Sandeep Das

ECOLOGY AND BEHAVIOUR OF AMPHIBIANS OF ERAVIKULAM NATIONAL PARK WITH SPECIAL REFERENCE TO BUSH FROGS

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Thesis submitted to the
University of Calicut in partial fulfilment of the
requirements for the Degree of

DOCTOR OF PHILOSOPHY in ZOOLOGY

By

Sandeep Das

Supervising Guide
Dr. P.S. EASA

KSCSTE - KERALA FOREST RESEARCH INSTITUTE, PEECHI

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Dr. P.S. EASA
Former Scientist
& Head Dept. of Wildlife Biology
Kerala Forest Research Institute
Peechi-680 653, Kerala, India

Mobile: +91 9446324070
Email: easaelephant@yahoo.com

CERTIFICATE

This is to certify that the thesis entitled **Ecology and Behaviour of Amphibians of Eravikulam National Park with Special Reference to Bush Frogs** is an authentic record of research work carried out by **Mr. Sandeep Das** under my supervision and guidance in KSCSTE- Kerala Forest Research Institute in partial-fulfilment of the requirements for the degree of Doctor of Philosophy of the University of Calicut. The work has not been previously submitted for the award of any degree, diploma, or other similar titles to any candidate of any university.

Dr. P.S. Easa

Thrissur
22 February 2022

Dr. P.S. EASA
Former Scientist
Department of Wildlife Biology
KSCSTE-Kerala Forest Research Institute
Thrissur, Peechi-680653

KSCSTE Kerala Forest Research Institute
P.O.Peechi
680 653
Thrissur, Kerala

DECLARATION

I, Sandeep Das do hereby declare that the thesis entitled **"Ecology and Behaviour of Amphibians of Eravikulam National Park with Special Reference to Bush Frogs"** is an authentic record of the research work carried out by me in KSCSTE - Kerala Forest Research Institute, under the guidance of **Dr P. S. Easa.** I further declare that no part of this thesis has been submitted previously for any other degree or diploma of this or any other university.

Sandeep Das

Thrissur
22 February 2022

To my family

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ABSTRACT

Amphibians face drastic population declines due to climate change, habitat destruction and emerging diseases, making them the most threatened animal group among vertebrates. Despite the higher rates in species discovery and higher values of species diversity, amphibians are yet to garner conservation attention unlike larger animal groups. Conservation prioritization largely depend on the IUCN Red List of Threatened Species and more than two third of the total amphibian species are either not assessed or not updated. Recent works on amphibians in the Western Ghats are broadly focussed on taxonomy. Majority of the Protected Areas of the Western Ghats don't have detailed information of their amphibians. Eravikulam National Park (ENP) known for its montane shola and grasslands, in particular was not investigated for amphibians other than a few taxonomic works. The current study attempted to understand the ecology and behaviour of amphibians in ENP.

Quadrat and Visual Encounter Surveys were employed to collect data on amphibian diversity and distribution from 2014 to 2018. The study reports 37 species indicating high diversity and high degree of endemism (36 sp.). Shola ecosystem was observed to have more species richness whereas grasslands dominated in the abundance. Rhacophoridae was the most represented family with higher species diversity in the *Raorchestes* genus. The surveys report several new distributional and elevational records for multiple species. Molecular phylogenetic study was employed and intraspecific variation between populations of *Raorchestes ochlandrae* north and south of the gap was determined. Ten QS in grasslands with greater vocalization activity of bush frogs for a total of 100 hours was done to understand whether there is any activity pattern in their acoustic signalling. Both *Raorchestes dubois* and *Raorchestes resplendens* were recorded

to show changes in their signalling behaviour in accordance with the other species. Vocalization of *Raorchestes resplendens* were recorded and 141 calls from 10 individuals were analysed and call characteristics were described.

The impact of prescribed burning practices on amphibians was studied through quadrat sampling in 18 plots before burning, burning and after burning from 2015 to 2018. Results indicated a significant negative impact on amphibian populations due to burning. The current study provides baseline information on diversity and distribution, temporal activity pattern in vocalisation, vocalization of *Raorchestes resplendens* and impact of prescribed burning practices.

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ACRONYMS

CWS	-	Chinnar Wildlife Sanctuary
EDGE	-	Evolutionarily Distinct and Globally Endangered
ENP	-	Eravikulam National Park
IUCN	-	International Union for Conservation of Nature
KFD	-	Kerala Forest Department
KFRI	-	Kerala Forest Research Institute
PA	-	Protected Area
PAST	-	PAleontological Statistics
QS	-	Quadrat Survey
SWG	-	Southern Western Ghats
VES	-	Visual Encounter Survey
ZSI	-	Zoological Survey of India
WG	-	Western Ghats

Chapter I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Amphibians are one of the most diverse groups of land vertebrates (Hoffmann *et al*., 2010). By having the ability to live both in land and water, they are commonly known as animals that can lead a dual mode of life. That doesn't mean that all animals that could live in land and water are amphibians. Amphibians, the descendants of the first terrestrial vertebrates possess several unique characteristics which makes them what they are. Their skin is highly permeable and lack scales unlike reptiles. They are vulnerable to even the smallest changes in the atmosphere because of the permeable skin. They are also ectothermic animals who can't maintain their body temperature on their own (Wells, 2010). Another peculiar feature is the absence of protective covering for their eggs (anamniotic). Hence amphibians always need protection from desiccation of their eggs and that's why most of them are seen in the vicinity of aquatic ecosystems and areas that are wet and humid. A vast majority of amphibians lay eggs in water which later hatch out into aquatic tadpoles (Altig and McDiarmid, 1999). The magic of metamorphosis in amphibians is well known and popular. The non-reproductive larva (tadpole) then stays in water for a certain period before which they metamorphosize into miniature adults. While in water, the tadpoles are voracious feeders adapted to rapid utilization of food sources stored in their body (Wassersug, 1975). However, the journey from water to land has resulted in the evolution of a variety of reproductive modes in amphibians including the most common and popular mode with free living tadpoles, viviparity where fertilization is internal, and the female give birth to miniature froglets/toadlets (eg. *Nimbaphrynoides occidentalis*) and direct –developing frogs where eggs hatch out into froglets without a tadpole stage (Crump, 2015).

Amphibians evolved about 365 mya during Late Devonian period and they diversified during carboniferous period also dubbed as the *age of amphibians* (Zhang *et al*., 2005; Carrol 2009; Wells, 2010).

The present-day amphibians (Lissamphibia) numbering 8327 species (Frost, 2021) belong to three orders *viz.* Anura comprising of frogs and toads, Gymnophiona with snake like, worm like legless amphibians also known as Caecilians and Caudata including the salamanders and newts. Frogs and toads (7347 sp.), the leaping forms are the most diverse group of amphibians followed by lizard-like salamanders and newts (766 sp.) and the least represented fossorial and aquatic Gymnophiona (214 sp.). Unlike order Anura and order Caudata, where the fertilization is largely external except some rare anurans *(Ascaphus montanus, A. truei, Nectophrynoides viviparous*), order Gymnophiona differ in having a copulatory organ in males called Phallodeum which aids in internal fertilization. From mountain tops, tropical rain forests to dry deserts, amphibians inhabit almost all habitats except oceans. Recently, fossil evidence including skull bones of frogs from anuran family Calyptocephalellidae were unearthed from the Antarctic region for the first time (Mörs *et al.,* 2020) thereby marking the presence of the most diverse group of amphibians in almost all parts of the world.

Frogs and toads are more diverse in the tropics whereas salamanders and newts are found towards the northern regions of the world above equator and almost completely absent in the south except for South America. Caecilians are the least represented ones, and they are restricted to the tropics compared to the other two groups (Wake and Koo, 2018). Just like the fishes and reptiles, amphibians are also cold-blooded vertebrates. Their poikilothermic habit forces them to be in wet, humid microclimatic conditions to prevent desiccation. The highly permeable amphibian skin plays a major role in respiration and in maintaining their body temperature.

Amphibians are the most threatened group of vertebrates in the world. Although the exact estimates are debatable, one third of the world's amphibians are threatened with extinction (Stuart *et al.*, 2004). Forty-one percent of the world's total amphibian species assessed belong to one or other of threatened categories of the IUCN Red List (IUCN, 2021). Amphibian decline got serious attention only in 1989, soon after the first World Congress in Herpetology. Reports of sudden decline in amphibian population in Western United States, Australia, Costa Rica, Ecuador and several other places resulted in conducting the IUCN-The World Conservation Union Global Amphibian Assessment (GAA) to gather information on amphibian distribution, habitat associations, abundance, population trends and threats for all the described amphibians (Stuart *et al*., 2004). The number of amphibian species that went extinct since 1500 AD is 35 (IUCN, 2021). But the actual numbers will be much more than that and several species are on the brink of imminent extinction (Mendelson *et al.,* 2006).

The reasons for the global amphibian decline are largely anthropogenic. Just like any other animal groups, habitat destruction is one of the major contributors towards amphibian decline (Brooks *et al*., 2002). Congruent to habitat destruction comes a lot of other deleterious factors including intensive farming, use of pesticides, fertilizers, linear intrusions, and increased number of vehicles on the road which pose a serious threat to amphibians during their breeding seasons (Beebee and Griffiths, 2005). Increase in human population simultaneously resulted in a high rate of pollution. Local extinctions and decline of amphibians were caused in several parts of the world by invasive alien species of fishes and amphibians (Kats and Ferrer, 2003). Changing climates altered breeding cycles of amphibians, breeding became less successful, and many species restricted to lower elevations started climbing to mountain tops (Beebee and Griffiths, 2005; Pounds *et al*., 1999). Ozone layer depletion has significantly increased the UV-B irradiation levels and several amphibian species were reported to have detrimental effects (Blaustein *et al*., 1994; Pahkala *et al*., 2003). At the same time, impact of UV-B irradiation is subject to discussion as they vary between species in accordance to different regions and microclimates (Beebee and Griffiths, 2005). Large number of amphibians are caught from the wild in several parts of the world as food resource, used in pet trade, educational and medical research (Jenson and Camp, 2003). Apart from the reasons discussed, drastic amphibian declines were also caused by diseases (Berger *et al*., 1998; Cunningham, 1996; Daszak *et al*., 2003; Lips, 1999). Large-scale amphibian mortalities and population reduction were caused in America, Europe, Australia, and New Zealand by the most dreaded fungus *Batrachochytrium dendrobatidis* (Berger *et al*., 1998). Most of these factors alone or combinedly play a major role in amphibian declines and while trying to address these issues towards amphibian conservation it must be a multifactorial approach.

India, one of the mega biodiversity countries, holds a significant percentage of global biodiversity wealth. India has 468 species (Frost, 2021) of amphibians from three major orders Anura, Caudata and Gymnophioana. Order Caudata is represented by the least number of species from the Family: Salamandridae Goldfuss,1820 with 2 species; *Tylototriton himalayanus* Khatiwada, Wang, Ghimire, Vasudevan, Paudel, and Jiang, 2015 and *Tylototriton verrucosus Anderson*, 1871. The second most represented order is Gympnophiona with 40 species within 3 families including Family: Chikilidae Kamei, San Mauro, Gower, Van Bocxlaer, Sherratt, Thomas, Babu, Bossuyt, Wilkinson, and

Biju, 2012, Family: Grandisoniidae Lescure, Renous, and Gasc, 1986 and Family: Ichthyophiidae Taylor, 1968. Order Anura, consisting of frogs and toads, is the most represented order with 418 species from 14 different families. Amphibians in the region recently gained much attention among the scientific community and in the last twenty years itself there had been novel descriptions of more than 50 species most of which are from Western Ghats (Biju *et al*., 2014; Biju *et al*., 2011, 2019; Biju and Bossuyt, 2003, 2006, 2009; Gururaja *et al*., 2007; Vijayakumar *et al*., 2014; Zachariah *et al*., 2011, Biju *et al*., 2019; Garg *et al*., 2019; Garg and Biju, 2016, 2019).

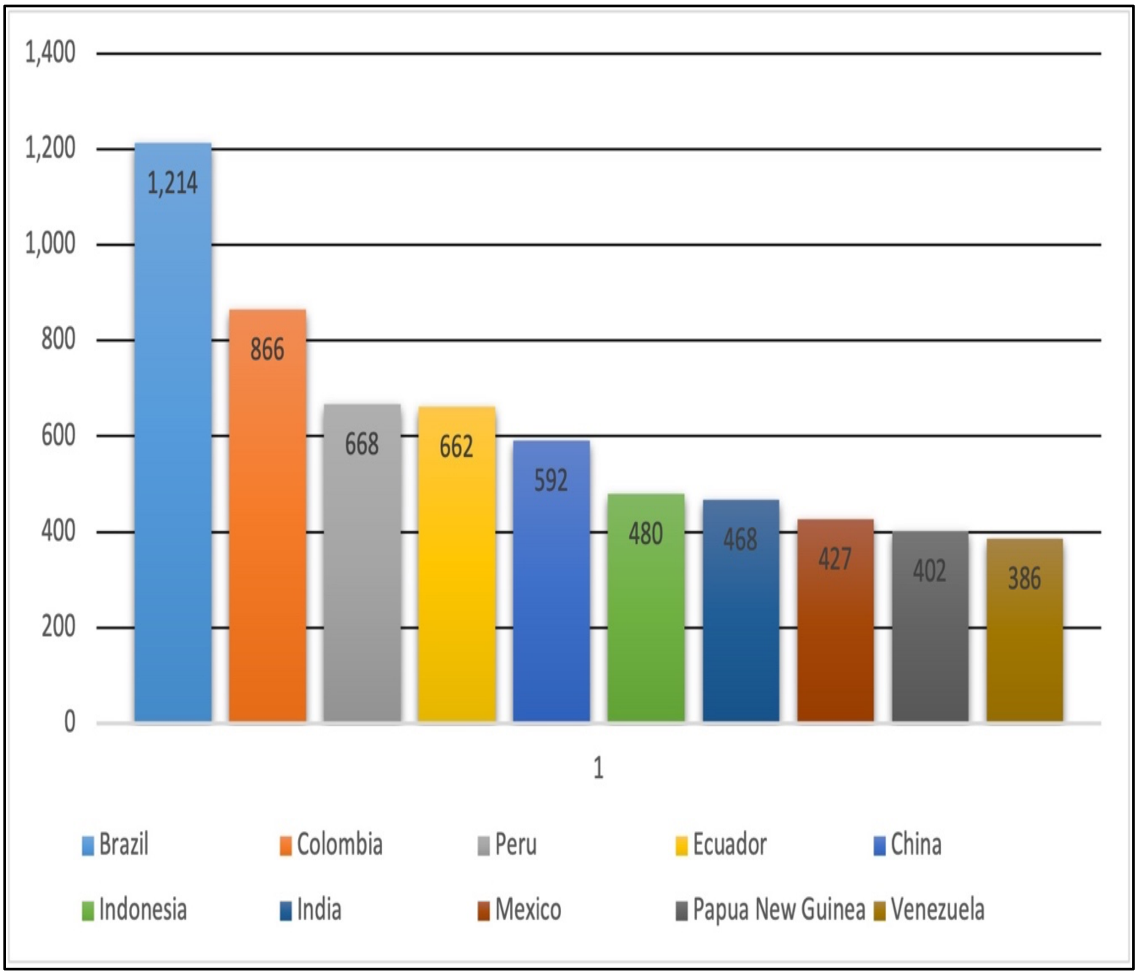

Figure 1: Top ten amphibian rich countries in the world (Amphibians Species of the World, Data taken on 16 May 2021)

Compared to the taxonomic studies, only a few works had been done on other aspects of amphibians including their ecology and behaviour (Bee *et al*., 2013; Gururaja *et al*., 2014; Seshadri *et al*., 2015; Thomas *et al*., 2014; Zachariah *et al*., 2012).

Even though India ranks 7th in the top ten amphibian rich countries (Fig. 1) in the world, only 14% of the total known species from the country are assessed for their threatened status in the IUCN Redlist (Tapley *et. al.,*2018). Most of the Protected Areas of the country in general do not have a comprehensive inventory of the lower forms of vertebrates, especially the amphibians. Moreover, major focus on the wildlife studies undergoing across the country are on larger charismatic species. Similar is the case in our study area Eravikulam National Park where most of the focus is on larger fauna including Nilgiri tahr. The Park with highly undulating terrain has three major vegetation types: grasslands, shrubs, and shola forests. At present, the Park authorities practice early/cold burning of selected plots in grasslands leading to a mosaic of burnt and unburnt areas. The practice is followed every year to facilitate growth of fresh grass to make available nutritious food for the Nilgiri tahr and other herbivores during the dry season and to help in fire protection. However, the impact of the practice on the lower forms of organisms has not been assessed.

The first ever study on Amphibians of Eravikulam National Park was by Radhakrishnan *et al*. (1996). Later, Andrews *et al*., (2005) reported 11 species from the park. A new species belonging to the genus *Nyctibatrachus* described from the park (Radhakrishnan *et al.,* 2007) was synonymized in 2011 in a revision of the Night Frog Genus *Nyctibatrachus* (Biju *et al*., 2011). Other than a couple of species descriptions including *Raorchestes resplendens* from Anamudi peak (Biju *et al*., 2010) and *Micrixalus frigidus*

(Biju *et al.*, 2014) from the park, not much is known about the ecology and behaviour of amphibians present in Eravikulam National Park.

The current study attempted to understand more about the ecology and behaviour of the amphibians of Eravikulam National Park with the following four objectives.

- Documentation of diversity and distribution of Amphibians of Eravikulam National Park
- Study of Temporal Activity Pattern in vocalization of Bush Frogs of Eravikulam National Park
- Call repertoire study of Critically Endangered *Raorchestes resplendens*
- Impact of prescribed fire management practices on Grassland inhabiting amphibians of Eravikulam National Park

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Chapter II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Biodiversity is in peril all over the world due to unparalleled loss of ecosystems. Millions of hectares of tropical forests are lost and visibly degraded each year (Achard *et al*., 2002). More than half of Earth's species are restricted to the tropics and a vast majority of this area holds high levels of diversity and high degree of endemism, which warrants immediate conservation attention. Myers (1988) suggested the concept of biodiversity hotspots and the Western Ghats and Sri Lanka was one among the areas that were in the list of areas that need attention in addition to the first ten global biodiversity hotspots). Western Ghats and Sri Lanka (WG/SL) is one of the four global biodiversity hotspots in India (Myers *et al*., 2000) and they are often considered as a single unit owing to their biogeographical history (Gunawardene *et al*., 2007). Loss of forest cover in the Western Ghats and Sri Lanka hotspot has been drastic where only 6.8% is left of the original extent of forest cover, 182,500 km^2 (Myers *et al*., 2000). Protected Areas in the Western Ghats in comparison to the total area, 160,000 km^2covers only up to 9 % (Gunawardene *et al*., 2007). Nonetheless they support higher levels of diversity in plants and vertebrates.

Himalayas and Western Ghats hold more than sixty percent of India's amphibian wealth. In fact, the hotspot also supports local amphibian endemism to a greater scale (Bossuyt *et al*., 2004) with Western Ghats having 91 % endemism. However, detailed investigation on amphibian diversity and ecology in highly specialized habitats of Western Ghats is seldom done (Vasudevan *et al*., 2001). It is in that perspective, Eravikulam National Park, one of the most unique landscapes in the Western Ghats with its undulating topography and distinctive habitats, was chosen to study its amphibians.

Pioneering works in the field of Amphibians of India were species descriptions and the first one was conducted by Schneider (1799) involving descriptions of several species including *Duttaphrynus melanostictus, Duttaphrynus scaber, Euphlyctis cyanophlyctis, Sphaerotheca breviceps and Uperodon systoma* with type locality as " India Orientali" which is probably Tranquebar, current Tharangambadi in Mayiladuthurai district of Tamil Nadu (Dubois, 1983; Bauer, 1998). Another historical work was by Daudin (1802), where he described *Hoplobatrachus tigerinus* from "Bengale", India. It was during the British Raj (1857 to1947), Indian batrachology witnessed a series of new species descriptions for the first time (Jerdon, 1853; Beddome, 1870, 1878; Gunther, 1876, 1876; Boulenger, 1882a and b).During his tenure as surgeon in the East India Company, Thomas Caverhill Jerdon travelled several parts of India and described 18 new amphibian species. While a vast majority of them were from the Western Ghats there were species descriptions from other parts of India too. In the Catalogue of reptiles inhabiting the Peninsula of India published in 1853, Jerdon described *Hoplobatrachus crassus*, *Minervarya agricola, Minervarya nilgirica, Minervarya rufescens, Sphaerotheca pluvialis, Micrixalus phyllophilus*, *Micrixalus saxicola*, *Microhyla rubra*, *Uperodon montanus*, *Clinotarsus curtipes*, *Indosylvirana flavescens, Ghatixalus variabilis*, *Pseudophilautus wynaadensis*, *Raorchestes glandulosus*, *and Raorchestes tinniens*. In 1870, Jerdon described *Ombrana sikimensis* from Darjeeling*, Hyla annectans* from Khasi Hills and *Rhacophorus malabaricus* from Malabar, the Western coast of India in Proceedings of Asiatic Society, Bengal.

Richard Henry Beddome, from his collections in India and Sri Lanka described two new species in Madras Monthly Journal of Medical Science in 1870 and *Melanobatrachus indicus* in 1878 as a new genus and species in the Proceedings of the Zoological Society of London. His extensive collections paved the way for several other species descriptions by different authors in future. Gunther (1876) described *Duttaphrynus beddomii* from Beddomes collection and *Rana temporalis,* a Ranid frog from Sri Lanka, which was thought to have occurred in Anamalai hills and nearby areas of Southern Western Ghats. The same publication in the *Proceedings of Zoological Society,* 1876 also had descriptions of many other species including *Pedostibes tuberculosus*, *Uperodon triangularis*, *Indirana brachytarsus*, *Walkerana diplosticta, Indirana beddomii*, *Ghatophryne ornata, Raorchestes beddomii* and *Raorchestes chalazodes.* Zoologist and Botanist George Albert Boulenger, who had described a large number of species during that period, was another scientist who had worked extensively on Beddome's collections. Some of his species descriptions from the Western Ghats include two new toads *Duttaphrynus parietalis* and *Duttaphrynus microtympanum*, two night frogs *Nyctibatrachus beddomii* and *Nyctibatrachus major* and a torrent frog from *Micrixalus fuscus* from Travancore, *Sphaerotheca dobsonii* from Mangalore*, Micrixalus silvaticus* from Malabar*,* two leaping frogs *Walkerana leptodactyla* and *Walkerana phrynoderma* from Anamalais*, Indirana semipalmata* with type locality as Malabar which is currently restricted to Idukki (Dahanukar *et al*.,2016)*, Raorchestes flaviventris* from Malabar and *Raorchestes signatus* from "Nilgherries" (= Nilgiri Hills), Tamil Nadu, India.

Starting from 1882 to 1920, Boulenger published around thirty nine species from India. Seventeen species were described in the publications Catalogue of the Batrachia Salientia s. Ecaudata in the collection of the British Museum, Catalogue of the Batrachia Gradientias. Caudata Batrachia Apoda in the collection of the British Museum in 1882. A single species *Rhacophorus lateralis* was described from Malabar in 1883. Another four species in 1887 including *Ingerophrynus macrotis, Limnonectes doriae, Humerana humeralis, Feihyla vittata*, two species *Amolops himalayanus* and *Indirana leithii* in 1888. *Micryletta inornata* was described in 1890 followed by another single species *Raorchestes travancoricus* in 1891. Two species *Boulenophrys parva* and *Chirixalus doriae* in 1893. A single species *Microhyla butleri* in 1900 and *Rana aurantiaca* in 1904 which is currently in the genus *Indosylvirana.* Species *Polypedates taeniatus* and *Ixalus* annandalii were published in 1906 currently known as *Raorchestes annandali* after several revisions. The rest of them including *Xenophrys major* and *Xenophrys robusta* were published in 1908, species such as *Bufoides kempi*, *Philautus garo* and *Philautus kempiae* in 1919 and the two species *Nanorana annandalii* and *Hylarana garoensis* got published in 1920.

H. S. Ferguson published a list of Travancore Batrachians in 1904 based on his collections from Ponmudi and Agasthyamala which are housed in the collections of the British Natural History Museum, London. C. R. Narayana Rao (1920) described *Nyctibatrachus sanctipalustris* from Brahamagiri Hills*, Indosylvirana montana* from Hill forests of Bhagamandala and *Minervarya mysorensis* from Jog in 1923. Later in 1937, Rao described twelve other species of anurans from different parts of the Western Ghats. A majority of his new species descriptions were from the Karnataka. Species such as *Duttaphrynus brevirostris, Micrixalus elegans, Nyctibatrachus kempholeyensis, Uperodon mormoratus* and *Indirana longicrus* were described from Kempholey of Mysore. Other species that were from Karantaka are *Minervarya sauriceps* from Jog forests which is currently a junior synonym of *Minervarya mysorensis, Sphaerotheca leucorhynchus* from Wattakole of Coorg*, Micrixalus kottigeharensis* Kottigehar, *Indosylvirana intermedia* from Sakleshpur and *Raorchestes charius* from chikamangalur.. The species that were contributed by Rao from other parts of Western Ghats includes, *Minervarya parambikulamana* from Parambikulam, Kerala, and *Uperodon anamalaiensis* from the plains of Anamalai hills. Rao, in collaboration with Thomas Nelson Annandale , founder director of Zoological Survey of India, published works on Indian tadpoles in 1916 and 1917. During his period in India from 1904 to 1924, Annandale conducted several expeditions across India and collected numerous amphibians along with fishes and reptiles. He described twelve amphibians including two caecilians, *Ichthyophis tricolor* and *Uraeotyphlus menoni* (Annandale, 1909, 1912, 1913, 1915 and 1919 a and b). In addition to batrachologists, scientists and collectors from other science backgrounds like marine biologist and ZSI former director Stanley Wells Kemp, entomologists T. Bainbrigge Fietcher, and F. H. Gravely, ichthyologist and surgeon Francis Day , collector Jean-Jacques Dussumier also contributed largely in building Indian amphibian collections and done several initial works before Independence (Biju, 2001). French herpetologist and taxonomist Alain Dubois made some significant contributions to Indian batrachology post Indian Independence by describing twelve species (1975a and b, 1978, 1979, 1981, 1984a and b, 1986a and b, 1987a and b, 1992, 1999, 2001, 2006, 2010, 2021), thirteen genus and two families; Micrixalidae and Ranixalidae.

Pillai alone and together with Chanda, Ravichandran and Pattabhiraman made a series of new descriptions post-independence. He described *Microhyla chakrapanii* from North Andamans, *Micrixalus nudis* from Chethalayam in Wayanad and *Minervarya murthi* (currently a junior synonym of *Minervarya nilgirica*) from Gudalur, Tamil Nadu in 1977, 1978 and 1979 consecutively. Pillai further described three more amphibians including two anurans *Duttaphrynus silentvalleyensis* and *Micrixalus thampi* in 1981, and a caecilian *Ichthyophis longicephalus* in 1986 from Silent Valley National Park, Kerala. Pillai and Pattabiraman (1981) added another species, *Blaira rubigina* a western ghat endemic torrent toad to the list of amphibians from Silent Valley. In addition to these, he described *Raorchestes shillongensis* from Shillong (Meghalaya) and *Odorrana mawphlangensis* from Mawphlang of Khasi Hills (Meghalaya) with Chanda in 1973 and 1977; *Micrixalus gadgili* with Pattabiraman in 1990 from Sabarigiri, Kerala; *Ichthyophis garoensis* from Gharo Hills in Meghalaya, *Uraeotyphlus interruptus* from Chengalam in Kerala and *Gegeneophis krishni* from Karnataka in 1999 with Ravichandran.

Inger *et al.* (1984) studied the Amphibian diversity of Ponmudi hills and described two new species of Night Frogs; *Nyctibatrachus aliciae* and *Nyctibatrachus minor.* Daniels (1992) published the distribution of amphibians of Western Ghats. A survey of reptiles and amphibians in Kerala part of Nilgiri Biosphere Reserve was done by Easa (1998). Another significant work by Easa on amphibians as a part of biodiversity documentation of Kerala was published as a handbook in 2003. Dubois and Ohler (1999) studied and updated the valid names of Bufonids and Indian amphibians and re-described them. Biju (2001) published a synopsis on frog fauna of Western Ghats in an occasional publication of Indian society for conservation biology. His contributions starting from 2001 till date are considered the most significant post-independence, involving around hundred new species descriptions from India and his works popularized batrachology as a promising research field in India. Bossuyt (2002) published a new species of bush frog, *Raorchestes* *griet* from Munnar. In 2003, Biju and Bossuyt described *Nasikabatrachus sahyadrensis*, a connecting link between the mesobatrachians and neobatrachians belonging to a new family Nasikabatrachidae. Popularly called the Coelacanth of frogs, it is said to be a living fossil revealing India's biogeographical link with Seychelles. Krishnamurthy (2003) studied amphibian assemblages on disturbed and undisturbed areas of Kudremukh National Park in 2003.

Andrews *et al.* (2005) studied the amphibians of Kerala, described its distribution and status and listed a total of 87 species of amphibians from Kerala, based on field work and published reports available till that time. Biju and Bossuyt (2005a, b, and c) described three new rhacophorids from the area *viz. Raorchestes ponmudi, Raorchestes graminirupes* and *Raorchestes nerostagona*. Biju *et al.* (2007) described *Nyctibatrachus minimus, a new* night frog from Wayanad, Kerala. A new species of reed inhabiting bush frog *Raorchestes ochlandra* was described by Gururaja *et al.* (2007) from Malabar Wildlife Sanctuary, Kerala.

Biju (2009) reported nesting behaviour of *Rhacophorous lateralis* from Western Ghats. Biju and Bossuyt (2009) reported the systematics and phlyogeny of *Philautus* (*Raorchestes*) in the Western Ghats along with the descriptions of 12 new species. The newly described species include *Raorchestes jayarami*, *Raorchestes kaikatti*, *Raorchestes chlorosomma, Raorchestes sushili*, *Raorchestes coonoorensis, Raorchestes marki*, *Raorchestes akroparallagi, Raorchestes chotta, Raorchestes munnarensis, Raorchestes chromasynchysi*, *Pseudophilauts amboli* and *Pseudophilautus kani*. Biju *et al.* (2010) described a new ground-dwelling rhacophorid; *Raorchestes resplendens* from the highest mountain peak (Anamudi) of the Western Ghats. Gururaja (2010) described a novel mode of reproduction in *Micrixalus saxicola* with detailed descriptions on foot-flagging behavior shown by the males of the species.

Zachariah *et al.* (2011) reported 10 new Rhacophorids (nine from the genus *Raorchestes* and one from *Polypedates, currently* Beddomixalus) from the Western Ghats. These included *Raorchestes agasthyaensis*, *Raorchestes crustai*, *Raorchestes johnceei*, *Raorchestes manohari*, *Raorchestes ravii*, *Raorchestes theuerkaufi*, *Raorchestes thodai*, *Raorchestes uthamani*, *Raorchestes kadalarensis* and *Polypedates bijui*. Biju *et al.* (2011) published a taxonomic review of the Night Frog of the genus *Nyctibatrachus* of the Western Ghats with description of 12 new species (*Nyctibatrachus danieli*, *Nyctibatrachus deveni*, *Nyctibatrachus gavi*, *Nyctibatrachus grandis*, *Nyctibatrachus acanthodermis, Nyctibatrachus indraneili*, *Nyctibatrachus jog*, *Nyctibatrachus periyar*, *Nyctibatrachus pillaii*, *Nyctibatrachus poocha, Nyctibatrachus shiradi* and *Nyctibatrachus vrijeuni)*. Bhatta *et al.* (2011) published a species of caecilian *Ichthyophis davidi*. Nair *et al.*, (2011a, and 2011b, 2012) made a series of publications on the endemic family Ranixalidae regarding the cryptic diversity and possibility of new and undescribed lineages, on microsatellite markers of the species *Indirana beddomii* and records of the *Batrachochytrium dendrobatidis* (Bd from Peppara) and *Ranavirus* (Rv) from Athirappilly and Malakkaappara in Kerala part of Western Ghats.

Seshadri *et al.* (2012) published a new species, *Raorchestes kakachi* from Kalakad Mundathurai Tiger Reserve. The reproductive strategies of the living fossil *Nasikabatrachus sahyadrensis* was published by Zachariah *et al.* (2012). Kamei *et al.* (2012) described a new family of Caecilians named Chikilidae from the North East followed by publication of three new species in the family in 2013. Abraham *et al.* (2013) published two new genus of frogs *Beddomixalus* and *Mercurana* from Western Ghats where *Polypedates bijui* was elevated to *Beddomixalus bijui*. The year 2014 witnessed descriptions of 14 new species in the genus *Micrixalus* and 7 new species in the genus *Hylarana* (Biju *et al.,* 2014 a and b). The effects of selective logging on frogs were also studied the same year by Seshadri (2014). Vijayakumar *et al.,* (2014) published nine new species of bush frogs of the genus *Raorchestes* and Vijayakumar *et al.* (2016) suggested glaciation, gradient and geography as multiple drivers towards the diversification of *Raorchestes* frogs in the Western Ghats. Meegaskumbura *et al*.(2015) studied the patterns and diversity of reproductive modes in Rhacophoridae. A large scale phylogeny on Microhylidae family involving several species from India was attempted by Peloso *et al*. (2016) along with several authors from around the world including Biju. In 2015, Thomas and Biju reported consumption as one of the major direct threats faced by the endangered Purple Frog. *Ichthyophis husaini* was reassessed and considered a junior synonym of *Ichthyophis garoensis* by Kamei and Biju (2016). Senevirathne, *et al.*, (2016) unearthed and described the fossorial tadpoles of the family Micrixalidae and Senevirathne, *et al*., (2016) studied the skeletal ontogeny of *Nasikabatrachus sahyadrensis*. Another genus *Frankixalus* described by Biju *et. al.*(2016) was later merged with the genus *Nasutixalus* by Sivongxay *et al.* (2016). Willaert *et al.*, (2016) gave a detailed description of a novel reproductive method involving a new mode of amplexus and unique mating strategy without contact in Bombay Night Frog, *Nyctibatrachus humayuni.* Mahony *et. al.,* (2017) discussed integrative approaches in time tree dating and studying the evolutionary history of frogs of Megophryne in the absence of fossil records.

Year 2017 to 2020 witnessed several new species descriptions from India, majority of them by Biju and his co-authors. Garg *et. al.* (2017a and b) described seven new species of *Nyctibatrachus* and four new species of *Fejervarya* (currently *Minervarya*) from the Western Ghats. Another new night frog species *Nyctibatrachus mewasinghi* was reported by Krutha *et. al.* (2017) from Malabar Wildlife Sanctuary. A complete revision of the genus *Microhyla* by Garg *et al.* (2018) resulted in a new species *Microhyla darreli.* Vijaykumar *et. al.,* (2019) described a new subfamily Astrobatrachinae under Nyctibatrachidae family, new genus *Astrobatrachus* and a species *Astrobatrachus kurichyana* from Kurichyaramala, Wayanad. Garg and Biju in 2019 erected a new genus and species *Mysticellus franki* from Wayanad.

Due to the lack of knowledge on what we have, in terms of amphibian diversity, in terms of lack of resources and dearth of batrachologists in the field, the attempts so far had been largely focussed on describing new species. The foregoing review clearly indicates that the information on the habitat requirement, associates and other ecological parameters are almost nil other than a few studies that are mentioned above. Even though the taxonomy is still in a flux, it's still better than the last couple of decades and we have a better understanding of Indian amphibians. However, very little is known about the diversity and distribution of amphibians in unique grassland and shola ecosystems of Eravikulam National Park. We don't know much about their ecology and behaviour in these landscapes as well as their responses to anthropogenic pressures caused by management practices like controlled burning.

Review of literature pertaining to all four objectives of the present study are given in the introduction part of the respective chapters.

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Chapter III

STUDY AREA

STUDY AREA

3.1. Eravikulam National Park

Eravikulam National Park (ENP), one of the most unique grassland-shola ecosystems in the Western Ghats was chosen for studying the diversity, distribution and selected aspects on the ecology and behavior of amphibians. Situated between 10º 05' - 10º 20' N Latitude and 77º 0' - 77º 10' E Longitude in the Anamalai hills,in the Devikulam Taluk of Idukki district, Kerala, India (Fig. 3.1).

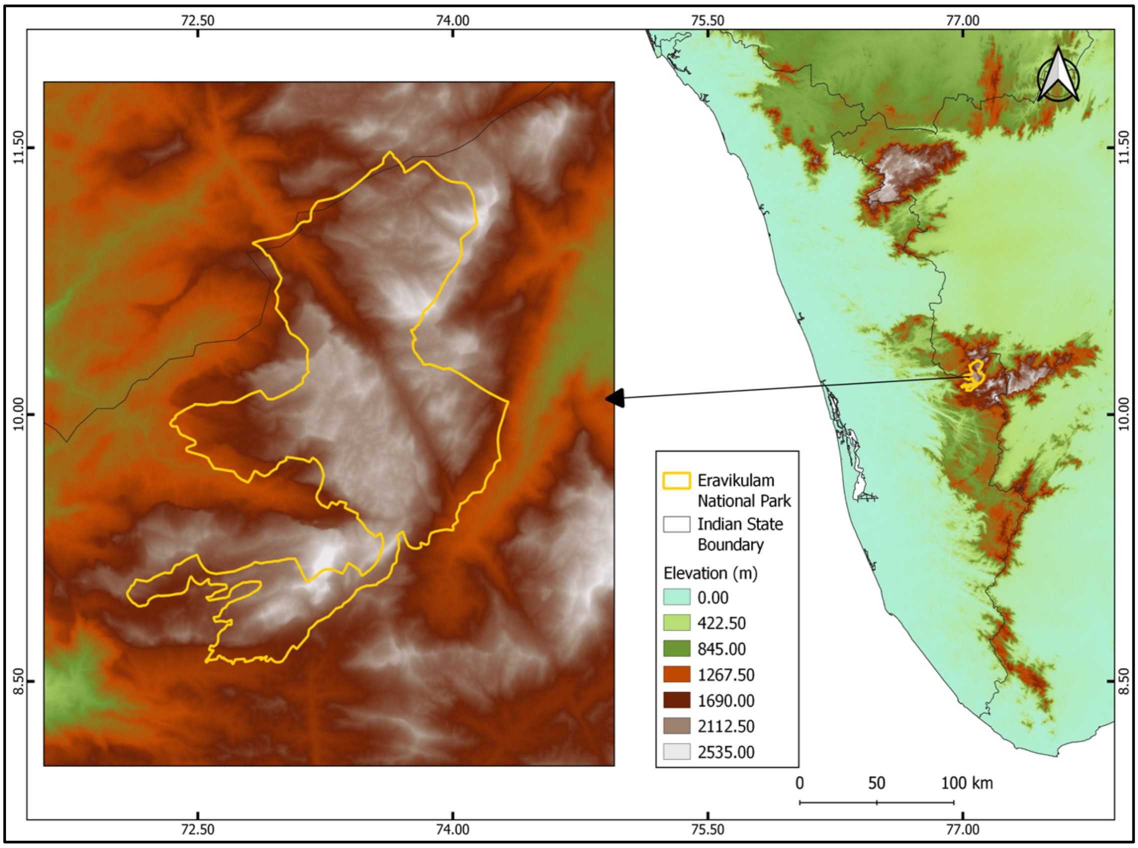

Figure 3.1 : Elevational profile of Eravikulam National Park

Eravikulam is a 97 km^2 protected area and a high rolling plateau popular for the endemic mountain goat *Nilgiritragus hylocrius,* Nilgiri tahr and the plietesial *Strobilanthus kunthiana* commonly known as Neelakurinji. With an average altitude of 2000 above mean sea level, the protected area is a mosaic of shola forests also known as southern montane wet forests or tropical forests (Champion and Seth, 1968; Nair and Khanduri,

2001) interspersed among large grasslands as one large sky island (Robin and Nandhini, 2012) unit and several small ones hosting a variety of unique flora and fauna. Even though it is famous for its scenic beauty with high rolling grasslands and shola ecosystem, only a small area, Rajamala in its boundary, is accessible to the common public for tourism activities. The name Eravikulam etymologically owes to the presence of the streams and pools, especially the large lake in the core area of the National Park.

3.2. Terrain

Once preserved as a game reserve by the Kanan Devan Hills Produce (KDHP) Company through the High Range Game Association, the area was taken back to the Government of Kerala in 1971 and declared as Eravikulam- Rajamala Wildlife Sanctuary (Fig.3.2) in 1975 in order to protect the Nilgiri tahr and its last remaining habitats. Later in 1978, it was elevated to National park status and became Eravikulam National Park. The ecological boundary of the park meets Anamalai Tiger Reserve in Tamilnadu, Chinnar Wildlife Sanctuary (CWS) under Munnar Wildlife Division (MWD) and Koodakkad proposed reserve under Marayur division in the north. The eastern boundary of the park extends to Koodakkad, the proposed reserves under Marayur division. Boundaries in south meets the grasslands of KDHP company, Mankulam Division and Munnar division and Anaimudi reserve forest under Munnar Division in the west. The hills within ENP range within 100 m to 600m including the highest peak in South India, Anamudi with an elevation of 2695m from the park's basal elevation of 2000 m core body comprising the high rolling plateau. The plateau is split into two by Turner’s Valley, the gorge found facing one of the core operational and camping points of the park named Eravikulam Hut.

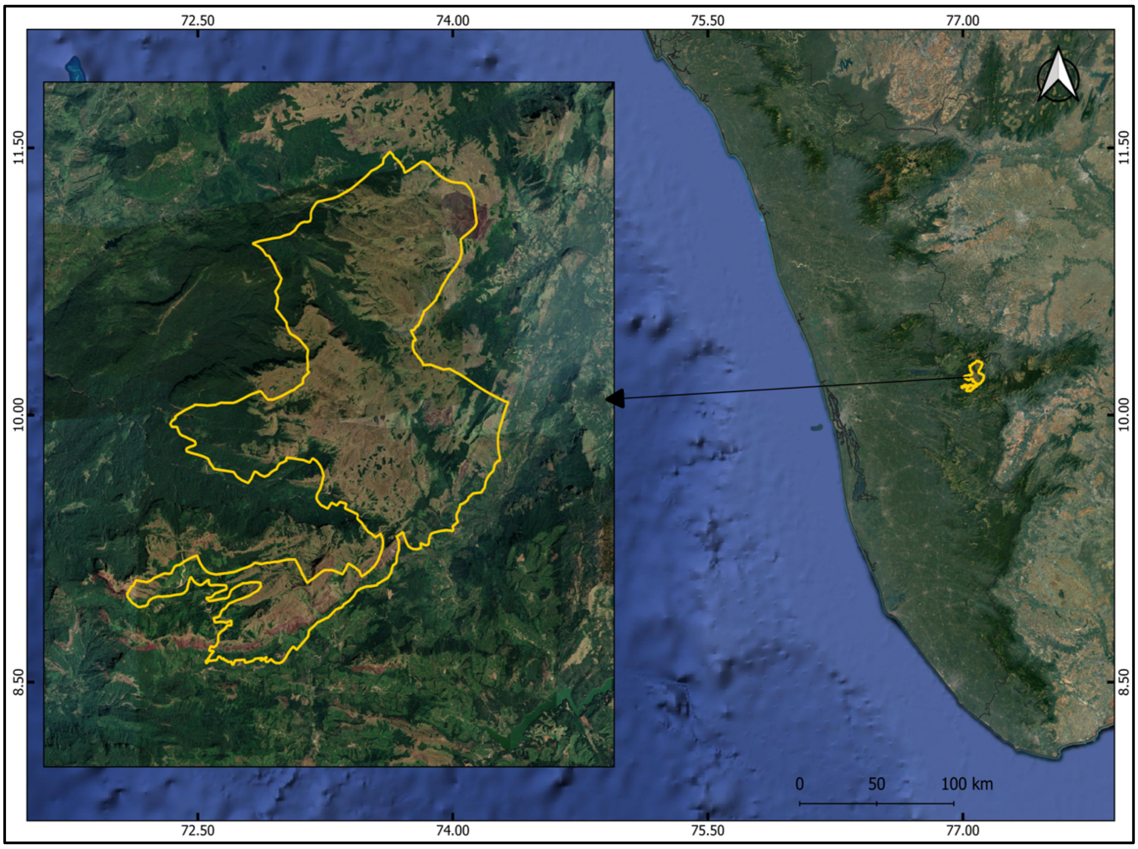

Figure 3.2: Eravikulam National Park

Another deep valley is the one between the Anamudi peak and the high rolling plateau which is in fact outside the boundary of ENP. The major habitats inside ENP includes grasslands also known as southern montane wet temperate grasslands which accounts for a total of 60% of the park followed by the shola ecosystems (25%) interspersed among the grasslands in the valleys and folds, shrub lands (7.5%) along the basal areas of the cliffs and the remaining areas has southern tropical hill forests (Menon, 1997).

3.3. Geology

Geologically ENP is characterized by presence of charnockite, garnet, wide variety of metamorphic rocks including silliminite, garnet biotic, hornblende biotic and granetic gneises, migmatites and granites of precambrian origin also known as Archean igneous origin (Suresh, 1998) and rocks comprise of silca, muscovite, feldspars with traces of ferro-magnesium minerals (KFD, 2012) . The soil is acidic with a pH range 4.13 – 5.34 consisting of little bit of gravel, sand(79.43 – 94.14 %), silt (1.43 – 11.00 %) and clay (2.71 – 5.57 %), rich in organic matter with a high percentage of organic carbon and nitrogen (Koshy, 1970; Jose *et al.,* 1994).

3.4. Climate

ENP harbours tropical montane climatic conditions owing to its high altitudinal influence over the tropical latitude. The park receives rain almost throughout the year except for the summer months. The rainfall pattern varies across different sites in the park and accounts for diverse microclimatic conditions supporting several flora and fauna endemic to these grassland shola ecosystems which are considered to have evolved even before the advent of humans. Monsoon rains dominate the annual weather cycle in ENP starting from pre-monsoon rains of March to May after summer and followed by the South-West and North-East monsoons in June to August with 60% of the total precipitation of the park and later in September to November (30%). In addition to the above mentioned three seasons it also experiences winter months during December to February when the temperature drops down to sub-zero values leading to moderate frosting in recent years (KFD, 2012). The Summer period records the mean daily maximum temperature and it drops down with the arrival of the monsoon clouds. Humidity goes as high as 83-100% during the monsoon months and comes as low as 59-62% during the winter months which is again subjected to further decline in relation to the higher wind speeds. High wind speed values (80 km/hr) were recorded during the monsoon (KFD, 2012) earlier whereas the present study recorded a wind speed of 93 km/hr at Anamudi summit in April 2016.

The park has a network of streams and the majority of them are perennial, supporting lots of life forms. The east flowing ones drain into the Pampa river and west flowing ones to Periyar and Chalakkudi river tributaries. Bheemanauda, a big artificial lake, marks its presence inside the park ever since it was given a protection status.

3.5. Flora and Fauna

Owing to its distinctive geography and climatic conditions ENP is rich in its floral and faunal composition. A total of 803 species of flora are recorded from the park including 727 angiosperms and 76 pteridophytes under 332 genres from 134 families with 505 dicotyledon and 222 monocotyledon species . 190 of the total recorded species as well as 18 out of the total 39 of the genus Impatiens recorded including the recently described one *Impatiens eravikulamensis* are endemic to ENP (KFD, 2012; Salish *et. al.,* 2019). Shola forests are dominated by common tree species like *Actinodaphne bourdellonii, Elaeocarpus recurvatus, Ixora notoniana, Ilex denticulata, I. wightiana, Michaelia nilagirica, Microtropis ramiflora, Pithecellobium subcoriaceum, Syzygium arnottianum* and *Symplocos pendula.* The common herbs and shrubs include *Gaultheria fragrantissima, Jasminum bignoneacium, Moonia heterophylla,, Smithia blanda, Valeriana hookeriana*, few species of *Strobilanthes* and shola edges with trees including *Berberis tinctoria, Ligustrum perrottettii, Mahonia leshenaultii, Rhododendron arboreum nilgiricum, Rhodomyrtus tomentosa and Vaccinium neilgherrense* (KFD, 2012). Grasslands have recorded as much as 308 species including species like *Andropogon lividus, Arundinella vaginata, Digitaria wallichiana and Arundinella mesophylla* whereas plateau and slopes majorly have *Chrysopogon zeylanicus* and *Sehima nervosum* (Karunakaran *et al.,* 1998) and the most dominant grassland species are

Eulalia phaeothrix and *Dicanthium polyptychum* (Rice, 1988) . The grasslands also have the *Strobilanthus kunthiana* which blooms once in 12 years.

Animal species wise ENP is rich in having 49 species of mammals, 132 species of birds, 13 species of reptiles, 20 species of amphibians, 4 species of fishes and 101 species of Butterflies (ZSI, 2002; Andrews *et al.,* 2005; KFD; 2012). The park is well known for Nilgiri Tahr and ENP being one of the largest protected habitats for the species in Kerala, holds a viable population (Easa and Alambeth, 2019; Easa *et al.,* 2010). Several other mammal species including carnivores like *Panthera tigris* (Tiger)*, Panthera pardus* (Leopard), *Prionailurus bengalensis* (Leopard Cat), *Felis chaus* (Jungle Cat), *Cuon alpinus* (Wild Dog), *Aonyx cinerea* (Asian small-clawed Otter) (Perinchery, 2008) omnivores such as *Melursus ursinus* (Sloth Bear), *Sus scrofa* (Wild Boar) and herbivores *Elephas maximus* (Elephant), *Bos gaurus* (Wild Gaur) and *Rusa unicolor* (Sambar Deer) inhabit the park.

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Chapter IV

DIVERSITY AND DISTRIBUTION OF AMPHIBIANS OF ERAVIKULAM NATIONAL PARK

DIVERSITY AND DISTRIBUTION OF AMPHIBIANS OF ERAVIKULAM NATIONAL PARK

4.1. Introduction

Biodiversity is the variety of life. It comprises of genetic diversity, species diversity and ecosystem or ecological diversity which are in turn highly interlinked (Gaston and Spicer, 2013). Our world is home to more than 1.4 million living species, of which more than half (750,000 species) are invertebrates, specifically insects while vertebrates constitute only a 3% (41000 species) of the total (Parker, 1982; Wilson, 1988; Gaston and Spicer, 2013). Despite the small percentage among the total living organisms, vertebrates are one of the dominant group of animals on earth owing to their key positions in the food web, their comparatively large body size and their abundance (Wiens, 2015). The greatest threats to biodiversity are linked to human activities. Biological diversity loss is at its highest level since Mesozoic era due to anthropogenic factors (Wilson, 1989).

Amphibians, are one of the most popular group of animals. Their dual mode of life is something that evoke curiosity among all irrespective of age and that earned a space for them in most of our human cultures (Hocking and Babbit, 2014). For centuries, they have been involved in a range of ecosystem services including regulating, cultural, provisioning and supporting services. They are an integral part of the food chain. Both the adult and tadpoles are involved in controlling invertebrates which could potentially lead to regulation of disease outbreaks (Brodman and Dorton, 2006; DuRant and Hopkins, 2008; Rubbo *et al*., 2011). Even though amphibian itself became a major pest and invasive species in Australia, introduction of Cane Toads to control the dung beetles was based on their ability to control invertebrate populations (Turvey, 2013). Amphibians are well known and acknowledged in many of our cultures. In some places they are celebrated as a part of the culture like the Puerto Rican Coqui frog (Hocking and Babbit, 2014). Some found their place in music, stories, art forms, jewelleries and in mythologies (Gibbons, 2003; DeGraaf, 1991).

Amphibian provisioning services includes their utilisation in the form of food and medicine. Frog legs are a delicacy and a source of protein across the world. Around 4716 metric tons of frog meat are consumed every year across the globe (Kusrini and Alford, 2006; Parker, 2011). Until recently a large amount of frogs, nearly 10 million were illegally smuggled out of India (Jensen and Camp, 2003). Use of amphibians in the field of medicine is another key provisional service from amphibians. They are widely used in several traditional medicines in the olden times, like using *Xenopus levis* in testing pregnancy and *Nasikabatrachus sahyadrensis* adult frogs for treatment for asthma and skin treatments (Thomas and Biju, 2015). The chemicals present in amphibian skins are unique and lot of research is happening in this aspect. They are being tested and used in curing cardiac related treatments, respiratory disorders, regeneration studies and even in HIV treatment (Hocking and Babbit, 2014). As supporting services, amphibians are involved in ecosystem engineering like effects on soil and temporary habitat changes caused by their burrowing mode of life and tadpoles feeding behaviour in aquatic systems influencing sedimentation (Ranvestel *et al*., 2004; Connelly *et al*., 2008; Burger e*t al.,* 2002).

Amphibians are the group of vertebrates that face a greater threat compared to all others (IUCN, 2021). Habitat loss, global warming and Infectious diseases directly result in greater loss of biodiversity including amphibians. This sharp decline in contrast with other animal groups alone attribute to higher level of attention amphibians receive in the field of conservation and research (Stuart *et al.,* 2004). With advancement of science and technology, the rate of amphibian discoveries also went high in the last couple of decades. More and more species are described every year from all over the world largely supported by the DNA barcoding system (Vences *et al.,* 2005). However, the status of these new species described are largely not assessed. Knowledge on the threat status of a total of 61.3 % of the present known amphibians are still not evaluated or the assessments are not up to date (Tapley *et al.,* 2018). Currently, the world has 8327 species of amphibians under three orders, Anura, Gymnophiona and Caudata (Frost, 2021). The tropical areas holds the highest diversity (Mittelbach *et al.,* 2007) with Brazil (1214 species) having maximum number of species (AmphibiaWeb, 2021).

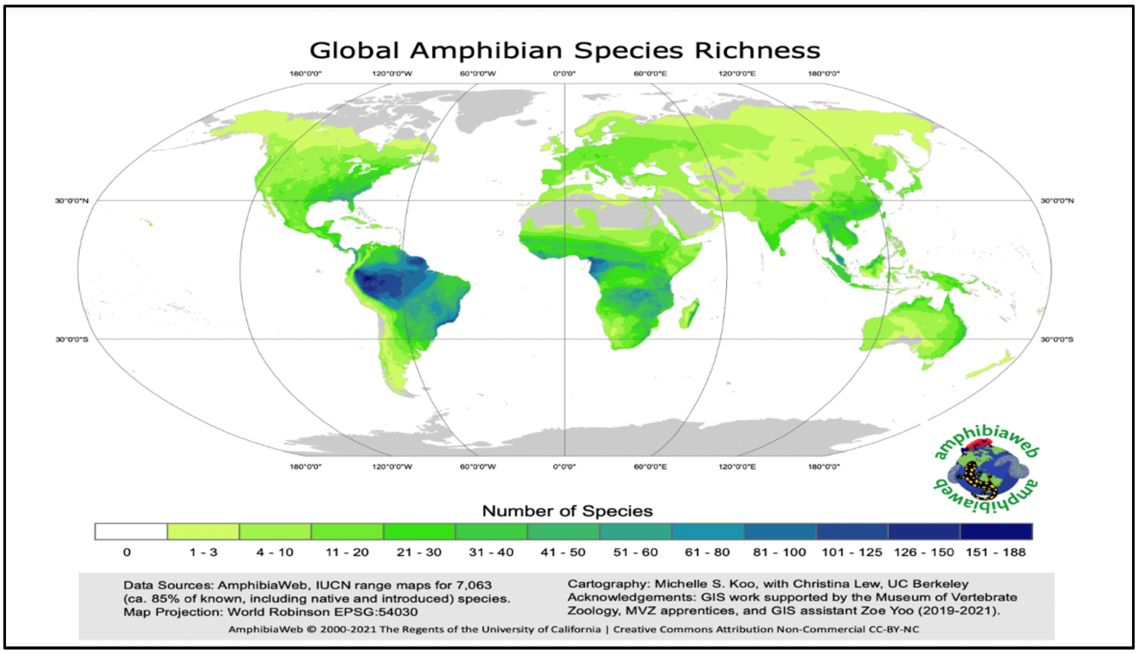

Figure 4.1 :Global amphibian species richness (From AmphibiaWeb)

India is home to 468 species of amphibians and majority of the research on amphibians are still focussed on taxonomic descriptions (Frost, 2021). The last two decade witnessed descriptions of more than 50 species of amphibians from the Western Ghats hotspot (Chapter I). A total of 258 amphibian species, which is more than half of total species in India are found in the Western Ghats (Dahanukar and Molur, 2020) and more than 90% of them are endemic to the region (Nameer *et al.*, 2015). Southern part of the Western

Ghats falling in 12-13^0 N latitude in fact is reported to have the highest species diversity majorly due to the extended rainfall and its well defined climatic regime. Detailed review of literature concerning amphibian studies in India and Western Ghats is given in Chapter II.

Studies other than taxonomic descriptions are limited to a few. Inger *et al.,* (1984) reported the amphibians and reptiles of Ponmudi and Pillai (1986) documented amphibians of Silent Valley . Daniels (1991, 1992, 2001) conducted several studies in Western Ghats and Eastern Ghats in Karnataka and Tamil Nadu and published a book on Amphibians of Peninsular India Daniels (2005). Herpetofaunal survey along with Ichthyofaunal studies of Kalakkad Mundathurai was done by Cherian *et al.* (1999). Bennet *et al.* (2000) did a study on frogs of Coorg. Similarly, another diversity and distribution study of amphibians of Northern Western Ghats was done by Dahanukar and Padhye (2005). After series of tadpole descriptions by Annandale (1905, 1908, 1909, 1910,1912,1913, 1915, 1919 a and b) and Annandale and Rao (1916), there were only a few studies on Anuran larvas from the Western Ghats (Girish and Saidapur, 1999; Pancharathna and Saidapur, 2005; Gurushankara *et al.,* 2006; Krishnamurthy *et al.,* 200; Girish and Krishnamurthy, 2009; Raj *et al.,* 2012).

Investigations on individual protected areas of Western Ghats, specifically Kerala are limited to a few studies. Easa (1998) surveyed amphibians and reptiles of Kerala part of Nilgiri Biosphere Reserve. Later, Shaji and Easa (1999) compiled a review on amphibian diversity of Kerala. Another work from Kerala on Amphibians was the survey of amphibians of Wayanad by Abraham *et al.* (2001). One of the comprehensive work on amphibians of Protected Areas (PA) of Kerala was done by Andrews *et al.* (2005). In that work, the authors reported the presence of 11 species of anurans in Eravikulam National Park (ENP). In addition to the faunal surveys undertaken in ENP by Zoological Survey of India and few new species descriptions (Radhakrishnan *et al.,* 1996; Radhakrishnan *et al.,* 2007; Biju *et al*., 2011; Biju *et al*., 2010; Biju *et al.*, 2014), no systematic studies on the diversity of amphibians of the PA is done so far. The current study aims to document the diversity and distribution of amphibians of ENP.

4.2. Methods

4.2.1. Study Area

The study was conducted in the largest National Park in Kerala, Eravikulam National Park. Situated in Munnar of Idukki district, Kerala, India, ENP is 97 km^2 large with its unique Shola Grassland ecosystem. With a basal elevation of 1800-2000m, it has one of the largest tract of Southern montane wet tropical forests also known as shola interspersed among high elevation grasslands in the Western Ghats. These sholas are isolated sky islands in the valleys and among depressions or folds in the mountains largely separated from the other habitats by vast grasslands. They are home to diverse unique flora and fauna found nowhere else in the world. Even though ENP is explored for large mammals, birds and vegetations, very few studies had been conducted on amphibians. Detail description on ENP is given in Chapter III.

4.2.2. Sampling

Diversity and distribution of amphibians of high altitude grassland shola ecosystems of Eravikulam National Park (ENP) were documented for four breeding (rainy) seasons from January 2015 to November 2018. Monsoon months were chosen for surveying

Figure 4.2 :Grassland and Shola forests of ENP

amphibians as they are more active and the encounter rates will be high during the period (Harikrishnan *et al.,* 2018).A combination of survey methods including quadrat surveys and visual encounter surveys were used to document the diversity and distribution (Krishnamurthy, 2003; Heyer *et. al.,* 1994; Sutherland, 2006). Survey was done equally in both grasslands and sholas day and night to document both diurnal and nocturnal species. The forest patches in Thattukaanam area, which is comparatively lower in terms of elevation were also considered as shola as they are contiguous with sholas of the high rolling plateau. To examine the extent of survey completeness, species accumulation curves were plotted.

