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COMMUNICATION

Presence of medium and large sized terrestrial mammals highlights the conservation potential of Patharia Hill Reserve in Bangladesh

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Abstract: Establishing and managing protected areas is a common approach for in situ conservation of wildlife and their habitats, but its effectiveness relies on the magnitude of anthropogenic pressures and their successful mitigation. Bangladesh is a densely populated country, and demand for land and natural resources is accelerated in and around the remaining forests. It is particularly of concern for an important transboundary hill forest, called the Patharia Hill Reserve in the northeastern Bangladesh, which appears to be the last resort for many important forest mammals. We conducted a camera-trap survey for assessing the occurrence of mammals in this tropical forest patch during January 2019 and July 2021. An effort of 2,805 trap-nights yielded 1,986 records of 22 medium and large-sized mammal species. We confirmed the presence of globally 'Vulnerable' Fishing Cat, Oriental Small-clawed Otter, Hog Badger, & Northern Pig-tailed Macaque and the globally 'Endangered' Phayre's Langur in this study. Our records include a number of species which are either the first record for the northeastern region or have been detected after several decades. In contrast, we could not detect several large carnivores which were recorded previously in the study site and in the Indian side of Patharia Hill Reserve. This study highlights the conservation value of this poorly known reserve forest, and presents a call for immediate action to maintain its function as a transboundary forest area.

Keywords: Activity pattern, Albino Hog Badger, mammals, semi evergreen forest, small carnivores, transboundary forest.

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Author contributions: MAR, AS, MSU, and MM conducted field survey with the help from MRKC, AH, and MAA. MAR and AS performed the analyses. MAA, AS and MAR prepared the draft of the manuscript. All authors contributed to editing the manuscript.

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INTRODUCTION

Protected areas have been mainstays of in situ conservation of wildlife and their habitats, and they have been generally effective in preventing large-scale land clearing (Bruner et al. 2001; Geldmann et al. 2019). However, deforestation is still observed in protected areas, combined with land conversion in buffer zones (Curran et al. 2004), which consequently, creates edge effects for mammal communities living therein (Kinnaird et al. 2003). Decline of mammalian species in protected areas due to hunting and other anthropogenic pressures has also been reported (Craigie et al. 2010; Harrison 2011). Such phenomena are particularly of concern where anthropogenic pressures from burgeoning human populations continue to increase around protected areas and remaining forests patches in tropical countries.

Bangladesh is considered a transitional zone for flora and fauna in southeastern Asia, being located in the Indo-Burma biodiversity hotspot. For the protection of its unique biodiversity, the Bangladesh government has established a network of protected areas comprising 20 national parks and 24 wildlife sanctuaries over decades (Bangladesh Forest Department 2022). However, Bangladesh is a densely populated country, and its population is still increasing (The World Bank 2022), thus demand for natural resources remains exceedingly high. In 1930, the country harboured four main forest areas, but currently only three remain covering only 12.8% of the entire land area: the Sundarbans in the southwest (mangrove), Chittagong and Chittagong Hill Tracts in the southeast, and greater Sylhet hilly forest areas in the north-east of Bangladesh (Reddy et al. 2016; Henry et al. 2021). Although the forest areas in Bangladesh continue to decline, yet the country is home to a total of 127 mammalian species, of which 60 species are known to occur exclusively in forests. Sadly, 11 species of mammals have been extirpated from the country over the last centuries, and around 40% of mammals are now threatened, and among them 19 are Critically Endangered (IUCN Bangladesh 2015).

The high deforestation rates in Chittagong Hill Tracts in the south-east, one of two main hilly forest areas of Bangladesh, has been reported in recent times (Reddy et al. 2016; Mamnun & Hossen 2021), suggesting that the remaining forest areas in the south-east and north-east becomes critically valuable for conservation of mammalian communities in the country. For instance, the northeast and southeast areas have been recognized as the last habitats for Asian Elephants *Elephas maximus* and other large mammals in Bangladesh (Islam et al. 2013; Ministry of Environment and Forests 2018). Despite of its importance of remaining forest areas in the northeast of the country, the status of mammalian communities in these areas is poorly known and scientific knowledge on them is scanty.

In the north-east, the past survey efforts for assessing mammalian species were limited to some protected areas, including the Lawachara National Park, Khadimnagar National Park and Rema-Kalenga Wildlife Sanctuary (Aziz 2011; Feeroz et al. 2011). It is reflected in the updated Red List of Bangladesh where one-third of the total national mammalian species have been categorised as ‘Data Deficient’ (IUCN Bangladesh, 2015). Besides the established protected areas in the north-east, one of the important and potential areas is Patheria Hill Reserve (PHR) forests are known to support diversity of wild animals. It is a transboundary hilly area bordered with India, and the importance of the area has been acknowledged in several studies, particularly for Asian Elephants, being carried out in the Indian sides (Talukdar et al. 2020a,b). These studies have identified PHR as one of the last habitats for many threatened animals including the Asian Elephant, Western Hoolock Gibbon *Hoolock hoolock*, Chinese Pangolin *Manis pentadactyla* and a range of other non-human primates (Talukdar & Choudhury 2017). Comparing with Indian side, the status of terrestrial mammals is poorly known Bangladesh, even though it is expected that the Bangladesh side of the PHR may harbour similar assemblage of mammalian fauna. As of today, only two studies focusing on medium- and large-sized terrestrial mammals were conducted in PHR: camera trap survey for two months with 300 camera-trap-nights (Rahman et al. 2021) and leech monitoring (Weiskopf et al. 2018).

Our knowledge of medium and large sized terrestrial mammals in the Bangladesh side of the PHR is largely lacking. To formulate conservation strategies to reverse the continued population decline of threatened mammals, field-based information on the occurrence of mammalian species is vital (Aziz et al. 2020). We therefore commissioned a survey using motion-trigger camera-traps to understand the current status of mammalian communities in the PHR of Bangladesh.

MATERIALS AND METHODS

Study area

The PHR is located within the Indo-Burma Biodiversity Hotspot and shared by Bangladesh and India across its border. Administratively, the PHR encompasses hilly

Figure 1. Map showing study sites and camera-trap locations in Patharia Hill Reserve of Bangladesh.

terrains with degraded forest landscapes across the Karimganj District of Assam in India and Moulvibazar District in Bangladesh. In Bangladesh, the Patharia forest was declared as a Reserve Forest in 1920 under the Forest Act, 1927, and managed by the Bangladesh Forest Department under four Forest Beats: Barolekha (1,303 ha), Madhabchhara (1,174 ha) and Samanbag (730 ha) under Barolekha Forest Range and Lathitila (5,510 ha) under Juri Forest Range (Figure 1) (Bangladesh Forest Department, 2021). Additionally, Madhabkunda Ecopark was declared covering an area of 265 ha demarcated from the Madhabchhara Forest Beat in 2001. The PHR comprises semi-evergreen vegetation that is largely degraded and remains as fragmented forest patches. The effort of restoration is on-going by the Forest Department and local villagers under the benefit sharing agreement. The area also includes community-shared orchards, tea gardens, streams and valleys. The remaining patches of the reserve forests include dominant plant species such as *Tectona grandis*, *Albizia procera*, *Anthocephalus chinensis*, *Artocarpus chaplasha*, *Dillenia pentagyna*, *Gmelina arborea*, *Terminalia bellirica*, *Tetrameles nudiflora*, *Vitex peduncularis, Aporosa dioica*, *Dipterocarpus turbinatus, Garcinia pedunculata, llex godajam, Lophopetalum fimbriatum*, *Mangifera sylvatica*, and *Quercus gomeziana*.

The PHR is characterised by tropical monsoon with a hot and wet summer and a cool and usually dry winter. It receives high rainfall, with an average annual rainfall of 2,372 mm. The forest area enjoys two distinct periods: dry period from November to February (average rainfall of >35 mm/month) and tropical humid monsoon period with regular precipitation from May to September (average rainfall of <370 mm/month). Temperature varies from 20°C to 33°C, but in winter it sometimes decreases to 11°C. Average annual temperature is 24.8°C. Lowest temperature were recorded from October to February. Humidity varied from 63% to 89% with an average of 80.6% (Talukdar & Choudhury 2017).

Although the PHR constitutes an important forest landscape of the remaining forest patches in northeastern Bangladesh, currently the PHR is highly disturbed due to agricultural activities, cattle grazing and extraction of timber and non-timber forest products by communities living inside and around the area. The communities living this area traditionally grow betel leaves, betel nuts, pineapple, lemon, and mangoes within the forest lands for their livelihoods. In the Lathitila, southern part of the PHR, at least 300 households live in the southwestern parts. Alongside orchard gardening, forest villagers grow stream-fed rice and other cash crops in valleys and on hill slopes. As a result, most of the forest fringes and south-west regions are either modified or degraded, and devoid of any natural vegetation.

Camera-trap surveys

We conducted camera-trapping in two phases, from 12 January of 2019 to 6 April 2020, and from 23 February to 8 July 2021. In the first phase, a total of 10 infrared camera-traps (Bushnell Core DS 30 MP No Glow) were deployed at Barolekha and Madhabchara forest beats, and camera-traps were relocated to increase survey coverage and species detectability. In the second phase, we increased our camera-traps to 24 (additional 14 Browning Dark Ops Pro XD) for extending our survey coverage over the Lathitila and Samanbag forest beats, approximately 87 km^2 in total. In the second phase, camera-traps were deployed for 1,407 trap-nights for Madhabchara and 523 trap-nights for Samanbag and Lathitila forest beats. Unfortunately, a data storage card was stolen from one camera in the first phase while two camera-traps during the second phase. Two camera-traps were removed after two months of deployment from the field due to malfunction in the second phase.

Camera-trap locations were selected based on in-situ assessment of active animal trails including signs of feeding, resting and scats. All available habitat types including natural patches of forest vegetation, degraded forest and valleys, streams, and bamboo groves were considered for camera-trap placement. Inter-distance between camera-traps ranged from 300 m to 500 m, with an average distance of 350 m.

We mounted each camera-trap approximately 2–5m above the ground on a tree, targeting medium- and small-sized mammals within camera exposure range. Undergrowth vegetation and twigs were trimmed from camera exposure range to allow uninterrupted capture of good quality images of the moving animal. No camera-trap stations were lured. Each camera-trap was set to operate for 24 hours with motion sensor mode for capturing three consecutive still images and a video afterwards for 10 seconds. Date and time were set to stamp on each recorded image for ease of keeping data in order.

Species identification

Small mammals (the body weight <1kg), mostly rodents, were excluded from the analysis except for porcupines. The occurrence of Large-toothed Ferret Badger *Melogale personata* has been documented in the region, however, the occurrence of Small-toothed Ferret Badger *Melogale moschata* was also reported in the Indian side of this region. Considering the distance from the national border in the area, we could not assume the ferret badger recorded in the survey is *Melogale personata* without tooth measurement. Here, we cautiously assigned all images of Ferret badger to *Melogale* spp.

Data analysis

We defined a camera trap record as a record if it occurred at least 30 minutes after a photograph of the same species at a given station. The total sampling effort is expressed as the total number of camera-trap-nights, one camera-trap-night being defined as a continuous 24 hr period of normal camera operation. Encounter rates were calculated as the number of records per 100 camera-trap-nights.

Activity patterns of species were examined using the time stamped on camera-trap images. The time stamp of camera traps provided the time and day when the photograph of the species was taken. Activity pattern were analyzed following Ridout & Linkie (2009). All analysis was conducted using the package “Overlap” in version 0.2.6 in R (Meredith & Ridout 2016).

RESULTS

Recorded species

Our survey efforts comprising 2,805 camera-trap-nights over two seasons produced 1,986 records of confidently identified species (Table 1). Twenty-two species representing four orders were photographed including one ‘Endangered’ species, namely, Phayre’s Leaf Monkey. The three most commonly detected species across two surveys were the Crab-eating Mongoose, Common Palm Civet, and Wild Boar. Golden Jackal, Fishing Cat, and Small Indian Mongoose were detected only in the 2019/2020 survey while Asian Golden Cat, Rhesus Macaque, and Phayre’s Leaf Monkey were recorded only in the 2020/2021 survey. The Asian Golden cat and Fishing Cat were detected only once during the study period. Overall, the order Carnivora accounted for 68% of all mammalian species, representing four species from each of the family Felidae, Mustelidae, & Viverridae, two species from Herpestidae, and one species from Canidae. Primates were represented by three species of macaques and one species of langur. Comparing to other two surveys in the same area, we have 14 species as new records, in particular for small carnivores. On the other hand, we have not detected Asian elephant and Capped langur in our camera trap survey although we found elephant footprints in the area. Comparing from interview surveys in the India side, the most striking results is that we have not detected large carnivores such as Leopards, Clouded Leopards, Dhole, and Black Bear throughout the two phases (Table 2).

Activity patterns

Activity patterns of species with >50 records are shown in Figure 2. Ferret Badger exhibited strong nocturnal patterns while Leopard Cat, Oriental Small-clawed Otter, Large Indian Civet, and Wild Boar showed its activity peaks both in the night time and around dawn and dusk. Crab-eating Mongoose exhibited diurnal patterns. Muntjac showed the peak of activity in the morning and increased again around dusk.

DISCUSSION

We confirmed the presence of 22 medium/ large-sized mammalian species, accounting for approximately 30% of forest-dwelling mammalian species in Bangladesh (IUCN Bangladesh 2015). Importantly, the list contains the globally Endangered Phayre’s Langur, and four globally ‘Vulnerable’ mammalian species comprising the Fishing cat, Oriental Small-clawed Otter, Hog Badger, and Northern Pig-tailed Macaque. Of note, 11 species of mammals detected in this study have been listed in the threatened category of the International Union for Conservation of Nature (IUCN Bangladesh 2015). Occurrence of such a high assemblage of threatened species within a relatively small patch of transboundary forest highlights the conservation value of this poorly known reserve forest in northeast Bangladesh.

Carnivores

Our data confirms the occurrence of 15 carnivore species in PHR. We could not detect any large carnivores during our survey as well as the previous camera trap study conducted in the site (Rahman et al. 2021), although there are records of bear and leopard from the region in the past (Pocock 1939; Sarker & Sarker 1984; IUCN Bangladesh 2015; Talukdar & Choudhury 2017). Even our camera trap setting had the bias towards the distance from water sources for fishing cat survey, we still have not detected any sign and footprint of large cats and bears during our survey in two successive dry seasons and one wet season. In addition, three camera trap stations were set in the area where local sightings of leopard were occurred five years ago of the survey. Non-detection of large carnivore in this survey is unlikely to be explained by only biases in camera trap stations.

Compared with large carnivores, a number of small carnivores detected in this study are particularly notable because of their rarity and on site record. One of such species is the Ferret Badger. Although they could belong to either Large-toothed or Small-toothed Ferret Badgers, this is the first record of the Ferret Badger in the wild in Bangladesh. A reasonable encounter rate of this species allows us to explain the activity pattern of Ferret Badger in the study site (Figure 2). It was exclusively nocturnal, which is consistent with the pattern in other area such

Table 1. Records of medium- and large-sized terrestrial mammals in Patharia Hill Reserve during the 2019–2020 and the 2020–2021 surveys.

Order	Family	Scientific name	English name	The 2019–2020 survey			The 2020–2021 survey			Species status	
				No. of independent records	Encounter rate	No. of camera trap stations detected	No. of independent records	Encounter rate	No. of camera trap stations detected	IUCN Red List	National status
Carnivora	Canidae	*Canis aureus*	Golden Jackal	3	0.43	1	0	0	0	LC	LC
	Felidae	*Prionailurus viverrinus*	Fishing Cat	1	0.15	1	0	0	0	VU	EN
		Felis chaus	Jungle Cat	1	0.15	1	2	0.10	2	LC	NT
		Catopuma temminckii	Asian Golden Cat	0	0	0	1	0.05	1	NT	VU
		Prionailurus bengalensis	Leopard Cat	39	5.64	9	39	1.85	17	LC	NT
	Herpestidae	*Herpestes urva*	Crab-eating Mongoose	238	34.39	11	551	26.08	25	LC	NT
		Herpestes auropunctatus	Small Indian Mongoose	5	0.72	1	0	0	0	LC	LC
	Mustelidae	*Melogale* spp.	Ferret Badger	33	4.77	2	18	0.85	7	LC	Not listed
		Arctonyx collaris	Hog Badger	4	0.58	2	11	0.52	7	VU	VU
		Aonyx cinerea	Oriental Small-clawed Otter	37	5.35	8	77	3.64	12	VU	EN
		Martes flavigula	Yellow-throated Marten	3	0.43	2	5	0.24	5	LC	VU
	Viverridae	*Paradoxurus hermaphroditus*	Common Palm Civet	157	22.69	9	192	9.09	15	LC	LC
		Viverra zibetha	Large Indian Civet	51	7.37	9	15	0.71	7	LC	NT
		Paguma larvata	Masked Palm Civet	1	0.15	1	1	0.05	1	LC	VU
		Viverricula indica	Small Indian Civet	34	4.91	3	2	0.10	2	LC	NT
Cetartiodactyla	Cervidae	*Muntiacus vaginalis*	Northern Red Muntjac	59	8.53	7	90	4.26	21	LC	EN
	Suidae	*Sus scrofa*	Wild Boar	43	6.21	9	143	6.77	13	LC	LC
Primates	Cercopithecidae	*Macaca assamensis*	Assamese Macaque	5	0.72	2	38	1.80	15	NT	EN
		Macaca leonina	Northern Pig-tailed Macaque	4	0.58	2	21	1.00	8	VU	EN
		Macaca mulatta	Rhesus Macaque	0	0	0	15	0.71	9	LC	VU
		Trachypithecus phayrei	Phayre's Leaf Monkey	0	0	0	3	0.14	2	EN	CR
Rodentia	Hystricidae	*Hystrix brachyura*	Malayan Porcupine	7	1.01	3	37	1.75	9	LC	LC

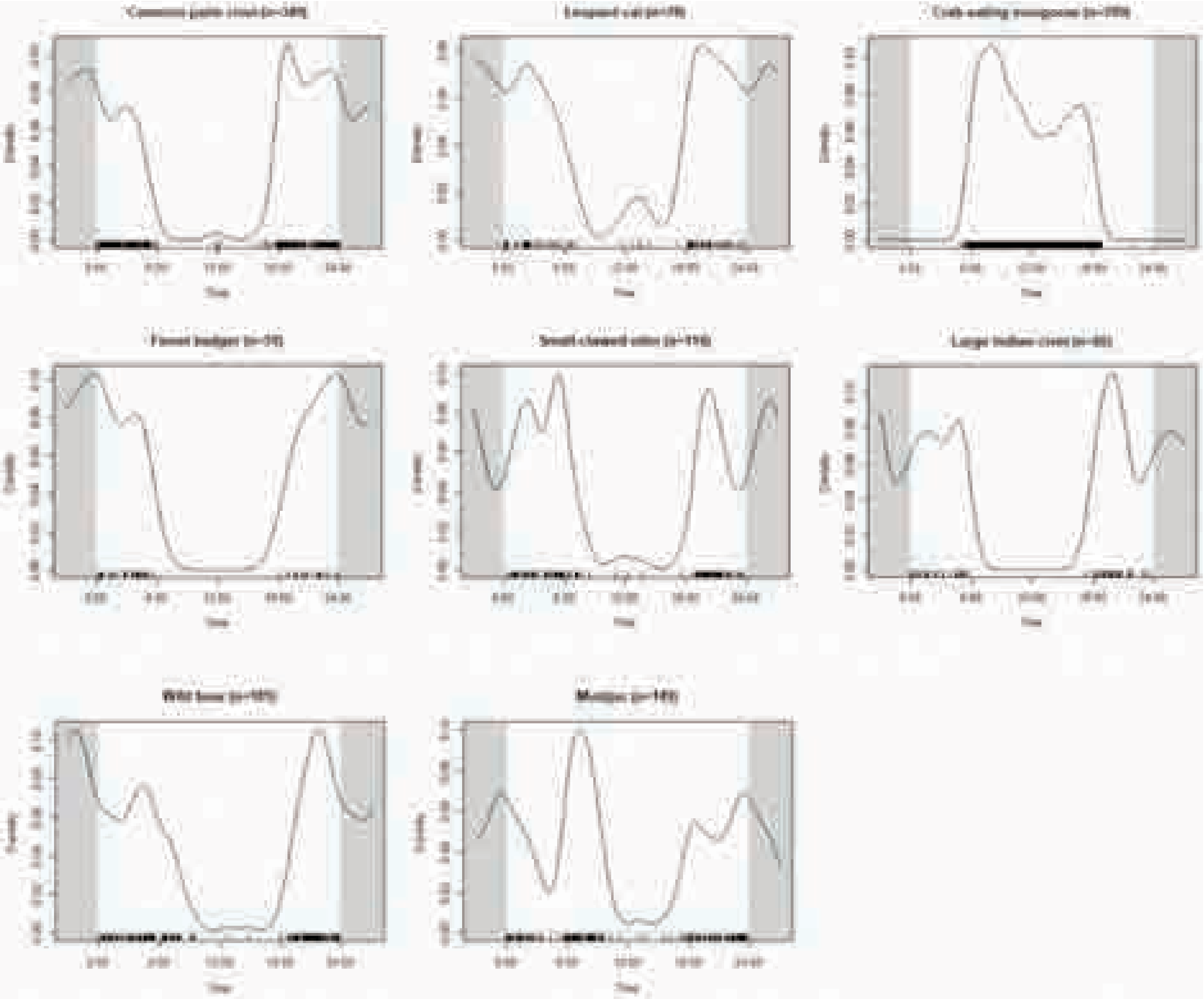

Figure 2. Activity patterns of eight mammal species detected >50 times during the 2019–2020 and 2020–2021 surveys in Patharia Hill Reserve, Bangladesh.

as Taiwan (Chen et al. 2009), China (Wang & Fuller 2003), and Cambodia (Suzuki et al. 2017). Interestingly, no Ferret Badger species was listed and evaluated in the updated Red List of Bangladesh (IUCN Bangladesh 2015), although there was a single record of Large-toothed Ferret Badger in 2008 from a small private zoo in the northeast Bangladesh (Islam & Belant 2008). Given the records of both badger species in areas of northeast India (Long & Killingley 1983; Choudhury 2013; Menon 2014), close to our study sites, both of the Ferret Badger species might occur in the PHR of Bangladesh.

In addition to Ferret Badger, three species of Mustelidae were detected. During our survey, the Oriental Small-clawed Otters was frequently photographed in streambed for both years. Our spatial configuration of the camera traps could increase the detectability of the otters along with streams as well as season, but the presence of the species warrants further investigation of the potential conservation significance of this area for this species. A recent study reports its sign from the Lathitila forest, adjacent to our study site (Akash et al. 2022a) and such hilly streams provide important habitat in India (Perinchery et al. 2011). It would be worth to explore the conservation importance of the PHR addition to the Sundarbans mangrove forest (Aziz 2018), considering the illegal demand for the species (Gomez et al. 2017; Uddin et al. 2022). Blandford (1888) reported the presence of Hog Badger in Sylhet, but the subsequent records of this species in Bangladesh were reported only from several locations in southeast Bangladesh (Feeroz et al. 2012), and uncertain records in Chittagong Hill Tracts (Khan 1984). Recently, it was reported from Sylhet region (Akash et al. 2022b). Interestingly, we found an albino individual captured

Table 2. Comparison of the records of small- and medium-sized mammals in Patharia Hill Reserve across four different surveys.

Order	Family	Scientific name	English name	Bangladesh side			Indian side
				This survey (Camera trap)	Rahman et al. (2021) (Camera trap)	Weiskopf et al. (2017) (Leech monitoring)	Talukdar & Chaudhury (2017) *2 (Interview and literature)
Carnivora	Canidae	*Canis aureus*	Golden Jackal	✔			✔
		Cuon alpinus	Dhole				✔
	Felidae	*Prionailurus viverrinus*	Fishing Cat	✔			✔
		Felis chaus	Jungle Cat	✔			✔
		Catopuma temminckii	Golden Cat	✔			✔
		Prionailurus bengalensis	Leopard Cat	✔	✔		✔
		Neofelis nebulosa	Clouded Leopard				✔
		Panthera pardus	Leopard				✔
	Herpestidae	*Herpestes urva*	Crab-eating Mongoose	✔			✔
		Herpestes auropunctatus	Small Indian Mongoose	✔			
	Mustelidae	*Melogale* sp.	Ferret Badger	✔	✔		✔
		Arctonyx collaris	Hog Badger	✔			✔
		Aonyx cinerea	Oriental Small-clawed Otter	✔			✔
		Lutrogale perspicillata	Smooth-coated Otter				✔
		Martes flavigula	Yellow-throated Marten	✔			
	Viverridae	*Paradoxurus hermaphroditus*	Common Palm Civet	✔	✔		✔
		Viverra zibetha	Large Indian Civet	✔			✔
		Paguma larvata	Masked Palm Civet	✔	✔		✔
		Viverricula indica	Small Indian civet	✔			✔
		Arctictis binturong	Binturong				✔
	Ursidae	*Ursus thibetanus*	Asiatic Black Bear				✔
Cetartiodactyla	Cervidae	*Muntiacus vaginalis*	Northern Red Muntjac	✔	✔	✔*1	✔
	Suidae	*Sus scrofa*	Wild Boar	✔	✔	✔	✔
Proboscidea	Elephantidae	*Elephas maximus*	Asian Elephant		✔		✔
Primates	Cercopithecidae	*Macaca* sp.	Macaque			✔	
		Macaca assamensis	Assamese Macaque	✔			✔
		Macaca leonina	Northern Pig-tailed Macaque	✔	✔		✔
		Macaca mulatta	Rhesus Macaque	✔			✔
		Macaca arctoides	Stump-tailed Macaque				✔
		Trachypithecus phayrei	Phayre's Leaf Monkey	✔			✔
		Trachypithecus pileatus	Capped Langur		✔		✔
	Lorisidae	*Nycticebus bengalensis*	Bengal Slow Loris				✔
	Hylobatidae	*Hoolock hoolock*	Western Hoolock Gibbon				✔
Rodentia	Hystricidae	*Hystrix brachyura*	Malayan Porcupine	✔	✔		✔
		Atherurus macrourus	Asiatic Bush-tailed Porcupine				✔
Lagomorpha	Leporidae	*Lepus nigricollis*	Indian Hare				✔
Pholidota	Manidae	*Manis pentadactyla*	Chinese Pangolin				✔

*1—Originally, the records of *Muntiacus muntjac* was reported, but it changed to *Muntiacus vaginalis* following the current phylogeography study on red muntjacs (Martins et al. 2017). *2—Following species were excluded from the list due to sceptical records based on known distribution: *Herpetes javanicus, Lutra lutra, Muntiacus muntjak,* and *Capricornis rubidus.*

in camera-traps for the first time in Bangladesh (Image 1J). The Yellow-throated Marten was detected during both seasons with only eight independent records. A previous camera trap study detected this species from another forest in the northeast region (Aziz 2011) but it remained undetected in the PHR (Rahman et al. 2021).

Four species of cats (Felidae) were detected during this survey. The globally and nationally threatened Fishing Cat was detected only once during the 2019–2020 dry season survey near the Indian border. Although camera traps were initially set for detecting Fishing Cats in the PHR forests, only a single record from two season surveys indicates that the cat could be relatively in low density in the PHR. The Asian Golden Cat was also photographed only one time. This may not accurately reflect their status due to our sampling bias towards water sources, but still plausible that the population of this secretive cat could be inherently low, considering the fact that the previous camera traps in this area did not detect this species (Rahman et al. 2021), even this species is likely to be found in various forests (Mittermeier & Wilson 2009; Zaw et al. 2014; Dhendup 2016). The Jungle Cat was recorded in a single camera-trap station placed in an area having degraded forests dominated by agricultural land. The Leopard Cat was relatively common in this area, and a Leopard Cat with her kitten was the photographed in the first week of May (Image 1E). Whilst we detected four cat species, only leopard cat was detected in the past by Rahman et al. (2021) from PHR, highlighting the importance of investing an increased number of trap-nights for recording elusive cats. Of four felid species, only Leopard Cat has reasonable number of records for the activity pattern, demonstrating that the cat is nocturnal and crepscular tendency, consistence with Grassman (2000), Lynam et al. (2013), Mukherjee et al. (2019).

Two species of Herpestidae were recorded in this survey. The Crab-eating Mongoose was the most frequently photographed species during this survey, and our biases in setting camera trap stations close to water resources streams is likely to have resulted in high encounter rate. This species has been found in this region, but currently no further ecological information is available beyond the presence data (Feeroz 2015a; Hasan et al. 2018). With a number of hilly streams, this area could be a source of the knowledge of this species such as investigating habitat requirements and population. The activity pattern of this species exhibits strongly diurnal, consistent with previous studies (Chen et al. 2009). Another mongoose species, the Small Indian Mongoose, was also photographed in daytime, but their distribution was restricted to the forest edges. The number of this species recorded was not enough to look at activity pattern.

Other medium- and large-sized mammals

Four species of primates were detected, and it is worth mentioning the records of two species. Firstly, our study confirmed the presence of Assamese Macaques from the PHR for the first time, which has been detected during both surveys, with a higher encounter rate in the 2020–2021 dry season. In the past, the Assamese Macaque was recorded only from two locations of the south-east and north-east hill forests. It was reported in 1995 from the Gazipur Tea Estate of Rajkandhi in the north-east, about 20 km south-west from our study site (Feeroz 2015b). No sighting reports of its occurrence appeared from that site afterwards. Interestingly, none of the protected areas in the northeast region are known to hold any population of this least known non-human primate (IUCN Bangladesh 2015). Secondly, we have three records of Phayre's Leaf Monkey which is Critically Endangered in Bangladesh. This species is confined to the south-east and north-east hill forests, and the deforestation of this area push the species over the brink to extinction (Kabir 2015). These records highlight the conservation significance of this forest for the primate in Bangladesh as one of the last remaining forests. It was also supported by our opportunistic records of a few groups of globally Endangered Western Hoolock Gibbon in the PHR.

Two ungulate species were detected during our survey, Northern Red Muntjac and Wild Boar. These two species were also recorded in two previous surveys (Weiskopf et al. 2018; Rahman et al. 2021). In Bangladesh, the Muntjac is categorized as Endangered due to limited distribution (Dey 2015), although globally it is identified as a Least Concern species (Timmins et al. 2016). In the PHR, the species was commonly detected in different environments from degraded areas to streambeds. The reasonable records produced the activity pattern of muntjac with the peaks in the morning, the evening and the night, which is consistence with previous studies (Gray & Phan 2011; Rasphone et al. 2020). On the other hand, Wild Pig showed crepuscular activity patterns that is inconsistent with previous studies where it was found diurnal (Rasphone et al. 2020). Wild Pig may reduce their diurnal activity when human disturbance is high (Keuling et al. 2008; Cremonesi et al. 2021; Aditya & Ganesh 2022), although the impact of human disturbance on activity pattern of Wild Pig is still not conclusive (Brivio et al. 2017). In the PHR, high human disturbance has

Image 1. Mammal species recorded during the 2019–2020 and 2020–2021 dry seasons in Patharia Hill Reserve, Bangladesh.
a—Golden Jackal *Canis aureus* | b—Fishing Cat *Prionailurus viverrinus* | c—Jungle Cat *Felis chaus* | d—Asian Golden Cat *Catopuma temminckii* | e—Leopard Cat *Prionailurus bengalensis* | f—Crab-eating Mongoose *Herpestes urva* | g—Indian Grey Mongoose *Herpestes edwardsii* | h—Ferret Badger *Melogale* sp. | i—Hog Badger *Arctonyx collaris* | j—Albino Hog Badger | k—Oriental Small-clawed Otter *Aonyx cinerea* | l—Yellow-throated Marten *Martes flavigula*.

Image 1. Mammal species recorded during the 2019–2020 and 2020–2021 dry seasons in Patharia Hill Reserve, Bangladesh.
m—Common Palm Civet *Paradoxurus hermaphroditus* | n—Large Indian Civet *Viverra zibetha* | o—Masked Palm Civet *Paguma larvata* | p—Small Indian Civet *Viverricula indica* | q—Northern Red Muntjac *Muntiacus vaginalis* | r—Wild Boar *Sus scrofa* | s—Assamese Macaque *Macaca assamensis* | t—Pig-tailed Macaque *Macaca leonina* | u—Rhesus Macaque *Macaca mulatta* | v—Phayre's Leaf Monkey *Trachypithecus phayrei* | w—Malayan Porcupine *Hystrix brachyura*.

been observed, but the magnitude of the hunting on these species remains unknown. Since the hunting pressure on ungulate species has been reported in other forest areas of Bangladesh and identified as the concern on the capacity for large carnivore conservation (Aziz et al. 2017), further work is required to investigate the hunting pressure and develop conservation strategy accordingly.

As an additional note, although not detected in our camera-traps, we observed a herd of five female individuals of Asian Elephants roaming in our study sites, which frequently cross the borders between Bangladesh and India (Talukdar et al. 2020a,b), highlighting the conservation significance of this transboundary forest areas.

Conservation implications

Our study revealed that the PHR is one of the richest mammalian hotspots in northeastern Bangladesh, with particular reference to the diversity of medium- and large-sized terrestrial mammals. The number of forest-based terrestrial carnivores detected is certainly higher than many protected areas in the northeastern region of the country (Aziz 2011; Rahman et al. 2021). The presence of Fishing Cat, Hog Badger, Ferret Badger, Oriental Small-clawed Otter and Assamese Macaque, among the others, is of particular importance considering their endangerment and rarity in Bangladesh.

However, the non-detection of large carnivore may suggest a worrying situation that the forest would result in unsuitable to accommodate large carnivores if no practical conservation measures are taken. The PHR is a highly disturbed habitat because of agricultural activities (e.g., betel leaf cultivation, orchards, paddy fields) in buffer zones. Elsewhere, reduction or local extinction of large carnivores has been observed within protected areas along with edge effects and habitat degradation originating from the land use change within and buffer zone of the protected area (e.g. Datta et al. 2008; Watson et al. 2015). Besides the demand for the agriculture land, illegal extraction of forest resources, cattle grazing and wildlife poaching are common in the PHR. Despite that the zoning strategy has been taken for the management of the PHR, anthropogenic pressure has been extended from social forestry zones. Intensive monitoring for wildlife poaching and illegal logging in the remaining natural patches within the PHR is needed. The restoration of the habitat is also urgently required, particularly in the areas close to the border. The remnant forest patches across the transboundary border of the PHR have been the last resort for mammals and have greater potential for the conservation of wildlife in general and mammalian fauna in particular. We recommend that the PHR be elevated to the status of protected area so that effective measures are ensured for the protection of wildlife and their habitats from further damage. Specifically, northeastern parts of Lathitila and southeastern parts of Madhabchhara forest beats should be demarcated for declaring a wildlife sanctuary for long-term conservation of wildlife and maintaining transboundary wildlife movement between Bangladesh and India. These conservation actions should be urgently considered given the rapid disappearance of forest habitats elsewhere in Bangladesh, and having the potential of large carnivores alongside diversified mammalian communities in the PHR.

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COMMUNICATION

Diversity and abundance of aquatic birds in Koonthankulam village pond, Tamil Nadu, India

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Abstract: The diversity of birds in Koonthankulam pond, located in Koonthankulam village (8.495N, 77.755E), Tirunelveli district, southern Tamil Nadu, was studied. A total of 90 species belonging to 21 orders, 42 families, and 73 genera were recorded. The study recorded seasonal migrants such as Black Ibis, Oriental White Ibis, Bar-headed Goose & Spoonbill and indigenous species including the Pond Heron, Cattle Egret, White-breasted Kingfisher, Red-wattled Lapwing, Rose-ringed Parakeet, Purple-rumped Sunbird, Hoopoe, and Indian Robin. The primary data were analyzed by principal component analysis, cluster, and analysis of variance. Analysis of variance showed that the Menhinick index is statistically significant P <0.05. A structural equation model was applied to analyze the physico-chemical parameters of water samples collected from the sampling site. Analysis of experimental data through the structural equation model indicates temperature and dissolved oxygen may indirectly affect bird diversity.

Keywords: Avian fauna, migrants, principal compound analysis, structural equation modeling.

Abbreviations: PCA—Principal compound analysis | ANOVA—Analysis of variance | SEM—Structural equation model | TDS—Total dissolved solids | DO—dissolved oxygen | CFI—Comparative Fit Index | TLI—Tucker-Lewis Index | RMSEA—Root Mean Square Error of Approximation | IUCN—International Union for Conservation of Nature | GFI—Goodness of Fit.

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Author contributions: SM implemented the field surveys and collected the data; wrote the first draft. DP designed data analysis and done by ES. NAN supervised the research and provided multiple revisions in the early stages of writing. All authors read and approved the final manuscript.

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INTRODUCTION

The process of urbanization has fragmented and degraded different types of habitats. One such habitat is ponds of varied sizes, especially in urban and semi-urban areas. Under such conditions, the existing ponds provide little hope for life and support for the survival of organisms. Wetlands are among the most productive ecosystems in the world and play vital roles in flood control, aquifer recharge, nutrient absorption, and erosion control. In addition, wetlands provide home for a huge diversity of wildlife such as birds, mammals, fish, frogs, insects, and plants (Buckton 2007). Among several organisms surviving in and around water bodies, birds occupy a significant position, as they are one of the critical ecosystem functionaries.

Birds play prominent roles in ecosystems, serving as pollinators (Stiles 1978), predators (Rudebeck 1950), scavengers (Roen 2005), prey (Rudebeck 1950), and regulators of pest populations (Peterson 1980). Their interactions are wide and varied with abiotic and biotic components of different ecosystems, i.e., they are not restricted to one particular system but also to adjacent systems as they enjoy the power of flight. India hosts around 1,353 species of birds (Praveen & Jaypal 2023). Analysis of avian diversity portrays the status of their aquatic habitats and neighboring ecosystems. As there is no detailed report on the diversity of Koonthankulam village pond, the present study was carried out to analyze seasonal variation in bird diversity and their relationship with water quality parameters.

MATERIALS AND METHODS

The study was carried out in Koonthankulam village pond (8.495N, 77.755E), Tirunelveli, southern Tamil Nadu, from January 2017 to November 2018. This pond is surrounded by agricultural fields, where different crops are grown throughout the year. Macro-invertebrates of the agricultural fields and grains scattered around after harvesting along with the pond allure avifauna to this region. Bird watching and recording have been carried out for six seasons (namely, spring, summer, early monsoon, late monsoon, early winter, and late winter) by point count protocol as per Newson et al. (2009). Observations were made using a binocular (Nikon 16x50 AculonA211), and photography was done with Canon 6D Mark II with zoom lenses. The birds recorded were identified by referring to Ali & Ripley (1981).

Physical and chemical parameters such as temperature, pH, total dissolved solids (TDS), conductivity, salinity, and dissolved oxygen (DO), were measured on the spot using a water analyzer (Systronic make 371). Other parameters (Hardness, magnesium, calcium, chloride, alkalinity, and acidity) were determined following the standard procedure from American Public Health Association (APHA) and Trivedy & Goel (1984). The map has been generated using the software QGIS 3.6.

Structural equation modeling (SEM) is a multivariate statistical tool that can be used to describe linear relationships among variables (McCune & Grace 2002; Grace 2006). SEM provides explicit regression estimations for all parameters (Byrne 2001). Structural equation modeling of groundwater physicochemical parameters data was used to characterize the groundwater quality and to identify the controlling factors on bird diversity. IBM SPSS AMOS 22.0 was used to analyze the structured model's fit and estimate the parameters of both observed and latent variables. Chi-square test, the root mean square error of approximation (RMSEA), and the goodness fit index are used as measures of model fit. A measure of minimum sample discrepancy is indicated by the value chi-square divided by the degrees of freedom (CMIN/df) (Belkhiri & Narany 2015). This measure was used to analyze the fit of the model. A value of less than 5 indicates the model's fit is adequate (Arbuckle 2012), less than 3 reflects that the model is acceptable (Kline 1998), whereas a value of 2 or less represents the model was fit as a good model. Goodness-of-Fit statistics (GFI) was calculated as the variance proportion accounted for by the estimated covariance (Tabachnick & Fidell 2007). The RMSEA provides a way to understand optimally chosen parameter estimates that would fit the covariance matrix (Byrne 1998). When the proposed structural model has a (comparative fit index) CFI>0.95 and an RMSEA <0.05, then the structural model is to be considered a good model (Byrne 2010). The diversity indices were calculated using PAST 3.14 software.

RESULT AND DISCUSSION

A total of 90 species of birds belonging to 21 orders and 42 families were recorded. Of this, 87 species of birds are of 'Least Concern', and three species are 'Near Threatened' (Table 1). Of these, 41 were waterbirds, and 49 were terrestrial birds (Table 1). Waterbirds constitute 12 species of waterfowl (swimmers), 25 species of waders, and three species were divers. Out of 49 terrestrial birds, 35 species were passerine birds, five were birds of prey,

Image 1. Map of the study area.

six were upland ground birds, two were night birds, and two were tree-clinging birds. It is evident from the data that the order Passeriformes is represented by the most families (Sturnidae, Motacillidae, Corvidae, Dicruridae, Estrildidae, Muscicapidae, Alaudidae, Motacillidae, Nectariniidae, Passeridae, Pycnonotidae, Muscicapidae, Monarchidae, and Leiothrichidae). In contrast, the highest numbers of species recorded were from Ardeidae, Anatidae, Cuculidae, Columbidae, Rallidae, and Threskiornithidae. The highest abundance index (9.2) was seen for *Anastomus oscitans*. *Bubo bubo* and *Pandion haliaetus* had a low abundance index (0.02). Of the 90 species, 26 species have more than 1 abundance index.

The distribution of various species was analyzed using the principal component analysis (PCA) method (Dauda et al. 2017). The distribution of the occurrence of various species and their variance-covariance matrices were analyzed through scatter diagrams generated from PCA, and the results were further evaluated by 95% ellipses (Figure 1). The results revealed that the species clustering differs with seasons. In spring, *Threskiornis melanocephalus* (THME), *Psittacula krameri* (PSKR), *Passer domesticus* (PADO), and *Corves splendens* (COSP) were found, while *Ardeola grayii* (ARGR), *Turdoides striata* (TUST), *Acridotheres tristis* (ACTR), *E. garzetta* (EGGA) and *T. melanocephalus* (THME) were recorded during summer. Similarly, *A. tristis* (ACTR), *E. garzetta* (EGGA), *C. splendens* (COSP), *Bubulcus ibis* (BUIB), and *Pseudibis papillosa* (PSPA) were recorded in early monsoon, while *A. oscitans* (ANOS), *P. papillosa* (PSPA), *Himantopus himantopus* (HIHI), *A. tristis* (ACTR), and *C. splendens* (COSP) were found during late monsoon. In early winter, *A. oscitans* (ANOS), *Anser indicus* (ANIN), *Mycteria leucocephala* (MYLE), and *C. splendens* (COSP) were recorded, which distinguish themselves from other species in abundance. More species were abundant in late winter than in other seasons. From the results, it could be seen that the pond has been dominated by *C. splendens* (COSP), *E. garzetta* (EGGA), *A. tristis* (ACTR), etc.

The similarity in the species composition and abundance among the six seasons analyzed by Bray-Curtis coefficient (Cluster analysis) clustered the

Table 1. Occurrence, IUCN Red List, and abundance index of avian communities in the Koonthankulam village pond, Tirunelveli, Tamil Nadu, India.

	Scientific Name	Family	Order	IUCN Red List status	Abundance index	Behavior category
1	*Accipiter badius*	Accipitridae	Accipitriformes	LC	0.15	Bird of prey
2	*Acridotheres tristis*	Sturnidae	Passeriformes	LC	5.33	Percher
3	*Alcedo atthis*	Alcedinidae	Coraciiformes	LC	0.24	Percher
4	*Amaurornis phoenicurus*	Rallidae	Gruiformes	LC	0.41	Swimmer
5	*Anas acuta*	Anatidae	Anseriformes	LC	0.31	Swimmer
6	*Anas arcuata*	Anatidae	Anseriformes	LC	0.51	Swimmer
7	*Anas crecca*	Anatidae	Anseriformes	LC	0.44	Swimmer
8	*Anas poecilorhyncha*	Anatidae	Anseriformes	LC	0.79	Swimmer
9	*Anas querquedula*	Anatidae	Anseriformes	LC	0.22	Swimmer
10	*Anastomus oscitans*	Ciconiidae	Ciconiiformes	LC	9.2	Wader
11	*Anhinga melanogaster*	Anhingidae	Suliformes	NT	0.46	Diver
12	*Anser indicus*	Anatidae	Anseriformes	LC	2.7	Swimmer
13	*Anthus rufulus*	Motacillidae	Passeriformes	LC	0.26	Percher
14	*Ardea cinerea*	Ardeidae	Pelecaniformes	LC	0.52	Wader
15	*Ardea purpurea*	Ardeidae	Pelecaniformes	LC	0.15	Wader
16	*Ardeola grayii*	Ardeidae	Pelecaniformes	LC	1.32	Wader
17	*Artamus fuscus*	Artamidae	Passeriformes	LC	2.7	Percher
18	*Athene brama*	Strigidae	Strigiformes	LC	0.25	Night bird
19	*Bubo bubo*	Strigidae	Strigiformes	LC	0.02	Night bird
20	*Bubulcus ibis*	Ardeidae	Pelecaniformes	LC	3.61	Wader
21	*Butorides striatus*	Ardeidae	Pelecaniformes	LC	0.53	Wader
22	*Calidris alpina*	Scolopacidae	Charadriiformes	LC	0.15	Wader
23	*Casmerodius albus*	Ardeidae	Pelecaniformes	LC	0.45	Wader
24	*Centropes sinensis*	Cuculidae	Cuculiformes	LC	0.29	Percher
25	*Charadrius dubius*	Charadriidae	Charadriiformes	LC	0.32	Wader
26	*Clamator jacobinus*	Cuculidae	Cuculiformes	LC	0.29	Percher
27	*Columba livia*	Columbidae	Columbiformes	LC	2.47	Upland ground
28	*Coracias benghalensis*	Coraciidae	Coraciiformes	LC	0.75	Percher
29	*Corves macrorhynchos*	Corvidae	Passeriformes	LC	1.28	Percher
30	*Corves splendens*	Corvidae	Passeriformes	LC	8.11	Percher
31	*Cuculus poliocephalus*	Cuculidae	Cuculiformes	LC	0.16	Percher
32	*Dendrocitta vagabunda*	Corvidae	Passeriformes	LC	0.39	Percher
33	*Dicrurus leucophaeus*	Dicruridae	Passeriformes	LC	0.44	Percher
34	*Dicrurus macrocercus*	Dicruridae	Passeriformes	LC	1.45	Percher
35	*Dinopium benghalense*	Picidae	Piciformes	LC	0.15	Tree clinging bird
36	*Dupetor flavicollis*	Ardeidae	Pelecaniformes	LC	0.06	Wader
37	*Egretta garzetta*	Ardeidae	Pelecaniformes	LC	4.22	Wader
38	*Egretta intermedia*	Ardeidae	Pelecaniformes	LC	1.59	Wader
39	*Eudynamys scolopacea*	Cuculidae	Cuculiformes	LC	0.34	Percher
40	*Euodice malabarica*	Estrildidae	Passeriformes	LC	1.85	Percher
41	*Falco peregrinus*	Falconidea	Falconiformes	LC	0.09	Bird of prey
42	*Francolinus pondicerianus*	Phasianidae	Galliformes	LC	0.57	Upland ground
43	*Fulica atra*	Rallidae	Gruiformes	LC	1.27	Swimmer
44	*Gallinula chloropus*	Rallidae	Gruiformes	LC	0.61	Swimmer
45	*Halcyon smyrnensis*	Alcedinidae	Coraciiformes	LC	0.57	Percher

	Scientific Name	Family	Order	IUCN Red List status	Abundance index	Behavior category
46	*Haliastur indus*	Accipitridae	Accipitriformes	LC	0.4	Bird of prey
47	*Himantopus himantopus*	Recurvirostridae	Charadriiformes	LC	1.85	Wader
48	*Hydrophasianus chirurgus*	Jacanidea	Charadriiformes	LC	0.78	Wader
49	*Lonchura punctulata*	Estrildidae	Passeriformes	LC	0.16	Percher
50	*Luscinia brunnea*	Muscicapidae	Passeriformes	LC	0.15	Percher
51	*Merops orientalis*	Meropidae	Coraciiformes	LC	0.53	Percher
52	*Merops philippinus*	Meropidae	Coraciiformes	LC	0.74	Percher
53	*Milvus migrans*	Accipitridae	Accipitriformes	LC	0.13	Bird of prey
54	*Mirafra cantillans*	Alaudidae	Passeriformes	LC	0.55	Percher
55	*Motacilla maderaspatensis*	Motacillidae	Passeriformes	LC	1.07	Percher
56	*Mycteria leucocephala*	Ciconiidae	Ciconiiformes	NT	3.18	Wader
57	*Nectarinia asiatica*	Nectariniidae	Passeriformes	LC	0.74	Percher
58	*Nectarinia zeylonica*	Nectariniidae	Passeriformes	LC	0.44	Percher
59	*Nycticorax nycticorax*	Ardeidae	Pelecaniformes	LC	0.88	Wader
60	*Oriolus oriolus*	Oriolidae	Passeriformes	LC	0.27	Percher
61	*Pandion haliaetus*	Pandionidea	Accipitriformes	LC	0.02	Bird of prey
62	*Passer domesticus*	Passeridae	Passeriformes	LC	2.15	Percher
63	*Pavo cristatus*	Phasianidae	Galliformes	LC	1.45	Upland ground
64	*Pelecanus onocrotalus*	Pelecanidae	Pelecaniformes	LC	0.4	Swimmer
65	*Pelecanus philippensis*	Pelecanidae	Pelecaniformes	LC	1.28	Swimmer
66	*Phaenicophaeus viridirostris*	Cuculidae	Cuculiformes	LC	0.28	Percher
67	*Phalacrocorax carbo*	Phalacrocoracidae	Phalacrocoracidae	LC	0.13	Diver
68	*Phalacrocorax niger*	Phalacrocoracidae	Phalacrocoracidae	LC	1.87	Diver
69	*Platalea leucorodia*	Threskiornithidae	Pelecaniformes	LC	0.75	Wader
70	*Plegadis falcinellus*	Threskiornithidae	Pelecaniformes	LC	0.81	Wader
71	*Porphyrio porphyrio*	Rallidae	Gruiformes	LC	0.52	Swimmer
72	*Pseudibis papillosa*	Threskiornithidae	Pelecaniformes	LC	1.88	Wader
73	*Psittacula krameri*	Psittacidae	Psittaciformes	LC	3.32	Percher
74	*Pteroclesnamaqua*	Pteroclidea	Pterocliformes	LC	0.09	Percher
75	*Pycnonotuscafer*	Pycnonotidae	Passeriformes	LC	0.16	Percher
76	*Sarkidiornis sylvicola*	Anatidae	Anseriformes	LC	0.87	Swimmer
77	*Saxicoloides fulicata*	Muscicapidae	Passeriformes	LC	0.34	Percher
78	*Stactolaema olivacea*	Lybiidae	Piciformes	LC	0.42	Tree clinging bird
79	*Streptopelia chinensis*	Columbidae	Columbiformes	LC	0.18	Upland ground
80	*Streptopelia decaocto*	Columbidae	Columbiformes	LC	0.3	Upland ground
81	*Streptopelia senegalensis*	Columbidae	Columbiformes	LC	0.19	Upland ground
82	*Tachybaptus ruficollis*	Podicipedidae	Podicipediformes	LC	0.72	Swimmer
83	*Tachymarptis melba*	Apodidae	Apodiformes	LC	2.5	Percher
84	*Terpsiphone paradise*	Monarchidae	Passeriformes	LC	0.19	Percher
85	*Threskiornis melanocephalus*	Threskiornithidae	Pelecaniformes	NT	6.21	Wader
86	*Tringa nebularia*	Scolopacidae	Charadriiformes	LC	0.36	Wader
87	*Turdoides striata*	Leiothrichidae	Passeriformes	LC	1.33	Percher
88	*Upupa epops*	Upupidae	Bucerotiformes	LC	0.32	Percher
89	*Vanellus indicus*	Charadriidae	Charadriiformes	LC	0.57	Wader
90	*Vanellus malabaricus*	Charadriidae	Charadriiformes	LC	0.48	Wader

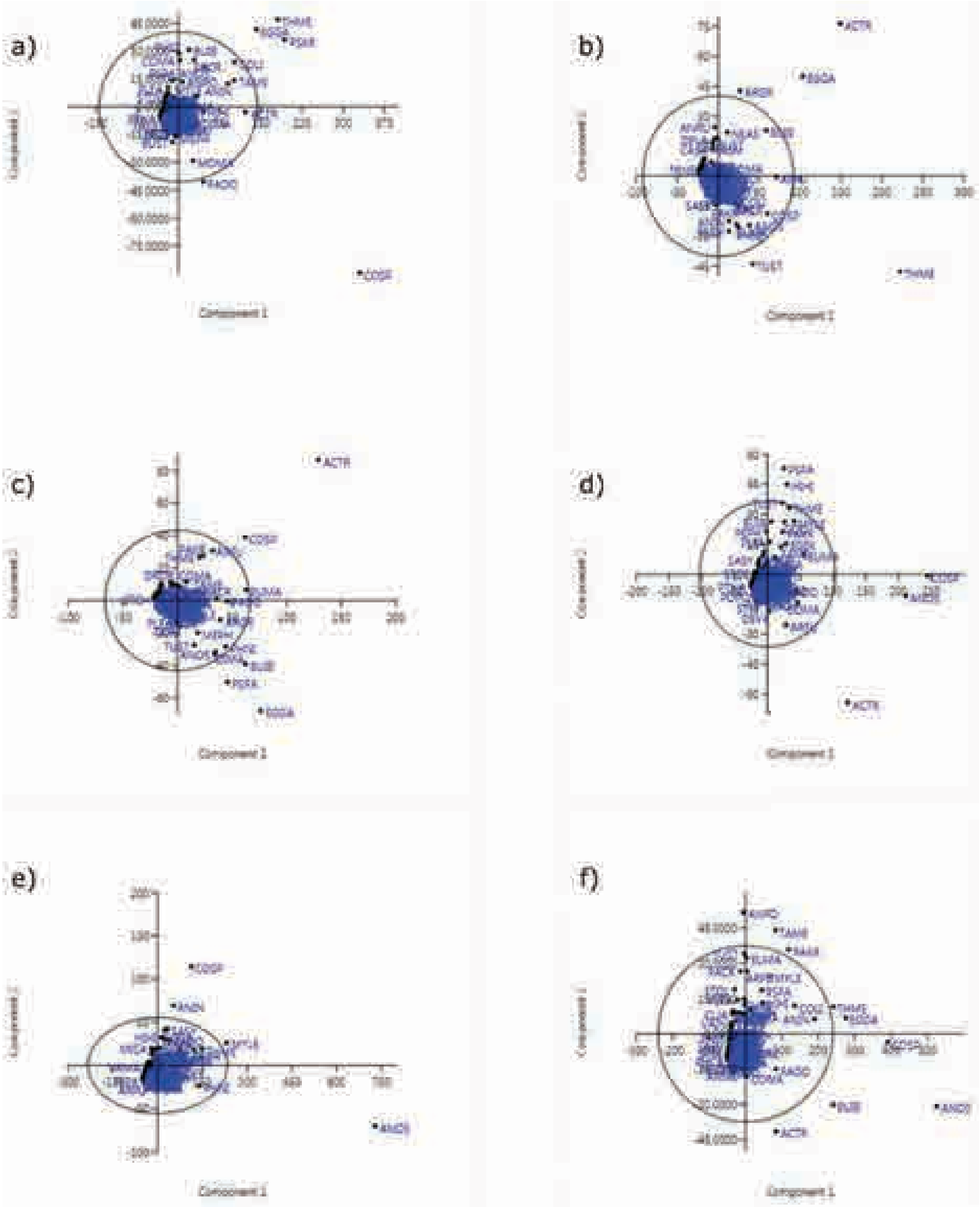

Figure 1. Scatter diagrams of bird species using PCA for a— spring| b— summer | c— early monsoon | d— late monsoon | e— early winter | f— late winter.

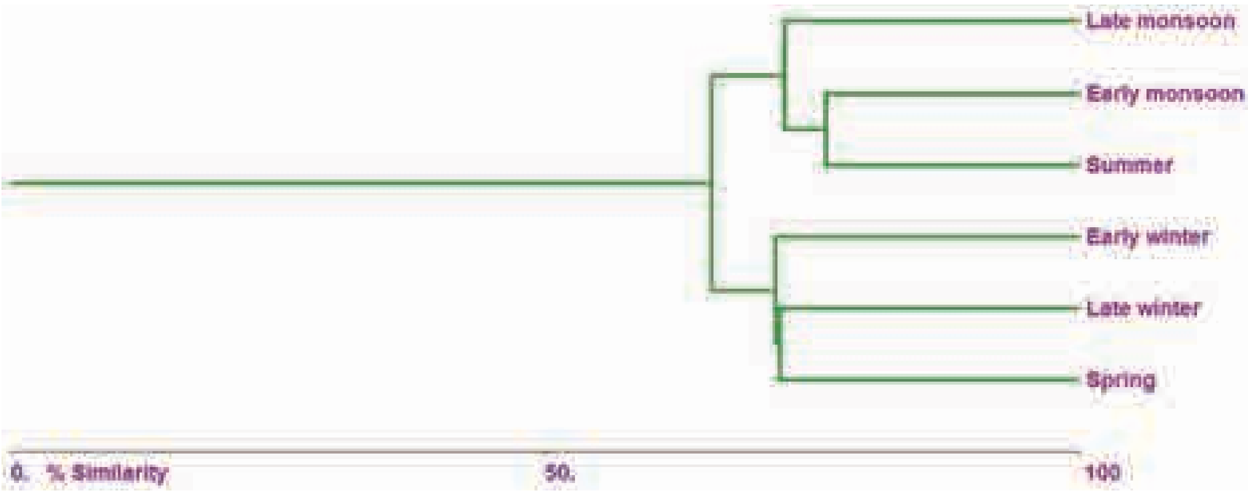

Figure 2. Single linkage cluster analysis of the population of birds among seasons using Bray-Curtis cluster analysis of similarity.

Table 2 Avifaunal diversity in different seasons of the Koonthankulam. *significant (P <0.05).

Biodiversity indices	Spring	Summer	Early monsoon	Late Monsoon	Early winter	Late winter	Sum of squares	F value
	Feb–Mar	Apr–May	Jun–Jul	Aug–Sep	Oct–Nov	Dec–Jan		
Taxa_S	84.33 ± 3.18	82.00 ± 1.53	83.33 ± 2.40	80.66 ± 0.33	84.00 ± 2.08	85.00 ± 1.73	39.111	0.61
Individuals	2980.33 ± 316.26	1902.66 ± 266.89	1408.33 ± 215.13	1315.00 ± 158.24	1741.66 ± 168.94	3027.33 ± 498.27	8665477.111	6.673*
Shannon_H	3.36 ± 0.19	3.53 ± 0.09	3.64 ± 0.09	3.70 ± 0.06	3.61 ± 0.11	3.55 ± 0.17	0.214	0.898
Buzas & Gibson's	0.35 ± 0.06	0.42 ± 0.03	0.46 ± 0.03	0.50 ± 0.03	0.45 ± 0.04	0.42 ± 0.06	0.038	1.24
Menhinick	1.56 ± 0.12	1.91 ± 0.11	2.25 ± 0.12	2.25 ± 0.13	2.03 ± 0.08	1.57 ± 0.09	1.435	7.811*
Chao-1	94.77 ± 3.09	101.27 ± 9.26	94.48 ± 5.46	90.89 ± 0.37	93.73 ± 1.51	93.57 ± 3.34	179.678	0.518

seasons into five, in the range of 55.25–100.00 (Figure 3). The five clusters show that each season has a different composition of the bird populations. The dendrogram showed that summer and early monsoon have a maximum similarity of 76.30. Two groups were identified among the six seasons. Early winter, late winter, and spring formed a group and early monsoon, late monsoon, and summer formed another group.

The number of individuals across seasons differed significantly (ANOVA, $F_{5,12}$ = 6.673, P <0.05; Table 2). A higher number of individuals was present in late winter (3027.33 ± 498.27), whereas the lowest number was recorded in late monsoon (1315.00 ± 158.24). The second-highest population of birds appeared in spring (2980.33 ± 316.26). This implies that the number of birds from December–March was high. The maximum Taxa_S was found in late winter (85.00 ± 1.73), whereas the minimum was in late monsoon (80.66 ± 0.33), with the range of Taxa_S over all the seasons being 5. The results reveal minimum deviation in species composition with high variation in population. However, the highest Shannon_H diversity (3.70 ± 0.06) was in late monsoon and the lowest (3.36 ± 0.19) in spring, indicating a more diverse and even species distribution in late monsoon. The Shannon_H diversity of birds among various seasons was not significantly different (ANOVA, $F_{5, 12}$ = 0.898, P >0.05).

The species evenness among the various seasons was measured by Buzas and Gibson's index. Evenness was maximum in late monsoon (0.50 ± 0.03) and minimum in spring (0.35 ± 0.06). However, the Buzas and Gibson's evenness indices across various seasons were not significantly different (ANOVA, $F_{5, 12}$ = 1.24, P >0.05). The richness was measured by the Menhinick species richness index. The Menhinick richness index differed significantly among the seasons (ANOVA, $F_{5, 12}$ = 7.811, P <0.05). Early (2.25 ± 0.12) and late monsoon (2.25 ± 0.13) have a high value of Menhinick richness index, and spring (1.56 ± 0.12) and late winter (1.57 ± 0.09) have a low value. The Chao-1 estimator was used to analyze singleton and doubleton species in the bird community. The maximum singleton and doubleton species occurred

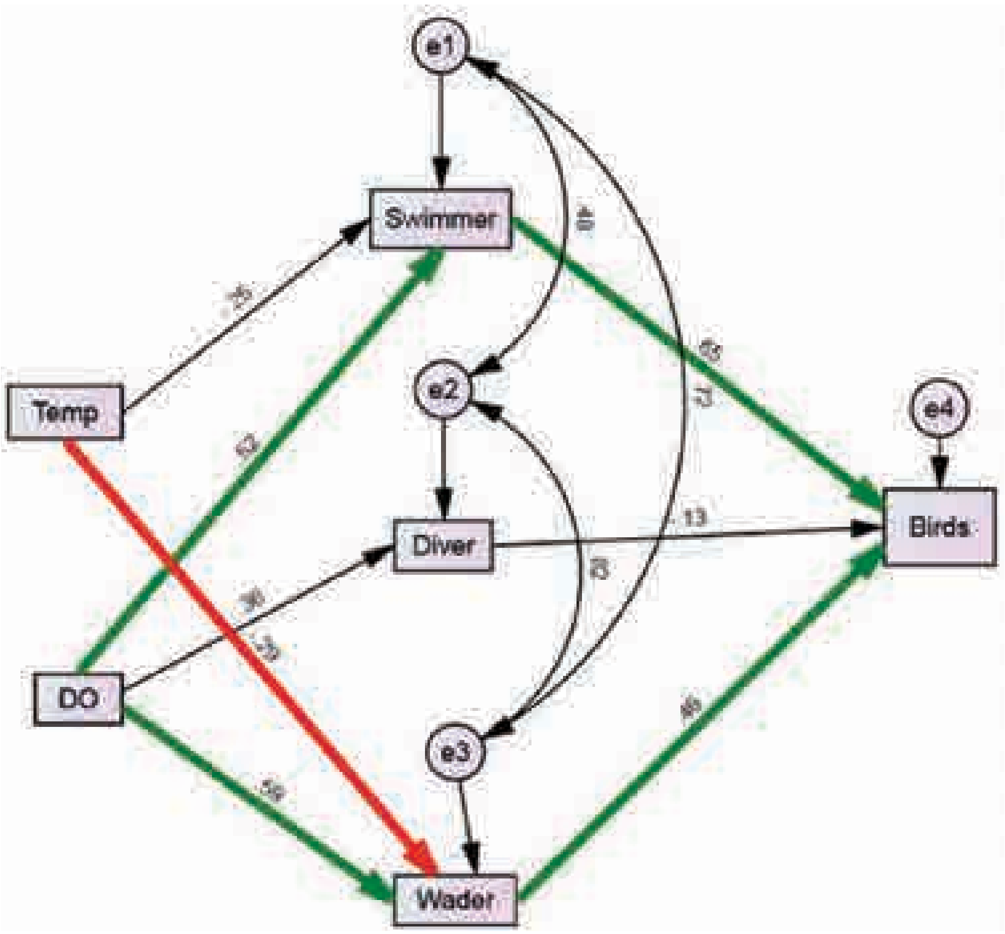

Figure 3. SEM of regression model.

in summer (101.27 ± 9.26) and the minimum in late monsoon (90.89 ± 0.37). Among all the seasons, the Chao-1 estimator was not significantly different (ANOVA, $F_{5,12} = 0.518$, $P > 0.05$).

A correlation analysis has been carried out for all physico-chemical parameters with swimmer, diver and wader species to understand the influence of the water-quality parameters on the bird population (Table 3). The following results were noticed in that analysis. The temperature was negatively correlated with swimmer and wader populations at $p = 0.01$, and DO was positively correlated with all the three water bird communities encountered in this study at $p = 0.05$. In addition to this, a SEM model was designed and analyzed to confirm that temperature and DO were the most important parameters that affect bird counts. Thus, a structural equation model has been framed with the following six parameters, temperature, DO, swimmer, diver, and wader abundances. Bird's abundance has been studied with reference to the effect of temperature using structural equation modeling, as in Duclos et al. (2017). Patra et al. (2010) used stepwise multiple regression analysis to study the physico-chemical parameters affecting the avifaunal abundance; e1, e2, and e3 are added to the SEM to reduce the error value between the variables. Temperature, DO, and errors are exogenous variables, and swimmer, diver, and wader birds are endogenous variables.

Figure 3 expresses the conceptual framework for the model. The fitness of a SEM is important to understand the reliability of the results. The measure CMIN/df (0.518) <2, GFI (0.95) >0.90, and RMSEA (0.00) <0.05 revealed that the model represented a realistic fit of the data. The regression equations for the four endogenous variables with standardized coefficients are

Birds = (-.13) Diver + (.65) Swimmer + (.46) Wader + (1) e4

Diver = (.36) DO + (1) e2

Swimmer = (.52) DO + (-.25) Temp + (1) e1

Wader = (.59) DO + (1) e3 + (-.29) Temp

Five path coefficients were significant at 0.05 (Table 4). From the significance of these path coefficients,

Table 3. Correlation of physico-chemical parameters, swimmer, diver, and wader. *significant at the level of 0.05 | **significant at the level of 0.01

	Swimmer	Diver	Wader	Tempera-ture (˚C)	pH	DO (ppm)	TDS (ppm)	Salinity (ppt)	Conductivity (uS)	Acidity (mg/l)	Alkalinity (mg/l)	Free Co2 (mg/l)	Chloride (mg/l)	Calcium (mg/l)	Total hardness (mg/l)	Magnesium (mg/l)	Nitrogen (mg/l)
Swimmer	1.000																
Diver	0.553*	1.000															
Wader	0.874**	0.721**	1.000														
Temperature (˚C)	-0.659**	-0.454	-0.692*	1.000													
pH	-0.168	-0.081	-0.014	0.463	1.000												
DO (ppm)	0.583*	0.585*	0.682*	-0.352	0.226	1.000											
TDS (ppm)	0.160	-0.127	0.088	-0.398	0.066	0.227	1.000										
Salinity (ppt)	-0.083	-0.225	-0.197	-0.028	0.313	-0.044	0.730**	1.000									
Conductivity(uS)	0.050	-0.012	0.008	-0.281	0.178	0.210	0.934**	0.861**	1.000								
Acidity(mg/l)	-0.259	-0.241	-0.341	0.248	0.670*	0.052	-0.349	0.067	-0.223	1.000							
Alkalinity (mg/l)	-0.110	-0.263	-0.165	0.344	0.662*	-0.019	0.055	0.344	0.105	0.647*	1.000						
Free Co2 (mg/l)	-0.361	-0.098	-0.460	0.347	0.508	-0.177	-0.471	0.068	-0.246	0.819**	0.461	1.000					
Chloride (mg/l)	0.212	-0.360	-0.025	0.298	0.329	-0.124	-0.147	0.089	-0.238	0.488	0.593*	0.410	1.000				
Calcium (mg/l)	-0.110	-0.417	-0.319	-0.402	-0.535	-0.405	0.376	0.316	0.320	-0.405	-0.537	-0.309	-0.160	1.000			
Total hardness (mg/l)	0.038	-0.432	-0.297	0.033	-0.085	-0.283	0.541	0.726**	0.529	-0.030	0.327	0.041	0.457	0.421	1.000		
Magnesium (mg/l)	0.033	-0.369	-0.209	0.016	-0.069	-0.201	0.519	0.679*	0.512	-0.151	0.316	-0.022	0.405	0.300	0.934**	1.000	
Nitrogen (mg/l)	-0.204	-0.067	-0.061	0.501	0.505	0.135	-0.337	-0.172	-0.337	0.620*	0.647*	0.386	0.414	-.603*	-0.239	-0.228	1.000

it is revealed that DO positively influences swimmer and wader counts, while temperature negatively influences wader counts. Duclos et al. (2017) reveal that temperature directly affects the abundance of birds. Waders have a negative direct effect from temperature. Dissolved oxygen positively influenced total avifaunal abundance (Patra et al. 2010). Both correlation analyses and SEM model confirmed that temperature and DO are the main parameters that affect bird count in this study area.

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Table 4. Regression weights between parameters of the SEM.

			Unstandardized Estimate	Standardized Estimate	S.E.	C.R.	P
Swimmer	<---	Temp	-12.284	-0.253	10.072	-1.220	0.223
Swimmer	<---	DO	61.548	0.516	28.169	2.185	0.029
Diver	<---	DO	17.555	0.363	13.003	1.350	0.177
Wader	<---	DO	243.994	0.586	90.907	2.684	0.007
Wader	<---	Temp	-49.556	-0.292	21.093	-2.349	0.019
Birds	<---	Swimmer	3.645	0.650	0.625	5.828	0.000
Birds	<---	Wader	0.739	0.460	0.244	3.024	0.002
Birds	<---	Diver	-1.779	-0.129	1.436	-1.239	0.215

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COMMUNICATION

Plastral deossification zones in the Endangered Spiny Hill Turtle *Heosemys spinosa* (Testudines: Geoemydidae) on Borneo

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Abstract: Studies of the evolution of turtle shell morphology have raised numerous questions. In this study, five adult females and two adult males of the Endangered Spiny Hill Turtle *Heosemys spinosa* from two localities in western Sarawak in East Malaysia (Borneo) were examined for the presence of plastral deossification zones, reported as indicative of kinesis in earlier studies. A total of 52 (34 females, 18 males) radiographs demonstrated distinct poorly-ossified areas across the hyo-hypoplastral and the hypo-xiphiplastral junctures in females of straight carapace length (SCL) 143.4–211.4 mm. The feature is here interpreted as a permanent plastral hinge, which was not observed in either of the males examined. The smallest female with a weak but discernible line at the contact of these bones had a SCL of 125 mm, suggestive of minimum size of maturity in *Heosemys spinosa* in this population.

Keywords: Biology, growth, maturity, osteology, plastron, reproductive biology, sulcus, turtle.

Bahasa: Kajian mengenai evolusi morfologi berkenaan cengkerang kura-kura merupakan topik yang menimbulkan pelbagai persoalan. Lima ekor betina dan dua ekor jantan yang matang dari spesies Terancam yang dikenali sebagai Spiny Hill Turtle (*Heosemys spinosa*), dari dua lokaliti di barat Sarawak (Malaysia Timur, Borneo) telah dikaji untuk kehadiran zon deossifikasi plastral, yang dilaporkan sebagai indikasi kinesis menurut kajian awal. Sebanyak 52 pengimejan radiografi menunjukkan kawasan ossifikasi yang kurang ketara di antara hyo-hypoplastral dan hypo-xiphiplastral dalam kumpulan kura-kura betina yang mempunyai straight carapace length (SCL) 143.4–211.4 mm. Ciri-Ciri ini dikategorikan sebagai engsel plastral yang kekal, dan tidak dilaporkan dari kumpulan kura-kura jantan yang dikaji. Betina yang terkecil menunjukkan garis yang kabur tetapi kelihatan di antara dua tulang ini pada size SCL 125 mm, yang seterusnya mencadangkan saiz kematangan minimum untuk populasi ini.

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Competing interests: The authors declare no competing interests.

Author details: Siti Nor Baizurah is a PhD graduate from UNIMAS, previously worked on the herpetofauna ecology for her postgraduate studies and her research interests include ecology, anthropogenic effects on ecosystem, human wildlife conflicts and wildlife management. Indraneil Das received a D.Phil in Zoology from the University of Oxford, and was a Fulbright Fellow at Harvard University. He is currently professor at the Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, where he conducts research on the systematics and ecology of the herpetofauna of Borneo and south-east Asia.

