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Hiding on jagged karst pinnacles: A new microendemic genus and species of a limestone-dwelling agamid lizard (Squamata: Agamidae: Draconinae) from Khammouan Province, Laos

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ABSTRACT

We describe a unique new species and genus of agamid lizard from the karstic massifs of Khammouan Province, central Laos. *Laodracon carsticola* **Gen. et sp. nov.** is an elusive medium-sized lizard (maximum snout-vent length 101 mm) specifically adapted to life on limestone rocks and pinnacles. To assess the phylogenetic position of the new genus amongst other agamids, we generated DNA sequences from two mitochondrial gene fragments (16S rRNA and *ND*2) and three nuclear loci (*BDNF*, *RAG1* and *c-mos*), with a final alignment comprising 7 418 base pairs for 64 agamid species. Phylogenetic analyses unambiguously place the new genus in the mainland Asia subfamily Draconinae, where it forms a clade sister to the genus *Diploderma* from East Asia and the northern part of Southeast Asia. Morphologically, the new genus is distinguished from all other genera in Draconinae by possessing a notably swollen tail base with enlarged scales on its dorsal and ventral surfaces. Our work provides further evidence that limestone regions of Indochina represent unique "arks of biodiversity" and harbor numerous relict lineages. To date, *Laodracon carsticola* **Gen. et sp. nov.** is known from only two adult male specimens and its distribution seems to be restricted to a narrow limestone massif on the border of Khammouan and Bolikhamxai provinces of Laos. Additional studies are required to understand its life history, distribution, and conservation status.

Keywords: Biodiversity; Endemism; Indochina; Karstic landscape; *Laodracon carsticola*; Phylogeny; Southeast Asia; Taxonomy

INTRODUCTION

Limestone karsts are sedimentary rock outcrops made of calcium carbonate. These habitats are widely recognized as unique centers of biodiversity and act as refugia for some of the most threatened species on the planet (Bannikova et al., 2014; Jenkins et al., 2005; Tolentino et al., 2020). The Asian continent hosts the highest number of karst habitats in the world, where they form vast landscapes covering large portions of southern China, Indochina, Sundaland, the

Philippine Archipelago, and the islands of Wallacea and Melanesia (Grismer et al., 2021b). Though widespread, karst formations are characterized by their fragmented nature, creating numerous towers, caves, and hills acting as isolated archipelagos of habitat. The fractured and eroded surfaces of limestone form numerous microhabitats with stable climatic conditions to which many taxonomic groups have specialized, resulting in extraordinarily high degrees of range-restricted endemism (Clements et al., 2006, 2008; Grismer et al., 2021a, 2021b; Poyarkov et al., 2018; Sodhi et al., 2014, 2010; Suwannapoom et al., 2018). As a result, limestone outcrops are considered "imperiled arks of biodiversity" because the stable environmental conditions within their habitats act as reservoirs for flora and fauna. This provides an opportunity for ancient and relic lineages to persist for long periods of time, even if global climate change and competition with other lineages have driven their extinction from adjacent areas (Clements et al., 2006). Recent studies have also shown that karst formations have helped to foster speciation in many limestone-specialized groups, including geckos (Grismer et al., 2021a, 2021b, 2022), various groups of invertebrates (Barjadze et al., 2015) and several plant lineages (Chen et al., 2018; Chin, 1977; Chung et al., 2014). Therefore, limestone karsts are now interpreted not only as biodiversity "museums" that harbor relictual endemic taxa, but also as biodiversity generators and platforms of speciation (Grismer et al., 2021a).

Lizards in the subfamily Draconinae are an exclusively Asian radiation that includes 31 genera and 256 species, comprising approximately 50% of the total diversity within the family Agamidae (Grismer et al., 2016; Uetz et al., 2023; Wang et al., 2018). Draconine lizards are diurnal omnivores exhibiting a wide range of arboreal to terrestrial lifestyles, and represent a key element of the subtropical and tropical ecosystems they inhabit (Grismer et al., 2016). Phylogenetic relationships within Draconinae have received significant attention by a number of recent studies (Karunarathna et al., 2020; Macey et al., 2000; Pal et al., 2018; Shaney et al., 2020), including the work of Grismer et al. (2016), who argued that the subfamily likely originated on the Eurasian mainland from where it has twice colonized the Indian subcontinent during the early to late Eocene. A more recent study by Wang et al. (2019a) analyzed phylogenetic relationships of Draconinae and demonstrated that the generic-level taxonomy of the subfamily still largely remains incomplete. This work resulted in several rearrangements, the most significant being the break-up of the genus *Japalura* Gray *sensu lato* into the revalidated genus *Diploderma* Hallowell and a new genus *Cristidorsa* Wang, Deepak, Datta-Roy, Lin, Jiang, Che & Siler. After this revision, *Japalura* was restricted to regions surrounding the Himalayan mountains, whereas all East Asian and northern Indo-Burmese species were transferred to *Diploderma* (Wang et al., 2019a). While this systematic revision promoted further taxonomic treatments within Draconinae, especially in the clade containing *Diploderma* and *Pseudocalotes*, all such studies have been geographically limited to China (Cai et al., 2022; Wang et al., 2016, 2019b). No attention has hitherto been given to adjacent countries south of China, such as Laos, even though the latter country contains wide swathes of habitat suitable for lizards in the *Diploderma–Pseudocalotes* clade. Considering the presence of several species of these two genera in adjacent Yunnan Province (such as *Diploderma menghaiense* and *Pseudocalotes kakhienensis*; Wang et al., 2022), taxonomic diversity of Laotian draconine lizards is likely underestimated.

In 2022 and 2023, during recent field surveys in the limestone massifs of Khammouan Province (hereafter, Prov.) in central Laos, we encountered two unusual agamid lizard specimens that superficially resembled members of the genera *Pseudocalotes* and *Diploderma*, as defined by Wang et al. (2019a). *Pseudocalotes* at present includes 22 species distributed in Southeast Asia and southern China, with three species recorded from Laos (Poyarkov et al., 2023). The genus *Diploderma* currently contains 43 recognized species, of which two-thirds have been described in the last decade (Uetz et al., 2023). Most *Diploderma* species have small, micro-endemic distributions (Cai et al., 2022; Liu et al., 2022; Wang et al., 2021b) and up to date no member of the genus has been recorded from Laos (Uetz et al., 2023; Wang et al., 2021b). A subsequent morphological comparison with members of *Diploderma*, *Pseudocalotes* and other Draconinae genera revealed a set of characters steadily distinguishing the newly collected Laotian agamid specimens from all other members of the subfamily. Furthermore, molecular phylogenetic analyses confirmed the placement of the Laotian karst agamid within Draconinae and demonstrated that the two specimens represent a previously unknown lineage of agamid lizards forming a sister taxon to *Diploderma*. Herein, we describe this putatively relict lineage as a new genus and new species.

MATERIALS AND METHODS

Sample collection
Field surveys were conducted by S. Xayyasith, S. Sitthivong, and P. Brakels within the frameworks of the National University of Laos (NUOL) project from August 2022 to February 2023 at The Rock Viewpoint tourist attraction within Nam Sanam-Phou Pha Marn Provincial Protected Area (hereafter PPA.), Khammouan Prov., central Laos (see Figure 1). Photographs were taken to document the colour pattern of the specimen in life prior to euthanasia. Liver tissues were stored in 95% ethanol and lizards were preserved in 75% ethanol. Specimens were subsequently deposited in the collections of the NUOL, Vientiane, Laos.

