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Six unrecorded species of *Russula* (Russulales) from Nagaland, India and their nutrient composition

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Abstract. *Kumar R, Tapwal A, Pandey S, Raja-Rishi R, Mishra G, Giri K. 2014. Six unrecorded species of Russula (Russulales) from Nagaland, India and their nutrient composition. Nusantara Bioscience 6: 33-38.* The young and matured carpophores of mushrooms were collected from the forests of Puliebzie, Zakhama, Pherma, Mankoi, Chungtia and Tigit located in different districts of Nagaland, one of the north-eastern states of India. All the species were found associated with *Pinus khasya, P. caribaea*, *P. patula, Cryptomeria japonica, Canarium resiniferum, Dipterocarpus macrocarpus* and *Shorea assamica.* The collected mushrooms were identified as *Russula* species on the basis of their macro and microscopic characteristics. The identified *Russula* species were also screened for their nutrient content. The protein and carbohydrate content was found to vary between 28.12 to 42.86, and 49.33 to 55 %, respectively. These species [*Russula aeruginea* Lindblad; -Fr., *R. alnetorum* Romagnesi, *R. brevipes* Peck, *R. fragrantissima* Romagnesi, *R. nobilis* Velen. and *R. ochroleuca* (Pers.) Fr Gray.] are new record from Nagaland state of India.

Key words: *Russula*, mushroom, Nagaland, nutrient content

INTRODUCTION

Nagaland is situated in the north-eastern part of India within the longitude of 93°15′ E to 25°6′ E and Latitude 25°10′ N to 27°4′ N. It comprises of eleven districts with an area of 16,579 sq.km. The altitude ranges between 194-3826 m and the forest cover is about 80.33% of state's geographic area (ISFR 2011). The prominent tribes of Nagaland are Chakhesang, Angami, Zeliang, Aoo, Sangtam, Yimchunger, Chang, Sema, Lotha, Khemungan, Rengma, Konyak, Pachury and Phom. The average annual rainfall ranges between 2000 and 2500 mm and the temperature during the summer ranges between 15 and 30ºC, while in winter it falls below 4ºC.

Cogent climatic conditions and diverse vegetation favor the growth of a variety of mushrooms in the forests. Most of these mushrooms (species of *Russula, Agaricus, Cantharellus, Boletus* etc.) form mutual symbiotic association with forest trees in the form of ectomycorrhiza, which is most important for their growth, nutrient absorption and protection of roots from pathogens (Marx 1997). The genus *Russula* is cosmopolitan and an ectomycorrhizal genus associated with a wide range of Gymnosperms and Angiosperms (Richardson 1970; Alexander 1981; Molina and Trappe 1982; Pillukat and Agerer 1992; Kraigher et al. 1995; Agerer 2002). Ectomycorrhiza create distinct features in roots of forest trees. These characters are preferentially dependent, influenced and fashioned by the fungal hyphae of these essentially important structures of the root system (Agerer 2002). Many species of fungi are normally involved in ectomycorrhizal association with a single tree or a single species may involve in this association with more than one tree (Marx 1997).

Wild mushrooms are richer sources of protein and have a lower amount of fat than commercial mushrooms (Barros et al. 2007). A large number of *Russula* species are better known for their antimicrobial and antioxidant activities and thus having medicinal significance (Mercan et al. 2006; Liu 2007; Turkoglu et al. 2007; Jain and Pande 2013). Twenty-three species of the genus *Russula* have been reported which are associated with different forest trees of Pakistan (Ahmad et al. 1997). The diversity of *Russula* species has been explored in different parts of the west district of Sikkim, India (Das 2010; Das et al. 2010; Das and Verbeken 2011, 2012). Recently, three new species, i.e. *R. sharmae, R. sikkimensis* and *R. dubdiana* were reported from Sikkim, India (Das et al. 2013) We collected, identified and analyzed the nutrient content of fifteen mushroom species from different forest of Nagaland recently (Kumar et al. 2013).

The diversity of *Russula* species has not been explored from Nagaland state of India yet. The present paper proposes six new records, i.e. *R. aeruginea* Lindblad; -Fr., *R. alnetorum* Romagnesi, *R. brevipes* Peck, *R. fragrantissima* Romagnesi, *R. nobilis* Velen and *R. ochroleuca* (Pers.) Fr. Gray from Nagaland state of India.

