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Multilocus phylogeny and revised classification for mountain dragons of the genus *Japalura s.l.* (Reptilia: Agamidae: Draconinae) from Asia

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Although the genus *Japalura s.l.* has long been recognized as paraphyletic based on limited genetic sampling, its problematic taxonomy has not been revised, and phylogenetic relationships among the majority of congeners remain unknown. Here we utilize a densely sampled dataset of both multilocus genetic and morphological data to provide the first phylogenetic inference of relationships among *Japalura s.l.* species. Our results show that *Japalura s.l.* is paraphyletic, consisting of four major clades that are scattered across the phylogeny of the subfamily Draconinae: the first clade from the western, central and middle-eastern Trans-Himalayas, the second clade from the far eastern Trans-Himalayas, the third clade from East Asia and the last clade from Indochina. To address this widespread paraphyly of the genus and to stabilize the taxonomy within the family Draconinae, we revise the current taxonomy and split *Japalura s.l.* into four genera. By doing so, we recognize two existing generic names, *Japalura sensu stricto* and *Pseudocalotes*, resurrect one name available in the literature, *Diploderma*, and describe one new genus, ***Cristidorsa*** **gen. nov.** We discuss phylogenetic relationships and taxonomy within *Japalura s.l.* and present a diagnostic key to all recognized genera of the subfamily Draconinae.

ADDITIONAL KEYWORDS: China – India – integrative taxonomy – lizard – new genus – Tibetan Plateau.

INTRODUCTION

Lizards of the Old World family Agamidae have long fascinated biologists with their extreme ecological and morphological variation, from winged, gliding arboreal species to spiny, desert specialists (Smith, 1935; Moody, 1980; Stuart-Fox & Ord, 2004). Within the family, dragon lizards of the subfamily Draconinae represent a remarkable radiation of reptiles distributed throughout Asia, with more than 214 species recognized (Pyron *et al.*, 2013; Grismer *et al.*, 2016b; Uetz & Hošek, 2017). The genus *Japalura* Gray, 1853 is one of the most speciose and most widespread genera in Draconinae, containing 34 recognized species that are distributed from Pakistan along the foothills of the Himalayas to the oceanic islands of Japan (Wang *et al.*, 2016; Ananjeva *et al.*, 2017; Rao *et al.*, 2017; Fig. 1). Members of the genus display a diverse spectrum of

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species-specific, sexually selected morphological traits, including ornamentation, coloration, crest morphology, limb length, and body size (Mahony, 2009; Manthey, 2010; Denzer *et al.*, 2016). Such common and high-levels of sexual dimorphisms among congeners were hypothesized to facilitate speciation and to maintain genetic integrity of sympatric species (Wang *et al.*, 2015, 2016). Furthermore, the unique association between the microendemic distributions of congeners and the major mountains and rivers in Asia also make the genus a great system for investigating the biogeographic history of the region (Yang & Rao, 1992; Zhao *et al.*, 1999; Wang *et al.*, 2016).

Yet despite the high diversity in the genus, and its importance in evolutionary and biogeographic studies, little is known about the phylogenetic relationships of *Japalura s.l.* both within the genus as well as with respect to other genera in the subfamily Draconinae. Although *Japalura* has long been suspected to be paraphyletic, based on morphological traits (Schmidt, 1927), it was not until 2000 that phylogenetic analyses using genetic data confirmed this hypothesis (Macey *et al.*, 2000). Using mitochondrial DNA (mtDNA) data only, Macey *et al.* (2000) showed that *Japalura s.l.* has two, well-supported, deeply divergent clades: the first clade, which is represented by the type species of the genus, *Japalura variegata*, and *J. tricarinata*, was inferred to be closely related to members of the genus *Draco*; and the second clade, which is represented by *J. flaviceps* and *J. splendida*, was recovered as a sister-group to members of the genus *Pseudocalotes* (Macey *et al.*, 2000). Subsequent studies that used the same sequence data of *Japalura* congeners as Macey *et al.* (2000) recovered similar phylogenetic relationships among draconine lizards, further highlighting the paraphyletic nature of *Japalura sensu lato s.l.* (Schulte *et al.*, 2004; Zug *et al.*, 2006).

Analysing a different mtDNA locus of another island endemic species of the genus, *Japalura polygonata*, Honda *et al.* (2000) also investigated the phylogenetic position of *Japalura* in the family Agamidae. Although results of this study failed to infer the exact placement of the *Japalura* species sequenced with strong support, Honda and colleagues showed that *J. polygonata* was more closely related to a clade containing the genera *Calotes* and *Aphaniotis* than to the clade containing the genera *Draco* and *Mantheyus* (Honda *et al.*, 2000). More recently, Pyron *et al.* (2013) conducted a higher level study of squamate reptiles that incorporated all available mtDNA data from previous studies. Incorporating all previously included species of *Japalura s.l.* (*J. flaviceps*, *J. polygonata*, *J. splendida*, *J. tricarinata* and *J. variegata*), not only did Pyron *et al.* (2013) recover the same two divergent clades of *Japalura* observed previously by Macey *et al.* (2000), but also, they recovered a third distinct clade represented by *J. polygonata*, which is sister to the South-East Asian agamid *Gonocephalus robinsonii* (now recognized as *Malayodracon robinsonii*). However, given the large amount of missing data and non-overlapping gene sampling among different clades, questions remain about the phylogenetic position of this third clade represented by *J. polygonata* (Pyron *et al.*, 2013).

Currently, most authors agree that *Japalura s.l.* is paraphyletic, and the genus *s.l.* represents multiple, deeply divergent clades; however, no studies have addressed the taxonomic instability of the genus *s.l.* (Macey *et al.*, 2000; Stuart-Fox & Owens, 2003; Mahony, 2010; Ananjeva *et al.*, 2011; Pyron *et al.*, 2013; Rao *et al.*, 2017). Furthermore, although authors have given names to all three clades recovered from previous phylogenetic studies (including *J. veriegata* species group/Himalayan Lineage, *J. splendida* group/Indochina Lineage and *J. polygonata* group/Island Lineage), these clade names were established based on five of 34 species of the genus *s.l.* only, leaving the exact group/lineage memberships of the remaining 29 congeners unknown (Stuart-Fox & Owens, 2003; Mahony, 2010; Ananjeva *et al.*, 2011).

Fortunately, broader genetic and morphological sampling across members of the genus *Japalura s.l.* has now been amassed, allowing for a more robust phylogenetic assessment of monophyly of the genus and phylogenetic positions of different clades within the genus *s.l.* with respect to other genera in the subfamily Draconinae. In this study, we employ a multilocus phylogenetic dataset to evaluate the phylogenetic relationship of *Japalura s.l.* and to address long-standing taxonomic problems at the generic level. Specifically, we are interested in testing the monophyly of *Japalura s.l.* estimating the number of distinct clades within this genus and testing whether the three proposed species groups based on limited phylogenetic sampling from previous studies represent monophyletic groups. Based on our results, we provide a revised nomenclatural classification scheme that recognizes the species diversity of *Japalura s.l.* as members of one of four distinct genera, revise the morphological diagnoses for each of the four genera, and present an updated key to all genera in the subfamily Draconinae. Finally, we point to future directions for phylogenetic and taxonomic studies of *Japalura s.l.* and, more broadly, the subfamily Draconinae in general.

TAXONOMIC HISTORY

The genus *Japalura s.l.* has a long, convoluted taxonomic history based on morphological data. First described by Gray in 1853, the genus *Japalura* was established based on the type species, *J. variegata*

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from Sikkim, Himalaya. Gray (1853) gave a vague definition of his new genus and stated that it was most similar to *Agama*, but differed by having a concealed tympanum. Ever since the initial description, different species have been placed into the genus *Japalura*, and numerous authors have attempted to refine the taxonomic definition of the genus based on newly included taxa. For example, Boulenger (1885) first added more diagnostic characteristics to *Japalura*, including having a compressed body shape, heterogeneous dorsal scales, a dorsal crest, a small gular pouch (or absent), an oblique fold, a feebly compressed tail and no pre-anal or femoral pores. Pope (1935) diagnosed *Japalura* from other agamid genera by having a body not depressed and possessing hidden tympana. In the same year, Smith (1935) provided a combined version of the diagnoses from Pope (1935) and Boulenger (1885) that was much more comprehensive. However, Smith's (1935) diagnosis of *Japalura* still cannot differentiate most of its members from other genera in the subfamily Draconinae, as many exceptions exist within the genus *s.l.* (e.g. *J. dymondi*, *J. kumaonensis*, *J. major*, *J. tricarinata* and *J. varcoae* have exposed tympana). Later, Inger (1960) further differentiated *Japalura s.l.* from the South-East Asian genus *Phoxophrys* Hubrecht, 1881 by having greatly overlapping supraciliaries, a relatively long head, the presence of hair-like sense organs on the head scales and a compressed tail at the base. However, those diagnostic characters again are not consistent among many of the congeners of *Japalura* (Ota, 1989b). Subsequent studies largely followed the diagnosis of the genus by Boulenger (1885), but these generic definitions remained ambiguous and impracticable, and failed to differentiate *Japalura s.l.* from other genera of Draconinae, such as *Pseudocalotes* (Zhao & Adler, 1993; Kästle & Schleich, 1998; Zhao *et al.*, 1999; Yang & Rao, 2008; Wang *et al.*, 2015).

Such an absence of practical morphological diagnoses of *Japalura s.l.* has resulted in uncertain systematic affiliations and unstable nomenclatures, not only for members of the genus *s.l.* but also for species in other genera (Pope, 1935; Smith, 1935; Inger, 1960; Kästle & Schleich, 1998; Yang & Rao, 2008; Mahony, 2010). Species that are currently recognized as members of *Japalura s.l.* have been confused and assigned to different, recognized agamid genera, including *Acanthosaura* Gray, 1831 (*J. dymondi* [Boulenger, 1906]; *J. varcoae* [Boulenger, 1918]; *J. major* [Boulenger, 1885]; *J. kumaonensis* [Annandale, 1907]), *Calotes* Cuvier, 1817 (*J. tricarinata* [Blyth, 1853]) and *Phoxophrys* (*J. gramahi* [Stejenger, 1924]); and species that are currently recognized in other recognized agamid genera have been placed into *Japalura s.l.* including *Aphaniotis* Boulenger, 1885 (*Aphaniotis ornata* [Lidth De Jeude, 1893]), *Phoxophrys* (*Phoxophrys nigrilabris* [Boulenger, 1885]; *P. robinsoni* [Boulenger, 1920]; Inger, 1960), and *Pseudocalotes* Fitzinger, 1843 (*Pseudocalotes kaulbacki*, [Smith, 1937]; Mahony 2010).

Moreover, several generic names were established, but later synonymized as subjective junior synonyms of *Japalura* based on morphological data alone, including *Diploderma* Hallowell, 1861 (type species *D. polygonontum*, from the Okinawa islands of Japan), *Oriotiaris* Günther, 1864 (type species *O. tricarinata*, from north-east India), and *Oreocalotes* Jerdon, 1870 (type species *O. major*, from the western Himalayas) (Schleich & Kästle, 2002; Mahony, 2010). Nonetheless, the evolutionary independence of most of those genera represented was not examined specifically under a comprehensive morphological or molecular phylogenetic framework (Macey *et al.*, 2000; Pyron *et al.*, 2013; Grismer *et al.*, 2016b).

