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# Distribution and habitat associations of the Critically Endangered frog *Walkerana phrynoderma* (Anura: Ranixalidae), with an assessment of potential threats, abundance, and morphology

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**Abstract**

**Distribution and habitat associations of the Critically Endangered frog *Walkerana phrynoderma* (Anura: Ranixalidae), with an assessment of potential threats, abundance, and morphology.** Little is known about *Walkerana phrynoderma,* a frog endemic to the Anamalai Hills of the Western Ghats of India. Baseline information (i.e., distribution, threats, habitat characteristics, activity patterns, and relative abundance) is provided for this species, with the aim of improving our understanding of the status of the species in the wild. Visual-encounter, transect, and time-activity budget surveys were conducted in and around the Anamalai Hills of the Western Ghats. The frog skin was swabbed to determine the presence/absence of *Batrachochytrium dendrobatidis,* and habitat and environmental characteristics were recorded at sites where *W. phrynoderma* was found. These data were compared with those of sites apparently lacking this species that had suitable habitat. *Walkerana phrynoderma* is restricted to evergreen forests between 1300 and 1700 m a.s.l. in the Anamalai Tiger Reserve and at Munnar; thus, its range was extended from the state of Tamil Nadu to the adjoining state of Kerala. Pesticide runoff and human disturbance are the most severe threats to the species; *B. dendrobatidis* was not detected. This nocturnal anuran prefers forest edges and is associated with well-shaded forest floors in cool areas near freshwater streams. *Walkerana phrynoderma* is rarely encountered whereas its congener, *W. leptodactyla,* is more common. The impact of anthropogenic disturbances, especially waste disposal and development of tourism infrastructure, should be evaluated. The land that is owned by the Forest Department peripheral to the protected areas could be designated as eco-sensitive sites to prevent changes in land use that could have an adverse effect on *W. phrynoderma.*

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**Keywords:** amphibian, Anamalai Hills, Cardamom Hills, EDGE species, *Indirana phrynoderma, Sallywalkerana phrynoderma.*

**Resumo**

**Distribuição e associações de habitat do anuro Criticamente Ameaçado *Walkerana phrynoderma* (Anura: Ranixalidae), com uma avaliação das ameaças potenciais, abundância e morfologia.** Pouco se sabe sobre *Walkerana phrynoderma,* um anuro endêmico dos Montes Anamalai dos Ghats Ocidentais da Índia. Com o objetivo de aumentar nosso conhecimento do status da espécie na natureza, fornecemos informações básicas (i.e., distribuição, ameaças, características do habitat, padrões de atividade e abundância relativa). Foram feitas investigações por encontro visual, transectos e orçamento temporal no interior e entorno dos Montes Anamalai dos Ghats Ocidentais. A secreção da pele dos animais foi amostrada para determinar a presença/ausência de *Batrachochytrium dendrobatidis,* e foram registradas as características do habitat e do ambiente nos locais onde *W. phrynoderma* foi encontrada. Esses dados foram comparados com os dos locais em que a espécie estava aparentemente ausente. *Walkerana phrynoderma* está restrita às florestas perenifolias situadas entre 1300 e 1700 m a.s.l. na Reserva Anamalai Tiger e em Munnar; dessa forma, sua distribuição foi extendida do estado de Tamil Nadu para o estado vizinho de Kerala. As ameaças mais severas a essa espécia são o uso de pesticidas e perturbações antrópicas; *B. dendrobatidis* não foi detectado. Esse anuro norturno prefere bordas florestais e está associado com chãos de florestas bem sombreadas em áreas frescas próximo a riachos. *Walkerana phrynoderma* é raramente encontrada, enquanto sua congênere, *W. leptodactyla,* é mais comum. O impacto de perturbações antropogênicas, especialmente deposição de lixo e desenvolvimento de infraestrutura turística, deveria ser avaliado. A área de propriedade do Departamento de Florestas na periferia das áreas protegidas poderia ser designada como locais ecossensíveis para prevenir mudanças no uso da terra que pudessem ter um efeito adverso sobre *W. phrynoderma.*

**Palavras-chave:** anfíbio, espécie-EDGE, *Indirana phrynoderma,* Montes Anamalai, Montes Cardamom, *Sallywalkerana phrynoderma.*

## Introduction

Amphibians are the most threatened vertebrate taxon as evidenced by their rapid global declines as a result of habitat loss, climate change, and infectious disease (Stuart *et al.* 2004, Skerratt *et al.* 2007, Sodhi *et al.* 2008, Baillie *et al.* 2010, Hoffmann *et al.* 2010). Most amphibian species (53.8%) occur in 25 biodiversity hotspots (Myers *et al.* 2000). The biodiversity hotspot of Western Ghats and Sri Lanka is one of the eight "hottest" hotspots as determined by high endemic species-to-area ratio and ongoing habitat loss. About 89% of the 229 amphibian species known from the Western Ghats of India are endemic (Frost 2017, Kanagavel *et al.* 2017a). This mountain range is a reservoir of unparalleled evolutionary history having served as Cenozoic refugium by providing ideal environment to the endemic genera such as *Micrixalus, Nyctibatrachus,* and *Walkerana* (Roelants *et al.* 2003, Dahanukar *et al.* 2016).

