Rec. zool. Surv. India: Vol. 121(3)/355–361, 2021
DOI: 10.26515/rzsi/v121/i3/2021/152628

ISSN (Online) : 2581-8686
ISSN (Print) : 0375-1511

'Endangered' or 'Near Threatened', distribution status of Karaavali Skittering frog from the west coast of peninsular India

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Abstract

The Karaavali skittering frog, *Euphlyctis karaavali* was described in 2016 from the western coastal plains of Karnataka, India with the IUCN Red list conservation status as 'Endangered'. In the present account, species distribution boundaries has been re-defined based on the field sampling and museum collections. On account of the present studies, IUCN Red list conservation status for the species is proposed as 'Near Threatened'. Discussions have been made on the morphological crypticity prevailing in the genus and the species complex *E. cyanophlyctis* since two centuries. Premature proposal of Red list conservation status during the new species descriptions and their implications on the biodiversity documentation studies are reviewed.

Keywords: Conservation, Distribution, Extent Of Occurrence (EOO), Genetic Identity, Skittering Frog, Taxonomy, West Coast of India

Introduction

The Karaavali Skittering frog, *Euphlyctis karaavali* was described by Priti *et al.* (2016) from the western coastal plain village Sanikatta, Uttara Kannada, Karnataka of Indian peninsular plateau based on the collections made during the year 2015. The distribution range of the species was thought to be coastal plain districts of Uttara Kannada, Udupi and Dakshina Kannada in Karnataka. Despite limited field surveys and collections, the IUCN Red List conservation status for the species was proposed as Endangered (EN) under B1ab (i)(iii)(iv).

Anoop *et al.* (2017), while establishing the complete mitochondrial genome of *E. karaavali* reported the species from Nedumangad, Thiruvananthapuram, Kerala further extending the range distribution to extreme southern western coastal plains of peninsular India without any comments on its IUCN Red List conservation status.

Although amphibian taxonomic research was initiated during the year 1799 with the description of *Duttaphrynus melanostictus* (as *Bufo melanostictus*), *Duttaphrynus scaber* (as *Bufo scaber*), *Euphlyctis cyanophlyctis* (as *Rana cyanophlyctis*), *Sphaerotheca breviceps* (as *Rana breviceps*) and *Uperodon systoma* (as *Rana systoma*) by Schneider, still the taxonomic identity and the distribution ranges are not clear for the species *D. melanostictus* and *E. cyanophlyctis*.

In the last decade, almost 130 species of amphibians were described within the political boundaries of India, of which IUCN Red List conservation status has been proposed for only four species and the rest of the species need systematic field study-based assessment (now treated as either Data Deficient or Not Assessed) (Dinesh *et al.*, 2019). During the description of *Raorchestes resplendens* Biju *et al.* (2010a) proposed the species conservation status as Critically Endangered; Chandramouli *et al.* (2016) proposed the status as Endangered for *Blythophryne beryet*; *E. karaavali* and *Microhyla laterite* were proposed the status as Endangered by Priti *et al.* (2016); Seshadri *et al.* (2016) respectively. The conservation status of *R. resplendens* by Biju *et al.* (2010a) could be justified due

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Article Received on: 22.05.2020 Accepted on: 04.08.2021

to high altitude preference of the species and limited availability of its habitat in the southern Western Ghats and of *B. beryet* by Chandramouli *et al.* (2016) for being an arboreal island species. Being low elevation water-associated species, the conservation status of *M. laterite* and *E. karaavali* needs reevaluation based on the large-scale field explorations.

In India seven species of *Euphlyctis* are reported (Frost, 2020), of which six species are described within the Indian political boundaries since the year 1799, wherein three species are described in the new millennia (Dinesh *et al.*, 2009; 2019). Until the description of *E. karaavali* by Priti *et al.* (2016), the species *E. hexadactylus* was thought to be very widespread in peninsular India due to high morphological character crypticity and now the species *E. hexadactylus* distribution is established to the east coast of peninsular India (Priti *et al.*, 2016; Frost, 2020).

Here we report multiple populations of *E. karaavali* based on field studies, National Zoological Collections and the genetic studies in the west coast of India with the reassessment of IUCN Red List conservation status for the species.

