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RESOURCE ARTICLE

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Exploring the vertebrate fauna of the Bird's Head Peninsula (Indonesia, West Papua) through DNA barcodes

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Abstract

Biodiversity knowledge is widely heterogeneous across the Earth's biomes. Some areas, due to their remoteness and difficult access, present large taxonomic knowledge gaps. Mostly located in the tropics, these areas have frequently experienced a fast development of anthropogenic activities during the last decades and are therefore of high conservation concerns. The biodiversity hotspots of Southeast Asia exemplify the stakes faced by tropical countries. While the hotspots of Sundaland (Java, Sumatra, Borneo) and Wallacea (Sulawesi, Moluccas) have long attracted the

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attention of biologists and conservationists alike, extensive parts of the Sahul area, in particular the island of New Guinea, have been much less explored biologically. Here, we describe the results of a DNA-based inventory of aquatic and terrestrial vertebrate communities, which was the objective of a multidisciplinary expedition to the Bird's Head Peninsula (West Papua, Indonesia) conducted between 17 October and 20 November 2014. This expedition resulted in the assembly of 1005 vertebrate DNA barcodes. Based on the use of multiple species-delimitation methods (GMYC, PTP, RESL, ABGD), 264 molecular operational taxonomic units (MOTUs) were delineated, among which 75 were unidentified and an additional 48 were considered cryptic. This study suggests that the diversity of vertebrates of the Bird's Head is severely underestimated and considerations on the evolutionary origin and taxonomic knowledge of these biotas are discussed.

KEYWORDS
amphibians, birds, DNA barcoding, fish, mammals, reptiles

1 | INTRODUCTION

The Earth's biosphere has been unevenly explored with some geographic regions relatively well surveyed and others lacking taxonomic baselines and awaiting large-scale screening of their biotas. The resulting gaps in taxonomic knowledge impede conservation planning and management, a situation particularly evident in species-rich tropical areas experiencing extensive infrastructure development (e.g., roads, dams and agriculture) and deforestation over the past few decades. This is particularly dramatic for highly endemic biotas of tropical insular systems that have become increasingly accessible to human activity and biological invasions and, as a consequence, are mostly endangered (Hoffman et al., 2010; Myers et al., 2000; Schipper et al., 2008). Southeast Asia exemplifies the stakes associated with the conservation of species-rich biota in insular tropical systems. Of the four main biogeographic provinces in this area, three (Sundaland, Wallacea, and the Philippines) have been recognized as biodiversity hotspots because they harbour an exceptional number of endemic species, and intense anthropogenic pressures threaten their existence (Hoffman et al., 2010; Myers et al., 2000; Thiault et al., 2019). Both Sundaland (Java, Sumatra and Borneo) and Wallacea (Sulawesi, Moluccas) have long captured the attention of biologists and served as early model systems in biogeographic studies (Mayr, 1944; Wallace, 1859). In particular, vertebrate assemblages in both regions have been actively documented over the last two decades, resulting in the description of hundreds of new species (Hubert et al., 2015) and the assessment of associated phylogenetic and biogeographic patterns (de Bruyn et al., 2013; den Tex et al., 2010; Esselstyn et al., 2013; Hutama et al., 2017; Lim et al., 2017; O'Connell et al., 2018; Pinheiro et al., 2017; Rowe et al., 2019; Wood et al., 2012).

In contrast, the northern part of Sahul, for example, the island of New Guinea, has received comparatively little attention, as a result of limited accessibility. Delimited from Wallacea to the west by the Lyddeker line, its component lineages show multiple affinities with the neighbouring Wallacea or Australia, while also displaying high levels of endemism (Crayn et al., 2015; Rowe et al., 2008; Unmack et al., 2013). The extent of this endemism is presumed to be vastly underestimated, because recent DNA-based species inventories purported large numbers of undescribed taxa (Kadarusman et al., 2012; Riedel et al., 2013). Unfortunately, the rapid acceleration of deforestation in New Guinea during the last decades (Austin et al., 2019; Filer et al., 2009; Nelson et al., 2014; Shearman & Bryan, 2011), is putting many species and their habitats at risk, calling for comprehensive inventories of fauna and flora to facilitate conservation planning.

In the last 15 years, DNA barcoding, the use of the mitochondrial cytochrome oxidase I gene as an internal species tag (Hebert et al., 2003; Hebert et al., 2003), has been providing a major boost to documenting biodiversity. Initially designed to overcome limits of morphology-based species-level identification, it has increasingly been accepted as a tool to capture species boundaries and as a foundation for automated molecular species identification and detection (April et al., 2011; Blagoev et al., 2015; Delrieu-Trottin et al., 2019; deWaard et al., 2019; Kerr et al., 2007). The utility of DNA barcoding, however, always depends on the taxonomic coverage of the associated DNA barcode reference library which, in turn, requires solid taxonomic knowledge of the biotas under scrutiny (Hubert & Hanner, 2015). Several studies have emphasized the benefits of integrating a standardized DNA-based approach into the inventories of poorly known faunas (de Araujo et al., 2018; Dahruddin et al., 2017; Milá et al., 2012; Monaghan et al., 2009; Riedel et al., 2013; Sholihah et al., 2020; Smith et al., 2005, 2008; Sonet et al., 2018; Tänzler et al., 2012; Vacher et al., 2020). Newly developed DNA-based species delimitation methods (Fujiwasa & Barraclough, 2013; Kekkonen et al., 2015; Monaghan et al., 2009; Puillandre et al., 2012; Ratnasingham & Hebert, 2013; Zhang et al., 2013) further speeded up the pace of species discovery by dramatically increasing the throughput and lowering analytical costs (Butcher et al.,2012; Riedel et al., 2013).

One of the more geologically intricate regions of the island of New Guinea is its northwest portion, known as the Bird's Head

Peninsula, particularly the Lengguru karstic massif, in the West Papua province. This massif originated from the subduction of the Australian and Pacific plates that resulted in the development of an accretion prism during the last 10 million years (Myr) (Hall et al., 2011; Lohman et al., 2011). Previous DNA-based inventories of freshwater fishes in this area resulted in the discovery of multiple new taxa (Kadarusman et al., 2012; Nugraha et al., 2015). The co-occurrence of multiple lineages of distinct biogeographic origin, suggests that the Bird's Head Peninsula has been colonized from several regions (Kadarusman et al., 2012; Unmack et al., 2013) and warrants a more thorough faunal inventory, in particular for freshwater fauna, for which freshwater-specific processes of isolation have led to extremely high levels of endemism, much higher than for other continental vertebrates (Leroy et al., 2019).

Between October and November 2014, a large multidisciplinary expedition to the Bird's Head Peninsula sampled mammals, birds, reptiles, amphibians and fishes across a diverse array of ecosystems, from mangroves to the "cloud" forest habitats, from lakes and rivers to caves of the Lengguru karst system. One of the main aims of the expedition was to conduct a DNA-based inventory of vertebrates in this relatively unchartered part of New Guinea and to extend the taxonomic coverage of the DNA barcode reference library. This survey resulted in 1005 records for 264 vertebrate molecular operational taxonomic units (MOTUs) that are presented and discussed in this publication.

2 | MATERIALS AND METHODS

2.1 | Sampling and collection management

The Lengguru expedition, conducted between the 17 October and the 20 November 2014, surveyed 35 sites in the Lengguru massif (http://www.lengguru.org/) and an additional set of 20 sites in the Western part of the Bird's Head Peninsula (Figure 1). Freshwater fishes were sampled using electrofishing gear and cast nets. Bats and birds were trapped using mist nets. Amphibians and reptiles were hand collected or captured with glue traps. Rodents were captured using non-lethal cage traps. Specimens were photographed, individually labeled and their provenance information recorded, including geocoordinates, collection date, and collectors. A muscle tissue or blood sample was taken from each captured specimen and fixed in 95% ethanol. Fish, amphibian and reptile voucher specimens were fixed in 5% formalin solution and subsequently transferred into a 70% ethanol solution. Mammals were preserved in 80% ethanol and birds were prepared on site as dried study skins by the Indonesian Institute of Sciences (LIPI) personnel. Both tissue and voucher specimens were deposited in the national collections at the Research Center for Biology (RCB) from LIPI.