MATERIALS AND METHODS

Sample collection and diversity analysis

The periodic surveys and collections were done in the forests of Lahorijan, Puliebzie, Zakhama, Pherma, Mankoi,

Chungtia, Nongkham, Namcha and Tigit (Figure 1) during the rainy (June to September) and winter (October to December) seasons during 2010-2011. The collected samples were wrapped in wax paper and brought to the laboratory for identification and proximate analysis. Morphological and anatomical characterization was carried out under Stereo microscope (Olympus BX 50) and Compound microscopes. The taxonomic identification was carried out consulting the available literatures (Zoberi 1973; Purakasthya 1985; Agerer 1991; Alexopolous et al. 1996; Adhikari 2000, 2004). The specimens were preserved in 2% formaldehyde and kept in the museum of Forest Protection Division, Rain Forest Research Institute, Jorhat, Assam by assigning identification numbers. The frequency and density of different species have been determined by the following formulas:

$$\text{Freq. of fungal species (\%)} = \frac{\text{Number of site in which the sp. is present}}{\text{Total number of sites}} \times 100$$

$$\text{Density} = \frac{\text{Total number of individuals of a particular species}}{\text{Total number of species}} \times 100$$

Nutrient content analysis

For proximate analysis, fruiting bodies were oven dried and powdered in a Moulinex blender. The fine powder was stored in the desiccators and utilized for proximate mineral and nutrient analysis following Anthrone method (Fasidi and Kadiri 1993).

Moisture content: The fresh and oven dried weight (80° C for 48 h) of each mushroom species was recorded and moisture content was determined (Raghuramulu et al. 2003) .

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight-dry weight}}{\text{Fresh weight}} \times 100$$

Dry matter content: Weight obtained after gradual oven drying from 35-60°C.

Crude fiber: The crude fiber content was calculated using the following equation:

Crude fiber (g/100 g sample) = [100-(moisture + fat)] × (We-Wa)/Wt of sample (Raghuramulu et al. 2003).

Protein content: 0.5 g of the powdered mushroom sample was extracted with 50 cm of 2% NaCl in a water-bath at 60°C for 1 h. The extract was filtered out and 50 cm of 3% copper acetate monohydrate were added to the filtrate to precipitate the protein content. The precipitated protein was then centrifuged and dissolves in 50 cm of 0.1 m NaOH. The quantity of protein in the alkaline solution was then determined using the folin-phenol method (Kadiri and Fasidi 1990).

Total carbohydrate estimation: The content of the available carbohydrate was determined by the following equation:

Carbohydrate (g/100 g sample) = 100-[(moisture + fat + protein + ash+ crude fiber) g/100 g] (Raghuramulu et al. 2003).

Ash content: The powdered mushroom sample (3.0 g) was ashed in a Gallenkamp furnance in previously ignited and cooled crucible of known weight at 550°C for 6 h. Fairly cooled crucibles were put in desiccators and weighed (Raghuramulu et al. 2003). The ash content (g/100g) was calculated using the following equation:

$$\text{Ash content} = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

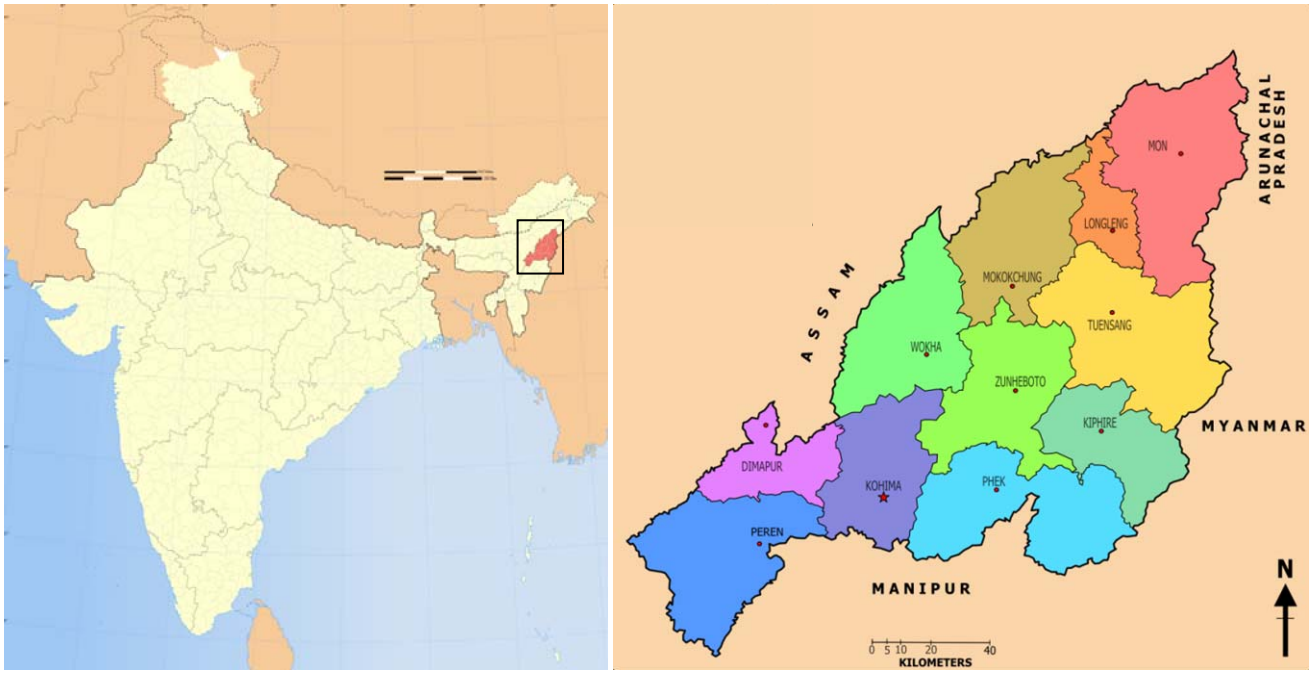

Figure 1. Study sites in the Nagaland state of India

Statistical Analysis: Experimental values are given as means ± standard deviation (SD). Statistical significance was determined by one-way variance analysis (ANOVA). Differences at $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Six *Russula* species were identified from different forest of Nagaland state, India. The macroscopic and microscopic characters like shape, size, color, texture, attachment of stipe, smell, spore print and spore size were recorded (Figures 1 and 2). The description of the collected specimens is as follows:

***Russula ochroleuca* (Pers.) Fr Gray. (ID No/RFRI/NL-000362) (Figure 2A, 3A)**

It was found in Lahorijan, Puliebzie, Zakhama forest range. The cap was 4-10 cm in diameter, convex with a depression. The color of the cap was yellow with eventually furrowed margins. The stem was 39-70x14-24 mm long and white. The color of the flesh was white. The gills were adnexed, creamy. The spore print was whitish to pale cream. The spores measure 8-10x7-8 µm, broadly ovoid with warts up to 1.2m high, joined by numerous fine lines forming a fairly well-developed network, cystidia were absent.

***Russula nobilis* Velen (ID No/RFRI/NL-000298) (Figure 2B, 3B)**

It was found in Pherma and Mankoi forest range. The cap was 3 to 9 cm in diameter, smooth, non-striate and bright red. The cap of this species generally remains convex with at most only a shallow central depression. The flesh was red or pink immediately beneath the cuticle; elsewhere the flesh was white. The gills were white and adnexed. The crowded gills of this species are very brittle indeed and easily crumble if they are handled. The spores measure 7-8x6-6.5 µm, ovoid with warts up to 0.5 µm tall and joined by narrow connectives in a nearly complete reticulum. The spore print was white.

***Russula alnetorum* Romagnesi (ID No/RFRI/NL-000351) (Figure 2C, 3C)**

It was found in leaf litter in the forest range of Mankoi and Chungtia. The cap was 3-5 cm, convex deviant flat centrally depressed; the surface was light violet in color. The gills were white, adnexed and narrow in front. The stem was 3-8 cm long, 2.5-4 cm thick, sturdy and solid, more or less equal, dry, smooth and whitish. The spore's measure 7-11x6.5-l0 µm, broadly ellipsoid to subglobose, ornamented with warts 0.7-1.7 µm high.

