Phylogenetic analysis of the endemic and threatened bat *Hipposideros pomona* K. Andersen, 1918 (Chiroptera: Hipposideridae) in the Western Ghats

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "**Phylogenetic analysis of the endemic and threatened bat *Hipposideros pomona* K. Andersen, 1918 (Chiroptera: Hipposideridae) in the Western Ghats**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar titles, of any other university or society

Place: Vellianikkara:
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CERTIFICATE

Certified that this thesis entitled "**Phylogenetic analysis of the endemic and threatened bat *Hipposideros pomona* K. Andersen, 1918 (Chiroptera: Hipposideridae) in the Western Ghats**" is a record of research work done independently by Mr. Abhin M Sunil (2020-17-004) under my guidance and supervision. It has not previously formed the basis for the award of any award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of Mr. Abhin M Sunil, a candidate for the degree of Master of Science in Forestry with major in Wildlife Sciences, agree that the thesis entitled "**Phylogenetic analysis of the endemic and threatened bat *Hipposideros pomona* K. Andersen, 1918 (Chiroptera: Hipposideridae) in the Western Ghats**"is a record of research work done independently by Mr. Abhin M Sunil (2020-17-004) may be submitted by Mr. Abhin M Sunil, in partial fulfilment of the requirement for the degree.

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Abhin M Sunil

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INTRODUCTION

1. Introduction

Bats are an essential part of biodiversity and account for more than half of tropical terrestrial mammals (Findley, 1993; Wilson and Reeder, 2005). Globally, there are more than 1,400 species of bats. (Simmons and Cirranello, 2022), with 133 species in India (Srinivasulu *et al.*, 2021). Bats can be classified into two broad categories, fruit bats and insect bats. Fruit bats are pollinators and seed dispersers, helping to restore forests, whereas insectivorous bats play a significant role in managing pest populations in agriculture and natural environments as well as regulating zoonotic disease vectors like malaria and dengue fever. (Fujita and Tuttle, 1991; Kunz *et al.*, 2011). Since bats are highly diverse and depend on various indicators of ecosystem health, they are considered good biological indicators (Russo and Jones, 2015). Although there are a large number of bat species and their ecological functions vary widely, the studies on the Indian bats are very few and molecular studies, in particular, are still in their infancy because of their nocturnal and flying nature, which makes their direct observation difficult (Findley, 1993). The diversity and variability of bat communities are under-explored. Hence understanding the cryptic diversity is crucial for evaluating the strength of the population and setting meaningful conservation priorities to withstand the threat of stochastic extinction.

Sixty-three species of bats in nine families have been reported from the Western Ghats (WG), of which five are threatened with the risk of extinction (Raman and Hughes, 2021). *Hipposideros pomona* K. Andersen, 1918 is a small to medium-sized insectivorous bat species endemic to the southern Western Ghats (SWG) (Srinivasulu and Srinivasulu, 2018a). According to IUCN, the species is endangered due to its restricted distributional range (Srinivasulu *et al.*, 2020a). It is mostly found in thick forests at medium to high altitudes in the WG. (Raman and Hughes, 2021).

Biogeographic barriers can act as a barrier to species dispersal, resulting in reproductive isolation and thus aiding speciation. The effect of the barriers depends on the motility of the animal. Actively moving and robust animals, such as higher vertebrates, may disperse across more minor barriers, while less mobile animals could be restricted due to the same.

The 30 km wide Palghat gap in the SWG acts as a barrier to the species movement for many forest depended species, consequently acting as a barrier for gene flow, which can result in speciation as well as increase the chances of gene pool isolation (Vidya *et al.*, 2005; Ram *et al.*, 2015; Robin *et al.*, 2017; Joshi *et al.*, 2018; Chattopadhyay *et al.*, 2021). This barrier could affect the movement between subpopulations of *H. pomona* in the south and north of the Palghat gap. From this hypothesis, The objective of the current study is to assess the cryptic diversity occurring between H. pomona subpopulations due to biogeographic barriers and To understand the genetic variability within the subpopulations of *H. pomona.*

Subpopulations of a species which are genetically distinct are three times more likely to go extinct than the whole species (Hughes *et al.*, 1997), which in turn causes loss of intraspecific genetic diversity and makes populations susceptible to extinction (Manel and Holderegger, 2013). Hence more studies on different bat species have to be conducted further to know the effects of population isolation across this barrier.

REVIEW OF LITERATURE

2. Review of Literature

2.1. Order Chiroptera

Chiroptera is one of the 29 currently extant mammalian orders. It represents one of the most speciose and ubiquitous orders of mammals, with over 1400 extant species (Simmons and Cirranello, 2022); In terms of species richness, Chiroptera is only second to Rodentia with almost 20% of all mammalian species. They are distributed worldwide except in the Arctic, Antarctic and certain oceanic islands (Bates and Harrison, 1997). Several features distinguish the order Chiroptera, the most notable of which are morphological characteristics related to the capacity to powered flight (e.g., wings, patagia, and rotated hindlimbs). Moreover, additional characteristics linked with flying, such as a high metabolic rate and particular qualities such as echolocation and longevity, distinguish the group among mammals (Speakman *et al.*, 2005). Chiroptera is classically divided into two suborders: Megachiroptera (Old World Fruit Bats) and Microchiroptera (laryngeally echolocating bats). Furthermore, Chiroptera contains at least four extinct clades closely related to Microchiroptera (Simmons and Conway, 1997). However, the use of molecular data and explicit phylogenetic methodologies in recent years has resulted in another suborder of Chiroptera, suborder Yinpterochiroptera, which includes the megabat family Pteropodidae as well as the microbat families Rhinolophidae, Rhinopomatidae, and Megadermatidae, and suborder Yangochiroptera, which includes the remaining microbat families (Springer *et al.*, 2001; Teeling *et al.*, 2002). Aside from taxonomic richness, Chiroptera species vary greatly in size, with the smallest, *Craseonycteris thonglongyai*, weighing approximately 2g and having a wingspan of 12-13cm. The largest, Pteropus, weighs up to 1.5kg and has a wingspan of over 2m (Bates and Harrison, 1997).

There are a number of unique biogeographic zones and biodiversity hotspots in South Asia. While mammalian endemism is mostly concentrated in peninsular India, notably the Western Ghats, mammalian diversity is predominately centred in northeastern India and the Western Ghats hotspots (Tamma *et al.*, 2016). Despite the fact that bats typically display these mammalian trends, their endemism in peninsular India is currently believed to be primarily at the subspecific level. Bat diversity in this region is often represented as cryptic clades with high diversity (Bates and Harrison, 1997; Chattopadhyay *et al.*, 2016).

2.2. Western Ghats

One of India's four designated global biodiversity hotspots is the Western Ghats mountain range (Myers *et al.*, 2000), with 63 species of bats found in the region (Raman *et al.*, 2020). Palghat Gap is a low-elevation area in the Western Ghats that is 500 million years old and has a width of 40 km at an average elevation of 140 m (Robin *et al.*, 2010).

There are several cryptic species in the order Chiroptera, most of which have only recently been discovered. The number of bat species currently described may be significantly underestimating the variety of bats since an increasing number of cryptic species are being described (Thabah *et al.*, 2006; Karuppudurai *et al.*, 2007; Kanagaraj *et al.*, 2010; Chinnasamy *et al.*, 2011; Chattopadhyay *et al.*, 2012, 2016, 2021; Ruedi *et al.*, 2012; Chakravarty *et al.*, 2020).

Several bat families have cryptic species discovered; however, the oriental family Hipposideridae seems to have exceptionally high levels of cryptic diversity (Pye, 1972; Jones *et al.*, 1993; Thabah *et al.*, 2006; Vallo *et al.*, 2008; Murray *et al.*, 2012). This family is represented by 90 insect bat species globally (Simmons and Cirranello, 2022), 16 species in the Indian subcontinent (Saikia, 2018b)), and nine in peninsular India (Raman and Hughes, 2021). Except for a few species, most have a broad distributional range throughout south and southeast Asia (Bates and Harrison, 1997). However, recent multivariate and phylogenetic analysis shows that some species previously considered widespread now have a restricted distribution (Zhao *et al.*, 2015; Raman, Padmarajan, Thomas, Sidharthan and Alice C. Hughes, 2020). *H. pomona* is an intriguing situation in this setting. It has significant cryptic diversity, and discoveries have shown species-level lineages throughout its distribution in South and Southeast Asia. (Srinivasulu and Srinivasulu, 2018b; Srinivasulu *et al.*, 2020b).