Morphological description
Measurements were taken to the nearest 0.1 mm with a

Figure 1 Distribution of *Laodracon carsticola* Gen. et sp. nov. in Nam Sanam-Phou Pha Marn PPA., Khounkham Dist., Khammouan Province, central Laos

Mitutoyo digital caliper. The descriptions of the morphological characteristics follow Wang et al. (2021b). The following morphometric characters were measured: snout-vent length (SVL): from the snout tip to anterior edge of cloaca; tail length (TAL): from the anterior edge of the cloaca to the tip of tail; head length (HL): from the tip of snout to the jaw joint; head width (HW): measured between the widest points of the head; head depth (HD): measured as the perpendicular distance at the temporal region of head; snout-eye length (SEL): measured between the tip of snout and anterior edge of orbital bone; fore-limb length (FLL): between the point of insertion at axillary to the tip of Finger IV, excluding the claw, measured as the limb straighten; hind limb length (HLL): between the point of insertion at groin to the tip of Toe IV, excluding the claw, measured as the limb straighten; Toe IV length (T4L): measured between the tip of Toe IV to the base between Toe III and IV, excluding the claw; trunk length (TRL): measured between the limb insertion points between axillary and groin; snout-tympanum distance (STD): distance from snout to anterior margin of tympanum; eye diameter (ED): vertical diameter of eye; tympanum diameter (TY): horizontal diameter of tympanum; nostril-orbit distance (NO): shortest distance from nostril to orbit; orbit diameter (DO): horizontal diameter of orbit; nuchal crest length (NCL); and nuchal crest height (NCH). In addition, the following scalation and meristic characters were recorded: supralabial scale count (SL): number of enlarged, modified labial scales from rostral to the corner of mouth; infralabial scale count (IL): number of enlarged, modified labial scales from mental to the corner of mouth; middorsal crest scale count (MD): number of modified crest scales longitudinally from the first nuchal crest to the scale above cloaca; ventral scale count (VS): number of ventral body scales counted in a straight line along the medial axis between the transverse gular fold and the anterior edge of cloaca; Finger IV subdigital lamellae count (F4S): number of subdigital lamellae scale from the base between Finger III and IV to the tip of Finger IV, excluding the claw; Toe IV subdigital lamellae count (T4S): number of subdigital lamellae scales from the base between Toe III and IV to the tip of Toe IV, excluding the claw; nasal–supralabials scale rows (NSL): number of horizontal rows of small scales between the first supralabial and the nasal; suborbital scale rows (SoR): number of longitudinal rows of scales between supralabials and inferior-most edge of orbit circle, excluding fine ciliary scales in the orbit; number of rostral scales (RC); number of nuchal crest spines (NCS); number of enlarged postmental scales (ESP); number of postmental scales (PM); femoral and preanal pores (FPP, present or absent); scales in frontal area (smooth or keeled); shape of mental scale (triangular or not, wider than long or longer than wide); presence of gular sac (present/absent); gular scales (keeled/granular); presence of gular markings (present/absent); presence of dorsal spines (present/absent); vertebral scales (keeled/smooth); vertebral scales enlarged (yes/no); scales on tail (keeled/smooth); gular fold on throat (transverse/longitudinal); coloration of throat. Measurements were taken on the left side of the specimen, and values for paired scalation characters (SL, IL, NSL, and SoR) were recorded on both sides of the body, with counts provided in left/right order. In addition, the following morphological characters were examined: a transverse gular fold across the neck (presence or absence), degree of gular

pouch development (absence, feeble, or strongly developed), and tympana state (exposed or concealed under fine scales).

Morphological characteristics were compared with other genera in the subfamily Draconinae (including *Diploderma*, *Pseudocalotes*, *Acanthosaura*, etc.), and were based on museum vouchers (Supplementary Table S1) and data from literature sources (Ananjeva & Stuart, 2001; Ananjeva et al., 2011; Denzer et al., 2015; Mahony, 2010).

Laboratory methods

For molecular phylogenetic analyses, we extracted total genomic DNA from ethanol-preserved femoral muscle tissue using standard phenol-chloroform-proteinase K extraction procedures with consequent isopropanol precipitation, leading to a final concentration of ~1 mg/mL (protocols from Hillis et al., 1996; Sambrook & Russell, 2001). We visualized isolated DNA in agarose electrophoresis with the presence of ethidium bromide. We measured the concentration of total DNA in 1 μL using a NanoDrop 2000 (Thermo Scientific, USA), and consequently adjusted the concentration to ca. 100 ng DNA/μL.

We amplified mtDNA fragments covering partial sequences of 16S rRNA and *ND2* mitochondrial DNA (mtDNA) genes. We also amplified partial fragments of *BDNF*, *c-mos* and *RAG1* nuclear genes (nuDNA). These markers have been widely used in recent phylogenetic studies of lizards within Draconinae (Grismer et al., 2016; Wang et al., 2018). We performed DNA amplification in 20 μL reactions using ca. 50 ng genomic DNA, 10 nmol of each primer, 15 nmol of each dNTP, 50 nmol additional MgCl$_2$, Taq PCR buffer (10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.1 mmol/L MgCl$_2$, and 0.01% gelatin), and 1 unit of Taq DNA polymerase. Primers used in PCR and sequencing of were obtained from previous studies and are summarized in Supplementary Table S2. PCR conditions included an initial denaturation step of 5 min at 94 °C and 43 cycles of denaturation for 1 min at 94 °C, primer annealing for 1 min with a TouchDown program from 65 °C to 55 °C reducing 1 °C every cycle, extension for 1 min at 72 °C, and a final extension step for 5 min at 72 °C.

The PCR products were loaded onto 1.5% agarose gels in the presence of ethidium bromide and visualized via electrophoresis. When distinct bands were produced, we purified the PCR products using 2 μL of a 1:4 dilution of ExoSapIt (Amersham, USA) per 5 μL of PCR product prior to cycle sequencing. The 10 μL sequencing reaction included 2 μL of template, 2.5 μL of sequencing buffer, 0.8 μL of 10 pmol primer, 0.4 μL of BigDye Terminator v3.1 Sequencing Standard (Applie Biosystems, USA), and 4.2 μL of water. The cycle sequencing used 35 cycles of 10 s at 96 °C, 10 s at 50 °C, and 4 min at 60 °C. We purified the cycle sequencing products by ethanol precipitation. We carried out sequence data collection and visualization on an ABI 3730xl Automated Sequencer (Applied Biosystems, USA). The obtained sequences were deposited in GenBank under accession numbers OR538398, OR544068, and OR544064–OR544066 (see Supplementary Table S3).

Phylogenetic analyses

To generate phylogenies, we used 16S rRNA, *ND2*, *BDNF*, *c-mos*, and *RAG1* sequences of the agamid from Laos, as well as the homologous sequences of 63 other agamids, including 39 out of 43 currently recognized *Diplopderma* species obtained from the earlier phylogenetic studies of the genus (e.g., Cai et al., 2022; Grismer et al., 2016; Liu et al., 2020,

2022; Wang et al., 2019a, 2021a, 2021b) and representatives of 22 out of 31 recognized genera of Draconinae. We also added homologous sequences of *Hypsilurus nigrigularis* (Amphibolurinae) and *Phrynocephalus mystaceus* (Agaminae), which were used as outgroup taxa. The information on GenBank accession numbers, museum vouchers, and localities of origin for sequences used in this study are summarized in Supplementary Table S3.