2.2 | DNA sequencing and international repositories

Genomic DNA was extracted from the muscle tissue samples using a Qiagen DNeasy 96 tissue extraction kit following the manufacturer's specifications. A 651-bp segment from the 5' region of the cytochrome oxidase I gene (COI) was amplified using the M13 tailed primers cocktails C_FishF1t1/C_FishR1t1 for fishes, C_VF1LFt1/ C_VR1LRt1 for mammals and reptiles (Ivanova et al., 2007), AmphF2_t1 (TGTAAAACGACGGCCAGTTTCAACWAAYCAYAAAGAYATYGG)/ AmphR3_t1 (CAGGAAACAGCTATGACTADACTTCWGGRTGDCC RAARAATCA) for amphibians (Prosser, unpublished data) and BirdF1_t1/ BirdR2_t1 for birds (Hebert et al., 2004). PCR amplifications were done on a Veriti 96-well Fast (ABI-AppliedBiosystems) thermocycler with a final volume of 10.0 µl containing 5.0 µl Buffer 2x, 3.3 µl ultrapure water, 1.0 µl each primer (10 µM), 0.2 µl enzyme

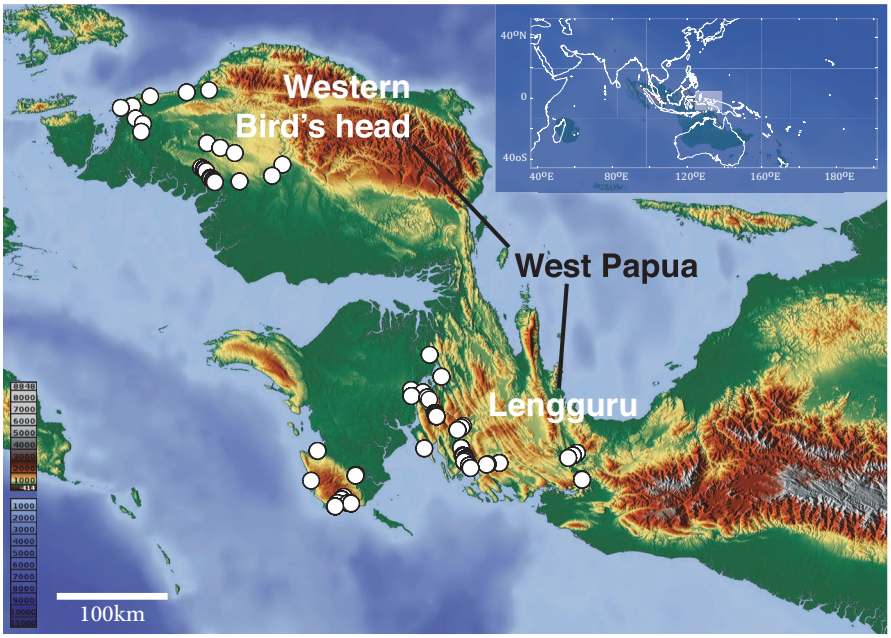

FIGURE 1 Distribution of the 35 collection sites in the Lengguru massif (West Papua, Indonesia) and the 20 collection sites in the western part of the Bird's head peninsula for the 1,046 samples analysed for this study. Map from https://maps-for-free.com/

TABLE 1 Summary statistics of the number of DNA barcode records assembled per class, family and genus

Systematics	N specimens
Actinopterygii	**343**
Atheriniformes	**225**
Atherinidae	12
Craterocephalus	12
Melanotaeniidae	213
Melanotaenia	213
Clupeiformes	**5**
Clupeidae	5
Nematalosa	5
Cypriniformes	**2**
Cyprinidae	2
Barbodes	2
Gobiiformes	**51**
Butidae	10
Butis	8
Ophiocara	1
Oxyeleotris	1
Eleotridae	20
Eleotris	6
Giuris	2
Mogurnda	12
Oxudercidae	21
Awaous	2
Lentipes	1
Redigobius	3
Sicyopterus	2
Stenogobius	2
Stiphodon	11
Kurtiformes	**1**
Apogonidae	1
Glossamia	1
Ovalentaria	**2**
Rhyacichthyidae	2
Rhyacichthys	2
Perciformes	**29**
Kuhliidae	4
Kuhlia	4
Leiognathidae	2
Leiognathus	2
Lutjanidae	2
Lutjanus	2
Terapontidae	6
Mesopristes	1
Pingalla	5
Toxotidae	15
Toxotes	15
Siluriformes	**20**
Ariidae	12
Neoarius	12
Plotosidae	8
Neosilurus	3
Porochilus	5
Syngnathiformes	**8**
Syngnathidae	8
Hippichthys	2
Microphis	6
Amphibia	**167**
Anura	**167**
Ceratobatrachidae	38
Cornufer	38
Hylidae	1
Nyctimystes	1
Limnodynastidae	1
Lechriodus	1
Microhylidae	99
Asterophrys	16
Austrochaperina	11
Callulops	10
Cophixalus	12
Hylophorbus	17
Oreophryne	16
Sphenophryne	7
Xenorhina	10
Pelodryadidae	14
Litoria	14
Ranidae	14
Papurana	14
Aves	**155**
Accipitriformes	**4**
Accipitridae	4
Accipiter	4
Columbiformes	**9**
Columbidae	9
Chalcophaps	8
Ptilinopus	1
Coraciiformes	**25**
Alcedinidae	25
Ceyx	16
Dacelo	2

(Continues)

(Continues)

TABLE 1 (Continued)

Systematics	N specimens
Syma	1
Tanysiptera	6
Passeriformes	**112**
Acanthizidae	18
Aethomyias	2
Gerygone	3
Origma	7
Sericornis	6
Artamidae	4
Melloria	4
Maluridae	1
Chenorhamphus	1
Melanocharitidae	24
Melanocharis	7
Toxorhamphus	17
Meliphagidae	18
Melilestes	5
Meliphaga	10
Myzomela	2
Xanthotis	1
Monarchidae	4
Arses	1
Symposiachrus	3
Oriolidae	4
Pitohui	4
Pachycephalidae	16
Colluricincla	6
Pachycephala	5
Pseudorectes	5
Paradisaeidae	2
Cicinnurus	1
Diphyllodes	1
Petroicidae	10
Heteromyias	1
Peneothello	2
Poecilodryas	1
Tregellasia	6
Phylloscopidae	2
Phylloscopus	2
Pomatostomidae	1
Garritornis	1
Psophodidae	3
Cinclosoma	3
Rhipiduridae	5
Rhipidura	5

(Continues)

TABLE 1 (Continued)

Systematics	N specimens
Psittaciformes	**5**
Psittaculidae	5
Alisterus	2
Micropsitta	3
Mammalia	**173**
Artiodactyla	**9**
Cervidae	1
Rusa	1
Suidae	8
Sus	8
Chiroptera	**135**
Emballonuridae	13
Emballonura	12
Mosia	1
Hipposideridae	44
Aselliscus	2
Coelops	1
Hipposideros	41
Pteropodidae	55
Dobsonia	12
Macroglossus	5
Nyctimene	9
Paranyctimene	2
Pteropus	4
Rousettus	11
Syconycteris	12
Rhinolophidae	7
Rhinolophus	7
Vespertilionidae	16
Miniopterus	10
Myotis	3
Pipistrellus	3
Diprotodontia	**6**
Macropodidae	2
Dorcopsis	2
Petauridae	2
Dactylopsila	1
Petaurus	1
Phalangeridae	2
Phalanger	2
Peramelemorphia	**7**
Peramelidae	7
Echymipera	7
Rodentia	**16**
Muridae	16

(Continues)

TABLE 1 (Continued)

Systematics	N specimens
Melomys	1
Paramelomys	4
Rattus	10
Uromys	1
Reptilia	**167**
Squamata	**167**
Agamidae	2
Hypsilurus	2
Boidae	2
Candoia	2
Colubridae	10
Boiga	1
Dendrelaphis	1
Rhabdophis	1
Stegonotus	5
Tropidonophis	2
Elapidae	2
Aspidomorphus	1
Micropechis	1
Gekkonidae	32
Cyrtodactylus	22
Gehyra	2
Gekko	4
Hemidactylus	2
Lepidodactylus	1
Nactus	1
Pythonidae	3
Apodora	1
Leiopython	1
Simalia	1
Scincidae	115
Carlia	6
Emoia	47
Eremiascincus	2
Lygisaurus	14
Sphenomorphus	39
Tiliqua	3
Tribolonotus	4
Typhlopidae	1
Ramphotyphlops	1
Total	**1005**

Phire Hot Start II DNA polymerase (5 U) and 0.5 μl of DNA template (~50 ng). Amplifications were conducted as follows: initial denaturation at 98°C for 5 min was followed by 30 cycles consisting of denaturation at 98°C for 5 s, annealing at 56°C for 20 s, and extension at 72°C for 30 s, followed by a final extension step at 72°C for 5 min. The PCR products were purified with ExoSap-IT (USB Corporation) and sequenced in both directions. Sequencing reactions were performed at the Centre for Biodiversity Genomics, University of Guelph, Canada, using the "BigDye Terminator v3.1 Cycle Sequencing Ready Reaction" and sequencing was performed on an ABI 3730xl capillary sequencer (Applied Biosystems), following standard protocols described in Hebert et al., (2013). Sequences and collateral information were deposited on BOLD (Ratnasingham & Hebert, 2007) and are available as a public data set DS-LENG (dx.doi.org/10.5883/DS-LENG, Table S1).

2.3 | Genetic distances and species delimitation

Kimura 2-parameter (K2P) (Kimura, 1980) pairwise genetic distances were calculated using the R package Ape 4.1 (Paradis et al., 2004). Maximum intraspecific and nearest-neighbour genetic distances were calculated using the matrix of pairwise K2P genetic distances and the R package Spider 1.5 (Brown et al., 2012). We checked for the presence of a barcode gap, for example, the lack of overlap between the distributions of the maximum intraspecific and the nearest-neighbour genetic distances, by plotting both distances and examining their relationships on an individual basis, instead of comparing both distributions independently (Blagoev et al., 2015). A neighbour-joining (NJ) tree was built based on K2P distances and used to visually inspect genetic distances and DNA barcode clusters (Figure S1).