Material and Methods

The primary set of distribution data was taken from published literature of Priti *et al.* (2016) and Anoop *et al.* (2017). Field sampling was done by Channakeshavamurthy and the team in and around Kozhikode, Kerala. Specimens collected were photographed in controlled conditions and euthanized with MS222, liver samples were dissected for molecular studies, and initial fixing was done in 4% formalin for 24 hours and preserved in 70% alcohol for further studies. Morphometric measurements were taken with Mitutoyo vernier callipers (to the nearest 0.1 mm). DNA extraction, PCR amplification for 16S rRNA, sequencing and phylogenetic analysis protocols are followed after Dinesh *et al.* (2015, 2017) for Dicroglossid frogs. Morphological character set abbreviations used in Table 1 is followed after Dinesh *et al.* (2015). In addition to this, old collection at ZSI/WGRC/Kozhikode (Radhakrishnan and Dinesh, 2013) and ZSI/WRC/Pune (un-published) were included in the present study (Figure 1). The minimum convex hull of the occurrences data for Extent of Occurrence (EOO) as per IUCN Red list criteria was calculated using GeoCAT-Geospatial Conservation Assessment Tool (Bachman *et al.*, 2011).

Results

Morphological (Figure 3) and morphometric data for our samples of *E. karaavali* were matching with the descriptions of the species by Priti *et al.* (2016) (Figure 1 & Table 1). Pair wise Genetic Distance (GD) for the 16S rRNA was 0.6 % for the populations reported by Priti *et al.* (2016) and 0.8 % for the population reported by Anoop *et al.* (2017) (Figure 2 & Table 2). Phylogenetic analysis showed a monophyletic clade for the species of *E. karaavali* (Figure 1) with 0.6% to 1% GD (Table 2).

Priti *et al.* (2016) have mentioned the GenBank accession numbers KU870372 and KU870374 for the 16S rRNA which is erroneous and it should be read as KU870372 and KU870373 as per the GenBank records.

In the present account, only fresh samples were checked for genetic identity and based on the morphological and morphometric characters museum collections of ZSI/WGRC/Calicut (ZSI/WGRC/V/A/283; ZSI/WGRC/V/A/285; ZSI/WGRC/V/A/311; ZSI/WGRC/V/A/340; ZSI/WGRC/V/A/408 and ZSI/WGRC/V/A/554 (Radhakrishnan and Dinesh, 2013) (Table 3) and ZSI/WRC/Pune (ZSI/WRC/A/102; ZSI/WRC/A/103; ZSI/WRC/A/247; ZSI/WRC/A/248; ZSI/WRC/A/249; ZSI/WRC/A/250; ZSI/WRC/A/346 and ZSI/WRC/A/350 (unpublished data) labeled as *E. hexadactylus* are treated as *E. karaavali*.

As per IUCN, Extent of Occurrence (EOO) is an important tool for assessing the conservation status of a taxon, where it maps all known occurrences of a taxon within its outermost geographical limits. EOO is measured by a minimum convex polygon whose internal angle does not exceed 180 degrees and contains all the sites of occurrence of the species (IUCN, 2012; Burgman and Fox, 2003). The minimum convex polygon measured for the EOO of *E. karaavali* is around 40516.126 sq km (Bachman *et al.*, 2011) and based on this we propose the conservation status as Near Threatened (NT) for the species (Figure 1). Wherever we have encountered the species in the field they were found to be abundant.

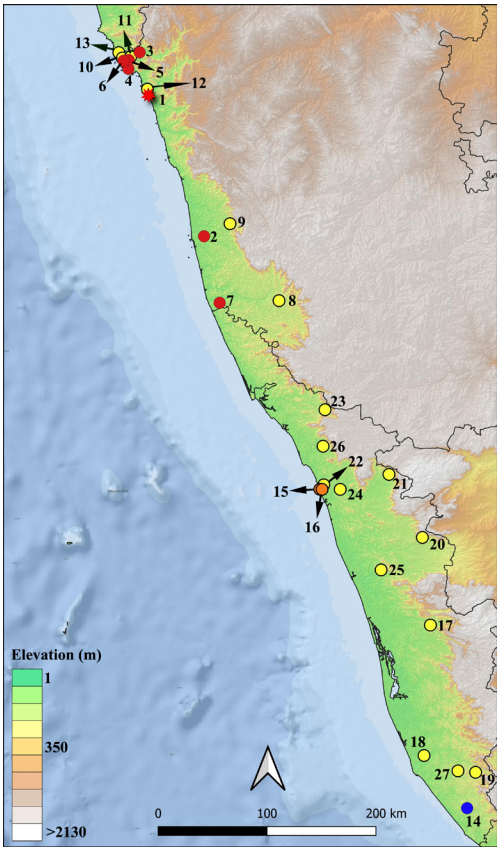

Figure 1. Map showing the distribution range of *E. karaavali* in the west coast of India (red star, type locality; red circle, other collection localities by Priti *et al.* (2016); blue circle, genetically identified by Anoop *et al.* (2017); orange circle, genetically confirmed sample in the present study; yellow circle, museum collection localities in the present study).

