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Phylogenomics, biogeography and taxonomic revision of New Guinean pythons (Pythonidae, *Leiopython*) harvested for international trade

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ABSTRACT

The large and enigmatic New Guinean pythons in the genus *Leiopython* are harvested from the wild to supply the international trade in pets. Six species are currently recognized (*albertisii*, *biakensis*, *fredparkeri*, *huonensis*, *meridionalis*, *montanus*) but the taxonomy of this group has been controversial. We combined analysis of 421 nuclear loci and complete mitochondrial genomes with morphological data to construct a detailed phylogeny of this group, understand their biogeographic patterns and establish the systematic diversity of this genus. Our molecular genetic data support two major clades, corresponding to *L. albertisii* and *L. fredparkeri*, but offer no support for the other four species. Our morphological data also only support two species. We therefore recognize *L. albertisii* and *L. fredparkeri* as valid species and place *L. biakensis, L. meridionalis, L. huonensis* and *L. montanus* into synonymy. We found that *L. albertisii* and *L. fredparkeri* are sympatric in western New Guinea; an atypical pattern compared to other Papuan species complexes in which the distributions of sister taxa are partitioned to the north and south of the island's central mountain range. For the purpose of conservation management, overestimation of species diversity within *Leiopython* has resulted in the unnecessary allocation of resources that could have been expended elsewhere. We strongly caution against revising the taxonomy of geographically widespread species groups when little or no molecular genetic data and only small morphological samples are available.

1. Introduction

Many of the world's biodiversity hotspots are tropical regions characterized by their remoteness and inaccessibility. These traits have impeded detailed specimen collection, creating challenges for taxonomic assessments (Heads, 2002; Reddy, 2014; Magurran, 2017). In some cases, small sample sizes for systematic revisions based on morphology may result in investigators overlooking cryptic taxa (Bickford et al., 2007). In other cases, small sample sizes for morphological assessments may result in observed variation among specimens being considered of diagnostic significance, resulting in the splitting of species that may indeed not warrant specific status (Donnellan et al., 2015; Hillis, 2019). The advance of molecular genetic techniques in phylogenetic systematics has helped to both reveal cryptic species and temper cases of natural variation being inadvertently misinterpreted and subsequently used to diagnose novel taxa (Larson, 1994; Donnellan et al., 2015).

The island of New Guinea is a region of immense biodiversity and complex topography with numerous areas of endemism (Beehler, 2007; Polhemus, 2007). However, the island is large, remote, and topographically complex, so sampling across the geographic range of some widespread species has been limited (Bruxaux et al., 2018). For example,

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Heads (2002), in his review of Papuan biogeography, noted: *'it is sometimes felt that the New Guinea biota is so hopelessly under-collected that valid generalizations about distribution there are impossible'*. In other cases, even if sampling for some species is geographically widespread, too few samples are available to adequately assess morphological variation for taxonomic purposes (McDowell, 1975).

The white-lipped pythons, *Leiopython* Hubrecht 1879, comprise a widespread and enigmatic species complex across New Guinea and many of its offshore islands (O'Shea, 1996). White-lipped pythons are harvested from the wild in West Papua and Papua, Indonesia, for the international pet trade (Natusch and Lyons, 2012). Two obvious morphological variants are recognized – a gold form (*L. albertisii*) from northern and western New Guinea, and a brown/black form (*L. meridionalis*) found in southern and eastern New Guinea (O'Shea, 1996; Schleip, 2014). These forms were shown to be separate taxa by Schleip (2008) based on morphology and mitochondrial DNA. In addition, Schleip (2008) described another four taxa from New Guinea using morphological data only – *L. biakensis*, *L. fredparkeri*, *L. huonensis*, and *L. montanus*. The morphological differences used to diagnose each species were subtle and included minor variations in head scalation and mean body scale counts. No ecological data accompanied the species descriptions, and the extent of the distribution of each taxon remains unknown (for a detailed description of each species and its distribution see Schleip [2008]). Without accompanying molecular genetic data from the additional taxa described, coupled with the relatively small and sparse geographic sample available to Schleip (2008), it is not known whether those taxa represent genuine evolutionary forms worthy of conservation action or whether they instead represent a series of morphological variants.

All species in the Family Pythonidae are listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Therefore, all species of *Leiopython* require non-detriment findings (i.e. risk assessments to help ensure wild harvest sustainability; CITES [2017]) to be undertaken before exports can occur. Moreover, customs and enforcement personnel in both importing and exporting countries are required to differentiate specimens of each species to accurately report trade transactions. The identification requirement is likely to be ineffective due to taxonomic uncertainty and by a paucity of morphological characters with which to identify each *Leiopython* species.

Here, we use next generation sequencing of 421 nuclear loci and complete mitochondrial genomes of *Leiopython* to comprehensively assess the molecular genetic diversity found within this clade and to examine the six species hypothesis proposed by Schleip (2008). In addition, we examine morphological data from specimens from New Guinea to test the diagnostic value of the morphological characters used to identify the currently recognized *Leiopython* species.

2. Materials and methods

2.1. Genetic specimen sampling, DNA sequencing and alignment

We obtained tissue samples for our molecular genetic analyses that covered a wide geographic spread encompassing all the six putative species proposed by Schleip (2008). All tissue samples are held in the Australian Biological Tissue Collection (ABTC); location data are presented in Fig. 1 and further genetic sample details are provided in Table S1 in Supplementary Material.