4.2.3. Quadrat Survey

Quadrat survey (QS) involves laying out multiple square plots within randomly selected habitats. Then these destined areas were actively searched for amphibians. Since the quadrats are placed at random in areas of interest and each plot constitute an independent sample, statistical deductions on species diversity, density and relative abundance can be made from the data, provided the quadrats are sufficiently large (Heyer and Berven, 1973; Heyer et al., 1994; Sutherland, 2006; Dodd, 2009). This method is used in inventory studies, understanding species assemblages and change in their composition over a period of time. The number of people involved in the survey and size of the quadrats laid for data collection varies depending upon the target group and the research questions. Plot size ranges from as small as 0.5 m^2 to very large plots.

In the present study, fifty quadrats of 10 x 10 m were laid in both shola and grassland ecosystems of ENP. The areas to be surveyed were decided prior to the survey and the quadrats were laid randomly based on spatial replication methods. In grasslands, the plots laid were thoroughly searched including all microhabitats such as grass clumps, base of grass to the top of the grass, among vegetation, swamps, under stones, inside water and along the banks. In shola plots, all possible microhabitats including litter, vegetation, bark, roots, leaves of trees, inside streams and reeds were thoroughly searched. Each quadrat is spatially separated at least 50 m to avoid recounting of individuals. Quadrats were sampled for one hour time period by two persons walking towards the centre of the plot so as to avoid animals escaping out of the plot. Animals encountered were bagged and kept in a moist cotton cloth bag and were released immediately at the same site of capture soon after completing the survey in each plot.

4.2.4. Visual Encounter Survey

Even though both the methods are more or less equal in terms of result, studies suggest Visual encounter surveys (VES) as comparatively better in getting more records of the amphibians in contrast to quadrat methods in tropical forests with diverse microhabitats (Crump and Scott Jr, 1994; Doan, 2004). Heyer *et al.,* (1994) suggested not to use VES surveys for density studies whereas this method is used in estimating species diversity, richness and relative abundances of selected areas. Data are considered independent (Doan, 2003; Rodel and Ernst, 2004) and VES surveys can be replicated over a period of time. Logistically simple and easier, VES has been used in large number of studies pertaining to amphibians.

VES surveys were done in Eravikulam both day (8:00 – 18:00 hrs) and night (18:00 - 01:00hrs) so as to record all the species present. A total of 101 hours VES effort was done with each individual VES effort timed for one hour. Existing trek paths inside the shola and grassland patches were used for the survey. Each and every microhabitat was rigorously searched for amphibians. Each VES effort was done at a distance not less than 50m from the previous one to avoid recounting of animals. Animals encountered were bagged and kept in a moist cotton cloth bag and later released immediately at the same site of capture soon after completing the survey in each plot.

4.2.5. Amphibian identification

All species encountered were photographed and identified based on diagnostic morphological characters as per the latest taxonomic literature (Boulenger, 1882 a and b; Biju *et al.,* 2010; Biju *et al.,* 2011; Gururaja, 2012; Biju *et al.,* 2012; Vijayakumar *et al.,* 2014; Abraham *et al.,* 2015; Garg and Biju, 2016; Garg *et al.,* 2018; Garg et al., 2019). Taxonomy follows Frost (2021). Specimens collected for further studies were preserved and deposited at Wildlife Museum of Kerala Forest Research Institute, Peechi, Kerala (KFRI/WLM A0035).

4.3. Analysis

4.3.1. Statistical analysis

Species accumulation curves were plotted from the data collected using QS and VES surveys. To estimate the total richness from the observed richness, software EstimateS

9.1.0 was used to plot the curves employing non parametric meters Chao 1 and Jacknife 1 (Colwell, 2013). Diversity indices including Simpsons, Shannon-Wiener, Fisher's alpha and Margalef were estimated for both shola and grasslands of ENP. To understand the similarity between the shola and grasslands, diversity t-test was done and later confirmed by perMANOVA test using Bray-Curtis similarity index with the software PAleontological STatistics (PAST) Version 4.03.

4.3.2. Molecular Phlyogenetics

DNA sequences were generated for three *Raorchestes ochlandrae* specimens from three locations including one from Thattukaanam of Eravikulam National Park (10.218434° N, 77.021008°E, 1557 m ASL) and two specimens from Vazhachal and Malamanda in Vagamon. The extracted genomic DNA from liver tissue samples were stored in absolute ethanol at -2^{0}C, using the DNeasy (QiagenTM) blood and tissue kit. Partial sequences of one mitochondrial (16s rRNA) gene were amplified. PCR conditions followed previously reported protocols (16s, primers 16Sar-L and 16Sbr-H: Palumbi et al. 1991). PCR condition was as follows: Fragments of 16s were amplified using an initial denaturation at 95°C for 5 min, followed by 39 cycles of denaturation at 95°C for 45 sec, annealing at 50.4°C for 45 sec and extension at 72°C for 1 min 30 sec. Final extension was at 72°C for 10 min. PCR reactions were carried out in 25µl reaction containing 11µl of Takara emerald RR310B mastermix, 12µl of PCR grade H2O, 0.5µl of each forward and reverse primers and 1µl (60-80ng) of template DNA. PCR amplifications were carried out in S1000TM Thermal Cycler (Bio-Rad, USA). Amplified PCR products were run on a 2% agarose gel and viewed with an Essential V4 (UVITEC Cambridge, UK) gel documentation system to confirm the PCR amplification. PCR products were purified and Sanger sequenced in both directions at Barcode Biosciences (Bangalore, India).

Bidirectional sequences were manually checked using the CHROMAS and aligned using ClustalW with default prior settings implemented in MEGA 7 (Higgins, *et al*. 1994; Tamura *et al*., 2011). Forty five sequences were downloaded for other *Raorchestes* sp and two outgroups (*Pseudophilautus* sp.) from the Genbank and accession numbers are given in the phylogenetic tree (Fig. 4. 83).

For the phylogenetic reconstruction, Maximum Likelihood (ML) analysis was performed using IQ-TREE (Nguyen *et al*., 2015), implemented in the web server version (http://iqtree.cibiv.univie.ac.at/) (Trifinopoulos *et al*., 2016). IQ-TREE server was used Modelfinder (Kalyanamoorthy *et al*., 2017) to find the best-fit evolutionary model for the single partition. Model finder suggested TIM2e+G4 as the best-fit model and the analysis was done using the same model. Support for internal branches in ML was quantified using bootstrap (1000 UltraFast bootstrap replicates).

4.3.3. Distribution mapping

GPS points for all species encounters were taken using Etrex Garmin 30X and map for individual species was created by software QGIS Desktop 3.14.0.

4.4. Results

Thirty seven species of amphibians under two orders, Anura and Gymnophiona were recorded from the park as a part of the study. While a majority (36 species) belonged to Anura, there was a single species (*Uraeotyphlus sp.*) from the order Gymnophiona. In addition to the twenty species of amphibians reported from Eravikulam National Part as a part of previous studies, seventeen species of amphibians including fourteen frogs, two toads and one caecilian were recorded from ENP during the present study. Thirty six species of amphibians were recorded from the shola compared to only six species from grasslands. Five species *viz. Duttaphrynus microtympanum, Nyctibatrachus deccanensis,* *Walkerana leptodactyla, Raorchestes beddomii* and *Raorchestes dubois* were found inhabiting both the grasslands and sholas (Fig.4.3). High elevation grassland specific species *Raorchestes resplendens* was found only in the grassland habitats.

Amphibians recorded belonged to ten different families. The tree frog family Rhacophoridae, comprised 54% of the total species recorded with twenty species followed by 11% by the night frog family Nyctibatrachidae with four species. The rest of the families Micrixalidae, Bufonidae, Microhylidae and Ranixalidae were represented by two species (4%) and families Dicroglossidae, Nasikabatrachidae, Ranidae and Ichthyophidae by a single species (2%) (Fig. 4. 4). Direct developing bush frogs from the genus *Raorchestes* of Rhacophoridae family was the most dominant group with fourteen species recorded from ENP (Fig. 4.5).

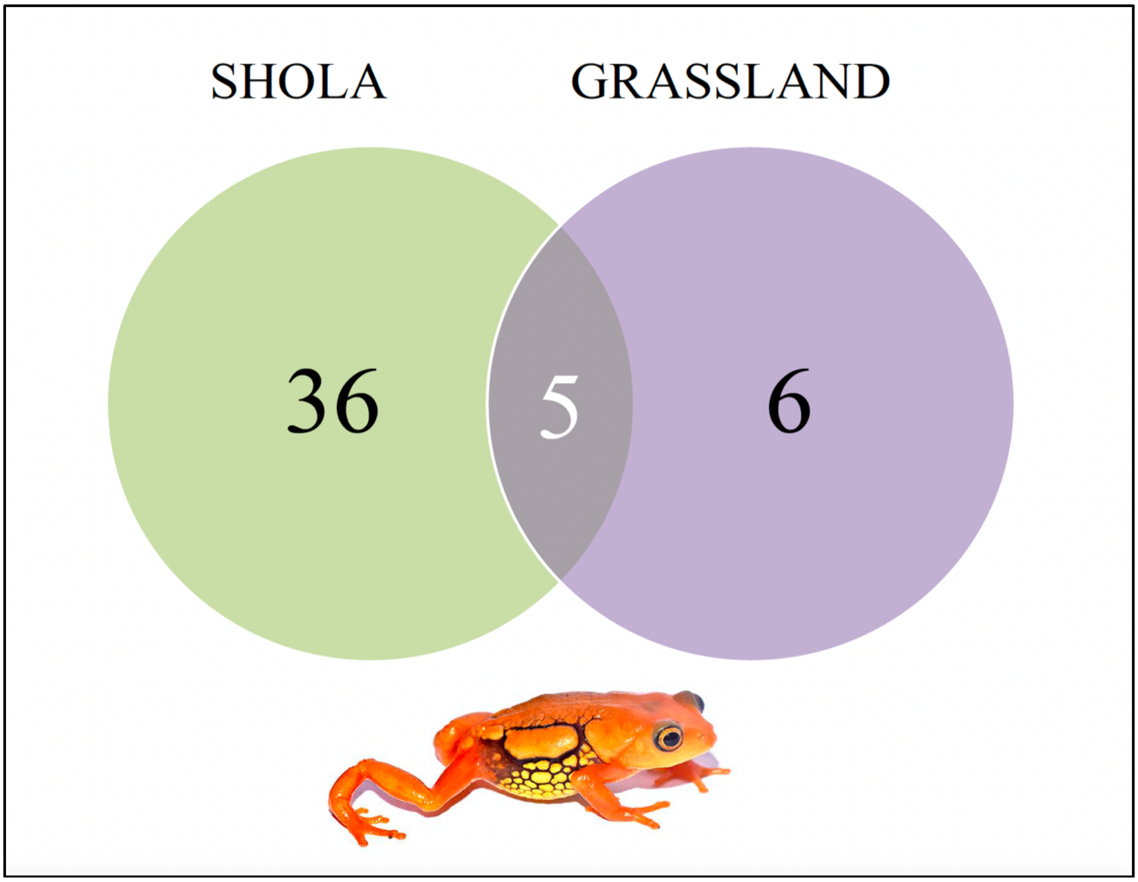

Figure 4.3: Venn diagram of number of amphibian species recorded from ENP

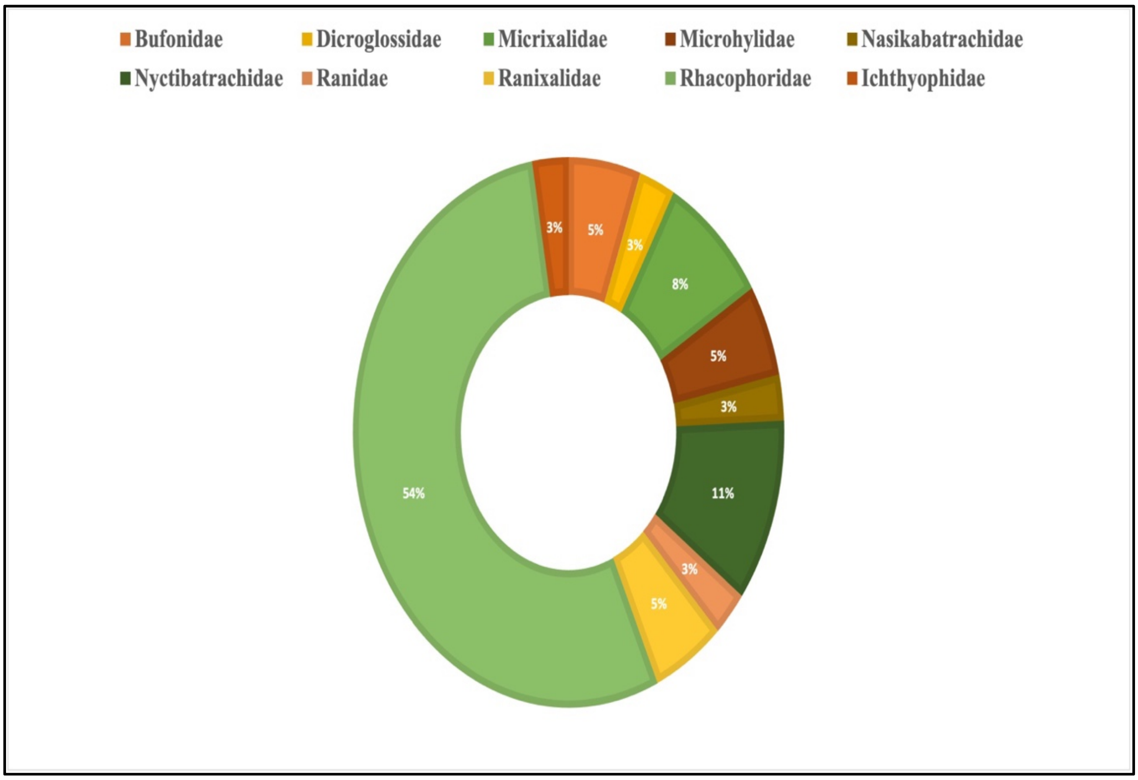

Figure 4.4: Amphibian families from ENP (N=37)

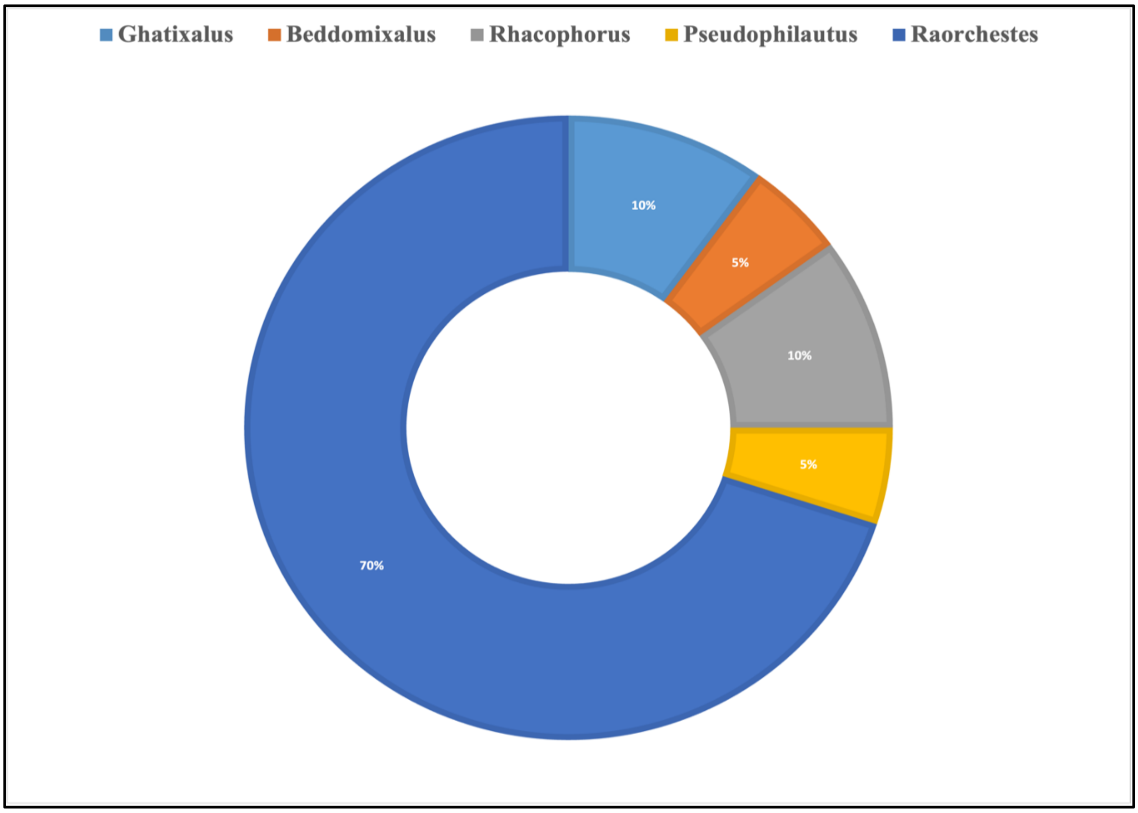

Figure 4.5: Tree frog genera of ENP

4.4.1. Amphibians of ENP and their distribution

Distribution of all thirty seven species recorded was studied in detail. Maps of each species with its distribution data inside ENP was plotted based on the thorough surveys throughout the grasslands and sholas. Even though species including *Micrixalus frigidus* and *Raorchestes resplendens* were described with ENP as the type locality, their distribution inside park was not clearly known. Several new records to the park as well as new distribution points of species were also recorded including the first record of *Melanobatrachus indicus* and *Nasikabatrachus sahyasrensis* from ENP as well as several new locations of *Raorchestes resplendens* within the park.

1. ***Duttaphrynus melanostictus* (Schneider, 1799),** Common Indian Toad

Order: Anura

Family: Bufonidae

IUCN Category: Least Concern

Endemism: Wide spread distribution

Wildlife Protection Act, 1972: Not listed

CITES: Not listed

Distribution: Widely distributed in India whereas inside ENP, they were rarely encountered. Found only from three locations including Thattukaanam and areas close to Tamil Nadu border which are low in elevation (1400- 1500m) comparatively to other regions of ENP. They were recorded only in the Shola patches and not in the grasslands.

Figure 4. 6: *Duttaphrynus melanostictus*

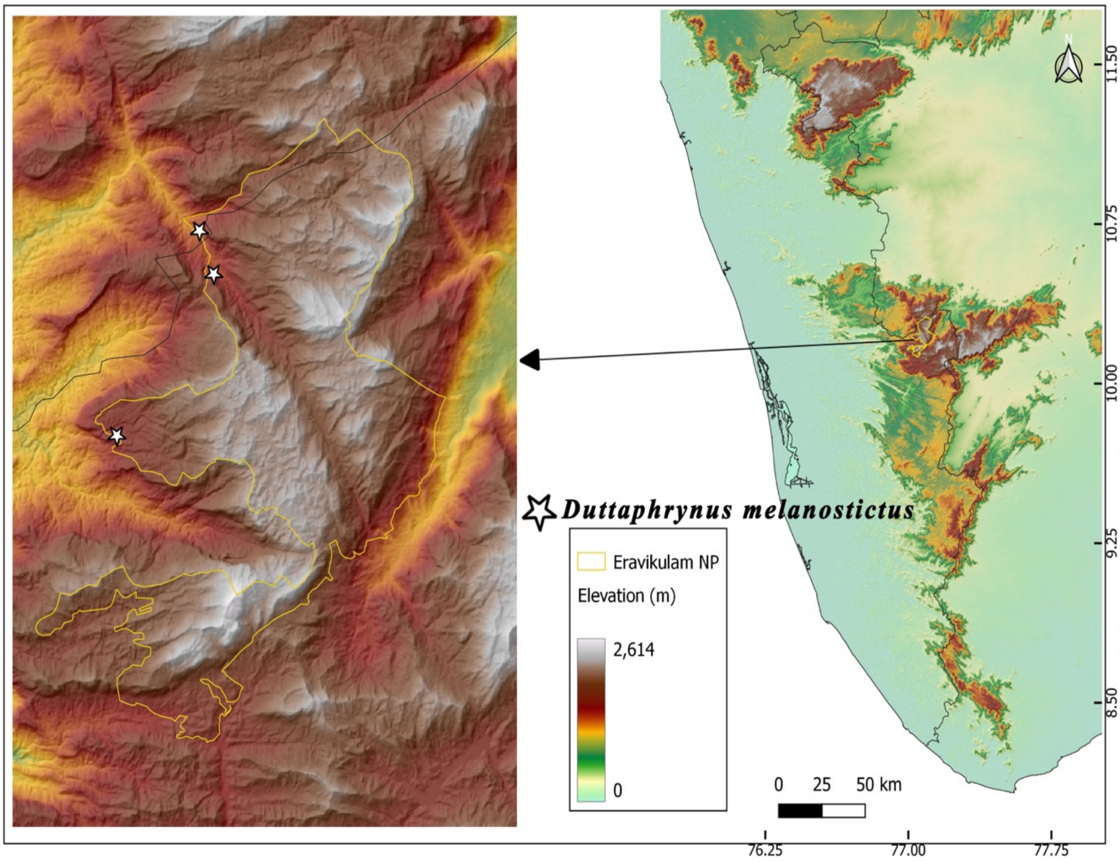

Figure 4. 7: Distribution map of *Duttaphrynus melanostictus* inside ENP

2. ***Duttaphrynus microtympanum* (Boulenger, 1882),** Small-eared Toad

Order: Anura

Family: Bufonidae

IUCN Category: Vulnerable

Endemism: Endemic to Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: So far recorded only from SWG in Kerala and Tamil Nadu. Inside Eravikulam National Park, it is the commonest Bufonidae recorded both from the shola and grasslands. Found at elevations ranging from 1400 m to 2500 m.

Figure 4. 8: *Duttaphrynus microtympanum*

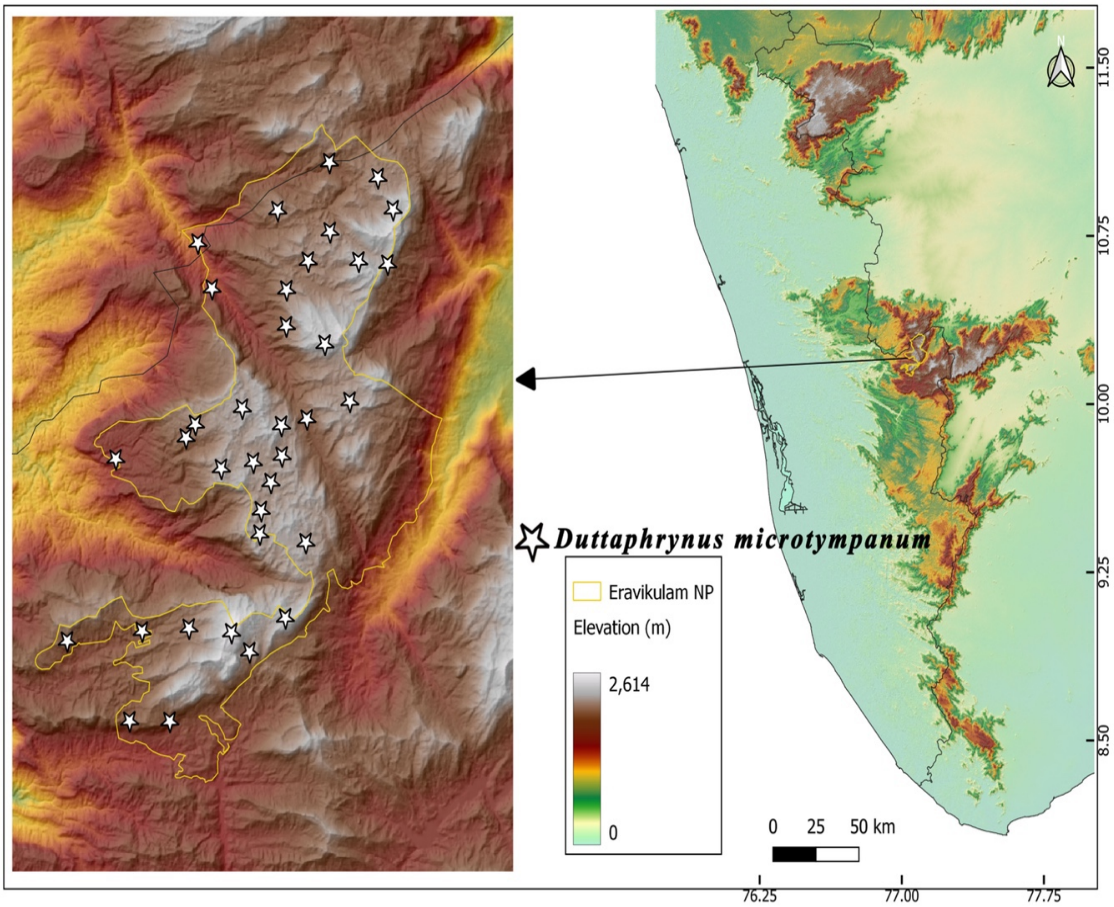

Figure 4. 9: Distribution map of *Duttaphrynus microtympanum* inside ENP

3. ***Minervarya brevipalmata* (Peters, 1871),** Short-webbed Frog

Order: Anura

Family: Dicroglossidae

IUCN Category: Data Deficient

Endemism: Endemic to Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Found from 10 different locations within ENP. All the records were within the shola habitat. Found from 1400- 2200 m inside the park

Figure. 4. 10: Calling *Minervarya brevipalmata* male

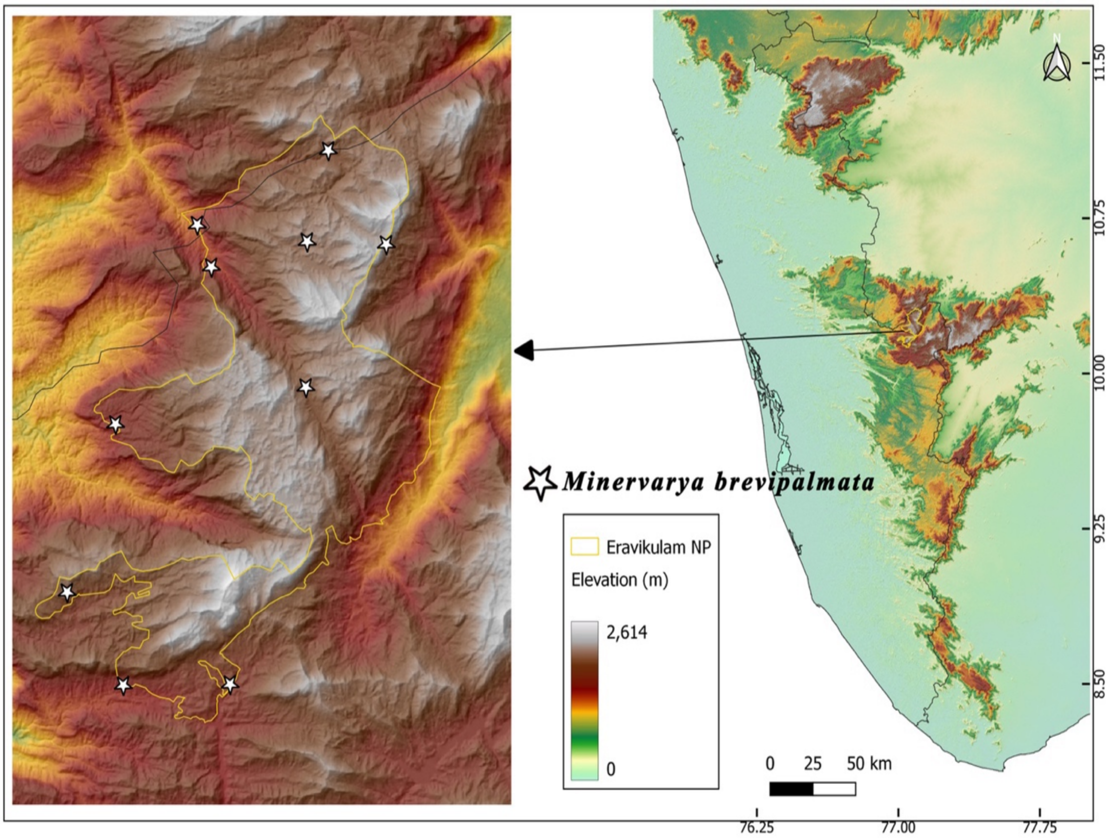

Figure. 4. 11: Distribution map of *Minervarya brevipalmata* inside ENP

4. ***Micrixalus adonis* Biju, Garg, Gururaja, Shouche, and Walujkar, 2014,** Beautiful Dancing Frog

Order: Anura

Family: Micrixalidae

IUCN Category: Not Evaluated

Endemism: Endemic to Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from six locations inside ENP largely from the shola patches in the lower elevation (1400 -1600 m) regions of the park.

Figure. 4. 12: *Micrixalus adonis*

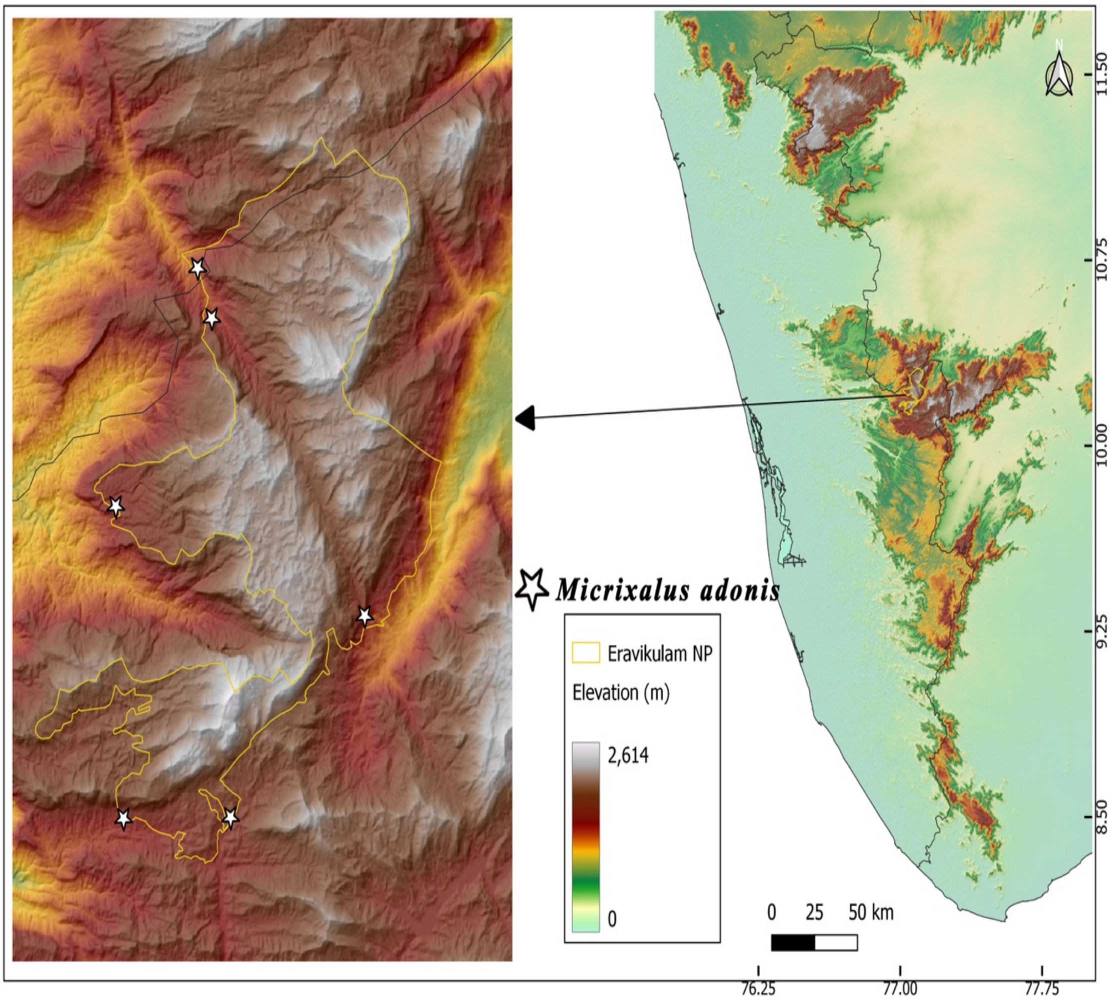

Figure. 4. 13: Distribution map of *Micrixalus adonis* inside ENP

5. ***Micrixalus frigidus* Biju, Garg, Gururaja, Shouche, and Walujkar, 2014** Cold Stream Dancing Frog

Order: Anura

Family: Micrixalidae

IUCN Category: Not Evaluated

Endemism: Endemic to Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Commonest species of endemic Micrixalidae family. Reported from nine locations inside ENP. Recorded only from high elevation (1800 -2300 m) shola patches.

Figure. 4. 14: ***Micrixalus frigidus***

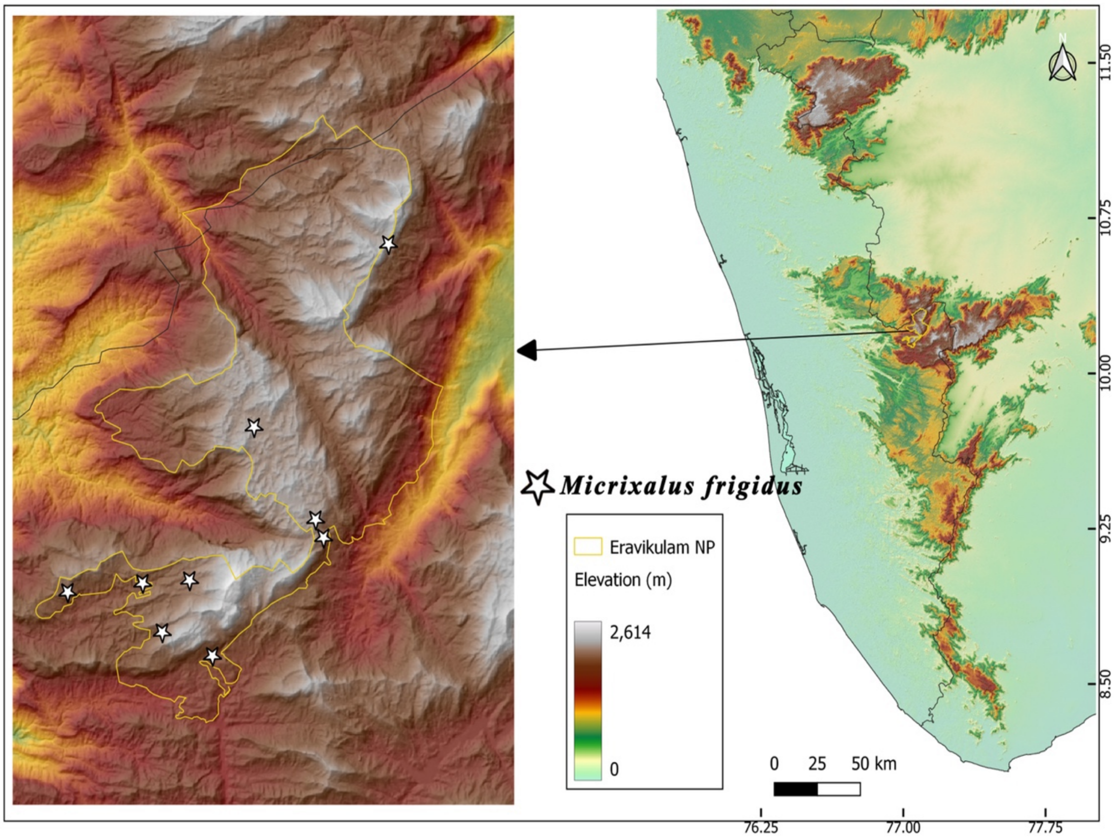

Figure. 4. 15: Distribution map of *Micrixalus frigidus* inside ENP

6. ***Micrixalus nigraventris* Biju, Garg, Gururaja, Shouche, and Walujkar, 2014**

Black-bellied Dancing Frog

Order: Anura

Family: Micrixalidae

IUCN Category: Not Evaluated

Endemism: Endemic to Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Reported from seven locations in the sholas inside ENP. Seen at more or less same elevational range of *Micrixalus frigidus*. A significant population of this species was observed from streams around Anamudi area.

Figure. 4. 16: *Micrixalus nigraventris* tin amplexus with *Walkerana leptodactyla*

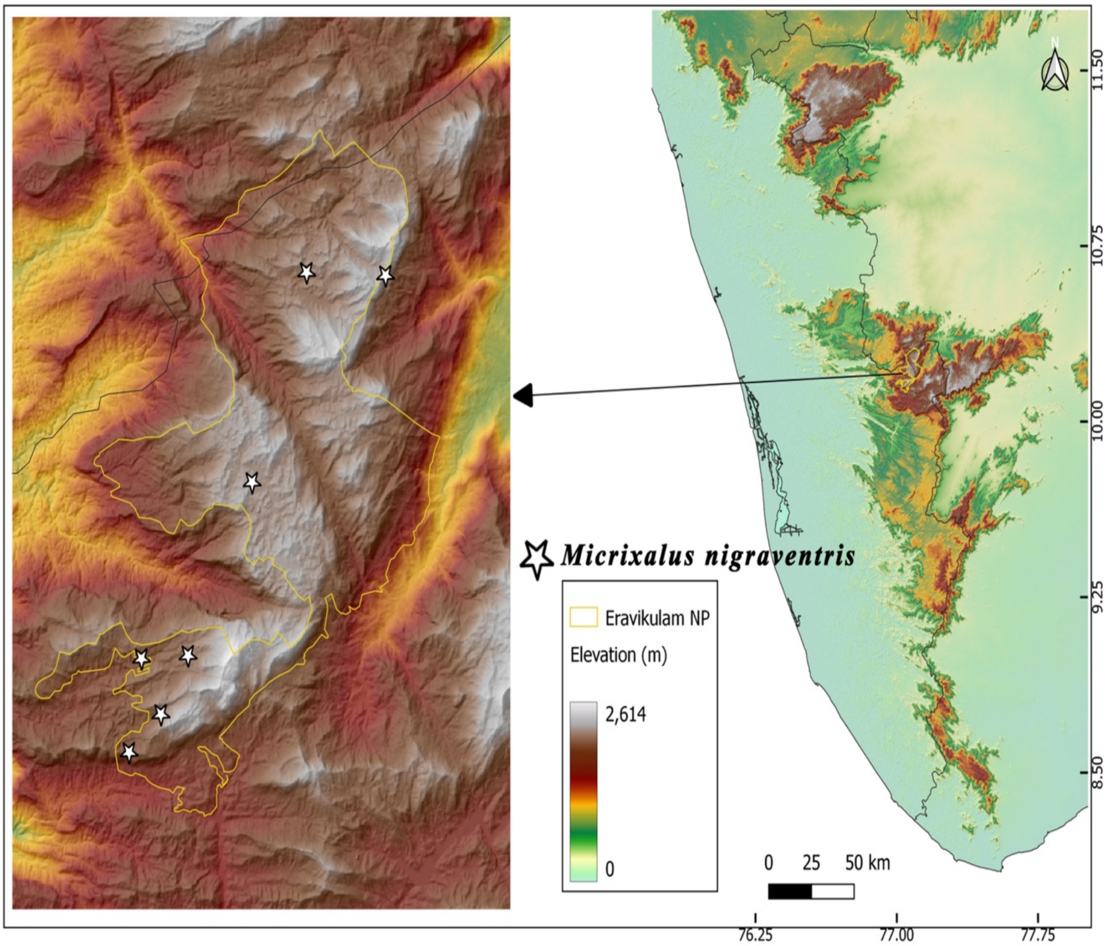

Figure. 4. 17: Distribution map of *Micrixalus nigraventris* inside ENP

7. ***Melanobatrachus indicus* Beddome, 1878,** Galaxy Frog

Order: Anura

Family: Microhylidae

IUCN Category: Endangered

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

CITES: Not listed

Distribution: The present study reports the first ever record of the species from ENP. It was found from five locations only from the shola patches inside ENP (1600 -2000m) .

Figure 4. 18: ***Melanobatrachus indicus***

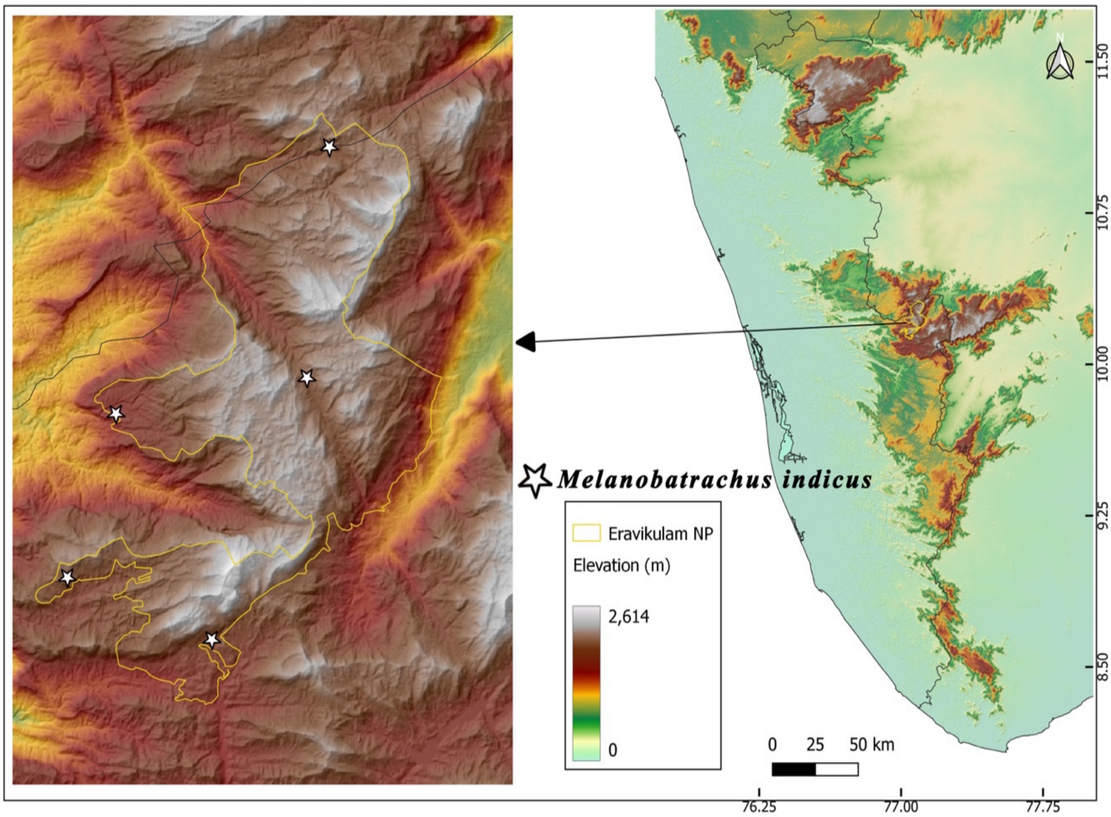

Figure 4. 19: Distribution map of *Melanobatrachus indicus* inside ENP

8. ***Uperodon montanus* (Jerdon, 1854),** Jerdon's Ballon Frog

Order: Anura

Family: Microhylidae

IUCN Category: Near Threatened

Endemism: Endemic to Kerala, Karnataka and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Species is widely distributed inside ENP. Recorded from 22 locations within the park. Found only in the shola patches (1400 -2200m).

Figure 4. 20: *Uperodon montanus*

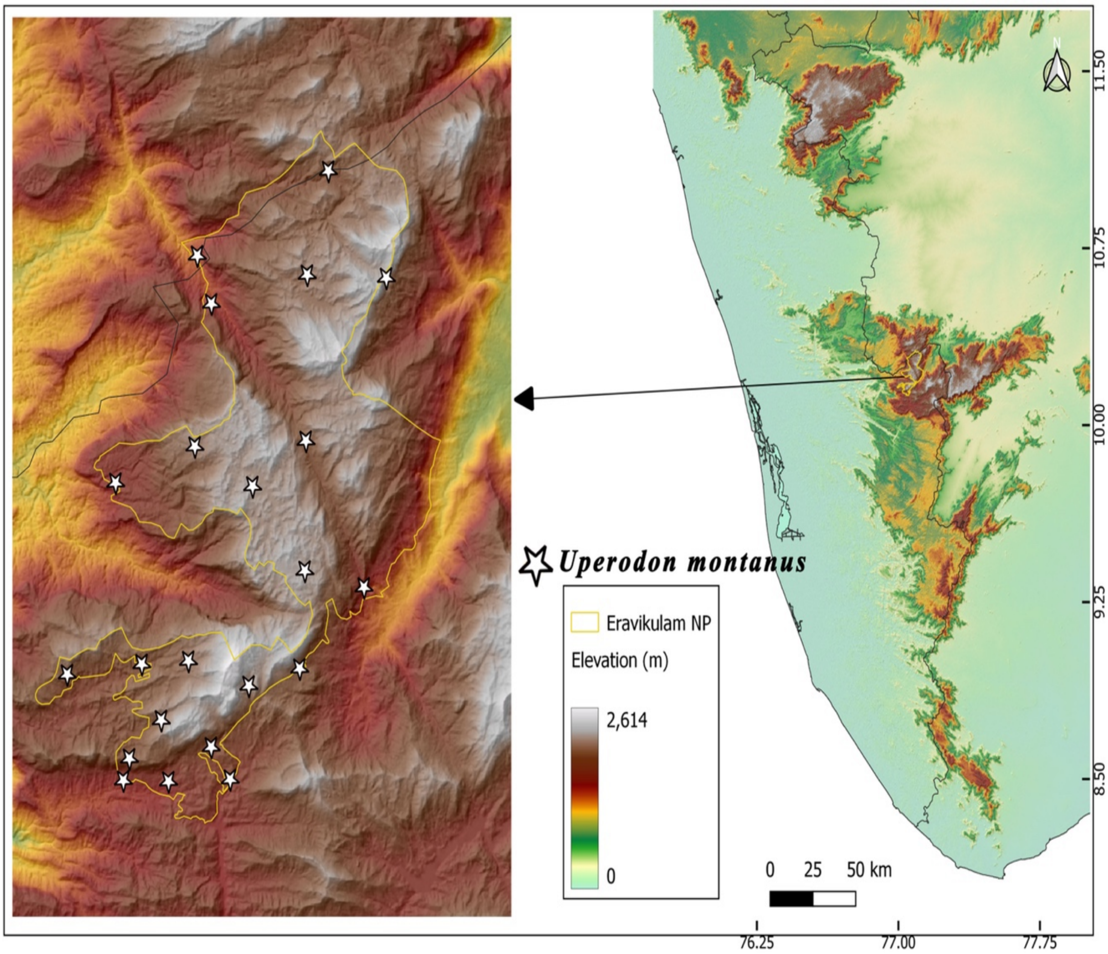

Figure 4. 21: Distribution map of *Uperodon montanus* inside ENP

9. ***Nasikabatrachus sahyadrensis* Biju & Bossuyt, 2003,** Purple Frog

Order: Anura

Family: Nasikabatrachidae

IUCN Category: Endangered

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: First record of the species from the park as well as the highest elevational record (1400m). Tadpole of the species was found from single locality close to Tamil Nadu border, inside ENP .

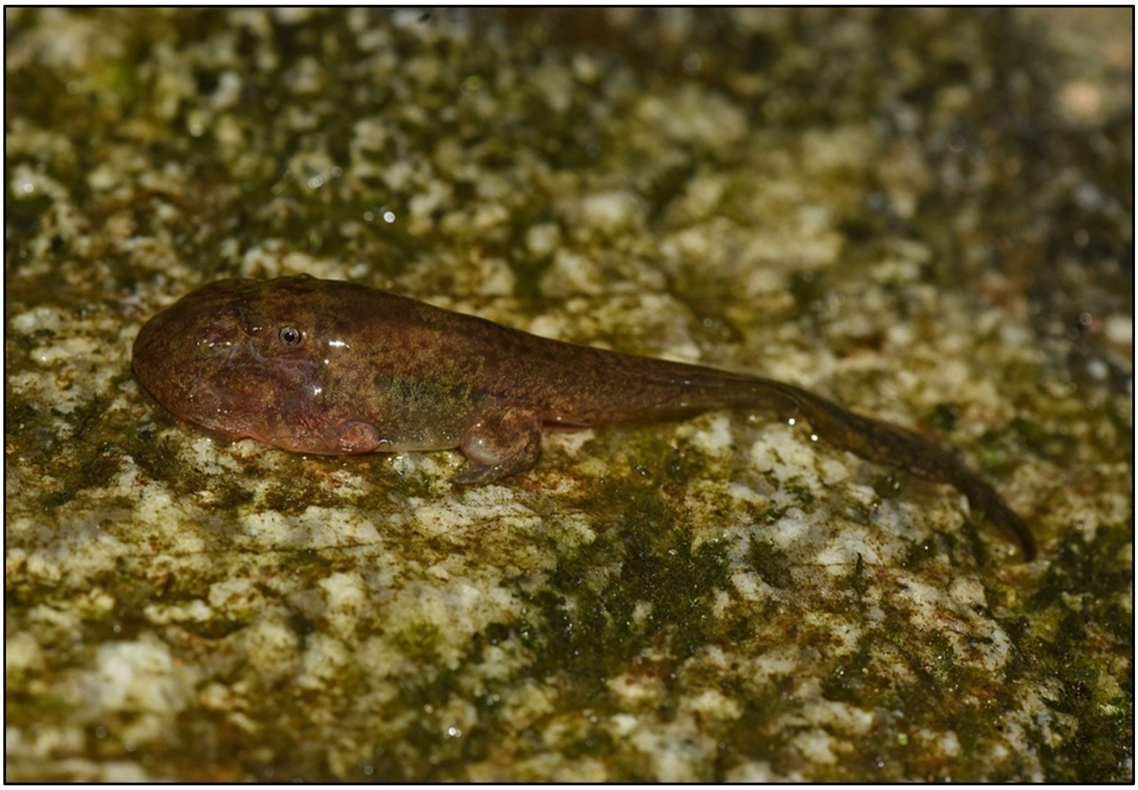

Figure 4. 22: Tadpole of *Nasikabatrachus sahyadrensis*

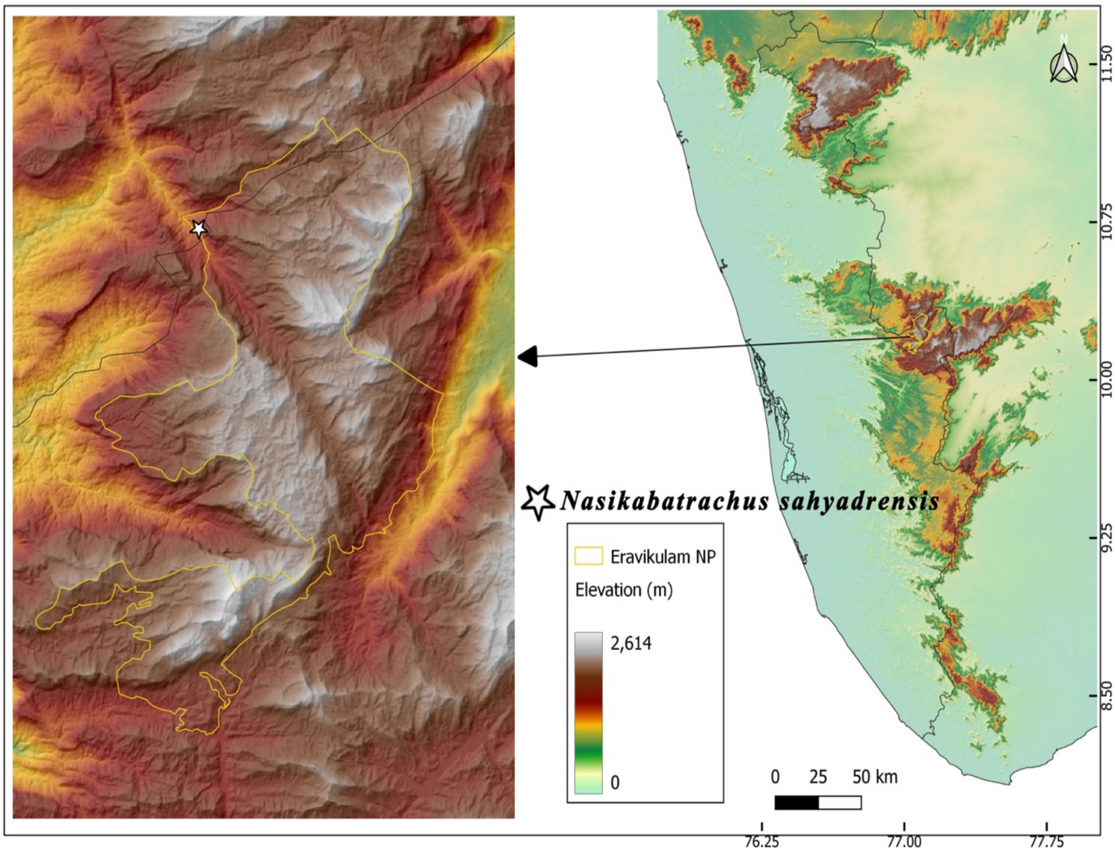

Figure 4. 23: Distribution of *Nasikabatrachus sahyadrensis* inside ENP

10. ***Nyctibatrachus acanthodermis*** **Biju, Bocxlaer, Mahony, Dinesh, Radhakrishnan, Zacharaiah, Giri & Bossuyt 2011,** Spinular Night Frog

Order: Anura

Family: Nyctibatrachidae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: First record of the species from the park and in fact a very common species found from twenty three locations inside ENP (1400 -2100 m). The species was recorded only in the streams of shola ecosystem.

Figure 4. 24: ***Nyctibatrachus acanthodermis***

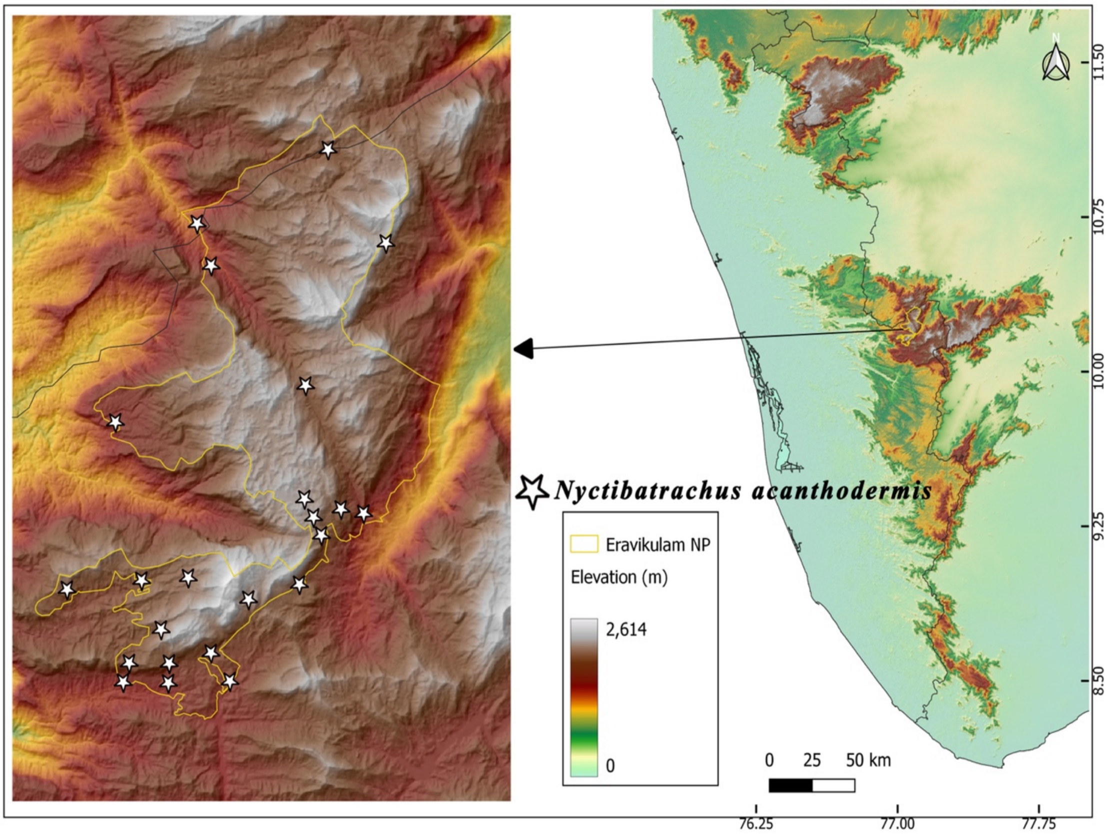

Figure 4. 25: Distribution map of *Nyctibatrachus acanthodermis* inside ENP

11. ***Nyctibatrachus anamallaiensis* (Myres, 1942),** Anamalai Night Frog

Order: Anura

Family: Nyctibatrachidae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Reported from twenty three locations inside ENP and recorded only in and around the streams and swampy areas of shola ecosystem at an elevational range of 1400 - 2200 m.

Figure 4. 26: Egg gaurding *Nyctibatrachus anamallaiensis*

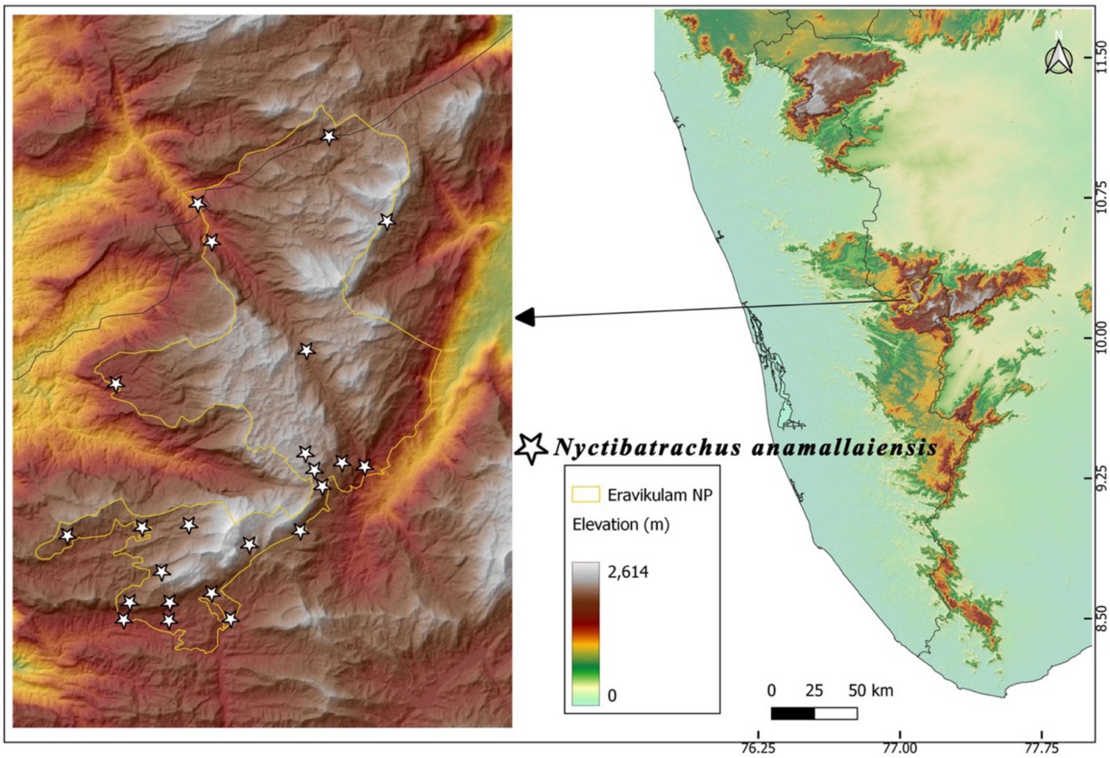

Figure 4. 27: Distribution map of *Nyctibatrachus anamallaiensis* inside ENP

12. ***Nyctibatrachus deccanensis* Dubois, 1984,** Deccan Night Frog

Order: Anura

Family: Nyctibatrachidae

IUCN Category: Vulnerable

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Commonest member of Nyctibatrachidae inside ENP with records from forty one locations. Found in and around the streams and swampy areas of both shola and grassland ecosystem in the high rolling plateau (1800 m and above).

