found after a tea plantation was sprayed with glyphosate in Sri Lanka (de Silva 2011). Most of our individuals with anomalies were found in tea and coffee plantations which are regularly subject to agrochemical pollutions where only a small part reaches their target organism. Due to the accumulation of pesticides in the soil (Annett et al. 2014; Herek et al. 2020) gymnophiona that are living in the soil most likely have a chronic exposure throughout their life. This chronic exposure to various pesticides might adversely affect their development and lead to anomalies in adult life stages.

A very wide range of agrochemicals is used in tea and coffee plantations in the Western Ghats (Daniels 2003; Gower and Wilkinson 2005; Hegde et al. 2019; Raghavendra and Venkatesha 2021), including malathion, chlorpyrifos and glyphosate. In anurans, malathion caused depigmentation in early development in *Microhyla ornata* under laboratory conditions (Pawar et al. 1983), and chlorpyrifos in environmentally relevant concentrations causes anophthalmy in *Dryophytes chrysoscelis* (Britson and Threlkeld 1998). The effects of glyphosate are controversial and are difficult to assess because of the dispersants and surfactants associated with its use (Lajmanovich et al. 2003; Jones et al. 2010; Relyea 2011; Yadav et al. 2013; Annett et al. 2014). In any case, its commercial formulation as Roundup has caused anomalies including abnormal eyes, mouth and pigmentation in larval amphibians in many laboratory studies (e.g. Herek et al. 2020) and in insects, it inhibits melanin synthesis with potential pathological affects (Smith et al. 2021). Furthermore, these pesticides may also act synergistically with each other and with other agrochemicals, such as nitrates, to increase amphibian pathologies (Britson and Threlkeld 2000; Egea-Serrano et al. 2012).

Farmers want to kill insects on the leaves of plants, but the pesticide will be washed off by rain. Thus farmers continue to spray the pesticide and it accumulates underground. Because caecilians live underground and feed on soil animals, they may retain more chemicals from the ground surface including pesticides. Hence, the anomalies observed in caecilians could be an indicator for such cryptic soil pollution.

In the absence of further evidence through experimental studies on caecilians, it remains uncertain if the anomalies observed by us were caused by exposure to agrochemicals, although evidence from a range of other studies and taxa supports this possibility (Henle et al. 2017a). The presence of anomalies could also indicate impairments in physiology, behavior and reproduction (Hegde et al. 2019; Karlsson et al. 2021), or even physical damage during cultivation, any of which might threaten caecilian populations (de Silva 2011; Egea-Serrano et al. 2012). To further elucidate the detrimental effects of agrochemicals on caecilians, laboratory-based research under environmentally relevant conditions is urgently needed, along with toxicological assessments of agrochemicals in the tissues of caecilians sampled from the field.

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