Ranixalidae Dubois, 1987 comprises two genera, *Indirana* and *Walkerana,* both of which are endemic to peninsular India (Dahanukar *et al.* 2016, Garg and Biju 2016). Ranixalid frogs are terrestrial, and associated with riparian, leaf-litter habitats in tropical wet, evergreen, and semi-evergreen forests (Nair *et al.* 2012). They have well-developed toe pads that enable them to adhere to wet rock surfaces where they breed in streams. They lay terrestrial eggs and have semi-terrestrial tadpoles that cling on to wet surfaces of steep rocks and trees (Roelants *et al.* 2003, Tapley *et al.* 2011, Gaitonde and Giri 2014). The three currently recognized species of

*Walkerana* occur south of the Palghat Gap, a major biogeographical barrier in the Western Ghats, and are threatened by loss of habitat as a result of land-use changes, road development, and harvesting of timber and firewood (Biju *et al.* 2004a, b, Biju and Dutta 2004, Dahanukar *et al.* 2016, IUCN 2017). Additionally, *Batrachochytrium dendrobatidis* Longcore, Pessier and D. K. Nichols, 1999 (*Bd*), which causes chytridomycosis, a disease associated with global amphibian population declines (Skerratt *et al.* 2007), has been reported from the Western Ghats (Nair *et al.* 2011, Dahanukar *et al.* 2013, Molur *et al.* 2015). *Bd* has been detected in two ranixalid frog species, *Indirana brachytarsus* (Günther, 1876) and *I. leithii* (Boulenger, 1888) (Nair *et al.* 2011, Dahanukar *et al.* 2013, Molur *et al.* 2015). Amphibian declines associated with this pathogen have not been reported in India; however, there has been relatively little longitudinal monitoring of amphibian populations in the region.

*Walkerana phrynoderma* (Boulenger, 1882) is Critically Endangered and assessed as a global priority for amphibian conservation owing to its evolutionary distinctiveness (Biju *et al.* 2004a, Isaac *et al.* 2012). The presence of the species is confirmed by only one record in the Anamalai Hills of the Western Ghats where its range is less than 100 km$^2$. The locality is the Anamalai Tiger Reserve and its surroundings (previously known as Indira Gandhi Wildlife Sanctuary) (Biju *et al.* 2004a, Garg and Biju 2016); another locality record (Dahanukar *et al.* 2016) probably is erroneous. Because of the presence of this frog, the Anamalai Tiger Reserve has been designated as one of 18 Alliance for Zero Extinction sites in India (AZE 2013).

Virtually nothing is known about the ecology, behavior, or morphology of this species, which is known only from five individuals (Dahanukar *et al.* 2016, Garg and Biju 2016). Reportedly, the elevational distribution is between 500 and 1750 m a.s.l. and the maximum snout–vent length (SVL) is 33.0 mm (Boulenger 1890, Biju *et al.* 2004a, Dahanukar *et al.* 2016, Garg and Biju 2016). The frog is thought to be threatened by local residents who collect wood for fuel (Biju *et al.* 2004a). Our goal is to generate baseline data (e.g., distribution, relative abundance, morphology, habitat preference, abiotic factors affecting distribution) for *Walkerana phrynoderma,* including potential threats to its existence to inform conservation management.

## Materials and Methods

### *Study Area*

The Anamalai Tiger Reserve is 958 km$^2$ in the state of Tamil Nadu with elevations ranging from 100–2659 m a.s.l. The vegetation varies from tropical evergreen to scrub and thorn forest (Kumaraguru *et al.* 2011, Rameshan *et al.* 2014). Munnar is a popular tourist destination with an area of 557 km$^2$ in the state of Kerala at an elevation of 1600 m a.s.l.; it adjoins the Anamalai Tiger Reserve. Munnar is within the jurisdiction of the Munnar Wildlife Division and Munnar Forest Division, that manage protected areas (PAs) and reserve forests in the region that occur across elevations of 1400 and 2600 m a.s.l. Most of the land composing Munnar, as well as parts of the Anamalai Tiger Reserve around Valparai, have been converted to tea, coffee, cardamom, and *Eucalyptus* plantations that are interspersed with fragments of tropical evergreen forests (Raman and Mudappa 2003). In Valparai, these plantations occupy 220 km$^2$, whereas the forest fragments cover about 100 km$^2$ (Raman and Mudappa 2003, Sridhar *et al.* 2008). The area has a rich anuran assemblage, with several new frogs—e.g., *Micrixalus nelliyampathi* Biju, Garg, Gururaja, Shouche, and Walukar, 2014, *M. frigidus* Biju, Garg, Gururaja, Shouche, and Walukar, 2014, *M. adonis* Biju, Garg, Gururaja, Shouche, and Walukar, 2014, *Raorchestes chlorosomma* (Biju and Bossuyt, 2009), *R. munnarensis* (Biju and Bossuyt, 2009), *R. resplendens* Biju, Shouche, Dubois, Dutta, and Bossuyt, 2010, and *Nyctibatrachus poocha* Biju, Van Bocxlaer, Mahony, Dinesh, Radhakrishnan,

Zachariah, Giri, and Bossuyt, 2011—having been discovered recently (Biju and Bossuyt 2009, Biju *et al.* 2010, 2011, 2014).

*Site-based Survey*

Visual-encounter surveys were undertaken by three to six persons who actively searched terrestrial habitats between 08:45 and 20:30 h across the known species range of *Walkerana phrynoderma* (Anamalai Tiger Reserve) and adjoining areas (Munnar) to determine exact locations where the species occurred (Figure 1). We opportunistically chose 38 locations to survey as much of the study area as possible across the different habitats and elevations (600–2100 m a.s.l.). These surveys totaled 108.8 person.hr and were conducted in Tamil Nadu (22 locations) in 2013 and at Kerala (16 locations) in 2014 during monsoon seasons (i.e., southwest monsoon from May–August; northeast monsoon from October–December). We recorded the anurans encountered at each site and, when possible, identified the genus and species. Weather parameters, habitat type, elevation, habitat-level threats, and total survey time were also recorded (Table 1). We used a Garmin GPS 62S to determine geographical coordinates and elevation, and a digital thermometer (Eurolab, to the nearest 0.1°C) to measure air and soil temperatures. We measured relative humidity with a digital hygrometer (TempTec CTH 288, to the nearest 1%). We collected water samples from the closest body of water at each location

