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10.11609/jott.2025.17.7.27171-27322
www.threatenedtaxa.org

26 July 2025 (Online & Print)
17(7): 27171-27322
ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher
Wildlife Information Liaison Development Society
www.wild.zooreach.org

Host
Zoo Outreach Organization
www.zooreach.org

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Cover: A Southern Rockhopper Penguin *Eudyptes chrysocome* stands on Tussock Grass on Westpoint Island. Painted in poster colors, this artwork is a reproduction of a photograph by Phillip Colla. Thanks to the photographer for the original image. © Pooja Patil.

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 July 2025 | 17(7): 27242–27248

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.9370.17.7.27242-27248

#9370 | Received 18 November 2024 | Final received 24 March 2025 | Finally accepted 24 June 2025

COMMUNICATION

Diet composition of three syntopic, ecologically divergent frogs (*Euphlyctis*, *Minervarya*, *Polypedates*) from paddy fields of Kohima, Nagaland, India

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Abstract: Monitoring indicator species like amphibians is crucial to assess habitat health. The diet of 129 anurans belonging to the three most abundant species found in the paddy fields of Kohima district in Nagaland, northeastern India—the aquatic *Euphlyctis adolfi,* the terrestrial *Minervarya nepalensis* and the arboreal *Polypedates himalayensis*—was studied. Results revealed 302 intact prey items belonging to 11 prey categories, gleaned through the stomach-flushing method. While Coleoptera was the most abundant prey found in all three species; Clitellata (terrestrial earthworms), Diptera, and Orthoptera were also important prey items. The high degree of overlap in the dietary niche of the three species despite their diverged microhabitat associations, could be the result of abundant prey items and the segregation of microhabitats. Lastly, as these frogs share a common prey base, they evidently segregate their foraging microhabitats to avoid competition.

Keywords: Aquatic, arboreal, class, index of relative importance, northeastern India, order, terrestrial.

Editor: S.R. Ganesh, Kalinga Foundation, Agumbe, India. **Date of publication:** 26 July 2025 (online & print)

Citation: Chase, T. & S. Kalita (2025). Diet composition of three syntopic, ecologically divergent frogs (*Euphlyctis*, *Minervarya*, *Polypedates*) from paddy fields of Kohima, Nagaland, India. *Journal of Threatened Taxa* 17(7): 27242–27248. https://doi.org/10.11609/jott.9370.17.7.27242-27248

Funding: None.

Competing interests: The authors declare no competing interests.

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Author contributions: First author (TC) collected the diet content from the field and identified the diet content for analysis and drafted the manuscript. Corresponding author (SK) supervised the work, checked the manuscript and communicated it to the journal.

Acknowledgements: We would like to thank Nisapi Pucho, Sedevikho Chase, and Methaheto Chase for providing accommodation which facilitated this work. We thank the people of Viswema, Nerhema, Kohima, Jotsoma, Khonoma, and Dzüleke for their support, and cooperation.

INTRODUCTION

Anurans (frogs & toads) are the most diverse order of amphibians and are ecological indicator species that require close monitoring (AmphibiaWeb 2025). India is home to a vast number of little-known, threatened, and endemic amphibians, despite harbouring a very high human population and this is particularly true for the northeastern India that is one of the country's three biodiversity hotspots (Dinesh et al. 2024). The Kohima District of Nagaland has a hilly terrain and very less naturally occurring standing water. Rice terrace cultivation is a widely practiced form of agriculture in this region. Paddy fields serve as crucial habitats for anurans, providing essential standing water for breeding and supporting tadpole development, especially in regions with limited natural aquatic environments (Elphick 2000). Despite the high anuran diversity in this region (Talukdar & Sengupta 2020), a comprehensive literature review revealed only three published studies on the diet of adult anurans in northeastern India, indicating a significant research gap in this area (Chanda 1993; Ao et al. 2001; Sarkar & Dey 2022). Despite the reduced habitat heterogeneity in paddy fields, resilient generalist species inhabit these fields (Piatti et al. 2010). Paddy fields serve as surrogate habitats for aquatic species (Elphick 2000), including anurans from surrounding areas (Seshadri et al. 2020).