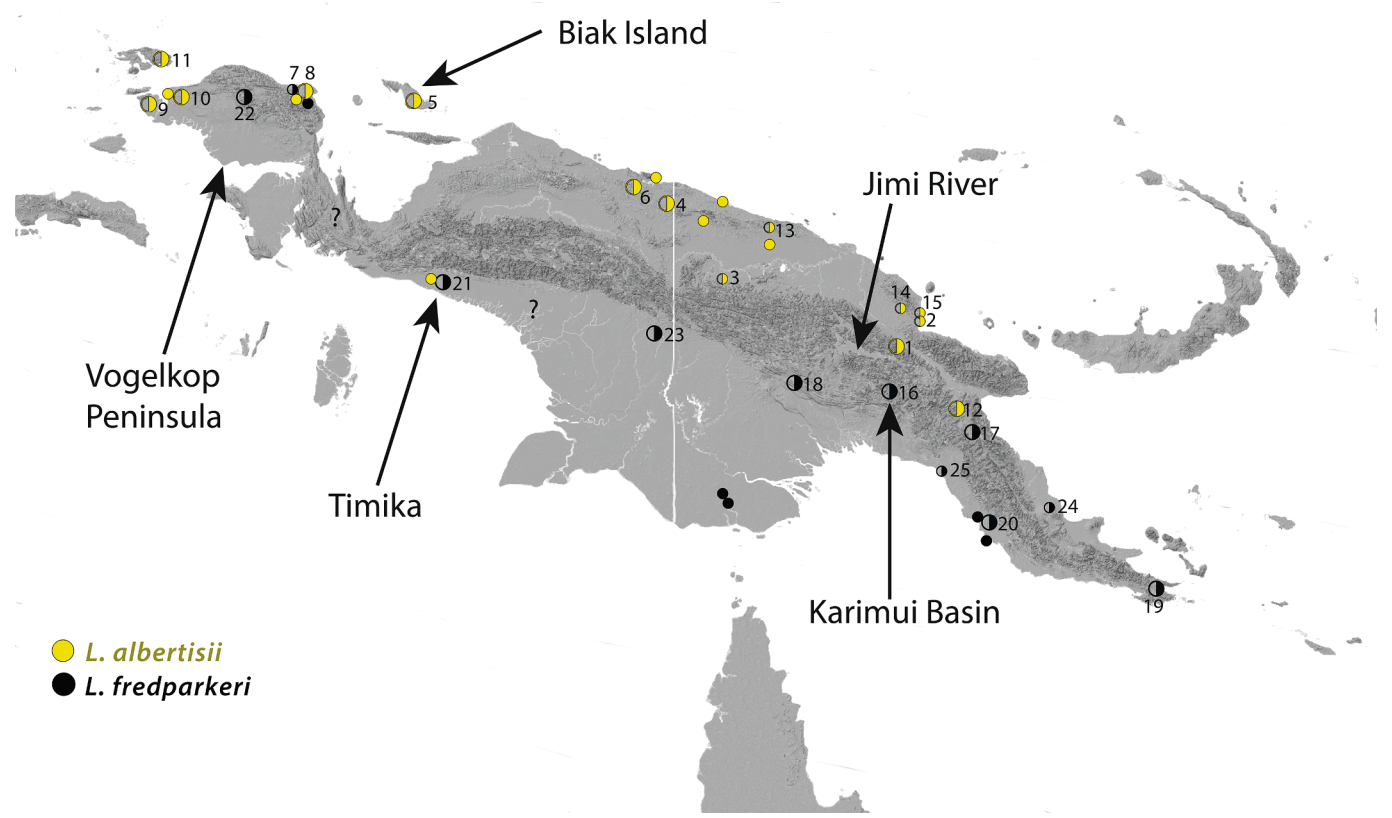

Fig. 1. Sampling localities for *Leiopython albertisii* and *L. fredparkeri*. Whole circles depict localities for which only morphological data were available. Large half-filled circles represent localities for which mtDNA, nDNA and morphological data were available. Small half-filled circles depict localities for which mtDNA and morphological data were available. Question marks indicate a lack of understanding about the extent of distribution of the two taxa identified in our study. Specimen numbers correspond to those provided in Table S1. Key regions discussed in the text are indicated by arrows.

We extracted DNA using a Qiagen DNeasy Blood & Tissue kit. The data were collected at the Center for Anchored Phylogenomics (www.anchoredphylogeny.com) at Florida State University using anchored hybrid enrichment (AHE; Lemmon et al., 2012), with library preparation, sequencing, assembling, orthology detection and alignment following Natusch et al. (2020). The final dataset comprised 421 loci, with an average length of 1861 bp per locus.

We also reconstructed the mitochondrial genomes from the raw reads retrieved as by-catch from the AHE sequence captures for each sample using MITObim version 1.9 (Hahn et al., 2013) using a script available at www.github.com/IanGBrennan/mitoGenome_Assembly. We used the mitochondrial genome of *Python regius* GenBank AB177878 (Dong and Kumazawa, 2005) as a reference. We aligned the sequences using MAFFT version 7.3 (Katoh and Standle, 2013) and then carefully inspected the alignment by eye. Furthermore, we sequenced *cytb* for additional samples that were not included in the AHE sequence captures to align with the mitochondrial genomes (see Table S1 in Supplementary Material for specimen information). To amplify via Polymerase Chain Reaction (PCR) and sequence *cytb* we followed the protocols described in Natusch et al., (2020).

2.2. Phylogenetic hypotheses

To estimate the optimal partitioning scheme and substitution model for each partition, for both the mtDNA and nDNA, we used the ModelFinder algorithm implemented in *IQ-Tree* v. 1.7 (Nguyen et al., 2015; Chernomor et al., 2016; Kalyaanamoorthy et al., 2017). The optimal partitioning schemes comprised 18 and five partitions for the nDNA and mtDNA alignments, respectively. We then used *IQ-Tree* to infer the maximum likelihood tree for each of the nDNA and mtDNA concatenated alignments and each locus separately, and assessed phylogenetic uncertainty with 1000 ultrafast bootstraps (Hoang et al., 2018), and the gene concordance factor (gCF) and the novel site concordance factor (sCF) for the nDNA data (Minh et al., 2018).

To estimate a species tree based on the multi-species coalescent we used *Astral III* v.5.6 (Zhang et al., 2017). This method infers a species tree using individual gene trees, which were generated for each nuclear locus using *IQ-Tree*. Astral III finds the species tree that has the maximum number of shared induced quartets among all the gene trees. It estimates branch lengths in coalescent units and branch support as local posterior probabilities (Sayyari and Mirarab, 2016).

2.3. Population structure

We assessed population genetic structure and admixture using the program *STRUCTURE* version 2.3 (Pritchard et al., 2000). *STRUCTURE* uses genetic markers such as single nucleotide polymorphisms (SNPs), which we extracted from the nuclear loci using the R package *phrynomics* (Leaché et al., 2015). Since we used several SNPs per locus, we implemented the linkage model with correlated allele frequencies. We performed five independent runs, with K (number of genetic clusters) of one to four. Each run comprised 500,000 MCMC generations with a burnin of 50,000 steps. We extracted the most likely value of K using the Evanno or ΔK method (Evanno et al., 2005) and the ln $\Pr(D|K)$ method (Pritchard et al., 2000) implemented in STRUCTURE HARVESTER (Earl and vonHoldt, 2012) and produced the genetic structure plots in CLUMPAK (Kopelman et al., 2015), which uses CLUMPP (Jakobsson and Rosenberg, 2007) and DISTRUCT version 1.1 (Rosenberg, 2004).