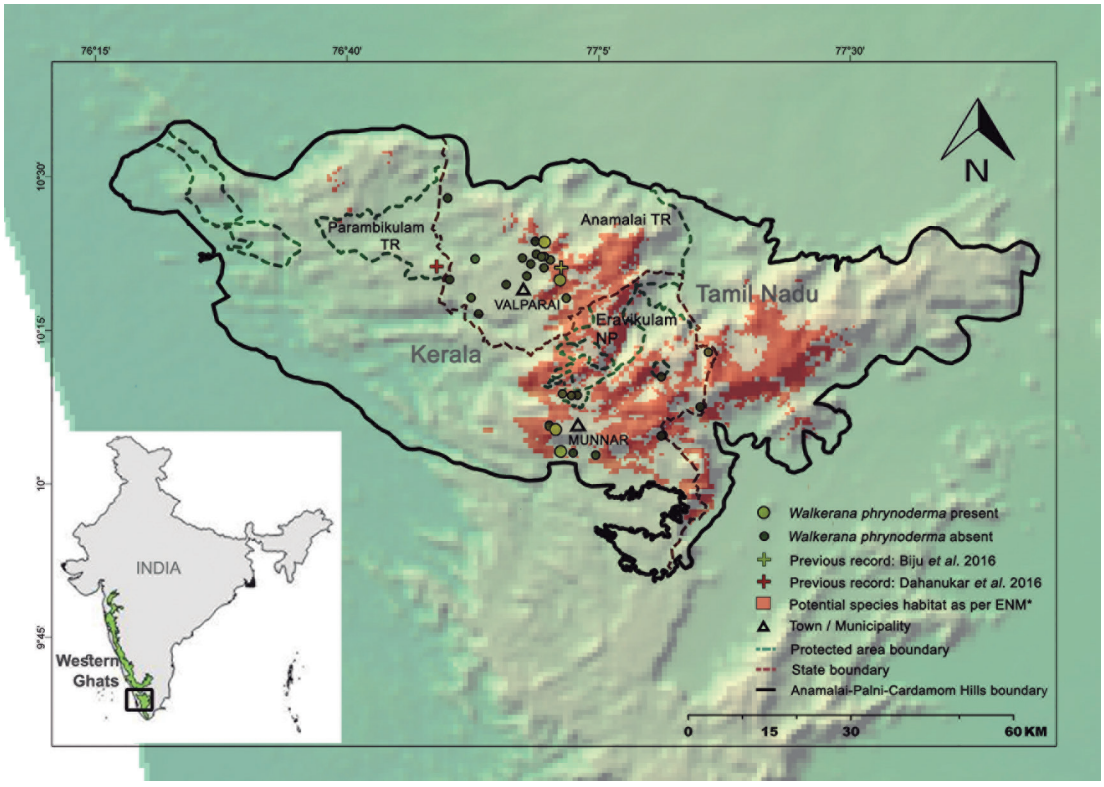


**Figure 1.** Distribution and potential habitat as per ENM (environment niche model) of the Warty-skinned Leaping Frog, *Walkerana phrynoderma* in the Anamalai, Palni, and Cardamom hills of the southern Western Ghats, India.

**Table 1.** Weather and habitat characteristics of sites occupied by *Walkerana phrynoderma* including the frog's basic morphology.

| | **Range** | **Mean ± SE** |
|---|---|---|
| Snout–vent length (cm) | 0.9–4.7 | 2.6 ± 0.2 |
| Mass (g) | 0.1–15.0 | 3.4 ± 0.7 |
| Elevation (m a.s.l.) | 1317–1703 | 1601.7 ± 13.7 |
| Air temperature (°C) | 15.4–21.5 | 18.1 ± 0.3 |
| Soil temperature (°C) | 15.9–20.7 | 18.2 ± 0.2 |
| Water temperature (°C) | 16.4–19.4 | 17.7 ± 0.2 |
| Humidity (%) | 65–100 | 85.5 ± 1.6 |
| Distance from closest water source (m) | 0.18–500.0 | 40.5 ± 16.8 |
| Distance from closest perennial stream (m) | 0.6–500.0 | 46.7 ± 16.6 |
| Bank height (cm) | 0.0–28.0 | 3.2 ± 1.6 |
| | **Percentage (%)** | |
| Rainfall | None = 50, Little = 23.3, Moderate = 20, Heavy = 6.7 | |
| Cloud cover | Overcast = 100 | |
| Wind | None = 50, Little = 43.3, Moderate = 6.7 | |
| Resting substrate | Mud = 26.7, Litter = 60, Moss = 13.3 | |

and tested them for nitrate (mg/L), nitrite (mg/L), ammonia (mg/L), pH (7.4–8.7), alkalinity (0–5.71 meq/L) and carbonate hardness (0–15.7 dKH) with Salifert® test kits following the manufacturer's guidelines. We recorded prevailing weather conditions (Table 1) and described any apparent habitat threats at each site. Because *W. phrynoderma* was found at only a few sites, a generalized linear model was used to interpret the influence of the weather and water parameters on our ability to detect the frog.

We constructed an environmental niche model with the Maximum Entropy Species Distribution Modeling software (Version 3.4.1) to identify the other areas in and around the Anamalai Hills where *W. phrynoderma* might occur. To estimate the geographical distribution of the species, we used the following raster layers: precipitation of driest month; temperature seasonality, and altitude from the WorldClim database (Hijmans *et al.* 2005); and land cover and maximum green vegetation fraction from the USGS database (Broxton *et al.* 2014). These layers were re-sampled to the WGS 1984 Transverse Mercator projection at a 1 $km^2$ resolution and snapped to the Anamalai Hills landscape that included the Cardamom and Palni hills. The threshold of occurrence in the model was set to minimum training presence to generate the most restrictive niche that contained all the sample localities. We omitted areas of the resulting distribution that were grasslands, wetlands, croplands, and urban areas where we did not encounter this species, despite intensive surveys.