Author contributions: SNB carried out the research work, providing data (ie: photographs, measurements) and drafted the manuscripts. ID coordinated the data and participate in writing and reviewing the manuscript. All author agreed to the final version.

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INTRODUCTION

Evolution of turtles has produced variations in shell morphology and pelvic elements (Rivera 2008; Williams & Stayton 2019) and shell kinesis, although female plastral kinesis remains speculative in most species. The degree of kinesis depend on specializations in muscle and ligament systems, and active kinesis usually involves modifications of kinetic structures that allow flexibility through voluntary muscle connections (Pritchard 2003). In passive kinesis, modifications of muscles are not necessary and may rely on pressures exerted over certain periods, including for respiration. For instance, pelvic kinesis in *Homopus signatus* (Hofmeyr et al. 2005), posterior plastral lobe kinesis in *Heosemys spinosa* (Mertens 1942, 1971), carapacial pankinesis in *Dogania subplana* (Pritchard 1993) and *Kinixys erosa* (Pritchard 2008) have been assumed on the basis of structure, although their mechanism and function is poorly known. Shell kinesis has been reported in smaller individuals of terrestrial or semi-terrestrial species as a possible adaptation to escape predation and aid locomotion (Berlant & Stayton 2017; Cordero et al. 2018). In contrast, posterior plastral lobe kinesis reported in *H. spinosa* has been hypothesized to be part of its reproductive strategy (Waagen 1984; Moll 1985), even though the shell was once considered as akinetic, and the possibility of plastral muscle specialization has been proposed (Bramble 1974). The hinge in *H. spinosa* reportedly develops in mature females (Moll 1985), resembling the hinge position displayed in the genus *Cuora*, albeit the kinesis in *H. spinosa* is limited to the posterior part of the plastron (Pritchard 1993).

Shell kinesis harbours numerous advantages, including predation survival, locomotion and facilitation of the passage of large eggs in small turtles. Accordingly, variation in the turtle shell has been speculated to be affected by multiple pressures, including phylogenetic, environmental and reproduction, in which plastral kinesis is one result of those pressures (Angielczyk et al. 2011). It is important to note that plastral modifications is usually expressed strongly in adults of both sexes in kinetic species (Ernst & Barbour 1989). Consequently, a number of studies (reviewed in Cordero & Quinteros 2015) discusses the adaptations of shell kinesis to habitat preferences, while sexually dimorphic kinesis must be related to reproduction strategy benefits, although a recent study suggests that the evolutionary structure of shell kinesis may stem from more complex relationships between ecological, phylogeny, and developmental processes in turtles (Cordero et al. 2018).

In the present study, we examine adults of the Spiny Hill Turtle from two free-ranging populations in Sarawak, East Malaysia (northwestern Borneo), in order to understand plastral kinesis (represented by plastral sulcus). Specifically, we will try to ascertain if the feature is restricted to females, the specific plastral bones associated with sulcus, the minimal size of animal that correlates with the development of the feature, and finally, if there are seasonality in variation of the feature.

MATERIALS AND METHODS

Data on plastral morphology were collected within a larger study on the spatial and thermal ecology of the Spiny Hill Turtle from two localities in western Sarawak, northern Borneo. The first was from Kubah National Park (headquarters at 1.6115^{0}N, 110.1964^{0}E, WGS 84), a protected area of 2,230 ha, located within the Matang Massif, the second from forests attached to a privately-owned farmland (1.3073^{0}N, 110.5037^{0}E, WGS 84), around the township of Serian (Image 1). Vegetation types represented include mixed dipterocarp forest, Kerangas (Bornean heath forest) and submontane forests (Hazebroek & Morshidi 2000). Data were obtained between 11 April 2017 and 30 January 2019 from five females that were fitted with temperature sensitive transmitters (Holohil^{TM} Ri2B and Holohil^{TM} PD-2T) for a study of spatial and thermal ecology, during which the present study was conducted. Animals were brought to a veterinary clinic for radiography and released at the point of encounter within a week.

Radiography procedures were conducted by a qualified veterinarian. A Sedecal Apr-Vet (Model E7239X) radiographic unit was used to produce three views per individual (dorsal, ventral and lateral positions). Each exposure was 78 kV (25 mA to 320 mA) for 0.08 sec, following which individuals were weighed using a digital scale (Camry/ ACS-3—JC31). General anaesthesia was not used in the procedure, and depending on the mobility of the individual turtle, manual restraints with tape was used. An Xscan Radiology Application (Version 2.10) was used to edit the image obtained, prior to examination.

OBSERVATIONS AND DISCUSSION

A total of 34 radiographic images were taken of five females, which displayed a distinct sulcus, presumably comprising connective tissue, across the midbody,

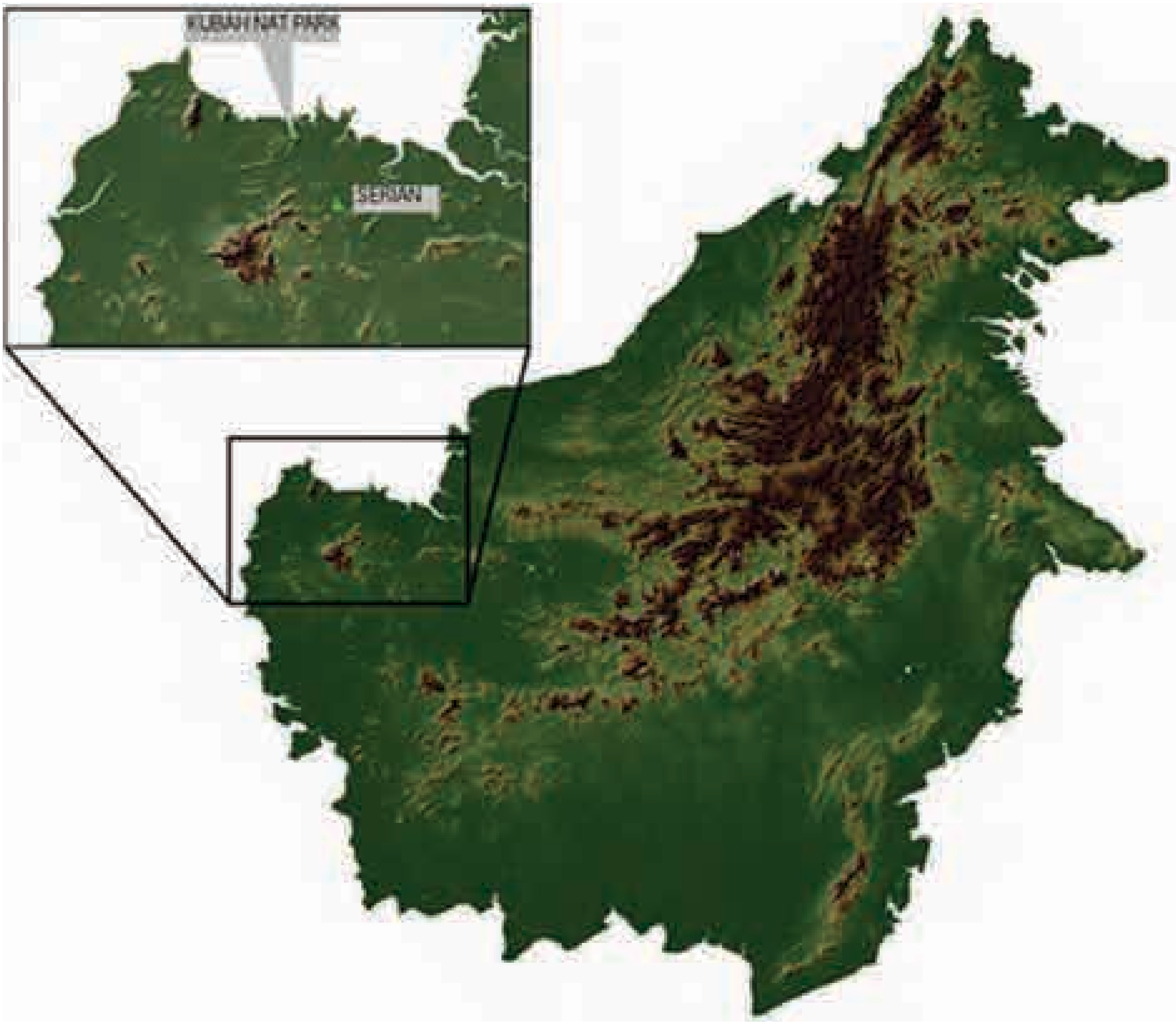

Image 1. Map of north-western Borneo; expanded area to top right show locations of study sites at Kubah National Park and the township of Serian, Sarawak State, East Malaysia.

specifically transversely between the hyoplastral-hypoplastral bones, and the hypoplastral & xiphiplastral bones, under the abdominal and femoral scute regions. None of the 18 radiographs of the two males showed evidence of a sulcus (refer to Image 5), which has been referred in the literature as indicative of a plastral hinge (Mertens 1942, 1971; Bramble 1974; Waagen 1984). The feature is known to exist only in adult females of the species (Moll 1985), presumably to facilitate oviposition and perhaps to allow the passage of large eggs (Yasukawa et al. 2001; Joyce et al. 2012). Other sexually dimorphic features have been listed in Baizurah & Das (2021). Images 2–4 indicate that kinesis of plastral elements may be shown by *H. spinosa* as seen in two females (SNB 638 and SNB 641) across time. The smallest female (mean SCL of 125.1 mm) was beginning to display presence of such a gap (Image 3) indicating possible size at maturity of females in *H. spinosa*. Examination of the radiographs of these individuals do not indicate a greater development of the hinge at any particular month, as might be expected if the hinge becomes functional only periodically, for the passage of the eggs.

Previous studies have discussed that pelvic aperture dimensions may be a limiting factor in reproductive output, and how it relates to sexual dimorphism in some turtles (Clark et al. 2001; Matysiak et al. 2017; Cordero et al. 2018). Apart from pelvic size variation and reduced relative plastral length in males in increasing reproduction output, plastral kinesis can serve a similar function in increasing reproduction output. Plastral kinesis is thought to reduce pelvic strain in females during egg-laying (Legler 1960; Yasukawa et al. 2001). The first

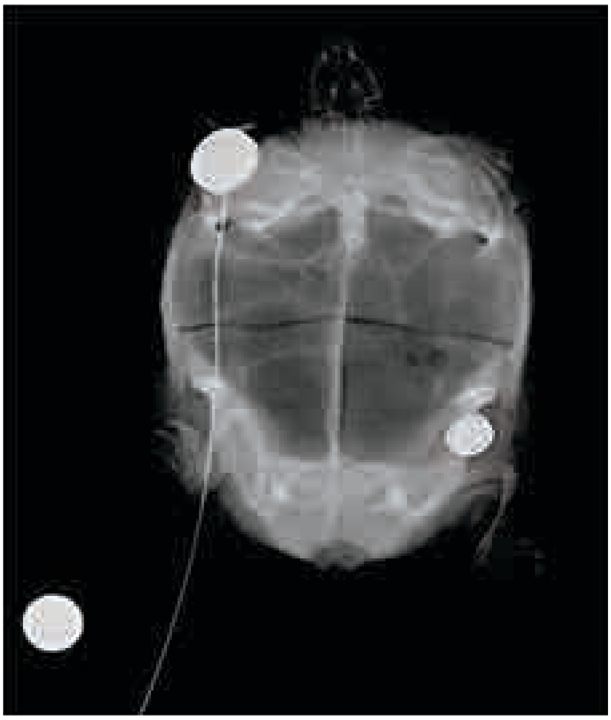

Image 2. Radiographs of plastron showing sulcus at juncture of hyo-hypoplastra of an adult (SCL 191 mm) female *Heosemys spinosa* (SNB 643). Image taken in November 2018. Also visible in this and the subsequent images are the externally-attached radio-transmitter, and an iButton, implanted for a larger study of movement and thermoregulation in the species.

Table 1. Details of *Heosemys spinosa* radiographically examined.

Identification	Sex	Total radiographs	Locality
SNB 637	Male	16	Kubah NP
SNB 638	Female	14	Kubah NP
SNB 639	Male	2	Kubah NP
SNB 641	Female	12	Kubah NP
SNB 642	Female	2	Kubah NP
SNB 643	Female	4	Kubah NP
SNB 640	Female	2	Serian

record of a plastral hinge in *H. spinosa* was by Mertens (1942, 1971), followed by the detailed examination of two females by Waagen (1984), who speculated that the structure is rather weak in adult females. Hence the likelihood that kinesis serves a probably protective mechanism is unlikely. It is important to note that these descriptions are not supported by histological data hence lacking the information needed to functionally validate female-specific plastral kinesis in *Heosemys*. Waagen (1984) described the hinge as consisting of fibrous tissues internally that did not appear to change with body size, although the possibility of temporal change was mentioned. As described in that study, the present one found that the structure does not remain distinct year round, the deossification of bones presumed related to oviposition, not affected by seasonal changes, as evidenced in our radiographs (see Images 1–3). However, we have no indication that any of our females were preparing to reproduce, and no mating behaviour was observed. Previous literature on reproduction of *H. spinosa*, albeit in captivity, stated that copulation is typically triggered by rain showers, and egg deposition usually occurs in March-July (Herman 1993; Goetz 2007). In Sarawak (northwestern Borneo), high humidity and temperatures are encountered throughout the year, and periods associated with high rainfall events occur between November and March, with the passage of the north-east monsoons, and a weaker one between May and September, coinciding with the south-west monsoons (Sa'adi et al. 2019).

Numerous functional traits usually emerge late in turtle ontogeny, including development of fibrous tissues, which are known to progress slowly via repatterning of tissue which is acquired over the growth period (Cordero et al. 2018). The gradual process may explain the changes in hyo-hypoplastral, and hypo-xiphiplastral regions we noted across time. For instance, hatchling plastron shape of kinetic-shelled species undergoes differentiation post embryonic stages, especially in area where the hinge presumably occurs, as they reached maturity, in contrast to akinetic species which undergoes plastron differentiation at extreme and posterior ends (Cordero et al. 2019). Lastly, extrinsic factors such as abundance of resources and rainfall are known to affect reproductive cycles in some species (Akani et al. 2005; Loehr et al. 2011; Graham et al. 2015). Our radiographic observations suggest that hyo-hypoplastral and hypo-xiphiplastral kinesis in *H. spinosa* is possibly influenced by reproductive needs, developing during ontogeny, and may not be associated with local climate.

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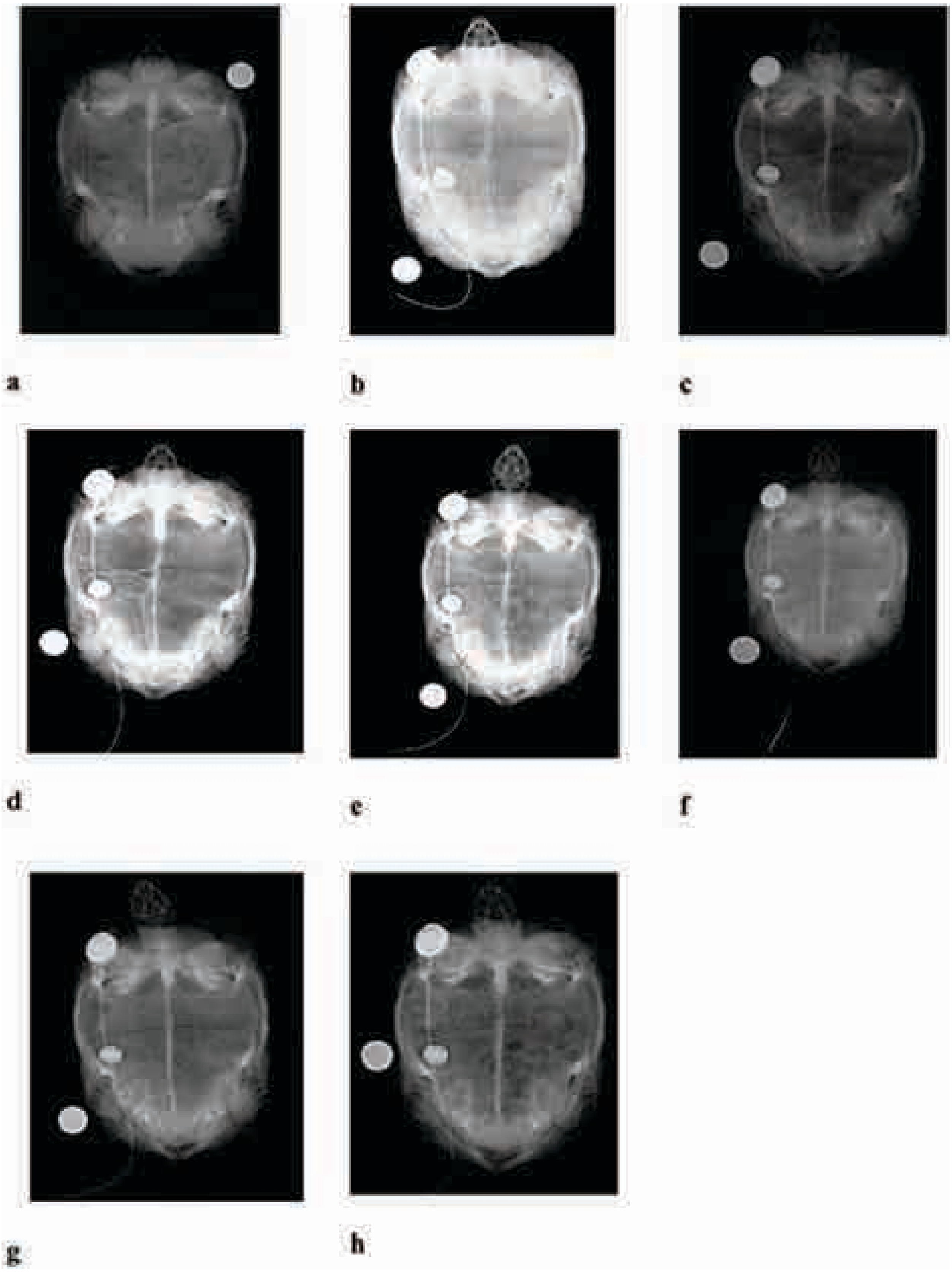

Image 3. Radiographs of plastron showing sulcus at junctures of hyo-hypoplastra and hypoplastra-xiphiplastra of a female *Heosemys spinosa* (SNB 638). Straight carapace length (SCL in mm) as follows: a) 191.33; b) 195.46; c) 198.12; d) 198.12; e) 201.3; f) 208.31; g) 210.06; h) 210.53. Diameter of scale markers = 22 mm. a) Aug-2017; b) Oct-2017; c) Dec-2017; d) Feb-2018; e) April 2018; f) Nov-2018.

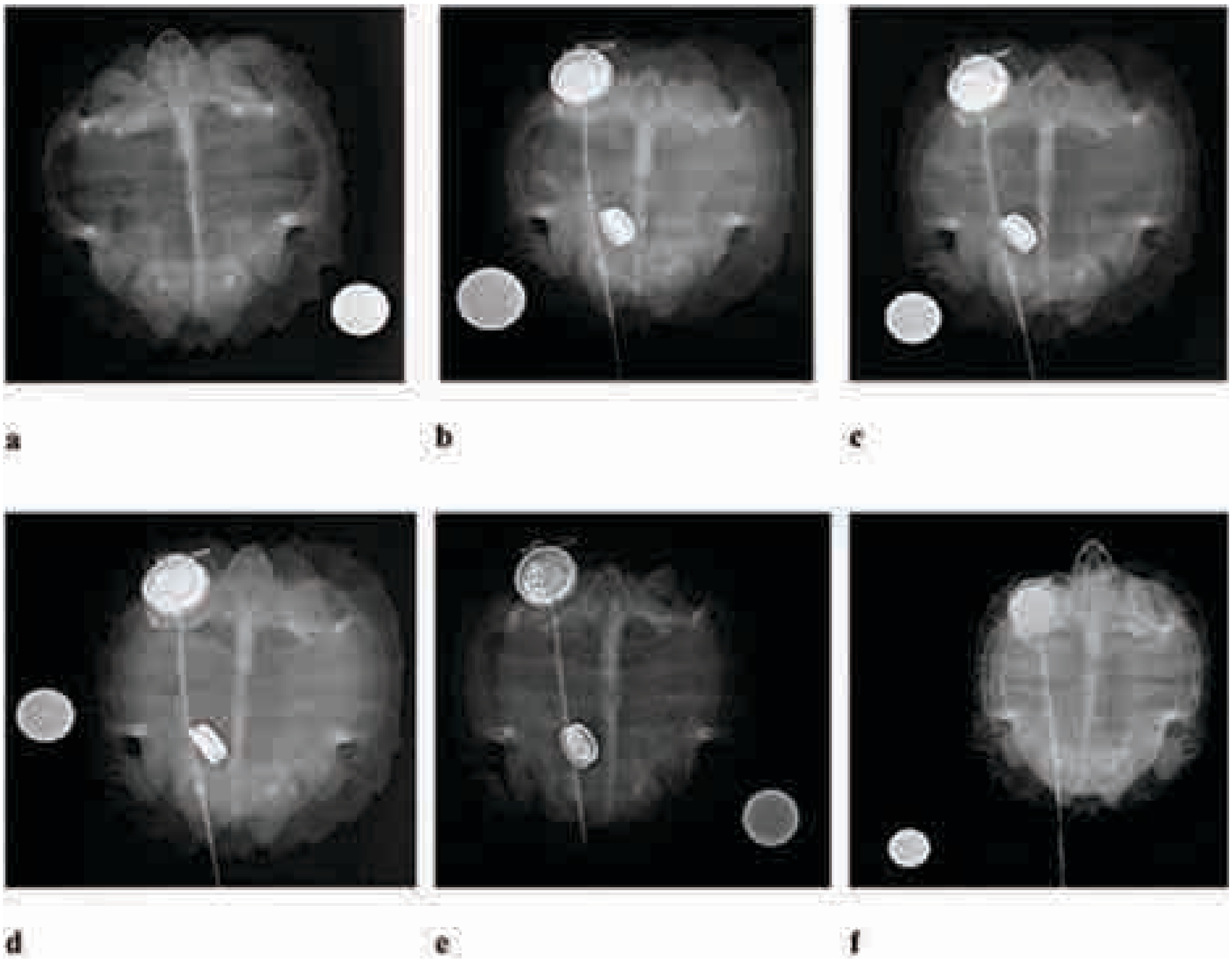

Image 4. Radiographs of plastron showing sulcus at junctures of hyo-hypoplastra and hypoplastra-xiphiplastra of a female *Heosemys spinosa* (SNB 641). Straight carapace length (SCL in mm) as follows: a) 125.1; b) 126.2; c) 126.96; d) 128.52; e) 131.07; f) 143.29. Diameter of scale markers: 22 mm.

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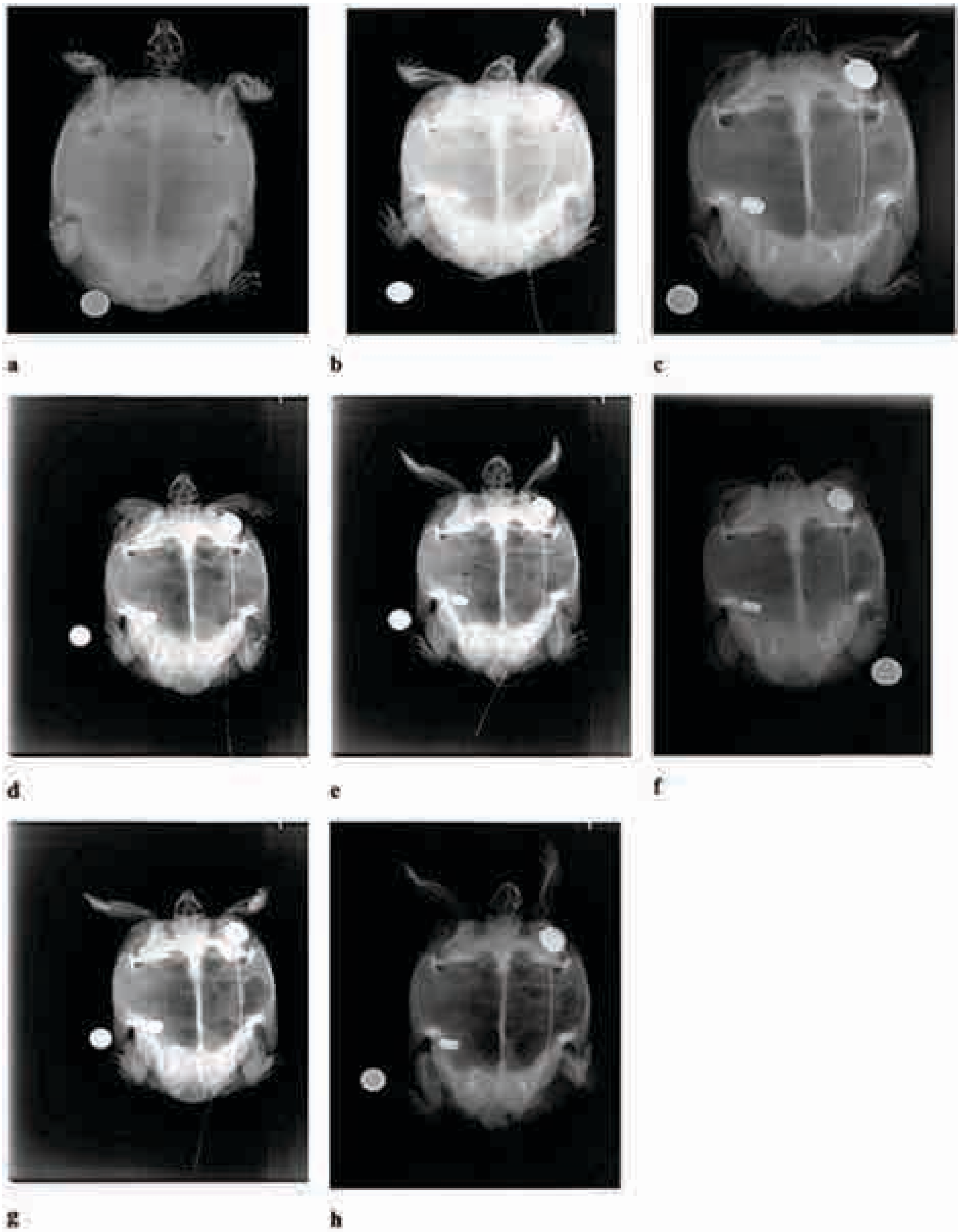

Image 5. Radiographs of plastron of a male *Heosemys spinosa* (SNB 637). Straight carapace length (SCL) 637 mm. Straight carapace length (SCL in mm) as follows: a) 198.2; b) 200.8; c) 205.1; d) 218.5; e) 223.19; f) 225.91; g) 231.27; h) 237.72. Diameter of scale markers: 22 mm.

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COMMUNICATION

Addition of four new records of pit vipers (Squamata: Crotalinae) to Manipur, India

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Abstract: In spite of the potential richness in biodiversity, Manipur is still one of the least explored areas in northeastern India. In this work, we report on our finding of four pit viper species, namely, *Trimeresurus mayaae*, *T. salazar*, *T. erythrurus*, and *Protobothrops jerdonii* in Manipur during our herpetological surveys in 2022. We affirm the identity of these specimens based on the combination of standard morphological characters and mitochondrial cytochrome b gene. Our study not only reports four new records of pit vipers for the first time in Manipur, but also extends their respective known geographical ranges. This information calls for further dissemination especially to health care sector, as it is on venomous snakes that could be of medical importance as regards human snakebites.

Keywords: morphology, new state report, northeastern India, phylogenetics, *Protobothrops*, range extension, systematics, *Trimeresurus.*

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Competing interests: The authors declare no competing interests.

Author details: Premjit Singh Elangbam is a BCom graduate currently engaging in wildlife photography & research in Manipur. He has contributed photographs and articles in many books on flora & fauna of Manipur especially Indian birds. He has involved in many efforts in conservation research conducted by Forest Dept., Govt. of Manipur. Now, he is secretary of Wildlife Explorers Manipur, an organization fully devoted to wildlife photography based on conservation & research in Manipur. Lal Biakzuala is an emerging researcher in the field of herpetological taxonomy and systematics, natural history, molecular phylogenetics, and conservation. He is currently pursuing his PhD degree focussing on the molecular systematics of elapid snakes, and is also currently working under ICMR funded project for estimating the incidence, mortality, morbidity and economic burden due to snakebites in India. Parag Shinde is a commerce graduate and has also finished a photography school. He is passionate about reptile photography, herpetological related field investigations and animal rescuing. H.T. Decemson is currently doing his Ph.D. work on primates and also engaged as JRF under the DST-SERB project on the taxonomy, ecology and distribution of amphibians and reptiles in Mizoram. He also extended his work on the herpetofauna of Manipur State in collaboration with the Department of Forest, Sanjenthong, Imphal, Government of Manipur. M. Mathipi Vabeiryureilai is an assistant professor with an expertise in cancer biology, evaluation of medicinal plants, taxonomy and molecular phylogeny of various taxa. H.T. Lalremsanga is a professor and a pioneer in the field of herpetology in Mizoram. Currently, he is teaching post-graduate students, guiding research scholars, and overseeing major projects funded by the Department of Science and Technology - Science and Engineering Research Board (DST-SERB) and Department of Biotechnology (DBT) under the Ministry of Science and Technology, the Defence Research and Development Organisation (DRDO) under the Ministry of Defence, Indian Council of Medical research (ICMR), The Habitats Trust (THT), Noida and the National Mission for Himalayan Studies (NMHS), Government of India.

Author contributions: PSE, HD, PS did the field-based investigations and sampling; LB performed data analyses, and wrote the first draft of the manuscript with HTL; MV carried out laboratory and technical works; HTL conceived the study, review and editing, and approval of the manuscript prior submission. All authors reviewed and agreed upon the final version.

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INTRODUCTION

Pit vipers are a group of front-fanged venomous snakes that comprise various genera of vipers having heat sensitive pit between each eye and nostril. Asian pit vipers that are distributed across southern and southeastern Asia are known to inhabit several types of habitats like wet, humid and montane forests, and also found in a few dry forest habitats (Gumprecht et al. 2004). Recent phylogeographic studies have largely addressed the taxonomic status of certain groups of pit vipers like *Trimeresurus* complex (Malhotra & Thorpe 1997, 2004; David et al. 2011; Mallik et al. 2021) and *Protobothrops* (see Guo et al. 2009). Geographical diversity as well as sexual dimorphism often led to inaccurate taxonomic assignment among the closely similar pit viper species (Malhotra & Thorpe 1997, 2004). Consequently, pit vipers are still regarded to contain several taxonomically complex species in India. Recently, many such species complexes were revised including those from the *Craspedocephalus malabaricus* and *C. macrolepis* complexes (Mallik et al. 2021), the *T. medoensis* complex (Rathee et al. 2022), and the *T. albolabris* complex (Chandramouli et al. 2020; Mirza et al. 2020; Vogel et al. 2022).

Manipur is a hilly Indian state that lies at the extreme east of the northeastern region of India bordering Nagaland in the north, Mizoram in the south-east, Assam in the west, and Myanmar towards the east and south-east. In spite of the potential richness in biodiversity, zoological exploration in Manipur is rather limited (Roonwal 1948; Singh 1995; Pawar & Birand 2001) and there exist only a handful of snake faunal reports from the area till date (e.g., Mathew 2005; Sinate et al. 2021, 2022; Elangbam et al. 2022). Although Wallach et al. (2014) listed Manipur as the potential range of *T. erythrurus*, there is no confirmatory records from the area, and none of the aforementioned literature listed pit vipers from the snake fauna of Manipur except *Ovophis monticola* (see Whitaker & Captain 2008). In this paper, we provide notes on our new addition of pit vipers from Manipur based on our field documentations during our herpetological surveys in 2022.

MATERIALS AND METHODS

Sampling and morphology

We conducted our field surveys after obtaining herpetological specimen collection permit from the Forest Department, Government of Manipur (permit no. #3/22/2018-WL [Vol-II]). Morphological characters of the newly collected specimens were taken. We followed Dowling's (1951) method for counting ventrals (Ve), and we excluded the terminal scute in counting the subcaudals (Sc). All the measurements are given in millimeter, and other acronym used for the morphological characters are: snout-vent length, SVL; tail length, TaL; inter-orbital distance (measured on cephalic scale which was counted on a straight line between the middle of the supraoculars), IOD; supralabials, SL; infralabials, IL; and the bilateral characters are given as left/right. We euthanized the live specimens using MS-222 according to the standardized protocol (Conroy et al. 2009), and the procedure was approved by the Institutional Animal Ethics Committee (IAEC) (Permission No. MZU-IAEC/2018/12). All the specimens collected during this survey were deposited in the Departmental Museum of Zoology, Mizoram University, India (MZMU). For molecular analyses, we dissected the liver tissue from the freshly euthanized specimens and stored in 95% ethanol at -20°C refrigerator. Distribution map was prepared using QGIS v3.16.2.

DNA extraction and amplification

We employed mitochondrial cytochrome b (cytb) gene for molecular based identification. For this purpose, genomic DNA was extracted from the liver tissue using QIAamp DNA Mini Kit following the standard protocol provided. We amplified the fragment of cytb gene using a published primer pairs (Snk) (Dubey et al. 2009) under the thermal conditions of 94˚C for 3 min for initial denaturation; 35 cycles for denaturation at 94˚C for 30 sec, annealing at 49˚C for 40 sec, and extension at 72˚C for 30 sec; with the final extension of 72˚C for 5 sec, and cooling at 4°C for 15 min. The amplified products were purified using ThermoFisher ExoSAP- IT PCR product clean-up reagent and sequenced at Barcode BioSciences, Bangalore, India using Sanger's dideoxy method. The newly generated sequences were submitted to NCBI GenBank database (Benson et al. 2018) and obtained the accession numbers (OQ968475–77).

Molecular analyses

We assembled the dataset mainly based on the published datasets of Mirza et al. (2023) for *Trimeresurus* and Guo et al. (2009) for *Protobothrops*, and we obtained the published nucleotide sequences from the NCBI GenBank database (Benson et al. 2018). We used the default parameter settings of MUSCLE (Edgar 2004) for performing nucleotide alignment in MEGA 11 (Tamura et al. 2021). The flanking gaps created by the samples with shorter nucleotide sequences are treated as missing data. We estimated the uncorrected p-distance using complete deletion for gaps/missing data in MEGA 11 (Tamura et al. 2021). We partitioned the assembled datasets (1,088 bp in length for *Trimeresurus*; 847 bp in length for *Protobothrops*) by codon position. The best partitioning schemes and nucleotide evolutionary models selected by PartitionFinder v2 (Lanfear et al. 2017) under the Bayesian Information Criterion are HKY+I for cytb pos1, TRN+G for cytb pos2, HKY+G for cytb pos3 in the dataset of *Trimeresurus*; HKY+I+G for cytb pos1 and pos3, and TIM+I+G for cytb pos2 in the dataset of *Protobothrops*. By implementing the selected models, we performed the partitioned Bayesian inference (BI) phylogeny separately for the two datasets in MrBayes v3.2.5 (Ronquist et al. 2012). We conducted four independent runs with one cold and three hot chains for 10 million generations and sampling every 5000 generations. We discarded 25% of the trees as burn-in cut off after examining the trace plots generated by the MCMC runs in Tracer v1.7 (Rambaut et al. 2018).

RESULTS AND DISCUSSION

In this report, we documented a total of four pit viper species from Manipur (Image 1), and we established their identity based on the combination of mitochondrial cyt b gene and their respective morphological characters, including key diagnostic features from relevant published literature (e.g., Guo et al. 2009; Zambre et al. 2009; Yang et al. 2011; Rathee et al. 2022; Mirza et al. 2020, 2023).

Trimeresurus mayaae Rathee et al., 2022 (Image 2)

The recently discovered Maya's Pit Viper, *T. mayaae* is a species genetically close to *T. medoensis* as well as morphologically similar to *T. gumprechti* of Indochina

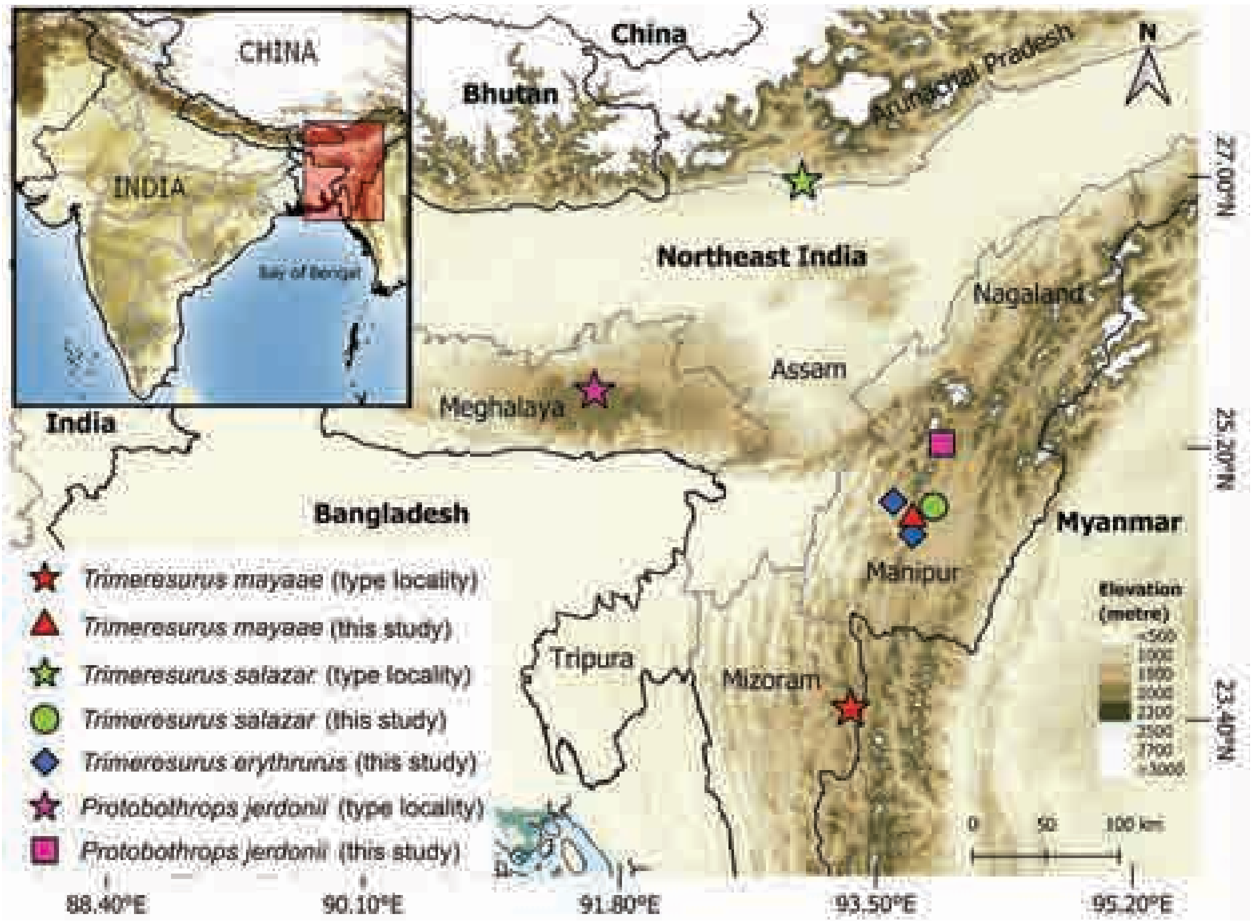

Image 1. Map showing the type localities and new collection sites of the four pit vipers in Manipur, India: *Trimeresurus mayaae* are shown in red star (type locality) and red triangle (this study); *T. salazar* in green star (type locality) and green circle (this study); *T. erythrurus* in blue diamonds (this study); *Protobothrops jerdonii* in purple star (type locality) and purple square (this study).

(Rathee et al. 2022 and references therein). Not much is known on the biology of this cryptic species, and its description is based on specimens originating from the Northeast Indian States of Mizoram and Meghalaya. This species, in fact represents the third new species of the genus from India, to be described in the past three years (Rathee et al. 2022).

On 17 July 2022, we collected both male (MZMU2971; Image 2a) and female (MZMU2970; Image 2b) individuals of *T. mayaae* from Sadu Chiru area nearby a waterfall in Kangpokpi District, Manipur (24.740°N, 93.747°E; 1,139 m). A female individual was found resting on top of tree branch at 2211 h and a male individual was found crawling on the ground about 6 m away from where a female was collected at 2237 h. The microhabitat of the collection site was predominantly covered by thorny shrubs like *Solanum incanum*, and bamboos such as *Melocanna bacifera* and *Chimonobambusa callosa.* These specimens were morphologically examined in the field and concorded to the descriptions and diagnostic keys of *Trimeresurus mayaae* fide Rathee et al. (2022). The two newly collected specimens are characterized by the absence of red and white post-ocular stripe in both specimens; iris greenish around the periphery and adjoined by a prominent rust color around the center in male, while it is greenish with partially blended by a rust color in female; snout-vent length (SVL) 430 mm in male, and 310 mm in female; tail length (TaL) 97 mm in male, and 61 mm in female; dorsal scale row (DSR) 19:19:15 in male, 21:19:15 in female; Ve 154 in male, 152 in female; Sc 58 in male, 53 in female; inter-orbital distance (IOD) measured in scale are 7 in male, and 8 in female; supralabials (SL) 9/9 (left/right) in male, 10/9 in female; infralabials (IL) 10/11 in male, 10/10 in female, and the first pair contact with each other. The new specimens updated the key diagnostic features in the lower limit of Ve in male i.e., 154 vs. 157–162 (Rathee et al. 2022) and female i.e., 152 vs. 153 (Rathee et al. 2022), and also in the lower limit of Sc in female i.e., 53 vs. 54–55 (Rathee et al. 2022). These specimens also represent the first record of the species from the Manipur State and extend the known distribution range by ca. 146 km aerial distance north-eastward from the type locality in Champhai, Mizoram and ca. 209 km aerial distance towards SE from the paratypes locality in Umroi, Meghalaya (Rathee et al. 2022) (Image 1).

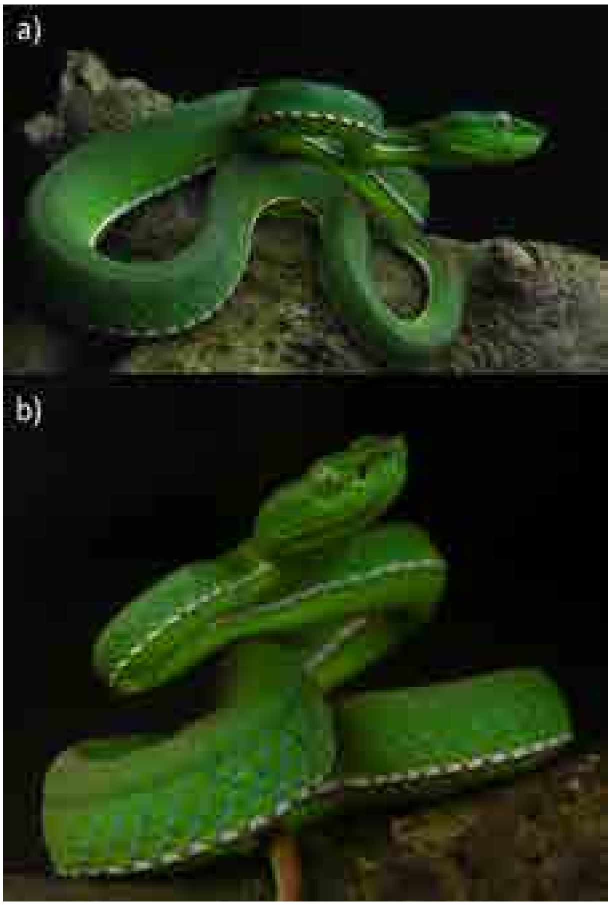

Image 2 . *Trimeresurus mayaae in* life from Sadu Chiru area nearby a waterfall in Kangpokpi District, Manipur: a—Male (MZMU2971) | b—female (MZMU2970). © Parag Shinde

Despite the fact that we are utilizing a single gene (cyt b) in this study, our reconstructed BI phylogram of *Trimeresurus* species considerably concorded to the tree topology, particularly on the clades of the subgenus *"Trimeresurus"* and *"Viridovipera"* fide Mirza et al. (2023) where they combined four mitochondrial genes (cyt b, 16S rRNA, 12S rRNA, and ND4). Accordingly, our cyt b gene tree depicted the new *T. mayaae* specimen (MZMU2970; Accession No. OQ968476) clustering alongside the type series of the species by a well-supported Bayesian posterior probability support (PP = 0.99), and is nested with the holotype from Mizoram (PP=1.00) (Figure 1). Moreover, the specimen (MZMU2970) is showing 0.0–0.7% intraspecific genetic distance with respect to the type series, and the least genetic distance was seen with the holotype (0.0%) from Mizoram, India (see Table S1).

Trimeresurus salazar Mirza et al., 2020 (Image 3)

The Salazar's Pit Viper, *T. salazar* is one of the recently discovered *Trimeresurus* in the past three years. The species has been confirmed so far from central-southern Nepal, Southern Bhutan (Vogel et al. 2022), Northeast Indian States of Arunachal Pradesh (type locality), Assam (Mirza et al. 2020), Meghalaya (Rathee

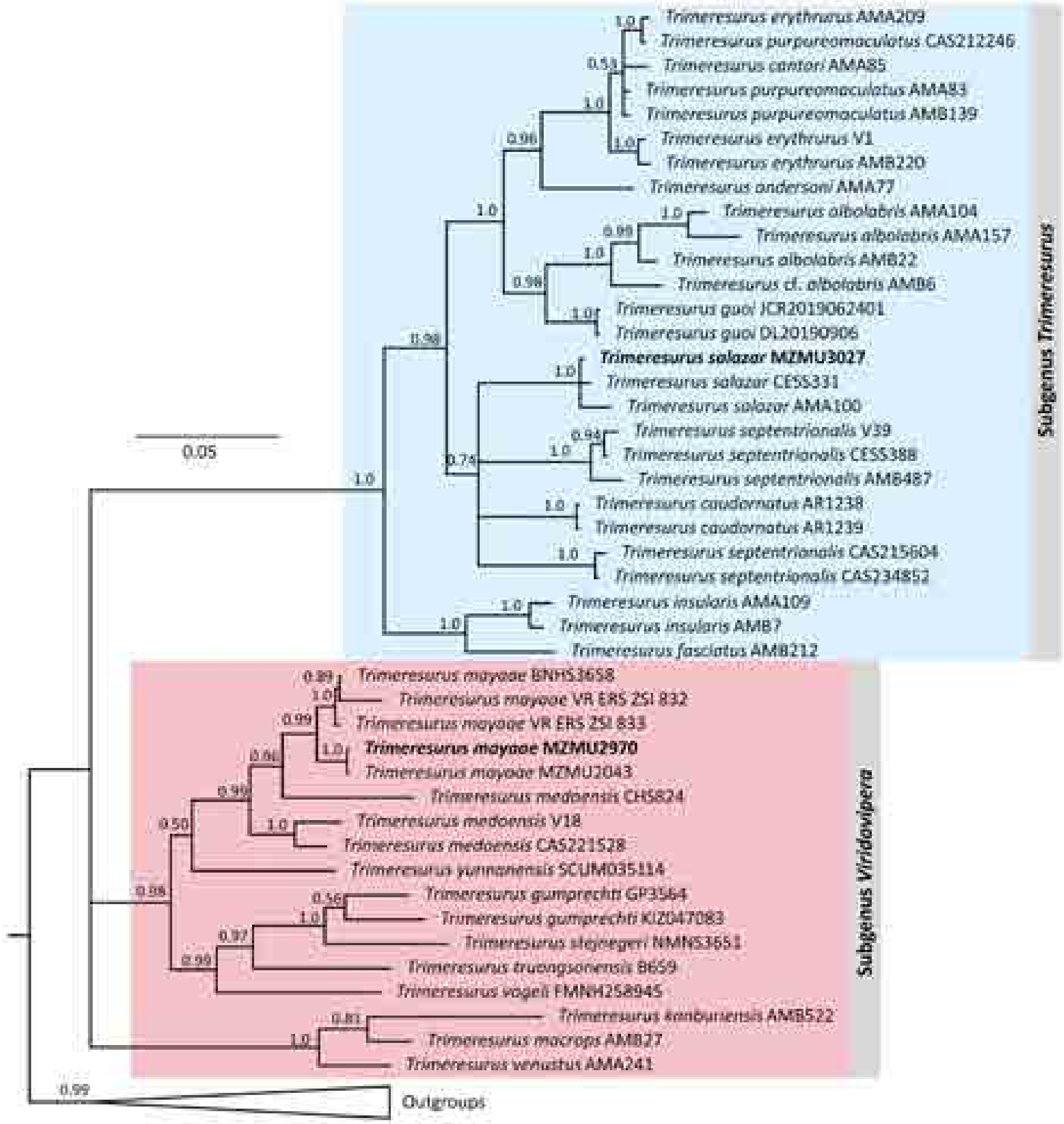

Figure 1. Bayesian inference phylogenetic tree inferred using mitochondrial cytochrome b gene of *Trimeresurus* species. The clades consisting the subgenera of *Trimeresurus* and *Viridovipera* fide Mirza et al. (2023) are shaded in blue and red, respectively. Numbers at the nodes represent the Bayesian posterior probability (PP) values. Sequences generated in this study are given in bold.

et al. 2021), Sikkim, Nagaland, and the other Indian States of Madhya Pradesh, Jharkhand, Chhattisgarh, Bihar and Northern West Bengal (Vogel et al. 2022). The species is distinguished from the congeners by the combined evidence of mitochondrial 16S and ND4 genes, external morphology, dentary and hemipenial morphology (Mirza et al. 2020).

On 17 October 2022 at ca. 0900 h, we encountered and captured a male individual coiled among bushes of tomato *Solanum lycopersicum* inside a plantation surrounded by *Mangifera indica*, *Lantana camara*, *Psidium guajava*, and *Melocanna bacifera* at the foothills of Langol Hill Reserve Forest, near the villages of Lamdeng and Khundi, Imphal West District, Manipur (25.227262°N, 93.939305°E; 2,170 m). The new specimen is collected ca. 253 km aerial distance southeastward from the type locality at Pakke Tiger Reserve, Arunachal Pradesh (Mirza et al. 2020) (Image 1). The other nearest occurrence record of the species is most likely from Hamren, Karbi Anglong District in Assam (CESS604) ca. 174 km aerial distance north-westward from the new collection site. Previously, the locality for CESS604 was mistakenly provided as "Aizawl, Mizoram, India" by Vogel et al. (2022) while it was actually captured from the Assam-Meghalaya interstate border road (25.829°N, 92.556°E, 449 m) (Lalnunhlua pers. comm.), and this clarification warrants the removal of Mizoram as part of the known range of *T. salazar* until there is further confirmatory work. The snake remained very active while capturing and under temporary captivity for morphological examination. Based on the morphological characters, we identified the specimen as a male *T. salazar* (MZMU3027) in agreement with literature mentioning diagnostic keys (Mirza et al. 2020; Rathee et al. 2020; Vogel et al. 2022). We obtained the following morphological attributes: SVL 475 mm; TaL 110 mm; DSR 21:19:15; Ve 165, Sc 65; IOD 12; SL 11/11, and the first SL fused with nasal scale; IL 12/12, and the first pair in contact with each other. Although the lateral stripe on head, one of the taxon's male key features fide Mirza et al. (2020) is not evident in our specimen, we considered the particular character as a phenotypically plastic trait as seen in *T. mayaae* (Rathee et al. 2022) and *T. popeiorum* (Mirza et al. 2023) rather than sexually dimorphic trait, and also in consideration of the low sampling size in the original description (three

Image 3. Male *Trimeresurus salazar* (MZMU3027) in life from Langol hill Reserve Forest, Imphal West District, Manipur. Inset showing the antero-lateral side of the head. © Premjit Singh Elangbam

Image 4. a—Uncollected female *Trimeresurus erythrurus* in life from Bishnupur District, Manipur | b—uncollected road killed *T. erythrurus from* Noney town, Noney District, Manipur. Inset showing the lateral view of the head. © Premjit Singh Elangbam

males and one female) (See Mirza et al. 2020). Even so, with respect to *T. erythrurus*, the species that usually lacks the red postocular stripe in males, the specimen (MZMU3027) is distinct in having 19 mid-dorsal scale rows vs. 23 in *T. erythrurus* (Mirza et al. 2020).

Our cyt b based BI phylogram also depicted a subclade of *salazar + septentrionalis +caudornatus* that was conceived by Mirza et al. (2023); and the new *T. salazar* specimen (MZMU3027; Accession No. OQ968477) clustered with the conspecies from Meghalaya, India (CESS331) and Nepal (AM A100) with a well-supported branch (PP = 1.00) (Figure 1). We uncovered 0.2–0.5% intraspecific genetic distance among *T. salazar* samples, and our sample shows 0.2% genetic distance with respect to both of them (see Table S1).

Trimeresurus erythrurus (Cantor, 1839) (Image 4)

The Red-tailed Pit Viper, *T. erythrurus* was originally described from the "Delta Gangeticum" which is apparently from the Indian Sunderbans of West Bengal state in the present time fide Deuti et al. (2021). The species is widely distributed in parts of Southern Asia including Bhutan (Wangyal 2014; Deuti et al. 2021 and references therein), Nepal, Bangladesh, Myanmar (Mahony et al. 2009; Wallach et al. 2014; Chan et al. 2022), and the Indian states of Andhra Pradesh (Kakinada), West Bengal (Deuti et al. 2021), and Northeast India viz. Arunachal Pradesh, Assam, Mizoram, Nagaland, and Sikkim (Whitaker & Captain 2008; Wallach et al. 2014); possibly in Meghalaya and Manipur (see Wallach et al. 2014).

On 23 July 2022, at ca. 0700 h, we documented a live individual of *T. erythrurus* from a forest trail at Bishnupur District, Manipur (24.632°N, 93.746°E; 920 m; Image 1) ca. 500 km aerial distance northeast from the type locality in Sunderbans (Deuti et al. 2021). Seeing the bulged abdomen, it was evident that the snake had recently fed, possibly upon small mammal or a bird (Image 4a). Morphologically, we identified the snake as female *T. erythrurus* in having golden-yellow colored eye; greenish dorsum; white lateral stripe on the head and the ventrolateral body; total length of 595 mm; Ve 168; Sc 51; and 23 mid-dorsal scale which are in agreement with the published diagnostic characters of the species (Chen et al. 2020; Mirza et al. 2020; Deuti et al. 2021). We subsequently released back the snake into the wild after taking measurements and scalation data. On 4 November 2022, at ca. 1100 h, we encountered additional uncollected road killed male individual at Noney town, Noney District, Manipur (24.851°N, 93.617°E; 510 m asl.) ca. 28 km aerial distance northwest from the point of our observation of the live individual. As the snake was badly damaged and large amount of the scales were eaten off by ants, we could barely examine the snake but nevertheless, obtained the key features like white lateral stripe on the head and ventrolateral body, 23 mid-dorsal scale rows, and Ve 158 (Image 4b). Given that we could not obtain genetic data for this species in Manipur, we advocate DNA studies on the Manipur population to further corroborate the present report.

Protobothrops jerdonii (Günther, 1875) (Image 5)

The widely distributed *P. jerdonii* is presently found in southwestern China, northeastern India, Nepal, northern Myanmar as well as northern Vietnam (see Guo

Image 5. Male *Protobothrops jerdonii* (MZMU3011) in life from Chawangkining, near Zaimeng Lake, Kangpokpi District, Manipur. © Parag Shinde

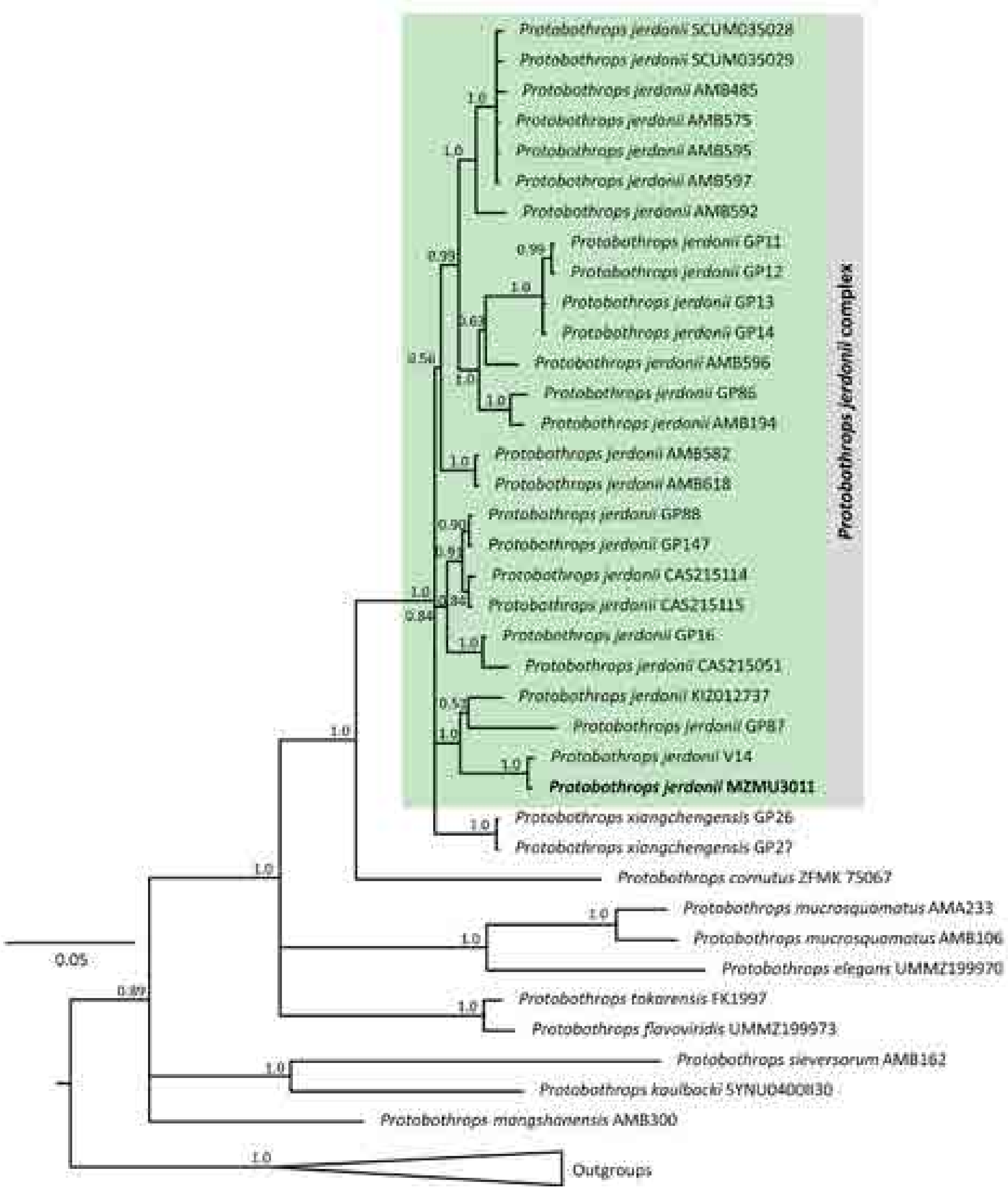

Figure 2. Bayesian inference phylogenetic tree inferred using mitochondrial cytochrome b gene of *Protobothrops* species. Numbers at the nodes represent the Bayesian posterior probability (PP) values. Sequence generated in this study are given in bold.

et al. 2009). The high level of variation in their ecology as well as morphology, particularly in the scale counts and coloration has led to a controversial intraspecific taxonomic treatment within *P. jerdonii* (Guo et al. 2009). This has led some authors to recognise the species as monotypic without any subspecies, while certain other authors treat the taxon to be polytypic (see Guo et al. 2009) with a total of five species or subspecies proposed so far within the *P. jerdonii* complex. They are, apart from the nominotypical form *jerdonii* Günther, 1875; *xanthomelas* Günther, 1889; *melli* Vogt, 1922; *meridionalis* Bourret, 1935; and *bourreti* Klemmer, 1963. Among these, only *jerdonii*, *bourreti* and *xanthomelas* were considered as valid subspecies by several authors like Hoge & Romano-Hoge (1983), Golay et al. (1993), Orlov et al. (2001), and Gumprecht et al. (2004).

On 18 July 2022 at 1949 h, we encountered the specimen (MZMU3011) at Chawangkining village nearby Zaimeng Lake, Kangpokpi District, Manipur (25.204°N, 93.940°E; 1,725 m). The snake was captured while crawling across a forest track covered by herbs and thorny shrubs like *Mikania micrantha*, *Mussaenda* spp., *Girardinia diversifolia*, and *Rubus* spp., and surrounded by bamboos such as *Melocanna bacifera* and *Chimonobambusa callosa.* The collection site of the new specimen is situated ca. 235 km aerial distance towards east from the type locality in Khasi Hill, Meghalaya fide Wallach et al. (2014) (Image 1). In comparing the descriptions and diagnostic keys from literature (e.g., Zambre et al. 2009; Yang et al. 2011), we identified the specimen as male *P. jerdonii* in having SVL 414 mm; TaL 77; Ve 171; Sc 66; SL 7/8; IF 11/11; IOD 8; DSR 21:21:16. In considering the subspecies recognized by Gumprecht et al. (2004), the Ve of our specimen (171) falls within the range of *P. jerdonii jerdonii* (160–170) fide Yang et al. (2011), the Sc of our specimen (66) enter the Sc range of both *P. jerdonii xanthomelas* (54–67) and *P. jerdonii bourreti* (65–72) fide Yang et al. (2011). Moreover, the basal body color of our specimen is yellowish brown, and a series of rhomboidal or irregularly shaped reddish-brown patches with black margins are present along the dorsal body. Based on the morphological data alone, we cannot certainly assign the new specimen (MZMU3011) to any of the three nominal subspecies following the recent most diagnostic keys fide Yang et al. (2011). Thus, we for now refrain from making subspecies level taxonomic identification for the specimen.

Our reconstructed cyt b gene tree also depicted paraphyly amongst *P. jerdonii* as conceived by Guo et al. (2009) where they sequenced four mitochondrial genes (cyt b, 12S rRNA, 16S rRNA, and ND4). Our sample (MZMU3011; Accession No. OQ968475) nested alongside the conspecific sequences from Northeast India (V14) with a high branch support (PP = 1.00), and these two formed a subclade with the other samples from China (GP87 and KIZ012737) with high support (PP = 1.00) (Figure 2). Our estimated genetic distance also showed 0.0–3.3% intraspecific distance between ours and the other *P. jerdonii* samples; the least genetic distance (0.0%) is seen with the Northeast Indian sample while the highest (3.3%) is seen with respect to the samples from Huili, Sichuan, China (GP11–GP14) (see Table S2).

CONCLUSION

As per the current knowledge, only *O. monticola* has been listed as present in Manipur (Whitaker & Captain 2008). In this work, we report the occurrence of four new records of pit vipers in Manipur for the first time, and simultaneously provide new information on their respective geographical distribution ranges. Considering the topology in our phylogenetic reconstruction and our *P. jerdonii* sample (MZMU3011) that disclosed the overlapping of the conventional taxonomic keys for the subspecies fide Yang et al. (2011), we are convinced that our study corroborated the taxonomic statement on the species by Guo et al. (2009) where they considered *P. jerdonii* as a monotypic and paraphyletic species. In the scenario of *T. mayaae*, both ours and the published phylogenies (Rathee et al. 2022; Mirza et al. 2023) depicting the nesting of *T. mayaae* next to *T. medoensis*. Nonetheless, combining the meristic data of the type series (Rathee et al. 2022) and the new specimens, *T. mayaae* is morphologically distinct in terms of the Ve counts of male i.e., 154–162 vs. 138–149 in *T. medoensis*, and female i.e., 152–153 vs. 141–143 in *T. medoensis*; also in the mid-dorsal scale row i.e., 19–21 in *T. mayaae* vs. 17 in *T. medoensis*.

The present work, however, provides evidence for the occurrence of four additional species of pit vipers in Manipur for the first time, and simultaneously provides new information on their respective geographical distribution ranges. Thus, currently, five species of pit vipers including *P. jerdoni*, *T. salazar*, *T. mayaae*, *T. erythrurus*, and *O. monticola* are known from Manipur (Whitaker & Captain 2004; this work). We emphasise that this new data on the occurrence of four more species of pit vipers in Manipur may be disseminated well in health care sectors. This is so because these venomous snakes may be of medical importance in terms of potential snakebites on humans (e.g., Kanwar 2018; Rai et al. 2021; Ravikar et al. 2023), more so in forest fringe areas.

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COMMUNICATION

Addition to the Odonata fauna of Tripura, India

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Abstract: The present study was conducted in nine different locations (forested areas and unclassified natural areas) of six administrative districts of Tripura State from March 2012 to May 2019 as part of the biodiversity exploration of the state for further addition of odonate fauna. In this communication, we report six species of Anisoptera and seven species of Zygoptera as new records from Tripura State. Among these 13 newly recorded species, one Anisoptera and two Zygoptera species are Data Deficient and the rest are Least Concern according to IUCN Red List.

Keywords: Damselfly, dragonfly, Insecta, amphibiotic, habitat preference, Indo-Burma biodiversity hotspot, range extension, data deficient, regional species pool, IUCN Red List.

Abbreviations: TU—Tripura University | ODO—Odonata | AESH—Aeshnidae | GOMP—Gomphidae | LIBE—Libellulidae | EUPH—Euphaeidae | PLAT—Platycnemididae | COEN—Coenagrionidae| IMG XXXX (4 digits)—raw image number of photograph.

Bengali: বর্তমান অধ্যয়নটি ত্রিপুরা রাজ্যের ছয়টি জেলার নয়টি ভিন্ন স্থানে (বনাঞ্চল এলাকায় এবং অশ্রেণীবদ্ধ প্রাকৃতিক এলাকায়) করা হয়েছে মার্চ ২০১২ থেকে মে ২০১৯ পর্যন্ত, রাজ্যের জীববৈচিত্র্য অন্বেষণের অংশ হিসাবে আরও নতুন প্রজাতির ফড়িং রাজ্যের 'ফড়িং তালিকায়' যোগ করার জন্য। এই কমিউনিকেশনে, আমরা ত্রিপুরা রাজ্যের 'ফড়িং তালিকায়' Anisoptera-র ছয়টি প্রজাতি এবং Zygoptera-র সাতটি প্রজাতি নিবন্ধন করি। IUCN রেড লিস্ট অনুযায়ী, এই ১৩টি নতুন নিবন্ধীকৃত প্রজাতির মধ্যে, একটি Anisoptera এবং দুটি Zygoptera প্রজাতি Data Deficient এবং বাকিগুলি Least Concern.

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Author contributions: DD and JM have collected the data and prepared the manuscript; BKA reviewed the manuscript and also allowed using laboratory facilities.

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INTRODUCTION

The insect order Odonata comprises of dragonflies (Anisoptera) and damselflies (Zygoptera). Members of this order are widely distributed, insectivorous, and amphibiotic. Egg and larval stages are aquatic and the adult stage is terrestrial. Odonata insects prefer hilly streams, and forested riparian habitats and most of the endemic and rare species are restricted to these habitats (Andrew et al. 2008, 2009; Subramanian 2009, 2012; Mitra et al. 2010; Subramanian et al. 2011; Koparde et al. 2015). Habitats like ponds, lakes, coastal marshes, natural & man-made water bodies, water logged areas, grass lands, and paddy fields support species richness and diversity (Subramanian 2009, 2012; Mitra et al. 2010; Subramanian et al. 2011; Majumder et al. 2014; Tiple & Khoparde 2015). The eastern India is one of the globally rich areas of odonates, and the forest streams and rivers of this region are natural habitats for many endemic and habitat-specialist species. In the eastern Himalaya, high endemism occurs in Khasi hills and Darjeeling-Sikkim Himalaya (Subramanian & Babu 2017). Tripura province, a small hilly State of northeastern India in Indo-Burma biodiversity hotspot zone (Myers et al. 2000), supports diverse odonate fauna (Clausnitzer et al. 2009) due to habitat diversity and micro habitat richness.

Of the 6,392 odonate species recorded across the world (Paulson et al. 2022), 488 are known from India (Subramanian & Babu 2017). India's odonate fauna include 186 species/ subspecies as endemic (Subramanian & Babu 2017). Lahiri (1977, 1987) made notable contributions to odonates of Meghalaya and Manipur (parts of north eastern India). Mitra (2002) did the most comprehensive work on odonates of north eastern India, including a review of all the literature available till then. Joshi & Kunte (2014) reported 69 species including one addition (*Calicnemia erythromelas* Selys, 1891) to the Indian Odonata fauna from Nagaland. Joshi et al. (2017) reported *Pseudothemis zonata* (Burmeister, 1839) and *Burmagomphus divaricatus* (Lieftinck, 1964) for the first time from Nagaland and Manipur, respectively. In Tripura, Srivastava & Sinha (2000) reported 35 species and Majumder et al. (2014) reported 53 species. In this paper, we report our findings on Odonates from Tripura State.

METHODS

Study area

The present study was conducted at nine different locations (forested and unclassified habitats, Table: 1) of Tripura, from March 2012–May 2019 as a part of biodiversity exploration study. Geographically, Tripura lies between 22.9333°N—24.53333°N & 91.1666°E—92.35°E, and in the meeting point of two biodiversity hotspots (Eastern-Himalaya hotspot in the east and Indo-Myanmar hotspot region in the west) (Myers et al. 2000). This small north-eastern hilly state (Tripura) has a tropical savanna climate and receives an annual rainfall ranges from 1800–2400 mm approximately; south-west monsoon spread over seven months almost (April–October) and average minimum temperatures are around 20–25 ^{0}C, maximum temperatures are around 30–35 ^{0}C with elevation range varying from 15 m (lowest) to 930 m (highest).

Field sampling and Identification

In this opportunistic study, field observations and photography of odonates were conducted mainly on clear sunny days between 08:00 h and 12:00 h during monsoon season to record maximum species of odonates (Majumder et al. 2014). Species were recorded though random walks along potential habitats like streams, bamboo forest, small forest patch, manufactured water bodies, and deep forest habitats and followed direct search technique (Sutherland 1996) and sighting is opportunistic. Comet optical binoculars 8 x 40 NV JL 77888 were used during field observations and photographs were taken by Canon EOS 50D, Canon Power Shot SX 530 HS, Canon Power Shot SX 200 IS, and SONY DSC-HX 200V camera models and YU 5010A mobile device. At first sight, photographs were taken and individuals were identified with the help of reference books (mentioned below in identification part). If only photography was not enough for identification, then specimens were captured and these were released back to their respective habitats, after visual observation and photographic identification in field was successful. In cases where field identification was not enough, then effort was made to collect at least one individual with insect net for accurate identification in the Ecology & Biodiversity Laboratory of Tripura University under permission from the authority of Tripura Biodiversity Board, Government of Tripura [letter no. F. 22/3(6)/for JBIC/I&P/B-D/07/2336 and 4972-76 dated 07.01.2009 and 29.06.2009], respectively. Morphological characters for identification of unidentified species were studied

Image 1. Google Earth image of India showing the Tripura state and different study sites of six administrative districts.

Table 1. Geoco-ordinates of the study sites.

District	Study sites	Latitude (N)	Longitude (E)	Elevation (meter)	Habitat types
Dhalai	Saikabari (SB); Study site-1	24.12^0	91.90^0	123	Perennial stream surrounded by moist-deciduous secondary forest
Gomati	Chabimura(CM); Study site-2	23.54^0	91.60^0	47	Riparian woody forest
	Dhajanagar (DN); Study site-3	23.54^0	91.46^0	107	Man-made freshwater pond
Khowai	Baramura Hill (BH); Study site-4	23.81^0	91.57^0	44	Secondary mixed-moist deciduous forest nearby small stream
	Sonatala (ST); Study site-5	24.04^0	91.61^0	33	Slow flowing drain water surrounded by herbs and shrubs
North Tripura	Jampui Hill (JH); Study site-6	23.99^0	92.28^0	176	Slow flowing stream surrounded by semi-evergreen forest
South Tripura	Trishna Wildlife Sanctuary (TWS); Study site-7	23.25^0	91.37^0	45	Natural freshwater lakes with submerged vegetation
West Tripura	Amtali (AT); Study site-8	23.77^0	91.26^0	27	Secondary scrub jungles
	Ishan Chandra Nagar (ICN); Study site-9	23.75^0	91.25^0	25	Man-made freshwater pond surrounded by scrub jungles

in laboratory using Zeiss Stemi 508 stereo zoom 8:1 microscope. Photographs of the observed habitat types explored in this study, helped in recording the habitat preference of different odonates.

Identification of recorded specimens was done with the help of identification keys provided by Fraser (1933, 1934, 1936); Mitra (2002); Subramanian (2009, 2014); Nair (2011), and with online databases, <www.

indianodonata.org> (Joshi et al. 2019)**.** The taxonomic list follows widely accepted systematics of Odonata by Dijkstra et al. (2013) and Subramanian & Babu (2017). Recordings of location parameters such as latitude, longitude, and elevation were done with the help of Garmin eTrex Vista GPS device. Conservation status of the recorded odonates of this study was according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN 2020).