2.4. Species delimitation

Analysis of gene trees, species trees and population genetic structure supported only two genetic clusters corresponding to the northern and western gold populations and to the southern and eastern brown/black populations (which according to the principle of priority would carry the names *L. albertisii* and *L. fredparkeri*, respectively). To provide a further test of this two species hypothesis we conducted a species delimitation analysis using the Bayesian program BPP v. 4.1. (Flouri et al., 2018). BPP implements the multi-species coalescent to compare the posterior probability of different species delimitation models (Rannala and Yang, 2013; Yang and Rannala, 2010). We fixed the topology based on our phylogenetic analyses, which only includes two putative species, and assigned conservative priors to ancestral population size (θ) and shallow divergence times (τ) with *inverse gamma* (3, 0.2) and *inverse gamma* (3, 0.002), respectively. We ran the reversible jump (rj) MCMC for 50,000 generations, with a burnin of 2,000 and a sampling frequency of four. We performed this twice to confirm convergence between the runs.

2.5. Morphological analysis

The taxonomy of Schleip (2008) is primarily based on morphological data (scalation). We expanded on Schleip's (2008) morphological dataset with more geographically comprehensive specimen sampling for the northern populations (Table 1; Table S2). To achieve this we gathered morphological data from 116 specimens of *Leiopython* representing five of the six species described by Schleip (2008). We assigned specimens to each putative species based on the locality descriptions provided by Schleip (2008), who did not consider any species to occur in sympatry. These specimens were held in museum collections that were not examined by Schleip (2008), specifically the: Australian Museum, Sydney (AMS); Australian National Wildlife Collection, Canberra (ANWC); Museum Victoria (MV), Museum Zoologicum Bogoriense, Bogor (MZB); Queensland Museum, Brisbane (QM); and the University of Papua, Manokwari (UPM). We further extended the sample set by examining specimens captured in the field alongside local villagers (*sensu* Natusch and Lyons, 2012). We only included specimens whose specific locality could be confirmed. In most cases, this meant excluding pythons held by middlemen or major collectors at transit ports.

We examined the 13 morphological characters used by Schleip (2008) to delimit species in his multivariate analysis (Table 1). We broadly tested the diagnostic ability of Schleip's (2008) morphological characters on our new dataset. We did not have access to specimens of *L. huonensis* described by Schleip (2008). In addition, we examined the heads of four specimens from Bulolo, which we included as *L. montanus* (the type locality, Wau, is only 12 km away and at the same elevation [1000 m] as the collection site at Bulolo). Because only the head was available, morphological data for these specimens was incomplete. Following Schleip (2008), we pooled the sexes for our analyses and used non-parametric Kruskal-Wallis tests to examine significant differences in individual morphological characters among taxa. To visualize differences in continuous morphological characters between the identified taxa, we first imputed the missing data using a random forest machine learning algorithm, using the R package *missForest* (Stekhoven and Bühlmann, 2012), and then performed a multivariate principal components analysis (PCA) using the R package *FactoMineR* (Lê et al., 2008). All other tests were performed using JMP Pro 14 (SAS Institute, Cary, NC). Raw data are available in Table S2.

3. Results

3.1. Phylogenetic hypotheses

All phylogenetic analyses of the molecular genetic data strongly support two main clades of *Leiopython*: a northern and a southern clade (Figs. 1 and 2 and supplementary material). The northern clade extends from Lae and the Markham River Valley in eastern Papua New Guinea along the north coast of New Guinea and into the Vogelkop Peninsula, Indonesia. The northern clade includes populations from Salawati and Waigeo Islands in the Raja Ampat Archipelago and Biak Island in the Cenderawasih Group (Fig. 1). The southern clade includes populations from the Northern and Milne Bay Provinces in far eastern Papua New

Table 1
Summary statistics for scale count and color trait comparisons between the six species of *Leiopython* described by Schleip (2008). Summarized data are sourced from A) Schleip (2008) and B) the present study. Data included are ranges, means and associated standards errors.

Character	*L. albertisii*		*L. biakensis*		*L. fredparkeri*		*L. huonensis*		*L. meridionalis*		*L. montanus*	
Data source	A	B	A	B	A	B	A	B	A	B	A	B
Sample size	30	95	2	2	13	1	15	0	22	14	5	4
DMB	43–51 47.1 ± 2.53	42–52 47.6 ± 2.7	45–47 46 ± 1.41	49–52 50.5 ± 2.12	47–51 49.1 ± 1.04	52 –	43–55 48.3 ± 2.99	NA –	45–52 48.1 ± 1.50	47–53 50.9 ± 1.7	49–54 50.6 ± 2.30	NA –
VEN	262–283 274.5 ± 4.83	259–282 273.7 ± 3.6	270–272 271 ± 1.41	270–274 272 ± 2.83	266–277 270.5 ± 3.15	270 –	258–282 268.2 ± 6.12	NA –	264–278 272.6 ± 4.30	258–275 268.6 ± 5.35	263–274 268.4 ± 4.45	NA –
SCA	65–79 71.5 ± 2.85	61–78 71.1 ± 3.5	65–70 67.5 ± 3.54	70–72 71 ± 1.41	63–76 69.8 ± 3.35	66 –	65–78 70.6 ± 3.94	NA –	64–77 68.7 ± 3.33	64–73 68.6 ± 2.87	62–70 66.0 ± 3.39	NA –
SPL	12–13 12.9 ± 0.28	12–15 12.9 ± 0.45	11–12 11.8 ± 0.35	13 –	12–14 13.0 ± 0.32	13 –	12–13 12.7 ± 0.45	NA –	12–14 13.1 ± 0.37	13 –	13 –	13 –
INL	15–17 15.9 ± 0.28	14–18 16.1 ± 1.3	14–16 15.3 ± 1.1	14 –	16–18 17.2 ± 0.59	17 –	15–17 16.1 ± 0.59	NA –	15–18 16.6 ± 0.66	15–18 17 ± 0.8	16–18 17.0 ± 0.35	16–17 16.25 ± 0.5
POC	2–4 3.0 ± 0.25	2–4 3.2 ± 0.4	3 –	3 –	2–4 3.1 ± 0.45	3 –	3–4 3.2 ± 0.24	NA –	2–4 3.0 ± 0.35	3 –	3–4 3.5 ± 0.35	3–4 3.25 ± 0.5
SLE	2–3 3.0 ± 0.18	2–3 2.9 ± 0.3	2 –	3 –	2–3 2.7 ± 0.43	3 –	2–3 2.9 ± 0.26	NA –	2–3 2.8 ± 0.37	2–3 2.9 ± 0.3	3 –	3 –
SOC	0 –	0 –	0 –	0 –	0–1 0.5 ± 0.52	0 –	0 –	NA –	0–1 0.2 ± 0.36	0 –	0 –	0 –
LOR	1 –	1–3 1.03 ± 0.23	1 –	1 –	1–2 1.1 ± 0.13	1 –	1 –	NA	1–2 1.1 ± 0.35	1–3 1.14 ± 0.53	2–3 2.3 ± 0.45	1–2 1.25 ± 0.28
PFR	1 –	0–1 0.99 ± 0.1	1 –	1 –	1 –	1 –	1 –	NA –	1–2 1.1 ± 0.35	1 –	2 –	1–2 1.25 ± 0.25
PAR	2 –	0–2 1.76 ± 0.46	2 –	2 –	2 –	2 –	1 –	NA –	1–2 1.0 ± 0.21	1–2 1.28 ± 0.47	1 –	1 –
PML	Yes (93%)	Yes (89%)	Yes (100%)	Yes (100%)	Yes (85%)	Yes (100%)	Yes (93%)	NA	Yes (96%)	Yes (43%)	No (100%)	No (25%)
SPT	Present	Present (93%)	Present	Present (100%)	Absent	Absent	Present	NA	Absent	Absent (100%)	Absent	Absent (100%)
Color	Gold		Gold		Black		Gold		Black		Black	