Together with genetic indicators, characteristics like echolocation call parameters have also been effective in identifying cryptic species. Several species exhibit species-specific traits in the construction of their echolocation calls (Karine and Kalko, 2001). In this context, Indian bat researchers have increased their emphasis on echolocation research in recent years (Thabah *et al.*, 2006; Chattopadhyay *et al.*, 2012, 2016, 2021; Wordley *et al.*, 2014; Chakravarty *et al.*, 2020; Raman and Hughes, 2021). Bats use echolocation calls to communicate, detect, navigate and locate prey (Li *et al.*, 2007; Kazial *et al.*, 2008; Yovel *et al.*, 2009; Knörnschild *et al.*, 2010; Jones and Siemers, 2011). These ultrasonic vocalisations are convenient for bat identification (Hayes, 2000). The echolocation calls of the H. *pomona* from western ghats were found to have an average frequency at maximum energy (FMaxE) of 122.4 ± 3.65 (kHz) (Raman and Hughes, 2021)

2.3. Ecological importance of Bats

2.3.1. Pollination and seed dispersal

Chiropterophily, or bat pollination, and Chiropterochory, or bat seed distribution, are mutualistic population interactions that support healthy ecosystem functioning and greater development and density of forest trees. Phyllostomidae (New World leaf-nosed bats) and Pteropodidae (Old World fruit bats) are the primary groups aiding pollination and seed dissemination. Fruit bats are a keystone species since they are islands' only significant pollinators and seed dispersers (Cox *et al.* 1991). In fragmented and damaged forest environments, bats are crucial for preserving the genetic continuity of plant populations (Galetti and Morellata, 19;94 Fleming *et al.*, 2009). In 2014, Prasad and Sunojkumar researched the coexistence of plants and fruit bats in Kerala, where bats provide food and a place to rest while also serving as dispersers and pollinators for plants.

2.3.2. Succession

According to Muscarella and Fleming (2007), the main way animals contribute to successional change in tropical ecosystems is through seed distribution. Successional change in the biotic community is the change in the relative abundance of the dominant species within a community (Muscarella and Fleming, 2007). Through the spread of early and late successional species, frugivorous basal flora facilitates regeneration in tropical forest fragments, disturbed regions, and disturbed areas (Medellin and Guona, 1999; Mascarella and Fleming, 2007)

2.3.3. Insect pest suppression

Insectivorous bats, through biological pest control and integrated pest management in the tropics, eat species from more than 15 orders of arthropods, including mosquitoes and significant agricultural pests. Lepidoptera, Coleoptera, Homoptera, Hemiptera, Orthoptera, Odonata, Diptera, Dermoptera, and Trichoptera are some of the major orders of arthropods that bats eat. Ephemeroptera, Blattodea, Mantodea, Neuroptera, and Araneae. Isoptera and Hymenoptera (Vanitharani and Chelladurai, 2005)

2.3.4. Soil fertility and nutrient distribution

Guano from bats is the major ingredient that can improve soil fertility and aid nutrient dispersion. According to Ferreira et al. (2007), it is a significant energy source and a fertiliser (Kuepper, 2003). Carbon, nitrogen, and organic matter source. Potassium and Phosphorous show the guano's fecundity spectrum (Ferreira *et al.,* 2000; Emerson and Roark, 2007). characteristics such as pH that are physical, chemical, and spatial. The primary factors influencing the amount of energy source and fertility of bat guano are the percentage of organic matter, moisture content, proximity to the cave entrance, area and form of the deposits, and age.

2.4. Echolocation in bats

One of the most amazing mammal species is the bat. Not only are they the only flying mammals, but they also have sophisticated echolocation abilities. Microbats and the Megachiropteran genus Rousettus have high frequency echolocation, but their primary sense of location is hearing. Although certain species emit and analyse main components as high as 150 KHz, the frequencies employed in echolocation by bats typically range between 25 KHz and 100 KHz (Grinnell, 1995). The prey detection range in the dark is determined by the insect's size, echo reflectivity, and, most critically, the frequency and strength of the echolocation sounds (Houston *et al.,* 2003, Surlykke and Kalko, 2008). Low frequency calls have a greater range than high frequency calls because atmospheric attenuation impacts them less. For particularly big insects and night migrating birds that certain bats prey upon, the extended, nearly constant frequency cries (between 10 and 20 kHz) of giant aerial haunting bats may have maximum detection distances upto several metres (Estoker *et al.,* 2009). However, because of the considerable absorption of ultrasound in air and the low target strength, particularly of minute insects, most bats' prey detection ranges are just a few metres (Holdered and von Helversen, 2003; Surlykke and Kalko, 2008).

Bats utilise echolocation to navigate and hunt, but it also serves to convey species identity, individual identity, sex, and group membership (Voigt-Heucke *et al.*, 2010; Kazialer *et al.*, 2008). (Voigt-Heucke *et al.*, 2010). The authors, Voigt-Heuckeer *et al.* (2010), were unaware of any other taxon in which an animal's ubiquitous behaviour, such as orientation, which is clearly shown for non-social purposes, also functions as a signal for its conspecifics.

2.5. Types of calls in bats

Regarding auditory communication, bats are unique because they have two distinct sound types: social cries, which are only utilised in interactions between bats, and echolocation calls, which are produced for navigation and preying. Social sounds, in contrast to ultrasonic echolocation screams, have a frequency lower than 20 kHz, making them theoretically audible to humans. Moreover, they frequently feature a multi harmonic structure (Fenton, 2003). Social calls have been demonstrated to be uniquely distinct for each individual (Carter *et al.,* 2008), or to be used more generally for a group (Racey and Swift, 1985), to help group foraging (Wilkinson and Boughman, 1998), for courtship displays (Behr and von Helversen, 2004), and territorial interactions (Behr *et al.,* 2006). Contrarily, echolocation has hitherto only been thought of as an acoustical instrument that allows bats to orient in the dark, which is necessary for the location of prey and night-time navigation (Griffin, 1958; Schnitzler *et al.,* 2003).

2.6. Ecology, biology and behaviour

Bats are nocturnal, spending the day roosting in caves, rock crevices, greenery, or different human-made buildings. Some bats live alone, while others live in colonies and can number millions. The bat's history with humans extends back to antiquity. They are recognised to significantly impact ecosystem health and the human economy (Fujita and Tuttle, 1991; Kunz *et al.,* 2011). Bats spend the whole night contributing to the ecosystem of a region with the same diversity and vitality as birds do during the day. So, every attempt to comprehend the variety and effectiveness of an ecological system must incorporate an understanding of bats there (Saikia, 2018a).

2.6.1. Feeding behaviour

The investigation of bat guano and faecal samples indicated that individual bats had a variety of diets while studying bats' feeding behaviour and diet. Typically, fruits and seeds make up most of the faeces of frugivorous bats. In insectivorous bats' case, most of their faeces contain parts of insects. Due to the enormous variety of insects that insectivores consume, studies on their eating patterns are more extensive than those on frugivorous bats. Many researchers also use bat guano to determine the diversity of a given region's arthropods. The insectivorous bats consumed any accessible insects 3 to 10 mm in length. Nematoceran Dipteria were by far the most prevalent insects found in light-trap samples, and they made up a significant amount of the diet all summer long. Significant amounts of Coleoptera, Trichoptera, Lepidoptera, Ephemeroptera, and Neuroptera were also devoured (Anthony and Kunz, 1977). At particular periods of the year, the bats choose alternate, more plentiful and/or more lucrative food, mostly by relocating from their primary feeding sites to other (generally transient) foraging grounds (Arlettaz, 1996).

Elisabeth (1995) examined pipistrelle bats' hunting habits and echolocation in her work, "Insect chase prey capture and echolocation in pipistrelle bats." The research shows that the forging patterns involved four stages: the search flight (before prey was discovered), approach flight (pursuit after prey was discovered), capture, and recovery of prey. These phases corresponded to the search phase, the approach phase, the terminal phase, and the pause that followed in echolocation behaviour. The common frugivorous bar *Cynapterus sphinx* consumes various wild and certain orchard tree species' fruits, flowers, and leaves in varied amounts. Food preferences and availability, which are influenced by yearly cycles of blooming and fruiting, are tied to the seasonal fluctuation in its diet. Typically, it picks the fruits from the tree and brings them to feeding perches, where it eats the soft parts, drinks the juice, drops the fibres and seals, and distributes the seeds (Bhat, 1994).

According to a study on the dietary variation of the Indian pygmy bat in different seasons, *Pipistrellus mimus*, from southern India, which involved collecting faecal samples twice a week below a maternity roost for one year found that it contains 26.3% coleopterans, 20.4% dipterans and 17.5% homopterans (Whitaker *et al.*, 1999). An investigation on the diets of *Rhinolophux rouxii, Hipposideros speoris,* and *Hipposideros at*er in the Peechi-

Vazhani Wildlife Sanctuary in Kerala reveals a greater intake of insect groups including Lepidoptera, Coleoptera, and Diptera (Aravind, 2014).

2.6.2. Breeding behaviour

Only a small portion of the 120 bat species that Bradbury (1977) classed by social structure (representing about 12% of the order) had their mating systems described. He said that year-round research is still needed to demonstrate the presence of monogamous families in any bat species. One male may mate with many females in more than 90% of the mammal species investigated. In contrast, the majority of bird species, even those once assumed to be monogamous, have had their mating systems disproven by several DNA investigations. Bats follow the typical mammalian pattern. Fewer but surprisingly, many bat species tend to be monogamous, whereas most appear to be polygynous. There are several bat species where females mate sequentially with many males, and there are other species where paternity outside of apparent mating groups has been observed. According to the information, bat females frequently reproduce more than once. Bats are classified into three structural association groups based on mating: single male/multiple female mating groups and single male/single female mating groups (McCracken and Wilkinson, 2000). Numerous bat species are promiscuous, meaning neither the partners before nor after mating have any ongoing relationships. Intensely structured and non-random procreative mating is possible (Thomas *et al.*, 1979).