RESULTS

Thirteen species of Odonata are reported as new records and added to the Odonata fauna of Tripura from this study. Of these, six dragonfly species belong to six different genera under three families, namely Aeshnidae, Gomphidae, Libellulidae, and seven damselfly species belonging to seven different genera under three families, namely Coenagrionidae, Euphaeidae, and Platycnemididae (bold marked species of Appendix 1). A detailed account of findings is given below:

Suborder: Anisoptera Selys, 1854

Family: Aeshnidae Rambur, 1842

1. *Periaeschna magdalena* (Martin 1909) (Image 3.1)

Material examined: 2 males (TU/ODO/AESH/IMG0358), 30.iii.2012, Chabimura, Photo: J. Majumder;1 male (TU/ODO/AESH/IMG0002), 22.xi.2012, Jampui Hill, Photo: J. Majumder.

Habitat: Slow flowing streams surrounded by woody riparian forest.

IUCN Red List status: Least Concern (Dow 2009)

Distribution: Bhutan, China, India and Viet Nam.

Comments: Genus *Periaeschna* and species *P. magdalena* are reported for the first time from Tripura. Fraser (1936) reported this species from Garo Hills, Meghalaya which is approximately 255 km and 265 km (in aerial distance) far from Chabimura (study site-2) and Jampui hills (study site-6), respectively.

Family: Gomphidae Rambur, 1842

2. *Macrogomphus montanus* Selys, 1869 (Image 3.2)

Material examined: 1 female (ODO/GOMP/IMG 6290), 30.v.2019, Sonatala, Photo: D. Datta.

Habitat: Dry shrub jungle near to human habitation.

IUCN Red List Status**:** Data Deficient (Subramanian 2010)

Distribution: Bangladesh, India, and Nepal.

Comments: Genus *Macrogomphus* and species *M. montanus* are reported for the first time from Tripura. Earlier, Fraser (1936) reported this species from Assam and Khan (2018) reported it from different locations including Khagrachari (approximately 110 km far from Sonatala, Study site- 5) from the Chittagong Division, southeastern Bangladesh.

Family: Libellulidae Rambur, 1842

3. *Tetrathemis platyptera* Selys, 1878 (Image 3.3)

Material examined: 1 male (TU/ODO/LIBE/IMG0001), 09.vi.2013, Dhajanagar, Photo: J.Majumder.

Habitat: Man-made freshwater pond surrounded by secondary vegetation.

IUCN Red List Status: Least Concern (Dow 2020)

Distribution: China, India, Indonesia, Lao People's Democratic Republic, Peninsular Malaysia, Myanmar, Thailand and Viet Nam.

Comments: Genus *Tetrathemis* and species *T. platyptera* are reported for the first time from Tripura. Earlier, Laltanpuii (2017) reported this species from different locations including Reiek (approximately 110 Km far from Dhajanagar, Study site-3) of Mamit District, Mizoram.

4. *Tramea limbata* Desjardins, 1832 (Image 3.4)

Material examined: 1 male (TU/ODO/LIBE/DSC02352), 01.xi.2013, Trishna WLS, Photo: J. Majumder.

Habitat: Natural freshwater lake with submerged vegetation.

IUCN Red List Status: Least Concern (Clausnitzer 2016)

Distribution: Botswana, Cameroon, Cape Verde, The Democratic Republic of the Congo, Cote d'Ivoire, Gabon, India, Liberia, Mali, Mauritius main island, Mayotte, Nepal, Nigeria, Oman, South Africa, Sri Lanka, Yemen and Namibia.

Comments: Genus *Tramea* and species *T. limbata* are reported for the first time from Tripura. Earlier, Laltanpuii (2017) reported this species from different locations including Lengpui (approximately 130 Km far from Trishna WLS, Study site- 7) of Aizawl District, Mizoram.

5. *Trithemis festiva* Rambur, 1842 (Image 3.5)

Material examined: 2 male (TU/ODO/LIBE/IMG0712), 12.vii.2016 Baramura Hill, Photo: D. Datta

Habitat: Slow flowing streamlets surrounded by secondary mixed moist deciduous forest

IUCN Red List Status: Least Concern (Dow 2009)

Distribution: Afghanistan, Cambodia, China, Cyprus, Greece, Hong Kong, India, Indonesia, Iran, Iraq, Lao People's Democratic Republic, Malaysia, Myanmar, Nepal, Pakistan, Papua New Guinea, Philippines, Russian Federation, Singapore, Sri Lanka, Taiwan, Province of

China, Thailand, Turkey, and Viet Nam.

Comments: Species *T. festiva* is reported for the first time from Tripura. Earlier, Rajesh Sanap recorded this species from Hatikuli Tea Estate, Golaghat District, Assam (posted in Odonata of India), approximately 600 Km far from Baramura Hills, Study site- 4.

6. *Zyxomma petiolatum* Rambur, 1842 (Image 3.6)

Material examined: 1 female (TU/ODO/LIBE/IMG2208); 03.viii.2017, Sonatala, Photo: D. Datta; 1 male (TU/ODO/LIBE/IMG0571), 24.iv.2018, Sonatala, Photo: D. Datta

Habitat: Shady areas with big trees and scrub jungles.

IUCN Red List Status: Least Concern (Subramanian & Dow 2017)

Distribution: Australia, Bangladesh, Brunei Darussalam, China, Hong Kong, India, Indonesia, Japan, Lao People's Democratic Republic, Malaysia, Myanmar, Nepal, New Caledonia, Palau, Papua New Guinea, Philippines, Seychelles, Singapore, Sri Lanka, Taiwan, Province of China, Thailand, Vanuatu, and Viet Nam.

Comments: Genus *Zyxomma* and species *Z. petiolatum* are reported for the first time from Tripura. Earlier, Takhelmayum & Gupta (2014) reported this species from Keibul Lamjao National Park (KLNP), Manipur, approximately 230 km far from Sonatala (Study site-5).

Suborder: Zygoptera Selys, 1854
Family: Coenagrionidae Kirby, 1890
7. *Aciagrion occidentale* Laidlaw, 1919 (Image 4.1)

Material examined: 1 female (TU/ODO/COEN/DSC00945), 13.viii.2013, Saikabari, Photo: J. Majumder; 1 female (TU/ODO/COEN/IMG0003), 11.viii.2013, Amtali, Photo: J. Majumder.

Habitat: Slow flowing small perennial streams surrounded by secondary bushy forest

IUCN Red List Status: Least Concern (Mitra 2010)

Distribution: Bangladesh, Cambodia, India, Sri Lanka, Thailand and Viet Nam.

Comments: *A. occidentale* is reported for the first time from Tripura. Earlier, Bora & Meitei (2014) reported this species from the ICAR Research Complex for NEH Region, Umaim, Meghalaya, which is approximately 170 Km and 220 Km far from Saikabari (study site-1) and Amtali (study site-8), respectively.

8. *Argiocnemis rubescens* Selys, 1877 (Image 4.2)

Material examined: 1 male (TU/ODO/COEN/IMG0158), 29.iii.2012, Chabimura, Photo: J. Majumder.

Habitat: Stagnant marshy water body with submerged vegetation and surrounded by scrub jungles.

IUCN Red List Status: Least Concern (Mitra 2017)

Distribution: Australia, Bangladesh, Brunei Darussalam, India, Indonesia, Lao People's Democratic Republic, Malaysia, Myanmar, Papua New Guinea, Philippines, Singapore, Thailand and Vietnam.

Comments: *A. rubescens*is reported first time from Tripura. Earlier, Boruah et al. (2016) reported this species from Kaziranga National Park (KNP), Assam, approximately 360 km far from Chabimura (study site-2).

9. *Mortonagrion aborense* Laidlaw, 1914 (Image 4.3)

Material examined: 2 females (TU/ODO/COEN/DSC04897), 15.iii.2013, IC Nagar, Photo: J. Majumder.

Habitat: Freshwater pond surrounded by shrubs and bamboo brakes.

IUCN Red List Status: Least Concern (Subramanian 2010)

Distribution: Bangladesh, India, Indonesia, Malaysia, and Thailand.

Comments: Genus *Mortonagrion* and species *M. aborense* are reported for the first time from Tripura. Earlier, Boruah & Saikia (2015) reported this species from Barpeta, Assam, approximately 285 Km far from IC Nagar (study site-9).

Family: Euphaeidae Jacobson & Bianchi, 1905
10. *Dysphaea walli* Fraser, 1927 (Image 4.4)

Material examined: 2 males (TU/ODO/EUPH/IMG8754), 17.vi.2013, Chabimura, Photo: J. Majumder.

Habitat: Alongside riverbank, surrounded by mature riparian mixed-moist deciduous forest.

IUCN Red List Status: Data Deficient (Dow 2019)

Distribution: Bangladesh, India and Myanmar.

Comments: Genus *Dysphaea* and species *D. walli* are reported for the first time from Tripura. An earlier observation (Anonymous 2020a) (www.indianodonata.org) by Somen Sarkar reported the species from Jeypore-Dehing Rain Forest, Dibrugarh District, Assam, approximately 570 km far from Chabimura (study site-2).

Family: Platycnemididae Tillyard, 1917
11. *Elattoneura campioni* Fraser, 1922 (Image 4.5)

Material examined: 2 females (TU/ODO/PLAT/DSC00750), 13.viii.2013, Saikabari, Photo: J. Majumder.

Habitat: Free flowing stream surrounded by mature secondary squashy semi-evergreen forest.

IUCN Red List Status: Data Deficient (Sharma &Dow 2010)

Distribution: India and Myanmar

Comments: Genus *Elattoneura* and species *E.*

campioni are reported for the first time from Tripura. Earlier, Boruah et al. (2016) reported this species from Kaziranga National Park (KNP), approximately 290 km far from Saikabari (study site-1).

12. *Prodasineura verticalis* Fraser, 1921 (Image 4.6)

Material examined: 2 males (TU/ODO/PLAT/DSC00579), 10.viii.2013, Saikabari, Photo: J. Majumder.

Habitat: Free flowing stream surrounded by secondary mixed-moist deciduous forest.

IUCN Red List Status: Least Concern (Dow 2010)

Distribution: Bangladesh, Brunei Darussalam, China, India, Indonesia, Lao People's Democratic Republic, Malaysia, Myanmar, and Thailand.

Comments: Genus *Periaeschna* and species *P. magdalena* are reported for the first time from Tripura. Earlier, Subramanian (2015) reported this species from northeastern India. An observation (Anonymous 2020b) published in Odonata of India (www.indianodonat.org) website by Rejoice Gassah from Dosdewa Forest Trail, Karimganj District, Assam, approximately 50 km far from Saikabari (study site-1).

13. *Pseudocopera ciliata* Selys, 1863 (Image 4.7 and 4.8)

Material examined: 2 males, (TU/ODO/PLAT/IMG0196), 29.iii.2018, Sonatala, Photo: D. Datta; 1

Image 2. Habitat photographs of study sites: 1—Perennial stream surrounded by moist-deciduous secondary forest (Saikabari, study site-1) | 2—Riparian woody forest (Chabimura, study site-2) | 3—Man-made freshwater pond surrounded by herbs and shrubs (Dhajanagar, study site-3) | 4—Slow flowing streamlet surrounded by secondary mixed moist deciduous forest (Baramura Hill, study site-4) | 5—Slow flowing drain surrounded by herbs and shrubs (Sonatala, study site-5) | 6—Slow flowing stream surrounded by semi-evergreen forest (Jampui Hill, study site-6) | 7—Natural freshwater lake with submerged vegetation (Trishna WS, study site-7) | 8—Secondary scrub jungles (Amtali, study site-8) | 9—Part of a pond surrounded by scrub jungles (IC Nagar, study site-9). © 2, 4, 5, 7—Dhiman Datta | © 1, 3, 6, 8, 9—Joydeb Majumder.

female (TU/ODO/PLAT/IMG6928), 26.v.2016, Sonatala, Photo: D.Datta

Habitat: Slow flowing drain water area near to pond or paddy field.

IUCN Red List Status: Least Concern (Dow 2018)

Distribution: Bangladesh, Cambodia, China, Hong Kong, India, Indonesia, Peninsular Malaysia, Myanmar, Taiwan, Province of China, Thailand and Vietnam.

Comments: Genus *Pseudocopera* and species *P. ciliata* are reported for the first time from Tripura. Earlier, Josh & Kunte (2014) reported this species from Intanki, Peren District, Nagaland which is approximately 255 km far from Sonatala (study site-5). All distances mentioned here are aerial distance between two selective areas.

DISCUSSIONS AND CONCLUSIONS

Before this study, quality field data on distribution and habitat preference of odonates was scanty from most of the eastern Himalayan range, particularly from the southern and eastern parts including the state Tripura of India. Present study is a substantial advancement of the odonate diversity of Tripura over the earlier studies (Srivastava & Sinha 2000; Majumder et al. 2014). After this study with addition of 13 species, the updated odonate fauna of Tripura state is represented by 75 species under 49 genera (28 Anisoptera genera and 21 Zygoptera genera) and nine families (4 Anisoptera and 5 Zygoptera); (Appendix 1). Three odonata species namely, *M. montanus*, *D. walli* and *E. campioni* showed Data Deficient as per IUCN Red List categories of threatened species. Among the 13 species reported here, 12 species are endemic to India except *D. walli* as per Subramanian & Babu (2017). *P. magdalena, Macrogomphus montanus, Tetrathemis platyptera, Tramea limbata, Trithemis festiva, Z. petiolatum, Aciagrion occidentale, Argiocnemis rubescens, Mortonagrion aborense, Elattoneura campioni, Prodasineura verticalis, Pseudocopera ciliata* are endemic to India recorded from this study. Members of *T. festiva* are found commonly near streams and those of *Zyxomma petiolatum* are common in shady areas surrounded by big trees and shrubs. Members of *Aciagrion occidentale, Argiocnemis rubescens, Mortonagrion aborense, P. ciliata* are very common in their respective study sites. However, *P. magdalena, M. montanus, T. platyptera, T. limbata, Dysphaea walli, E. campioni, P. verticalis* are found to be comparatively rare in their study sites. The documenting of the regional species pool from this part of India has benefited the inclusion of previously unrecorded odonate species, which will help future researchers in the understanding of species biology, distributional ranges, and prospective habitats.

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Image 3. Photographs of new records of dragonflies: 1—*Periaeschna magdalena* (male) | 2—*Macrogomphus montanus* (female) | 3—*Tetrathemis platyptera* (male) | 4—*Tramea limbata* (male) | 5—*Trithemis festiva* (male)|6—*Zyxomma petiolatum* (male). © 2, 5, 6—Dhiman Datta | © 1, 3, 4—Joydeb Majumder.

Image 4. Photographs of new records of damselflies: 1—*Aciagrion occidentale* (female) | 2—*Argiocnemis rubescens* (male) | 3—*Mortonagrion aborense* (female) | 4—*Dysphaea walli* (male) | 5—*Ellatoneura campioni* (female) | 6—*Prodasineura verticalis* (male) | 7—*Pseudocopera ciliata* (female) | 8—*Pseudocopera ciliata* (male). © 7, 8—Dhiman Datta | © 1–6—Joydeb Majumder.

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Appendix 1. Updated checklist of dragonflies and damselflies recorded from Tripura. It contains species from all the studies done so far from Tripura (Srivastava & Sinha 2000, Majumder et al. 2014) along with the present study (in bold fonts).

	Scientific name with author and year of description	Family
	Dragonflies	
1	*Gynacantha subinterrupta* Rambur, 1842	Aeshnidae
2	***Periaeschna magdalena*** Martin, 1909	Aeshnidae
3	*Ictinogomphus rapax* Rambur, 1842	Gomphidae
4	***Macrogomphus montanus*** Selys, 1869	Gomphidae
5	*Paragomphus lineatus* Selys,1850	Gomphidae
6	*Acisoma panorpoides* Rambur, 1842	Libellulidae
7	*Aethriamanta brevipennis* Rambur, 1842	Libellulidae
8	*Brachydiplax chalybea* Brauer, 1868	Libellulidae
9	*Brachydiplax farinosa* Krüger, 1902	Libellulidae
10	*Brachydiplax sobrina* Rambur, 1842	Libellulidae
11	*Brachythemis contaminata* Fabricius, 1793	Libellulidae
12	*Camacinia gigantea* Brauer, 1867	Libellulidae
13	*Cratilla lineata* Brauer, 1878	Libellulidae
14	*Crocothemis servilia* Drury, 1773	Libellulidae
15	*Diplacodes nebulosa* Fabricius, 1793	Libellulidae
16	*Diplacodes trivialis* Rambur, 1842	Libellulidae
17	*Lathrecista asiatica* Fabricius, 1798	Libellulidae
18	*Neurothemis intermedia* Ris, 1919	Libellulidae
19	*Neurothemis fulvia* Drury, 1773	Libellulidae
20	*Neurothemis intermedia* Rambur, 1842	Libellulidae
21	*Neurothemis tullia* Drury, 1773	Libellulidae
22	*Onychothemis testacea* Laidlaw, 1902	Libellulidae
23	*Orthetrum brunneum* Fonscolombe, 1837	Libellulidae
24	*Orthetrum chrysis* Selys, 1891	Libellulidae
25	*Orthetrum glaucum* Brauer, 1865	Libellulidae
26	*Orthetrum luzonicum* Brauer, 1868	Libellulidae
27	*Orthetrum pruinosum* Burmeister, 1839	Libellulidae
28	*Orthetrum Sabina* Drury, 1770	Libellulidae
29	*Orthetrum triangulare* Selys, 1878	Libellulidae
30	*Palpopleura sexmaculata* Fabricius, 1787	Libellulidae
31	*Pantala flavescens* Fabricius, 1798	Libellulidae
32	*Potamarcha congener* Rambur, 1842	Libellulidae
33	*Rhodothemis rufa* Rambur, 1842	Libellulidae
34	*Rhyothemis variegata* Linnaeus, 1763	Libellulidae
35	***Tetrathemis platyptera*** Selys, 1878	Libellulidae
36	*Tholymis tillarga* Fabricius, 1798	Libellulidae
37	***Tramea limbata*** Desjardins, 1832	Libellulidae
38	*Trithemis aurora* Burmeister, 1839	Libellulidae
39	***Trithemis festiva*** Rambur, 1842	Libellulidae
40	*Trithemis kirbyi* Selys, 1891	Libellulidae
41	*Trithemis pallidinervis* Kirby, 1889	Libellulidae
42	*Urothemis signata* Rambur, 1842	Libellulidae
43	***Zyxomma petiolatum*** Rambur, 1842	Libellulidae
44	*Epophthalmia vittata* Burmeister, 1839	Macromiidae
	Damselflies	
45	*Neurobasis chinensis* Linnaeus, 1758	Calopterygidae
46	*Vestalis gracilis* Rambur, 1842	Calopterygidae
47	*Vestalis smaragdina* Selys, 1879	Calopterygidae
48	*Aristocypha quadrimaculata* Selys, 1853	Chlorocyphidae
49	*Libellago lineata* Burmeister, 1839	Chlorocyphidae
50	***Aciagrion occidentale*** Laidlaw, 1919	Coenagrionidae
51	*Aciagrion pallidum* Selys, 1891	Coenagrionidae
52	*Agriocnemis femina* Brauer, 1868	Coenagrionidae
53	*Agriocnemis lacteola* Selys, 1877	Coenagrionidae
54	*Agriocnemis pygmaea* Rambur, 1842	Coenagrionidae
55	*Agriocnemis splendidissima* Laidlaw, 1919	Coenagrionidae
56	***Argiocnemis rubescens*** Selys, 1877	Coenagrionidae
57	*Ceriagrion cerinorubellum* Brauer, 1865	Coenagrionidae
58	*Ceriagrion coromandelianum* Fabricius, 1798	Coenagrionidae
59	*Ceriagrion olivaceum* Laidlaw, 1914	Coenagrionidae
60	*Enallagma parvum* Selys, 1876	Coenagrionidae
61	*Ischnura rubilio* Selys, 1876	Coenagrionidae
62	***Mortonagrion aborense*** Laidlaw, 1914	Coenagrionidae
63	*Onychargia atrocyana* Selys, 1865	Coenagrionidae
64	*Pseudagrion australasiae* Selys, 1876	Coenagrionidae
65	*Pseudagrion microcephalum* Rambur, 1842	Coenagrionidae
66	*Pseudagrion rubriceps* Selys, 1876	Coenagrionidae
67	***Dysphaea walli*** Fraser, 1927	Euphaeidae
68	*Euphaea ochracea* Selys, 1859	Euphaeidae
69	*Calicnemia imitans* Lieftinick, 1948	Platycnemididae
70	*Coeliccia didyma* Selys, 1863	Platycnemididae
71	*Copera marginipes* Rambur, 1842	Platycnemididae
72	*Copera vittata* Selys, 1863	Platycnemididae
73	***Elattoneura campioni*** Fraser, 1922	Platycnemididae
74	***Prodasineura verticalis*** Fraser, 1921	Platycnemididae
75	***Pseudocopera ciliata*** Selys, 1863	Platycnemididae

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COMMUNICATION

Occurence and distribution of two new libellulids (Odonata: Insecta) of the Kashmir Valley, India: *Orthetrum sabina* (Drury, 1770) and *Palpopleura sexmacaluta* (Fabricius, 1787)

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Abstract: Odonates from the Kashmir Himalaya have been least studied with only 22 species reported from this region. After a long gap of 41 years, the present work forms the first observations on occurence and distribution of two new odonates from the Kashmir valley. Two dragonflies *Orthetrum sabina* (Drury, 1770) and *Palpopleura sexmaculata* (Fabricius, 1787) belonging to the family Libellulidae are reported for the first time from this region. The findings open new insights about phenology, distribution patterns, behaviour, and the effects of climate change on Himalayan Odonata.

Keywords: Anisoptera, Blue-tailed Yellow Skimmer, Green Marsh Hawk, habitat characterization, Jhelum basin, paddy fields, presence, Zygoptera

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Author contributions: TG designed the study, conducted surveys, data collection, data analysis and wrote the manuscript. MK contributed in data analysis, wrote and reviewed the manuscript.

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INTRODUCTION

Globally, 6,392 species of odonates belonging to 693 genera have been documented (Paulson et al. 2021), of which 588 taxa are known from the Indian Subcontinent (Kalkman et al. 2020). Not much is known about the ecology and distribution of odonates from the Kashmir region of northwestern Himalaya, India. The earliest record of Odonata provides a checklist of 15 species (Calvert 1898) from the erstwhile Kashmir, followed by 22 species by Fraser (1933, 1934, 1936). Studies from Kashmir (Singh & Baijal 1954; Asahina 1978; Kumar & Prasad 1981) have either reported synonyms or previously-described species like *Anaciaesna kashmirense,* or species of doubtful identity *Coenagrion kashmirus* (Chowdhary & Das 1975) which require further investigation. After 1981, no new contributions have been made to the checklist of Odonata of Kashmir. Even the studies from Kashmir (Riyaz & Sivasankaran 2021; Qureshi et al. 2022) have made no mention of new species. A recent study of Paray & Mir (2023) makes a mention of *O. sabina* but provides little information about its distribution and other attributes which are very important for establishing confirmed presence of new odonates from any area. We report latest additions to odonate fauna along with their confirmed presence, distribution records, phenology and habitat specificity based on elaborate studies.

Orthetrum sabina (Drury, 1770), commonly known as Slender Skimmer or Green Marsh Hawk, is an Indo-Malayan species, widely distributed in Ethiopian, Oriental, and Australian regions (Mitra 2002; Subramanian 2005). Its broad distribution stretches from Australia, Japan, and Samoa in the east to Somaliland – northern Africa (Boudot et al. 2009), including central Asia (Fraser 1936) and most of the Indian Subcontinent (Kalkman et al. 2020). *Palpopleura sexmaculata* (Fabricius, 1787), Blue-Tailed Yellow Skimmer or Asian Widow, is widely spread throughout the Oriental region (Mitra 2002; Subramanian 2005; Nair 2011) to Tibet and throughout Malaysian and Indo-China to China (Fraser 1936), excluding Sri Lanka and Andaman & Nicobar Islands (Kalkman et al. 2020). There being no confirmed record of these two species from the Kashmir region, this study establishes their presence in Kashmir Valley and adds two new libellulids to the Odonata of Kashmir.

Study area

Kashmir Valley (33–35 °N & 73–75.2 °E) is in the northwestern Himalayan region of the Indian Subcontinent (Dar et al. 2012). It is an oval-shaped valley approximately 135 km long and 32 km wide. This valley is surrounded by the main Himalayan range in the north-east and the Pir Panjal mountain range in the south-west (Drew 1875; Wadia 1931). Pir Panjal acts as a natural barrier (Rashid et al. 2011), dividing Kashmir valley from the Jammu division and separating various biogeographic elements between the Indian mainland and the valley (Puri 1943), covering an area of 15,520.3 km^2. The valley plains have long stretches of paddy and low-lying water bodies on either side of river Jhelum, which flows south–north of the valley. For this study, the lower plains of the valley; wetlands, and paddy fields forming a stretch of 300 km^2, within an elevation gradient of 1,500–2,000 m is the intensive study area (ISA) (Image 1).

METHODS

Based on an accidental sighting of one of the dragonfly species reported in this study, opportunistic surveys were carried out in the ISA. To ascertain the occurrence of these records, 90 opportunistic surveys were carried out in 2018–2020, during the summer months (June–October) in the potential habitats of the ISA. Surveys were conducted covering a total area of 5 km^2 /day from early morning to late afternoon, as most odonates are active during this time (Paulson 2019; Resende 2002). For census of active and teneral odonate individuals similar survey methods have been used in the past (Ubukata 1974) and are still being used (Renner et al. 2015). Once the dragonflies were sighted, individuals were either photographed, caught live using insect nets, and identified using available literature (Fraser 1933, 1934, 1936) and standard odonates field guides (Subramanian 2005; Mitra 2006; Nair 2011).

RESULTS

An annotated list of Odonata from the Kashmir Valley during the study period (2018–2020) from ISA is shown in Table 1. A total of 26 species, including two new records, belonging to a total of seven families of suborder Zygoptera (4) and Anisoptera (3) were observed. Fifteen species of the family Libellulidae were observed.

Orthetrum sabina (Drury, 1770)

The map (Image 1) shows the locations of *O. sabina*. A total of 44 individuals were sighted including mating

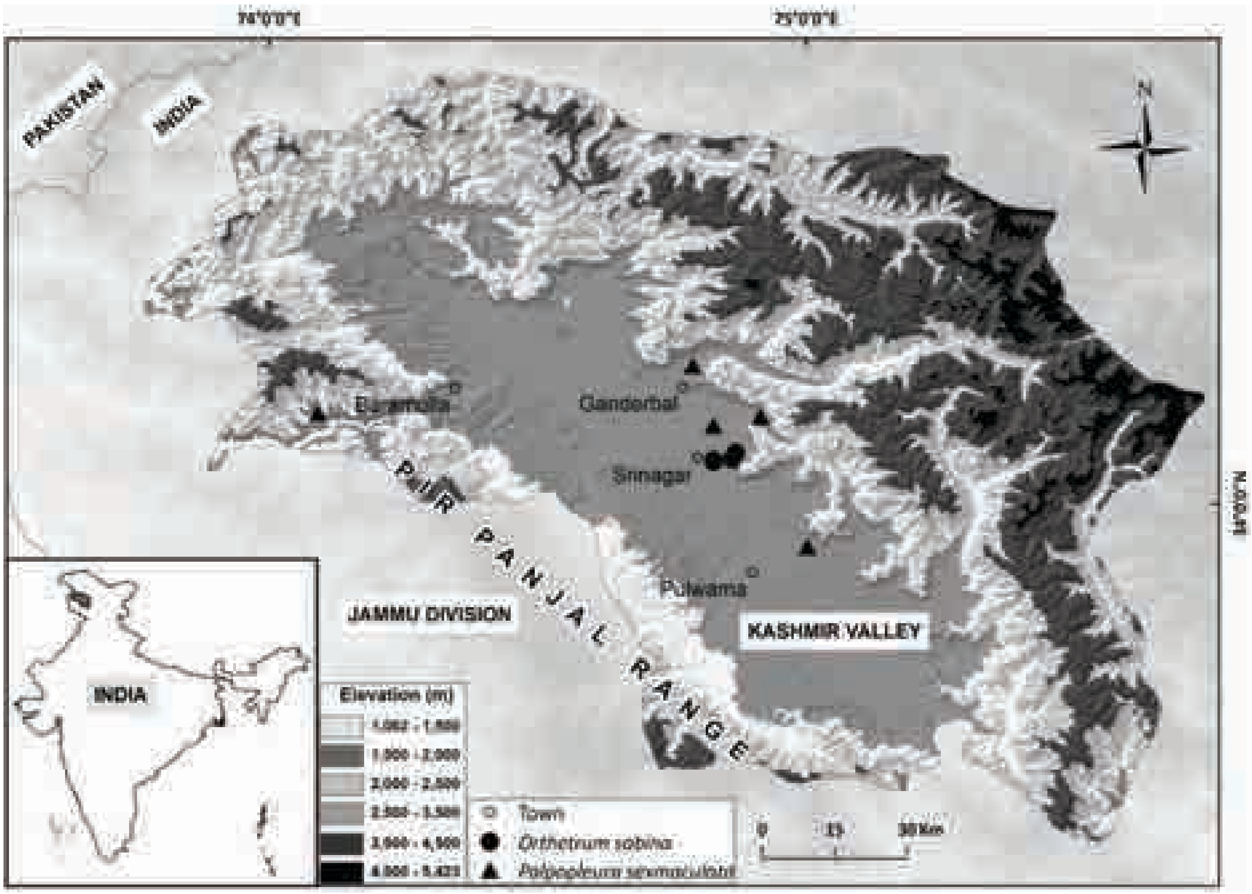

Image 1. Intensive study area (1,500–2,000 m elevation) and locations of two libellulids, *Orthertum sabina* and *Palpopleura sexmaculata*, from Kashmir Valley, India.

Image 2. *Orthetrum sabina* (Drury, 1770) (male) taken at Kashmir Golf Course, Srinagar, Jammu & Kashmir.

Image 3. Habitat of *Orthetrum sabina* (Drury, 1770) at Kashmir golf course, Srinagar, Jammu & Kashmir.

pairs during 2018–2020. These were observed from three different locations (Kashmir Golf Course 34.077785 °N, 74.826818 °E; Police Golf Course 34.082128 °N 74.861290 °E; and Royal Springs Golf Course 34.091302 °N, 74.868227 °E) within 5 km distance during July–September. Observations were made during peak sunshine hours. Image 2 and 3 shows the identified individual as *O. sabina* within its habitat. In successive years the species have been observed from the same areas in July–September, mostly around Dal Lake (Table 2).

Palpopleura sexmaculata (Fabricius, 1787)

The map (Image 1) shows the locations from where

Table 1. Annonated list of Odonata during the study period (2018–2020) from the intensive study area.

Order Odonata Fabricius, 1793
Suborder Zygoptera Selys, 1854
A. Family: Lestidae Calvert,1907
1. *Lestes barbarus* (Fabricius, 1798)
2. *Sympecma paedisca* (Brauer, 1877)

B. Family: Synlestidae Tillyard, 1917
3. *Megalestes major* Selys, 1862

C. Family: *Chlorocyphidae* Cowley, 1937
4. *Rhinocypha quadrimaculata* Selys, 1853

D. Family: Coenagrionidae Kirby, 1890
5. *Enallagma cyathigerum* (Charpentier, 1840)
6. *Ischnura inarmata* Calvert, 1898

Suborder Anisoptera Selys, 1854
E. Family: Aeshnidae Leach, 1815
7. *Aeshna juncea* (Linnaeus, 1758)
8. *Aeshna mixta* Latreille, 1805
9. *Anax ephippiger* (Burmeister, 1839)
10. *Anax parthenope* (Selys, 1839)

F. Family: Cordulegasteridae Hagen, 1875
11. *Cordulegaster brevistigma* Selys, 1854

G. Family: Libellulidae Leach, 1815
12. *Crocothemis erythraea* (Brullé, 1832)
13. *Crocothemis servilia* (Drury, 1770)
14. *Libellula quadrimaculata* Linnaeus, 1758
15. *Orthetrum brunneum* (Fonscolombe, 1837)
16. *Orthetrum glaucum* (Brauer, 1865)
17. *Orthetrum luzonicum* (Brauer, 1868)
18. *Orthetrum sabina* (Drury, 1770)
19. *Orthetrum triangulare* (Selys, 1878)
20. *Palpopleura sexmaculata* (Fabricius, 1787)
21. *Pantala flavescens* (Fabricius, 1798)
22. *Selysiothemis nigra* (Vander Linden, 1825)
23. *Sympetrum commixtum* (Selys, 1884)
24. *Sympetrum fonscolombii* (Selys, 1840)
25. *Sympetrum meridionale* (Selys, 1841)
26. Sympetrum *striolatum* (Charpentier 1840)

P. sexmaculata was found. The first individual was observed in paddy fields near Harwan (34.158821 °N, 74.913766 °E). The specimen was collected, identified, and confirmed as *P. sexmaculata*, (Image 4). Most of the observations have been from paddy fields in July–August. This species was also observed from Mallabagh, Srinagar (34.142^{0}N, 74.826^{0}E), Nunar, Ganderbal (34.254^{0}N, 74.787^{0}E), Bijhama, Uri (34.166^{0}N, 74.089^{0}E), and Awantipora, Pulwama (33.919^{0}N, 75.001^{0}E). A total of 183 individuals along with mating pairs were observed during the study period (Table 2). Image 5, shows the habitat of this species.

Image 4. Photograph of *Palpopleura sexmaculata* (Fabricius, 1787) (female) taken at Bijhama, Uri, Jammu & Kashmir.

Image 5. Habitat of *Palpopleura sexmaculata* (Fabricius, 1787) at Bijhama, Uri, Jammu & Kashmir.

DISCUSSION

Distributed throughout the Indian subcontinent, *O. sabina* has never been reported from the Kashmir Valley. All the previous studies limit its distribution to the southern part of the Pir Panjal range of the Himalaya. Found near warm waters, covered and open canopies, this dragonfly perches for a long time on branches, twigs, rocks, or ground (Subramanian 2005; Nair 2011). This species is known to be a voracious predator (Subramanian 2005; Emiliyamma et al. 2007) preying on a range of flying insects and odonates including conspecifics. From our observations, we found the males perching on concrete structures near small water bodies. This species was found in undisturbed habitats, perched motionless generally on rocks or cement surfaces, mostly large open golf courses, gardens around Dal Lake, and adjoining areas with elevations ranging from 1585–1595 m. Individuals of *O. sabina* were found solitary and never found associated with any other species of dragonfly. The presence of

Table 2. Year-wise summary (2018–2020) of the number of individuals observed of *O. sabina* and *P. sexmaculata*.

Species	Year-wise number of individuals observed			Location and elevation (m)	Habitat features
	2018	2019	2020		
O. sabina	11	8	25	a) 34.07778 °N, 74.82681 °E (1585) b) 34.08212 °N 74.86129 °E (1590) c) 34.09130 °N, 74.86822 °E (1595)	- Reeds, rocks or parapets, undisturbed shallow water bodies. - Canopy absent.
P. sexmaculata	57	38	88	a) 34.15882 °N, 74.91376 °E (1675) b) 34.14297 °N, 74.82615 °E (1590) c) 34.25444 °N, 74.78783 °E (1690) d) 34.16621 °N, 74.08976 °E (1690) e) 33.91933 °N, 75.00148 °E (1590)	- Paddy fields with knee-high grasses and water. - Low-lying aquatic grass lands dominated by monocots.

mating pairs suggests that this species breeds here too. And thereby, confirming its presence in this region.

Previous distribution records of *P. sexmaculata* do not include the Kashmir Valley of the northwestern Himalayan region. Even though it is known to be distributed in higher altitudes of Tibet, Indo-China–China (Fraser 1936), very little evidence is available on its distribution from northwestern Himalaya. Known to occur in large colonies in marshy spots and watered rice fields where they breed (Kumar & Prasad 1981; Subramanian 2005), this small dragonfly is a weak flyer for short distances (Nair 2011). The study area has numerous rice fields, and most of our observations are from paddy fields from across the valley. Since our observations are from July–August, paddy fields are well-watered and possibly form the best breeding grounds for this dragonfly. Swarms of *P. sexmaculata* were always found associated with other species such as *Pantala flavescens, Libellula quadrimaculata, Crocothemis servilia,* and *Sympetrum fonscolombii*. Our results suggest the continuous occurrence of both these species during 2018–2020 from different parts of the study area. The findings hint towards climate change induced spatio-temporal distribution, habitat shifts, and range extension of Odonates (Cancellario et al. 2022). Both these are Oriental species whose distribution is limited by the southern slopes of the Himalaya, but their dispersion extends further north in the transitional zone between the Oriental and the Palaearctic region (Heiser & Schmitt 2013). Kashmir valley falls in this transition zone hence explaining the presence of these two dragonflies here.

As this study was carried out in a militarised border zone of India, security was always a concern. Few of the potential habitats could not be surveyed due to security restrictions. Due to limited resources available, little attempt was made to collect larvae or exuvia to study the emergence of these dragonflies. Similarly, studies to understand winter survival weren't conducted which could reveal important information on longevity and survival through successive years. It was challenging to quantify whether the same individual was encountered through successive years of the study duration. Further investigations need to be carried out to understand the migration of odonates and their range extensions across different geographic regions.

CONCLUSION

This is the first confirmed addition to the odonates of Kashmir Valley in four decades with established presence, mating records, and their distribution. The records of new species could lead into new insights into the dispersion of odonates. Further research is mandated to understand the spatiotemporal distribution of odonates in the Kashmir Valley region.

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COMMUNICATION

Rayed Thistle Fly *Tephritis cometa* Loew (Diptera: Tephritidae) a new record to India

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Abstract: *Tephritis cometa* Loew, 1840, known to infest field thistle or Canada thistle or creeping thistle *Cirsium arvense,* a medicinal plant used as a tonic and to treat disease such as diarrhea and tuberculosis; it is diuretic. *Cirsium arvense* is a perennial flowering plant belonging to the family Asteraceae and is native to Europe, northern Africa, and western Asia. We report the first record of *Tephritis cometa* Loew, 1840 in southern Asia from Kashmir, India. The identified specimen of *Tephritis cometa,* commonly known as Rayed Thistle Fly was found on *Cirsium arvense,* a grass (Asteraceae) at the site, SKU2 in district Kulgam. However, the actual host plant is unknown. This paper includes morphological studies, key identification, diagnosis, and its distribution.

Keywords: Kulgam, methyl eugenol, yellow simple bottle trap, southern Asia.

Urdu:

ٹفریٹس کومٹا لوی 1890، ایک ایسا کیڑا ہے جو کہ کنیڑا تھسل یا کریپنگ تھسل، سرسیم اروینس پودے پے بیٹھ کر اس پہ انڑے دیتا ہے۔پھر یہ انڑے لاروے می تبدیل ہوتے ہے اور اس پودے کو کھاتے ہے۔ سرسیم اروینس بہت ہی مفید پودا ہے جو انسان کی سحت کیلے بہت کارگر ثابت ہے۔ یہ ایک ایسا پودا ہے جو کہ بہت ساری بیمار یو جیسے دست، تپ دق اور پیشاب کی بیماری کیلے علاج ہوتا ہے۔ یہ پودا ایسٹیریسی فیملی سے وابستہ ہے جس می پھول کھلتے رہتے ہے اور یہ یورپ، شمالی افریقہ اور مغریبی ایشیاہ می بنیادی طور پر پایا جاتا ہے۔ ٹفریٹس کومٹا لوی 1890، کو ہم نے پہلی بار جنوبی ایشیاہ کے کشمیر انڑیا کے ایک ظلع کلگام کے سایٹ ایس۔کے۔یو 2 می دریافت کیا ہے۔در اصل کشمیر می یہ کس پودے پہ اپنا گزر بسر کرتی ہے یہ ابھی ہم نہی جان پاے۔ اس پیپر می اس کیڑے کی بنیادی پہچان، بیرونی جسم کے بارے می اطلاعات اور کھاں کھاں پایا جاتا ہےّ سب شامل ہے۔

اشارتی الفاظ: کلگام، میتھاعیل اوجینال، زرد رنگ کے بوتل والے ٹراپ، جنوبی ایشیاہ

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Author contributions: Conceptualization, RA, BH, & TA; methodology, BH, RA, & TA; formal analysis, RA; field survey, RA; data curation, RA; writing-original draft preparation, RA; All authors have visualized and reviewed the manuscript.

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INTRODUCTION

There are roughly 4,500 species of true fruit flies (Diptera: Tephritidae), and about 100 of them are pests of commercially farmed fruits (Norrbom et al. 1999; Korneyev & Dilberk 2000). Tephritids are known to traverse enormous distances and are invasive in all continents (Duyck et al. 2004). These notorious insects are phytophagous, and some of them can be harmful pests or employed as weed-controlling bioagents (White & Elson-Harris 1992). The majority of them are members of a few sizable genera, such as *Bactrocera* and *Dacus*, which are widespread throughout zoogeographic regions.

Tephritis Latreille is the third largest genus in the Tephritinae and the sixth largest genus in the Tephritidae with roughly 170 species (Norrbom et al. 1999; Korneyev & Dirlbek 2000). The majority of *Tephritis* species consume various species of the same genus and are commonly referred to as stenophagous or monophagous (Korneyev 2016). Some characteristic features such as two dark frontal setae; dorso-central setae located on or just posterior to transverse suture; two orbital setae, anterior setae acuminate and dark (brown or blackish), posterior setae typically lanceolate and pale (whitish or yellowish; in 2 species brown or black); flat scutellum with two sets of setae, with apical setae that are 0.5–0.6 times longer than basal setae; wing pattern varies greatly between species, typically reticulate with a well-developed apical fork, but can also be stellate or even banded (Freidberg & Kugler 1989; Merz 1994) set *Tephritis* apart from the other genera in the subfamily Tephritinae.

Korneyev (2013), described eleven species of the genus Tephritis, mostly from the Palaearctic Middle East, with an unusual banded wing pattern with four new records: *Tephritis afrostriata*, *T. cameo*, *T. gladius*, and *T. ochroptera*. Korneyev & Evstigneev (2019), redescribed six species from western Palaearctic region in which *T. conura* (Loew, 1844), *T. hendeliana* Hering, 1944, and *T. hyoscyami* (Linnaeus, 1758) are from Europe, *T. anthrax* Korneyev & Evstigneev, 2019 from Caucasian and Transcaucasia mountains, while other two, *T. cardualis* Hardy, 1974 and *T. atokoptera* Agarwal & Kapoor, 1988, from Pakistan and India, respectively. Among the nine species of Tephritidae that are recorded for the first time from Transcaucasia are two new species, *T. oedipus* Hendel, 1927 and *T. hendeliana* Hering, 1944 (Evstigneev & Glukhova 2022).

T. cometa Loew, (1840), belongs to the tribe Tephritini, family Tephritidae and has been identified as a frequent pest of *Cirsium arvense*, *C. vulgare*, and *C. lappaceum*. Typically, the larvae of *T. cometa* eat the flowers of *Cirsium* species, while some species induce the formation of galls in roots or stems of Asteraceae (Freidberg 1984; Merz 1994). The records of *T. cometa* for the first time were reported from Flanders and Belgium (Nobby 2001), Foum El Gherza Dam, a wet land habitat in Biskar region (Deghivhe-Diab et al. 2021), Iran (Namin et al. 2010), Turkey (Kutuk 2006; Kutuk & Katranci 2021), and Europe, Israel, Afghanistan, Kazakhstan, Mangolia, & China (Norrbom et al. 1999).

India is located at the intersection of the Afro-tropical, Indo-Malayan, and Paleo-Arctic realms and, therefore, possesses characteristic elements from all three. The combination of these three diverse realms gives the nation a rich and distinctive biological variety, due to which, India is one among world's 12 mega-diverse nations (Sinha et al. 2010). The Kashmir Himalaya is a part of the Himalayan biodiversity hotspot that has a serious threat of invasion by foreign insect species. Despite harmful impacts of these insect species on native biodiversity and economic and ecological systems of the region, a field-based intensive sampling study for management of fruit fly with pheromone application technology (PAT) is lacking, which merits urgent research attention. Therefore, it is necessary to recognise the fruit flies that can severely harm a variety of horticultural, agricultural and other plant species. Furthermore, the majority of *Tephritis* species infest the flowerheads and, in certain cases, the stems of Asteraceae hosts, which may lead to the development of galls (Freidberg 1984). As a result, our objective is to investigate pheromone application technology for the management of all known and undiscovered fruit flies that have travelled thousands of miles to establish in the Kashmir Himalaya.

MATERIALS AND METHODS

Survey and trap installation

Kashmir, the northernmost geographical region of the Indian subcontinent where apricots are grown on 2,880 ha, cherry on 2,713 ha, plum on 1,427 ha and peach on 714 ha of land (Daily Hindustan Times 5 August 2022). In Kashmir Valley's fruit and vegetable production zones, a total of 30 sites, three from each district, were chosen for survey through 2019–2021 (Image 1). A total of 94 para-pheromone-filled traps were placed over all the selected areas to catch fruit flies. All the implanted traps were constructed using simple cold drink bottles, which contained methyl eugenol inside hanging rubber

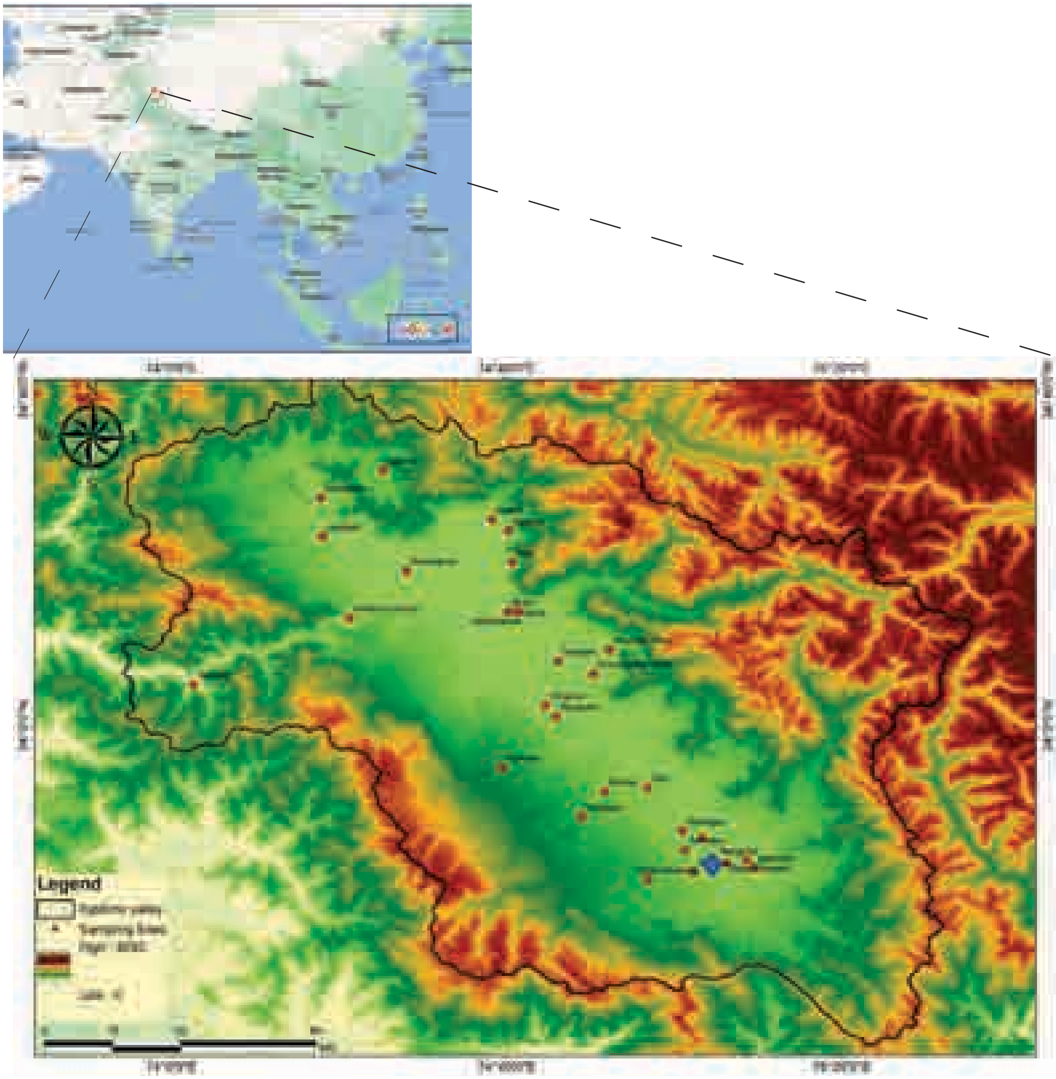

Image 1. Sampling sites selected for the management of tephritid fruit flies in Kashmir, India.

septa and SPLAT at the bottom. Three equally spaced holes, each measuring 1.5 cm^2, were drilled into the basic bottle trap using a hot T-type handle spanner and a glue gun for the entry of fruit flies.

Collection and identification

All the installed traps were checked and refreshed fortnightly. The voucher specimens were deposited at the Zoology Museum, University of Kashmir. For identification, Hering (1944), Freidberg & Kugler (1989), and Merz (1994) were consulted and further confirmation by Prof. Drew, director, ICMF, Griffith University, Queensland, Australia.

Morphological studies

The present work is totally based on morphological studies following Kutuk (2006), Mohamadzade et al. (2015), and Gharajedaghi et al. (2011a). Studied morphological characters show differences in wing patterns among different species and are presented in the plate (Image 2). Photographs of wing and habitus were taken using a Leica S9D Stereo zoom microscope at the Entomology Research Laboratory, Department of Zoology, University of Kashmir.

RESULTS

According to the results of our morphological study, *Tephritis cometa* has now crossed into India's territory and may be a severe threat to several grass plants, particularly the country's *Cirsium* spp.

Material examined: 2 male, ZoKU-Art/06209, 14.VIII.2021, Tarigam, Kulgam, Jammu & Kashmir, India,

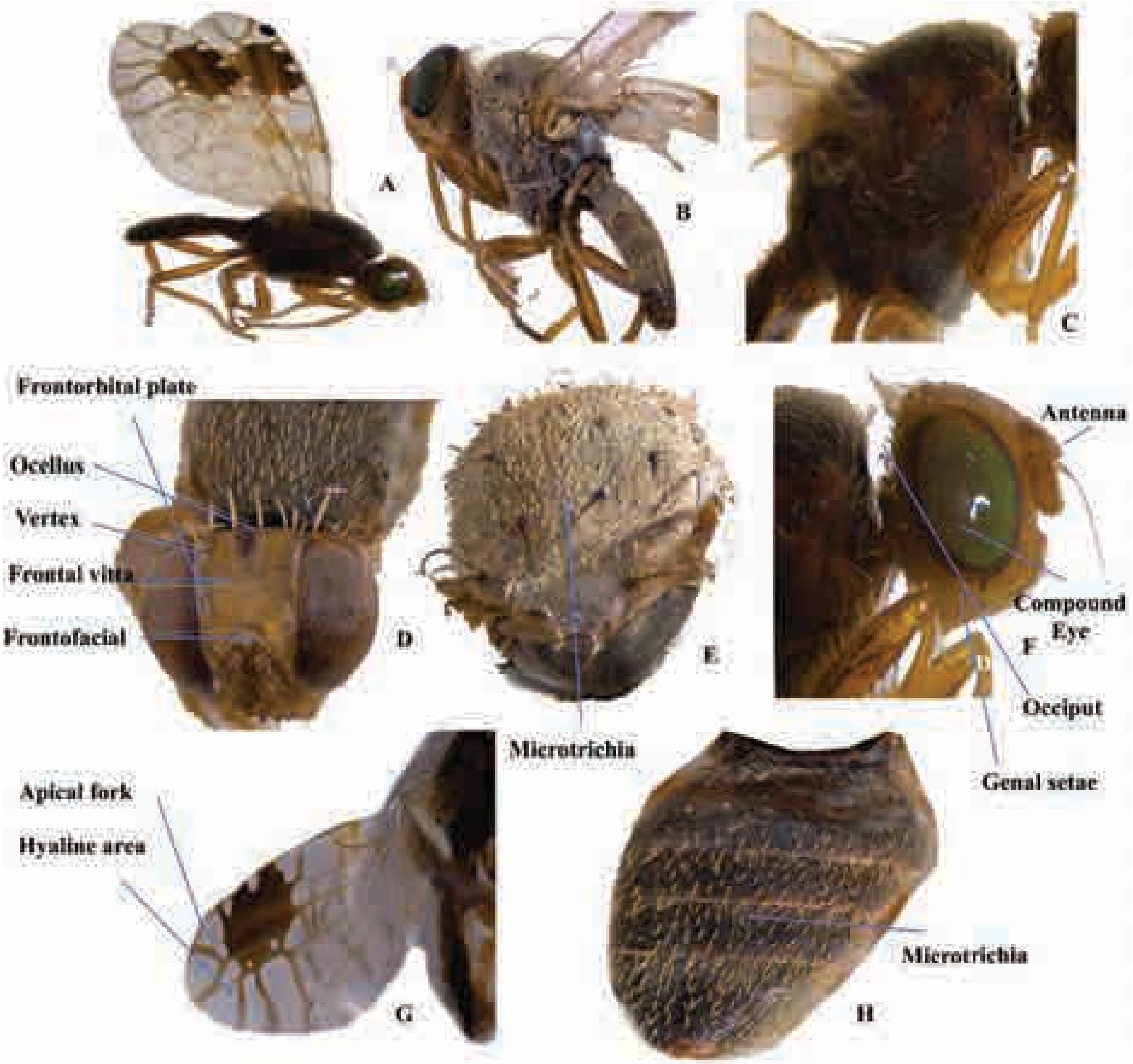

Image 2. *Tephritis cometa*: A—Habitus | B—Lateral habitus | C—Lateral thorax | D—Frontal habitus Ommatidia | E—Dorsal thorax | F—Ommatidia | G—Wing | H—Abdomen. © Rayees Ahmad.

33.7081°N & 75.0380°E, 1,569.14 m, McPhail trap 2, A. Rayees.

Diagnosis: A small fly of about 5–6 mm with distinctively patterned wings. Ocellus with star shaped mark and five ocellar and frontal setae. Frons about 1.8 times as long as eye width, third segment of antenna about 1.7 times as long as wide: apical fork of wing present; branches of apical fork widen distinctly towards wing margin; two or more hyaline areas present in cell m; basal half of wing mainly hyaline; cell r1 with small subapical hyaline spot; branches of apical fork uniformly narrow along their entire length; hyaline spot of cell r_{2+3} continuous with basal indentation of cell r1 (usually 3 hyaline spots), (Image 2). The species is collected along with *Bactrocera* spp. in the MacPhail pheromone trap containing methyl eugenol and SPLAT gel.

Remarks: The genus *Tephritis* has general characteristics making it easily identifiable from other Tephritid fruit flies due to the presence of long and thick inner vertical setae.

Head: The head is predominantly yellow to brown; yellowish on the occiput and ocellar tubercle; brown anterior orbital setae; white postorbital setae; first flagellomere yellow; basal half on arista yellow; palpus mostly yellow, brownish at apex; wide fronto-facial angle.

Table 1. Occurrence of *Tephritis cometa* at SKU site in Kulgam District of southern Kashmir Zone, India.

Zones	Southern Kashmir				Central Kashmir			Northern Kashmir		
Sites Year	Site SAN	Site SKU	Site SPU	Site SSP	Site CBD	Site CGA	Site CSR	Site NBN	Site NBR	Site NKU
2019	-	-	-	-	-	-	-	-	-	-
2020	-	-	-	-	-	-	-	-	-	-
2021	-	+	-	-	-	-	-	-	-	-
Total Specimens	0	2 males	0	0	0	0	0	0	0	0

+—present | -—absent | 2—number of specimens trapped.

Thorax: The majority of the setae are brown and acuminate; setulae are numerous usually in pairs on the margin of the scutellum; the basal scutellar seta are longer than the apical scutellar seta; yellow halter; the ground colour is often black; the microtrichia are grey; the scutellum is shiny and silvery.

Legs: Numerous yellow setulae; tarsi usually brown.

Wing: Branches of apical fork widen distinctly towards wing margin; two or more hyaline areas present in cell m; basal half of wing mainly hyaline; cell r_1 with small subapical hyaline spot; branches of apical fork uniformly narrow along their entire length; hyaline spot of cell r_{2+3} continuous with basal indentation of cell r1.

Abdomen: Ground colour usually black; microtrichia numerous and shiny.

Measurements: Body: 5–6 mm; male wing: 4–5 mm.

Habitat: Various habitats such as horticulture and agriculture crop including grassland and areas where Californian Thistle occur (Freidberg 1984; Merz 1994; Deghivhe-Diab et al. 2021).

Distribution: West and Middle Asia, Europe, Israel, Afghanistan, Russia, Kazakhstan, Mangolia, & China (Norrbom et al. 1999), Estonia, Latvia, Lithuania, Ukraine, Moldova, Azerbaijan, Georgia, Armenia, Kazakhstan, Uzbek, Tajikistan, Kirghis, Turkomanas, Switzerland, England, Anglia, Germany, & Turkey (Foote 1984; White 1988; Freidberg & Kugler 1989; Merz 1994; Kutuk & Ozgur 2003; Kutuk 2006), Netherland, France, & Iran (Namin et al. 2010; Gharajedaghi et al. 2011b), Flanders & Belgium (Nobby 2001), and Foum El Gherza Dam, a wet land habitat in Biskar region (Deghivhe-Diab et al. 2021).

Host plants: Numerous authors have described a variety of hosts, including *Circium gaillardotii, C. vulgare, C. arvense,* and *C. palustre* (White 1988; Freidberg & Kugler 1989; Merz 1994). We caught it at the SKU2 site in the Kulgam district where there were fruits including *Malus domestica* Borkh., *Pyrus* spp. L. and *Prunus persica* L., vegetables- *Cucurbita pepo* L., *Capsicum* sp. L., *Solanum lucopersicum* L., *S. melongena* L., and grasses- *Cirsium arvense* Scop.*, Bromus inermis* Leyss., *Cynodon dactylon* L., *Impatiens* sp. L., *Mentha longifolia* L., and *Plantago major* L. were present. However, the actual host is not known here in Kashmir Himalaya.

India status: Reported for the first time in India.

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COMMUNICATION

New state records of some Dermaptera De Geer, 1773 (Insecta) species in India

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Abstract: The insect order Dermaptera is globally composed of approximately 2,000 species distributed over 245 genera and 16 families, of which approximately 285 species composed within 72 genera and 16 families are known from India until now. A recent examination of some unidentified Dermaptera species housed in the National Zoological Collection of the Zoological Survey of India revealed new state records for six Dermaptera species in India. All the six species are dealt herein with material examined, diagnostic characters, distribution, remarks, and photographs of adult male and female, penultimate sternite and ultimate tergite with forceps, and male genitalia.

Keywords: Distribution, earwigs, oriental, orthopteroid insects, taxonomy.

Editor: Anonymity requested.

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Author contributions: TD–conceptualization, primary taxonomic identification, morphological description, capturing photographs, writing original draft. KGE–conceptualization, methodology, verification of taxonomic identification, data analysis, review and editing, resources, finalization of the original draft, and supervision. SKS–conceptualization, methodology, verification of taxonomic identification, data analysis, review and editing, resources, preparation of photographic plates, finalization of the original draft, and supervision.

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INTRODUCTION

Insects of the order Dermaptera together with Orthoptera and Phasmatodea are commonly grouped as 'orthopteroid insects' because of their characteristic interrelationship (Deepak & Ghosh 2018). Elongated flattened bodied earwigs are the most common members of this order (Deepak & Ghosh 2018).

The order Dermaptera is known by approximately 1,900 species distributed over 245 genera and 16 families (Hopkins et al. 2017). In India, the order is recorded by 285 species belonging to 72 genera and seven families (Srivastava 1986, 1988, 1989, 1993, 1997, 2003a,b, 2005a,b, 2008a,b, 2013; Julka & Purohit 1984; Hegde et al. 2015; Deepak & Ghosh 2018; Karthik et al. 2022). A recent examination of the unidentified specimens housed in the National Zoological Collection of the Zoological Survey of India (ZSI) and labeled as collections of different states of India along with respective verbatim data revealed many new distributional records for different states of India.

The present communication reports new distribution records of six species of dermapterids in India.

MATERIAL AND METHODS

In the present study, some unidentified Dermaptera specimens collected by many scientists from different parts of the country and housed in the National Zoological Collection of ZSI, some of which date back to 1964–65, were examined. The specimens were found preserved in 70% ethanol.

The specimens were examined under stereo-zoom trinocular microscope (LIECA EZ4) and identified up to species level using the keys of Srivastava (1988, 2003, 2013) and other relevant literature. The male genitalia were dissected from the penultimate sternite with the help of forceps and examined for confirming species identity. The photographs of dorsal habitus of adults and male genitalia were captured with the camera (LIECA M205 A) attached to the stereo-zoom microscope. The photographic plates were compiled in Adobe Photoshop CS3.

RESULT

Of the examined species, *Euborellia compressa* (Borelli, 1907) presents new records for Haryana; *Nala lividipes* (Dufour, 1829) for Andhra Pradesh, Gujarat, Haryana, and Jharkhand; *Labidura riparia* (Pallas, 1773) for Bihar, Gujarat, Haryana, Jharkhand; *Forcipula quadrispinosa* (Dohrn, 1863) for Haryana; *F. trispinosa* (Dohrn, 1863) for Madhya Pradesh; and *Echinosoma parvulum* Dohrn, 1863 for Jharkhand.

Systematic account

Superfamily Anisolaboidea Sakai, 1982
Family Anisolabididae Verhoeff, 1902
Subfamily Anisolabidinae Verhoeff, 1902
Genus *Euborellia* Burr, 1910

Euborellia compressa (Borelli, 1907)

(Images 1A,B; 2A,B; 3A)

Anisolabis compressa Borelli, 1907, *Boll. Musei Zool. Compo R. Univ. Torino,* 25(558): 3.

Euborellia compressa Burr, 1915, *J. R. micr Soc.,* 1915: 548, pl. 12, fig. 8.

Material examined: 23 exs. (10 males, 13 females), India, Haryana (vill. Bajaura and its surroundings, Mandi Kulu Road, Kulu subdivision), 07 February 1965, A. Husain, ZSI Registration No. 37887/H5.

Diagnosis (male): Body colour black to reddish-brown; sides of pronotum, antennae, and legs yellow; head yellowish-brown in male and reddish-brown in female; ultimate tergite dark brown in both sexes. Head triangular, longer than broad; frons slightly convex with its hind margin faintly emarginated in the middle. Eyes shorter than postocular area. Pronotum quadrate, slightly longer than broad, widened posteriorly with straight lateral margin. Prozona slightly raised and metazona flat. Abdomen elongated; 5th–9th segments with oblique carina; ultimate tergite smooth, transverse, gradually sloped backwards and obliquely and weakly concave above the base of forceps; penultimate sternite transverse, posteriorly narrowed with its hind margin subtruncate in the middle. Forceps asymmetrical, remote at base, gradually tapering with its internal margin serrated.

Male genitalia (Image 3A): Paramere as long as broad, square-shaped with distinct oblique median membrane; external apical angle sharp and tips obtuse; distal lobes with denticulate chitinous pads; virga not visible.

Remarks: *Euborellia compressa* (Borelli, 1907) and *E. annulipes* (Lucas, 1847) are closely related with regard to external morphology except the following characters. *E. compressa* (Borelli, 1907) has clear yellow legs while *E. annulipes* (Lucas, 1847) has black bands on legs. The paramere of *E. annulipes* (Lucas, 1847) is rectangular while it is square in *E. compressa* (Borelli, 1907).

Distribution: India (Himachal Pradesh, Haryana (new record), Jammu & Kashmir, Tamil Nadu, Uttarakhand, Uttar Pradesh, and West Bengal), Pakistan (Northwestern Province, Chitral), Uganda, and Zaire.

Threatened status: *Euborellia compressa* (Borelli, 1907) is not assessed on the IUCN Red List. Rapid habitat destruction both at the global and local level may lead to reduction in population size of the species.

Family Labiduridae Verhoeff, 1902
Subfamily Nalinae Steinmann, 1975
Genus *Nala* Zacher, 1910

Nala lividipes **(Dufour, 1829)**

(Images 1C, D; 2C, D; 3B)

Forficula pallipes Dufour, 1820, *Ann gener des Sc. Phys. de Bruxelles,* 4: 316, pl. 116, figs. 7a & b.

Forficula dufouri Desmarest, 1820, *Fauna fr. Orth.,* pl. 1, fig. 7.

Forficula lividipes Dufour, 1829, *Annls Sci. Nat.,* 13: 340.

Forficesila castanea Serville, 1839, *Hist. Nat. Ins. Orth., Paris*: 26.

Forficesila meridionalis Serville, 1839, *Hist. Nat. Ins. Orth., Paris*: 26.

Forficesila vicina Lucas, 1846, *Expl. Alger,* 3: 5, pl. 1, fig. 2, 2a.

Labidura lividipes vicina Bormans, 1900, *Das Tierriech*, 11: 36.

Labidura inconspicua Kirby, in Distant, 1900, *Ins. Transvaal.,* 1: 12, pl. 1, fig. 1.

Echinosoma? obscurum Kirby in Distant, 1900, *Ins. Transvaal.,* 1: 12, pl. 1, fig. 2.

Labaidura lividipes Bormans and Krauss, 1900, *Das Tierreich,* 11: 36.

Paralabidura lividipes Burr, 1910, *Trans. ent. Soc. Lond.*: 184.

Nala lividipes Burr, 1911, *Genera Insect.,* 122: 36.

Labidura australica Mjöberg, 1913, *Ent. Tidskr.*: 27.

Material examined: 03 exs. (2 males & 1 female), India, Andhra Pradesh, Madhavaram, 10 October 1963, B. Nath, ZSI Registration No.: 41004/H5. 01 ex. (01 male), India, Gujarat, Morbi, Mesariya, 07 December 2017, H. S. Banyal & Party, ZSI Registration No.: 40998/H5. 02 exs. (01 male & 01 female), India, Haryana, Hisar, 06 September 1963, R. K. Bhatnagar, ZSI Registration No.: 39245/H5. 02 exs. (01 male & 01 female), India, Jharkhand, Latehar, Auranga river, 23 October 2021, Emily & Party, ZSI Registration No.: 37880/H5. 08 exs. (03 males, 05 females), India, Jharkhand, Betla National Park, 28 October 2021, Emily & Party, ZSI Registration No.: 37881/H5.

Diagnosis (male): Body colour dull blackish-brown; pronotum dark brown; elytra and wings yellowish-brown with the apical part of wing bright yellow; legs yellow with femur and tibiae having black bands. Head quadrate with its hind margin emarginate in the middle. Eyes as long as the postocular area. Pronotum slightly longer than broad, posteriorly widened with its margin round. Abdomen finely pubescent. Penultimate sternite triangular with slight emargination present in the middle of hind margin. Forceps gently incurved, tapering, with the apices pointed; inner margin ventrally crenulate with a distinct tooth at apical third.

Male genitalia (Image 3B): Parameres three times longer than broad, narrowed apically with pointed acute tips; external margin covex; virga thin, tubular with distinct basal vesicle.

Remarks: This species is closely related with *Nala nepalensis* (Burr, 1907) but can be easily distinguished by the structure of their forceps.

Distribution: India (Andhra Pradesh (new record), Arunachal Pradesh, Chattisgarh, Delhi, Gujarat (new record), Haryana (new record), Himachal Pradesh, Jammu & Kashmir, Jharkhand (new record), Karnataka, Madhya Pradesh, Odisha, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttarakhand, Uttar Pradesh, and West Bengal), Bhutan, Nepal, and Sri Lanka.

Threatened status: *Nala lividipes* (Dufour, 1829) is not assessed on the IUCN Red List. Rapid habitat destruction both at the global and local level, however, may lead to reduction in population size of the species.

Subfamily Labidurinae Burr, 1909
Genus *Labidura* Leach, 1815

Labidura riparia **(Pallas, 1773)**

(Images 1E, F; 2E, F; 3C)

Forficula riparia Pallas, 1773, *Reise Russ, Reichs,* 2: 727.

Forficula pallipes Fabricius, 1775, *Syst. Ent.,*: 270.

Forficula maxima Villiers, 1780, *Linn. Ent.,* 1: 427.

Forficula bilineata Herbst, 1786, *Arch. Insektengesch.,* 7 & 8: 103.

Forficula gigantea Fabricius, 1787, *Mant. Ins.,* 1: 224.

Forficula bidens Olivier, 1791, *Encyl. Méthod.,* 3: 466.

Forficula crenata Olivier, 1791, *Encyl. Méthod,* 3: 467.

Forficula flavipes Fabricius, 1793, *Ent. Syst.* 2: 2.

Forficula erythrocephala Fabricius, 1793, *Ent. Syst.,* 2: 4.

Forficula rufescens Beauvois, 1805, *Ins. Afr. Amer. Orth.*: 35.

Forficula morbida Serville, 1831, *Ann. Sci. nat.* 22: 35.

Image 1. Dorsal habitus of: A—*Euborellia compressa* (Borelli, 1907), male | B—*Euborellia compressa* (Borelli, 1907), female | C—*Nala lividipes* (Dufour, 1829), male | D—*Nala lividipes* (Dufour, 1829), female | E—*Labidura riparia* (Pallas, 1773), male | F—*Labidura riparia* (Pallas, 1773), female | G—*Forcipula quadrispinosa* (Dohrn, 1863), male | H—*Forcipula quadrispinosa* (Dohrn, 1863), female | I—*Forcipula trispinosa* (Dohrn, 1863), male | J—*Forcipula trispinosa* (Dohrn, 1863), female | K—*Echinosoma parvulum* Dohrn, 1863, male | L—*Echinosoma parvulum* Dohrn, 1863, female. © Tanusri Das.

Forficula affinis Guérin-Méneville, in Ramon, 1836, *Hist. Cuba Ins.,* 7: 330, pl. 12, fig. 2.

Forficesila gigantea Burmeister, 1838, *Hand. Ent.,* 2: 751.

Forficula bivittata Klug, in Burmeister, 1838, *Hand. Ent.,* 2: 751.

Forficula suturalis Burmeister, 1838, *Hand. Ent.,* 2: 752.

Forficula marginella Costa, 1839, *Atti. Accad. Bourbon,* 4: 50.

Forficesila terminalis Serville, 1839, *Hist. Nat. Orth.*: 25.

Forficula icterica Serville, 1839, *Hist. Nat. Orth.*: 25.

Forficula bicolor Motschulsky, 1846, in Fischer, 1846, *Horae ent. Ross.,* 4: 354.

Forficula fischeri Motschulsky, in Fischer, 1846, *Horae ent. Ross.,* 4: 354.

Forficula amurensis Motschulsky, 1858, *Bull. Soc. nat. Moscou,* 32: 499.

Labidura riparia Dohrn, 1863, *Stettin. ent. Ztg.,* 24: 313.

Labidura bengalensis Dohrn, 1863, *Stettin. ent. Ztg.,* 24: 312.

Labidura serville Dohrn, 1863, *Stettin. ent. Ztg.,* 24: 316.

Labidura auditor Scudder, 1876, *Proc. Boston Soc. nat. Hist.,* 18: 252.

Labidura granulosa Kirby, 1891, *J. Linn. Soc. Zool.,* 23: 511.

Labidura pulviailis Kirby, 1891, *J. Linn. Soc. Zool.,* 23: 512.

Labidura clarki Kirby, 1891, *J. Lin. Soc. Zool.,* 23: 512.

Labidura distincta Rodzianko, 1897, *Wein ent. Ztg.,* 16: 153.

Apterygida huseinae Rehn, 1901, *Proc. Acad.* Nat. *Sci. Philad.*: 273.

Labidura trunctata Kirby, 1903, *Ann. Mag. nat. Hist.,* 7 (11): 67.

Tomopygia sinensis Burr, 1904, *Trans. ent. Soc. Lond.*: 288.

Labidura dubronyi Borg, 1904, *Ark. Zool.,* 1: 565.

Labidura karschi Borg, 1904, *Ark. Zool.,* 1: 565.

Labidura mongolica Rehn, 1906, *Proc. U.S. natn. Mus.,* 29: 503, fig. 2.

Labidura riparia hurculeana Semenov, 1908, *Ent. Obozr.*: 171.

Labidura leucotarsata Mjöberg, 1913, *Ent. Tidskr.,* 34: 27.

Labidura confusa Capra, 1921, *Annali Mus. civ. Stor. nat. Giacomo Doria,* 53: 157.

Labidura cryptera Liu, 1946, *J. West China Border Res. Soc.,* 16: 20, fig. 6.

Labidura? elegans Liu, 1946, *J. West China Border Res. Soc.,* 16: 20, fig. 4.

Diplatys himalayanus Baijal and Singh, 1954, *Agra Univ. J. Res. (Sci.),* 3 (2): 455, figs. 1–4.