The nucleotide sequences were initially aligned in MAFFT v.6 (Katoh et al., 2002) with default parameters; the alignment was subsequently checked by eye in BioEdit 7.0.5.2 (Hall, 1999) and slightly adjusted. The mean uncorrected genetic *P*-distances between sequences were calculated with MEGA 7 (Kumar et al., 2016). Phylogenetic trees were estimated for the combined mitochondrial DNA fragments (16S rRNA and *ND2*) and nuclear gene (*BDNF*, *c-mos*, and *RAG1*) datasets. An approximately unbiased tree-selection test (AU-test; Shimodaira, 2002) using Treefinder ver. March 2011 (Jobb, 2011) did not reveal statistically significant differences between mtDNA and nuDNA topologies and thus, both datasets were concatenated in the subsequent analyses. Individual trees inferred separately from mtDNA and nuDNA datasets are presented in Supplementary Figure S1. The optimum partitioning schemes for alignments were identified with PartitionFinder 2.1.1 (Lanfear et al., 2012) using the greedy search algorithm under an Akaike Information Criterion (AIC) and are presented in Supplementary Table S4. When the same model was proposed for different codon positions of a given gene, they were treated as a single partition.

Phylogenetic trees were inferred using Bayesian Inference (BI) and Maximum-Likelihood (ML) approaches. We conducted BI in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run with one cold chain and three heated chains for one million generations, with sampling every 100 generations. We performed five independent MCMCMC runs, and the initial 2 500 trees were discarded as burn-in; we checked that effective sample sizes (ESS) were all above 200 by exploring the likelihood plots using TRACER v1.6 (Rambaut & Drummond, 2007). We assessed confidence in tree topology by the frequency of nodal resolution (posterior probability for Bayesian analysis; BI PP) (Huelsenbeck et al., 2001). Nodes with BI PP values of 0.95 and above were considered strongly supported, nodes with values of 0.90–0.94 were considered as well-supported, and the BI PP values below 0.90 were regarded as no support (Wilcox et al., 2002).

We conducted ML analysis using the IQ-TREE web server (Nguyen et al., 2015; Trifinopoulos et al., 2016) and employed one-thousand bootstrap pseudoreplicates via the ultrafast bootstrap (ML UB; Hoang et al., 2018) approximation algorithm. Nodes having ML UB values of 95% and above were considered strongly supported, while nodes with values of 90–94% were regarded as well-supported, and the ML UB node values below 90% were considered as no support (Minh et al., 2013).

RESULTS

### Partitions, substitution models and sequence characteristics

Our combined dataset was composed of 7 418 bp (including 1 891 bp of *ND2* and flanking tRNA genes, 1 217 bp of 16S

rRNA gene, 680 bp of *c-mos* gene, 743 bp of *BDNF* gene, and 2 887 bp of *RAG1* gene). The concatenated mtDNA and nuDNA dataset included 64 samples, including 62 representatives of Draconinae and two outgroup taxa (see Supplementary Table S3). Information on fragment lengths and variability is summarized in Supplementary Table S4. Analyses from PartitionFinder 2.1.1 proposed six different partitions in total, which are also summarized in Supplementary Table S4.

Phylogenetic relationships of Draconinae

Phylogenetic trees obtained using ML and BI analyses of the concatenated mtDNA and nuDNA dataset were congruent apart from the topologies of several poorly supported nodes. The ML tree that was generated from the dataset (see Figure 2) inferred the following set of phylogenetic relationships:

1. *Mantheyus phuwuanensis* (Manthey & Nabhitabhata) (Clade I, see Figure 2) was strongly suggested as a sister taxon to all the remaining Draconinae genera, which formed a well-supported monophyletic group (100/1.0; hereafter node
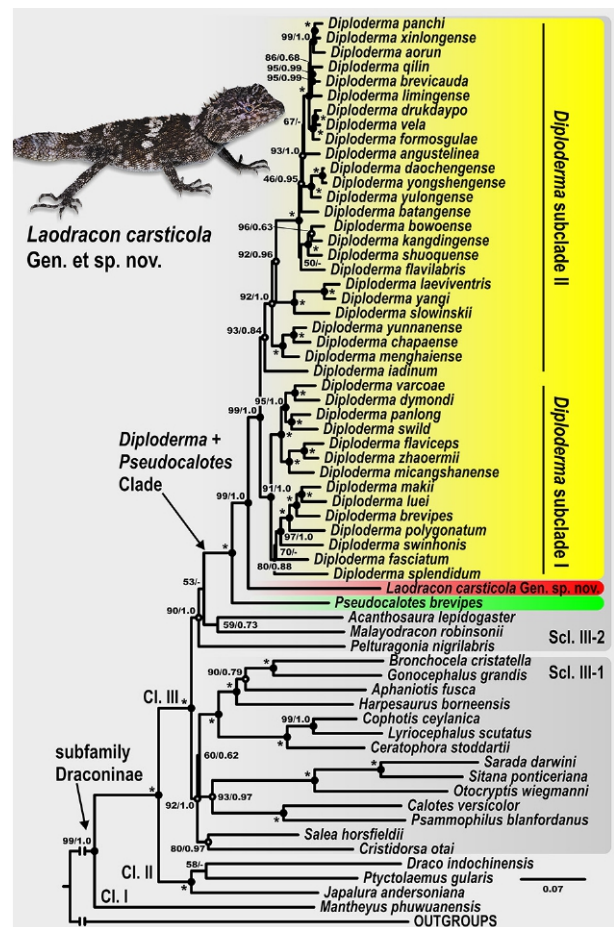
Figure 2 Maximum Likelihood topology of Draconinae agamas based on 7 418 bp of concatenated *ND2*, 16S rRNA, *c-mos*, *BDNF* and *RAG1* gene sequences

Values at nodes correspond to UFB/BI PP, respectively; asterisk (*) indicate strongly supported nodes (BPP=1.0; UFB=100%); black circles represent nodes with BPP and UFB support >0.95 and 95%, respectively; white circles represent nodes with BPP support >0.90 only or UFB >90% only; nodes lacking circles are not supported. For specimen and sequence data see Supplementary Table S3. Photograph by Nathanaël Maury.

support values are given for BI PP/ML UB, respectively).

2. Within the latter group two major clades were revealed: Clade II which comprised the genera *Japalura* sensu stricto, *Ptyctolaemus* Peters and *Draco* Linnaeus (100/1.0), and Clade III which included all the remaining genera of Draconinae (100/1.0). Basal nodes within Clade II remained essentially unresolved, while Clade III was divided in two Subclades III-1 and III-2 which got moderate support in ML analysis but were strongly supported in BI analysis (92/1.0 and 90/1.0, respectively; see Figure 2).

3. Subclade III-1 included 14 genera of Draconinae from Southeast and South Asia, with phylogenetic position of *Salea* Gray and *Cristidorsa* remaining unclear (tend to form a poorly supported clade; 80/0.97). The genera *Calotes* Cuvier and *Psammophilus* Fitzinger forming a clade (100/1.0) which together was suggested as a sister taxon to a clade including genera *Otocryptis* Wagler, *Sitana* Cuvier*,* and *Sarada* Deepak, Karanth & Giri (100/1.0). Sri-Lankan genera *Ceratophora* Gray, *Cophotis* Peters, and *Lyriocephalus* Merrem formed a strongly supported clade (100/1.0) with sister relationships to a clade including Southeast Asian genera *Harpesaurus* Boulenger, *Aphaniotis* Peters, *Gonocephalus* Kaup, and *Bronchocela* Kaup (100/1.0).