Several methods have been proposed for delineating species based on DNA sequences (Kapli et al., 2017; Pons et al., 2006; Puillandre et al., 2012; Ratnasingham & Hebert, 2013). Each of these methods has different properties, particularly when dealing with singletons (i.e., delimited lineages represented by a single sequence) or heterogeneous speciation rates among lineages (Luo et al., 2018). A combination of different approaches is increasingly used to overcome potential pitfalls arising from uneven sampling (Kekkonen & Hebert, 2014; Kekkonen et al., 2015; Limmon et al., 2020; Shen et al., 2019; Sholihah et al., 2020). We used six different sequence-based methods of species delimitation to identify MOTU: (i) Refined single linkage (RESL) as implemented in BOLD and used to generate Barcode index numbers (BIN) (Ratnasingham & Hebert, 2013), (ii) Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012), (iii) Poisson tree process (PTP) in its single (sPTP) and multiple rates version (mPTP) as implemented in the stand-alone software mptp_0.2.3 (Kapli et al., 2017; Zhang et al., 2013), and (iv) general mixed yule-coalescent (GMYC) in its simple (sGMYC) and multiple rate version (mGMYC) as implemented in the R package Splits 1.0–19 (Fujisawa & Barraclough, 2013). A final delimitation scheme was established based on a majority-rule consensus among the six delimitation analyses performed.

Both RESL and ABGD use DNA alignments as input files while a maximum likelihood (ML) tree was used for PTP and an ultrametric tree was used for GMYC. The ML tree was reconstructed using RAxML (Stamatakis, 2014) based on a GTR+I+Γ substitution model. The ultrametric tree was reconstructed using the Bayesian approach

TABLE 2 Summary statistics of genetic distances including minimum, maximum and average K2P distances within-MOTU, among MOTUs within genus and among MOTUs within family for fishes, birds, mammals, amphibians and reptiles

Level	Taxa	n	N taxa	Comparisons	Min dist (%)	Mean dist (%)	Max dist (%)	SE dist (%)
Within species	Fishes	229	27	2215	0.00	0.37	11.14	0.00
	Birds	140	30	426	0.00	0.43	2.48	0.00
	Mammals	140	28	457	0.00	0.64	17.52	0.00
	Amphibians	115	14	580	0.00	1.78	10.87	0.00
	Reptiles	79	14	308	0.00	1.98	17.40	0.01
Within genus	Fishes	172	5	8541	0.65	9.75	18.02	0.00
	Birds	38	5	81	0.00	8.77	12.87	0.04
	Mammals	82	7	434	0.00	18.66	24.94	0.01
	Amphibians	47	3	191	15.23	21.56	25.99	0.02
	Reptiles	45	4	178	2.81	15.16	24.52	0.05
Within family	Fishes	57	4	279	11.39	17.79	24.99	0.01
	Birds	131	10	625	6.14	14.24	19.85	0.01
	Mammals	123	6	1254	12.51	20.42	26.95	0.00
	Amphibians	88	2	1339	20.65	25.58	50.00	0.00
	Reptiles	86	5	915	13.67	24.81	36.34	0.00

implemented in BEAST 2.4.8 (Bouckaert et al., 2014) based on a strict-clock model using a genetic distance of 1.2% per million year (Bermingham et al., 1997). A preliminary analysis indicated that 50 million steps was a sufficient length for the Markov chains to reach ESS>200 for all estimated parameters. Thus, two Markov chains of 50 million steps each were run independently on the entire vertebrate data set using the Yule pure birth model tree prior and a GTR+I+Γ substitution model. Both runs were merged using LogCombiner 2.4.8 (Bouckaert et al., 2014) and sGMYC and mGMYC analyses were conducted on 10 chronograms sampled along the merged runs using the complete DNA sequences data set following Hubert et al., (2019). The final delimitation scheme for sGMYC and mGMYC was established based on a majority-rule consensus of all 10 replicates. Further Bayesian chronograms for visual inspection of the topologies and inferred divergence times were built independently for fishes, mammals, birds, amphibians and reptiles using the same parameters. Both runs were combined independently for each group using LogCombiner 2.4.8 and the maximum credibility tree was constructed using TreeAnnotator 2.4.7 (Bouckaert et al., 2014). Sampling coverage at the MOTU level was examined for the five vertebrate groups through accumulation curves generated with BOLD (Smith et al., 2009).

3 | RESULTS

Sequencing yielded a total of 1005 COI sequences out of 1140 samples. The product lengths for the various primer combinations were as follows: 652 bp for C_FishF1t1/C_FishR1t1, 658 bp for AmphF2_t1/AmphR3_t1, 657 bp for C_VF1LFt1/C_VR1LRt1, and 694 bp for BirdF1_t1/COIbirdR2_t1. Amplification failures were randomly distributed among species, and at least one individual of each species was successfully sequenced. Average sequence length for all DNA barcodes was 649 bp, and no stop codons were detected suggesting that these sequences correspond to functional coding regions. A total of 21 orders representing 61 families and 136 genera were sequenced, including nine orders representing 17 families and 29 genera of fishes, one order with six families and 13 genera of amphibians, five orders comprising 18 families and 37 genera of birds, five orders with 12 families and 27 genera of mammals, and one order representing eight families and 26 genera of reptiles (Table 1). The number of specimens identified to the species level largely varied among classes: 70% of the 343 fish specimens, 70% of the 167 amphibian specimens, 100% of the 155 bird specimens, 85% of the 173 mammal specimens and 53% of the reptile specimens. Intraspecific, interspecific within genus and interspecific within family genetic distances largely vary among classes (Table 2).

The MOTU delimitation analyses yielded varying numbers of MOTUs depending on the algorithm used for all classes (Figures 2–3, Table S1). Numbers of delimited MOTUs were 54 for RESL, 66 for ABGD, 44 for sPTP, 38 for mPTP, 51 for sGMYC and 65 for mGMYC for fishes; 45 for RESL, 46 for ABGD, 43 for sPTP, 33 for mPTP, 44 for sGMYC and 49 for mGMYC for birds; 51 for RESL, 67 for ABGD, 51 for sPTP, 27 for mPTP, 52 for sGMYC and 69 for mGMYC for mammals; 43 for RESL, 47 for ABGD, 37 for sPTP, 31 for mPTP, 38 for sGMYC and 47 for mGMYC for amphibians; and 63 for RESL, 70 for ABGD, 59 for sPTP, 40 for mPTP, 59 for sGMYC and 67 for mGMYC for reptiles (Table S1). The final consensus consisted of 59 MOTUs for fishes, 46 MOTUs for birds, 53 MOTUs for mammals, 43 MOTUs for amphibians and 63 MOTUs for reptiles. Thus, a total of 264 MOTUs was added to the DNA barcode reference library. Distributions of both maximum intraspecific

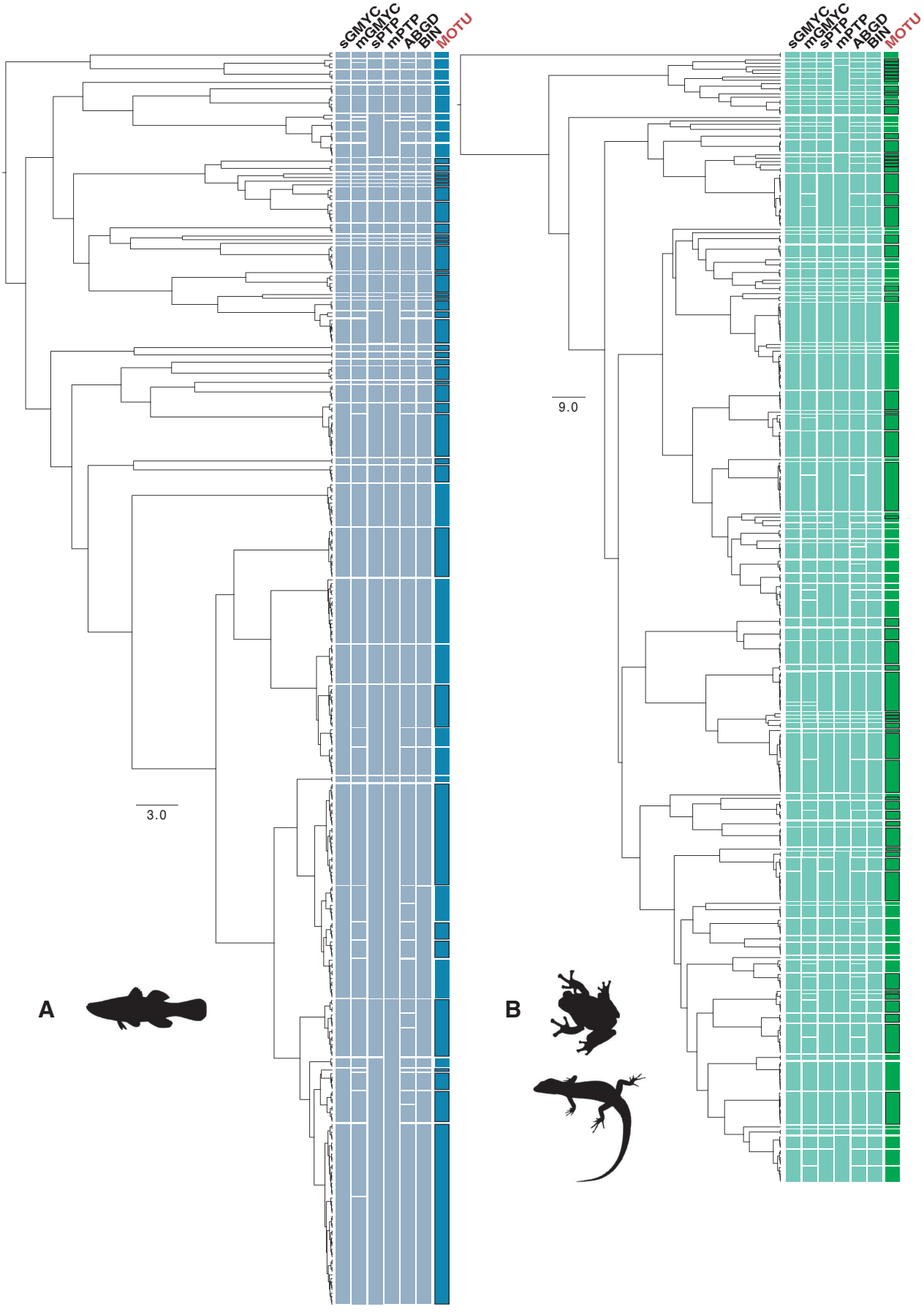
sGMYC
mGMYC
sPTP
mPTP
ABGD
BIN
MOTU
3.0
A
9.0
B