Harems are often described as clusters of females mating with a single male. Due to its vagueness and anthropocentric implications of male supremacy and female subservience, this usage of the name "harem" has drawn criticism (Lewis, 1992).

While there has been significant progress in understanding bat mating systems, there needs to be more advancement in our knowledge of the ecological processes influencing the evolution and preservation of these systems. According to popular wisdom, resource distribution has a greater impact on female dispersion patterns than male dispersion patterns and vice versa (Bradbury and Vehrencamp, 1977).

Research on bat mating systems needs to catch up in using gene markers compared to studies on other species, such as birds. McCracken and Bradbury conducted the first genetic investigation of a bat mating system in 1977.

Female bats may hold viable sperm for up to 200 days, and social groupings might number just one individual or hundreds of thousands. In bats, the danger of sperm competition is positively correlated with the relative testis mass. Reduced confidence in paternity may be one of the costs of living in groups, and the size of the social group may also affect the degree of sperm competition (Hosken, 1997).

Compared to male and non-reproductive female bats, female reproductive bats are more limited by their thermoregulatory and energy requirements. Fertile females may have a smaller geographic range than other bats due to restrictions. Latitude and elevation are likely to have an impact on these restrictions. The relative quantity of fertile females and elevation were significantly inversely correlated. As the elevation rose, there were fewer fertile females overall. High-altitude settings that increase thermoregulatory costs and reduce foraging effectiveness may prevent reproductive females from roosting and foraging (Racey and Entwistle, 2000).

2.6.3. Roosting behaviour

Bats reside in their roost surroundings for more than half of their lifetimes. The state and activities of roosts are very important to their ecosystem. The qualities of a roost are crucial to a species' success. It offers a habitat appropriate for mating, hibernating, raising offspring, and protection from inclement weather and predators (Kunz, 1982). In particular, caves, rock crevices, tree holes, and buildings are preferred dark locations for the micro-chiropteran bats to sleep. The largest variety of roosting places may be found in caves with a wide heat range, complicated structural layouts, and varied elevations (Tuttle and Stevenson, 1978). Bats utilise a variety of man-made structures, including residences, historical sites, temples, mosques, and other buildings.

They utilise cracks in the walls, chimneys, attics, and areas under tiles or other roofing components inside these places. Some bats solely utilise one kind of roost, such as caves, while others could switch up their roosts yearly.

The Indian flying fox, *Pteropus medius*, typically roosts in bigger trees, the Leshenault's Rousette, *Rousettus leshenaultii,* typically roosts in buildings and caves; and the Greater short-nosed fruit bat, *Cvnopterus sphinx*, is typically found in structures, trees, and other vegetation. The bulk of bat colonies are found close to water sources, which shows that the bats choose their day roost locations to escape excessive temperatures during the day. *Hipposideros lankadiva, H. fulvus, Taphozous melanopogon, T. nudiventris, Lyroderma lyra, Rhinopoma microphyllum*, and *R. hardwickii* were among the insectivorous bat species that were proportionately common in a range of artificial structures. The species was discovered to roost in locations with plenty of fresh water. The daytime roosting places for *Pipistrellus coromandra* and *P. ceylonicus* include trees, building roofs, gaps between logs, house ceilings and walls, and spaces between hut roof tiles. Hipposideros fulvus and H. lankadiva used the cellars of older homes to nest. It depends on the presence of water and shade and prefers cold, moist environments. *T nudiventris* nests in cracks in roofs and walls and occasionally hang from roofs and building ceilings.

According to research on the roosting ecology of insect foraging bats, several species, including *H. fulvus, H. lankadiva* mainly roost on buildings while species like *T. nudiventris, T. melanopogan, and R. hardwickii* shared roosts. The relationship between bats and artificial structures appears to be regionally variable (Elangovan and Kumar, 2015).

The findings of a study based on *Barbastella barbastellus* roost selection reveals that the trees in woodlands that are not managed were preferred for roosting, and woodlands which were subjected to limited harvest activities were used in proportion to availability, and open and pasture mixed woodlands, were completely avoided. The choice was based on the state of the trees; dead trees were favoured, and higher trees were chosen as roosts. Cavity selection depended on cavity type, height, and entry orientation; roost cavities were often found beneath loose bark, higher up than random cavities, and more usually faced south (Russo *et al.,* 2004).

The timing of the sun setting and rising was initially used to explain when bats emerged from their chambers at twilight and when they returned (Erkett. 1978). Later, it was thought that various environmental elements, including weather, moonlight, and ambient light levels (Elangovan and Marimuthu, 2001; Thies *et al.*, 2006), may affect bat activity. Bats avoid circumstances where they could be preyed upon by raptorial birds, such as those where they emerge in the early hours of the morning or under a full moon (Welbergen, 2006). However, insectivorous bats frequently take a chance on predation when their prey is abundant at night to maximise their foraging possibilities (Jones and Rydell, 1994; Rydell *et al.*, 1996).

The curtain creeper (*Vononia scandens*) and the mast tree (*Polyalthia longifolia*) are used as building materials by *Cynopterus sphinx* to create partially enclosed chambers (stem tents) where they may sleep. Male singletons are largely, if not completely, responsible for building and maintaining stem tents. A single male C. sphinx in V. scandens can sever up to 300 tiny to medium-sized stems, forming a hollow that is somewhat flattened and bell-shaped. In P. longifolia, a male can make an entrance or exit portal as well as a place to roost by cutting a few small to medium-sized branches and several leaf petioles (Balasingh *et al.*, 1995).

In 2003, Singaravelan and Marimuthu published the first study describing a cave as the day roost of Salim Ali's fruit bat, *Latidens salimalii*. It was previously unknown that this species, mist-netted frequently in the High Wavy Mountains, roosts throughout the day in vegetation, trees, or structures, much like sympatric fruit bats. The High Wavy Mountains are the first place this species has ever roosted. In the cave's deepest corners, most inhabitants nested in groups. *L. salimalii*'s only known night roost was not more than a kilometre from its day roost.

Many bat species in the Philippines have a reasonably stable environment because of the steady microclimate in caves. Still, human encroachment on this habitat threatens many of the species that roost there. The diversity and conservation status of cave bats are mostly unknown and undiscovered worldwide. The bats' diminishing conservation status impacts the caverns, local disturbances like bat hunting for bush meat, and unrestricted tourism (Tanalgo and Tabora, 2015).

2.6.4. Habitat selection and preference

Energy conservation is the main issue bats face. This is accomplished through the metabolic economy, which is made feasible by the common feature of chiropterans with a "poor" mechanism for controlling body temperature. Bats are homoeothermic species that can fly great distances, yet when they are at rest, their body temperatures tend to resemble the ambient temperature. They are known as heterothermic mammals for this reason. Bats' metabolic needs are significantly lowered when they are sleeping. Bats could not continue without food for extended periods if they kept their body temperatures as high while sleeping as when flying because food stores would soon catabolise despite the lower surface area to volume ratio. The choice of an appropriate environment for resting is one of the most crucial variables in maintaining a bat population. Therefore, physiological reactivity in connection to habitat choice is mostly responsible for energy conservation (Twente, 1955).

The availability of suitable roost sites between logged and uncut stands may be a significant driver of bat Myotis species and silver-haired bats, according to research on the selection of habitats by bats in Ontario. According to habitat models, the snag was not significantly influencing bats' habitat choice in uncut stands. Forest managers should use wood harvest techniques that preserve relic old-growth forests to preserve bat habitats (Jung *et al*., 1999).

Increased moon visibility had a detrimental effect on flight activity, although insect abundance and ambient temperature were substantially and positively correlated with bat activity. These variables worked best as a unit to explain overall bat activity and bat activity while hunting in open areas and at the edges of habitats. The strongest predictor of activity by species foraging in settings with many obstacles in the forest was the interplay between temperature and light intensity (Meyer *et al*., 2004).

Wetlands are widely acknowledged as an essential habitat type for the preservation of several migratory bird species. The significance of foraging environments for supporting migrating bats has, until recently, received little attention, even though many bats are also long-distance travellers. The Nathusius pipistrelle (*Pipistrellus nathusii*), a migratory bat, has specific habitat selection tendencies in the southernmost region of the Iberian

Peninsula, where this particular species both breeds and hibernates. *P. nathusii* preferred the riparian and natural wetlands (Phragmites reed beds) while avoiding the human-modified aquatic habitats (rice paddies). Since agricultural land conversion decreases the availability of this species' preferred foraging habitat, it is likely to be harmful (Flaquer *et al.,* 2009).

Out of 50 species in the Western Ghats, 47% spend the night in caves, 31% in fissures, 8% in dry tree logs, and 13% in trees and other vegetation. In bamboo thickets, just one species of bird roosts. Seventeen species (31%) were restricted to high altitude woods, primarily found in natural caves. In contrast, roosting sites for 27 species (52%) were documented in buildings in forests, rural or urban areas nearby forests. The majority of these species have been observed roosting in small groups of a few to roughly 25 people. Only 14 species (or 27% of all species) develop colonies of hundreds to thousands of individuals (Korad *et al.,* 2007).