4. Subclade III-2 included the representatives of five Draconinae genera from Southeast Asia and East Asia. The phylogenetic relationships between the genera *Pelturagonia* Mocquard, *Malayodracon* Denzer, Manthey, Mahlow & Böhme, and *Acanthosaura* Gray remained essentially unresolved, while the genera *Pseudocalotes* and *Diploderma* formed a strongly supported clade (100/1.0). The new agamid from Laos was strongly suggested as a sister taxon to the genus *Diploderma* (99/1.0).

5. The phylogenetic relationships within *Diploderma* were sufficiently resolved, with two moderately supported subclades revealed within the genus; for content of each subclade see Figure 2.

Taxonomic account
Our analyses unambiguously placed the phylogenetic position of the newly discovered agamid from Laos as a member of the *Diploderma*+*Pseudocalotes* Clade, and strongly suggested that it represents a sister taxon of the genus *Diploderma* (Figure 2). Morphologically, the Laotian agamid shares several characters considered to be diagnostic for members of *Diploderma* by Wang et al. (2019a), including dorsolaterally arranged scales on the dorsum that are enlarged and parallel to the vertebral crest; nuchal and dorsal crest scales relatively short and thick, not elongated into lanceolate spines; post-occipital and post-orbital spines absent; gular scales homogeneous in size, not decreasing in size medially; scales on jaws subequal in size; dorsal scales heterogeneous in size and shape, not regularly imbricate. At the same time, the specimen from Laos possesses a combination of morphological characters which separates it from members of the genus *Diploderma*, as well as all other genera within Draconinae, particularly its distinctively swollen tail base and by having enlarged heterogeneously-shaped keeled scales on the dorsal, lateral, and ventral surfaces of the tail base.

A fully resolved taxonomic framework should ideally reflect phylogenetic relationships, while also maintaining stability and reducing the need for additional revisionary changes in the future (Vences et al., 2013; Wake, 2013). To avoid subjective instability, Vences et al. (2013) proposed several Taxon Naming Criteria (TNCs) for the assignment of Linnaean ranks to clades, and recognized three Priority TNCs (monophyly, clade stability, and phenotypic diagnosability) and several Secondary TNCs (time banding, biogeography, adaptive zone, and hybrid viability). The present evidence strongly supports the monophyly of the clade containing *Diploderma* and the newly discovered agamid from Laos, with the latter forming a lineage sister to *Diploderma*. In this case, three taxonomic decisions are available: (1) assign the agamid from Laos to the genus *Diploderma*; (2) recognize the newly discovered lineage as a subgenus of *Diploderma*; or (3) describe the agamid from Laos as a new genus. Following the TNCs of Vences et al. (2013), we argue that substantial evidence supports the third decision, and provide justification for this act below.

All three Priority TNCs are satisfied by recognizing the agamid lineage from Laos as a separate genus. The phylogenetic relationships within the *Diploderma*+*Pseudocalotes* clade are fully resolved based on the analyses from mtDNA and nuDNA data and dense sampling encompassing most of the genera in Draconinae. Moreover, the monophyly of *Diploderma sensu stricto* is robustly supported within all phylogenetic results. Both factors satisfy the first two Priority TNCs of clade stability and monophyly. The presence of a unique combination of morphological traits, such as the presence of a distinctly swollen tail base with heterogeneously shaped keeled scales, also distinguishes the Laos agamid from members of *Diploderma* and *Pseudocalotes*, thus satisfying the third Priority TNC of diagnosability.

Aside from the three Priority TNCs, our decision to recognize a new genus for the new Agamid from Laos satisfies several Secondary TNCs. Though we did not perform divergence time-estimates in the present study, it appears that the age of the radiation between *Diploderma* and the Laotian agamid is substantial. Several recent studies demonstrated that the split between *Diploderma* and *Pseudocalotes* likely occurred in late Eocene–early Oligocene (ca. 37.4 Ma in Grismer et al., 2016; ca. 33.5 Ma in Karunarathna et al., 2020), while the onset of intrageneric radiation of these genera took place during Oligocene (ca. 25.5–23.3 Ma in Grismer et al., 2016; ca. 21.7 Ma in Karunarathna et al., 2020). The divergence between *Diploderma* and the Laotian agamid may have taken place during the early Oligocene, which is notably older than the ages of several other draconine genera (e.g., the split between *Sitana* and *Otocryptis* (27.0 Ma), or the split between *Ceratophora* and *Lyriocephalus*+*Cophotis* (28.1 Ma); divergence estimates from Grismer et al., 2016). Furthermore, the new genus and species from Laos is known exclusively from limestone karst pinnacle habitats, which may represent a unique ecological specialization in Draconinae. To date, karst specialization has not been reported in any members of this lizard subfamily, including all species of *Diploderma*. Therefore, the recognition of the agamid from Laos as a distinct genus satisfies the Secondary TNCs of time banding and adaptive zone.

The cumulative evidence suggests that recognizing the Laotian agamid as a separate genus would not only enhance the diagnosability of the genera within the *Diploderma*+*Pseudocalotes* clade, but also better reveal possible unique ecological specialization and distinct evolutionary history. Therefore, we formally describe the newly discovered draconine lineage from Laos as a new genus below.

Laodracon **Gen. nov. Brakels, Sitthivong, Wang, Nguyen & Poyarkov**

Diagnosis: A genus of the subfamily Draconinae characterized by the following characteristics: body size moderate (male SVL 94.5–101.6 mm); tail base distinctively swollen at base; tail scales at swollen base distinctively enlarged dorsally and ventrally, heterogeneous dorsally and laterally, but homogeneous ventrally, all keeled and protruding with keels mostly misaligned; tympanum exposed; dorsal scales heterogeneous, strongly keeled, enlarged scales arranged in somewhat irregular dorsolateral series close to vertebral crest; nuchal and dorsal crest scales relatively short and thick, not elongated into lanceolate spines; post-occipital and post-orbital spines absent; gular scales mostly homogeneous in size, not decreasing in size medially; scales on the lateral surfaces of jaws subequal in size across gular region; no preanal or femoral pores.

Distribution: To date, *Laodracon* **Gen. nov.** is known only from a single limestone outcrop at the Rock Viewpoint tourism concession area within Nam Sanam-Phou Pha Marn PPA., Khounkham District (hereafter Dist.), Khammouan Province, Laos (Figure 1). This limestone massif represents the northernmost portion of the larger Central Indochina Limestone landscape which extends from southern part of Bolikhamxai Prov. in the north-west and extends into Phong Nha-Ke Bang National Park, Quang Binh Prov., Vietnam, in the south-east. Therefore, it is possible that *Laodracon* **Gen. nov.** also occur in other karst area within this landscape in central Laos and adjacent areas of Vietnam.

Etymology: The generic name "*Laodracon*" is a Latinized noun in masculine nominative singular form, which gives reference to its area of occurrence in central Laos. The word "dracon" is derived from Greek "δράκων" (drakon) meaning "dragon" or a "basilisk" and refers to mythological serpents; it is widely used as a part of genus-level names within the subfamily Draconinae. We recommend the name "Laos Karst Dragons" as common name of the new genus in English.

Comparison with other Draconinae genera in East and Southeast Asia: *Laodracon* **Gen. nov.** differs from its sister genus *Diploderma* by having a different tail shape at base (distinctively swollen at tail base vs. not swollen at all or only slightly swollen) and a distinct shape and arrangement of scales at the swollen site on the tail (scales distinctively enlarged, heterogeneous dorsally and laterally and homogeneous ventrally, all keels on individual scales cariante, protruding and misaligned vs. not enlarged or only slightly enlarged dorsally, mostly homogeneous, all keels carinate and not protruding; Figure 3). Additional comparisons between the type species of the new genus *Laodracon* **Gen. nov.** with 18 species of *Diploderma* distributed in Indochina, southern China and Myanmar are detailed below.