To date, a total of 34 species are recognized as members of the genus *Japalura s.l.* in the recent literature (Yang & Rao, 2008; Mahony, 2010; Manthey *et al.*, 2012; Cai *et al.*, 2015; Wang *et al.*, 2016; Ananjeva *et al.*, 2017; Rao *et al.*, 2017), including: *J. andersoniana* Annandale, 1905; *J. bapoensis* (Yang, Su & Li, 1979); *J. batangensis* Li, Deng, Wu & Wang, 2001; *J. brevicauda* Manthey, Denzer, Hou & Wang, 2012; *J. brevipes* Gressitt, 1936; *J. chapaensis* Bourret, 1937; *J. dasi* (Shah & Kästle, 2002); *J. dymondi* (Boulenger, 1906); *J. fasciata* Mertens, 1926; *J. flaviceps* Barbour & Dunn, 1919; *J. grahami* (Stejneger, 1924); *J. hamptoni* Smith, 1935; *J. iadina* Wang, Jiang, Siler & Che, 2016; *J. kumaonensis* (Annandale, 1907); *J. laeviventris* Wang, Jiang, Siler & Che, 2016; *J. luei* Ota, Chen & Shang, 1998; *J. major* (Jerdon, 1870); *J. makii* Ota, 1989; *J. micangshanensis* Song, 1987; *J. ngoclinensis* Ananjeva, Orlov & Nguyen, 2017; *J. otai* Mahony, 2009; *J. planidorsata* Jerdon, 1870; *J. polygonata* (Hallowell, 1861); *J. sagittifera* Smith, 1940; *J. slowinskii* Rao, Vindum, Ma, Fu & Wilkinson, 2017; *J. splendida* Barbour & Dunn, 1919; *J. swinhonis* Günther, 1864; *J. tricarinata* (Blyth, 1853); *J. varcoea* (Boulenger, 1918); *J. variegata* Gray, 1853; *J. vela* Wang, Pan, Hou, Siler & Che, 2015; *J. yunnanensis* Anderson, 1878; *J. yulongensis* Manthey, Denzer, Hou & Wang, 2012; and *J. zhaoermii* Gao & Hou 2002.

MATERIAL AND METHODS

Taxon sampling

We collected 25 out of 34 species of the genus *Japalura s.l.* from Mainland China, Taiwan and India [Field collection permits (Mainland China: BBCJ-2014-001; Taiwan: Forest Bureau No. 1061700632); Wang *et al.*, 2015, 2016, 2017, 2018a, 2018b; Supporting

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Information, Appendix S1]. Lizards were euthanized after capture in the field following the protocols of corresponding institutions in their home countries (USA and Mainland China: IACUC R17-019; Taiwan: IACUC of NTNU No. 106027), and liver or muscle tissues were collected from each individual and preserved in 95% ethanol. Specimens were fixed in buffered 10% formalin and subsequently transferred to 70% ethanol for long-term preservation. Vouchered specimens were deposited in research institutions, including the Museum of Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ), National Museum of Natural Science (NMNS) and Center for Ecological Sciences, Indian Institute of Science (CESG).

Additional tissue samples were also obtained through museum loans from the California Academy of Sciences, USA (CAS), Museum of Comparative Zoology, USA (MVZ) and Sam Noble Oklahoma Museum of Natural History (OMNH) (Supporting Information, Appendix S1). Additionally, we collected novel sequence data for six outgroup species of agamids representing four genera from Mainland China and the Philippines (Supporting Information, Appendix S1), and we incorporated existing genetic sequence data of four species of *Japalura s.l.* and 17 species of outgroup agamids were obtained from GenBank (Supporting Information, Appendix S2).

MORPHOLOGICAL DATA COLLECTION

We examined a total of 217 specimens representing 27 species of *Japalura s.l.* and 31 specimens representing 11 species from seven genera of outgroup agamid diversity (Supporting Information, Appendix S3). Following previous studies (Moody, 1980; Mahony, 2010; Grismer *et al.*, 2016a), and our prior knowledge on morphological characteristics of Draconinae (Mahony, 2010; Wang *et al.*, 2018b), we chose a suite of pholidosis characteristics to examine for all specimens, as they are shown to be most useful in delimiting generic-level diversity (abbreviations given in parentheses): presence of modified scales on dorsal body (MDS), defined as presence of scales on dorsal body that are modified in shape, size or texture (e.g. enlarged, keeled, flat or raised in conical shape); degree of modification of dorsal modified scales (DMDS), defined as the level of modification compared to the ground or normal scales on dorsal body, defined as strongly modified or weakly modified; presence of dorsolateral ridges (PDR), defined as presence of regularly and closely arranged, lateral rows of enlarged, keeled dorsal scales on the body; presence of V-shaped ridges on dorsal body (PVR), defined as presence of enlarged, keeled scales that are arranged in V-shape ridges on dorsal body; and presence of head spines (PS), defined as presence of elongated spines on the post-orbital, occipital and supratympanic regions of head.

In addition, standard morphometric measurements of agamid lizards were taken by KW for a subset of male specimens only (53 specimens representing 21 species of *Japalura s.l.* and 19 specimens representing eight species from six genera of outgroup taxa within Draconinae) (Supporting Information, Appendix S4). Male specimens only were chosen for the statistical analyses, because males are generally more diverse in morphology; and by choosing one sex only, we remove the confounding impacts from sexual dimorphism from the analyses, which is evident in most Draconinae genera (Zug *et al.*, 2006; Manthey, 2012; Deepak *et al.*, 2016; Wang *et al.*, 2016). The following morphological characters and their measurement methods followed Wang *et al.* (2016) and included: snout–vent length (SVL), tail length (TAL), head width (HW), head length (HL), snout–eye length (SEL), fore-limb length (FLL), hind-limb length (HLL) and trunk length (TRL). In addition, length of tallest nuchal crest (CL), measured from the base to the tips of the tallest nuchal crest scale, was also recorded. All raw measurements were standardized to relative ratios by either dividing against SVL or HL, and the ratios include HL/SVL, TRL/SVL, TAL/SVL, FLL/SVL, HLL/SVL, CL/HL, SEL/HL and HW/HL. Only these resulting ratios were used for statistical analyses (statistical methods, see below).

STATISTICAL ANALYSES FOR MORPHOLOGICAL DATA

Principle Component Analysis (PCA) and Discriminant Analysis of Principle Components (DAPC) were performed on the relative ratios of measurements to determine whether *Japalura s.l.* occupied unique morphospace with respect to morphologically similar Draconinae genera, and whether the morphological clustering coincided with the phylogenetic clades recovered from our molecular dataset. PCA was performed on the relative ratios of continuous morphometric measurements, using the prcomp command in R, v.3.2.1. All PCA data were natural log transformed prior to analysis and scaled to their standard deviation in order to normalize their distribution. DAPC were performed on the principle components with eigenvalues greater than one (Kaiser, 1960), using R, v.3.2.1 (Supporting Information, Appendix S5).

MOLECULAR DATA COLLECTION

Total genomic DNA was extracted from liver or muscle tissues using Fujita's Guanidine Thyocyanate protocol (Esselstyn *et al.*, 2008). We targeted three nuclear loci, including Brain Derived Neurotrophic Factor (*BDNF*), Oocyte Maturation Factor (*CMOS*) and RNA Fingerprint Protein 35 (*R35*), and one mitochondrial

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gene, NADH dehydrogenase subunit 2 (*ND2*). As a result of amplifying *ND2*, we also obtained a short fragment of the cytochrome *c* oxidase subunit I (*COI*) gene and components of eight flanking transfer RNA genes (tRNA^{Gln}, tRNA^{Ile}, tRNA^{Met}, tRNA^{Trp}, tRNA^{Ala}, tRNA^{Asn}, tRNA^{Cys} and tRNA^{Tyr}). Genes were targeted and amplified using both published and newly designed primers and PCR protocols (Table 1).

Amplified products were visualized on 1.5% agarose gels, and clean, single-banded products were purified with 1 µL of a 20% dilution of ExoSAP-IT (US78201, Amersham Biosciences). Cycle sequencing reactions were carried out using ABI Prism Big-Dye Terminator chemistry (Ver. 3.1; Applied Biosystems), and purified with Sephadex (NC9406038, Amersham Biosciences) in Centri-Sep 96 spin plates (CS-961, Princeton Separations). Purified products were sent to Eurofins Genomics for analysis. All novel sequences were deposited in GenBank (Supporting Information, Appendix S1).

ALIGNMENT AND PHYLOGENETIC ANALYSIS

Continuous gene sequences were assembled and edited using GENEIOUS, v.10.0.6. Alignments were produced initially using MUSCLE (Edgar, 2004), with minor subsequent manual adjustments. Alignments and resulting topologies are deposited in Data Dryad (doi:10.5061/dryad.683h4j5). To assess phylogenetic congruence between the mitochondrial and nuclear data, we inferred the phylogeny for each gene independently using likelihood and Bayesian analyses. As no strongly supported incongruences between mitochondrial and nuclear data were observed, we concatenated the two datasets for the final analyses.

We conducted concatenated, partitioned Bayesian analyses in the program MRBAYES, v.3.2.1 (Ronquist & Huelsenbeck, 2003). All protein-coding genes were partitioned by codon positions, except for *COI*, which is treated as a single partition due to its short length (<40 bp), and the mitochondrial tRNAs were combined and treated as a single partition as well (Table 2). The best models of nucleotide substitution were selected for each partition by the Akaike Information Criterion (AIC), as implemented in JMODELTEST2, v.2.1.10 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012). A rate multiplier model was used to allow substitution rates to vary among subsets. Default settings were used for all other model parameters. Two independent Markov chain Monte Carlo analyses were run, each with four Metropolis-coupled chains, a melting temperature of 0.02 and an exponential distribution with a rate parameter of 25 as the prior branch lengths (Marshall, 2010). All Bayesian analyses were run for 6 000 000 generations, with parameters and topologies sampled every 3000 generations. Stationarity and convergence were assessed with TRACER, v.1.6.0 (Rambaut, Suchard & Drummond, 2013), and we confirmed convergence of tree splits with AWTY (Nylander *et al.*, 2008). Although runs reached stationarity after 5 million generations, we conservatively discarded the first 15% of samples as burn in, resulting in a total of 1700 topologies from the posterior distribution for both runs.