The ten species of bats studied in the Western Ghats of India (*Hesperoptenus tickelli, Miniopterus fuliginosus, Miniopterus pusillus, Myotis horsfieldii, Pipistrellus ceylonicus, Megaderma spasma, Hipposideros pomona, Rhinolophus beddomei, Rhinolophus rouxii,* and *Rhinolophus lepidus*) were mostly found due to their high mobility, which allows them to take advantage of even small or isolated foraging areas, small scale habitat variables and distances to habitat features were the most effective predictors of bat occurrence. Examples of such variables include the percentage of tea plantation cover and distance to water. Most species responded favourably to coffee plantations grown in natural shade and to forest remnants, but negatively to tea plantations that had undergone more extensive modification. Even though plantations cause bats to react unfavourably, environments where tea predominates that also have stretches of woodland and shaded coffee may harbour a wide variety of bat species (Wordley *et al.,* 2015).

2.7. Threats, habitat management and conservation of bats

Including numerous bat species restricted to the north of the Peninsula in peninsular Malaysia (Corbet and Hill, 1992) would cause the predicted species richness to be overestimated. In Surveys on Peninsular Malaysia (Lekagul and McNeely, 1988; Koopman, 1989; Corbet and Hill, 1992; Zubaid, 1993; Francis, 1995; Kingston *et al.,*

2003) indicate a decline in mMicrochiropteradiversity towards the southern end of the peninsula. Only 15 of the 24 Microchiroptera species and 6 Megachiroptera species identified for the little tropical island of Singapore, according to recent studies of its bat fauna, are still alive. Since considerable land transformation or habitat loss (95%) and biota loss happened early in Singapore's colonial history before detailed surveys of bats were performed, these documented declines in chiropteran species richness almost definitely understate the total losses (David *et al*, 2006). One of the biggest challenges to the bat population in Jordan was the unchecked use of pesticides. Since the founding of the Kingdom, large quantities of DDT and other organophosphorus insecticides have been used across the nation to control the vectors of malaria and leishmania, particularly in the Jordan Valley, where caves have unintentionally been sprayed to kill resting mosquitoes and sandflies. The Ministry of Agriculture in Jordan did not believe bats to be pests and did not target them, however, the application of pesticides caused a dramatic fall in some populations of the Egyptian Fruit Bat, *Rousettus. aegyptiacus*. Other significant factors contributing to the loss of the bat population include habitat damage brought on by deforestation, urbanisation, agricultural expansion, road development, and tourism. A task force connected to the regional nature conservation groups should be established as part of Jordan's bat education and protection initiatives. The members of this task team ought to be experts in conservation, academics, and environment lovers. Providing adequate training programmes for conservation enthusiasts based on bat conservation in nations with experience in this area is important. Another conservation initiative involves educational campaigns targeted at Jordanian public schools and carried out in partnership with the ministry of education. Increasing public awareness of the necessity for conservation in general and of bat conservation in, in particular the goal. Work must be started to broaden bat conservation laws' legal protections and increase existing laws' enforcement (Zuhair *et al.,* 2005)

Elisabeth *et al* (1995) study of Neotropical bats in the canopy revealed that this trend is influenced by vertical stratification, species-specific variations in food, foraging tactics, roost locations, and sample bias. They also evaluated the variations in catch rates of several better-sampled species in high and low nets. Changes in the forest's structure are likely to impact the structure and function of the nearby bat populations because of how differently various bat species utilise space. This knowledge is particularly crucial in light of the requirement to create and implement conservation-oriented initiatives to preserve biodiversity. A study of recent advancements in approaches for bat inventorying reveals that such investigations require a mix of techniques, including mist netting and acoustic monitoring. A study on the causes of the significant drop in the number of bats in southern Belgium reveals a sharp decline in the numbers of several bat species, including *Rhinolophus ferrumequinum, R. hipposideros, Barbastella barbatellus, Myotis dasycneme,* and *M. myotis*. Over the past fifty years, the bat variety in these hibernacula has plummeted by half. The Walloon Region's extensive ecosystem degeneration is reflected in the study's observation of a 50% loss in species variety, which also represents the cause of a significant shift in how these ecosystems operate (Thierry *et al.,* 2009). The greatest hazards to bats in the islands of Fiji were listed as roost disruption, roost loss, hunting, imported predators, and deforestation. Some of the proposed conservation projects in the Fiji Islands include awareness campaigns, the eradication of imported pests like domestic cats, and the development of natural orchards (Palmeirim *et al.* 2005). The condition is particularly important when talking about the survival of bats in the Indo-Malayan region. Of the 260 bat species known, one is extinct, fourty three are threatened with risk of extinction (Hutson *et al.,* 2001). In Pakistan one bat species is threatened. 4 are Vulnerable, 9 are Near Threatened, 18 are Least Concern, 7 are Data Deficient, and One is Not Evaluated (Walker and Molur, 2003). The primary causes of hazards to bats in Pakistan include the loss of their natural habitat brought on by a rise in human population and human activities including deforestation, pesticide usage, industrial activity, loss of structures, or modifications to the design of their roofs. These human activities are also the main causes of their population decline. Even very few changes to the ecosystem, such as removing important landscape features like tree lines, hedgerows, and canals that bats frequently use during flight, causing them to quit their roosts and maternity colonies. Similar to the last example, much is known about certain bats to say that they are not threatened. Still, it is difficult to determine the status of others who are seldom encountered. This makes it exceedingly challenging to determine which species in Pakistan are vulnerable or require specific conservation efforts (Mahmood-ul-Hassan and Nameer, 2006).

Research on the protection of bat cave roosts in Yucatan, Mexico, examined the highly nested pattern of species distribution and how caverns that sustain diverse populations of many species also serve as roosts for multiple threatened species. The most important locations for bat preservation are found in the vast networks that are so alluring to tourists, which made it difficult to carry out the conservation strategy. Plans for protecting cave ecosystems must be a part of any conservation efforts for Yucatan's bats. Due to the state's unique social and historical characteristics, such conservation strategies must also be tied to preserving ancient monuments, managing caves as tourist destinations, and using sport speleologists (Arita,1996). The recent Nipah outbreak in the Kerala region and the Covid-19 pandemic has also added to the existing threat to bats ((Plowright et al., 2019; Lu et al., 2021).

MATERIALS AND METHODS

3. Materials and Methods

3.1. Study Area

The study was conducted on forests across the Kerala part of SWG, where H. pomona was previously reported (Raman *et al.,* 2020)

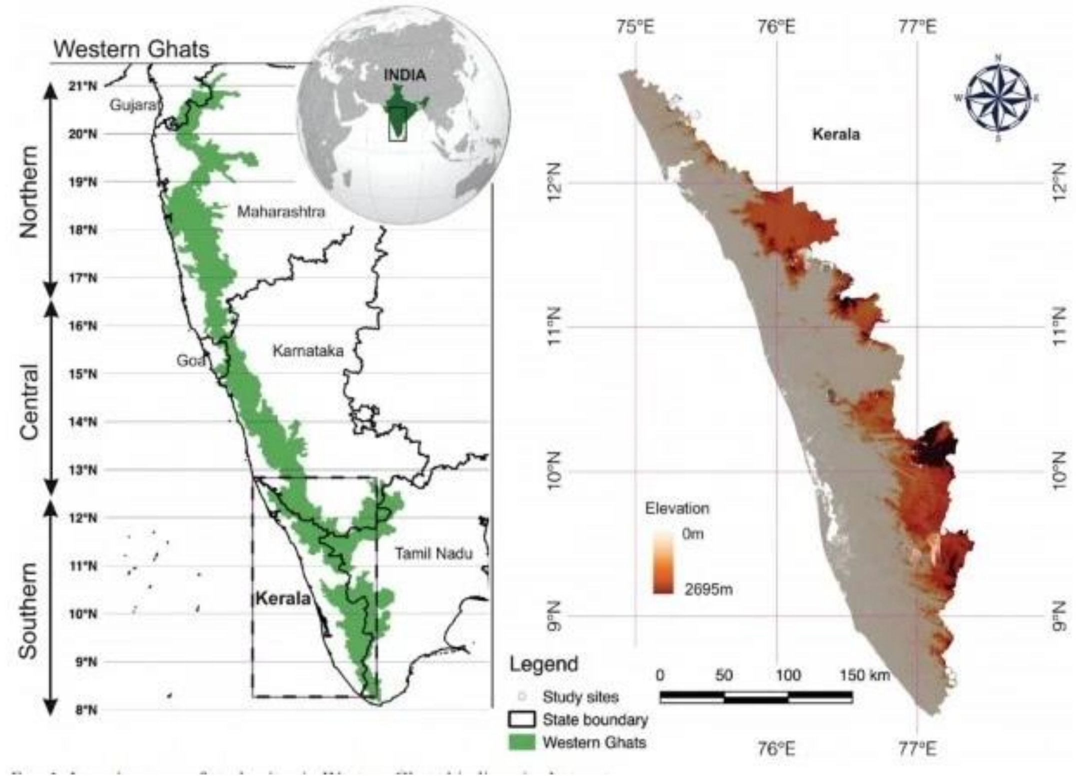

Plate 1 Study area

3.2. Methods

3.2.1. Bat sampling

Bat sampling was carried out using harp traps of dimension 1.5m width, 2.2m height, 7.5cm between four frames and 2.5 cm between vertical monofilament fishing lines (Raman and Hughes, 2020). Sixteen standard morphological measurements were taken on the bats captured in the harp trap using a digital vernier calipers (with precision 0.1mm) (Bates and Harrison, 1997). External measures included FA - forearm length, HBL- head to body length, EAR - ear length, TL - tail length, TIB - tibia length, HF - hindfoot length, THUMB- thumb length, 3MT - third metacarpal, 4MT - fourth metacarpal, 5MT - fifth metacarpal, 1PH3MT - first phalanx of third metacarpal, 2PH3MT - second phalanx of third metacarpal, 1PH4MT - first phalanx of fourth metacarpal, 2PH4MT - second phalanx of fourth metacarpal, 1PH5MT- first phalanx of the fifth metacarpal, and 2PH5MT- second phalanx of fifth metacarpal.