From the closely related genus *Pseudocalotes*, *Laodracon* **Gen. nov.** differs from by having a differential head shape (head wide and robust, HW 77% HL vs. head slender and narrow, HW<70% HL), a differential shape of nuchal crests (tall triangular shape vs. elongated lanceolate shape), more suborbital scale rows and a different shape of suborbital scales (4 rows, subequal in size vs. single or multiple rows, but medial row distinctively enlarged), and differential shape of gular and lateral jaw scales (homogeneous in size vs. decreasing in size medial posteriorly on gular, but increasing in size laterally on lateral jaw).

Comparisons of the new genus with other Draconinae genera occurring in the mainland Southeast Asia are summarized in Supplementary Tables S5, S6. Apart from the genus *Phoxophrys*, the new genus *Laodracon* **Gen. nov.** can be easily distinguished from the remaining genera in Draconinae by having a distinctively swollen tail base with enlarged, heterogeneous, strongly keeled, misaligned, protruding scales (vs. not swollen at base, scales either subequal in size, not protruding, or keels aligned and carinate). Furthermore, the new genus can be distinguished from *Acanthosaura* and *Calotes* by the absence of orbital and temporal spines (vs. presence); from *Aphaniotis* and *Pseudocophotis* by having an exposed tympanum (vs.
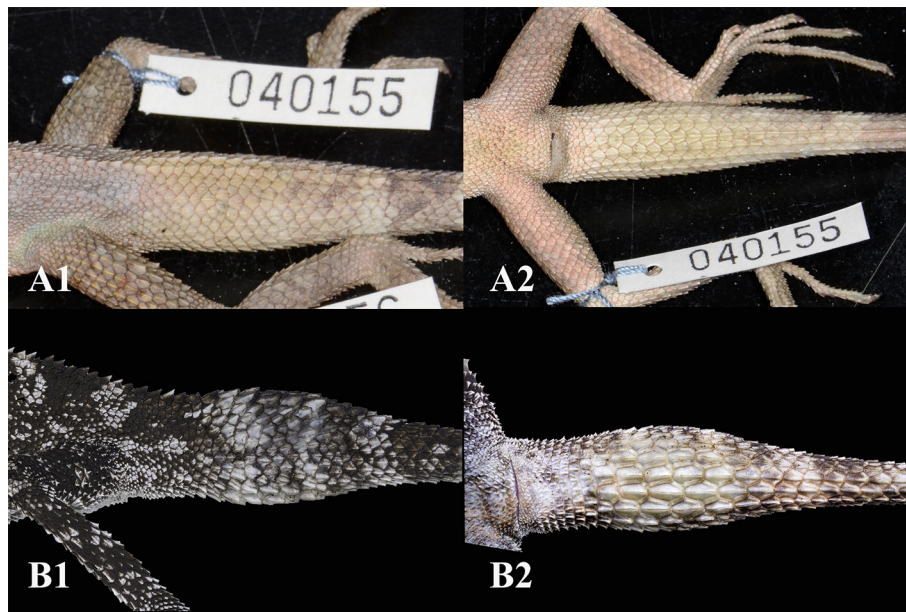
Figure 3 Comparison on the diagnostic characters on tails between *Diploderma* and *Laodracon carsticola* Gen. et sp. nov.

A, B: Tail basis of *D. splendidum* (A: Kunming Institute of Zoology 040155) and *Laodracon carsticola* Gen. et sp. nov. (B: National University of Laos R.2022.01, holotype) is shown in dorsolateral (1) and ventral (2) views. Photographs by Kai Wang (A), and Nathanaël Maury (B).

concealed); from *Bronchocela* by having differential shape of dorsal scales (heterogeneous vs. homogenesous) and a shorter tail (TAL/SVL<3.0 vs. >3.0); from *Cristidorsa* and *Japalura* by the absence of V-shaped ridge of enlarged scales on the dorsum (vs. presence); from *Draco* by the absence of prolonged ribs, supporting a wing-like patagium (vs. presence); from *Mantheyus* by the absence of femoral pores and lacking a pouch-like skin extension on the belly (vs. presence); from *Ptyctolaemus* by the absence of three longitudinal gular folds on the each side of the throat area forming a U-shaped pattern (vs. presence); from *Gonocephalus* and *Malayodracon* by having dorsolateral series of enlarged scales (vs. absence).

Type species: The new genus currently contains a single species *Laodracon carsticola* **sp. nov.,** which we formally describe below:

***Laodracon carsticola* sp. nov.**

(Figures 2–5, Supplementary Figure S2; Table 1; Supplementary Tables S5, S6)

Holotype: Adult male NUOL R.2022.01 (Figure 4), collected on 6 October 2022 by P. Brakels, S. Xayyasith, and S. Sitthivong on the summit of a limestone outcrop near the Rock Viewpoint, within Nam Sanam-Phou Pha Marn PPA., Khounkham Dist., Khammouan Prov., central Laos (N18.174°, E104.487°; at an elevation of 450 m a.s.l.).

Paratype: Adult male NUOL R.2022.02 (Supplementary Figure S2), collected on 2 February 2023 by the same collectors and at the same location as the holotype.

Diagnosis: The new species is defined by a combination of generic characters of *Laodracon* **Gen. nov.** listed above, and additionally is characterized by the following combination of morphological traits: tail long, TAL/SVL 2.11; head robust, HW/HL 0.77; hind limbs thin, long, HLL/SVL 0.73; transverse scale rows around midbody 34; subdigital lamellae under fourth finger 15/15; subdigital lamellae under fourth toe 28/28; gular pouch present; transverse gular fold present, distinct; scales on ventral, lateral and dorsal surfaces of head and body strongly keeled; dorsal background coloration black, with three white cross-bands formed by a series of large blotches and irregular white spots; gular with thin, dark gray reticulated patterns and a median blue gular spot.

Description of holotype: Adult male in a good state of preservation; measurements and scale counts of holotype presented in Table 1. Body slightly compressed laterally (Figure 4C), SVL 101.6 mm; tail, forelimbs, and hindlimbs long and slender (TAL/ SVL 2.11, FLL/SVL 0.46, HLL/ SVL 0.73); head triangular, longer than wide (HW/HL 0.77, HD/HW 0.77, SEL/HL 0.3); snout projecting (Figure 4D), snout tip obtuse in dorsal (Figure 4F) and lateral (Figure 4D) aspects. Rostral scale rectangular, bordering five small postrostral scales. Nasal scale separated from rostral by two scales but in contact with first supralabial (Figure 4D); single group of enlarged, keeled scales at anterior and posterior edges of the orbit (Figure 4D); enlarged translucent scale above pineal foramen absent (Figure 4F). Tympanum exposed; supralabials slightly keeled, nine on both sides, four scale rows between orbit and supralabials. Mental pentagonal, contacting three scales posteriorly, of which median smallest (Figure 4E);

Table 1 Measurements and counts of the type series of *Laodracon carsticola* Gen. et sp. nov. (For character abbreviations see Materials and methods)