Partitioned Maximum Likelihood analyses were conducted using RAxML-VI-HPC, v.8.2.10 (Stamatakis, 2014) on the concatenated dataset using the same partitioning strategy as for Bayesian analysis. The more complex model (GTR + Γ) was applied for all subsets (Table 2), with 1000 replicate ML inferences run. Each

Table 1. Summary of primers and annealing temperatures employed in this study

Gene	Primer name	Primer sequence	Annealing temperature (ºC)	Referred study
ND2	L4160	CGATTCCGATATGACCARCT	52	Macey *et al.*, 2000
	H4980	ATTTTTCGTAGTTGGGTTTGRTT	52	Macey *et al.*, 2000
	Jap_264F	GGGCCCATACCCCRAAAAC	55	Present study
	Jap_1559R	GGATTAATGCCCTCTGGATT	55	Present study
	L4437b_F	AAGCAGTTGGGCCCATACC	55	Macey *et al.*, 2000
	H5540_R	TTTAGGGCTTTGAAGGC	55	Macey *et al.*, 2000
	Jap_70F	CCACCAAACAACTACACCTA	55	Present study
	Jap_1559R	GGATTAATGCCCTCTGGATT	55	Present study
BDNF	BDNF.F	CCCCAATGAAAGAAGTGASCCTC	55	Crottini *et al.*, 2009
	BDNF.R	TGGGTAGTTCGGCACTGAGAATTCC	55	Crottini *et al.*, 2009
CMOS	G73.1	GGCTRTAAARCARGTGAAGAAA	55	Whiting *et al.*, 2003
	G74.1	GARCWTCCAAAGTCTCCAATC	55	Whiting *et al.*, 2003
R35	R35.F	GACTGTGGAYGAYCTGATCAGTGTGG	55	Whiting *et al.*, 2003
	R35.R	GCCAAAATGAGSGAGAARCGCTTCTG	55	Whiting *et al.*, 2003

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Table 2. Models of evolution selected by Akaike Information Criterion (AIC) and applied for partitioned, phylogenetic analyses

Gene	Partition	AIC model	Number of characters (bp)
BDNF	1st codon	GTR+Γ	176
	2nd codon	HKY	176
	3rd codon	HKY+Γ	175
CMOS	1st codon	JC+Γ	181
	2nd codon	HKY+Γ	180
	3rd codon	HKY	180
R35	1st codon	HKY+Γ	213
	2nd codon	GTR	213
	3rd codon	HKY+Γ	213
tRNAs (Gln, Ile, Met, Trp, Ala, Asn, Cys, Tyr)	Combined, single partition	GTR+Γ	613
ND2	1st codon	GTR+Γ	345
	2nd codon	GTR+Γ	345
	3rd codon	GTR+Γ	345
COI	Single partition	GTR+Γ	33

inference was initiated with a random starting tree, and nodal support was assessed with 1000 bootstrap pseudoreplicates (Stamatakis, Hoover & Rougemont, 2008).

To root the resulting phylogenetic trees, *Hydrosaurus pustulatus* (subfamily Hydrosaurinae) was chosen as the outgroup of subfamily Draconinae, based on published higher level phylogenetic studies of squamate reptiles (Pyron *et al.*, 2013).

Hypothesis testing

We tested taxonomic hypotheses proposed in previous studies to address the following questions: (1) Does our expanded dataset support the previous shown relationship between *Malayodracon* and island species of *Japalura s.l.* by Pyron *et al.* (2013)? (2) With expanded taxa sampling, does our dataset support the previous shown sister relationship between the genus *Calotes* and *Salea* by Grismer *et al.* (2016b)? (3) With the exclusion of the controversial species, *J. bapoensis*, do congeners from each of the three general geographic regions (the Himalayas, Indochina/Mainland East Asia and East Asian Islands) form a monophyletic group, as suggested by previously proposed species groups (e.g. Himalayan Species Group, Indochinese Species Group and Island Species Group)? (4) Does *J. bapoensis* form a monophyletic group with remaining species of *Japalura s.l.* in mainland East Asia?

To do so, we estimated posterior probabilities of different topologies that correspond to each of the above question using a Bayesian approach. We constrained corresponding topologies for each hypothetical scenario and estimated the posterior probability of supporting such hypotheses using the proportion of post-burn-in trees that are consistent with the corresponding hypothesized topology. This was accomplished by filtering the total number of post-burn-in trees for each constrained topology using PAUP*, v.4.0b10 (Swofford, 1999).

RESULTS

Phylogenetic results

We did not observe any incongruent clades with strong statistical support [≥0.95 posterior probability (PP) or ≥70 bootstrap support (BS)] between nuclear and mitochondrial gene trees. Therefore, we felt justified in concatenating all data into one matrix, containing 3388 nucleotides for subsequent analyses. Characteristics of nucleotides, as well as the inferred best-fit models of sequence evolution selected by Akaike Information Criterion (AIC), are summarized in Table 2.

The results of Maximum Likelihood and Bayesian analyses are largely consistent with each other. However, the placements of an outgroup (*Acanthosaura lepidogaster*) are different between the two analyses (in ML analyses, *Acanthosaura* is recovered as sister to the clade containing *Malayodracon*, *Pseudocalotes*, *Sitana* and clade O of *Japalura s.l.* (BS = 53; Fig. 2). The subfamily Draconinae is recovered with strong support, *Mantheyus phuwuanensis* as the first branching lineage [1.00/100 (PP/BS, respectively, herein noted in the same order)]. Both analyses support the same relationships among focal clades with strong support (≥0.99/≥80), with the results supporting the paraphyly of *Japalura s.l.* Four divergent clades are recovered within the *Japalura s.l.* (Fig. 2, clades C, G, N and O): (1) *J. andersoniana*, *J. kumaonensis*, *J. tricarinata* and

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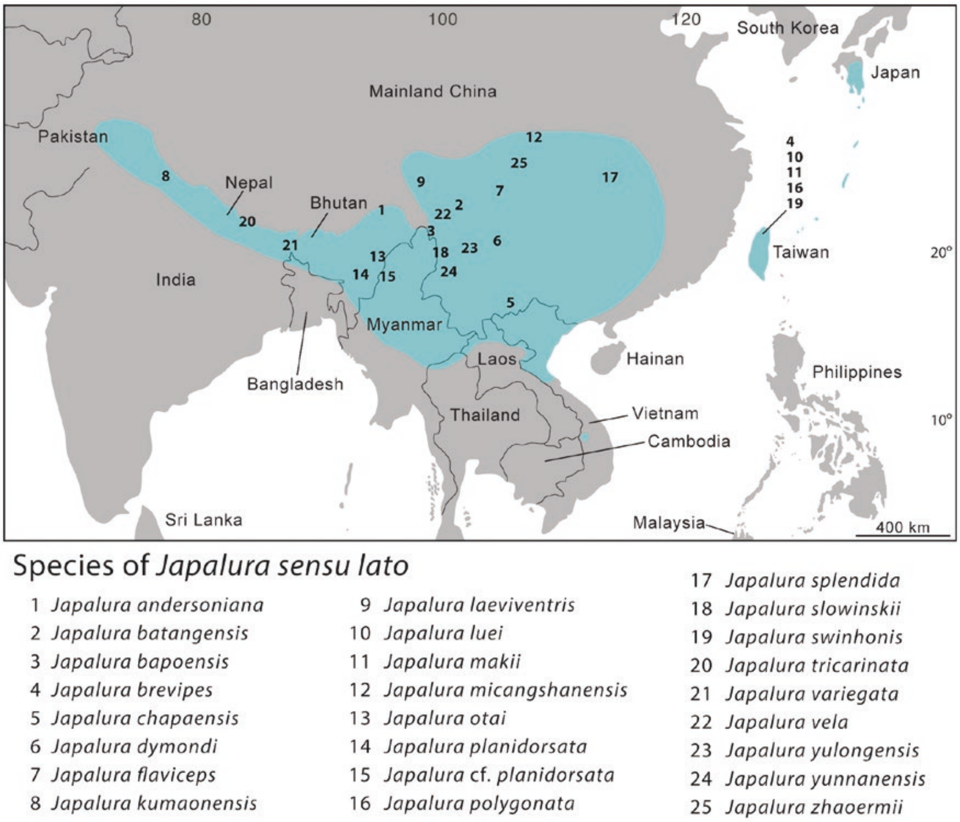

Figure 1. Distribution of *Japalura s.l.* in Asia. Shaded area (light blue) represents the estimated distribution of *Japalura s.l.* (the isolated record in south-central Vietnam represents the questionable taxon *Japalura ngoclinensis*; see Discussion); solid black lines represent national boundaries (political boundaries along the Himalaya are not included to accommodate disputed, political issues surrounding this region); and numbers represent geographic locations of sampled species from this current study, each corresponding to the listed taxon in the figure key shown below the map.

J. variegata form a monophyletic group with strong support (Fig. 2, clade C, 1.00/83), which form a strongly supported group with *Draco* and *Ptyctolaemus* (Fig. 2, clade A, 1.00/91); (2) *J. otai* and *J. planidorsata* form the second, more distantly related clade (Fig. 2, clade G, 1.00/100) that is sister to the genus *Salea* (0.99/69; Fig. 2); (3) *J. bapoensis* is nested within *Pseudocalotes* with strong support (Fig. 2, clade M, 1.00/100); and (4) all the remaining species from Mainland China and East Asian islands form a monophyletic group (Fig. 2, clade O, 1.00/100) that is sister to the genus *Pseudocalotes* (1.00/100). Furthermore, two well-supported sub-clades (Fig. 2, sub-clade P, 1.00/100; and sub-clade Q, 1.00/100) were recovered within clade O from Mainland China and East Asian Islands (Fig. 2).

Although population sampling is not the focus of this paper, we included multiple populations of the same species when samples were available. At the species level, most species are recovered as monophyletic lineages, except *J. planidorsata* and *J. flaviceps* in clades G and O, respectively (Fig. 2). The sampled Myanmar individuals of *J.* cf. *planidorsata* are paraphyletic with respect to *J. otai*, and the GenBank sequence of *J.* cf. *flaviceps* is not recovered with topotypic individuals of the species, but instead is supported as part of a monophyletic group with topotypic material of *J. zhaoermii* (Fig. 2).