Data recorded for each of 30 *Walkerana phrynoderma* include the following: elevation, weather parameters, SVL, mass, activity, habitat type (e.g., forest edge / within forest, forest type and stream), substrate, stream-bank height, distance from nearest perennial stream and

distance from the water source that was nearest to the individual, which could range from a pool to a perennial stream. Each frog was measured with dial Vernier calipers (RSK, to the nearest 0.02 mm), and mass was determined with a mini digital weight balance (B001L9CHP0, to the nearest 0.1 g). Detailed morphological measurements (Appendix I) were taken for 17 adult *W. phrynoderma* as per Gopalan *et al.* (2012). Individual frogs were classified as adult (> 3 cm), subadult (< 3 cm and > 0.1 g) or metamorph (< 0.1 g). A measuring tape (to the nearest 0.1 cm) was used to measure habitat-related distances. We conducted ANOVA and Kruskal-Wallis tests to determine whether frogs of different age classes had varied habitat requirements with respect to elevation, weather, habitat, stream bank height, distance from perennial stream and distance from water source.

*Swabbing and Detection of* Bd *Infection*

During the period 2013–2015, eight *Walkerana phrynoderma* (Munnar = 5, Valparai = 3) and 12 *W. leptodactyla* (Boulenger, 1882) (Munnar = 6, Valparai = 6) were skin-swabbed in the field and then immediately released at the point of capture. Our aim was to determine the presence of *Bd* in the focal species, as well as in the widespread and more abundant congener. Frogs were swabbed on the ventral surface—viz., five swab strokes on both thighs, shank, and hind feet and five strokes on drink patch. Powder-free nitrile gloves were used to handle specimens and new gloves were used with each specimen. The swabs were preserved in 70% ethanol. We attempted to extract *Bd* DNA from swabs following Goka *et al.* (2009) with modifications (Dahanukar *et al.* 2013). Quantitative PCR based on SYBR Green method was carried out using primer pair ITS1-3 Chytr and 5.8S Chytr (Boyle *et al.* 2004) following the protocol of Dahanukar *et al.* (2013). Serially diluted DNA extracted from $10^6$ *Bd* zoospores were used as standards. All samples were run in duplicates.

*Habitat Survey*

To understand the role of the habitat type on the occurrence of *Walkerana phrynoderma*, detailed vegetation and soil analyses were undertaken in February 2015. Four sites were identified with the site-based survey—at two, *W. phrynoderma* was known to occur, and two other sites the species had not been detected. All the sites had similar habitats and were located at about the same elevation (1428–1587 m a.s.l.). Three plots (5 × 5-m) were opportunistically chosen at each site and data described below were recorded from each plot. We measured the diameter of tree trunks at breast height (DBH); if the DBH was 15 cm or greater, the plant was considered to be a tree, or to be a sapling if the DBH was between 5 and 15 cm. Shrubs were classified as woody-stemmed, short plants less than 50 cm high, and seedlings were green herbaceous stems less than 50 cm high. We counted the trees in each 5 × 5-m plot and the number of saplings, shrubs, and seedlings in a randomly selected 2 × 2-m subplot within the tree plot. Canopy cover was calculated by viewing it through a graduated glass plate (100, 1 × 1-cm squares) attached to a cardboard cylinder. The number and percent of squares that were completely covered by the canopy were recorded. Soil litter of a 10 × 10cm subplot was weighed to calculate litter density (g) with an electronic scale (Weiheng®, to the nearest 0.01 kg). The soil pH and nutrients, organic carbon (g), total nitrogen (%), available phosphorus (kg/ha) and available potassium (µg per sample) were calculated as per Motsara and Roy (2008). The parameter data from the three plots were either averaged (all soil characteristics and canopy cover) or added (all vegetation characteristics except canopy cover) to reflect the characteristics of the entire site. The means of the parameters for the sites where *W. phrynoderma* were present and the sites lacking the species were used for qualitative comparisons because the sample sizes were too small for robust statistical comparisons.

*Time-activity Budget Survey*

We observed adult *Walkerana phrynoderma* in the rainy season (August–September) in 2014 between 07:00 and 19:30 h to document their behavior, which was recorded as a time-activity budget. This time-period was chosen as per the conditions of our official research permissions and safety concerns due to the presence of large megafauna. The activities of each individual frog (i.e., resting, moving, and feeding) and the duration of each activity were recorded during half-hour periods; we employed the focal animal-sampling technique in which three different observers alternate between the half-hour periods (Altmann 1974, Smart *et al.* 2014). If visual contact was lost, observations were recorded up to the point it was lost. Frogs were not marked for identification and those observed on different days were treated as different individuals. We used red light to observe frogs after 18:00 h to minimize disturbance (Dayananda and Wickramsinghe 2013). Frog activities were categorized according to the different time-periods (Morning = 07:00–12:00; Afternoon = 12:01–17:00; Evening = 17:01–19:30) and analyzed with a one-way ANOVA to test whether the time period affected activity in *W. phrynoderma.*

*Transect Survey*

To estimate the relative abundance and the factors that influence the number of *Walkerana phrynoderma* found, three 70-m transects, each subdivided into seven 10-m segments were surveyed. Each transect was established in a different habitat within the species range (Appendix II) and were marked across existing routes/pathways to minimize habitat disturbance and comply with the terms of research permission. Each transect segment was actively searched up to a height of 0.3 m for 5 min; these surveys were repeated seven times from 18:00–19:30 h by two observers from August–November 2014. Each observer searched one side (2 m) of the transect line and observers switched sides at every 10-m segment. The numbers of individuals of each species/genus encountered were recorded in each segment, and climatological data were recorded at the start and end of each survey.

