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## A ground-dwelling rhacophorid frog from the highest mountain peak of the Western Ghats of India

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**A new rhacophorid frog is described from Eravikulam National Park in the Western Ghats of India. The species is morphologically dissimilar from any known member of this family in having a bright orange to reddish colouration, multiple macroglands on the body and extremely short limbs. Phylogenetic analyses of mitochondrial genes indicate that this new frog is nested in a radiation of shrubfrogs that had its origin on the Indian subcontinent, and which is here recognized as a distinct genus, *Raorchestes* gen. nov. The new species, *Raorchestes resplendens* sp. nov. is likely restricted to less than 3 sq. km on the summit of Anamudi, and deserves immediate conservation priority.**

**Keywords:** Endotrophic development, macroglands, mitochondrial phylogeny, *Raorchestes* gen. nov., *Raorchestes resplendens* sp. nov., Western Ghats.

THE Rhacophoridae constitute a radiation of about 300 species of frogs<sup>1</sup> with a distribution in the tropical regions of Asia and Africa. A large part of their taxonomy has traditionally been based on the variety of reproductive strategies, such as the use of foam nests or development without going through a free-swimming tadpole stage. Molecular evidence has recently solved the phylogenetic position of many rhacophorid species<sup>2–7</sup> and has resulted in the erection of new genera, such as *Gracixalus*<sup>3</sup>, *Feihyla*<sup>4</sup>, *Liuixalus*<sup>5</sup> and *Ghatixalus*<sup>6</sup>. Although rhacophorids find breeding microhabitats from the soil to the forest canopy, calling males are often found in vegetation layers<sup>8</sup>, and the family as a whole is therefore usually considered arboreal. Here we describe an exceptional glandular rhacophorid with extremely short limbs from Anamudi summit (2695 m asl) in the Western Ghats of India. We performed molecular analyses to infer its phylogenetic position within the family and studied its reproduction and development.

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We constructed a data matrix of 3370 bp mtDNA containing a part each of 12S rRNA, tRNA<sup>VAL</sup>, 16S rRNA, ND1, tRNA<sup>ILE</sup>, tRNA<sup>GLN</sup> and tRNA<sup>MET</sup> genes for a variety of rhacophorid frogs to infer the phylogenetic position of the new species within the family (GenBank accession number GU808563 for BNHS 5301). Maximum parsimony (MP) and maximum likelihood (ML) analyses were executed with the software PAUP\* 4.0b10<sup>9</sup>, Bayesian analyses and calculation of posterior probabilities were performed with MrBayes<sup>10</sup>. Our results largely agree with previous studies<sup>6,11,12</sup> and show that frogs traditionally classified in the genus *Philautus*<sup>1</sup> do not form a monophyletic group. Consistent with recent analyses<sup>5,12</sup>, they form a clade with a main distribution on the Sunda shelf (*Philautus sensu stricto*), and a group with its centre of diversification on the Indian subcontinent. MP, ML and Bayesian analyses all place the new species in the latter clade of direct developing frogs, a group that in itself consists of two reciprocally monophyletic clades (Figure 1, left): the largest part of direct developing frogs form a distinct radiation with a notably large diversity in the Western Ghats. Its sister clade is a mainly Sri Lankan radiation, which has known a single dispersal event to the Indian mainland, resulting in few extant species in the Western Ghats<sup>11</sup>. Although the name *Kirtixalus* has been proposed for the latter clade<sup>13</sup>, the older name *Pseudophilautus* Laurent 1943 is available for this island radiation<sup>14,15</sup> and its nested Indian members<sup>11</sup>. The type species of *Pseudophilautus*, *Ixalus temporalis* Günther 1864, is considered to be extinct<sup>16</sup>, but clearly is a member of the Sri Lankan radiation of direct developing frogs. *Pseudophilautus* can be defined in a phylogenetic framework as the most inclusive clade including the species