Scale counts: DMB = Dorsal midbody rows; VEN = Ventral scales; SCA = Subcaudal scales; SPL = Surpralabial scales; INL = Infralabial scales; POC = Postocular scales; SLE = Supralabials entering the eye; SOC = Subocular scales; LOR = Loreal scales; PFR = Prefrontal scales; PAR = Parietal scale pairs; PML = Parietal scales that border the frontal scale in contact at the midline. Color trait: SPT = Whitish spot on the postoculars. Color = coloration of dorsal surface.

Guinea through the Gulf Province, including highland populations from Wau and the Karimui Basin, and extends as far west as Timika in southern Papua, Indonesia (Fig. 1). In addition, the molecular genetic data confirmed the presence of the southern clade on the Vogelkop Peninsula in the vicinity of Manokwari and the Maybrat Regency (Fig. 1). The main discordance between the nDNA and mtDNA data is the placement of the sample from the Markham Valley, which was part of the northern clade in nDNA and the southern clade in mtDNA (Fig. 2). Relationships among populations within each clade are discordant between trees, which is consistent with them being conspecific populations connected by gene flow.

3.2. Population structure and species delimitation

The genetic structure estimated from the allele frequencies of the nDNA SNPs with *STRUCTURE* comprised two clusters (K) according to the Evanno or ΔK method, and one according to the ln Pr($D|K$) method (Fig. 2). With two clusters, specimens from the northern and southern clades show highly distinct genetic structure with little inferred admixture.

Our Bayesian analysis of species delimitation based on the multi-species coalescent, run on *BPP*, shows unequivocal support for the split between the northern and southern clades as different species, with a posterior probability of 1.0 for that model. Our analyses show no support for any other clades within *Leiopython*. We apply the names *L. albertisii* and *L. fredparkeri* to the northern and southern clades, respectively, according to the principle of priority.

3.3. Morphology

In the Principal Components Analysis, the first two principal component (PC) axes represent 19.39 and 18.57% of the variation respectively (Fig. 3). Although PCs 3–5 explain another 37.9% of the variation, this is not partitioned by species so we do not plot these PCs here (see Table S3). PC1 is mostly explained by ventral (loading of 0.63) and dorsal midbody (loading of −0.69) scale counts, and separates *Leiopython fredparkeri* with higher dorsal midbody scales and fewer ventral scales than *L. albertisii* (Fig. 3; Table S3). However, these differences are by no means unequivocal and make it challenging to diagnose species based on scalation alone. The most obvious difference between the two taxa are the gold (*L. albertisii*) vs black (*L. fredparkeri*) dorsal colouration (Table 2).

Our expanded morphological dataset was consistent with some of the morphological patterns described by Schleip (2008), but several of his findings were not supported (Table 1). Specifically:

All of the *Leiopython albertisii* examined by Schleip (2008) had two pairs of parietal scales, as well as white markings on the post-ocular scales, but we found that this was not always the case (Table 1).