Spongiphora nainitalensis Baijal and Singh, 1954, *Agra Univ. J. Res. (Sci.),* 3 (2): 456, fig. 5–9.

Erotesis jeolikotensis Baijal and Singh, 1954, *Agra Univ. J. Res. (Sci.),* 3 (2): 458, figs. 10–13.

Elaunon nainitalensis Baijal and Singh, 1954, *Agra Univ. J. Res. (Sci.),* 3 (2): 460, figs. 16–19.

Labidura orientalis Steinmann, 1979, *Acta zool. hung.,* 25 (3–4): 422, figs. 12–13.

Material examined: 01 ex. (01 male), India, Bihar, Nalanda, Rajgir, 13 March 1973, Bhola Nath & party, ZSI Registration No.: 38991/H5. 10 exs. (03 males, 06 females & 01 Nymph), India, Gujarat, Jamjodhpur, 20 January 2017, H.S. Hegde, ZSI Registration No.: 40999/H5. 02 exs. (01 male, 01 female), India, Haryana, Karnal, 20 September 1963, R.K. Bhatnagar, ZSI Registration No.: 38992/H5. 01 ex. (01 male), India, Jharkhand, Latehar, Auranga river, 23.x.2021, Emily & Party, ZSI Registration No.: 37883/H5. 01 ex. (01 female), India, Jharkhand, Betla National Park; 28 October 2021, Emily & Party, ZSI Registration No.: 37882/H5.

Diagnosis (male): Body colour brown with head, pronotum, elytra, wings and abdomen blackish-brown; antennae, sides of pronotum, and middle of elytra yellow; legs pale yellow with dark joints; ultimate tergite and forceps yellowish-brown with its apical part dark brown; penultinate sternite dark brown. Head quadrate. Pronotum straight at sides; postero-lateral angle and hind margin round. Prozona tumid and metazona depressed. Elytra and wings well developed. Ultimate tergite transverse with spines or disc present at the base of forceps. Penultimate sternite triangular. Forceps long, tapering gradually and internally provided with two pairs of teeth. Forceps variously shaped.

Male genitalia (Image 3C): Parameres flat, three to four times longer than broad, with its margin staright, and bears a membranous epimerite at the apex; virga distinctly visible, basally with a vesicle and an inner sinuous tube.

Remarks: The species exhibit extreme variations in general body colouration, size and shape of pronotum, texture and extent of elytra and wings, and shape of ultimate tergite and forceps (specially the inner armature).

Distribution: India (Arunachal Pradesh, Assam, Bihar (new record), Chattisgarh, Gujarat (new record), Haryana (new record), Himachal Pradesh, Jharkhand

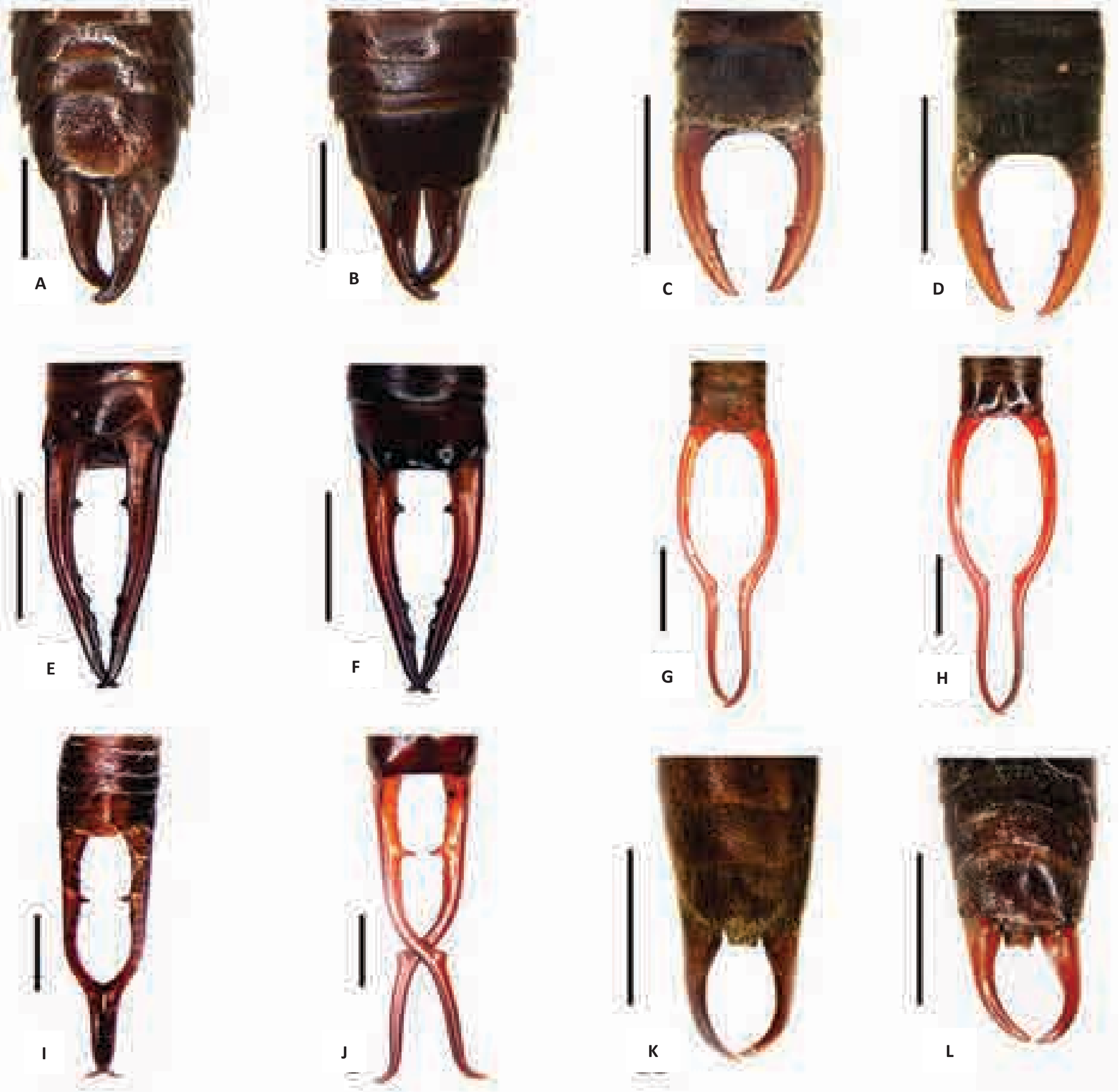

Image 2. Penultimate sternite with forceps (A, C, E, G, I, K) and Ultimate tergite with forceps (B, D, F, H, J, L) of: A, B—*Euborellia compressa* (Borelli, 1907) | C, D—*Nala lividipes* (Dufour, 1829) | E, F—*Labidura riparia* (Pallas, 1773) | G, H—*Forcipula quadrispinosa* (Dohrn, 1863) | I, J—*Forcipula trispinosa* (Dohrn, 1863) | K, L—*Echinosoma parvulum* Dohrn, 1863. © Tanusri Das.

(new record), Karnataka, Madhya Pradesh, Manipur, Meghalaya, Mizoram, Odisha, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttarakhand, Uttar Pradesh, West Bengal, Delhi, and, Jammu & Kashmir) and cosmopolitan.

Threatened status: *Labidura riparia* (Pallas, 1773) is not assessed on the IUCN Red List. However, rapid habitat destruction both at the global and local level may lead to reduction in population size of the species.

Genus *Forcipula* Bolivar, 1897

Forcipula quadrispinosa (Dohrn, 1863)

(Images 1G, H; 2G, H; 3D)

Labidura quadrispinosa Dohrn, 1863, *Stettin. ent. Ztg.,* 24: 311.

Forcipula quadrispinosa Bolivar, 1897, *Annls. Soc. ent. Fr.,*66: 283.

Labidura morosa Kirby, 1891, *J. Linn. Soc.,* 23: 513.

Forcipula jacobsoni Burr, 1908, *Ann. Mag. nat. Hist.,* (8) 1: 47.

Forcipula simplex Bey-Bienkjo, 1970, *Zool. Zh.,* 49: 1815.

Genitalata mahajani Kapoor, 1974, *Zool. J. Linn. Soc.,* 155: 83, figs. 1-4.

Material examined: 02 exs. (01 male, 01 female), India, Haryana, Badshapur, 28 April 1964, S. K. Mitra, ZSI Registration No. 40349/H5.

Diagnosis (male): Body clour dull brown with head and abdomen blackish-brown; elytra and wings reddish-brown; legs yellow. Pronotum longer than broad with raised prozona and dipressed metazona. Elytra and wings well developed. Abdomen provided with sharp incurved spines at the sides of second to fifth segments. Ultimate tergite depressed medially, with its hind and parallel margin straight, oblique above the base of forceps. Penultimate sternite triangular, narrowed posteriorly with rounded hind margin. Forceps strongly arcuate in the basal half, internally crenulated then slightly bend inwards and straight with gradually tapering tip.

Male genitalia (Image 3D): Parameres about five times longer than broad, apically round with short epimerite; virga distinct.

Remarks: *Forcipula quadrispinosa* (Dohrn, 1863) and *F. trispinosa* (Dohrn, 1863) are closely related species and can be distinguished by the shape of their forceps and male genitalia.

Distribution: India (Assam, Bihar, Chattisgarh, Himachal Pradesh, Jammu & Kashmir, Karnataka, Madhya Pradesh, Odisha, Haryana (new record), Rajasthan, Sikkim, Tamil Nadu, Telangana, Uttarakhand, Uttar Pradesh, and West Bengal), Bhutan, Indo China, Mauritius, Myanmar, Nepal, Philippines, Reunion Island, southern China, and Thailand.

Threatened status: *Forcipula quadrispinosa* (Dohrn, 1863) is not assessed on the IUCN Red List. Rapid habitat destruction both at the global and local level, however, may lead to reduction in population size of the species.

Forcipula trispinosa (Dohrn, 1863)

(Images 1I, J; 2I, J; 3E)

Labidura trispinosa Dohrn, 1863, *Stettin. ent. Ztg.,* 24: 310.

Forcipula trispinosa Bormans and Krauss, 1900, *Das Tierreich,* Lief 11: 30.

Labidura (?) *pugnax* Kirby, 1891, *J. Linn. Soc. (Zool.),* 25: 510, pl. 12, fig. l.

Forcipula trispinosa var. *minor* Burr, 1910, *Fauna British India Dermaptera*: 93, pl. 3, fig. 260.

Forcipula trispinosa sub. sp. *minor* Brindle, 1966, *Ann. Mag. nat. Hist.,* (13) 9: 268.

Forcipula pugnax var *parallela* Borelli, 1912, *Bull. Mus. Hist. nat. Paris*: 14.

Forcipula walkeri (néc Kirby) Kapoor and Malla, 1978, *J. inst.* Sc., 1: 2.

Forcipula afghana Steinmann, 1980, *Acta zool. hung.,* 26 (1–3): 243.

Material examined: 02 exs. (01 male, 01 female), India, Madhya Pradesh, Shadol, Narmada River, Amarkantak, 27 May 1963, S. Chakrapany, ZSI Registration No.:38830/H5.

Diagnosis (male): Body colour blackish-brown; elytra, wings and forceps reddish-brown. Head quadrate with its hind margin emarginate medially. Pronotum longer than broad; prozona raised and metazona depressed. Elytra and wings well developed. Abdomen presented with spines on each side of second to fourth segments. Ultimate tergite transverse. Penultimate sternite triangular. Forceps elongate; internally serrated with a pair of tooth present at basal one third, afterwards bent at apical one third; pointed apically.

Male genitalia (Image 3E): Parameres long, apically narrowed with acute tip, epimerite short; virga distinctly visible with large basal vesicle.

Remarks: *Forcipula trispinosa* (Dohrn, 1863) and *F. quadrispinosa* (Dohrn, 1863) are closely related species and can be distinguished by the shape of their forceps and male genitalia.

Distribution: India (Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Himachal Pradesh, Jammu & Kashmir, Maharashtra (new record)**,** Madhya Pradesh (new record), Meghalaya, Mizoram, Punjab, Sikkim, Uttarakhand, Uttar Pradesh, and West Bengal), Afghanistan, Bhutan, Nepal, and Pakistan.

Threatened status: *Forcipula trispinosa* (Dohrn, 1863) is not assessed on the IUCN Red List. Rapid habitat destruction both at the global and local level, however, may lead to reduction in population size of the species.

Superfamily Pygidicranoidea Popham, 1965
Family Pygidicranidae Verhoeff, 1902
Subfamily Echinosomatinae Burr, 1910
Genus *Echinosoma* Serville, 1839

Echinosoma parvulum Dohrn, 1863

(Images 1K, L; 2K, L; 3F)

Echinosoma parvulum Dohrn, 1863, *Stettin. ent. Ztg.,* 24: 66.

Material examined: 04 exs. 4 (03 males, 01 female), India, Jharkhand, Latehar, 23 October 2021, Emily & Party, ZSI Registration No.: 38982/H5.

Diagnosis (male): Body colour dull brown with short pubescence present all over; legs yellowish with

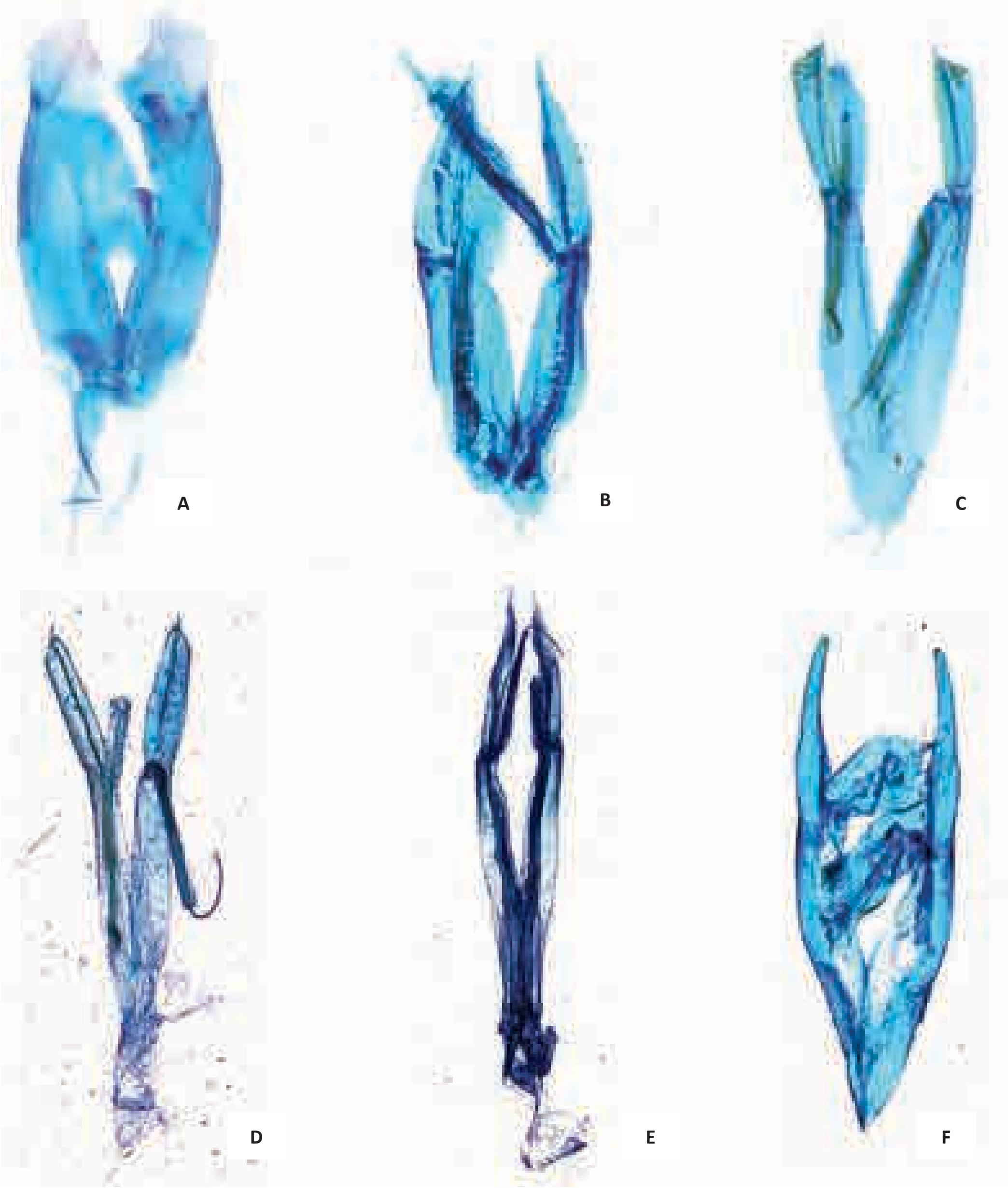

Image 3. Male genitalia of: A—*Euborellia compressa* (Borelli, 1907) | B—*Nala lividipes* (Dufour, 1829) | C—*Labidura riparia* (Pallas, 1773) | D—*Forcipula quadrispinosa* (Dohrn, 1863) | E—*Forcipula trispinosa* (Dohrn, 1863) | F—*Echinosoma parvulum* Dohrn, 1863. © Tanusri Das.

black bands. Head pentagonal. Pronotum broader than long; prozona raised and metazona depressed. Elytra well developed and wings absent. Forceps small, bow shaped, with its apical end pointed.

Male genitalia (Image 3F): Parameres as long as proparameres, with faintly undulate external margin and rounded tip; distal lobes longer than parameres; virga short.

Remarks: The species can be easily distinguished from *Echinosoma andamanensis* Srivastava, 1988 by the shape of various parts of male genitalia and virga and also the shape of female pygidium.

Distribution: India (Jharkhand (new record), Karnataka, Madhya Pradesh, Tamil Nadu, Uttarakhand, and Uttar Pradesh) Sri Lanka, and Vietnam.

Threatened status: *Forcipula trispinosa* (Dohrn, 1863) is not assessed on the IUCN Red List. Rapid habitat destruction both at the global and local level, however, may lead to reduction in population size of the species.

DISCUSSION

Earwigs are generally nocturnal insects and take shelter in small, dark, and moist areas during the daytime. Some species show wider distribution throughout the country while some are found only in specific regions. For example, *Nala lividipes* (Dufour, 1829), *Labidura riparia* (Pallas, 1773), *Forcipula quadrispinosa* (Dohrn, 1863), and *F. trispinosa* (Dohrn, 1863) are the dominant species of the country as are widespread throughout many states. Moreover, *Nala lividipes* (Dufour, 1829) and *Labidura riparia* (Pallas, 1773) are also common inhabitant of agricultural crop fields of the country. Another frequently available species is *Euborellia compressa* (Borelli, 1907) which shelters inside the bark of rotting logs as well as in moist ground. On the contrary, *Echinosoma parvulum* Dohrn, 1863 is a relatively less distributed species in India. Indian Dermaptera fauna represents only about 15% (285/1,900) of the world fauna, indicating that the group is not well studied in the country. This shortfall of documentation demands for a comprehensive taxonomic exercise on Dermaptera fauna of the country.

Of the 285 Dermaptera species known from India and the oriental region, 165 are known only from India, exhibiting a high endemism of about 58 percent.

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COMMUNICATION

Moth diversity of Guindy, Chennai, India and DNA barcoding of selected erebid moths

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Abstract: In this study, diversity of moths has been documented from Chennai, the capital city of Tamil Nadu. During the study, over 100 specimens were collected from which 59 moth species were identified from the commercial hub of Chennai, Guindy. The species identified belonged to 52 genera, 11 families, and 25 subfamilies. Erebidae was a front runner, followed by Crambidae, Geometridae, Sphingidae, and Noctuidae. Furthermore, Eupterotidae, Uraniidae, Nolidae, Lasiocampidae, Pterophoridae, and Thyrididae were the least recorded families. Among 26 erebids, 14 species were subjected for identification through mitochondrial cytochrome oxidase subunit 1 gene to resolve the ambiguity. The sequences resulted were deposited in GenBank and BOLD system where they received accession numbers and process IDs. Further, phylogenetic analysis categorized *Metanastria hyrtaca* Cramer, 1782 in a separate clade.

Keywords: Barcode, biodiversity, conservation, Erebidae, moths.

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Competing interests: The authors declare no competing interests.

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Author contributions: S. Bhuvaragavan: Conceptualization, sample collection, investigation, interpretation, preparation of manuscript draft and funding acquisition. M. Meenakumari: Investigation and data curation. R. Nivetha: Interpretation of results, data curation and preparation of manuscript draft. S. Janarthanan: Project administration, supervision, funding acquisition, review and edit of manuscript.

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INTRODUCTION

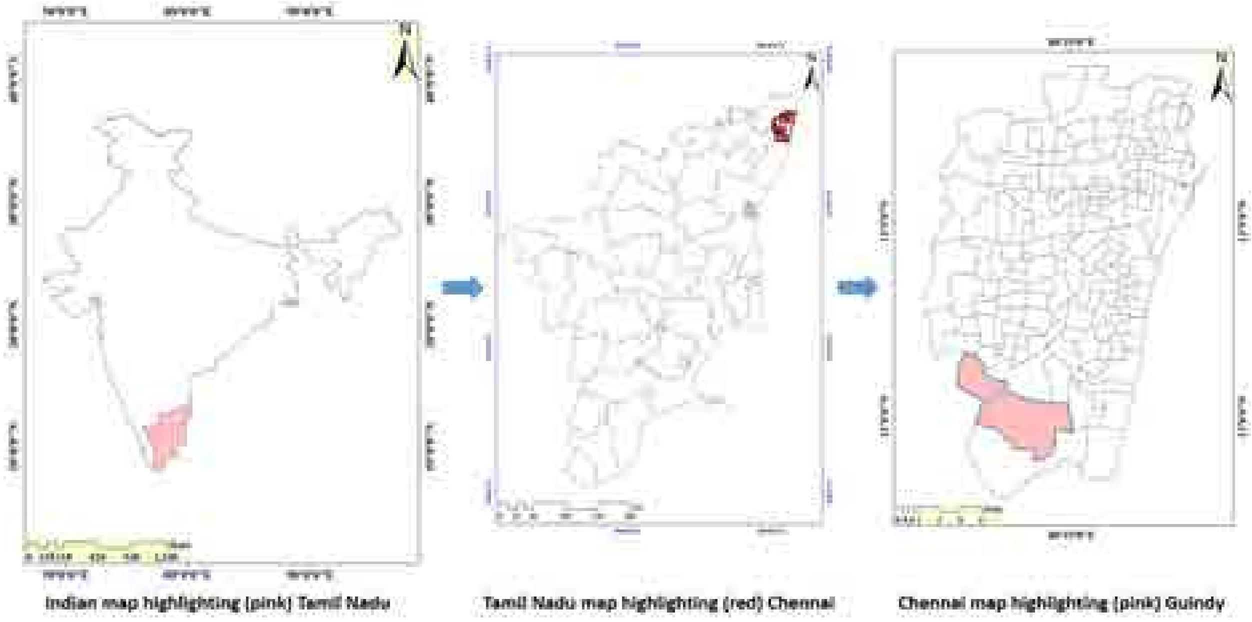
With about 1.2 million species, arthropods continue to be a dominant group in the earth's biodiversity. Their significance in sustaining the health of an ecosystem by furnishing livelihood and nutrition to human communities is far-reaching (Chakravarthy & Sridhara 2016). Nevertheless, insects are contemplated to be a potential group for understanding the effects of habitat attributes and environmental gradients on faunal diversity (Watt et al. 1997; Humphrey et al. 1999; Dey et al. 2017). Lepidoptera, which encompasses butterflies and moths, constitutes one of the three most species-rich insect orders and the largest evolutionary radiation of herbivorous animals comprising around 175,000 described species (Cover & Bogan 2015). However, another 125,000 to 150,000 species are thought to await description (Goldstein 2017). It exhibits close association with vegetation, their depletion and ensuing regeneration and is accordingly regarded as an indicator taxon (Summerville et al. 2004; Dey et al. 2015). Moths, being the most prominent terrestrial invertebrates, represent the majority of the order Lepidoptera consisting 158,570 described species (Zhang 2013). An estimation of about 15000 species of Lepidoptera belonging to 84 families are reported from India (Chandra et al. 2019). They form a critical facet of the terrestrial ecosystem by serving as nocturnal pollinators, herbivores of crops and prey for numerous species (Wagner et al. 2021). Many angiospermous plants that largely depend on animal-assisted pollination are critically associated with moth species (Wahlberg et al. 2013). Erebidae is the most prominent moth family consisting of 24,569 species belonging to 18 subfamilies (Nieukerken et al. 2011). Most of them are phytophagous as larvae and few are nectar suckers as adults (Terra & Ferreira 2020). The economic importance of family Erebidae can be attributed to the fact that it includes a significant number of major and minor pest species, and therefore their distributional knowledge is highly significant for the economy of any country (Bin-Cheng 1994). Furthermore, exploring the changes in the pattern associated with moth distribution and abundance in different local habitats constitutes a significant element of global biodiversity monitoring and conservation (Dennis et al. 2019).

Classification of organisms is a prerequisite for understanding their distribution and diversity in any habitat. Classification of closely related lepidopteran species based on wing patterns and other morphological attributes posses' difficulties and imprecision those are amenable to change as a function of environment and prevalence of several biotypes. Over the last few years, DNA barcodes are known to answer elemental ecological questions that govern community assemblage, processes of macroevolution, species conservation and incorporation of molecular tools along with morphology, which can add value to the existing information on moth diversity (Dey et al. 2019). A cytochrome oxidase subunit 1 (COI) gene identification system is contemplated to be more reliable, economical and a quick fix to the problems involved in species identification (Hebert et al. 2003). Since Hebert et al. (2003), order Lepidoptera has been regarded as a model group for DNA-barcoding studies (Goldstein 2017). Several studies have been carried out to investigate the moth diversity in peninsular India, yet Tamil Nadu has only fewer studies especially minuscule information in Chennai metropolitan, as follows. Reports of 154 species of noctuid moths from the Tamil Nadu part of Western Ghats, 67 species of erebid moths and 105 moth species from Maruthamalai hills are notable among them (Sivasankaran & Ignacimuthu 2014). Close to 135 species have been recorded in Valmiki Nagar, Chennai (Nagarajan et al. 2021). Besides being an ecologically significant group, they are less explored, finding their way into the present biodiversity conservation scenario (Dey et al. 2015). Despite rich lepidopteran diversity existing in India, attempts that are made to generate DNA barcode data of moths in India are very scarce (Dey et al. 2019; Kumar et al. 2019). Urban areas are considered significant drivers of biodiversity change due to expressively transformed landscape changes and rapid anthropogenic actions (Zari 2018). Declines in the diversity and abundance of moth population are reported over the past few years due to explicit factors like loss of habitat, fragmentation, pollution, urbanization and other related anthropogenic practices (Dennis et al. 2019; Hallmann et al. 2020). There is a research gap in knowledge of how the aforementioned explicit factors impact the diversity and abundance of population of moths in an urban environment. Consequently, an attempt was made to generate a preliminary checklist of moth fauna from Guindy, a commercial hub in Chennai and further species authentication of selected erebid moths to resolve ambiguity in identification using mitochondrial COI gene.

MATERIALS AND METHODS

Study area

The study was conducted in Guindy, one of the largest Southern neighbourhoods of Chennai, Tamil



Indian map highlighting (pink) Tamil Nadu

Tamil Nadu map highlighting (red) Chennai

Chennai map highlighting (pink) Guindy

Figure 1. Map depicting the study area (Guindy, Chennai, Tamil Nadu) generated using ArcGIS (v10.8) software.

Nadu (Figure 1). It is located between 13.010236° N latitude and 80.215652° E longitude. Guindy National Park is situated inside the city covering an area of 2.70 km^2 lies between 12.99° N, 80.23° E and 13.00° N, 80.21° E consisting of single habitat type, dry evergreen woodland.

Sample collection and identification

Moth species were collected using traps consisting of light source (Mercury vapour light) during night from places in and around Guindy, Chennai. The collected specimens were identified by their morphological characters using manuals of Bell & Scott (1937) and Hampson (1892, 1895, 1896). They were killed using chloroform, pinned using entomological pins and stretched on spreading board. Later, they were oven-dried at 52°C and were preserved in the insect box. The stretched specimens were photographed using Nikon camera after drying.

Genomic DNA extraction, PCR amplification (COI gene) and sequencing

Species authentication was carried out using the mitochondrial COI gene to resolve ambiguity in identifying 14 selected Erebid individuals. Total genomic DNA from individual species was extracted from the legs using the phenol-chloroform method. DNA extracted were then resuspended in Tris- etheylenediaminetetraacetic acid (EDTA) buffer (TE buffer) and stored at -20°C until further use. The lepidopteran specific COI primers of Hebert et al. (2003) [Forward primer - F: 5′ -ATTCAACCAATCATAAAGATATTGG-3′; Reverse primer - R: 5′- TAAACTTCTGGATGTCCAAAAAATCA-3′] were used to amplify regions of COI from 14 species of moths belonging to the Erebidae family that exhibited uncertainty in their identification using taxonomic keys. PCR amplification was carried out in a total volume of 10 µl consisting of Ampliqon-Taq DNA Polymerase 2x Master Mix RED, lepidopteran specific COI primers of Hebert et al. (2003), template DNA and sterile water (MyGene Series, Peltier Gradient Thermal Cycler). The reaction mixture was initially denatured for 5 min at 94°C followed by 35 cycles of denaturation at 94°C for 1 min, annealing of 56°C for 1 min, extension of 72°C for 1 min and a final extension cycle of 72°C for 7 min. It was then stored at 4°C. A control reaction was prepared without template DNA. A 1.2% agarose gel stained with ethidium bromide was used to examine the amplified gene product. It was then gel purified and sequenced using the Sanger dideoxynucleotide sequencing protocol (AgriGenome Labs, Kochi). Sequences were then analysed with the National Centre for Biotechnology Information (NCBI) Blast Server and submitted in NCBI GenBank and Barcode of Life Data (BOLD) system to obtain corresponding accession numbers and process IDs.

Phylogenetic analysis

A phylogenetic tree was constructed using MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms to study the evolutionary relationship among various species identified (Kumar et al. 2018). The Neighbour-Joining method was used to infer the evolutionary history, and the Kimura 2-parameter method was used to compute evolutionary distances (Kimura 1980). Bootstrap analysis was also performed using MEGA X (10000 replicates). The available (database) mitochondrial COI gene sequences of morphologically-identified species (38) (among the 45 species) were retrieved from NCBI for constructing phylogenetic tree along with COI gene-based identified species (14) in this study. Multiple sequence alignment was carried out before the construction of the phylogenetic tree using CLUSTALW multiple alignment available as accessory application in BioEdit software. All the sequences were then subjected to evolutionary analysis by phylogenetic tree construction using neighbour-joining method mentioned above.

RESULTS

Distribution profile of moth fauna from Guindy, Chennai

59 species were identified, and a checklist was constructed along with their scientific name, common name, family and subfamily (Table 1, Image 1–7). The 59 species identified belonged to 52 genera and 11 families such as Erebidae, Crambidae, Geometridae, Sphingidae, Noctuidae, Eupterotidae, Lasiocampidae, Nolidae, Pterophoridae, Thyrididae and Uraniidae (Figure 2). As a result of the comparative distribution, family Erebidae was higher in numbers with a total of 26 species (21 genera and 25 species), followed by the families such as Crambidae with 10 species (9 genera and 10 species), Geometridae with 8 species (7 genera and 6 species), Sphingidae with 5 species (5 genera and 5 species) and Noctuidae with 4 species (4 genera and 3 species); while families viz. Eupterotidae, Lasiocampidae, Nolidae, Pterophoridae, Thyriridae and Uraniidae accounted for single species each. The Family Erebidae was observed to be a species-rich group in Guindy, Chennai.

Mitochondrial COI gene amplification

The lepidopteran specific COI primers of Hebert et al. (2003) did amplify COI gene from all the 14 erebid species. The product was then gel purified, sequenced, and analysed. To resolve ambiguity in identification of Erebid moths, the DNA barcoding was adopted and the sequence results identified 14 different species of Erebidae which includes *Achaea janata* (Linnaeus, 1758), *Achaea mercatoria* (Fabricius, 1775), *Amata passalis* (Fabricius, 1781), *Asota caricae* (Fabricius, 1775), *Creatonotos gangis* (Linnaeus, 1763), *Erebus caprimulgus* (Fabricius, 1781), *Erebus macrops* (Linnaeus, 1768), *Eudocima materna* (Linnaeus, 1767), *Eudocima phalonia* (Linnaeus, 1763), *Hypocala deflorata* (Fabricius, 1794), *Olepa schleini* (Witt et al. 2005), *Perina nuda* (Fabricius, 1787), *Sphingomorpha chlorea* (Cramer, 1777) and *Utetheisa pulchelloides* (Hampson, 1907). The representative amplified COI gene is presented in Figure 3. The nucleotide sequences of mitochondrial COI gene from all the 14 species were deposited in GenBank and BOLD system where they received individual accession numbers and process IDs, respectively (Table 2).

Phylogenetic analysis

MEGA X: Molecular Evolutionary Genetics Analysis was used to construct a phylogenetic tree to infer the evolutionary relationship among various identified species of moths. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) was shown next to the branches. The Neighbourhood joining method was used instead of maximum parsimony or maximum likelihood approaches because of its accuracy, rapidity and optimum assumptions (Hong et al. 2021). The results of the phylogenetic analysis are shown in Figure 4, with *Apis mellifera* being the outgroup. *Metanastria hyrtaca* (Cramer, 1782) formed a separate clade, and all other species were clustered in another clade.

DISCUSSION

Species identification is a prerequisite in estimating biodiversity in an area and perceiving knowledge on species ecology. Thus, explicit identification is obligatory to gain insights into any species' diversity and distribution profile in any place under study. Morphological identification and taxonomic keys are important methods used extensively (Sviridov & Leuschner 1986). Notably, among the various moths collected in this study, moths belonging to Erebidae family dominated others. Presumably, their polyphagous nature could be the impetus for their wide distribution, making them fit to survive in any resource condition (Zahiri et al. 2012). A similar domination pattern of erebid moths was also observed in the Northern part of the Western Ghats (Shubhalaxmi et al. 2011; Gurule & Nikam 2013). These are then accompanied by species belonging to the family Crambidae, the second most prominent family,

Table 1. Checklist of moth fauna from Guindy, a commercial hub in Chennai.

	Family	Subfamily	Species (Common name)	Author & year
1	Crambidae	Pyraustinae	*Maruca vitrata* (Bean pod borer)	Fabricius, 1787
2	Crambidae	Pyraustinae	*Omphisa anastomosalis* (Sweetpotato vineborer)	Guenée, 1854
3	Crambidae	Pyraustinae	*Spoladea recurvalis* (Beet Webworm Moth)	Fabricius, 1775
4	Crambidae	Spilomelinae	*Botyodes asialis*	Guenée, 1854
5	Crambidae	Spilomelinae	*Cnaphalocrocis medinalis* (Rice leaf roller)	Guenée, 1854
6	Crambidae	Spilomelinae	*Cnaphalocrocis poeyalis* (Lesser rice- leafroller)	Boisduval, 1833
7	Crambidae	Spilomelinae	*Diaphania indica* (Cucumber Moth)	Saunders, 1851
8	Crambidae	Spilomelinae	*Haritalodes derogata* (Cotton leaf roller)	Fabricius, 1775
9	Crambidae	Spilomelinae	*Isocentris filalis*	Guenée, 1854
10	Crambidae	Spilomelinae	*Palpita vitrealis* (Jasmine Moth)	Rossi, 1794
11	Erebidae	Aganainae	*Asota caricae* (Tropical Tiger Moth)	Fabricius, 1775
12	Erebidae	Arctiinae	*Amata passalis* (Sandalwood defoliator)	Fabricius, 1781
13	Erebidae	Arctiinae	*Creatonotos gangis* (Baphomet Moth)	Linnaeus, 1763
14	Erebidae	Arctiinae	*Olepa schleini*	Witt et al. 2005
15	Erebidae	Arctiinae	*Utetheisa pulchelloides* (Heliotrope Moth)	Hampson, 1907
16	Erebidae	Calpinae	*Eudocima materna* (Dot-underwing Moth)	Linnaeus, 1767
17	Erebidae	Calpinae	*Eudocima phalonia* (Common fruit-piercing Moth)	Linnaeus, 1763
18	Erebidae	Catocalinae	*Achaea janata* (Castor semi-looper)	Linnaeus, 1758
19	Erebidae	Erebinae	*Achaea mercatoria*	Fabricius, 1775
20	Erebidae	Erebinae	*Dysgonia stuposa*	Fabricius, 1794
21	Erebidae	Erebinae	*Erebus caprimulgus*	Fabricius, 1781
22	Erebidae	Erebinae	*Erebus macrops* (Common Owl Moth)	Linnaeus, 1768
23	Erebidae	Erebinae	*Lacera noctilio*	Fabricius, 1794
24	Erebidae	Erebinae	*Ophiusa tirhaca* (Green Drab)	Cramer, 1777
25	Erebidae	Erebinae	*Pericyma cruegeri* (Poinciana looper)	Butler, 1886
26	Erebidae	Erebinae	*Sphingomorpha chlorea* (Sundowner Moth)	Cramer, 1777
27	Erebidae	Hypeninae	*Hypena obacerralis*	Walker, 1859
28	Erebidae	Hypocalinae	*Hypocala deflorata*	Fabricius, 1794
29	Erebidae	Lymantriinae	*Artaxa digramma*	Boisduval, 1844
30	Erebidae	Lymantriinae	*Euproctis scintillans* (Lymantriid Moth)	Walker, 1856
31	Erebidae	Lymantriinae	*Euproctis similis* (Yellow-tail Moth)	Fuessly, 1775
32	Erebidae	Lymantriinae	*Laelia exclamationis*	Kollar, 1848
33	Erebidae	Lymantriinae	*Laelia litura* (Tussock Moth)	Walker, 1855
34	Erebidae	Lymantriinae	*Olene mendosa* (Brown Tussock Moth)	Hübner, 1823
35	Erebidae	Lymantriinae	*Perina nuda* (Clearwing Tussock Moth)	Fabricius, 1787
36	Erebidae	Scoliopteryginae	*Anomis* spp.	Hübner, 1821
37	Eupterotidae	Eupterotinae	*Eupterote bifasciata* (Giant Lappet Moth)	Kishida, 1994
38	Geometridae	Ennominae	*Iridopsis larvaria* (Bent-lined Gray)	Guenée, 1858
39	Geometridae	Ennominae	*Chiasmia eleonora*	Cramer, 1780
40	Geometridae	Ennominae	*Chiasmia* spp.	Cramer, 1780
41	Geometridae	Ennominae	*Macaria multilineata* (Many-lined Angle)	Packard, 1873
42	Geometridae	Ennominae	*Cleora* spp.	Curtis, 1825
43	Geometridae	Geometrinae	*Thalassodes veraria*	Guenée, 1858
44	Geometridae	Geometrinae	*Nemoria bistriaria* (Red-fringed Emerald)	Hübner, 1818

	Family	Subfamily	Species (Common name)	Author & year
45	Geometridae	Sterrhinae	*Idaea sylvestraria* (Dotted Border Wave)	Hübner, 1799
46	Lasiocampidae	Pinarinae	*Metanastria hyrtaca* (Hairy caterpillar)	Cramer, 1782
47	Noctuidae	Hadeninae	*Chasmina candida*	Walker, 1865
48	Noctuidae	Heliothinae	*Helicoverpa armigera* (Cotton Bollworm)	Hübner, 1808
49	Noctuidae	Noctuinae	*Spodoptera litura* (Tobacco Cutworm)	Fabricius, 1775
50	Noctuidae	Noctuinae	*Mythimna* spp.	Ferdinand Ochsenheimer, 1816
51	Nolidae	Nolinae	*Nola analis*	Wileman & West, 1928
52	Pterophoridae	Pterophorinae	*Geina periscelidactyla* (Grape Plume Moth)	Fitch, 1855
53	Sphingidae	Macroglossinae	*Hippotion boerhaviae* (Hippotion Sphinx Moth)	Fabricius, 1775
54	Sphingidae	Macroglossinae	*Nephele hespera* (Crepuscular Hawkmoth)	Fabricius, 1775
55	Sphingidae	Sphinginae	*Acherontia lachesis* (Greater death's head Hawkmoth)	Fabricius, 1798
56	Sphingidae	Sphinginae	*Agrius convolvuli* (Convolvulus Hawkmoth)	Linnaeus, 1758
57	Sphingidae	Sphinginae	*Psilogramma increta* (Plain grey Hawkmoth)	Walker, 1864
58	Thyrididae	Striglinae	*Striglina scitaria* (Daincha leaf webber)	Walker, 1862
59	Uraniidae	Microniinae	*Micronia aculeata* (Asian Spotted Swallowtail Moth)	Guenée, 1857

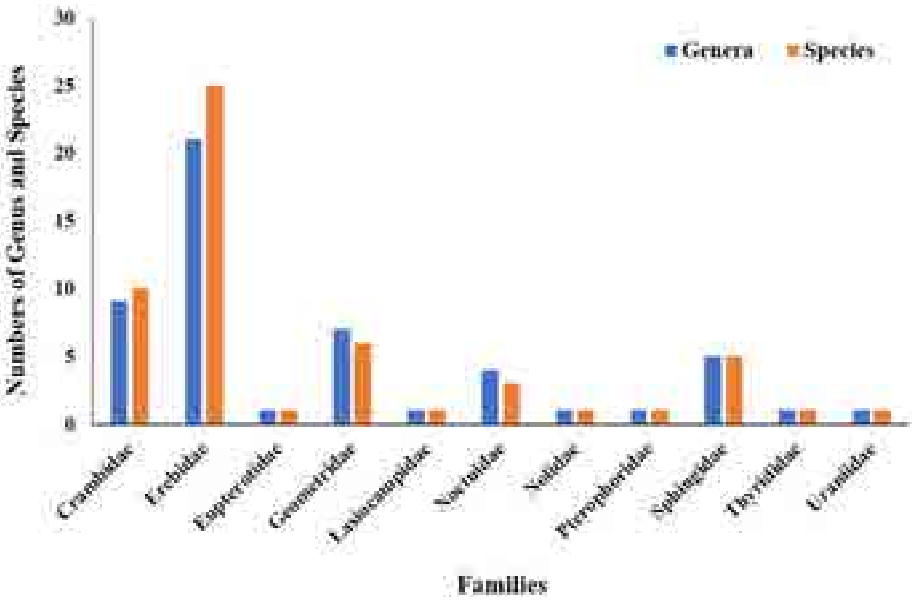


Figure 2. The species richness of moth fauna in relation to their families from Guindy, Chennai.

which is attributed to the phytophagous, detritivorous, coprophagous, parasitic habits of their larvae and ability to feed on roots, stems or grasses (Nayak & Ghosh 2020). This is followed by the distribution of Geometridae, the next abundant moth family. Comparatively, the least documented families were Eupterotidae, Uraniidae, Nolidae, Lasiocampidae, Pterophoridae and Thyrididae. Twenty-six species belonging to 18 genera of family Pterophoridae were identified and examined from the Shiwalik hills of North-West India (Pooni et al. 2019). In an attempt to document the moth fauna of Goa, *Collinsa decoratalis* (Warren, 1986), a thyridid moth, was reported as a new record from the Western Ghats. In addition to this, the uraniid moth *Pseudhyria rubra* (Hampson, 1891) was also reported for the first time from Goa (Gurule & Brookes 2021). Estimated diversity and distribution of moths in Nanda Devi Biosphere Reserve, Shendurney and Ponmudi in Agastyamalai Biosphere Reserve, Tawang district (Arunachal Pradesh) recorded that the most abundant family was Geometridae

(Chandra & Sambath 2013; Dey et al. 2015; Sondhi et al. 2018). Geometrid moths were found in abundance at tea plantations of North-East India (Sinu et al. 2013). However, Erebidae was the most profusely distributed family in Vagamon hills (Western Ghats), Dehradun and Devalsari, North East Jharkhand, Midnapore town (West Bengal) and Banaras Hindu University, Varanasi (Sondhi & Sondhi 2016; Singh et al. 2017; Nayak & Ghosh 2020; Nayak & Sasmal 2020).

Family Erebidae is copiously found in a diverse habitat, which includes predominantly polyphagous species and pests. The discovery of the species *Asota paliura* (Swinhoe 1893) belonging to the family Erebidae from India was also reported (Rajan & Shamsudeen 2020). A tentative list of Erebidae from the Tamil Nadu part of Western Ghats is documented as well (Sivasankaran & Ignacimuthu 2014). In addition, based on the survey made in Tamil Nadu at different localities, the genus *Othreis* (Synonym *Eudocima*) (Linnaeus, 1763) was one among the two genera of predominant fruit piercers, which is by far the most harmful and a severe pest on citrus, guava, pomegranate, grapes, fig, sapota, mango, papaya, and tomato in India (Ramkumar et al. 2010). An endemic Indian moth, *Gurna indica* (Moore, 1879) of the Erebidae family, was rediscovered after 125 years (Kalawate et al. 2019). An attempt has been made to document the species of Erebid moths from Aligarh, Uttar Pradesh, India (Farooqui et al. 2020). In addition, the discovery of *Asota paliura* (Swinhoe, 1893) (Lepidoptera: Erebidae) represents a new record from India (Rajan & Shamsudeen 2020). Similarly, *Pericyma cruegeri* (Butler, 1886) was also reported for the first time in India (Singh & Ranjan 2016). New additions of eight species to the known Indian fauna of the family Erebidae was also accounted (Kirti et al. 2017). Recently, moth diversity and preliminary checklist of moths from different regions of Rajasthan including Sariska Tiger Reserve were reported (Dar et al. 2021a,b; Jamal 2021). Additionally, there is also first report of Oleander Hawkmoth, *Daphnis nerii* (Linnaeus, 1758) from India (Dar et al. 2022).

DNA barcoding is a proven tool used for expeditious and unambiguous identification of species, thus circumventing the problems associated with morphology-based identification of species (Hebert & Gregory 2005). PCR amplification of short fragments within the barcoding region of the COI gene has been comprehensively used to identify different species. Sustainable identification relies mainly upon the construction of a system that utilizes DNA sequences as taxon barcodes. The mitochondrial COI gene was

Table 2. GenBank accession numbers and BOLD process IDs for erebid species authenticated using mitochondrial COI gene.

	Species	GenBank accession number	BOLD Process ID
1	*Achaea Janata*	MW421768	DBEM007-21
2	*Achaea mercatoria*	MW425700	DBEM008-21
3	*Amata passalis*	MW425697	DBEM002-21
4	*Asota caricae*	MW425696	DBEM001-21
5	*Creatonotos gangis*	MW425695	DBEM014-21
6	*Erebus caprimulgus*	MW435024	DBEM009-21
7	*Erebus macrops*	MW425705	DBEM010-21
8	*Eudocima materna*	MW425702	DBEM005-21
9	*Eudocima phalonia*	MW425701	DBEM006-21
10	*Hypocala deflorata*	MW407951	DBEM012-21
11	*Olepa schleini*	MW425704	DBEM003-21
12	*Perina nuda*	MW425699	DBEM013-21
13	*Sphingomorpha chlorea*	MW425703	DBEM011-21
14	*Utetheisa pulchelloides*	MW425698	DBEM004-21

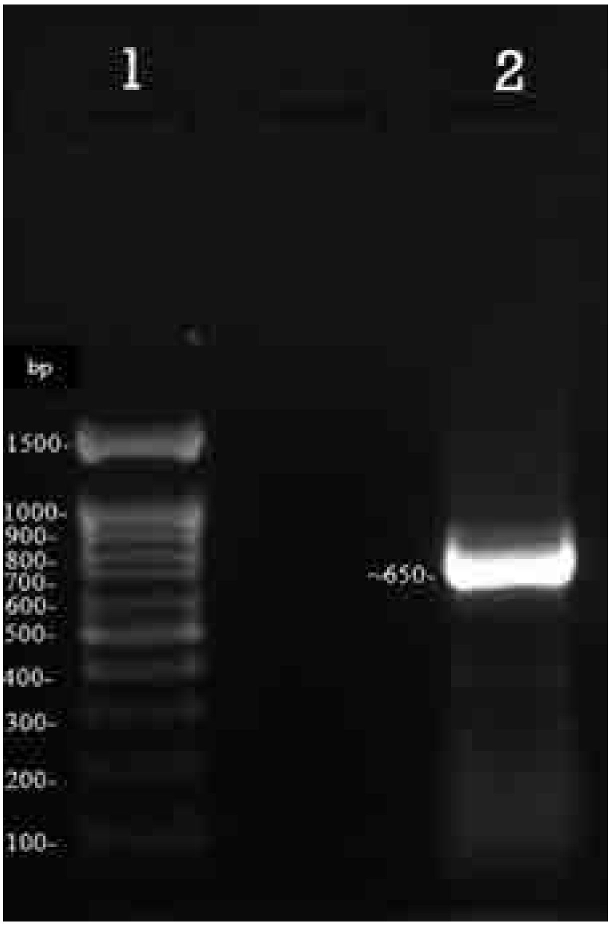


Figure 3. Electrophoresis of representative mtCOI gene: Lane 1—DNA ladder | Lane 2—Amplified product.

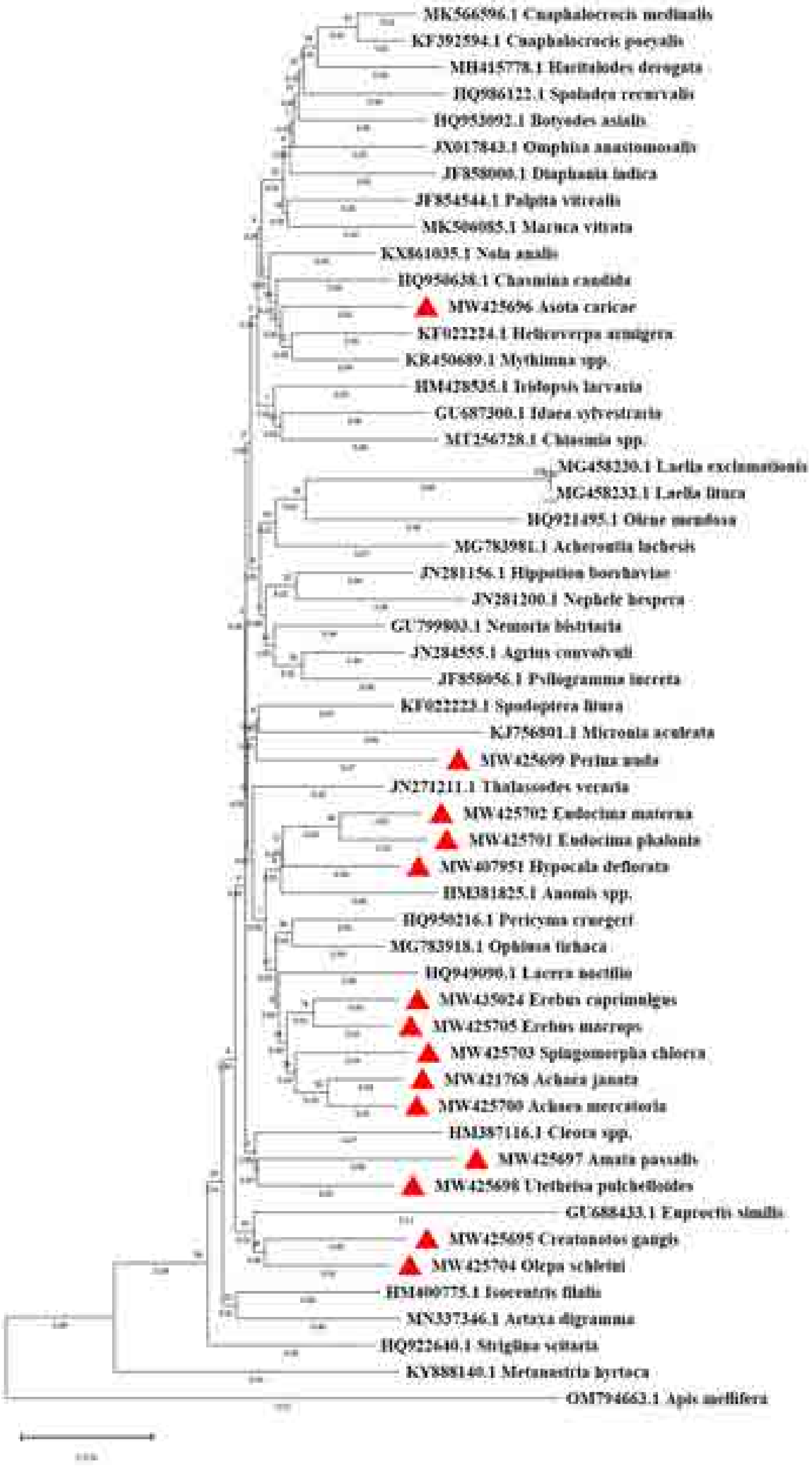


Figure 4. Phylogenetic tree based on mitochondrial COI gene sequences (MEGA X). The available (database) mitochondrial COI gene sequences of morphologically-identified species (38) (among the 45 species) were retrieved from NCBI for constructing phylogenetic tree along with COI gene-based identified species (14) (denoted in triangle) in this study.

established to serve a crucial role in the global bio-identification system for animals (Hebert et al. 2003). DNA barcoding is considered a definitive method for identifying insects (Jalali et al. 2015). COI DNA barcodes were used to distinguish among species of three lepidopteran families in north-western Costa Rica (Hajibabaei et al. 2006). A DNA Barcoding reference library of about of 113 species of geometrid moths from Western Himalaya was constructed which can effectively provide information on geographical distribution and basis for their conservation (Dey et al. 2019). Another study in Namdapha National Park, East Himalaya, produced a DNA barcode sequence of 44 Geometridae moths (Kumar et al. 2018). Further, a study concluded that a two-step barcoding analysis pipeline could swiftly characterize insects' biodiversity and explicate species boundaries for taxonomic complexes (Jin et al. 2018). Thus, the DNA barcoding tool can be used to discriminate constructively among various species in the lepidopteran family (Hajibabaei et al. 2006). To resolve ambiguity in some erebids, we used mitochondrial COI gene for identification of species. This assisted in the precise identification of the 14 erebid species. Phylogenetic studies can provide clues on the evolutionary relatedness among various groups of organisms.

The collection site of this study also covers the area in the University of Madras. Many urban universities like Banaras Hindu University have developed many strategies to monitor, manage and conserve biodiversity (Nayak & Ghosh 2020). In addition, universities have an eccentric potentiality to embrace a biophilic design inside the campus which aids in reconfiguring urban residents to the biosphere and serve as an excellent source for biodiversity-based research in urban (Liu et al. 2021). Further, the study can be extended to cover many urban areas to comprehend the effect of urbanization on the distribution profile of moths.

The distribution profile of a species depends significantly on the biogeographical region in which they occur (Gaston 1994). Artificial light pollution due to the imprudent use of artificial light was reported to cause temporal and spatial disorientation, biorhythms desynchronization, and desensitization of visual systems, affecting the moth physiology and behaviour (Nayak & Ghosh 2020). In addition, LED lights have been found to lower the risk of urban areas becoming ecological traps (White et al. 2016). Spatial habitat heterogeneity is essential to sustain the gamma diversity of macro-moth species (de Miranda et al. 2019). Urban green areas were indicated in a finding to support a wide array of moths (Paul 2021). A maiden comprehensive annotated checklist of moths of Delhi with 234 species that were not previously reported were added (Komal et al. 2021). Consequently, the number of described species may or may not constitute the definite number of species occurring in an area. Nevertheless, this documentation can provide particulars on their distribution and their conservation status.

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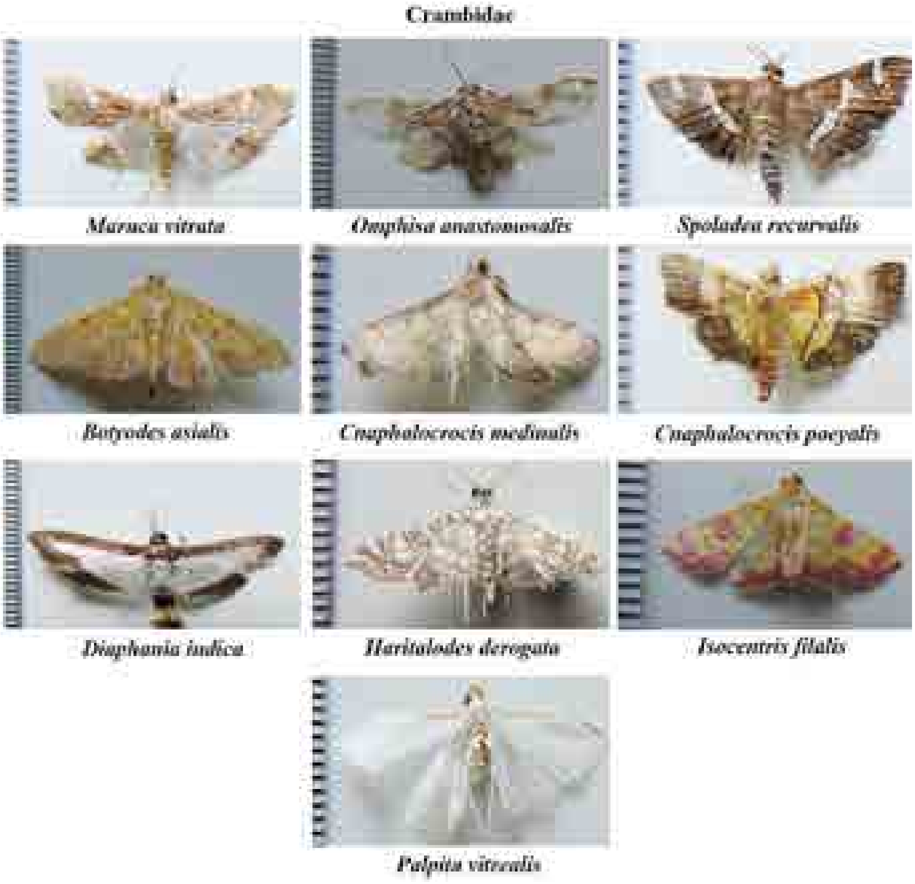

Image 1. Moths of Guindy: Crambidae.

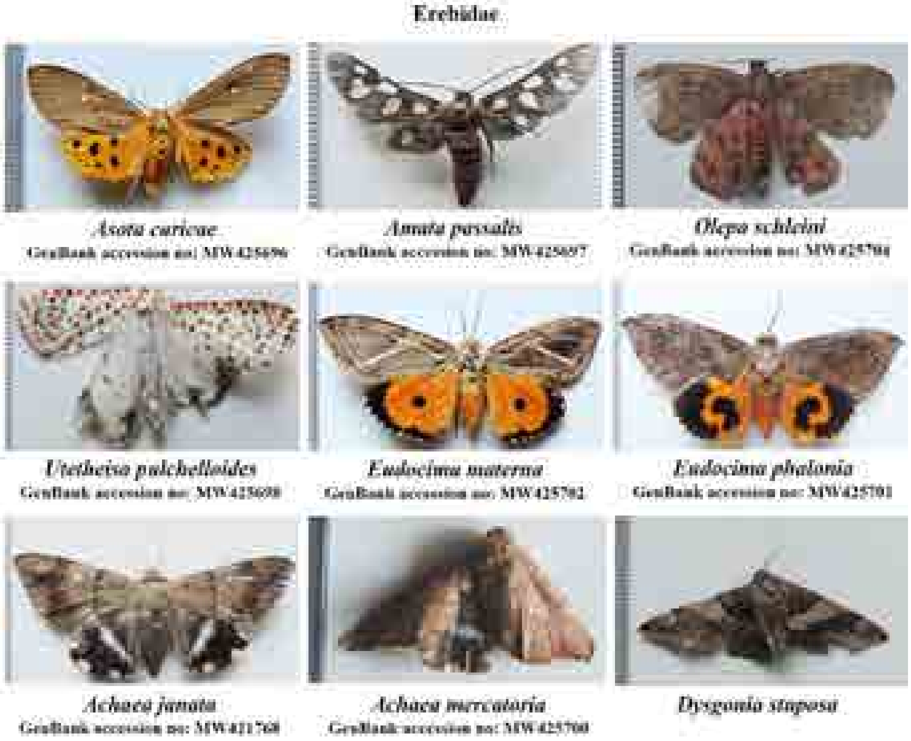

Image 2. Moths of Guindy: Erebidae.

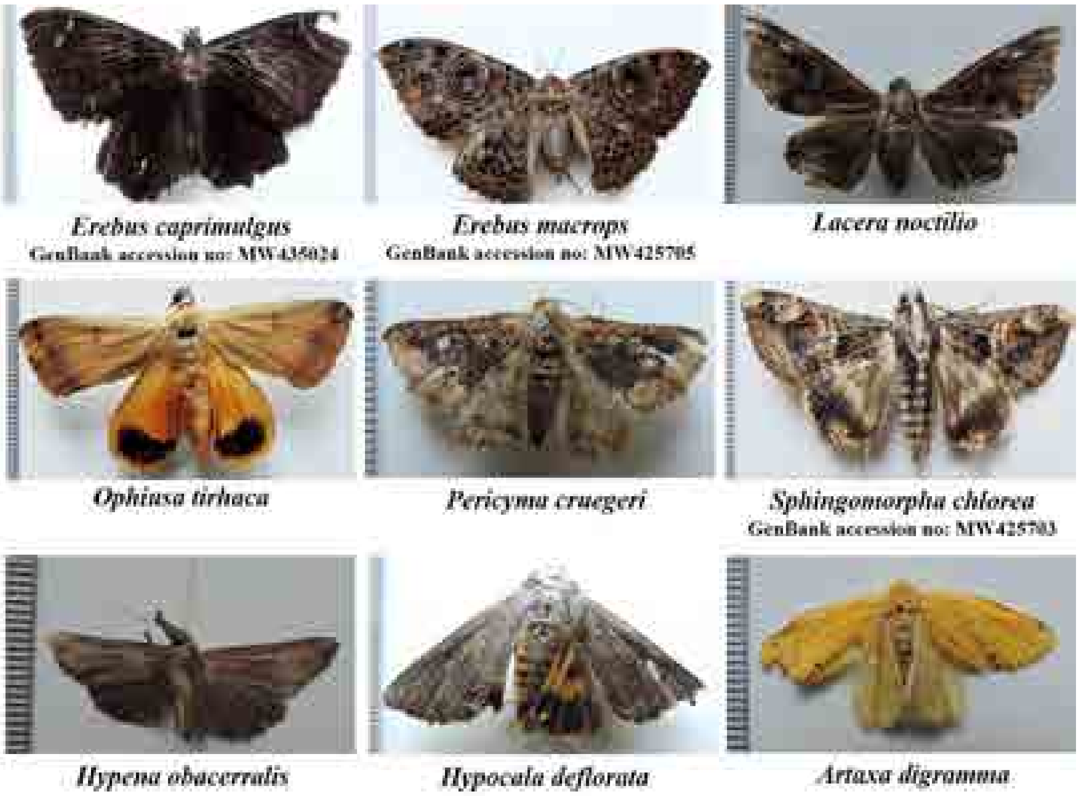

Image 3. Moths of Guindy: Erebidae.

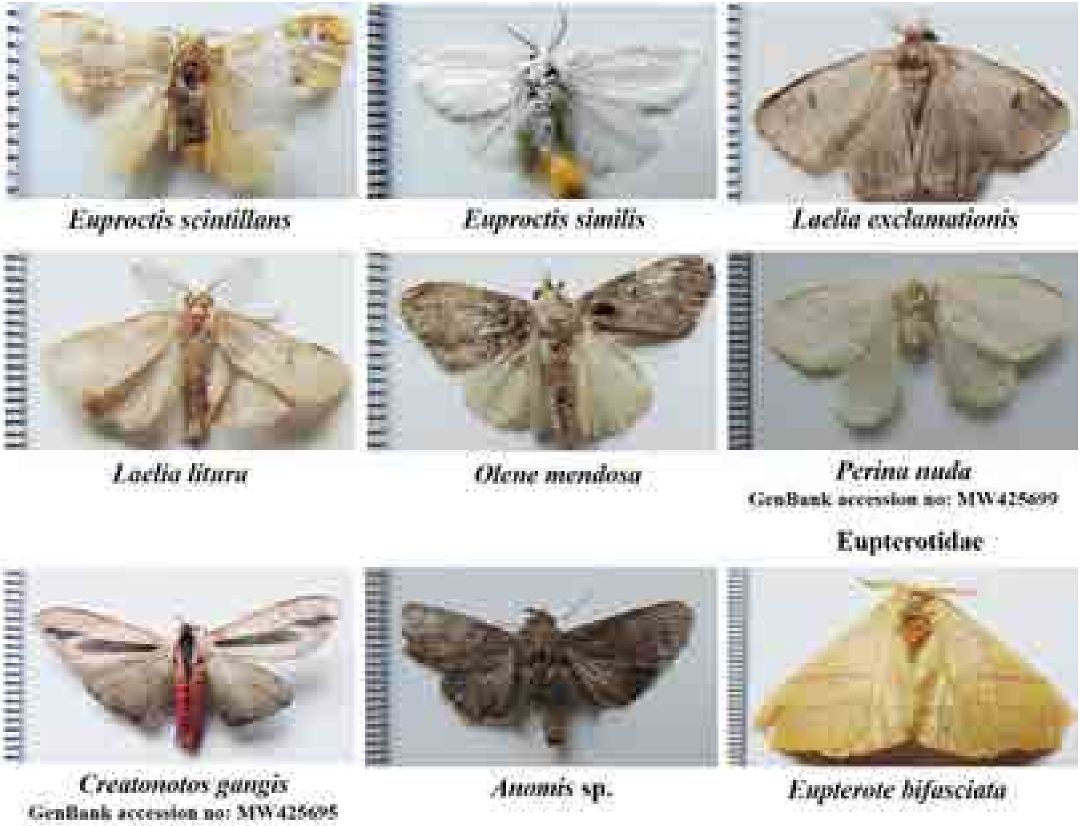

Image 4. Moths of Guindy: Erebidae and Eupterotidae.

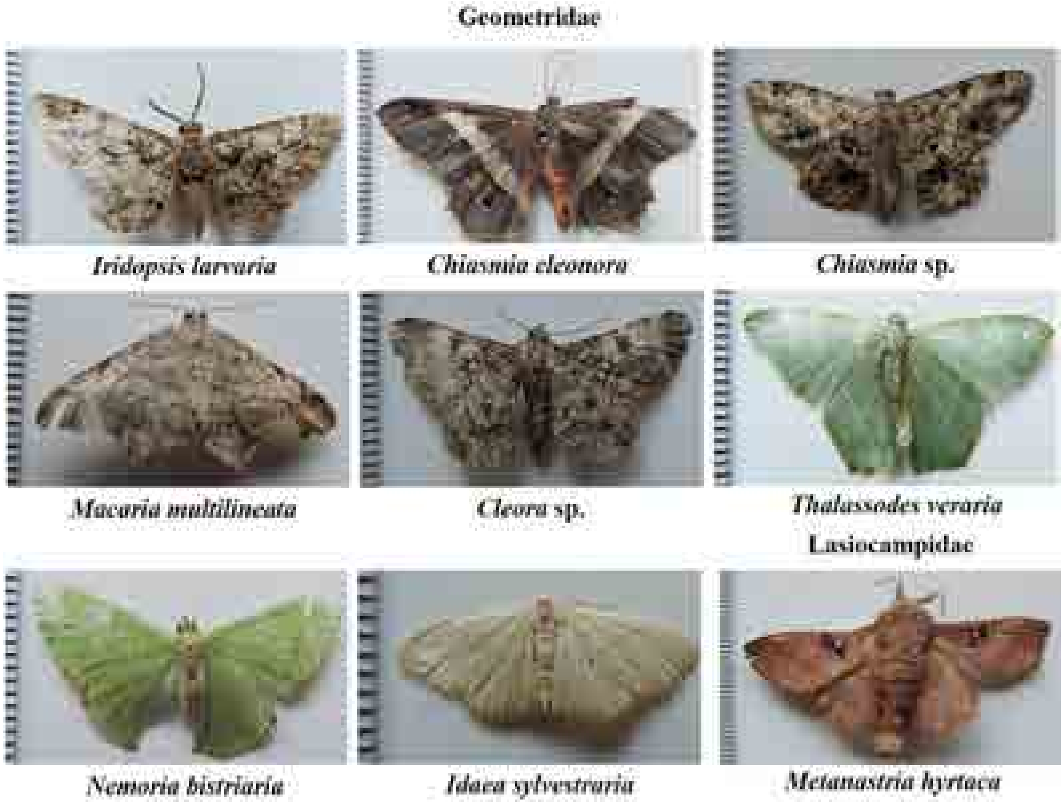

Image 5. Moths of Guindy: Geometridae and Lasiocampidae.

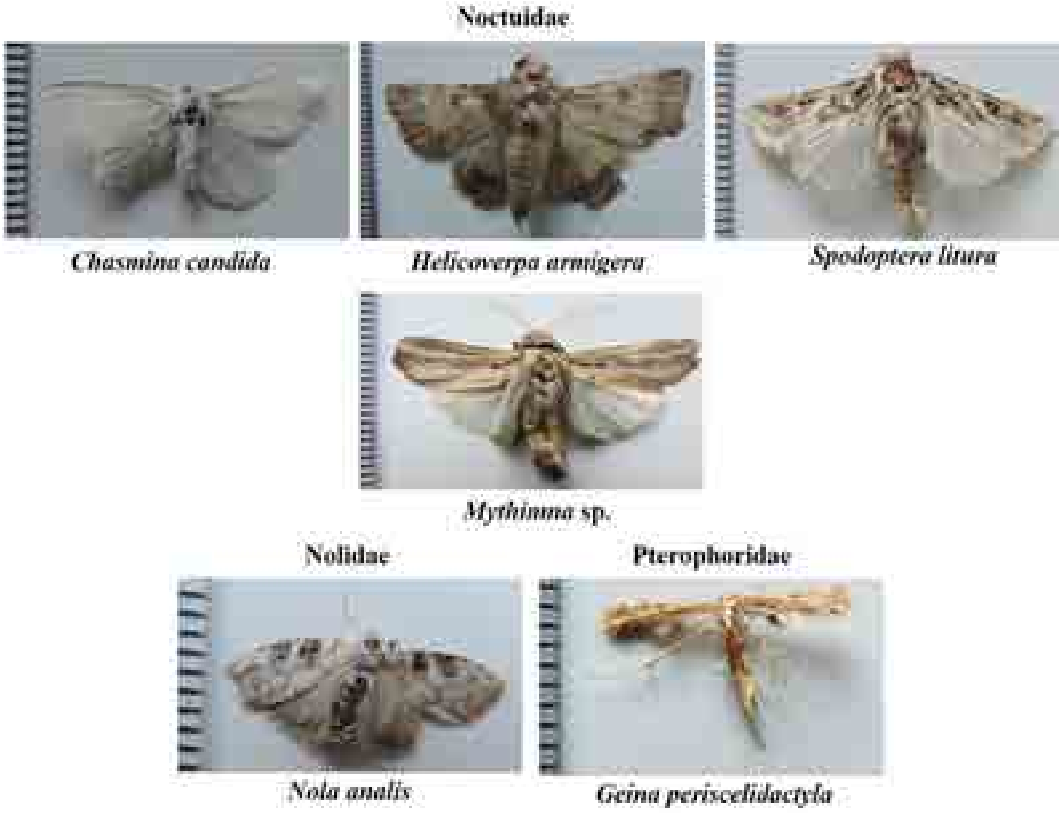

Image 6. Moths of Guindy: Noctuidae, Nolidae and Pterophoridae.