Specimen ID	NUOL R.2022.01	NUOL R.2022.02	Specimen ID	NUOL R.2022.01	NUOL R.2022.02
Type status	Holotype	Paratype	Type status	Holotype	Paratype
Sex	Male	Male	Sex	Male	Male
Measurements (mm)			Scalation		
SVL	101.6	94.5	SL	9/9	9/9
TAL	214.2	204.4	IL	8/8	8/8
TRL	54.7	42.1	NSL	5/5	5/5
HW	22.5	21.3	SoR	5/5	8/8
HL	29.2	29.1	RC	1	1
HD	17.3	17.4	MD	34	26
STD	26.6	24.4	NCS	9 (2-5-2)	12 (2-8-2)
ED	3.4	3.4	F4S	15/15	15/15
TY	3.1	3.2	T4S	22/22	26/26
SEL	11.3	11.1	VS	28 (keeled)	27 (keeled)
NO	9.3	8.8	ESP	4/4	4/4
DO	6.8	6.4	PM	3	3
FLL	46.6	46.1	FPP	Absent	Absent
HLL	74.2	73.9	Frontal scales	Keeled	Keeled
T4L	13.7	13.8	Mental scale	Triangular, wider than long	Triangular, wider than long
NCL	12.4	14.6	Gular sac	Present	Present
NCH	3.4	3.7	Gular scales	Keeled	Keeled
TAL/SVL	210.8%	216.3%	Gular markings	Present	Present
FLL/SVL	45.9%	48.8%	Dorsal spines	Present	Present
HLL/SVL	73.0%	78.2%	Vertebral scales strongly keeled	Yes	Yes
TRL/SVL	53.8%	44.6%	Vertebral scales enlarged	Yes	Yes
SEL/HL	38.7%	38.1%	Scales on tail	Keeled	Keeled
HW/HL	77.1%	73.2%	Fold on throat	Transverse	Transverse
HD/HW	76.9%	81.7%	Throat color	Blue	Blue
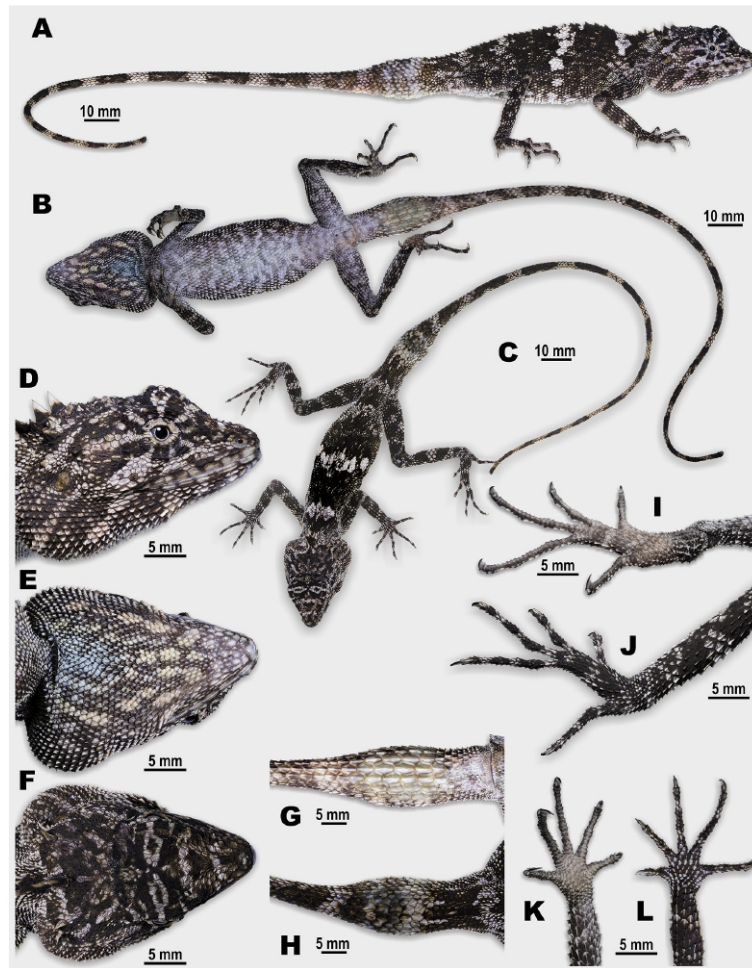
Figure 4 Holotype of _Laodracon carsticola_ Gen. et sp. nov. (NUOL R.2022.01), adult male, in life
A: General lateral view; B: General ventral view; C: General dorsal view; D: Head in lateral aspect; E: Head in ventral aspect; F: Head in dorsal aspect; G: Tail base in ventral aspect; H: Tail base in dorsal aspect; I: Volar aspect of right foot; J: Opisthenar aspect of left foot; K: Volar aspect of left hand; L: Opisthenar aspect of right hand. Scale bar equals to 10 mm for A–C, to 5 mm for D–L. Photographs by Nathanaël Maury.

infralabials keeled, eight on both sides, first pair of infralabials not in contact; scales on throat imbricate, homogeneous in size, strongly keeled, slightly smaller or equal to ventrals; transverse gular fold present (Figure 4E); gular pouch developed.

Body scales on dorsum heterogeneous in size and shape, forming two irregular lateral rows of closely arranged slightly enlarged, strongly keeled dorsal scales (Figure 4C); each enlarged dorsal scale bearing single lateral keel; axillary scales fine, much smaller than remaining dorsals; 34 transverse scale rows around midbody. Nuchal crest scales tall, pointed-triangular, slightly larger, and clearly separated from dorsal crest scales (Figure 4A); dorsal crest scales triangular, pointing posteriorly, each bearing two lateral keels, much lower than nuchals, slightly raised posteriorly (Figure 4A). Ventral body scale count 28, homogeneous, all strongly keeled, imbricate, arranged regularly transverse rows. Dorsal, ventral limb scales strongly keeled, mostly homogeneous, except smaller scales around knee and elbow areas; 15/15 subdigital lamellae under fourth finger; 28/28 subdigital lamellae under fourth toe. Tail base distinctively swollen. At swollen site, dorsal and lateral scales heterogeneous in size, protruding, distinctively keeled, keels misaligned; ventral scales homogeneous, distinctively enlarged, in different shape to dorsal and lateral ones, keels carinate; 20 scales across the tail base; remaining tail scales

much smaller, homogeneous, strongly keeled, carinate.
Color of holotype: In life, head background coloration dorsally black, turning slate-grey on head sides and ventral surfaces (Figure 4D–F). Cilliary scales white, nine whitish streaks radiating from the orbit, posterior two reaching the tympanal area. Two large white ocelli on dorsal surfaces of the orbits (Figure 4F). Nasals, supralabials, and infralabials greyish with black blotches; the scale rows between the supralabials and the orbits white with black spots. Tympanum brown. Iris bluish-grey (Figure 4D). Throat dark-grey with light-orange reticulations and a large bluish medial gular spot (Figure 4E). Nuchal scales black with bright white tips (Figure 4D). Dorsal background coloration jet black with occasional white speckling and three irregular white crossbars formed by large white blotches extending from dorsal crest to axilla, ventral scales at mid-body level, and groin; respectively (Figure 4A, C). Body flanks brownish-black with white speckling. Belly bluish-grey with black marbled pattern forming indistinct transverse bars or rows of blotches (Figure 4B). Dorsal crest scales black or white. Limbs dorsally black and ventrally dark grey with transverse whitish bands or speckling, pattern continued on hands and feet (Figure 3I–L). Digits black dorsally with transverse white spots. Tail base dorsally bluish-grey with brown spots, ventrally off-white, lacking dark markings (Figure 4G, H). Tail dorsally brownish-black with 13 transverse off-white to beige bands getting narrower towards

the tail tip (Figure 4A). After six months in preservative the coloration pattern did not change; the bluish tint of the iris and gular regions faded to grey.