Figure 4. 28: ***Nyctibatrachus deccanensis***

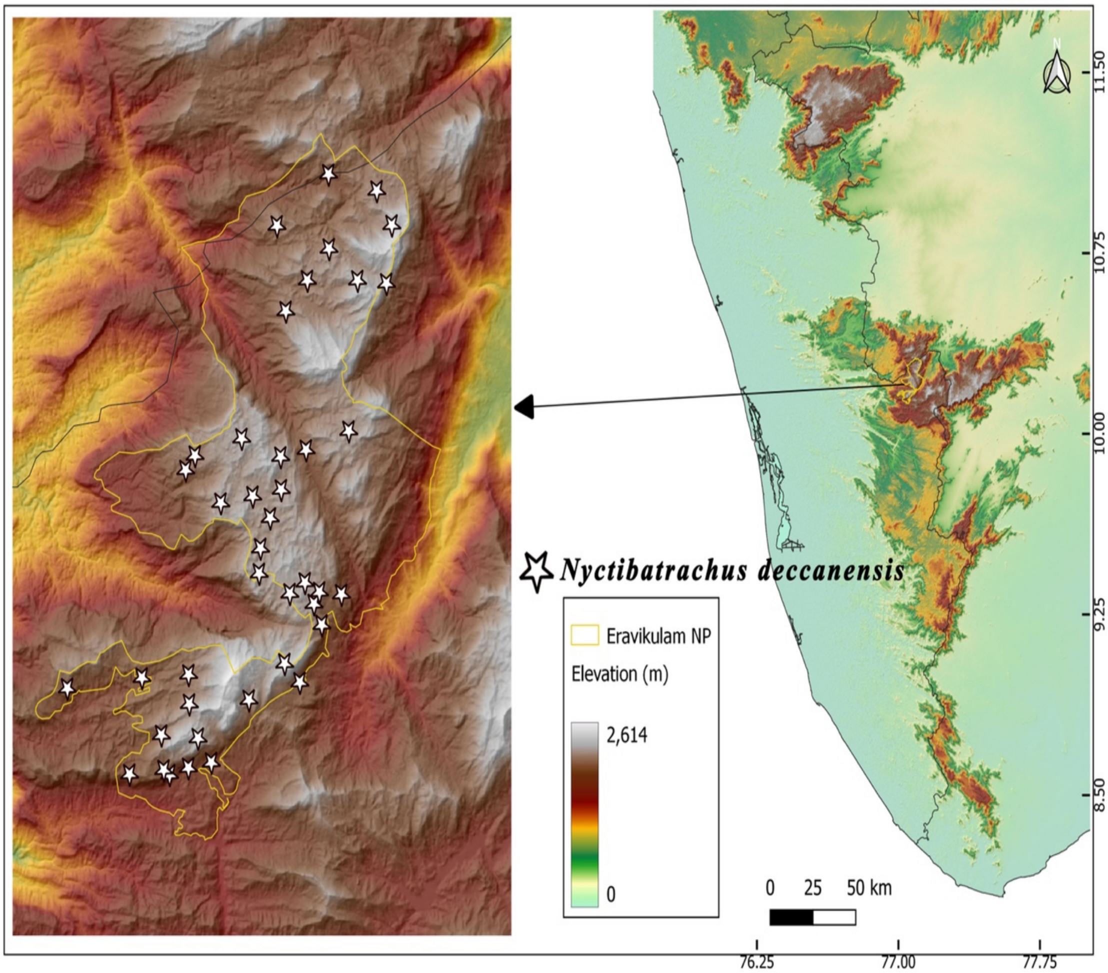

Figure 4. 29: Distribution map of *Nyctibatrachus deccanensis* inside ENP

13. ***Nyctibatrachus poocha* Biju, Bocxlaer, Mahony, Dinesh, Radhakrishnan, Zacharaiah, Giri & Bossuyt 2011,** Meowing Night Frog

Order: Anura

Family: Nyctibatrachidae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from seventeen locations in sholas of ENP at an elevational range of 1400-2200 m

Figure 4. 30: ***Nyctibatrachus poocha***

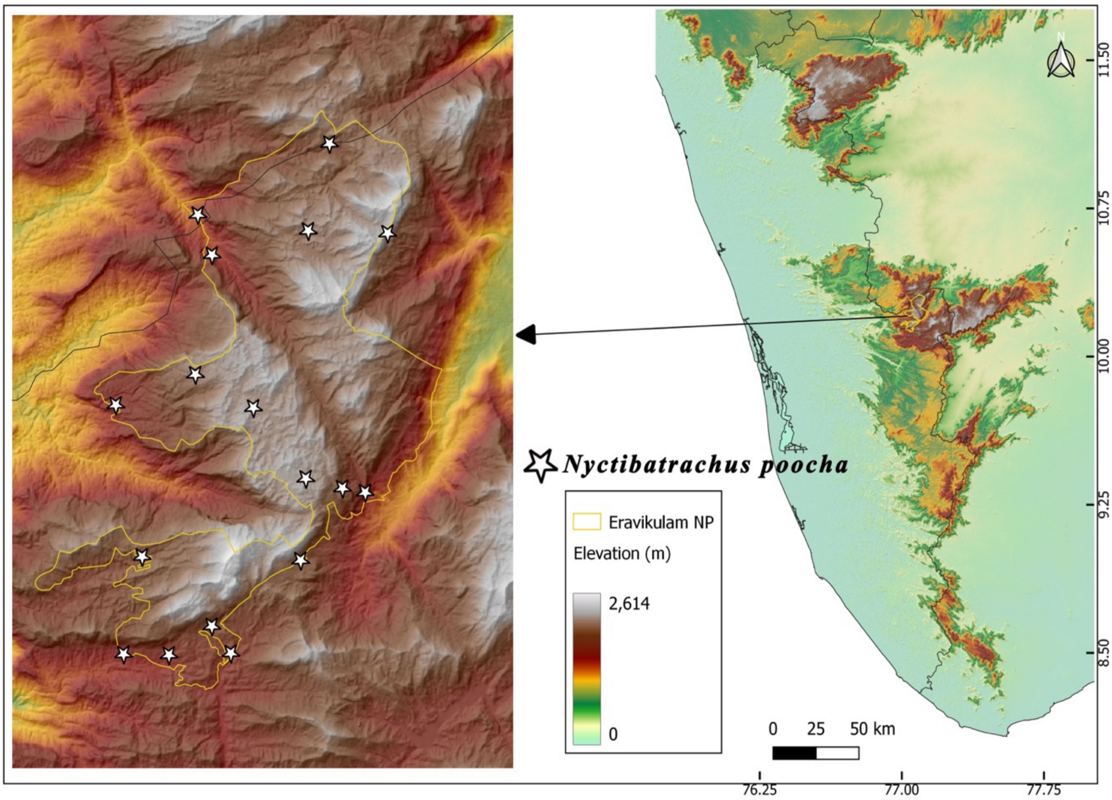

Figure 4. 31: Distribution map of *Nyctibatrachus poocha* inside ENP

14. ***Indosylvirana sreeni* (Biju, Garg, Wijayathilaka, Senevirathne & Meegaskumbura 2014),** Sreeni's Golden-backed Frog

Order: Anura

Family: Ranidae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded only from four low elevation sholas of ENP at 1400 -1500 m

Figure 4. 32: *Indosylvirana sreeni*

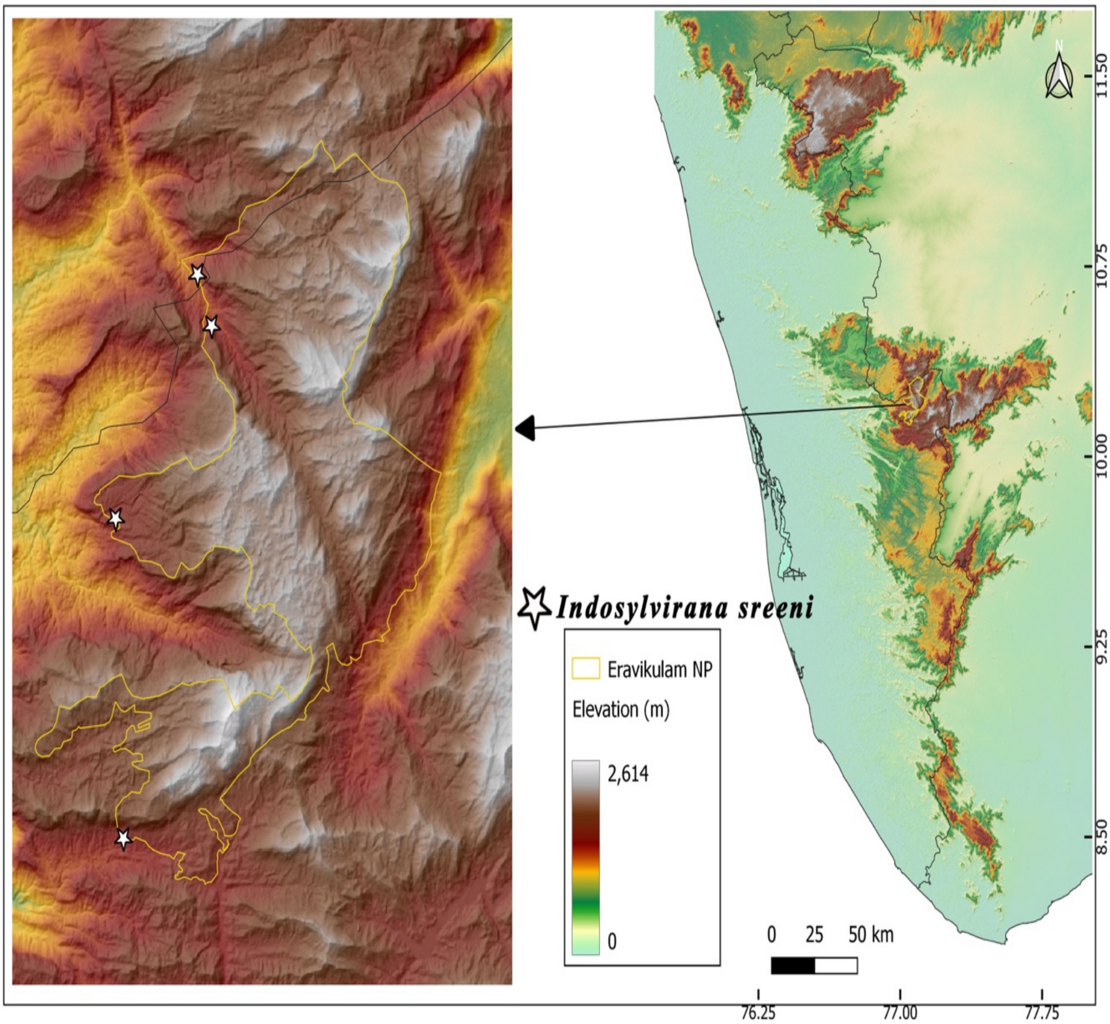

Figure 4. 33: Distribution map of *Indosylvirana sreeni* inside ENP

15. ***Walkerana leptodactyla* (Boulenger, 1882),** Boulenger's Leaping Frog

Order: Anura

Family: Ranixalidae

IUCN Category: Endangered

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Schedule IV

Distribution: One of the most widely distributed species from ENP with records from forty two locations inside shola and grasslands of ENP at an elevational range of 1400 - 2200 m.

Figure 4. 34: ***Walkerana leptodactyla***

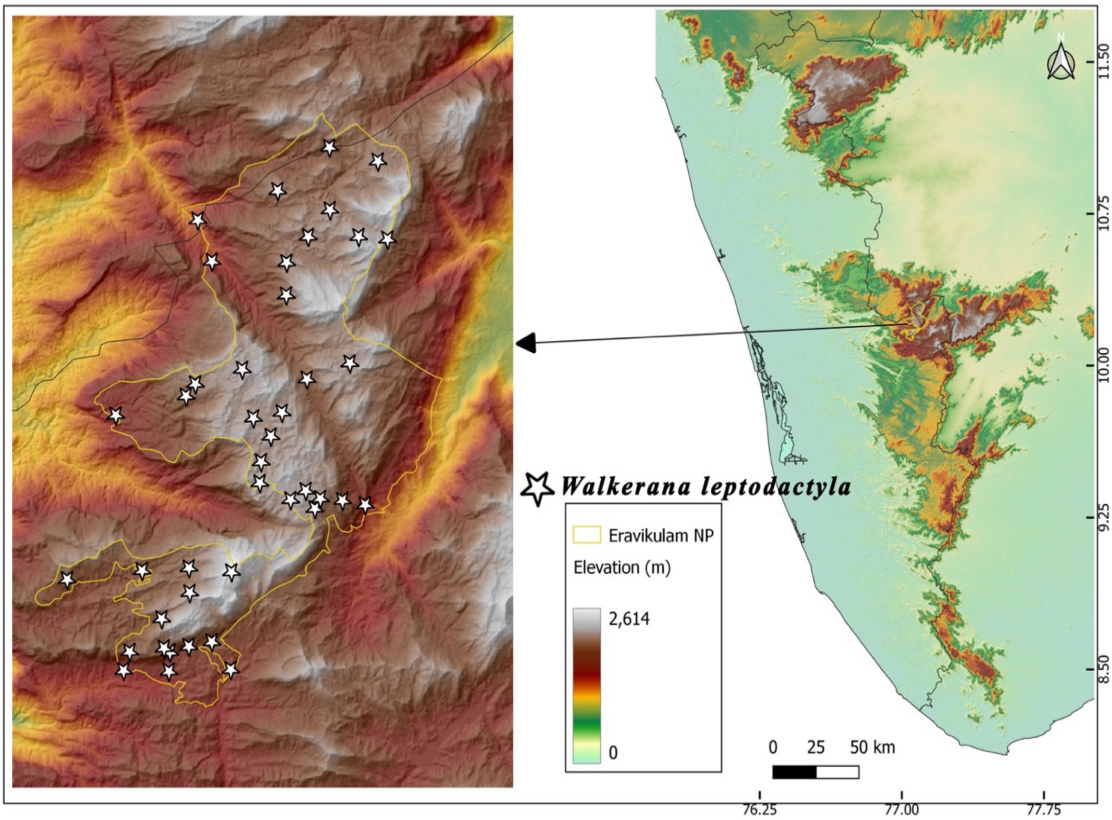

Figure 4. 35: Distribution map of *Walkerana leptodactyla* inside ENP

16. ***Walkerana phrynoderma* (Boulenger, 1882),** Toad-skinned Leaping Frog

Order: Anura

Family: Ranixalidae

IUCN Category: Critically Endangered

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Schedule IV

Distribution: First record for the park from four locations inside ENP at an elevational range of 1400-1700m within ENP.

Figure 4. 36: *Walkerana phrynoderma*

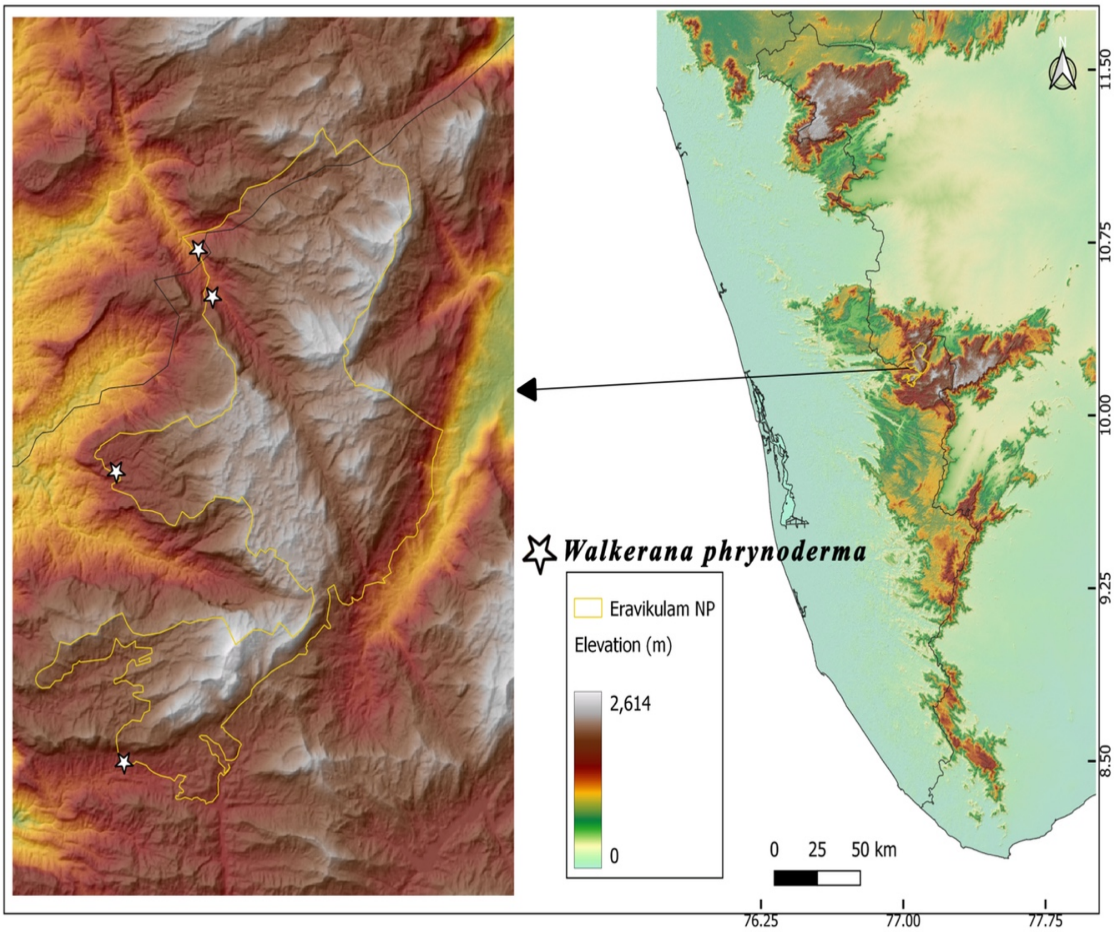

Figure 4. 37: Distribution map of *Walkerana phrynoderma* inside ENP

17. ***Beddomixalus bijui* (Zachariah, Dinesh, Radhakrishnan, Kunhikrishnan, Palot, Vishnudas, 2011),** Kadalar Swamp Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from three locations inside swamp habitats of sholas in ENP

Figure 4. 38: *Beddomixalus bijui*

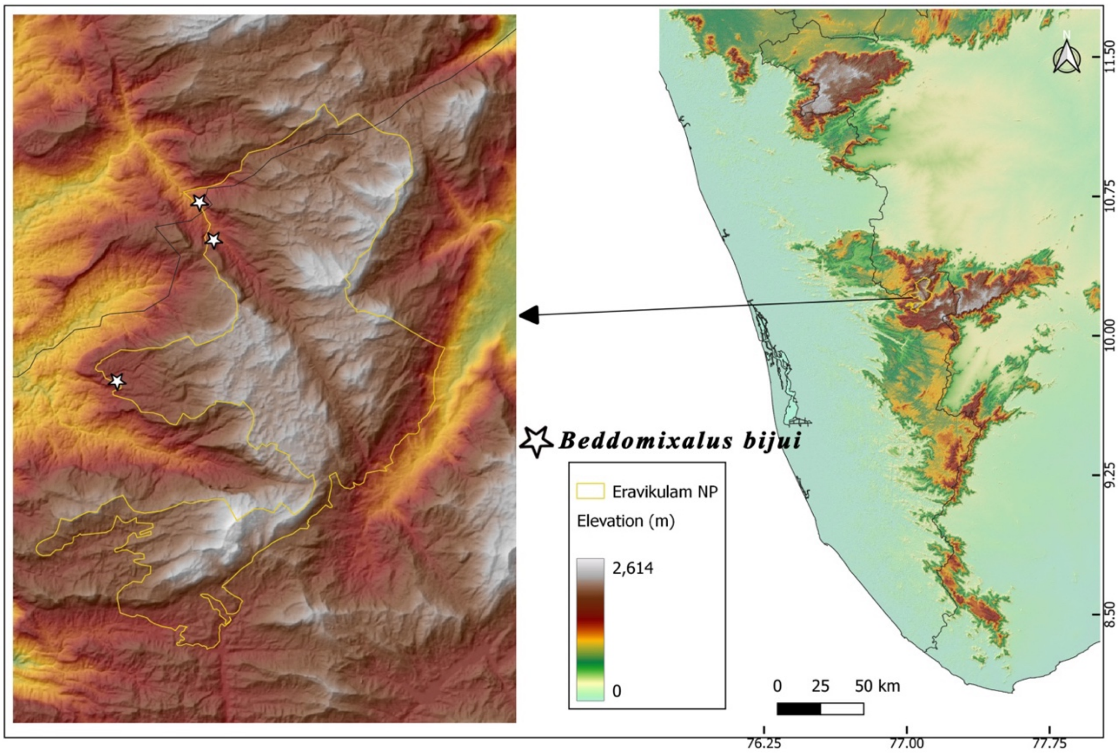

Figure 4. 39: Distribution map of *Beddomixalus bijui* inside ENP

18. ***Ghatixalus asterops* Biju, Roelants & Bossuyt, 2008,** Ghat Tree Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Most widely distributed species of ENP were recorded from twenty different locations at an elevational range of 1600 -2200 m.

Figure 4. 40: *Ghatixalus asterops*

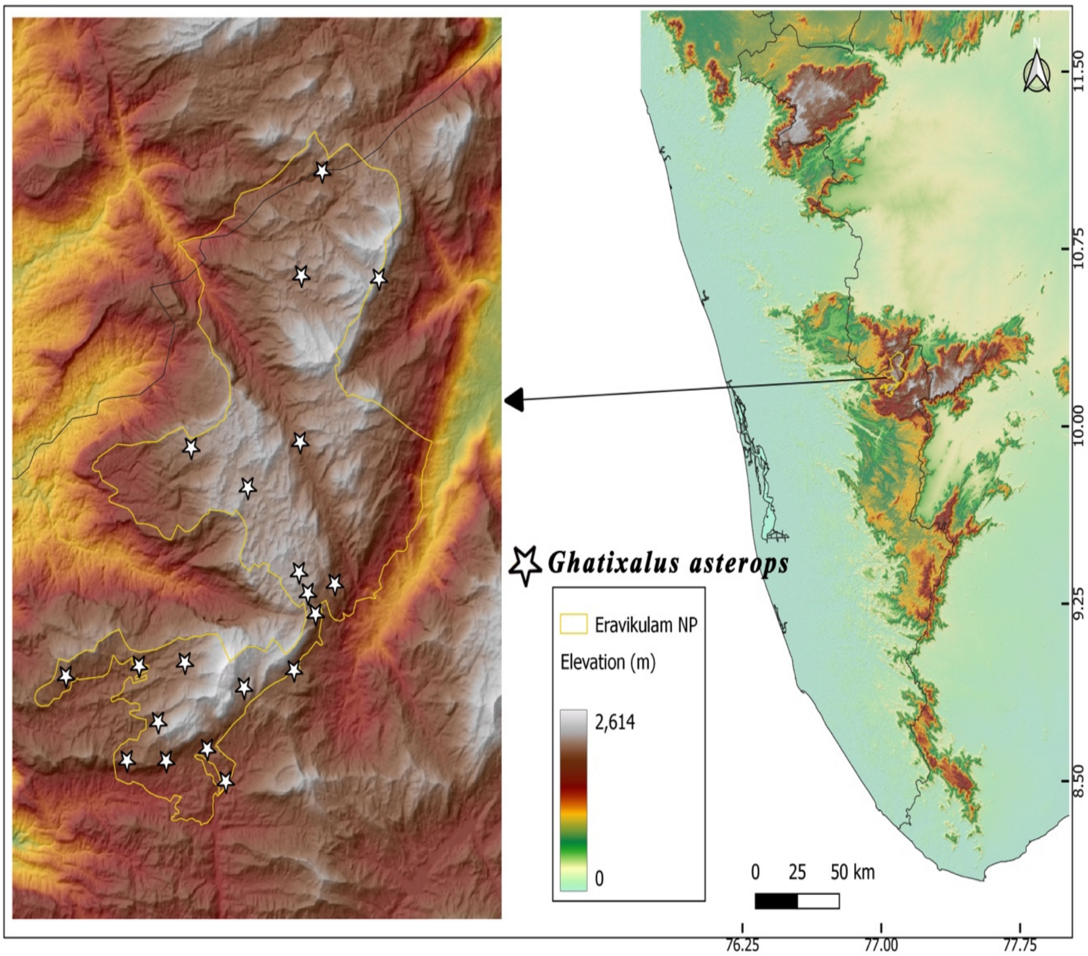

Figure 4. 41: Distribution map of *Ghatixalus asterops* inside ENP

19. ***Ghatixalus magnus* Abraham, Mathew, Cyriac, Zachariah, Raju, and Zachariah, 2015,** Large Ghat Tree Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Sympatric to *Ghatixalus asterops, G. magnus* was recorded from fifteen locations from ENP at an elevational range (1600 -2300 m)

Figure 4. 42: ***Ghatixalus magnus***

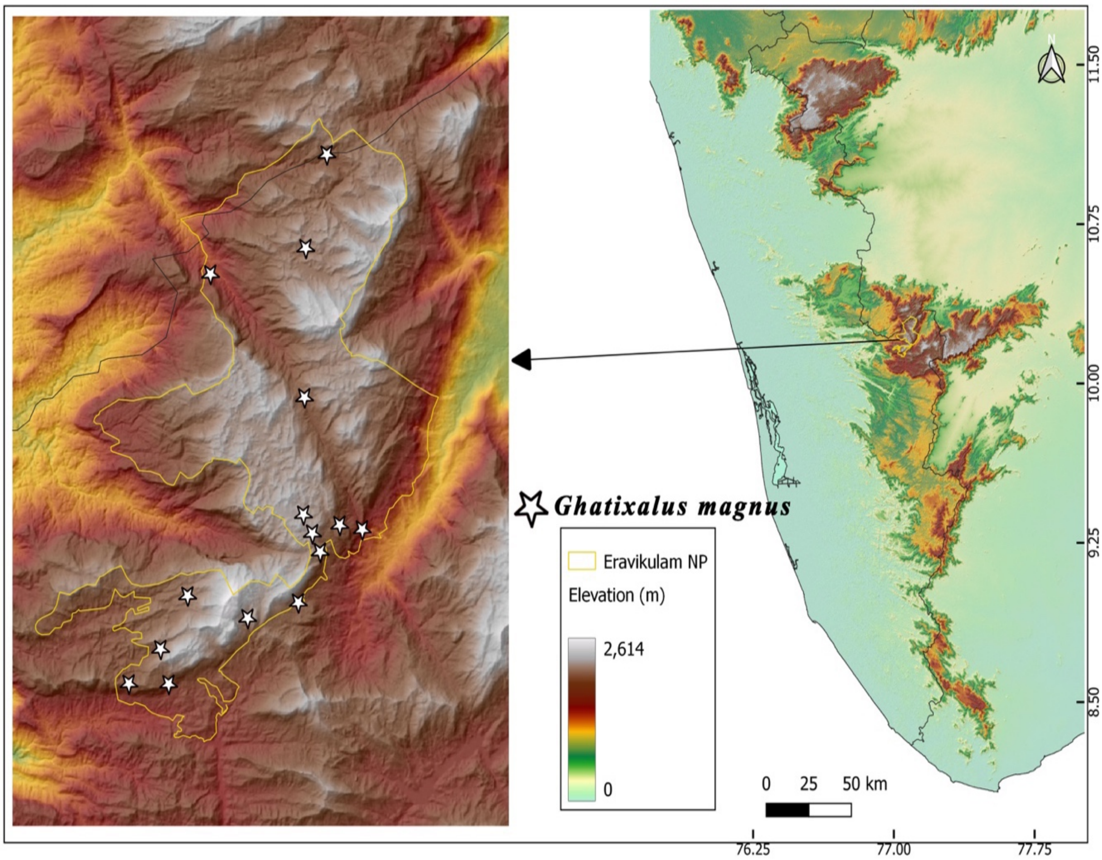

Figure 4. 43: Distribution map of *Ghatixalus magnus* inside ENP

20. ***Raorchestes beddomii*** **(Gunther, 1876),** Beddome's Bush Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Near Threatened

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from thirty one locations from ENP. Found both in the grasslands and shola ecosystem (1400 -2300 m).

Figure 4. 44: Calling *Raorchestes beddomi* male

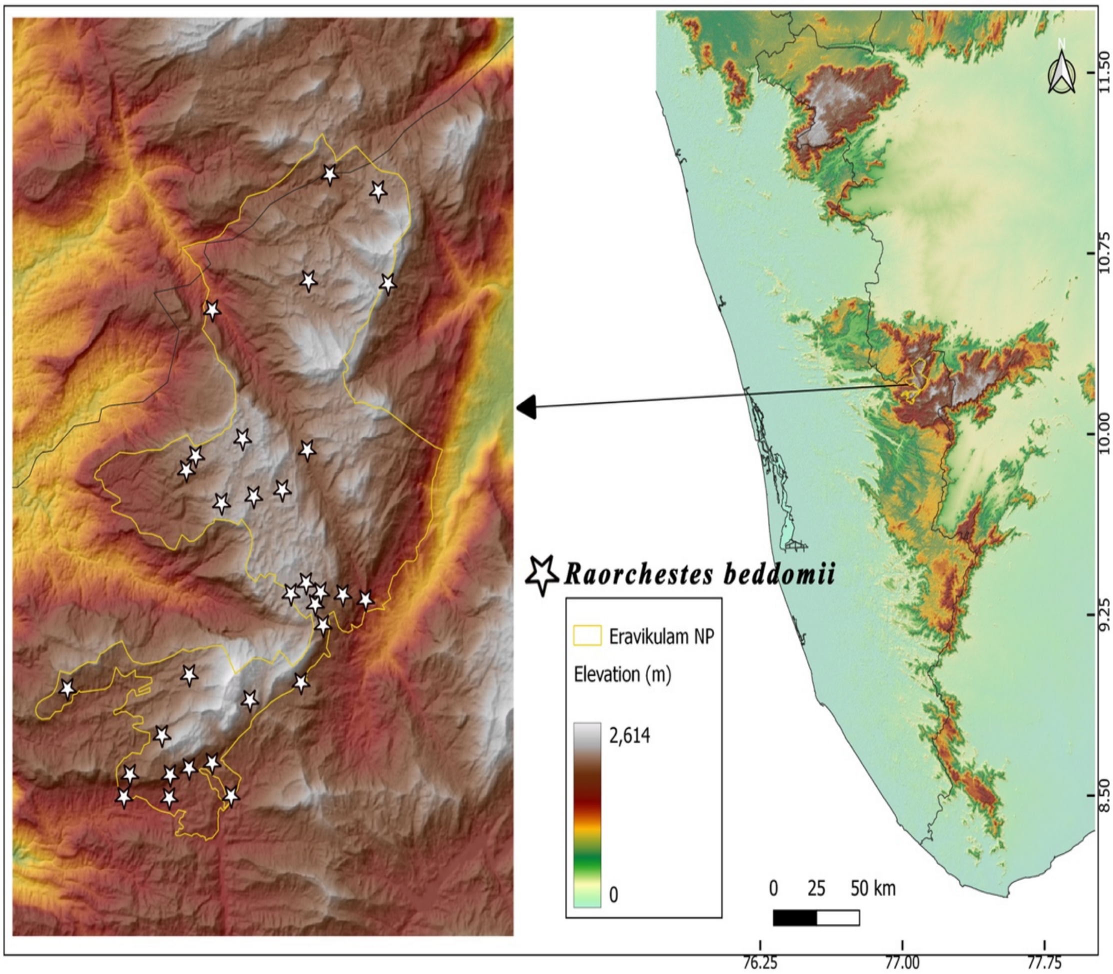

Figure 4. 45: Distribution map of *Raorchestes beddomi* inside ENP

21. ***Raorchestes blandus* Vijayakumar, Dinesh, Prabhu, and Shanker, 2014,** Pleasant Bush Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from three locations in shola ecosystem from ENP in low elevation areas (1400-1600m) including Thattukanam and bordering areas of Tamil Nadu.

Figure 4. 46: Calling *Raorchestes blandus* male

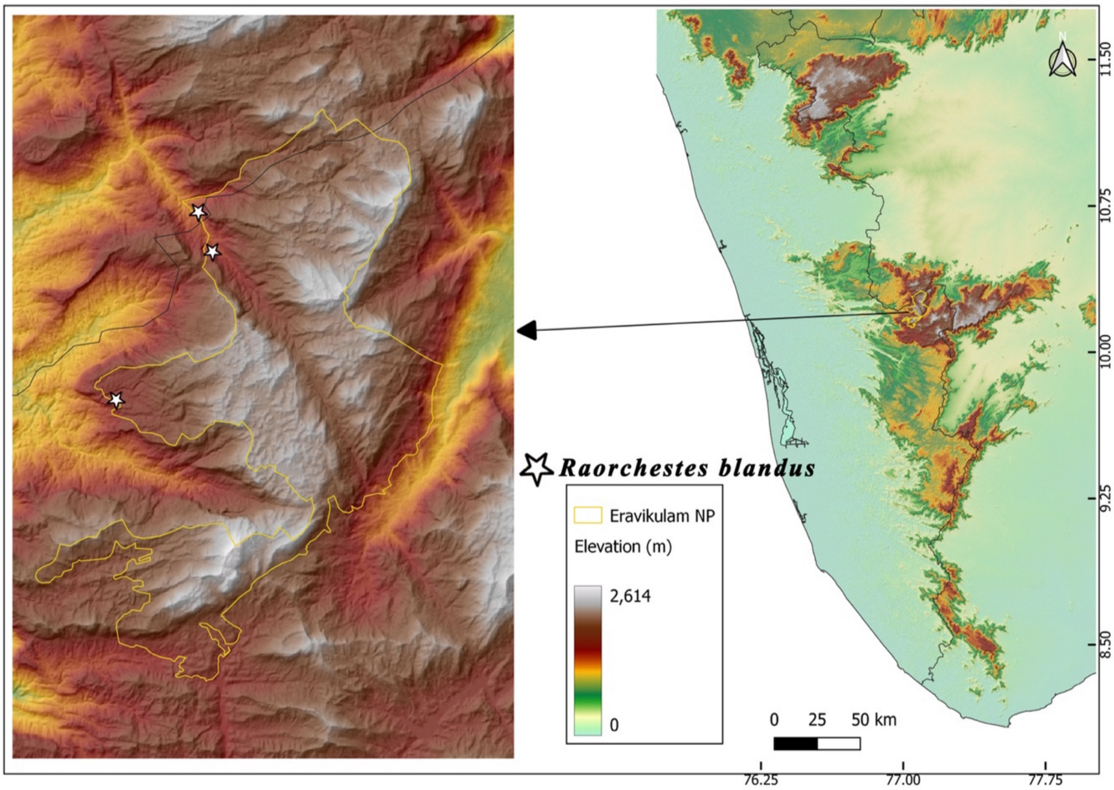

Figure 4. 47: Distribution map of *Raorchestes blandus* inside ENP

22. ***Raorchestes chlorosomma* (Biju & Bossuyt, 2009),** Green-eyed Bush Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Critically Endangered

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from eight locations in ENP at an elevational range of 1400 - 2200 m. They were found inhabiting bushes, shrubs and trees from 1 to 2 m in height from the ground and were only recorded from shola ecosystems

Figure 4. 48: Calling *Raorchestes chlorosomma* male

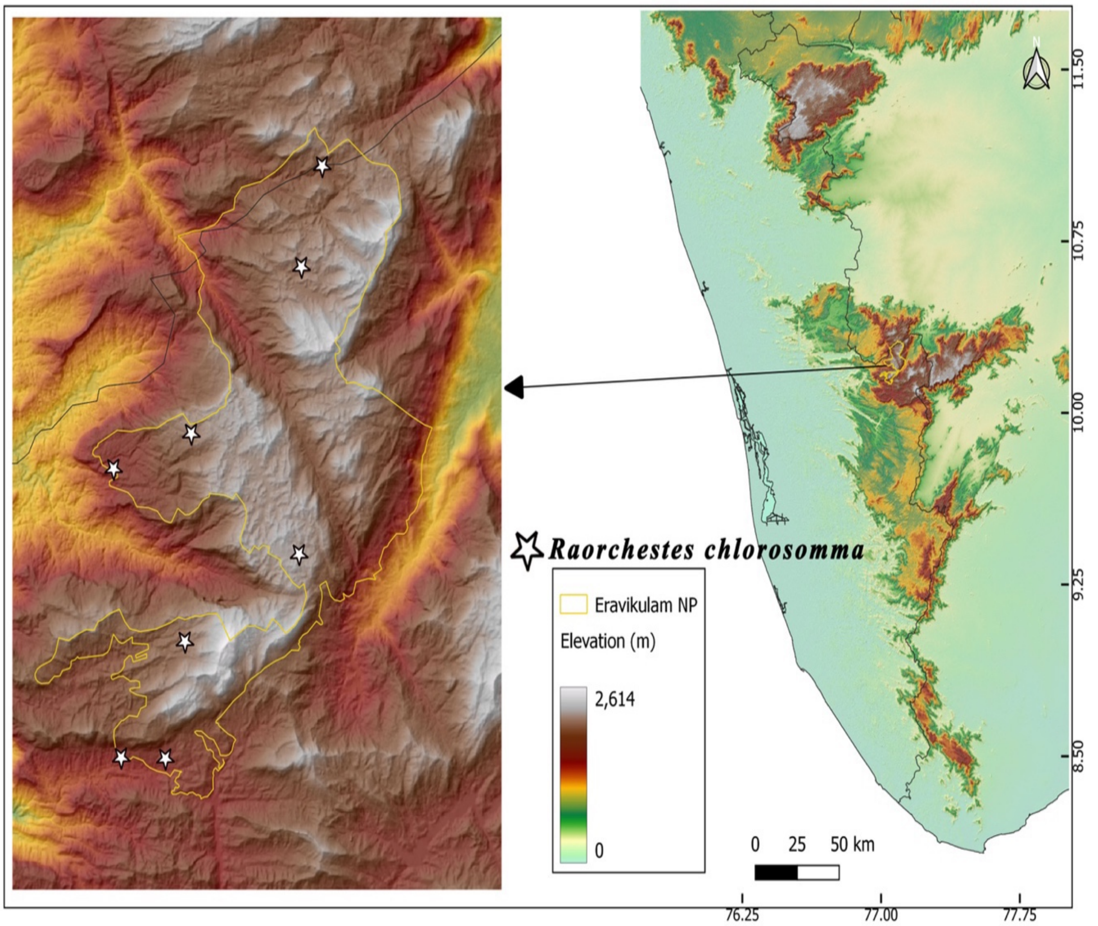

Figure 4. 49: Distribution map of *Raorchestes chlorosomma* inside ENP

23. ***Raorchestes drutaahu* Garg, Suyesh, Das, Bee, and Biju, 2021,** Fast-calling Shrub Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from single location, Thattukaanam of ENP. It was considered as an unidentified species until recently till it was formally described from adjacent Kadalar forests

Figure 4. 50: *Raorchestes drutaahu*

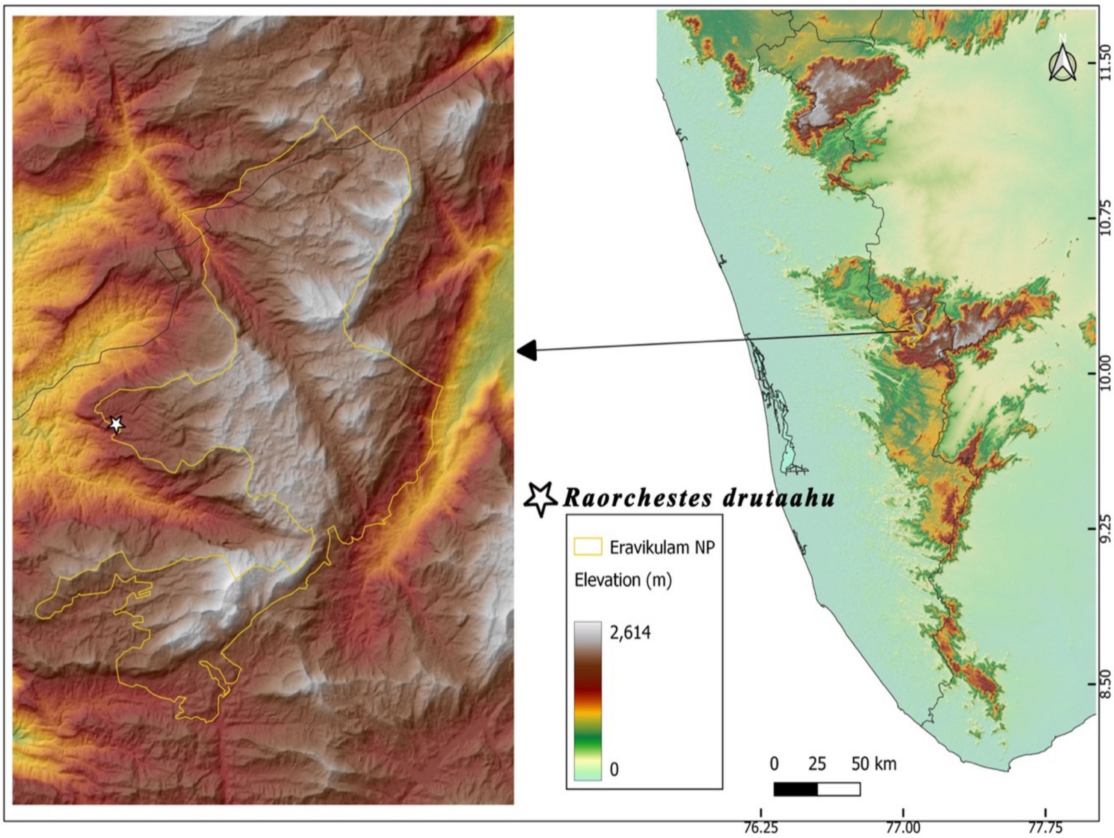

Figure 4. 51: Distribution map of *Raorchestes drutaahu* inside ENP

24. ***Raorchestes dubois* (Biju & Bossuyt, 2006),** Kodaikanal Bush Frog
Order: Anura

Family: Rhacophoridae

IUCN Category: Vulnerable

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from twenty five locations of ENP, *Raorchestes dubois* is the most common species. Even though largely found inhabiting grasslands including Anamudi, a few record were also from shola and its edges (1400 – 2695 m)

Figure 4. 52: *Raorchestes dubois*

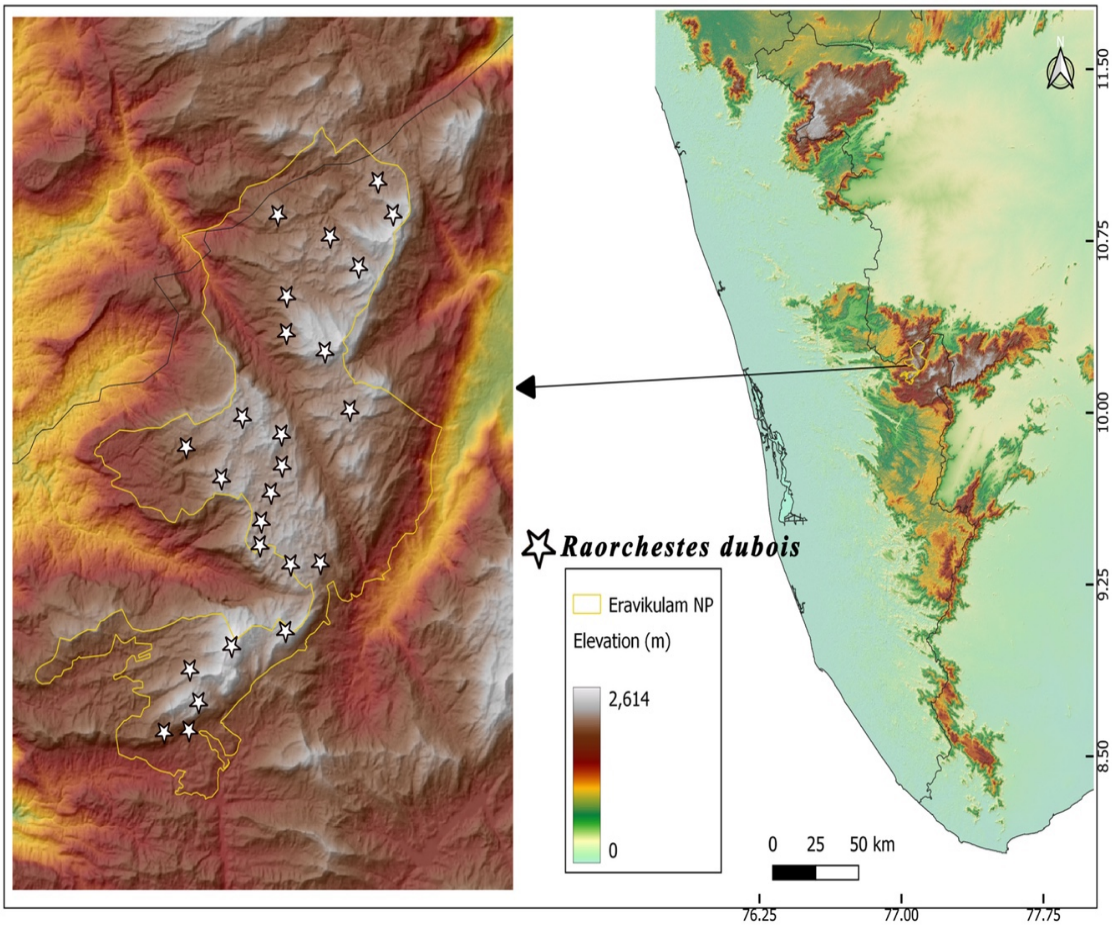

Figure 4. 53: Distribution map of *Raorchestes dubois* inside ENP

25. ***Raorchestes flaviventris* (Boulenger, 1882),** Yellow-bellied Bush Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Data Deficient

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from three locations of ENP (1400-1600). This is the first record of the species from ENP. They were recorded only from the shola forest patches.

Figure 4. 54: Calling *Raorchestes flaviventris* male

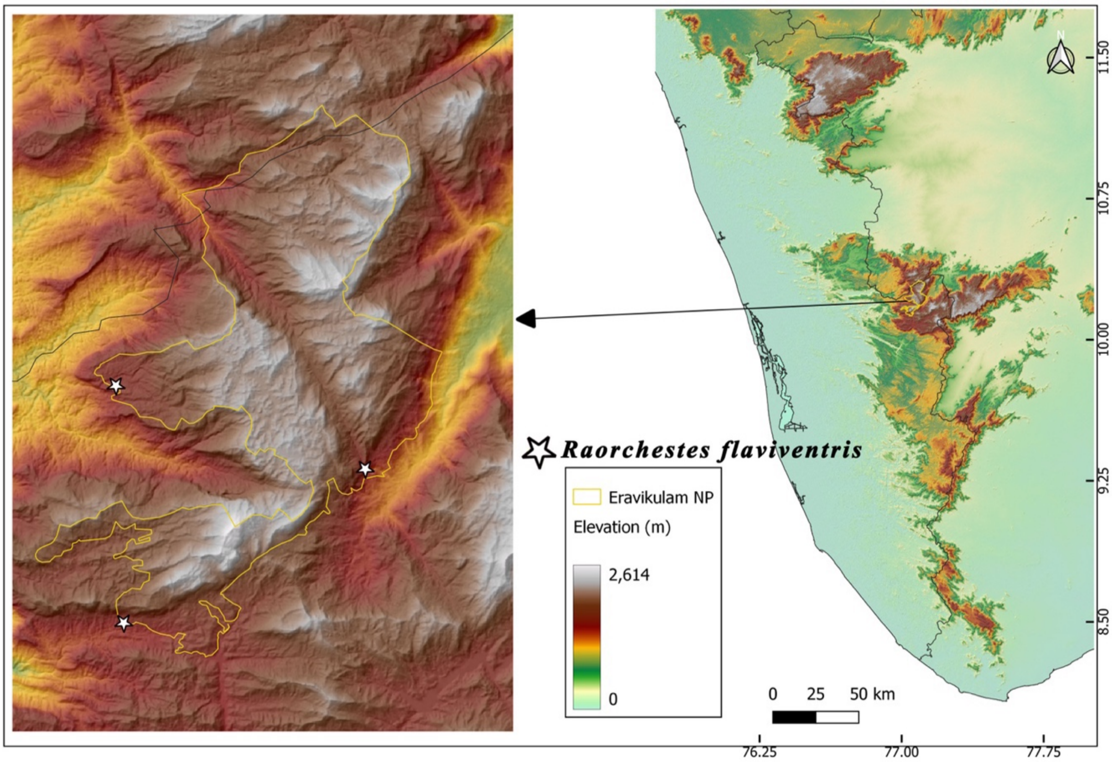

Figure 4. 55: Distribution map of *flaviventris* inside ENP

26. ***Raorchestes griet* (Bossuyt, 2002),** Griet Bush Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Critically Endangered

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from eight shola locations of ENP at an elevational range of 1400 -2000 m. Found mostly at not more than 1.5 m height they inhabit shrubs and grasses in the shola forests and its immediate surroundings

Figure 4. 56: Calling *Raorchestes griet* male

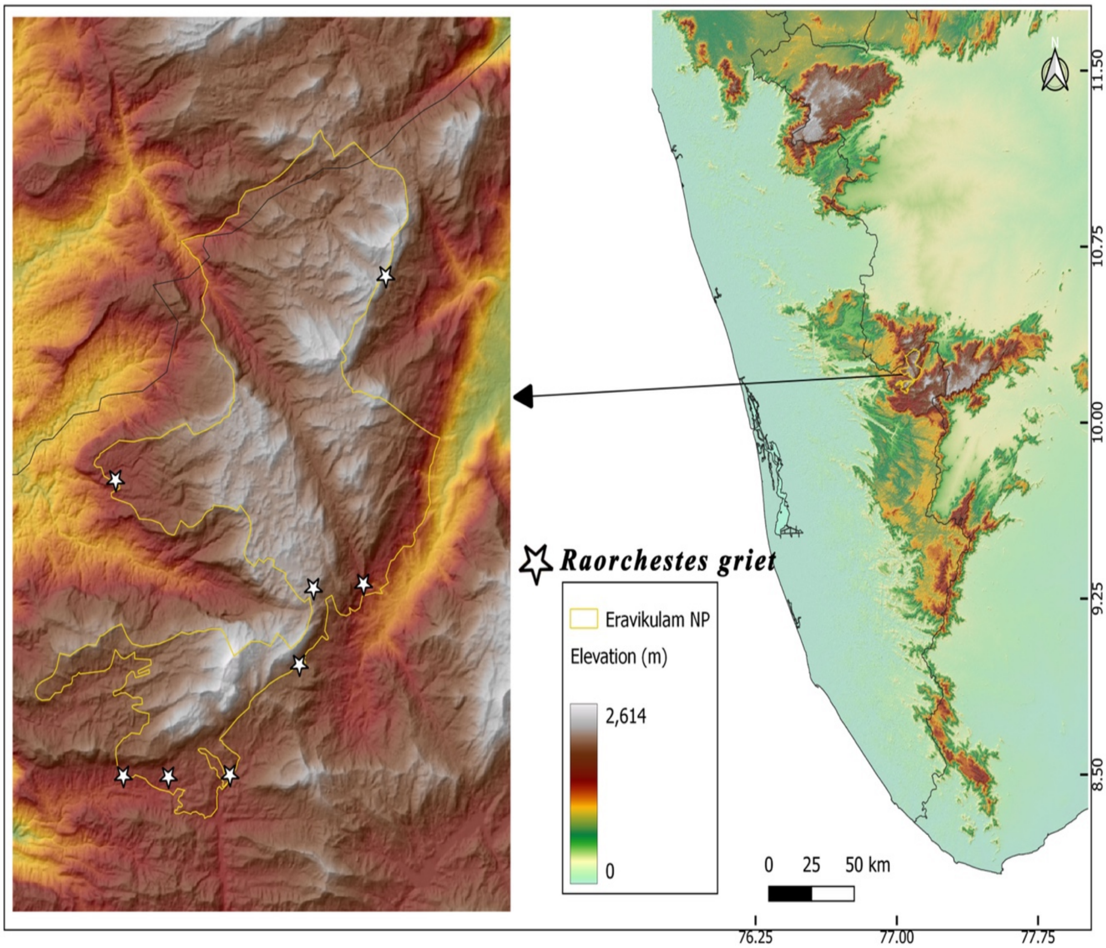

Figure 4. 57: Distribution map of *Raorchestes griet* inside ENP

27. ***Raorchestes jayarami* (Biju & Bossuyt, 2009),** Jayaram's Bush Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: One of the common species in the boundaries and low elevation regions of ENP. Recorded from eight shola locations starting from 1400 m to 2200 m.

Figure 4. 58: Calling *Raorchestes jayarami* male

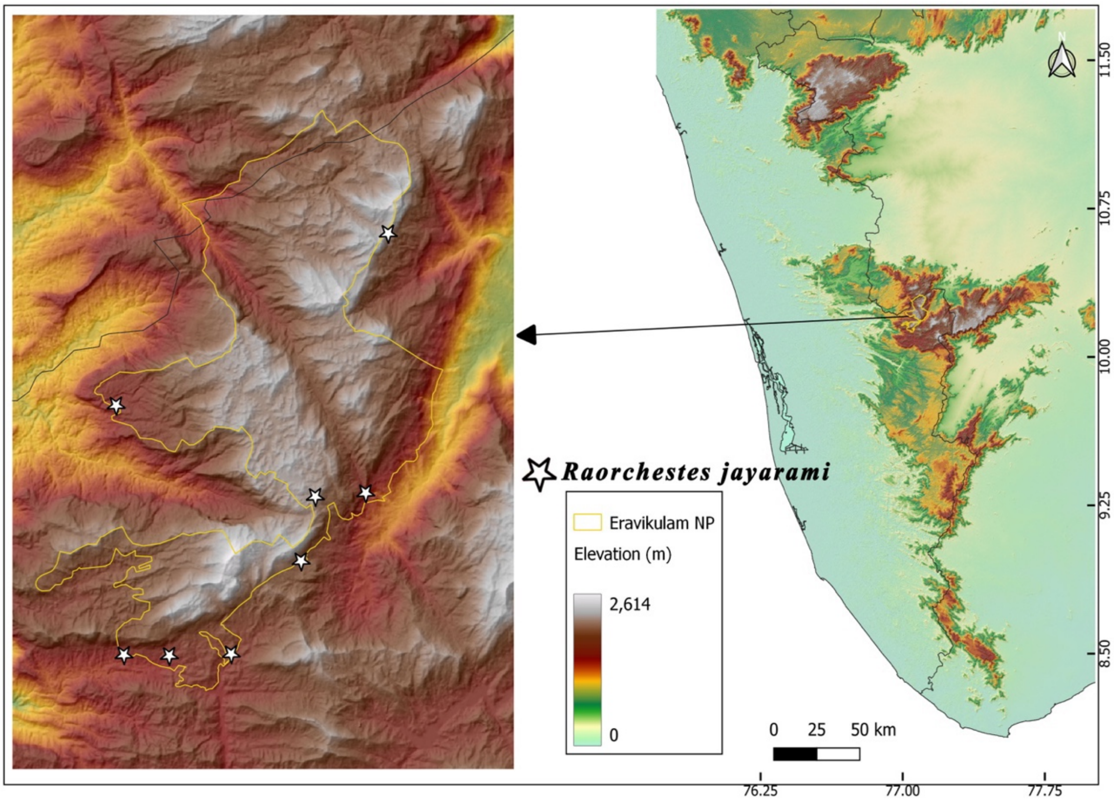

Figure 4. 59: Distribution map of *Raorchestes jayarami* inside ENP

28. ***Raorchestes kadalarensis* Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot, and Kalesh, 2011,** Kadalar Bush Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: First record from ENP. Recorded from fourteen shola locations from 1400 to2000 m

Figure. 4. 60: *Raorchestes kadalarensis*

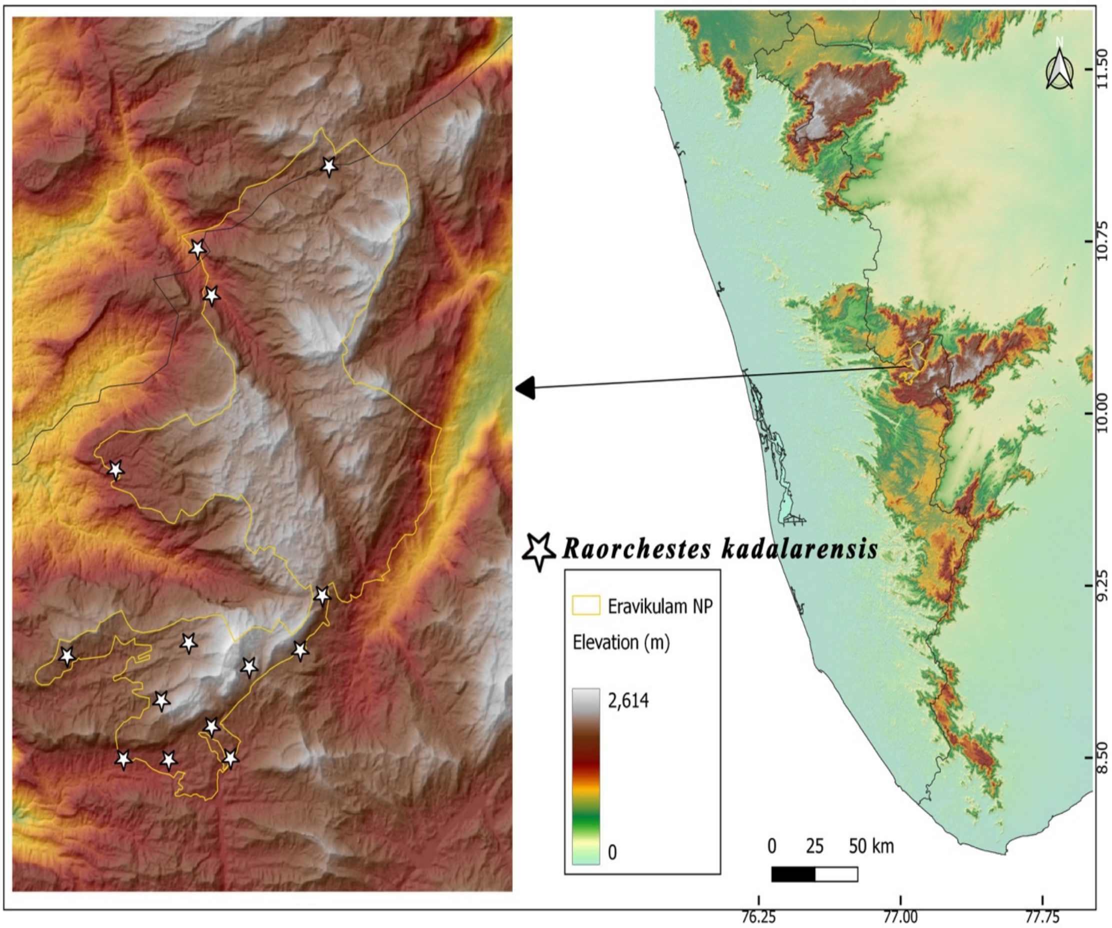

Figure 4. 61: Distribution map of *Raorchestes kadalarensis* inside ENP

29. ***Raorchestes munnarensis* (Biju & Bossuyt, 2009),** Munnar Bush Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Critically Endangered

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from fourteen shola locations of ENP at an elevational range of 1400 - 2200 m. They were reported only from inside shola forests and not from the grasslands.