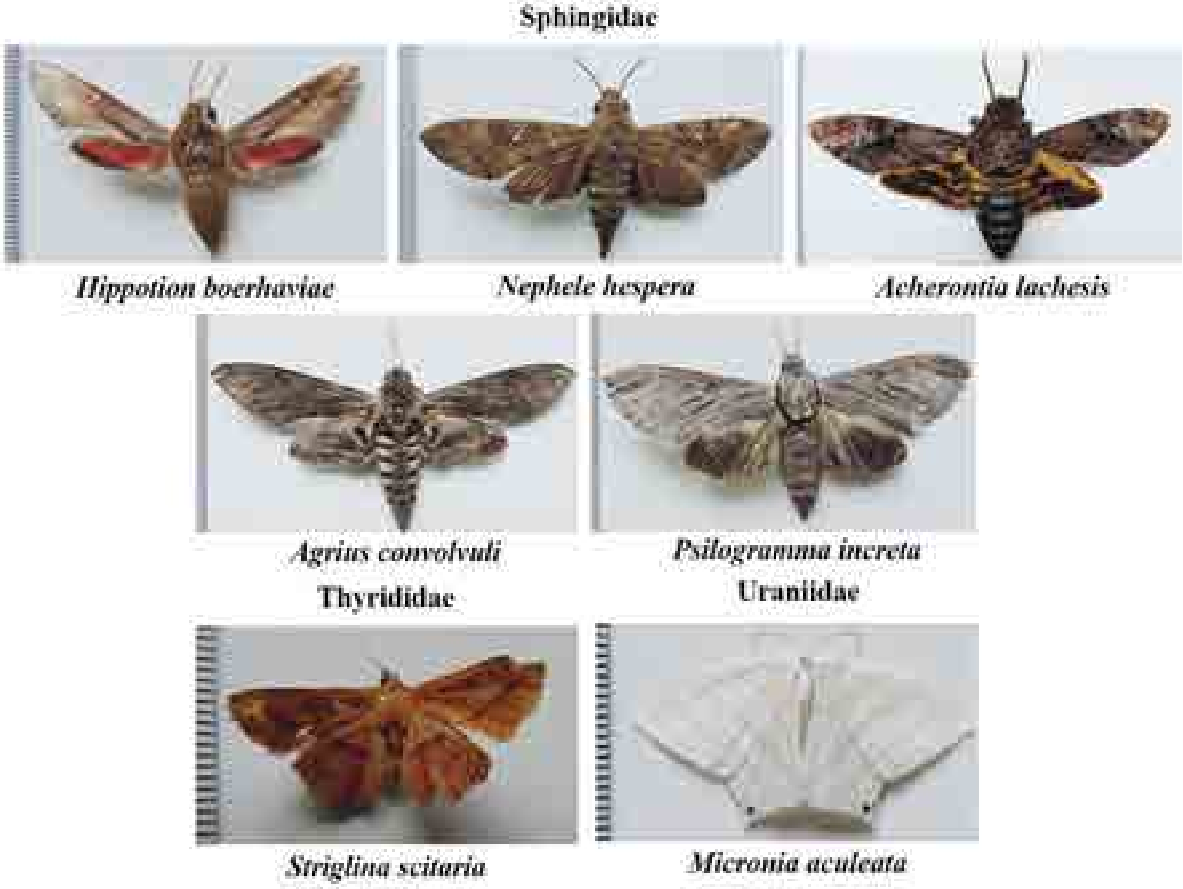

Image 7. Moths of Guindy: Sphingidae, Thyrididae, Uraniidae.

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COMMUNICATION

New record of the sphingid moth *Acherontia styx* Westwood, its parasitoid *Trichogramma achaeae* in Jasmine *Jasminum sambac* L., and its bioecology

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Abstract: The sphingid moth larvae are voracious defoliators of several crops. The present study was conducted to investigate damage of *Acherontia styx* on Jasmine, their seasonal incidence, and bioecology. Larval instars fed on jasmine leaves and flowers, with the highest defoliation being 35% and highest flower damage 47% during September. The lifecycle consisted of egg, five larval instars, pupal, and adult stages. The eggs were pearly white in colour and emerged larvae were cylindrical with a prominent anal horn. In subsequent moults the larvae continued to feed and become larger until they turned a reddish colour and ceased feeding prior to pupating in the soil. Adult moths were robust and swift fliers. The total life cycle from egg to adult was 56 days for males and 59 days for females. *Trichogramma achaeae* Nagaraja & Nagarkatti was noticed and documented as a potential egg parasitoid of the sphingid moth, a new record. A natural parasitization of eggs was noticed under field conditions, with changed colour of eggs to deep black from which a tiny parasitic wasp emerged, identified as *T. achaea.*

Keywords: Bioecology, egg parasitoid, flower damage, hawk moth, larval instars, lifecycle, seasonal incidence.

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INTRODUCTION

The sphingid moth *Acherontia styx* Westwood (Sphingidae: Lepidoptera) is newly recorded as a predator of *Jasminum sambac* L. in the present study. Sphingids are among the largest, most easily recognized, and best known among Lepidopterans. Their streamlined body, narrow wings, and rapid flight are reminiscent of hawks, hence the common name hawk moth. They hover in flight among flowers, hence they are also referred to as hummingbird moths. Most species are nocturnal, extremely strong fliers, and well known as long distance migrants (Bruce et al. 2017). They have a well-developed proboscis longer than the body to imbibe nectar. Sphingid larvae are easily recognized by their fleshy caudal horn or button, and 6–8 annulets on each body segment (Akito et al. 2009).

The present investigations on the nature and extent of damage, seasonal incidence and the bioecology of hawk moth, *A. styx* and a new record of an egg parasitoid are reported in this paper.

MATERIALS AND METHODS

Studies on the feeding damage, seasonal incidence, and exploration of natural parasitization of hawk moth, *A. styx* were undertaken in the field in ambient conditions during June 2015–May 2016 at the botanical garden premises, Tamil Nadu Agricultural University, Coimbatore. The bionomics of the pest was investigated under laboratory conditions.

Nature of damage: Field investigations were carried out to assess the seasonal fluctuation or temporal variation of jasmine hawk moth with local variety of jasmine 'Ramnad Local'. The damage of hawk moth on jasmine plants was assessed by counting total number of defoliated leaves and flowers per plant and expressed as percent damage of leaves and flowers. The total number of larvae present was also counted per plant.

Seasonal incidence: The hawkmoth incidence was recorded on five randomly selected plants per plot at fortnightly intervals, continuously for one year (March 2016–February 2017). The per cent incidence of hawkmoth was calculated by counting the total number of leaves and buds and the defoliated leaves and bored buds. The mean population of hawkmoth of jasmine was correlated with the following weather parameters, viz., maximum and minimum temperature, sunshine hours, rainfall, relative humidity, and wind velocity. The weather data of Coimbatore was collected from the meteorological observatory of Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore.

Natural parasitization: The eggs laid by adult moth on leaves and buds were monitored under field conditions. Eggs with changed colour (slight ash colour) were identified as parasitized and brought to the laboratory along with leaves and buds in air tight bags. Parasitized eggs were placed in plastic containers with lids covered tightly by muslin cloth and monitored for any emergence of the parasitoid.

Bioecology: For laboratory experiments, field collected eggs were placed over fresh leaves inside an oviposition cage. The neonate larvae after hatching were allowed to feed on the fresh jasmine leaves placed in the trays. The trays were cleaned on daily basis to remove the frass. As the larvae were voracious feeders on the leaves of jasmine, care was taken to ensure daily supply of fresh leaves for the developing larvae. During the pre-pupal stage, the larva was transferred to plastic containers filled with sand. These containers were placed in a rearing cage. The moths on emergence from the pupae were fed with ten percent sugar solution in cotton swabs, provided in penicillin vials as feed. The sexes were examined by different morphological characters and moths were kept under constant watch for studying mating, oviposition, and egg laying behaviour. Five pairs of adults were allowed for oviposition in another oviposition cage with a 250 ml conical flask with fresh jasmine branches. Leaves with the eggs were collected on daily basis and placed over fresh leaves and the biology was studied.

RESULTS

Nature of Damage: The adult moth laid pearly white eggs on the upper and lower surface of leaves and the outer surface of flower buds. The neonate larva hatched from the eggs, defoliated the young leaves and also the flowers. The larva exhaustively defoliated the leaves and also fed on the flowers. In the early stage of infestation, the plant slowly dried and died without producing any branch or shoot. The matured larva preferred to feed on the flowers than leaves causing severe damage to the plant. In the later stages of attack, infested shoots stop growing with only veins seen in the whole plant and the plant gradually died (Image 1).

Extent of damage: The percentage damage of leaves and flowers by *A. styx* was tabulated in Table 1. Maximum percentage damage to leaves caused by larvae of *A. styx* was 35.3% during September followed by 29.4 %

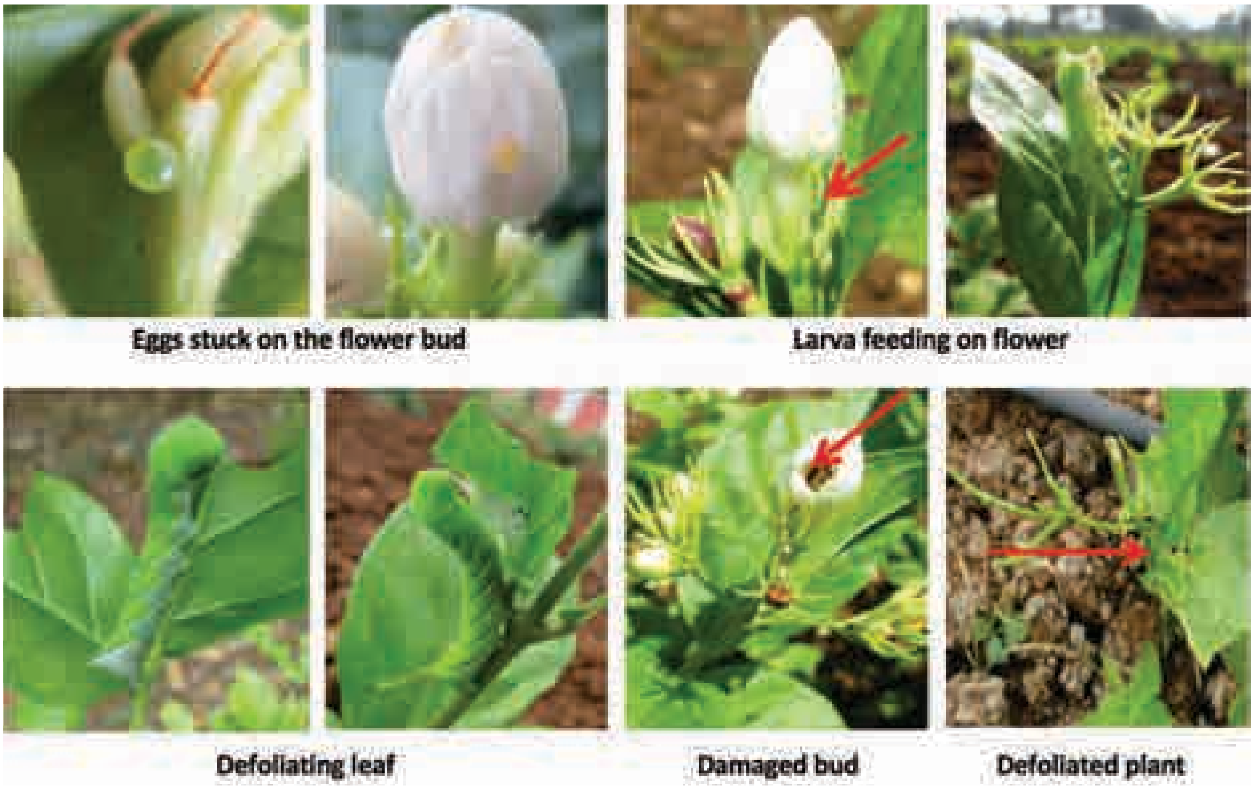

Image 1. Symptoms of predation by the hawk moth *Acherontia styx* on Jasmine. © I. Merlin K. Davidson.

in February, 26.4% in October and 21.3% in November. The least percentage of the leaves was observed in the month of August, which clearly depicted that during summer months the incidence was less. Maximum percent damage to flowers/ buds was in September with 46.8% damage followed by February 38.9%, October 37.8%, and November 30.7% (Figure 1).

Seasonal Incidence: The multiple linear regression analysis of *A. styx* was correlated in Table 2. A positive correlation was observed with maximum temperature (r = +0.530), but had a negative correlation with relative humidity (-0.677), rainfall (r = -0.553), and wind velocity (r = -0.251) with the incidence of *A. Styx* (Figure 2). The maximum (T_{max}) and minimum (T_{min}) temperature had significant contribution towards their abundance with the R^2 value 0.523 and a 1 °C increase in maximum temperature (T_{max}), 0.144% increase and with 1 °C decrease in minimum temperature (T_{min}) 0.259% decrease in *A. styx* incidence could be predicted.

Natural Parasitization: The eggs of *A. styx*, with slightly changed ash colour present in leaves and bud stalk, monitored in the laboratory, changed dark black color in two days confirming the parasitization (Image 2). After two days tiny parasitoids were found flying in the containers where parasitized eggs were placed. The emerged wasps were submitted to the division of germplasm collection and characterization, ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India for identification. The wasps were identified as *Trichogramma achaeae* (syn. *Trichogramma achaea*) Nagaraja & Nagarkatti (Image 3).

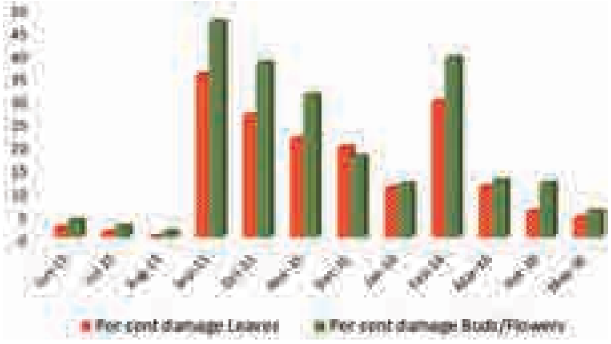

Figure 1. Extent of predation of Jasmine by the hawk moth, A. styx

Bioecology: The biology of *A. styx* was studied extensively by rearing on *J. sambac* leaves as host under invitro conditions. The lifecycle consisted of egg, five larval instars, pupal, and adult stages (Image 4). The biological parameters are enumerated in Tables 3 & 4 and detailed below.

Egg: The shiny white pearl like eggs were laid singly on the upper surface of leaves. An adult female lived 3–6 days and laid only 3–10 eggs at different intervals. Freshly laid eggs were white in colour and measured 0.70–0.80 mm, slowly changed to greenish color but they turned yellow during the incubation period when they grow from 0.90–0.97 mm. The eggs turned greenish in two days and turned bright yellow as the development progressed. The incubation period varied from 2–4 days with subsequent hatching of eggs. The egg period lasted for 5–6 days, in an average of 5.4 days with 94.7% hatchability.

Larval stages

Neonate larva: There were five larval instars in the larval stage of *A. styx*, apart from the neonate larva, which was the newly hatched larvae from the egg after completion of incubation. The neonate larva was tiny, white, cylindrical white colored instar with a conspicuous projection, anal horn, at the hind end of the abdomen. This stage fed on its own egg case immediately after hatching and after 10–15 minutes on the leaves. The nascent larva measured 3.3–4.0 mm x 0.4–0.5 mm whereas fully fed larva before moulting to the 1st instar grew to 4.5-5.5 mm x 0.6–0.7 mm. The neonate larva was tiny, whitish with long black anal horn.

First instar larva: The first instar larvae were yellowish green in colour with anal horn and measured 6.0–11.0 x 0.5–1.3 mm. The fully grown larvae measured 11.0–20.0 x 0.7–2.0 mm with yellow green head and thorax; and dark green abdomen. The anal horn was dark black. Three pairs of thoracic legs on 1st and 3rd thoracic segment and four pairs of prolegs on 6th–9th abdominal segments were observed. A fifth pair of prolegs was seen on the 13th abdominal segment. The larva fed on leaves but remained inactive prior to moulting. The first instar lasted for 3–4 days in an average of 3.50±0.5 days.

Second instar larva: The second instar larva was greenish with lateral yellow oblique lines in the sides of the abdomen and started eating voraciously. The anal horn turned greenish. The larva measured 20.0–35.0 x 2.0–4.0 mm. The fully fed larva measured 35.0–40.0 x 4.0–6.0 mm prior to moulting. The anal horn was dark reddish-black and measured 3.5–5.5 mm long and has a width of 0.3–0.4 mm. The legs displayed minute black spots. The second instar larva lasted for 3–4 days in an average of 3.6±0.7 days.

Table 1. Extent of damage of hawk moth, *Acherontia styx* in jasmine.

	Months	Percent predated	
		Leaves (%)	Buds/flowers (%)
1	June 2015	2.10	3.60
2	July 2015	1.10	2.30
3	August 2015	-	1.10
4	September 2015	35.30	46.80
5	October 2015	26.40	37.80
6	November 2015	21.30	30.70
7	December 2015	19.30	17.30
8	January 2016	10.70	11.50
9	February 2016	29.40	38.90
10	March 2016	11.0	12.30
11	April 2016	5.60	11.70
12	May 2016	4.20	5.60

*Mean of three observations

Table 2. Multiple linear regression analysis for the prediction of hawk moth, *Acherontia styx* in jasmine.

Weather parameter	Correlation coefficient
Intercept (a)	28.83*
Maximum temperature (T_{max})(°C)	0.144
Minimum temperature (T_{min}) (°C)	-0.259
Relative humidity (%)	-0.718*
Rainfall (mm)	-0.032
Sunshine (hrs)	2.034*
Wind Velocity(Km/hr)	0.343
R^2	0.523

Regression equation:
$Y2 = 28.83^{*} + 0.144X1 - 0.259X2 - 0.718^{*}X3 - 0.032^{*}X4 + 2.034^{*} X5 + 0.343X6$

Third instar larva: The third instar larva was more plumpy and caused extensive defoliation. The larva had yellowish oblique bands lined with a bluish-green outer layer with prominent black spiracles. The larva was quite big in size, 40.0–50.0 mm x 6.0–6.5 mm when newly moulted and 50.0–60.0 mm x 6.5–7.0 mm when fully fed. The anal horn was greenish and the tip was yellowish and measured 5.0–6.5 mm x 0.4–0.5 mm. The body colour was green with light yellow oblique lines on the abdomen. Tiny tubercles were present laterally on the terga. It voraciously fed on the leaves and branches and almost entire plant was denuded within 24 hours. The third instar lasted for 4–5 days in an average of 4.5±0.52 days.

Fourth instar larva: The fourth instar larva was greenish yellow and stout with lateral yellow marking and prominent black spiracles in each abdominal segment measuring 60.0–65.0 mm x 7.0–7.5 mm having

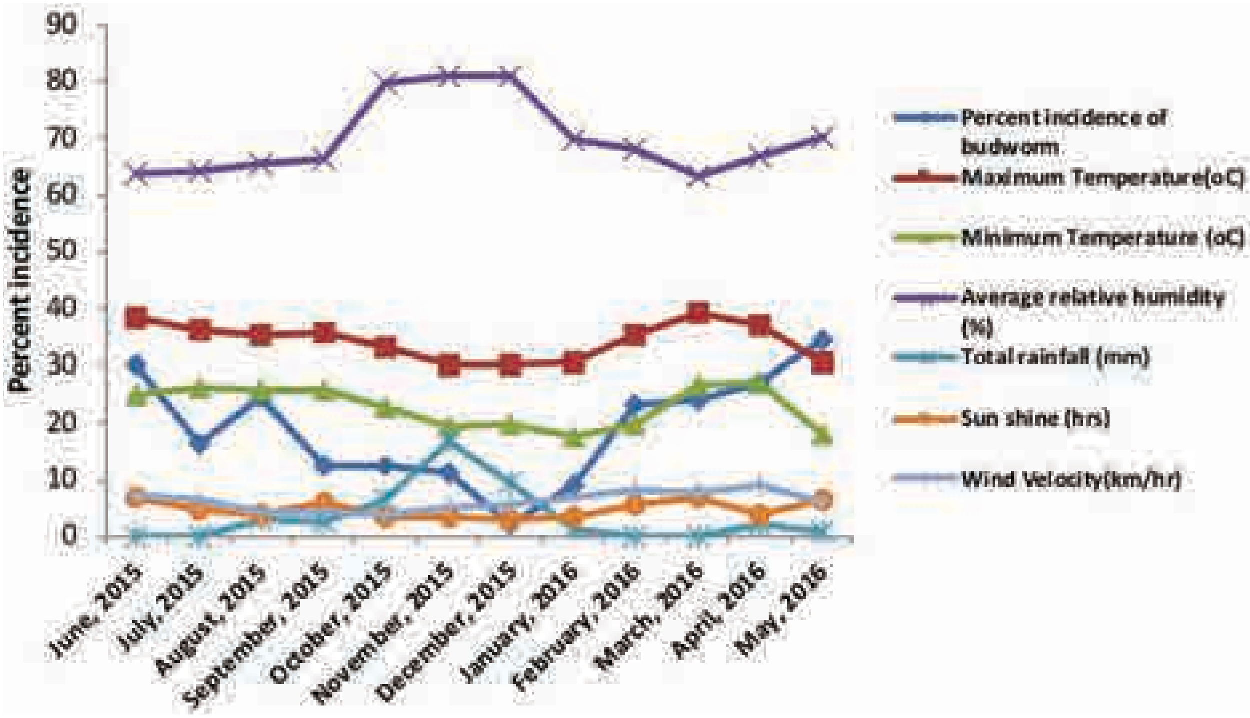

Figure 2. Influence of weather parameters on seasonal incidence of the hawk moth, *Acherontia* styx.

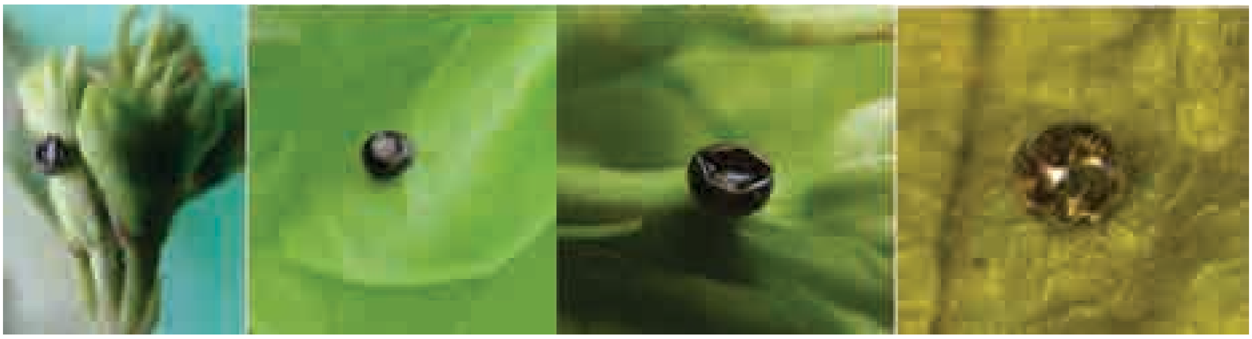

Image 2. Parasitized eggs of the hawk moth *Acherontia styx in* bud stalk and leaves. © I. Merlin K. Davidson.

Image 3. *Trichogramma achaeae* emerged from parasitized *Acherontia styx* eggs. © I. Merlin K. Davidson.

Table 3. Mean sizes of different life stages of hawkmoth, *Acherontia styx*.

Stage		Length (mm)		Width (mm)	
		Range	Mean ±SD	Range (mm)	Mean ±SD
Egg	Freshly laid			0.70–0.80	0.75±0.03
	Full grown			0.90–0.97	0.94±0.02
Neonate larve	Young larva	3.3–4.0	3.64±0.30	0.40–0.50	0.45±0.04
	Full grown	4.5–5.5	5.04 ±0.04	0.60–0.70	0.68±0.07
	Anal horn	2.3–3.0	2.68±0.31	0.2–0.25	0.33±0.01
First instar larva	Young larva	6.0–11.0	8.26±1.95	0.5–1.3	0.9 ± 0.31
	Full grown	11.0–20.0	15.2±3.83	0.7–2.0	1.32±0.53
	Anal horn	2.8–3.2	2.18±0.18	0.2–0.3	0.24±0.04
Second instar larva	Young larva	20.0–35.0	26.4±6.02	2.0–4.0	2.9±0.87
	Full grown	35.0–40.0	37.4±2.07	4.0–6.0	4.74±0.78
	Anal horn	3.5–5.0	4.18±0.55	0.3–0.4	0.34±0.04
Third instar larva	Young larva	40.0–50.0	45.4±4.33	6.0–6.5	6.3±0.16
	Full grown	50.0–60.0	54.8±4.15	6.5–7.0	6.72±0.19
	Anal horn	5.0–6.5	5.76±0.614	0.4–0.5	0.43±0.015
Fourth instar larva	Young larva	60.0–65.0	63.2±1.5	7.0–7.5	7.5±0.34
	Full grown	65.0–75.0	68.3±1.9	7.6–8.0	7.82±0.15
	Anal horn	6.5–7.5	6.96±0.34	0.5–0.6	0.72±0.19
Fifth instar larva	Young larva	65.0–75.0	69.4±3.85	7.5–8.5	7.88±0.311
	Full grown	75.0–85.0	79.6±3.71	8.5–10.0	9.22±0.53
	Anal horn	7.5–8.5	7.96±0.39	0.6–0.75	0.68±0.05
Pre-pupa		50.0–62.0	57.8±3.19	5.0–5.30	5.21±0.07
Pupa		40.0–42.0	41.1±0.53	4.5–5.0	4.68±0.16
Adult male		30.0–32.0	31.6±0.22	35.0–36.0	35.28±0.19
Adult female		37.0–38.0	37.46±0.29	39.0–40.0	39.48±0.25

a cylindrical shape. The anal horn changed its colour to yellow and measured 6.5–7.5 mm x 0.5–0.6 mm in size. One pair of spiracles is situated laterally on the thorax and seven pairs on abdomen (4th–10th segments). The last pair of spiracles was seen on the 11th segment. The yellow oblique lateral stripes on segments 5–11 were sharply defined, edged above with dark purple region. The anal horn was yellowish in color, true legs black, prolegs, and claspers green shaded. Spiracles were oval, yellowish with a central black slit, bordered greenish. The fully fed larva before moulting was of 65.0–73.0 mm x 7.6–8.0 mm size. The last instar larvae voraciously fed on the leaves which lasted for 4–5 days in an average of 4.8 days.

Fifth instar larva: The fifth instar larva was a colourful plump cylindrical creature, appeared yellowish-green and turned reddish-yellow as the stage progressed. The newly moulted larva measured 65.0–75.0 x 7.5–8.5 mm. The full fed caterpillar measured 75.0–85.0 x 8.5–10.0

Table 4. Biological parameters of the hawk moth, *Acherontia styx* of jasmine.

	Particulars	Period (days)
1	Egg period	5.40±0.527
2	Hatching per centage	94.70±1.11
3	Larval period First instar Second instar Third instar Fourth instar Fifth instar	 3.50±0.52 3.60±0.70 4.50±0.52 4.80±0.44 4.70±0.44
4	Pre-pupal period	4.50±0.52
5	Pupal period	19.7±2.23
7	Female Adult longevity Male Adult longevity	18.9±32.95 12.8±1.32
8	Total life cycle -Females Total life cycle -Males	58.6±4.97 55.8±2.10
9	Pre-oviposition period	1.30±0.50
10	Ovi-position period	2.10±0.33
12	Sex ratio	1:0.96
13	Fecundity	6.10±1.20

Image 4. Biology of Hawk Moth *Acherontia atyx*. © I. Merlin K. Davidson.

mm with dark yellow anal horn of 7.5–8.5 x 0.6–0.75 mm. The larva completely turned reddish, sluggish, and stopped feeding into its process of pupation. The mean larval period varied from 19.75–20.00 days in field conditions.

Cannibalism: Cannibalism was observed in older larvae, frequently in 4th and 5th instar larvae. When different larval instars were present in the tray, the older larvae attacked the younger instar larvae and sucked the fluid oozing out from the injured terga of the thoracic region. Thereafter, the injured larvae were totally consumed leaving the head capsule along with the prothorax. Moreover, during moulting process, the exuviae were completely consumed by the molted caterpillars.

Pre-Pupa: The larvae were pupated in soil. The full-grown last/5th instar larva stopped feeding and burrowed deep in soil with head forwards. It formed a cell like structure for pupation, shrunk in size, and curved to a semilunar shape. Then abdominal and thoracic legs were deformed and finally the head capsule was casted out and a pre pupa was formed. It is conical, dark yellowish-green coloured measuring 50.0–62.0 x 5.0–05.3 mm. The prepupal stage lasted for 4–5 days in an average of 4.5±0.52 days.

Pupa: The pre-pupa turned to a conical, soft, shining blood red coloured pupa with two black eyes on the anterior end, which was the head region measuring 40.0–42.0 x 4.5–5.3 mm. Distinct marks were present in the abdominal segments and the terminal segment ending into a spine like structure. The presence of genital and anal pores in the 8th and 9th abdominal segments respectively in male and on 7th and 9th abdominal segments in the female represented the sexual dimorphism. The pupa was obtect, stout, and dark reddish-brown. The pupal period varied between 17 and 23 days, in an average of 19.7 ±2.23days.

Adult: The Adult moths were large, robust, heavily built with a wing span of 35.0–40.0 mm. They were commonly called as a hawk moth, sphinx moth or death's head moth in the basis of their structural and behavioral characters. The moths were swift fliers resembling hawk. The forewing of moth was ornamented with a mixture of dark blotchy brown and grey patterns with black wavy markings and a protruding yellow spot on each wing. The abdomen was yellow in colour, the hind wings were yellowish-grey with black marks and large vertical line. A pair of large, black, and transparent eyes and a pair of thin feathery antennae were present on the lateral sides of head of both sexes. Adult males measured 30.00–32.00 mm in length and 35.00–36.00 mm in width with an expanded wing (both wing span about 7 cm). Females were longer, being 37.00–38.00 mm in length and 39.00–40.00 mm (mean 39.90±0.21 mm) in width with an expanded wing (both wing span about 8 cm) (Table 3). Males were smaller in size than the females. A shiny greyish tuft on thorax with one pair of black dots in males distinguished them from the females. Females were larger in size and had shiny reddish grey tuft like a human skull on the thorax. The adult moth had dark brownish fore wings and yellow hind wings with black markings, with a characteristic, skull-like marking on the thorax. The adult female lived for 18.9 days and males for 12.8 days. The ratio of male to female was 1:0.96.

Total lifecycle: The total life cycle from egg to adult was 55.8 ± 2.10 days for males and 58.6 ± 4.97 days for females.

DISCUSSION

Jasmine is a traditional flower crop belonging to the olive family (Oleaceae), cultivated throughout the tropical and subtropical parts of the world for its sweet-scented fragrant flowers (Ranadas et al. 1985; Bose & Yadav 1989; Kanniamal & Divya 2016). The commercial production of jasmine is affected by various pests. The hawk moth, *A. styx* Westwood was a sporadic pest, but voracious feeder of jasmine crop at larval stage, observed for the first time.

The incidence of hawkmoth was noticed in jasmine plant for the first time. The larvae were voracious feeders of leaves and caused severe defoliation perpetrating substantial damage at times. The adult moth laid eggs singly on both the side of leaves as well as flower buds. The emerged larva fed on the leaves as well as on the flowers. The matured larvae severely defoliated the plant leaving the vein alone. A single larva was capable of skeletonizing the whole plant. The present work was a novel approach in Tamil Nadu Agricultural University, Coimbatore, which has not been studied before or explored elaborately. However, some works on the bioecology of hawk moth, *A. styx* was reported by Mehta & Verma (1968), Lefroy (1990), Rai et al. (2001) Biswas et al., (2001), Sharma & Choudhary (2005), Atwal & Dhaliwal (2005) in *Sesamum indicum*, and Kumar et al. (2012) in an alternate host, *Clerodendrum phlomidis*.

The hawkmoth, *A. styx*, is newly recorded as a voracious predator of Jasmine in the present study during the year 2016. *J. sambac* as a larval host plant of *A. styx* was reported by Bangpai et al. (2017) in Thailand. The adult moth laid eggs in the adaxial and abaxial side as well as on the bud surface. The emerged larvae severely defoliated the leaves and fed on the flowers with the plants left with the stem and veins alone. This nature of the feeding of *A.styx* was reported previously by several workers (Ahirwar & Gupta 2010; Kumar et al. 2012; Devi & Ramaraju 2016).

The incidence of A. *styx* was found to be maximum in the month of September followed by February, October, and November. This finding is in line with Premdas (2017) who stated that the peak infestation of *A. styx* in terms of symptoms of infestation was observed in the 2nd week of February at 40 DAS in 1st sowing and the 3rd week of February at 33 DAS in the 2nd sowing with regard to sesame crop. The results also coincided with Bondre et al. (2016) in sesame crop who stated that the peak activity of the hawk moth were recorded during the 2nd week of October and reached its ultimate in a week (0.14 larvae/ plant) and declined progressively until the crop matures. Virani (2010) also stated that the incidence of this sphinx moth (*A. styx*) appeared after the 6th week of sowing, i.e., the 3rd week of March with a minimal infestation of 0.24 larvae per plant.

The multiple linear regression analysis of hawk moth, *A. styx* showed a positive correlation with maximum temperature (r = +0.530), but had a negative correlation with relative humidity (-0.677), rainfall (r = -0.553) and wind velocity (r = -0.251), which is in agreement with Ahirwar et al. (2009), who stated that maximum had a positive correlation but minimum temperature and rainfall had a negative correlation with the incidence of *A. Styx.*

A new record of natural parasitization was recorded in the eggs of hawkmoth, *A. styx* in jasmine. The ash coloured parsitized eggs slowly changed to black (Hoffman et al. 2002) from which a tiny parasitic wasp emerged which was identified as *Trichogramma achaea*. *T. achaeae* is a very tiny parasitic wasp in managing tiny lepidopteran larva, *Tuta absoluta*. *T. achaeae* laid its eggs in the eggs of a butterfly or moth. After an egg has been parasitized, it turns black. An adult *T. achaeae* is about 0.3 mm in size. The egg parasitoid *T. achaeae* has been recognized as a potential candidate parasitoid for the managing tomato pinworm, *T. absoluta* (Cabello et al. 2009; Oliveria et al. 2017; Zouba et al. 2013). Parasitism of *H. zea* eggs by *T. achaeae* in corn fields up to 60% and eggs of lepidopteran species belonging to family Lycaenidae was reported by Wright & Richard (2011). Eggs of *Achaea janata* (Noctuidae) being parasitized by *T. achaeae*, was also reported (Wright & Richard 2011; Krishnamoorthy 2012).

The binomics of *A. styx* was studied in detail. Eggs are oval, translucent and yellowish-green color usually singly on a host plant leaf, which was previously reported by Bangpai et al. (2017). The newly hatched larvae fed their egg shell as their first meal which was in line with the findings of Bangpai et al. (2017). The neonate larva emerged in 2–3 days as reported by Rai et al. (2001); Sharma & Chowdary (2005); Atwal & Dhaliwal (2005). The larval period was usually longer and lasted for around two months or more, which coincided with the findings of Mehta & Verma (1968); Lefroy (1990); Rai et al. (2001); Atwal & Dhaliwal (2005) and Sharma & Choudhary (2005). The full-grown 5th instar larvae burrow about deep in the soil and form an oval cell for pupation, which is in agreement with the previous findings (Lefroy 1990; Rai et al. 2001; Atwal & Dhaliwal 2005). The pupal period existed for 15–21 days which was witnessed in previous studies too (Sharma & Choudary 2005). The full-grown caterpillar was bright green in colour with light oblique yellow strips on each side and an anal horn, which was also reported by Mehta & Verma (1968); Lefroy (1990); Rai et al. (2001). A generation of hawk moth, *A. styx* lasted for 58.6 days, which is in conformity with Kumar et al. (2012), who found that the total life of hawk moth is 52.6 days in its new alternate host *C. phlomidis* Linneaus. Analogous results were corroborated by Mehta & Cerma (1968), Atwal & Dhaliwal (2005) and Sharma & Chowdary (2005).

CONCLUSION

The hawk moth, *A. styx* as a predator of Jasmine is reported for the first time. All the stages of larva defoliated the leaves and fed on flowers too. The incidence was severe in February and October. The pearly eggs lasted between 5–6 days and the total larval period was between 22–23 days. It took 4–5 days to get prepared for pupation in soil for around 20 days. The adult female lived for around 18–19 days and male for around 12–13 days with a total life cycle of 58–59 days for female and 55–56 days for males. A new record of natural egg parasitizion by the parasitic wasp, *T. achaea* was noticed, which could aid in the natural parasitization of the pest.

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COMMUNICATION

Identification and phylogenetic analysis of various termite species distributed across southern Haryana, India

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Abstract: Termites are highly abundant and vital insects that directly and indirectly influence local soils. The present study investigated the morphological and molecular phylogenetics of termite species collected from study fields in southern Haryana, India, from 2020 to 2021. Samples were subjected to integrated systematic analyses, taking into account the mandible features of soldiers for morphological systematics and 16S rRNA gene-based phylogeny for molecular systematics. Based on the external phenotype and relations to reference sequences in NCBI GenBank, 21 species were identified; these included five species each from *Odontotermes* and *Microcerotermes*, four species from *Coptotermes*, two species each from *Microtermes* and *Eremotermes*, and one species each from *Amitermes*, *Angulitermes,* and *Neotermes*. 16S rRNA gene sequences were utilized to construct phylogenetic trees to explore the relatedness among identified species. The results of the different molecular phylogenetic approaches including maximum parsimony, maximum likelihood, and neighbor-joining revealed nearly identical topological relations between the species and grouping of individuals in relation to their genera. The maximum parsimony tree based on mandible features has been found to be effective in clustering multiple species of a given genus in a clade in at least three termite genera.

Keywords: 16S rRNA gene, Kalotermitidae, mandible, molecular analysis, morphological taxonomy, phylogenetic tree, Rhinotermitidae, Termitidae.

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INTRODUCTION

Isopterans are one of the most significant insect groups, serving as key decomposers of wood and other materials, and termites can also be serious pests of wood and other crops (Bignell & Eggleton 2000; Ackerman et al. 2007; Pooja et al. 2017; Govorushko 2019; Korb et al. 2019). Termites are eusocial insects, with colonies exhibiting caste specialization and division of labour. Around 3,106 species are listed worldwide, of which 337 have been reported from India and 39 from Haryana State (Bignell et al. 2010; Krishna et al. 2013; Pooja et al. 2017; Paul et al. 2018; Effowe et al. 2021; Bhanupriya et al. 2022a,b). Since termites are highly diverse it is important to identify them properly and classify them into well-defined groups, typically via multiple approaches.

Classical systematics has been used to classify termite species using morphological features of worker and soldier castes (Donovan et al. 2000; Aldrich et al. 2007; Rocha et al. 2019), which is useful to the genus level. To accurately discern species, a combination of molecular and morphological approaches have proven useful (Wallman & Donnellan 2001; Austin et al. 2005; Yeap et al. 2007), especially in cases where only partial or damaged samples are available (Judith & Nicola 2008). Molecular systematics based on mitochondrial DNA sequences has proven especially effective in unravelling termite taxonomy (Wells & Sperling 2001; Roy et al. 2006). Studies have been carried out using a variety of mitochondrial genes, including those for cytochrome oxidases and ribosomal RNAs (e.g., 12S and 16S rRNA; Murthy 2020). Mitochondrial genes tend to vary more rapidly than those in nuclear DNA, and they are inherited maternally (Behura 2006). 16S rRNA-based trees have been used to understand the taxonomy and evolution of termite species (Kambhampati et al. 1996; Vidyashree et al. 2018).

The present study was designed to characterize termite species collected from southern Haryana, India, based on morphological and molecular data. Termites were classified using phylogenetic trees built on the basis of 16S rRNA gene sequences, and maximum parsimony trees based on soldier mandible features. The results of molecular identification and morphological assessments are compared.

METHODS AND METHODS

Collection of Termite Samples

A total of 168 termite samples (soldiers and workers) were collected from several localities of southern Haryana, India, situated between 28.25° N & 76.29° E during a 2-year study period (Table 1; Figure 1), via random sampling (Bhanupriya et al. 2022a,b) of microhabitats that included dung cakes, common rush, vegetation, leaf litter, tree bark, stumps, mounds, bamboo fencing and tree logs. Collections were completed at three-month intervals from March 2020 to November 2021. Each sample contained around 50 individuals and their distribution in study sites was mapped based on the collective data of the current study. The voucher specimens were well-maintained in 10ml of 70% ethanol mixed with 2–3 drops of glycerol in 20 ml glass vials for morphological and molecular analysis. Samples kept in the vials were labeled with the day and date of collection, name of the study sites and source of isolation, and retained in the Department of Zoology, IIHS, Kurukshetra University, Kurukshetra, India.

Morphological identification of termite species

Identification of specimens was done using identification keys (Roonwal & Chhotani 1989; Chhotani 1997; Krishna et al. 2013) based upon different diagnostic characteristics of soldier caste: head length, head width, head shape, mandible length, mandible plus head length, body length, body width, body-colour, tibial spur, tarsal segments and antennae segments (Wang et al. 2009; Bhanupriya et al. 2022a,b). These measurements were examined under the light compound microscope and photographs were also collected. And complete analysis of body measurements was performed by calculating mean and SD.

Parsimony tree based on mandible features

For construction of parsimony tree based on mandible characters, observations were made on features like mandible without a tooth, mandible with serrations, serrated mandibles without any large tooth, mandibles strongly incurved at distal half, mandible with incurved apex, cylindrical mandibles, tooth present at mid of the mandible, tooth present at the near tip of the mandible, tooth present at near base of the mandible, left mandible with a single tooth, right mandible with a single tooth, left mandible with six marginal teeth, right mandible with two teeth, left mandible with four crenulations, right mandible with two crenulations, right mandible with three crenulations and right mandible

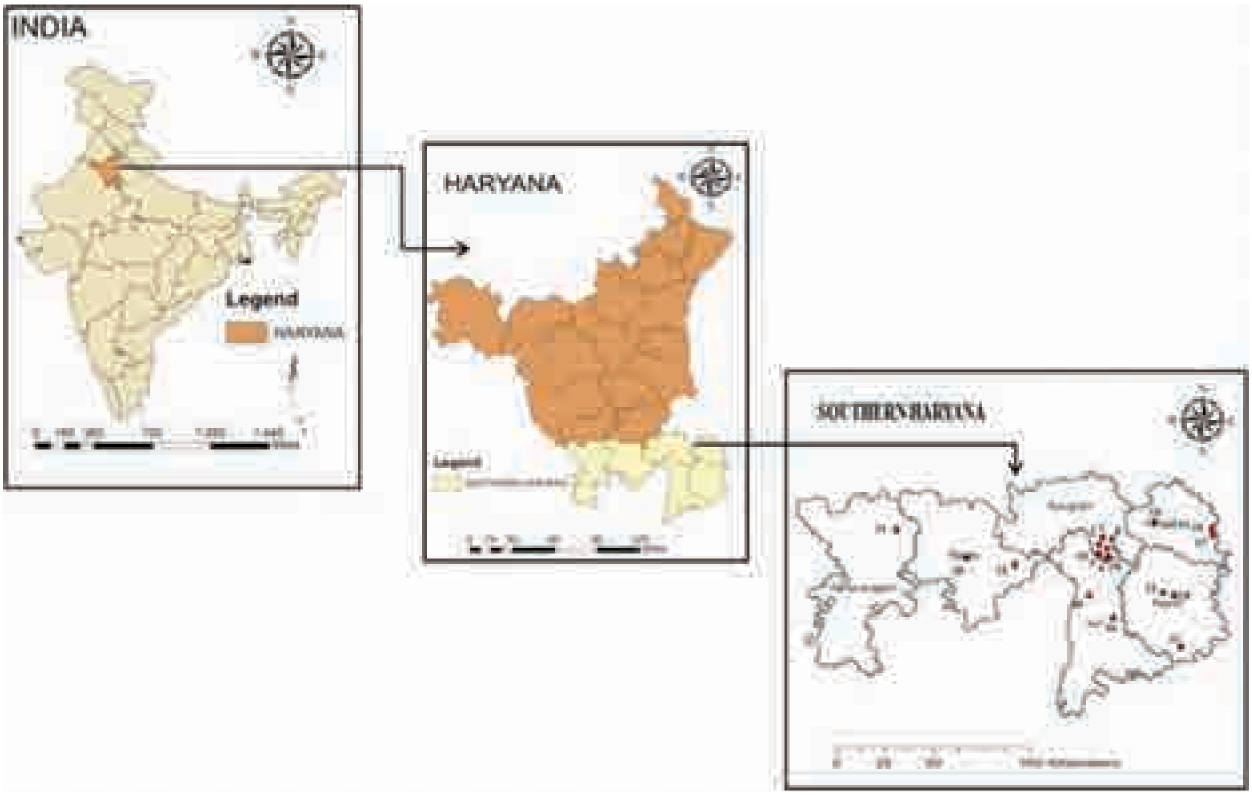


Figure 1. Various locations where termite samples included in this study were collected. Numbers (depicting species) mentioned in Table 1.

with four crenulations. The presence and absence of the above mentioned characters were assigned values of 1 and 0 respectively, and the strings of 1's and 0's were used as vectors to define a particular termite species. Maximum parsimony tree was constructed based on these strings of 1's and 0's using PAST 4.10 software.

Molecular identification

Extraction of Genomic DNA, PCR and Sequencing

The genomic DNA of termites was isolated from worker castes using Qiagen mini kits as per manufacturer instructions. The polymerase chain reaction was conducted using 16S rRNA gene-specific forward and reverse primers (16SF 5'-CGCCTGTTTATCAAAAACAT-3' 16SR 5'- CCGGTCTGAACTCAGATCACGT-3') by the protocol of Szalanski et al. (2004) with some modifications. Approximately, 500 bps amplicon of 16S rRNA gene was obtained for each termite species. The PCR reaction was performed in 0.2 ml of PCR tubes with a 25 µl reaction mixture consisting of 5 µl genomic DNA, 12.5 µl PCR mix, 1.0 µl primers (16SF & 16SR), and 6.5 µl of nuclease-free water. The PCR reactions were repeated in thermal cycles 40 times. In thermal cycles initial denaturation occurred at 95^{0} C for 5 minutes, denaturation, annealing, and extension at 94^{0} C, 55^{0} C, and 72^{0} C, respectively, for 30 seconds each, followed by final extension at 72^{0} C for 7 minutes. The sequence of amplified DNA segments was approved using both the primers using Applied Biosystems BigDye Terminator V3.1 Cycle Sequencing kit. These sequences were further copied and analyzed using ChromasPro v 1.34.

Data Analysis

The 16S rRNA gene sequences were assembled using ChromasProV3.1 sequence assembly software. The assembled sequences were edited to remove uncertain bases and revised sequences were subjected to BLAST analysis in the NCBI database, for molecular identification. Since these were partial sequences, the overall identification of termites relied on both molecular as well as morphological characters. Hereafter, these 16S rRNA gene sequences were submitted to NCBI to get the accession numbers (Table 1). The 21 sequences were aligned using MAFFT (Katoh et al. 2019) and trimmed and edited in Jalview (Procter et al. 2021). *Cryptocercus* (cockroach) mtDNA sequences were included to be used as the outgroups. To explore the genetic relatedness of given termite species, phylogenetic (NJ, ML and MP) trees were constructed using the PHYLIP package version 3.695 (Felsenstein 2008).

Table 1. Source of collection of termites, geographical coordinates of the location, and GenBank accession numbers of the sequenced 16S rRNA gene fragments.

	Source of collection	Latitude & Longitude	Date of collection	Molecular identity	Accession No.
1	Kikar tree	28.12352 N 77.89224 E	28.vi.2020	*Amitermes belli*	MZ269706
2	Mango tree	28.225932 N 77.082438 E	24.iv.2020	*Coptotermes gestroi*	OK606100
3	Woody thing	28.226763 N 77.084916 E	22.vi.2020	*Microtermes mycophagus*	OK606129
4	Sheesam wood	28.2288656 N 77.0883974 E	23.vi.2020	*Coptotermes kishori*	OL335912
5	Wood	28.2287955 N 77.0885184 E	23.vi.2020	*Coptotermes heimi*	OK606090
6	Woody thing	28.23808 N 77.04488 E	25.vi.2020	*Angulitermes akhorisainensis*	OL780326
7	Wood log	28.2233547 N 77.0803697 E	02.vi.2020	*Eremotermes paradoxalis*	OL335913
8	Common rush	28.39208 N 77.01185 E	28.vi.2020	*Microcerotermes newmani*	ON385997
9	Peepal tree	28.39208 N 77.28196 E	28.vi.2020	*Coptotermes emersoni*	OK181907
10	Woody thing	28.09059 N 77.01185 E	30.viii.2021	*Odontotermes obesus*	OL721750
11	Cattle dung	27.45184 N 77.82596 E	03.vii.2020	*Eremotermes neoparadoxalis*	OL335910
12	Soil mound	28.35496 N 77.2624 E	11.vii.2020	*Odontotermes redemanni*	OL454814
13	Wooden block	28.22636 N 77.80092 E	06.viii.2020	*Odontotermes guptai*	OL335911
14	Common rush	28.8804 N 77.4924 E	17.iii.2021	*Microcerotermes raja*	OL470522
15	Common rush	28.3324 N 77.4812 E	23.v.2021	*Microcerotermes cameroni*	OL470529
16	Common rush	28.0088 N 77.1048 E	18.iii.2021	*Microcerotermes baluchistanicus*	OL454819
17	woody logs	28.3072 N 77.4848 E	23.v.2021	*Microtermes obesi*	OL454826
18	Kikar tree	28.239467 N 77.051106 E	29.viii.2021	*Odontotermes parvidens*	OL454829
19	Sheesham tree	28.201364 N 76.72994 E	04.ix.2021	*Neotermes kemneri*	OL780345
20	Kikar tree	28.225001 N 76.545022 E	04.ix.2021	*Odontotermes assmuthi*	OL721753
21	Common rush	28.325773 N 76.277785 E	14.x.2021	*Microcerotermes beesoni*	OM241964

500 replicates of the DNA sequence alignment were generated using Seqboot. For constructing MP tree, the output of Seqboot was fed to the program Dnapars and the resultant MP trees were obtained. The majority rule consensus tree was built from MP trees using Consense program. For constructing ML tree, the output of Seqboot was fed to the program Dnaml and ML trees were obtained from the datasets which were fed to Consense to arrive at majority rule consensus ML Tree. For constructing NJ tree, the output of Seqboot was fed to the program Dnadist to compute the distance matrices for the given datasets. The output of Dnadist was fed to the program Neighbor to obtain the NJ trees from the given datasets. The output of Neighbor was fed to Consense to construct the majority rule consensus NJ tree.

RESULTS AND DISCUSSION

Taxonomic Account of Termites:

Based on aforementioned morphological keys, 168 termite samples were identified into 21 species (Image 1) belonging to three families (Termitidae, Rhinotermitidae, and Kalotermitidae), four subfamilies (Amitermitinae, Termitinae, Macrotermitinae, and Coptotermitinae) and eight genera (*Amitermes, Eremotermes, Microcerotermes, Angulitermes, Odontotermes, Microtermes, Coptotermes*, and *Neotermes*) as shown in Table 2. Species *M. baluchistanicus* is an arid zone species that is restricted to only Rajasthan (Rathore & Bhattacharyya 2004). Parihar (1981) reported that this species destroyed the guar crop. In the Nuh region of Haryana, *M. baluchistanicus* has been discovered for the first time.

Morphological tree

Investigation of the intra and intergenic relatedness in termites was carried out on the basis of mandible features of soldier castes by using parsimony analysis (Image 1). The mouthparts (mandibles) of termites are sclerotized structures that are adapted according to the substrate on which they feed. These adaptations are helpful for mechanically breaking down the hardwood substrates of their diet (Wilson & Jessica 2019). Therefore, mandibles are significantly important in

Table 2. Body parameters of the soldier castes of the studied termite species (n = 5).

	TBL**	HL	HML	ML	HW	BW	Mandible features
Amitermes belli	5.02±0.24*	1.13±0.11	1.9±0.14	0.77±0.08	1.18±0.26	1.04±0.12	single tooth on each mandible
Coptotermes gestroi	4.87±0.31	1.47±0.03	2.38±0.08	0.91±0.06	1.18±0.06	1.09±0.06	right mandible with 3 crenulations
Microtermes mycophagus	4.3±0.6	0.92±0.08	1.48±0.11	0.58±0.05	0.83±0.12	0.84±0.05	single tooth on each mandible
Coptotermes kishori	4.5±0.45	1.15±0.05	1.93±0.05	0.78±0.05	1.01±0.09	1.02±0.08	right mandible with 4 weaker crenulations
Coptotermes heimi	5.58±0.42	1.33±0.05	2.14±0.20	0.82±0.16	1.18±0.08	1.04±0.04	right mandible with 4 crenulations
Angulitermes akhorisainensis	4.56±0.19	1.34±0.04	2.69±0.05	1.36±0.04	0.94±0.05	0.9±0.03	Mandibles long, rod like, distally pointed and incurved
Eremotermes paradoxalis	3.84±0.24	0.95±0.08	1.76±0.15	0.81±0.07	0.68±0.07	0.72±0.08	single tooth on each mandible
Microcerotermes beesoni	4.8±0.4	1.32±0.13	2.13±0.2	0.81±0.12	0.84±0.12	0.82±0.08	finely serrated, a prominent denticle present near the base of each mandible
Coptotermes emersoni	5.18±0.55	1.1±0.1	1.96±0.12	0.86±0.05	0.96±0.12	1.08±0.08	2 teeth on right mandible
Odontotermes assmuthi	5.64±0.4	1.57±0.12	2.3±0.16	0.75±0.07	1.21±0.05	1.18±0.08	Left mandible with single tooth
Microcerotermes newmani	4.56±0.4	1.2±0.13	2±0.2	0.8±0.12	0.9±0.12	0.8±0.08	coarsely serrated
Eremotermes neoparadoxalis	3.84±0.22	0.77±0.06	1.75±0.11	0.88±0.04	0.73±0.11	0.67±0.06	single tooth on each mandible
Odontotermes redemanni	4.64±0.41	1.21±0.07	1.92±0.15	0.79±0.07	1±0.1	0.87±0.05	Left mandible with single tooth
Neotermes kemneri	8.04±0.27	2.55±0.13	4.19±0.13	1.64±0.04	1.92±0.11	2.46±0.12	right mandible with 2 and left with 6 teeth
Odontotermes guptai	4.92±0.08	1.04±0.12	1.64±0.18	0.6±0.08	0.95±0.05	0.91±0.06	Left mandible with single tooth
Microcerotermes baluchistanicus	4.72±0.58	1±0.07	1.96±0.05	0.96±0.05	0.67±0.08	0.88±0.08	coarsely serrated, with a larger tooth-like serration in the middle
Microcerotermes raja	4.76±0.28	1.18±0.11	2.01±0.14	0.83±0.05	0.81±0.01	0.8±0.1	Coarsely serrated, without any large tooth.
Microcerotermes cameroni	4.98±0.22	1.52±0.16	2.41±0.18	0.89±0.04	0.94±0.09	0.82±0.08	coarsely serrated with one larger tooth-like serration near the middle
Microtermes obesi	4.3±0.35	0.98±0.08	1.52±0.09	0.54±0.04	0.83±0.08	0.88±0.08	single tooth on each mandible
Odontotermes parvidens	6±0.38	1.99±0.07	3.17±0.25	1.23±0.11	1.76±0.21	2.34±0.24	Left mandible with single tooth
Odontotermes obesus	5.14±0.70	1.32±0.13	2.2±0.16	0.88±0.11	1.15±0.05	1.14±0.09	Left mandible with single tooth

*All values represented in mm | **TBL—Total Body Length | HL—Head Length | HML—Head Mandible Length | ML—Mandible Length | HW—Head Width | BW—Body Width.

feeding biology, i.e., pulling, cutting, scraping, pounding, and grinding the wooden structures (Matsuoka et al. 1996; Wilson & Jessica 2019).

As termites are cryptic species, hence for identification, soldiers' mandible features were counted as a valuable or noticeable parameter for their characterization (Donovan et al. 2000; Engel et al. 2009). This is also because of the higher range of disparity displayed by soldier caste in their conspicuous morphological characters associated with the head and mandibles compared to either the alate or worker castes (Ishikawa et al. 2008; Wang et al. 2009; Ahmed et al. 2011; Ke et al. 2017). Wang et al. (2009) identified five species of the genus *Reticulitermes* (*R. flavipes, R. virginicus, R. arenincola, R. tibialis,* and *R. hageni*) by utilizing soldiers and alates body features. One species, i.e., *Heterotermes indicola* (Mahapatro & Kumar 2013), two species of the genus *Neotermes* (*N. koshunensis* and *N. sugioi*) (Yashiro et al. 2019) and seven species of three genera (*Odontotermes, Macrotermes,* and *Microtermes*) (Kassaye et al. 2021) were also identified with soldiers and Imago's morphological features. Vidyashree et al. (2018) also utilized soldiers' features and characterized 12 species of termites from the Western

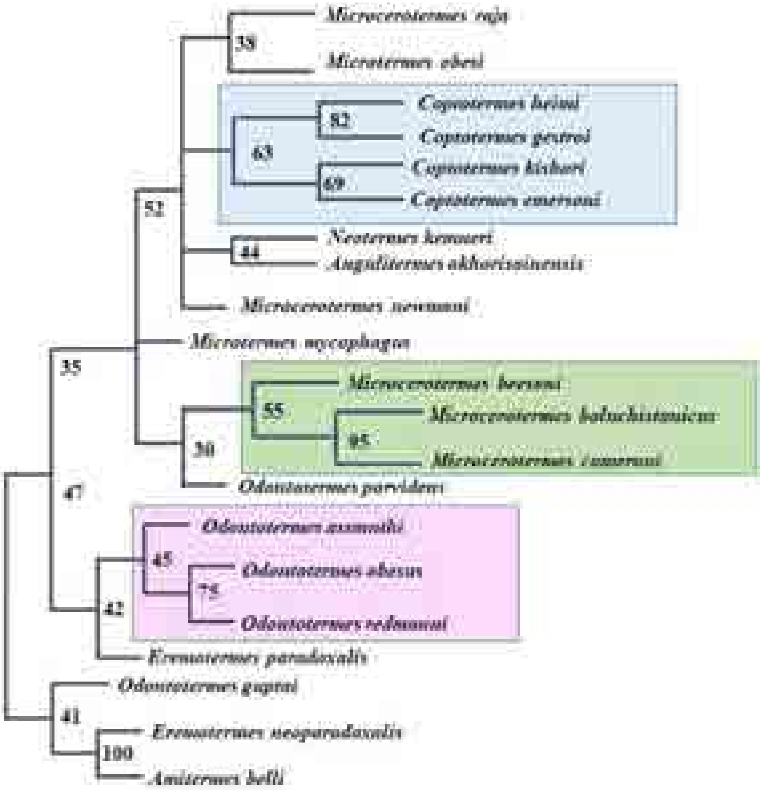


Figure 2. Maximum Parsimony tree based on mandibular features of termites soldiers. (*Bootstrap values obtained from 100 replicates datasets displayed at nodes; 100=100%)

Ghats of Karnataka, India.

In earlier phylogenetic studies, imago-worker's mandible characters were utilized for parsimony analysis. However, this usually resulted in inconsistencies in the results, which were more apparent in the family Termitidae (Ahmad 1950). Similarly, mandibular forms seem to have changed numerous times with modifications in feeding strategies like in the case of the *Nasutitermes* group (Donovan et al. 2000; Chiu et al. 2018; Govorushko 2019). Hence, traditional morphological phylogenies are difficult to rely upon always, since they have been built on restricted subsets of characters, e.g., the mandibles of worker-imago caste (Ahmad 1950) and the worker gut (Johnson 1979; Noirot 1995; Donovan et al. 2001). Rocha et al. (2017) also had the same view that workers' mandible characters alone are not good enough as phylogenetic markers. But according to Carrijo et al. (2020) termite species can be easily identified with the combination of the soldier as well as worker caste characters, as they showed in the classification of *Heterotermes longiceps* species.

Termites can also be classified on the basis of soldier's mandibles into different types of defense categories including Biting/Crushing, Slashing (Rhinotermitidae, Serritermitidae, and Termitidae), Slashing/ Snapping, Symmetrical Snapping (*Termes, Cavitermes, Homallotermes, Dentispicotermes,* and *Orthognathotermes*), Asymmetrical Snapping (*Capritermes* and *Neocapritermes*), Piercing (*Amitermes, Armitermes, Rhynchotermes,* and *Rhinotermes*), Vestigial (Nasutitermitinae and Rhinotermitidae), Ejected Secretion (*Nasutitermes*) and Vestigial Labral Brush (*Acorhinotermes*) (Prestwich 1984). But Rocha et al. (2017) reconstructed the classification of termites into three categories (biting/crushing, piercing and slashing) based on their defense behavior.

In the present study, the importance of mandibular features in soldiers was investigated in determining the taxonomy of termite species. The soldier caste is the main caste on which consistent amount of termite taxonomic work has been focused (Seid et al. 2008; Kuan et al. 2020; Amina et al. 2020). The MP tree based on mandible features exhibited certain clusters which were consistent with the DNA based tree, as it can be observed that the species of *Microcerotermes, Odontotermes*, and *Coptotermes* are falling in their respective clades with significant bootstrap values (Figure 2); earlier investigators have also emphasized on the importance of mandible features in the determination of taxonomic position of different termite species (Donovan et al. 2000; Carrijo et al. 2020). So, the tree based on mandible features though not completely defined the relationship between different termite families, but at genus level, proper clustering of three genera was obtained. First cluster comprised species of genus *Coptotermes* (*C. kishori, C. heimi, C. gestroi*, and *C. emersoni*), second comprised *Odontotermes* species (*O. obesus, O. assmuthi*, and *O. redemanni*) and the third comprised species of the genus *Microcerotermes* (*M. beesoni, M. baluchistanicus*, and *M. cameroni*). The clustering of rest of the species included in our study was chiefly dependent on the morphometric features.

Rocha et al. (2017) also notified such anomalies when he reported that, based on head morphology, *Rhynchotermes nasutissimus* and *Uncitermes teevani* came closer, though, phylogenetically, *U. teevani* is closer to *Labiotermes labralis*. Inward et al. (2007) commented that the defense morphologies in termites vary from species to species and evolved autonomously among all Isopterans. Similarly, Hare (1937) stated that soldiers of a few genera—*Microcerotermes, Termes*, and *Nasutitermes* (Termitidae)—lack a marginal tooth in their mandibles, while in some others, including *Amitermes, Eremotermes*, and *Odontotermes*, a distinct tooth is present at the edge of the soldier mandible (Chhotani 1997). Such observations point to the fact that there might have been a convergent evolution in the case of mandible features where similarities are indicative more of similar defense behavior and other habits rather than phylogenetic.

MOLECULAR IDENTIFICATION

Sequence analysis

About 500 bps of PCR products of 16S rRNA gene were sequenced for 21 species. These sequences were BLAST-searched in NCBI databases to determine the identity of termite samples. The sequences of these 21 species were deposited to the NCBI GenBank and the accession number of each termite species was obtained (Table 1).

Nucleotide-composition analysis

The nucleotide composition in the mt16S rRNA gene fragment was calculated for the 21 termite species using MEGA11 software (Tamura et al. 2021). It exhibited considerably high frequencies of A+T base composition that ranged from 61.08 to 68.56% compared to G+C composition (31.44–38.92 %). These nucleotide arrangements showed bias towards adenine and thymine in their composition which is consistent with data on 16S rRNA mitochondrial gene studies in various insects (Kambhampati et al. 1996; Vidyashree et al. 2018). The individual nucleotide frequencies for each species have been plotted in Supplementary Figure S3. High AT frequencies have also been reported by other groups like Kambhampati et al. (1996); Vidyashree et al. (2018), Austin et al. (2002), Ohkuma et al. (2004), and Murthy (2020).

Table 3. Maximum composite likelihood estimates the pattern of nucleotide substitution.

	A	T	C	G
A	-	5.34	3.69	**9.54**
T	6.75	-	**12.52**	2.85
C	6.75	**18.08**	-	2.85
G	**22.61**	5.34	3.69	-

* transitional substitutions were 22.61, 18.08, 12.52, and 9.54, and transversional substitutions were 6.75, 5.34, 3.69, and 2.85.

The entire gene analysis of investigated termite species was done using the maximum composite likelihood (MCL) estimates method. The MCL estimates calculate the probability of substitution of one base with another base (Tamura et al. 2021). Substitution rates were assessed using MEGA11 (Tamura et al. 2021). The rates of different transitional substitutions were 22.61, 18.08, 12.52, and 9.54, and the rates of transversional substitutions were 6.75, 5.34, 3.69, and 2.85 (Table 3). The nucleotide frequencies were found to be 36.24% (A), 28.65% (T), 19.84% (C), and 15.28% (G), respectively. The transition and transversion rate ratios were obtained as $k1 = 3.35$ (purines) and $k2 = 3.388$ (pyrimidines). The overall transition/transversion bias *(R)* came out to be 1.513, where $R = [A*G*k1 + T*C*k2]/[(A+G)*(T+C)]$.

Distance analysis

Based on sequence alignment, the divergence was calculated to investigate the intergenic variations among termite species by using MEGA11 software. The sequences of 16S rRNA gene from the termite species under this study were used to calculate pairwise genetic distance values (Kimura 2 parameter) using MEGA11 (Table 4). The K2P distance matrix values in species of the *Odontotermes* genus ranged from 0.031 to 1.256. It was found to be lowest (0.031) between *O. redemanni* and *O. obesus* and highest (1.256) between *O. parvidens* and *O. obesus*. The K2P interspecific distances in the genus *Coptotermes* ranged 0.005–1.015, recorded maximum (1.015) between *C. gestroi* and *C. emersoni,* and minimum (0.005) between *C. kishori* and *C. heimi*. However, divergence in genus *Microcerotermes* was ranged highest (0.081) between *M. raja* and *M. beesoni* and lowest (0.0)

Table 4. Pairwise genetic distances (Kimura 2-parameter) between species under study.

	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-																				
2	0.173	-																			
3	0.219	0.176	-																		
4	1.128	0.830	1.106	-																	
5	1.166	0.839	1.138	0.005	-																
6	0.974	0.715	0.96	0.145	0.151	-															
7	1.078	0.836	1.066	0.167	0.171	0.134	-														
8	0.943	0.719	0.983	0.143	0.137	0.013	0.123	-													
9	1.268	1.015	1.36	0.014	0.033	0.166	0.2	0.160	-												
10	1.162	0.924	1.235	0.159	0.171	0.163	0.186	0.159	0.159	-											
11	0.941	0.72	0.965	0.138	0.136	0.007	0.123	0.014	0.161	0.156	-										
12	1.072	0.804	1.044	0.15	0.164	0.124	0.047	0.120	0.17	0.168	0.122	-									
13	1.305	0.43	1.23	0.128	0.121	0.114	0.128	0.107	0.128	0.031	0.114	0.13	-								
14	1.167	0.760	1.133	0.260	0.252	0.24	0.251	0.238	0.292	0.314	0.230	0.243	0.272	-							
15	0.151	0.145	0.23	1.193	1.144	0.984	1.181	1.047	1.318	1.21	1.04	1.127	1.21	1.257	-						
16	0.946	0.688	0.982	0.154	0.154	0.076	0.123	0.065	0.179	0.162	0.068	0.116	0.137	0.246	1.077	-					
17	0.991	0.669	0.965	0.167	0.166	0.087	0.134	0.081	0.197	0.12	0.078	0.133	0.178	0.244	1.129	0.025	-				
18	0.984	0.719	0.946	0.138	0.150	0.007	0.118	0.014	0.175	0.155	0.0	0.122	0.114	0.228	1.048	0.070	0.077	-			
19	1.089	0.798	1.111	0.165	0.158	0.135	0.188	0.145	0.173	0.140	0.143	0.154	0.135	0.229	1.103	0.142	0.16	0.144	-		
20	0.162	0.167	0.228	1.216	1.218	1.047	1.15	1.023	1.343	1.256	1.056	1.170	1.238	1.197	0.045	1.037	1.072	1.066	1.15	-	
21	0.144	0.187	0.227	1.17	1.190	1	1.125	0.985	1.288	1.167	1.00	1.125	1.216	1.182	0.062	1.016	1.055	1.031	1.105	0.041	-

1—*Amitermes belli* | 2—*Coptotermes gestroi* | 3—*Microtermes mycophagus* | 4—*Coptotermes kishori* | 5—*Coptotermes heimi* | 6—*Angulitermes akhorisainensis* | 7—*Eremotermes paradoxalis* | 8—*Microcerotermes beesoni* | 9—*Coptotermes emersoni* | 10—*Odontotermes obesus* | 11—*Microcerotermes newmani* | 12—*Eremotermes neoparadoxalis* | 13—*Odontotermes redemanni* | 14—*Neotermes kemneri* | 15—*Odontotermes guptai* | 16—*Microcerotermes baluchistanicus* | 17—*Microcerotermes raja* | 18—*Microcerotermes cameroni* | 19—*Microtermes obesi* | 20—*Odontotermes parvidens* | 21—*Odontotermes assmuthi.*

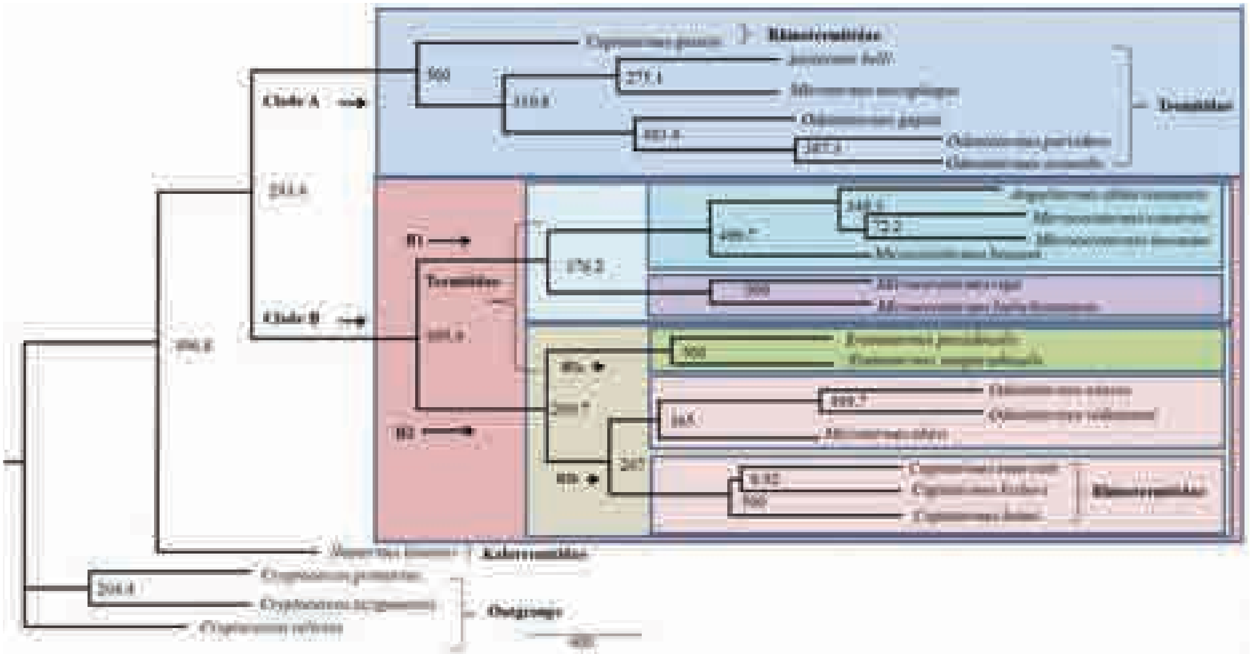


Figure 3. Phylogenetic tree built on Maximum Parsimony method to know the relatedness between isopterans; *Cryptocercus* sequences being taken as an outgroups. (*Bootstrap values obtained from 500 replicates datasets displayed at nodes; 500 = 100%).

between *M. cameroni* and *M. newmani* (Table 4).

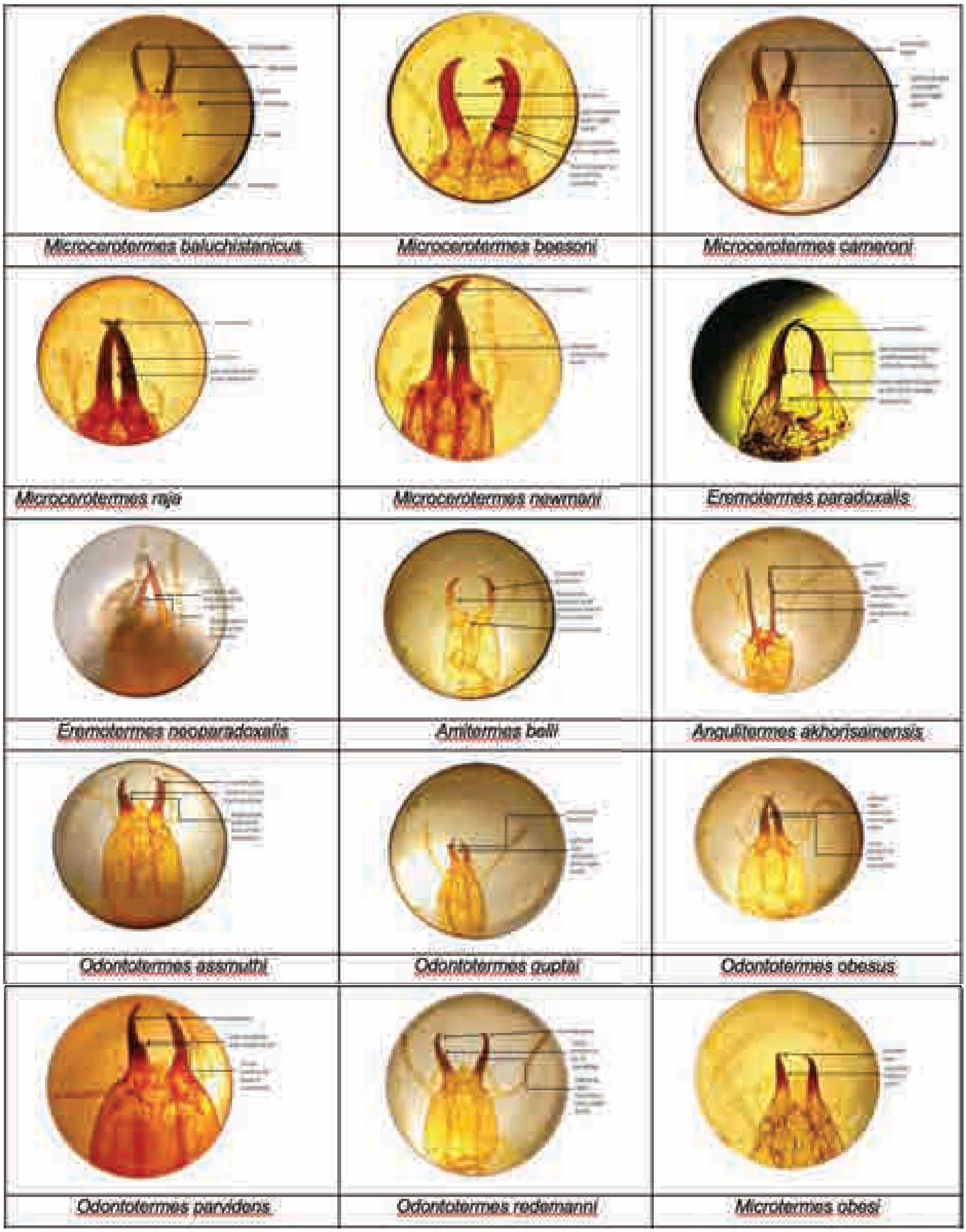
Phylogenetic analysis

The molecular phylogenetic trees were constructed from the aligned sequences of mt16S rRNA gene using maximum likelihood (ML), neighbor-joining (NJ), and maximum parsimony (MP) methods taking *Cryptocercus* mt gene sequences as an outgroup. All the methods yielded highly similar results and genetic relatedness between different termite species was established (Figure 3, Supplementary Figures S1 & S2).