FIGURE 2 Bayesian chronograms based on a 1.2% of genetic divergence per million years including DNA-based species delimitation derived from sGMYC, mGMYC, sPTP, mPTP, ABGD, RESL and final delimitation schemes based on majority rule consensus among the six methods for fishes (blue), and amphibians and reptiles (green). The black outlined boxes correspond to MOTUs derived from specimens identified to the species level. Regular boxes indicate MOTUs derived from specimens identified to the genus level.

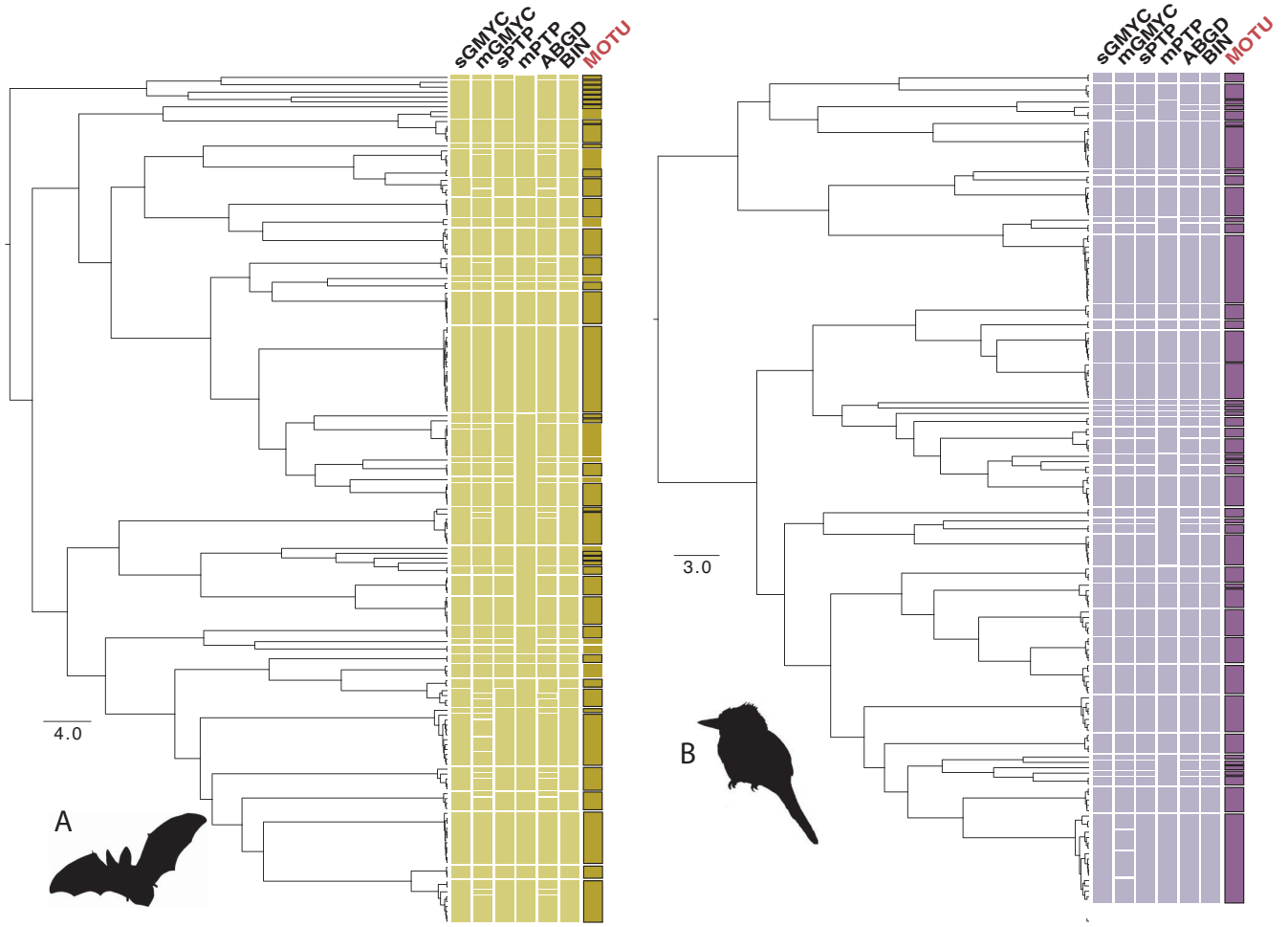

FIGURE 3 Bayesian chronograms based on a 1.2% of genetic divergence per Million years including DNA-based species delimitation derived from sGMYC, mGMYC, sPTP, mPTP, ABGD, RESL and final delimitation schemes based on majority rule consensus among the six methods for mammals (yellow) and birds (purple). The black outlined boxes correspond to MOTUs derived from specimens identified to the species level. Regular boxes indicates MOTUs derived from specimens identified to the genus level.

distance and distance to the nearest neighbour for MOTUs overlapped in a few cases for all classes (Figure 4a, 4b, 4g, 4h); however, a DNA barcoding gap was observed in most cases with only a few exceptions for fishes (Figure 4i). The proportion of MOTUs that could be assigned to species varied among classes with 71% for fishes, 100% for birds, 77% for mammals, 65% for amphibians and 48% for reptiles (Table S1). The unidentified MOTUs displayed varying trends of divergence to the nearest neighbour ranging from 0.6% to 22.5% for fishes, 3.5% to 20% for mammals, 1.2% to 23.5% for amphibians and 1.9% to 37% for reptiles (Table 3). Several cases of morphologically unrecognized MOTUs assigned to the same species were detected in all groups (Table 4). As observed for unidentified MOTUs, patterns of genetic distances displayed varying trends, with distances to the nearest neighbour ranging from 1.9% to 10.8% for fishes, 1.9% to 7.5% for amphibians, 1.5% to 2.1% for birds, 2.7% to 8.7% for mammals and 2.7% to 12% for reptiles (Table 4).

MOTU accumulation curves (Figure 5) indicate that the sampling is nearly representative for fishes and amphibians, with a plateau being almost reached; however, curves are far from reaching a plateau for birds and mammals, suggesting that the number of MOTUs recovered in this study underestimates the true vertebrate diversity in the Lengguru massif. The phylogeographic patterns were mostly congruent across groups in terms of spatial distribution and divergence (Figures 6–7). Multiple cases of closely related lineages that originated during the Pleistocene and occurring in sympatry or at neighbouring sites are detected for Fishes (Figure 6), reptiles (Figure 7a–7c) and amphibians (Figure 7d–7f), suggesting a contribution of in situ diversification to the