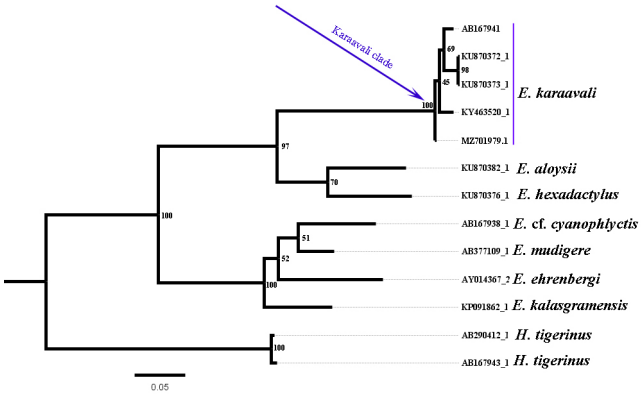

Figure 2. Maximum likelihood tree for the genus *Euphlyctis* based on 573 bp of 16S rRNA.

Figure 3. *E. karaavali* in life from Calicut township, Kozhikode, Kerala.

Table 1. Morphometric details of the original descriptions of *Euphlyctis karaavali* and the present collections.

	Priti *et al.*, 2016 (male, n=5)	**Priti *et al.*, 2016 (female, n=1)**	**ZSI/WRC/A (male, n=3)**	**ZSI/WRC/A (female, n=2)**
	Average (range)	**Average**	**Average (range)**	**Average (range)**
SVL	61.9 (53.5-70.9)	106.3	60.1 (55.5-67.1)	94.3 (94.2-94.3)
HW	23.2 (20.3-25.9)	40.3	22.4 (21.1-23.4)	35.5 (35.0-36.0)
HL	22.4 (19.8-24.5)	36.6	24.3 (21.4-27.7)	36.0 (35.5-36.5)
IN	3.5 (3.0-4.1)	5.8	2.5 (2.3-2.7)	2.3 (2.8-3.1)
NE	5.6 (4.6-6.2)	10.7	5.1 (4.8-5.5)	7.9 (7.7-8.0)
MN	18.2 (16.7-19.6)	30.7	20.9 (19.5-23.4)	31.0 (30.8-31.2)
MFE	13.4 (12.4-14.8)	21.5	17.3 (16.0-19.5)	23.8 (23.3-24.2)
MBE	8.2 (7.68.0-8.7)	14.0	11.2 (10.8-11.5)	16.2 (15.5-16.8)
SL	10.1 (8.8-11.5)	17.4	9.4 (8.3-10.8)	14.8 (14.4-15.1)

Table 1. Contd.

	Priti *et al.*, 2016 (male, n=5)	Priti *et al.*, 2016 (female, n=1)	ZSI/WRC/A (male, n=3)	ZSI/WRC/A (female, n=2)
	Average (range)	Average	Average (range)	Average (range)
EL	7.3 (6.7-8.5)	9.3	7.2 (6.5-8.3)	7.9 (7.8-7.9)
IUE	3.2 (2.4-4.0)	5.3	3.2 (2.7-3.5)	4.6 (4.3-4.8)
UEW	4.5 (4.0-5.6)	7.2	4.1 (3.5-4.9)	5.9 (5.6-6.1)
IFE	8.3 (7.2-9.3)	14.6	8.4 (7.5-9.9)	12.2 (12.1-12.3)
IBE	13.8 (12.6-16.1)	21.9	13.6 (12.6-15.2)	18.8 (18.4-19.1)
TYD	4.8 (4.4-5.2)	6.8	5.3 (4.5-6.4)	6.4 (6.0-6.7)
FLL	9.9 (8.0-11.9)	19.2	12.3 (11.3-13.9)	17.0 (16.2-17.7)
HAL	14.7 (13.3-15.7)	26.1	14.2 (13.7-14.9)	21.8 (20.7-22.9)
FL1	5.2 (4.7-5.9)	8.2	5.4 (5.2-5.5)	8.9 (8.8-9.0)
FL2	4.3 (3.9-4.7)	7.5	4.4(4.3-4.7)	7.8 (7.3-8.2)
TFL	6.5 (6.0-6.9)	11.5	7.1 (7.0-7.2)	10.6 (10.5-10.7)
ShL/FL	24.2 (13.3-31.0)	50.2	30.8 (28.3-33.5)	46.7 (46.0-47.3)
TiL	27.2 (23.1-29.8)	48.7	27.2 (26.2-29.0)	42.8 (41.7-43.9)
FOL	30.3 (26.8-34.4)	53.3	30.0 (27.3-32.7)	44.8 (42.1-47.5)
FTL	17.0 (15.2-18.8)	22.3	17.3 (16.0-19.0)	25.6 (24.2-27.0)
ITL	5.2 (4.9-5.6)	13.3	5.3 (4.8-6.1)	8.6 (8.5-8.6)
IMT	3.3 (2.4-4.3)	6.1	3.4 (2.9-4.4)	4.9 (4.6-5.2)