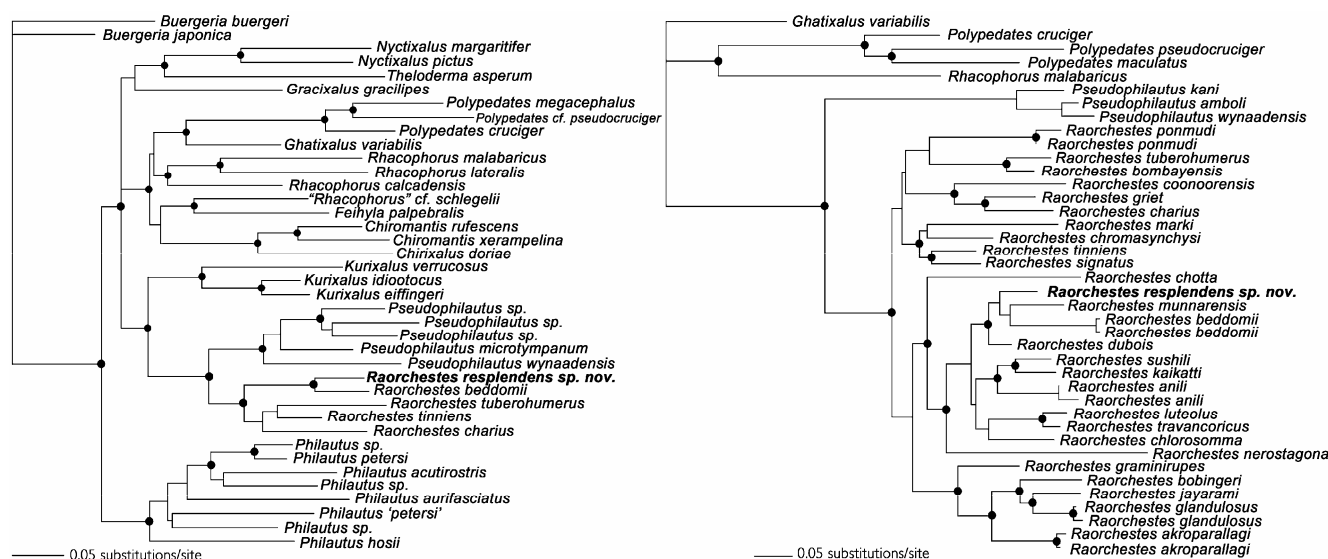
*Ixalus temporalis* Günther 1864 and excluding the species *I. glandulosus* Jerdon 1854. To distinguish species of the mainland radiation, we here suggest recognizing this remarkable and fairly large radiation (Figure 1, left) as a distinct genus.

***Raorchestes* gen. nov.** Type species, *Ixalus glandulosus* Jerdon 1854

**Etymology:** The generic epithet is derived from the name ‘Rao’ and the scientific nomen ‘*Orchestes*’. The former is chosen in honour of C. R. Narayan Rao, in recognition of his contribution to Indian batrachology. The latter is based on the first generic nomen coined for frogs of the *Philautus* group, *Orchestes* Tschudi 1838.

**Diagnosis:** We distinguish different kinds of taxonoses<sup>17</sup> and define the new genus according to two of them: **Idiognosis** (brief characterization of a taxon through characters): relatively small frogs, adult snout-vent length between 15 and 45 mm; vomerine teeth absent; large gular pouch transparent while calling; largely nocturnal species; direct development without free-swimming tadpoles in all species for which the development is known. **Entexognosis** (characterization of a taxon in a phylogenetic framework, through inclusion and exclusion of taxa or organisms): the most inclusive clade including *Ixalus glandulosus* Jerdon 1854 and excluding *I. temporalis* Günther 1864.