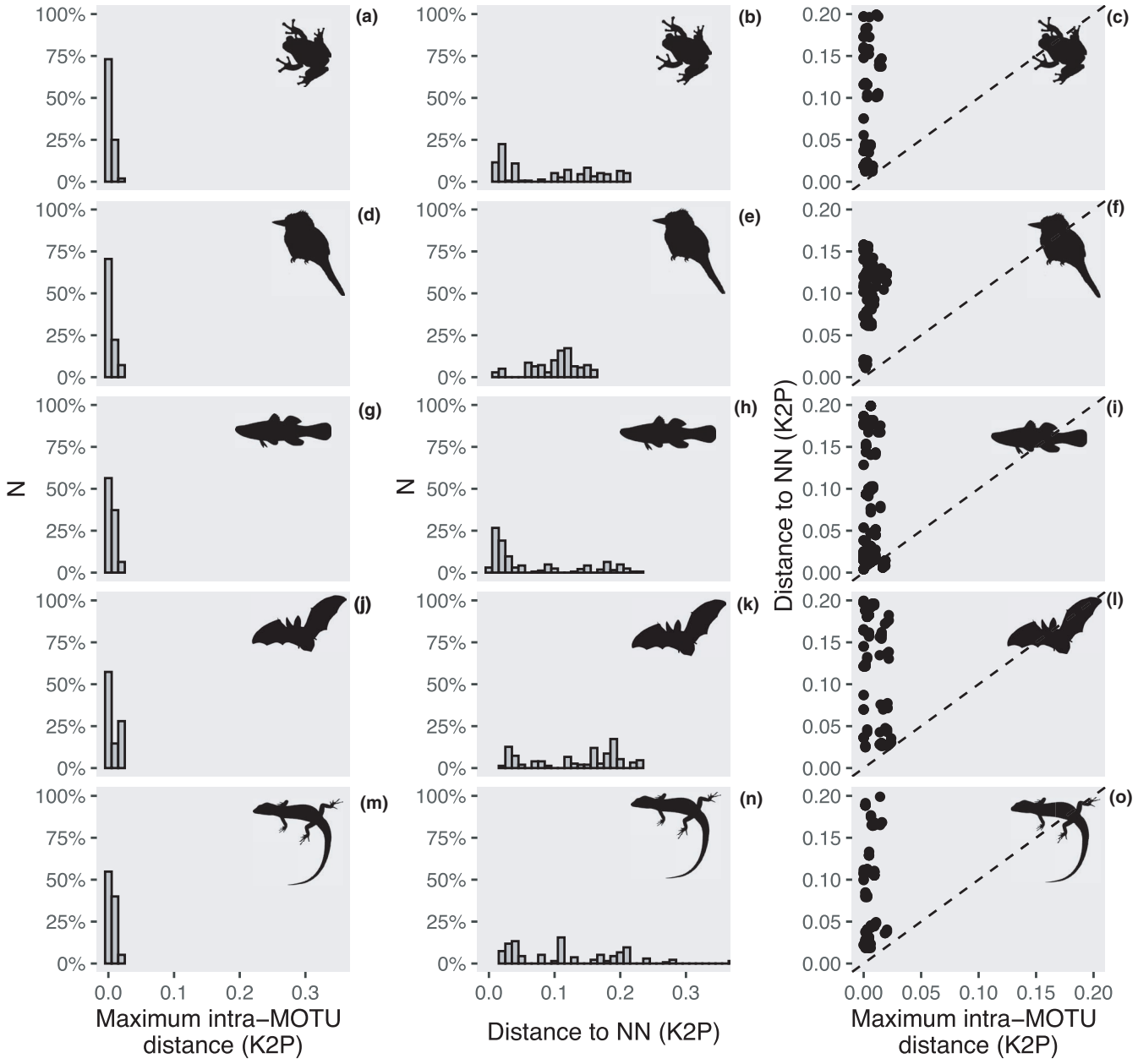

FIGURE 4 Distribution of genetic distances below and above MOTU boundaries for amphibians (a, b, c), birds (d, e, f), fishes (g, h, i), mammals (j, k, l) and reptiles (m, n, o). (a, d, g, j, m) Distribution of maximum intra-MOTU distances (K2P). (b, e, h, k, n) Distribution of nearest neighbour distances (K2P). (c, f, i, l, o) Relationship between maximum intra-MOTU and nearest neighbour distances. Points above the diagonal line indicate species with a barcode gap. MOTUs boundaries correspond to the final delimitation scheme derived from the majority rule consensus among the six delimitation methods.

diversity build-up in the area. For reptiles and amphibians, however, several cases of deep divergence, tracing back to the Miocene, between unidentified or cryptic MOTUs were detected (Figures 7a–5f).

4 | DISCUSSION

Ever since the seminal publications on DNA barcoding (Hebert, Cywinska, et al., 2003; Hebert & Gregory, 2005), numerous studies showed how DNA barcoding accelerated the development of molecular diagnostic tools for automated species identification in well-known faunas (April et al., 2011; Blagoev et al., 2015; Kerr et al., 2007; Knebelsberger et al., 2014; Shen et al., 2019). It also significantly helped in speeding up inventories and species discovery (Butcher et al., 2012; Hebert et al., 2004; Monaghan et al., 2009; Riedel et al., 2013; Smith et al., 2005, 2007, 2008; Tänzler et al., 2012). Our study confirms the benefits of integrating DNA barcoding into the taxonomic workflow of a biodiversity inventory in species-rich, yet poorly documented, biotas. This study contributed 1,005 new DNA barcode records to the reference

TABLE 3 List of the unidentified MOTUs including their genus assignment, consensus MOTU and BIN numbers, maximum within-MOTU K2P distance and K2P distance to the nearest neighbour

Taxa	Genus	MOTU	BIN	Distance max. (%)	Distance NN (%)
Amphibians	*Austrochaperina*	MOTU073	BOLD:ADN9008	0	11.6
Amphibians	*Austrochaperina*	MOTU074	BOLD:ADN9009	0.3	11.6
Amphibians	*Hylophorbus*	MOTU078	BOLD:ADN1146	-	14.8
Amphibians	*Hylophorbus*	MOTU080	BOLD:ADN4756	0.5	3.5
Amphibians	*Hylophorbus*	MOTU081	BOLD:ADO0048	0.2	1.2
Amphibians	*Hylophorbus*	MOTU082	BOLD:ADO3938	0.8	1.2
Amphibians	*Hylophorbus*	MOTU083	BOLD:ADO4150	0	14.8
Amphibians	*Lechriodus*	MOTU070	BOLD:ADO3037	-	22.8
Amphibians	*Oreophryne*	MOTU071	BOLD:ADN0004	0	19.7
Amphibians	*Oreophryne*	MOTU083	BOLD:ADO4150	0	17.3
Amphibians	*Oreophryne*	MOTU084	BOLD:AED5052	-	23.5
Amphibians	*Oreophryne*	MOTU085	BOLD:AED5053	0.2	17.3
Amphibians	*Oreophryne*	MOTU087	BOLD:ADO1683	1.3	19.7
Amphibians	*Xenorhina*	MOTU075	BOLD:ADN1930	-	18.8
Amphibians	*Xenorhina*	MOTU094	BOLD:ADO4474	1.5	13.7
Fishes	*Glossamia*	MOTU042	BOLD:ADM0638	-	21.8
Fishes	*Hippichthys*	MOTU057	BOLD:ADN0840	0.4	22.5
Fishes	*Melanotaenia*	MOTU002	BOLD:AAY9627	0	5.4
Fishes	*Melanotaenia*	MOTU003	BOLD:ABY7305	0	1.9
Fishes	*Melanotaenia*	MOTU004	BOLD:ABY8664	1.9	0.6
Fishes	*Melanotaenia*	MOTU006	BOLD:ACE4002	0	1.5
Fishes	*Melanotaenia*	MOTU007	BOLD:ADL9884	0	1.5
Fishes	*Melanotaenia*	MOTU008	BOLD:ADM3156	0.4	9.1
Fishes	*Melanotaenia*	MOTU009	BOLD:ADM8161	0.2	3.6
Fishes	*Microphis*	MOTU058	BOLD:ADN5559	1.5	16.7
Fishes	*Microphis*	MOTU059	BOLD:ADO3837	0.4	16.7
Fishes	*Neoarius*	MOTU051	BOLD:AAJ9962	1.5	7.7
Fishes	*Neoarius*	MOTU052	BOLD:ADL9301	0	3.8
Fishes	*Neoarius*	MOTU053	BOLD:ADM1229	0	2.3
Fishes	*Neoarius*	MOTU054	BOLD:ADM1230	0	2.3
Fishes	*Neosilurus*	MOTU055	BOLD:ADM9105	0	17.8
Mammals	*Coelops*	MOTU157	BOLD:ADJ4695	-	15.7
Mammals	*Echymipera*	MOTU191	BOLD:ADJ2534	-	3.5
Mammals	*Echymipera*	MOTU192	BOLD:ADJ2965	-	3.5
Mammals	*Hipposideros*	MOTU158	BOLD:ADI7709	-	14.5
Mammals	*Hipposideros*	MOTU159	BOLD:ADI7931	-	16.5
Mammals	*Hipposideros*	MOTU160	BOLD:ADJ0463	0	3.6
Mammals	*Melomys*	MOTU195	BOLD:ADI9381	-	14.7
Mammals	*Miniopterus*	MOTU179	BOLD:ADJ2078	1.6	15.4
Mammals	*Nyctimene*	MOTU170	BOLD:ABV8204	0.5	15.9
Mammals	*Pipistrellus*	MOTU183	BOLD:ADJ5548	-	20
Mammals	*Pipistrellus*	MOTU184	BOLD:ADJ5623	0	20
Reptilia	*Boiga*	MOTU205	BOLD:ADN8334	-	18.2
Reptilia	*Emoia*	MOTU239	BOLD:ADN9150	-	17.7

(Continues)

TABLE 3 (Continued)