The total number of amphibians observed, the proportion of each species, and the numbers of *Walkerana phrynoderma, W. leptodactyla, Micrixalus* sp., and *Raorchestes* sp. found were compared among transects. The latter taxa were chosen because their higher abundances provided larger sample sizes for analyses; other species were excluded. Transect-level differences were explored with Kruskal-Wallis tests. The relationship between climatological data and total amphibian abundance, as well as the abundance of *W. phrynoderma* were examined by linear regression analyses. The means of the weather data recorded during the beginning and end of the survey were calculated and utilized for this analysis.

**Results**

*Site-based Survey*

*Walkerana phrynoderma* occurred at four of 38 sites surveyed (2 each in Tamil Nadu and Kerala, 1321–1649 m a.s.l.; Figure 1). The environmental niche model (training data AUC = 0.990) predicted an area of approximately 940 km$^2$ including the Eravikulam National Park and Kodaikanal Wildlife Sanctuary (Palni Hills). Of the factors that could affect the presence / absence of *W. phrynoderma,* only air temperature and the amount of ammonia in the stream water differed significantly (Table 2). Air temperature was lower at sites occupied by *W. phrynoderma* (17.8 ± 0.9°C vs. 19.7 ± 0.4°C). Ammonia was higher at sites occupied by *W. phrynoderma* (0.25 ± 0 mg/L vs. 0.09 ± 0.1 mg/L). The greatest threats in all survey locations are the potential for pesticide runoff from surrounding tea, coffee, and cardamom plantations ($N$ = 27 locations), degraded habitat ($N$ = 9), roads ($N$ = 7), firewood

collection ($N$ = 3), livestock grazing ($N$ = 3), and garbage and solid waste ($N$ = 3). The four locations where *W. phrynoderma* occurs are threatened by potential pesticide runoff ($N$ = 4), firewood collection ($N$ = 2), livestock grazing ($N$ = 1), degraded habitat ($N$ = 1), roads ($N$ = 1), and garbage and solid waste ($N$ = 1).

*Walkerana phrynoderma* are small frogs with a maximum SVL of 4.7 cm (Table 1). Although predominantly brownish in color, some adults are reddish. The tympanum usually is 75% the eye diameter (Appendix I) and its distinctiveness varies. The extent of the dorsal tuberculation varies, with larger individuals having less coverage. The dorsum has a characteristic W-shaped ridge composed of large tubercles, which is distinct in subadult and adult frogs. The venter is predominantly black with white speckles; the patterns vary individually. The toes lack webbing. The sex of the individuals cannot be determined by external examination. *Walkerana phrynoderma* were found only in tropical montane evergreen forests; four metamorphs were encountered near a stream at the edge of an evergreen forest and montane grassland during the monsoon in August 2013. *Walkerana phrynoderma* was restricted to a high montane altitudinal zone and usually found associated with soil litter (Table 1). They were found in the vicinity of water sources (40.5 ± 16.8 m) or away from them; the frogs were not seen in water (Table 1). Most (70%) frogs were encountered at forest edges, rather than deep within the forest (30%). All were found resting on the substrate and were not active during the day.

Different life stages (adults = 11, subadults = 11, metamorphs = 8) of *W. phrynoderma* are found on different substrates (Kruskal-Wallis Test, $\chi^2$ = 7.5, $df$ = 2, $p$ = 0.02). Metamorphs usually were associated with moss (50%) and litter (37.5%), subadults with bare soil (54.5%) and litter (45.5%), and adults with litter (81.8%). The distance of the frogs from the closest perennial stream also varies with life stage (Kruskal Wallis Test, $\chi^2$ = 6.2, $df$ = 2, $p$ = 0.04). Metamorphs were found closer to streams (17.3 ± 11.9 m) than subadults (37.6 ± 8.3 m), and adults were found farthest from streams (77.4 ± 43.2 m).

**Table 2.** Generalized linear models to interpret the effect of weather and water-quality parameters on presence / absence of *Walkerana phrynoderma*.

| | ***B*** | ***Std Error*** | ***Wald Chi-square*** | ***p*** |
|---|---|---|---|---|
| Intercept | 5.58 | 10.31 | 0.29 | 0.59 |
| Elevation | > 0.01 | > 0.01 | 1.60 | 0.21 |
| Air temperature | 0.08 | 0.04 | 4.28 | 0.04 |
| Soil temperature | -0.03 | 0.03 | 0.94 | 0.33 |
| Humidity | > -0.01 | 0.01 | 0.24 | 0.62 |
| pH | -0.63 | 1.40 | 0.20 | 0.65 |
| Ammonia | -1.45 | 0.46 | 9.75 | > 0.01 |
| Nitrate | 0.01 | 0.01 | 0.29 | 0.59 |
| Alkalinity | 1.63 | 1.23 | 1.76 | 0.19 |
| Carbonate hardness | -0.57 | 0.43 | 1.79 | 0.18 |

*Habitat Survey*

*Walkerana phrynoderma* was found on well-shaded forest floors, which is inferred from the canopy cover and the number of saplings and shrubs. Even though there were fewer trees at sites with *W. phrynoderma,* the canopy cover resembled that of sites lacking this species (Table 3). Consequently, at the sites where *W. phrynoderma* occurred, there were more saplings and fewer seedlings (Table 3), and the soil contained less nitrogen, phosphorus, and potassium.

*Time-activity Budget Survey*

In a period of 6 days, a total of 11.85 h was spent observing the activity of *Walkerana phrynoderma*. Individuals usually rested either in, or on, leaf litter (11.84 h), and were observed moving 0.01 h. Vocalizations were not heard, and no significant differences were found between the activities undertaken and the different time periods from 07:00–19:30 h.