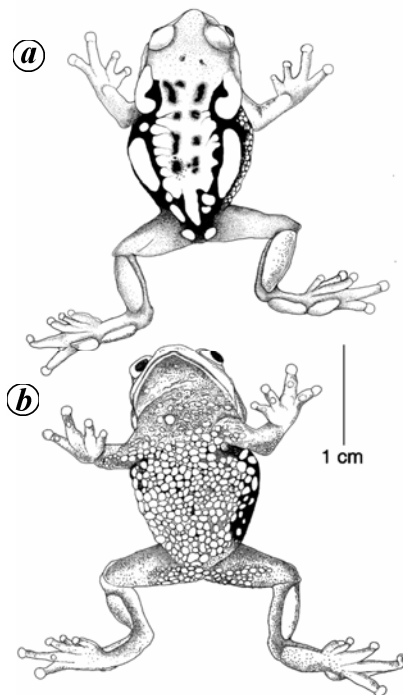
**Distribution:** Most species are from the Western Ghats of India, but recent molecular phylogenetic analyses supporting a nested position for ‘*Philautus*’ *menglaensis*, *longchuanensis* and *gryllus* in this clade<sup>12,15</sup> indicate that the range of *Raorchestes* extends at least to southern China, Laos and Vietnam<sup>1</sup>. *Raorchestes* is absent from Sri Lanka.



**Figure 1.** Left, Maximum likelihood tree based on a 3370 bp data matrix of mtDNA, showing the nested phylogenetic position of *Raorchestes resplendens* in the genus. Right, Maximum likelihood tree for known *Raorchestes* species (all except *R. ochlandrae*) of the Western Ghats, based on a 1488 bp data matrix of mtDNA. Black circles indicate Bayesian posterior probabilities above 95%.

**Table 1.** Morphometric measurements (all in mm; range, mean and standard deviation) of *Raorchestes resplendens* sp. nov. Abbreviations are defined in the text

	Male (N = 5)					Female (N = 2)							
	BNHS 4087	BNHS 5301	BNHS 5302	BNHS 5303	ZSIC A9140	Range	Mean	SD	BNHS 5304	BNHS 5305	Range	Mean	SD
SVL	24.0	24.5	22.7	24.0	24.5	22.7–24.5	23.9	0.7	28.3	25.2	25.2–28.3	26.8	2.2
HW	9.4	9.6	8.6	9.2	9.5	8.6–9.6	9.3	0.4	10.6	10.2	10.2–10.6	10.4	0.3
HL	9.1	8.9	8.1	8.7	9.1	8.1–9.1	8.8	0.4	10.1	9.8	9.8–10.1	10.0	0.2
SL	3.0	3.3	3.0	3.4	3.4	3.0–3.4	3.2	0.2	3.6	3.6	3.6	3.6	0.0
EL	2.4	2.6	2.7	2.6	2.6	2.4–2.7	2.6	0.1	2.7	2.8	2.7–2.8	2.6	0.1
IUE	2.7	3.1	2.9	2.9	2.8	2.7–3.1	2.9	0.1	3.3	3.4	3.3–3.4	3.4	0.1
UEW	1.6	1.3	1.3	1.5	2.1	1.3–2.1	1.6	0.3	1.3	1.3	1.3	1.3	0.0
FLL	4.8	5.0	4.7	4.6	5.0	4.6–5.0	4.8	0.2	5.4	5.3	5.3–5.4	5.4	0.1
HAL	6.0	6.3	5.8	6.6	6.3	5.8–6.6	6.2	0.3	6.9	6.6	6.6–6.9	6.8	0.2
ShL	8.3	8.2	7.8	8.2	8.3	7.8–8.3	8.2	0.2	9.8	8.7	8.7–9.8	9.3	0.8
TL	8.8	9.1	8.3	9.0	9.4	8.3–9.4	8.9	0.4	10.6	9.1	9.1–10.6	9.9	1.1
TFOL	12.9	12.6	11.8	12.8	13.2	11.8–13.2	12.7	0.5	14.7	12.4	12.4–14.7	13.6	1.6
FOL	8.5	9.1	7.0	8.5	8.9	7.0–9.1	8.4	0.8	1.1	9.1	9.1–10.1	9.6	0.7