Figure 4. 62: ***Raorchestes munnarensis***

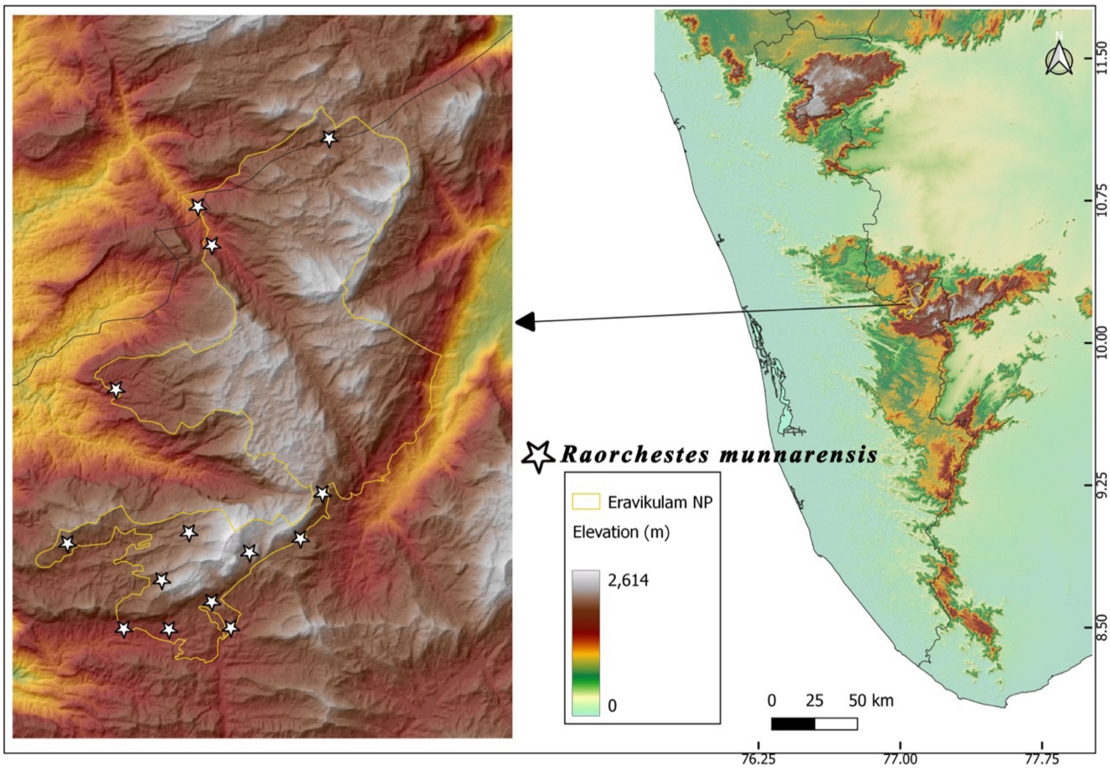

Figure 4. 63: Distribution map of *Raorchestes munnarensis* inside ENP

30. ***Raorchestes keirasabinae*** **Garg, Suyesh, Das, Bee, and Biju, 2021,** Keira's Shrub Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from single shola in Thattukaanam (1400 m) of ENP. This species was considered as *Raorchestes nerostagona* until recently when it was given a separate species status by Garg *et al.,* 2021.

Figure 4. 64: *Raorchestes keirasabinae*

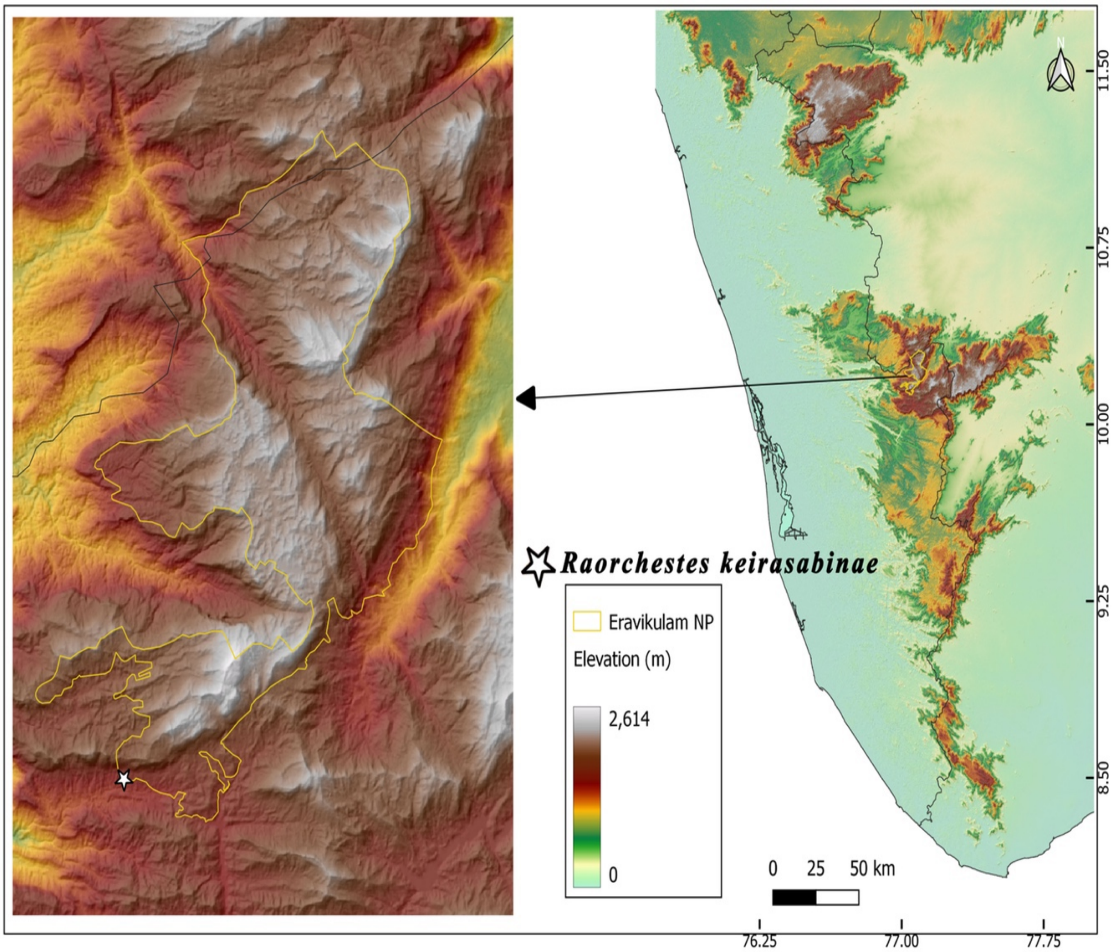

Figure 4. 65: Distribution map of *Raorchestes keirasabinae* inside ENP

31. ***Raorchestes ochlandrae* (Gururaja, Dinesh, Radhakrishnan & Ramachandra, 2007),** Ochlandrae Reed Bush Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Data Deficient

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from reed patches inside two shola locations of ENP at 1400m elevation.

Figure. 4. 66: *Raorchestes ochlandrae*

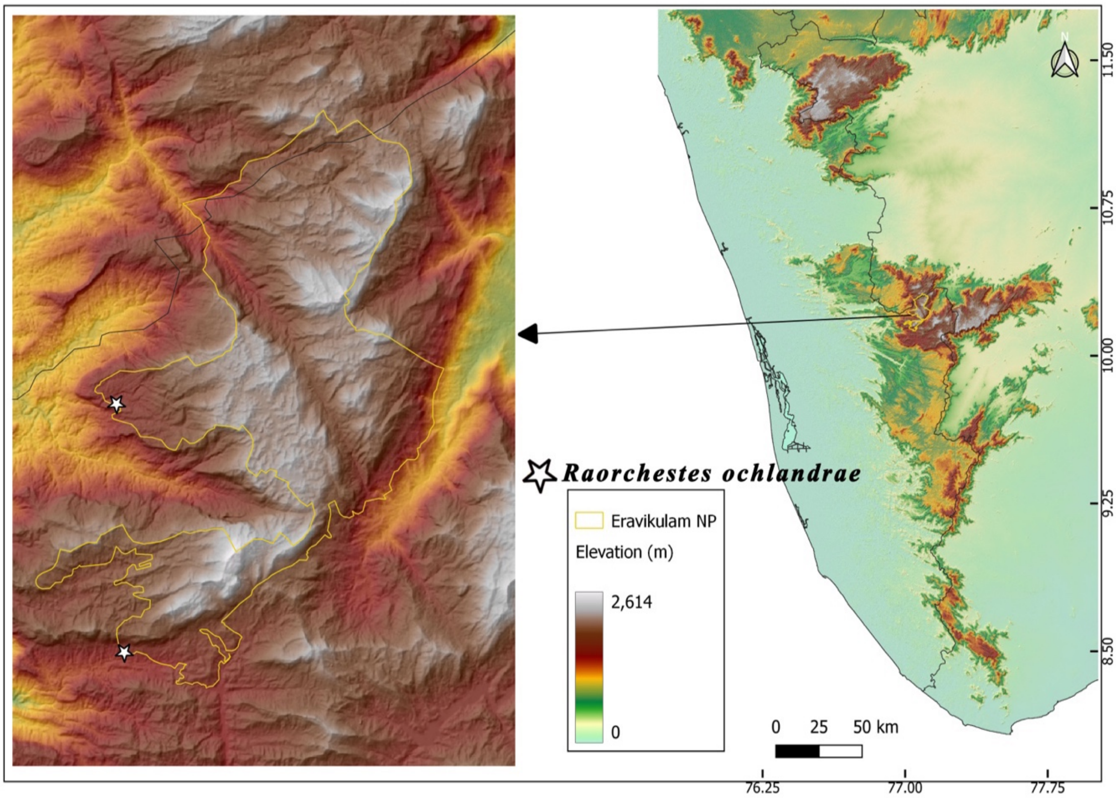

Figure 4. 67: Distribution map of *Raorchestes ochlandrae* inside ENP

32. ***Raorchestes resplendens* Biju, Shouche, Dubois, Dutta, and Bossuyt, 2010,**

Resplendent Shrub Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Critically Endangered

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Initially known only from two locations inside (Anamudi and Poovar) ENP, currently recorded from twenty five locations throughout ENP. They are reported only from the grasslands at an elevational range starting from 1800 m to 2695 m on top of Anamudi summit.

Figure 4. 68: *Raorchestes resplendens*

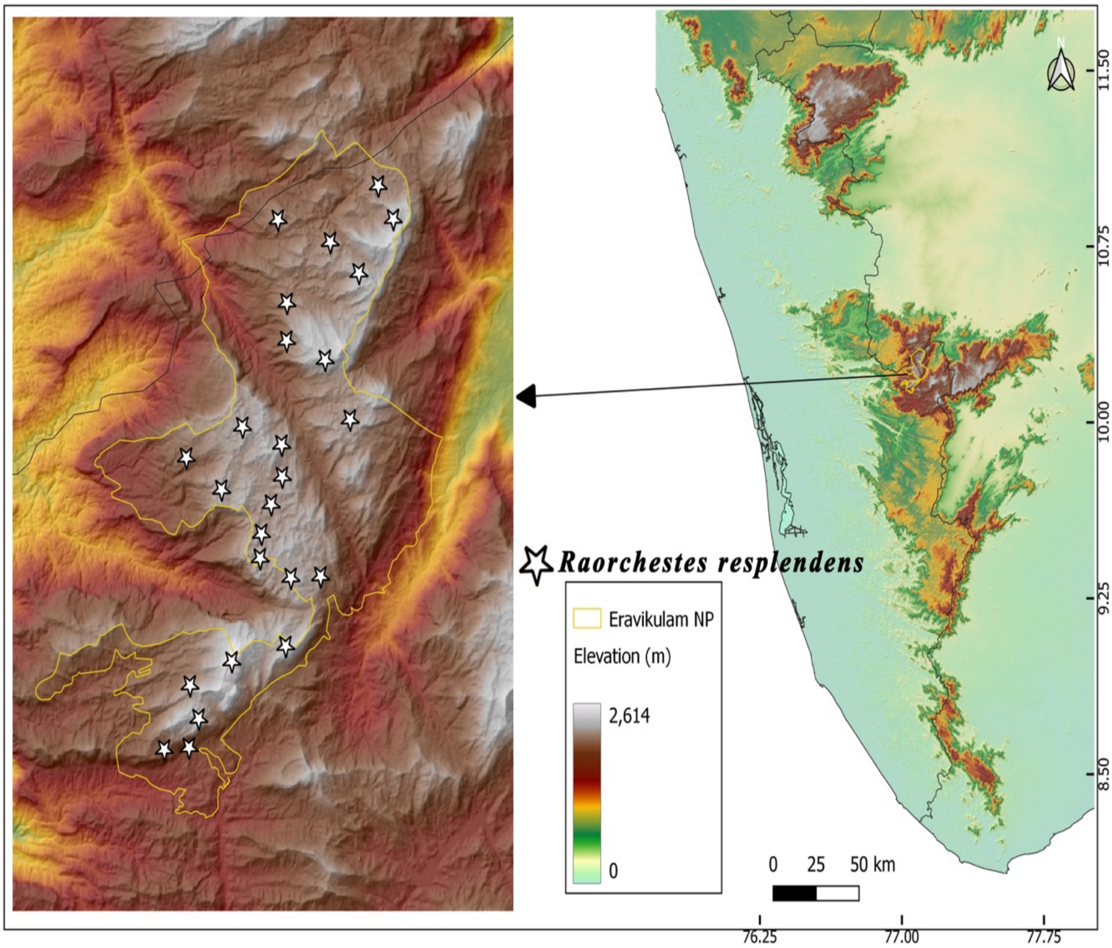

Figure 4. 69: Distribution map of *Raorchestes resplendens* inside ENP

33. ***Raorchestes sushili* (Biju & Bossuyt, 2009),** Sushil's Bush Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Critically Endangered

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from four locations inside shola habitats of ENP ranging from 1400 m to 1600 m.

Figure 4. 70: *Raorchestes sushili*

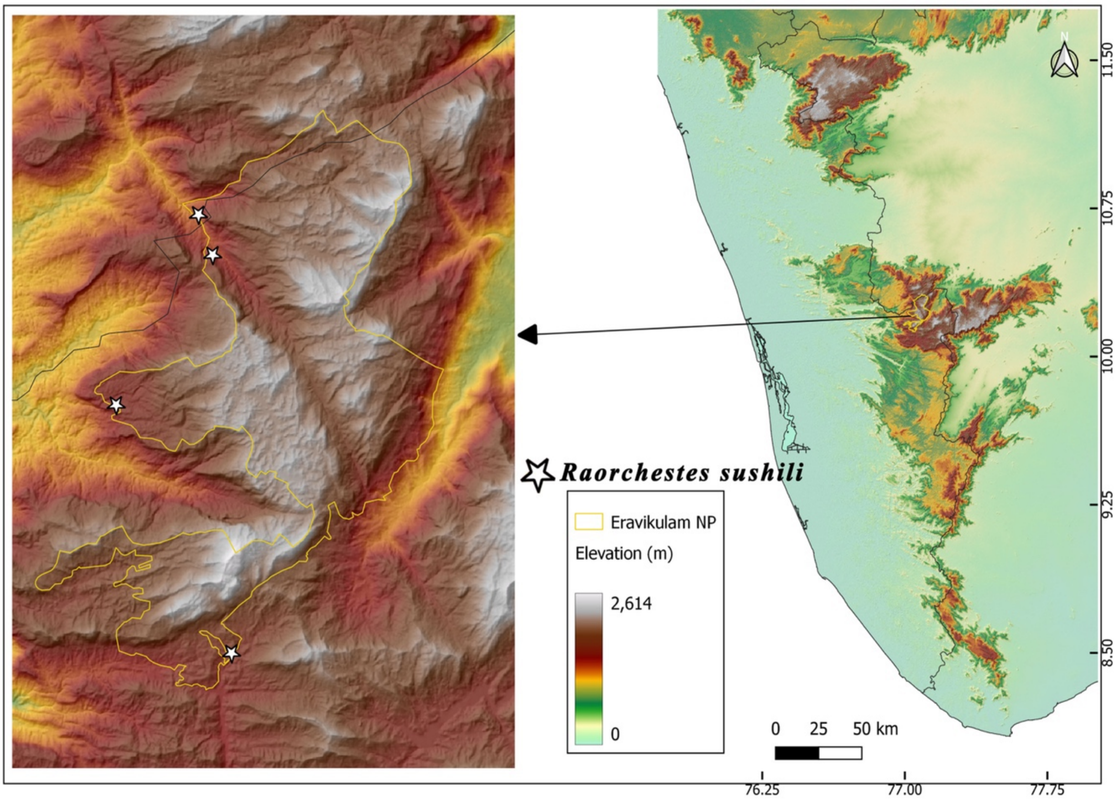

Figure 4. 71: Distribution map of *Raorchestes sushili* inside ENP

34. ***Raorchestes uthamani* Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot, and Kalesh, 2011,** Uthaman's Bush Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Not Evaluated

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded for the first time from a location north of its type locality Gavi. In ENP, they were recorded from two locations inside shola habitats (1400-1600 m).

Figure 4. 72: *Raorchestes uthamani*

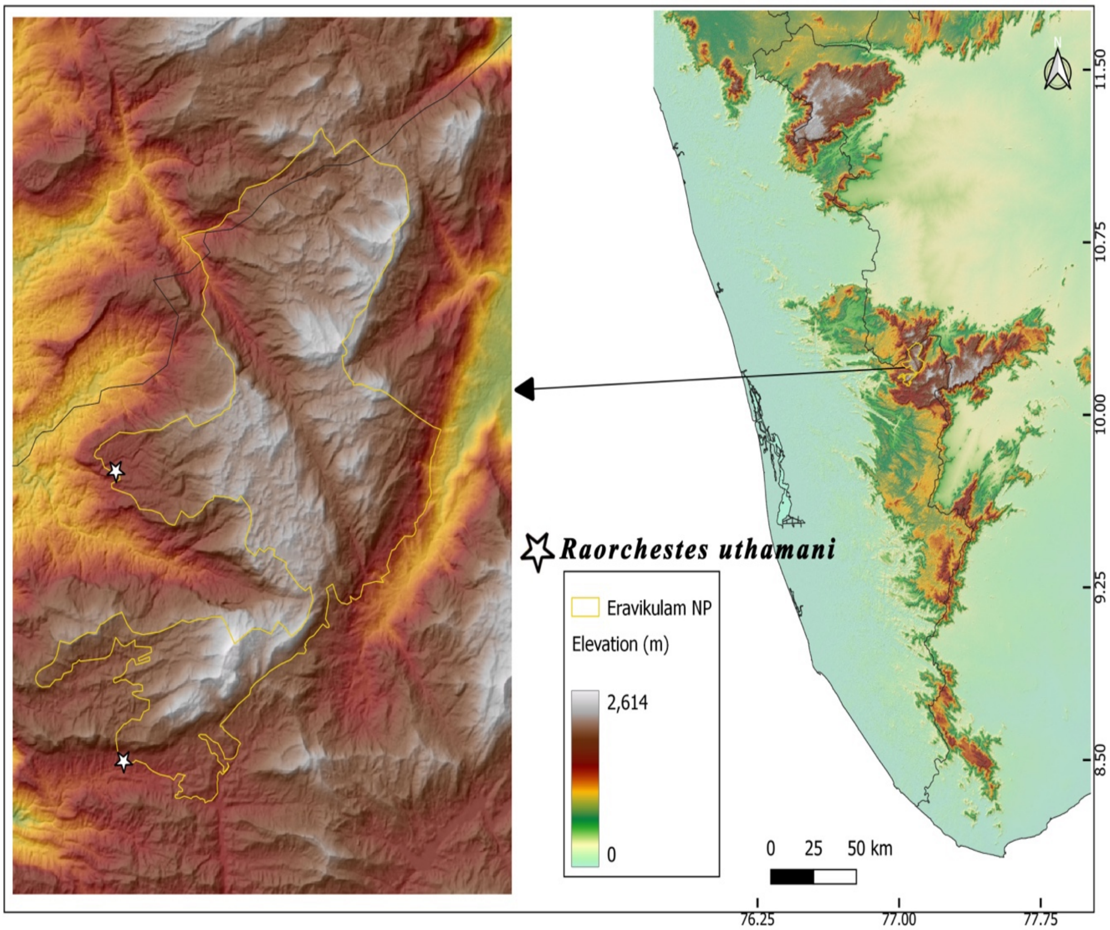

Figure 4. 73: Distribution map of *Raorchestes uthamani* inside ENP

35. ***Rhacorphorus calcadensis* Ahl, 1927,** Kalakkad Tree Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Endangered

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from three locations inside shola habitats of ENP at 1400-1600 m elevations.

Figure 4. 74: ***Rhacorphorus calcadensis***

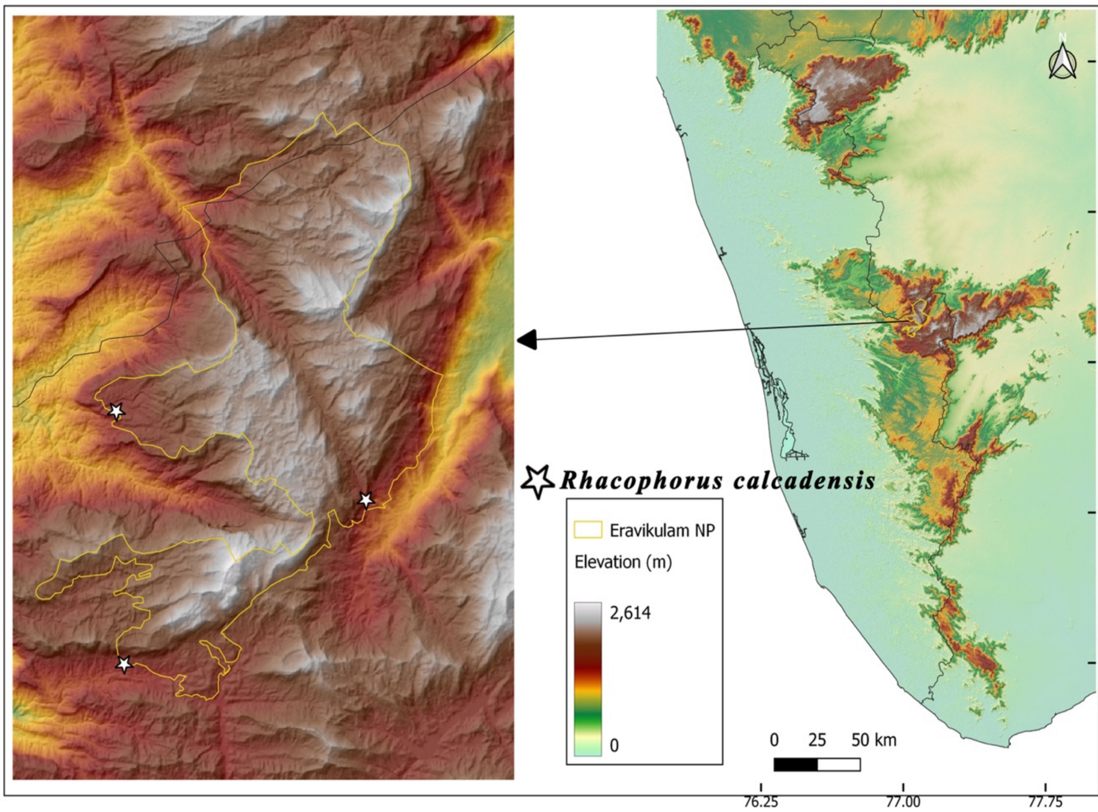

Figure 4. 75: Distribution map of ***Rhacorphorus calcadensis*** **inside ENP**

36. ***Rhacorphorus pseudomalabaricus* Vasudevan & Dutta, 2000,** Anamala Gliding Frog

Order: Anura

Family: Rhacophoridae

IUCN Category: Critically Endangered

Endemism: Endemic to Kerala and Tamil Nadu part of Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: Recorded from eight locations inside ENP starting from 1400m to 2000 m. Found only in the sholas and not from the grasslands.

Figure 4. 76: *Rhacorphorus pseudomalabaricus*

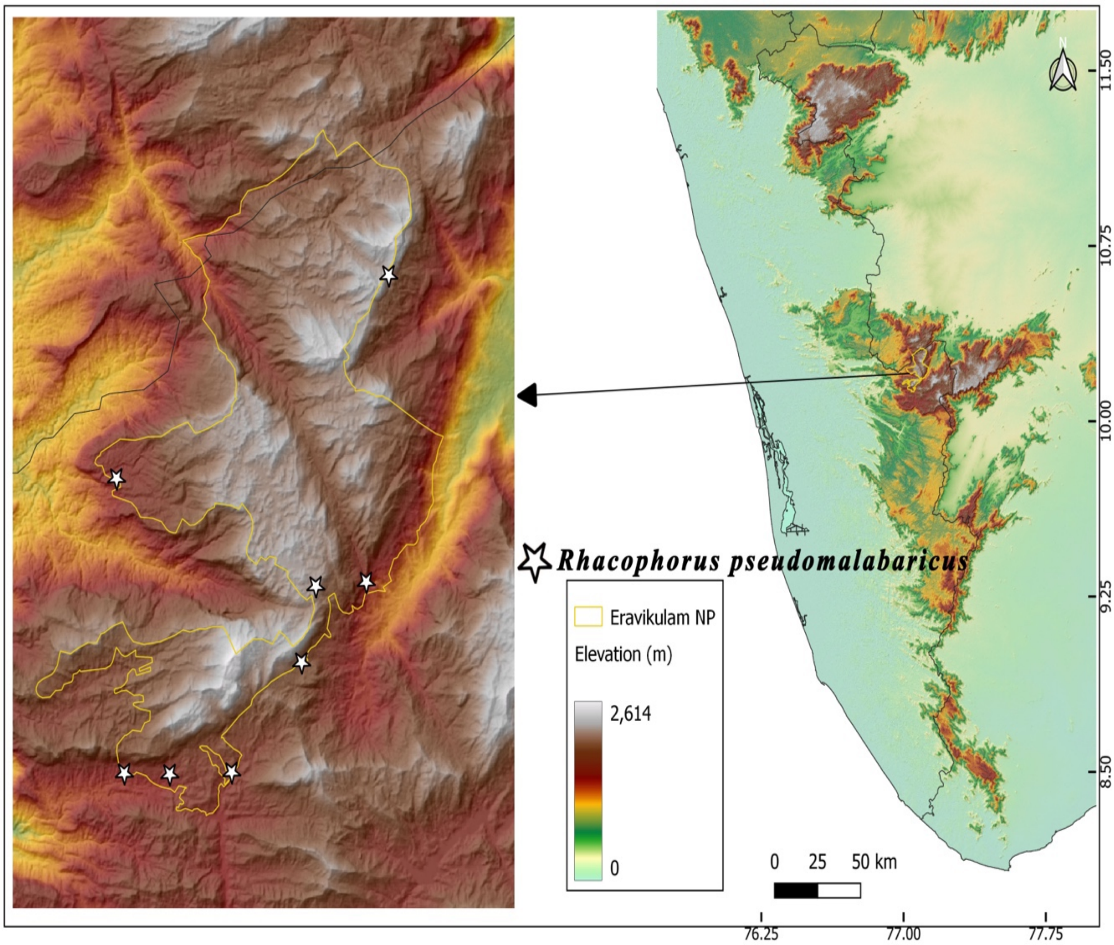

Figure 4. 77: Distribution map of *Rhacorphorus pseudomalabaricus* inside ENP

37. **Uraeotyphlus sp. ,** Caecilian

Order: Gymophiona

Family: Ichtyophidae

IUCN Category: Not Applicable

Endemism: Endemic to Western Ghats

Wildlife Protection Act, 1972: Not listed

Distribution: First ever record of a Caecilian from ENP as well as first record of a *Uraeotyphlus sp.* at 2000 m elevation. Recorded from a single location in ENP close to Chinnar Wildlife Sanctuary.

Figure 4. 78: ***Uraeotyphlus sp.***

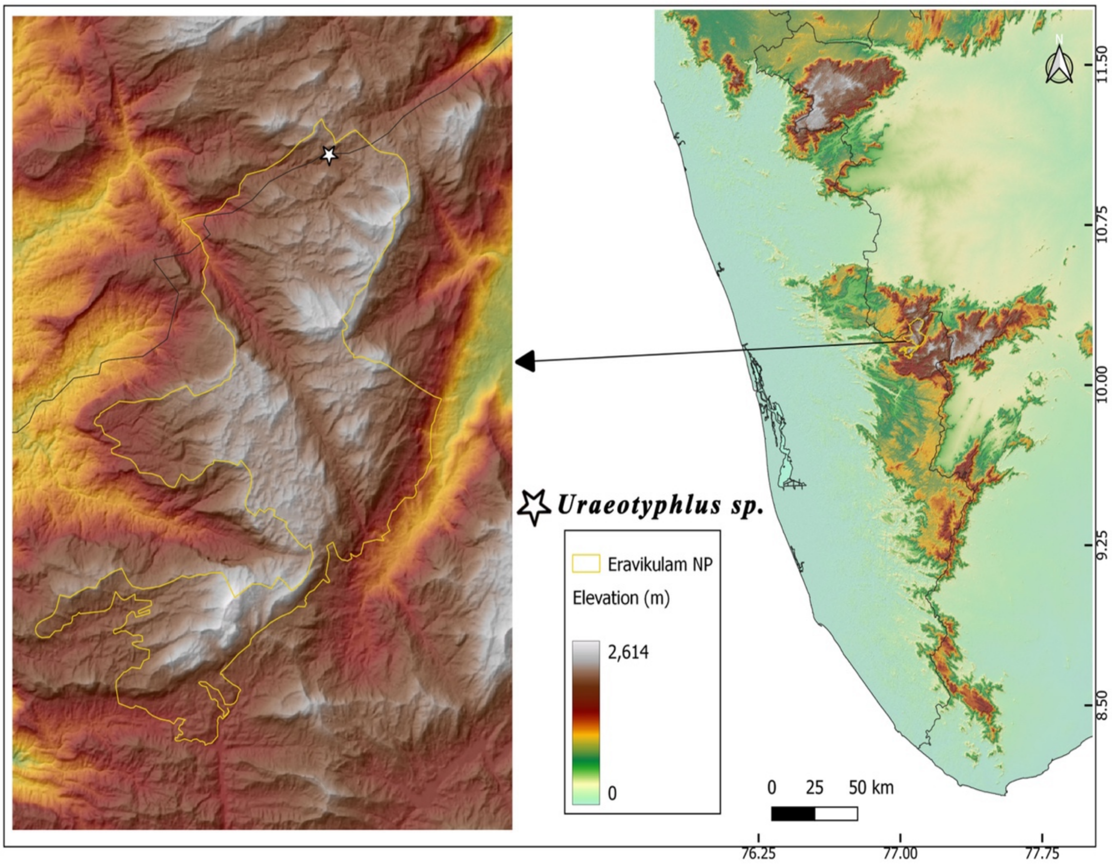

Figure 4. 79: Distribution map of *Uraeotyphlus sp*. inside ENP

4.4.2. Quadrat Survey

Twenty five species were recorded in 51 quadrats employed in shola and grassland ecosystem of Eravikulam National Park. The species accumulation curve became nearly stable and approached an asymptote at 51 surveys. The observed species richness (S Mean – 25) resembled estimated species richness (Chao 1 Mean -25). However there was slight variation in the estimated richness, Jack 1 Mean (27.95) to the mean species richness (Fig. 4.80) .

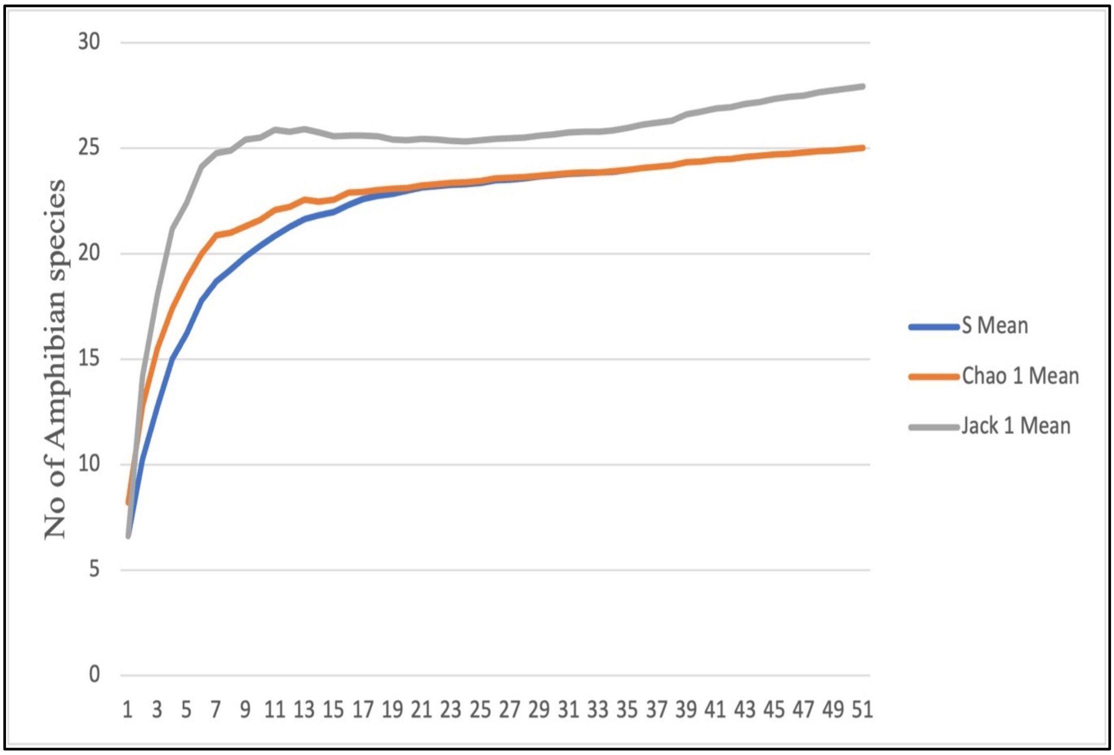

Figure 4. 80: Amphibian species accumulation curve of QS surveys

4.4.3. Visual Encounter Survey

The 101 VES surveys included 51 in the sholas and 50 in the grasslands. Thirty seven species were recorded including 36 from the sholas and 6 from the grasslands (Table 4.2) with five species common in both the habitats. The species accumulation curve reached an asymptote at 101 VES surveys. The mean species richness (S Mean – 37) are congruent to the estimated species richness (Chao 1 Mean -37 and Jack 1 Mean -37.9) indicating sufficient sampling effort. Higher number of species and better resemblance in the observed and estimated species richness lead to use of data collected using VES surveys in further analysis including diversity indices and for testing the diversity between shola and grassland habitats (Fig. 4.80)

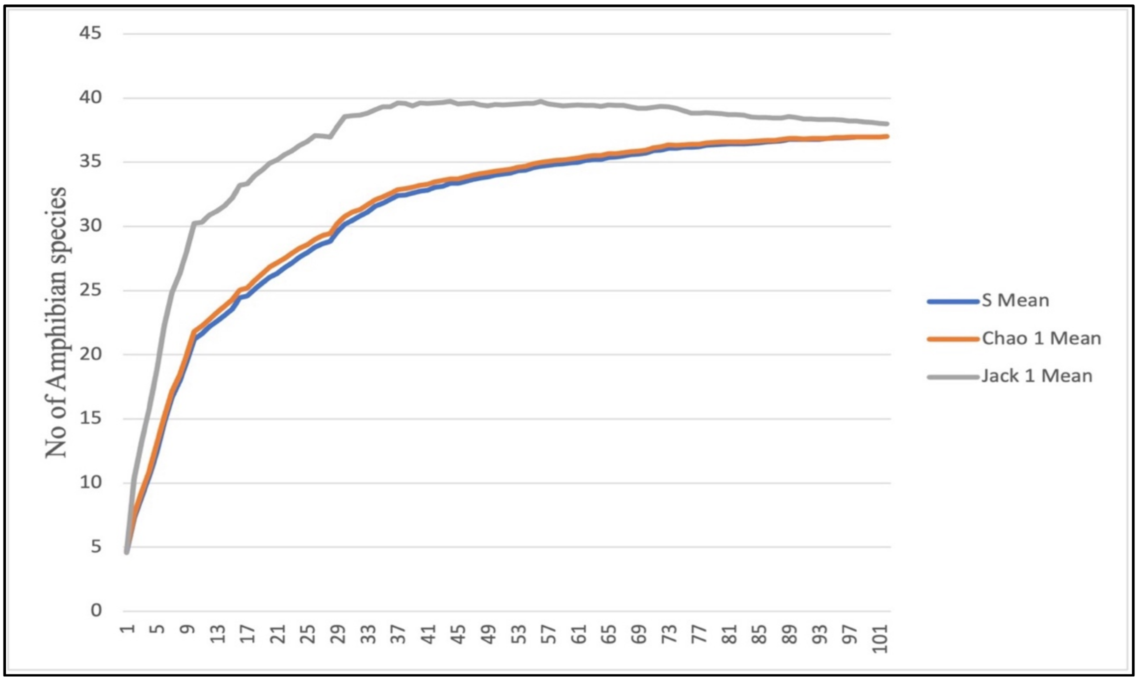

Figure 4. 81: Amphibian species accumulation curve of VES surveys

4.4.4. Species Diversity and abundance

Simpsons, Shannon-Wiener, Fisher's alpha and Margalef diversity indices were calculated for both shola and grassland ecosystems. All indices indicate higher diversity in ENP with shola ecosystem accounting for most of the diversity, whereas grasslands reported comparatively very low diversity (Table

4.2 & 4.3).

A perMANOVA test using Bray-Curtis similarity index was attempted to confirm the results of the diversity t -test. Before conducting the test, the data was relativized by maximum. The results showed that the amphibian diversity in the shola ecosystem and grasslands have a significant difference (p=0.0001)

From 101 VES surveys , 37 species with a total of 3575 individual amphibians were recorded. Shola ecosystem was found to be more diverse than grasslands and whereas grasslands had higher species abundance (1999 individuals) with only 6 species in comparison to the 1596 individuals in shola with 36 species. (Table 4.1)

Table 4. 1: Number of amphibian species recorded (encounter rates as specimens/sampling hour in brackets) in ENP

Species	ENP	Shola	Grasslands
Duttaphrynus melanostictus	10 (0.1)	10 (0.1)	0
Duttaphrynus microtympanum	109 (1.07)	48 (0.94)	61(1.20)
Minervarya brevipalmata	40 (0.4)	40 (0.78)	0
Micrixalus adonis	34 (0.33)	34 (0.67)	0
Micrixalus frigidus	119 (1.17)	119 (2.33)	0
Micrixalus nigraventris	29 (0.28)	29 (0.57)	0
Melanobatrachus indicus	53 (0.52)	53 (1.03)	0
Uperodon montanus	12 (0.12))	12 (0.23)	0
Nasikabatrachus sahyadrensis	9 (0.09)	9 (0.18)	0
Nyctibatrachus acanthodermis	22 (0.22)	22 (0.43)	0
Nyctibatrachus anamallaiensis	132 (1.3)	132 (2.59)	0
Nyctibatrachus deccanensis	205 (2.1))	101 (1.98)	104 (2.03)
Nyctibatrachus poocha	65 (0.64))	65 (1.27)	0
Indosylvirana sreeni	31 (0.30)	31 (0.61)	0
Walkerana leptodactyla	136 (1. 33)	70 (1.37)	65 (1.27)
Walkerana phrynoderma,	6 (0.06)	6 (0.11)	0
Beddomixalus bijui	38 (0.37)	38 (0.74)	0
Ghatixalus asterops	51 (0.5)	51 (0.1)	0
Ghatixalus magnus	11 (0.11)	11 (0.22)	0
Raorchestes drutaahu	8 (0.08)	8 (0.16)	0
Raorchestes beddomii	66 (0.65)	35 (0.69)	31 (0.61)
Raorchestes blandus	3 (0.03)	3 (0.06)	0
Raorchestes chlorosomma	30 (0. 30)	30 (0.59)	0

Raorchestes dubois	1299 (12.74)	212 (4.16)	1087 (21.31)
Raorchestes flaviventris	7 (0.07)	7 (0.14)	0
Raorchestes griet	44 (0.43)	44 (0.86)	0
Raorchestes jayarami	119 (01.17)	119 (2.33)	0
Raorchestes kadalarensis	60 (0.59)	60 (1.18)	0
Raorchestes munnarensis	82 (0.80)	82 (1.61)	0
Raorchestes keirasabinae	5 (0.05)	5 (0.10)	0
Raorchestes ochlandrae	11 (0.11)	11 (0.21)	0
Raorchestes resplendens	651 (06.38)	0	651 (12.76)
Raorchestes sushili	16 (0.16)	16 (0.31)	0
Raorchestes uthamani	4 (0.04)	4 (0.08)	0
Rhacophorus calcadensis	22 (0. 22)	22 (0.43)	0
Rhacophorus pseudomalabaricus	35 (0.34)	35 (0.69)	0
Uraeotyphlus sp.	1 (0.02)	1 (0.1)	0

Table 4. 2: Diversity indices of amphibians of ENP

	Eravikulam National Park	
	Shola	Grassland
No. of amphibian species	36	6
Individuals	1576	1999
Simpson_1-D	0.943	0.5933
Shannon_H	3.142	1.133
Margalef	4.754	0.6579
Fisher_alpha	6.563	0.7621

Table 4. 3: Amphibians species recorded from Shola and grasslands of ENP

		Habitats	
Sl. No	**Species**	**Shola**	**Grassland**
1	*Duttaphrynus melanostictus*	✓	
2	*Duttaphrynus microtympanum*	✓	✓
3	*Minervarya brevipalmata*	✓	
4	*Micrixalus adonis*	✓	
5	*Micrixalus frigidus*	✓	
6	*Micrixalus nigraventris*	✓	
7	*Melanobatrachus indicus*	✓	
8	*Uperodon montanus*	✓	

9	*Nasikabatrachus sahyadrensis*	✓	
10	*Nyctibatrachus acanthodermis*	✓	
11	*Nyctibatrachus anamallaiensis*	✓	
12	*Nyctibatrachus deccanensis*	✓	✓
13	*Nyctibatrachus poocha*	✓	
14	*Indosylvirana sreeni*	✓	
15	*Walkerana leptodactyla*	✓	✓
16	*Walkerana phrynoderma*	✓	
17	*Beddomixalus bijui*	✓	
18	*Ghatixalus asterops*	✓	
19	*Ghatixalus magnus*	✓	
20	*Raorchestes drutaahu*	✓	
21	*Raorchestes beddomii*	✓	✓
22	*Raorchestes blandus*	✓	
23	*Raorchestes chlorosomma*	✓	
24	*Raorchestes dubois*	✓	✓
25	*Raorchestes flaviventris*	✓	
26	*Raorchestes griet*	✓	
27	*Raorchestes jayarami*	✓	
28	*Raorchestes kadalarensis*	✓	
29	*Raorchestes munnarensis*	✓	
30	*Raorchestes keirasabinae*	✓	
31	*Raorchestes ochlandrae*	✓	
32	*Raorchestes resplendens*		✓
33	*Raorchestes sushili*	✓	
34	*Raorchestes uthamani*	✓	
35	*Rhacophorus calcadensis*	✓	
36	*Rhacophorus pseudomalabaricus*	✓	
37	*Uraeotyphlus sp.*	✓	_

4.4.5. Threatened and Endemic amphibians

Large proportion of the species recorded in the study are not assessed even after very long period since their descriptions. Seventeen out of the total 37 species are not assessed or not evaluated (NE) as per IUCN. Seven species, *viz. Walkerana phrynoderma, Raorchestes chlorosomma, Raorchestes griet, Raorchestes munnarensis, Raorchestes resplendens, Raorchestes sushili* and *Rhacophorus pseudomalabaricus* falls in the most threatened category, Critically Endangered. *Melanobatrachus indicus, Nasikabatrachus* *sahyadrensis, Walkerana leptodactyla* and *Rhacophorus calcadensis* are in the Endangered category and three *viz. Duttaphrynus microtympanum, Nyctibatrachus deccanenesis* and *Raorchestes dubois* are in Vulnerable category. Among the rest, three species belong to Data Deficient, 2 in Near threatened and a single species in the Least Concern Category.

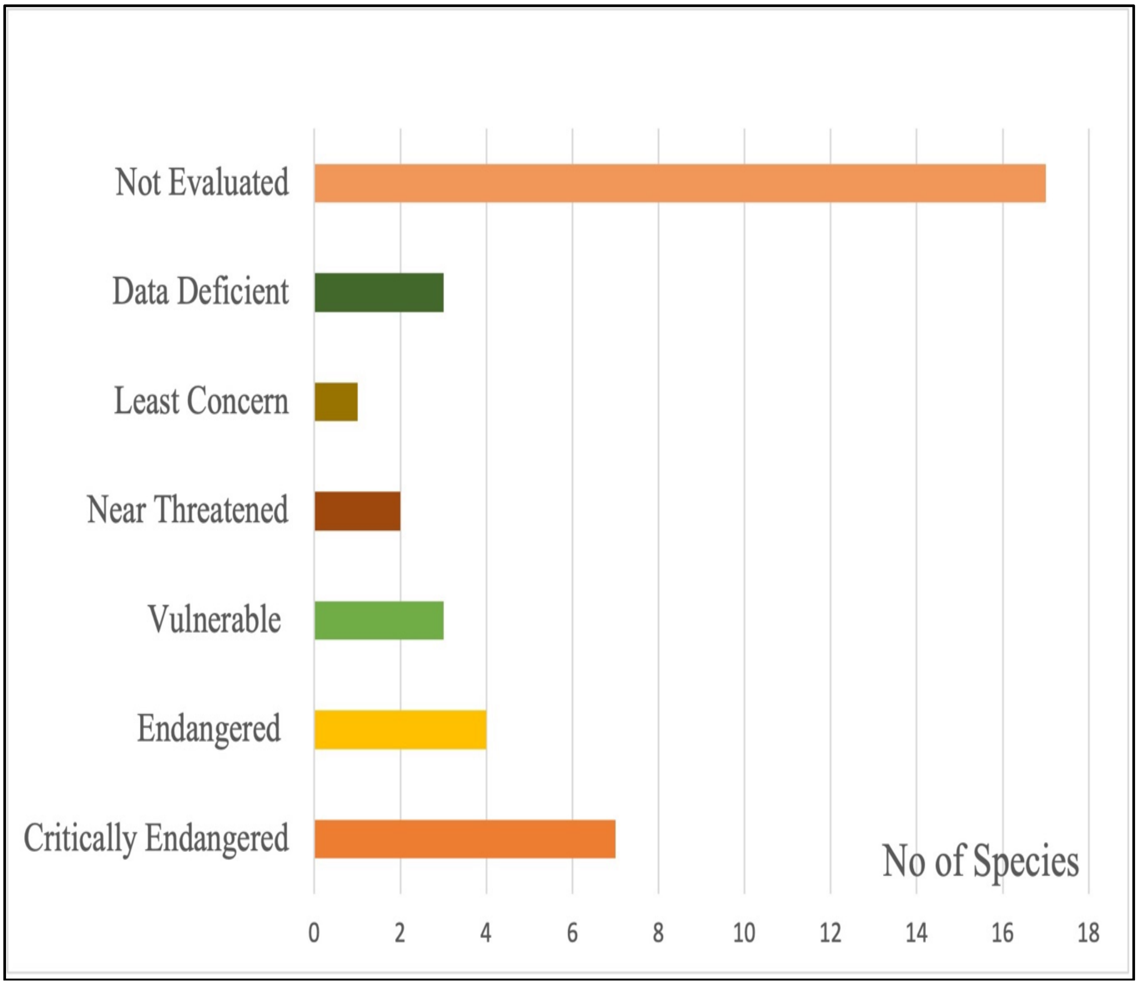

Figure 4. 82: Amphibians in different threatened categories

When it comes to endemism, 97% (36 sp.) of the total 37 species reported from ENP are endemic to Western Ghats. Except for a single species, *Duttaphrynus melanostictus* all the other species recorded are endemic to southern Western Ghats.

4.4.6. Molecular Phlyogenetics

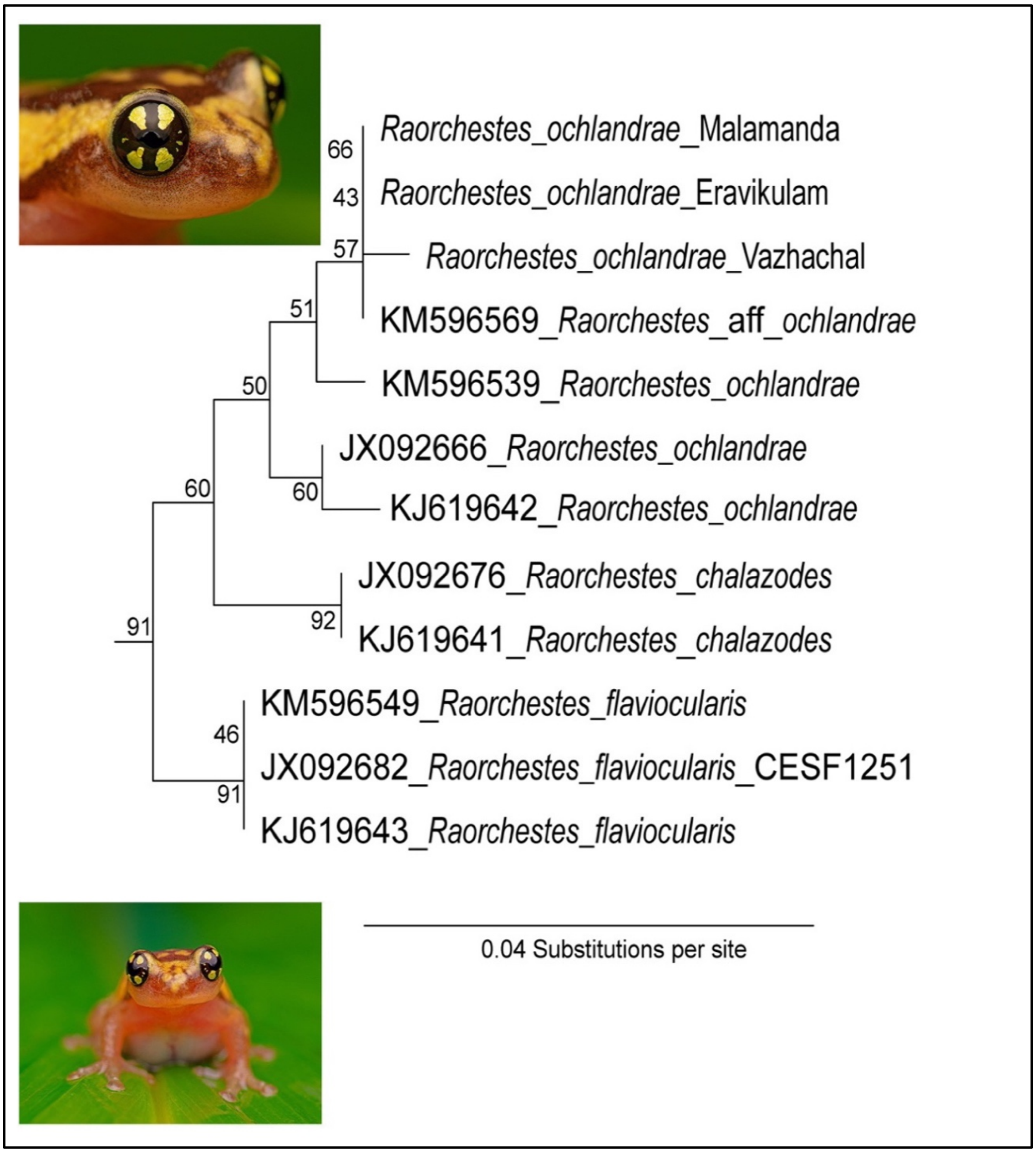

Figure 4. 83: Phylogenetic tree showing relationships among Ochlandrae clade

Raorchestes ochlandrae was known from its type locality, Malabar Wildlife Sanctuary. Recently, there had been several records of the species from localities south of Palghat gap. Our surveys also found the species inside ENP and to confirm the identity of the species we conducted a molecular (ML) study. In our ML analysis, the newly generated samples of *R. ochalndrae* from different locations including the sample from Eravikulam National Park, nests well within the ochlandrae clade (Fig. 4.83). Samples from the north and south of Palghat gap form a separate cluster in the phylogeny. However, this can be attributed to the geographical separation among these populations. The uncorrected genetic pairwise distance between the samples of *R. ochlandrae* ranges between 0.2% and 0.8% in the mitochondrial 16S gene, showing an intraspecific variation owing to the geographic isolation.

4.5. Discussion

Thirty seven species of amphibians were recorded from Eravikulam National Park. The species accumulation curves plotted based on our VES data suggests maximum effort and a result in concordance with the estimated species richness. In general, the temperature, water, energy requirements, habitat heterogeneity and ecological history are considered the key factors that drive the gradients of global species diversity and distribution (Hawkins *et al*., 2003; Currie *et al*., 2004). Amphibians are ectothermic vertebrates that are highly dependent on temperature and water (Wells, 2010) and hence their diversity levels are largely governed by water and temperature coupled with the regional history (Buckley and Jetz, 2007). Among the shola and grassland ecosystems in ENP, shola recorded more number of species, which can be attributed to the higher levels of habitat heterogeneity and the temperature conditions prevailing. Sholas are known to have high floristic diversity (Jose *et al.,* 1994), which explains the diverse microhabitat presence and the temperature conditions in shola forests are also ideal with micro-climates well within 31^0c to 16^0c supporting amphibian populations (Thomas and Sankar, 2001).

Studies suggest that the grasslands and the sholas have a long evolutionary history and the monsoon climate conditions played a key role in shaping it (Thomas and Palmer, 2007). Both these ecosystems are considered as ecological climaxes with well-defined and contrasting microclimates (Jose *et al.,* 1994). Shola ecosystem provides wet climate regime as well as wide range of niche for the highly microhabitat specific amphibians whereas grasslands are comparatively less diverse. This habitat heterogeneity in turn supports amphibians with space, food resources and necessary environmental parameters for their survival. Vegetation inside shola are majorly tropical origin whereas shola boundaries and grasslands are more of sub-tropical to temperate origins (Meher-Homji, 1967). Grassland habitats of Eravikulam comprise 60% of the total area of the park and that alone explains the higher amphibian abundance with less diversity (6 species). Similar situations of low amphibian species diversity with high abundance is known from humid subtropical lowland forest of southern Japan (Watanabe *et al.,* 2005) and the difference is generally attributed to the composition of the animal community and the difference in the functionality of the total system (Scott, 1976), in this case the grasslands. Also the probability of detecting amphibian species in rather monotonous grassland niche is higher owing to greater visibility during the VES.

Among the amphibian families recorded, Rhacophoridae represented by four genera accounts for 20 out of 37 species recorded. The family comprises of species that utilize an arboreal niche for their survival and the shola ecosystem with lot of similar microhabitats support the same. Globally, the family has 445 species under two subfamilies Buergeriinae (6 sp.) and Rhacophorinae (440 sp.) distributed widely in the southern Asia and sub Saharan Africa. The genus *Raorchestes,* commonly called as Bush Frogs or Shrub frogs are direct developing frogs without free swimming tadpoles and 15 out of the total 20 species recorded from the family belongs to this group (Biju *et al.,* 2010). As the common name denotes, they are adapted to live in shrubs, bushes and trees.

Such preference in habitats helps them in diversifying areas like ENP with enough microhabitats.

The critically endangered *Raorchestes resplendens* are restricted to the montane grassland ecosystems of ENP (Das *et al.,* 2020). The species was thought to be present only in two locations, Anamudi and Poovar within the park. But our surveys found them from 25 locations throughout ENP and its adjacent areas in Munnar. ENP is the largest contiguous habitat and one of the three protected areas where this endemic species is found. Legs that are smaller compared to their sister species of the shrub frog of the genus *Raorchestes* is an adaptation to their largely terrestrial life. This is reflected in their preference for the grassland ecosystem of ENP and it proves the uniqueness of the habitat.

Similar to *Raorchestes resplendens,* the present study reports several new distributional records as well as new elevational records for species from ENP. An interesting record is the encounter of a single tadpole of *Nasikabatrachus sahyadrensis*, an Evolutionarily Distinct and Globally Endangered Species (EDGE), which is a new distribution record and its highest elevational record (1414m). However, no adults were recorded during the study. It is assumed that the tadpoles might have migrated upstream from its breeding site, which is contiguous with the stream habitat. Records of *Melanobatrachus indicus* from shola forests of ENP that are contiguous to montane tropical evergreen forests provides new information about the distribution of the EDGE species. ENP is the second National Park after Mathikettan Shola National Park in Kerala from where the species is reported and it adds to the conservation value of the species. Previous studies have reported them only from a maximum of 1700 m (Palot and Sureshan, 2015) whereas the current study have reports at elevational range1800 m – 2000 m. Likewise, records of recently described *Raorchestes drutaahu,* canopy specialist *Raorchestes keirasabinae, Raorchestes blandus,* *Raorchestes flaviventris* and reed specialist *Raorchestes uthamani* are new to the list of amphibians of the park.

Report of the species *Raorchestes ochlandrae* from ENP is a new distributional record and elevational record (1400 -1600 m) for the species south of its type locality, Malabar Wildlife Sanctuary (Gururaja *et al.,* 2007). Current record, far away from its type locality was investigated and confirmed using molecular techniques especially since the group is one of the most diversified amphibian groups in India. Even though geographically separated by the Palghat gap, the species still nests with the *Raochestes ochlandrae.* Single records of *Nasikabatrachus sahyadrensis* and *Uraeotyphlus sp.* could be attributed to their fossorial life- history trait which in turn effect the detection of the species (Biju and Bossuyt, 2003; Wells, 2010)

Munnar landscape and its pristine grasslands has undergone large scale habitat destruction in the form of monoculture plantations and large-scale burning practice since the British rule (Chandran, 1997; Easa *et al,* 2010; Sandeep *et al,* 2019) as well as recent changes mainly due to tourism activities. Coupled with these threats comes global warming which will lead to serious consequences to the grassland and shola ecosystem and its inhabitants (Sukumar *et al.,*1993). Long term studies are required to understand more about the ecology and behaviour of the species which are currently unknown. This calls for baseline information to start with. With diverse microhabitats and its unique climate regime, ENP supports high diversity, abundance and high degree of endemism (97%). At a time when increasing human pressure is also taking its toll on the ecosystem, knowledge on the diversity and distribution of the most threatened species groups like amphibians is crucial in conservation.

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Chapter V

STUDY OF TEMPORAL ACTIVITY PATTERN IN VOCALISATION OF BUSH FROGS OF ERAVIKULAM NATIONAL PARK

STUDY OF TEMPORAL ACTIVITY PATTERN IN VOCALISATION OF BUSH FROGS OF ERAVIKULAM NATIONAL PARK

5.1. Introduction

Darwin was one of the first who allocated a sense of beauty to animals like that of humans that was seldom looked at until the 20th century (Cronin, 1992). Even though discussion on amphibian mate selection was infinitesimal in his book, Darwin did mention about the fight among the male frogs for potential mates and proposed sexual selection as a probable evolutionary reason in the formation of calls in frogs (Darwin, 1871 and 1874). Twentieth century witnessed several studies using anurans as model organisms in testing hypothesis about mate choice and sexual selection (Noble and Noble, 1923; Noble and Aronson, 1942; Emlen, 1968 and 1976; Heusser, 1969; Wiewandt, 1969; Wells, 1977 and 1978; Howard, 1978; Fellers, 1979a and b; Arak, 1983; Ryan, 1985 and 1991; Gerhardt, 1988 and 1994; Halliday and Tejedo, 1995; Sullivan, *et al,* 1995; Grafe *et al.,* 1999; Gerhardt and Huber, 2002). Sexual selection encompass the disposition of behavioural strategies employed by individuals to acquire mates. Female invests higher level of energy in production of eggs with lot of energy reserves including yolk, essential in the embryonic development and additional nutritional resources for developing embryos that are retained inside the body or the ones that are hatched. Males are subjected to lower energy investment that only supports the sperms to reach the eggs even when they produce millions of sperms (Wells, 2007). Female reproductive success is largely governed by energy availability (Trivers, 1972) and that resulted in them becoming a finite resource and a reason for competition among males to win over a mate (Clutton-

Brock and Vincent, 1991; Parker and Simmons, 1996). Simultaneously, the male success is dependent on diverse traits that augment the ability of males to locate, attract and eventually mate with a female. In addition to the morphological traits also known as secondary sexual characters like larger size, presence of weapons in males for fighting with conspecific males or difference in colour from their females, males also employ much better strategy of vocalisation to attract potential females (Wells, 2007).

Vocalization is one of the major characteristic features of an anuran. It is very vital in anuran breeding success and has a significant role in mate selection (Bailey, 1991; Ryan and Rand, 1993; Sueur, 2002; Gerhardt and Huber, 2002; Wells, 2007, Henry and Wells, 2010). Analogous to the song of a male bird, anurans use their calls in impressing and winning a mate and at the same time deterring males of their own kind from their territory (Huxley, 1927). When a frog makes a call, the air that's pushed into the lungs with support of muscles of the buccal cavity is forced back (De Jongh and Gans, 1969) to the buccal cavity through the larynx. This is made possible with the help of contraction of the external oblique muscles and the internal oblique muscles (Martin, 1972; Martin and Gans, 1972; Gans, 1973; Taigen *et al.,* 1985; Marsh and Taigen, 1987) also called transverse muscles (Ecker, 1889; Duellman and Trueb, 1986). Both are situated in the thoracic and abdominal cavities. These muscle layers are connected to rectus abdominus muscle, which is also involved in the vocalization process. Sound is produced by the action of vocal chords when the air from the lungs passes through the larynx and the muscles in the laryngeal region is vital . Majority of the species produce sound during the exhalation of air from the lungs. But frogs of the genus *Bombina* do it during inspiration and some species in the genus *Discoglossus* produce sound both during inspiration and expiration (Schneider, *et al.*1988;Lörcher, 1969; Weber, 1974). Many researchers have attempted to compare the results obtained from the study on bird calls to frogs and one such idea was the relation of the frequency of frog calls to their habitat structure (Littlejohn, 1977; Telford, 1982).