While some taxa demonstrate a restricted trophic niche, relying on a limited range of prey items, others exhibit a broader diet, consuming a diverse assemblage of prey organisms. Primarily, anurans feed on arthropods and they can be important pest control agents in agro-ecosystems (Khatiwada et al. 2016). Anurans play a crucial role in the food chain due to the diet they consume and also because they are prey to animals in the higher trophic levels. Niche overlap does not equate to an increase in competition among species when there are enough resources for all species (Pianka 1974). Niche partitioning studies can give insights into a community's species diversity, abundance, and distribution (Toft 1985). Information on diet helps in the understanding of ecology, natural history (Donnelly 1991), niche partitioning (Toft 1985), and community structure (Toft 1980). The present study focussed on the following two parameters: (i) to assess the composition of anurans in paddy fields; (ii) to compare the diet of the three most abundant species observed in the local paddy fields, with respect to three syntopic, ecologically-dissimilar frog species.

MATERIALS AND METHODS

Study species

Three co-occurring or syntopic frog species that have divergent habitat utilisation patterns were chosen for the study. They were: the aquatic skittering frog *Euphlyctis adolfi* (Günther, 1860), the terrestrial cricket frog *Minervarya nepalensis* (Dubois, 1975) and the arboreal tree frog *Polypedates himalayensis* (Annandale, 1912). These species depend on stagnant water for breeding and other vital life processes including metamorphosis (Chanda 2002). These species use the water from embankments for breeding during summer. While *E. adolfi* primarily inhabits water, *M. nepalensis*, and *P. himalayensis* occur primarily in the periphery of embankments on land, and on vegetation, respectively. For taxonomic definitions of the studied frog species see Sanchez et al. (2018), Saikia et al. (2020), and Dufresnes et al. (2022).

Study sites

Six paddy fields, one each from five villages and one sub-urban locality in Kohima District, Nagaland, were surveyed. The six paddy fields were located in Nehrema Village, Kohima Town, Viswema Village, Jotsoma Village, Khonoma Village, and Dzüleke Village. The closest paddy fields were 2.46 km apart.

Sampling

Sampling was carried out from March to June, i.e., pre-monsoon to monsoon during 2021–2022. Stomach-flushing was done following Solé et al. (2005) immediately after capture of each individual frog from 1800 h to 2100 h. Following the stomach-flushing, all individuals were released back into the environment. Each stomach was flushed thrice. The stomach content was stored in 70% ethanol in screw cap vials. Diet content of 129 individuals of anurans belonging to three species- *Euphlyctis adolfi* (n = 45), *Minervarya nepalensis* (n = 51), and *Polypedates himalayensis* (n = 33) were examined during the study. Diet contents were identified up to the order level under a dissecting microscope. Partially digested food items, stones, and plant materials were categorized as miscellaneous and were not considered for analysis. A significant amount of diet contents observed was either partially digested or partially eaten; hence, intact bodies of prey items were a representation of the total prey consumed. Identification keys for diet contents were taken from Gibb & Oseto (2006). Prey items were measured with Mitutoyo 505–730 dial calipers (0.02 mm accuracy). Data analysis was