**Figure 2.** *Raorchestes resplendens* sp. nov. holotype. *a*, Dorsal view; *b*, Ventral view.

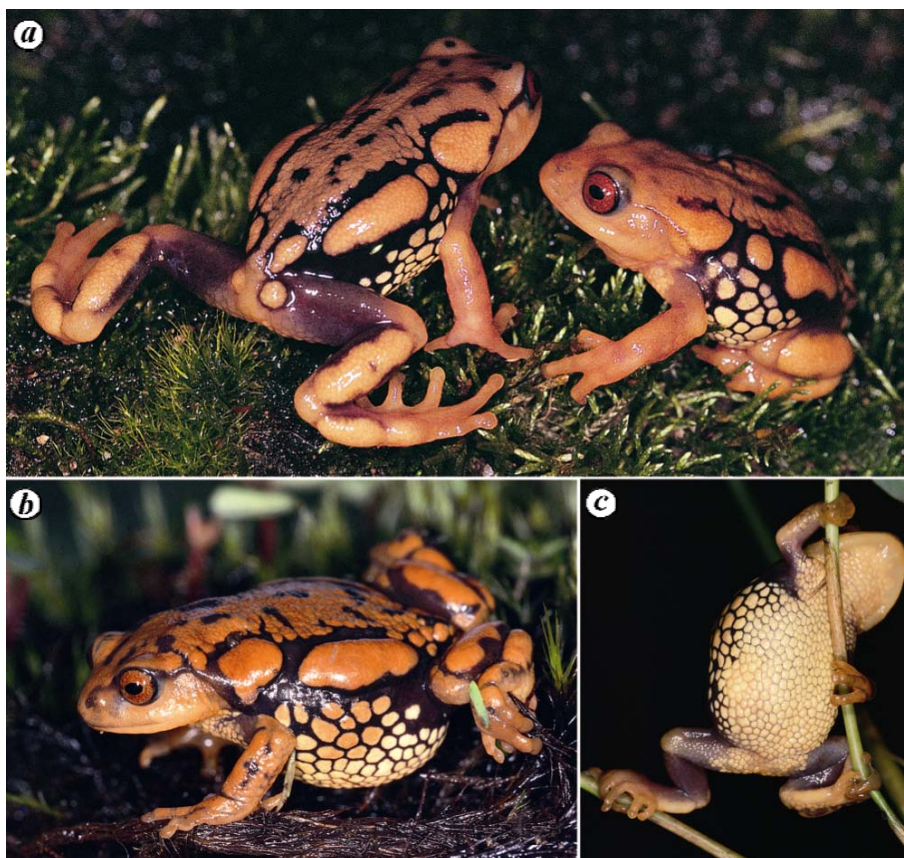
*Raorchestes resplendens* sp. nov. *Holotype*: BNHS 4087 (Figures 2, 3a left animal), an adult male collected by SDB at an altitude of 2695 m asl, at Anamudi summit, 10°10'N 77°05'E, Idukki District, Eravikulam National Park, Kerala, India. This specimen is kept in the Bombay Natural History Society (BNHS), Mumbai, Maharashtra, India. *Paratypes*: four adult males, BNHS 5301, 5302, 5303 and ZSIC A9140 (Kolkata, West Bengal, India); two adult females, BNHS 5304 and 5305. All were collected along with the holotype (Table 1).

*Etymology*: The species epithet '*resplendens*' is a Latin term meaning 'bright-coloured, glittering, resplendent'. It refers to the bright red or orange colouration. Suggested vernacular name: 'resplendent shrubfrog'.