Taxa	Genus	MOTU	BIN	Distance max. (%)	Distance NN (%)
Reptilia	*Emoia*	MOTU240	BOLD:ADO3330	0.2	10.7
Reptilia	*Emoia*	MOTU232	BOLD:ADM9606	0	10.9
Reptilia	*Emoia*	MOTU233	BOLD:ADM9607	-	10.9
Reptilia	*Emoia*	MOTU234	BOLD:ADN0860	0.5	12.9
Reptilia	*Emoia*	MOTU235	BOLD:ADN0861	1.6	16.5
Reptilia	*Emoia*	MOTU236	BOLD:ADN5383	0.8	16.4
Reptilia	*Emoia*	MOTU237	BOLD:ADN9148	-	16
Reptilia	*Emoia*	MOTU238	BOLD:ADN9149	-	12.9
Reptilia	*Emoia*	MOTU239	BOLD:ADN9150	0	2.2
Reptilia	*Emoia*	MOTU240	BOLD:ADO3330	0.6	1.9
Reptilia	*Emoia*	MOTU241	BOLD:ADO4309	0.2	1.9
Reptilia	*Eremiascincus*	MOTU244	BOLD:ADO2936	-	9.8
Reptilia	*Eremiascincus*	MOTU245	BOLD:ADO2937	-	9.8
Reptilia	*Gehyra*	MOTU221	BOLD:ADO7212	0.2	24
Reptilia	*Hypsilurus*	MOTU202	BOLD:ADN7192	0	37
Reptilia	*Lepidodactylus*	MOTU224	BOLD:ADE2841	-	23
Reptilia	*Nactus*	MOTU225	BOLD:ADO5284	-	24
Reptilia	*Ramphotyphlops*	MOTU264	BOLD:ADN5549	-	31
Reptilia	*Rhabdophis*	MOTU207	BOLD:ADN7031	-	10
Reptilia	*Sphenomorphus*	MOTU258	BOLD:ADO0519	-	8.7
Reptilia	*Sphenomorphus*	MOTU259	BOLD:ADO0520	0.9	16.5
Reptilia	*Sphenomorphus*	MOTU260	BOLD:ADO0521	-	21.2
Reptilia	*Sphenomorphus*	MOTU252	BOLD:ADN1408	0.2	8.5
Reptilia	*Sphenomorphus*	MOTU253	BOLD:ADN1409	0.6	17.5
Reptilia	*Sphenomorphus*	MOTU254	BOLD:ADN5384	-	8.5
Reptilia	*Sphenomorphus*	MOTU255	BOLD:ADN5577	-	19.7
Reptilia	*Sphenomorphus*	MOTU256	BOLD:ADO0517	-	15.8
Reptilia	*Sphenomorphus*	MOTU257	BOLD:ADO0518	-	4.3
Reptilia	*Sphenomorphus*	MOTU258	BOLD:ADO0519	0.9	10.5
Reptilia	*Sphenomorphus*	MOTU259	BOLD:ADO0520	1.1	4.3
Reptilia	*Sphenomorphus*	MOTU260	BOLD:ADO0521	-	8.7

library for the Bird's Head Peninsula, including 264 MOTUs whose delimitation was corroborated by most DNA-based delimitation methods applied (Figures 2–3). The distances to the nearest-neighbour are usually exceeding maximum intra-MOTU distances by an order of magnitude of 12 (Table 2); and a barcode gap is generally observed (Figure 4). A single case of DNA barcode sharing is observed in mammals, when a specimen of *Sus scrofa* was nested within *S. verrucosus*. This was expected, considering the reported introgression among wild *Sus* species, as well as between domesticated and wild lineages (Scandura et al., 2011). Along the same line, species delimitation analyses failed to separate a single species pair, including *Rattus praetor* and *R. tanezumi* (mammals, MOTU199).

Several cases of MOTUs displaying small genetic distances among them were detected among fishes, amphibians and reptiles (Figure 5) including some newly discovered MOTUs and/or multiple MOTUs within one species entity delineated based on morphology. Several cases of large conflicts between PTP and other algorithms were associated to cases of multiple MOTUs displaying small genetic distances among them. In particular, none of the *Melanotaenia goldiei* and *M. mairasi* MOTUs were found by the sPTP and mPTP algorithms, resulting in the lowest estimate of numbers of MOTUs for all methods (Table S1). MOTUs of these two *Melanotaenia* groups displayed much lower K2P genetic distance to their nearest neighbours than in other fish lineages. Similar discrepancies between PTP and other methods such as GMYC were previously described (Luo et al., 2018; Shen et al., 2019), with PTP being less effective when large number of species and varying divergence levels were involved. Several similar cases were also observed for amphibians (e.g., *Asterophrys pullifer* MOTUs, *Hylophorbus* spp.), reptiles (e.g., *Cyrtodactylus irianjayensis*

TABLE 4 List of MOTUs assigned to the same species based on morphological characters, including MOTU and BIN numbers, maximum within-MOTU K2P distance (percent) and K2P distance to the nearest neighbour (percent)

Taxa	Genus	MOTU	BIN	Distance max.	Distance NN
Amphibia	*Cornufer papuensis*	MOTU060	BOLD:ADN5223	0.8	1.4
Amphibia	*Cornufer papuensis*	MOTU061	BOLD:ADN6050	-	6.5
Amphibia	*Cornufer papuensis*	MOTU062	BOLD:ADN9748	0.3	1.4
Amphibia	*Cornufer bimaculatus*	MOTU063	BOLD:ADO0083	0	7.5
Amphibia	*Cornufer bimaculatus*	MOTU064	BOLD:ADO0084	-	7.5
Amphibia	*Cornufer bimaculatus*	MOTU065	BOLD:ADO0085	-	3.9
Amphibia	*Cornufer bimaculatus*	MOTU066	BOLD:ADO0272	-	3.9
Amphibia	*Cornufer punctatus*	MOTU067	BOLD:ADN3984	0.6	4.3
Amphibia	*Cornufer punctatus*	MOTU068	BOLD:ADN5222	0.2	4.3
Amphibia	*Asterophrys pullifer*	MOTU088	BOLD:ADO1683	0.2	2.1
Amphibia	*Asterophrys pullifer*	MOTU089	BOLD:ADO2921	0	3.7
Amphibia	*Asterophrys pullifer*	MOTU090	BOLD:ADO2922	0.2	2.1
Amphibia	*Sphenophryne cornuta*	MOTU091	BOLD:ADN1659	-	2.4
Amphibia	*Sphenophryne cornuta*	MOTU092	BOLD:ADO0053	0	1.9
Amphibia	*Sphenophryne cornuta*	MOTU093	BOLD:ADO0994	0	1.9
Amphibia	*Litoria infrafrenata*	MOTU098	BOLD:AAN2556	0.3	2
Amphibia	*Litoria infrafrenata*	MOTU099	BOLD:ADN6283	0.3	2
Aves	*Melilestes megarhynchus*	MOTU121	BOLD:AAF2363	0	1.5
Aves	*Melilestes megarhynchus*	MOTU122	BOLD:AAF2363	0.3	1.5
Fishes	*Giuris margaritaceus*	MOTU028	BOLD:AAK3399	-	10.8
Fishes	*Giuris margaritaceus*	MOTU029	BOLD:ADM7171	-	10.8
Fishes	*Mogurnda mogurnda*	MOTU030	BOLD:AAD3229	0	2.1
Fishes	*Mogurnda mogurnda*	MOTU031	BOLD:AAD3229	1	2.1
Fishes	*Mogurnda mogurnda*	MOTU032	BOLD:AAD3229	0	2.5
Fishes	*Toxotes oligolepis*	MOTU049	BOLD:ADM9057	0.4	1.9
Fishes	*Toxotes oligolepis*	MOTU050	BOLD:ADM9058	0.2	1.9
Mammals	*Sus scrofa*	MOTU150	BOLD:AAA3445	2.4	2.7
Mammals	*Sus scrofa*	MOTU151	BOLD:AAA3445	-	2.7
Mammals	*Nyctimene albiventer*	MOTU171	BOLD:ABV8022	0.3	4.3
Mammals	*Nyctimene albiventer*	MOTU172	BOLD:ADI7768	2	4.3
Mammals	*Syconycteris australis*	MOTU176	BOLD:ABV9982	-	2.7
Mammals	*Syconycteris australis*	MOTU177	BOLD:ABV9982	1.7	2.7
Mammals	*Dorcopsis muelleri*	MOTU185	BOLD:ADI7672	-	5.7
Mammals	*Dorcopsis muelleri*	MOTU186	BOLD:ADI9488	-	5.7
Mammals	*Echymipera kalubu*	MOTU193	BOLD:ADI7714	0.2	2.5
Mammals	*Echymipera kalubu*	MOTU194	BOLD:ADJ1796	-	2.5
Mammals	*Paramelomys platyops*	MOTU197	BOLD:ADI8691	0	8.7
Mammals	*Paramelomys platyops*	MOTU198	BOLD:ADJ4724	-	8.7
Reptiles	*Cyrtodactylus irianjayaensis*	MOTU213	BOLD:AED5725	-	10.5
Reptiles	*Cyrtodactylus irianjayaensis*	MOTU214	BOLD:AED5297	-	12
Reptiles	*Cyrtodactylus irianjayaensis*	MOTU215	BOLD:ADN4791	0	10.7
Reptiles	*Cyrtodactylus irianjayaensis*	MOTU216	BOLD:ADN4792	-	12
Reptiles	*Cyrtodactylus irianjayaensis*	MOTU217	BOLD:ADN9189	-	10.5
Reptiles	*Cyrtodactylus sermowaiensis*	MOTU219	BOLD:ADO1322	0.5	2.7
Reptiles	*Cyrtodactylus sermowaiensis*	MOTU220	BOLD:ADO1323	0.2	2.7

(Continues)

TABLE 4 (Continued)