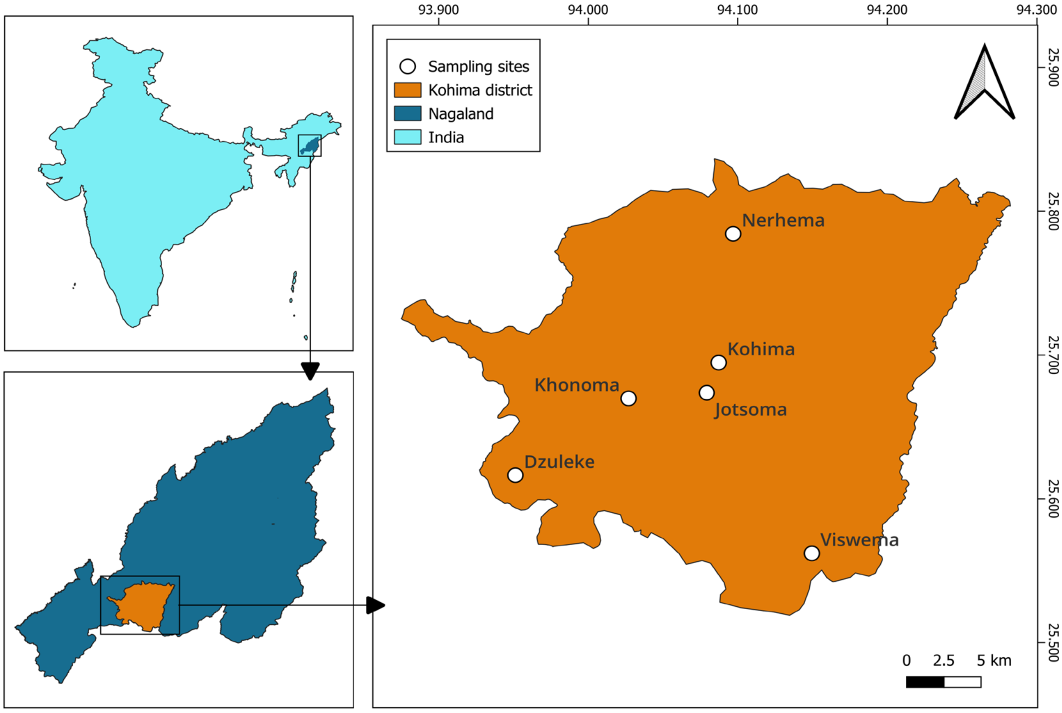

Figure 1. Map showing the study sites in Kohima District, Nagaland, northeastern India.

done using MS Excel and RStudio.

Data analysis

Vacuity index was measured as the proportion of empty stomachs to the total number of individuals of each species sampled. The volume of prey items was calculated using the formula for ellipsoid bodies (Colli & Zamboni 1999):

$$V = \frac{4}{3}\pi \left(\frac{L}{2}\right)\left(\frac{W}{2}\right)^2$$

Where, V is the volume, L is the length, and W is the width of a prey item.

The importance of diet contents was determined by ranking them using the index of relative importance (IRI) (Pinkas 1971):

$$IRI = (N + V)F$$

Where IRI = index of relative importance, N = numerical percentage, V = volumetric percentage, and F = frequency of occurrence percentage. Trophic niche breadth was calculated using the pliang non-Wiener index (Shannon & Weaver 1949):

$$H' = \sum P_i log\,(P_i)$$

Where H' is the Shannon-Weaver index, p_i is the proportion of individuals found to consume prey *i*. The H' value was standardized using the evenness index (Shannon & Weaver 1949):

$$J' = \frac{H'}{ln(n)}$$

Where J' is the measure of evenness and n is the number of species. Species were paired to calculate niche breadth by following Pianka's niche breadth formula:

$$O_{jk} = \frac{\sum_i^n p_{ij} p_{ik}}{\sqrt{\sum_i^n p_{ij}^2 \sum_i^n p_{ik}^2}}$$

Where $\hat{O}_{jk}$ is Pianka's measure of niche overlap, $\hat{P}_{ij}$ is the proportion of i^{th} resource used by j^{th} species and $\hat{P}_{ik}$ is the proportion of i^{th} resource used by k^{th} species.

RESULTS

Out of the 169 individual anurans belonging to the three species that were examined, 129 individuals contained food items in their stomachs. A total of 302 intact prey items were recovered which belonged to three classes (Insecta, Clitellata and Malacostraca) and 11 categories (Araneae, Coleoptera, Diptera, Orthoptera, Blattodea, Hemiptera, Lepidoptera (larva), Hymeniptera, Trichoptera, Clitellata, Decapoda), respectively. It was observed that several individuals had empty stomachs: 21 individuals of *Minervarya nepalensis* (vacuity index

= 29.58%), 14 individuals of *Euphlyctis adolfi* (vacuity index = 23.73%), and five individuals of *Polypedates himalayensis* (vacuity index = 13.16%). Partially digested prey was observed in several individuals of anurans while intact prey was relatively fewer. Results showed that *E. adolfi* consumed prey of eight categories while *M. nepalensis* and *P. himalayensis* consumed prey of nine categories, respectively. Statistical analysis revealed that the difference in the total number of prey consumed among the species was not significant (Kruskal-Wallis chi-squared = 2, df = 2, p = 0.3679).