Female's mate selection is based on various traits among which vocalization is of greater significance which in turn acts a driver towards the evolution of the male's call (Henry and Wells, 2010; Bee et. al., 2012). An ecosystem with several sympatric species is subjected to vocalization from multiple species and the calls from those species act as an evolutionary pressure in the formation of the acoustic signals as well as the structure of the assemblage (Duellman and Pyles, 1983;Gerhardt, 1994a). Multiple species calls can also lead to the reduction in the clarity of calls perceived by females of a single species due to the interference from the surrounding calls and also in confusing the female frog in choosing their own species (Gerhardt, 1994a and b). Hence, such sympatric species with similar breeding periods exhibit partitioning spatially and temporally in order to avoid competition and interference in acoustic signalling. Studies demonstrate acoustic partitioning, spatial segregation and temporal partitioning as three techniques adopted by anurans in order to reduce or circumvent the signal interference between two or more species.

In acoustic niche partitioning, also called spectral stratification, different species partition themselves acoustically by having different spectral and temporal call properties (Littlejohn, 1977; Drewry and Rand, 1983; Bourne and York, 2001; Martins et al., 2006). In response to the competition among multiple species in acoustic signalling vocalising anurans eventually evolve to have a unique acoustic niche in order to communicate in a clamorous environment. Different species of the genus *Eleutherodactylus* have evolved to have different dominant frequencies in their vocalization as a result of spectral separation like several other anuran communities from different parts of the world (Drewry and Rand, 1983; Duellman, 1967 and 1978; Dubois, 1977; Hödl, 1977; Elzen and Kruelen, 1979; Humphries, 1979; Schlüter, 1979, 1980 a, b and c, 1981; Telford, 1982; Garcia-Rutledge and Narins, 2001). However, acoustic partitioning does not necessarily ensure a complete interference free acoustic atmosphere for the frogs owing to multiple variables. These variables include difference in number of species occurring together in one area from site to site, correlation of body size difference to interspecific difference in the dominant frequencies, presence of species in aggregations which shows broad frequency overlaps and anuran sensitivity to acoustics that are outside their frequency range (Crump, 1974; Dubois, 1977; Hödl; 1977; Garcia-Rutledge and Narins; 2001).

Spatial segregation is achieved through species-specific male assemblages and by usage of different microhabitats as calling sites (Bourne and York, 2001; Chek *et al*., 2003; Schmidt *et al*., 2012). Multiple species when found together in close proximities often partition themselves by calling from different microhabitats and from different heights of the vegetation they are in (Fouquette, 1960; Duellman, 1967; Hödl, 1977; Littlejohn, 1977; Mac Nally, 1979; Telford, 1982; Garcia-Rutledge and Narins, 2001). Puerto Rican Coqui frogs *Eleutherodactylus locustus* and *E. brittoni*, North American Tree frog species *Hyla versicolor* and *H. chrysoscelis* are some examples for species that exhibit spatial segregation in vocalization. They used similar microhabitats as calling sites when found independently whereas different sites when in close proximity of the second species (Drewry and Rand, 1983; Ptacek, 1992). In general, related species are observed to have calling sites that overlap to a greater degree when they are found distantly and they barely overlap when found in propinquity.

A third technique of partitioning of calling time, also known as temporal partitioning, involves separation in their calling time, sometimes in an hourly time scale (Bourne and York, 2001; Garcia-Rutledge and Narins, 2001; Kronfeld-Schor and Dylan, 2003; Diwakar and Balakrishnan, 2007). A classic example in this case is reported in three tree frog species of Panama that are found together in the same ecosystem. Not only *Hyla ebraccata, H. microcephala* and *H. phlebodes* inhabit the same habitat, they share a common calling microhabitats but also have overlapping acoustic properties and frequency in their calls. Acoustic and spatial partitioning hardly works in this case and frogs adapted themselves to partition based on specific time intervals by calling in rows when other species are silent (Schwartz and Wells, 1983a and b). The interactions between species within an ecosystem are a result of intraspecific interactions. Proneness to a change in calls or songs of a species ,in response to sympatric ones depends on the similarity of acoustic signals of multiple species with a higher degree of call frequency overlap. To a greater extent it is studied in birds and some insects (Cody and Brown, 1969; Latimer and Broughton, 1984).

In the current study, we aim to understand temporal partitioning in the vocalization of Bush frogs of the genus *Raorchestes* that are sympatric in Eravikulam National Park (ENP).

5.2. Methods

5.2.1. Sampling

Call surveys were done to select sites for data collection. ENP is dominated by grasslands and the two *Raorchestes* species, *Raorchestes dubois* (Fig. 5. 1) and *Raorchestes resplendens* (Fig. 5. 2) that inhabit the grasslands were chosen for the study based on their abundance and ease in data collection. Vocalization in anurans happens only during their breeding season, which begins with the pre-monsoon showers starting from May and ending with the south west monsoon in August in the case of our study subjects. Based on the reconnaissance surveys on vocalisation activity of both the species, sampling was done during the peak breeding season of June - July months of 2015 and 2016 when large number of male individuals were found signalling acoustically for mates. Ten plots of 20 x 20 m dimensions were laid randomly in areas with maximum presence of vocalizing individuals of both the species. Marshy grassland habitats of Eravikulam Hut and Anamudi areas were selected for laying 5 plots each.

Figure 5. 1: Vocalizing *Raorchestes dubois*

All the males inside the study plot at a given point of time were counted hourly. Ten hour sampling effort was done in each of the ten plots starting from 18:00 hours to 04:00 hours on different dates. Calls recorded at approximately 0.5m distance using ZOOM H4nSP Handy Recorder were confirmed later. Dry and wet bulb temperatures and humidity in the centre of the plot was taken every hour using dry and wet bulb thermometers and Kestrel 3500 hand-held weather station. Wind speed and the presence/absence of mist in the area were also recorded using the weather station to understand whether there is any change in activity pattern in response to change in those parameters. Vocalizations of both the species are unique and unmistakable once familiar and hence sampling was done by listening to the calls every hour by walking along the grid lines.

Figure 5. 2: ***Raorchestes resplendens***

5.2.2. Analysis

Statistical analysis of temporal partitioning was done using XL Stat and MS Excel. Proportion of males calling per hour was calculated and was plotted against the time interval to generate graphs. Smooth curves were generated using the above software, which works on the principle of data interpolation to map the peak calling activity of the individual versus the time interval (per hour).

5.3. Results

Only two species of *Raorchestes viz. Raorchestes dubois* and *Raorchestes resplendens* were recorded sympatric in the grasslands of the study site. Both the species occupy almost similar microhabitats for acoustic signalling. *Raorchestes dubois* calls from tips of grass clumps and they sit exposed whereas *Raorchestes resplendens* calls inside the grass clumps making them less separated acoustically. Their spectral separation is also little considering the similar call properties including a dominant frequency of 2.5-2.7 khz in both the species. Data collected from ten different sample plots were analysed and proportion of calling males were plotted against time for each individual plots. Our results from those graphs shows that there is considerable variation in the temporal activity pattern of both the species starting from 18:00 hours to 04:00 hours.

Data from the plot 1 in the Anamudi area of ENP shows that *Raorchestes dubois* and *Raorchestes resplendens* are temporally partitioned in their vocalization. The green line denotes calling activity curve of *Raorchestes dubois* and the orange one represents *Raorchestes resplendens. Raorchestes dubois* starts their calling activity by 18:00 hrs, it goes to a first peak at 20:00 hrs and goes down before it peaks again at around 03:00 hrs (Figure. 5.3).

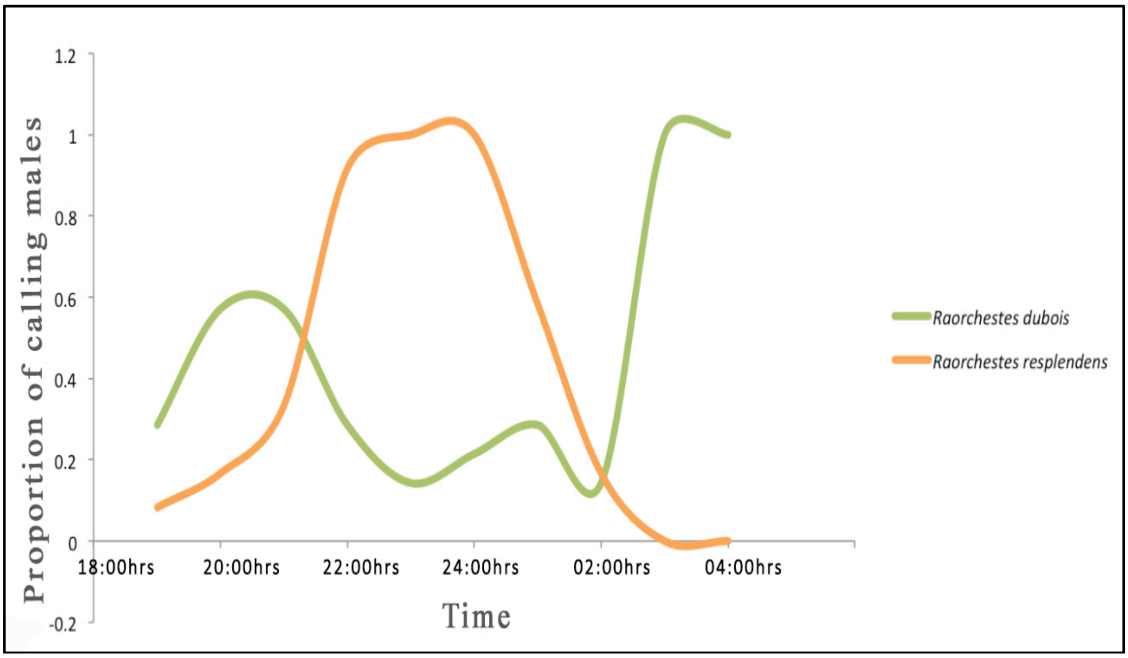

Figure 5. 3: Proportion of calling males versus time of Plot 1 in Anamudi

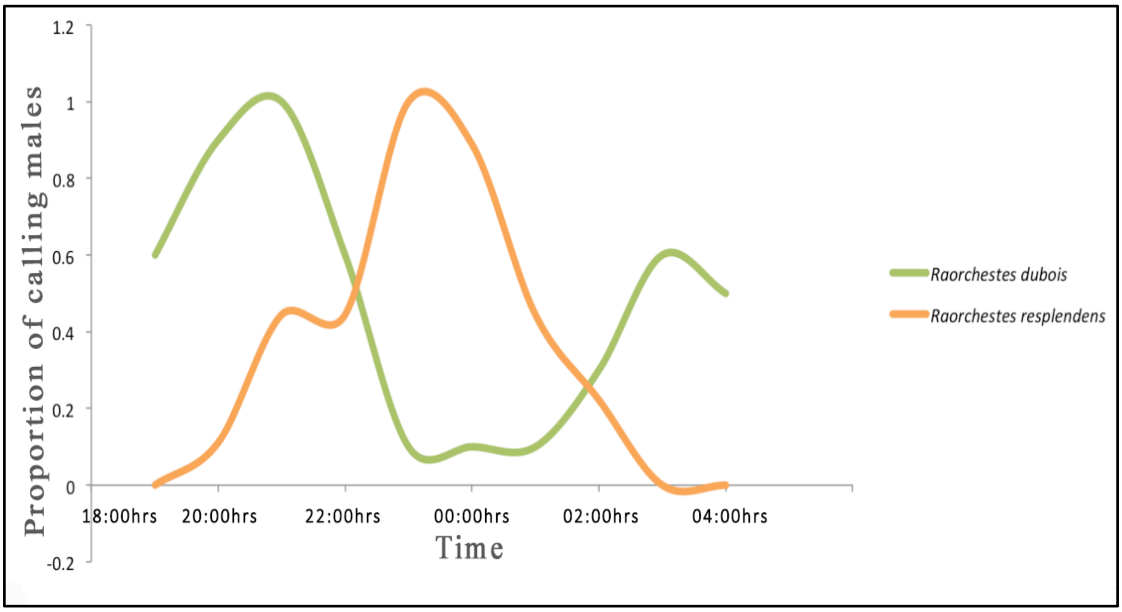

Figure 5. 4: Proportion of calling males versus time of Plot 2 in Anamudi

The second species *Raorchestes resplendens* called majorly between 20:00 hrs to 02:00 hrs with a broad peak between 22:00 hrs and 24:00 hrs (Figure. 5.3). Data from the rest of the plots were also congruent to the finding from the plot 1 irrespective of the location (Figure. 5.3- Figure. 5.12).

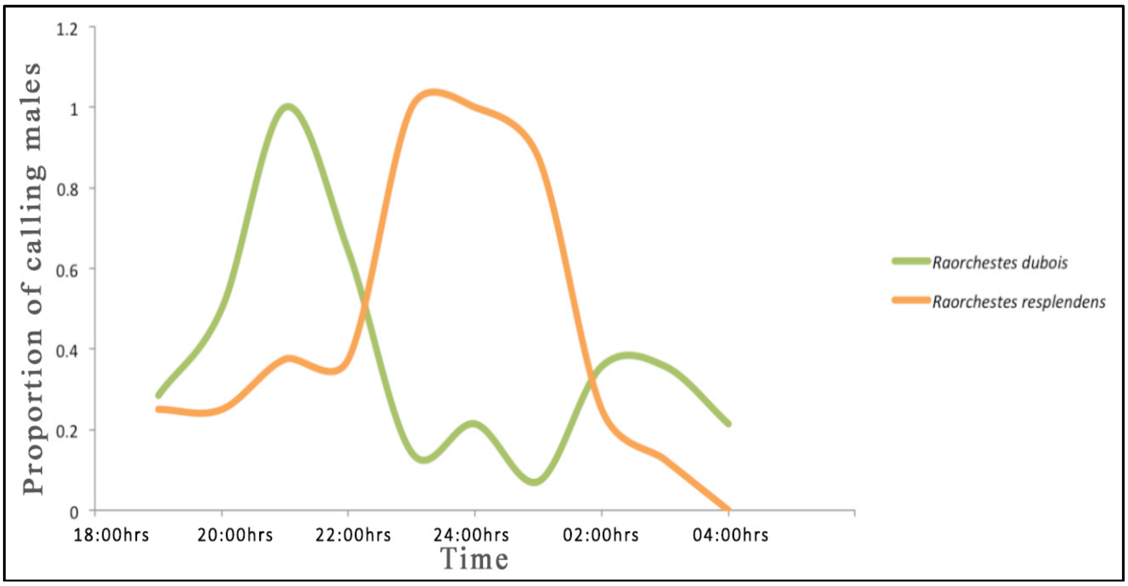

Figure 5. 5: Proportion of calling males versus time of Plot 3 in Anamudi

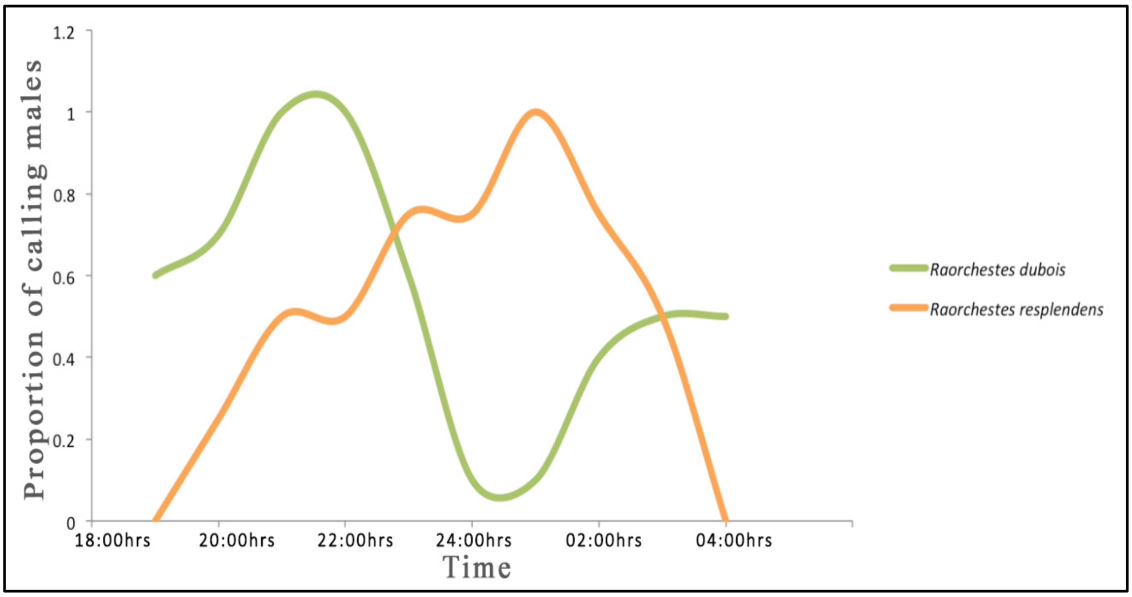

Figure 5. 6: Proportion of calling males versus time of Plot 4 in Anamudi

Minor variations in the duration of the peak activity was observed among the two species within the whole data set (Fig. 5.7, Fig.5.9, Fig 5.12). These minor variations might be due to the ecological parameters including low temperatures, presence of heavy mist and higher wind speeds. Plots that showed considerable variation in the activity pattern with the rest of the data experienced higher wind speed in intervals coupled with presence of heavy mist. This explains the difference in the peak activity periods and the partitioning in activity between the two species in the grasslands. Data on proportion of calling males from all the plots were merged and plotted against time (Fig. 5.13).The rectangular bars in the Figure. 5.13 shows calling activity of the two species and has been constructed by adding 10% on either side of the calling activity curve along the x (time) axis.

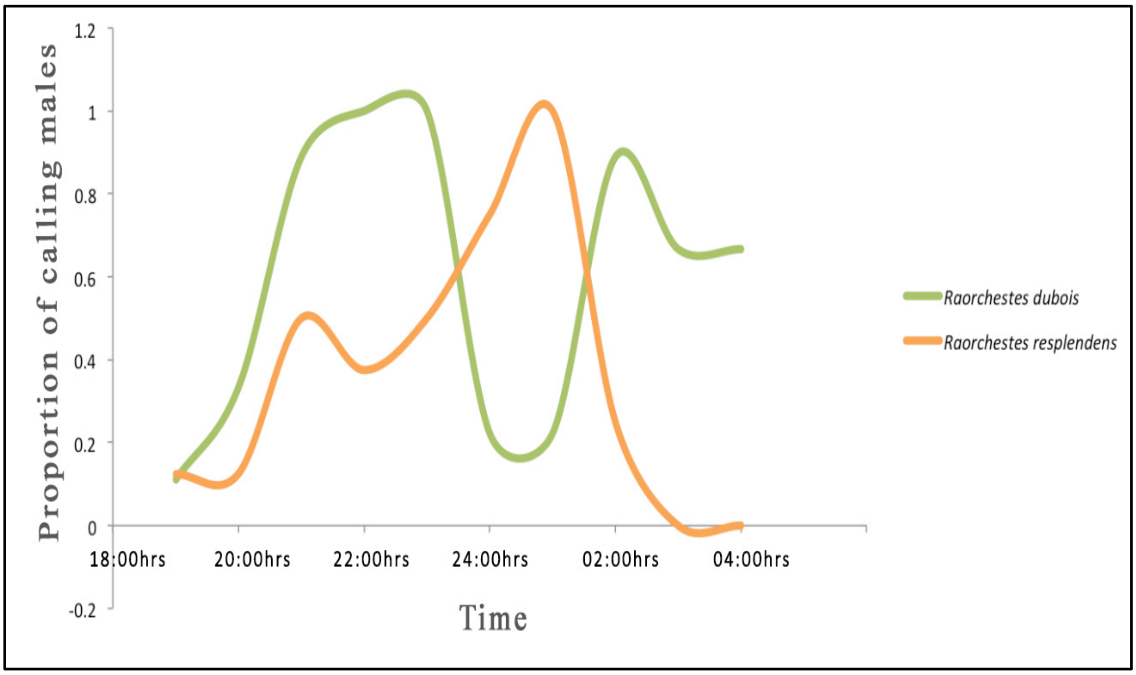

Figure 5. 7: Proportion of calling males versus time of Plot 5 in Anamudi

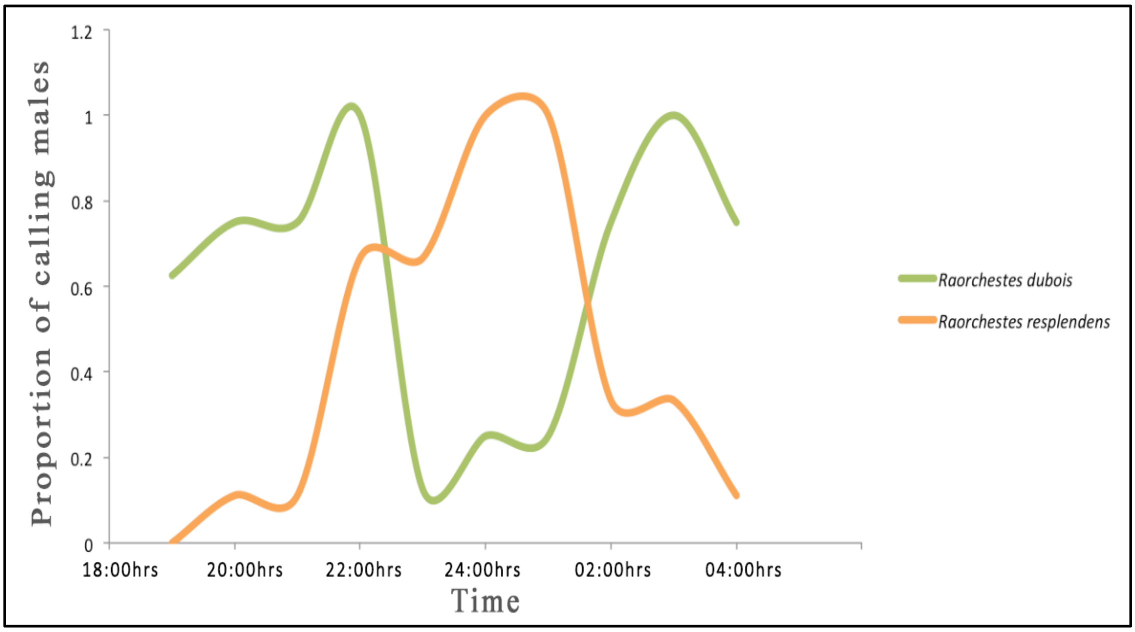

Figure 5. 8: Proportion of calling males versus time of Plot 1 in Eravikulam Hut

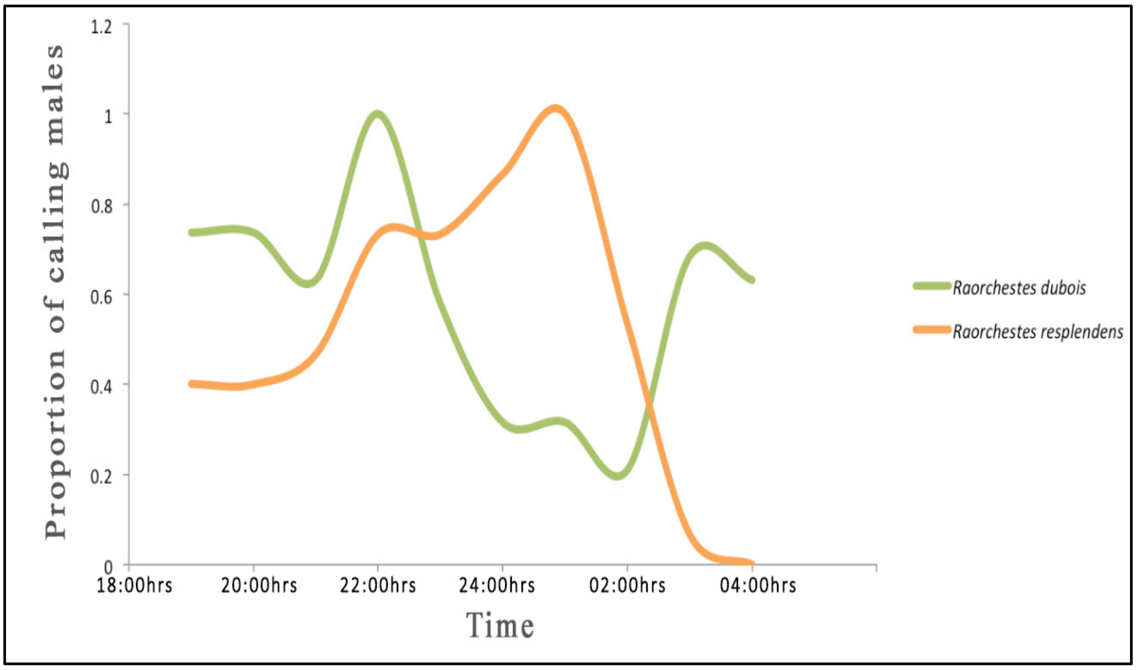

Figure 5. 9: Proportion of calling males versus time of Plot 2 in Eravikulam Hut

The differences in temporal activity for each species were calculated by measuring the difference between their times of peak calling activity. Results indicate *Raorchestes dubois* vocalize with two calling activity peaks and remain rather silent during the peak calling activity of *Raorchestes resplendens* which has only one peak. (Fig. 5.13) Greater activity in *Raorchestes resplendens* were recorded between 23:00 hrs - 24:00hrs after which the activity slowly recedes. *Raorchestes dubois* starts calling comparatively much earlier compared to *Raorchestes resplendens* and that explains a slighter earlier peaking (21:00 hrs) of calling activity in the species. The latter begin active calling only later compared to the first and peaks by around 23:00 hrs. While *Raorchestes resplendens* are found only in the open marshy grassland habitats that are protected, in other words undisturbed , *Raorchestes dubois* are found in bushes and shrubs along human habitations. Their calling activity when not in the vicinity of *Raorchestes resplendens* is rather different to what that is observed inside ENP. Habitat outside the protected area harbours various forms of signal interference, majority of them being anthropogenic and that largely govern the activity pattern in *Raorchestes dubois* in those areas.

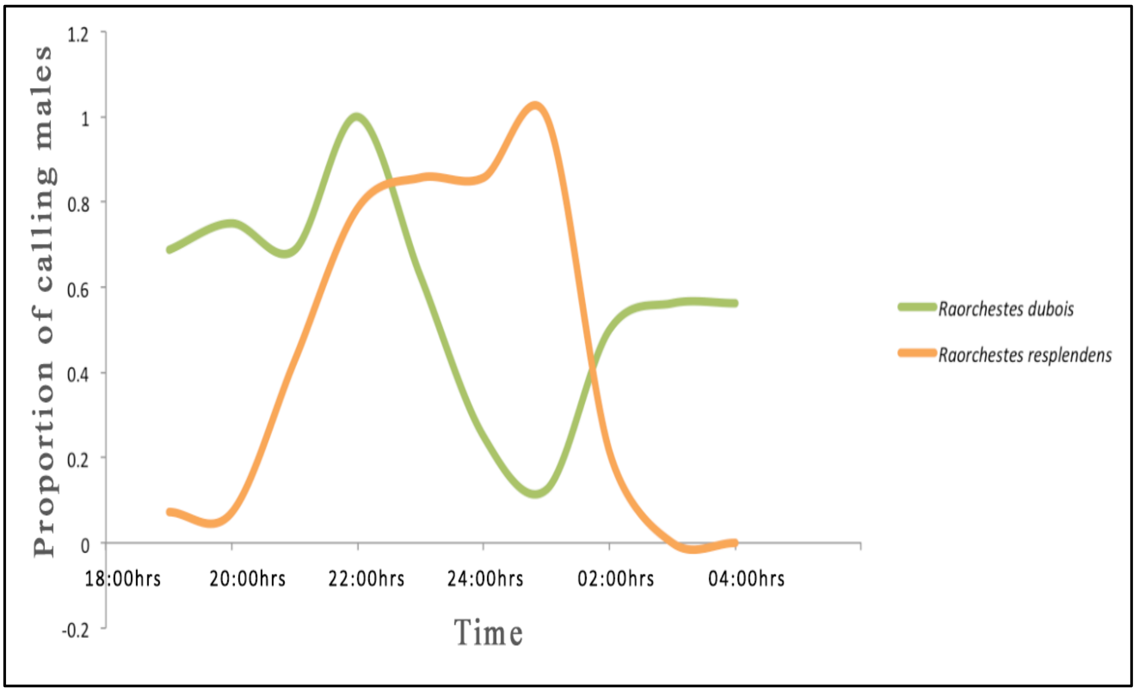

Figure 5. 10: Proportion of calling males versus time of Plot 3 in Eravikulam Hut

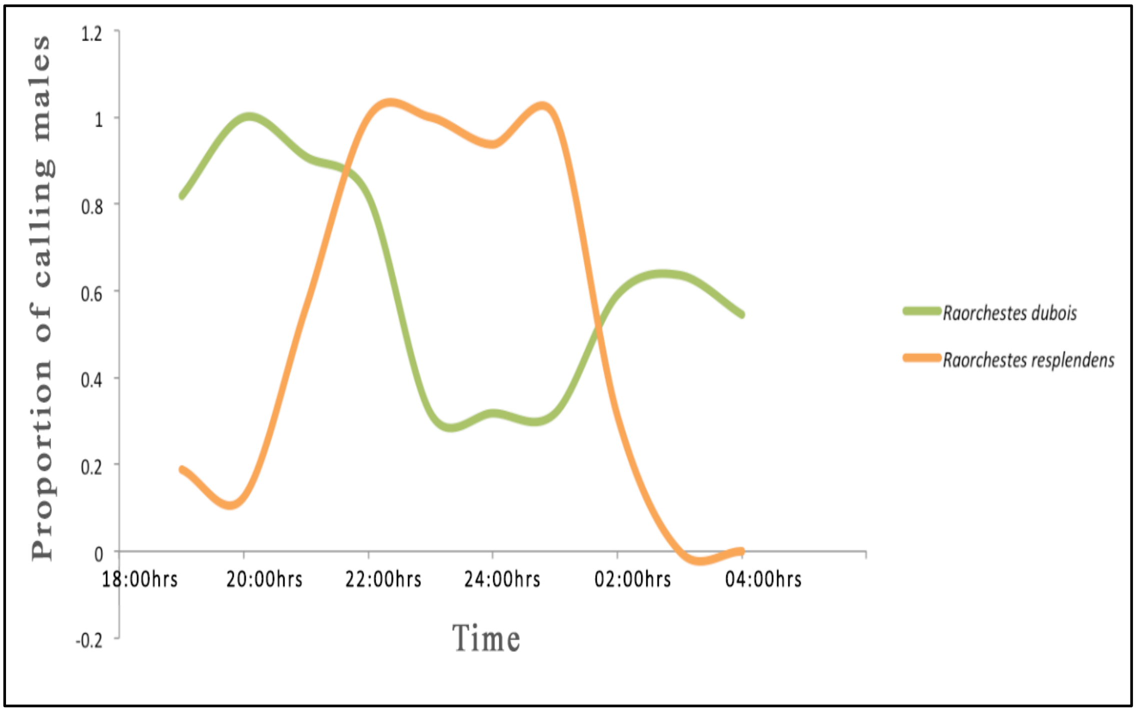

Figure 5. 11: Proportion of calling males versus time of Plot 4 in Eravikulam Hut

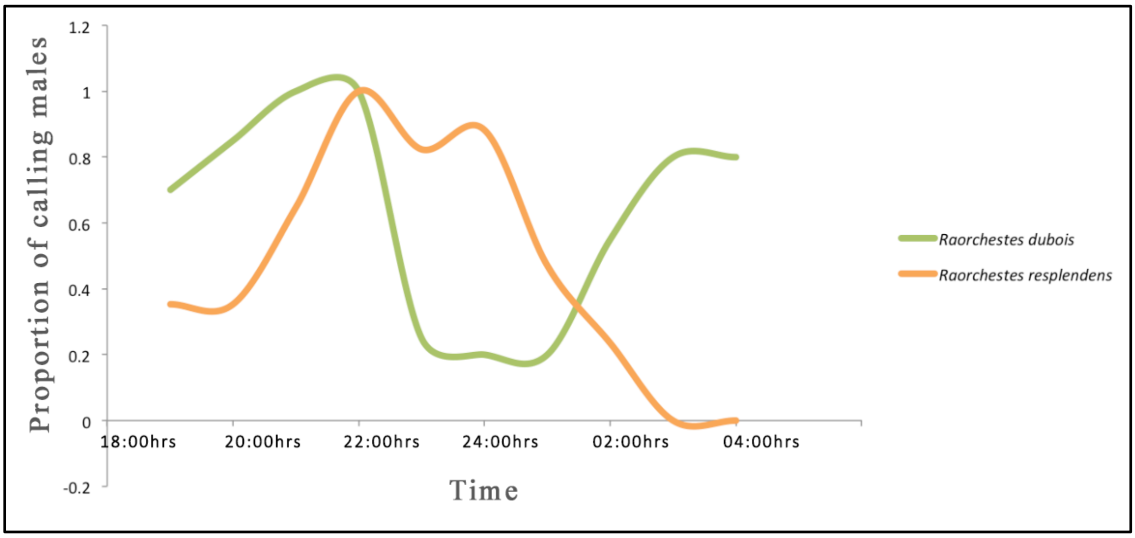

Figure 5. 12: Proportion of calling males versus time of Plot 5 in Eravikulam Hut

Table 5.1 : Proportion of calling males of *Raorchestes dubois* and *Raorchestes resplendens* at one hour time intervals from 18:00 hrs to 04:00 hrs

Proportion Calling	***Raorchestes dubois***	***Raorchestes resplendens***
18-19 hrs	0.70	0.20
19-20 hrs	0.89	0.23
20-21 hrs	1	0.50
21-22 hrs	1	0.83
22-23 hrs	0.46	0.94
23-24 hrs	0.28	1
00-01 hrs	0.25	0.91
01-02 hrs	0.54	0.34
02-03 hrs	0.81	0.07
03-04 hrs	0.74	0.01

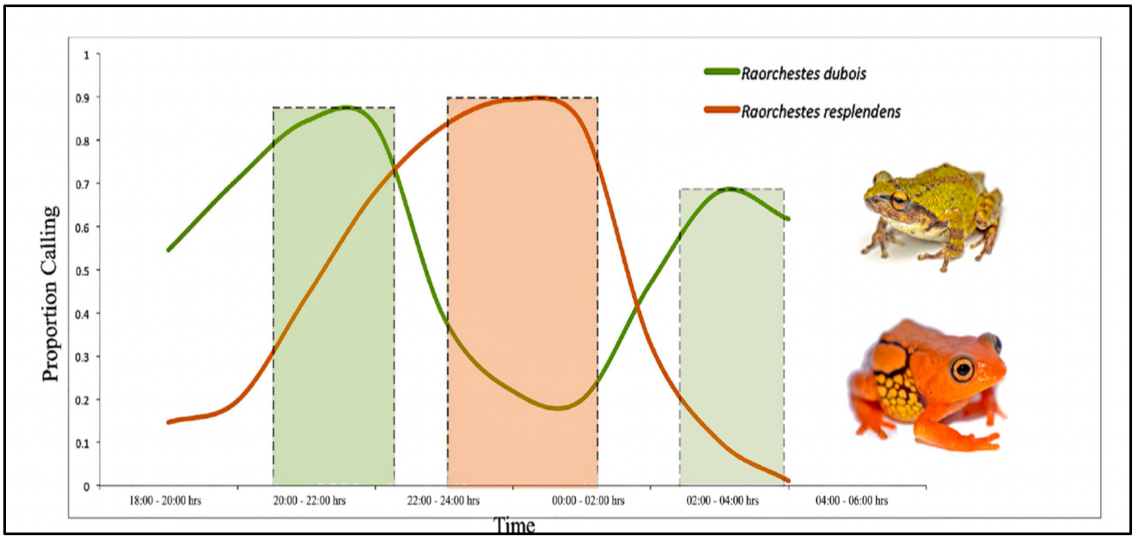

Figure 5. 13 Proportion of calling males versus time of all 10 Plots in ENP

5.4. Discussion

Evolution of anuran calls and their acoustic behaviour are driven by varied factors pertaining to the physical and biological environments. Position of the vocalising individual, habitats and microhabitats that they chose to call, presence of interferences like waterfalls or loud torrents, intraspecific and interspecific competition in signalling, female selection and even predators involve in the shaping of anuran call properties. Sympatric species often partition themselves in order to avoid signal interference from an individual or individuals that has occupied a proximal spot for vocalising. This not only causes problems for the females in locating mates of their own species but also prevents the males from attracting them (Bourne and York, 2001; Wells, 2007; Schwartz and Wells, 1983a and b). Frogs with similar frequency range when present together shows behavioural adaptations to stay away from acoustic interferences at intraspecific level. This may sometimes lead to interspecific interactions as well. In such instances, calls of one species might slowdown or completely stop the calls of another species and resumes once the first species stops. They time their activity in accordance with the activity of their sympatric species and calls in silent intervals of the active species and change their calling time in response to individual calls. Some of the studies demonstrate the complete stopping of calls as a strategy by anurans to overcome signal interference from other species(Littlejohn and Martin, 1969).

Results of the present study also confirms the hypothesis that sympatric species with very little overlapping of call frequencies and with hardly any spatial stratification generally adopts temporal partitioning in their vocalisation activity pattern (Wells, 2007). Such behaviour is normally congruent to the previous findings on frogs that they take turns in acoustic signalling in response to the activity of their sympatric species. In this case, *Raorchestes dubois* and *Raorchestes resplendens* demonstrate this behaviour by having different activity peak timings and also in being active when the other species is comparatively quieter. Phelps *et al.* (2006) reported that male *Physalaemus pustulosu*s increased their calling activity in presence of calls from a sympatric *Leptodactylus labialis.* The results from the present study indicates temporal partitioning as response to acoustic signal interference caused by different species in the same habitat. One possible explanation for the increased activity among frog calls in presence of another is to avoid the risk of predation. This study also suggests the role of environmental factors playing a vital role in anuran vocalization pattern and their change in response to the change in ecological parameters including wind speed and presence of mists. Sampled plots in Anamudi area experienced greater wind speed (up to 76 km/hr) in comparison with the plots of the Eravikulam Hut areas (<50 km/hr) which explains the minor changes in the call peaks in Anamudi plot data (Fig. 5.7, Fig.5.9, Fig 5.12) from the rest of the data.

Raorchestes dubois and *Raorchests resplendens* are small sized anurans. They occupy grass clumps in open marshy grassland ecosystem for acoustic signalling which are always exposed to all sorts of immediate changes in the environmental parameters such as increase in the wind speed inhibiting/stalling their acoustic behaviour momentarily or for greater period until the situations are perfect. When birds and insects are often used as model organisms to study temporal partitioning, amphibians are scarcely used. Current study is one of the very few attempts from Western Ghats and first from ENP to understand more about the acoustic signalling behaviour among frogs of the genus *Raorchestes* and acts as foundation to future research in vocalization studies.

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Chapter VI

CALL REPERTOIRE STUDY OF CRITICALLY ENDANGERED *RAORCHESTES RESPLENDENS*

Call repertoire study of Critically Endangered *Raorchestes resplendens*

6.1. Introduction

Amphibians are the most threatened group of vertebrates with 41% of the total assessed species under threatened categories (IUCN, 2016). Considering the total number of new species described between 2004 and 2016, India ranks second globally with 155 new species (Tapley *et al*., 2018). Of these, 75% are from the Western Ghats and Sri Lanka Biodiversity Hotspot (Myers *et al*., 2000; Mittermeier *et al*., 2004). Despite the number of species described from the Western Ghats is on the rise, detailed studies on individual species are scarce.

One of the prominent characteristics of anurans is their ability in vocalization and are majorly attributed to males. Understanding vocal behaviour is crucial in studying the evolutionary history of frog communications, which in turn plays a key role in their conservation and systematics. It is also crucial towards understanding the role of vocalisation in their social and sexual behaviour, to resolve taxonomic ambiguities among cryptic species and towards conserving the species (Vences *et al.,* 2010; Bardeli *et al.,* 2010; Blumstein *et. al,* 2011; Bee *et al.,* 2013a). Male frogs and toads call to attract potential mates, deter conspecific males and in establishing their own territory (Wells, 2010). These territories, which are in fact breeding or nesting sites, are often used in parental care by either of the parents. Early works on frog calls were mostly basic descriptions on calls in onamatopoeic words supported with behavioural notes. Wright (1914) described seven different characteristics of bullfrog's call based on anecdotal evidence. First ever collection of frog songs were published as an album by ornithologist Arthur A. Allen from Cornell University and Peter Paul Kellog (Allen and Kellogg, 1948). A comprehensive review on acoustic communication in anurans based on the evolutionary and ecological framework was done for the first time by Bogert (1960). He classified anuran vocal repertoires into six categories *viz.* mating calls, territorial calls, male release calls, female release calls, distress calls and warning calls which were later modified and perfected (Littlejohn, 1977; Wells, 1977a and b, 1988).

Generally frogs are observed to have single noted calls whereas some rhacophorid frogs have characteristic multiple calls. One of the most diverse groups of frogs in India with the greatest number of species described since 2004 is the genus *Raorchestes* known to be a genus of direct developing rhacophorid frogs (Biju *et al*., 2010; Dinesh *et al.,* 2020). Only a few attempts were made to describe the vocal repertoire of species of the genus *Rarochestes* in the Western Ghats. Works on the species *Raorchestes graminirupes, Raorchestes kakachi, Raorchestes ghatei* and *Raorchestes travancoricus* are to name a few (Bee *et al.,* 2013b; Padhye *et al.,* 2015; Seshadri *et al*. 2012; Rajkumar *et al.;* 2016).

Raorchestes resplendens Biju, Shouche, Dubois, Dutta & Bossuyt, 2010 is a medium-sized, ground-dwelling, Western Ghats endemic bush frog. Its prominent orange colouration and large glands, bordered with black make it distinct from other species (Fig. 6.1). The species belongs to the *beddomii* clade (Vijayakumar *et al*., 2014) and is restricted to the Anamalai massif of Western Ghats. The species is known only from its type locality, a three square kilometer patch of habitat on the Anamudi summit, the highest peak (2,695m) in Western Ghats in Eravikulam National Park (ENP) and Poovar, a site approximately 20km north-east of Anamudi summit (Biju *et al*., 2010; Joseph *et al*., 2012). Joseph *et al.* (2012) suggested the possibility of a wider distribution of the species within ENP. *Raorchestes resplendens* is assessed as Critically Endangered (IUCN/SSC Amphibian Species Specialist Group, 2011).

The vocalisation of *Raorchestes resplendens* was studied in Eravikulam National Park (ENP, 10.083–10.333 0N & 77.00—77.166 0E) in Kerala, India. More than 60% of the park, with undulating terrain, is dominated by grasslands (Fig. 6. 2) with shola patches in the valleys. Anamudi, Eravikulam Hut, Kolukkumala, Saambamala, Kaatumala, Poovaar areas in ENP and areas adjacent to ENP in Munnar landscape including Meeshapulimala were also chosen for collecting data on *Raorchestes resplendens* call recordings.

Figure 6.1. *Raorchestes resplendens* in its habitat

6.2. Methods

6.2.1. Sampling

A combination of survey methods including Visual Encounter Surveys (VES), call surveys and scan searches (Heyer *et al*.,1994; Krishnamurthy 2003; Halliday 2006) were

Figure 6.2. Marshy grassland habitat of *Raorchestes resplendens* in ENP

used between January 2015 and December 2018 to document the distribution of *Raorchestes resplendens*. During the breeding season (May−September), surveys were undertaken from 18.00 to 02.00 h, as bush frogs are known to be most active at night (Biju *et al*., 2010). Morning and evening surveys were also conducted from 08.00 to 13.00 hrs and from14.00 to17.00 hrs to record diurnal activity, if any. Surveys were done in shola-grassland ecosystems above 1,700m especially inside ENP from where the species was first described and reported. To avoid repeated count and getting maximum distribution range of the species, the surveys were spatially replicated.

Calls of *Raorchestes resplendens* were recorded at approximately 0.5m distance using ZOOM H4nSP Handy Recorder from various locations in ENP. Ten to twenty calls were recorded for each individual (n=10 males). Ambient temperature and snout vent length (SVL) was taken immediately after the recording using Kestrel 3500 hand-held weather station and a Mitutoyo digital vernier caliper.

6.2.2. Analysis

Analyses of the calls were done using Raven v1.4 software (Cornell Laboratory of Ornithology, Ithaca, NY, USA) (Bee *et al.,* 2013a and b; Thomas *et al.,* 2014). Temporal and spectral parameters of calls were measured following definitions of Bee *et al*. (2013a and b). Six call properties: call duration (ms)—time between the beginning of first pulse and the end of last pulse in a call; call rise time (ms)—time between the beginning of first pulse and the peak of pulse of maximum amplitude; call fall time (ms)—time between the peak of pulse of maximum amplitude and end of last pulse; inter-call interval—time between end of a call to the beginning of the next call; call rate—number of calls delivered per minute and overall dominant frequency were analyzed.

6.3. Results

6.3.1. Call Description

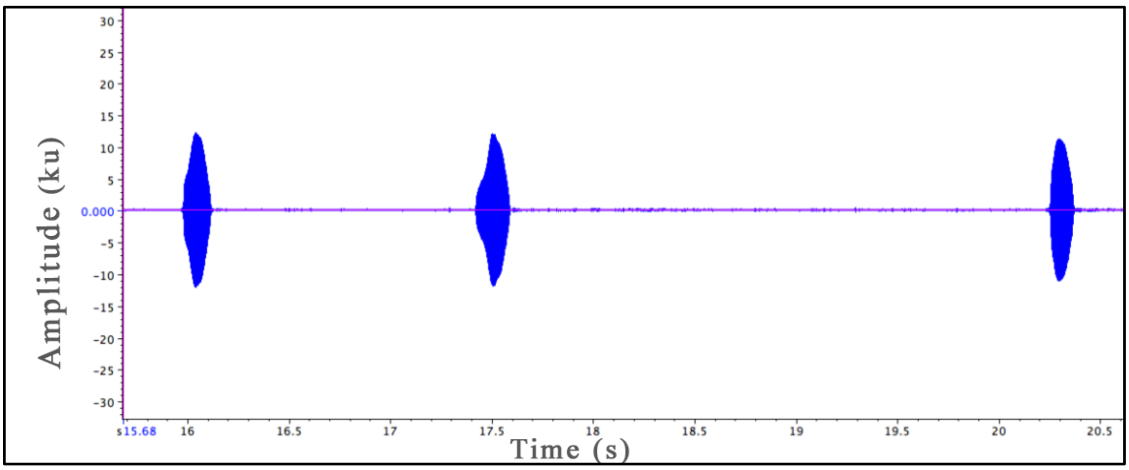

Figure 6.3. Wave form of *Raorchestes resplendens* call in the 5s timeframe

Raorchestes resplendens male individuals were observed to call actively from 18.00 to 02.00 hrs during their peak breeding season in May–September. A total of 141 calls from 10 males were analyzed for the description of vocalization. Temperature ranged between 16 and 20^0 C during all the recordings. Calls were relatively simple (Figs. 6.3 and 6.4). The advertisement call (https://doi.org/10.6084/m9.figshare.12781229.v1) had non-pulsatile temporal structure unlike published calls of other bush frogs including *R. flaviocularis, R. silentvalley, R. lechiya, R. travancoricus* (Bee *et al.,* 2013a, b; Vijaykumar *et al*., 2014; Rajkumar *et al.,* 2016; Zachariah *et al.,* 2016). Advertisement calls typically ranged between 58.9 and148.8 ms in duration (Table 6.1). On an average, the interval between two calls was 2.9 ± 3.6 s, and these intervals were uncorrelated with SVL or mass (Table 6.2). The call rise time (= 46.3 ms ± 29.4 ms; Table 6.1) was slightly shorter than call fall time (= 56.7 ms ± 16.8 ms; Table 6.1). The calls were typically delivered at a rate of 21.5 calls/minute (Table 6.1). The spectrum was characterized by a single broad peak with mean dominant frequency of 2.5 KHz (Fig. 6.5, Table 6.1).

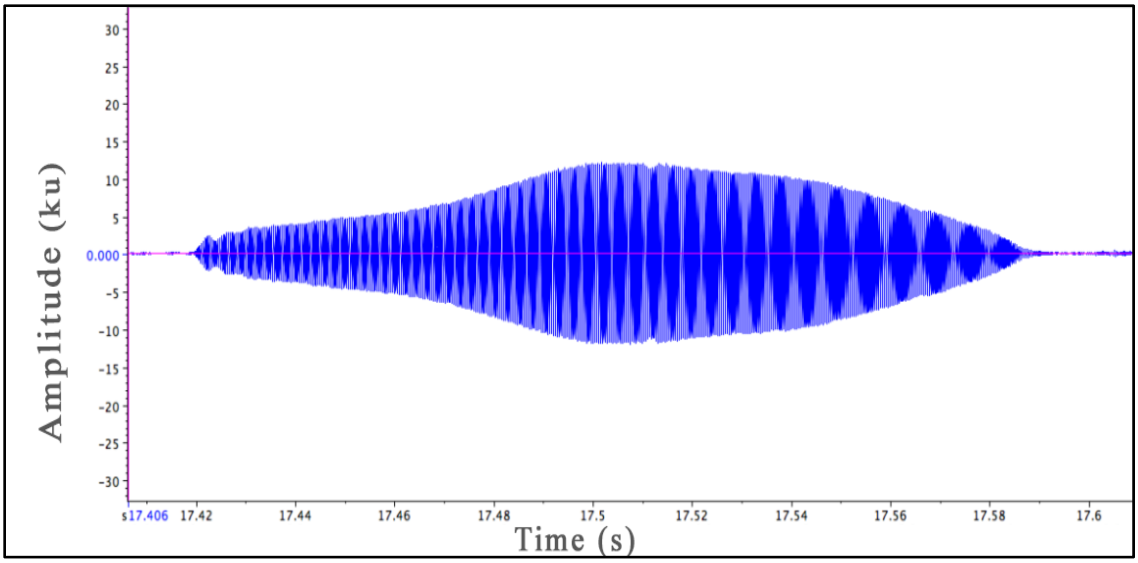

Figure 6. 4. Wave form of *Raorchestes resplendens* call in 2s time frame

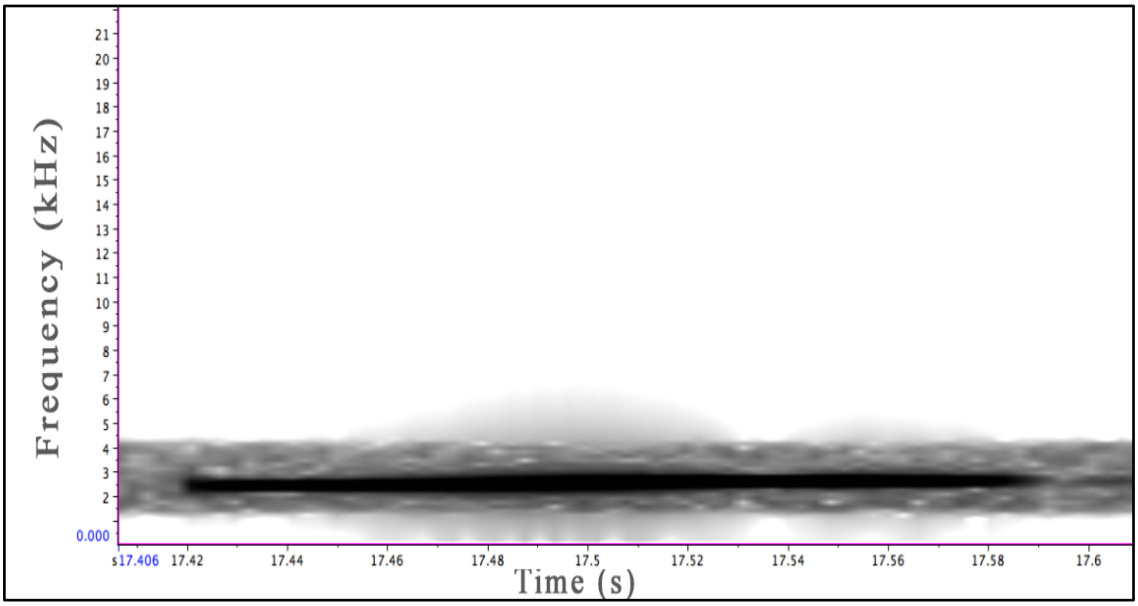

Figure 6. 5. Spectrogram of *Raorchestes resplendens* call

Table 6.1.Call characteristics of 141 calls of *Raorchestes resplendens* from 10 males

Call Properties	Mean	SD	Minimum	Maximum
Call Duration (ms)	103	37.3	58.9	148.8
Call Rise Time (ms)	46.3	29.4	11.9	80.5
Call Fall Time (ms)	56.7	16.8	39.9	69.8
Intercall Interval (s)	2.9	3.6	1.4	4.9
Overall Dominant Frequecny (KHZ)	2.5	0.1	2.4	2.8
Call Rate (Calls/min)	21.5	7.9	16.1	41.4

Table 6.2. Correlation of call properties

Call Properties	SVL	Mass
Call Duration (s)	-.3143	-.2042

	p=.376	p=.571
Call rise time (s)	-.2457	-.1687
	p=.494	p=.641
Call Fall time (s)	-.3669	-.2200
	p=.297	p=.541
Intercall Interval (s)	.0973	.0382
	p=.789	p=.916
Overall Dominant Frequency (kHz)	-.3496	-.5580
	p=.322	p=.094
Calls/Min	.2291	.2723
	p=.524	p=.447

6.4. Discussion

Advertisement calls earlier known as mating calls are the vocal signals produced by a frog or a toad in literally advertising their presence to the females for breeding. The fact that these vocalizations serve more than one type of message resulted in the much more general name, advertisement calls (Gerhardt, 1992). These calls in longer durations have the capacity to stimulate hormone productions in females (Lea *et al.,* 2001), in individual identification (Davis, 1987) and preserving spacing among conspecifics, sizing and understanding opponents or rival males (Whitney and Krebs, 1975; Wells , 1977a; Fellers, 1979). Studies also show that in species like *Eleutherodactylus coqui,* the same call conveys different messages to different sexes of the same species (Narins and

Capranica, 1978). The current study provide information on the vocalisation of *Raorchestes resplendens* for the first time.

Raorchestes resplendens call from the basal area of the grass clumps and that explains their low frequency vocalisation like several other frogs and birds that call closer to or from the ground (Wells, 1977a). Another interesting feature observed in the current study is that none of the call properties were related to the body size (SVL) or mass (Table 6.2), which warrants collection and analysis of even more calls from a larger population size and suggests further research on the topic. The surrounding atmosphere, the vegetation, the microclimatic conditions and other physical parameters that are around a vocalising animal including its position, always has a toll on the effectiveness of the signals transmitted from a call (Wiley and Richards, 1978; Richards and Wiley, 1980; Narins and Zelcik, 1988). One of the reasons for their absence in their several remaining habitats in and around Munnar other than Protected Areas might be the numerous interferences in the form of vehicles and anthropogenic sounds that can distort their audio signals before being received by the males or females of the species .

Vocalisation is a beneficial source in identification of species and studying its distribution as it is unique for each species. Very little information was available on the species owing to its secretive lifestyle and shy behaviour. Only a handful of records were known including its type locality and another published records within the national park. But the present study on the vocalisation of *Raorchestes resplendens* helped in confirming the presence and absence of the species much easier during their peak breeding season (May–September). A species that is comparatively rare and difficult to find were recorded from areas they were not known before and detailed mapping of the distribution of the species were made possible. The study hasn't got a single record of the species inside the shola forests. Our findings on the distribution of the *Raorchestes resplendens* are also concordant with the fact that frogs common in open areas with high pitched calls and lower dominant frequency (Zimmerman, 1983) are rarely seen or even not seen in the forests just like *R. resplendens*. As is seen, *Raorchestes resplendens* vocalize and breed from the base of grass clumps, which is a uniform environment compared to the densely vegetated shola patches with diverse microhabitats and a higher degree of interferences that can cause greater attenuation of the call.

The data on distribution was shared with the International Union of Conservation of Nature's second Global Amphibian Assessment (GAA2) in August – October 2020. As a result, the species which was considered Critically Endangered earlier has been down listed based on the data gathered on their distribution using their vocalisation information. The study clearly marks the role and advantage of vocalisation studies towards the conservation and management of a species.

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Chapter VII

IMPACT OF PRESCRIBED FIRE MANAGEMENT PRACTICES ON GRASSLAND INHABITING AMPHIBIANS OF ERAVIKULAM NATIONAL PARK

IMPACT OF PRESCRIBED FIRE MANAGEMENT PRACTICES ON GRASSLAND INHABITING AMPHIBIANS OF ERAVIKULAM NATIONAL PARK

7.1. Introduction

Scientists around the world have been voicing concerns over the growing populations and the obvious increase in the anthropogenic factors leading to another mass extinction. Studies have always shown a steep increase in the rate of threats that affect biodiversity. Most of these threats come under the larger problems like habitat destruction, global climate change and its varied consequences, arrival and introduction of new pathogens and diseases.

The Earth witnessed around five mass extinctions in its history where it lost a lot of its life forms in a short span of time (Jablonski, 1995; Erwin, 2001). When changing climates was the major causative factor for all those extinction events, the current threats to biodiversity are largely human driven. According to the World Conservation Union (IUCN) Red List of threatened species, more than forty thousand species of animals are threatened with extinction (IUCN, 2021).

Among all the animal groups, amphibians are the ones with forty one percentage of the known species in the world that are threatened and are likely to be one of the major group that are already in a race towards extinction (Wake and Vredenburg, 2008). The general understanding on their threatened status and the notion that they require immediate help, amphibians have been getting a lot of attention than other groups in the last couple of decades. A large number of factors lead to amphibian declines. But the most important ones are global climate change, habitat destruction and infectious disease (Stuart *et al.,* 2004; Pechmann and Wake, 2005; Pounds *et al.,* 2006). These animals are generally adapted to live in wet and humid conditions owing to their vascularised moist skin and cutaneous respiration. Any change in the surrounding environments, quality of water and air can have serious impacts on their life (Vitt *et al.,* 1990; Murphy *et al.,* 2000). Species inhabiting the tropical regions are more susceptible even to the slightest change in the atmosphere as they are normally subjected to very little temperature variations.

A total of thirty five species of amphibians have gone extinct, out of which nine went extinct after 1980 (Stuart *et al.,*2004) and more than hundred species haven't been seen for a long time since 1980 as per the records of the Global Amphibian Assessment (GAA) (IUCN, 2004). While some of them were rediscovered (Rajkumar *et al.,* 2016) as a result of exhaustive surveys, captive breeding programs and conservation research, a vast majority of them are still not seen in the wild.

Compared to other group of animals, amphibians have witnessed more evolutionary events than other taxa starting from the Devonian period about 400 million years ago. They have survived several mass extinctions, went through toughest climate spells, witnessed formation of mountains, continental movements and many more. Some of the present day amphibian lineages are quiet old including Giant salamanders of the genus *Dicamptodon, w*hich date back to Miocene and *Nasikabatrachus sahyadrensis* dating back to Cretaceous periods (Hime *et al.,* 2020). It is interesting to note that a group of organisms that have gone through a lot of dramatic changes over a long period is currently threatened by changes in the atmosphere. But the rate at which these dramatic changes occur in the environment compounded with human interference took it to a much more faster rate than the olden times and that is what made them more vulnerable. With the increase in human population, came the need for space and it resulted in speeding up of land conversion for agriculture and developments. Rate of habitat destruction increased over a period of time and eventually led to decline of many animals including amphibians. The history of conservation date back to the nineteenth century (Haila, 2012) in the United States and efforts were made to preserve the remaining life forms and nature from further decline. Subsequently, numerous conservation areas, like conservation reserves, sanctuaries and national parks were formed for conservation of nature and wildlife across the world. However, the focus was majorly on larger animals, particularly mammals (Entwistle, 2000) and the smaller life forms including amphibians, reptiles and several other invertebrates were neglected. Some of them were even considered as pests. Ever since conservation became a practice, management of those dedicated areas and the wildlife also became a part of it. Open habitats and grasslands were the first ones that were widely managed owing to their less complex structure and wide distribution. These grasslands have evolved into the present shape by several millions of years of climatic conditions, fire and grazing by the herbivores (Anderson, 1982).