The bat species identification was made using the existing and locally developed anatomical key (Bates and Harrison, 1997; Raman *et al.*, 2020).

Plate 2 Hipposideros pomona © Sreehari Raman

Plate 3 Harp trap

Plate 4 Setting trap in the field

Plate 5 Bat handling

3.2.2. Tissue sampling

Wing punches of 3 mm diameter were taken with a sterile biopsy punch, preserved in molecular grade ethanol, and stored in a deep freezer until extraction.

Plate 6 Taking wing punch © Sreehari Raman

3.2.3. DNA extraction

DNA extraction using the standard protocol. Polymerase Chain Reaction performed to amplify the mitochondrial cytochrome oxidase 1 (COI) (Ivanova *et al.*, 2006), mitochondrial cytochrome b (Cyt b) (Wang *et al.*, 2003; Guillén-Servent and Francis, 2006; Li *et al.*, 2007; Vallo *et al.*, 2008; Sun *et al.*, 2009). PCR products were outsourced for sequencing.

Plate 7 Extracting DNA in Forensic Lab, Dept. of wildlife sciences, KAU

3.2.4. Acoustic sampling

Recordings were made from both mosquito net flying cages (2m x 2m x 2m) and hand-release. Using the programme BatSound Touch, a M500-384 ultrasound detector was used to record the echolocation calls of the bats flying inside the net. From all calls, eight parameters were calculated. Spectrograms were used to determine the start frequency (Fstart), end frequency (Fend), frequency maximum (Fmax), and frequency minimum (Fmin), oscillograms were used to determine the call duration (D) (ms), inter-pulse interval (IPI) (ms), frequency of maximum energy (FmaxE) (kHz), and duty cycle

(DC) was calculated by dividing the call duration by the inter-pulse interval and multiplying the result by 100.

Plate 8 Mosquito net for recording echolocation calls

3.3. Data Analysis

3.3.1. Phylogenetic Analysis

Sequences were aligned in Molecular Evolutionary Genetics Analysis Version 11 (MEGA 11) (Tamura *et al.*, 2021). Phylogenetic reconstructions were performed using the maximum likelihood (ML) method and the Neighbour Joining Method. A statistical parsimony network of haplotypes was created using Population Analysis with Reticulate Trees (POPART) (Leigh and Bryant, 2015).

3.3.2. Acoustic analysis

Statistical analyses were conducted in R (R Development Core Team, 2003). The frequency of maximum energy (FmaxE) (kHz) of the subpopulations in the north and south were compared and statistically tested using Welsh two-sample t-test.

3.3.3. Morphometric analysis

Statistical analyses were conducted in R (R Development Core Team, 2003). For classification analysis, one way ANOVA was used to find significant variables ($p<0.05$). Statistically sound variables were selected for Principal Component Analysis (PCA)

RESULTS

4.Results

4.1. Phylogenetic Reconstruction

After DNA extraction, amplification and sequencing, a 652 base pair long mitochondrial cytochrome oxidase 1 gene was obtained from sample no. XTBG_54. This sample was collected from the Sholayar forests of Thrissur district, Kerala, located south of the Palghat gap. This sequence was used to construct a topology tree with available samples in the National Centre for Biotechnology Information (NCBI) database. Four CO1 samples from the database viz. NC_018540.1:5342-6886 *Hipposideros armiger* (Gene ID: 13539867), NC_061963.1:5341-*6885 Hipposideros pomona* (Gene ID: 71461813), and NC_061568.1:5340-6884 *Hipposideros cervinus* (Gene ID: 71432569) along with sample XTBG_54 sequence was aligned in MEGA 11 and trimmed to 652 bp length. The phylogenetic tree was constructed in MEGA 11 using both the maximum likelihood method and neighbour-joining method, which showed a clear difference in the XTBG_54 sample with the *H. pomona* sequence available in the NCBI database. These sequences were then used to create a statistical parsimony network of haplotypes in POPART software.

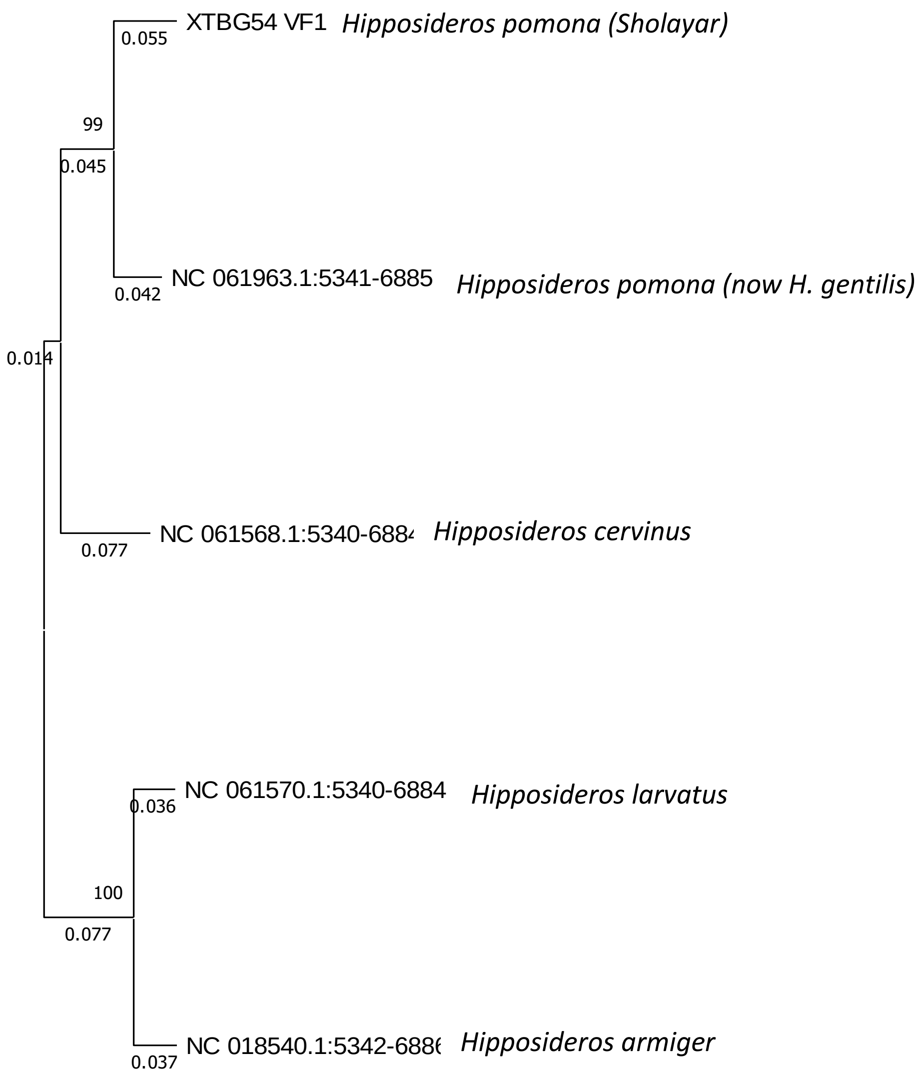

Figure 1 Phylogenetic tree using maximum likelihood method

Figure 2 Phylogenetic tree using Neighbour-joining method

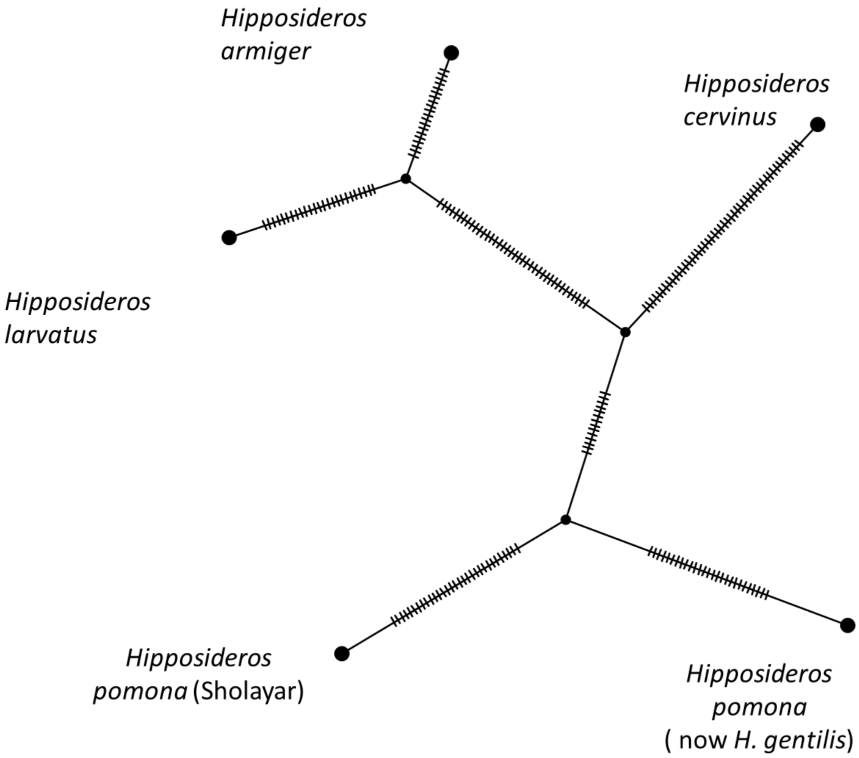

Figure 3 Statistical parsimony network of haplotypes of compared gene sequences

4.2. Acoustics

The frequency of maximum energy (FmaxE) (kHz) of 26 echolocation sound samples (14 from north of the Palghat gap and 12 from south) were analysed with welch two-sample t-test. The call samples of subpopulations were statistically distinct (p= 0.04), with 119.63 kHz mean in the northern sub-population and 123.26 kHz mean in the southern sub-population with a 95% confidence interval [0.1456184, 7.1284076]