Table 2. GenBank details for the Maximum likelihood tree (Figure 2).

Sl. No.	Species	GenBank accession number	Sampling location
1.	*E. karaavali*	MZ701979.1	Calicut, Kozhikode
2.	*E. karaavali*	KU870372.1	Kodanga, Uttara Kannada
3.	*E. karaavali*	KU870373.1	Sanikatta, Uttara Kannada
4.	*E. karaavali*	KY463520.1	Nedumangad, Thiruvananthapuram
5.	*E. karaavali*	AB167941.1	Adyar, Mangalore
6.	*E. aloysii*	KU870382.1	Kodanga, Uttara Kannada
7.	*E.* cf. *cyanophlyctis*	AB167938.1	Madikeri
8.	*E. ehrenbergi*	AY014367.2	Arabian Peninsula
9.	*E. hexadactylus*	KU870376.1	Puducherry
10.	*E. kalasgramensis*	KP091862.1	Kalasigram, Bangladesh
11.	*E. mudigere*	AB377109.1	Mudigere, Chickkamagaluru

Table 3. Collection locality details used for mapping and EOO calculations for *E. karaavali*.

Sl. No.	Source data	Locality	Latitude	Longitude
1.	Priti *et al*., 2016	Sanikatta, Uttara Kannada, Karnataka	14.551	74.338
2.	Priti *et al*., 2016	Kodanga, Udupi, Karnataka	13.373	74.802
3.	Priti *et al*., 2016	Baire, Uttara Kannada, Karnataka	14.907	74.265
4.	Priti *et al*., 2016	Chendia, Uttara Kannada, Karnataka	14.769	74.171
5.	Priti *et al*., 2016	Kadwada, Uttara Kannada, Karnataka	14.843	74.174
6.	Priti *et al*., 2016	Tariwada, Uttara Kannada, Karnataka	14.836	74.130
7.	Priti *et al*., 2016	Konaje, Mangaluru, Karnataka	12.818	74.932
8.	Field sightings only	Kowkradi, Dakshina Kannada, Karnataka	12.836	75.427
9.	Field sightings only	Kuchchur, Udupi, Karnataka	13.478	75.018
10.	Field sightings only	Kanasgiri Uttara Kannada, Karnataka	14.860	74.145
11.	Field sightings only	Near Taleband Uttara Kannada, Karnataka	14.846	74.179
12.	Field sightings only	Near Gokarna Beach Uttara Kannada, Karnataka	14.544	74.315
13.	Field sightings only	Polem beach, Goa	14.905	74.091
14.	Anoop *et al*., 2017	Nedumangad, Thiruvananthapuram, Kerala	8.603	76.995
15.	ZSI/WGRC/ Calicut collections	Kozhikode Beach Road, Kozhikode, Kerala	11.263	75.767
16.	ZSI/WGRC/Calicut collections	ZSI Calicut, Kozhikode, Kerala	11.262	75.786
17.	ZSI/WGRC/Calicut collections	Thattekad BS, Ernakulam, Kerala	10.130	76.689
18.	ZSI/WGRC/Calicut collections	Sasthamcotta (DB College), Kollam, Kerala	9.042	76.636
19.	Field sightings only	Kulathupuzha, Kollam, Kerala	8.902	77.066
20.	Field sightings only	Dhoni reserve forest, Palakkad, Kerala	10.861	76.622
21.	Field sightings only	Vazhikkadavu, Malappuram, Kerala	11.387	76.344
22.	ZSI/WGRC/V/A/283	Kottooli, Kozhikode, Kerala	11.271	75.792
23.	ZSI/WGRC/V/A/285	Neerkadavu, Kannur, Kerala	11.926	75.810
24.	ZSI/WGRC/V/A/311	Kanniparamba, Kozhikode, Kerala	11.262	75.937
25.	ZSI/WGRC/V/A/340	Machad, Thrissur, Kearala	10.590	76.278
26.	ZSI/WGRC/V/A/408	Janakikkadu, Kozhikode, Kerala	11.622	75.794
27.	ZSI/WGRC/V/A/554	Vallamvetti, Kollam, Kerala	8.914	76.919