As evident in the maximum parsimony tree, all the termite sequences are distinctly different from the *Cryptocercus* sequences, which cluster separately at the base of the tree. *Amitermes belli*, *C. gestroi*, *M. mycophagus*, *O. guptai*, *O. parvidens,* and *O. assmuthi* together form a well-bootstrap value (500) supported clade (Clade A) in the MP tree as well as in ML and NJ trees. *Neotermes kemneri* associates with this clade in both NJ and ML trees, which could be indicative of relatedness between *Neotermes kemneri* and various members of this clade. In clade A, species *O. guptai, O. parvidens, O. assmuthi, M. mycophagus,* and *A. belli*, all belong to the same family Termitidae, and *C. gestroi* belongs to the family Rhinotermitidae; their clustering being strongly supported by 100% bootstrap value. Our findings were broadly consistent with those of Vidyashree et al. (2018) (based on 16S rRNA) who stated that the species of genera *Microtermes* and *Odontotermes* (belonging to family Termitidae and subfamily Macrotermitinae) form a major cluster together as they showed higher resemblance with each other on morphological basis.

The rest of the termite sequences are clustered together in a large clade (Clade B) which is well supported with bootstrap values in all the trees examined. This clade could be further subdivided into two subclades, i.e., B1 and B2 with a 495.4 bootstrap value at the node joining them. Subclade B1 having six members of two genera (*Microcerotermes* and *Angulitermes*) that belong to the same family Termitidae, i.e., *Angulitermes akhorisainensis*, *M. cameroni*, *M. newmani*, *M. beesoni*, *M. raja,* and *M. baluchistanicus*. Species *M. raja* and *M. baluchistanicus* are highly related with a 500 bootstrap value, while *Angulitermes akhorisainensis*, *M. cameroni*, *M. newmani,* and *M. beesoni* are also clustered together at 499.7 bootstrap value. The same relationships are observed in ML and NJ trees as well.

Subclade B2 having members, i.e., *E. paradoxalis*, *E. neoparadoxalis*, *O. obesus*, *O. redemanni*, *M. obesi*, *C. emersoni*, *C. kishori,* and *C. heimi*. Therefore, subclade B2 comprises species from three subfamilies (Amitermitinae, Coptotermitinae, and Macrotermitinae) and four genera (*Eremotermes, Coptotermes, Odontotermes,* and *Microtermes*) of two families, Termitidae and Rhinotermitidae. B2 is further subdivided



Microcerotermes baluchistanicus

Microcerotermes beesoni

Microcerotermes cameroni

Microcerotermes raja

Microcerotermes newmani

Eremotermes paradoxalis

Eremotermes neoparadoxalis

Amitermes belli

Angulitermes akhorisainensis

Odontotermes assmuthi

Odontotermes guptai

Odontotermes obesus

Odontotermes parvidens

Odontotermes redemanni

Microtermes obesi

Image 1. Micrographs of mandibles of soldier caste of 21 morphologically identified species.

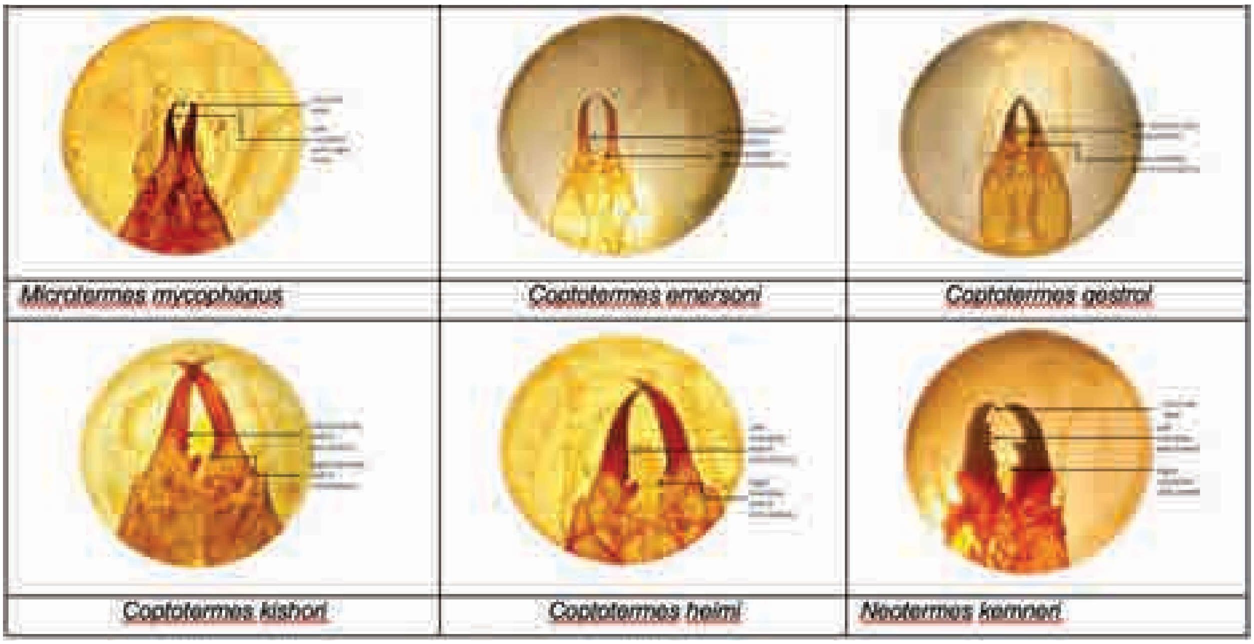


Image 1 contiued. Micrographs of mandibles of soldier caste of 21 morphologically identified species.

into two sub-subclades, i.e., B2a and B2b. In the subclade B2a, *E. paradoxalis* and *E. neoparadoxalis* are clustered together with 100% bootstrap value. However, in B2b, members of two families named Rhinotermitidae and Termitidae clustered together with almost 42% bootstrap value. Rhinotermitidae family is represented by *C. emersoni, C. kishori,* and *C. heimi,* whereas the Termitidae family is represented by *O. redemanni, O. obesus,* and *M. obesi.* Species *O. obesus*, *O. redemanni,* and *M. obesi* could also be considered related to each other since this clustering is common in both MP and ML trees, and the cluster is well supported with bootstrap values in the ML tree.

Species of genera *Coptotermes*, *Odontotermes*, and *Microtermes* were clustered on separate clades, i.e., A and B2. One possible explanation of this separate clustering comes from the morphological features of the members of these two groups, which differ in the location of tooth on the either side of mandibles and shape of the head. In *O. redemanni* and *O. obesus,* mandibles are sickle shaped and head is oval shaped. *M. mycophagus* possesses one tooth like projection near the tip of the mandibles while *M. obesi* don't have tooth. *O. obesus* is always found to cluster with the species *M. obesi* (Vidyashree et al. 2018), whereas, species of the genus *Microcerotermes* of subfamily Amitermitinae tend to fall in a separate cluster (Bourguignon et al. 2014; Vidyashree et al. 2018).

Findings from the present investigation broadly validate the results of Thompson et al. (2000) and Ohkuma et al. (2004) who described Asian termite's phylogeny, based on COII gene, taking 31 genera of Termitidae and Rhinotermitidae families.

The present work was designed to study the morphology and carry out genetic analysis of different termite species belonging to the family Termitidae, Rhinotermitidae and Kalotermitidae based on the mt16S rRNA gene. This integrated analysis was done to solve the problem that occurs in the identification of these puzzling species (Austin et al. 2005, 2012; Yeap et al. 2007; Ke et al. 2017; Ghesini et al. 2020). Significant similarities were observed in certain cases regarding the clustering of individual species in both the phylogenetic tree and the tree based on mandible features, for example, species of genus *Microcerotermes* and *Coptotermes* formed separate clades in mandible-based tree just like in the phylogenetic tree. Rhinotermitidae family clustered distinctly from Termitidae which is in equivalence with morphological identification (Vidyashree et al. 2018).

Lee et al. (2005) also verified morphological and phylogenetic analyses of Malaysian termites of the Termitidae family (Isoptera) with COII gene sequence. The first few combined studies at both the molecular and morphological levels between the major groups of isopterans were performed by Lo et al. (2004) and Inward et al. (2007). Their analysis showed Kalotermitidae, Hodotermitidae and Termitidae to be monophyletic, while Rhinotermitidae and Termopsidae were found to be paraphyletic. This was further verified by Legendre et al. (2008) using seven gene sequences (12S rDNA, 16S rDNA, 18S rDNA, 28S rDNA, COI, COII, and cytb) establishing phylogenetic connections between the termite species. Their findings revealed that the genera of Rhinotermitidae (*Heterotermes, Reticulitermes* and *Coptotermes*) forms a sister group with the Termitidae. Rhinotermititdae and Termitidae members exhibited sister relations in our investigation as well (Figure 3; Clade B). In another study, Rhinotermitidae family shared paraphyletic relations with the family Termitidae whereas Kalotermitidae was found to be monophyletic with the Rhinotermitidae, Serritermitidae and Termitidae (Bourguignon et al. 2014). In the present research with limited members of termite species, members of Kalotermitidae, Rhinotermitidae and Termitidae also showed common ancestry (Figure 3; Clade A)

Overall, from our studies, it could be concluded that morphological and molecular systematics both considered together generates a better template for termite identification and classification.

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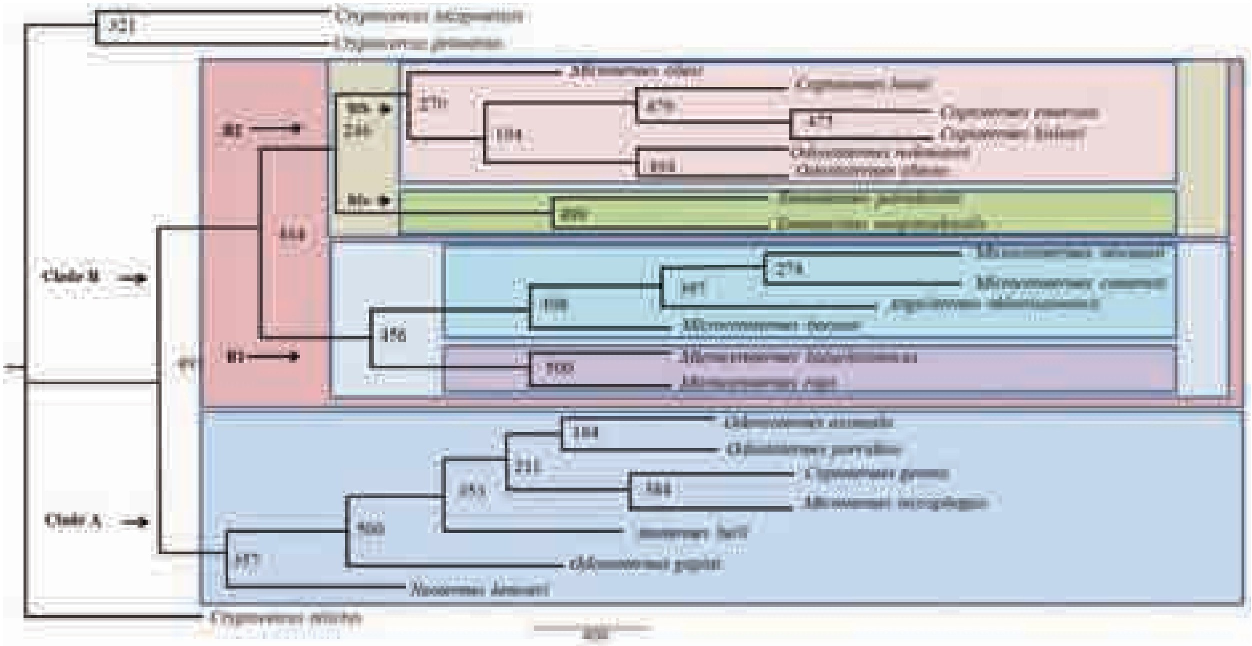

Figure S1. Phylogenetic tree built on neighbor joining method to know the relatedness between isopterans; *Cryptocercus* sequences being taken as an outgroups. (*Bootstrap values obtained from 500 replicates datasets displayed at nodes; 500 = 100%)

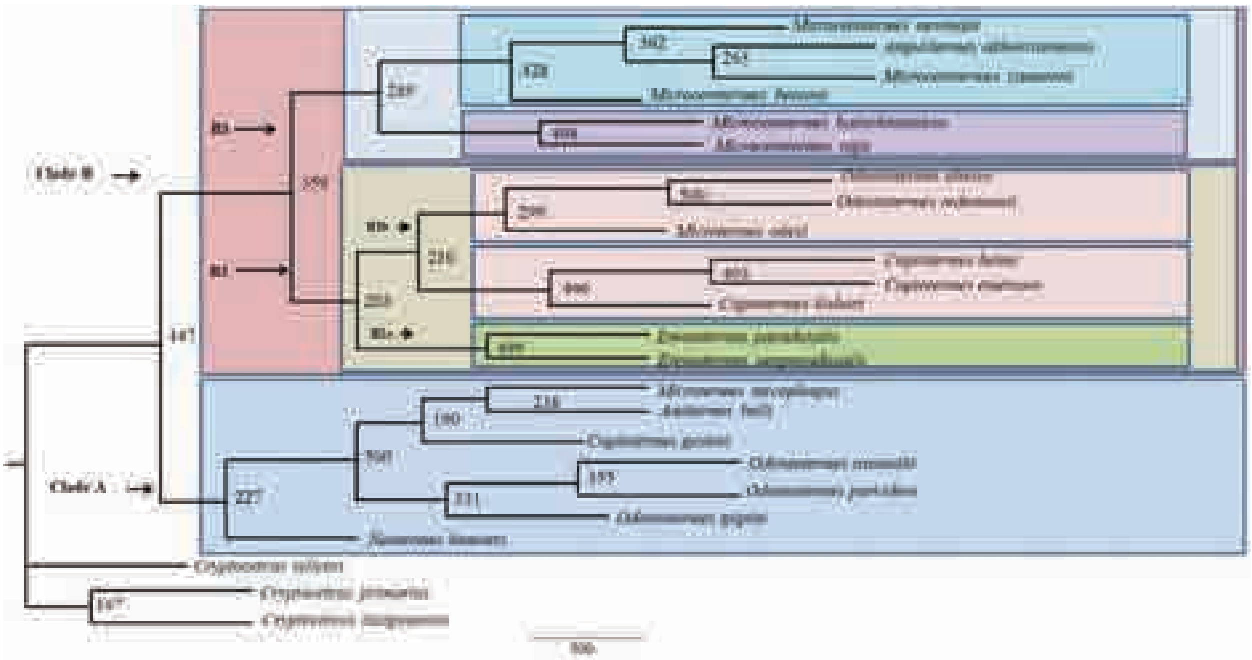

Figure S2. Phylogenetic tree built on maximum likelihood method to know the relatedness between isopterans; *Cryptocercus* sequences being taken as an outgroups. (*Bootstrap values obtained from 500 replicates datasets displayed at nodes; 500 = 100%)

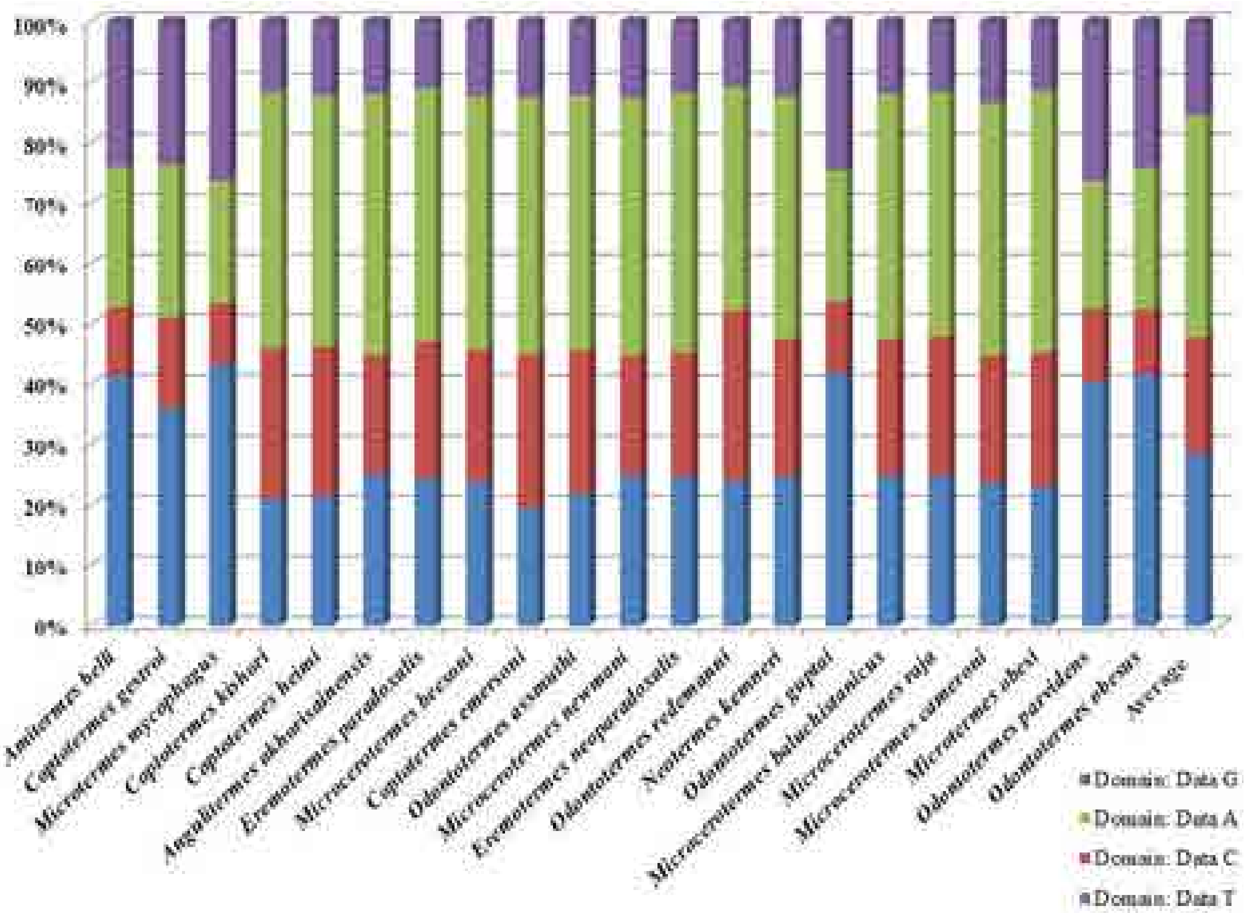

Figure S3. Graph displaying percentage of nucleotide composition in studied termite species.

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COMMUNICATION

Survey of Black Band Disease-affected scleractinian corals via drone-based observations in Okinawa, Japan

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Abstract: Diseases are a growing global threat to scleractinian corals. This study used a relatively inexpensive commercial drone flown simultaneously along with reef walking/snorkeling to confirm the presence of cyanobacterial Black Band Disease (BBD) on a reef in subtropical Okinawa, Japan. At the surveyed reef, the scleractinian corals included encrusting forms (12.57% ± 5.72 coverage), followed by massive/submassive (6.09% ± 4.05) and tabular corals (2.66% ± 1.19). Our survey is unique in that drone research has not been previously applied to BBD surveys, followed by ground truthing. Thus, this study can be regarded as an example of using drones during coral disease assessments.

Keywords: coral disease, subtropical, coral reef, *Montipora*.

Japanese abstract: イシサンゴ類の病気は世界的な脅威となっている。本研究では、亜熱帯域である沖縄島のサンゴ礁リーフにおいて、歩行およびシュノーケリングによって目視で観察すると同時に、比較的安価な市販のドローンを飛行させ、シアノバクテリアのブラックバンド病（BBD）の感染率を算出した。本調査地で観察されたイシサンゴ類のうち、BBDが確認された面積の割合は、被覆状サンゴが (12.57±5.72%)と最も高く、次いで塊状サンゴ（6.09±4.05%）、テーブル状サンゴ（2.66±1.19%）であった。これまでの研究ではドローンを用いた観察手法が野外でBBDの識別に用いられたことはなく、本研究で適用可能なことが初めて示唆された。本研究の結果は、ドローンと目視観察を組み合わせてBBDの感染率を評価した研究の一例として位置づけられる。

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Author contributions: RRD—conceptualization, writing - original draft, reviewing and editing, investigation, visualization, data curation, formal analysis, validation, funding acquisition, software. PTK—conceptualization, writing - review and editing, investigation, methodology, visualization, software. SHK—writing - review and editing, resources. JDR—writing - review and editing, funding acquisition, supervision.

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INTRODUCTION

Coral diseases are one of the major factors implicated in the decline of shallow water coral reef ecosystems worldwide (Sutherland et al. 2004; Hazraty-Kari et al. 2021). Among the diseases recognized to date, cyanobacterial Black Band Disease (BBD) is one of most well-studied diseases, affecting at least 24 scleractinian, one hydrozoan, and six gorgonian species in the Atlantic and Indo-Pacific oceans/seas (Antonious 1973; Bruckner 2015; Roff 2016). Within the Japanese archipelago, BBD has been reported from both mesophotic and shallow reefs affecting six coral genera: *Montipora*, *Acropora*, *Echinopora*, *Pachyseris*, *Goniastrea* and *Gardineroseris* (Wada et al. 2017; Kubomura et al. 2018; Das et al. 2022a) and among these, the encrusting form of genus *Montipora* can be considered one of the primary hosts in the region (Wada et al. 2018; Das et al. 2022a).

Coral diseases have been widely studied by field-based in situ observations, which obviously provide several benefits such as ease of observation and the need for few pieces of equipment. However, such observer-based data are also prone to bias and inconsistency, as well as often only being able to cover only limited areas. An alternate method of observation to cover large areas is remote sensing-based disease assessments (Kabiri et al. 2013; Maynard et al. 2015), although such methods have their own limitations such as interference via clouds and dust (Purkis 2018). Recently, consumer-grade drones have been increasingly used to monitor coral reef ecosystems (Casella et al. 2017; Kabiri et al. 2020), and this relatively low-cost equipment can clearly increase the ease of monitoring coral reefs (Murfitt et al. 2017). While the commercialization of cheap drones is recent, the concept existed earlier with suggestions of utilizing high-resolution cameras along with multiple spectrum/hyper-spectral sensors on hexacopters, and the usage of hydrogen balloons (Rützler 1978; Kabiri et al. 2014). Here, we utilized such drone technology to detect and identify cyanobacterial BBD infecting individual coral colonies on a nearshore intertidal habitat in Okinawa, Japan.

MATERIALS AND METHODS

On a clear day on the 09th of September 2021, we flew a commercial drone (Mini2, DJI Technology Co. Ltd.) equipped with a 1/2.3” inch CMOS sensor camera (12 MP) over the shallow nearshore reefs of Sesoko Island (off central western Okinawajima Island), near Sesoko Station, University of the Ryukyus (26.6340°N, 127.8641°E) (Das & Yamashiro 2018; Das et al. 2022a). The reef in this area is comprised of various morphotypes and species of the genus *Montipora*, such as *Montipora* cf. *aequituberculata*, *Montipora* cf. *digitata*, and encrusting *Montipora* cf. *informis*, *Montipora* cf. *efflorescens*, and other *Montipora* spp. (Yamashiro et al. 2000; Baird et al. 2018; Das et al. 2022a). *Montipora* spp. in Okinawa have been recently reported to be easily infected by BBD (Das et al. 2022a). The time and the day of our survey were chosen based on low wind and tidal conditions. We identified BBD-infected encrusting *Montipora* colonies from drone imagery. Simultaneously, reef walking and snorkeling was conducted to locate and confirm BBD-infected colonies initially identified by the drone imagery.

In this study, a total number of 148 images (4000 x 2250 pixels) and four videos (1920 x 1080 p; obtained between 9000 h to 1600 h) were taken from different heights between 30 to <100 m.

Aerial photographs were converted from initial .jpg to .tiff format. Three photographs were specifically chosen based on clarity and low sea surface reflection (sun glint), and in each of them a 5x5m quadrat was delineated. Additionally, wherever possible, in-situ images of the infected colonies were taken during reef walking/snorkeling (Olympus TG-5 camera/PT-058 housing). The drone images were then uploaded into open-sourced Coral-Net software (Beijbom et al. 2012) for benthic analyses. The benthic components were classified as tabular, encrusting, foliose, massive/submassive hard corals, dead coral, hard substrate/sand, and others. A total of 175 randomly generated points were created in each of the three 5x5 quadrats and were categorized accordingly. The categories were then identified visually based on the above-mentioned classification. Automatic annotation was not applicable since a minimum of twenty images are required to train the AI-based classification algorithm in Coral Net to provide satisfactory results (Chen et al. 2021).

RESULTS AND DISCUSSION

Benthic analyses revealed encrusting hard corals had the highest percentage cover within quadrats (12.57% ± 5.72 SD), followed by massive/submassive (6.09% ± 4.05 SD) and tabular corals (2.66% ± 1.19 SD) (Supplementary Table 1). Soft corals along with other benthos accounted for 23.05% ± 4.01 SD, while dead corals accounted for 2.48% ± 2.16 SD. A total of six encrusting *Montipora* colonies with BBD were identified through these drone images and videos. The dead white bare skeleton along

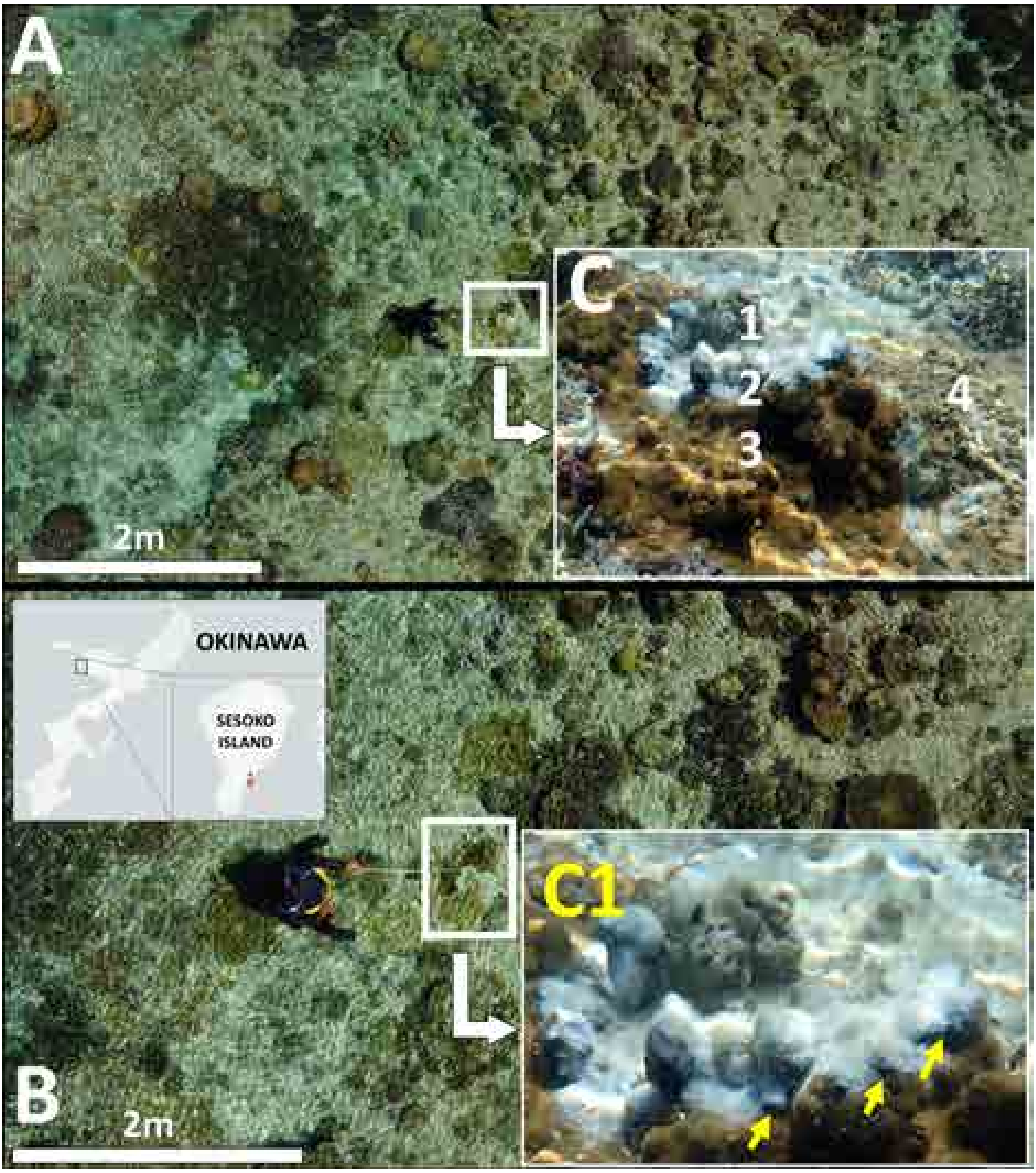

Image 1. A—Aerial view of the study area with a BBD-infected encrusting *Montipora* colony (white arrow) | B—Close-up image with BBD colony (White box) | C—In situ image of BBD infected colony (1—Recently dead coral skeleton | 2—Progressing BBD | 3—Healthy coral | 4—Healthy *Montipora* colony competing for space) | C1—Close-up image, yellow arrows indicating progressing black band. The black band is not as thick and wide probably due to low tide and direct exposure to sunlight. © Image A and B: PT-K; Image C and C1: RRD.

with neighboring healthy tissue was clearly visible through the high-resolution images (Image 1A–C, C1; 2A, B, B1). To our knowledge, this is the first study where cyanobacterial BBD affected areas were surveyed through drones.

Globally, coral reef health has been successfully monitored through the usage of commercial drones. Within the Persian Gulf, commercial drones have been used to map coral reefs, revealing coral mortality and bleaching (Kabiri et al. 2020). In a similar study at

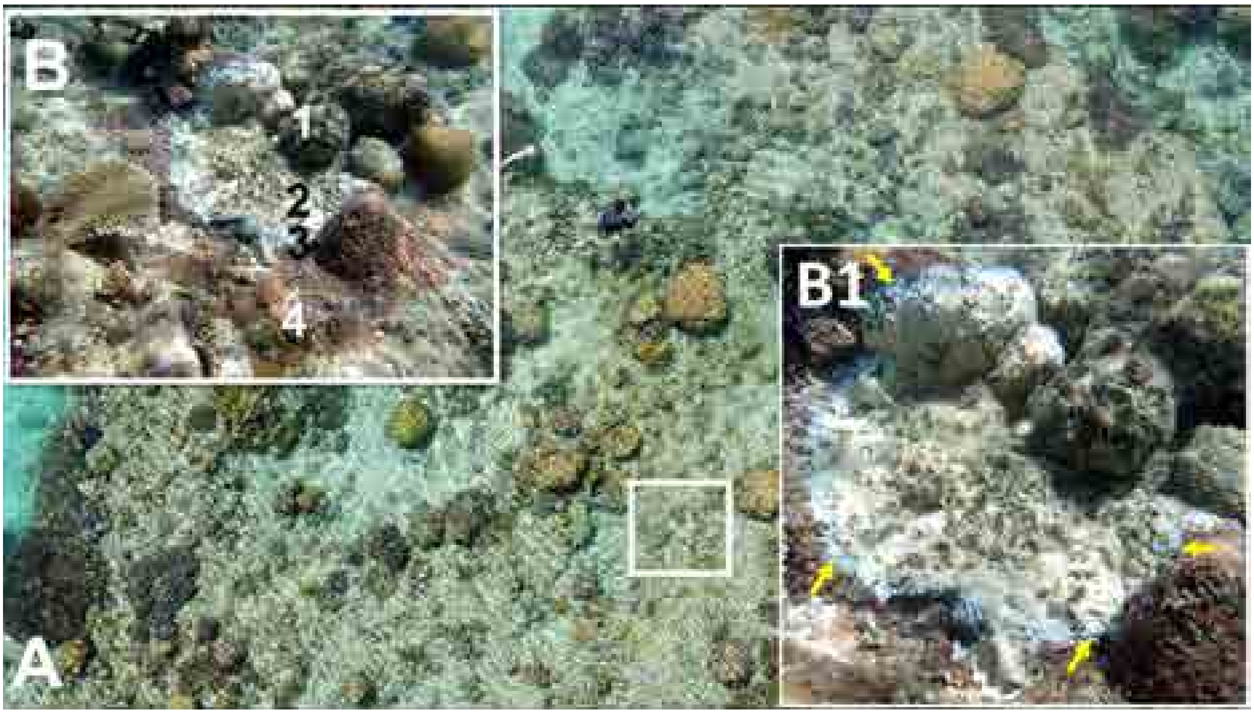

Image 2. A—Aerial view of the study area with BBD colony | B—Close up of the same colony (white box) via Olympus TG5 camera (1—Dead coral skeleton overgrown by turf algae | 2—Recently dead coral skeleton | 3—Progressing BBD | 4—Healthy coral) | B1—Further zoomed image of (B), arrows indicating the progressing black band. © Image A: PT-K; Image B and B1: RRD.

Kaneohe Bay, Hawaii, coral bleaching was observed using drones (Levy et al. 2018). In the current study, we specifically focused on a readily observable disease (BBD) within a specific study area, combined with simultaneous in situ verification.

A fully developed BBD has a thick microbial consortium, generally black/dark in color, the dark coloration from the dominant cyanobacterium *Roseofilum reptotaenium* (Hutabarat et al. 2018). Wada et al. (2017), in research at Akajima, Okinawa (~40 km from Sesoko Is.), further showed four different types of BBD patterns; black bands; grey bands; mottled black bands, and an absence of bands, all affecting encrusting *Montipora*. At greater depths of >30m, BBD appears purple-black due to the lack of sulfide oxidizing (SO) bacteria *Beggiatoa* sp. (Kubomura et al. 2018). These SO bacteria form a major population within shallow water BBD bacterial mats and are thought to be responsible for the whitish coloration of BBD during the night (Richardson 1996).

In future studies, there remains a necessity to focus on more diverse coral genera which are affected by numerous other diseases and pathogens. Thus, this work demonstrates the potential of incorporating drones while concurrently doing field observations under appropriate conditions (low wind, low surface sunlight reflection, etc.). Additionally, drone usage will be very effective if diseases have reached epizootic levels within a given reef. Further, recurrent observations of the same reef could also provide time-series datasets. Additionally, we were able to cover a large reef area in much shorter time than when compared to snorkeling/free swimming methods. The drone was flown over an area of 7,000 m^2 with approximately 75 m^2 (n = 3 of 5 X 5 m quadrats) was considered for analyses of coral percent coverage. It can be argued that drone-based observations are only limited to shallow reef ecosystems, but reefs in such shallow waters are often diverse and are among the most threatened by anthropogenic factors (Richards et al. 2015). We suggest the use of similar methodology to understand other forms of coral diseases, such as the coral-killing sponge *Terpios hoshinota* (“Black Disease”), which threatens intertidal reefs in many areas of southern Japan (e.g., Reimer et al. 2010, 2011) and elsewhere in the Indo-Pacific (e.g., Montano et al. 2015; Das et al. 2020).

Finally, we provide a few considerations and suggestions for utilizing drones to monitor coral reefs. Drone flight is feasible only under optimal environmental conditions, with successful flights and good-quality image acquisition hampered by strong winds, rain, or even too much sunshine. Additionally, even if drones can cover large areas, it is possible to overlook colonies that have just begun to exhibit early signs of infection. For instance, BBD initially develops as a cyanobacterial patch (Sato et al. 2009), and because these early phases may not have considerable tissue loss, such colonies can be easily overlooked. Similar issues may exist in the case of other diseases and should be considered before research planning. Another issue is that drones can only clearly view very shallow or intertidal reefs, and to reach deeper reefs, submersible drones would be necessary (Das et al. 2022b). Finally, if there is a large population of marine birds in the survey region, drone flights should be carefully monitored or should be flown at a suitable height.

Therefore, based on these limitations and recommendations, we conclude that drones can be regarded as tools to be included when or if the weather conditions are suitable. Such technologies can significantly improve the efficiency of surveys of coral diseases and aid in creating effective management strategies for the preservation of the coral reef ecosystems.

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Supplementary Table 1. Percent coverage of benthic categories obtained from CoralNet.

Image ID	Image name	Annotation status	Points	51_tabular_hard_coral	Hard Coral (encrusting)	Hard Coral (foliose)	Hard Coral (massive/submassive)	Dead coral	Hard Substrate/Sand	Other
2293735	Drone-Q1-RRD-Sesoko-5x5-DJI_0385.JPG	Confirmed	175	4	18.286	1.143	1.714	4	47.429	23.429
2293736	Drone-Q2-RRD-Sesoko-5x5-DJI_0346.JPG	Confirmed	175	2.286	12.571	0	6.857	0	59.429	18.857
2295906	Drone-Q3-RRD-Sesoko-5x5-DJI_0451.JPG	Confirmed	175	1.714	6.857	0	9.714	3.429	51.429	26.857
		Average		**2.666666667**	**12.57133333**	**0.381**	**6.095**	**2.4763333**	**52.76233333**	**23.048**
		SD		**1.189592087**	**5.714500007**	**0.659911358**	**4.05406993**	**2.163488**	**6.110100927**	**4.0136**

Supplementary Video 1. In situ observation of BBD-infected encrusting *Montipora* colony.

Supplementary Video 2. In situ observation of BBD-infected encrusting *Montipora* colony.

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COMMUNICATION

Trace elements in *Penaeus* shrimp from two anthropized estuarine systems in Brazil

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Abstract: This study measured concentrations of trace elements (Al, As, Cd, Cu, Fe, Mn, Ni, and Pb) in the muscle of pink shrimps (genus *Penaeus*) from two anthropized estuarine systems in Brazil: Guanabara Bay (GB) and Sepetiba Bay (SB). Concentrations were highest in the less anthropized SB site, where shrimps showed higher assimilation rates that can be explained by their higher trophic position compared to shrimps from GB. These results reinforce the role of food sources as the main route of trace elements for the aquatic animals.

Keywords: Coastal systems, metals, metalloid, pink shrimps, stable isotope.

Portuguese abstract: Este estudo verificou que a concentração de elementos traço (Al, As, Cd, Cu, Fe, Mn, Ni e Pb) no músculo de camarões-rosa (gênero *Penaeus*) é variável entre dois sistemas estuarinos antropizados no Brasil (Baía de Guanabara - BG e Baía de Sepetiba - BS), sendo maior no local menos antropizado (BS). As regressões entre as concentrações de elemento traço e os valores de δ^{15}N mostraram maiores taxas de assimilação nos camarões da BS, o que pode ser explicado pela sua posição trófica mais elevada em relação aos camarões da BG. Os resultados reforçam o papel das fontes alimentares como a principal rota de elementos traço para os animais aquáticos.

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Competing interests: The authors declare no competing interests.

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Author contributions: Di Beneditto, A.P.M.: project administration, funding acquisition, conceptualization, investigation, writing - original draft, writing - review & editing. Pestana, I.A.: formal analysis, writing - review & editing. de Carvalho, C.: shrimps sampling, writing - review & editing.

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INTRODUCTION

Trace elements such as metals and metalloids can accumulate in all compartments of aquatic environments (Brown & Depledge 1998), where their concentrations reflect both natural levels and anthropogenic contamination. When they enter human food chains, elements such as Cd, Hg, and Pb can cause neurological and kidney damage (WHO 2019), while As (metalloid) is linked to several types of cancers (Palma-Lara et al. 2020). Shrimps and other crustaceans accumulate trace elements from water, sediment, and food sources at levels beyond those necessary for nutrition and metabolism (Rainbow 2002; Boudet et al. 2019). Food is the main route of trace elements for both invertebrates and vertebrates (Rainbow 2002; Di Beneditto et al. 2021; Kehrig et al. 2022)

The pink shrimps *Penaeus brasiliensis* Latreille, 1817 and *P. paulensis* Perez Farfante, 1967 are sympatric in the southwestern Atlantic, where juveniles inhabit estuaries while adults live in marine waters (Neto 2011). Carvalho et al. (2021) investigated the stomach contents and the niche breadth of juvenile pink shrimps in two anthropized estuarine systems located in Sepetiba Bay and Guanabara Bay, southeastern Brazil (~23°S) (Figure 1). These areas are proximal coastal nurseries and fishing sites for both species. Niche analysis revealed pink shrimps from Guanabara Bay occupied a lower trophic position and showed greater trophic diversity in comparison to pink shrimps from Sepetiba Bay (Carvalho et al. 2021). The authors verified that interspecific differences in feeding preferences are negligible within the same estuarine system.

Based on findings of Carvalho et al. (2021) and the premise that food sources are the main route of trace elements for the animals, we made two predictions: i) the concentration of trace elements in shrimps *Penaeus* is variable between the two estuarine systems, following the spatial difference of the trophic niche, and ii) pink shrimps from Sepetiba Bay have higher trace element concentrations, since their trophic position is higher than in Guanabara Bay.

METHODS

Since *P. brasiliensis* and *P. paulensis* have similar feeding habits and niches in estuarine systems, we grouped them as *Penaeus* shrimps. Juvenile pink shrimps were sampled through fisheries inside Guanabara Bay (n = 80 individuals) and Sepetiba Bay (n = 67 individuals); herein referred as GB and SB, respectively (Figure 1). The samplings were done in 2021—2022. In GB, shrimps were caught in a fishing site in the central portion of the bay (8 km^2, 12—15 m deep), 10 km from the bay entrance (Figure 1). In SB, the fishing site included an area of approximately 6 km^2 and 10 m deep, 20 km from the bay entrance and 3 km from its northeastern shore, in front of the Guandu River mouth (Figure 1).

GB is more anthropized than SB. It comprises 384 km^2 with a drainage basin of 4,080 km^2 (55 small-river inputs). In surrounding areas, there are almost 12 million inhabitants, 6,000 industries and intensive inputs of domestic sewage and industrial effluents (Cordeiro et al. 2021). The SB comprises 450 km^2 with a drainage basin of 2,065 km^2. The SB surrounding areas have 400 industries, mainly chemical and metallurgic plants, and a population of approximately 2.0 million (Costa et al. 2011).

After sampling, shrimps were stored in transparent clean plastic bags and kept in cold storage during transportation. In the laboratory, the abdominal muscle (edible portion) of each shrimp was removed, stored in a dry sterile bottle, frozen (-20ºC), freeze-dried and homogenized to a fine powder using a mortar and pestle.

The trace elements considered in this study are Al, As, Cd, Cu, Fe, Mn, Ni, and Pb, which were determined using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) 720 ES (Varian Liberty Series II). Briefly, freeze-dried muscle (0.5 g) was solubilized in 10 mL of 65% HNO_3 and heated in a digester block. The samples were resuspended in 5 mL of 0.5% HNO_3 at 60 °C, filtered and brought to a final volume of 20 mL with 0.5% HNO_3. An analytical control solution was prepared to check for contamination. A reference material (*DORM-4* fish protein, National Research Council of Canada) was analyzed to test the precision and accuracy, and the recovery values were above 95%. The coefficients of variation among analytical replicates were < 10%. The concentrations were determined in mg kg^{-1} of dry weight.

We used the ratio of nitrogen stable isotope ($\delta^{15}N$) to evaluate the trophic position of the pink shrimps. Dry muscle sample (0.4 mg) of each shrimp was analysed using an organic elemental analyzer (Flash 2000, Thermo Scientific) coupled to a mass spectrometer (Delta V Advantage Isotope Ratio Mass Spectrometer, Thermo Scientific) through the ConFlo-VI interface (Model BR30140, Thermo Scientific). The reference value for nitrogen was the atmospheric nitrogen. Samples were analyzed using analytical blanks and urea analytical standards (IVA Analysentechnik-330802174). Analytical control and reproducibility were done for every 10 samples using a certified isotopic standard (Elemental

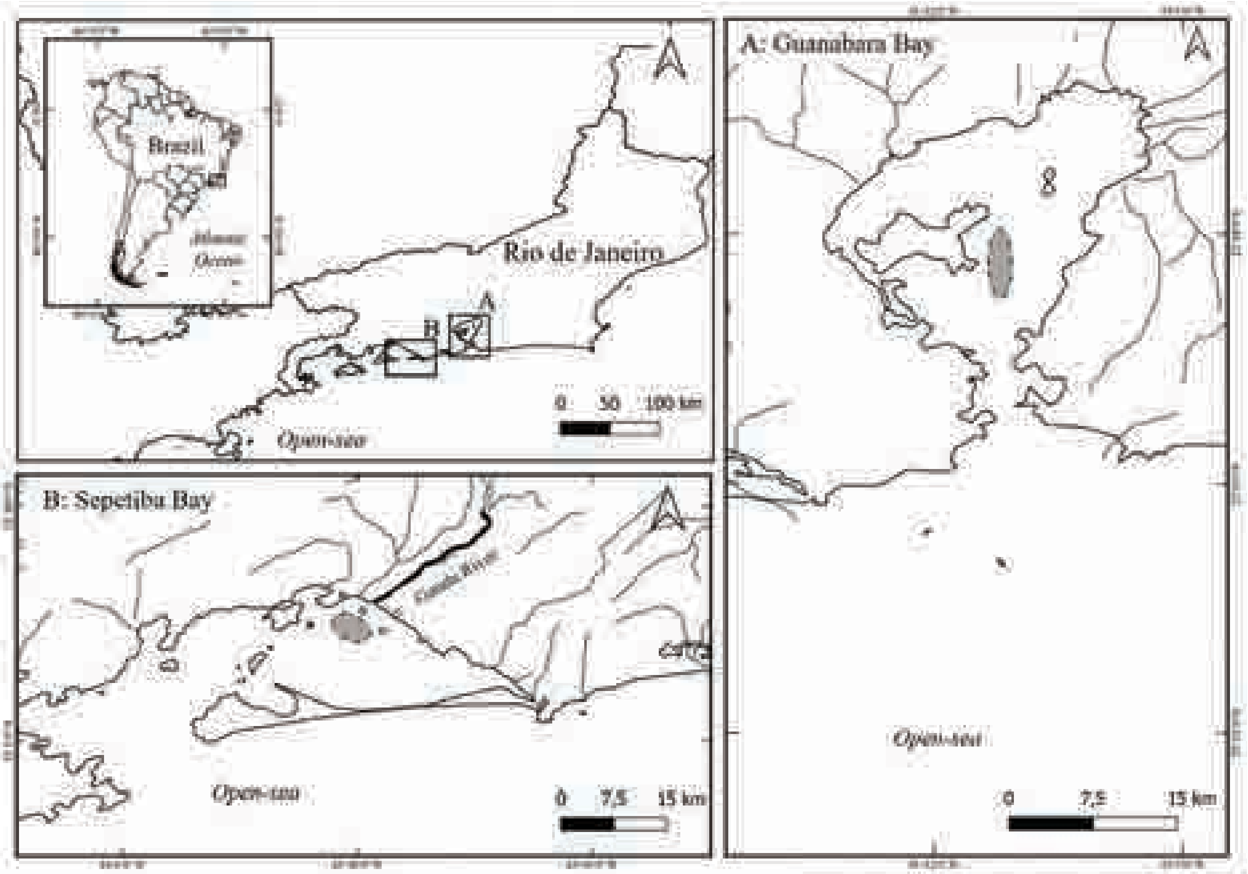

Figure 1. Guanabara Bay (A) and Sepetiba (B) Bay, southeastern Brazil, and the fishing sites of the pink shrimps inside the bays (gray ellipse areas). Extract from Di Beneditto et al. (2021).

Microanalysis Protein Standard OAS) and based on triplicates for every 10 samples (± 0.3‰ for $\delta^{15}N$). The isotopic result was presented as parts per thousand (‰).

Table 1. Median ± interquartile range values of Al, As, Cd, Cu, Fe, Mn, Ni, and Pb (mg·kg-1 dry weight), and δ15N (‰) in the muscle of pink shrimps from two anthropized estuarine systems in Brazil. Lowercase letters a and b indicate ANOVA differences at at *p* <0.05.

Estuarine system Elements	Guanabara Bay (GB)	Sepetiba Bay (SB)
Al	16.8 ± 25.5^b	42.2 ± 47.3^a
As	3.1 ± 2.1^b	5.1 ± 3.6^a
Cd	0.02 ± 0.01^a	0.02 ± 0.01^a
Cu	8.5 ± 4.8^a	9.6 ± 6.2^a
Fe	10.8 ± 16.9^b	35.8 ± 31.4^a
Mn	0.8 ± 1.0^b	1.5 ± 1.3^a
Ni	0.1 ± 0.2^b	0.2 ± 0.3^a
Pb	0.1 ± 0.04^a	0.1 ± 0.1^a
$\delta^{15}N$	7.5 ± 6.6^b	12.2 ± 0.9^a

RESULTS AND DISCUSSION

The concentrations of Al, As, Cd, Cu, Fe, Mn, Ni, and Pb, and the values of $\delta^{15}N$ in the muscle of the pink shrimps were greater in SB than GB (Table 1). This finding was statistically supported by the ANOVA results (R Core Team 2022) for most elements (Table 1). ANVISA (2021) and FAO/WHO (1991) have established the maximum tolerable limits of some trace elements (e.g., As, Cd, Cr, Cu, Hg and Pb) for food products. In this study, the trace elements determined in the pink shrimps were below these limits.

The difference in $\delta^{15}N$ values reinforces the findings of Carvalho et al. (2021): pink shrimps from SB are in higher trophic position than GB (p <0.001). The isotopic difference between the pink shrimps from the two estuarine systems was 4.7%, which is high enough to distinguish different trophic levels between them. According to Post (2002), differences in $\delta^{15}N$ values ranged from 2 to 5% indicate different trophic levels.

Regressions between trace element concentrations

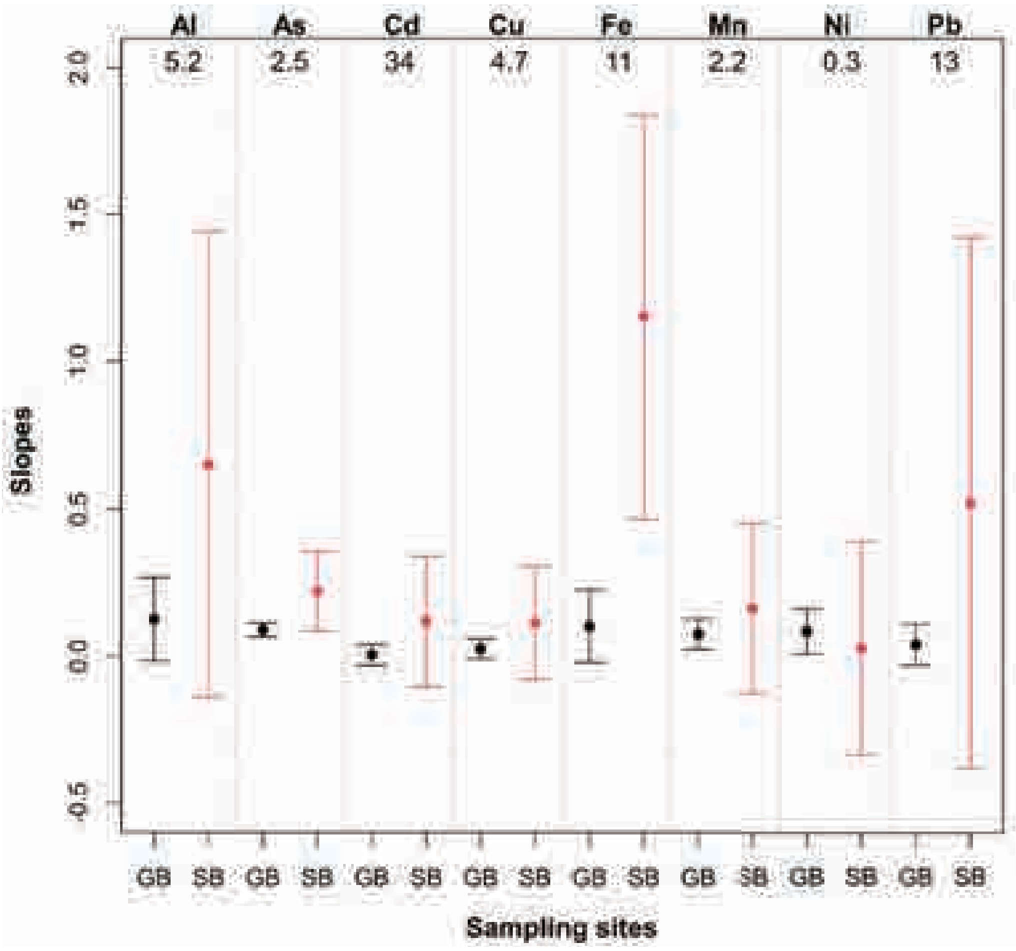

Figure 2. Slopes obtained from regressions between trace elements concentrations and δ15N values in pink shrimps from two anthropized estuarine systems in Brazil. The symbols (black circle to Guanabara Bay - GB, and red circle to Sepetiba Bay - SB) and bars represent each slope and its 95% confidence interval, respectively. Numbers below the trace elements are the slope ratio between the estuarine systems (SB/GB).

and δ^{15}N values were adjusted (R Core Team 2022) to verify the assimilation rate of each element according to the trophic position (i.e., their slopes). Mathematical transformations were done whenever necessary to meet the regression assumptions (normality, linearity, homoscedasticity) using a maximum likelihood function (Venables & Ripley 2002). The absence of overlapping in the 95% confidence intervals of the slopes can be interpreted as a significant difference between the two estuarine systems (p <0.05). Meanwhile, the high variance in the regressions associated with SB, as showed by the confidence intervals (Figure 2), made it difficult to detect significant differences (except for Fe). Since this is a statistical problem and not a conceptual one, the data interpretation was based on the magnitude of difference in slopes (numbers at the top of Figure 2). The slope ratios between the estuarine systems (SB/GB) were greater than 1 (except for Ni), revealing a greater assimilation rate of the elements in SB. Considering all elements, the average assimilation rate was 9.2 ± 11.0 times greater in SB compared to GB (Figure 2). The results confirmed the two-hypothesis: the concentration of trace elements is variable between the pink shrimps, following the spatial difference of their trophic niches; and pink shrimps from SB have higher trace element concentrations than in GB due to their higher trophic position.

Trace elements have different availability in the environment and different physiological pathways in the consumers (Boudet et al. 2019; Kolarova & Napiórkowski 2021), which may explain different concentrations in the pink shrimps between and within estuaries. The level of anthropization is higher in GB than SB (Costa et al. 2011; Cordeiro et al. 2021); thus, it could be expected that both the trace element concentrations and assimilation rates are higher in the pink shrimps from GB. However, the trophic position drove the assimilation rates, reinforcing the role of food sources as the main route of trace elements to the aquatic animals (Rainbow 2002; Di

Beneditto et al. 2012; Kehrig et al. 2022).

Carvalho et al. (2021) recommended attention to monitoring the pink shrimps from SB due to their smaller trophic breadth and lower $\delta^{15}N$ range, which may increase their sensitivity to changing habitat. The data on trace elements in the shrimps' muscle reinforce this recommendation, once their exposure to trace elements, including hazardous elements, can increase in a changing habitat.

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COMMUNICATION

Aquatic Hemiptera inhabiting rice fields in Karaikal, Puducherry, India

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Abstract: The present investigation was taken up to record the diversity and abundance of aquatic hemipterans in an irrigated rice ecosystem during kharif 2019 and rabi 2019–2020. The aquatic hemipterans were collected using a D-frame dip net at weekly intervals. This study revealed 21 species under 13 genera and nine families of Hemiptera in irrigated rice fields during kharif 2019 and rabi 2019–2020. *Micronecta scutellaris* (Stål, 1858) and *Anisops sardeus* Herrich-Schäffer, 1850 were the dominant species in both seasons. Corixidae and Notonectidae were the most abundant families. Species diversity was maximum in kharif 2019, while species richness was maximum during rabi 2019–2020. Multiple linear regression indicated that all the physicochemical characteristics and weather parameters together were responsible for significant variation in the occurrence of Corixidae (53.60%) and Notonectidae (82.40%). Based on our research, we found that rice fields serve as suitable habitats and play a crucial role in supporting diverse range of aquatic hemipterans.

Keywords: Biodiversity indices, correlation, irrigated rice ecosystem, kharif, multiple regression, rabi, relative abundance.

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Author details: Dr. M Kandibane works as the professor and Head, Department of Agricultural Entomology, PAJANCOA&RI, Karaikal, U.T. Puducherry. He has 17 years of experience in teaching, research and extension activities. He has guided 18 post graduate entomology students within in a period of eight years. L. Gopianand is a PhD student at the Department of Entomology, Faculty of Agriculture, Annamalai University, Chidambaram. He is interested in insect systematics and ecology.

Author contributions: MK—concept, supervision & reviewing; LG—field sampling, collection of data, analysis and preparation of manuscript.

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INTRODUCTION

Rice *Oryza sativa* L. is a major cereal crop and is consumed as a staple food by the majority of the population in India (Priya et al. 2019). It is also a major crop cultivated in the Cauvery delta region of Tamil Nadu, including Karaikal district (which lies at the tail end of the delta region), Union Territory of Puducherry. Aquatic insects can thrive and form a wide food chain in the rice ecosystem due to the availability of water during the entire growing season in irrigated rice fields (Bambaradeniya et al. 2004). Terrestrial insects were the primary focus in the majority of studies on insect diversity which related to rice fields (Jauharlina et al. 2019). However, the aquatic fauna of irrigated rice fields was extensively studied in China (Zhu et al. 2017), India (Gopianand & Kandibane 2022), Indonesia (Wakhid et al. 2020), Japan (Natuhara 2013), Philippines (Yano et al. 1981), and Thailand (Maneechan & Prommi 2023). Cochard et al. (2014) reported 39 aquatic arthropods belonging to seven orders were collected from rice fields in the central part of Thailand. A total of 45 aquatic insect species from 20 families and seven orders were recorded in rice fields in Bogor, West Java, Indonesia (Wakhid et al. 2020).

Globally, around 4,656 species of aquatic and semiaquatic Heteroptera are recorded, which constitutes three infraorders, 20 families, and 326 genera (Polhemus & Polhemus 2008). In India, a total of 325 species have been recorded, which constitutes 84 genera and 18 families of aquatic and semi-aquatic Hemiptera (Basu & Subramanian 2017). A total of 20 species, comprising 15 genera, and nine families of the aquatic and semi-aquatic Heteroptera were reported in Puducherry (Thirumalai & Kumar 2005). Some of the aquatic hemipterans are natural predators in rice fields, such as the species belonging to the families, Gerridae, Hydrometridae, Mesoveliidae and Veliidae, which have been reported to prey on brown planthopper (Heong & Hardy 2009). A total of eight families of aquatic hemipterans (i.e., Hydrometridae, Mesoveliidae, Micronectidae, Notonectidae, Veliidae, Nepidae, Gerridae, and Pleidae) were recorded in the rice fields at the Khon Kaen province, northeastern Thailand during June to October 2015 (Thongphak & Iwai 2016).

Considering the importance of the hemipterans in ecosystem functioning as prey, predators, scavengers, and bioindicators (Steward et al. 2022), it is essential to know the available aquatic hemipterans in the rice ecosystem. So, there is an immense scope to study the community structure of aquatic hemipterans in rice ecosystem. Hence, the present investigation was taken up to record the diversity and abundance of aquatic hemipterans in an irrigated rice ecosystem of Pandit Jawaharlal Nehru College of Agriculture and Research Institute (PAJANCOA&RI), Karaikal in two seasons.

MATERIALS AND METHODS

The study was undertaken during kharif 2019 and rabi 2019–2020. The aquatic hemipterans were collected from the irrigated rice fields at weekly intervals from July 2019 to February 2020 in the eastern farm of PAJANCOA&RI (10.9488°N, 79.7813°E & 4 m) (Figure 1). The kharif cropping season starts from July to October and the rabi season is from October to March. The study area is expected to receive an average annual rainfall of 126 cm, plus irrigation water from the Mettur dam of Tamil Nadu.

The aquatic hemipterans were collected with a D-frame dip net 12” wide x 10” long (305 x 254 mm) and 22” in depth, made up of white nylon cloth with a 500 µm mesh. The handle was about 30” in length and 32 mm in diameter. The collections were carried out in the early morning from 0060 h–0090 h at weekly intervals from after the transplanting to before harvest. A total of 25 sweeps were made in 25 selected sites in the rice fields at random (Figure 1). The net was passed through the standing water in the rice fields and then shaken in the standing water to remove silt and mud. The leftover contents of the net including the trapped aquatic insects were transferred to a white pan 27.5 x 35 x 5.5 cm with about 2 cm of water in it, and the aquatic insects were sorted out after complete washing. Most of the surface swimming insects like riffle bugs were collected by dragging a dip net on the water surface (half submerged) and then they were picked up by hand and put into vials containing 70% ethanol (Wakhid et al. 2020; Gopianand & Kandibane 2022). The collected specimens after sorting out to family level were stored in 30 ml vials containing 70% ethanol with a few drops of glycerine and preserved in insect storage boxes for identification to species level (Walker et al. 1999). The collected aquatic Hemiptera were identified with the standard literature of Bal & Basu (1994) and Thirumalai (2004). Identification of aquatic hemipterans was done by Dr. K.A. Subramanian (Scientist-D), Zoological Survey of India. All images of identified aquatic hemipterans were captured with Nikon D5300 DSLR camera and Leica EZ4E stereo zoom microscope.

The weekly average meteorological parameters were

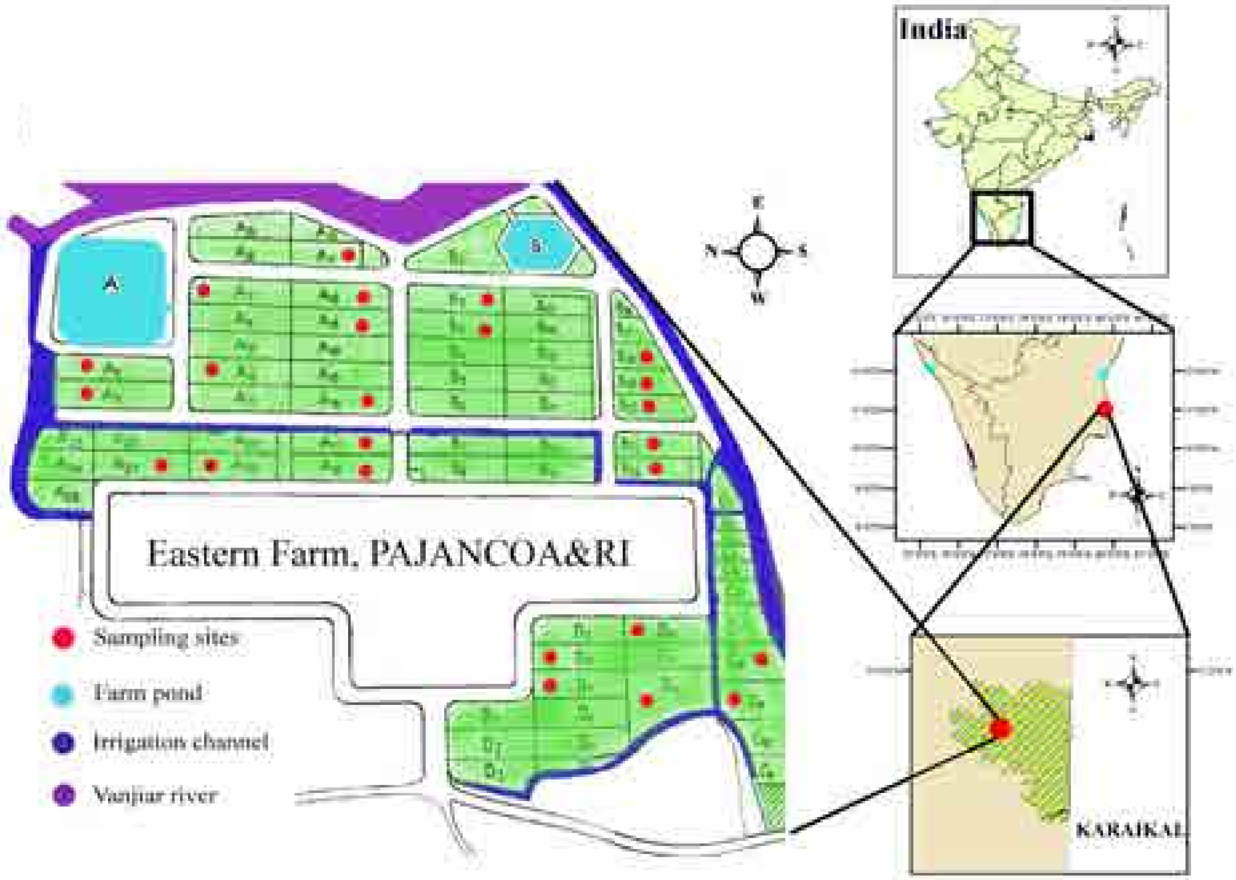

Figure 1. Study area (PAJANCOA & RI, Karaikal, U.T. of Puducherry, India) with sampling sites.

calculated from the daily meteorological data of the study area was obtained from the agrometeorological observatory, Department of Agronomy, PAJANCOA&RI, Karaikal. Physico-chemical parameters such as water temperature (WT), pH and electrical conductivity were recorded using a mercury bulb thermometer, digital pH meter (pH 700- Eutech instruments), and conductivity meter (CON 700-Eutech instruments), respectively. Based on the relative abundance % (RA), the species were classified as Subrecedent (<1), Recedent (1.1–3.1), Subdominant (3.2–10), Dominant (10.1–31.6), and Eudominant (> 31.7) (Engelmann 1978).

The diversity indices were analysed namely Simpson's index of dominance (D) calculated using the formula

$$D = \sum \frac{[N_i(N_i-1)]}{[N_t(N_t-1)]}$$

(Where, N_i = the number of individuals in the ith species; N_t = the total number of individuals in the sample) and Simpson's index of diversity (λ): 1 – D (Simpson 1949). Shannon diversity index was determined by $H' = \Sigma P_i \ln P_i$(Where, $P_i = n/N$; P_i = the proportion of individuals in the ith species; n = number of individuals belonging to ith species; N = Total number of individuals) (Shannon 1948). Evenness indices are determined by (E) = H / ln S (Where, H = Shannon's index; S = Total number of species) (Ludwig & Reynolds 1988). Menhinick's richness index is calculated from the ratio of the number of taxa to the square root of sample (Menhinick 1964). Margalef index was determined by using the formula: R1 = (S-1) / ln N (Where, S = Total number of species; N = Total number of individuals) (Margalef 1963). Equitability-J was calculated using $J = H / H_{max}$ (where, H is the Shannon's diversity index; H_{max} is different for each community and depends on species richness) (Pielou 1975). The Berger-Parker index $d = N_{max} / N$ (where N_{max} is the number of individuals in the most abundant species) (Berger & Parker 1970). Correlation and multiple regression were used to predict the effect of physicochemical characteristics and weather parameters on the abundance of aquatic hemipterans. All analyses were done with PAST version 4.0 (Hammer et al. 2001) and Agricolae R package version 1.4.0 (Mendiburu 2015).

RESULTS

A total of 21 aquatic hemipteran species were recorded. During kharif 2019, a total of 2,743 individuals were collected, which comprised 13 species under nine genera and eight families (Image 1 a–u). As per Engelmann scale, *Micronecta scutellaris* (Stål, 1858) and *Anisops sardeus* Herrich-Schäffer, 1850 were eudominant species with 43.5 and 37.1% relative abundance, respectively. Among the eight families, Corixidae was the most abundant family (47.7%), followed by Notonectidae (45.5%) (Table 1). A total of 4,608 individuals of aquatic hemipterans were collected during rabi 2019–2020 which comprised 17 species under 13 genera and nine families (Image 1 a–u). Among the 17 species, *A. sardeus* and *M. scutellaris* (were the eudominant species with the highest relative abundance of 46.4% and 42.4%, respectively. Out of the nine families Notonectidae and Corixidae were eudominant with the highest relative abundance of 47.2% and 43.7%, respectively (Table 1).

In both seasons, the diversity index values for dominance (D) were less than one and it was due to the higher representation of two dominant species, i.e., *A. sardeus* Herrich-Schäffer, 1850 and *M. scutellaris* (Stål, 1858). Based on the dominance indices values, higher dominance was recorded in the rabi season compared to the kharif season. The highest values for diversity indices of Simpson index (λ) (0.667), Shannon diversity index ($H^{'}$) (1.441), and Menhinick (0.248) were recorded in kharif 2019. Higher Shannon index values indicate higher diversity. In this case, the kharif season had slightly higher diversity and the rabi season had higher species richness. The maximum dominance based on the Margalef index (1.897) and Berger Parker index (0.464) was recorded in rabi 2019–2020 (Table 1). The evenness—e^H/S and equitability—J values were calculated for the kharif and rabi seasons, resulting in 0.325 and 0.202 for evenness, and 0.562 and 0.435 for equitability—J, respectively (Table 1). The higher evenness observed in the kharif season suggests a more balanced distribution of species abundances compared to the rabi season.

The family Corixidae showed a significant ($p<0.05$) negative correlation (-0.63) with electrical conductivity (EC), atmospheric temperature (-0.62), water temperature (-0.58) and positive significant correlation with relative humidity (0.76). The family Mesoveliidae expressed a significant negative correlation (-0.66) with water temperature throughout the study period. The family Notonectidae alone showed a positive significant ($p<0.05$) correlation with relative humidity (0.76) and rainfall (0.66). The families Notonectidae, Pleidae and Veliidae showed significant negative correlation with pH (-0.62, -0.64, -0.58) and EC (-0.75, -0.61, -0.63), respectively (Table 2). Multiple linear regression also indicated that all the physico-chemical characteristics together were responsible for significant variation in the occurrence of Corixidae (53.60%) and Notonectidae (82.40%) (Table 2).

DISCUSSION

Nine species of aquatic Hemiptera were recorded in both seasons, and four species of Kharif 2019 were not recorded during Rabi 2019–2020. It indicated that the nine species of aquatic Hemiptera were common species found in irrigated rice ecosystems, and had the ability to survive under the fluctuating environmental conditions. The other four species, which had favourable climatic conditions during Kharif 2019, appeared only in that season, and not in rabi, due to unfavourable environmental conditions (Table 1). Das & Gupta (2012) reported 14 species of aquatic Hemiptera in Assam. Thirumalai & Kumar (2005) recorded 20 species of aquatic and semi-aquatic Hemiptera from the wetlands of Karaikal and Pondicherry. In the present study, the maximum diversity index was recorded during rabi season (mid-November to April), which is in contrast with the results of Das & Gupta (2012) who stated that Shannon's index value for pre-monsoon (0.93), monsoon (0.87), post-monsoon (0.86), and winter (0.92) during March 2007 to February 2008 in Bharambaba temple pond, Assam.