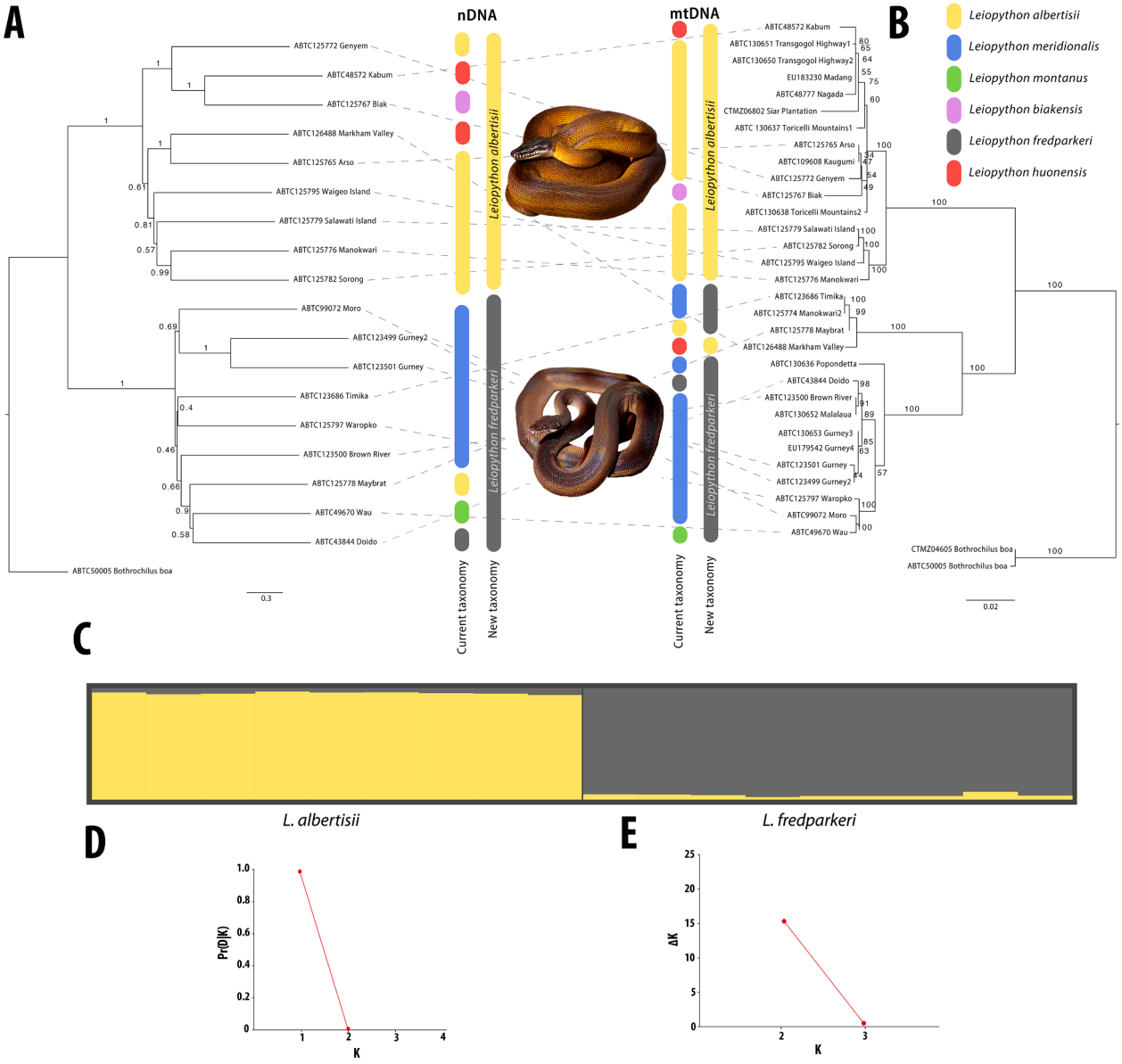

Fig. 2. (A) nDNA species tree inferred with *Astral III* and (B) mtDNA ML concatenated gene tree estimated with *IQ-Tree* for *Leiopython*. Colored bars depict the current and new proposed taxonomies according to the legend at the top right. Branch support values for the nDNA species tree are in local posterior probability and for the mtDNA gene tree they correspond to ultrafast bootstrap/gene concordance factors/site concordance factors. (C) Genetic Bayesian clustering of the18 individuals based on the allelic frequencies at nuclear loci using *STRUCTURE*, identified one cluster according to the ln Pr($D|K$) method (D) and two clusters (K) by the Evanno or ΔK method (E).

Schleip (2008) found that *Leiopython biakensis* is separated from *L. albertisii* in having higher mean subcaudal and supralabial scale counts and lower mean ventral scale counts, but our data did not support this (independent Kruskal-Wallis tests; all $P > 0.05$; Table 1). In addition, both *L. biakensis* examined for our study had three supralabials contacting the orbit (as opposed to two in Schleip, 2008; Table 1).

We found specimens of *L. fredparkeri* to have parietal scales bordering the frontal scale to be in contact at the median line in only 42% of specimens as opposed to 96% observed by Schleip (2008) (Table 1). Photographs of specimens of *L. fredparkeri* from Timika examined by Schleip (2008) showed two pairs of parietals not seen in specimens from other sites. In contrast, only one of the five specimens from Timika examined by us possessed two pairs of parietals.

The characters used by Schleip (2008) to differentiate *L. montanus* from other *Leiopython* taxa are the presence of higher loreal scale counts and a second pair of prefrontal scales. In the four specimens from Bulolo examined by us, the first condition was satisfied in only two cases and the second condition in only one case.

4. Discussion

Our robust molecular dataset provides strong support for *L. albertisii* and *L. fredparkeri*, but not for the other species described by Schleip (2008). Here, we first offer explanations for our conclusion, and finish by discussing what our data reveal and its implications for Papuan biogeography and the conservation of *Leiopython* species.

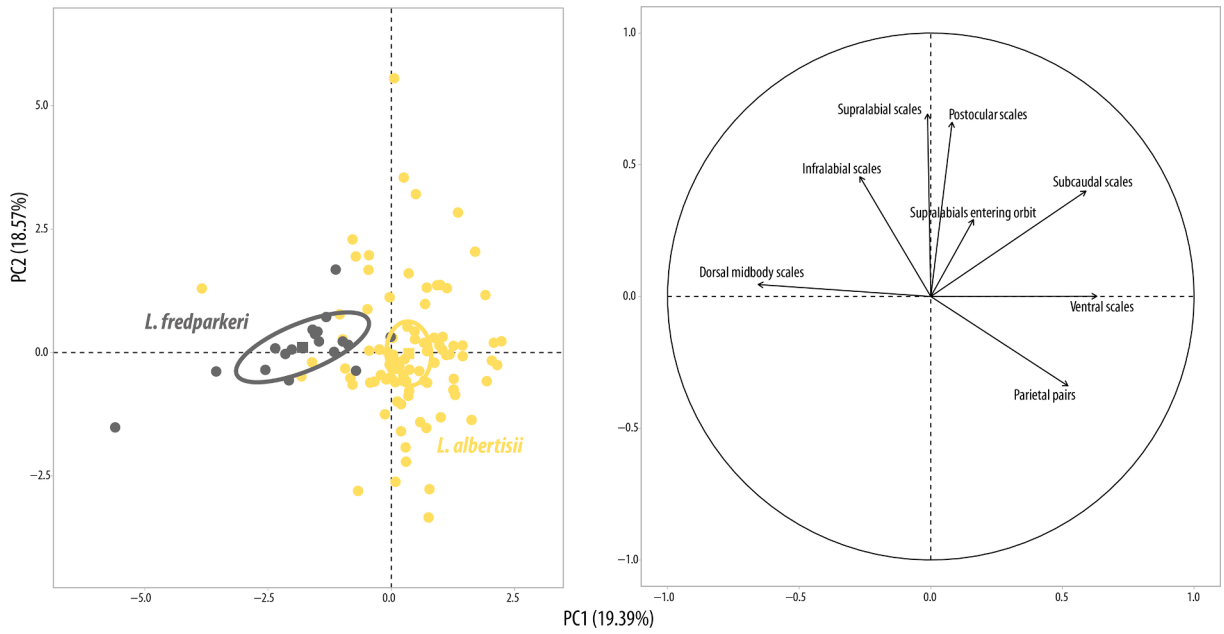

Fig. 3. Left: Principal Component Analysis (PCA) plot for morphometric variables. Ellipses correspond to the 95% confidence interval around the centroid of each species. Right: Variable factor map illustrating the variable loadings for each Principal Component (PC).