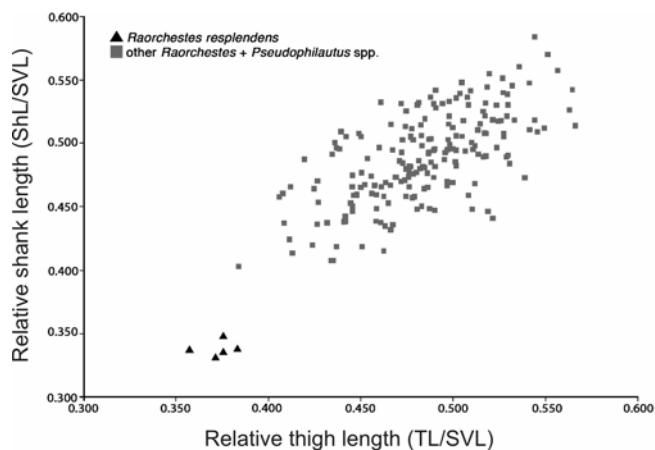
*Diagnosis*: *Raorchestes resplendens* can be distinguished from all members of the genus by its multiple prominent glandular swellings; laterally behind the eyes, on the side of the dorsum, on the anterior side of the vent, on the dorsal side of the forearms and shanks, and on the posterior side of tarsus and metatarsus (Figures 2 and 3). Additionally, the colour of the iris is invariably bright red (Figure 3) and the legs are extremely short (Figure 4).

*Phylogeny*: To infer a more precise phylogenetic position of *R. resplendens*, we constructed a 1488 bp data matrix covering part of the 16S rRNA, part of the ND1, the complete tRNA<sup>ILE</sup> and tRNA<sup>GLN</sup>, and part of the tRNA<sup>MET</sup> genes for all known extant species (except for *R. ochlandrae*) from the Western Ghats<sup>2</sup>. MP, ML and Bayesian are in agreement with previous analyses<sup>2</sup>. Additionally, *R. resplendens* comes out as a close relative of *R. beddomii*, *R. munnarensis* and *R. dubois* (Figure 1, right). The low support for relationships within this clade however indicates that either of the latter three species may be the sister taxon of *R. resplendens*.

*Description*: The description (all measurements in mm) of the holotype follows terminology used elsewhere<sup>2,18–20</sup>. Medium sized frog (SVL 24); head slightly wider than long (HL 9.1; HW 9.4; MN 7.4; MFE 6.6; MBE 4.1); outline of snout in dorsal view nearly rounded, its length (SL 3.0) larger than the horizontal diameter of the eye (EL 2.4); canthus rostralis indistinct; loreal region vertically acute; distance between posterior margins of eyes (IBE 8.0) 2.1 times in distance between anterior margins of eyes (IFE 3.8); interorbital area (IUE 2.7) larger than upper eyelid (UEW 1.6); tympanum (TYD 1.3) rather distinct, rounded; vomerine ridge absent;



**Figure 3.** *Raorchestes resplendens* sp. nov. in life. **a**, Holotype (BNHS 4087, left-hand side); paratype (BNHS 5302, right-hand side); **b**, Paratype (BNHS 5304), profile; **c**, Paratype (BNHS 5304), ventral side, showing granulation.



**Figure 4.** Plot of relative thigh length ( $x$ -axis, TL/SVL) versus relative shank length ( $y$ -axis, ShL/SVL) for 200 males of Indian *Raorchestes* and *Pseudophilautus* species. The plot shows a wide ecomorph diversity, likely corresponding to different microhabitat preferences. The extremely short legs of *Raorchestes resplendens* reflect its terrestrial ecology.

tongue cordate, without a papilla; supratympanic fold from posterior corner of upper eyelid to base of forelimb. Forelimb length (FLL 4.8) shorter than hand (HAL 6.0);

tips of fingers with discs, with distinct circummarginal grooves; webbing on fingers absent; fingers without lateral dermal fringe; subarticular tubercle indistinct, prepollex oval; supernumerary tubercles absent, nuptial pads absent. Hind limbs moderately short; shank about 3 times longer (ShL 8.3) than wide (ShW 2.6), shorter than thigh length (TL 8.8), slightly shorter than distance from base of inner metatarsal tubercle to tip of toe IV (FOL 8.5; TFOL 12.9); tips of toes with discs and distinct circummarginal grooves; webbing reduced, reaching just below the 2nd subarticular tubercle on inside of toe IV and up to the 2nd subarticular tubercle on outside of toe IV; dermal fringe along toe V absent; subarticular tubercles rather indistinct; supernumerary tubercle absent; inner metatarsal tubercle indistinct.