*Transect Survey*

Anuran encounter rates differed among the three transects (Kruskal Wallis test, $\chi^2 = 6.9$, $df = 2$, $p = 0.03$); the most frogs were found in the forest edge transect inside PA and the fewest in the forest transect (Figure 2). *Walkerana leptodactyla* was the most common species in all transects, followed by *Raorchestes* sp., *W. phrynoderma,* and *Micrixalus* sp. (Figure 2). The total numbers of *W. phrynoderma* (Kruskal-Wallis test, $\chi^2 = 12.2$, $df = 2$, $p < 0.01$), *W. leptodactyla* (Kruskal Wallis test, $\chi^2 = 10.9$, $df = 2$, $p < 0.01$), and *Micrixalus* sp. (Kruskal-Wallis test, $\chi^2 = 11.7$, $df = 2$, $p < 0.01$) differed in the three transects. The fewest *W. phrynoderma* were found in the forest interior transect in contrast to the other transects (Figure 2). The most *W. leptodactyla* were found in the forest edge transect outside PA, and the least in the forest interior transect. Most *Micrixalus* sp. were found in the forest edge transect inside PA and the fewest in the forest edge outside PA (Figure 2).

**Table 3.** Vegetation and soil characteristics of sites with and without *Walkerana phrynoderma.*

| | **Sites with *W. phrynoderma* (*N* = 2)** | **Sites without *W. phrynoderma* (*N* = 2)** |
|---|---|---|
| Canopy cover (%) | 93 ± 4 | 93 ± 1 |
| Tree number | 5.5 ± 1.5 | 12.0 ± 3 |
| Sapling number | 44 ± 19 | 31.5 ± 9.5 |
| Shrub number | 28 ± 10 | 20.5 ± 11.5 |
| Seedling number | 76 ± 28 | 150 ± 50 |
| Litter weight (g) | 0.18 ± 0.02 | 0.25 ± 0.05 |
| Soil pH | 4.6 ± 0.01 | 4.7 ± 0.44 |
| Organic carbon (g) | 0.51 ± 0.28 | 0.47 ± 0.05 |
| Total nitrogen (%) | 38.36 ± 0.04 | 54.97 ± 2.09 |
| Available phosphorus (kg/ha) | 0.16 ± 0.02 | 0.24 ± 0.04 |
| Available potassium (µg per sample) | 35.75 ± 1.72 | 40.62 ± 5.12 |

Weather conditions did not affect the total number of *W. phrynoderma* encountered, but it did influence the total number of anurans found (Table 4). Substrate temperature is inversely correlated with the total number of frogs found.

Bd *Infection Detection*

All 20 samples (8 *Walkerana phrynoderma* and 12 *W. leptodactyla*) tested negative for *Bd* with less than one zoospore equivalent for both the runs of quantitative PCR.

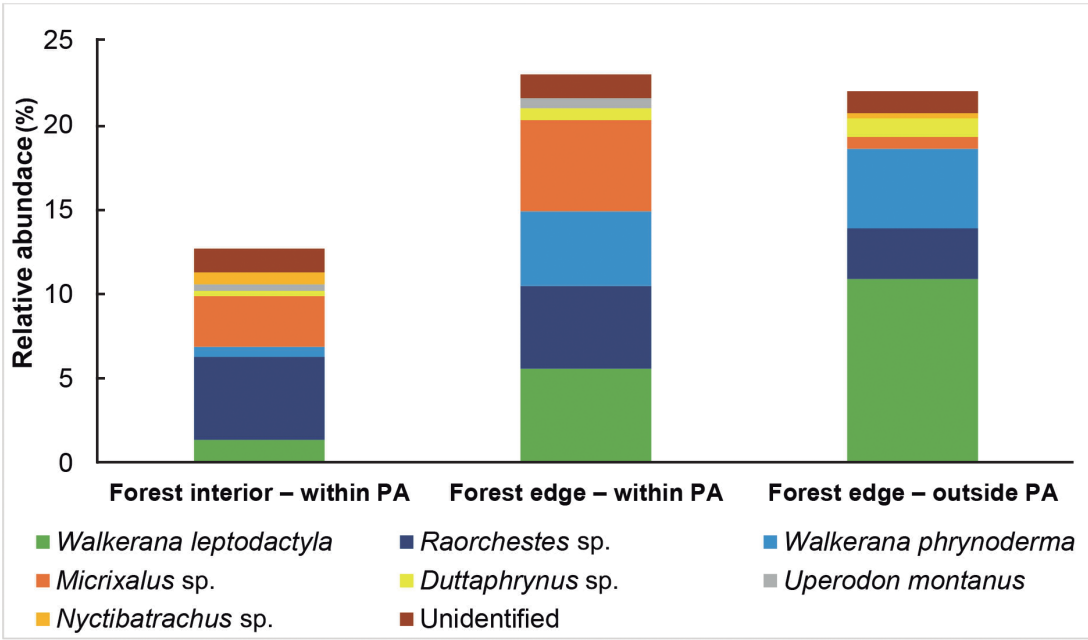


**Figure 2.** Relative abundance of anurans found along the three transects in the Anamalai Hills; "unidentified" refers to frogs that moved away before they could be identified.