Table 2
Summary statistics for scale count and color trait comparisons between the two species of *Leiopython* identified in the present study. Data from this study are pooled with data from Schleip (2008). Data included are ranges, means and associated standards errors.

Characters	*L. albertisii*	*L. fredparkeri*
Sample size	125	36
Dorsal midbody rows	42–52	47–53
	47.5 ± 2.81	49.15 ± 2.04
Ventral scales	259–283	258–275
	273.9 ± 4.4	270.8 ± 5.2
Subcaudal scales	61–79	64–77
	71.2 ± 3.5	68.7 ± 3.1
Supralabial scales	12–15	12–14
	12.9 ± 0.46	13.1 ± 0.3
Infralabial scales	14–18	15–18
	16.1 ± 0.80	16.7 ± 0.7
Postocular scales	2–4	2–4
	3.2 ± 0.38	3.0 ± 0.27
Supralabials entering the eye	2–3	2–3
	2.9 ± 0.28	2.8 ± 0.34
Subocular scales	0	0–1
	–	0.12 ± 0.3
Loreal scales	1–3	1–3
	1.02 ± 0.26	1.13 ± 0.46
Prefrontal scales	0–1	1–2
	0.98 ± 0.11	1.06 ± 0.28
Parietal scale pairs	0–2	1–2
	1.79 ± 0.43	1.13 ± 0.38
Parietal scales bordering frontal in contact at the midline	Yes (90%)	Yes (69%)
Whitish spot on the postoculars	Present (95%)	Absent (100%)
Dorsal colouration	Gold	Black/brown

4.1. Systematics

Phylogenetic analyses of our molecular genetic data nested a specimen (AMS R115346) collected at the type locality of Schleip's (2008) *L. fredparkeri* (Karimui Plateau) within other specimens of *L. meridionalis* from southern New Guinea. Possession of two pairs of parietal scales was the only morphological character with which Schleip (2008) distinguished *L. fredparkeri* from *L. meridionalis* (with a single pair of parietals in Schleip's data). Yet Schleip (2008) also reported some specimens of *L. meridionalis* with two parietal pairs, and we recorded 29% of specimens (4 of 14 examined) with two pairs (Table 1). More broadly, Schleip (2008) justified the description of *L. fredparkeri* by an assumed allopatric distribution, suggesting that a ring of mountains separate the Karimui Plateau from the lowlands below. Although the area is a mountainous zone, the Tua River, a tributary of the Purari River, penetrates the Karimui Plateau from the southern lowlands (see Fig. S2 in Supplementary Material). We see little reason why specimens of this large and mobile python would be geographically isolated in this area, either now or historically. Furthermore geographic distribution is an extrinsic property of species, and is effectively uninformative as primary evidence for species boundaries. Finally, because Schleip (2014) replaced the names *L. hoserae* and *L. bennettorum* with *L. meridionalis* and *L. montanus* (respectively) in 2014, the principle of priority favours *L. fredparkeri* (described in 2008; Schleip 2008) as the appropriate name for the southern taxon. Both *L. meridionalis* and *L. montanus* are thus relegated to junior synonyms of *L. fredparkeri*.

Schleip (2008) partly justified the description of *L. huonensis* by implying differences in ecological and environmental conditions for its distribution compared to other *Leiopython*. The localities from which type specimens were collected (Lae, Finschhafen) are lowland sites, with no obvious barriers between them and nearby sites containing *L. albertisii*. Our molecular sample from the southern side of the Markham Valley is only ~60 km from the *L. huonensis* type locality of Lae, and our sample from Kabum is only ~50 km up the Markham River Valley from the specimen of *L. huonensis* from Dumpu (Schleip, 2008). Although temperature and rainfall can change over small areas in New Guinea (McAlpine et al., 1983), *L. albertisii* is distributed across a vast geographic and altitudinal range that experiences strikingly different climatic conditions. We are aware of no obvious barriers to gene flow in the intervening areas that might restrict *Leiopython* from the Huon Peninsula from crossing the Markham River valley. Moreover, Schleip (2008) reported specimens from the Wabag and the Jimi River Region of northern PNG (near to our Kabum sample) as "indistinguishable" from *L. huonensis*. This raises questions about whether *L. huonensis* does show

morphological differentiation from *L. albertisii* at nearby sites, and does not lend strong support for environmental differentiation as implied evidence of species status for Huon Peninsula *Leiopython*.

Schleip (2008) described specimens from Biak Island as separate species based on two individuals with lower mean ventral scale counts and the possession of only two supralabials entering the orbit. The two further specimens from Biak that we examined both had three labials entering the orbit. Schleip (2008) also separated this species from *L. albertisii* by comparison with a sub-sample of *L. albertisii* from the Vogelkop Peninsula. Repeating this analysis with specimens from the Vogelkop, from the rest of northern New Guinea, and with all specimens pooled, revealed no significant difference in ventral scale numbers between any population. Although pythons from Biak extrinsically are reproductively isolated owing to the oceanic nature of the island, the absence of demonstrable morphological and molecular divergence from mainland populations means that we do not consider the Biak population to warrant specific status. Although another python taxon from Biak has been separated taxonomically from its mainland relatives due to genetic and morphological divergence (Natusch et al., 2020), the possibility of dispersal to Biak is presumably a process that has been operating since the island achieved its current proximity to the New Guinean mainland (Cowie and Holland, 2006).

The situation for *L. montanus* is similar. Five specimens were used to describe this taxon. Examination of specimens from Bulolo – a site 12 km away in the same valley, with no obvious barriers to gene flow – does not support the scale arrangements used by Schleip (2008) to distinguish this taxon. Moreover, Schleip (2008) describes examining three specimens of *L. meridionalis* that possess two pairs of pre-frontal scales and could be confused with *L. montanus*. Importantly, *L. montanus* is not supported by molecular data. Nevertheless, the presence of *L. fredparkeri* at Wau is intriguing. This population is on the northern watershed of the Owen Stanley Ranges, where, based on the distribution of other taxa, one would expect to find the northern taxon, *L. albertisii*.