Taxa	Genus	MOTU	BIN	Distance max.	Distance NN
Reptiles	*Lygisaurus novaeguineae*	MOTU246	BOLD:ADN7325	0.3	3.6
Reptiles	*Lygisaurus novaeguineae*	MOTU247	BOLD:ADN8226	2	3.6
Reptiles	*Lygisaurus novaeguineae*	MOTU248	BOLD:ADO2052	-	6.1

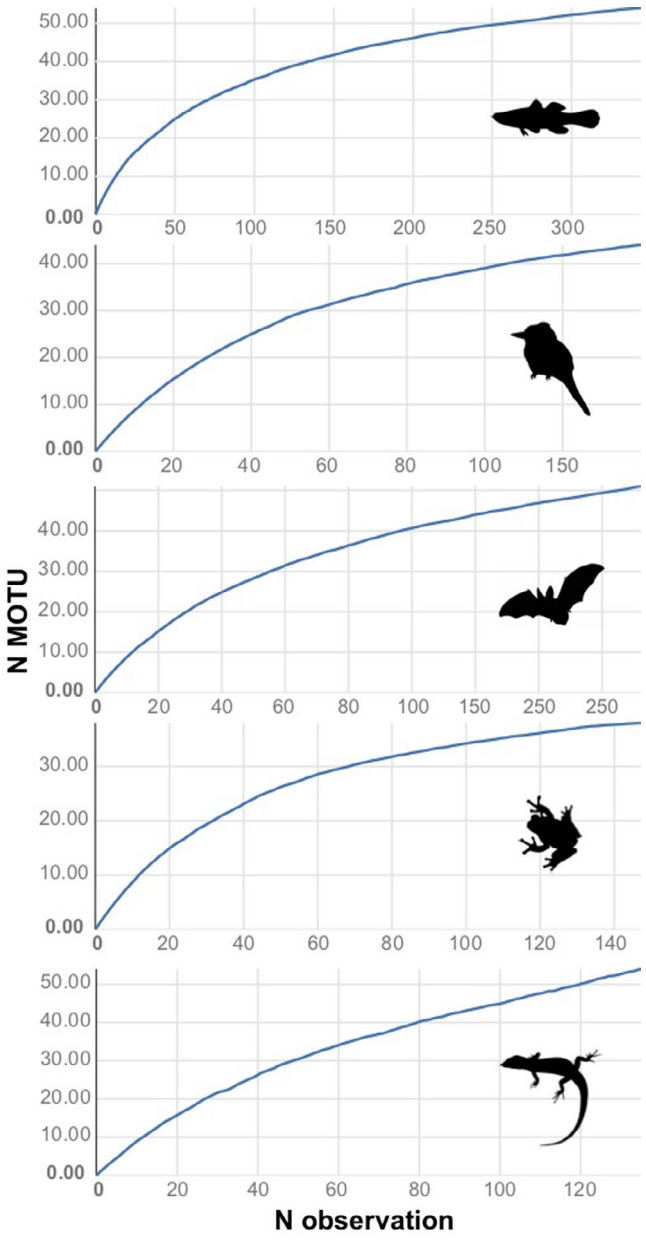

FIGURE 5 MOTU accumulation curves for fishes, birds, mammals, amphibians and reptiles. The x-axis varies among classes.

MOTUs, *Emoia* spp.) and mammals (e.g., *Hipposideros* spp.). Along the same line, GMYC is known to produce excessive splitting if based on maximum credibility trees without collapsing sequences into haplotypes. Here, applying GMYC algorithms to sampled trees along the merged Markov chain, using the complete DNA barcode data set, yielded numbers of MOTUs that compared favourably to other methods, with mGMYC providing generally a better match to the final consensus. These results confirm the benefits of combining several species delimitation methods and using a consensus instead of a single method when it comes to avoiding artefacts (Blair & Bryson, 2017; Delrieu-Trottin et al., 2020; Kekkonen & Hebert, 2014; Kekkonen et al., 2015; Shen et al., 2019). Aside from these particular cases, methods were largely congruent and resulted in the delimitation of MOTUs with homogeneous maximum intraspecific K2P distance distribution across classes (Figure 4).

The proportion of MOTUs that were only identified to the genus level is high for fishes, amphibians and reptiles with a total of 75 MOTUs not identified to the species level (Table 3). This trend is markedly different from previous molecular studies of vertebrates in the neighbouring biogeographical provinces of Sundaland and Wallacea, where identification to the species level is much more common (Arida, 2017; Bernstein et al., 2020; Conte-Grand et al., 2017; Dahruddin et al., 2017; Sholihah et al., 2020). Along the same line, the number of cryptic MOTUs is substantial with 48 MOTUs delimited within 19 species across the five classes (Table 4), the number of cryptic lineages within species ranging from two in most cases to five in *Cyrtodactylus irianjayensis* (reptiles). This trend was expected for some genera such as *Melanotaenia* for which DNA-based methods already helped to discover multiple new species in the Western parts of the island of New Guinea (Kadarusman et al., 2012; Nugraha et al., 2015). We found seven new MOTUs of *Melanotaenia*, which are now awaiting description (Table 3). Multiple cases of high and formally undescribed diversity were also detected here such as for the amphibian genera *Hylophorbus* (five MOTUs) and *Oreophryne* (four MOTUs), the reptile genera *Emoia* (12 MOTUs) and *Sphenomorphus* (12 MOTUs) and the bat genus *Hipposideros* (3 MOTUs). The result for amphibians was expected as previous similar efforts in tropical species-rich and poorly explored areas, for example, the Amazon, yielded similar results (Fouquet et al., 2007; Vacher et al., 2020). This study highlights that diversity of the continental vertebrate biotas of the Bird's Head Peninsula is probably largely underestimated. Species accumulation curves are still far from reaching a plateau, especially for birds, mammals and reptiles. This vast diversity is confined to very restricted areas. Our inferences indicate that some of the MOTUs (*Melanotaenia golidiei* group, *Melanotaenia mairasi* group, *Asterophrys pullifer*, *Hylophorbus* spp., *Sphenomorphus* spp. and *Emoia* spp.) may have diversified during the Pleistocene and are distributed in the periphery of the Lengguru massif (Figures 6–7). These particular cases, also pointed out by conflicting PTP and GMYC species delimitation results, suggest a recent in situ origin through allopatric speciation on small spatial scales. This trend was expected considering the complex geological history of the Lengguru massif that experienced an intense orogenic activity over the past 5 million years (Bailly et al., 2009; Villeneuve et al., 2010). In

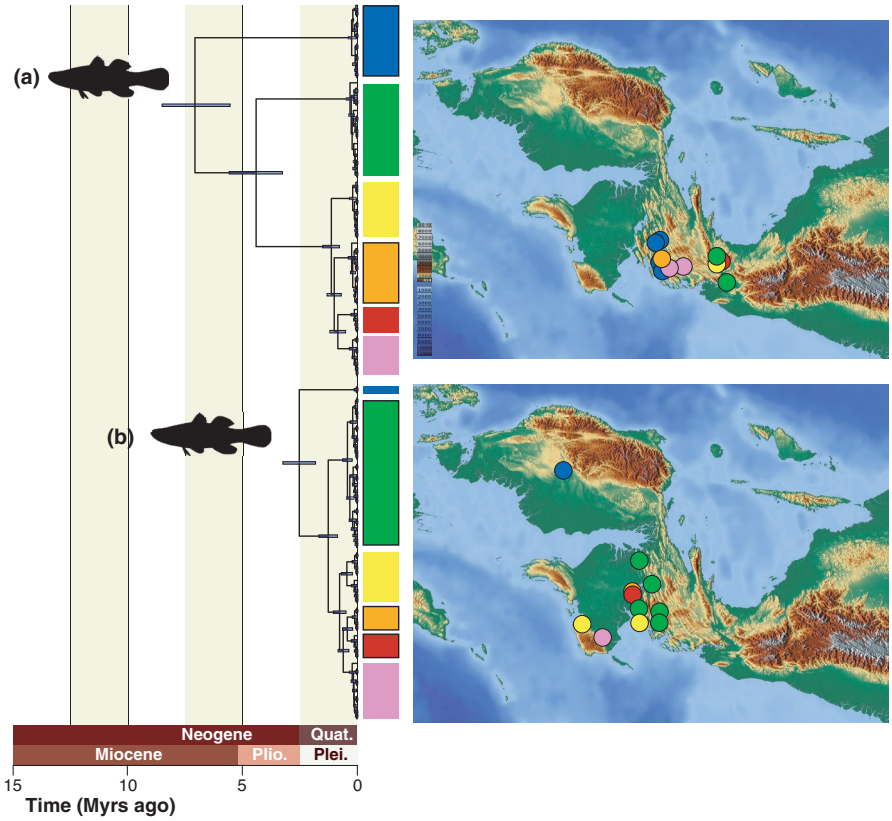

FIGURE 6 Phylogeographic patterns among selected groups of fishes. MOTUs are represented according to the final delimitation schemes based on majority rule consensus among the six methods. The black outlined boxes correspond to MOTUs derived from specimens identified to the species level. Regular boxes indicate MOTUs derived from specimens identified to the genus level. (a) *Melanotaenia mairasi* group including *M. mairasi* (blue), *M. goldiei* (orange), MOTU008 (green), MOTU007 (yellow), MOTU006 (red), MOTU003 (pink). (b) *Melanotaenia ammeri* group including *M. ammeri* (green), *M. arguni* (orange), *M. veoliae* (red), MOTU002 (blue), MOTU004 (yellow) and MOTU005 (pink).

addition, karsts are highly fragmented landscapes that foster geographic isolation and promote endemism (Clements et al., 2006; Polhemus & Allen, 2006). The present study further suggests that the build-up of species diversity in the Lengguru massif probably originated through a combination of immigration and in situ diversification over the course of its geological history as previously reported, for example, for the genus *Melanotaenia* (Kadarusman et al., 2012; Unmack et al., 2013). This calls for an increased effort to document further New Guinea's biota and to develop rapidly a critical mass of biological expertise in Indonesia Papua, particularly in times of ongoing deforestation and habitat loss (Austin et al., 2019; Filer et al., 2009; Nelson et al., 2014; Novotny & Molem, 2020; Shearman & Bryan, 2011).