**Table 4.** Linear regression analysis describing the effect of weather parameters on the total number of anurans encountered during the transect survey.

| | *B* | *Std Error* | *Beta* | *T* | *p* |
|---|---|---|---|---|---|
| Constant | -75.2 | 70.8 | | -1.1 | 0.31 |
| Air temperature | -4.3 | 3.4 | -0.36 | -1.2 | 0.24 |
| Soil temperature | 8.2 | 3.1 | 0.80 | 2.7 | 0.02 |
| Humidity | > 0.1 | 0.2 | -0.01 | -0.1 | 0.97 |
| Rainfall | -3.5 | 3.0 | -0.35 | -1.2 | 0.27 |
| Wind | 0.7 | 3.4 | 0.05 | 0.2 | 0.85 |
| Cloud cover | 4.7 | 4.5 | 0.30 | 1.0 | 0.32 |

## Discussion

*Distribution, Habitat, and Activity of* Walkerana phrynoderma

The range of *Walkerana phrynoderma* extends outside the PA network to Munnar in the Anamalai Hills of southern Western Ghats—30.5 km south of its recorded locality in the Anamalai Tiger Reserve. The species will still be assessed as Critically Endangered (B1ab(i,ii,iii) + 2ab(i,ii,iii)) as per the IUCN Redlist because its distribution is restricted to an area estimated to be 26.3 $km^2$ and an area of occupancy of 5 $km^2$ that is severely fragmented and predicted to diminish in size as a result of ongoing anthropogenic activities. As per this study, the species is known from one severely fragmented locality, where the area, extent, and quality of its habitat is expected to decline owing to the potential runoff of chemical effluents from tea plantations and development of large-scale tourism infrastructure. The altitudinal range of *W. phrynoderma* is much more restricted than previously thought; we only found the species at elevations between 1300 and 1700 m a.s.l. Other field studies have recorded the species at 928 m (Parambikulam; Dahanukar *et al.* 2016) and 1750 m a.s.l. (Valparai, the same field site as ours; Garg and Biju 2016). The locality reported by Dahanukar *et al.* (2016) should be verified because the individual depicted in the image appears to be under severe stress and its habitat seems to be unnatural and different from the type locality. Moreover, our study involved a meticulous survey across 38 different locations from elevations of 600–2100 m a.s.l. It included the protected area adjacent to Parambikulam (Topslip, Anamalai Tiger Reserve), the location where *W. phrynoderma* was thought to occur previously as reported by Biju *et al.* (2014) but we did not find the species at 900 m a.s.l. Also, Garg and Biju (2016) did not encounter it at 955 m a.s.l. in Parambikulam. Our results suggest that *W. phrynoderma* occupies a highly restricted range in the Anamalai Hills and the adjoining Cardamom Hills of the Western Ghats. The geographical distribution model must be interpreted with caution given the limited sample size. However, it does provide information on other regions where populations of *W. phrynoderma* could occur and we suggest that these areas should be surveyed as a priority.

In general, frogs of the genera *Indirana* and *Walkerana* are terrestrial and live on the forest floor amidst leaf litter, short grass, and wet rocks close to streams (Nair *et al.* 2012). However, adult *W. phrynoderma* are less dependent on proximity of streams or water as is *I. brachytarsus* (Daniels 2005), at least during the time of year that we undertook our surveys. The difference in habitat preference between metamorphs, sub-adults, and adults of *W. phrynoderma* might reflect ontogenic shifts in foraging habitat preferences, varying mobility in different habitats, different structural support required from their habitat, or natal site fidelity (Beard *et al.* 2003, Haggarty 2006, Valdez *et al.* 2016). The expected breeding sites of *Walkerana* / *Indirana* (wet rock surfaces) were searched opportunistically in the habitat of *W. phrynoderma,* but tadpoles of the species were not observed. The reproductive biology and vocalization of this species are unknown. Although they may have the same reproductive mode as ranixalid frogs, given the association of metamorphs and streams, this cannot be confirmed and warrants further study. *Walkerana phrynoderma* is a nocturnal species even though it can be found by day (Garg and Biju 2016) when it is not active. When these frogs are not moving, they are well camouflaged and difficult to detect on the terrestrial substrate.

*Walkerana phrynoderma* was found on well-shaded forest floors with the same amount of canopy cover as the sites lacking the species. The difference is that the habitats occupied by *W. phrynoderma* had fewer, but larger trees with wider crowns; this, combined with higher elevations may result in lower air temperatures that are more favorable for the species. The areas also have nutrient-poor soils, and higher ammonia content in the water.

*Relative Abundance*

*Walkerana phrynoderma* seems to be restricted to a few discontinuous fragments of tropical evergreen forest, where it apparently is not the most common frog in the anuran assemblage in the habitat. Globally, there have been numerous studies investigating the microclimatic variation between forest edges and interiors (Chen *et al.* 1993, Ewers and Banks-Leite 2013, Baltzer *et al.* 2014). Different habitats and degree of disturbance seem to affect the overall relative abundance of amphibians because the forest edges support a higher relative abundance of certain species in this case and *W. phrynoderma* specifically. The apparent edge effect should be interpreted cautiously as the denser vegetation in the forest interior could have an impact on detection probability. Moreover, these results could be seasonally biased because the surveys were undertaken during monsoon seasons, and the abundances of amphibians in the forest edges and interior are influenced by such seasonality (Schlaepfer and Gavin 2001).