Euphlyctis adolfi consumed the highest number of prey followed by *P. himalayensis* and *M. nepalensis*. *Polypedates himalayensis* on average consumed the highest number of prey per individual (Table 1). There was a statistically significant difference between the total number of prey consumed by the individuals of the three species (Kruskal-Wallis test = 28.232, df = 2, p <0.05). Coleoptera was the most common prey item in all the three species (relative occurrence: 34.88% relative occurrence in *E. adolfi*, 32% in *M. nepalensis* and 48.98% in *P. himalayensis*).

Table 1. Average prey consumed per individual of each species.

Frog species	No. of anurans	No. of prey (n)	Mean	SD
E. adolfi	45	129	2.867	2.06
M. nepalensis	51	75	1.471	1.17
P. himalayensis	33	98	2.97	1.49

Table 2. Niche breadth values measured with Shannon-Weaver index and evenness measure.

Frog species	H'	J'
M. nepalensis	1.87	0.851
E. adolfi	1.67	0.805
P. himalayensis	1.59	0.722

Table 3. Niche overlap values measured with Pianka's measure.

Frog species	*M. nepalensis*	*E. adolfi*	*P. himalayensis*
M. nepalensis	1	0.728	0.949
E. adolfi	0.728	1	0.765
P. himalayensis	0.949	0.765	1

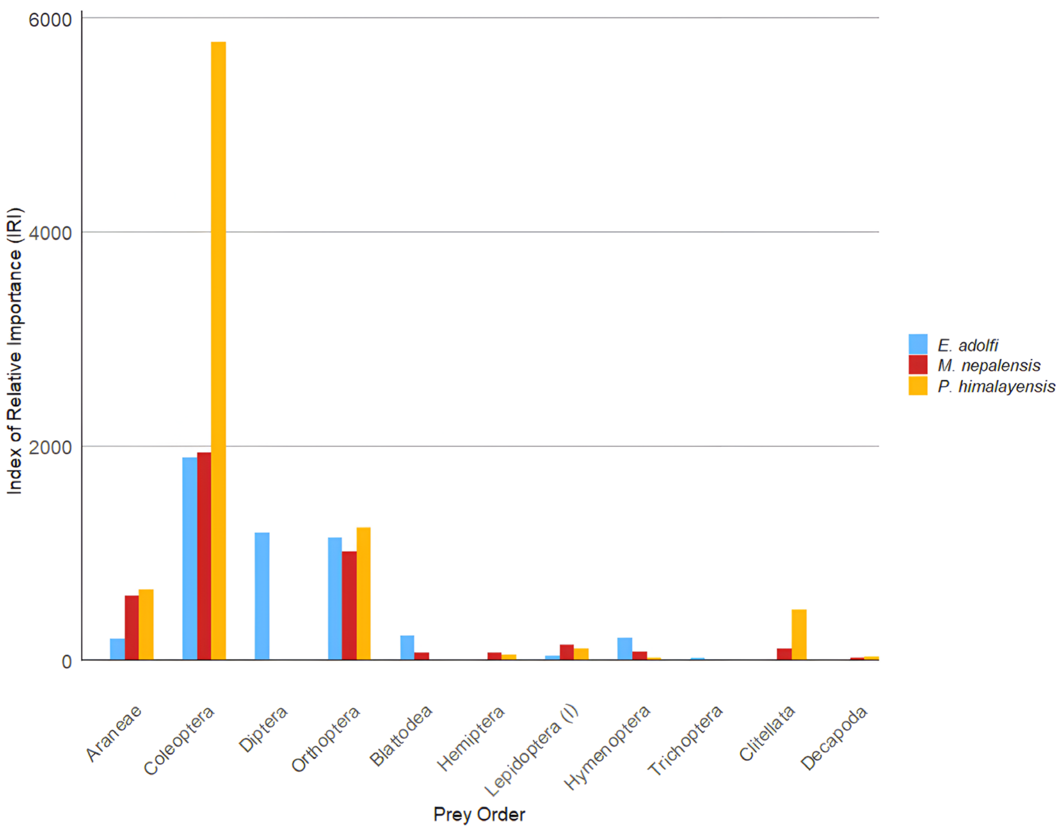

Figure 2. Index of relative importance values across prey orders of *Euphlyctis adolfi*, *Minervarya nepalensis* and *Polypedates himalayensis*.