Skin of snout smooth; between eyes shagreened; upper eyelids sparsely granular; anterior and posterior part of back and flanks with symmetrical glandular swellings: a kidney-shaped gland behind eye, a rather long and wide latero-dorsal gland all along upper part of flank, a small rounded gland on anterior side of vent; dorsal parts of limbs with glandular swellings: a long gland all along dorsal part of forelimbs (from elbow to base of hand); a rather long gland on dorsal part of shank, on posterior



**Figure 5.** *a–g*, *Raorchestes resplendens* developmental stages in life. *a*, Eggs after 3 h; *b*, neural fold at 30 h; *c*, head, limb bud and tail differentiation at 80 h and beginning of embryo rotation; *d*, hind limb differentiation at 140 h; *e*, elongation of toe and forelimb differentiation at 230 h; *f*, froglet with reduced tail and mouth differentiated into upper and lower lips at 380 h and tail and yolk considerably reduced in this stage; *g*, fully mobile froglets measuring SVL  $4.5 \pm 0.7$  mm emerged after 540 h (about 22 days).

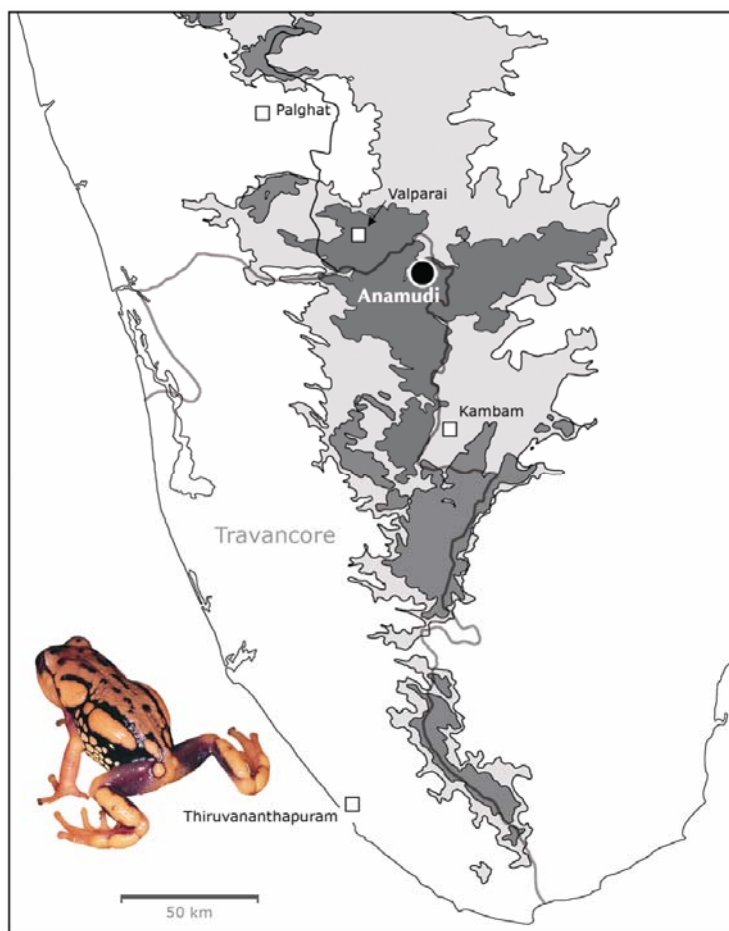
side of tarsus, and on posterior side of metatarsus of toe V; a few less prominent glands throughout the dorsal and lateral parts of the body (Figure 3*a*, left); ventral side of throat slightly granular, belly coarsely granular (oval to hexagonal); thigh and lower arm sparsely granular.