Anisops sardeus Herrich-Schäffer, 1850 was the eudominant species with the highest relative abundance during rabi 2019–2020. It was inferred that the high relative abundance of *A. sardeus* might be due to the continuous stagnation of rainwater in the rice fields till the harvest of crops. During rabi 2019–2020, it was observed that *A. sardeus* mainly feeds on the larvae of chironomids, mosquitos, fallen leaf-folder larvae (*Cnaphalocrocis* spp.) and other rice pests. It was also found that the canopy of the rice did not allow sunlight to fall over the surface of the water, which favours the abundance of aquatic hemipterans in the rice crop (Kandibane et al. 2007). Moustafa et al. (2017) reported that *A. sardeus* was abundant in rice fields of Egypt, due to high content of organic matter. Tripole et al. (2008) noted that a high density of Notonectidae was found in winter and rainy season, which had favourable environmental conditions for their abundance relative

Table 1. Diversity and dominance of aquatic hemipterans in an irrigated rice ecosystem.

	Taxa	kharif 2019		rabi 2019–2020		Total
		RA (%)	Status of dominance*	RA (%)	Status of dominance*	
I	Belostomatidae (Water bugs)					
1.	*Diplonychus annulatus* (Fabricius, 1781) (Image 1a, nymph)	-	-	0.13	Subrecedent	6
2.	*D. rusticus* (Fabricius, 1871) (Image 1b)	0.44	Subrecedent	0.48	Subrecedent	34
3.	*Lethocerus indicus* (Lepeletier & Serville, 1825) (Image 1c)	-	-	0.07	Subrecedent	3
II	Corixidae (Water boatmen)					
1.	*Micronecta scutellaris* (Stål, 1858) (Image 1d)	43.46	Eudominant	42.43	Eudominant	3147
2.	*M. ludibunda* Breddin, 1905 (Image 1e)	4.23	Subdominant	-	-	116
3.	*Sigara pectoralis* Fieber, 1851 (Image 1f)	-	-	1.24	Recedent	57
III	Gerridae (Water striders)					
1.	*Aquarius adelaidis* (Dohrn, 1860) (Image 1g)	1.1	Recedent	0.48	Subrecedent	52
2.	*Limnogonus fossarum* (Fabricius, 1775) (Image 1h)	0.97	Subrecedent	0.78	Subrecedent	63
3.	*L. nitidus* (Mayr, 1865) (Image 1i)	0.15	Subrecedent	-	-	4
4.	*Rhagadotarsus kraepelini* Breddin, 1905 (Image 1j)	-	-	0.28	Subrecedent	13
IV	Hydrometridae (Water measurer)					
1.	*Hydrometra greeni* Kirkaldy, 1898 (Image 1k)	1.46	Recedent	0.93	Subrecedent	83
V	Mesoveliidae (Water treaders)					
1.	*Mesovelia vittigera* Horváth, 1895 (Image 1l)	1.49	Recedent	0.56	Subrecedent	67
2.	*M. horvathi* Lundblad, 1933 (Image 1m)	-	-	0.50	Subrecedent	23
VI	Nepidae (Water Scorpions)					
1.	*Laccotrephes griseus* (Guérin-Méneville, 1835) (Image 1n)	0.77	Subrecedent	-	-	21
2.	*Ranatra elongata* Fabricius, 1790 (Image 1o)	-	-	0.46	Subrecedent	21
3.	*R. varipes* Stål, 1861 (Image 1p)	-	-	0.55	Subrecedent	27
VII	Notonectidae (Back swimmers)					
1.	*Anisops sardeus* Herrich-Schaeffer, 1849 (Image 1q)	37.11	Eudominant	46.35	Eudominant	3154
2.	*A. lundbladiana* Lansbury, 1962 (Image 1r)	3.46	Subdominant	0.82	Subrecedent	133
3.	*A. nasutus* Fieber, 1851 (Image 1s)	4.96	Subdominant	-	-	136
VIII	Pleidae (Pygmy back swimmer)					
1.	*Paraplea frontalis* (Fieber, 1844) (Image 1t)	-	-	3.2	Subdominant	146
IX	Veliidae (Riffle bugs)					
1.	*Microvelia douglasi* Scott, 1874 (Image 1u)	0.40	Subrecedent	0.74	Subrecedent	45

Diversity indices	kharif 2019	rabi 2019–2020
Species richness	13	17
Individuals	2743	4608
Dominance- D	0.333	0.397
Simpson’s diversity index (λ)	0.667	0.604
Shannon diversity index (H')	1.441	1.233
Menhinick’s richness index	0.248	0.250
Margalef’s richness index	1.516	1.897
Evenness_e^H/S	0.325	0.202
Equitability- J	0.562	0.435
Berger-Parker dominance index	0.435	0.464

*—As per Engelmann scale (Engelmann 1978) | Relative abundance % (RA): <1—Subrecedent | 1.1–3.1 —Recedent | 3.2–10—Subdominant | 10.1–31.6—Dominant | > 31.7—Eudominant.

Table 2. Correlation and regression for aquatic Hemiptera with weather parameters in rice ecosystem.

Family	pH	EC	AT	WT	RH	RF	Regression equation	R^2
Belostomatidae	-0.37	-0.36	-0.47	-0.51	0.38	0.21	$Y = 19.82 -0.23X_1 +0.62X_2 -0.15X_3 -0.36X_4 -0.05X_5$	0.309#
Corixidae	-0.50	-0.63*	-0.62*	-0.58*	0.61*	0.35	$Y = -36.45 -14.61X_1 -28.57X_2 +1.56X_3 +2.07X_4 +2.14X_5 +0.05X_6$	0.536*
Gerridae	-0.01	-0.26	-0.05	-0.16	0.10	0.09	$Y = -27.73 +1.31X_1 -2.89X_2 +1.01X_3 -0.74X_4 +0.11X_5 +0.01X_6$	0.220#
Hydrometridae	-0.05	-0.08	-0.02	-0.15	0.05	-0.09	$Y = -3.41 +0.35X_1 -0.35X_2 +0.40X_3 -0.52X_4 +0.03X_5$	0.074#
Mesoveliidae	-0.25	-0.24	-0.27	-0.66*	0.29	-0.01	$Y = -3.67 -0.44X_1 +0.04X_2 +0.16X_3 -0.37X_4 +0.07X_5$	0.145#
Nepidae	-0.30	-0.30	-0.33	-0.30	0.34	0.33	$Y = -3.88 -0.55X_1 -0.34X_2 +0.17X_3 -0.08X_4 +0.08X_5 +0.01X_6$	0.202#
Notonectidae	-0.62*	-0.75*	-0.85*	-0.77*	0.76*	0.66*	$Y = 378.48 -18.67X_1 -14.13X_2 -6.42X_3 +1.49X_4 +0.72X_5 +0.05X_6$	0.824*
Pleidae	-0.64*	-0.61*	-0.53	-0.55	0.40	0.11	$Y = 81.85 -2.25X_1 -6.56X_2 -1.10X_3 +0.10X_4 -0.19X_5 -0.02X_6$	0.432#
Veliidae	-0.58*	-0.63*	-0.38	-0.40	0.37	-0.06	$Y = -8.14 -0.49X_1 -2.91X_2 +0.33X_3 +0.01X_4 +0.07X_5 -0.01X_6$	0.386#

*—Significant at $p<0.05$ | #—Not significant | X_1—water pH | X_2—Electrical conductivity (EC) | X_3—Air temperature (AT) | X_4—Water temperature (WT) | X_5—Relative humidity (RH) | X_6—Rainfall (RF) | R^2—coefficient of determination.

to other aquatic hemipterans. Streams & Newfield (1972) reported the winter populations of Notonectidae with high distributions among a large number of water bodies in which there was a continuous stagnation of water in England. The second eudominant family Corixidae had the highest abundance during study period. Savage (1989), reported that Corixidae is the pioneer in quickly colonizing new habitats, including newly transplanted rice fields. This might be the possible reason for the population establishment in the rice crop. Purkayastha & Gupta (2015), reported that *Micronecta scutellaris* (Stål, 1858) belonging to the family Corixidae was eudominant (37.29%) during winter in a flood plain ecosystem of Assam. Bao et al. (2021) observed that the family Corixidae was the most abundant hemipteran in the rice fields of Uruguay. The above studies are in conformity with the present findings. In our findings, the optimal water level in the rice fields maintained with the support of Mettur dam water and abundant rain, resembles a permanent wetland habitat in kharif and rabi season, respectively. Therefore, the rice fields serve as suitable habitats for aquatic hemipterans.

CONCLUSION

These aquatic hemipterans are economically significant to the rice ecosystem because they are predators of rice predators like the Brown Planthopper *Nilaparvata lugens* (Stål) and Green Leafhopper *Nephotettix* spp. From this observation, we conclude that physicochemical characteristics and weather parameters directly influence the distribution pattern of aquatic hemipterans in rice fields. Although the application of agro-chemicals and other regular operations significantly reduce the biodiversity, these rice fields serve as a temporary wetland in the absence of natural wetlands, providing habitat for the conservation of several aquatic hemipteran species as well as other macro-invertebrates.

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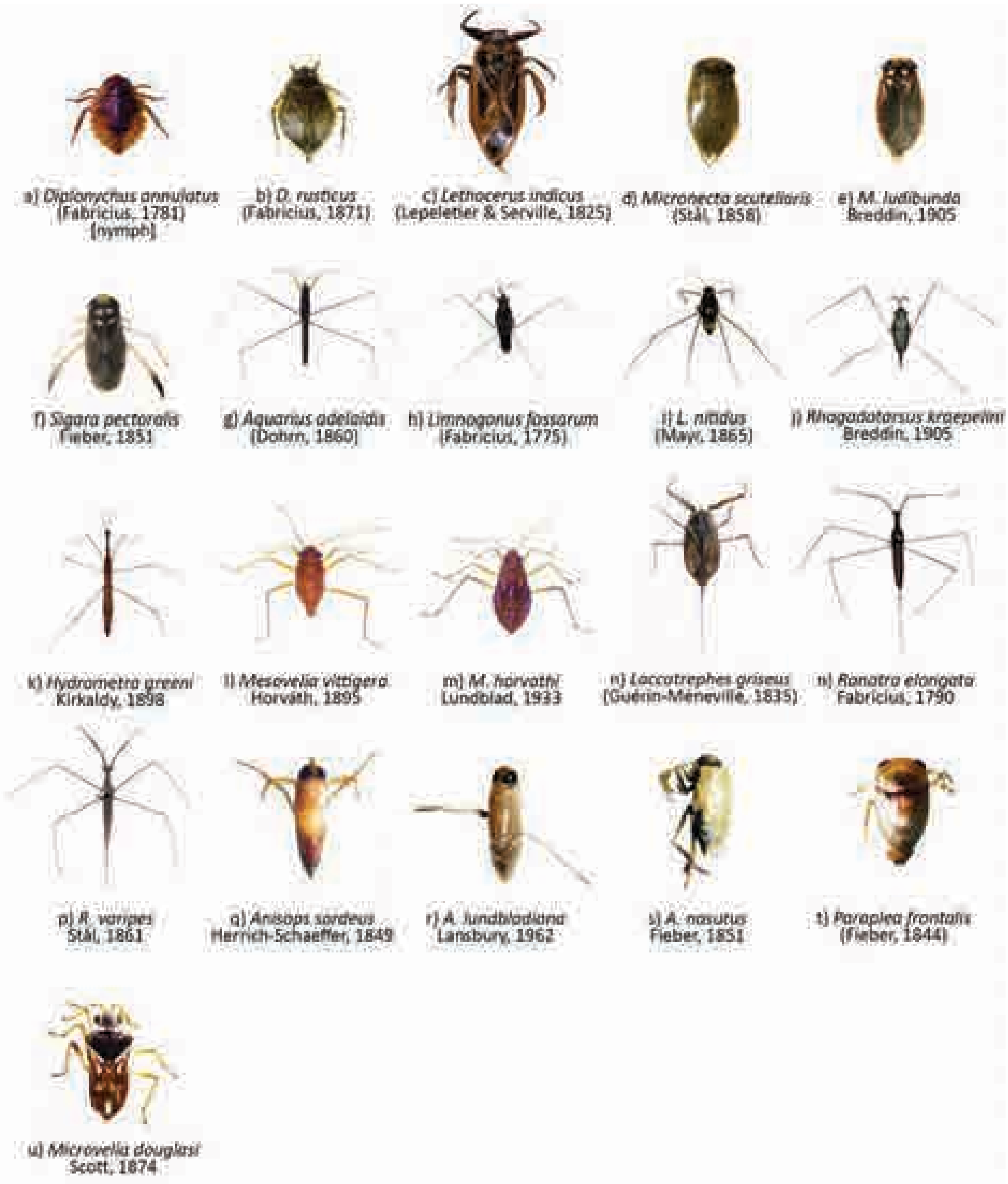

a) *Diplonychus annulatus* (Fabricius, 1781) (nymph)
b) *D. rusticus* (Fabricius, 1871)
c) *Lethocerus indicus* (Lepeletier & Serville, 1825)
d) *Micronecta scutellaris* (Stål, 1858)
e) *M. ludibunda* Breddin, 1905
f) *Sigara pectoralis* Fieber, 1851
g) *Aquarius adelaidis* (Dohrn, 1860)
h) *Limnogonus fossarum* (Fabricius, 1775)
i) *L. nitidus* (Mayr, 1865)
j) *Rhagadotarsus kraepelini* Breddin, 1905
k) *Hydrometra greeni* Kirkaldy, 1898
l) *Mesovelia vittigera* Horváth, 1895
m) *M. horvathi* Lundblad, 1933
n) *Laccotrephes griseus* (Guérin-Méneville, 1835)
o) *Ranatra elongata* Fabricius, 1790
p) *R. varipes* Stål, 1861
q) *Anisops sardeus* Herrich-Schaeffer, 1849
r) *A. lundbladiana* Lansbury, 1962
s) *A. nasutus* Fieber, 1851
t) *Paraplea frontalis* (Fieber, 1844)
u) *Microvelia douglasi* Scott, 1874

Image 1. Aquatic hemipterans collected from rice fields of Karaikal, Puducherry, India. © L Gopianand.

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COMMUNICATION

Leaf defoliation and *Tabernaemontana rotensis* (Asterids: Gentianales: Apocynaceae) flower induction and fruit development

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Abstract: *Tabernaemontana rotensis* (Kaneh.) P.T. Li is an attractive small tree that is endemic to the islands of Guam and Rota. Conservation efforts of the threatened population are constrained by lack of research. Understanding the ecology of flower and fruit development is fundamental to successful conservation of threatened angiosperms. This study determined the extent of flower induction following tropical cyclone defoliation, tested the efficacy of 10% urea sprays as a defoliant to induce flowering, and quantified the resulting fruit expansion to determine ontogeny traits. A total of 512 inflorescences were observed, half following tropical cyclones and half following defoliation with urea. Fruit length was measured every five to seven days until seed dispersal. The mean length of time between defoliation and initial flower anthesis was 29 days, and did not differ between tropical cyclone defoliation and urea solution aerosol defoliation. Four stages of observable fruit development were identified following anthesis. Linear increases in ovary length occurred for two weeks, maximum ovary length occurred at about day 30, color break from green to orange began at about day 60, and seed dispersal occurred at about day 90. Defoliation treatment did not influence the timing of these stages. The results indicated that tropical cyclone and urea solution defoliation consistently generated mast flowering after about one month with mast seeding about three months later. Conservationists may use this new knowledge to predictably schedule seed harvests at about four months following a natural or anthropogenic defoliation event. Many *Tabernaemontana* species are exploited for traditional medicine, and the use of defoliation to manipulate phenology of these species may benefit the practitioners of this trade.

Keywords: Conservation biology, crop regulation, defoliation, endangered plants, Guam, masting, tropical cyclone.

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INTRODUCTION

Of the world's described tree species, about 58% are endemics constrained to single countries (Beech et al. 2017). Islands contain many endemic species, representing taxa that are among the most threatened globally (Caujapé-Castells et al. 2010). An ecological understanding of all aspects of reproductive biology is required for development of effective conservation practices for threatened endemic tree species, therefore, an understanding of the reproductive biology of island species is of utmost importance for developing successful conservation programs (Kaiser-Bunbury et al. 2010). *Tabernaemontana rotensis* is restricted to two adjacent islands in a single archipelago (USFWS 2015), with most of the population existing on the single island of Guam (USFWS 2020). Protecting habitat of *T. rotensis* from ongoing land use change may not be enough to recover the species, as knowledge of fruit set, fruit ontogeny, seed quality, seed storage, and regeneration behaviors are also critical.

The native flora of the Mariana Islands includes several Apocynaceae tree species, including *Tabernaemontana rotensis*. The endemic range of the small tree includes the islands of Guam and Rota, and the species was listed as threatened under the United States Endangered Species Act in 2015 (USFWS 2015). The known global population declined about 25% between the 2015 listing and the 2020 conservation status update for the species (USFWS 2020).

The primary threat to this tree species has been identified as habitat loss and fragmentation due to land use change (USFWS 2015). For example, an ongoing military construction program will permanently convert more than 2,000 ha within northern Guam *T. rotensis* habitat (USFWS 2020). This Guam case study is germane to contemporary global extinction risk factors, as land use change is the greatest threat causing tree extinctions (BGCI 2021) and will remain a major driver of plant extinctions into the future (Pereira et al. 2012). Spatial patterns of the population reveal an allopatric distribution of several sub-populations. For example, only three sub-populations contain at least 25 mature trees, and a reported 98% of these mature trees occur in a single sub-population (USFWS 2020). This geographical clustering of the *T. rotensis* population adds to the extinction threats. Endemic plants that are spatially constrained are generally faced with the greatest extinction risks (Enquist et al. 2019; Nic Lughadha et al. 2020; Staude et al. 2020).

The forests of the Mariana Islands are often disturbed by tropical cyclones (Stone 1971; Marler 2001). Masting events have been observed in *T. rotensis* trees following tropical cyclones, but this plant response has not been adequately investigated to confirm its consistency. A full ecological understanding of this phenomenon and the dynamic changes during fruit ontogeny would benefit conservation and horticultural decisions.

Horticulturists are ideally equipped to conduct pragmatic investigations aimed at understanding plant ecology and improving conservation approaches (Marler 2017). The horticulture industry exploits horticultural defoliants for various purposes, including inducement of flowering in some species. One commonly used defoliant for managing guava trees, for example, is urea aerosol sprays with concentrations of 3% to 30% (Chapman et al. 1979; Singh et al. 2002, 2018; Samant et al. 2020).

Tabernaemontana rotensis responds well to horticultural management and serves as an attractive urban landscape tree. Trees in managed gardens in Guam and Philippines and several in situ locations in Guam provided the experimental sites for this study. The objectives were to confirm the extent and timing of mast flowering after tropical cyclones, determine if urea leaf sprays could generate defoliation that induced mast flowering, then measure fruit development from anthesis to the seed dispersal stage for the two defoliation sources. I hypothesized that urea solution defoliation of *T. rotensis* trees would elicit mast flowering and fruiting in a manner similar to tropical cyclone defoliation. The new knowledge will directly inform conservation decisions.

MATERIALS AND METHODS

Study Locations

Northern Guam was used for all tropical cyclone observations and three of the urea defoliation studies. The latitudinal range was N13.493°–13.611°, and the longitudinal range was E114.837°–144.926°. The elevation range was 74–180 m. Climate is classified as Köppen: Af, with mean temperature of 27.7°C, mean monthly rainfall of 19.8 cm, and mean relative humidity of 77.3%. The soils for the *in situ* observations were primarily Clayey-skeletal, gibbsitic, nonacid, isohyperthermic Lithic Ustorthents, and the soils for the controlled urea spray study were Clayey, gibbsitic, nonacid, isohyperthermic, Lithic Ustorthents.

The conservation research garden in the Philippines was located at N15.165°, E120.505° at 234 m. Climate is classified as Köppen: Am, with mean temperature of 29.7°C, mean monthly rainfall of 9.4 cm, and mean relative humidity of 82.7%. The soils were Coarse loamy, isohyperthermic, Typic Untipsamment.

Flower Induction

Tabernaemontana rotensis trees on Guam were observed following several tropical cyclone events to confirm the circumstantial observations that indicated mast fruiting occurred in response to tropical cyclones. These were Typhoon Tingting on 28 June 2004, Typhoon Chaba on 21 August 2004, Typhoon Francisco on 16 October 2013, and Typhoon Dolphin on 15 May 2015. Disparity in tree damage among habitats is a common feature of tropical cyclones, and results from spatial differences in topography and forest structure (Marler et al. 2016; Zhang et al. 2022).

Data Collection

In order to include a control treatment, various habitats were visited following each of these four tropical cyclones to find habitats with *T. rotensis* trees that were not defoliated. Eight trees that were defoliated and eight trees that were not defoliated were visited beginning three weeks after each tropical cyclone, then every three to four days thereafter until open flowers were observed.

The trees that were not defoliated did not exhibit any flower production throughout the dates of the study. The trees that were defoliated rapidly exhibited flowers, and dates of initial anthesis in each inflorescence were recorded for eight inflorescences per tree for a total of 256 inflorescences for the entire investigation of typhoon-defoliated trees. Characteristics of the four natural defoliation replications are described in Table 1.

A solution of 10% urea was sprayed as an aerosol over the entire canopy of eight in situ trees averaging 2.4 m in height in May 2012 as an initial test as a defoliant. Although most of the leaves were damaged and many abscised in accordance with objectives, the indiscriminate application also damaged stem apices such that vigorous vegetative regrowth occurred from lateral buds. Eight trees with a mean height of 2.7 m were sprayed in June 2012 by strategically spraying each stem by beginning with the oldest leaves and continuing in an apical direction until reaching the youngest fully expanded leaves. The remaining apical leaves were not sprayed, but were pruned on each petiole with shears. This procedure avoided damage to the stem apices with the urea solution, and copious inflorescences were included in the regrowth. These eight trees served as the first urea-defoliation replication for this investigation, and eight untreated trees in the same habitat were included as controls. Characteristics of the resulting four urea defoliation replications are described in Table 1. The procedure was repeated with a second set of 16 in situ trees exhibiting a mean height of 2.8 m in August 2012, with half of them serving as controls. In order to confirm the efficacy of this treatment for inducing flowers in cultivation, 16 *T. rotensis* trees with a mean height of 2.6 m and growing in a border planting of a commercial farm in Dededo, Guam were used as the third replication, with eight trees serving as controls. The urea application was applied in September 2012. These trees were sourced from a northeast Guam locality. Finally, 16 trees averaging 2.4 m in height and growing in an Angeles City, Philippines research garden were used as a fourth replication, with eight trees serving as controls. These trees were sourced from the same Guam locality. The urea application was applied in September 2015.

The growth of all control trees throughout the course of the study did not include any inflorescence production. Each of the eight urea-defoliated trees within the four replications were observed every three to four days to record leaf abscission and regrowth responses. Dates of initial anthesis for eight inflorescences per tree were recorded for a total of 256 inflorescences.

Table 1. The influence of defoliation of *Tabernaemontana rotensis* in Guam and Philippines on flower induction (eight trees per replication, eight inflorescences per tree) and fruit growth (four trees per replication, eight fruits per tree).

Defoliation replication	Date	Location	Community type
Natural defoliation events			
Typhoon Tingting	vi.2004	Guam	in situ
Typhoon Chaba	viii.2004	Guam	in situ
Typhoon Francisco	x.2013	Guam	in situ
Typhoon Dolphin	v.2015	Guam	in situ
Urea defoliation events			
Forest trees	vi.2012	Guam	in situ
Forest trees	viii.2012	Guam	in situ
Agroforestry	ix.2012	Guam	circa situ
Research garden	ix. 2015	Philippines	ex situ

Fruit Development

Developing fruits from the induced mast flowering events were observed and measured to more fully characterize reproductive behavior of this endemic tree. Four in situ trees following each tropical cyclone described in section 2.1 were selected for naturally defoliated trees. Eight inflorescences per tree were marked and the length of one fruit per inflorescence was measured. For each marked fruit, the fruit length was measured to the nearest mm every five to seven days until seed dispersal. Therefore, 32 fruits were observed for each defoliation replication for a total of 128 fruits for the growth following tropical cyclone defoliation. Four of the cultivated trees for each of the four events for the urea-defoliated study in section 2.1 were used for observing fruit development for horticulturally defoliated trees. Eight inflorescences from each tree were selected, and one fruit per inflorescence was marked for the measurements. All decipherable ontogeny events were observed and recorded.

Statistics

For flower induction data, the number of days between the defoliation date and the initial flower anthesis for an inflorescence was calculated from the calendar dates that were recorded. Each defoliation event was treated as a replication. The number of days for the 64 inflorescences within one defoliation replication were averaged to calculate the mean number of days for each replication. The differences between tropical cyclone versus urea defoliation treatments were determine by t test, n = 4.

Fruit growth quantified as ovary length as a function of time was fitted with non-linear regression, and every ovary measured followed the model $y = A(1-e^{Bx})$ where y signified fruit length and x signified days since anthesis. For each defoliation replication, all data from the 32 inflorescences were combined and the data were fitted individually per replication to obtain the values for A and B in the regression model. Thereafter, the influence of defoliation treatment on fruit ontogeny was determined by subjecting parameter A and parameter B to t test, n = 4.

For fruit development, the number of days between anthesis readily observable developmental events was recorded for each observed fruit. These events included the date that linear ovary extension ceased, the date that maximum ovary length occurred, the date that color break from green to orange began, and the date the ovary split open for seed dispersal. The mean of the 32 fruits for each of four defoliation replications was calculated for each of the developmental stages. The influence of defoliation treatment on each developmental event was subsequently determined by t test, n = 4.

RESULTS

The individual *T. rotensis* flower is attractive and the corolla is comprised of five homogeneously distorted petals (Image 1A). Petals are reflexed for most of their length, but are flat near the apex with undulating margins.

Flower Induction

The four tropical cyclones that served as defoliation events consistently generated regrowth within one to two weeks. The trees that received urea sprays exhibited leaf damage within one day and regrowth within one to two weeks. Observance of developing inflorescences occurred within two to three weeks for both defoliation treatments (Image 1B). Mast flowering with initial anthesis occurred within one month. Flowering continued for several weeks depending on the size of the inflorescence (Image 1C). The first flower to reach anthesis required 28.9 ± 0.3 days after the defoliation event, and was not influenced by defoliation treatment ($t = 0.171$, $P = 0.433$).

Fruit Development

At anthesis the two halves of the *T. rotensis* ovary appear as if they are united within the corolla, and they retain this appearance until about 8 mm in length. At this stage, the entire corolla tube abscises intact, revealing one prominent style and stigma. Immediately following this event, the ovary splits into two distinct halves separated by an acute angle. The angle separating the two halves increases until the halves are oriented directly opposite each other on the pedicel at about one month following anthesis.

The pattern of fruit length as a function of time was remarkably consistent among the replications and defoliation treatments. Parameter A of the non-linear regression model did not differ between the two defoliation treatments ($t = 0.242$, $P = 0.408$). Similarly, parameter B did not differ between the defoliation treatments ($t = 0.342$, $P = 0.372$). Therefore, the data from all replications in the study were represented by a single model (Image 2).

There were four observable developmental events that were identified during fruit growth. The increase in fruit length was linear immediately after anthesis, then growth increment deviated from linearity by slowing down at about two weeks (Image 2, Stage A). Defoliation treatment did not influence the timing of this ontogenetic trait ($t = 0.092$, $P = 0.465$). The ovaries were bright green initially and did not change in color

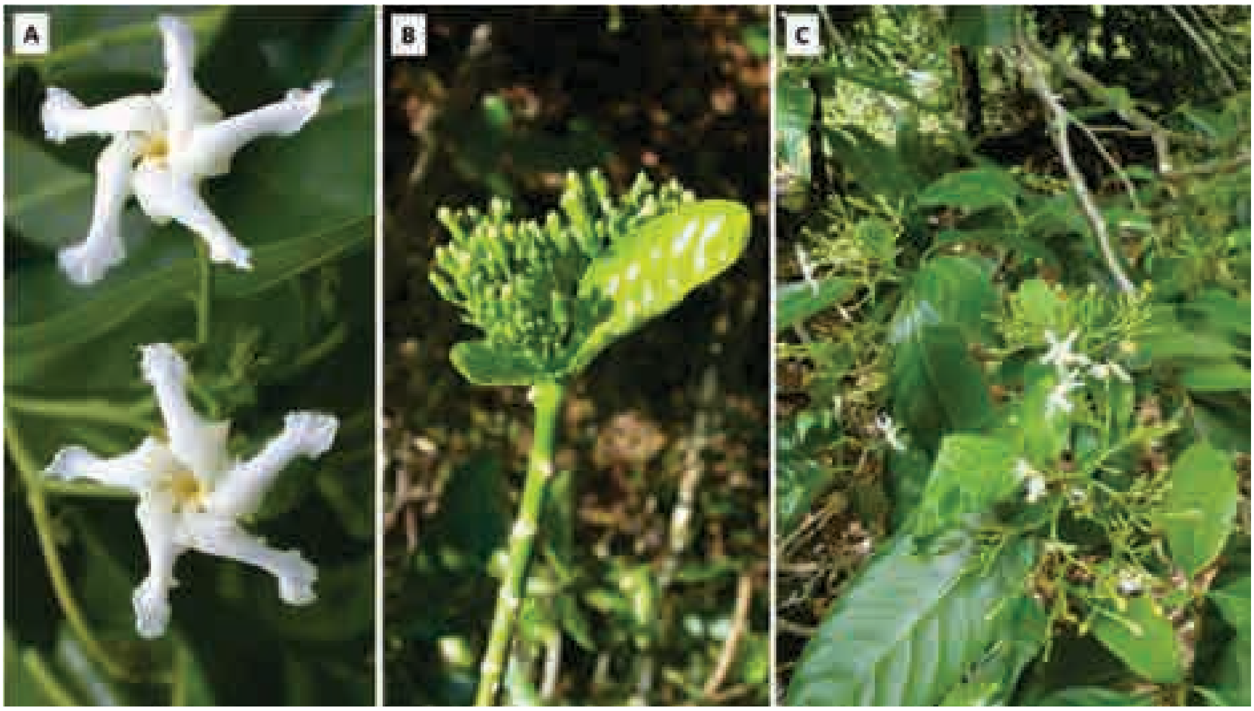

Image 1. *Tabernaemontana rotensis*: A—The attractive flower is produced in inflorescences comprised of very few individual flowers up to more than 100 flowers | B—Appearance of developing inflorescence at three weeks after induction by a urea defoliation event | C—Appearance of inflorescence at five weeks after a defoliation event, showing some flowers prior to anthesis, some flowers at anthesis, and some flowers past anthesis. © Thomas Marler.

until fruits reached maximum length at 29–34 days after anthesis (Image 2, Stage B). The date of maximum fruit length was not influenced by defoliation treatment (*t* = 0.293, *P* = 0.389). Fruit color morphed from bright green to dull green during the second month of fruit growth and a muted orange color break occurred 55–63 days after anthesis (Image 2, Stage C). The timing of this color break was not influenced by defoliation treatment (*t* = 0.281, *P* = 0.394). Color development progressed during the final month of fruit growth until a bright orange phenotype characterized the fruits as they split open to expose seeds at 88–94 days after anthesis (Image 2, Stage D). The window of time between anthesis and the opening of the ovary to expose seeds was not influenced by defoliation treatment (*t* = 0.461, *P* = 0.330).

DISCUSSION

The timing of first anthesis following defoliation and duration of each stage of fruit development exhibited notable stability among the individual trees, experimental sites, seasons, and years in this study. The results were consistent with the hypothesis that urea solution defoliation would generate plant responses similar to tropical cyclone defoliation. There was a remarkable stability in timing of reproductive behavior. Anthesis began at one month after defoliation and seed dispersal occurred at about three months after anthesis. The field work also revealed two general observations. First, variation in mature ovary length of *T. rotensis* fruits appeared to be constrained, with most ovaries maturing at 38–43 mm in length. The general observations of thousands of fruits during this Guam study revealed that ovary diameter or circumference, which were not directly measured, may be a more variable fruit trait. Second, many angiosperms are plagued by vulnerability to fruit abscission at critical developmental stages, a phenomenon called “June drop” for many fruit crops (Rieger 2006). *Tabernaemontana rotensis* did not exhibit this ontogenetic trait, and every flower that set fruit appeared to be able to support the developing ovary to maturity.

Masting is a common behavior among some tree species, and many benefits of masting to tree regeneration and community assembly are understood (Koenig 2021). For *T. rotensis*, synchrony of flowering among many sympatric trees appears to be associated with the seed masting behavior. This synchrony may increase mate availability which may improve out-

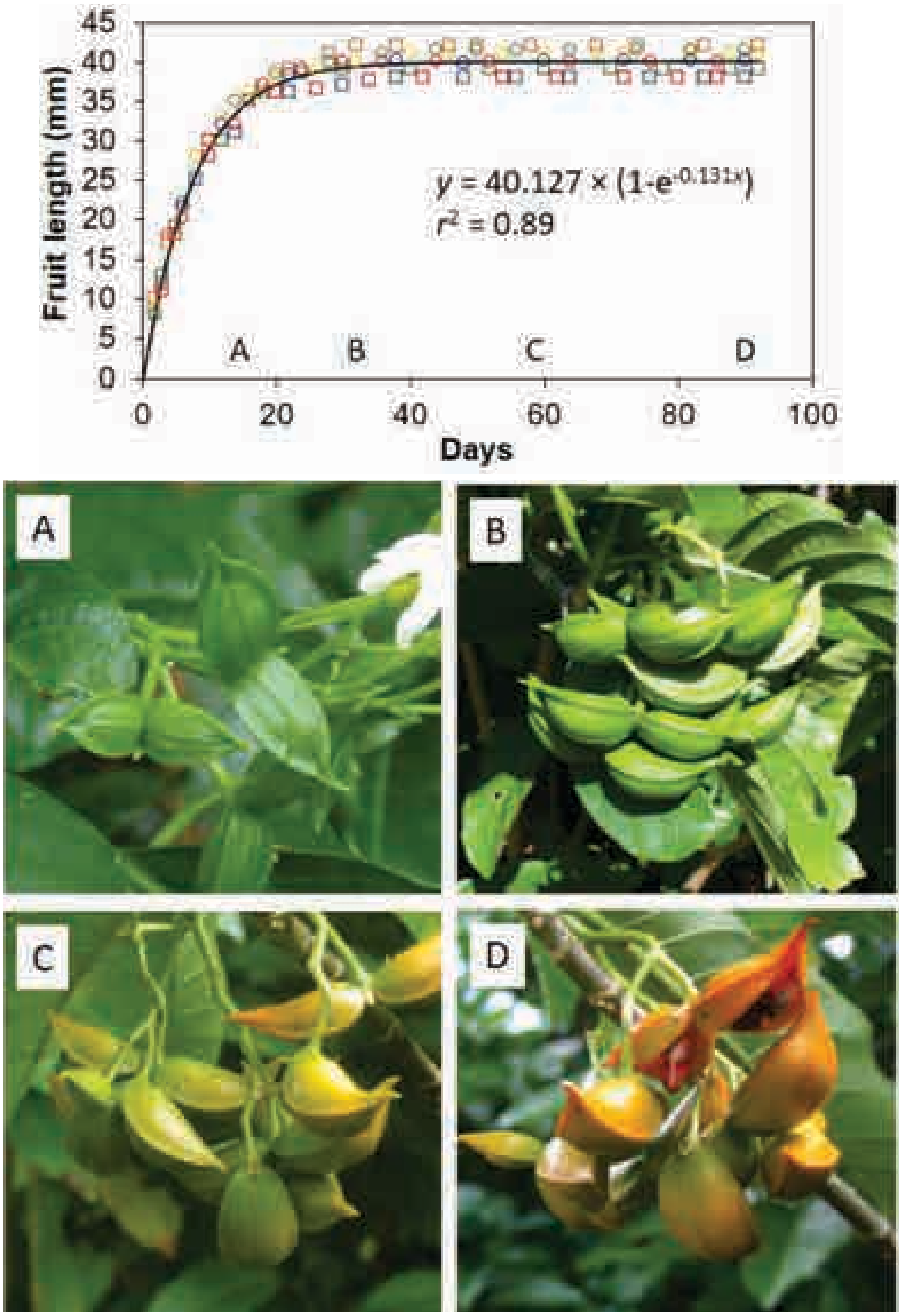

Image 2. ***Tabernaemontana rotensis*** **fruit length as influenced by time. Circle markers represent in situ trees naturally defoliated by tropical cyclones. Square markers represent in situ or cultivated trees defoliated by urea solution aerosol spray. Each marker color represents one replication that is the mean of eight fruits per tree on four trees. Upper case letters on the horizontal axis represent developmental stages depicted by the photographs. © Thomas Marler.**

crossing and increased the percentage of flowers that become pollinated. More research is needed to determine if defoliation-induced flowering also generates increased seed count per fruit and increased seed viability.

Masting behavior is often linked to natural disturbances which synchronize population phenology (Vacchiano et al. 2021). Understanding how natural events affect seed production may be challenging, but my findings indicated that tropical cyclone defoliation events may consistently lead to mast fruiting of *T. rotensis* about four months after the disturbance. These findings illuminate a facet of the ecology of the region that may be directly influenced by climate change factors, as most models predict greater intensity of future tropical cyclones (Marler 2014). The use of horticultural defoliation treatments that do not damage stem apices also consistently generated masting after about four months. This relatively fixed maturation duration may be exploited by conservationists to schedule seed harvesting events by recording the date of the natural or anthropogenic defoliation events, then scheduling fruit harvest events after about four months.

Several avenues of further study are warranted. First, a dose response curve of urea throughout the entire published 3% to 30% concentration range (Chapman et al. 1979; Singh et al. 2002, 2018; Samant et al. 2020) may reveal the most efficacious dosage for *T. rotensis*. An inadvertent benefit from this horticultural protocol is the nitrogen that is transferred to the soil along with abscised leaves may act as fertilizer. Therefore, urea dosages that are greater than the minimum required for defoliation may be beneficial as a conservation action. Second, tree size may influence masting behaviors, especially for small, young trees that may not produce seeds as consistently as large trees (Bogdziewicz et al. 2020b). The range in tree size was purposefully constrained in this pilot study for logistical reasons. More research may be warranted to determine if the masting behaviors and timing of fruit ontogeny stages are consistent among a range of *T. rotensis* tree size categories. Third, the mechanisms of defoliation caused by urea are not fully understood, but likely involve osmotic stress due to reduced osmotic potential on the laminae surfaces. Productivity of this tree species is mostly limited by phosphorus within in situ settings (Marler 2021). The use of triple superphosphate solutions to impose laminae surface osmotic stress may reveal a response similar to that of urea solutions. Studies designed to determine dosage and efficacy may also reveal if translocation of phosphorus from leaves to stems may occur prior to leaf abscission. If this is shown to occur, the increased stem nutrient pool would be available for translocation into post-defoliation regrowth. Fourth, numerous biology questions were beyond the scope of this paper, and remain to be studied. These include all aspects of pollination biology, histological changes of the ovary from fertilization through seed maturation, and seed dispersal strategies. Fifth, the influences of climate change on plant masting behavior are being actively studied (Bogdziewicz 2022; Bogdziewicz et al. 2020a). The manner in which climate change influences the reproductive behaviors of this tree species are not known, but may be studied in the future using my findings as a historical benchmark. The results herein illuminate the fact that disturbance of a forest community by a tropical cyclone may provide some beneficial outcomes to some species. Climate change predictions indicate more intensity of future tropical cyclones (Marler 2001). The regeneration and recruitment dynamics of native forests that are frequently subjected to tropical cyclone disturbance may be unique when compared to forests in regions that do not experience tropical cyclones (Chao et al. 2022). A comprehensive look at the full spectrum of *T. rotensis* plant and population responses to tropical cyclones may reveal many interesting aspects of biology and ecology in the face of frequent large-scale disturbances.

This small, handsome Apocynaceae tree continues to be subjected to the anthropogenic threats that caused the federal listing on the U.S. Endangered Species Act (USFWS 2015). The habitat loss due to land use change (USFWS 2020) will not subside in the foreseeable future because of the expansive military buildup occurring on Guam (Marler 2013). Formal recovery programs for endangered tree species depend on experimental and observational studies to provide knowledge to inform conservation decisions. The current study adds to a growing body of horticultural and ecological literature on this species. The seeds of *T. rotensis* rapidly lose viability during storage in ambient conditions, and respond well to full sun as germination and seedling growth conditions (Marler et al. 2015). The CO_2 efflux from *T. rotensis* stems exhibits a pattern that is dependent on the diel cycle, and is greatest during the photoperiod at about 2.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and least during the nocturnal period at about 1.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Marler & Lindström 2020). Green and senesced leaves of *T. rotensis* contain relatively high magnesium, manganese, and nickel concentrations in comparison to 24 sympatric plant species (Marler 2021). Leaf nutrient resorption and senesced leaf traits indicate that *T. rotensis* leaf litter is relatively labile, with more rapid decomposition and nutrient turnover predicted than for most of Guam's sympatric species (Marler 2021).

The genus *Tabernaemontana* contains about 100 described species growing in tropical and subtropical latitudes (Silveira et al. 2017). Although a considerable amount of research has been devoted to the genus, the focus has been on pharmacological properties of the plant organs (Naidoo et al. 2021). As a result, horticulture, ecology, and conservation questions have not been adequately answered. In the absence of research on a species of interest, the use of surrogate taxa for research may provide valuable information (e.g. Marler et al. 2021). Therefore, my findings on masting behaviors and fruit development may inform management decisions for other closely related *Tabernaemontana* species. The extensive literature on phytochemicals and ethnomedicinal uses of *Tabernaemontana* species provides a potential avenue for expanding the conservation efforts of *T. rotensis*. The closely related *Tabernaemontana pandacaqui* Lam. is among the species that have been studied for its medicinal value (Taesotikul et al. 1990). Medicines extracted from trees are integral to the well-being of millions of people, and research on this aspect of tree value is critical for decision-makers to understand the urgent need for conserving the world's trees (Rivers et al. 2022). Therefore, a dedicated research program focused on identifying the medicinal uses of *T. rotensis* has potential for successful outcomes and may add justification for convincing decision-makers about the value of conserving this island endemic tree. There is a sense of urgency to this goal, as the known global *T. rotensis* population declined about 25% between 2015 and 2020 (USFWS 2020).

CONCLUSION

Defoliation of *T. rotensis* trees caused synchronized inflorescence production with anthesis occurring after about one month, maximum fruit length after about two months, color break of pericarp tissue from green to orange after about three months, and seed dispersal after about four months. Tropical cyclones provide the main source of natural defoliation within the endemic range of the species, and climate change may alter regeneration and recruitment behaviors of this tree species via the predicted changes in tropical cyclone intensity. An aerosol spray of urea solution provided the experimental source of defoliation, with plant responses that were similar to tropical cyclone defoliation. Conservationists may use this new knowledge to manually induce mast flowering events and accurately predict mast seed production windows of time following natural and anthropogenic defoliation. This benefits conservation projects designed to salvage plants from a planned construction site because practitioners can use this knowledge to force scheduled seed production rather than wait for a natural flowering event.

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SHORT COMMUNICATION

First record and DNA barcode of a scarab beetle, *Adoretus kanarensis* Arrow, 1917 (Coleoptera: Scarabaeidae: Rutelinae), from Maharashtra, India

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Abstract: *Adoretus kanarensis* Arrow, 1917, composed within the subfamily Rutelinae of family Scarabaeidae, was described from India about 107 years ago. These conspicuous, attractive, and tiny leaf chafers are recorded as predators of several agricultural crops. The mt DNA Barcodes of many *Adoretus* Dejean, 1833 species are available in global databases. In this work, first DNA barcode of *A. kanarensis* has been reported. Morphological details with photographs of the species along with a checklist of the genus *Adoretus* from Maharashtra is provided. The mt COI DNA sequences of *Adoretus* species recorded until now for the genus that are listed in GenBank are analyzed.

Keywords: Chafers, dung beetle, Nashik, new record, polyphagous pest, survey.

The genus *Adoretus* Dejean, 1833 is distributed in Africa, Asia, and Europe (Anonymous 2023). It is a polyphagous and economically important genus, due to its potential damage to a wide variety of host plants including important agricultural cash crops like sugarcane, tobacco and ornamental plants (McQuate & Jameson 2011). There are about 460 species (Krajcik 2007) reported in the world, of which 47 are reported in India (Arrow 1917), few literature is available on this genus from India (Chandra 2009; Chandra et al. 2012; Ghosh et al. 2020). Some of the species of this genus are of biosecurity concerns (McQuate & Jameson 2011). The accurate identification based on the morphological characters are important for undertaking proper control measures. Beetles of the subfamily Rutelinae are not dung beetles in the true sense. They are phytophagous and commonly known as May or June beetles or shining leaf chafers (Sreedevi et al. 2017). The pioneering work on this group in India was undertaken by Arrow (1917) and Balthasar (1963, 1974). Since the fauna has not been revised, few scattered publications are available on this particular group from India, without detailed distribution records (Kumar & Pandey 2023).

The present study was undertaken to document the

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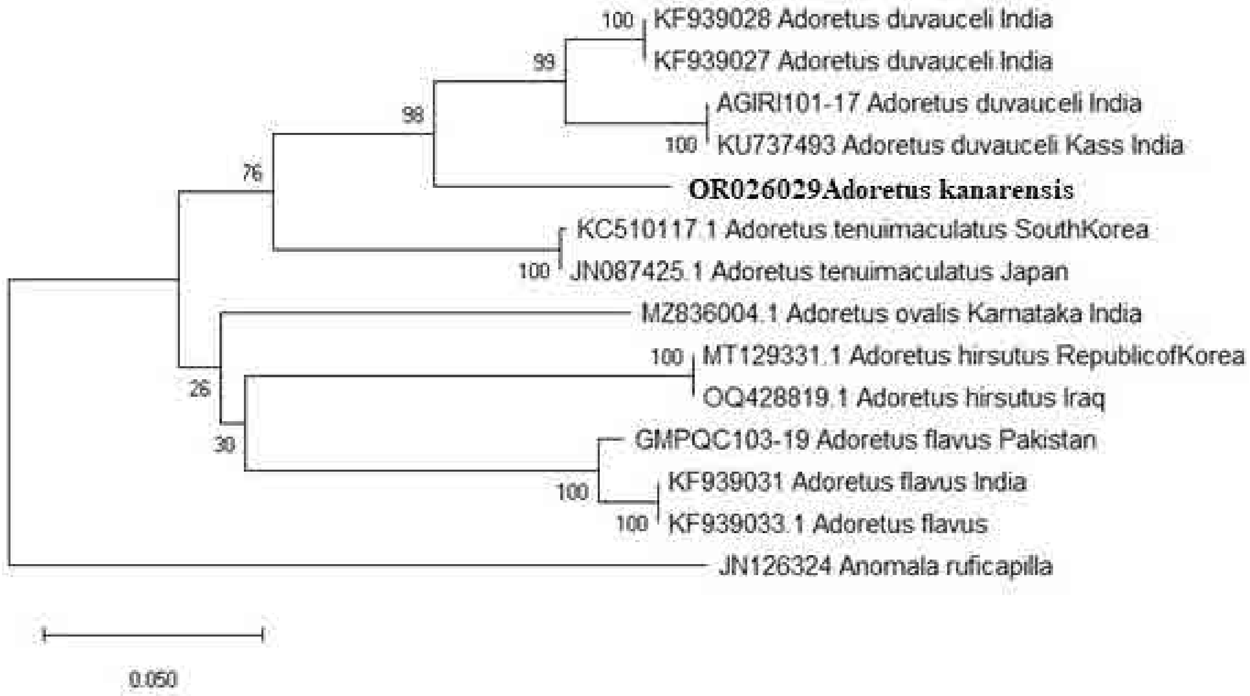

Figure 1. Maximum likelihood tree for the species of *Adoretus kanarensis* based on the 578 bp of mitochondrial COI DNA gene sequences.

first record of *A. kanarensis* from Maharashtra since Arrow (1917) noted the distribution as Bombay: North Kanara, indicating that North Kanara was included at that time when Bombay was the Bombay presidency during British India. Considering this, the present report forms the first report of *A. kanarensis* from Maharashtra, India based on voucher specimen with first DNA barcode for the country.

Though *Adoretus* species are widely distributed, only 12–13 mitochondrial cytochrome oxidase subunit (COI) sequences are currently available in global database. Hence, during one of our experiments to generate mt DNA barcodes for the coleopteran species, we report the first mt DNA barcode of *A. kanarensis* from Maharashtra, India.

MATERIAL AND METHODS

Sampling of dung beetles

Specimen for the present study was collected at night using light trap. The map of the collection locality was prepared using open free QGIS software. The details of collection locality are given under material examined and also shown in Figure 1.

Preservation and Identification

The collected specimen was euthanized in the vapours of ethyl acetate and brought to the laboratory for further studies. For morphological identification, the specimen was studied under Leica EZ4E stereomicroscope. The identification was done following the keys of Arrow (1917). Further, the voucher specimen was deposited in the National Repository of Zoological Survey of India, Western Regional Centre, Pune, Maharashtra (India).

DNA isolation, PCR and Sequencing

The ethanol preserved tissue was used for DNA isolation. DNA from the tissues of the beetle was extracted from metathoracic leg using DNeasy kit (Qiagen), according to the manufacturer's protocol. The obtained DNA was amplified using polymerase chain reaction (PCR) using ABI thermocycler. Following primers (Meyer et al. 2005) were used for amplification of COI gene: dgLCO F1 5'GGTCAACAAATCATAAAGAYATYGG 3' and dgHCO R1 5'TAAACTTCAGGGTGACCAAARAAYCA 3'. PCR reaction was carried out in total volume of 25 µl containing 2 µl DNA template, 10 pmol of each primer and 2 µl of dNTP and 0.2 µl of Taq polymerase (Bangalore GeNei). Thermo-cycling conditions were as follows:

One initial cycle of 1 min at 95˚C followed by five cycles of 95˚C for 1 min, then denaturation 35 cycles of 95˚C for 1 min, annealing at 52°C for 40 s, extension at 72˚C for 1 min 15 s, with final extension of 72˚C for 5 min.

From each PCR reaction, 2 µL of the PCR product was visualized on a 2% agarose gel stained with ethidium bromide, together with a GeneRuler 100 bp Plus DNA

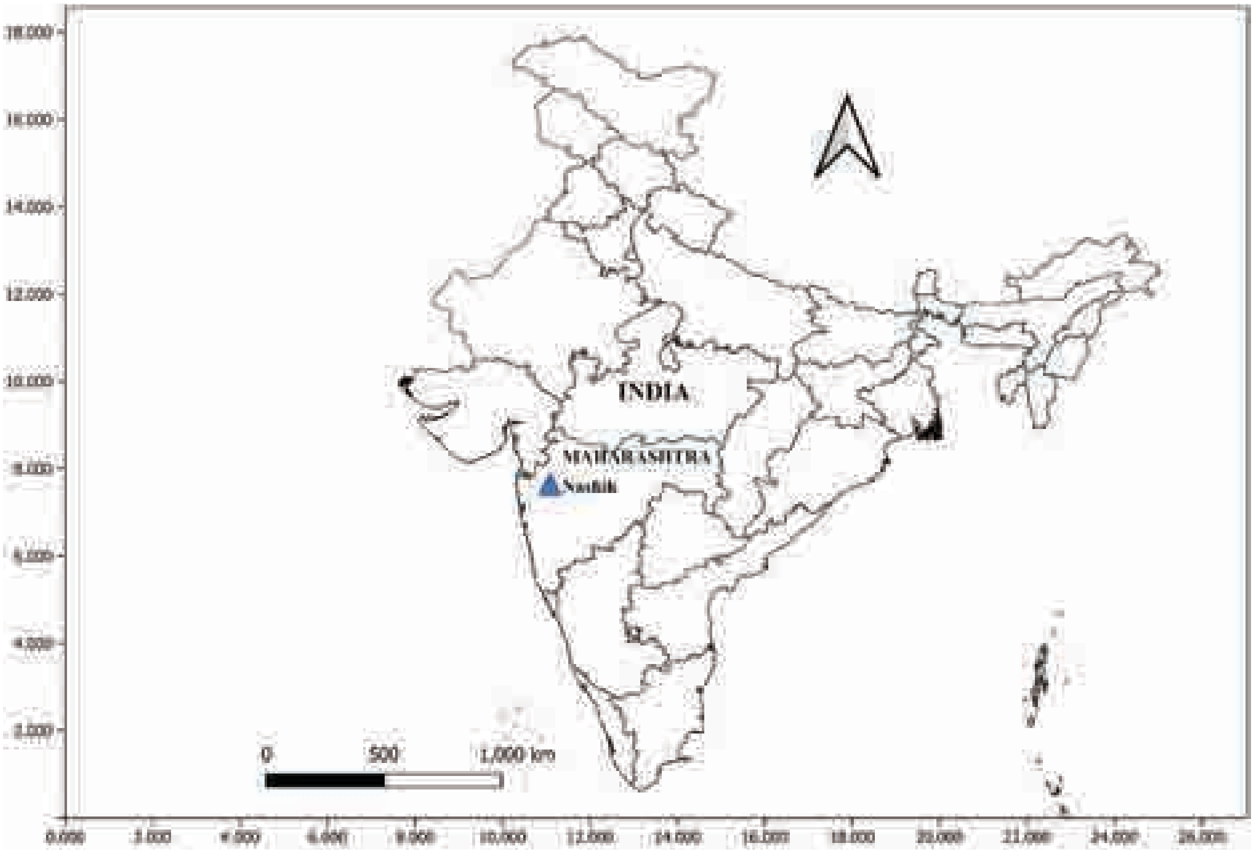

Figure 2. Collection locality of *Adoretus Kanarensis* from Maharashtra, India.

Ladder (Thermo Scientific). The obtained PCR products were sequenced with both, the forward and reverse, primers using an automated sequencer (3730 DNA analyzer, ABI, Hitachi).

Data analysis

Sequence was edited to remove ambiguous base calls and the forward and the reverse sequences were assembled using Chromas Pro version 1.34 (Technelysium Pty Ltd., Tewantin, Queensland, Australia). FASTA format of *Adoretus kanarensis* sequences was used for performed BLAST search at NCBI and species identification tool at Barcode of Life Data System (BOLD). All the obtained sequences were aligned and manually edited using BioEdit version 7.2.6. The Maximum Likelihood method and General Time Reversible model (GTR) model of base substitution was used to calculate pairwise genetic distance in MEGA X version 10.0.5. Additionally, to check the performance of DNA barcoding, sequences were downloaded from NCBI and BOLD (Table 2, Supplementary data) for some species of same genus submitted from different geographical areas. Only sequences which formed monophyletic clades with the sequences studied here were selected, to avoid use of sequences from wrongly identified species. These sequences along with our data were used to generate trees using MEGA X (Nei & Kumar 2000; Kumar et al. 2018).

RESULTS AND DISCUSSIONS

Morphologically, the collected sample was identified as *Adoretus kanarensis* Arrow, 1917 (Figure 3).

Systematic account

Order **Coleoptera Linnaeus, 1758**
Suborder **Polyphaga Emery, 1886**
Super family **Scarabaeoidea Latreille, 1802**
Family **Scarabaeidae Latreille, 1802**
Subfamily **Rutelinae MacLeay, 1819**
Genus ***Adoretus* Dejean, 1833**
***Adoretus kanarensis* Arrow, 1917**

Material examined: ZSI-WRC-ENT-1/2856, 27.i.2017, 01 Female, Sinner, Nashik district (19.8543 N, 73.9922 E), Maharashtra, coll. Pranil Jagdale.

Image 1. Adult female of *Adoretus kanarensis* Arrow, 1917. © Pranil Jagdale.

Table 1. Checklist of the genus *Adoretus* Laporte, 1840 from Maharashtra, India.

	Species	Distribution	References
1	*Adoretus caliginosus* Burmeister, 1844	India (Karnataka, Haryana, Himachal Pradesh, Maharashtra, West Bengal, Punjab, Sikkim, Tamil Nadu); Myanmar; Pakistan.	Arrow 1917; Mittal 1981; Chandra 1986; Ghosh et al. 2020; Schoolmeester 2023
2	*Adoretus excisus* Ohaus, 1914	India (Maharashtra, Madhya Pradesh, West Bengal).	Arrow 1917; Schoolmeester 2023
3	*Adoretus incurvatus* Ohaus, 1914	India (Bihar, Haryana, Himachal Pradesh, Maharashtra, Tamil Nadu).	Arrow 1917; Mittal 1981; Chandra 1986; Ghosh et al. 2020; Schoolmeester 2023
4	*Adoretus kanarensis* Arrow, 1917	India (Karnataka, Maharashtra (present study), Madhya Pradesh).	Arrow 1917
5	*Adoretus lasiopygus* Burmeister, 1855	India (Assam, Haryana, Bihar, Chhattisgarh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal); Bangladesh; Bhutan; Nepal; Sri Lanka.	Arrow 1917; Mittal 1981; Chandra 2000; Ghosh et al. 2020
6	*Adoretus limbatus* Blanchard, 1850	India (Bihar, Chhattisgarh, Haryana, Jammu & Kashmir, Madhya Pradesh, Maharashtra, Odisha, Uttar Pradesh, West Bengal); Bangladesh; Myanmar; Thailand.	Arrow 1917; Mittal 1981; Chandra 1986; Ghosh et al. 2020
7	*Adoretus lobiceps* Arrow, 1917	India (Gujarat, Madhya Pradesh, Maharashtra).	Arrow 1917; Chandra 2000.
8	*Adoretus pusillus* Arrow, 1917	India (Maharashtra)	Arrow 1917; Schoolmeester 2023
9	*Adoretus stoliczkae* Ohaus, 1914	India (Maharashtra, Madhya Pradesh); Pakistan.	Arrow 1917; Chandra 2000
10	*Adoretus sorex* Arrow, 1917	India (Maharashtra & Tamil Nadu), Sri Lanka	Arrow 1917; Schoolmeester 2023
11	*Adoretus versutus* Harold, 1869	India (Andaman & Nicobar Islands, Bihar, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Tamil Nadu, Uttarakhand, West Bengal); Bangladesh; Fiji; Indonesia; Malaysia; Mauritius; Sri Lanka.	Arrow 1917; Mittal 1981; Chandra 1986; Chandra 2000; Ghosh et al. 2020

Table 2. Details for the mt DNA COI sequences utilized in the construction of the phylogenetic tree.

	GenBank Accession No./ BOLD ID	Locality	Species name as per NCBI	Publication details as per NCBI
1	JN087425	Japan	*Adoretus tenuimaculatus* Waterhouse, 1875	Unpublished
2	OQ428819	Iraq	*Adoretus hirsutus* Ohaus, 1914	Unpublished
3	KC510117	South Korea	*Adoretus tenuimaculatus* Waterhouse, 1875	Unpublished
4	KF939031	South Korea	*Adoretus hirsutus* Ohaus, 1914	Unpublished
5	MT129331	Republic of Korea	*Adoretus hirsutus* Ohaus, 1914	Kang et al. 2021
6	KU737493	India	*Adoretus duvauceli* Blanchard, 1851	Unpublished
7	AGIRI101-17	India	*Adoretus duvauceli* Blanchard, 1851	Unpublished
8	GMPQC103-19	Pakistan	*Adoretus flavus* Arrow, 1917	Unpublished
9	KF939033	India	*Adoretus flavus* Arrow, 1917	Unpublished
10	MZ836004	India	*Adoretus ovalis* Blanchard, 1851	Unpublished
11	KF939027	India	*Adoretus duvauceli* Blanchard, 1851	Unpublished
12	KU939028	India	*Adoretus duvauceli* Blanchard, 1851	Unpublished
13	**OR026029**	India	*Adoretus kanarensis* Arrow, 1917	Present study

Diagnosis

Female (Image 1): Length, 10 mm; width, 5 mm. Bright brownish-yellow, moderately shining. The lateral margins of head, pronotum, broad sutural line reaching anteriorly till the humeral callus and posteriorly not reaching the margins and extremities of tibia and complete tarsus are dark reddish-brown. Head transversely rugose, small, with the clypeus broadly rounded. The pygidium has a bare apical area. Legs are slender, the front tibia is armed with three strong teeth, the larger claw of the front and middle feet is cleft, and the shorter hind claw is more than half the length of the longer.

DNA Barcode diagnosis

In this research study, *A. kanarensis* was identified using available literature and sequence of COI gene isolated from an adult female. No matches were found among the already-existing entries in the BOLD database after analysis with the BOLD Identification tool. The genetic difference between the two samples was over 10%, indicating that the examined species has not yet been recorded in BOLD. GenBank's BLAST analysis yielded the same outcome.

The preliminary molecular analysis was carried out using available material from NCBI GenBank (Table 2). As expected, *A. kanarensis* nested within the genus *Adoretus* with the outgroup taxon *Anomla ruficapilla* Burmeister, 1855 using Maximum Likelihood method with General Time Reversible model. In the current study, *A. kanarensis* formed a monophyletic clade with *A. duvauceli* Blanchard, 1851 species.

Since the species *A. kanarensis* is of economic significance, the present mt DNA barcode data generated is expected to be helpful in building a reliable DNA barcode library for the country intimated with a voucher specimen and helpful in addressing the taxonomic problems as the morphological characters are cryptic.

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SHORT COMMUNICATION

New record of *Lucilia cuprina* (Wiedemann, 1830) (Diptera: Calliphoridae) from the Trans-Himalayan Region, cold arid desert of Kargil Ladakh, India

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Abstract: *Lucilia* spp. commonly known as the green bottle fly, is cosmopolitan in distribution and well documented from different parts of the world. They have medical, forensic and veterinary importance. In the present study, *Lucilia cuprina* (Wiedemann 1830) is documented for the first time from the Trans-Himalayan region of Kargil Ladakh. It was found that this fly is fairly widespread in the study area. These flies show strong positive correlation with the temperature and weak positive correlation with the relative humidity. Therefore, fly abundance was recorded maximum in midsummer (July & August) and least in April & October; however, no fly activity was seen during winter months from November through March.

Keywords: Distribution, abundance, occurrence, green bottle fly, forensic importance.

Abbreviations: RH—relative humidity | up calyp—upper calypters | low calyp—lower calypters | ant—antenna | ant spir—anterior thoracic spiracle | gen—gena | gr amp—greater ampulla | ketter—ketatergite | post spit—posterior thoracic spiracle | hind cox—hind coxa | sub scl—subcostal sclerite | bas cost—basicostae | h cal—humerous callus | ntl—notopluron | in ver set—inner vertical setae.

One of the green bottle flies, *Lucilia cuprina*, is found all over the world. Being a synanthropic fly it is more common in human surroundings, around areas like slaughterhouses, meat stores, latrines, and garbage disposal sites. The adult female lays eggs on dead and decomposing organic matter wherein the larval stages are completed. The 3rd instar larvae stop feeding and pupate in the soil; however, adults are free-living (Falk 2016). This fly species has been found to be useful in forensic science, causes myiasis in humans and other vertebrates, and acts as a mechanical vector for a variety of pathogens such as nematodes, helminths, protozoans, fungus, bacteria, and viruses (Heath 1982; Stevens & Wall 1996; Fetene & Worku 2009; Akbarzadeh et al. 2015; Hasson 2017; Tomberlin et al. 2017).

This species is well documented from the Oriental Region of the Indian subcontinent, but no records of this fly are known from the Trans-Himalayan region and its adjoining areas including Himachal Pradesh and Jammu & Kashmir (Nandi 2002; Nandi & Sinha 2004; Bharti 2011). Here we report *L. cuprina* for the first time from the Trans-Himalayan region of Kargil Ladakh and report its distribution and seasonal abundance. The current study will be useful in examining the other aspects of this fly in the Trans-Himalayan area, including its potential use in medicine, forensics, and veterinary science.

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Competing interests: The authors declare no competing interests.

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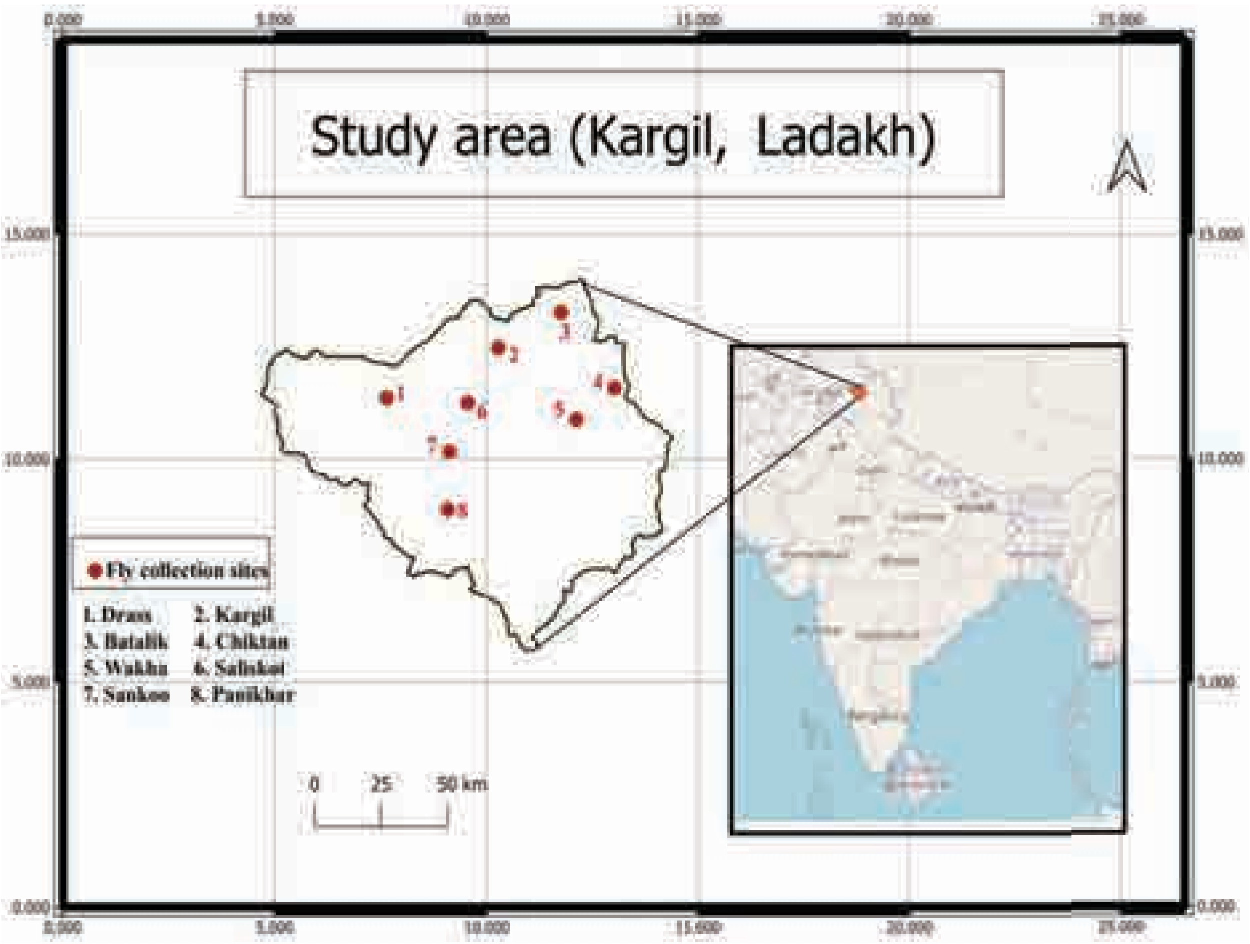

Figure 1. Map of study area Kargil Ladakh.

Material and Methods

Kargil is a district under the administration of UT, Ladakh, India in Trans-Himalayan region, situated 30 to 35 degree N and 75 to 77 degree E, with an area of about 14,036 km^2. The survey was conducted for three consecutive years from April 2018 to March 2021. To ease the survey, based on geography, topography, and climatic condition, the study area was divided into eight main sites, viz.: Drass, Kargil town, Batalik, Chiktan, Wakha (Shargole), Saliskot, Sankoo, and Panikhar (Figure 1). The survey was carried out on a monthly basis by using plastic bottle traps baited with 100 g unwashed day old goat/sheep stomach (Hussain et al. 2022, 2022). On each visit, three traps were installed in all the above mentioned study sites at a distance of about 100 m for three hours extending from 1100 h to 1400 h, around places like slaughterhouses, local latrines, meat shops, and waste dumping areas. The survey was not conducted during the winter months (November to March) as the climatic conditions were not feasible and no fly activity was observed at average temperatures below 4°C. The trapped flies were killed using ethyl acetate/chloroform. Based on their morphology *L. cuprina* was sorted out, counted and identified up to species level by using available keys (Wallman 2001; Carvalho & Mello-Patiu 2008; Whitmore et al. 2020). The total number of flies captured from each site/visit were pooled and drawn against each month to access the seasonal abundance. Photographs were captured using Leica S9i stereo-zoom binocular microscope fitted with camera and edited with Adobe Photoshop 7.0. Data was analysed using software SPSS 16.0 and graphs were plotted using software Origin pro 8. Climatic data of the district Kargil was obtained from the Indian Metereological Department, Metereological Center, Rambagh, Srinagar, Jammu & Kashmir UT, India.

Results And Discussions

Lucilla cuprina (Wiedemann, 1830)

Type-locality: China. Type in the Leyden Museum

Type species: *Lucilia acutifolia*

Material examined: India: Ladakh: Kargil town, 4♀ :1♂, 34.56°N, 76.13°E, 2,672 m, 11.vi.2018, M. Hussain; Drass, 2♀ :1♂, 34.41°N, 75.77°E, 3,081 m, 18.v.2018, M. Hussain; Batalik, 2♀, 34.66°N, 76.34°E, 2,814 m, 11.v.2018, M. Hussain; Chiktan, 3♀, 34.46°N, 76.52°E, 3,294 m, 18.vi.2018, M. Hussain; Wakha, 2♀, 34.37°N, 76.39°E, 3,371 m, 18.vi.2018, M. Hussain; Trespone, 1♀, 43.41°N, 76.03°E, 2,849 m, 16.vii.2018, M. Hussain; Sankoo, 3♀, 34.28°N, 75.96°E, 2,985 m, 16.vii.2018, M. Hussain; Panikhar, 1♀, 34.13°N, 75.95°E, 3,229 m, 16.vii.2018, M. Hussain.

Diagnosis

Body metallic green; gena white with black hairs; posterior slope of humeral callus with 0–4 hairs; notopleuron surface between last notopleuron seta and edge of notopleuron with 2–5 hairs; central occipital area below each inner vertical seta with one setula; ketatergite bar; wings hyaline; basicostae bright yellow; stem vein bar above; lower calypters bar above; frontoclypeal membrane dark brown; width of frontal stripe (frontal vitta) as wide as parafrontal plate; color of the fore femora dark metallic green (Image 2–9).

During the present study 1,176 flies were captured from April 2018 to March 2021, of which Kargil town represented a maximum of 202 (17.18%) followed by Chiktan 173 (14.71%), Sankoo 154 (13.1%), Batalik 138 (11.73%), Saliskot 137 (11.64%), Drass 135 (11.47%), Wakha 129 (10.1%), and Panikhar 108 (9.18%) which indicates that this species is widely distributed across the Trans-Himalayan region which coincides with the distributions of *L. sericata* (Hussain et al. 2022).

Being a cold blooded animal, the activity of *L. cuprina* is directly influenced by climatic factors like temperature, humidity, rainfall, and snowfall. Kargil, being a part of a cold climate desert, shows great variation in the seasonal temperature ranging from -35˚C during midwinter to 40°C during midsummer (Behera et al. 2014). During the study it was recorded that this species showed a strong positive correlation with temperature (r = 0.868) and a weak positive correlation with relative humidity (r = 0.276). *Lucilia* spp. overwinters in both the larval and pupal stages (Wall et al. 2000; Rosati 2014). During the present study it was recorded that adult *L. cuprina* begin to appear in April with an average temperature of 13.95±1.4°C (Mean±SE), and reached its highest peak in August with an average temperature of 23.81±1.0°C (Mean±SE) and was not observed during winter months from November through March during which the ambient average temperature remained below 1.24±1.8°C to -6.12±2.3°C (Mean±SE) (Figure 2). It was found that this species was most abundant in August, which recorded 119.00±14.0 (Mean±SE) followed by July with 111.60±4.4 (mean±SE) and the least (4.33±0.66; mean±SE) was recorded in the month of April. Statistical analysis (ANOVA, Duncan test) showed that there was no significant difference in the fly abundance in July and August; whereas, these two months showed significant difference in fly-abundance from rest of the months. These results corroborate with those of Brundage et al. (2011) and Hussain et al. (2022).

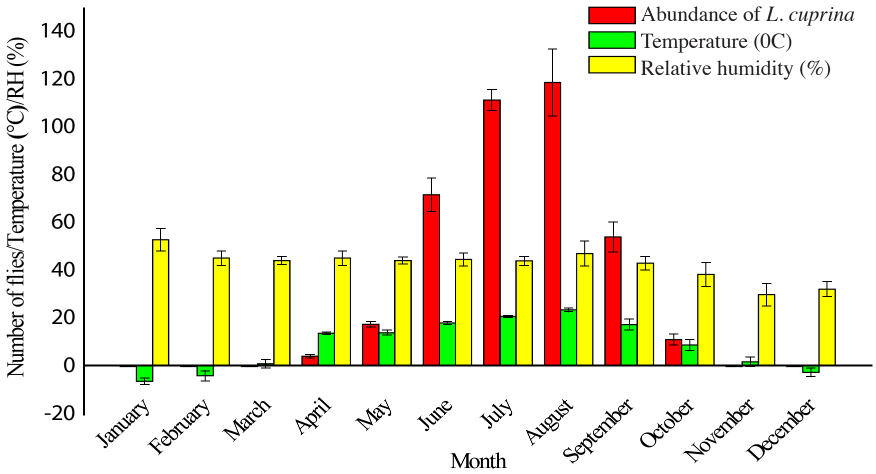

Figure 2. Seasonal abundance of *L. cuprina* in Kargil Ladakh from April 2018 to March 2021.