*Threats Faced by* Walkerana phrynoderma

A major threat to this species is habitat loss resulting from subsistence wood collection by local communities (Biju *et al.* 2004a). This, along with habitat fragmentation, are obvious and important threats, and this study emphasizes another—viz., the close proximity of *Walkerana phrynoderma* to tea plantations, where anurans may be exposed to pesticide runoff. Chemical pollution is a major threat to amphibians and there is evidence of the susceptibility of amphibians to the toxic effects of nitrogenous compounds (Beebee and Griffiths 2005, Egea-Serrano *et al.* 2009). At organic coffee plantations in the Western Ghats, the relative abundance of amphibians was found to be higher than at coffee plantations that used fertilizers and pesticides (Rathod and Rathod 2013). Pesticide runoff is associated with population declines (Davidson and Knapp 2007), deformities (Taylor *et al.* 2005, Gurushankara *et al.* 2007a, b), and reduced immune response, each of which renders anurans more susceptible to diseases (Carey *et al.* 1999, Christin *et al.* 2004). *Bd* was detected in the Western Ghats relatively recently, but we do not know whether infection leads to lethal chytridiomycosis in amphibians in this region (Nair *et al.* 2011, Dahanukar *et al.* 2013, Molur *et al.* 2015). *Bd* has been found to infect species of the genus *Indirana* (Nair *et al.* 2011, Dahanukar *et al.* 2013, Molur *et al.* 2015), but it has not been found in *W. phrynoderma;* thus, chytridiomycosis may not threaten the species currently. However, our sample size was limited. Further, the range of *W. phrynoderma* is coincident with an area where there is a high probability of chytrid presence owing to cool ambient temperatures (Molur *et al.* 2015). Thus, long-term population monitoring and pathogen surveillance should be undertaken so that any population decline is detected early and suitable conservation interventions can be implemented in time.

*Conservation Measures*

The actual effect of pesticides, livestock grazing, and firewood collection by local communities on the population of *Walkerana phrynoderma* must be determined to formulate appropriate conservation measures. Existing tourism-induced disturbances (especially waste disposal) should be reviewed by the Forest Department within and outside protected areas (Reserve Forests) with the aim of reducing the impact of tourism on the habitat of a species with highly specific environmental requirements. The development of new tourism infrastructure (trekking routes, buildings, and roads) within Forest Department-owned land should be avoided if possible. Once calculated, organizations should strictly adhere to the tourist-carrying capacity of sites, given that this Critically Endangered species has a limited distribution. The Forest Department-owned land outside the protected areas (Reserve Forests) could be declared as eco-sensitive to

prevent management-induced, land-use changes that may have a detrimental effect on populations of *W. phrynoderma.* This is especially critical given that the abundance of *W. phrynoderma* outside the protected area is similar as that of protected areas; thus, these unprotected sites should be monitored to ensure that crucial tracts of habitat are protected in the range of *W. phrynoderma.* Last, the Forest Department should ensure that its front-line staff is able to identify and monitor at least key amphibian species (Kanagavel *et al.* 2017b). Official, annual amphibian-monitoring programs such as those that exist for megafauna (Kumara *et al.* 2012) should be initiated to provide data to track the status of threatened anurans and undertake appropriate conservation interventions when necessary.

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**Appendix I.** *Morphometric data for adult* Walkerana phrynoderma *(10 = Valparai, 7 = Munnar) as per Gopalan* et al. *(2012) compared with data reported in the literature.*

| | **Current study (*N* = 17)** | | **Garg and Biju 2016** | **Dahanukar *et al.* 2016** |
|---|---|---|---|---|
| | Range (mm) | Mean ± SE (mm) | (*N* = 1) | Mean ± SE (mm, *N* = 3) |
| Snout–vent length | 23.7–46.6 | 32.7 ± 1.4 | 33.9 | 24.8 ± 6.4 |
| Head length | 9.8–18.4 | 12.9 ± 0.6 | 13.1 | 9.9 ± 2.5 |
| Head width | 10.7–19.6 | 14.0 ± 0.6 | 13.7 | 9.6 ± 2.4 |
| Maximum eye diameter | 3.1–5.5 | 4.0 ± 0.2 | 4.0 | 2.9 ± 0.8 |
| Eyelid width | 2.7–4.3 | 3.2 ± 0.1 | 3.3 | 2.3 ± 0.5 |
| Interorbital distance | 2.3–4.8 | 3.2 ± 0.2 | 2.9 | 2.6 ± 0.5 |
| Tympanum diameter | 1.9–3.8 | 2.8 ± 0.1 | 2.1 | 1.4 ± 0.4 |
| Eye–snout distance | 4.1–8.4 | 5.7 ± 0.3 | - | - |
| Eye–naris distance | 1.9–4.2 | 2.9 ± 0.2 | - | 2.2 ± 0.5 |
| Naris–snout distance | 2.2–4.4 | 3.0 ± 0.2 | - | 2.3 ± 0.5 |
| Internasal distance | 3.0–6.3 | 4.3 ± 0.2 | - | 3.2 ± 0.8 |
| Upper arm length | 4.3–11.1 | 6.6 ± 0.4 | - | 5.5 ± 1.4 |
| Forearm length | 5.6–10.2 | 7.3 ± 0.4 | 6.2 | 5.2 ± 1.5 |
| Hand length | 5.2–13.0 | 8.2 ± 0.4 | 8.3 | 5.9 ± 1.5 |
| Thigh length | 13.8–28.1 | 19.1 ± 0.9 | 18.4 | 13.4 ± 3.2 |
| Shank length | 15.0–31.4 | 20.7 ± 1.0 | 20.1 | 15.0 ± 4.2 |
| Tarsus length | 8.4–15.4 | 10.8 ± 0.7 | - | 7.1 ± 2.1 |
| Total foot length | 16.2–28.1 | 18.8 ± 1.1 | 17.8 | 17.5 ± 5.3 |

**Appendix II.** *Description of the habitat at transects setup in* Walkerana phrynoderma*'s range.*

| | **Habitat** | **Elevation (m a.s.l.)** | **Site status** |
|---|---|---|---|
| 1 | Partially paved road that forms an edge between two evergreen forest patches of primary vegetation. | 1597 | Within protected area |
| 2 | Evergreen forest interior that lacks a paved or well-defined path. | 1551 | Within protected area |
| 3 | Boundary path between a Reserve Forest and privately owned land. The area is abandoned and no longer cultivated. The upper canopy is primary vegetation, whereas the lower is secondary. | 1559 | Outside protected area |