**Colour of holotype:** In life (Figure 3*a*, left): The predominant colour of dorsum is deep brick red, which sharply contrasts with the black filling irregular furrows on the dorsum; the dorsal parts of limbs, fingers, toes and tips are uniform deep brick red; glandular swellings are surrounded with black patches; posterior lateral abdominal area, groin and inner side of the thighs are black; ventral side light yellow, with irregular oval to hexagonal granulation; iris bright red. In alcohol, the dominant brick red colour changed into brownish grey which contrasts with the black furrows; lateral side light yellowish; limbs are reddish grey; ventral side light greyish white.

**Breeding and development:** Breeding observations were conducted in the natural habitat without handling the animals. During a late afternoon (16 h) in July, a pair of *R. resplendens* in amplexus was located on the ground in bamboo thickets. Initial amplexus was inguinal for a period of about 3 h during which time the female was almost motionless. After that, the amplexus became axillary, the female started to search for a suitable oviposition site, and at 20 h, eggs were burrowed under the moss covered forest floor, deep inside the base of bamboo clumps. The male and female left the nest immediately after completion of spawning. Several other clutches were located within a radius of 7 m, and all were at the base of bamboo clumps (number of eggs: 18–28,  $N = 7$ ).

The female, male and egg clutches were collected and developmental stages were observed under laboratory conditions with a temperature difference from natural conditions of maximum 3°C. As in other species of this genus, *R. resplendens* undergoes direct development, i.e. complete development in the eggs, without a free-swimming larval stage. After oviposition (eggs:  $N = 24$ , diameter  $4.1 \pm 0.4$  mm), the female's oviduct still contained mature embryos ( $N = 18$ , diameter  $3.8 \pm 0.5$  mm). This indirectly suggests that the female may mate with multiple males and/or may breed more than once in a single season. The developmental time from embryo to hatching varied from 22 to 27 days ( $N = 7$ ). Descriptions of embryo morphology are based on observation of living and preserved material. The major features of eight stages are illustrated in Figure 5*a–g*.

**Comments on morphology:** The combination of a unique morphology and nested phylogenetic position of the new species points to a quick morphological divergence from congeners. The most striking character of *R. resplendens* is the presence of numerous macroglands. These are absent in all species of *Philautus*, *Pseudophilautus* and *Raorchestes* presently known and thus are derived characters. The presence, distribution and size of the macroglands shows no sexual dimorphism, so we hypothesize that the primary function will not be involved in reproduction, but this needs further testing. The quick morphological evolution is also visible when plotting relative limb length of *Pseudophilautus* and *Raorchestes* species (Figure 4). These measurements indicate that the new species has much shorter limbs than



**Figure 6.** Map showing the type locality of *Raorchestes resplendens* at Anamudi, in the Western Ghats. Map modified from Inger *et al.*<sup>21</sup>. Shaded contours showing above 300 m asl (light) and above 1000 m asl (dark).

its congeners and members of the sister clade, pointing to a rapid morphological evolution that is dissociated from molecular evolution. These short limbs have resulted in a much more pronounced crawling behaviour in this species compared to its congeners.

*Comments on conservation concern:* *R. resplendens* is known only from the type locality (Figure 6). The highest temperature reported for the Anamudi summit is 30°C in May 1980 and the lowest is -3°C in June 1980 (ref. 22). The vegetation, climate and temperature of the hills are different from other regions of the Western Ghats, and share several floristic elements with Himalayas<sup>22–24</sup>. Most importantly, despite intensive searches in suitable habitats close to the type locality, we were unable to locate this species in any other place. It is therefore possible that the entire range of this species does not exceed a few square kilometres. We suggest immediate action to make this highly endangered species a top priority for conservation.

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