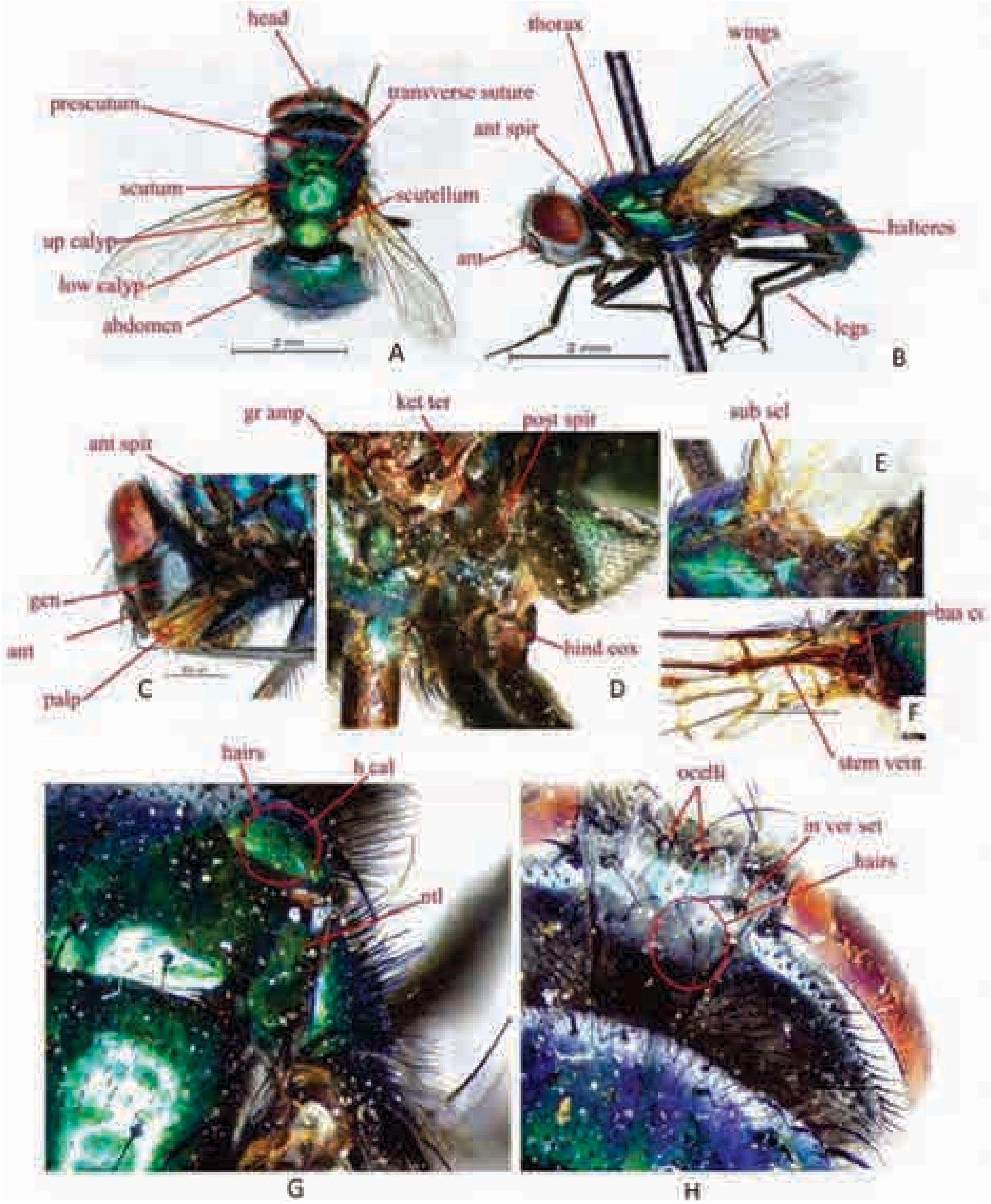

Image 1. *Lucilla cuprina*: A—Body, dorsal view | B—Body, lateral view | C—Head lateral view | D—Thorax, postero-lateral view | E—Wing base, ventral view | F—Wing, dorsal view | G—Thorax, dorsal view | H—Head, dorsal view.

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SHORT COMMUNICATION

On the occurrence of *Nitella myriotricha* A.Braun ex Kützing, 1857 ssp. *acuminata* D.Subramanian, 1999 (Charophyceae: Charales: Characeae), from eastern India

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Abstract: *Nitella myriotricha* A.Braun ex Kützing ssp. *acuminata* D.Subramanian 1999 has been recorded from different localities of West Bengal, eastern India. It is the first report of the taxon outside its original locality of occurrence at Kummidipoondi (Tamil Nadu) by Subramanian in 1999. The possibilities of endemism and origin of variations in this species have been discussed.

Keywords: Charophyceae, eastern India, endemism, new record, *Nitella myriotricha* spp. *acuminata*.

Nitella Agardh (1824) is a cosmopolitan genus of Charophyta that grows in undisturbed aquatic ditches, lakes, and bogs. It is easily identified due to the position of its reproductive organs even in macroscopic form. In comparison to its sister genus *Chara* L. it prefers to grow in low nutrient, soft and slightly acidic waters (Bryant & Stewart 2011; Graham et al. 2016). In contrast to *Chara* it is either slightly lime-encrusted or un-encrusted genus (John & Rindi 2015). It is less rigid, excorticated and lack spines. If we consider the position of reproductive organs it is just reverse to that of *Chara.* In both the genera sex organs are lateral in position, in *Chara,* nucule is above & globule is below while position is just reverse in *Nitella.*

Nitella Agardh (1824) is recognized by 237 species, four subspecies & 27 forms World over (Guiry & Guiry 2022). In India, it is represented by 74 taxa belonging to 38 species & infraspecific taxa (Sundaralingam 1957; Subramanian 2002; Gupta 2012). *Nitella myriotricha* A.Braun ex Kützing is a species unique for the presence of mucilage cloud around the thallus (Wood 1962; Wood & Imahori 1965). Currently, three subspecies are recognized under this species, viz., *Nitella myriotricha* ssp. *acuminata* D. Subramanian, 1999, *N. myriotricha* ssp. *incurvata* D. Subramanian 1999, and *N. myriotricha* ssp. *huillensis* A.Braun & Welwitsch. Of these *N. myriotricha* ssp. *huillensis* has been elevated to the species level, *N. huillensis* (A.Braun & Welwitzch) T.F.Allen, 1968 (WoRMS 2022).

Certain other taxa of *Nitella*, viz., *N. batrachosperma* (Reichenbach) A.Braun, *N. hyalina* (DeCandolle) C.Agardh, *N. pseudoflabellata* A.Braun f. *multipartita* (T.F.A.) R.D.W, *N. pseudoflabellata* A.Braun var. *mucosa* (Nordst.) Bailey, *N. pseudoflabellata* A. Braun f. *imperialis* T.F.A., and *N. wattii* J.Groves also hold mucilaginous cloud (Sundaralingam 1957; Wood & Imahori 1965; Krause

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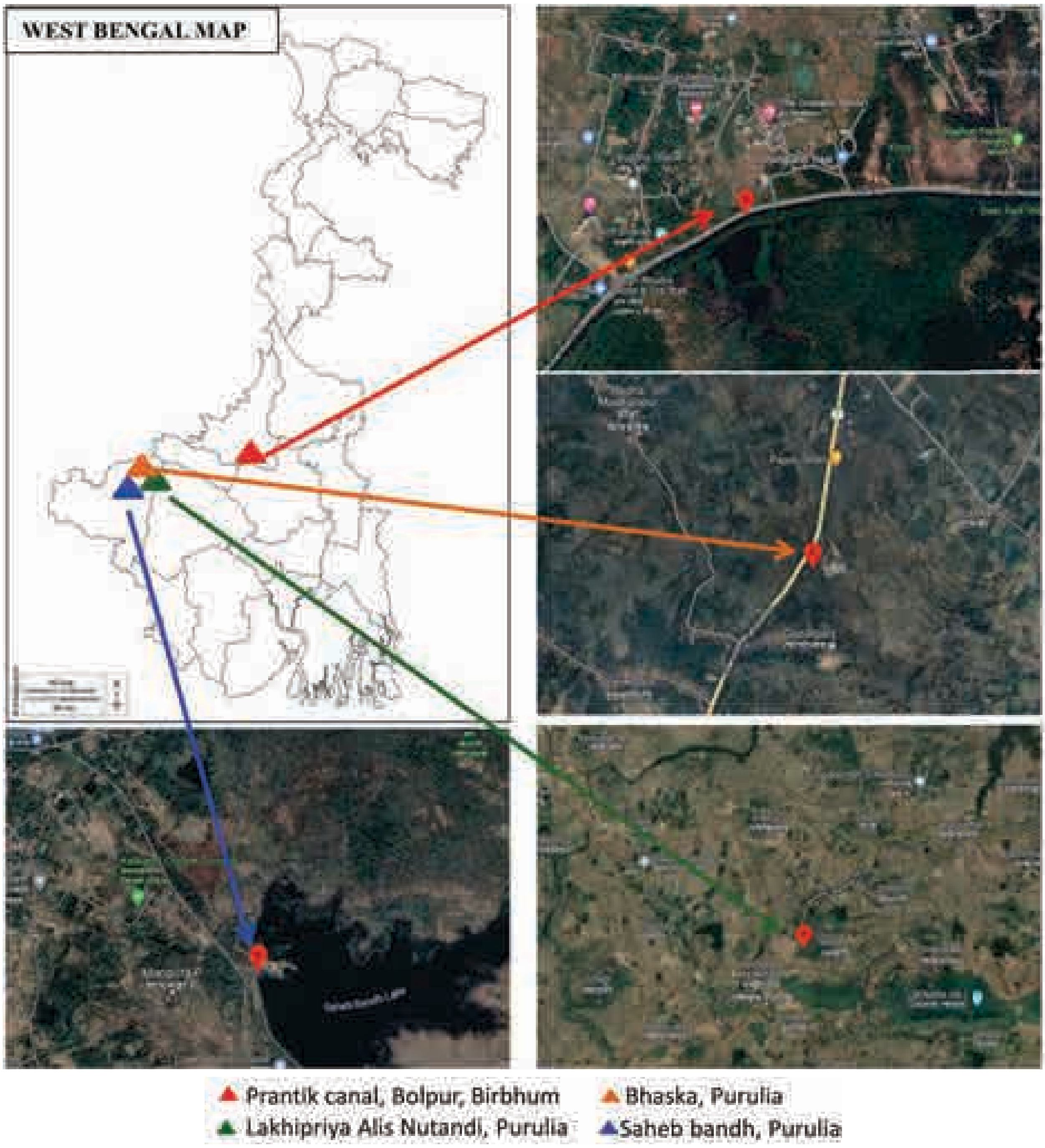

Image 1. The different collection spots in West Bengal. Source: Google.com.

1997; Subramanian 2002) but our specimen fits well with *Nitella myriotricha* ssp. *acuminata* D.Subramanian.

During the systematic study on the charophytes of eastern India the authors recorded *N. myriotricha* ssp. *accuminata* D.Subramanian from several localities of West Bengal (Image 1).

MATERIAL AND METHODS

The samples were collected from Prantik canal, Bolpur, Birbhum District, West Bengal, India. Similar populations of the taxon were also recorded from Bhaska, Lakhipriya Alis Nutandi & Saheb Bandh, Purulia District, West Bengal. Ten samples from these four localities have been studied. Samples were washed vigorously by gentle cleans in running water and preserved in 4%

formalin. Conductivity, pH, water temperature, salinity, TDS, and habitat were also recorded at the time of sample collection. Multi-parameter PCSTestr™ 35 device was used to measure the ecological data. Dissolved sodium, potassium, and calcium of the water sample were measured with the help of flame photometer128 and dissolved oxygen was measured with titration method. A small portion of the sample was taken on a clean petridish and set under the Zeiss Stemi 508 stereozoom research microscope having digital camera attachment with standard scale. Microphotography was done and measurements were taken with the Zeiss Zen 2.6 software. Detailed taxonomic study was done by microscopic observations for identification of species. The identification of species was done with the help of standard monograph (Subramanian 2002).

RESULTS

Nitella myriotrichia* A.Braun ex Kützing ssp. *acuminata
[Subramanian D., 2002. Plate 14 Figs.- 1–4]
(Image 1 A–F)

Plant body 8–12 cm in height; monoecious; bright green in colour; whole plant body entirely covered with dense mucilaginous cloud; axes 400–630 µm in diameter, internodes longer than branchlet, 6 branchlets, unequal in length; 7 dactyles, acute in shape; nucule and globule are present at the second and third branchlet nodes: no sex organs at shorter branchlets; antheridium large, one per node, orange in colour, 320–470 µm in diameter; oogonium large, 332 µm long, 240 µm broad, yellowish green in colour, convolutions 8–9; corona cells in two tiers 40–43 µm in broad & 42–45 µm long.

Our specimen appears to be narrower, may be due to different climatic regime.

A comparative account of the specimen with the holotype is given (Table 1):

Distribution in India: Tamil Nadu (Subramanian 1999, 2002).

Ecological data (Table 2): GPS Location -23°41'24.5"N, 87°40'02.1"E;23°29'00.0"N,86°42'14.9"E;23°36'46.9"N, 86°48'14.3"E; 23°34'36.1"N, 86°41'56.8"E.

DISCUSSION

As far as the distribution of *Nitella myriotricha* is concerned, it is known from very few localities of the world, viz.: *N. myriotricha* A.Braun ex Kützing (Australia- Casanova 2009); Queensland (Phillips 2002; Bostock & Holland 2010); *N. myriotricha* ssp. *acuminata* D.Subramanian (Tamil Nadu, India, Subramanian 1999, 2002); *N. myriotricha* ssp *incurvata* D.Subramanian (Tamil Nadu, India, Subramanian 1999, 2002). Since *N. myriotricha* ssp. *huillensis* A.Braun & Welwitzch (1868) has been considered as a separate species, i.e., *N. huillensis* (A. Braun & Welwitzch) T.F. Allen, 1888; only three taxa of this species is now recognized. As per IUCN Red List (Mani 2013) *N. myriotricha* ssp. *acuminata* is considered synonymous to the main species. From the above records it appears that the species has a very restricted distribution. According to IUCN Red List (Mani 2013) it is treated a species under 'Least Concern' (LC) category. We have studied well developed plants that tallies with the description of Subramanian (1999, 2002). Our plants have been found inhabiting the ponds and ditches of dry lateritic soils of Purulia and Birbhum districts of West Bengal where ecological conditions are unique having alkaline pH. It is our observation that such specific ecological niche is the preferred situation of these plants. But the plants are not infrequent in these regions. Our taxon is known from India only, wherever the original taxon is known from Australia & New

Table 1. Comparative account of the specimen with the holotype.

Name of the species	Height	Axis	Branchlets	Dactyles	Antheridium	Oogonium	Corona cells
Holotype *Nitella myriotricha* ssp. *acuminata* D. Subramanian	Plant body 9–10 cm high	1,100 µm	Not mentioned	Not mentioned	450–500 µm in diameter	300 µm long, 280 µm broad	Not mentioned
Our specimen	Plant body 8–12 cm high	400–630 µm	6 in number	7 in number	320–470 µm in diameter	332 µm long, 240 µm broad	Two tiers, 42.743 µm in diameter, 44.960 µm in length

Table 2. Ecological data

Locality	pH	TDS (ppm)	Salinity (ppm)	W.T. (ºC)	Cond. (µs)	Na (ppm)	Ca (ppm)	K (ppm)	D.O. mg/L (ppm)
Prantik Canal	7.71	144	87.4	20.4	204	15.43	51.96	1.30	8

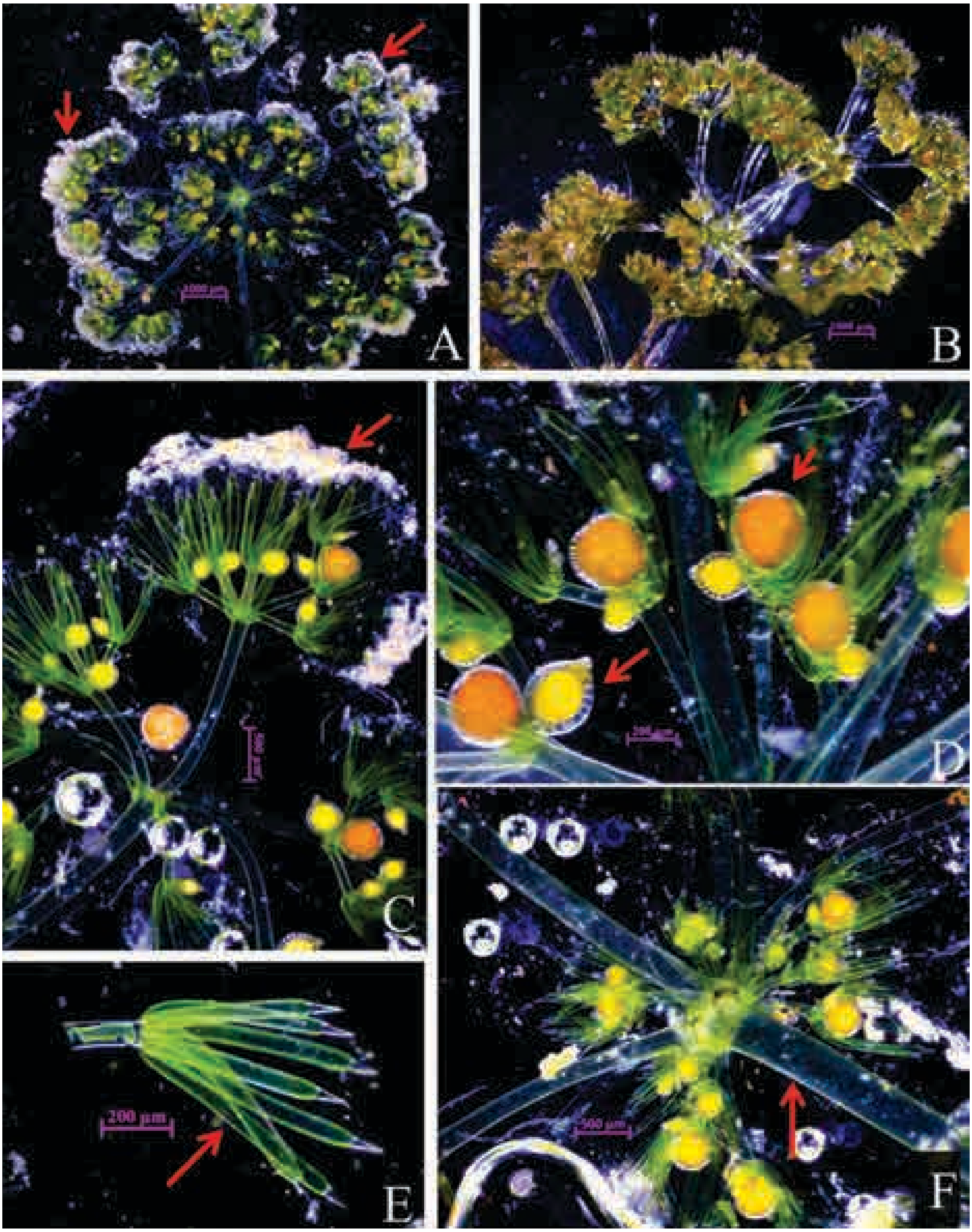

Image 1. ***Nitella myriotricha*** **A.Braun ex Kützing ssp.** ***acuminata*****: A,C—arrows showing mucilaginous cloud | B—showing whole plant body | D—Arrow showing antheridia and oogonia | E—arrow showing dactyl with the pointed apex | F—arrow showing the smooth transparent main axis and node. © Jai Prakash Keshri.**

Zealand. It reveals two possibilities: 1. Endemism may be a key factor for its occurrence, 2. Continental drift may be another factor when actually the Indian and Australian subcontinents were united (Wegner 1924, 1929; Demhardt 2005) and separation of these subcontinents may have provided opportunity to the Indian species to evolve into two new subspecies. The possibility of endemism is guessed due to the fact that the occurrence of the species has been noted in India and Australia only. If we apply the Vavilov's (1926) principle here the origin of this species may have occurred in Indian subcontinent because variations into subspecies have been noted in India only.

CONCLUSION

In India, species of Characeae are poorly described and more extensive floristic studies need be undertaken, particularly in remote areas of the country. Only few taxa have been reported from southern India. But in eastern India particularly in West Bengal floristic study is very poor. In this study *Nitella myriotricha* ssp. *acuminata* have been collected from Prantik, Bolpur, Birbhum District, West Bengal. This is the first report of the taxa outside the original site. A mucilaginous cloud was present over here. This is the unique identifying character of this species.

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NOTE

Dark Clouds Ahead? Anecdotal evidence for an illegal live trade in Sunda *Neofelis diardi* and Indochinese *N. nebulosa* Clouded Leopards (Mammalia: Carnivora: Felidae)

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The Sunda Clouded Leopard *Neofelis diardi* and Indochinese Clouded Leopard *N. nebulosa* are medium-sized felids native to tropical southern and southeastern Asia. The only two extant members of their genus, both species exhibit morphological adaptations consistent with arboreality (Van Valkenburgh 1987; Hubbard et al. 2009) and share a common ancestry with other extant pantherine felids (Johnson et al. 2006). Collectively, clouded leopards occur from Nepal east to Vietnam and south through the Indo-Malay Archipelago (*N. nebulosa*) into Sumatra and Borneo (*N. diardi*) (Hearn et al. 2015; Grassman et al. 2016). Both species were historically treated as a single phenotypically variable species until recently, when closer molecular and morphological scrutiny yielded evidence of two distinct species (Buckley-Beason et al. 2006; Kitchener et al. 2006).

Clouded leopards primarily inhabit tropical forests across their range, particularly lowland dipterocarp forests. Limestone forests, upland tropical evergreen and deciduous forest, lowland seasonal and mixed deciduous forest, and peat swamp forests (Rabinowitz et al. 1987; Rabinowitz 1988; Rabinowitz & Walker 1991) are also considered suitable habitats. They appear able to persist in fragmented, isolated forests, and forests where

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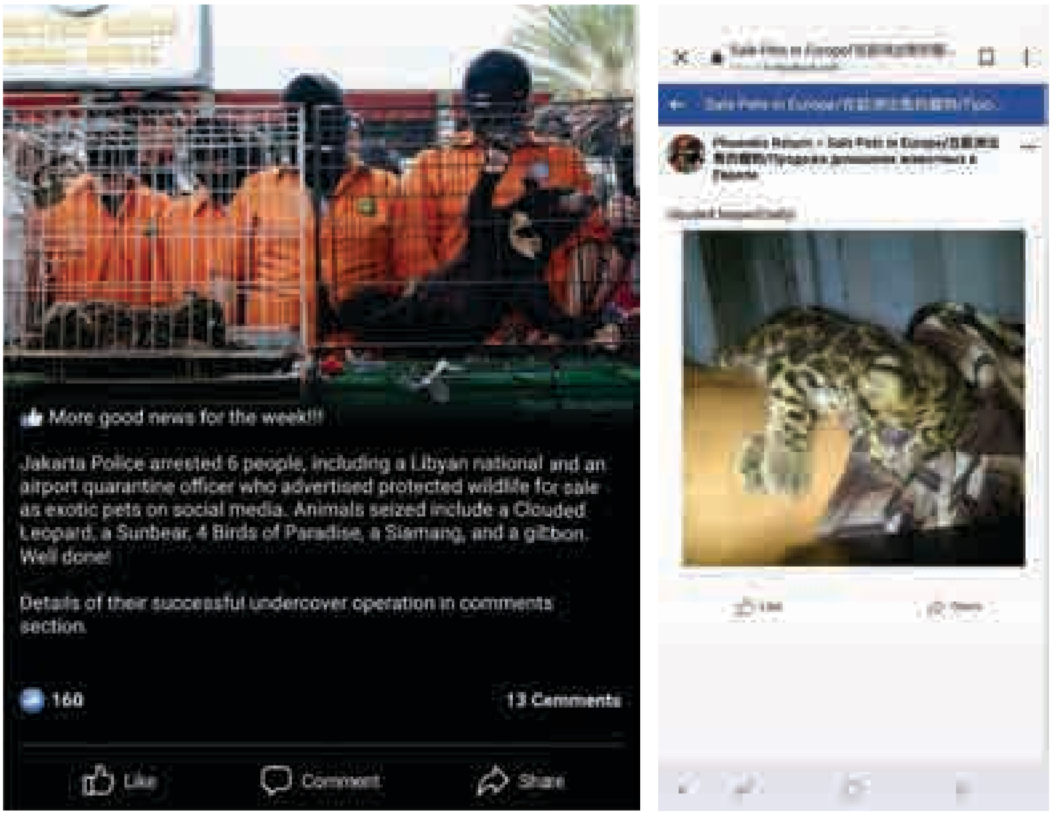
selective logging occurs (Mohd-Azlan & Lading 2006; Brodie & Giordano 2012; Brodie et al. 2015). Though their prey has never been investigated in detail, it is likely that clouded leopards prey largely upon ungulates, and primates both on the ground and in the canopy; data gathered anecdotally thus far at least supports this (Matsuda et al. 2008; Morino 2010; Giordano & Rustam 2016; Giordano 2022). Threats to the survival of both species include deforestation, particularly for oil palm, indiscriminate poaching via snaring, and intentional hunting for illegal trade in skins and parts (Hearn et al. 2015; Grassman et al. 2016; Giordano 2022). Both are CITES Appendix I species, with international trade in live animals or their parts prohibited without explicit official permission by the governments of range countries. They are protected legally from hunting by national wildlife legislation across their range.

In 2019, we began an investigation of the trade in clouded leopards and their parts in Asia. This was partly inspired by social media posts we discovered of a live Sunda Clouded Leopard apparently kept by an exotic pet dealer (Image 1). Our online investigations continued with respect to how and where clouded leopards are sold on social media and other digital platforms, irrespective of laws and policies prohibiting such activities. Although these investigations are still ongoing, we discovered more than one-half dozen social media posts and local language news articles, that we believe are indicative of an illegal live trade in Sunda Clouded Leopards in Indonesia (Image 2a,b). In addition, also discovered at least one older incident that involved young Indochinese Clouded Leopards (Image 3) originating from the Lao PDR, which were confiscated along the national borders of Thailand and Myanmar. Several of these incidents were supported or accompanied by photos of a clouded leopard in a small cage, consistent with those often used at markets, or at a post-confiscation publicity event coordinated by law enforcement for the media. Our work expands somewhat on an aspect of D'Cruze & Macdonald (2015), which highlighted records of live trade in clouded leopards as exotic pets, and possibly for exotic food dishes (e.g., IUCN SSC Cat Specialist Group 1987). Their data also highlighted the changing trends in CITES records of the 'purpose' of live-traded clouded leopards over several decades. They further characterized a major shift toward 'commercial use', which constituted more than half of all trade in the decade and a half leading up to 2013. Our work therefore expands somewhat on D'Cruze & Macdonald (2015) and provides new evidence of the species allegedly for illegal sale as an exotic pet on social media.

New evidence of live trade in other carnivores from Indonesia, and possibly Malaysia, has also emerged recently, and can provide further context for a possible emerging live trade in Sunda Clouded Leopards. Some species for which fewer historical records of trade exists, for example, such as 'Vulnerable' Small-clawed Otters *Aonyx cinereus* and Smooth-coated Otters *Lutrogale perspicillata* (de Silva et al. 2015; Wright et al. 2015), are among those species recently identified as threatened by increased live trade (Gomez & Bouhuys 2018). This appears in large part to be driven by their popularity and portrayal as pets on social media (Harrington et al. 2019). Similarly, the Otter Civet *Cynogale bennettii*,

Image 1. Screen grab of a 'pet' Sunda Clouded Leopard *Neofelis diardi* on Facebook in Indonesia.



More good news for the week!!!

Jakarta Police arrested 6 people, including a Libyan national and an airport quarantine officer who advertised protected wildlife for sale as exotic pets on social media. Animals seized include a Clouded Leopard, a Sunbear, 4 Birds of Paradise, a Siamang, and a gibbon. Well done!

Details of their successful undercover operation in comments section.

Image 2. Screen grabs of Facebook postings: a—wildlife seized by Jakarta police, including a Sunda Clouded Leopard and a Sun Bear. © Sindophoto | b—a posting purportedly showing a Sunda Clouded Leopard kitten for sale in Europe.

Image 3. Two Clouded Leopards confiscated by Thai authorities en route from Laos. Here they are being temporarily cared for by the local zoo. © Terry Whittaker.

a small 'Endangered' viverrid (Ross et al. 2015) also native to southeastern Asia, was recently observed for sale on an Indonesian Facebook group (Bouhuys 2019), the first known report for the species. Live trade in all carnivores across the region, including mongoose, ferret badgers, and civets (Shepherd 2012; Nijman et al. 2014) has largely been unregulated to date, and many species appear to be increasing in popularity as 'novelty pets' (Bouhuys 2019). Changes in the demand for different species are known to occur through time, as evidenced by Indonesia's primate markets (Nijman et al. 2015). Such patterns, particularly as influenced by social media, may have implications for future live trade in Sunda Clouded Leopards. Interestingly, other than the one record we present of two live Indochinese Clouded Leopards, we uncovered few unequivocal records of live trade in the species, whether as pets or otherwise. Given however there exists evidence of larger felids like leopards and tigers kept locally as pets in the region, including in Myanmar (Min 2020), it is possible we just haven't yet uncovered adequate evidence supporting more widespread live trade in Indochinese Clouded Leopards. In contrast to the live pet trade in cheetahs in the Middle East (Tricorache et al. 2018; Spee et al. 2019), the cultural origins of which may relate to their historical use for hunting other prey (Masseti 2009), some live trade in Sunda Clouded Leopards could be related to local opportunistic hunting, which appears widespread on Borneo (Mohd-Azlan et al. 2017). The incidental killings of mother Clouded Leopards for example, could pave the way to more opportunities for selling kittens or cubs at regional markets. Regardless of the pathway to the marketplace, there is real concern that social media activity risks normalizing the keeping of threatened and endangered species as exotic pets (Moloney et al. 2020), and thus the expansion of potential markets.

We recommend that additional international attention and investment of resources, including local, national, and international law enforcement activities, focus their efforts on the growing trade in clouded leopards. We further note that the potential threats to human safety in the trafficking of live animals in the form of disease, particularly given the potential of cats and other carnivores to facilitate transmission of coronaviruses (Bell et al. 2004; Matharvarajah & Dellaire 2020), should further serve as incentive to end all illegal trade in these species.

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NOTE

Further photographic record of Asiatic Brush-tailed Porcupine *Atherurus macrourus* Linnaeus, 1758 (Mammalia: Rodentia: Hystricidae) from Manas National Park, Assam, India

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Three species of porcupines occur in southern Asia: Indian Crested Porcupine *Hystrix indica*, Himalayan Crestless Porcupine *Hystrix brachyura*, and Asiatic Brush-tailed Porcupine *Atherurus macrourus* (Molur et al. 2005). *Hystrix indica* is found in most of the forested regions in India (Agrawal 2000) but is restricted to only a few locations in northeastern India (Talukdar et al. 2019). *Hystrix brachyura* is found in the forested areas of northeastern India (Agrawal 2000) and northern Bengal (Mallick 2012) but is absent from the other parts of India. *Atherurus macrourus* is distributed across southern and southeastern Asia (Molur 2020). In India, the species was reported from Namdapha National Park (Agrawal 2000; Datta et al. 2008a), Pakke Wildlife Sanctuary in Arunachal Pradesh (Datta et al. 2008b), Intanki NP in Nagaland (Kumar & Kaul 2013), Ngengpui WS in Mizoram (Forest clearance 2013), and Khasi Hills of Meghalaya (Agrawal 2000). In Assam, sporadic records of the species were reported in Karbi Anglong, North Chachar Hills, Garampani WS, Nameri NP, and Manas NP (Choudhury 2013). Despite continuous and extensive camera trap surveys and other ecological research on carnivores and their prey base every year (Goswami & Ganesh 2014; Lahkar et al. 2018, 2020a,b), there have been few photographic evidences and published records of the species from Manas NP (Choudhury 2013; Jhala et al. 2020). The current note presents the recent photographic record of *A. macrourus* in MNP.

Camera traps (n = 554) were deployed in a grid size of 1X1 km^2 for studying the Clouded Leopard ecology at MNP from April 2017 to May 2019. MNP lies on the borders of the Indo-Gangetic and Indo-Malayan biogeographic realms and is located at the foothills of the Bhutan Himalayas in the Bhabar area of western Assam (26.7460°N, 91.0203°E) (Wikramanayake et al. 2001). Contiguous with Royal Manas National Park (RMNP) in Bhutan, the park forms one of the large areas of conservation significance in southern Asia. The

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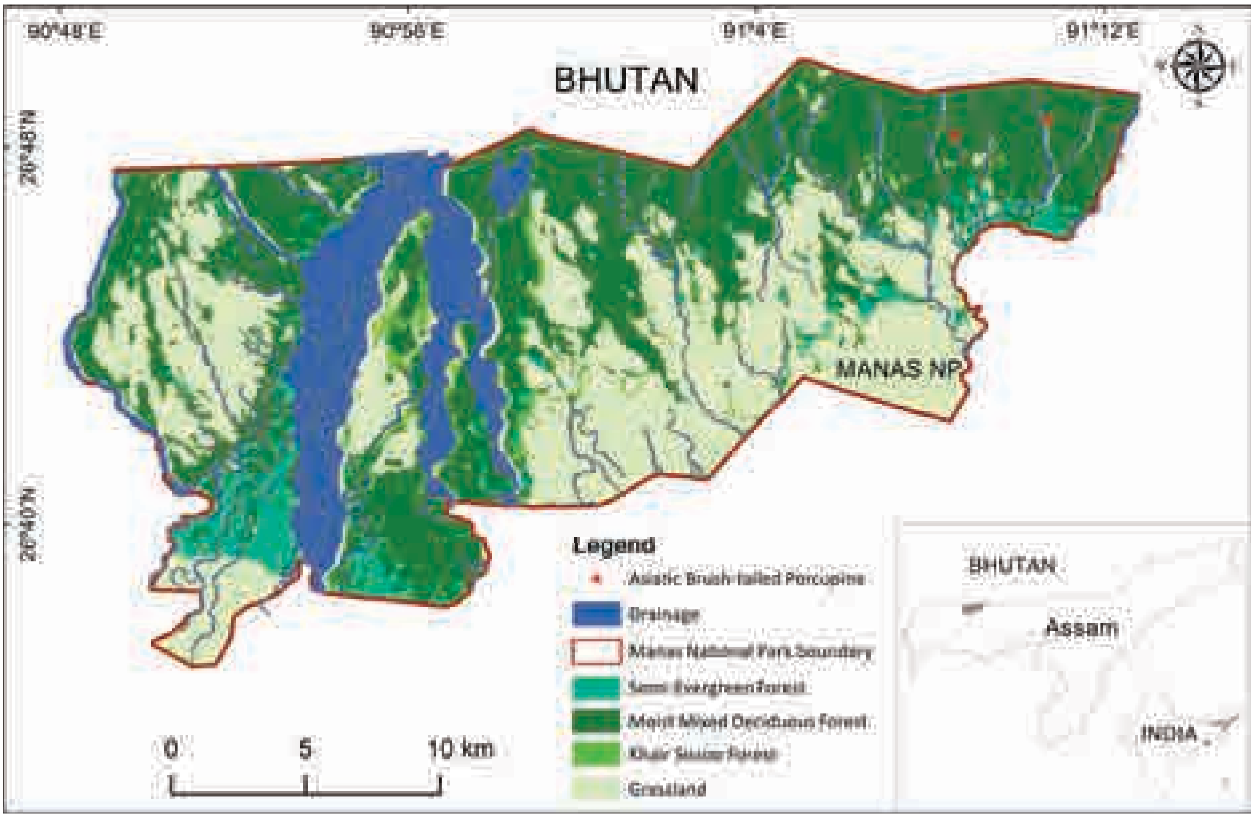

Figure 1. Locations where photos of Asiatic Brush-tailed Porcupine in Manas National Park, Assam, India, were captured.

vegetation of MNP can be broadly classified into eastern wet alluvial grasslands, moist deciduous, and semi-evergreen forests (Champion & Seth 1968).

At each location, a single Cuddeback X-Change colour camera (Cuddeback, Green Bay, WI, USA) was affixed to a tree at approximately 30–45 cm above the ground. Data from more than 500 camera-trap stations were available for analysis, and the Asiatic Brush-tailed Porcupine was recorded from two stations (91.1998°N, 26.8018°E and 91.1623°N, 26.7962°E) with three independent captures (Figure 1 & Image 1). All captures occurred at night (2145 h, 0331 h, and 2031 h), indicating nocturnal behaviour (Image 1). The species was photo-captured in the Bhuyapara range, the eastern part of the NP with a moist mixed deciduous forest type dominated by *Albizia procera*, *Terminalia bellirica,* and *Bombax ceiba* (Figure 1). However, the species also inhabit other forests, such as tropical wet evergreen, tropical semi-evergreen, and sub-tropical broadleaf hill (Talukdar et al. 2019). As reported by the field director, Mr. H.K. Sarma, Manas Tiger Reserve, the previous photographic evidence was recorded in the park by the front-line staff with the help of Aaranyak, an NGO, in 2018 (Jhala et al. 2020).

Our recent photographic evidence complements the previous occurrence and sporadic records of Asiatic Brush-tailed Porcupine in Manas National Park. Since the presence of the Asiatic Brush-tailed Porcupine in Manas has not yet been published, Manas is not indicated in the IUCN Red List distribution map for this species (Molur 2020). Though classified as 'Least Concern' on the IUCN Red List of Threatened Species, the species is showing a decline in population across its range primarily due to habitat loss, jhum (shifting) agriculture, small-scale logging, subsistence harvesting for food, and accidental mortality (Molur et al. 2005). The Asiatic Brush-tailed Porcupine, one of the least studied species in India, opens up the scope for further studies and highlights the need for conservation efforts to protect them from extinction.

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NOTE

Predation of the Nicobar Shrew *Crocidura nicobarica* by a Cattle Egret *Bubulcus ibis*

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Crocidura is the most speciose currently recognized genus of mammals, with about 200 species (Burgin et al. 2018). India is known for the presence of 12 *Crocidura* species, distributed in the mainland and Andaman & Nicobar Islands (Kamalakannan et al. 2021). Currently, the Andaman & Nicobar (AN) Islands are known to house only a single genus *Crocidura* shrew, with five endemic species, namely, the Andaman Shrew *C. andamanensis*, the Andaman Spiny Shrew *C. hispida*, Jenkin's Shrew *C. jenkinsi*, the Nicobar Shrew *C. nicobarica*, and the most recent discovery, the Narcondam Shrew *C. narcondamica* by Kamalakannan et al. (2021), of which, *C. nicobarica* is only known from the Great Nicobar Island (Molur et al. 2005; Menon 2014; Kamalakannan et al. 2021). Due to the restricted insular habitat and the associated limited population size, the *C. nicobarica* has been categorised as 'Critically Endangered' by the IUCN Red List of Threatened Species (Molur 2016).

The Great Nicobar Island is the largest of the 11 islands of the Nicobar group of Islands, situated at the southernmost portion of the Andaman & Nicobar Islands, India (7.0346°N & 93.7842°E). The Great Nicobar Island is home to several endangered and endemic vertebrate fauna including the endangered Giant Leatherback Sea Turtle *Dermochelys coriacea*, the endemic Nicobar Megapode *Megapodius nicobariensis*, and the endemic Nicobar Treeshrew *Tupaia nicobarica* (Saha 1980; Miller 1902; Pande et al. 1991; Kamalakannan et al. 2020). The mammalian fauna of the Great Nicobar Island comprises mostly bats and few non-volant mammals; the only known wild larger mammals are the Wild Boar *Sus scrofa* and the Nicobar Long-tailed Macaque *Macaca fascicularis umbrosus* (Bates & Harrison 1997; Molur et al. 2002, 2005; Aul et al. 2014). The region is composed of deciduous, moist, & evergreen forest, grasslands, and perennial rivers & streams (Rodgers & Panwar 1988).

Insects and small vertebrates are the primary diets of Cattle Egret *Bubulcus ibis* (Vega-Sánchez et al. 2022; Talbi et al. 2023), but predation of *Crocidura* shrew is rarely observed. Here, we recorded a Cattle Egret preying on a shrew at the littoral forests near Govind Nagar, Great Nicobar Islands (6.9997°N & 93.9086°E; Figure 1); it was an opportunistic observation, while watching the wetland birds. It was also observed that the Cattle Egret flipped the shrew back up on the ground and kept on biting on the head and neck regions with its bills and subsequently gobbled it down in one motion. The bird was identified based on its white plumage, short, thick neck, a sturdy yellow bill, and a hunched

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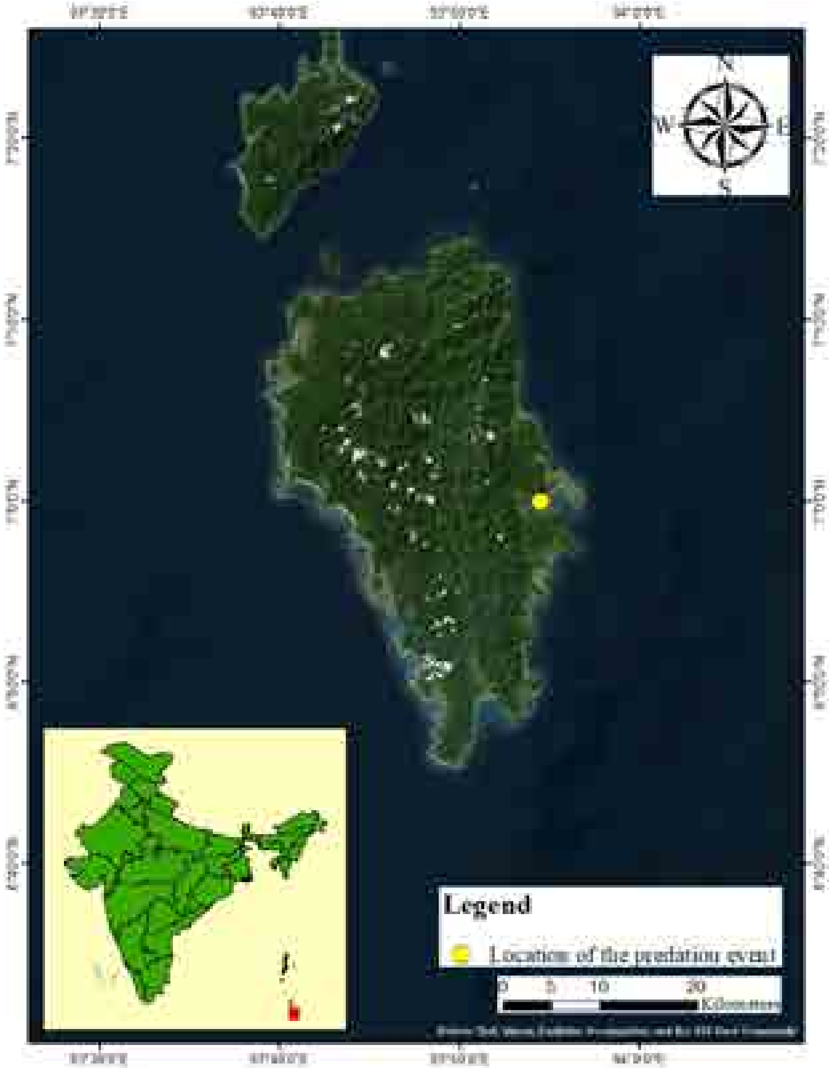

Figure 1. Map showing the location of predation of the Nicobar Shrew by a Cattle Egret in the Great Nicobar Islands, India.

posture; the mammal was identified as a shrew based on its long snout, grey dense dorsal pelage and medium-sized tail, and further as *C. nicobarica,* because it is the only shrew species known from the Great Nicobar Island (Image 1). Some individuals of the *C. nicobarica* have previously been observed in field surveys conducted at this location. The video of the predation event can be accessed at the following link: https://osf.io/m7dxr/). *C. nicobarica* is a comparatively large-sized shrew (head and body length: 10.7–12 cm; tail length: 9–9.5 cm), characterised by a bristly sooty brown dorsal fur with a slender tail (Menon 2014). They inhabit the leaf litter tropical moist deciduous forests.

The equilibrium between foraging gain and predation risk is the fundamental trade-off in animal life (Mazza et al. 2019). Predation instances on shrews are rarely observed due to its secretive behaviour, releasing pungent and musky odour when it is threatened (Nagorsen 1996; Jung 2016). Jung (2016) has reported a similar predation event on a Western Water Shrew *Sorex navigator* by a Belted Kingfisher *Megaceryle alcyon* from Yukon, Canada. Further studies on these species' natural history of both *C. nicobarica* and *B. ibis* in the Great Nicobar Island will yield more information about their potential interactions and their ecological relations and functions.

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Image 1. Predation of the Nicobar Shrew by a Cattle Egret from the Great Nicobar Islands, India.

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NOTE

War prompts distress symptoms in Israeli Blind Snake

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Case study

The effects of armed conflict on wildlife are an often sidelined consequence of war. The rare studies on this subject mainly deal with population declines caused by bombs & chemicals, habitat alteration (both collateral & strategic), increase in exploitation by displaced people, and decrease in enforcement (reviewed in Gaynor et al. 2016). Behavioral reactions to sudden noises of military origin were studied in endotherms (Weisenberger et al. 1996; Krausman et al. 1998; Maier et al. 1998, Conomy et al. 1998; Goudie & Jones 2004), but all focused on aircraft engines, not blasts, only two studies included physiological indices of stress, such as heart rate (Weisenberger et al. 1996) and more recently, in a lizard, blood corticosterones (Kepas et al. 2023). Study of reptiles' reaction to anthropogenic noise in general is minimal (Shannon et al. 2016).

On 11 May 2021, following recent tensions and violent events in Jerusalem, hundreds of rockets were fired into Israel, including Tel Aviv, where our laboratory is located. Most rockets were intercepted, some hit their targets, in both cases producing thunderous explosions. Six hours later, another bombardment was fired. Throughout that time, we were conducting unrelated measurements of the metabolic rate of a Syrian Blind Snake *Xerotyphlops syriacus*, which resulted in a coincidental documentation of its physiological response to the rocket barrages.

Xerotyphlops syriacus (Jan, 1864) is a nocturnal and fossorial blind snake of the family Typhlopidae inhabiting Lebanon, Syria, Jordan, Israel, the Palestinian Territories, and Egypt (Bar et al. 2021). The individual we investigated was caught in Tel Aviv University (under permit #2021/42720 from the Nature and Parks Authority). It was kept in a terrarium with moist earth but no food to ensure a post-absorptive state during the study. After a week it was placed in a 50 ml metabolic chamber connected to a LICOR LI-7000 CO2/H2O analyzer (LICOR, Lincoln, NE, USA) which was kept dark and at a stable 20°C. Dry air flowed through the chamber at 50 ml/min, whereupon exiting CO_2 was measured. An empty identical chamber was used to calibrate baseline gas levels, to which measurement automatically switched for 15 minutes at pre-programmed intervals, without affecting the airflow or any other aspect of the chamber housing the snake. An hour after the first measurement, a second was made using the same protocol except for temperature, which

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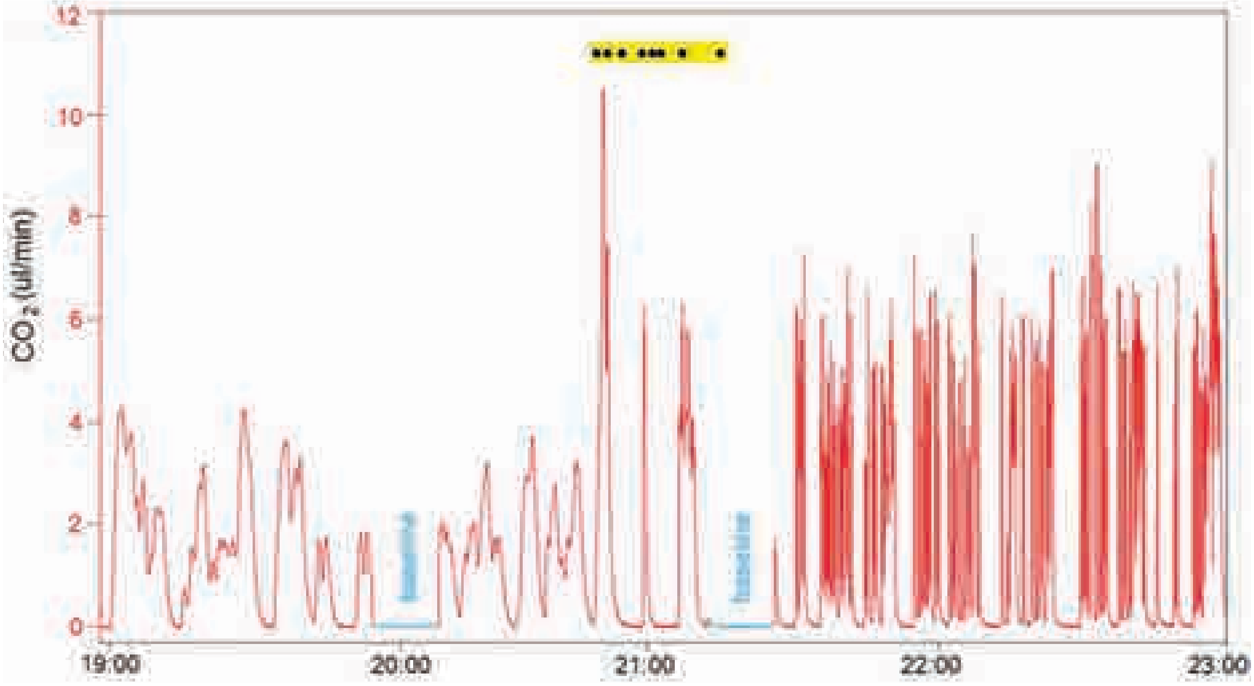

Figure 1. Carbon dioxide production of *Xerotyphlops syriacus* before, during, and after the first barrage (in yellow). Inset is a detail of the barrage itself, each black dot indicates a round of several rockets that hit or were intercepted in North Tel Aviv. Blue lines denote the pre-set baseline intervals, which come from a different channel. Chamber temperature is 20°C.

was cooled down to 12°C. The snake was rehydrated when the experiment ended, and released the following day back where it was found. We analyzed the data with Expedata 1.9.20, using the baseline to correct drift and shift. Measurements were conducted under ethics permit #18616 from the TAU Ethics Committee. Sunset was at 1929 h, and the room and surrounding floor were empty of people by the beginning of this recording. Everything was pre-programmed to run automatically so we could see real-time results from afar.

At 2047 h, concurrently with the explosion of the first round of rockets, we witnessed a sharp change in the gas exchange pattern (Figure 1), from the usual smooth pattern to a series of rapid peaks. The amplitude of the peak at the first explosion was 2.8 times the maximum pre-explosion peak. Eleven sharp CO_2 peaks in the span of 200 seconds (5.88-fold the prior frequency) instantly followed the penultimate rocket round, which hit nearest to the laboratory. In the last round we measured only the first seconds, because of the automatic switch to the baseline channel, but the start of a rise in CO_2 is detectable. Between the end of the rocket barrage (2116 h) and the predetermined end of the measurement (2300 h), O_2 uptake returned to basal levels but respiration rates stayed high. During the second measurement that night, with colder temperature, we witnessed two respiration peaks of starting at 0251 h and 0301 h (Figure 2). Each of these peaks, concurrent with the two new rounds of explosions, had an amplitude three times higher and a total area tenfold above the average for peaks in the hour before.

We interpret the change in gas exchange patterns of *X. syriacus* as a response to the explosions outside. While the physiological symptoms of fear and distress in reptiles are difficult to confirm (Lambert et al. 2019), relaxed breathing is usually taken as an indicator of well-being in reptiles (Warwick et al. 2013) and other animals (Carstens & Mober 2000). Nearly every round of rockets was followed by a sharp peak in gas exchange, too closely matched to be dismissed as a coincidence. The overall metabolic rate was not higher after the explosions ended but breathing changed to high frequency bouts of unusually high, interspaced peaks, which persisted for hours. This response was exhibited by a blind snake with no external ears, inside a sealed, padded chamber, within an incubator located in a closed, windowless second-floor room of a concrete building. Animals in nature are much less shielded from the sound of explosions and the ensuing destruction. Wildlife is understandably not the focus when politics descend into violence, but is doubtlessly also grievously affected.

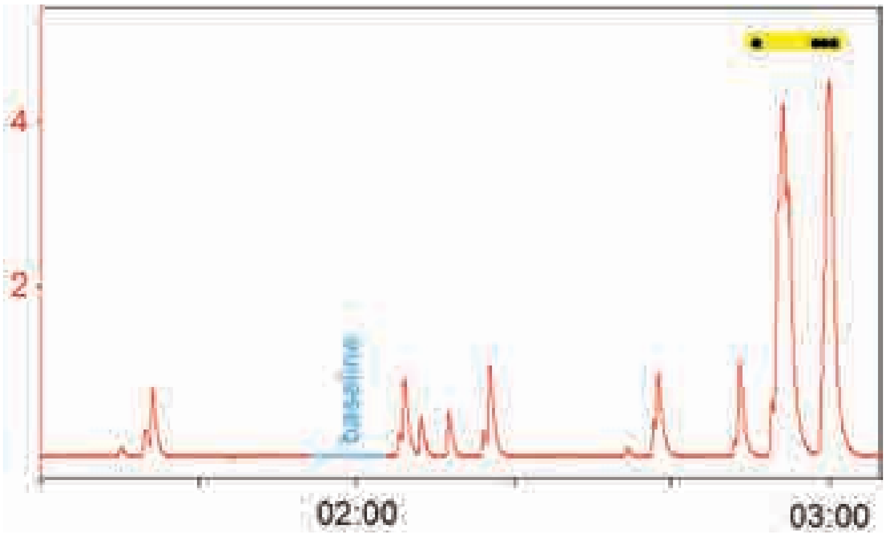

Figure 2. Carbon dioxide production (y-axis units are ul/min CO_2) of *Xerotyphlops syriacus* before and during the second barrage (in yellow). Each black dot indicates a round of several rockets that hit or were intercepted in North Tel Aviv. Blue lines denote the pre-set baseline intervals, which come from a different channel. Chamber temperature is 12°C.

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NOTE

Further distribution records of *Varadia ambolensis* (Stylommatophora: Helicarionoidea) from the state of Goa

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The Western Ghats hotspot of India has 270 species of land snails of which 76% are endemic to this region (Aravind et al. 2005) and 40% are micro-gastropods (Aravind et al. 2008). *Varadia amboliensis* is a helicarionoidean species of land snail endemic to the northern and central Western Ghats. It is the only known large semi-slug species having broad densely tuberculated shell lobes and a depressed, discoid shell with a disproportionately large body whorl and colour ranging from golden-brown to reddish-yellow (Bhosale et al. 2021). We, herein, report new occurrence records for the recently describe *V. amboliensis* from the state of Goa. Identity of the individuals were confirmed based on the characters as described by Bhosale et al. (2021); the shell surface was smooth and glossy, with irregular, faint collabral striae. Surface of mantle was densely covered by small, irregular tubercles that appear lighter on top, mantle with two broad shell lobes and two dorsal lobes, living individuals were glossy grey or greyish-white with irregular dark mottling; head and tail dark grey or blackish with tentacles tending to be paler at their tips. Tail consisting of large slit-like caudal pit; caudal horn prominent when extended.

On 2 September 2021 we observed an individual of *V. amboliensis* moving on a tar road at Netravali Wildlife Sanctuary (15.062^0N & 74.240^0E) during our field visit at 0042 h (Image 2). In another such instance, on 22 September 2021, during our field visit at Collem, Goa (15.333^0N & 74.259^0E), we found eight individuals of *V. amboliensis* on the rocks, dead wood logs and shrubs of *Strobilanthes* sp., alongside a slow flowing stream at 1900 h (Image 3). During our herpetological survey on 20 October 2021, at 2345 h in Sural Village, Goa (15.668^0N & 74.173^0E), we encountered 12 individuals of *V. amboliensis* on an embankment and on the forest floor during our field visit in the area (Image 4).

All of the above records were opportunistic sightings in semi evergreen patches, during our herpetological field visits, which indicates that dedicated surveys could yield more such records. Currently, this species is known from only a few localities and our sightings will add to the distribution data of this species.

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Editor: Basudev Tripathy, Zoological Survey of India, Pune, India. **Date of publication:** 26 June 2023 (online & print)

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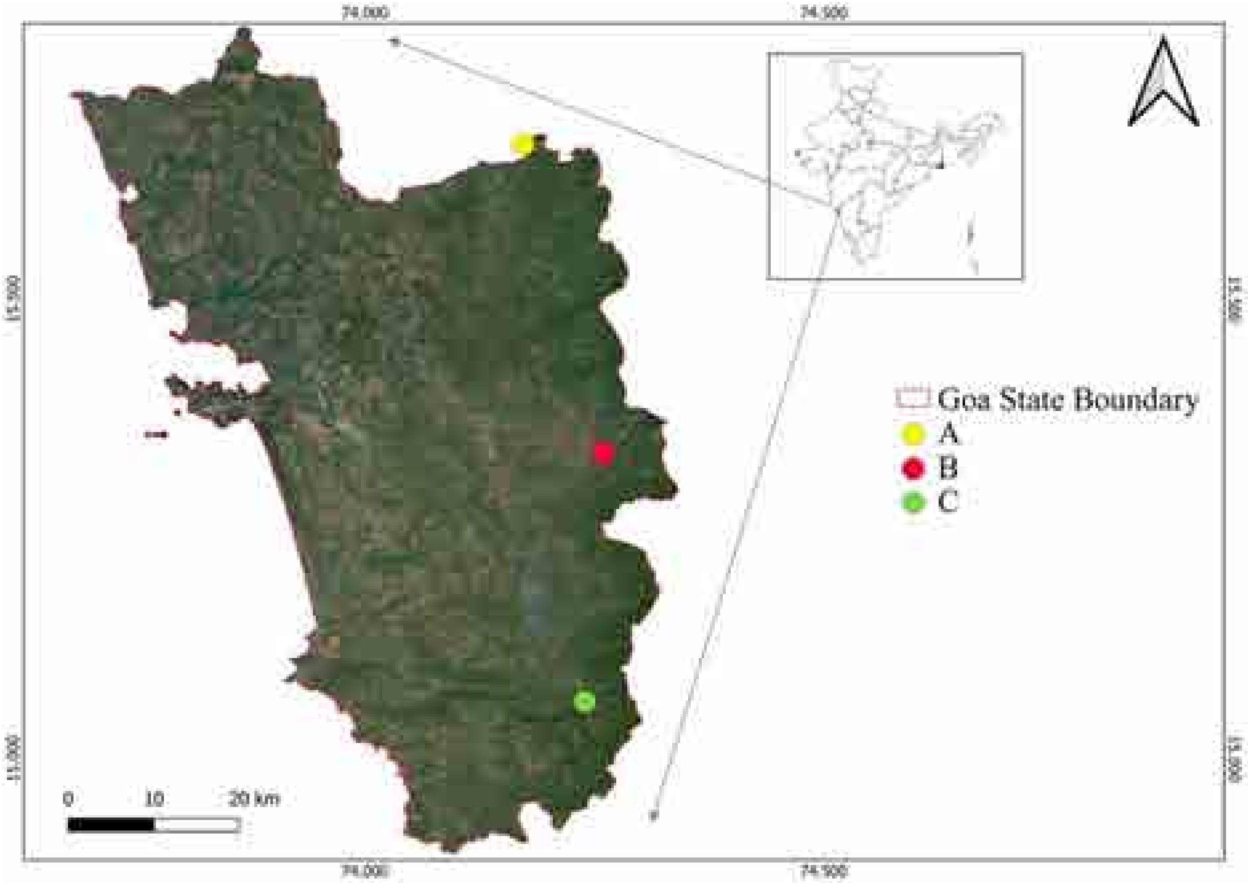

Image 1 . Distribution map showing *Varadia ambolensis* occurrences at: A—Netravali Wildlife Sanctuary | B—Collem village | C—Sural Village in the state of Goa.


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Image 2. *Varadia ambolensis* photographed in Netravali Wildlife Sanctuary on 2 September 2021 at 0042 h.


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Image 3. *Varadia ambolensis* photographed in Collem village, Goa on 22 September 2021 at 1900 h.


© Mayur Gawas

Image 4. *Varadia ambolensis* photographed in Sural village, Goa on 20 October 2021 at 2345 h.

S.K. Kadam, D.V. Muley & D.C. Raheem (2021). *Varadia*, a new helicarionoidean semi-slug genus from India's Western Ghats (Stylommatophora: Helicarionoidea). *European Journal of Taxonomy* 757(1): 50–79. https://doi.org/10.5852/ejt.2021.757.1413

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NOTE

Eleocharis acutangula ssp. *neotropica* D.J.Rosen (Cyperaceae): a new record for southern Western Ghats, India

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The genus *Eleocharis* R.Br. comprises about 300 species distributed in tropical and temperate regions of the world (Govaerts et al. 2021). In India, the genus is represented by 28 species (Prasad et al. 2020) in which 19 are reported from the Western Ghats (Nayar et al. 2014). As part of taxonomic revision of the genus *Eleocharis* in the Western Ghats, the authors collected some interesting specimens from the wetlands of the Kollam district of Kerala. On critical examination and scrutiny of literature, it was identified as *Eleocharis acutangula* (Roxb.) Schult. ssp. *neotropica* D.J.Rosen.

Scirpus acutangulus Roxb. was described by Roxburgh (1820) based on specimens from India. Schultes (1824) transferred it in to *Eleocharis* and made the combination *Eleocharis acutangula* (Roxb.) Schult. Later, Svenson (1939) and Browning et al. (1997) suggested the existence of variability among specimens of *E. acutangula* from different geographical areas. Rosen et al. (2007) suggested sufficient variation existed among specimens of worldwide collections of *E. acutangula* and they segregate into three infraspecific taxa: *E. acutangula* ssp. *acutangula, E. acutangula* ssp. *breviseta* D.J.Rosen, and *E. acutangula* ssp. *neotropica* D.J.Rosen based on multivariate statistical analysis, ecological and distributional data. Recently, Prasad et al. (2020) in their checklist placed both the subspecies as the synonym of *E. acutangula* ssp. *acutangula*. But the specimens which were collected from Kollam district of Kerala is found quite distinct from subspecies *acutangula* and detailed taxonomic studies with fresh collections and original protologue (Rosen et al. 2007) clearly depict the existence of characters of ssp. *neotropica* and needs a taxonomic recognition as subspecies and therefore we reinstated its status.

Eleocharis acutangula (Roxb.) Schult. is a widespread species found in a wide range of habitats throughout India (Prasad et al. 2020). Scrutiny of literature revealed that subsp. *neotropica* was not reported from southern Western Ghats earlier but known from northern Western Ghats regions of India (Chandore 2015). Hence, the present collection extends its known distribution range to the southern Western Ghats, forming an addition to the flora of Kerala state. A detailed description along with photographs of the species is provided here for easy identification. The key characters to distinguish these two subspecies are mentioned in the Table 1.

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Image 1. *Eleocharis acutangula* ssp. *neotropica*: A—Habitat | B—Individual plants | C—Rhizome | D—Spike | E—Glume | F—Nut | G—SEM of Nut. © Kavya K. Nair & A. R. Viji

Table 1. Diagnostic characters of *E. acutangula* ssp. *neotropica* and ssp. *acutangula*.

E. acutangula ssp. *neotropica*	*E. acutangula* ssp. *acutangula*
Longest perianth bristle up to 4 mm long, soft, flexous, spinulose to below the middle	Longest perianth bristle up to 3.2 mm long, stiff, spinulose throughout or smooth
Nut neck weakly constricted	Nut neck markedly constricted
Style base up to 0.6 times long as wide	Style base up to 1.1 times long as wide
Mature Nut yellow green tinged with amber	Mature Nut dark amber to dark brown

Taxonomic treatment

Eleocharis acutangula (Roxb.) Schult. subsp. *neotropica* D.J.Rosen, J. Bot. Res. Inst. Texas 1(2): 886.2007.

Type: Peru, Departamento de Loreto, Maynas, Iquitos, prolongacion Yavari, Versailles-Paina, open annually burned grassland, 23 March 1974, McDaniel & Rimachi 18552 (Holotype:- MO 324923 image!)

Perennial herbs; rhizomes short with fibrous root. Culms triquetrous, tufted, erect, 45–80 × 0.3–0.4 cm internally spongy, soft, with incomplete transverse septa, pale green. Leaf reduced to sheath, 2, ca.15 cm long, mucronate at apex, brownish. Inflorescence, a terminal spike, cylindric, 3–3.5 × 0.28–0.3 cm, many-flowered, acute. Glumes oblong-ovate, ca. 5 × 3.7 mm, all fertile, abaxially many veined with adaxially distinguishable prominent midvein, apex acute. Hypogynous bristles 6, 2–2.4 times longer than nut, unequal, retrorsely scabrid, ca. 4 mm long. Stamens 3; anthers linear, 1–1.5 mm long. Style 3 fid. Nut biconvex, shining yellow tinged with amber, ca. 1.5 × 1.6 mm, style base persistent, dark brownish, conical, ca. 0.6 mm long (Image 1).

Flowering & Fruiting: June to August.

Specimen collected: 5110, 12 vii 2022. India, Kerala, Kollam District, Nilamel (8.8238^{0}N, 76.8824^{0}E), coll. Kavya K. Nair & A. R. Viji, TBGT

Note: The Scanning Electron Microscopic studies on the nut revealed the presence of elongated hexagonal epidermal cells with ca. 15 rows of transverse cells on one side of the nut. The periclinal walls are thick and straight in appearance.

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BOOK REVIEW

Putting wetland science to practice: a review

Review by Tiasa Adhya [1] & Partha Dey [2]

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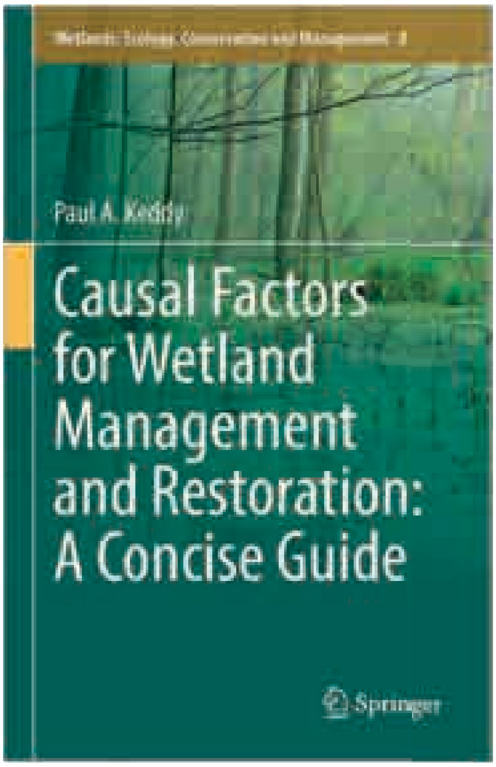

In these unprecedented times, we find ourselves amidst the sixth mass extinction while simultaneously embarking on the United Nations Decade on Ecosystem Restoration, spanning from 2021 to 2030. As if the stakes were not high enough, this timeframe serves as the ultimate countdown for achieving the Sustainable Development Goals (SDGs), vociferously advocated by scientists as the last window of opportunity to prevent the catastrophic climate change. Amidst this critical juncture, no other ecosystem plays a more pivotal role in achieving SDGs and buffering our civilization against climate change as wetlands. With their ability to regulate floods, purify and store water, produce oxygen, sequester carbon, provide food and showcase unrivalled biodiversity, wetlands stand as the veritable champions of our planet. It is in this regard that Dr. Paul Keddy's 'Causal factors for Wetland Management and Restoration: A Concise Guide' could not be more timely. Through this insightful book, he seeks to equip park managers, landscape architects, consultants, planners and engineers with a succinct and invaluable resource that will enable them to make tangible differences in the conservation and revitalization of wetlands.

General public and policy makers, mostly, assume that wetlands are just waterbodies due to a weak understanding of what wetlands truly are. Dr Keddy, at the onset, irons out this deficit and provides a solid ecological foundation by defining what a wetland is. It is "an ecosystem that arises when inundation by water produces soils dominated by anaerobic processes, which, in turn, forces the biota, particularly rooted plants, to adapt to flooding." Inundation is the predominant feature that characterizes wetlands. These could be seasonally inundated with an ephemeral presence of water for half the year and look like grasslands during the other half. Flooding cuts off aerial oxygen supply to the soil. Wetland life forms must thus adapt to survive both inundation and low oxygen levels. Such wetlands can be categorized into six major types: swamp, marsh,

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Acknowledgements: We acknowledge the continued inspiration provided by the Fishing Cat, an apex predator of wetlands. The species has been guiding us in our journey and we hope to not only learn with it but also to dedicate ourselves towards its conservation.

bog, fen, wet meadow and aquatic –each experiencing different intensities and duration of inundation.

The second chapter of the book is what might be Dr. Keddy's notable and a seminal contribution to the budding field of wetland ecology—'the causal factor approach'. This approach revolves around identifying and understanding the environmental factors that govern the very essence of wetlands—their composition and functionality. Therefore, this chapter provides a key to a small set of essential tools, offering a simplified lens to comprehend the complexities of wetland ecosystems. In areas where wetlands remain relatively unscathed, managers can focus on preserving the existing state by safeguarding the causal factors at play. In other areas, where wetlands have suffered damage, tweaking these causal factors will be paramount in the pursuit of restoring them to their desired state. The trifecta of flooding, nutrients and natural disturbances (through biomass removal) emerge as the pivotal forces, wielding significant influence over the rich tapestry of wetland variation.

Within the realm of the 'causal factor approach', duration of flooding is the most important factor creating a variety of wetland types along a spectrum of varying water depths – from permanently flooded to temporarily flooded. The link between ecological concepts and their application in conservation is also swiftly described. For instance, it has been advised that while creating new wetlands, a variety of water depths and gentle slopes should be incorporated into the design that will then sustain a matrix of different wetland vegetation types and provide a sanctuary to a plethora of life forms. Such concepts are especially important to perceive wetlands as ecosystems that are implicitly tied to the fate of rivers. Even subtle fluctuation in water depths created by the varying duration of floods holds the power to metamorphose a wetland from a marsh to a swamp, orchestrating a shift from a plant community dominated by tree-less, herbaceous forms to a reign of woody plants. This understanding provides the basis for realizing the effect of dams and river diversion projects on the persistence of wetlands. A single glance at the easily graspable conceptual diagrams that characterize the book helps us to answer questions and anticipate the far-reaching consequences of policies that continue to change the wet landscapes.

The latter portion of the book delves into a myriad of other factors that mould the character of wetlands, including the intriguing dynamics of plant competition, the impact of herbivory, the transformative effects of burial or sedimentation, the influence of salinity, the ramification of roads, the challenges posed by invasive species, and the crucial interplay with human population size. Throughout these chapters, the author skillfully provides tailored guidelines for wetland managers with different objectives — whether their aim is to maintain the existing state of wetlands or to embark on the ambitious journey of restoring them to a desired condition.

As we approach the book's culmination, the concluding chapters weave together a comprehensive synthesis of concepts, shedding light on the interconnectedness of individual wetlands within a vast global network. We come to understand that each wetland, be it nestled within a protected area, situated along a buffer zone, or forming part of a landscape-spanning corridor, contributes to a greater whole, a magnificent continuum that extends far beyond the boundaries of any single site. The last chapter serves as an invaluable exercise for wetland managers, presenting a thought-provoking series of questions that once answered by invoking the general principles introduced in the beginning, will pave the way towards achieving practical objectives in the protection and restoration of local wetlands.

Most river basins of Asia are severely degraded and fragmented because of river diversion projects. Based on the principles elucidated in the book, it might be worthwhile to predict how the dynamics of wetlands and wetland complexes strung together to the fate of the rivers might be affected due to decreasing water flow and increasing sedimentation. This will set the stage to examine corresponding impact on the ecological community and society. For instance, what happens when an aquatic wetland progresses towards a marsh and a marsh towards a swamp? Understanding the nature and pace of such a change will enable us to perceive the effect of this change on both biodiversity and dependent human communities (especially fishing communities) as well as the consequence for larger societies. This might then expedite restoration efforts if the change and/or degradation of wetland ecological functions has alarming consequences for biodiversity and society.

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