Discussion

Due to similar body size and similar body colour patterns, it is difficult to distinguish the individuals of *Euphlyctis* in the field as well as museum collections, and this could be one of the reasons all the old collections of the west coast were identified as *E. hexadactylus* which was originally described from the east coast. Among the eight species known from the genus, specific genetic identity is confirmed only for the five species *E. hexadactylus* (east coast of India), *E. ehrenbergii* (Arabian peninsula), *E. aloysii* (Western Ghats), *E. mudigere* (Western Ghats), *E. kalasgramensis* (India, Bangladesh) and *E. karaavali* (west coast of India) and there is need to fix the taxonomy and genetic identity of the species *E. ghoshi* (Manipur, northeast India) and *E. cyanophlyctis* (described from "India orientali").

Interestingly the species *E. cyanophlyctis* was described more than two centuries ago and the taxonomic status and genetic identity of the species is still in a state of flux. The species was described during 1799 by Schneider from "India orientali" (which could be current West Bengal during the

rule of the East India Company (from 1757 to 1858), but Bauer (1998) considered the type locality as 'Tranquebar', India (present Tharangambadi, Mayiladuthurai, Tamil Nadu). Priti *et al.* (2016) considered the genetic samples of *Euphlyctis* by Kurabayashi *et al.* (2005) from Madikeri as *E. cyanophlyctis* which is described from the east coast as the case of *E. hexadactylus* (and *E. karaavali*), where it is very unlikely species described from the west coast of India having distribution in the west coast and Western Ghats. With this backdrop the genetic sequences used by Kurabayashi *et al.* (2005) and Priti *et al.* (2016) the nomen *E. cyanophlyctis* needs further taxonomic resolution.

Recently Mirza *et al.* (2019) have stressed upon the problems associated with the premature consideration of the species as "Endangered" under the IUCN classification and funding agency biases for such labels for the species during project allocations. 'Unsound taxonomy' is a bidirectional threat for amphibian studies in general as many a times funds might be allocated to species which might not be under immediate threat (Mirza *et al.*, 2019) and when a species is assigned a 'threatened category' during the description itself, it is difficult to get the local forest permits to study the biology and ecology of such species where specimen collections are essential.

One optional way to encourage other studies is to consider the IUCN conservation status to be 'Data Deficient' (Biju *et al.*, 2010a & 2010b; Bhatta *et al.*, 2011; Kotharambath *et al.*, 2012, 2015; Garg *et al.*, 2017; Dinesh *et al.*, 2017; Raj *et al.*, 2018; Vineeth *et al.*, 2018; Phuge *et al.*, 2019; Biju *et al.*, 2019; Vijayakumar *et al.*, 2019) during the species description and to raise the fund support for taking up of next level of studies.

Acknowledgements

Our special thanks to Dr. Kartik Shanker of the Evolutionary Ecology Lab at Centre for Ecological Sciences (CES), Indian Institute of Science (IISc), Bangalore, for the support in molecular studies as a part of 'Open Taxonomy Initiative'. We thank Mr. Amith Hegde for sharing the field observations data on the northern West Coast of Karnataka. KPD and BHCKM are grateful to the Director, ZSI, Kolkata; the Officer-in-Charge, ZSI, WRC, Pune and the Officer-in-Charge, ZSI, WGRC, Kozhikode for the help and support. KPD is thankful to DBT and SERB (SR/FR/LS-88/210/09.05.2012) for the fellowship and assistance to conduct part of this work. PD is grateful to the Principal and the Head, Department of Zoology, Mount Carmel College, Autonomous, Bengaluru, for the support. AG would like to thank the department of Atomic Þnergy, Government of India and NISER, Bhubaneswar for facilities.

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