More broadly, Schleip (2008) notes numerous exceptions, variations in scalation, and/or aberrant specimens. In particular, Schleip (2008) relied heavily on parietal scale arrangements of the head to distinguish taxa. Although some broad patterns are clear, the variation – even within single populations of one taxon – was great enough for us to be unable to usefully diagnose the different species (McDowell, 1975; Table 1). This is unsurprising. The head and body scales of many species of python from single localities (e.g., *Leiopython*, *Simalia* – with samples $>$ 500) vary considerably, underpinning the need to examine large samples to prevent making type II errors (D. Natusch unpubl. data, 2020). Some of these traits even display environmental plasticity, such as incubation temperature affecting the fragmentation of head scales (Brown et al., 2017). We thus strongly caution against using morphological data from wide-ranging species with small sample sizes to delimit species. It may indeed be the case that more than two species of *Leiopython* – diagnosable by unique scale arrangements - occur in New Guinea. However, as evidenced here, the inclusion of molecular data will be essential to accurately elucidate the systematics of the group.

4.2. Biogeography

Although our knowledge of the distribution of the two *Leiopython* taxa in New Guinea is incomplete, the species are broadly separated to the north and south by New Guinea's central mountain range; a pattern consistent with many other Papuan taxa, i.e., rainbow fish (McGuigan et al., 2000), birds (Beehler, 2007), turtles (Georges et al., 2014), lizards (Tallowin et al., 2018; Tallowin et al., 2020) and green pythons (Natusch et al., 2020). In addition to the specimens from Wau (see above), the notable exceptions to this general observation are specimens of *L. fredparkeri* occurring as far west as Manokwari and the Maybrat Regency of the Vogelkop Peninsula. In the Vogelkop Peninsula, *L. fredparkeri* occurs in broad sympatry with *L. albertisii*. Whether these species confine themselves to separate habitats or specific niches is unknown. *Leiopython albertisii* may occur also to the south in the vicinity of Timika; but this is based on only two specimens held in the Museum Zoologicum Bogoriense (MZB), which appeared golden in colour and one of which possessed white markings on the post-ocular scales indicative of *L. albertisii*. This possible zone of contact in southern Papua has also been observed in *Morelia viridis* (Natusch et al., 2020). Together with specimens from Timika, *L. fredparkeri* from the Vogelkop showed considerable phylogeographic structure in their mtDNA. However, because of our limited geographic sampling, poor understanding of distributional limits, and lack of structure within our nDNA, we are reluctant to designate specimens from these populations as separate taxa. Further detailed work will be required to assess their status.

Schleip (2008) reported several specimens of Leiopython from Merauke (8° 28′S, 140° 20′E) on the southern coast of Papua, Indonesia, and used one specimen in his molecular analysis. These specimens are all reported to have originated from the pet trade. Problematically, however, the habitat surrounding Merauke comprises tropical woodland, an unsuitable habitat for *Leiopython* (see below), and in decades of specimen collection there *Leiopython* have not been recorded (D. Natusch unpubl. data, 2020). We caution using specimens collected from the live animal trade as reference samples in systematic revisions, since the purported collection locality frequently reflects a major transport hub rather than a direct collection location.

Although our population structure analysis revealed no significant signs of admixture between the two identified taxa, the discordance between mtDNA and nDNA in an individual from the Markham Valley possibly indicates mitochondrial introgression between *L. albertisii* and *L. fredparkeri*. Dating analyses suggests recent divergence of the two taxa (likely during the Pliocene; Esquerré et al., 2020); and even though our analyses reveal two clearly defined lineages within *Leiopython*, these are probably at early stages of speciation. Denser population genetic sampling is necessary to understand patterns of gene flow between the two species.

4.3. Conservation

Morphological and molecular support for only two species of *Leiopython* rather than six has significant implications for studies of ecology and evolution (Esquerré and Keogh, 2016; Esquerré et al., 2017; Esquerré et al., 2020), but most notably for their conservation. Our revision will make it easier for customs officials around the world to identify the species of *Leiopython*. Moreover, the national CITES Scientific Authorities of Indonesia and Papua New Guinea will not be required to undertake non-detriment findings for international trade in all six taxa, significantly reducing the management and administrative burden and the unnecessary allocation of precious conservation funds. Nevertheless, such resource use has already taken place. Both CITES and the IUCN Red List of Threatened Species have recognized and assessed all six species described by Schleip (2008). Because the species are poorly known, and described from discrete localities (giving the impression of small distributions), two are already listed as vulnerable and in need of conservation action (IUCN, 2020). These assessments will need to be revised in light of our findings.

The finding that four species described by Schleip (2008) represent morphological variants rather than valid species again offers a cautionary tale. Both taxonomists and the scientific community need to rely on more thorough datasets and provide suitable justification for the recognition of novel taxa. Without such rigor, we risk spending considerable time and resources attempting to manage and conserve phylogenetic diversity that does not exist or is not under threat.

5. Taxonomy

Based on molecular and morphological results and the discussion presented above, we do not consider *L. biakensis, L. meridionalis, L. huonensis* or *L. montanus* to be valid species. We consider *L. biakensis* and

L. huonensis to be junior synonyms of the northern species, *L. albertisii*, and *L. meridionalis* and *L. montanus* to be junior synonyms of the southern species, *L. fredparkeri*. Here, we offer re-descriptions of the two species of *Leiopython* justified in our study to aid differentiation by both herpetologists and regulatory personnel tasked with their management.

Leiopython albertisii Peters & Doria 1878
Leiopython gracilis Hubrecht 1879
Leiopython biakensis Schleip, 2008
Leiopython huonensis Schleip, 2008

Diagnosis: *Leiopython albertisii* is distinguished from *L. fredparkeri* by its dark yellow or golden dorsal colouration (*versus* dark brown or black in *L. fredparkeri*); presence of small specks of white patterning on the postocular scales in most specimens; and generally two parietal scale pairs (as opposed to a single pair in most *L. fredparkeri*). Compared to *L. fredparkeri*, *L. albertisii* possesses a shorter and narrower head size relative to its body (Natusch and Lyons, 2012). *Leiopython albertisii* also differs from *L. fredparkeri* in having a lower mean number of dorsal midbody rows and a higher mean number of ventral scales (259–283 *versus* 258–275, respectively). However, an overlap in the ranges of the scale counts, coupled with small sample sizes for this wide-ranging taxon, renders these aspects of scalation of doubtful diagnostic value. Importantly, *L. albertisii* is easily differentiated from *L. fredparkeri* on the basis of molecular genetic data.