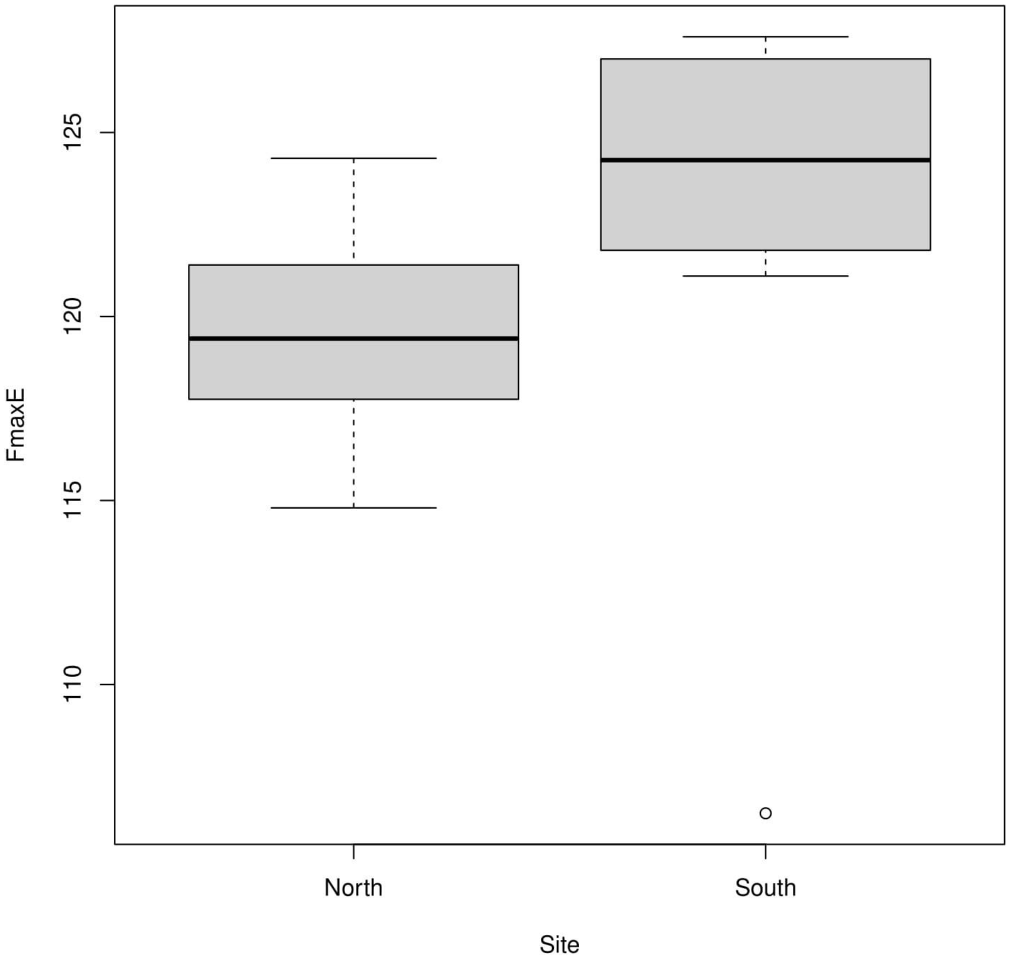

Figure 4 Boxplot of acoustics samples from north and south subpopulations of H. pomona across the Palghat gap

4.3. Morphometric Analysis

Sixteen morphometric parameters of 12 samples were used for classification analysis, of which three variables were significantly different for two subpopulations viz. Forearm length (FA), length of first phalanx of third metacarpal (1PH3MT), length of fourth metacarpal (4MT). These were analysed with welch two-sample t-test.

4.3.1. Forearm Length

The FA of samples of subpopulations were statistically distinct (p= 0.03) with 39.61 mm mean in the northern sub-population and 42.25 mm mean in the southern sub-population with a 95% confidence interval [0.4054659, 4.8705341]

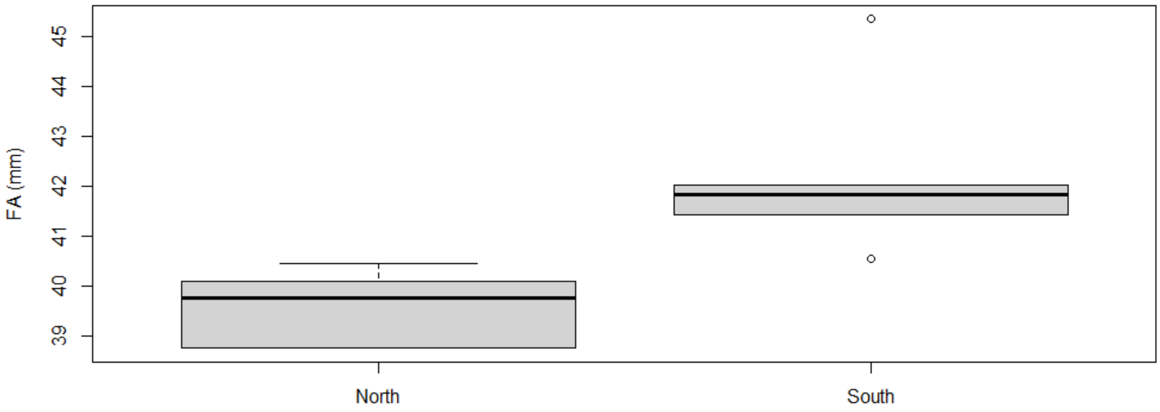

Figure 5 Boxplot of Forearm lengths of north and south subpopulations of H. pomona across the Palghat gap

4.3.2. Length of the first phalanx of 3rd Metacarpal

The 1PH3MT of samples of subpopulations were statistically distinct (p= 0.05) with 16.2 mm mean in the northern sub-population and 17.04 mm mean in the southern sub-population with a 95% confidence interval [-1.65621102,0.017944]

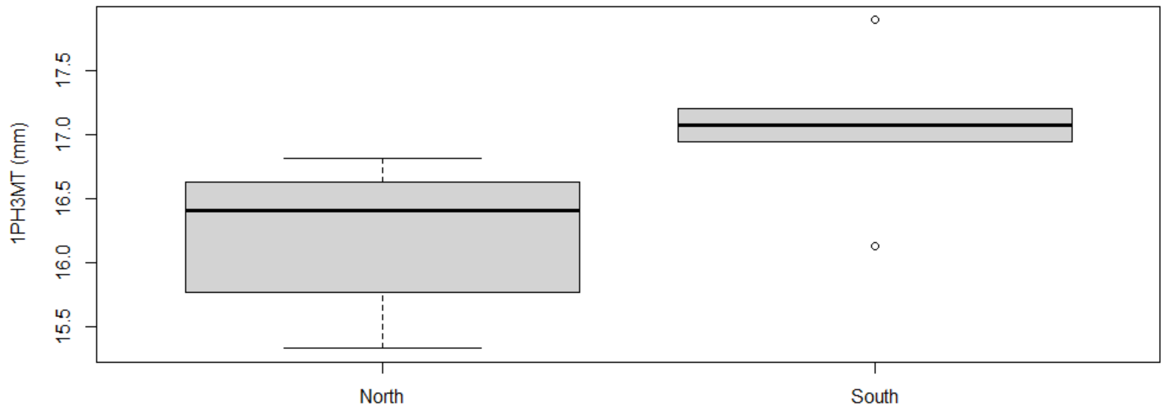

Figure 6 Boxplot of lengths of first phalanx of 3^{rd} metacarpal of north and south subpopulations of H. pomona across the Palghat gap

4.3.3. Length of fourth Metacarpal

The 4MT of samples of subpopulations were statistically distinct (p= 0.02) with a 30.76 mm mean in the northern sub-population and 32.14 mm mean in the southern sub-population with a 95% confidence interval [0.2153706,2.5486294]