***Russula aeruginea* Lindblad;-Fr. (ID No/RFRI/ NL-000352) (Figure 2D, 3D)**

It was found in Puliebzie, Zakhama and Pherma forest range. The cap was 4-9 cm across, convex then flattening or depressed, grass-green, sometimes with yellowish or brownish tinges, without any violaceous tints, with rusty spots, center darker, smooth or radially veined, peeling halfway; margin often furrowed. The stem was 40-80x7-20 mm, white to yellowish, fairly firm. Flesh white. The gills were free. The spore print was cream. Spores elliptic, with rounded warts up to 0.6µ high, some joined by fine lines to form a very incomplete network with 0-2 meshes, 6-10x5-7 µm; cystidia cylindrical to spindle-shaped, without septa.

***Russula fragrantissima* Romagnesi (ID No/RFRI/ NL-000285) (Figure 2E, 3E)**

It was found in Nongkham, Namcha and Tigit forest range. The cap was 7-20 cm across, sub globose, slowly expanding with incurved margin, thick, fleshy; yellowish-brown and tuberculate-striate at the margin. The gills were adnate, close, pale yellow and narrow in front. The stem was 70-150x15-60 mm long, firm, hollow, colored as cap. The spore print was pale orange-yellow. The spores measure 6-9x5.5-7.7 µm; broadly elliptic, warts up to 1 µm with partial to complete reticulum.

***Russula brevipes* Peck (ID No/RFRI/ NL-000282) (Figure 2F, 3F)**

Russula brevipes Peck, is widely distributed throughout Nagaland. It was found very common in Puliebzie, Zakhama, Pherma, Mankoi, Chungtia and Namcha forest range. It can easily be identified by its large size and white coloration which does not stain when handled. It is also known as short-stem *Russula*. The fungus color was whitish to dull-yellow. The cap ranged from 7 to 30 cm (3-12 in) in diameter, whitish to dull-yellow in color and funnel-shaped with a central depression. The gills were narrow and thin, recurrent in an attachment, nearly white when young but becoming pale yellow to buff in age, and sometimes forked near the stipe. The stem was 3-8 cm long, 2.5-4 cm thick, sturdy and solid, more or less equal, dry, smooth, whitish. The spore print was white. The spore's measure 8-11x6.5-l0 µm, broadly ellipsoid to subglobose, ornamented with warts 0.7-1.7 µm high.

Frequency of species and proximate analysis

The maximum frequency occurrence was exhibited by *Russula fragrantissima* (66.6%) followed by *Russula aeruginea* (58.3%) and *Russula ochroleuca* (41.6%). The minimum frequency occurrence was observed with *Russula alnetorum* (25%). The frequency and density of different *Russula* species are shown in Table 1.

The nutrient composition of the selected mushroom species is shown in Table 2. Fresh mushrooms contained about 90% moisture and 10% dry matter and dry mushrooms contained about 90% dry matter and 10% moisture (Chang and Buswell 1996). The moisture content of the collected mushroom samples ranges from 87.11-93.90%. *R. ochroleuca, R. aeruginea* and *R. fragrantissima* have higher moisture content in comparison to other species. The dry matter content ranged from 4.10-4.62%. Crude fibres were recorded in the range of 9.81-12.13% and recorded minimum for *R. fragrantissima* and maximum for *R. brevipes.* The protein contents were ranged from 28.93% to 39.1% of dry weight (Ragunathan et al. 2003; Sanmee et al. 2003). The highest (43%) and lowest protein content (28%) was recorded in *R. ochroleuca* and *Russula alnetorum,* respectively. The carbohydrate content of edible mushrooms usually ranges from 40.6% to 53.3% of dry weight (Khanna et al. 1992;

Figure 2.A. *Russula ochroleuca*, **B.** *Russula nobilis*, **C.** *Russula alnetorum*, **D.** *Russula aeruginea*, **E.** *Russula fragrantissima*, **F.** *Russula brevipes*