One of the most accepted practice of managing the grasslands and open habitats in those times was the use of fire as a management tool (Edwards, 1984; Cutler, 1979). From the primordial periods to the recent times, fire has acted as one of the strongest ecological forces in shaping this world. When it's a boon to a wide range of animals in the form of new food and open hunting grounds for herbivores, carnivorous mammals and birds, fire is a bane to lesser life forms like amphibians, reptiles and invertebrates that are slow moving, sedentary and microhabitat specific. Such animals that are restricted to specific habitats in small populations are highly vulnerable to chance extinctions as a result of short-term events like fire (Wells, 2010). The biological response of the inhabitants in an ecosystem to the fire incidents differ according to the time, size, frequency and the intensity (Pyne, 1982).

The negative and positive impacts of controlled burning as a part of habitat management practice had been widely studied on amphibians world-wide. Various factors decides the impact of burning on amphibians. It depends on the time (season) and place of burning, the community structure of present in that area and also the habitat type. While some of the studies reported fire as a disruptive event on terrestrial ecosystems affecting amphibians, some showed no affects and some with positive affects like the improvement of suitable habitats (Means and Campbell, 1981; Kirkland *et al.,* 1996; McLeod and Gates, 1998; Ford *et al.,* 1999; Russell *et al.,* 1999; Moseley *et al.,* 2003; Pilliod *et al.,* 2003; Greenberg and Waldrop, 2008; Certini *et al.,* 2021). The cold highland forests as well as wet tropical forests provide enough moist microhabitats for the amphibians and hence they are high in amphibian diversity and abundance (Scot, 1976, 1982; Péfaur, 1980). Amphibians greatly depend on the soil moisture and moist conditions in their refuge sites (Rosenthal, 1954; Wyman, 1988). Soil moisture is also a very essential factor in the survival of amphibian life-history stages.

Even though there are studies on impact of fire on ecosystems from Indian Sub-continent, most of them were focussed on soil properties and vegetation (Goldammer 1990; Puyravaud *et.al.* 1995; Swarupanandan *et. al.* 2001; Balagopalan *et.al.* 2002; Saha 2002; Saha and Howe, 2003; Hiremath and Sundaran 2005; Kodandapani *et al.* 2008; Saravanan *et. al.* 2014) than on faunal elements (Johnsingh, 1986). Frequent fire incidents as a consequence of uncontrolled human activities inside and outside protected areas are considered as a serious threat to amphibians along with other animals in the Western Ghats (Kodandapani *et al.,* 2004). A study on impact of fire in Chinnar Wildlife Sanctuary (CWS) and Panthanthod of Mannarkad Forest Division reported negative impacts of fire on soil properties, its microorganisms and vegetation. The study also showed that the conditions didn't recover to its initial stage even after 17 years (Balagopalan *et al.,* 2002). Fire incidents, large or small when occur continuously in a location leads to the decline in soil fertility eventually resulting in threatening ecosystem productivity and resilience (DeBano, 2000). Recent studies on the impact of controlled burning on soil components in the montane grasslands of ENP revealed similar results and suggested an irreversible damage to the natural composition of soil (Sandeep *et al.,* 2019). Another study from Eravikulam National Park and Parambikulam Tiger Reserve on grasshoppers reported prescribed fire management practice as disastrous to them and even suggested adopting small scale burnings so as to minimise the impact and provide fresh grass to the flagship endemic mountain goat species, Nilgiri Tahr (Bhasker *et al.,* 2019). Even though the detrimental impact of fire on biodiversity is studied in detail in diverse habitats (Andersen, 2021; Ondei, 2020), the use of fire as a management tool continued due to various reasons, sometimes focussing solely on the management and conservation of single species (Easa *et. al.,* 2010). Eravikulam National Park, one of the very few unique grassland ecosystems in Western Ghats is subjected to controlled burning even from the British period (Davidar, 1978). Controlled burning is practiced during the winter months in order to facilitate growth of fresh and nutritious grass for the endangered mountain goat, Nilgiri tahr (*Nilgiritragus hylocrius*) as well as to avoid accidental fires during the summer season. The indigenous community Muthuvans had been doing it from the British period and they were employed for carrying out the burning (Easa *et al*., 2010;

KFD, 2012). Burning is practiced every year in alternate patches in order to get a mosaic of fresh grass and old grass throughout the park. The park is divided into several 50 ha grids and they are burnt in a 3 year rotation (KFD, 2012). However, there is no systematic data available on the influence of burning on other biodiversity of ENP as well as the positive or negative effects on Nilgiri tahr itself.

The current study investigates the impact of prescribed fire management practices on grassland inhabiting amphibians of Eravikulam National Park.

7.2. Methods

7.2.1. Study Area

Figure 7. 1: Controlled burning in Sambamala hill

The study was conducted in the grasslands of Eravikulam National Park. Detailed description on ENP is given in Chapter III. Controlled burning towards the management of grasslands in ENP has a long history starting from the time when it was used as a game reserve for hunting by the colonials. The practice is still continued even after the formation of the National Park in order to provide fresh palatable grass for Nilgiri tahr. Burning is practiced every year in the winter months (December-February). Grasslands are burnt in alternate patches in order to get a mosaic of fresh grass and old grass throughout the park. The park has a dedicated control burning regime, which is divided into several 50 ha grids. The girds are categorised under the labels 1, 2 and 3 and they are burned in a 3 year rotation.

Figure 7. 2: Kambipaalam hill after a day of burning

Grids of a single group is burnt in an year followed by the second and the third in the consecutive years so as to ensure coverage of the entire grassland of ENP in 3 year time. For the data collection, two hills that are accessible from Eravikulam Hut base camp and the ones where burning was done during the study period *viz.* Sambamala and Kambipaalam were chosen.

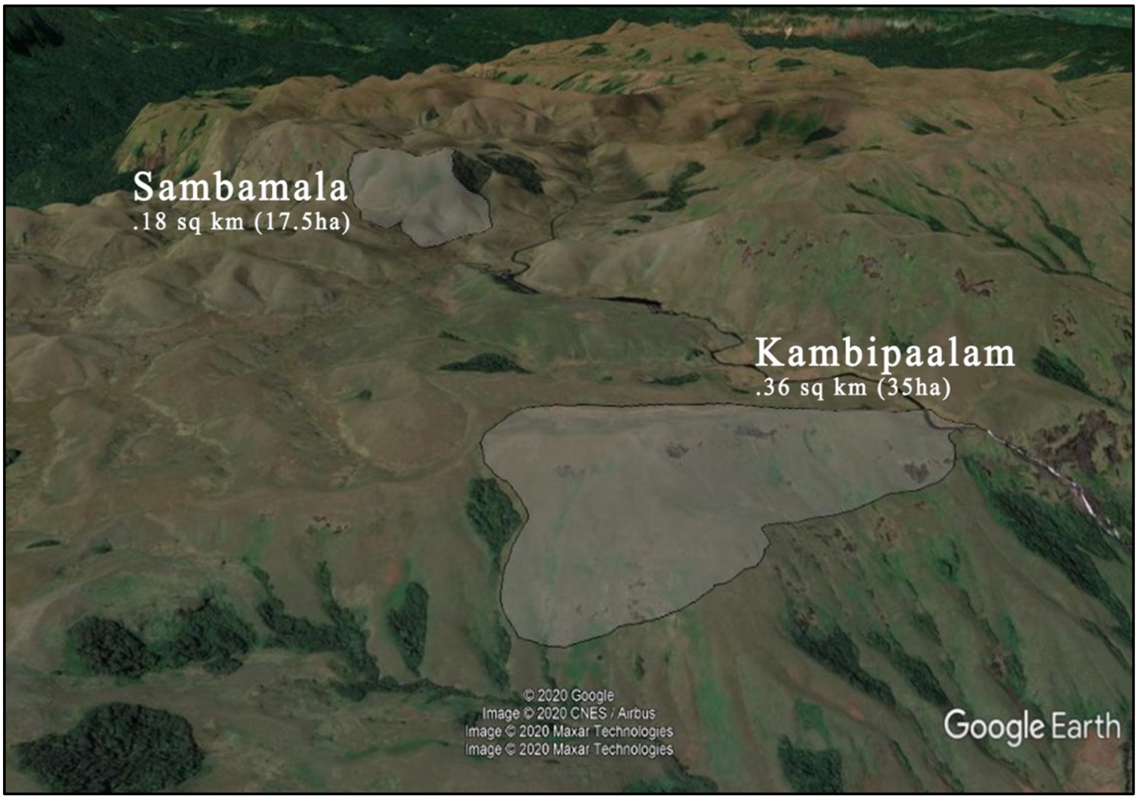

Figure 7. 3: Extent of area burnt in Sambamala and Kambipaalam

7.2.2. Sampling

Data on the abundance of amphibians in the grasslands of Eravikulam National Park that are subjected to controlled burning management practice was collected from 2015 to 2018. Eighteen 10 x 10 m quadrat plots including 6 pre-burned plots (before burning), 6 unburned plots (Control) and 6 burned plots (Experiment) were laid in the two hills Sambamala and Kambipaalam in ENP. In the chosen sites, a total of 17.5 ha was burnt on one aspect of the Sambamala hill where as a total of 35 ha area of the complete crest area of Kambipaalam was burnt. The control plots were laid in the hills adjacent to the areas that were not burnt atleast in the last 3 years. Data from pre-burned plots were collected an year before burning whereas burned plots were surveyed immediately after burning as well as during the breeding seasons. Amphibians are generally seen and encountered better during their breeding seasons and they are generally less active during other periods which can also effect the data collection. Hence all the plots were sampled during May-June months when amphibian activity was at its peak for understanding abundance of amphibians and the impact of burning on all four years starting from 2015 to 2018. Control plots (unburned) were taken alongside the experimental plots (burned). While 2015 data marked the unburned period, 2016 has data on the year of burning and data on post burning were taken in the years 2017 and 2018. Temperature data during fire incidents were recorded using thermocouples (TCAV-L).

In order to avoid recounting of animals as a result of movements between the plots, a minimum of 50 m distance was always maintained between all the plots sampled. Surveying the plot from two sides involving two persons (me and my field assistant) and walking towards the centre further avoided animals from escaping out of the plot, instead walk towards it. Animals encountered were bagged and kept in a moist cotton cloth bag and they were released immediately at the same site of capture soon after complete surveying of each plots.

The grasslands of Eravikulam National Park is home to six species of anurans and these species are unmistakable in the field. Doubtful animals were photographed and confirmed later based on the species description works (Boulenger, 1882 a and b; Biju *et al.,* 2011; Garg and Biju, 2016, Biju *et al.,* 2012, Vijayakumar *et al.,* 2014) and taxonomy follows Frost (2021).

7.2.3. Analysis

The data were collected on the number of individuals recorded (absolute abundances) per plots in all seasons from pre-burnt, unburnt and burnt plots. The data wereanalysed for the impact of burning on abundance of frogs by testing for significant difference in abundance (averaged across all years) between control plots (where no burning was performed) and burnt plots (where burning was performed) separately in Kambipaalam and Sambamala hills using two sample t-test assuming unequal variances. Attempts were also made to quantify an overall significant difference in abundance of frogs (averaged across all years) in control and burnt plots using one-way ANOVA for both the hills (Kambipaalam and Sambamala). Before using t-test and one-way ANOVA, the normality of datasets was tested using Shapiro-Wilk test. All statistical tests were performed using R 4.0.2 software.

7.3. Results

Frog abundance (t-test with unequal variances) between control and burnt plots was significant in both Kambipaalam (- $p < 0.01$) and Sambamala hills (- $p<0.01$; - $p<0.05$) except in 2015 (Figs. 7.4 & 7.6). When all the years were analysed together for significant differences in frog abundance between control and burnt plots using One-way ANOVA, significant differences were observed in control and burnt plots in both Kambipaalam and Sambamala hills (Figs. 7.5 & 7.7).

Burning caused approximately 10 times and 7 times decrease in frog abundance as compared to the control plots in Kambipallam Hills (- $p < 0.001$) and Sambamala Hills (- $p < 0.001$), respectively (Figs. 7.4 & 7.6). When averaged across all the years, burning caused approximately 2.5 times and 2 times decrease in frog abundance in Kambipallam Hills and Sambamala Hills respectively (Figs. 7.5 & 7.7). The impact of burning on individual species is also concordant to the above mentioned results where none of the species had a significant difference in the level of impact in comparison to the others.

There was no difference between control and experiment plots in the abundance of amphibians before burning (2015).

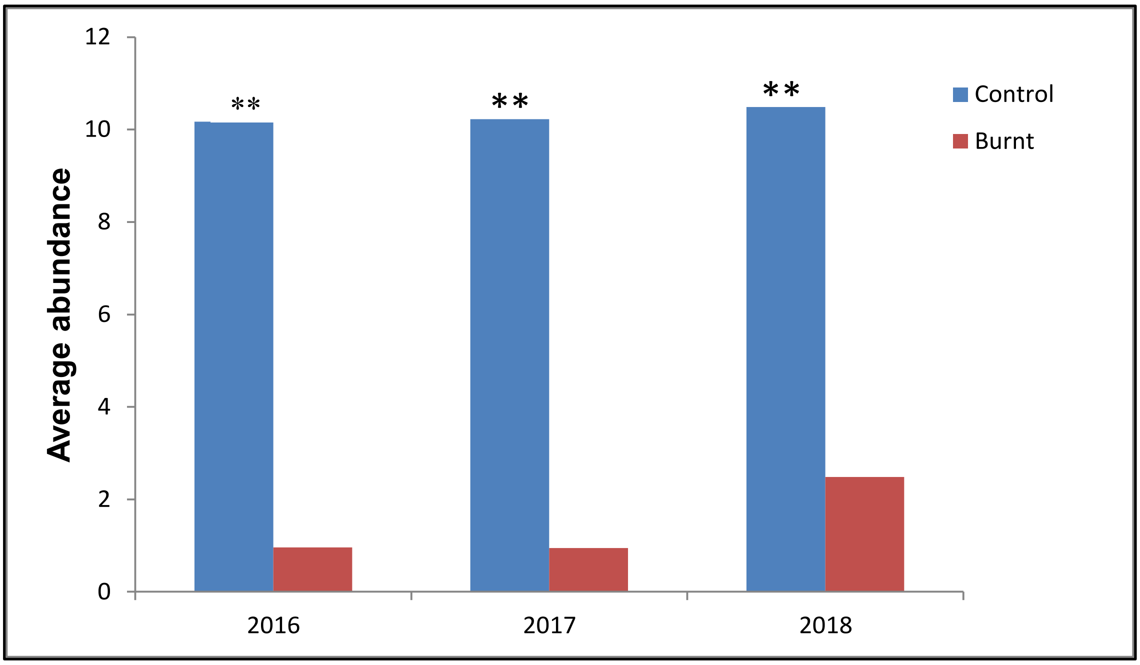

Figure 7. 4: Average abundance of frogs in control and burnt plots in Kambipaalam hill (- p < 0.01)**

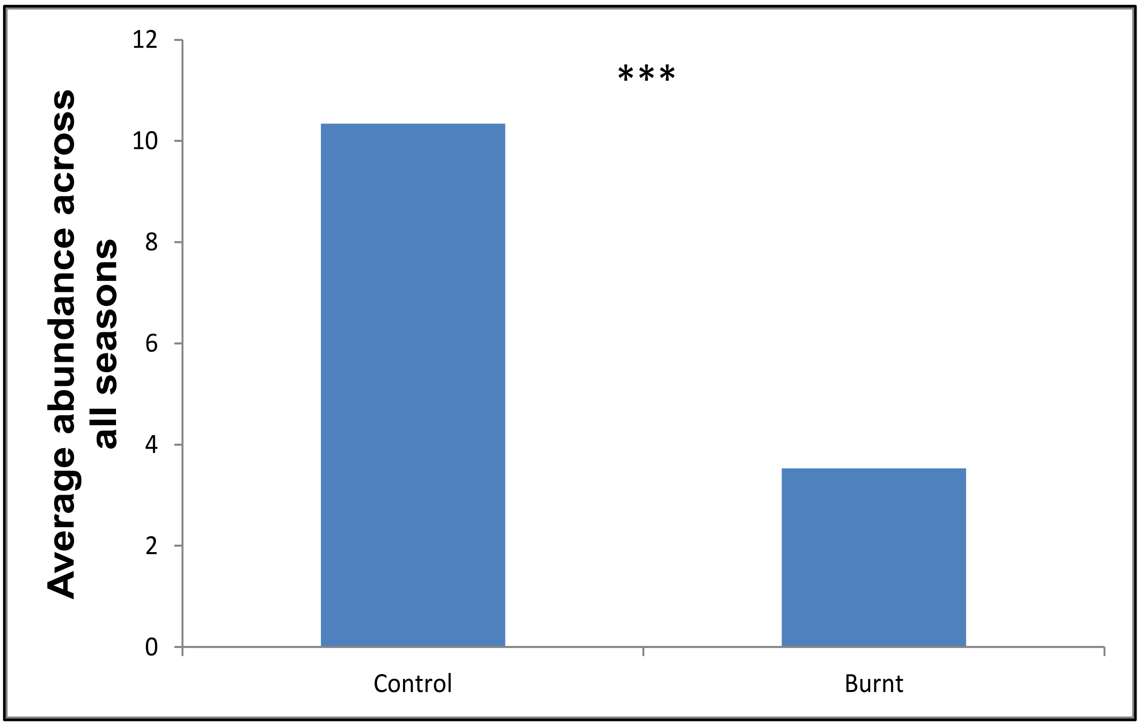

Figure 7. 5: Average abundance of frogs across all seasons in Kambipaalam hill (* - p < 0.001)**

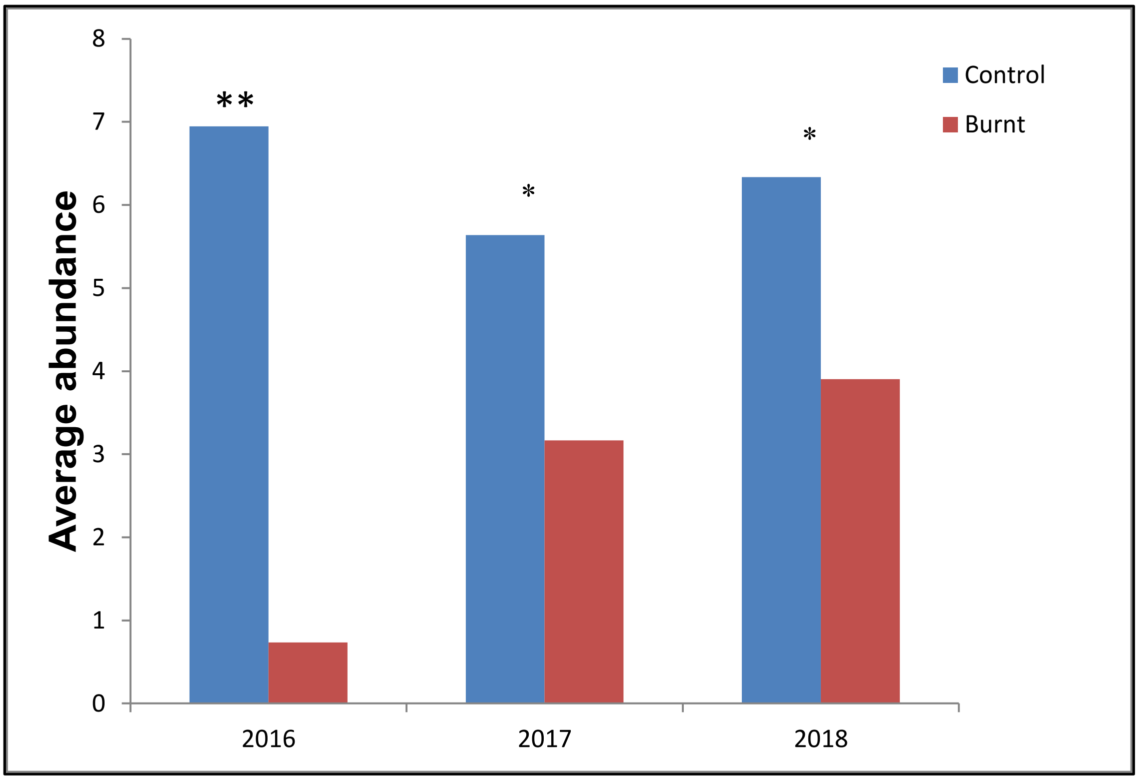

Figure 7. 6: Average abundance of frogs in control and burnt plots in Sambamala (- p<0.01; * - p<0.05)**

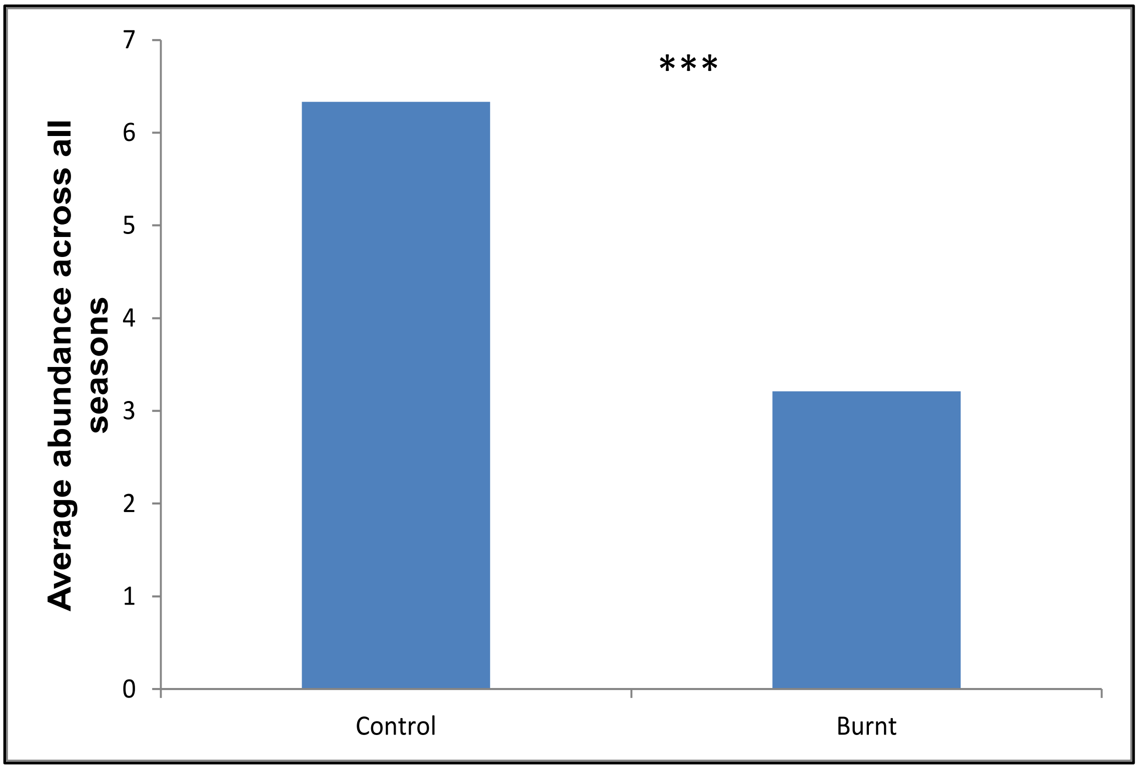

Figure 7. 7: Average abundance of frogs across all seasons in Sambamala hill (* - p < 0.001)**

The amphibian abundance remained more or less same in Kambipaalam hill for first year after burning (2017) and a slight increase in the amphibian abundance was recorded in the second year (2018) suggesting amphibian recolonisation. The frog abundance increased 0.98 times in 2017 and 2.6 times in 2018 from the initial abundance in 2016 and it was still not back to half of the initial stage even after the second year after burning (Fig.7.4). However, Sambamala hill which was only partially burnt, reported recolonisation at a better rate, 4.3 times more amphibian abundance in the first year (2017) post burning compared to 2016. It was gradually increased to 5.3 times more of the initial amphibian abundance in the next year(2018) (Fig. 7.6).

7.4. Discussion

Grasslands of Eravikulam National Park recorded less diversity in comparison to the Sholas and was found to have only six species of amphibians including *Duttaphrynus microtympanum, Nyctibatrachus deccanensis, Walkerana leptodactyla, Raorchestes beddomii, Raorchestes dubois* and *Raorchestes resplendens* out of total thirty six species recorded from ENP (Chapter IV) . Our results indicate that controlled burning does have a significant impact on these amphibians. The difference in decrease of amphibian abundance in response to burning in the two study sites, Kambipaalam and Sambamala concludes that there is a significant impact on large-scale burning. When the Kambipaalam hill was completely burned (35 ha), including the crest of the hill and all sides, Sambamala was burnt only on one side of the hill (17.5 ha). This difference is heavily reflected in the data where the decrease in average abundance of amphibians are too higher in Kambipaalam hill than Sambamala hill. Species recovery in both sites also depends on the extent of the area burnt to the physical properties of the hills such as habitat continuity, presence of marshy swamps and rock faces. In Kambipaalam hill, the rate of recovery of amphibian abundance was very low and slow compared to Sambamala hill which recorded comparatively better and faster recovery. This may have achieved by the recolonising of species through utilising the microhabitat connectivity from other aspects of the same hill. At the same time, Kambipaalam hill became more or less deserted owing to its near complete and large extent of burning. Burning in small patches or portions of the hill leaving pockets of habitats undisturbed can act as corridors and facilitate faster re-colonisation. However, the burnt plots never returned to the initial state of amphibian abundance even in the Sambamala hill where only a portion of the large hill was burnt. This emphasises the fact that controlled fires are indeed a threat to amphibians of ENP. Montane grasslands of ENP is unique in having narrow temperature ranges as well as its largely persistent wet and humid atmosphere. Amphibians living in such conditions will struggle to survive when subjected to the slightest of the changes in the rainfall patterns, soil moisture and micro climatic conditions.

Fire has direct and indirect effects on ecosystems and its animals. Direct effects are largely dependent on the severity of the burning and the duration of the fire (Neary *et al.,* 1999). Fire, even though controlled mostly lasts for longer durations from 4 to 5 hours depending on the size of the selected areas. While the threshold temperatures that can disrupt biological systems in soil during fire goes from a minimum of 49^0 c in small mammals to 94^0 c in mycorrhizae (DeBano *et al.,* 1998), the temperature recorded among the grass clumps during fire ranged from 300 to 360^0c. The grass clamps, one of the main refuges of the amphibians of ENP were recorded to have ~ 50 -100^0c inside, soon after the fire. However, the temperature dropped down to the atmospheric temperature in around 2 hour time since the burnings were mostly done during the winter months when the park experiences its minimal temperatures. Studies suggest that smaller animals sometimes exhibit panic at fire incidents and could result in higher rates of mortality (Lyon *et al.,*1978). Nonetheless, only a few dead individuals were encountered during the study period and it is assumed that a larger percentage of animals gets caught and burnt in the fast moving, long lasting and high temperature fire.

Amphibians moving through burnt areas and ashes towards unburnt areas were also recorded during the study. Immediately after the burning, the areas witnessed a lot of predators including several bird species such as Kestrel, Black-shouldered Kite, Common Buzzard, mongoose species such as Brown Mongoose and Stripe-necked Mongoose, Jungle Cat and Leopard Cats and many more. Small and slow moving animals including amphibians and reptiles who take refuge in the grass clumps suddenly become exposed to these predators and gets hunted. Hence the survivors of the fire will migrate to neighbouring microhabitats or towards wet and swampy marshes.

Figure 7. 8: *Raorchestes resplendens* moving through the ashes

Many pockets in the grassland areas post fire started to have invasion of ferns and the Western Ghat endemic *Strobilanthus kunthiana.* The effect of these invasions on amphibian communities are not clearly studied. But it could possibly create problems in the long run when the more exposed ferns take over rather closed microhabitat of grass clumps. The park has been experiencing continuous fire ever since it has been managed. The park has a long history of fire and we still don't have enough information on their influence on animals over a longer period of time.

There are cases where fires are beneficial for amphibians in maintaining habitats. If the fires are suppressed in the longleaf pine savannas, the habitat will be soon replaced by hard wood forests resulting in the decline of the amphibians present (Means *et al.,* 2004). Similarly, there are multiple studies on fire being beneficial for amphibian and reptile communities but none from the tropics (Mushinsky, 1984; Greenberg *et al.,* 1994; Litt *et al.,* 2001). A management practice suitable for one ecosystem might not produce the same result for all others. Systematic studies should be undertaken before implementing a management practice into conserving a habitat and it shouldn't be based on single species conservation and management. Nilgiri tahr is reported to benefit from the controlled burning practice and ENP is one of the protected areas in the Western Ghats with largest population of the species (Easa *et al*., 2010). Conserving the Nilgiri tahr at the cost of several other organisms and the ecosystem should be scientifically tested. Otherwise, the advantages of the management activity get outweighed by the disadvantages over a longer period. Study suggests small scale burnings of much longer intervals allowing enough time for the habitat and its animals to recover could ensure the benefit to most of the biota including less attended amphibians rather than a single species.

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Chapter VIII

CONCLUSIONS

CONCLUSIONS

Conservation of biodiversity is of utmost importance in the present world. Growing human populations along with other factors including global warming, habitat destruction and emergence of infectious diseases are negatively impacting life on earth at a much greater rate than ever before. Much of the conservation attention are focussed on large and charismatic animals while a vast majority of lesser life forms that significantly contribute to biodiversity are largely neglected.

Despite being one of the high diverse group among land vertebrates, amphibians face a greater level of threat towards extinction. Adapted to variety of microhabitats, they live in almost all ecosystems. Microhabitat specificity, dependence on water, temperature and other environmental variables makes them vulnerable to a greater level of threats making them the most threatened group of vertebrates on earth. As nature's best control agents of invertebrates, in having a pivotal role in the food chain as prey and predator, as indicators of healthy aquatic and terrestrial habitats and their use in the field of medicine makes them as important as the larger and charismatic animals of the ecosystem.

Conservation of amphibians have been receiving a lot of importance lately, owing to their alarming threat status. Amphibian conservation primarily requires detailed information whereas the group is still in a taxonomic flux and as a result the studies are focussed on the taxonomic aspect to a greater extent. Information on the ecology and behaviour of species and ecosystems are imperative in conservation.

Western Ghats, one of the hottest hotspots in the world supports more than half of total amphibian species in India and 90% of them are found nowhere else in the world. Even then the effort on amphibian conservation in this part of India is minimal. Positive changes involving initiatives like having flagship amphibians for protected areas and awareness campaigns had been happening in Kerala part of Western Ghats recently.

The current study on ecology and behaviour of amphibians was done in Eravikulam National Park. ENP is one of the most unique ecosystem in the Western Ghats. Also called as sky islands, that are geographically isolated by higher elevations, ENP holds the largest fragments of montane grasslands and shola forest ecosystem in the Southern Western Ghats(SWG). Like most of other areas, this protected area was also established primarily for conservation of the endangered Nilgiri tahr and its habitat. In fact the current management practices also are focussed on the same. Scientists have discovered several amphibian species from ENP but no studies on the ecology and behaviour was attempted on amphibians of the park. As a part of this study, diversity and distribution, temporal partitioning of vocalising activity of two species of sympatric bush frogs, vocalization study and the impact of prescribed burning management practice inside ENP were studied.

8.1. Documentation of diversity and distribution of Amphibians

The study reports thirty seven species of amphibians including a single record from the family Gymnophiona. Shola ecosystems were found to have 31 species that are exclusively found only in the sholas whereas grasslands had only one species (*Raorchestes resplendens)* exclusive to it. Shola has greater diversity with a total of 36 species compared to the grasslands with only 6 species. The unique climate conditions and high micro-habitat diversity accounts for the greater diversity in sholas. Grasslands, however seemed to support a greater abundance despite having a low diversity. The bush frog species adapted to live in bushes and shrubs, *Raorchestes dubois* and *Raorchestes* *resplendens* were the most abundant ones reported from the grasslands. Results includes 20 additional species to the list of amphibians from ENP based on the previous studies. First record of species such as *Nasikabatrachus sahyadrensis* which ranks 3rd globally in the in the list of evolutionarily distinct and globally endangered (EDGE) species, *Walkerana phrynoderma* at 22nd and *Melanobatrachus indicus* at 109th rank highlights the significance and the need for conservation of ENP. The most diverse family recorded was Rhacophoridae with 20 species and the genera *Raorchestes* with 15 species indicates high diversity in the arboreal microhabitats in the shola forests. Distribution of all 37 species found from the park was also mapped.. New elevational and distribution records of several species add to the current understanding. While 14 out of 37 species recorded belong to the threatened categories of IUCN Red List, another 17 are not even assessed. This emphasise the urgent need for updating the IUCN red list assessment so as to ensure conservation attention to the species. Molecular phylogenetic study on the species *Raorchestes ochlandrae* further helped in confirming its identity and in understanding the intraspecific variation between populations north and south of the gap.

8.2. Temporal Activity Pattern in vocalization of Bush Frogs

Vocalisation plays crucial role in amphibian breeding. They announce their presence to their mate and their rivals through their calls. However, species that co-occur in similar habitats with similar calls will face problems due to the overlapping of their calls. On such occasions multiple species when present together partition themselves temporally, so that they can be clearly heard and approached by potential mates. *Raorchestes dubois* and *Raorchestes resplendens*, two of the most abundant and sympatric species recorded from the grasslands of ENP was chosen to study the temporal activity pattern in vocalisation. Hundred-hour long study from 10 plots shows the influence of activity pattern of one species on the other. When *Raorchestes resplendens* was observed to have a single peak in its calling activity, *Raorchestes dubois* had two peaks without overlapping the peak of the first. Present study is the first attempt from ENP and will act as foundation to similar studies on temporal activity pattern in acoustic signalling behaviour of bush frogs.

8.3. Call repertoire study of Critically endangered *Raorchestes resplendens*

Vocalisation of amphibians has a pivotal role in amphibian systematics and conservation. Call of amphibian species are widely used in taxonomic descriptions as it is unique to individual species and can be used as a tool towards amphibian identification. Calls that sound similar to human ears might be different to amphibians and this helps them in distinguishing one another. Call characteristics of critically endangered and montane grassland endemic species *Raorchestes resplendens* was studied from ENP. They live inside grass clumps and are largely terrestrial. The species was considered rare and found only from 2 locations within ENP and this is largely attributed to the habitat they live in and the difficulty in locating them. But with the detailed study of the call, the presence and absence of the species was made easier, and it further helped in recording the distribution of the species from several locations from ENP.

8.4. Impact of prescribed fire management practices on Grassland inhabiting amphibians of Eravikulam National Park

Eravikulam National Park is subjected to large-scale controlled burning as a part of management practice mostly to provide food for the charismatic mammal, Nilgiri tahr. Even though management practices focussed solely on single and large charismatic species has been negatively criticized since a long time, such practices still exist in different parts of the world.

In the case of ENP itself, the practice of cold burnings in the winter months has a long history without much understanding on the impact on other floral and faunistic elements in the park. Recent studies suggest significant negative impact on soil and on grasshopper populations in ENP. The present study looked at the consequences of burning on amphibian populations of the grasslands of the park. Results indicate a significant decrease in amphibian populations in both study areas from the park. However, the rate of decrease in amphibian abundance varied with the size, aspect and habitat contiguity. Smaller patches with proper habitat connectivity showed less impact on populations compared to large areas with less habitat connectivity. Burnt areas experience a sharp decline soon after the burning. Sampling plots with sufficient habitat contiguity showed better and faster recolonization of amphibians compared to larger areas that were burnt. Our results are also in agreement to previous studies on impact of controlled burning in grasslands of ENP and suggest minimising the damage by reducing the extent of burning to smaller plots and supporting the system to revive by choosing areas with habitat connectivity.

8.5. Recommendations

- Eravikulam National Park is rich in terms of amphibian diversity. Some of the species recorded from the study were rarely encountered. Seasonal surveys and long-term monitoring of amphibians are also essential in their conservation.
- Conservation attention should be given to *Raorchestes resplendens* as ENP is the largest contiguous habitat for the species. Management activities including

controlled burning, maintenance of fire lines and trek paths should be done with proper care without affecting the habitat of the species.

- Reducing the size of areas subjected to controlled burning practice as well as increasing the burning intervals are suggested to minimize the impact and support recolonisation
- It is also suggested to have an amphibian ambassador to the park to create awareness and raise interest among the general public and the park managers in order to have a positive behavioural change towards amphibian conservation

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COMMUNICATION

New locality records and call description of the Resplendent Shrub Frog *Raorchestes resplendens* (Amphibia: Anura: Rhacophoridae) from the Western Ghats, India

Sandeep Das [1], K.P. Rajkumar [2], K.A. Sreejith [3], M. Royaltata [4] & P.S. Easa [5]

[1–5] Forest Ecology & Biodiversity Conservation Division, Kerala Forest Research Institute, Peechi, Kerala 680653, India.
[1,2] University of Calicut, Thenhipalam, Malappuram District, Kerala 673635, India.
[1] sandeep.koodu@gmail.com (corresponding author), [2] rajkp16@gmail.com, [3] kalpuzhasreejith@gmail.com, [4] royalgis.gdr@gmail.com, [5] easaelephant@yahoo.com

Abstract: The Resplendent Shrub Frog, *Raorchestes resplendens* Biju, Shouche, Dubois, Dutta, & Bossuyt, 2010 is a Critically Endangered species endemic to the Western Ghats and was considered to be restricted to a three-square kilometer patch atop Anamudi summit. In this study, we report 36 new locations of the species from the Anamalai massif of the southern Western Ghats. Niche-based prediction modelling suggests that the species is restricted to Anamalai massif. The call description of this frog is also provided for the first time. The preferred microhabitat of the frog is *Chrysopogon* grass clumps in the marshy/swampy montane grassland ecosystem. Restricted to a small area with controlled burning management practiced in its habitat, *R. resplendens* needs immediate attention.

Keywords: Anamalai, Critically Endangered, ground-dwelling bush frog, new distribution record, vocalization.

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Author details: Sandeep Das is Research Scholar at the Kerala Forest Research Institute, Peechi. He has been working on amphibians of Kerala since 2010 and is interested in their ecology and behavior. He is also a ZSL EDGE fellow working on the endangered Purple Frog *Nasikabatrachus sahyadrensis*. Rajkumar, K.P. is a Research Scholar at the Kerala Forest Research Institute, Peechi. His current work deals with the faunal and floral inventory of marshy grasslands and diversity in Kerala. He is also a ZSL EDGE fellow working on the Galaxy Frog Melanobatrachus indicus. Dr. Sreejith K.A. is actively involved in field oriented studies on ecosystem dynamics of tropical forests in the Western Ghats. By maintaining and monitoring a large number of permanent plots, he is trying to elucidate the spatial and temporal variation of biodiversity and its dynamics. Royaltata, M. is a Research Scholar at the Kerala Forest Research Institute, Peechi. With experience in GIS and Remote Sensing, he is interested in their application in forest management, species distribution, niche-modelling,assessment of soil water and SWAT in the states of Tamil Nadu and Kerala. Dr. Easa, P.S. has nearly forty years of experience in wildlife research, conservation and management, and has worked on diverse groups of animals. He has been with the Kerala Forest Research Institute, Peechi and is also associated with a number of Research programs of numerous national and international institutions

Author contribution: PSE & SD conceived the study; SD, KPR carried out field work; all authors equally contributed to the data compilation, analysis and writing the manuscript

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INTRODUCTION

There are currently 8,134 described species of amphibians (Frost 2020) and an average of 144 species described every year starting from 2004–2015 (Tapley et al. 2018). At the same time, amphibians are the most threatened group of vertebrates with 41% of the total assessed species under threatened categories (IUCN 2016). Considering the total number of new species described between 2004 and 2016, India ranks second globally with 155 species (Tapley et al. 2018). Of these, 75% are from the Western Ghats and Sri Lanka Biodiversity Hotspot (Myers et al. 2000; Mittermeier et al. 2004). One of the most diverse groups of frogs in India with the greatest number of species described since 2004 is the genus *Raorchestes* known to be a genus of direct developing rhacophorid frogs (Biju et al. 2010).

Raorchestes resplendens Biju, Shouche, Dubois, Dutta, & Bossuyt, 2010 is a Western Ghats endemic, medium-sized, ground-dwelling bush frog. Its prominent orange colouration and large glands, bordered with black make it distinct from other species of *Raorchestes*. The species belongs to the *beddomii* clade (Vijayakumar et al. 2014) and is restricted to the Anamalai massif of Western Ghats. The species is known from only from its type locality, a three square kilometer patch of habitat on the Anamudi summit, the highest peak (2,695m) in Western Ghats in Eravikulam National Park (ENP) and a site approximately 20km north-east of Anamudi summit (Joseph et al. 2012). Joseph et al. (2012) suggested the possibility of a wider distribution of the species within ENP. *Raorchestes resplendens* is assessed as Critically Endangered (IUCN SSC Amphibian Species Specialist Group 2011).

In this study, we provide information on the distribution of the species inside and outside the protected area network based on surveys undertaken in 2015–2018. In addition, we also predict the probable distribution of the species using niche-based modelling. We also provide the first ever description of the vocalization of *R. resplendens.*

STUDY AREA

Eravikulam National Park (ENP, 10.083–10.333 ^{0}N & 77.00—77.166 ^{0}E) in Kerala, India. This 97km^2 national park is one of the few remaining undisturbed patches of the montane shola-grassland ecosystem in the Western Ghats. The high elevation protected area located in the Kannan Devan Hills of Idukki District has a base elevation of approximately 2,000m. ENP experiences tropical montane climate with average annual rainfall of 5,000–6,500 mm. More than 60% of the park area is dominated by grasslands with shola patches in the valleys.

MATERIALS AND METHODS

A combination of survey methods including visual encounter surveys, call surveys, and scan searches (Heyer et al. 1994; Krishnamurthy 2003; Halliday 2006) were used between January 2015 and December 2018 to document the distribution of *R. resplendens*. During the breeding season (May–September), surveys were undertaken from 18.00–02.00 h, as bush frogs are known to be most active at night (Biju et al. 2010). Morning and evening surveys were conducted from 08.00–13.00 h and 14.00–17.00 h to record diurnal activity, if any. Surveys were done in shola-grassland ecosystems above 1,700m especially inside ENP from where the species was first described and reported. To avoid repeated count and getting maximum distribution range of the species the surveys were spatially replicated.

Calls of *R. resplendens* were recorded at approximately 0.5m distance using ZOOM H4nSP Handy Recorder from four locations in ENP, including Anamudi, Kolukan, Bheemanoda, and Sambamala area. Ten to 20 calls were recorded for each individual (n=10 males). Ambient temperature and snout vent length (SVL) was taken immediately after the recording using Kestrel 3500 hand-held weather station and a Mitutoyo digital vernier caliper. Analyses of the calls were done using Raven v1.4 software (Cornell Laboratory of Ornithology, Ithaca, NY, USA) (Bee et al. 2013a,b; Thomas et al. 2014). Temporal and spectral parameters of calls were measured following definitions of Bee et al. (2013a,b). Six call properties: call duration (ms)—time between the beginning of first pulse and the end of last pulse in a call; call rise time (ms)—time between the beginning of first pulse and the peak of pulse of maximum amplitude; call fall time (ms)—time between the peak of pulse of maximum amplitude and end of last pulse; inter-call interval—time between end of a call to the beginning of the next call; call rate—number of calls delivered per minute; and overall dominant frequency were analyzed for the current study.

Prediction of distribution and calculation of extent of occurrence (EOO): Maximum entropy species distribution modelling software (Maxent) version 3.4.1 was used to predict the distribution of *R. resplendens* in Anamalai Hills. We used approximately 30 arc seconds of data for altitude, precipitation, average temperature and 19 bioclimatic variables available at the WorldClim website (http://www.worldclim.org/); 30-m resolution raster dataset layers were georectified to WGS 1984 43 North Zonation. Geographical coordinates and elevation of each location were recorded using Garmin Montana 680 and a map with sight records and the potential distribution was plotted using ArcGIS. The EOO and area of occupancy (AOO) (IUCN 2012) were calculated using the geospatial conservation assessment tool, GeoCAT (Bachman et al. 2011). The EOO was also calculated from species distribution model by overlaying fishnet squares over the prediction map. Each square covered an area of 4km^2. Squares with medium, high, and very high prediction values were included to calculate the EOO since there were no records of the species from areas of medium to very low prediction even after intensive surveys.

RESULTS AND DISCUSSION

Prior to our study the Critically Endangered (CR) *R. resplendens* (Image 1) was known to occur only inside ENP from two locations, Anamudi summit and Poovar. The present study reports 36 new locations for the species including four from outside ENP (Table 1 and Image 2). The four new locations outside ENP are Njandalamala of Chinnar Wildlife Sanctuary, a location south-east of ENP in Munnar Forest Division, a location near the south-west boundary of ENP in Munnar Forest Division, and one location in the adjacent Anamalai Tiger Reserve of Tamil Nadu lying close to the north-west boundary of ENP. The record from near Konalar, Anamalai Tiger Reserve is the lowest elevational record (1,896m) for the species whereas Anamudi Peak (2,695m) is the highest. The previously reported lowest elevational record was from Poovar (2,522m).

During the three-year study period from within ENP limits *R. resplendens* was encountered 637 times. This makes the species the second most encountered *Raorchestes* species in the grasslands of ENP after *Raorchestes dubois* (1,438 times). The unique ground-dwelling habit favored by *R. resplendens* could be the reason they evaded researchers for such a long time. They seem to be very sensitive to light and retreat into grass clumps whenever there is an artificial source of light. Contrary to the tiny bamboo thicket (*Arundinaria densifolia*) habitat preferred by the *R. resplendens* recorded on Anamudi summit, the majority of the individuals observed elsewhere were found actively calling and breeding in marshy/swampy grasslands (Image 3) alongside a water source in the valleys of the montane grasslands rather than on peaks.

At 21.20h on 28 May 2015, a single male was observed calling within a grass clump (*Chyrsopogon* sp.), 5cm above the ground at a marshy area on the base of Sambamala Hill (Image 1). Further investigation resulted in reporting 21 individuals (14 calling males and 7 females) on the same day from the same habitat patch. A single male specimen was collected and preserved in the wildlife museum of Kerala Forest Research Institute,

Image 1. *Raorchestes resplendens* in its habitat.

Table 1. Sighting locations of *Raorchestes resplendens* from southern Western Ghats.

	Location	Area	Lat.	Long.	Elevation
1	Njandalamala	Chinnar WS, KL	10.313642°	77.141561°	2346m
2	Munnar Division	Munnar Forest Division, KL	10.093747°	77.202883°	2587m
3	Rajamala Tourism Zone	Eravikulam, KL	10.143794°	77.037753°	1905m
4	Naaykollimala	Eravikulam, KL	10.142961°	77.036047°	1909m
5	Wireless Station Rajamala	Eravikulam, KL	10.149767°	77.044744°	2238m
6	Umayamala	Eravikulam, KL	10.163153°	77.072042°	2169m
7	Mesthirikettu	Eravikulam, KL	10.184550°	77.088272°	2174m
8	Range Point	Eravikulam, KL	10.187094°	77.085794°	2203m
9	Bheemanoda	Eravikulam, KL	10.195603°	77.084517°	2228m
10	Kallupaalam	Eravikulam, KL	10.194811°	77.077353°	2243m
11	Kallupaalam 2	Munnar Forest Division, KL	10.190761°	77.072839°	2173m
12	Bheemanoda 2	Eravikulam, KL	10.196908°	77.086600°	2204m
13	Bheemanoda 3	Eravikulam, KL	10.192550°	77.090950°	2200m
14	Varayattumala 1	Eravikulam, KL	10.204817°	77.085856°	2212m
15	Varayattumala 2	Eravikulam, KL	10.208128°	77.088392°	2237m
16	Kambipaalam Mala	Eravikulam, KL	10.217369°	77.081100°	2216m
17	Eravikulam	Eravikulam, KL	10.209414°	77.075336°	2199m
18	Eravikulam 2	Eravikulam, KL	10.218831°	77.078683°	2178m
19	Eravikulam 3	Eravikulam, KL	10.221906°	77.079378°	2156m
20	Sambamala Base	Eravikulam, KL	10.216506°	77.071711°	2200m
21	Sambamala	Eravikulam, KL	10.213450°	77.065103°	2266m
22	Anamudi View Near Kolukan	Eravikulam, KL	10.218089°	77.059017°	2229m
23	Kolukkan	Eravikulam, KL	10.227481°	77.047964°	2110m
24	Campamala	Eravikulam, KL	10.225033°	77.074289°	2329m
25	Erumapetti	Eravikulam, KL	10.231128°	77.089286°	2269m
26	Turners Valley	Eravikulam, KL	10.222319°	77.089286°	1901m
27	Chinna Mannumudi	Eravikulam, KL	10.228486°	77.094269°	2247m
28	Kudimala	Eravikulam, KL	10.215919°	77.109719°	2049m
29	Near Varattukulam	Eravikulam, KL	10.236183°	77.100469°	2182m
30	Kaatumala	Eravikulam, KL	10.254211°	77.097894°	2526m
31	Kaatumala 1	Eravikulam, KL	10.258489°	77.101667°	2271m
32	Kaatumala 2	Eravikulam, KL	10.267222°	77.090308°	2050m
33	Poovar 1	Eravikulam, KL	10.286419°	77.084633°	1984m
34	Konalar	Grass Hills, TN	10.321906°	77.070497°	1896m
35	Border Grass Hills	Eravikulam, KL	10.309903°	77.092350°	2096m
36	Border Chinnar	Eravikulam, KL	10.299444°	77.113611°	2092m
37	Poovar (Previous record)	Eravikulam, KL	10.273414°	77.086064°	2040m
38	Anamudi (Previous record)	Eravikulam, KL	10.168367°	77.059954°	2695m

KL—Kerala | TN—Tamil Nadu | WS—Wildlife Sanctuary.

Peechi, Kerala (KFRI/WLM/A0035). The size was small in comparison with the details given in published information and from those field-measured earlier during the study. The measurements of the preserved specimens are as follows: snout vent length (SVL) 20.76mm small; head slightly wider than long (HW) 7.88mm, (HL) 7.44mm; snout length (SL) 2.63mm larger than horizontal diameter of the eye (EL) 2.43mm; snout

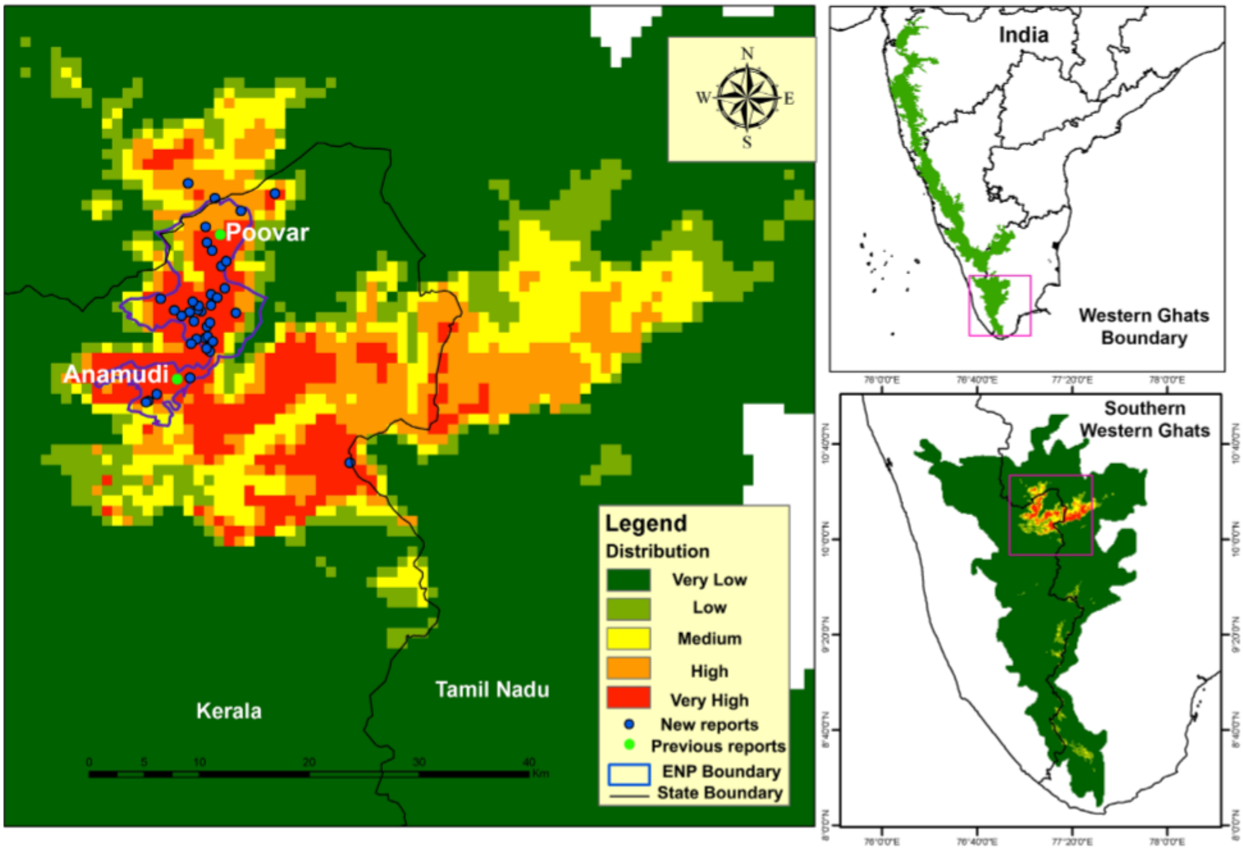

Image 2. Distribution of the *Raorchestes resplendens* and prediction based on niche-modelling.

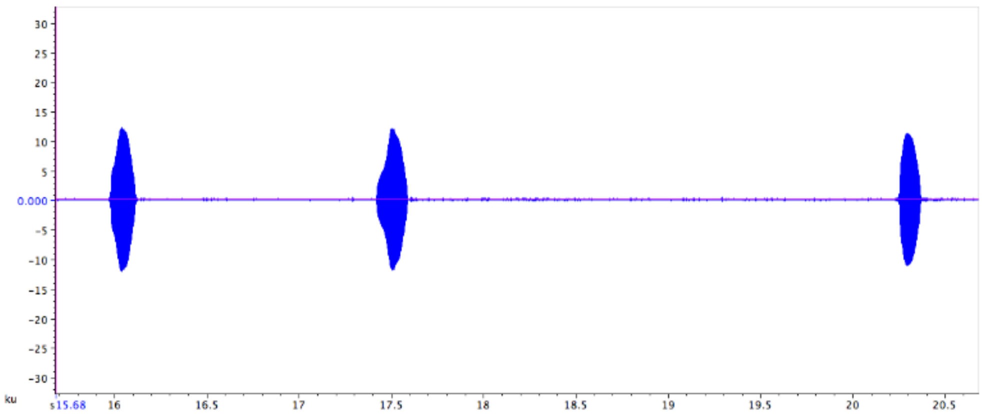

Figure 1. Wave form of *Raorchestes resplendens* call in 5s time frame.

rounded in dorsal view; minimum distance between upper eyelids (IUE) is 2.82mm and maximum width of upper eyelid (UEW) is 1.43mm. Distinct and rounded tympanum. Forelimb (FLL) 4.44mm shorter than hand length (HAL) 4.733mm; fingers with discs and distinct circum-marginal grooves; webbing absent on fingers and absence of nuptial pads. Unlike many of the species in the genus *Raorchestes*, the hind limbs are moderately short for this species; shank length (ShL) 5.37mm shorter than thigh length (TL) 7.01mm; foot length (FOL) 7.06mm shorter than distance from the base of inner metatarsal tubercle to the tip of toe IV. Toes with discs and distinct circum-marginal grooves and reduced webbing. Dorsum with large orangish glands whereas the creamy white ventrum is granular.

Call Description

Raorchestes resplendens males were observed

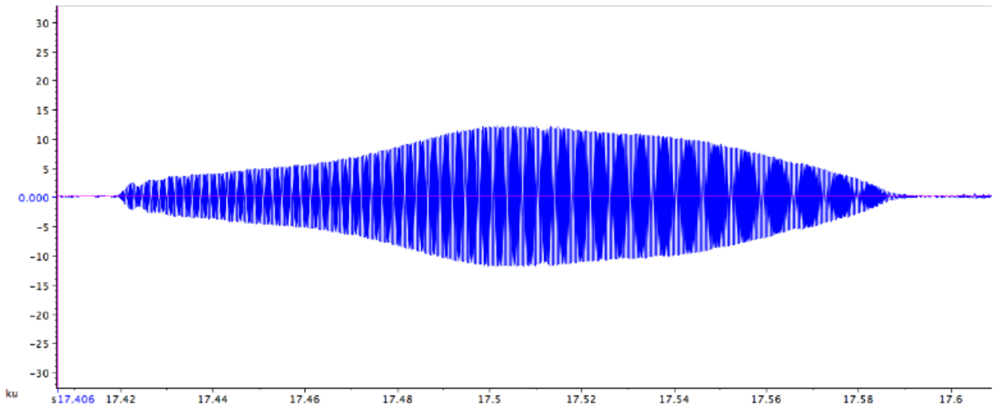

Figure 2. Wave form of *Raorchestes resplendens* call in 2s time frame

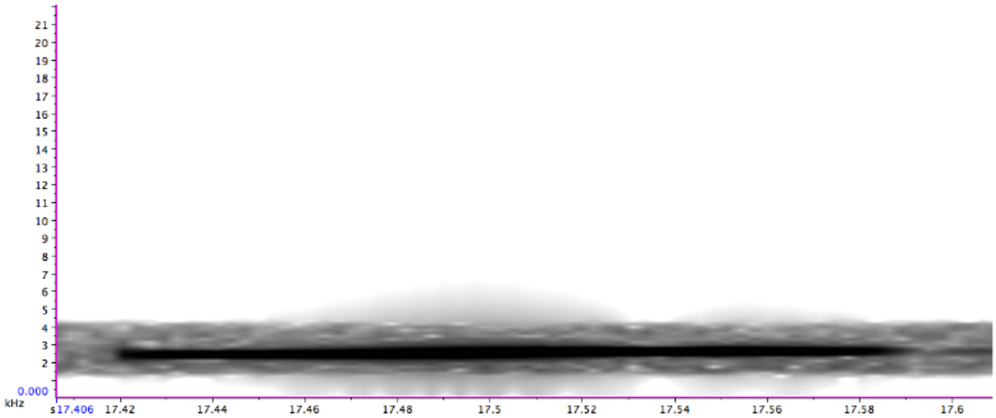

Figure 3. Spectrogram of *Raorchestes resplendens* call

actively calling from 18.00–02.00 h, during their peak breeding season in May–September. A total of 141 calls from 10 males were analyzed for the description of vocalization. Temperature ranged between 16–20 °C during all recordings. Calls were relatively simple (Figure 1 & 2). The advertisement call (https://doi.org/10.6084/m9.figshare.12781229.v1) had non-pulsatile temporal structure unlike published calls of other bush frogs including *Raorchestes graminirupes*, *R. flavіocularis, R. silentvalley, R. lechiya, R. travancoricus*, and *Pseudophilautus kani* (Bee et al. 2013a,b; Vijaykumar et al. 2014; Rajkumar et al. 2016; Zachariah et al. 2016). Advertisement calls typically ranged between 58.9–148.8 ms in duration (Table 2). On an average, the interval between two calls was 2.9 ± 3.6 s, and these intervals were uncorrelated with SVL or mass (Table 2). The call rise time ($\overline{X}$ = 46.3 ms ± 29.4 ms; Table 2) was slightly shorter than call fall time ($\overline{X}$ = 56.7 ms ± 16.8 ms; Table 2). The calls were typically delivered at rate of 21.5 calls/minute (Table 2).

The spectrum was characterized by single broad peak with mean dominant frequency of 2.5 KHz (Figure 3, Table 2).

Distribution

The niche-based prediction model of distribution in the southern Western Ghats suggests that the species is restricted to montane grasslands of Munnar-Valparai area of Anamalai massif. The EOO and AOO calculated using GeoCAT are 289km^2 and 84km^2, respectively. The approximate EOO calculated based on the prediction using minimum convex polygon was ~272km^2 with the majority of the area being within ENP and the calculated EOO does not include areas where our model suggested a low, very low likelihood of occurrence as there were no actual observations of the species in these areas (Image 2). The species habitat is well-protected as its distribution largely occurs within protected areas. The areas outside the protected area network owned by the Kerala Forest Department where the species occurs could be further designated as eco-sensitive zones to prevent management-based habitat modifications

Table 2. Call characteristics of 141 calls of *Raorchestes resplendens* from 10 males.