MORPHOLOGICAL RESULTS

For continuous morphometric data, PCA and DAPC analyses show members of *Japalura s.l.* occupy distinct regions of morphospace compared to other genera (Fig. 3). Furthermore, four groups were recovered within *Japalura s.l.* in morphospace, which coincide with the four major clades of the genus *s.l.* recovered from the genetic data (Fig. 3). For PCA analysis, the first four components account for 78% of the total variance: PC1 loads most heavily on relative fore-limb (FLL/SVL) and hind-limb length (HLL/SVL) (25% of the total variance); PC2 loads most heavily on relative crest length (CL/HL) and snout length (SEL/HL) (23% of total variance); PC3 loads most heavily on relative trunk length (TRL/SVL) and head length (HL/SVL)

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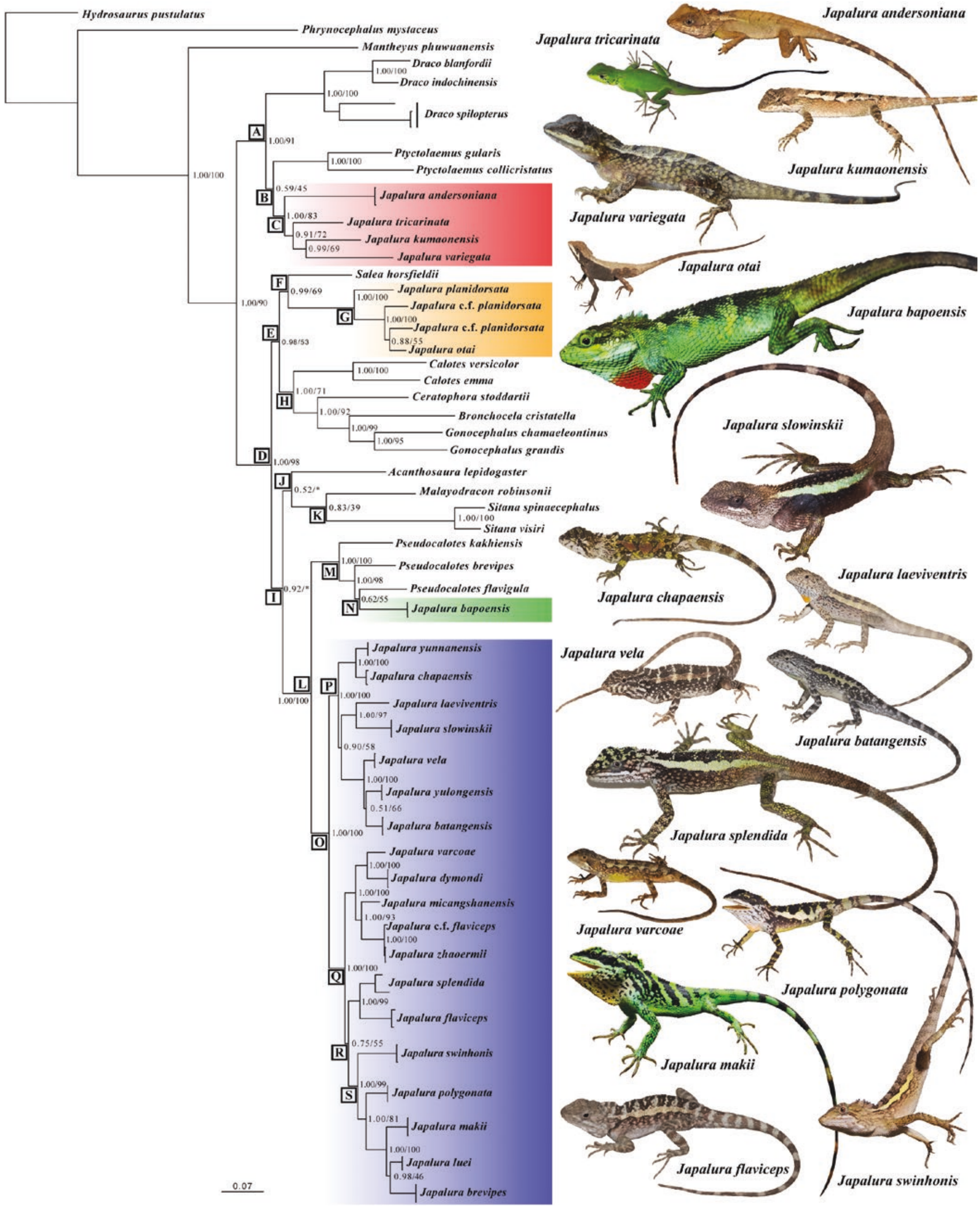

Figure 2. Phylogenetic relationships among *Japalura sensu lato* based on both Maximum Likelihood and Bayesian Analyses of two mitochondrial genes (*COI* and *ND2*), tRNAs and three nuclear genes (*BDNF*, *CMOS*, and *R35*). Maximum Likelihood bootstrap and Bayesian posterior probability values are included at all nodes, except (1) terminal nodes that unify multiple individuals of the same species [which all have 1.00/100 support (Bayesian/Maximum Likelihood)]; and (2) non-conflicting (either kind of analysis yielded significant supports) yet inconsistent nodes between two types of analyses (e.g. nodes unifying clades I and J), in which only the Bayesian posterior probability is given. Photos of selected species of *Japalura s.l.* are included (scaled to reflect relative sizes of each species) with their current taxonomic names (vs. revised taxonomic names, summarized in taxonomic accounts in the Discussion).

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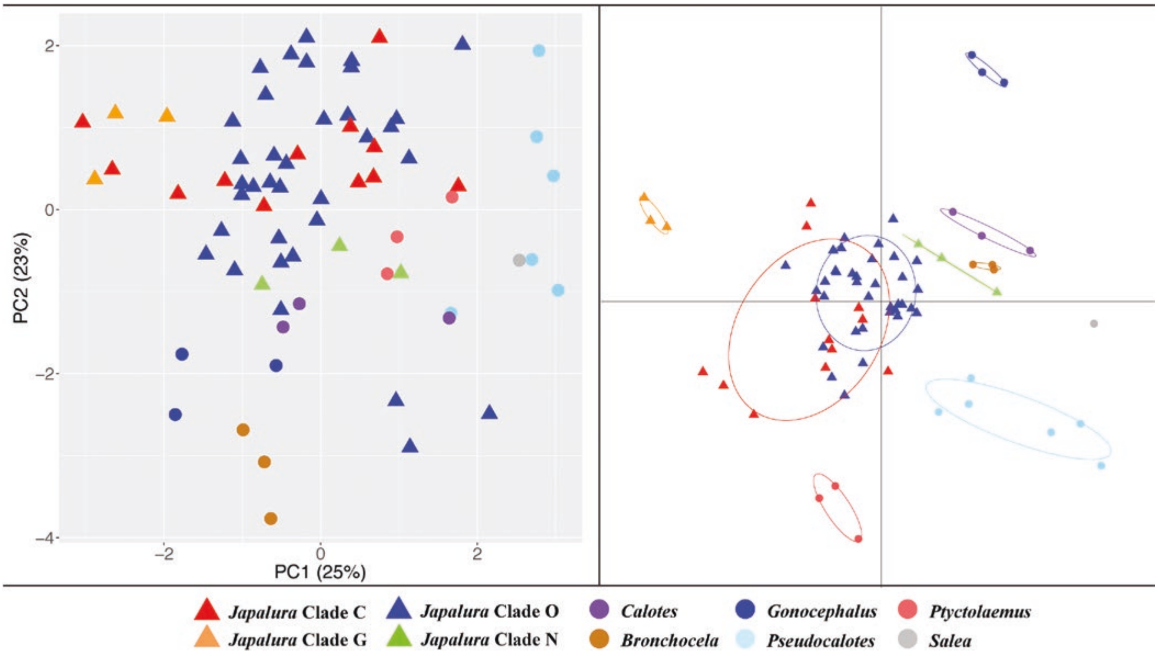

Figure 3. Principle component analysis (PCA) and discriminant analysis of principle component (DAPC) of continuous morphometric measurements and ratios of *Japalura s.l.* and morphologically similar genera. Colour code of each clade of *Japalura s.l.* coincides with the one used for the same clade in Figure 2.

(16% of the total variance); and PC4 loads most heavily on relative tail length (TAL/SVL) and head length (HL/SVL) (14% of the total variance) (Table 3; Supporting Information, Appendix S4). The DAPC further characterize these results and show the visual clustering of four different clades of *Japalura s.l.* in morphospace (Fig. 3). In addition, each of the four genetic clades/morphological clusters can also be diagnosed readily using a combination of discrete pholidosis characteristics (Fig. 4; Table 3).

TOPOLOGY TESTING

Our topological tests do not support the monophyly of the clade containing the East Asian *Japalura s.l.* and *Malayodracon robinsonii* (H1, PP = 0.00) and the clade containing *Calotes* and *Salea* (H2, PP = 0.00) (Table 4). For *Japalura s.l.* species from the three geographic regions, with the exclusion of the controversial species, *J. bapoensis*, the Bayesian statistical tests show no support for the monophyly of the Himalayan species group (H3, PP = 0.00), nor the monophyly of the remaining mainland Indochinese species group (H4, PP = 0.00). Only the monophyly of East Asian species (together both mainland and island species) is statistically supported (H5, PP = 1.00). As for the controversial species, *J. bapoensis*, we found no statistical support for its monophyly with respect to the remaining East Asian species (H6, PP = 0.00) (Table 4).

DISCUSSION

NEW CLASSIFICATION OF *JAPALURA S.L.*

Our morphological and phylogenetic results both support observations of paraphyly among members of *Japalura s.l.* in previous studies (Figs 2, 3; Table 3; Macey *et al.*, 2000; Mahony, 2010). We recover two previously recognized clades in similar phylogenetic positions in the inferred topologies (*J. variegata* clade and *J. splendida* clade), and we confirm the generic placement of *J. bapoensis* in the genus *Pseudocalotes* as in previous studies (Macey *et al.*, 2000; Schulte *et al.*, 2004; Zug *et al.*, 2006; Pyron *et al.*, 2013; Grismer *et al.*, 2016b; Fig. 2). Furthermore, with the exclusion of *J. bapoensis*, this study supports the previously assumed monophyly of all East Asian species, including both mainland and island lineages (H5, PP = 1.00; Fig. 2, clade O, 1.00/100; Table 4; Ananjeva *et al.*, 2011).

In addition to observing consistent patterns with previous research, our results reveal novel relationships of draconine genera with the expanded taxonomic and genetic coverage. First, analyses recover two distinct clades from the previously proposed Himalayan species group: clade C, which corresponds to the previously discovered Himalayan clade by Macey *et al.* (2000) and Pyron *et al.* (2013), and clade G, revealing relationships observed for the first time (Fig. 2). Although species of both clades are from the Himalayan region, they are recovered in distantly

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Table 3. Comparisons of discrete characters among the four clades of *Japalura s.l.* and morphologically similar genera within Draconinae. Focal clades of *Japalura s.l.* refer to those supported in Figure 2

Characters	*Japalura s.l.*				Outgroup genera				
	Clade C	Clade G	Clade O	Clade N	*Calotes*	*Gonocephalus*	*Malayodracon*	*Pseudocalotes*	*Salea*
SSDB	HT	HT	HT	HT	HM	HT	HT	HT	HT
AESD	DLR & VR	DLR & VR	Irregular	DLR	/	Irregular	Irregular	Irregular	Irregular
ECSN	A	P	A	A	A	A	A	A	A
KSL	K	K	S	K	K	K	K	S	S
SOR	Multiple	Multiple	Singular	Multiple	Multiple	Multiple	Singular	Mostly singular	Singular
RSSOR	Subequal	Subequal	N/A	Subequal or one row enlarged	Subequal	Subequal	N/A	N/A or one row enlarged	N/A
HS	A	A	A	A	P	A	A	A	A
SCS	LT	LT	TT	LT	L	L	TT	TT or L	L
GP	FB or WD	FB	WD	FB or WD	WD	WD	WD	WD	WD

Abbreviations of morphological characteristics include: size and shape of dorsal body scales (SSDB); arrangements of enlarged dorsal scales (AESD); enlarged conical scales on nape (ECSN); keel status of loreal scales (KSL); suborbital scale rows (SOR); relative size of suborbital scale rows (RSSOR); head spines (HS); shape of crest scales (SCS); gular pouch (GP). Abbreviations of character state include: heterogeneous (HT); homogeneous (HM); dorsolateral ridge (DLR); V-shaped ridge (VR); A (absent); P (present); low triangular (LT); tall triangular (TT); lanceolate (L); feebly developed (FB); WD (well developed). 'N/A' indicates not applicable.

related groups within Draconinae with strong support (Fig. 2). Statistical topology tests further support the phylogenetic results, rejecting the assumed monophyly of the Himalayan congeners (PP = 0.00; Table 4; Stuart-Fox & Owens, 2003; Mahony, 2010; Ananjeva *et al.*, 2011; Wang *et al.*, 2018b). Second, we reject the previously hypothesized sister relationship between island *Japalura* species and *Malayodracon* (Pyron *et al.*, 2013). The phylogenic results support a closer relationship between East Asian species of *Japalura s.l.* (Mainland and Island) and *Pseudocalotes* (Fig. 2, clade L), and topology tests reject the null hypothesis of reciprocal monophyly between island *Japalura s.l.* and *Malayodracon* (Table 4).