5 | CONCLUSIONS

The present study highlights major biodiversity knowledge gaps in the Bird's Head Peninsula, and confirms the utility of standardized DNA-based species delimitation methods in aiding biodiversity inventories. Applied to poorly surveyed faunas, such as those of the island of New Guinea, they facilitate the discovery of previously unknown biodiversity and highlight priorities for further taxonomic study. Here, a total of 123 MOTUs, corresponding 75 unidentified and 48 unrecognized MOTUs based on an initial screening of their morphology, are waiting a re-examination of their morphological characters and potentially a formal description. This number of potential new species is high and still underestimated for several groups such as reptiles, a trend that further points to the need to improve our knowledge of this biodiversity-rich island. Our study clearly shows how much we still do not know about the nonmarine vertebrate diversity of the Bird's Head Peninsula. Given that many species are still awaiting discovery and that we are looking at an accelerated loss of forest and other suitable habitat in West Papua, the need for priority conservation is paramount.

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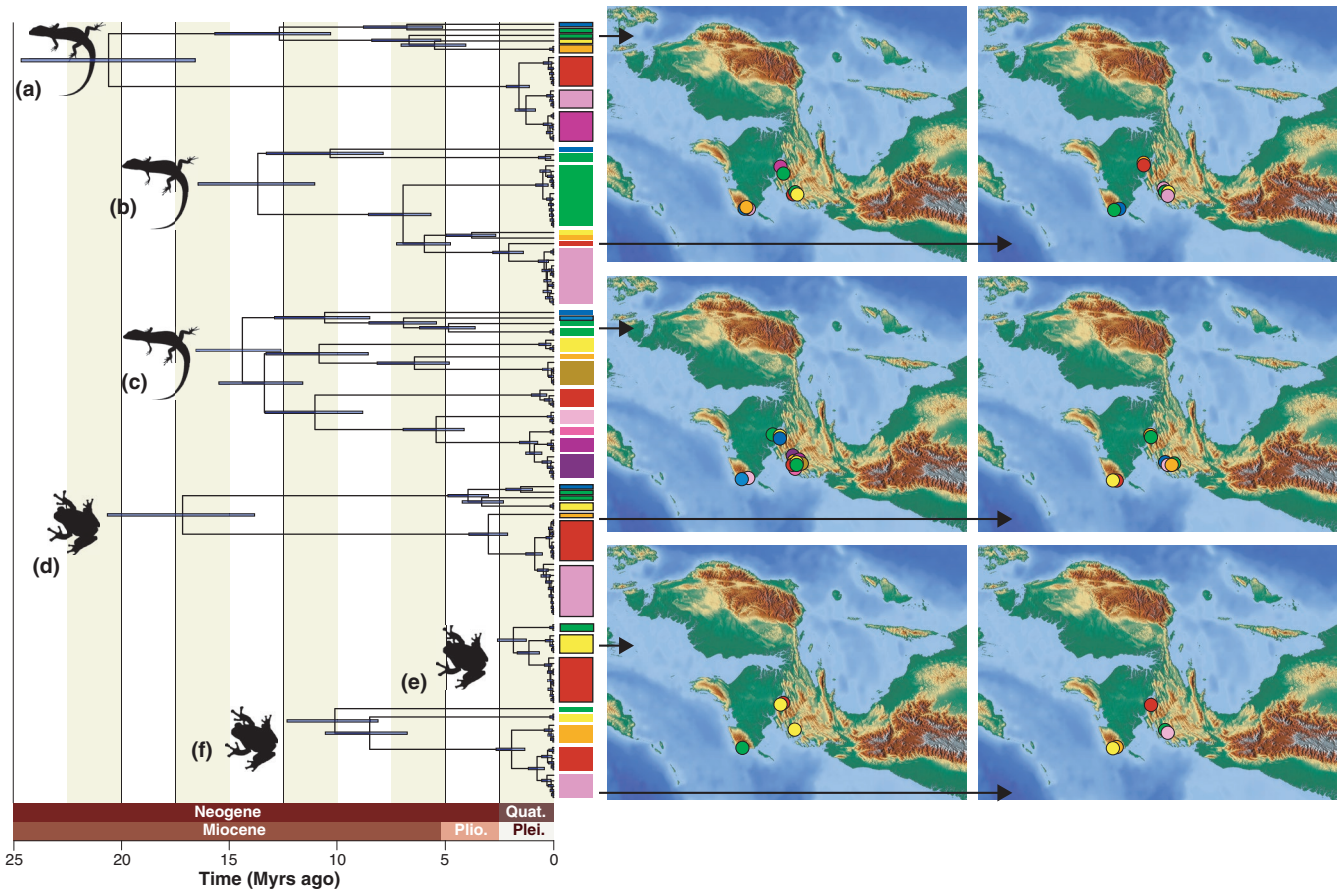

FIGURE 7 Phylogeographic patterns among selected groups of reptiles (a, b, c) and amphibians (d, e, f) displaying high diversity of closely related MOTUs occurring on restricted spatial scales. MOTUs are represented according to the final delimitation schemes based on majority rule consensus among the six methods. The black outlined boxes correspond to MOTUs derived from specimens identified to the species level. Regular boxes indicate MOTUs derived from specimens identified to the genus level. (a) *Cyrtodactylus* spp. including *Cyrtodactylus irianjayaensis* MOTU212 (yellow), MOTU213 (dark green), MOTU214 (orange), MOTU215 (blue), MOTU216 (light green), *Cyrtodactylus marmoratus* (red) and *Cyrtodactylus sermowaiensis* MOTU218 (purple) and MOTU219 (pink). (b) *Sphenomorphus* spp. including MOTU255 (blue), MOTU249 (dark green), MOTU257 (light green), MOTU248 (yellow), MOTU259 (orange), MOTU256 (red) and MOTU258 (pink). (c) *Emoia/Sphenormorphus* spp. including *Emoia jakati* (light blue,) MOTU236 (dark blue), MOTU232 (dark green), MOTU235 (yellow), MOTU237 (orange), MOTU233 (brown), MOTU234 (red), MOTU230 (pink), MOTU238 (light purple), MOTU240 (purple) and MOTU239 (dark purple). (d) *Cornufer* spp. including *Cornufer bimaculatus* MOTU063 (blue), MOTU065 (dark green), MOTU064 (light green) and MOTU063 (yellow), *Cornufer papuensis* MOTU061 (orange), MOTU062 (red) and MOTU060 (pink). (e) *Asterophrys pullifer* including MOTU089 (green), MOTU088 (yellow) and MOTU090 (red). (f) *Hylophorbus* spp. including MOTU079 (green), MOTU083 (yellow), MOTU080 (orange), MOTU081 (red) and MOTU082 (pink).

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AUTHOR CONTRIBUTIONS

Laurent Pouyaud, Gono Semiadi, Régis Hocdé, Jacques Slembrouck, and Kadarusman organized the expedition and designed the survey; Awal Riyanto, Philippe Gaucher, Amir Hamidy, Antoine Fouquet, Evy Arida, Mulyadi, W, Christophe Cochet, Jacques Slembrouck, Laurent Pouyaud, Marc Legendre, Régis Hocdé, Kadarusman, Sopian Sauri, Edy H.P. Melmambessy, AA, Aksamina M. Yohanita, Apandi, Gono Semiadi, Nanang Supriatna, Sigit Wiantoro, Christophe Thébaud, H, Borja Mila, Hadi Wikanta, Mohammad Irham and Suparno conducted the field sampling and curated the specimens; Nicolas Hubert, Hadi Dahruddin, Yuli Sulistya Fitriana, Evy Arida, Hidayat Ashari, Alex Borisenko, Antoine Fouquet and Dirk Steinke conducted the sequencing and quality control; Nicolas Hubert, Dirk Steinke, Alex Borisenko, Erwan Delrieu-Trottin, Yuli Sulistya Fitriana, Gono Semiadi, Antoine Fouquet and Evy Arida submitted data records on BOLD and analysed the data. All authors contributed in drafting and revising the manuscript.

DATA AVAILABILITY STATEMENT

Sequence data and associated collection information have been made available on the Barcode of Life Datasystem (BOLD) in the data set "DS-LENG: DNA barcode reference library of some West Papua vertebrates" (dx.doi.org/10.5883/DS-LENG).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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