Call character	Mean	SD	Minimum	Maximum
Call duration (ms)	103	37.3	58.9	148.8
Call rise time (ms)	46.3	29.4	11.9	80.5
Call fall time (ms)	56.7	16.8	39.9	69.8
Intercall interval (s)	2.9	3.6	1.4	4.9
Overall dominant frequency (KHz)	2.5	0.1	2.4	2.8
Call rate (calls/min)	21.5	7.9	16.1	41.4

(Kanagavel et al. 2018). The absence of the species at Anamudi National Park and adjacent areas could be due to the absence of grassland habitats.

The report of the species from areas other than Eravikulam National Park including Chinnar Wildlife Sanctuary, grass hills of Anamalai Tiger Reserve, and areas of Munnar Forest Division ensures better conservation possibilities as these areas are under protection by the Kerala and Tamil Nadu forest departments. Controlled cold burning of grasslands in November–February months before the grass gets dry (Image 4), practiced as a part of habitat management programme in Eravikulam National Park (Kerala Forests & Wildlife Department 2013), is observed to be detrimental to slow-moving reptiles and amphibians due to mortality during the fire and exposed habitat without thick grasses (Image 5) after fires. It was also observed that the mortality is

Image 3. Marshy grassland habitat of *Rarochestes resplendens*.

Image 4. Control burning in montane grasslands.

Image 5. *Raorchestes resplendens* moving through burnt grassland.

comparatively less and recolonization in smaller animals is faster in areas where mosaic pattern is followed while burning (Bhaskar et al. 2019). A further reduction in the size of the burnt areas in mosaic pattern would ensure better protection to the herpetofauna. More sampling efforts and systematic approach is required to understand more about the specific threats faced by the *Raorchestes resplendens*. The management practice of controlled burning, however, might be a threat that needs immediate attention which is specific to ENP, one of its major habitat.

Information on the call of the species will be helpful in further studies as the species is very hard to detect which might be the possible reason for detecting the species from only two locations after the initial description of species in 2010 and the knowledge of the distribution extent can lead to proper conservation action plans for the Critically Endangered species.

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Short Communication

A checklist of amphibians of Kerala, India

Sandeep Das

Forest Ecology and Biodiversity Conservation Division, Kerala Forest Research Institute (KFRI), Peechi, Kerala 680653, India
sandeep.koodu@gmail.com

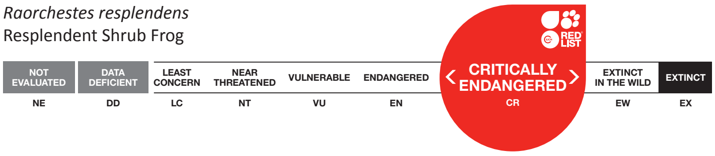
Abstract: A checklist of amphibians of Kerala State is presented in this paper. Accepted English names, scientific binomen, vernacular names in Malayalam, IUCN conservation status, endemism, Indian Wildlife (Protection) Act schedules, and the appendices in the CITES, pertaining to the amphibians of Kerala are also given. The State of Kerala has 151 species of amphibians, 136 of which are endemic to Western Ghats and 50 species fall under the various threatened categories of IUCN.

Keywords: Endemism, CITES, Malayalam name, vernacular name, Western Ghats, Indian Wildlife (Protection) Act.

Globally, there are about 7,356 known amphibian species belonging to three living orders (Frost 2015). From January 2004 until March 2015 the world has witnessed the discovery and description of 1,786 species of amphibians (Amphibia Web 2015). At the same time, 36 species of amphibians are extinct and over 1,957 species are threatened (Monastersky 2014). The golden era of amphibian systematics in India was during the British Raj from the 1850s to 1925 (Biju 2001), followed by the period from 2000 to 2015 (Amphibia Web 2015), when vast number of amphibians were described from our country. Of the 384 species found in India (Dinesh et al. 2015), 154 were described between 2000 and March 2015, among which 111 are from the Western Ghats. At the current pace, with new technologies, tools, and more taxonomists working on amphibians in the country, it is likely that several new species will get described in the near future.

Dutta (1997) published the first checklist on Indian amphibians with 212 species. Later Das & Duttta (1998) updated the list with English names. Another update on the list was done by Daniels (2001). Chanda (2002) was the first to publish a Handbook on Indian Amphibians with brief accounts followed by Daniels (2005) with 238 species of amphibians from Peninsular India in his book *Amphibians of Peninsular India*. Dinesh et al. (2009) published an annotated checklist of amphibians of India as an occasional paper of Zoological Survey of India with a total of 248 species including all new species described till 2009. Gururaja (2012) published a pictorial guide to

Raorchestes resplendens
Resplendent Shrub Frog



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frogs and toads of the Western Ghats. Dinesh and his colleagues have periodically updated the checklist of amphibians of India and a recent updated version of the list was made available online in 2015 with 384 species.

Studies on amphibians in Kerala date back to the descriptions in *Fauna of British India* volumes by Boulenger (1882, 1890, 1892), followed by a list of *Batrachians of Travancore* (Kanyakumari to present Munnar (Devikulam Taluk)) by Ferguson (1904) and tadpole descriptions by Annandale & Rao (1916, 1917). Other significant work on amphibians of Kerala are Pillai & Pattabiraman (1981, 1990), Inger (1984), Biju (2001), Biju & Bossuyt (2003), Easa (2003) and Sivaprasad (2013).

Since then several taxonomic revisions and new species have been described by Biju & Bossuyt (2005a, 2005b, 2005c), Biju et al. (2007), Gururaja et al. (2007), Biju & Bossuyt (2009), Biju et al. (2010), Zachariah et al. (2011a, 2011b), Biju et al. (2011), Abraham et al. (2013), Biju et al. (2014a, 2014b), Vijayakumar et al. (2014), Peloso et al. (2014). In addition, a checklist of amphibians of Kerala, with 104 species was published by Dinesh et al. (2010). Seventy-three species of amphibians have been described from India after 2010 and no updated checklists on the amphibians of Kerala have been made since 2010. The present paper attempts to provide an exhaustive, comprehensive and up-to date list with valid nomenclature of amphibians known from Kerala. The taxonomy and nomenclature follows Frost (2015). Majority of the vernacular names are freshly coined as there were no known existing names.

In this monograph, 151 species of amphibians in 11 families and two orders are listed. Out of which 136 are endemic to Western Ghats. Fifty species of amphibians of Kerala, fall under the various threatened categories of IUCN, and five are Near Threatened. Nineteen species fall under the schedules IV of Indian Wildlife (Protection) Act and two species come under appendix II of CITES.

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Table 1. The checklist of amphibians of Kerala

	English name	Species name	Authority	Malayalam name	Vernacular name	IUCN	END	WPA	CITES
	I. ORDER ANURA								
	1. Family BUFONIDAE (toads)								
1	Beddome's Toad (Travancore Toad)[1]	*Duttaphrynus beddomii*	(Gunther, 1875)	തെക്കൻ ചൊറിത്തവള	Tekkan Cheāṟittavaḷa	EN	WG		
2	Common Indian Toad (Common Asian Toad)[1]	*Duttaphrynus melanostictus*	(Schneider, 1799)	ചൊറിത്തവള	Cheāṟittavaḷa	LC			
3	Small-eared Toad (Southern Hill Toad)[1]	*Duttaphrynus microtympanum*	(Boulenger 1882)	ചെറുചെവിയൻ	Cheṟuceviyan	VU	WG		
4	Ridged Toad (Indian Toad)[1]	*Duttaphrynus parietalis*	(Boulenger, 1882)	കാട്ടു ചൊറിത്തവള	Kāṭṭucheāṟittavaḷa	NT	WG		
5	Ferguson's Toad[1]	*Duttaphrynus scaber*	(Schneider, 1799)	കുഞ്ഞൻ ചൊറിത്തവള	Kuññan Cheāṟittavaḷa	LC			
6	Silent Valley Toad (South Indian Hill Toad)[1]	*Duttaphrynus silentvalleyensis*	(Linnaeus, 1758)	സൈലൻറ് വാലി ചൊറിത്തവള	Silent Valley Cheāṟittavaḷa	DD	KL		
7	Malabar Torrent Toad (Black Torrent Toad , Ornate Toad)[2]	*Ghatophryne ornata*	(Gunther, 1876)	തീവയറൻ നീർചൊറിയൻ	Tīvayaṟan Nīrceāṟiyan	EN	WG		
8	Red Stream Toad (Kerala Stream Toad)[3]	*Ghatophryne rubigina*	(Pillai & Pattabiraman, 1981)	ചെമ്പൻ അരുവിയൻ	Chempan Aruviyan	VU	WG		
9	Malabar Tree Toad (Warty Asian Tree Toad)	*Pedostibes tuberculosus*	Gunther 1875	മരചൊറിയൻ	Marachēāṟiyan	EN	WG		
	2. Family DICROGLOSSIDAE (fork-tongued frogs)								
10	Skittering Frog (Indian Skipper Frog)[4]	*Euphlyctis cyanophlyctis*	(Schneider, 1799)	ചാട്ടക്കാരൻ	Chāṭṭakkāran	LC		Sch. IV	
11	Indian Pond Frog (Green Pond Frog)[4]	*Euphlyctis hexadactylus*	(Lesson, 1834)	വയൽ തവള	Vayal Tavaḷa	LC		Sch. IV	App. II
12	Jerdon's Bullfrog (Carnatic Peters frog)[4]	*Hoplobatrachus crassus*	(Jerdon, 1853)	ആട്ടുമാക്കാച്ചി	Āṭṭumākkācchi	LC		Sch. IV	
13	Indian Bullfrog[4]	*Hoplobatrachus tigerinus*	(Daudin, 1803)	നാട്ടുമാക്കാച്ചി	Nāṭṭumākkācchi	LC		Sch. IV	App. II
14	Minervarya Frog[32]	*Minervarya sahyadris*	Dubois, Ohler & Biju, 2001	ചിലുചിലപ്പൻ	Chiluchilappan	EN	WG		
15	Indian Burrowing Frog[5]	*Sphaerotheca breviceps*	(Schneider, 1799)	ചെറുകാലൻ കുഴിത്തവള	Cheṟukālan Kuḻittavaḷa	LC			
16	Short-webbed Frog (Peter's Frog, Pegu Wart Frog)[6, 33]	*Zakerana brevipalmata*	(Peters, 1871)	ചതുപ്പൻ ചിലപ്പൻ	Chatuppan Chilappan	DD	WG	Sch. IV	
17	Kerala Warty Frog (Verrucose Frog)[6, 33]	*Zakerana keralensis*	(Dubois, 1980)	കേരള ചിലപ്പൻ	Kēraḷa Chilappan	LC		Sch. IV	
18	Nilgiris Wart frog (Nilgiri Frog)[6, 33]	*Zakerana nilagirica*	(Jerdon, 1853)	നിലഗിരി ചിലപ്പൻ	Nilagiri Chilappan	EN	WG	Sch. IV	
19	Parambikulam Wart Frog[7, 33]	*Zakerana parambikulamana*	(Rao, 1937)	പറമ്പികുളം ചിലപ്പൻ	Paṟampikuḷaṁ Chilappan	DD	KL		
20	Rufescent Burrowing Frog (Reddish Burrowing Frog)[8, 33]	*Zakerana rufescens*	(Jerdon, 1853)	ചെങ്കൽ ചിലപ്പൻ	Cheṅkal Chilappan	LC	WG	Sch. IV	
	3. Family MICRIXALIDAE (dancing frogs)								
21	Munnar Torrent Frog (Beautiful Dancing Frog)	*Micrixalus adonis*	Biju, Garg, Gururaja, Shouche, & Walujkar, 2014	മൂന്നാർ പിലിഗിരിയൻ	Munnār Piligiriyan	NE	KL		

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22	Elegant Torrent Frog (Elegant Dancing Frog)[9]	*Micrixalus elegans*	(Rao,1937)	കൊടുകു പിലിഗിരിയൻ	Keāṭukhu Piligiriyan	DD	WG		
23	Cold Stream Torrent Frog (Cold Stream Dancing Frog)	*Micrixalus frigidus*	Biju, Garg, Gururaja, Shouche, & Walujkar, 2014	ആനമല പിലിഗിരിയൻ	Ānamala Piligiriyan	NE	WG		
24	Dusky Torrent Frog (kalakad Dancing Frog)[10]	*Micrixalus fuscus*	(Boulenger, 1882)	അഗസ്ത്യമല പിലിഗിരിയൻ	Agastyamala Piligiriyan	NT	WG		
25	Gadgil's Torrent Frog (Gadgil's Dancing Frog)	*Micrixalus gadgili*	Pillai & Pattabiraman, 1990	ഗാട്ഗിൽ പിലിഗിരിയൻ	Gāṭgil Piligiriyan	EN	KL		
26	Kallar Torrent Frog (Kallar Dancing Frog)	*Micrixalus herrei*	Myers, 1942	കല്ലാർ പിലിഗിരിയൻ	Kallār Piligiriyan	NE	WG		
27	Kurichiyar Torrent Frog (Kurichiyar Dancing Frog)	*Micrixalus kurichiyari*	Biju, Garg, Gururaja, Shouche, & Walujkar, 2014	കുറിച്യർ പിലിഗിരിയൻ	Kuṟichyar Piligiriyan	NE	KL		
28	Mallan's Torrent Frog (Mallan's Dancing Frog)	*Micrixalus mallani*	Biju, Garg, Gururaja, Shouche, & Walujkar, 2014	ശെന്തുരുണി പിലിഗിരിയൻ	Śenturuṇi Piligiriyan	NE	KL		
29	Nelliyampathi Torrent Frog (Nelliyampathi Dancing Frog)	*Micrixalus nelliyampathi*	Biju, Garg, Gururaja, Shouche, & Walujkar, 2014	നെല്ലിയാമ്പതി പിലിഗിരിയൻ	Nelliyāmpati Piligiriyan	NE	KL		
30	Black-bellied Torrent Frog (Black-bellied Dancing Frog)	*Micrixalus nigraventris*	Biju, Garg, Gururaja, Shouche, & Walujkar, 2014	കരിവയറൻ പിലിഗിരിയൻ	Karivayaṟan Piligiriyan	NE	WG		
31	Naked Torrent Frog (Naked Dancing Frog)	*Micrixalus nudis*	Pillai, 1978	വയനാട് പിലിഗിരിയൻ	Vayanāṭ Piligiriyan	VU	KL		
32	Pink-thighed Torrent Frog (Nilgiri Torrent Frog,Nilgiri Dancing Frog)[10]	*Micrixalus phyllophilus*	(Jerdon, 1854)	ചെങ്കാലൻ പിലിഗിരിയൻ	Cheṅkālan Piligiriyan	VU	WG		
33	Sairandhri Torrent Frog (Sairandhri Dancing Frog)	*Micrixalus sairandhri*	Biju, Garg, Gururaja, Shouche, & Walujkar, 2014	സൈരന്ധ്രി പിലിഗിരിയൻ	Sairandhri Piligiriyan	NE	KL		
34	Sali's Torrent Frog (Sali's Dancing Frog)	*Micrixalus sali*	Biju, Garg, Gururaja, Shouche, & Walujkar, 2014	പൊന്മുടി പിലിഗിരിയൻ	Peānmuṭi Piligiriyan	NE	KL		
35	Wayanad Torrent Frog (Wayanad Dancing Frog, Malabar Tropical Frog)[11]	*Micrixalus saxicola*	(Jerdon, 1854)	വടക്കൻ പിലിഗിരിയൻ	Vaṭakkan Piligiriyan	VU	WG		
36	Forest Torrent Frog (Forest Dancing Frog)[10]	*Micrixalus silvaticus*	(Boulenger, 1882)	കാട്ടു പിലിഗിരിയൻ	Kāṭṭu Piligiriyan	DD	KL		
37	Thampi's Torrent Frog (Silent Valley Dancing Frog)	*Micrixalus thampii*	Pillai, 1981	സൈലൻറ് വാലി പിലിഗിരിയൻ	Silent Valley Piligiriyan	DD	KL		
	4. Family MICROHYLIDAE (narrow-mouthed frogs)								
38	Black Microhylid Frog (Malabar Black narrow-mouthed frog, Orange Black Tubercled Indian Microhylid)	*Melanobatrachus indicus*	Beddome, 1878	ചോലക്കറുമ്പി	Cēālakkaṟumpi	EN	WG		
39	Ornate Narrow-mouthed Frog (Ornated Pygmy Frog)[12]	*Microhyla ornata*	(Dumeril & Bibron, 1841)	സ്വർണ കുറുവായൻ	Svarṇa Kuṟuvāyan	LC	WG		
40	Reddish Narrow-mouthed Frog[12]	*Microhyla rubra*	(Jerdon, 1854)	ചെമ്പൻ കുറുവായൻ	Cempan Kuṟuvāyan	LC			
41	Sholigari Microhylid	*Microhyla sholigari*	Dutta & Ray, 2000	ഷോളിഗാരി കുറുവായൻ	Ṣēāḷigāri Kuṟuvāyan	EN			
42	Anamalai Balloon Frog (Anamalai Balloon Frog)[13]	*Uperodon anamalaiensis*	Rao, 1937	ആനമല ബലൂൺതവള	Ānamala Balūṇ Tavaḷa	DD	WG		
43	Indian Balloon Frog[14]	*Uperodon globulosus*	(Gunther, 1864)	ബലൂൺ തവള	Balūṇ Tavaḷa	LC			

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44	Jerdon's Balloon Frog[15]	*Uperodon montanus*	(Jerdon, 1854)	ചോല ബലൂൺതവള	Cheāla Balūṇ Tavaḷa	NT	KL		
45	Marbled Balloon Frog[16]	*Uperodon systoma*	(Schneider, 1799)	വെണ്ണക്കൽ ബലൂൺതവള	Veṇṇakkal Balūṇ Tavaḷa	LC		Sch. IV	
46	Painted Frog[17]	*Uperodon taprobanica*	Parker, 1934	ചിത്ര തവള	Chitra Tavaḷa	LC			
47	Malabar Balloon Frog[18]	*Uperodon triangularis*	(Gunther, 1875)	മലബാർ ബലൂൺതവള	Malabār Balūṇ Tavaḷa	VU	WG		
48	Variegated Balloon Frog[19]	*Uperodon variegata*	(Stoliczka, 1872)	വർണ്ണ ബലൂൺതവള	Varṇṇa Balūṇ Tavaḷa	LC			
	5. Family NASIKABATRACHIDAE (purple frog)								
49	Purple Frog (Pig Nose Frog)	*Nasikabatrachus sahyadrensis*	Biju & Bossuyt, 2003	പാതാള തവള	Pātāḷa Tavaḷa	EN	WG		
	6. Family NYCTIBATRACHIDAE (night frogs)								
50	Spinular Night Frog	*Nyctibatrachus acanthodermis*	Biju, Bocxlaer, Mahony, Dinesh, Radhakrishnan, Zachariah, Giri & Bossuyt 2011	മുള്ളൻ രാത്തവള	Muḷḷan Rāttavaḷa	NE	KL		
51	Aliciae's Night Frog	*Nyctibatrachus aliciae*	Inger, Shaffer, Koshy & Bakde, 1984	അലിസി രാത്തവള	Alisi Rāttavaḷa	EN	WG		
52	Anamallai Night Frog[20]	*Nyctibatrachus anamallaiensis*	(Myers, 1942)	ആനമല രാത്തവള	Ānamala Rāttavaḷa	NE	WG		
53	Beddome's Night Frog (Pygmy Wrinkled Frog)[20]	*Nyctibatrachus beddomii*	(Boulenger, 1882)	ബെടോം രാത്തവള	Beṭēāṁ Rāttavaḷa	EN	WG		
54	Anamallai Night Frog[4]	*Nyctibatrachus deccanensis*	Dubois, 1984	ചോല രാത്തവള	Cheāla Rāttavaḷa	VU	WG	Sch. IV	
55	Deven's Night Frog	*Nyctibatrachus deveni*	Biju, Bocxlaer, Mahony, Dinesh, Radhakrishnan, Zachariah, Giri & Bossuyt 2011	ദേവനി രാത്തവള	Dēvani Rāttavaḷa	NE	KL		
56	Gavi Night Frog	*Nyctibatrachus gavi*	Biju, Bocxlaer, Mahony, Dinesh, Radhakrishnan, Zachariah, Giri & Bossuyt 2011	ഗവി രാത്തവള	Gavi Rāttavaḷa	NE	KL		
57	Indraneil's Night Frog	*Nyctibatrachus grandis*	Biju, Bocxlaer, Mahony, Dinesh, Radhakrishnan, Zachariah, Giri & Bossuyt 2011	വയനാട് രാത്തവള	Vayanāṭ Rāttavaḷa	NE	KL		
58	Indraneil's Night Frog	*Nyctibatrachus indraneili*	Biju, Bocxlaer, Mahony, Dinesh, Radhakrishnan, Zachariah, Giri & Bossuyt 2011	ഇന്ദ്രനീലി രാത്തവള	Indranīli rāttavaḷa	NE	WG		
59	Kempholey Night Frog[20]	*Nyctibatrachus kempholeyensis*	(Rao, 1937)	കെംഫോളേ രാത്തവള	Kemphēāḷē Rāttavaḷa	DD	WG		
60	Malabar Night Frog (Malabar Night Frog, Large Wrinkled Frog)	*Nyctibatrachus major*	Boulenger, 1882	പെരും രാത്തവള	Peruṁ Rāttavaḷa	VU	WG		
61	Miniature Night Frog	*Nyctibatrachus minimus*	Biju, Bocxlaer, Giri, Roelants, Nagaraju & Bossuyt, 2007	കുഞ്ഞൻ രാത്തവള	Kuññan Rāttavaḷa	DD	KL		
62	Kerala Night Frog	*Nyctibatrachus minor*	Inger, Shaffer, Koshy & Bakde, 1984	കേരളാ രാത്തവള	Kēraḷā Rāttavaḷa	EN	KL		

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63	Periyar Night Frog	*Nyctibatrachus periyar*	Biju, Bocxlaer, Mahony, Dinesh, Radhakrishnan, Zachariah, Giri & Bossuyt 2011	പെരിയാർ രാത്തവള	Periyār Rāttavaḷa	NE	KL		
64	Pillai's Night Frog	*Nyctibatrachus pillai*	Biju, Bocxlaer, Mahony, Dinesh, Radhakrishnan, Zachariah, Giri & Bossuyt 2011	പിള്ള രാത്തവള	Piḷḷa Rāttavaḷa	NE	WG		
65	Meowing Night Frog	*Nyctibatrachus poocha*	Biju, Bocxlaer, Mahony, Dinesh, Radhakrishnan, Zachariah, Giri & Bossuyt 2011	പൂച്ചത്തവള	Pūcchattavaḷa	NE	WG		
66	Kalakad Night Frog	*Nyctibatrachus vasanthi*	Ravichandran, 1997	കളക്കാട് രാത്തവള	Kaḷakkāṭ rāttavaḷa	EN	WG		
67	VUB Night Frog	*Nyctibatrachus vrijeuni*	Biju, Bocxlaer, Mahony, Dinesh, Radhakrishnan, Zachariah, Giri & Bossuyt 2011	വിയുബി രാത്തവള	Vi Yu Bi Rāttavaḷa	NE	WG		
	7. Family RANIDAE (true frogs)								
68	Bicoloured Frog (Malabar Frog)[4]	*Clinotarsus curtipes*	(Jerdon, 1853)	കാട്ടുമണവാട്ടി	Kāṭṭumaṇavāṭṭi	NT	WG	Sch. IV	
69	Fungoid Frog (Malabar Hills Frog)[30]	*Hydrophylax malabarica*	(Tschudi, 1838)	മണവാട്ടിത്തവള	Maṇavāṭṭittavaḷa	LC		Sch. IV	
70	Boulenger's Golden-backed Frog (Small Wood Frog, Trivandrum Frog)[30]	*Indosylvirana aurantiaca*	(Boulenger, 1904)	ബൊലെഞ്ചർ മണവാട്ടിത്തവള	Beāleñcar Maṇavāṭṭittavaḷa	VU	KL	Sch. IV	
71	Don's Golden-backed Frog[31]	*Indosylvirana doni*	(Biju, Garg, Mahony, Wijayathilaka, Senevirathne & Meegaskumbura, 2014)	ഡോണി മണവാട്ടിത്തവള	Ḍēāṇi Maṇavāṭṭittavaḷa	NE	KL		
72	Yellowish Golden-backed Frog[30]	*Indosylvirana flavescens*	(Jerdon, 1853)	മഞ്ഞ മണവാട്ടിത്തവള	Mañña Maṇavāṭṭittavaḷa	NE	WG		
73	Indian Golden-backed Frog[31]	*Indosylvirana indica*	(Biju, Garg, Mahony, Wijayathilaka, Senevirathne & Meegaskumbura, 2014)	ഇന്ത്യൻ മണവാട്ടിത്തവള	Intyan Maṇavāṭṭittavaḷa	NE	WG		
74	Rao's Intermediate Golden-backed Frog[30]	*Indosylvirana intermedius*	(Rao, 1937)	റാവു മണവാട്ടിത്തവള	Ṟāvu Maṇavāṭṭittavaḷa	NE	WG		
75	Large Golden-backed Frog[31]	*Indosylvirana magna*	(Biju, Garg, Mahony, Wijayathilaka, Senevirathne & Meegaskumbura, 2014)	വലിയ മണവാട്ടിത്തവള	Valiya Maṇavāṭṭittavaḷa	NE	WG		
76	Sreeni's Golden-backed Frog[31]	*Indosylvirana sreeni*	(Biju, Garg, Mahony, Wijayathilaka, Senevirathne & Meegaskumbura, 2014)	ശ്രീനി മണവാട്ടിത്തവള	Śrīni Maṇavāṭṭittavaḷa	NE	WG		
77	Urban Golden-backed Frog[31]	*Indosylvirana urbis*	(Biju, Garg, Mahony, Wijayathilaka, Senevirathne & Meegaskumbura, 2014)	നാട്ടു മണവാട്ടിത്തവള	Nāṭṭu Maṇavāṭṭittavaḷa	NE	KL		
	8. Family RANIXALIDAE (leaping frogs)								
78	Beddome's leaping Frog (Beddome's Indian frog)[21]	*Indirana beddomii*	(Gunther, 1875)	ബെടോം പാറത്തവള	Beṭēāṁ Pāṟattavaḷa	LC	WG	Sch. IV	

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79	Anamallais Leaping Frog (Anamallais Indian frog)[21]	*Indirana brachytarsus*	(Gunther, 1875)	ആനമല പാറത്തവള	Ānamala Pāṟattavaḷa	EN	WG	Sch. IV	
80	Spotted Leaping Frog (Malabar Indian Frog)[22]	*Indirana diplosticta*	(Gunther, 1875)	പുള്ളി പാറത്തവള	Puḷḷi Pāṟattavaḷa	EN	WG	Sch. IV	
81	Boulenger's Leaping Frog (Boulenger's Indian Frog)[21]	*Indirana leptodactyla*	(Boulenger, 1882)	ബൊലെഞ്ചർ പാറത്തവള	Beāleñcar Pāṟattavaḷa	EN	WG	Sch. IV	
82	Toad skinned Leaping Frog (Kerala Indian Frog)[4]	*Indirana phrynoderma*	(Boulenger, 1882)	ചൊറിയൻ പാറത്തവള	Cheāṟiyan Pāṟattavaḷa	CR	WG	Sch. IV	
83	South Indian Frog[4]	*Indirana semipalmata*	(Boulenger, 1882)	ചെറുകാലൻ പാറത്തവള	Cheṟukālan Pāṟattavaḷa	LC	WG	Sch. IV	
	9. Family RHACOPHORIDAE (tree frogs)								
84	Kadalar Swamp Frog	*Beddomixalus bijui*	(Zachariah, Dinesh, Radhakrishnan, Kunhikrishnan, Palot & Vishnudas, 2011)	ആനമല ചതുപ്പൻ	Ānamala Chatuppan	NE	KL		
85	Ghat Tree Frog (Star-eyed Tree Frog)[23]	*Ghatixalus asterops*	Biju, Roelants & Bossuyt, 2008	ചോല മരത്തവള	Chēāla Marattavaḷa	DD	WG		
86	Green Tree Frog[23]	*Ghatixalus variabilis*	(Jerdon, 1853)	പച്ചചോല മരത്തവള	Paccha Chēāla Marattavaḷa	EN	WG		
87	Myristica Swamp frog	*Mercurana myristicapalustris*	Abraham, Pyron, Ansil, Zachariah & Zachariah, 2013	തെക്കൻ ചതുപ്പൻ	Tekkan Chatuppan	NE	KL		
88	Common Indian Tree Frog (Chunam Frog)[24]	*Polypedates maculatus*	(Gray, 1834)	തവിട്ടുമരത്തവള	Taviṭṭumarattavaḷa	LC			
89	Charpa Tree frog	*Polypedates occidentalis*	Das & Dutta, 2006	ചാർപ്പ തവിട്ടുമരത്തവള	Chārppa Taviṭṭumarattavaḷa	DD	WG		
90	False Hour-glass Tree Frog (Yellow Tree Frog)	*Polypedates pseudocruciger*	Das & Ravichandran, 1998	ഘടികാര തവിട്ടുമരത്തവള	Ghaṭikāra Taviṭṭumarattavaḷa	LC	WG		
91	Kani Bush Frog[9]	*Pseudophilautus kani*	(Biju & Bossuyt, 2009)	കാണി കരിയിലത്തവള	Kāṇi Kariyilattavaḷa	LC	KL		
92	Jerdon's Bush Frog[25]	*Pseudophilautus wynaadensis*	(Jerdon,1853)	വയനാടൻ കരിയിലത്തവള	Vayanāṭan Kariyilattavaḷa	EN	WG		
93	Agasthyamala Bush Frog	*Raorchestes agasthyaensis*	Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot & Kalesh, 2011	അഗസ്ത്യൻ ഇലത്തവള	Agastyan Ilattavaḷa	NE	WG		
94	Variable Bush Frog[9]	*Raorchestes akroparallagi*	(Biju & Bossuyt, 2009)	പച്ച ഇലത്തവള	Paccha Ilattavaḷa	LC	WG		
95	Anil's Bush Frog[9]	*Raorchestes anili*	(Biju & Bossuyt, 2006)	അനിലി ഇലത്തവള	Anili Ilattavaḷa	LC	WG		
96	Archaic Bush Frog	*Raorchestes archeos*	Vijaykumar, Dinesh, Prabhu & Shanker, 2014	പുള്ളി ഇലത്തവള	Puḷḷi Ilattavaḷa	NE	WG		
97	Golden-eyed Frog	*Raorchestes aureus*	Vijaykumar, Dinesh, Prabhu & Shanker, 2014	സ്വർണ്ണക്കണ്ണി ഇലത്തവള	Svarṇṇakkaṇṇi Ilattavaḷa	NE	WG		
98	Beddome's Bush Frog[26]	*Raorchestes beddomii*	(Gunther, 1876)	ബെഡോം ഇലത്തവള	Beṭēāṁ Ilattavaḷa	NT	WG		
99	Pleasant Bush Frog	*Raorchestes blandus*	Vijaykumar, Dinesh, Prabhu & Shanker, 2014	ബ്ലാണ്ടസ് ഇലത്തവള	Blāṇṭas Ilattavaḷa	NE	WG		
100	Bob Inger's Bush Frog [9]	*Raorchestes bobingeri*	(Biju & Bossuyt, 2005)	ബോബിങ്ങർ ഇലത്തവള	Bēābiṅṅar Ilattavaḷa	VU	WG		

	English name	Species name	Authority	Malayalam name	Vernacular name	IUCN	END	WPA	CITES
101	Chalazodes Bubble Nest Frog[26]	*Raorchestes chalazodes*	(Gunther, 1876)	പച്ച ഈറ്റത്തവള	Paccha Īṟṟattavaḷa	CR	WG		
102	Seshachar's Bush Frog[9]	*Raorchestes charius*	(Rao, 1937)	സെഷാചർ ഇലത്തവള	Seṣāchar Ilattavaḷa	EN	WG		
103	Green-eyed Bush Frog[9]	*Raorchestes chlorosomma*	(Biju & Bossuyt, 2009)	പച്ചക്കണ്ണി ഇലത്തവള	Pacchakkaṇṇi Ilattavaḷa	CR	WG		
104	Small Bush Frog[9]	*Raorchestes chotta*	(Biju & Bossuyt, 2009)	കുഞ്ഞൻ ഇലത്തവള	Kuññan Ilattavaḷa	DD	KL		
105	Confusing Green Bush Frog[9]	*Raorchestes chromasynchysi*	(Biju & Bossuyt, 2009)	കുറിച്യാർമല ഇലത്തവള	Kuṟicyārmala Ilattavaḷa	VU	WG		
106	Bark Bush Frog	*Raorchestes crustai*	Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot & Kalesh, 2011	പട്ട ഇലത്തവള	Paṭṭa Ilattavaḷa	NE	WG		
107	Kodaikanal Bush Frog[9]	*Raorchestes dubois*	(Biju & Bossuyt, 2006)	കൊടൈ ഇലത്തവള	Keāṭai Ilattavaḷa	VU	WG		
108	Yellow-bellied Bush Frog	*Raorchestes flaviventris*	(Boulenger, 1882)	മഞ്ഞവയറൻ ഇലത്തവള	Maññavayaṟan ilattavaḷa	DD	WG		
109	Glandular Bush Frog (Beautiful Bush Frog, Pretty Bush Frog)[26]	*Raorchestes glandulosus*	(Jerdon, 1853)	മാനന്തവാടി ഇലത്തവള	Mānantavāṭi Ilattavaḷa	VU	WG		
110	Ponmudi Bush Frog[9]	*Raorchestes graminirupes*	(Biju & Bossuyt, 2005)	പൊന്മുടി ഇലത്തവള	Peānmuṭi Ilattavaḷa	VU	WG		
111	Griet Bush Frog[9]	*Raorchestes griet*	(Bossuyt, 2002)	ഗ്രീറ്റ് ഇലത്തവള	Grīṟṟ Ilattavaḷa	CR	WG		
112	Jayaram's Bush Frog[9]	*Raorchestes jayarami*	(Biju & Bossuyt, 2009)	ജയറാം ഇലത്തവള	Jayaṟāṁ Ilattavaḷa	NE	WG		
113	Johnceei's Bush Frog	*Raorchestes johnceei*	Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot & Kalesh, 2011	ജോൻസീ ഇലത്തവള	Jēānsī Ilattavaḷa	NE	WG		
114	Kadalar Bush Frog	*Raorchestes kadalarensis*	Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot & Kalesh, 2011	കടലാർ ഇലത്തവള	Kaṭalār Ilattavaḷa	NE	KL		
115	Kaikatti Bush Frog[9]	*Raorchestes kaikatti*	(Biju & Bossuyt, 2009)	കൈകാട്ടി ഇലത്തവള	Kaikāṭṭi Ilattavaḷa	CR	KL		
116	Kakachi Bush Frog	*Raorchestes kakachi*	Seshadri, Gururaja & Aravind, 2012	കാക്കാച്ചി ഇലത്തവള	Kākkācchi Ilattavaḷa	NE	WG		
117	White Patch Bush Frog	*Raorchestes leucolatus*	Vijaykumar, Dinesh, Prabhu & Shanker, 2014	പാണ്ടൻ ഇലത്തവള	Pāṇṭan Ilattavaḷa	NE	WG		
118	Beautiful Reed Bush Frog (Manohar's Bush Frog)	*Raorchestes manohari*	Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot & Kalesh, 2011	മനോഹരി ഈറ്റത്തവള	Manēāhari Īṟṟattavaḷa	NE	WG		
119	Mark's Bush Frog[9]	*Raorchestes marki*	(Biju & Bossuyt, 2009)	മാർക്കി ഇലത്തവള	Mārkki Ilattavaḷa	CR	WG		
120	Munnar Bush Frog[9]	*Raorchestes munnarensis*	(Biju & Bossuyt, 2009)	മൂന്നാർ ഇലത്തവള	Munnār Ilattavaḷa	CR	WG		
121	Water Drop Frog (Kalpetta Bush Frog)[9]	*Raorchestes nerostagona*	(Biju & Bossuyt, 2005)	നീർത്തുള്ളി തവള	Nīrttuḷḷi Tavaḷa	EN	WG		
122	Ochlandrae Reed Bush Frog[9]	*Raorchestes ochlandrae*	(Gururaja, Dinesh, Palot, Radhakrishnan & Ramachandra, 2007)	ഈറ്റത്തവള	Īṟṟattavaḷa	DD	WG		
123	Large Ponmudi Bush Frog[9]	*Raorchestes ponmudi*	(Biju & Bossuyt, 2005)	വലിയ ഇലത്തവള	Valiya Ilattavaḷa	CR	WG		

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124	Ravi's Bush Frog	*Raorchestes ravii*	Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot & Kalesh, 2011	രവി ഇലത്തവള	Ravi Ilattavaḷa	NE	WG		
125	Resplendent Shrub Frog (Anamudi Bush Frog)	*Raorchestes resplendens*	Biju, Shouche, Dubois, Dutta & Bossuyt, 2010	ആനമുടി ഇലത്തവള	Ānamuṭi Ilattavaḷa	CR	WG		
126	Star-eyed Bush Frog[26]	*Raorchestes signatus*	(Boulenger, 1882)	നക്ഷത്രക്കണ്ണി ഇലത്തവള	Nakṣhatrakkaṇṇi Ilattavaḷa	EN	WG		
127	Sushil's Bush Frog[9]	*Raorchestes sushili*	(Biju & Bossuyt, 2009)	സുഷിലി ഇലത്തവള	Suṣhili Ilattavaḷa	CR	WG		
128	Theuerkauf's Bush Frog	*Raorchestes theuerkaufi*	Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot & Kalesh, 2011	തെർകൊഫ് ഇലത്തവള	Terkeāph Ilattavaḷa	NE	WG		
129	Nilgiri Bush Frog[25]	*Raorchestes tinniens*	(Jerdon, 1853)	നിലഗിരി ഇലത്തവള	Nilagiri Ilattavaḷa	EN	WG		
130	Travancore Bush Frog[27]	*Raorchestes travancoricus*	(Boulenger, 1891)	നീലക്കണ്ണി ഇലത്തവള	Nīlakkaṇṇi Ilattavaḷa	EN	WG		
131	Kudremukh Bush Frog[9]	*Raorchestes tuberohumerus*	(Kuramoto & Joshy, 2003)	കുദ്രേമുഖ് ഇലത്തവള	Kudrēmukh Ilattavaḷa	DD	WG		
132	Uthaman's Reed Bush Frog	*Raorchestes uthamani*	Zachariah, Dinesh, Kunhikrishnan, Das, Raju, Radhakrishnan, Palot & Kalesh, 2011	ഉത്തമനി ഈറ്റത്തവള	Uttamani Īṟṟattavaḷa	NE	KL		
133	Kalakad Tree Frog	*Rhacophorus calcadensis*	Ahl, 1927	കളക്കാട് പച്ചിലപ്പാറാൻ	Kaḷakkāṭ Pacchilappāṟān	EN	WG		
134	Small Tree Frog	*Rhacophorus lateralis*	Boulenger, 1883	മഞ്ഞക്കരയൻ പച്ചിലപ്പാറാൻ	Maññakkarayan Pacchilappāṟān	EN	WG		
135	Malabar Gliding Frog (Malabar Flying Frog, Malabar Tree Frog)	*Rhacophorus malabaricus*	Jerdon, 1870	പച്ചിലപ്പാറാൻ	Pacchilappāṟān	LC	WG		
136	Malabar False Tree frog	*Rhacophorus pseudomalabaricus*	Vasudevan & Dutta, 2000	പുള്ളിപച്ചിലപ്പാറാൻ	Puḷḷi Pacchilappāṟān	CR	WG		
	II. ORDER GYMNOPHIONA								
	10. Family ICHTHYOPHIIDAE (asiatic tailed caecilians)								
137	Beddome's Caecilian	*Ichthyophis beddomei*	Peters 1879	വരയൻ കുരുടി	Varayan Kuruṭi	LC	WG		
138	Bombay Caecilian	*Ichthyophis bombayensis*	Taylor, 1960	തടിയൻ കുരുടി	Taṭiyan Kuruṭi	LC	WG		
139	Kodagu Striped Caecilian	*Ichthyophis kodaguensis*	Wilkinson, Gower, Govindappa and Venkatachalaiah, 2007	കൊടഗു കുരുടി	Keāṭagu kuruṭi	DD	WG		
140	Long-headed Caecilian	*Ichthyophis longicephalus*	Pillai, 1986	മൂക്കൻ കുരുടി	Mūkkan Kuruṭi	DD	KL		
141	Three-colored Caecilian	*Ichthyophis tricolor*	Annandale, 1909	ത്രിവർണ കുരുടി	Trivarṇa Kuruṭi	LC	KL		
142	Chengalam Caecilian	*Uraeotyphlus interruptus*	Pillai & Ravich&ran, 1999	ചെങ്ങളം കുരുടി	Cheṅṅaḷaṁ Kuruṭi	DD	KL		
143	Malabar Caecilian[28]	*Uraeotyphlus malabaricus*	(Beddome, 1870)	മലബാർ കുരുടി	Malabār Kuruṭi	DD	KL		
144	Menon's Caecilian	*Uraeotyphlus menoni*	Annandale, 1913	മേനോൻ കുരുടി	Mēnēān Kuruṭi	DD	KL		
145	Narayan's Caecilian	*Uraeotyphlus narayani*	Seshachar, 1939	നാരായൺ കുരുടി	Nārāyaṇ Kuruṭi	DD	KL		

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146	Oommen's Uraeotyphlus	*Uraeotyphlus oommeni*	Gower & Wilkinson, 2007	ഉമ്മൻ കുരുടി	Um'man Kuruṭi	DD	KL		
147	Red Caecilian[28]	*Uraeotyphlus oxyurus*	(Dumeril & Bibron, 1841)	ചെമ്പൻ കുരുടി	Chempan Kuruṭi	DD	KL		
	11. Family INDOTYPHLYIDAE (common caecilians)								
148	Periya Peak Caecilian[29]	*Gegeneophis carnosus*	(Beddome, 1870)	പേരിയ കുരുടി	Pēriya Kuruṭi	DD	KL		
149	Malabar Cardomom Gegeneophis	*Gegeneophis primus*	Kotharambath, Gower, Oomen & Wilkinson, 2012	ഏലക്കാടൻ കുരുടി	Ēlakkāṭan Kuruṭi	DD	KL		
150	Ramaswami's Caecilian	*Gegeneophis ramaswamii*	Taylor, 1964	രാമസ്വാമി കുരുടി	Rāmasvāmi Kuruṭi	LC	WG		
151	Tejaswini Gegeneophis	*Gegeneophis tejaswini*	Kotharambath, Wilkinson, Oommen, and Gower, 2015	തേജസ്വിനി കുരുടി	Tējasvini kuruṭi	NE	KL		

[1] Formerly in the genus *Bufo*
[2] Formerly in the genus *Bufo*, *Ansonia*
[3] Formerly in the genus *Ansonia*
[4] Formerly in the genus *Rana*
[5] Formerly in the genus *Tomopterna*
[6] Formerly in the genus *Rana*, *Fejervarya*
[7] Formerly in the genus *Tomopterna*, *Fejervarya*
[8] Formerly in the genus *Pyxicephalus*, *Rana*, *Tomopterna*, *Fejervarya*
[9] Formerly in the genus *Philautus*
[10] Formerly in the genus *Ixalus*
[11] Formerly in the genus *Polypedates*, *Ixalus*
[12] Formerly in the genus *Engystoma*
[13] Formerly in the genus *Ramanella*
[14] Formerly in the genus *Cacopus*, *Systoma*
[15] Formerly in the genus *Hylaedactylus*, *Ramanella*
[16] Formerly in the genus *Rana*, *Ramanella*
[17] Formerly in the genus *Kaloula*
[18] Formerly in the genus *Callula*, *Kaloula*, *Ramanella*
[19] Formerly in the genus *Hylaedactylus*, *Callula*, *Kaloula*, *Ramanella*
[20] Formerly in the genus *Nannobatrachus*
[21] Formerly in the genus *Polypedates*, *Rana*
[22] Formerly in the genus *Ixalus*, *Rana*
[23] Formerly in the genus *Polypedates*, *Rhacophorus*
[24] Formerly in the genus *Hyla*, *Rhacophorus*
[25] Formerly in the genus *Phyllomdesua*, *Ixalus*, *Philautus*
[26] Formerly in the genus *Ixalus*, *Philautus*
[27] Formerly in the genus *Ixalus*, *Philautus*.
[28] Formerly in the genus *Cecilia*
[29] Formerly in the genus *Epicrium*
[30] Formerly in the genus *Rana, Hylarana*
[31] Formerly in the genus *Hylarana*
[32] Recently the genus *Minervarya* got synonymised with *Fejervarya* according to Dinesh et al. (2015)
[33] Recently the genus *Zakerana* got synonymised with *Fejervarya* according to Dinesh et al. (2015)

Image 1. ***Duttaphrynus beddomii***

Image 2. ***Pedostibes tuberculosus***

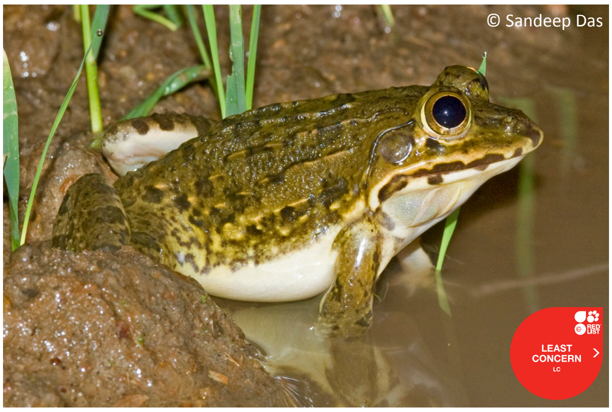

Image 3. ***Hoplobatrachus crassus***

Image 4. ***Micrixalus phyllophilus***

Image 5. ***Melanobatrachus indicus***

Image 6. ***Uperodon montanus***

Image 7. *Nasikabatrachus sahyadrensis*

Image 8. *Nyctibatrachus minimus*

Image 9. *Indosylvirana magna*

Image 10. *Indirana diplosticta*

Image 11. *Beddomixalus bijui*

Image 12. *Raorchestes crustai*

Image 13. *Raorchestes resplendens*

Image 14. *Ichthyophis longicephalus*

2015 available at http://mhadeiresearchcenter.org/resources (online only)(accessed: 27 March 2015).

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Phylogenomics Reveals Ancient Gene Tree Discordance in the Amphibian Tree of Life

PAUL M. HIME[1,2,*,†], ALAN R. LEMMON[3,*,†], EMILY C. MORIARTY LEMMON[4], ELIZABETH PRENDINI[5], JEREMY M. BROWN[6], ROBERT C. THOMSON[7], JUSTIN D. KRATOVIL[2,8], BRICE P. NOONAN[9], R. ALEXANDER PYRON[10], PEDRO L. V. PELOSO[5,11], MICHELLE L. KORTYNA[4], J. SCOTT KEOGH[12], STEPHEN C. DONNELLAN[13,14], RACHEL LOCKRIDGE MUELLER[15], CHRISTOPHER J. RAXWORTHY[5], KRUSHNAMEGH KUNTE[16], SANTIAGO R. RON[17], SANDEEP DAS[18], NIKHIL GAITONDE[16], DAVID M. GREEN[19], JIM LABISKO[20,21], JING CHE[22,23] AND DAVID W. WEISROCK[2,*]

[1]*Biodiversity Institute, University of Kansas, Lawrence, KS 66045, USA;* [2]*Department of Biology, University of Kentucky, Lexington, KY 40506, USA;* [3]*Department of Scientific Computing, Florida State University, Tallahassee, FL 32306, USA;* [4]*Department of Biological Science, Florida State University, Tallahassee, FL 32306, USA;* [5]*Division of Vertebrate Zoology: Herpetology, American Museum of Natural History, New York, NY 10024, USA;* [6]*Department of Biological Sciences and Museum of Natural Science, Louisiana State University, Baton Rouge, LA 70803, USA;* [7]*School of Life Sciences, University of Hawai'i, Honolulu, HI 96822, USA;* [8]*Department of Entomology, University of Kentucky, Lexington, KY 40546, USA;* [9]*Department of Biology, University of Mississippi, Oxford, MS 38677, USA;* [10]*Department of Biological Sciences, The George Washington University, Washington, DC 20052, USA;* [11]*Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, 66075-750, Brazil;* [12]*Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, 2601, Australia;* [13]*South Australian Museum, North Terrace, Adelaide 5000, Australia;* [14]*School of Biological Sciences, University of Adelaide, Adelaide 5005, Australia;* [15]*Department of Biology, Colorado State University, Fort Collins, CO 80523, USA;* [16]*National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bengaluru 560065, India;* [17]*Museo de Zoología, Escuela de Biología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador;* [18]*Forest Ecology and Biodiversity Conservation Division, Kerala Forest Research Institute, Peechi, Kerala 680653, India;* [19]*Redpath Museum, McGill University, Montreal, Quebec H3A 0C4, Canada;* [20]*The Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, The University of Kent, Canterbury, Kent, CT2 7NR, UK;* [21]*Island Biodiversity and Conservation Centre, University of Seychelles, PO Box 1348, Anse Royale, Mahé, Seychelles; and* [22]*State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Kunming 650223, China; and* [23]*Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming 650223, China*

**Correspondence to be sent to: Biodiversity Institute, University of Kansas, Lawrence, KS 66045, USA;*
E-mail: paul.hime@ku.edu.
Paul M. Hime and Alan R. Lemmon contributed equally to this article.

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Abstract.—Molecular phylogenies have yielded strong support for many parts of the amphibian Tree of Life, but poor support for the resolution of deeper nodes, including relationships among families and orders. To clarify these relationships, we provide a phylogenomic perspective on amphibian relationships by developing a taxon-specific Anchored Hybrid Enrichment protocol targeting hundreds of conserved exons which are effective across the class. After obtaining data from 220 loci for 286 species (representing 94% of the families and 44% of the genera), we estimate a phylogeny for extant amphibians and identify gene tree–species tree conflict across the deepest branches of the amphibian phylogeny. We perform locus-by-locus genealogical interrogation of alternative topological hypotheses for amphibian monophyly, focusing on interordinal relationships. We find that phylogenetic signal deep in the amphibian phylogeny varies greatly across loci in a manner that is consistent with incomplete lineage sorting in the ancestral lineage of extant amphibians. Our results overwhelmingly support amphibian monophyly and a sister relationship between frogs and salamanders, consistent with the Batrachia hypothesis. Species tree analyses converge on a small set of topological hypotheses for the relationships among extant amphibian families. These results clarify several contentious portions of the amphibian Tree of Life, which in conjunction with a set of vetted fossil calibrations, support a surprisingly younger timescale for crown and ordinal amphibian diversification than previously reported. More broadly, our study provides insight into the sources, magnitudes, and heterogeneity of support across loci in phylogenomic data sets.[AIC; Amphibia; Batrachia; Phylogeny; gene tree–species tree discordance; genomics; information theory.]

Understanding the extent of gene tree-species tree discordance in empirical studies can have important ramifications beyond the resolution of the species tree (Page and Charleston 1997; Degnan and Rosenberg 2006). Gene tree discordance has numerous causes (Degnan and Rosenberg 2009; Edwards 2009), and when a product of incomplete lineage sorting (ILS) or introgressive hybridization, can affect downstream interpretations about organismal evolution by obscuring divergence time estimates (Burbrink and Pyron 2011), or inducing apparent (but spurious) substitution rate variation (Mendes and Hahn 2016). A focus on the species tree and not on its discordant gene trees can lead to inaccurate interpretations of character evolution (Hahn and Nakhleh 2016), including artifactual patterns of diminishing molecular convergence over time (Mendes et al. 2016).

Gene tree discordance has been studied most intensively at relatively shallow phylogenetic scales (Maddison 1997; Pollard et al. 2006; Carstens and Knowles 2007; Avise and Robinson 2008; White et al. 2009). However, simulation results suggest that given particular combinations of effective population size and speciation rates, patterns of ILS may persist over tens or hundreds of millions of years (Oliver 2013). In line with these expectations, some empirical studies have purportedly found a signature of ILS at deep phylogenetic scales on the order of tens of millions of years (Pollard et al. 2006; Ebersberger et al. 2007; Hobolth et al. 2011; Salichos and Rokas 2013;

Suh et al. 2015; Sun et al. 2015). Although considerable attention has been paid to the overall reconstruction of very deep phylogenetic relationships using total evidence approaches (e.g., Rokas and Carroll 2006; Dunn et al. 2008), few studies have intensively examined the potential for gene tree–species tree discordance at deep timescales on a gene-by-gene basis. At these deep phylogenetic scales, distinguishing true discordance from gene tree estimation error can be challenging (Gatesy and Springer 2014).

Loci that are genealogically concordant with the species tree can lose phylogenetic information over time through multiple substitutions at sites, potentially leading to the spurious estimation of a discordant gene tree. Signals from actual discordance may similarly erode over time. Consequently, genes with high information content may be required to detect and quantify gene tree discordance at deep scales (Townsend 2007; Salichos and Rokas 2013). Scrutinizing signal from individual gene alignments and their contributions to the overall estimate of the species tree is also important (e.g., Brown and Thomson 2016). The topology of the gene tree alone may hide signal that is distributed across sites within a gene, signal that can be extracted to evaluate alternative gene-tree topologies and their support for a species-tree hypothesis (Arcila et al. 2017). Individual genes may be subject to systematic error due to a wide range of phenomena (Rannala and Yang 2008), and evidence for deep discordance may also provide insight into demographic histories.

Early amphibian evolution represents a unique empirical challenge for analyses of deep genealogical discordance (San Mauro et al. 2005; Siu-Ting et al. 2019). Here, we examine these issues using phylogenomic data assembled from extant amphibians (Lissamphibia) to estimate phylogenetic history across this clade and focus on the contentious relationships among the three deeply diverged amphibian orders. Amphibians are a diverse and imperiled class of vertebrates, with more than 8100 species described to date (AmphibiaWeb 2020; Frost 2020). Three amphibian orders are recognized: Anura (frogs and toads, approximately 7200 species), Caudata (salamanders and newts, approximately 750 species), and Gymnophiona (caecilians, approximately 200 species). Despite extensive investigation, phylogenetic affinities of some amphibian groups remain unresolved, including disagreement about deep interordinal relationships among the three extant amphibian orders, as well as relationships among more shallowly diverged lineages (Larson and Wilson 1989; Feller and Hedges 1998; Frost et al. 2006; Roelants et al. 2007; Fong et al. 2012; Chen et al. 2015; Jetz and Pyron 2018; Siu-Ting et al. 2019). While much research has centered on the use of morphology and paleontological approaches to resolve deep amphibian relationships (e.g., Carroll 2007; Sigurdsen and Green 2011; Pardo et al. 2017; Matsumoto and Evans 2018; Schoch 2019), here we place our focus on molecular phylogenetic approaches.

Although the monophyly of each amphibian order is not disputed (Frost et al. 2006; Roelants et al. 2007; Anderson et al. 2008; Pyron and Wiens 2011), relationships among them have remained murky. Three possible topologies exist (assuming a monophyletic Amphibia): the Batrachia hypothesis (e.g., Haeckel 1866; Duellman and Trueb 1994; Zardoya and Meyer 2001; Frost et al. 2006; Anderson et al. 2008; Siu-Ting et al. 2019), which places frogs and salamanders as each other's closest relatives, the Procera hypothesis (Feller and Hedges 1998; Siu-Ting et al. 2019), which suggests a sister relationship between salamanders and caecilians, and the Acauda hypothesis (named here), which proposes that frogs and caecilians form a clade.

Although different regions of the genome may support different topologies, a single species tree exists for interordinal amphibian relationships; thus, the question of the relationships between frogs, salamanders, and caecilians becomes a model selection problem. The Batrachia and Procera hypotheses have received the most support in previous studies (Frost et al. 2006; Roelants et al. 2007; Pyron and Wiens 2011; Siu-Ting et al. 2019), although recent genome-scale analyses with modest numbers of taxa (Fong et al. 2012; Chen et al. 2015) have found some loci that appear to support each of the alternative hypotheses. These studies (Fong et al. 2012; Chen et al. 2015) have even revealed some loci that appear to support the nonmonophyly of amphibians with respect to amniotes, further widening the hypothesis space for the deep evolution of Amphibia (Supplementary Fig. S1 available on Dryad at https://doi.org/10.5061/dryad.9kd51c5dc).

The technical hurdles to generating genome-scale data sets for phylogenetic inference in amphibians are still nontrivial, due primarily to their deep evolutionary divergences and inordinately large genomes (e.g., salamander genomes are ~15–120 Gb; Gregory 2020; Weisrock et al. 2018). Targeted sequence-capture approaches have grown in popularity in recent years (e.g., Bi et al. 2012; Faircloth et al. 2012; Lemmon et al. 2012) and have the potential to streamline the sequencing of large numbers of loci in parallel. Yet, the constraints imposed by sequence divergence across broad taxonomic scales limit the applicability of sequence capture approaches when capture probes are designed from a single or a few model taxa. Additionally, in clades with extreme variation in genome size, sequence capture efficiency can be highly variable because target loci are effectively diluted in large genomes.

Recent studies using large molecular data sets in amphibians have demonstrated the power of genomic data for resolving relationships, divergence times, and reconstructing shared patterns of diversification across amphibians (Roelants et al. 2007; Peloso et al. 2016; Feng et al. 2017; Irisarri et al. 2017; Streicher et al. 2018; Jetz and Pyron 2018; Hutter et al. 2019; Siu-Ting et al. 2019). However, to date no study has combined dense sampling from the nuclear genome with comprehensive taxon sampling at the subfamily level across all three orders of extant amphibians. To address these needs across extant amphibians, we designed a taxon-specific variation of the Anchored Hybrid Enrichment (AHE) protocol (Lemmon et al. 2012). By including probes designed from multiple model species spanning the three orders, we provide a novel sequence capture system for effectively targeting and sequencing hundreds of nuclear protein-coding loci across all extant amphibian species. We employed this capture system to collect AHE data and reconstruct the phylogeny of extant amphibians, assess the degree of deep-time genealogical discordance across their genomes, and evaluate the alternative hypotheses described above.

Materials and Methods

Amphibian-Specific Sequence Capture Probe Design

We designed a probe set that targets amphibian-specific orthologs for 366 of the 512 AHE loci developed by Lemmon et al. (2012). This probe set is designed from a diverse array of representatives of each of the three amphibian orders. Following Barrow et al. (2018), Heinicke et al. (2018), and Yuan et al. (2019), we mined the publicly available genome sequence for the model frog *Xenopus tropicalis* (Hellsten et al. 2010) and published transcriptomes for the salamanders *Ambystoma mexicanum* (Wu et al. 2013) and *Notophthalmus viridescens* (Abdullayev et al. 2013). To increase taxon representation in our probe design, we also developed and mined genomic resources *de novo* for six additional frogs (*Ascaphus montanus*, *Gastrophryne carolinensis*, *Mixophyes schevilli*, *Pseudacris feriarum*, *Pseudacris nigrita*, and *Rana sphenocephala*), one salamander (*Desmognathus fuscus*), and one caecilian (*Ichthyophis multicolor*), as well as transcriptomic resources for two additional salamanders (*Cryptobranchus alleganiensis* and *Ensatina eschscholtzii*). For each of these 13 amphibian taxa, we identified putative orthologs for the 366 amphibian-specific AHE loci from Lemmon et al. (2012) and designed RNA capture probes specific to these loci. Although not all of the 366 target loci were identified in all 13 model taxa, each locus was represented by, on average, 11.1 model taxa. Because it targets a subset of protein-coding exons, this probe set represents $<1\%$ of the *Xenopus* genome. After retaining a set of 220 putatively single-copy orthologous loci (see below in *Nuclear Locus Assembly and Alignment*), we identified human orthologs for these loci (see Supplementary Table S1 available on Dryad).