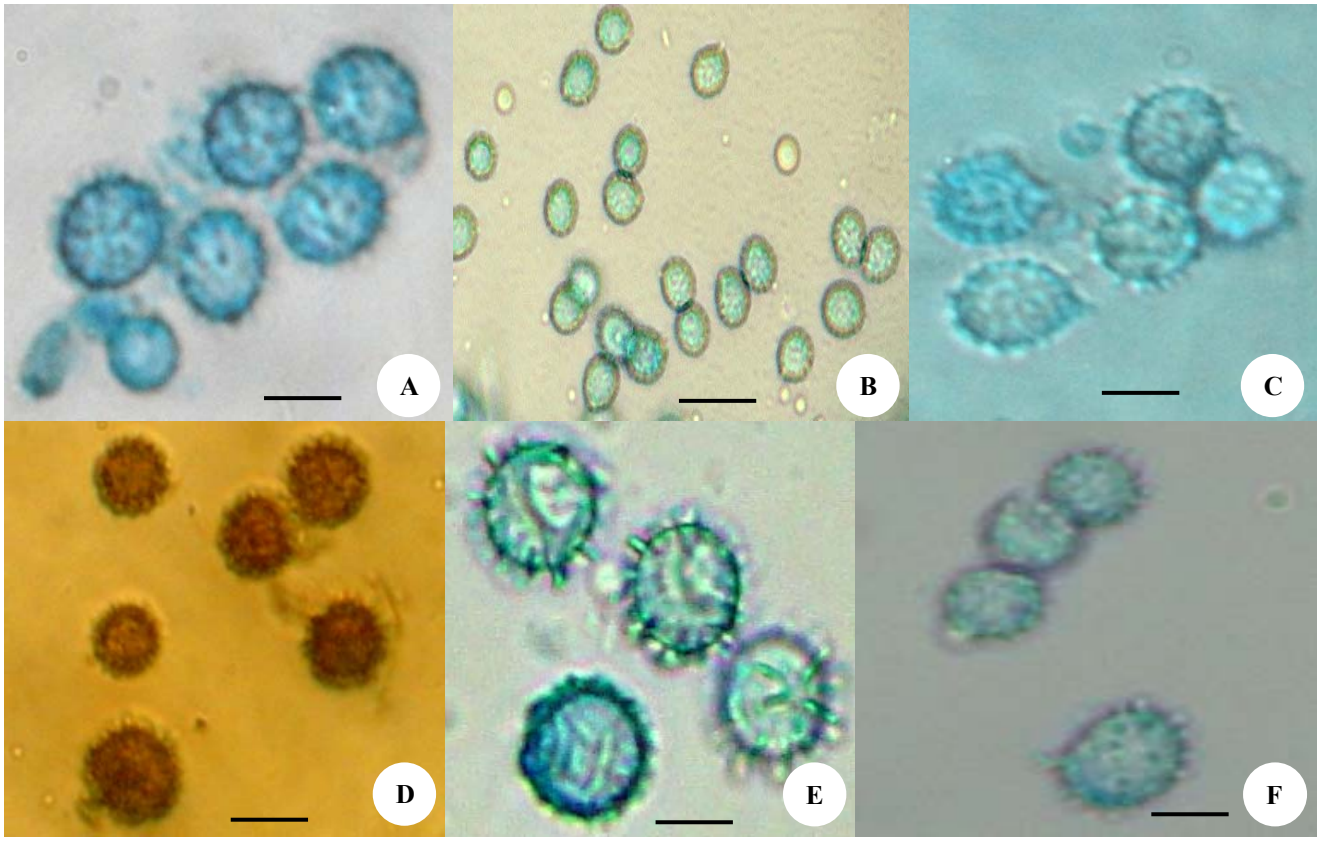

Figure 3.A. *Russula ochroleuca*, **B.** *Russula nobilis*, **C.** *Russula alnetorum*, **D.** *Russula aeruginea*, **E.** *Russula fragrantissima*, **F.** *Russula brevipes*. Bar = 10 um.