The recognition of all four clades of *Japalura s.l.* as a single genus would render most genera of the subfamily Draconinae paraphyletic, thus, further complicating an existing, long-held taxonomic problem. Therefore, in an effort to revise taxonomy to best reflect evolutionary histories of each clade of *Japalura s.l.* in nomenclature, we recognize four genera that each corresponds to the evolutionary clade recovered in our phylogenetic and morphological analyses.

The first clade (Figs 2, 3, clade C) includes the type species of three currently or historically recognized generic names, namely *Japalura sensu stricto* Gray (1853) (type species *J. variegata*), *Oriotiaris* Günther (1864) (type species *O. tricarinata*) and *Oreocalotes* Jerdon, 1870 (type species *O. major*) (Fig. 2). However, we recognize *Japalura sensu stricto* to represent this clade for the following reasons: (1) *Japalura sensu stricto* has temporal priority over the other two generic names; (2) recognizing the synonym *Oriotiaris* as a valid genetic name would render itself and *Japalura sensu stricto* both paraphyletic (Fig. 2); (3) for *Oreocalotes*, although we do not have access to the genetic materials of the type species, the morphology of the type species (*O. major* or *J. major* as currently recognized) falls within the morpho-cluster of species of *Japalura sensu stricto*, and the type species is morphologically most similar to *J. kumaonensis* (Wang *et al.*, 2018b), which is a confirmed member of *Japalura sensu stricto* (Figs 2, 3). Therefore, we treat both *Oriotiaris* and *Oreocalotes* as junior synonyms of *Japalura sensu stricto*.

Of the remaining three clades (clades G, N and O; Fig. 2), for clade N, we follow Mahony (2010) and transfer *Japalura bapoensis* to the genus *Pseudocalotes*, and conservatively consider it as a subspecies of *P. kingdonwardi* (see discussion below on its species status). Second, we resurrect the only available generic name in the literature, *Diploderma*, for clade O (with its type species *D. polygonatum polygonatum* Hallowell, 1861). Lastly, as clade G has no generic name available in the literature, we describe it as a new genus, *Cristidorsa* gen. nov. (described below). Based on the revised

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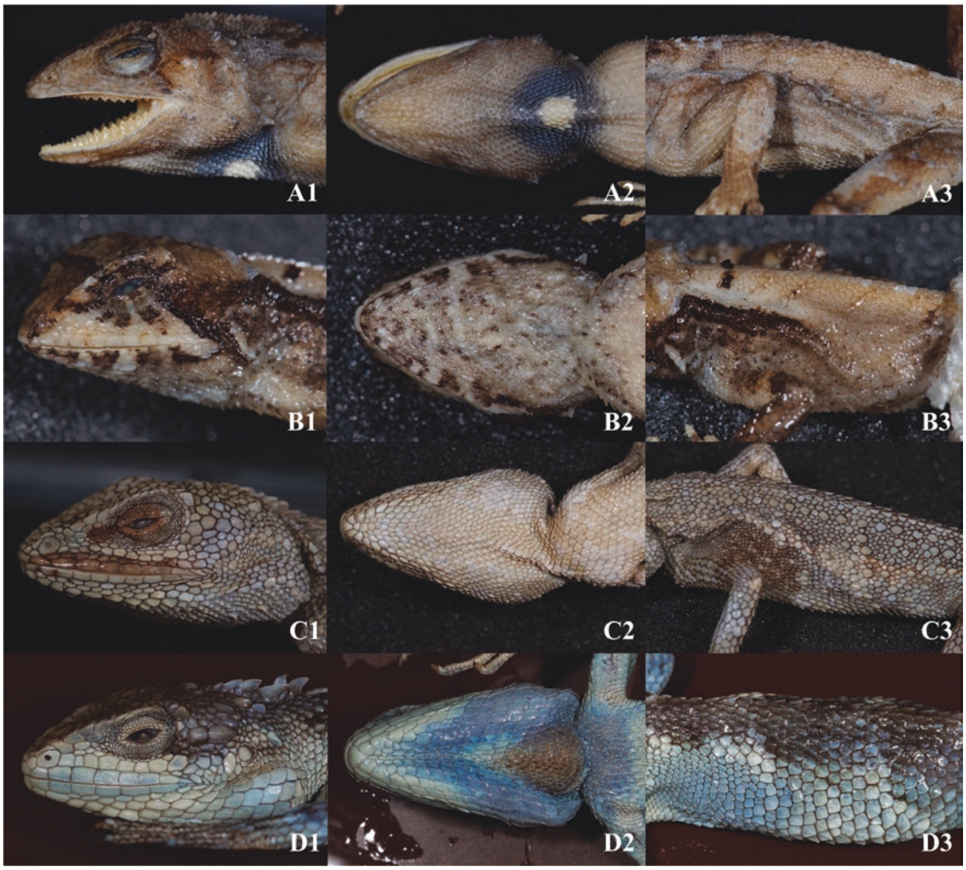

Figure 4. Close-up photographs of the head in lateral (1), and ventral (2), views, and body in lateral view (3), of representative species (A–D) of the four clades of *Japalura s.l.* recovered in phylogenetic and morphological analyses, showing diagnostic pholidosis characteristics: A, clade C: *Japalura andersoniana*, KIZ 011156; B, clade G: *Japalura* cf. *planidorsata*, CAS 233296; C, clade O: *Japalura polygonata*, MCZ 21244; and, D, clade N: *Japalura bapoensis*, CAS 241965.

Table 4. Bayesian topology tests conducted for previously hypothesized relationships of *Japalura s.l.* and other genera within the subfamily Draconinae

	Hypothesis	Description of constraint	Posterior probability
Does *Malayodracon* form monophyletic group with island *Japalura s.l.*?			
H1	Monophyly of island *Japalura* + *Malayodracon*	All sampled *Japalura* from Taiwan and *Malayodracon robinsonii*	0.00
Does *Calotes* form a sister relationship with *Salea*?			
H2	Monophyly of the clade containing *Calotes* + *Salea*	All sampled species of *Calotes* and *Salea*	0.00
With the exclusion of *Japalura bapoensis*, do congeners from each of three regions form a monophyletic group?			
H3	Monophyly of Himalayan species group	All sampled species from the Himalayan region	0.00
H4	Monophyly of Indochinese species group	All sampled species from mainland east Asia	0.00
H5	Monophyly of Indochinese species + island species	All sampled species from mainland east Asia and Taiwan	1.00
Does *Japalura bapoensis* belong to the east Asian clade of *Japalura s.l.*?			
H6	Monophyly of *J. bapoensis* + remaining Indochinese + island species	All sampled species from mainland east Asia (including *J. bapoensis*) and Taiwan	0.00

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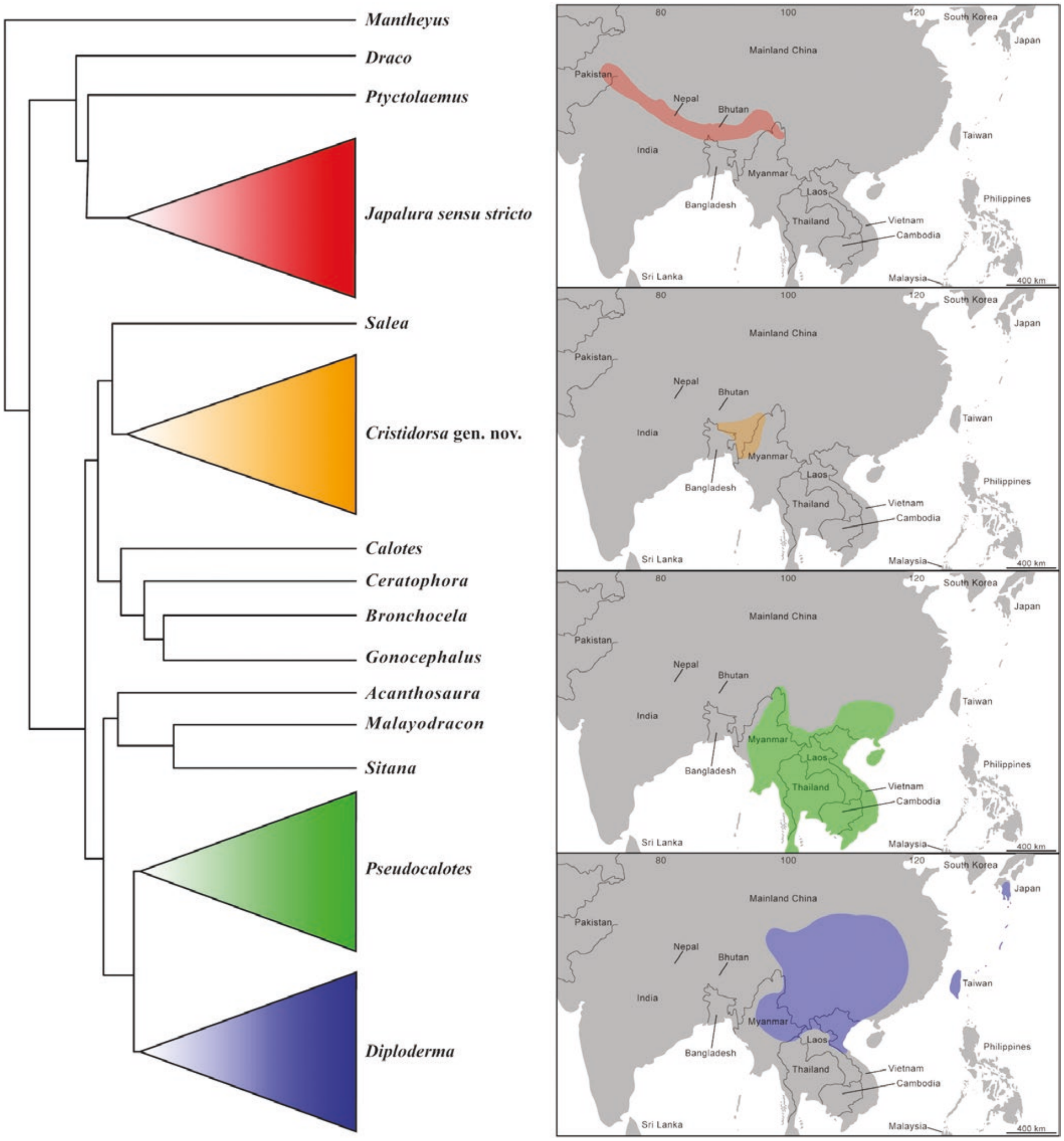

Figure 5. Simplified, inferred topology summarizing the new proposed classification scheme and recognized geographic distributions of the four focal clades of *Japalura s.l.* (*Japalura*, ***Cristidorsa* gen. nov.**, *Pseudocalotes* and *Diploderma*).

taxonomy and associated morphological diagnoses summarized in this study for each of the four clades, we propose a new classification scheme for all recognized members of *Japalura s.l.*

TAXONOMIC ACCOUNTS

JAPALURA GRAY, 1853

Etymology: The Latin name '*Japalura*' may be derived from a locality name in India, and the term is feminine gender. We suggest the English common name as 'Himalayan Dragon', and the Chinese name as '攀蜥' (pronounced as 'Pan-Xi').