Niche breadth and niche overlap

Dietary niche breadth was broadest in *M. nepalensis* and narrowest in *P. himalayensis* (Table 2). Niche overlap was highest between *M. nepalensis* and *P. himalayensis* and lowest between *M. nepalensis* and *E. adolfi* (Table 3). There was a high degree of overlap in the dietary niche of the three species.

Index of relative importance

Coleoptera (beetles) were the most abundant prey order found to be consumed by all three species studied. Prey categories Coleoptera, Orthoptera, and Clitellata were the highest contributors to the IRI value by volume for *M. nepalensis* (Table 5). In *P. himalayensis*, the diet volume was contributed mostly by class Clitellata (terrestrial earthworms) (Table 6). On the other hand, the largest volume contributors to the diet of *E. adolfi* were the orthopterans (Table 4). For all three species, coleopterans had the highest score for the Index of Relative Importance (IRI). Other important prey orders for *E. adolfi* were Diptera and Orthoptera. Orthoptera and Araneae were the highest contributors to IRI values in both *M. nepalensis* and *P. himalayensis.* The total prey volume was the highest in *E. adolfi* (568.36 cm^3, n = 45), while *M. nepalensis*, and *P. himalayensis* had similar volume (189.95 cm^3, n = 51 and 276.41 cm^3, n = 33, respectively).

DISCUSSION

Each of the three studied species have wide distribution across northeastern India (Chanda 2002; Ao et al. 2003; Dinesh et al. 2024) and was found to be the most abundant species in paddy field habitats in the studied areas. Due to their resilience and generalist behaviour, these species can thrive in this altered habitat. Other co-occurring species, viz., *Hyla annectans, Duttaphrynus melanostictus, Microhyla* sp., *Zhangixalus burmanus*, and *Zhangixalus smaragdinus* were excluded from this study due to small sample size present in our observations.

The vacuity index reveals a relatively high proportion of individuals with empty stomachs. A similar study found that anurans feed at a lower intensity during drier periods (Das 1996a). The high degree of dietary niche overlap is attributable to the similarity of IRI ratings of prey items among the three species. Coleoptera was the most important prey order according to the IRI values across all species. Diptera and Orthoptera ranked second and third in IRI values for *E. adolfi* respectively; while Orthoptera and Araneae ranked second and third

Table 4. Index of relative importance and its variables for *Euphlyctis adolfi*.

Prey Order / Class	Volume (%)	Frequency (%)	Number (%)	IRI
Araneae	3.19	15.56	9.30	194.38
Coleoptera	9.41	42.22	34.88	1870.27
Diptera	6.74	31.11	30.23	1150.36
Orthoptera	42.20	20	9.30	1030.05
Blattodea	28.96	11.11	4.65	373.50
Hemiptera	0	0	0	0
Lepidoptera (larva)	5.94	4.44	2.33	36.73
Hymenoptera	3.05	13.33	6.98	133.68
Trichoptera	0.50	6.67	2.33	18.83
Clitellata	0	0	0	0
Decapoda	0	0	0	0

Table 5. Index of relative importance and its variables for *Minervarya nepalensis*.

Prey Order / Class	Volume (%)	Frequency (%)	Number (%)	IRI
Araneae	12.41	19.61	17.33	583.29
Coleoptera	22.07	35.29	32.00	1908.42
Diptera	0	0	0	0
Orthoptera	20.62	25.49	20.00	1035.47
Blattodea	4.55	7.84	5.33	77.53
Hemiptera	8.30	5.88	4.00	72.35
Lepidoptera (larva)	6.25	7.84	9.33	122.21
Hymenoptera	0.85	9.80	6.67	73.69
Trichoptera	0	0	0	0
Clitellata	23.14	3.92	2.67	101.20
Decapoda	3.54	3.92	2.67	24.34

Table 6. Index of relative importance and its variables for *Polypedates himalayensis*.