Variation: Measurements and scale counts of paratype are presented in Table 1; paratype male NUOL R.2022.02 shown in Supplementary Figure S2. Overall, the paratype NUOL R.2022.02 is quite similar to the holotype in external morphology and coloration; with exception of the following characters: supralabials are separated from orbit by 8/8 scale rows; 33 middorsal scales, 26 of which pointed; nuchal crest consisting of 12 enlarged scales, of which eight middle the largest; toe IV with 26/26 subdigital lamellae; ventral scales keeled, in 27 rows. The paratype has a partially everted hemipenes, paired smooth organs lacking spines or papillae (Supplementary Figure S1I).

Natural history notes: Little is known about the biology of *Laodracon carsticola* **sp. nov.**. This species is currently only known from a single locality within the Nam Sanam-Phou Pha Marn PPA., Khounkham Dist., Khammouan Prov., Laos. Individuals were observed climbing or perching on the tops of sharp karst pinnacles (Figure 5). Only a few sightings of the new species have been made. The first individual was observed and photographed on 27 August 2022 around 1500h by S. Xayyasith during a bird watching excursion (Figure 5B). The second individual (the holotype) was observed on 6 October 2022, around 1630h by a local guide while guiding a group of tourists at a zipline course, and subsequently collected. The paratype male was collected on 2 February 2023, at 1400h at the same spot on the top of a limestone pinnacle. These three individuals were spotted while crawling on steep karst pinnacles around 50–70 m above the ground. The body color of the new species—a contrasting combination of black and white blotches—corresponds with the color of the limestone karst rocks on which it lives and likely serves as a disruptive coloration. Indeed, when perching on top of limestone karsts, individuals of the new species become almost invisible in the mosaic of sharp shadows and light spots (Figure 5B) and are difficult to spot unless they start moving. Almost all body scales of the new species bear pronounced keels and carinae, which might assist in climbing steep limestone rocks or perhaps serve as wedging points into karstic cracks and crevices for defense. This agamid species appears to be rock-dwelling and is only sighted on top of the karst pinnacles of the limestone mountains high above the ground (ca. 30–70 m), similar to some arboreal canopy-dwelling agamids, that rarely ever venture near the ground. The local people said this species is rare and was never recorded outside the limestone habitat on the ground. According to the local people and the staff of the Nam Sanam-Phou Pha Marn PPA, the new species is active in the late morning (0900h to 1100h) and late afternoon (1500h to 1700h), with the peak activity period being the latter interval. The diet of the new species likely consists of ants (Formicidae), which were numerous during the daytime.

Other reptile species that were recorded around the type locality (though on different elevations above the ground) of *Laodracon carsticola* **sp. nov.** include *Cyrtodactylus lomyenensis*, *Gekko khunkhamensis* Sitthivong et al., *Lycodon banksi*, *Protobothrops sieversorum*, and *Trimeresurus truongsonensis*.

Comparisons with *Diploderma* species occurring in southern China and Indo-Burma: Besides the morphological characters which define *Laodracon* **gen. nov.**, *Laodracon carsticola* **sp. nov.** differs from all members of *Diploderma* from adjacent regions except for *Diploderma dymondi*, *D. swild*, *D. panlong*, *D. varcoae*, and *D. slowinskii* by having an exposed tympanum (vs. concealed); and from all but *D. fasciatum*, *D. makii*, and *D. luei* by a different dorsal
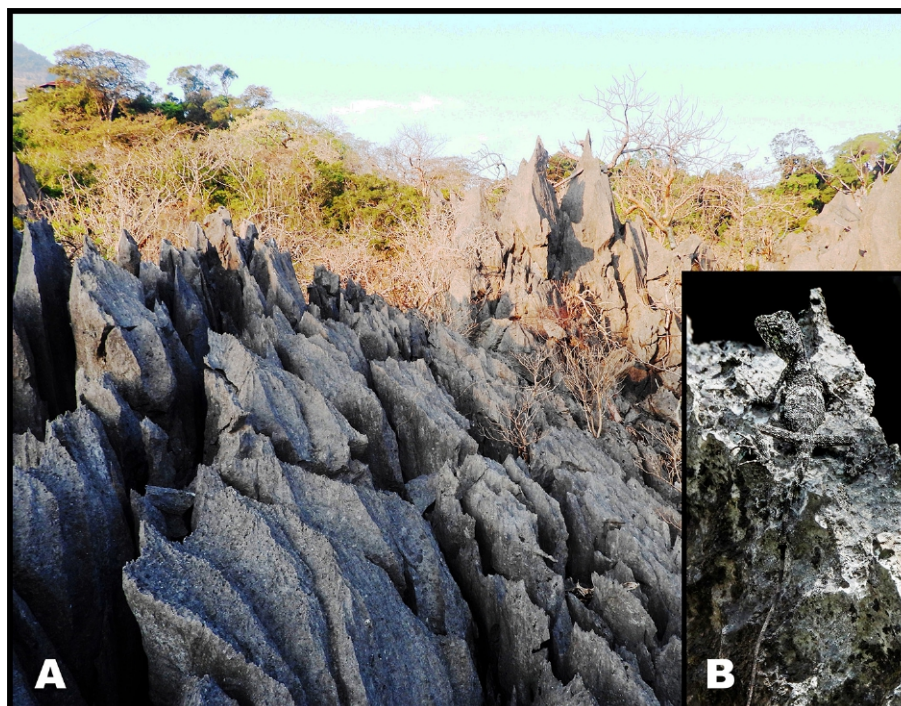
Figure 5 Natural habitat of *Laodracon carsticola* Gen. et sp. nov. in Nam Sanam-Phou Pha Marn PPA., Khounkham Dist., Khammouan Province, central Laos

A: Macrohabitat of the new species on karst pinnacles. B: A specimen of *Laodracon carsticola* Gen. et sp. nov. *in situ* (not collected) showing disruptive camouflage that hides the lizard while it perches on limestone. Photographs by Santi Xayyasith.

ornamentation pattern in males (transverse bands vs. dorsolateral stripes).

For *Diploderma* that also have an exposed tympanum, the new species differs by having a distinct body ornamentation and coloration (black in background, with white transverse bands vs. white, gray, or brown in background, with green or yellowish green dorsolateral stripes) and by the presence of a reticulated dark gular pattern with a blue gular spot (vs. absence of gular spot or reticulated pattern, or gular spot in different color). In the three species of *Diploderma* with transverse bands on dorsum, *Laodracon carsticola* **sp. nov.** differs from *D. fasciatum* by having homogeneous gular scales (vs. heterogeneous); and from *D. luei* and *D. makii* by having a more robust head (HW 0.77 HL vs. <0.7) and by the presence of transverse gular fold (vs. absence).

Distribution and biogeography: The new species is currently known only from the type locality within the limestone massif near the Rock Viewpoint, Nam Sanam-Phou Pha Marn PPA., Khounkham Dist., Khammouan Prov., Laos. It may also be distributed in adjacent limestone mountain areas such as Phou Nok Kok Provincial Protection Forest and Phou Hin Poun National Protected Area.

Conservation status: To date, *Laodracon carsticola* **sp. nov.** is known only from a very narrow area within Nam Sanam-Phou Pha Marn PPA., Khammouan Prov., Laos. Further research is required to clarify the extent of its distribution, population size and trends, and conservation status. We preliminary suggest the new species should be considered as Data Deficient (DD) following the IUCN's Red List categories (IUCN Standards and Petitions Committee, 2019).