Type species: Japalura variegata, Gray, 1853.

Diagnosis: Lizards of the genus *Japalura sensu stricto* differ from closely related genera by possessing the following morphological characteristics: (1) head width moderate, HW mostly <70% of HL; (2) nuchal and dorsal crest scales relatively low and thick, not significantly elongated into lanceolate spines, CL/HL<10%;

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(3) post-orbital and post-occipital spines absent; (4) gular scales mostly homogeneous in size; (5) size of scales on lateral jaw subequal in size across gular region; (6) dorsal scale significantly heterogeneous in size and shape, not regularly imbricate; (7) pair of distinctively enlarged, conical scales on nape above shoulder absent; (8) paravertebral rows of enlarged scales present on dorsolateral body; and (9) V-shaped ridges present along dorsal midline, formed by enlarged, keeled scales.

Phylogenetic definition: We define *Japalura sensu stricto* using the maximum crown-clade definition, which includes species that share a more recent common ancestor to *Japalura veriegata* than *Draco volans* or *Ptyctolaemus gularis*.

Included species: Based on our phylogenetic results, we assign the following species to the genus *Japalura sensu stricto*: *J. andersoniana*, *J. kumaonensis*, *J. tricarinata* and *J. variegata*. Following our morphological results and proposed morphological diagnoses, we also assign *J. dasi*, *J. major* and *J. sagittifera* into this genus, pending future phylogenetic studies.

Geographic distribution: Members of the genus are distributed along the southern foothills of the Himalayas, including north-eastern Pakistan (*J. kumaonensis* and *J. major*), northern and north-eastern India (*J. andersoniana*, *J. kumaonensis*, *J. major*, *J. sagittifera*, *J. tricarinata* and *J. variegata*), Nepal (*J. dasi*, *J. tricarinata* and *J. variegata*), Bhutan (*J. andersoniana*, *J. tricarinata* and *J. variegata*), southern parts of the Tibet Autonomous Region of China (*J. andersoniana* and *J. tricarinata*), and northern part of Myanmar (*J. sagittifera*). Congeners may also be found in north-western Bangladesh (Fig. 5).

CRISTIDORSA

WANG, DEEPAK, DATTA-ROY, LIN, JIANG, CHE & SILER
GEN. NOV.

Etymology: The Latin term '*Cristidorsa*' means 'ridged dorsum', which describes the distinct, characteristic ridges on the dorsal surface of the body in the new genus. The generic name is feminine and it consists of two parts, namely '*Cristi-*' (meaning 'ridged') and '*-dorsa*' (meaning 'dorsum'). We suggest the English common name as 'Ridged Dragons' and the Chinese name as '棱背蜥' (pronounced as 'Leng-Bei-Shi').

Type species: Cristidorsa otai (Mahony, 2009).

Diagnosis: Lizards of the genus '*Cristidorsa*' differ from other closely related Draconinae genera by having the following morphological characteristics: (1) head robust and relatively wide, HW/HL mostly >70%; (2) scales of lateral head keeled; (3) nuchal and dorsal crest feeble, CL/HL<5%; (4) post-occipital and post-orbital spines absent; (5) gular scales mostly homogeneous in size; (6) size of scales on lateral jaw subequal in size across gular region; (7) single pair of distinctively enlarged conical scales present on nape above shoulder, one scale on each side of vertebral crest; (8) dorsal scale significantly heterogeneous in sizes and shapes; (9) distinct, dorsolateral rows of enlarged, keeled scales present on dorsum; and (10) V-shaped ridges present along dorsal midline, formed by enlarged, keeled scales.

Phylogenetic definition: We define *Cristidorsa* using the maximum crown-clade definition, which includes species that share a more recent common ancestor with *Cristidorsa otai* than with *Salea horsfieldii*.

Included species: Based on our phylogenetic results, we assign *C. otai* and *C. planidorsata* to the genus *Cristidorsa*.

Geographic distribution: Members of the genus *Cristidorsa* are distributed on the south-east extreme of the Himalayan foothills, including north-east India (*C. otai* and *C. planidorsata*), and the north-west part of Myanmar [*C. otai*; see discussion on taxonomic status of the Myanmar population below (Fig. 5)].

DIPLODERMA HALLOWELL, 1861

Etymology: The Latin generic name '*Diploderma*' consists of two parts, '*Diplo-*' means 'double' or 'many', and '*-derma*' means 'skin', and the whole word is in a neuter gender. As the previous generic name '*Japalura*' and most species names of the genus *s.l.* are feminine, most names of species that are now assigned to *Diploderma* need their gender changed to neutral (except for existing neutral-gender names like *brevipes* or *flaviceps*, Latin nouns like *vela* or names derived from peoples' names, i.e. *dymondi*, *luei*, *makii*, *swinhonis*, *varcoae* and *zhaoermii*). We suggest the English common name of the genus as 'Mountain Dragon', and the Chinese common name as '龙蜥' (pronounced as 'Long-Xi').

Type species: Diploderma polygonatum Hallowell, 1861.

Diagnosis: Lizards of the genus *Diploderma* differ from closely related genera by having the following morphological characteristics: (1) scales of lateral head keeled; (2) nuchal and dorsal crest scales relatively short and thick, not elongated into lanceolate spines, CL/HL mostly <10%; (3) post-occipital and post-orbital spines absent; (4) gular scales mostly homogeneous in

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size, not decreasing in size toward the centre; (5) scales on lateral jaw subequal in size across gular region; (6) dorsal scale significantly heterogeneous in size and shape, not regularly imbricate; (7) paravertebral dorsolateral ridges of body present in most species, formed by enlarged, keeled scales (except in *D. swinhonis* and *D. leui*); and (8) V-shaped ridges along dorsal body midline absent in all but one species (except in *D. swinhonis*).

Phylogenetic definition: We define *Diploderma* using the maximum crown-clade definition, which includes species that share a more recent common ancestor with *Diploderma polygonatum* than with *Pseudocalotes tymanistriga* and *Acanthosaura lepidogaster*.

Included species: Based on our phylogenetic results, we assign the following species into the genus *Diploderma*: *D. batangense*, *D. brevipes, D. chapaense*, *D. dymondi*, *D. flaviceps*, *D. laeviventre*, *D. luei*, *D. makii*, *D. micangshanense*, *D. polygonatum* (and all of its subspecies), *D. slowinskii*, *D. splendidum*, *D. swinhonis*, *D. varcoae*, *D. vela*, *D. yulongense*, *D. yunnanense* and *D. zhaoermii*.

According to our proposed morphological diagnoses, we also assign *Diploderma brevicaudum*, *D. fasciatum*, *D. grahami*, *D. hamptoni* and *D. iadinum* to this genus, pending on future phylogenetic confirmations. In total, 24 species of *Japalura s.l.* are reclassified into the genus *Diploderma* (for discussion on the taxonomic status of *D. ngoclinense* see below).

Geographic distribution: Members of the genus are distributed across mainland East Asia, East Asian islands and northern Indochina, including central, southern and south-western Mainland China (*D. batangense*, *D. brevicaudum*, *D. chapaense*, *D. dymondi*, *D. fasciatum*, *D. flaviceps*, *D. grahami*, *D. iadinum*, *D. laeviventre*, *D. micangshanense*, *D. slowinskii*, *D. splendidum*, *D. vela*, *D. yulongense*, *D. yunnanense* and *D. zhaoermii*) and Taiwan (*D. brevipes*, *D. luei*, *D. makii*, *D. polygonatum* and *D. swinhonis*), the southern islands of Japan (*D. polygonatum*), central and eastern Myanmar (*D. hamptoni*), northern Vietnam (*D. fasciatum* and *D. chapaense*) and northern Thailand (*D. yunnanense*). Congeners such as *D. yunnanense* may also be distributed in Myanmar and northern Laos close to the Chinese border (Fig. 5).

PSEUDOCALOTES FITZINGER, 1843

Type species: Pseudocalotes tympanistriga (Gray, 1831).

Diagnosis: Pseudocalotes can be diagnosed from other Draconinae genera by having: (1) scales of lateral head smooth, especially loreal and subocular region; (2) scales of lateral jaw enlarged; (3) scales of central gular region distinctively smaller than others of throat; (4) sub-orbital scale row usually one, or multiple but with the middle one significantly enlarged; (5) post-orbital and post-occipital spines absent; (6) nuchal crest scales mostly elongated and tall, CL/HL mostly >10%; (7) dorsal crest scales feeble and low; (8) dorsal body scales heterogeneous in size and shape; (9) enlarged dorsal body scales not arranged in paravertebral, dorsolateral or V-shaped ridges.

Phylogenetic definition: We define *Pseudocalotes* using the maximum crown-clade definition, which includes species that share a more recent common ancestor with *Pseudocalotes tympanistriga* than with *Diploderma polygonatum* and *Acanthosaura lepidogaster*.

Included species: A single species of *Japalura s.l. Pseudocalotes kingdonwardi bapoensis*, is reclassified into the genus *Pseudocalotes*. Currently, the genus includes 22 recognized species in total (Grismer *et al.*, 2016a; Harvey *et al.*, 2017).

Geographic distribution: The genus is distributed in South-East Asia and Indochina, including southern Mainland China, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Sumatra, Thailand and Vietnam (Fig. 5).