Table 1. Frequency of occurrence and density of *Russula* species

Name of the species	Family	Association with	Use	Freq. (%)	Density
Russula aeruginea Lindblad et Fr.	Russulaceae	*Pinus patula*	Edible	58.3	108.3
Russula alnetorum Romagnesi	Russulaceae	*Shorea assamica/ Dipterocarpus macrocarpus/ Alnus incana*	Unknown	25.0	66.6
Russula brevipes Peck	Russulaceae	*Pinus khasya/ Shorea assamica*	Edible/ medicinal	25.0	25.0
Russula fragrantissima Romagnesi	Russulaceae	*Cryptomeria japonica/ Canarium resiniferum*	Inedible	66.6	133.3
Russula nobilis Velen.	Russulaceae	*Pinus khasya/ Pinus caribaea*	Inedible	33.3	33.3
Russula ochroleuca (Pers.) Fr Gray.	Russulaceae	*Shorea assamica / Dipterocarpus macrocarpus*	Edible/ medicinal	41.6	58.3

Table 2. Proximate composition (g/100g) of Wild Mushrooms of *Russula* species (mean ±SE)

Mushrooms	Moisture	Dry matter	Crude fibre	Protein	Carbohydrates	Ash
Russula aeruginea Lindblad et Fr.	93.33±0.44	4.33±0.21	11.20±0.13	37.55±0.25	55.00±0.26	7.05±0.02
Russula alnetorum Romagnesi	84.30 ±1.44	4.10±0.07	11.98±0.30	28.12±0.13	49.33±0.25	8.09±0.05
Russula brevipes Peck	87.13±1.13	4.21±0.12	12.13±0.09	32.76±0.21	52.07±0.16	7.97±0.10
Russula fragrantissima Romagnesi	95.17±0.88	4.62±0.02	9.81±0.07	36.30±0.30	53.23±2.05	7.07±0.04
Russula nobilis Velen.	87.83±1.15	4.21±0.05	11.28±0.14	35.14±0.12	54.83±0.19	7.0±0.06
Russula ochroleuca (Pers.) Fr Gray.	93.93±1.31	4.42±0.02	10.14±0.05	42.86±0.49	52.91±0.19	7.28±0.01
CD ($p < 0.05$)	2.624	0.300	0.302	0.799	2.59	0.143
SE (m)	0.822	0.094	0.095	0.250	0.81	0.045

Ragunathan et al. 1996). In the present study, *Russula* species have carbohydrate content between 55-49.33%. The ash content has exhibited quite variation from 8.09-7% in different species.

Several dozen species of wild fungi are sold in the market of north-eastern India and most are ectomycorrhizal (Tanti et al. 2011). Kumar et al. (2013) collected fifteen edible/ medicinal mushrooms of Nagaland and worked out them for protein, crude fiber, carbohydrate and ash content. Tapwal et al. (2013) made a collection of 30 macrofungal species belonging to 26 genera from wet evergreen forests of Assam, northeastern India and investigated their ecological relationship with higher trees and documented their utilization as per available literature. Several authors have described the taxonomy of mushrooms from various regions of the world, but an analysis reveals that 60% of the newly described fungi are from the tropics, including mushrooms and up to 55% of the mushroom species have proved to be undescribed (Hawksworth 2001). Except a dozen of species cultivated on a large scale, all the macrofungal species grow in the natural habitat and their harvest is being undertaken for the benefit in different countries including India. Nowadays the anthropogenic activity has made countries all over the world to show serious concern about the dwindling biodiversity being lost at the rate never known before. Therefore, exploration, systematics and conservation of wild mushrooms have received more attention in the present day world. The proteins of wild edible mushroom contain considerable amounts of non-essential amino acids like alanine, arginine, glycine, glutamic acid, aspartic acid, proline and serine (Manzi and Pizzoferrato 2000). The add-value arising from mushrooms are bioactive materials which lead to an increase in its consumption and therefore, stimulating the commercialization of edible species. Having some sound knowledge of macrofungal diversity at the community and species level in the natural forests of North-eastern India is an essential component for developing and understanding their overall ecology as well as assessing the crucial niche relationships of the various elements that make up the forest biota, including the macrofungi themselves.

CONCLUSION

The current environmental issues of global warming and climate change would adversely affect the regeneration and growth pattern of the delicate fungi which requires a specific micro-climate. Consequently, higher nutritional quality and unique flavor of these mushrooms are likely to be lost if these wild edibles are not properly documented. A huge gap exists with respect to our knowledge of macrofungal diversity existing in the forests of north-eastern India is currently a serious constraint of being able to develop any type of understanding. We hope that it may serve as a starting point for future studies. Our previous and present study is a step to decipher the macrofungal diversity, specifically in the biodiversity rich forests of Nagaland for proper planning management and conservation of biodiversity. We hope that it can serve as a starting point for future studies.

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