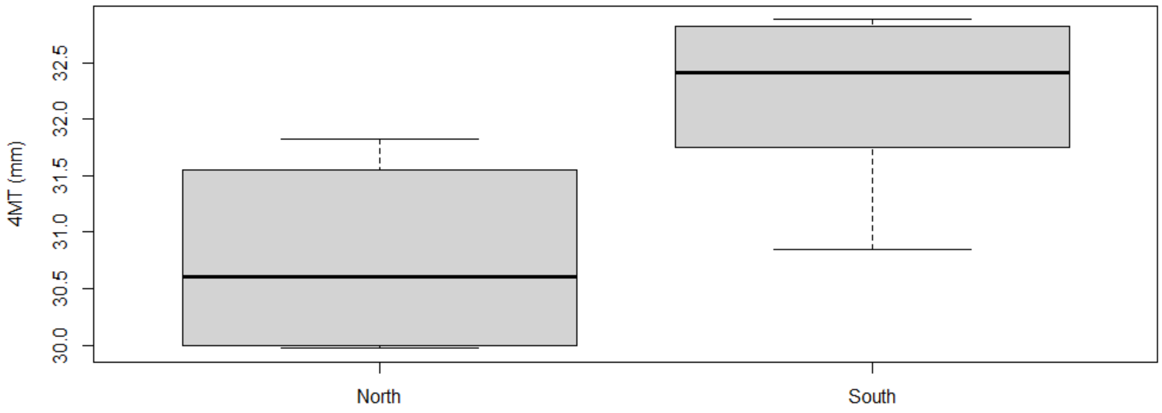

Figure 7 Boxplot of lengths of fourth metacarpal of north and south subpopulations of H. pomona across the Palghat gap

4.4. Principal Component Analysis

Sixteen morphometric parameters of 12 samples were used for classification analysis, of which four variables were significantly found sound based on one-way ANOVA (p-value $\leq$ 0.1) viz. Forearm length (FA), length of first phalanx of third metacarpal (1PH3MT), length of fourth metacarpal (4MT), and length of first phalanx of fourth metacarpal (1PH4MT). Principal component analysis was done on these four variables. Components PC1 and PC2 explained more than 80% of the variance. A Biplot of PC1 and PC2 components was generated to show the relationship of variables with the components. PCA1 and PC2 were plotted for both subpopulations with a 95% confidence interval. The result shows that both subpopulations are cryptic, and the morphology of the subpopulations are more or less similar.

	PC1	**PC2**	**PC3**	**PC4**
Standard deviation	1.5807	0.8892	0.7288	0.42391
Proportion of Variance	0.6247	0.1976	0.1328	0.04493
Cumulative Proportion	0.6247	0.8223	0.9551	1