We designed a set of 120 base pair (bp) RNA probes tiled across each of these loci for each of the 4061 locus-by-model-taxon combinations. Each locus consists of an evolutionarily conserved core exonic region flanked by more variable regions on either side. Our probes for each model taxon covered these core regions and also extended into the flanks in order to increase the lengths of captured loci across diverse taxa. Across all 13 model taxa and 366 target loci, the region covered by our probes was 1,090 bp per locus on average. In practice, longer assemblies are generated from this type of data because the use of paired-end sequencing allows for the extension of sequenced regions beyond the core conserved regions covered by the probes. This set of 57,750 unique 120-mer probes was synthesized by Agilent Technologies and sequences of these probes are included in this study's Dryad package (https://doi.org/10.5061/dryad.9kd51c5dc).

Taxon Sampling for Amphibian Phylogenetics

We assembled tissues and/or genomic DNA, nearly all from museum vouchers, for a set of 286 amphibian species broadly covering family- and subfamily-level diversity and performed targeted sequence capture using our amphibian-specific probe set. Including eight of the 13 model taxa used in the probe kit design, our ingroup taxa consisted of 15 species and nine families of caecilians (out of 214 species and 10 families), 41 species and 10 families of salamanders (out of 740 species and 10 families), and 230 species and 52 families of frogs (out of 7,193 species and 54 families) (detailed in Supplementary Table S2 available on Dryad; species and family counts follow AmphibiaWeb.org, last accessed April 13, 2020). Within each of the three amphibian orders, we attempted to sample representatives from each recognized family, and from multiple subfamilies in the case of particularly diverse families (taxonomy used here follows AmphibiaWeb 2020). We sampled taxa in rough proportion to the species richness of their respective families, but we were also constrained by the availability of tissues and the quality of genomic DNA available for sequencing. To guide taxon choice, we consulted previously published phylogenies and, where possible, attempted to include similar taxonomic coverage of the different amphibian families.

We specifically attempted to sample deeply divergent lineages, as well as taxa that would break up long branches deep in the amphibian phylogeny or potentially provide resolution of recalcitrant nodes. Capturing large numbers of loci from the deeply divergent salamander *Siren intermedia* was problematic due to the combination of an extremely large genome, and deep divergence from any of the model probe taxa. Because of the importance of *Siren* for resolving salamander phylogeny, we sequenced a multi-tissue transcriptome for this species and mined orthologs from this assembly to include with our alignments. For the early-branching caecilian *Rhinatrema bivittatum*, we mined a published transcriptome sequence (Irisarri et al. 2017) for orthologs to our target loci.

Outgroup choice can have important downstream implications for phylogenetic inference (e.g., Wilberg 2015; Grant 2019), so to counter the potential for long branch attraction and to better estimate model parameters and divergence times for deep branches, we included multiple outgroup taxa. We included gene sequences mined from available genomic resources on GenBank from four amniote outgroups (*Anolis carolinensis*, taxon ID: 28377; *Chrysemys picta*, taxon ID:

8478; *Gallus gallus*, taxon ID:9031; and *Homo sapiens*, taxon ID 9606) and the coelacanth (*Latimeria chalumnae*, taxon ID: 7897). To identify putatively orthologous sequences to our target loci for these five outgroup taxa, we performed blastn searches with the NCBI BLAST algorithm (Johnson et al. 2008) against our target loci.

Genomic Library Preparation and High-Throughput Sequencing

Genomic DNA was extracted using DNeasy silica column kits (Qiagen). We performed 2% agarose gel electrophoresis for each sample to confirm the presence of high molecular weight genomic DNA. Library preparation and enrichment were performed at Florida State University's Center for Anchored Phylogenomics (www.anchoredphylogeny.com). Each sample was fragmented with a Covaris sonicator to a mean fragment size of 300–500 bp. DNA samples with signs of degradation were subjected to less fragmentation during library preparation. Individual samples were uniquely barcoded by ligation of 8 bp single index oligonucleotides and then pooled together in batches of 12–16 for multiplexed target capture. Capture reactions were carried out following Lemmon et al. (2012). The enriched, captured products were amplified by low-cycle PCR with high fidelity polymerase. Resulting genomic libraries were bead-cleaned and pooled for paired-end 150 bp sequencing on the Illumina platform (12–24 caecilians and salamanders per Illumina lane, and 24–60 frogs per lane) and sequenced across 14 lanes of an Illumina HiSeq2500 sequencer at the Florida State University Translational Lab.

Nuclear Locus Assembly and Alignment

Loci were assembled using a quasi-*de novo* strategy employing spaced kmer matching and extension assembly from merged paired-end Illumina reads (as in Rokyta et al. 2012; Prum et al. 2015; Hamilton et al. 2016). Orthology was established using a neighbor-joining algorithm based on pairwise distance matrices that accommodate high variation at third codon positions (see Hamilton et al. 2016). Ortholog filtering resulted in a set of 220 putatively single-copy nuclear loci for which all five outgroup taxa were also sampled. Of this set of 220 loci, 195 contained representatives of each of the three amphibian orders (Supplementary Tables S3 and S4 available on Dryad). We used the approach of Pyron et al. (2016) to recover both allele copies for diploid individuals (and multiple allele copies for ploidy levels greater than two). In the case of phase ambiguities, nucleotides were randomly resolved to one of their potential states. Although we generated phased data for every individual, we retained only one randomly chosen haplotype for each individual at each locus in order to greatly reduce the computational burden of downstream analyses. Multiple sequence alignment was performed in a nested procedure. We first performed four separate alignments for frogs, salamanders, caecilians, and the amniote and *Latimeria* outgroups in MAFFT v7.221 (Katoh and Standley 2013) with the L-INS-i parameter settings. These subalignments were then combined using the MAFFT—merge function.

Preliminary examination of gene trees for these alignments revealed that some taxa had clearly erroneous placements in a small number of gene trees (e.g., a salamander placed within frogs, or a caecilian nested within amniotes). These taxa were typically characterized by very long branch lengths. Further scrutiny revealed that in nearly every case, large numbers of ambiguous or missing sites apparently drove this pattern. This effect of missing data was, however, not unexpected (Lemmon et al. 2009). To clean up these alignments, we implemented a taxon-filtering procedure for each locus that culled any ingroup taxon with greater than 50% missing and/or ambiguous sites across an alignment, or with a terminal gene tree branch length greater than five times the average branch length for that tree. This filtering procedure removed less than 1% of the taxon-by-locus combinations and greatly improved the consistency of estimated gene trees.

Culled alignments were examined by eye to correct obvious misalignment issues (e.g., large gaps preceded by a single leading nucleotide), and to establish reading frames across protein-coding portions of each locus. Between zero and two base pairs were trimmed from the upstream ends of each alignment so that the first nucleotide represented the first codon position and the last nucleotide represented the third codon position of each locus. Establishment of reading frame and alignment corrections were performed in Geneious R8 (Kearse et al. 2012) with hydrophobicity display enabled for translated amino acid sequences. In many cases, manual alignment correction substantially increased polarity conservation, especially around gaps where the alignment may be particularly uncertain.

MtDNA Assembly and Sample Vetting

To verify the identities of samples, we assembled complete and partial mitochondrial genomes for nearly all taxa from off-target bycatch reads. These mitochondrial data served as integrated "barcodes" with which we could verify the integrity of our taxon identifiers and flag potential cases of misidentified taxa. Raw read data were reassembled *de novo* with trinityrnaseq v2.0.3 (Grabherr et al. 2011) and the resulting assemblies were mined for mtDNA regions using blastn searches against known mitogenomes. This procedure identified six instances of apparent pairwise transposition of sample labels, corroborated by aberrant placements in preliminary gene trees (e.g., placement of two taxa appeared transposed). This mislabeling likely occurred after DNA extraction but prior to library preparation. When mtDNA confirmed sample switching, taxon identifiers for these pairs of taxa were amended accordingly.

Gene Tree Estimation

Based on strict orthology assessment, we retained 220 loci for gene tree estimation. Individual locus alignments had on average 5.88% missing sites (range 0.36–19.75% missing sites). Because these AHE loci are exclusively protein-coding, we used a codon-based partitioning strategy for identifying and modeling substitution variation. For each locus, we used the corrected Akaike Information Criterion (AIC_c) calculated in PartitionFinder2 (Lanfear et al. 2016) to simultaneously select among possible partitioning schemes and models of molecular evolution using the greedy search algorithm (Lanfear et al. 2012). We chose partitioning schemes for each locus using AIC_c for use during gene tree estimation (detailed in Supplementary Table S3 available on Dryad). Maximum likelihood (ML) estimates of each individual gene tree were obtained in RAxML v8.2.11 (Stamatakis 2014) under several different topological constraints. For each RAxML analysis, 500 rapid bootstraps were conducted followed by a series of 20 slow ML optimization steps before the full ML analysis with branch length optimization. For each locus, we performed 17 separate ML tree searches. The first ML search was for a completely topologically unconstrained analysis. The second ML search was performed using a topological constraint that enforced amphibian monophyly but did not enforce any further topological constraints. These monophyletic-Amphibia constraint trees were used in downstream analyses of the support across loci for either the Batrachia, Procera, or Acauda hypotheses. Additionally, we inferred ML trees for each of the 15 backbone constraints representing the 15 possible tree topologies relating frogs, salamanders, caecilians, amniotes, and the outgroup *Latimeria* (Supplementary Fig. S1 available on Dryad). These 15 constrained trees were used in downstream analyses of the support for all competing interordinal models (see below). For 15 backbone constraints, intraordinal amphibian monophyly and the monophyly of amniotes were both enforced, but no constraints were imposed on relationships within the amphibian orders or within amniotes.

Species Tree Estimation

We employed several methods to reconstruct the topology of the amphibian species tree. As a first pass, we concatenated all 220 loci together into a single alignment with 291,282 nucleotide characters for 291 taxa (286 amphibians, four amniotes, and the *Latimeria* outgroup). Overall, this concatenated alignment was 86.4% complete and had 199,328 variable sites and 176,483 parsimony-informative sites. We also explored optimal partitioning schemes for the concatenated alignment in two different ways. One approach combined first, second, and third codon positions across all loci into their own respective subsets and identified an optimal partitioning scheme based on a greedy search in PartitionFinder2 where aggregate first, second, and third codon positions each comprised their own partitions. Next, we used the rcluster algorithm (Lanfear et al. 2014) in PartitionFinder2 to heuristically search model space for 660 maximum potential partitions from 220 loci each partitioned by codon position resulting in 76 separate partitions. The best partitioning schemes for both approaches were used for concatenated ML phylogeny estimation in RAxML. RAxML analyses employed 500 rapid bootstrap replicates, followed by 20 slow ML optimization steps before the full ML analysis with branch length optimization. We enforced a single topological constraint on these concatenated ML analyses by setting *Latimeria* as the outgroup to all other taxa.

To account for different coalescent histories among loci, we performed species tree estimation in ASTRAL2 v5.6.1 (Mirarab and Warnow 2015) using the best ML tree for each of the 220 loci from the unconstrained gene tree analysis. Using ASTRAL, we produced trees with local posterior estimates of branch support (Sayyari and Mirarab 2016), as well as estimates of the effective number of genes supporting each bipartition and the relative support for each of the alternative quartet resolutions. Although ASTRAL is technically not a coalescent method, it is statistically consistent with the coalescent model for increasing numbers of loci and sites (Mirarab and Warnow 2015).

Divergence Time Estimation

We estimated divergence times in the MCMCTree program from PAML v4.9f (Yang 2007) using a set of 19 fossil calibrations, which were also applied by Feng et al. (2017) (see Supplementary Table S5 available on Dryad and Supplementary Fig. S2 available on Dryad). These fossil calibration points cover many of the deep branches within tetrapods and within amphibians. We began by estimating the substitution rate for each of the 220 loci partitioned by codon position in the PAML program baseml under a GTR+Γ model of nucleotide substitution with five discretized rate categories. For each locus, the gene tree topology was fixed to that from the unconstrained RAxML gene tree analyses and the root age for the divergence between *Latimeria* and tetrapods was set to 450 million years ago (Ma) (Benton et al. 2015). Based on all loci and codon positions, we estimated a mean substitution rate of 0.899 substitutions per billion years and used this estimate to parameterize a diffuse gamma Dirichlet prior on locus rates (rgene_gamma) as $\Gamma(1\ 1.11)$.

We used the concatenated alignment of 220 loci, partitioned into aggregate first, second, and third codon positions as input for MCMCTree and fixed the tree topology to the ASTRAL species tree estimate. We then applied 19 fossil calibration points to constrain nodes such that 95% of the prior probability mass fell between the lower and upper (soft) bounds of each fossil calibration, with 2.5% prior density extending above and below these bounds. Next, we obtained maximum likelihood estimates (MLEs) of branch lengths by approximate likelihood and calculated the gradient and Hessian matrices at MLEs of branch lengths with the usedata=3 option. Using inferred ML branch lengths, we estimated divergence times in MCMCTree with the usedata=2 option, uncorrelated rates across loci, and a GTR+Γ model of nucleotide substitution. MCMCTree analyses used two independent MCMC chains with a 1,000,000 generation burnin, subsequently sampling every 100 generations until 10,000 samples were collected. The outputs of these analyses were combined and summarized together using the print=-1 setting in MCMCTree to calculate the posterior mean divergence times and 95% highest posterior density (HPD) credible intervals on divergence times. Effective sample size (ESS) values of parameters were calculated for the combined results in Tracer v1.7.1 (Rambaut et al. 2018). We also performed fossil cross-validation analyses (Near and Sanderson 2004) of our divergence time estimates to quantify the sensitivity of our results to individual fossil calibration points by systematically omitting one of each of the 19 fossil calibrations in each run. The time-calibrated phylogeny was visualized with the MCMCtreeR package (Puttick 2019).

Support across Loci for Interordinal Amphibian Relationships

One hundred ninety-five loci included representatives of all three amphibian orders and were thus suitable for investigating support for competing interordinal models (Supplementary Fig. S1 available on Dryad and Supplementary Table S4 available on Dryad). We initially quantified support across these 195 loci for the three competing interordinal models that support a monophyletic Amphibia (Batrachia, Procera, and Acauda). We counted how many of these constrained ML gene trees recovered topologies consistent with Batrachia, Procera, or Acauda. For each of these 195 genes, we also calculated the proportion of gene tree bootstrap replicates that supported Batrachia, Procera, or Acauda and plotted these values across loci. Additionally, we used a model-selection framework to calculate the relative support for each of these three competing interordinal topologies. For each locus, we compared the log likelihoods of constrained gene trees and calculated the Akaike Information Criterion (AIC; Akaike 1974) for each topological model relative to the best model [the model with the lowest $-\ln(L)$]. AIC was calculated as $2k - 2\ln(L)$, where k is the number of free parameters in the model of sequence evolution and the tree (e.g., branch lengths) and L is the maximum likelihood under the constraints for a given hypothesis. There is an additional unknown number of free parameters associated with topological freedom in our hypotheses (i.e., we constrain monophyly of orders, but not relationships within orders) that we do not include in k, because the appropriate number is unclear. However, this value should be the same across hypotheses, because each has the same amount of topological freedom, therefore the associated penalty term would cancel when calculating differences in AIC scores. These differences between the best model and the other i competing models for each locus were calculated as $|\text{AIC}_{\text{min}} - \text{AIC}_i|$. Because k is identical across hypotheses, calculating these differences in AIC is equivalent to examining the log of the maximum likelihood ratio between the best model and each alternative. We also repeated these analyses for the full 15-model set depicted in Supplementary Figure S1 available on Dryad.

To further quantify support for competing interordinal models for amphibian relationships, we conducted approximately unbiased (AU) tests of topology (Shimodaira 2002) across the 15 backbone constraints. To examine support across loci for the interordinal amphibian relationships, we applied the method of gene genealogy interrogation (GGI; Arcila et al. 2017). For each locus, we inferred an ML topology and then plotted the cumulative number of loci supporting each alternative topological hypothesis, rank ordered by decreasing statistical significance of the AU P-value. We performed AU tests in PAUP* (v4a.151) (Swofford 2003).

Results

Nuclear Locus Assembly and Alignment

After filtering for potential paralogs and missing data in alignments, we retained a set of 220 loci, of which 195 had representatives of all three amphibian orders present. This resulted in total of 291,282 aligned nucleotide positions across all loci. On average, individual frogs, caecilians, and salamanders had 214, 165, and 146 loci, and 97%, 75%, and 66% of sites in the alignments present, respectively.

Gene Tree Estimation

Codon-based partitioning analyses and substitution model selection for individual loci identified 217 loci for which a single partition containing all codon positions and a GTR+Γ model of sequence evolution was applied (Supplementary Table S3 available on Dryad). The remaining three loci (L123, L262, and L285) were best modeled with two partitions, one combining first and second codon positions and the second partition comprising third codon positions and a GTR+Γ model. RAxML bootstrap support (BS) for individual gene trees was relatively high, with an average BS value of 73.8 across all branches in all gene trees (median value of 90). Individual unconstrained gene trees varied according to which of the 15 possible interordinal models of amphibian and amniote relationships were present in the ML tree. Among unconstrained gene trees, 143 loci identified a monophyletic Amphibia, 67 loci recovered Batrachia, 38 loci supported Procera, and 38 loci supported Acauda. All gene trees are included in this study's Dryad accession.

Topology and Timescale for the Amphibian Tree of Life

Concatenated RAxML and ASTRAL analyses produced similar estimates of the species tree (normalized Robinson–Foulds distance = 0.027), and we present the ASTRAL species tree here (Fig. 1; concatenated RAxML tree in Supplementary Fig. S3 available on Dryad). In both estimates of the species trees, we recovered a monophyletic Amphibia, monophyly of each amphibian order, and a frogs-sister-to-salamanders relationship (the Batrachia hypothesis), all with BS = 100. MCMCTree results indicated that the Amniota-Lissamphibia split occurred 347–352 Ma, during the early Carboniferous, that the ancestor of caecilians and an ancient batrachian diverged 287–303 Ma, in the late Carboniferous or early Permian, and that frogs split from salamanders by 269–275 Ma, during the middle Permian (Fig. 2).

The estimated phylogenetic relationships within each of the three amphibian orders were largely consistent with those from previous studies, with most interfamilial branches receiving maximal bootstrap support in both the RAxML and ASTRAL analyses (Supplementary Figs. S3 and S4 available on Dryad, respectively). Within caecilians, we recovered the deepest divergence event as being between the Rhinatrematidae and all other caecilians examined (Figs. 1 and 2, Supplementary Figs. S3 and S4 available on Dryad), with this divergence occurring 102–130 Ma in the early Cretaceous. All remaining family-level relationships within caecilians were resolved with high branch support (Supplementary Figs. S3 and S4 available on Dryad).

Our results unequivocally supported an initial divergence event in crown salamanders between Cryptobranchioidea (Cryptobranchidae and Hynobiidae) and a clade comprising Salamandroidea and Sirenidae (Fig. 1, Supplementary Figs. S3 and S4 available on Dryad), with a divergence occurring 148–171 Ma in the early to middle Jurassic (Fig. 2). After divergence with Sirenidae, the first split within Salamandroidea involved a clade comprising Ambystomatidae and Dicamptodontidae and Salamandridae, and a clade comprising successive divergence events splitting off Proteidae, Rhyacotritonidae, Amphiumidae, and Plethodontidae.

Within frogs, we recovered a nonmonophyletic Archaeobatrachia (Figs. 1 and 3, Supplementary Figs. S3 and S4 available on Dryad). At the root of the frog clade, the Leiopelmatoidea (Leiopelmatidae and Ascaphidae, Green and Cannatella 1993) diverged from all other frogs 205–223 Ma in the late Triassic (Fig. 3). Additional archaeobatrachian superfamily-level groups were also resolved, including Bombinatoroidea (Bombinatoridae and Alytidae) and the Pipoidea (Rhinophrynidae and Pipidae), and Pelobatoidea, the clade containing (Scaphiopodidae, Pelodytidae, Pelobatidae, and Megophryidae). Our results suggest that *Leiopelma* and *Ascaphus* diverged 187–209 Ma in the early Jurassic, and that Neobatrachia split from Pelobatoidea (Scaphiopodidae, Pelobatidae, and Pelodytidae) 169–185 Ma in the middle Jurassic.

Neobatrachia was recovered as monophyletic in all analyses (Fig. 1), and all methods supported *Heleophryne* as sister to all other neobatrachians (Figs. 1 and 3, Supplementary Fig. S3 and S4 available on Dryad). The remaining neobatrachian lineages formed two clades: (Hyloidea, (Myobatrachidae, *Calyptocephalella*)) and (Ranoidea, (*Sooglossus*, *Nasikabatrachus*)). Within Ranoidea, we recovered a clade comprising (Microhylidae, (Natatanura, Afrobatrachia)). Within Neobatrachia, we found that Heleophrynidae split from all other neobatrachians 140–152 Ma in the late Jurassic or early Cretaceous (Fig. 3). Our results also suggest that the major neobatrachian crown lineages Hyloidea, Ranoidea, Afrobatrachia, and Natatanura arose 67–77 Ma, 99–111 Ma, 81–92 Ma, and 61–69 Ma, respectively (Figs. 3 and 4). We found support for a sister relationship between *Nasikabatrachus* and *Sooglossus* and estimated the divergence time between these two deeply divergent anuran families at 62–93 Ma (Fig. 4). This particularly large range of dates for this node may be associated with an elevated percentage of missing loci for *Nasikabatrachus*. Within Ranoidea, we recovered Microhylidae sister to a clade containing Afrobatrachia and Natatanura (Fig. 4). Posterior mean divergence times, upper and lower bounds of the 95% HPD confidence intervals, and ESS values for all nodes are provided in Supplementary Table S6 available on Dryad. Our divergence time estimation results were largely robust to the sensitivity analyses wherein we reran the MCMCTree analyses excluding a single fossil calibration in each run. Results of the exclusion analyses are provided in Supplementary Table S7 available on Dryad.

Placement of Nasikabatrachidae and Sooglossidae

Our final ASTRAL and concatenated RAxML analyses both supported a sister relationship between the two deeply divergent anuran families Nasikabatrachidae and Sooglossidae (Supplementary Fig. S5 available on Dryad). Initial ASTRAL and RAxML analyses based on gene alignments that were not filtered for missing sites (see Materials and Methods section) disagreed with respect to the placement of *Nasikabatrachus*, with ASTRAL analyses placing it as sister to all Microhylidae and RAxML supporting a placement sister to *Sooglossus*. However, upon further scrutiny of individual gene alignments, we noted a pattern of short, gap-laden loci supporting *Nasikabatrachus* as sister to the microhylid genus *Oreophryne*, whereas loci with fewer missing sites supported the conventionally accepted placement of *Nasikabatrachus* sister to *Sooglossus* (Supplementary Fig. S5 available on Dryad). Final analyses that filtered out individual sequences with > 50% of sites missing for any locus resulted in ASTRAL species tree placement of *Nasikabatrachus* and *Sooglossus* as sister clades.

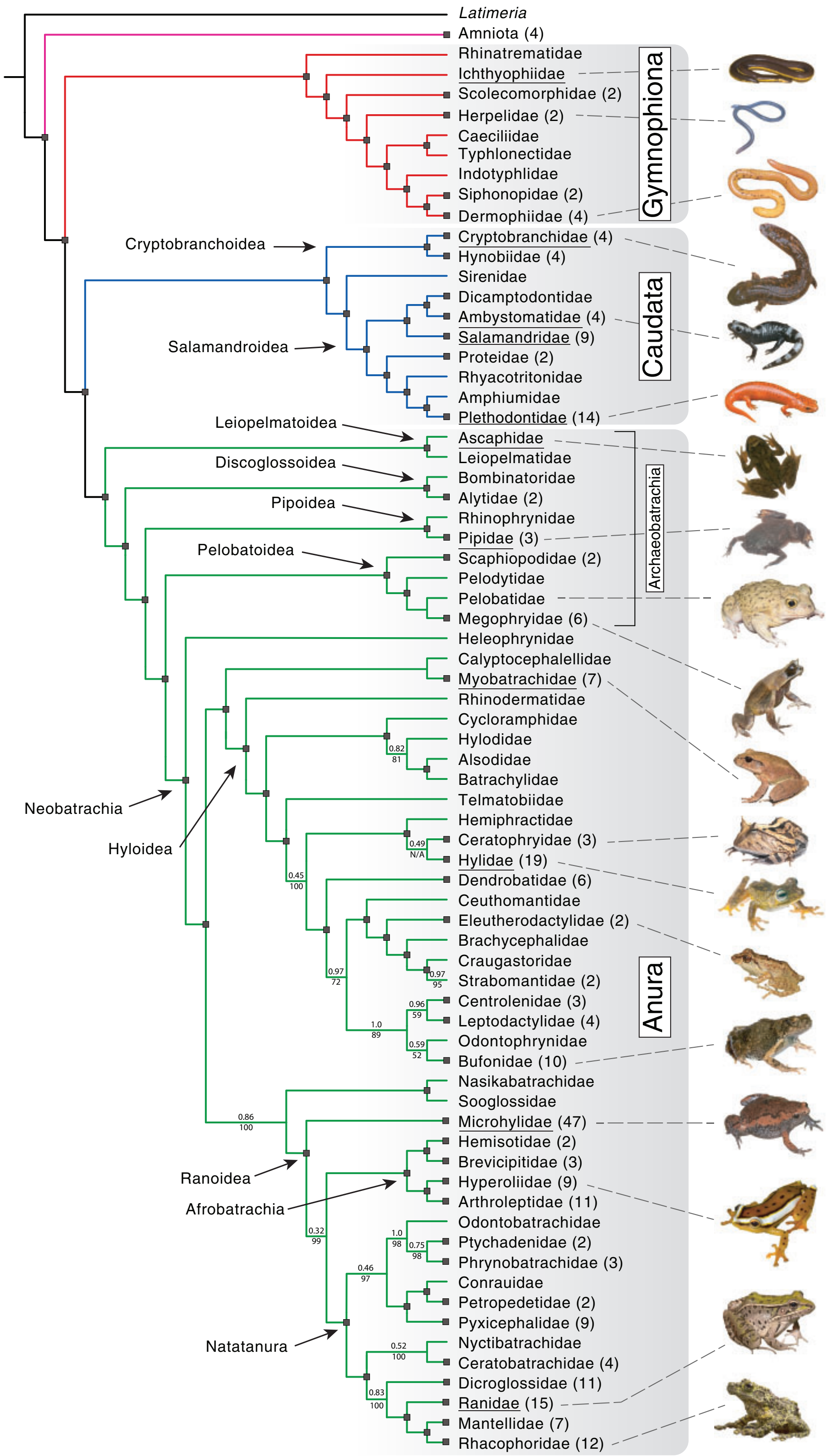

FIGURE 1. Topology of family-level amphibian relationships inferred from ASTRAL and RAxML analyses. Gray boxes denote branches with ASTRAL local posterior values of 1 and RAxML bootstrap percentages of 100. For branches without unanimous support, upper values indicate ASTRAL local posterior probabilities and lower values indicate RAxML bootstrap percentages. N/A indicates that a branch was recovered by ASTRAL but not by RAxML. Terminal branches without support values are represented by a single taxon from a given family. Values in parentheses after a family name indicate the number of species sampled if greater than one. Families from which species were selected for probe design are underlined. Dashed gray lines connect exemplar photos to their respective families. [Color figure can be viewed in the online pdf version]

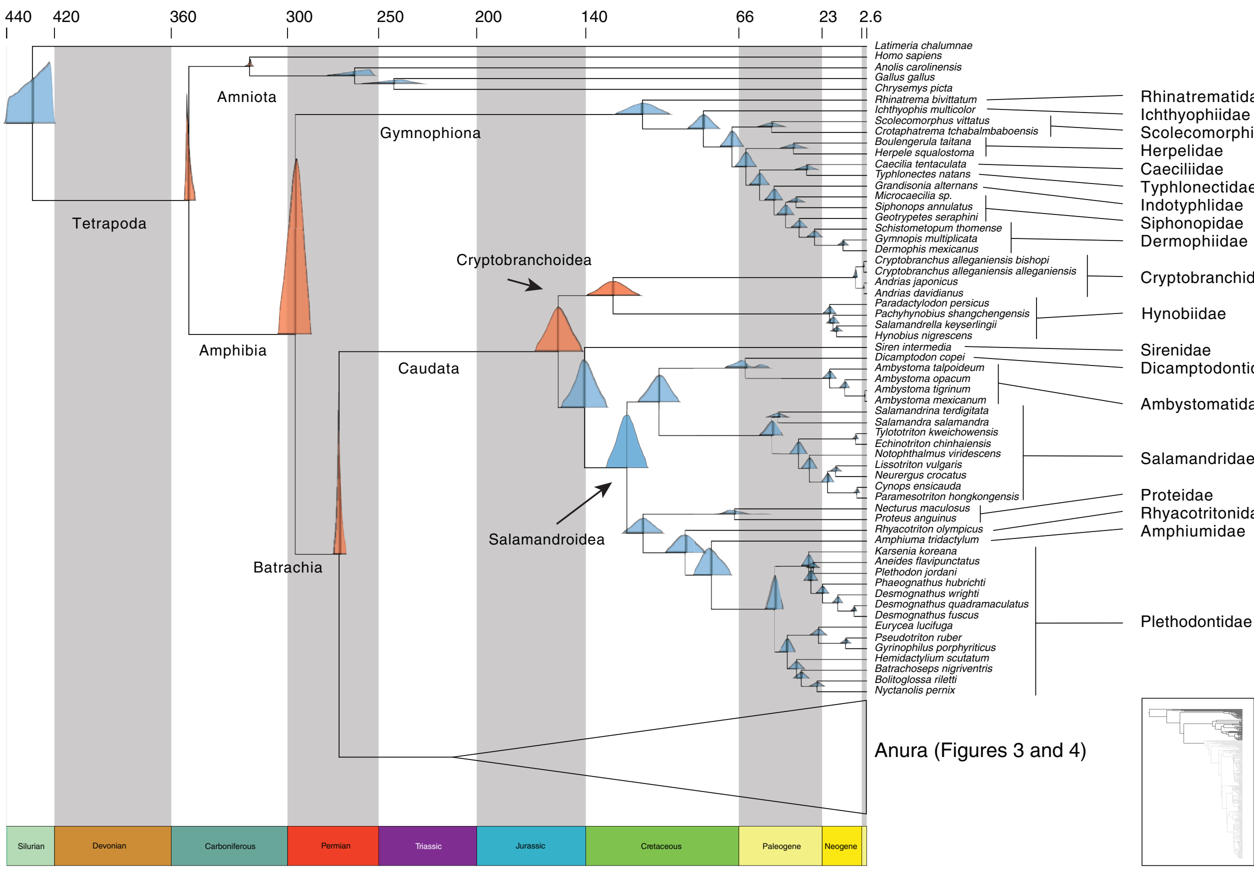

FIGURE 2. Time-calibrated phylogeny for Amphibia. Divergence times among amniotes, caecilians, and salamanders. Divergence times across Amphibia were inferred in MCMCTree with 19 fossil calibration points. Posterior distributions are plotted for each node, representing the 95% highest posterior density confidence interval of divergence times. Fossil calibrated nodes are denoted with orange posterior distributions and noncalibrated nodes are shown in blue. The tree topology is based on the results of the ASTRAL analysis. The heights of the posterior distributions are scaled to their corresponding branches. [Color figure can be viewed in the online pdf version]

Gene Tree Discordance and Genomic Support for Deep Amphibian Relationships

When considering only the Batrachia, Procera, and Acauda interordinal models, each of the three alternative interordinal hypotheses for a monophyletic Amphibia was supported by different subsets of the genome. Quantifying bootstrap support for interordinal models involving a monophyletic Amphibia revealed substantial variation across loci (Fig. 5a). A greater number of gene trees exhibited high bootstrap support for the Batrachia hypothesis relative to the Acauda or Procera hypotheses. However, each of these three interordinal hypotheses had at least three gene trees with BS > 90 at the interordinal nodes. Of 195 loci with at least one member of each amphibian order included, 89 loci supported Batrachia, 51 supported Procera, and 55 supported Acauda, respectively. Similarly, a rank-ordered plot of BS by interordinal hypothesis revealed multiple loci with low to moderate bootstrap support for each hypothesis (Fig. 5a). In fact, only 28 loci had BS > 90 for any of the deep interordinal nodes, suggesting that many loci might lack sufficient information content for resolving these deepest nodes in the amphibian phylogeny (Siu-Ting et al. 2019).

Measures of ΔAIC-based model support across the full set of 15 models that also account for the possibility of a nonmonophyletic Amphibia found very strong support for models involving a monophyletic Amphibia, with ΔAIC values as high as 1,400 against some nonmonophyletic models. Of the 195 interordinal-informative loci, 144 loci supported monophyly of Amphibia (68 loci supported Batrachia, 38 loci supported Procera, and 38 loci supported Acauda). The remaining 51 loci supported one of the 12 alternative models of a nonmonophyletic Amphibia (Supplementary Fig. S6 available on Dryad). The raw log likelihoods of the 15 constrained models across 195 loci used as input for AIC-based measures of support for the 3- and 15-hypothesis analyses are included in Supplementary Table S8 available on Dryad.

Results from GGI analyses (Fig. 6) demonstrated that the majority of loci support a monophyletic Amphibia, although support for either the Acauda, Procera, or Batrachia hypotheses varied across loci. Approximately unbiased test P-values represent how strongly competing topological models can be rejected

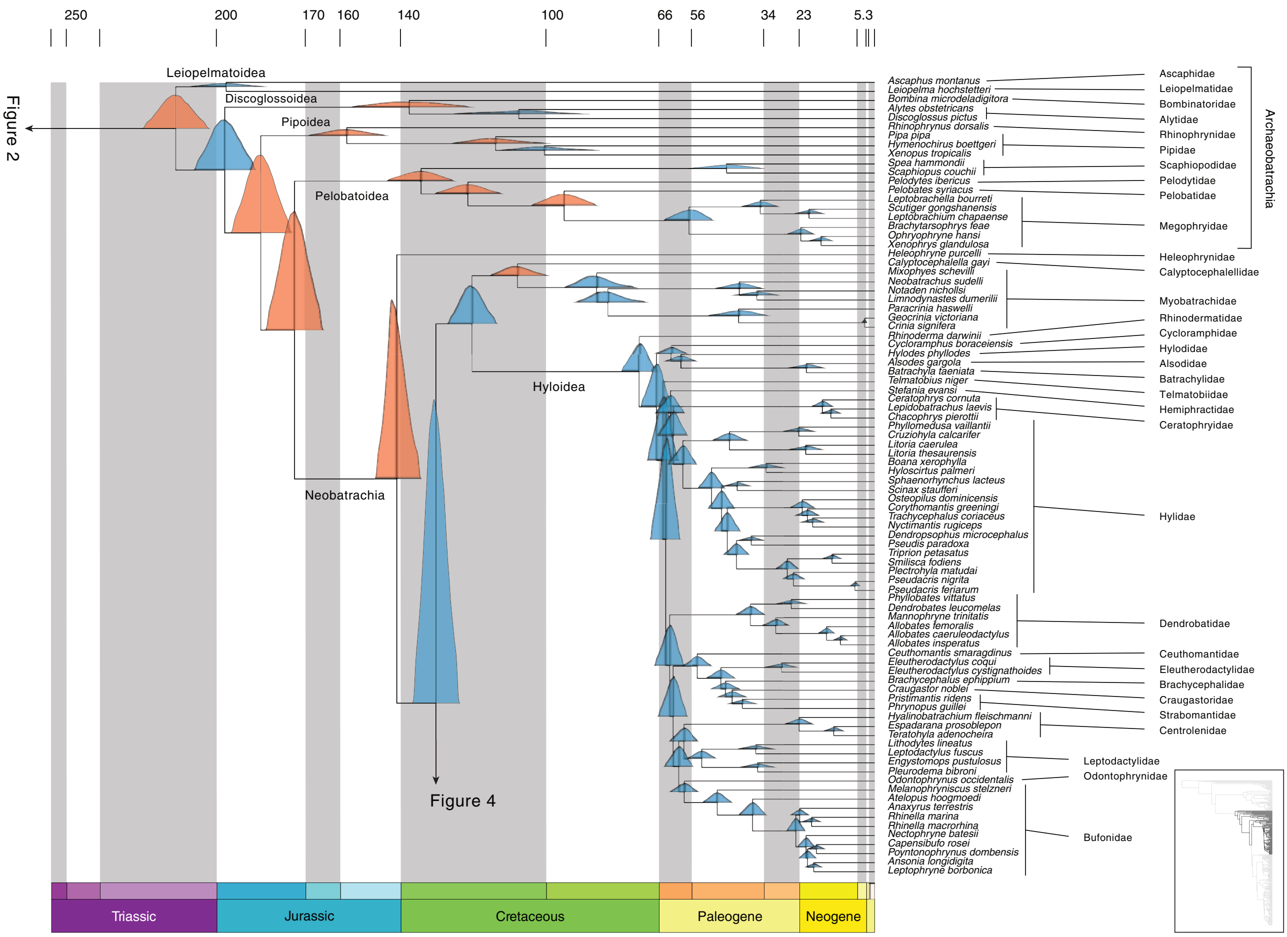

FIGURE 3. Time-calibrated phylogeny for Anurans I. Divergence times for a portion of Anura, containing the Archeobatrachia and some lineages and clades of Neobatrachia. Posterior distributions are plotted for each node, representing the 95% highest posterior density confidence interval of divergence times. Fossil calibrated nodes are denoted with orange posterior distributions and noncalibrated nodes are shown in blue. The tree topology is based on the results of the ASTRAL analysis. The heights of the posterior distributions are scaled to their corresponding branches. [Color figure can be viewed in the online pdf version]

relative to the preferred model, with a canonical value of 0.05 reflecting strong support. Very few loci had strong AU support ([1 - AU *P*-value] $>$ 0.95), but most gene trees supported the Batrachia hypothesis (Fig. 6). Across loci supporting each of the 15 possible interordinal topologies rank-ordered by decreasing AU test values, support dropped precipitously for Procera and Acauda and nononophyletic Amphibia models. Notably, only a small number of loci supporting any of the 15 possible models received support above our threshold of $P \leq 0.05$. Figure 6a shows GGI support for the three monophyletic Amphibia models and the 12 aggregated nonmonophyletic Amphibia models, whereas Figure 6b depicts GGI support across the 12 alternative models individually.

Discussion

Using data generated with a newly developed, amphibian-specific Anchored Hybrid Enrichment probe set, we produced the most comprehensive phylogenomic inference of the amphibian Tree of Life to date, both in terms of taxa (286 species, ~95% of amphibian families) and genetic loci (220 nuclear loci). Although there were few surprising topological relationships uncovered by this work (see below), we provide the most robust assessment to date of amphibian relationships and their divergence times, placing all major amphibian lineages together in a comprehensive phylogenomic framework. We recognize that future study is warranted in several clades (e.g., Plethodontidae, Microhylidae) to better resolve more shallow-scale relationships.

Overall, our species tree results firmly corroborate the monophyly of Amphibia and strongly supported the Batrachia hypothesis, grouping frogs and salamanders as a clade. Perhaps the most surprising aspect of our results was the identification of significant variation across the genome in the strength of support for competing hypotheses about the deepest divergences in the evolutionary history of amphibians. Some loci even exhibited strong support for a nonmonophyletic Amphibia. In the context of a monophyletic Amphibia,

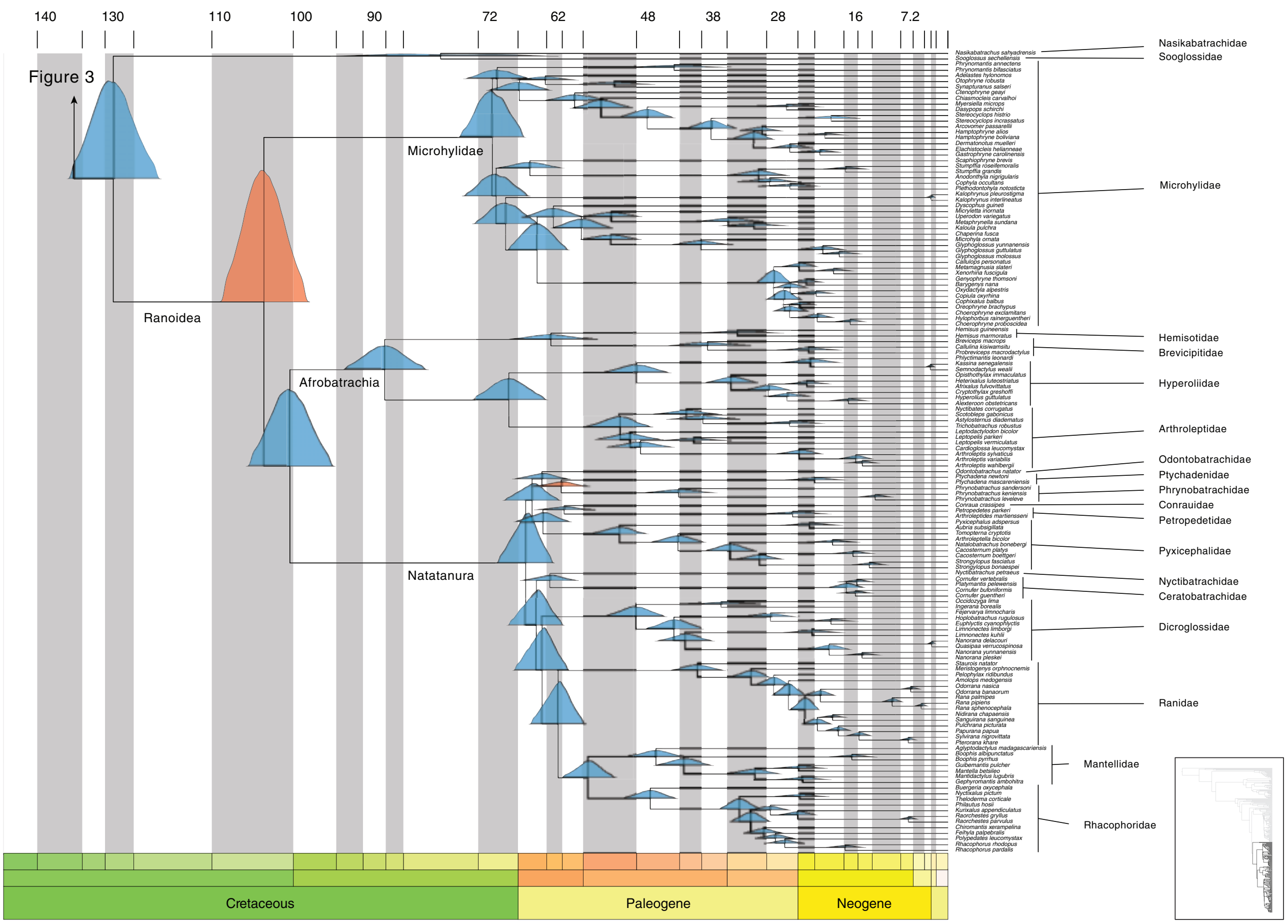

FIGURE 4. Time-calibrated phylogeny for Anurans II. Divergence times for the remaining portion of Anura, containing the remaining clades from Neobatrachia. Posterior distributions are plotted for each node, representing the 95% highest posterior density confidence interval of divergence times. Fossil calibrated nodes are denoted with orange posterior distributions and noncalibrated nodes are shown in blue. The tree topology is based on the results of the ASTRAL analysis. The heights of the posterior distributions are scaled to their corresponding branches. [Color figure can be viewed in the online pdf version]

each of the three possible interordinal hypotheses received strong support from at least some loci (see below). Yet, at the species-tree level, our genome-wide data strongly supported the monophyly of Amphibia and a sister relationship between frogs and salamanders (the Batrachia hypothesis).

Collectively, our results imply either the persistence of patterns of evolutionary mechanisms that contribute to genealogical discord (e.g., ILS or gene flow) over extremely deep timescales, and/or systematic error in phylogenetic estimates from some loci, leading to strongly supported, but inaccurate, estimates of gene trees. The observed heterogeneity in gene tree topologies in deep evolutionary history is obscured by species tree analyses, potentially leading to oversimplification of genomic evolutionary history. Our findings underscore the importance of conducting an in-depth examination of genome-wide phylogenetic signal as opposed to utilizing summary approaches such as gene concatenation or species tree analyses alone.

Our results further support recent analyses presented by Burbrink et al. (2020) for squamates. In that study, genomic interrogation using machine learning showed strong overall congruence for a single topological resolution of lizard and snake relationships that is obscured for some early nodes by apparent ILS, as well as poor or degraded phylogenetic signal in some loci. These congruent results across taxa and timescales for similarly constructed genomic data sets suggests that these processes of signal decay and genealogical discordance driven by rapid radiations and short internodes may be a ubiquitous feature of early branches in the Tree of Life (e.g., Rokas and Carroll 2006; Dunn et al. 2008). However, approaches such as ours and those of Burbrink et al. (2020) nonetheless show the tractability of resolving strong support for these relationships given sufficient data.

Evaluating Interordinal Amphibian Models and Deep ILS

Deep-time gene tree–species tree discordance was evident from multiple analyses. Bootstrap results provided some indication for variation across loci in

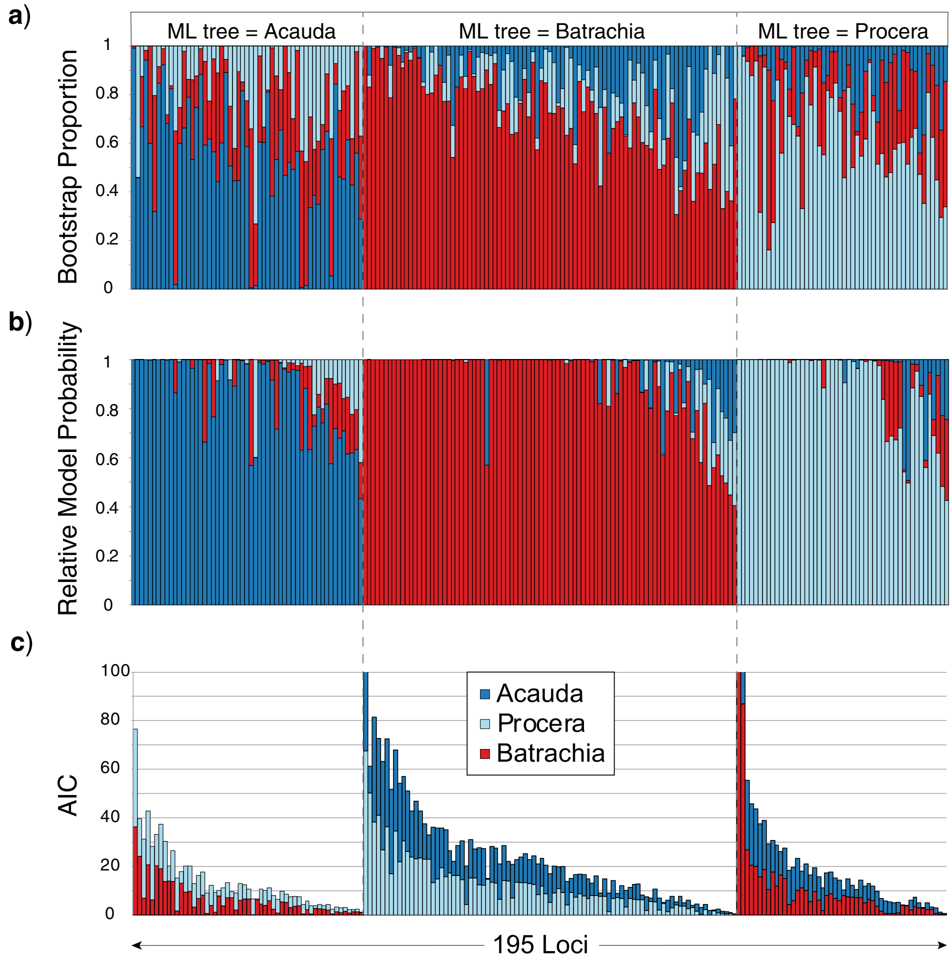

FIGURE 5. Evaluation of the magnitude and direction of support for competing models of a monophyletic Amphibia. a) Proportions of RAxML gene tree bootstrap replicates supporting the three possible interordinal topologies, assuming a monophyletic Amphibia. b) Relative model probabilities among three possible interordinal models. c) Differences in Akaike Information Criterion (ΔAIC) between best model and alternative models quantifying the magnitude of support against the two alternative models for interordinal amphibian relationships (bars are stacked for clarity and values are not cumulative). In all three panels, 195 loci with representatives of each amphibian order are plotted along the horizontal axis. Alternative models for Acauda, Batrachia, and Procera are colored in dark blue, red, and light blue, respectively. [Color figure can be viewed in the online pdf version]

support of different interordinal models involving a monophyletic Amphibia. Many loci were equivocal in their bootstrap support for the reconstruction of any of the three interordinal models involving a monophyletic Amphibia. Yet, each of the three interordinal hypotheses was reconstructed from at least a small number of loci with high bootstrap support. For example, three loci supported an Acauda gene tree with BS $>$ 90 and eight loci supported a Procera gene tree with BS $>$ 90, both in contrast to the robust species tree reconstruction of a Batrachia relationship (with 17 loci with concordant gene trees at BS $>$ 90). The bootstrap represents a bounded measure of branch support, with the ability to tell us something about the direction, but not the magnitude, of support for a particular hypothesis.

The use of GGI/AU tests and information-theoretic relative model probabilities provided two explicit statistical approaches for comparing competing interordinal models, although these provided somewhat contrasting results. GGI/AU tests were quite conservative, with small numbers of loci statistically supporting all of the three monophyletic amphibian models. For example, a total of just four loci supported a Batrachia gene tree topology with an AU test value $\geq$0.95, and no loci produced gene trees with significant AU Test support for Acauda or Procera topologies. While the GGI analyses indicated that Batrachia is favored for gene tree relationships among orders (roughly half of all loci have "best" gene trees consistent with Batrachia), most loci lacked definitive signal this far back in the evolutionary history of amphibians.

Our GGI results for the deep (250–300 Ma) divergences in Amphibia mirror those found in other anciently diverging clades (such as early animal relationships), in that very few individual gene trees are statistically significant for any one model (Arcila et al. 2017). In

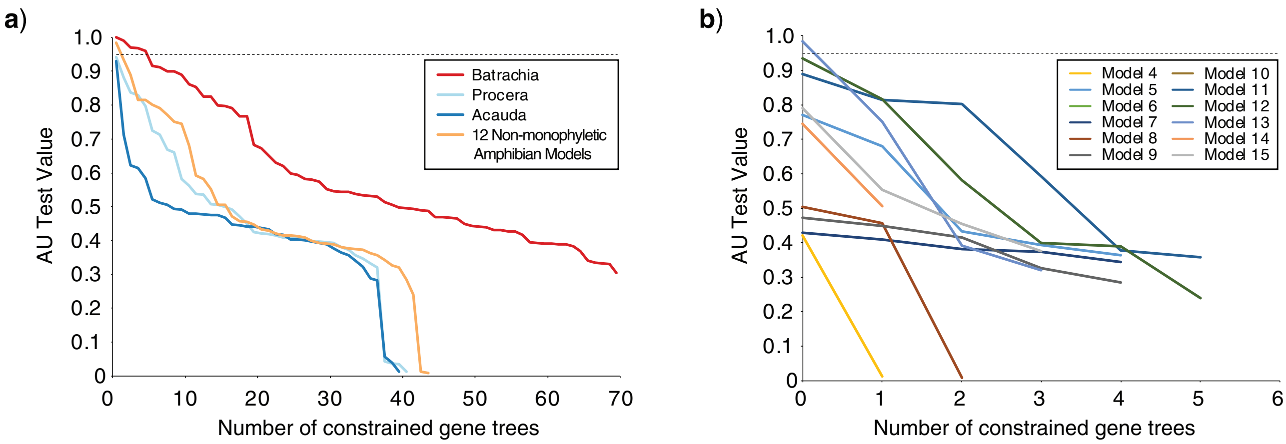

FIGURE 6. Gene genealogy interrogation with the approximately unbiased (AU) test across 195 loci. Results from AU tests performed for each of 195 loci with representatives of each amphibian order. Loci are binned according to which of the 15 possible topologies are supported by unconstrained RAxML gene trees, and then ranked according to the AU test *P*-value supporting their preferred hypothesis. a) Most unconstrained gene trees support a monophyletic Amphibia, but very few are significantly better than alternative trees at a threshold of 0.95. Alternative models for Acauda, Batrachia, and Procera are colored in dark blue, red, and light blue, respectively, and loci supporting any of the 12 models for a nonmonophyletic Amphibia are colored in orange. b) Loci supporting a nonmonophyletic Amphibia are plotted by which of the alternative models are supported. Model numbers correspond to Supplementary Figure S1 available on Dryad. [Color figure can be viewed in the online pdf version]

contrast to the conservative GGI/AU tests, information-theoretic relative model probabilities provided stronger support across loci for all three monophyletic amphibian models. For example, nearly all of the loci supporting each of the three monophyletic amphibian models received relative model probabilities greater than 0.5 and many loci supported one particular model with a relative model probability of 1.0. However, both the GGI/AU tests and the relative model probabilities, like the bootstrap support values, are bounded metrics of support ranging from 0 to 1 and, thus, may potentially obscure some patterns of support across loci.

In contrast, all three of our competing interordinal models had best gene trees that were supported with ΔAIC > 40 (ΔAIC > 2 is typically viewed as strong support for one model over another; Burnham and Anderson 2002). We note, however, that in cases where the degree of topological constraint varies among hypotheses, AIC may be challenging, because the appropriate number of free parameters is unknown. Nonetheless, many studies may benefit from focusing on a set of topological hypotheses with the same degree of topological freedom, as we do here.

When considering all results, we find strong support for amphibian monophyly and the Batrachia hypothesis at the species tree level, and substantial support for discordant gene trees across this deep evolutionary split. Our interpretation of this genealogical discord is ultimately based on the extremely strong measures of ΔAIC support for particular models. We find no correlation between the best supported interordinal models and numbers of taxa from each order, percent missing data across loci, GC content, or the variability of loci (Supplementary Fig. S7 available on Dryad). Several potential and nonmutually exclusive explanations may account for this observation. Our detection of strongly supported discordant gene trees may reflect one of the potential evolutionary phenomena known to yield such patterns (Maddison 1997), including ILS, horizontal transfer, natural selection, saturation, or a gene duplication and loss resulting in errors in orthology assignment (Siu-Ting et al. 2019).

We are unable to definitively discriminate among these possible mechanisms of genealogical discordance. However, the preservation of a historical demographic signal of ILS seems most likely because the number of loci supporting Batrachia is roughly equal to the numbers of loci supporting Procera and Acauda, and because the number of loci supporting these two minor topologies (Procera and Acauda) are also roughly equal, a specific prediction of ILS (Degnan and Rosenberg 2009). Nearly 12 myr separate the 95% credible intervals for the divergences between the lower bound of the divergence between Gymnophiona and the ancestor to Batrachia and the upper bound of the divergence between Caudata and Anura (Fig. 2), and it might be surprising to consider a pattern of ILS persisting over such a long period of time. However, at least one simulation study has demonstrated the potential for ILS to persist over tens to hundreds of millions of years (Oliver 2013). At least some empirical evidence has indicated that the preservation of deep ILS may be present in other major vertebrate clades (e.g. birds, Poe and Chubb 2004; Edwards et al. 2005). Although we observe a pattern of gene tree discordance that is consistent with expectations of ILS. Regardless of the specific mechanism(s) underlying deep gene tree discordance, a sole focus on the estimation and analysis of the species tree topology (rather than also scrutinizing individual gene tree topologies) may lead to a failure to consider more nuanced aspects of evolutionary history (Hahn and Nakhleh 2016).

Our detection of genealogical discordance across the amphibian genome also suggests that if one were to sample only a few loci at random, the genealogical variation across loci could lead to either weak support for deep nodes or spurious evidence for an alternative interordinal model. At least one other empirical amphibian study has reported discordance across loci, including a finding similar to ours, namely that some loci may support a nonmonophyletic Amphibia (Fong et al. 2012), and stochastic sampling effects combined with deep ILS may together account for the variation across previous studies in terms of which interordinal amphibian topologies have been supported.

Effects of Missing Data in Phylogenomic Data Sets

Our study also emphasized the importance of scrutinizing the effects of large amounts of missing data from individual gene alignments on species tree topologies. We found the effects of missing data to be a particularly important issue in the resolution of relationships among an important set of frog clades, which is expected when branches are very strongly supported (Lemmon et al. 2009). Our initial analyses led to a disagreement between RAxML and ASTRAL trees with respect to the placement of *Nasikabatrachus* (Supplementary Fig. S5a available on Dryad). Although the concatenated ML tree strongly supported the canonical placement (Biju and Bossuyt 2003) of *Nasikabatrachus* as sister to *Sooglossus* (BS = 100), the initial ASTRAL tree supported a surprising and previously unreported sister relationship between *Nasikabatrachus* and the family Microhylidae, with relatively strong support (ASTRAL local posterior = 0.71). Upon closer examination of the placement of *Nasikabatrachus* in the 194 gene trees containing both *Nasikabatrachus* and *Sooglossus*, we found that loci with greater proportions of missing sites ($n = 98$ loci) consistently favored *Nasikabatrachus* as sister to the microhylid *Oreophryne*, whereas loci with fewer missing data tended to favor traditional placements (Biju and Bossuyt 2003; Feng et al. 2017) of *Nasikabatrachus* sister to *Sooglossus* ($n = 94$ loci) (Supplementary Fig. S5b available on Dryad). Subsequent filtering of individual gene alignments to exclude taxa with $> 50\%$ of sites missing brought the ASTRAL and RAxML topologies into agreement (Supplementary Fig. S5c available on Dryad). Thus, ASTRAL was likely misled by the fact that gene trees supporting the novel placement of *Nasikabatrachus* slightly outnumbered gene trees supporting the previously identified placement as sister to *Sooglossus*. Other studies have also reported conflicting placements for taxa with large amounts of missing data when using either supermatrix (e.g., Lemmon et al. 2009) or species tree methods (Hosner et al. 2016; Moyle et al. 2016). An early expectation for genome-scale phylogenetics was that massive genetic data sets, regardless of levels of missing data, would provide unwavering reconstructions for even the most recalcitrant branches of the Tree of Life through the sheer size of the data. However, whereas adding more genetic loci to species tree analyses can increase the accuracy of the resulting inferences, it is becoming clear that phylogenomic data, on their own, are no panacea for difficult phylogenetic problems (Jeffroy et al. 2006; Brown and Thomson 2016; Shen et al. 2017; Walker et al. 2018).

Genomic Perspectives on the Amphibian Tree of Life

Beyond the firm establishment of the Batrachia relationship at the base of the amphibian Tree of Life, our study also cemented a number of important relationships between and within family-level lineages. A notable exception is that our results support a sister clade relationship between Afrobatrachia and Natatanura (local posterior support = 0.32), with the two as sister of Microhylidae (Fig. 4). This result contrasts with previous hypotheses of amphibian phylogenies based on relatively small amounts of DNA sequence data (e.g., less than 20 loci, Frost et al. 2006; Pyron and Wiens 2011) but agrees with more recent genomic studies (Feng et al. 2017; Siu-Ting et al. 2019; Yuan et al. 2019). Some amphibian clades received relatively high bootstrap support from concatenated RAxML analyses but are nonetheless not well supported by ASTRAL analyses. Within some families (e.g., Plethodontidae and Microhylidae), phylogenetic relationships are less well supported than relationships between families, suggesting some recalcitrant portions of the amphibian phylogeny still remain to be addressed.

Our divergence time estimates for Lissamphibia and Batrachia are somewhat younger than in Roelants et al. (2007), but largely in line with Feng et al. (2017). However, perhaps the most surprising aspects of our study are the relatively younger divergence times among families within each of the three amphibian orders. The Late Cretaceous-Paleogene boundary has been suggested as a critical epoch for amphibian evolution, reflected in the origin and early diversification of several hyperdiverse clades (i.e., Hyloidea, Microhylidae, Afrobatrachia, Natatanura, Salamandridae, and Plethodontidae) (Roelants et al. 2007; Pyron 2014; Feng et al. 2017). Our divergence time estimates, based on the most comprehensive amphibian dataset assembled to date, push the date of origin and initial diversification of frogs, caecilians, and salamanders, later by several million years, and may thus reshape the way we think about the evolution and biogeography of amphibians. This is especially the case concerning hypotheses of continental vicariance and dispersal (e.g., Pyron 2014) and the emphasis currently placed on the Cretaceous–Paleogene boundary (Feng et al. 2017). Understanding the main drivers of amphibian diversification in the Paleogene and Neogene, which contributed so significantly to the radiation that we see today, will be a major focus of future systematics research on amphibians.

Supplementary Material

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.9kd51c5dc. The sequence data generated in this study are also available from NCBI under BioProject accession PRJNA627509.

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