PHYLOGENETIC RELATIONSHIPS OF *JAPALURA S.L.*

Generic-level relationships

The results of this comprehensive phylogenetic analysis of the genus *Japalura s.l.* confirm previous observations of paraphyly (Fig. 2; Macey *et al.*, 2000; Schulte *et al.*, 2004; Zug *et al.*, 2006; Pyron *et al.*, 2013). Although we recover four distinct genera within *Japalura sensu lato*: *Japalura s.l.*: *Cristidorsa*, *Pseudocalotes* and *Diploderma* (clades C, G, N and O, respectfully; Fig. 2), and strongly support the monophyly of *Japalura sensu stricto*, we cannot resolve the intergeneric relationships among *Japalura sensu stricto*, *Draco* and *Ptyctolaemus* (clade B; 0.587/45; Fig. 2). This result is consistent with previous studies, which have recovered *Japalura sensu stricto* both as sister to *Draco* (Schulte *et al.*, 2004) or *Ptyctolaemus* (Pyron *et al.*, 2013). Therefore, at this time, no conclusion can be made with confidence regarding the intergeneric relationships among the three genera—future

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studies with expanded taxonomic and genomic datasets are needed.

Without taxonomic representation of the new genus *Cristidorsa*, previous genomic studies supported a sister relationship between *Calotes* and *Salea*, and together, this clade was observed to be reciprocally monophyletic with a clade consisting of *Bronchocela*, *Ceratophora*, *Cophotis*, *Gonocephalus* and *Lyriocephalus* (Grismer *et al.*, 2016b). In contrast, with our expanded taxon sampling, our data support the new genus *Cristidorsa* (Fig. 2, clade G) as the sister-group to *Salea* (Fig. 2, clade F, 0.99/69), and together with *Bronchocela*, *Calotes*, *Ceratophora* and *Gonocephalus*, forms a large and diverse clade (Fig. 2, clade E, 0.985/53). Furthermore, our Bayesian topology tests reject the hypothesized monophyly of a *Calotes* + *Salea* clade (Table 4, H2, PP = 0.00). We suspect that incomplete taxonomic sampling may have resulted in the relationships recovered in previous studies (Grismer *et al.*, 2016b). However, continued research on this diverse group of agamid lizards is needed.

Using a supermatrix of 12 genetic loci, the resulting topology of Pyron *et al.* (2013) did not show support for the monophyly of the sampled East Asian species of *Japalura s.l.* The sampled East Asian mainland species (*D. flaviceps* and *D. splendidum*; both were recognized as *Japalura* at the time) were not the closest sister-group to the East Asian island species, *D. polygonatum* (recognized as *Japalura polygonata* at the time; Pyron *et al.*, 2013). Instead, Pyron *et al.*'s (2013) results supported *D. polygonatum* as most closely related to *Malayodracon robinsonii* (recognized as *Gonocephalus robinsonii* at the time) from South-East Asia. However, it should be noted that the only available mitochondrial loci at the time for the mainland and island species were non-overlapping [*ND2* for mainland species (*D. flaviceps* and *D. splendidum*) and 16S and 12S for island species (*D. polygonatum* and *Malayodracon robinsonii*); Pyron *et al.*, 2013]. Additionally, it is recognized that *D. polygonatum* is morphologically distinct from *Malayodracon* and distributed in distinct biogeographic regions (Denzer *et al.*, 2016; Fig. 2). Therefore, the unexpected relationships observed previously may be an artefact of incomplete gene coverage. With expanded, overlapping genetic datasets across sampled Draconinae genera, our results recover different relationships from those observed in Pyron *et al.* (2013). Although all island species of *Diploderma* from Taiwan and Japan are recovered as a monophyletic group (Fig. 2, clade S), they are nested within a larger clade containing species from mainland East Asia (Fig. 2, clade O), with the whole group as sister to *Pseudocalotes* with strong statistical support (1.00/100; Fig. 2). Additionally, our results do not support *Malayodracon* as sister to *Diploderma polygonatum*, with the null hypothesis of this sister relationship rejected with strong statistical support (Table 4).

For the generic placement of *J. bapoensis* (Yang, Su & Li, 1979; Yang & Rao, 2008; Mahony, 2010; Cai *et al.*, 2015), analyses support the recent generic designation of this species in the genus *Pseudocalotes* (Mahony, 2010). First described as a subspecies of *Pseudocalotes kingdonwardi* (considered as *Calotes kingdonwardi* at the time; Smith, 1935; Yang *et al.*, 1979; Zhao *et al.*, 1999), *P. k. bapoensis* was later transferred to the genus *Japalura* based on morphological characteristics and subsequently elevated to full species status as *J. bapoensis* (Yang & Rao, 2008). However, the morphological diagnosis of the genus *Japalura* provided by Yang & Rao (2008) (e.g. heterogeneous dorsal scales, well-developed nuchal crests, presence of shoulder fold, feebly developed gular pouch, and small and exposed tympanum) cannot differentiate *Japalura s.l.* from *Pseudocalotes* (Mahony, 2010). In contrast, the proposed morphological characteristics of *J. bapoensis* match the revised diagnoses of *Pseudocalotes*, including having a single, enlarged suborbital scale row, distinctively smaller scales in the center of the throat, enlarged scales toward the lateral jaw, and distinctively large nuchal crest scales (Fig. 3; Yang & Rao, 2008; Mahony, 2010). Furthermore, the results of phylogenetic analyses in this and previous studies recover *J. bapoensis* as nested within the genus *Pseudocalotes* with strong support (*J. bapoensis* was treated as *Pseudocalotes kingdonwardi* in Grismer *et al.*, 2016b) (Fig. 2, clade M, 1.00/100). Therefore, on the basis of morphological and phylogenetic evidence, we consider *J. bapoensis* as a member of the genus *Pseudocalotes*. As neither additional specimens nor genetic data of the nominate subspecies, *P. k. kingdonwardi*, are available, we cannot evaluate whether *P. k. bapoensis* deserves full species status at this time. Therefore, following previous published work (Mahony, 2010), we conservatively treat *bapoensis* as a subspecies of *P. kingdonwardi*, pending future analyses should specimens and genetic material of *P. k. kingdonwardi* become available.

Finally, it is important to note that our new classification aims to provide a suite of testable hypotheses for future phylogenetic work, and the new classifications for non-sampled species represent our best attempt at a more stable taxonomic structure. Many species, such as *Japalura sagittifera* and *Diploderma hamptoni*, are known from poorly surveyed regions, and lack vouchered specimens and genetic samples. Therefore, our new classification of these understudied taxa is based on the

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best available morphological data alone, which may be subject to convergence and warrants additional investigations. Additionally, future phylogenomic studies are needed to better resolve the higher level relationships of *Japalura s.l.* To facilitate future taxonomic work, we provide updated morphological diagnoses to all genera of the subfamily Draconinae below [modified from Smith (1935) and Zhao *et al.* (1999)].

Diagnostic key to all genera of subfamily Draconinae

1a.	Femoral pores present	*Mantheyus*
1b.	Femoral pores absent	2
2a.	Ribs much prolonged, supporting wing-like expansion	*Draco*
2b.	Ribs not elongated into wing-like structure	3
3a.	Four toes on hind limbs; males with prominent, retractable dewlaps	4
3b.	Five toes on hind limbs	5
4a.	Enlarged scales present on posterior thigh, flank scales heterogeneous	*Sitana*
4b.	Enlarged scales absent on posterior thigh, flank scales homogeneous	*Sarada*
5a.	Distinct bony supra-orbital arch present; globular protuberance present on snout	*Lyriocephalus*
5b.	Above structures all absent	6
6a.	Series of long, lanceolate spines present along medial line on gular; gular appendage projecting forward beyond snout	*Hypsicalotes*
6b.	No large spine series along medial line on gular; gular appendage absent or cannot extend forward beyond snout	7
7a.	Distinct, elongated rostral appendage present (always in males, sometimes a mere tubercle in females)	8
7b.	No rostral appendage, or appendage short, not elongated	10
8a.	Dorsal body scales heterogeneous	*Ceratophora*
8b.	Dorsal body scales mostly homogenous	9
9a.	Dorsal and nuchal crests tall; dorsal crest scales enlarged in distinct, large triangles	*Harpesaurus*
9b.	Nuchal crest low; dorsal crest indistinct	*Thaumatorhynchus*
10a.	Three parallel longitudinal gular folds on each side of medial throat, curving and converging posteriorly into U-shape pattern	*Ptyctolaemus*
10b.	Gular folds not in above shape	11
11a.	Dorsal body scales homogeneous	12
11b.	Dorsal body scales heterogeneous	15
12a.	Lateral skin fold present from posterior jaw to shoulder on each side; tail long TAL >300% SVL	*Bronchocela*
12b.	Lateral skin folds absent from posterior jaw to shoulder; tail relatively short, TAL <300% SVL mostly	13
13a.	Body compressed dorsally; dorsal crest feeble	*Psammophilus*
13b.	Body not compressed dorsally; dorsal crests relatively well developed	14
14a.	Lateral gular sac pockets present; granular scutellation of gular sac	*Complicitus*
14b.	Lateral gular sac pockets absent; scales of gular not in granular shape	*Calotes*
15a.	Tail markedly swollen at base, flattened above, with dorsolateral keels formed by enlarged, angular scales	*Phoxophrys*
15b.	Tail not markedly swollen at basee, or swollen but rounded above	16
16a.	Post-occipital and post-orbital spines present; tympanum exposed	17
16b.	Spines on dorsal head absent; tympanum exposed or concealed	18
17a.	Nuchal and dorsal crest short; tail long, TAL >185% SVL; lateral gular fold weakly developed	*Oriocalotes*
17b.	Nuchal and dorsal crest much longer; tail short, TAL<180% SVL mostly; lateral gular fold strongly developed	*Acanthosaura*
18a.	Enlarged dorsal body scales arranged in regular rows or ridges	19
18b.	Enlarged dorsal body scales arranged randomly	21

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19a. Enlarged scales in dorsolateral ridges or rows only (except in *D. swinhonis*, which also in V-shape rows) ... *Diploderma*
19b. Enlarged scales in both lateral and V-shaped ridges or rows ... 20
20a. Pair of enlarged, conical scales present on nape; head relatively wide and robust, HW >70% HL mostly ... *Cristidorsa* **gen. nov.**
20b. Enlarged conical scale pair on nape absent; head relatively narrow and slender, HW <70% HL mostly ... *Japalura*
21a. Ventral body scales heterogeneous in size and shape ... *Mictopholis*
21b. Ventral body scales homogeneous in size and shape mostly ... 22
22a. Nuchal crest scales low, only slightly differentiated; body and limbs slender; neck distinct and long ... 23
22b. Nuchal crest scales tall, elongated in lanceolate spines; body and limbs relatively robust; neck robust and relatively short ... 24
23a. Toe V not longer than Toe I; males usually with distinctively developed dewlaps extending to anterior venter ... *Otocryptis*
23b. Toe V longer than Toe I; males with much smaller lateral gular folds ... *Aphaniotis*
24a. Suborbital scales in multiple rows, subequal in size; dorsal scales fine, smaller than ventrals; distinct transverse gular fold present; nuchal crest separated from nuchal scales by one or more rows of enlarged, supporting scales ... *Gonocephalus*
24b. Suborbital scales in singular row, or multiple rows but one row distinctively enlarged; dorsal scales larger or smaller than ventrals; transverse gular fold absent or indistinct; nuchal crest not separated from nuchals by enlarged, supporting scales ... 25
25a. Majority of dorsal body scales smaller than ventrals ... 26
25b. Majority of dorsal body scales larger than ventrals ... 29
26a. Lateral gular fold strongly developed with rounded tip, extending to anterior region of venter when folded; dorsal body scales muricate ... *Malayodracon*
26b. Lateral gular fold less developed without an obvious tip, not extending beyond throat when folded; dorsal body scales more heterogeneous with rhombic scales ... 27
27a. Head tall, robust, distinctively raised on occipital region with distinct cranial ridges ... *Dendragama*
27b. Head more compressed dorsally, much more slender, not distinctively raised on occipital region ... 28
28a. Tympana exposed ... *Pseudocalotes*
28b. Tympana concealed ... *Pseudocophotis*
29a. Tail prehensile, short, TAL <135% SVL; tympanum concealed ... *Cophotis*
29b. Tail not prehensile, long, TAL >160% SVL; tympanum exposed ... 30
30a. Dorsal body scales less heterogeneous; dorsal body scales more rounded; tail short, TAL <175% SVL ... *Lophocalotes*
30b. Dorsal body scales more heterogeneous; dorsal body scales more elongated; tail long, TAL >200% SVL ... *Salea*