Description: *Leiopython albertisii* is a large and robust python species growing to almost 2.5 m in total length and ~ 3.5 kg in mass in wild (Natusch and Lyons, 2012). The head is black or dark brown dorsally and is distinct from the neck. The jaws are white ventrally with thin black bars on the anterior edges of the supralabial and infralabial scales. A white postocular spot is present in most specimens (McDowell, 1975; Schleip, 2008). The body is dark yellow or golden dorsally, becoming lighter laterally and fading to white or cream on the ventral surface. The head and body are strongly iridescent. The sexes do not appear to be sexually dimorphic (Natusch and Lyons, 2012).

Distribution and Ecology: *Leiopython albertisii* is distributed across the north of New Guinea from Sorong in the west, across the Vogelkop Peninsula to Manokwari, the Bomberai Peninsula and the Vogelkop Isthmus, east through Nabire and northern Papua New Guinea to Lae and the Huon Peninsula. This species may also extend south of New Guinea's central cordillera in the vicinity of Timika on the south coast of Papua, Indonesia (based on examination of gold specimens with white postocular markings deposited in the MZB). The species also occurs on several offshore islands such as Misool, Waigeo, Salawati, Biak, Supiori, Yapen, Massau and Emirau islands. There is no evidence that the species occurs on New Britain or New Ireland. *Leiopython albertisii* inhabits a range of rainfall and altitude zones to at least 1500 m, and occupies several habitats: rainforests, mangrove forests, swamps, scrubby vegetation near beaches, and village gardens (O'Shea, 1996; D. Natusch, unpubl. data 2020). In these habitats, *L. albertisii* is semi-aquatic, and is commonly found in close proximity to rainforest streams or large rivers and swamps where it appears to be analogous in habits with *Liasis fuscus*. *Leiopython albertisii* is crepuscular to nocturnal, and has been observed hunting from the water (D. Natusch, pers. obs.). Diet records show that adults prey almost exclusively on small mammals (rodents, bandicoots). One juvenile specimen has been recorded to prey on a scincid lizard, and reptiles may be common dietary items for young snakes (Natusch and Lyons, 2012). Although perhaps not as common as other Papuan python species (e.g., *Simalia amethistina*, *Morelia viridis*, *Morelia spilota*), *L. albertisii* is easy to locate in New Guinea and is by no means 'rare' – even in areas where it is harvested for trade.

Leiopython fredparkeri Schleip, 2008
Leiopython meridionalis Schleip, 2014
Leiopython montanus Schleip, 2014

Diagnosis: *Leiopython fredparkeri* is distinguished from *L. albertisii* by dark grey, brown or black dorsal colouration (*versus* dark yellow or golden in *L. albertisii*); absence of small specks of white patterning on the postocular scales in all specimens; generally a single pair of parietal scales (as opposed to two pairs in most *L. albertisii*); and a higher mean number of dorsal midbody scales and fewer ventral scales than *L. albertisii*. Relative to its body length, *L. fredparkeri* has a longer and wider head than *L. albertisii* and preliminary data suggest that *L. fredparkeri* grows to a larger maximum body size (Natusch and Lyons, 2012). *Leiopython fredparkeri* is easily differentiated from *L. albertisii* on the basis of molecular genetic information.

Description: *Leiopython fredparkeri* is a large and robust python species growing to > 2.5 m in total length and ~ 3.5 kg in mass in wild (Natusch and Lyons, 2012). The head is black dorsally and distinct from the neck. The jaws are white ventrally with thin black bars on the anterior edges of the supralabial and infralabial scales (McDowell, 1975; Schleip, 2008). The body is dark grey or brown to black, becoming lighter laterally and fading to white or cream on the ventral surface. The head and body are strongly iridescent. The sexes do not appear to be sexually dimorphic.

Distribution and Ecology: *Leiopython fredparkeri* is distributed across the south of New Guinea from Milne Bay, coastal Morobe Province and the Owen Stanley Ranges along the south coast and through Gulf and Western Provinces in PNG and into southern Papua, Indonesia. The species occurs in the vicinity of Timika and also occurs west of the Vogelkop Isthmus in the vicinity of Maybrat Regency and Manokwari on the Vogelkop Peninsula. The species does not appear to occur in the west of the Vogelkop and is unknown to occur on the Bomberai Peninsula. *Leiopython fredparkeri* occurs in several high elevation sites such as the Tive Plateau in the vicinity of Karimui, and north of New Guinea's central range in the vicinity of Wau and Bulolo. *Leiopython fredparkeri* occurs in a variety of closed-forest habitats and village gardens, and, like *L. albertisii*, is closely associated with water bodies (O'Shea, 1996). Although *L. fredparkeri* is found in some areas of suitable habitat within New Guinea's Trans-Fly region, the species is absent from much of this area (including in the vicinity of Merauke in southern Papua) because of the presence of unfavourable woodland habitat where it appears to be replaced by *Liasis fuscus* (D. Natusch, unpubl. data, 2020). Despite several claims that *Leiopython* occur on islands in the Torres Straight (see Schleip, 2008 and references therein), there are no verified records of *Leiopython* from Australian territory and we consider them absent from Boigu and Saibai Islands. Despite strong connections to southern New Guinea there are no records of any *Leiopython* species from the Aru Archipelago in Maluku Province, Indonesia (D. Natusch, unpubl. data 2020). In general, *L. fredparkeri* is less well known than *L. albertisii* but is likely to share a similar diet, activity patterns and general ecology (see Natusch and Lyons [2012] for additional information).

Credit authorship contribution statement

Daniel J.D. Natusch: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition. **Damien Esquerré:** Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Jessica A. Lyons:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing. **Amir Hamidy:** Investigation, Writing - review & editing. **Alan R. Lemmon:** Formal analysis, Investigation, Resources, Data curation, Writing - review & editing. **Emily Moriarty Lemmon:** Formal analysis, Investigation, Resources, Data curation, Writing - review & editing. **Awal Riyanto:** Investigation, Writing - review & editing. **J. Scott Keogh:** Methodology, Resources, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition. **Stephen Donnellan:** Conceptualization, Methodology, Resources, Data curation, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2020.106960.

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