Prey Order / Class	Volume (%)	Frequency (%)	Number (%)	IRI
Araneae	7.53	30.30	13.27	630.21
Coleoptera	29.69	72.73	48.98	5721.20
Diptera	0	0	0	0
Orthoptera	13.72	39.39	17.35	1223.66
Blattodea	0.98	3.03	1.02	6.05
Hemiptera	3.76	6.06	4.08	47.50
Lepidoptera (larva)	4.83	9.09	6.12	99.53
Hymenoptera	0.28	6.06	3.06	20.24
Trichoptera	0	0	0	0
Clitellata	30.18	12.12	4.08	415.30
Decapoda	9.05	6.06	2.04	67.22

in IRI values for *M. nepalensis* and *P. himalayensis*, respectively. Clitellata was absent in the diet of *E. adolfi* owing to the anuran's aquatic habitat. Though *P. himalayensis* is a tree frog, it is often observed on the ground in paddy fields during the breeding period. We have observed that they consume prey of Clitellata (terrestrial earthworms) during this period.

Das (1996) reported that the related, peninsular Indian species *P. maculatus* feeds both on ground and trees and classified it as a terrestrial feeder. *Polypedates himalayensis* have been reported to deposit eggs on forest floors. Individuals of this species were observed calling from holes in the ground and paddy fields (Rangad et al. 2012), indicating that this species spends its breeding period on ground, descending from the nearby bushes. Therefore, niche overlap values indicate a high degree of overlap in the diet of these anurans. Diptera and Trichoptera were found only in *E. adolfi* while Clitellata, Hemiptera, and Decapoda were found only in *M. nepalensis* and *P. himalayensis.* The decapod prey items observed were freshwater shrimps.

Although several studies have reported the presence of stones and plant materials in the diet of anurans, the cause for ingesting such materials has not been ascertained (Modak et al. 2018; Bahuguna et al. 2019). The presence of such materials may be attributed to accidental ingestion. This study also reveals that all the three observed species lack specialization in the food intake and are hence considered generalists in their feeding habit. Previous studies on *E. adolfi* also reported that coleopterans occupied the highest volume percentage amongst all arthropod prey items consumed (Das & Coe 1994; Das 1996b).

It was observed that although there is a high dietary niche overlap among the species, the three species occupied different microhabitats, thus minimizing the chances of competition between species. *E. adolfi* individuals were primarily observed swimming or floating on water. *Polypedates himalayensis* were recorded from microhabitats with less water, such as wet soil, and moist edges of embankments within paddy fields. *Minervarya nepalensis* individuals were observed to be wide-ranging, their microhabitats overlapping between *E. adolfi,* and *P. himalayensis*. Within the embankments, *M. nepalensis* was seen at the edges and did not swim / float unless while escaping from the observer.

CONCLUSION

In this study eight species of anurans were recorded from paddy fields; out of which three were studied for their diet preferences. The study site has a hilly terrain with several torrential streams. The landscape has limited areas of wetland habitats, which make paddy fields a vital refuge for anurans as they require wetlands for breeding, larval development, and a source of food for both adults, and tadpoles. While some species may use the paddy field areas for breeding only, the studied species have been found outside their breeding period in this habitat. This indicates that these three species are resilient generalists (Piatti et al. 2010). Among the three species, *E. adolfi* was the only species that had been studied previously (Das & Coe 1994). The present study revealed a high degree of overlap of prey among the three species with a low number of ingested prey.

The niche overlap and coexistence of the species suggest two hypotheses. Firstly, the interspecific competition caused by the niche overlap is not enough to drive any species to competitive exclusion due to the abundance of prey base. Secondly, the existing competition has not lasted long enough for species to evolve different diets. These have been supported by Pianka (1974) and Piatti & Souza (2011). Although the dietary niche overlap is high among the species, the overall niche may be differentiated according to observations in microhabitat usage. Future studies are recommended to include prey diversity studies and extend the sampling period through the monsoon to the post-monsoon seasons. To determine the overall niche differentiation among these three syntopic frog species, we suggest the inclusion of other niche dimensions such as aural niche, in addition to spatial, and trophic niches studied here.

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NAAS rating (India) 5.64

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www.threatenedtaxa.org

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

July 2025 | Vol. 17 | No. 7 | Pages: 27171–27322
Date of Publication: 26 July 2025 (Online & Print)
DOI: 10.11609/jott.2025.17.7.27171-27322

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