SPECIES-LEVEL RELATIONSHIPS AND TAXONOMY

Japalura

Within *Japalura*, relationships of sampled taxa are largely resolved (Fig. 2). Interestingly, despite morphological similarity between *J. kumaonensis* and *J. tricarinata* (Wang *et al.*, 2018b), *J. kumaonensis* is most closely related to *J. variegata* (0.99/69; Fig. 2). As many of the Himalayan regions lack detailed surveys for *Japalura* diversity, including north-east India, Bhutan, Nepal, and north-western Bangladesh, many congeners are still poorly studied. In fact, several recognized species are known from a few vouchered specimens only with no genetic samples (e.g. *J. dasi*, *J. major* and *J. sagittifera*). Not only may these remote regions harbour undescribed diversity, but also, given the wide variation in ornamentation observed among populations (Bhosale, Das & Manthey, 2013; Wang *et al.* 2018a), understudied taxa such as *J. andersoniana*, *J. kumaonensis*, *J. tricarinata* and *J. variegata* may represent species complexes and contain cryptic diversity. Future research should focus on the collection of additional, vouchered genetic data of these poorly studied lineages and close examination of *Japalura* populations across the Himalayan region.

Cristidorsa

For congeners from Myanmar, both our morphological and molecular data support the previous hypothesis that

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Myanmar populations of *Cristidorsa* cf. *planidorsata* were misidentified, and are, in fact, *C. otai*. When discussing the taxonomy of Myanmar populations of *C. planidorsata* (considered as *J. planidorsata*), Mahony (2009) stated specifically that the only available specimen (juvenile) of *C. planidorsata* examined from Myanmar resembled the morphology of *C. otai*. However, given the lack of access to adult specimens from Myanmar, Mahony (2009) conservatively treated the Myanmar population as *C.* cf. *planidorsata*, but called for further investigation on the taxonomic status of these populations. Based on our data, the individuals of previously identified *C.* cf. *planidorsata* from Myanmar are not monophyletic with respect to the topotypic *C. planidorsata*, but instead, form a monophyletic group with topotypic *C. otai* from north-east India (Fig. 2, clade G). In addition, the morphological characteristics of the Myanmar populations match the diagnoses of *C. otai* and differ from *C. planidorsata*, including having flattened and posteriorly pointing conical scales on the temporal region of the head, shorter body lengths TRL 41.8–47.4% SVL, longer tails TAL 154.6–190.8% SVL, and shorter lip-stripes ending anterior to the limb insertion (Mahony, 2009). Therefore, we diagnose the Myanmar populations of *C.* cf. *planidorsata* as *C. otai*, and recommend that records of the former species in Myanmar be corrected to represent the latter.

Diploderma

Recently, Ananjeva *et al*. (2007) described a new species of *Japalura*, *J. ngoclinensis*, on the basis of three female specimens collected from the central highlands of Vietnam, far outside of the range of *Japalura s.l.* recognized at the time. Although there are no genetic data available for the species, we can assign it to the genus *Diploderma* based on our revised morphological diagnosis. The issue that remains is in regard to the taxonomic validity of *D. ngoclinense*. Despite the fact that the type specimens of the species are morphologically most similar to *D. splendidum*, a brief comparison was made to the latter species using one morphological characteristic only (Ananjeva *et al.*, 2017). The authors argued that the new species differs from *D. splendidum* by the absence of a transverse gular fold (Ananjeva *et al.*, 2017). However, based on the photographs of the type series in the original description, all type specimens of *D. ngoclinense* do have a shallow transverse gular fold (particularly distinct on the paratype, VNMH 3110) (Ananjeva *et al.*, 2017: figs 2–4), identical to that seen in preserved specimens of *D. splendidum* [USNM 35522 (holotype), CIB 2588, 2591, 2596, 72468, 72469 from Chongqin, China]. Based on the available data, the only differentiating characteristic between *D. ngoclinense* from *D. splendidum* is the mid-dorsal scale count, which is higher in *D. ngoclinense* (54–56 vs. 44–52 in *J. splendidum*; Ananjeva *et al.*, 2017). However, such a difference is based on a small sample size of three specimens of *D. ngoclinense*, and mid-dorsal scale counts are known to vary in *Diploderma* (Ota, 1989a; Manthey *et al.*, 2012; Wang et al., 2016, 2018). In addition to the suspicious distribution of *D. ngoclinense* (isolated and 1000 km south of the most southern-known range of the genus; Ananjeva *et al.*, 2017), we question the taxonomic validity of *D. ngoclinense*. Future field confirmation, detailed morphological comparisons and phylogenetic analyses are needed to validate the taxonomic status and distribution of this species.

Historically, almost all *Diploderma* diversity in central and south-west China was thought to be a single species, *D. flaviceps* (Zhao *et al.*, 1999). Although numerous new species have been described from the *D. flaviceps* complex, including *D. batangense*, *D. brevicaudum*, *D. flaviceps*, *D. iadinum*, *D. laeviventre*, *D. micangshanense*, *D. vela*, *D. yulongense* and *D. zhaoermii* (Song, 1987; Li *et al.*, 2001; Gao & Hou, 2002; Manthey *et al.*, 2012; Wang et al., 2015, 2016, 2017), many outlier records of *D. flaviceps* in south-west China have not been examined, some of which may represent either cryptic diversity in the genus or misidentifications (Wang *et al.*, 2016). For example, the genetic sequence data of an MVZ specimen from Wenchuan, north-west Sichuan Province, China (MVZ 216622) was recorded as *D. flaviceps* upon deposition in GenBank, and subsequently has been used for numerous phylogenetic studies (GenBank accession no. AF128500; Macey *et al.*, 2000; Schulte *et al.*, 2004; Zug *et al.*, 2006; Pyron *et al.*, 2013; Grismer *et al.*, 2016b). However, according to the museum record, the collection locality of this specimen is east of Wenchuan, Sichuan Province, China, which is geographically proximate to the type locality of a different congener, *D. zhaoermii* (Gao & Hou, 2002). Furthermore, our phylogenetic analyses recover this individual of *D.* cf. *flaviceps* as nested within topotypic material of *D. zhaoermii* (Fig. 2). Additionally, as *D. flaviceps* is not recognized to occur outside the upper Dadu River Valley and its direct tributaries (Manthey *et al.*, 2012; Wang *et al.*, 2016), we suspect that the *Diploderma* specimen (MVZ 216622, genetic sequence on GenBank AF128500) was misidentified, and it represents the species *D. zhaoermii* instead. Poorly studied groups and continuous taxonomic revisions create problems such as this for museum and online databases, and numerous changes to the taxonomy of the *D. flaviceps* complex in China are no exception. We propose major natural history collections update the taxonomy of *Japalura s.l.*, particularly members of the *D. flaviceps* complex from south-west China.

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CONCLUSIONS

Utilizing multilocus and morphological datasets, we have addressed a long-standing issue concerning the paraphyletic nature of *Japalura s.l.* reclassifying the genus into four genera. Revision of this widespread group further clarifies the phylogenetic relationships among genera in the subfamily Draconinae. However, several questions remain that were beyond the scope of this study. First, interspecific phylogenetic relationships of each of the four genera within *Japalura s.l.* are still not well understood. Second, without a fully resolved phylogeny of these major clades within *Japalura s.l.* it will remain difficult to test finer scale biogeographic hypotheses concerning some of the most interesting landscapes on earth, including the trans-Himalayan Mountains and the Hengduan Mountain Region in south-west China. Third, genetic samples remain unavailable for many genera within Draconinae, including *Harpesaurus*, *Mictopholis*, *Oriocalotes* and *Pseudocophotis*. Finally, focused phylogenetic studies are still needed to resolve species-level relationships in other genera (i.e. *Acanthosaura*, *Gonocephalus* and *Pseudocalotes*). Together, these problems perpetuate questions about interspecific relationships and the taxonomic validity of some species recognized currently. As such, Asian Dragon Lizards continue to represent an enigmatic vertebrate radiation in need of a broad spectrum of studies aimed at better understanding everything from species-level diversity to evolutionary patterns of diversification.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix S1. Summary of specimens corresponding to genetic samples included in the study, including GenBank accession numbers and vouchered specimen information. CAS = California Academy of Sciences, USA; MVZ = Museum of Comparative Zoology, USA; OMNH = Sam Noble Oklahoma Museum of Natural History, USA; KIZ = Kunming Institute of Zoology, Chinese Academy of Sciences, China; ZISP = Zoological Institute, St. Petersburg, Russia; WHT = Wildlife Heritage Trust, Sri Lanka; BNHS = Bombay Natural History Society, India; TNHC = Texas Memorial Museum, USA; LSUMZ = Louisiana Museum of Natural History, USA; USNM = National Museum of Natural History, USA; FMNH = Field Museum of Natural History, USA; CES = Center for Ecological Sciences, Indian Institute of Science, India. "/" indicates only tissue samples were taken without voucher specimens.

Appendix S2. Genbank sequences used in the phylogenetic analyses.

Appendix S3. Specimens examined. Museum abbreviations include: Museum of Natural History (BMNH), Kensington, London, UK; Museum of California Academy of Sciences (CAS), San Francisco, CA, USA; Center for Ecological Sciences, Indian Institute of Science (CESG), Bengaluru, Karnataka, India; Chengdu Institute of Biology, Chinese Academy of Sciences (CIB); Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ), Kunming, Yunnan, China; University of Kansas Biodiversity Institute (KU), Lawrence, KS, USA; Field Museum of Natural History (FMNH), Chicago, IL, USA; and Museum of Comparative Zoology (MCZ), Cambridge, MA, USA.

Appendix S4. Specimens measured for the principal component analyses of continuous morphological characteristics. Museum abbreviations as in caption of Appendix S3.

Appendix S5. Summary statistics for the principal component analyses of continuous morphological characteristics for *Japalura s.l.* and morphologically similar Draconinae genera. Abbreviations are listed in Material and Methods.

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