Table 1 Importance of components

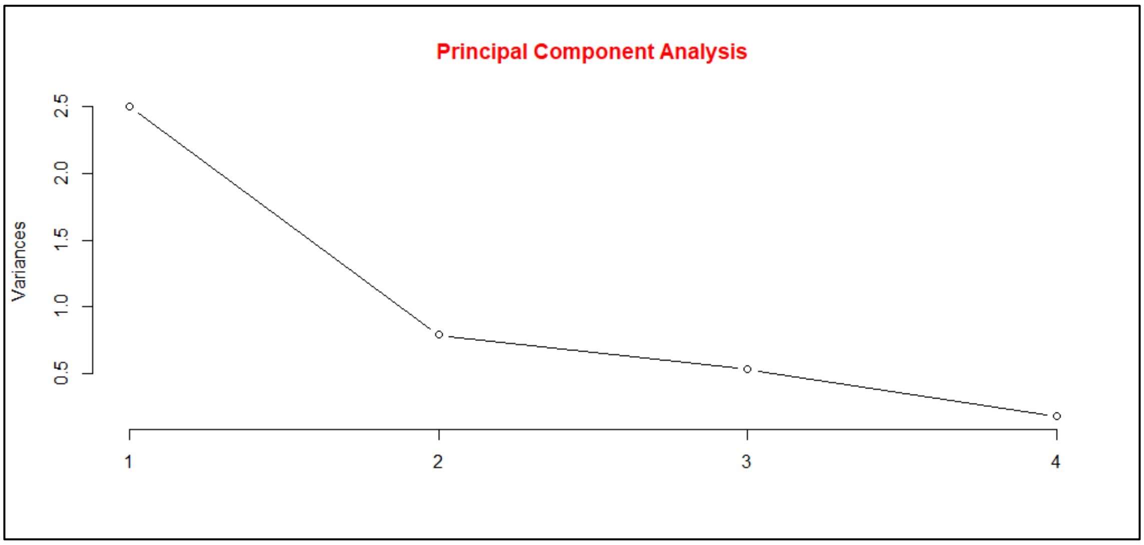

Figure 8 Proportion of variance of different components in PCA

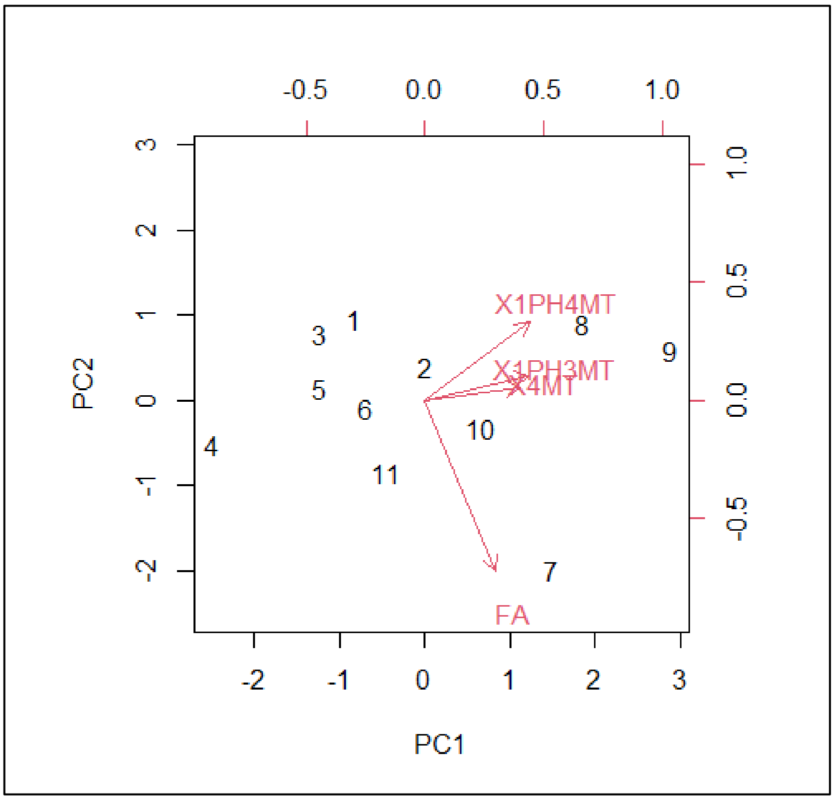

Figure 9 Biplot of PC1 and PC2 components

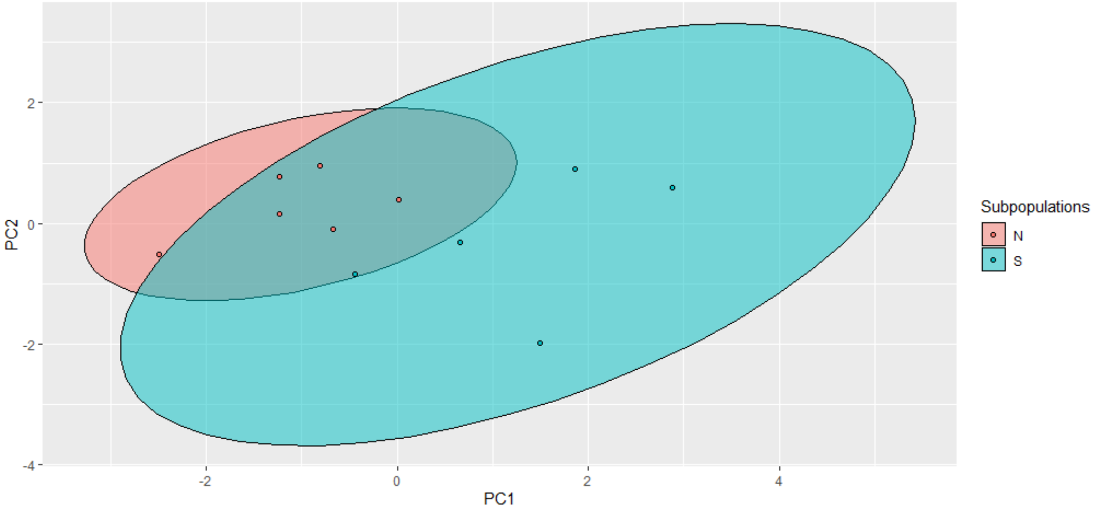

Figure 10 PCA of north and south subpopulations with 95% confidence interval

DISCUSSION

5. Discussion

5.1. Phylogenetics of *Hipposideros pomona*

Since its establishment, *Hipposideros pomona*'s taxonomic standing has been unclear. Knud Andersen's work on species related to *Hipposideros bicolor* led to the description of two new species: H. pomona in southern India and H. gentilis from northeast India, Burma, and the west coast of Sumatra. (Andersen, 1918; Hill, 1963; Douangboubpha *et al.*, 2010). Andersen (1918) based his diagnosis *of H. pomona* on possessing broader than usual noseleaf, horseshoe and sella. In contrast to *H. gentilis*, which had not broader than usual noseleaf, horseshoe and sella. In addition, he described three other subspecies of *H. gentilis*—sinensis, atrox, and major. Hill (1963) included pomona, gentilis, sinensis, atrox and major as subspecies of *H. bicolor*. Later, *H. pomona* was described as distinct species, including forms, gentilis, and sinensis, and assigned atrox and major to *H. bicolor* group (Hill *et al.*, 1986). With more research, Douangboubpha et al. (2010) suggested that *H. pomona* may really be two separate species, with *H. pomona*'s range limited to peninsular India and *H. gentilis*' distribution encompassing the taxa gentilis and sinensis extending from northeastern India into Southeast Asia. Based on differences in the morphometrics of the southern Indian specimens from those of *H. pomona gentilis* and the structure of the baculum of the southern Indian specimen of *H. pomona* from that of Assam and Southeast Asia, the peninsular Indian population was considered distinct by Srinivasulu and Srinivasulu (2018). Due to the disjunct distribution of the two species, the rest of the population was regarded as *H. gentilis*. The current known distribution of *H. pomona* is restricted to the southern Western Ghats, and the species was elevated to endangered status in IUCN red list due to the current restricted range (Srinivasulu and Srinivasulu, 2018b; Raman *et al.*, 2020; Srinivasulu *et al.*, 2020a). However, no genetic studies have been conducted yet to confirm the split. Present study results confirm that *H. pomona* and *H. gentialis* are genetically distinct species.

5.2. Palghat gap and clinal variation in sub-populations

Naturalists, biogeographers, and evolutionary biologists have long been intrigued by natural interruptions or gaps in geography, such as gaps in a hill or mountain range.Such gaps, like Palghat gap in the Western Ghats, shape the distribution of organisms, with various species or populations on either side of the gap. (Vidya *et al.*, 2005; Ram *et al.*, 2015; Robin *et al.*, 2017; Joshi *et al.*, 2018; Chattopadhyay *et al.*, 2021). Asian elephants (*Elephas maximus*) are among animals that most impressively display genetic difference across the Palghat gap. The Nilgiri, Annamalai, and Periyar elephant populations in Southern India were all sampled by Vidya *et al.* (2005), who discovered that subpopulations differ across the Palghat gap. Ram *et al.* (2015) examined the genetic diversity and population structure of the Lion-tailed Macaque (*Macaca silenus*) using mtDNA sequencing data and discovered two reciprocally monophyletic groupings that correspond to the populations north and south of the Palghat gap with strong branch support. Robin *et al*. (2017) evaluated the phylogeographic patterns of the complete community of highland birds (species occurring above 1400 m above sea level) in the Western Ghats and discovered that the Palghat gap has an impact on the phylogenic structures of 10 of the 23 species. Chattopadhyay et al. (2021) found clinal variation (north to south gradient) in phenotype within *Rhinolophus lepidus* populations across the Palghat gap in the Western Ghats. For the fruit bat *Cynopterus sphinx,* clinal phenotypic variation has also been documented throughout peninsular India. (Storz *et al.*, 2001). Wordley et al. (2014) recorded the echolocation calls of H. pomona from the Valparai Plateau in Tamil Nadu, where the average frequency at maximum energy (FMaxE) is 126.337 ± 1.25 kHz. Raman and Hughes (2021) recorded echolocation calls of pomona from various parts of SWG, Kerala and reported a FMAXE of 123.2 ± 3.49 kHz. The present study compared the FMAXE of H. pomona subpopulations in the north and south of the Palghat gap and found significant variation in calls with 119.63 kHz mean in the northern sub-population and 123.26 kHz mean in the southern sub-population, indicating they are phenotypically distinct.

5.3. Cryptic diversity among Hipposiderid bats

Although bats follow these general mammalian trends of high endemism in Western Ghats, they are reported to have a great deal of subspecific endemism frequently as part of vast, polytypic super-species composites that may have significant amounts of cryptic species-level variety (Bates and Harrison, 1997; Chattopadhyay *et al.*, 2012, 2016, 2021; Srinivasulu and Srinivasulu, 2018a). Chattopadhyay et al. (2021) observed evidence of clinal variation (north-south gradient) in phenotype within *Rhinolophus lepidus* populations across the Palghat gap with significant variation in acoustics (Peak Frequency) and morphology. In the present study, though the acoustics (FMAXE), Forearm length (FA), length of first phalanx of third metacarpal (1PH3MT), and length of fourth metacarpal (4MT) show significant differences among north and south subpopulations of taxon pomona, the PCA using significant morphometric parameters shows that they are highly cryptic and hard to distinguish based on morphology alone.

SUMMARY

6. Summary

Peninsular India is an important region for mammalian diversification and harbours major biogeographic barriers. The Palghat gap is such a barrier which is accounting for diversification in the southern Western Ghats. It is a known biogeographic barrier for many species, including mammals, birds, amphibians, reptiles, etc. However, little is known about the role of this region in the diversification of bats though it harbours high chiropteran diversity. In this study, I used genetic, acoustic, and morphometric parameters to assess the diversification of *Hipposideros pomona* bats in the Kerala part of the southern Western Ghats. The salient findings are summarised below.

1. Phylogenetic analysis of *H. pomona*, which was recently split from H. gentilis solely based on phenology, was done by comparing mitochondrial cytochrome oxidase 1 genome, which showed a clear difference between the two species
2. Acoustic and Morphometric analysis of the *H. pomona* subpopulations in the north and south of the Palghat gap revealed that they are phenologically distinct.
3. The Principal Component Analysis done on the morphometric parameters revealed that the subpopulations of *H. pomona* across the Palghat gap are morphologically cryptic
4. Further genetic studies have to be done to confirm the cryptic diversity occurring within these subpopulations across the Palghat gap biogeographic barrier

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Phylogenetic analysis of the endemic and threatened bat *Hipposideros pomona* K. Andersen, 1918 (Chiroptera: Hipposideridae) in the Western Ghats

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ABSTRACT

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8. ABSTRACT

Hipposideros pomona K. Andersen, 1918 is a small to medium sized insectivorous bat species endemic to the southern Western Ghats (SWG). According to IUCN, the species is endangered due to its restricted distributional. It is found mainly in thick forests of mid to high elevations of the SWG. Biogeographic barriers can act as a barrier to species dispersal, resulting in reproductive isolation and thus aiding speciation. The effect of the barriers depends on the motility of the animal. Actively moving and robust animals, such as higher vertebrates, may disperse across more minor barriers, while less mobile animals could be restricted due to the same. The 30 km wide Palghat gap in the SWG acts as a barrier to the species movement for many forest depended species, consequently acting as a barrier for gene flow, which can result in speciation as well as increase the chances of gene pool isolation. This barrier could affect the movement between subpopulations of *H. pomona* in the south and north of the Palghat gap.

The current study was conducted in Kerala part of the SWG, on the locations where *H. pomona* was previously recorded. Bat sampling was carried out using harp traps of dimension 1.5m width, 2.2m height, 7.5cm between four frames and 2.5 cm between vertical monofilament fishing lines. Sixteen standard morphological measurements were taken on the bats captured in the harp trap (to the nearest 0.1mm) using a digital vernier calliper. Wing punches of 3 mm diameter were taken with a sterile biopsy punch, preserved in molecular grade ethanol, and stored in a deep freezer. DNA extraction was carried out using the standard protocol. Polymerase chain reaction performed to amplify the mitochondrial cytochrome oxidase 1 (COI), mitochondrial cytochrome b (Cyt b). Bat acoustic recordings were made using an ultrasound detector and analysed using the software BatSound Touch. Phylogenetic analysis was performed using software Molecular Evolutionary Genetics Analysis Version 11 (MEGA 11) and Population Analysis with Reticulate Trees (POPART). Morphometric analyses, acoustic analysis and principal component analysis (PCA) of morphometric parameters were done in R software.

The current known distribution of *H. pomona* is restricted to the SWG, and the species was elevated to endangered status in IUCN red list due to the current restricted range. However, no genetic studies have been conducted to confirm the split. Present

study results confirm that *H. pomona* and *H. gentilis* are genetically distinct species. Within the SWG, the Palghat gap is a potential biogeographic barrier for *H. pomona*. The present study compared the forearm length (FA), length of first phalanx of third metacarpal (1PH3MT), and length of fourth metacarpal (4MT) – (Morphometry), and frequency of maximum energy FmaxE (Acoustics) of *H. pomona* subpopulations in the north and south of the Palghat gap and found significant variation, indicating that they are phenotypically distinct. However, the PCA result indicated that these subpopulations are cryptic.

Overall, phylogenetic analysis of *H. pomona*, which was recently split from *H. gentilis* solely based on morphology, was done by comparing mitochondrial cytochrome oxidase 1 genome, which showed a clear difference between the two species. Acoustic and morphometric analysis of the *H. pomona* subpopulations in the north and south of the Palghat gap revealed that they are phenotypically distinct. The PCA done on the morphometric parameters revealed that the subpopulations of *H. pomona* across the Palghat gap are morphologically cryptic.