Etymology: The specific epithet "*carsticola*" is a noun in the nominative case singular used in apposition, derived from German "*Karst*" (originally from Latin "*carsus*") used for "limestone landscapes" and "*cola*", a Latin word meaning "resident", "inhabiting" or "dwelling in". The name is given in reference to the limestone mountain habitat of the new species. We recommend the names "Khammouan Karst Dragon" and "*Chi Pom Pou Hin Poun Khammouane*" for the common names of the new species in English and Lao languages, respectively.

DISCUSSION

Limestone areas are universally recognized as unique biodiversity hotpots. By providing a diversity of microhabitats with very stable climatic conditions, karstic landscapes act as unique environments for survival of numerous ancient lineages and are referred to as "arks of biodiversity" (Clements et al., 2006). At the same time, limestone areas are also important drivers of speciation for certain organisms, especially for gekkonid lizards (Grismer et al., 2021a, 2021b, 2022). During the last two decades several previously unknown karst-adapted lineages of frogs (Milto et al., 2013; Nguyen et al., 2008; Orlov et al., 2009; Suwannapoom et al., 2018), snakes (Orlov et al., 2004; Ziegler et al., 2000), geckos (Grismer et al., 2021a, 2021b, 2022), insectivore mammals (Bannikova et al., 2014), rodents (Jenkins et al., 2005; Musser et al., 2005), and even birds (Fuchs et al., 2018; Woxvold et al., 2009) were discovered from the limestone areas of the mainland Southeast Asia, including Laos. Among the primarily terrestrial or arboreal subfamily Draconinae, there have been no species reported which are specifically adapted to life in karst formations. A Draconine *Mantheyus phuwuanensis*, a species inhabiting central Laos and northeastern Thailand,

occurs on large sandstone boulders along streams and demonstrates several morphological and behavioral features which are believed to serve as adaptations for fitting into rock crevices (Ananjeva & Stuart, 2001). However, this lizard is specifically restricted to sandstone massifs and does not occur in karstic habitats. In this study, we report on a new unique limestone-specialized species and genus of Draconinae, which is phylogenetically clustered in one group with predominantly arboreal genera *Diploderma* and *Pseudocalotes*.

Our phylogeny generally agrees with the evolutionary relationships of earlier studies that examined lizards in Draconinae (Grismer et al., 2016; Karunarathna et al., 2020; Macey et al., 2000; Wang et al., 2019a). With increasing international collaborations and wider adoptions of integrative taxonomic approaches, the generic level taxonomy of the subfamily Draconinae has experienced major revisions in recent years, including the resurrection of a previously synonymized genus (*Diploderma*), reclassifications of several species, and even the description of a new genus *Cristidorsa* (Gowande et al., 2021; Wang et al., 2019a). Our discovery of another new genus of Draconinae further highlights the need of more through phylogenetic and taxonomic studies on this radiation of agamid lizards in Asia.

Previous studies have shown that diversity of the genus *Diploderma* are mainly concentrated in the montane regions in southwestern China, and only three species extend their distribution into the northern edges of Indochina, namely *D. chapaense*, *D. fasciatum*, and *D. menghaiense* (Liu et al., 2020; Wang et al., 2019a, 2022). Little is known about any *Diploderma*, especially the Indochinese species, so our discovery of a new genus and species which morphologically resembles *Diploderma* further emphasizes the overlooked diversity of agamid lizards in this region. Much of Laos, Cambodia, and even Vietnam remain under-surveyed for herpetological diversity, particularly in specialized habitat types such as the karst landscapes where our new genus and new species reside. Given the abundant taxonomic discoveries from karst landscapes in recent years (Grismer et al., 2021a, 2021b, 2022; Milto et al., 2013; Nguyen et al., 2008; Orlov et al., 2004, 2009; Suwannapoom et al., 2018; Ziegler et al., 2000), we suspect that there is more additional undiscovered diversity hidden in these habitats and further international investigations that will formally quantify and describe such species are needed.

Compared to the previous studies, our phylogeny provides new insights on the phylogeny of Draconinae. Similar as the earlier works, we recognize three major clades within the subfamily: *Mantheyus phuwuanensis* is strongly suggested as a sister lineage to all other members of Draconinae, which are subdivided into two major clades, one of which includes *Draco*, *Ptyctolaemus*, and *Japalura*, and the other one including all the remaining genera. Compared to previous studies, our phylogeny achieved a better resolution of the latter clade containing the remaining draconine genera, which is subdivided into two major subclades Subclade III-1 and Subclade III-2 (see Figure 2), though both have only moderate node support levels. Within Subclade III-1, our analyses strongly supported monophyly of a group including Sri-Lankan genera *Ceratophora*, *Cophotis,* and *Lyriocephalus* and Southeast-Asian genera *Harpesaurus*, *Aphaniotis*, *Gonocephalus*, and *Bronchocela*, in accordance with the results of Grismer et al. (2016). Other strongly supported

groups within the Subclade III-1 included the *Sarada+Sitana+Otocryptis* clade and the *Calotes+Psammophilus* clade. Phylogenetic relationships within Subclade III-2 remained largely unresolved (Figure 2), with the exception of the clade containing *Diploderma+Pseudocalotes*, where *Pseudocalotes* was strongly supported as a sister taxon of a clade including *Laodracon* **Gen. nov**. and *Diploderma*. Though based on a larger taxon sampling, our results suggested the presence of two main subclades within *Diploderma*, agreeing with the earlier results of Wang et al. (2018). Within the *Diploderma* subclade I, species distributed on East Asian islands (*Diploderma makii* (Ota), *D. luei* (Ota, Chen & Shang), *D. brevipes* (Gressitt), *D. polygonatum* Hallowell, and *D. swinhonis* (Günther)) form a strongly supported group; the second group which got high node support levels includes species from the western part of southern China (*Diploderma varcoae*, *D. dymondi* (Boulenger), *D. panlong* Wang, Che & Siler, *D. swild* Wang, Wu, Jiang, Chen, Miao, Siler & Che, *D. flaviceps* (Barbour & Dunn), *D. zhaoermii* (Gao & Hou), and *D. micangshanense* (Song)). Within the *Diploderma* subclade II, which is composed of taxa from mainland China (including Xizang (Tibet)) and northern Indo-Burma, *D. iadinum* was suggested as a sister taxon to a group joining all the remaining species. Monophyly of the clades including species from Yunnan Prov. of China and the adjacent parts of Indochina was strongly supported (*D. yunnanense*, *D. chapaense*, and *D. menghaiense*). Another strongly supported clade included taxa from Xizang and Yunnan Prov. of China (*D. laeviventris* (Wang, Jiang, Siler & Che), *D. yangi* Wang, Zhang & Li, and *D. slowinskii*). Phylogenetic relationships within the remaining group of *Diploderma* subclade II, which in our analysis is represented by 18 species of *Diploderma* from China, were not sufficiently resolved.

Lastly, when Wang et al. (2019a) resurrected *Diploderma* Hallowell, 1861 as a full genus, they correctly noted the neutral gender of the genus name. However, we found some inconsistencies in the changes in species-level names of *Diploderma* as originally proposed by Wang et al. (2019a, 2021b). In particular, Wang et al. (2019a, 2021a, 2021b) proposed the names "*Diploderma laeviventre*" and "*Diploderma flavilabre*" treating the species names of these taxa as if they were adjectives in neutral gender, adjusting them to the neutral genus name "*Diploderma*". However, it is obvious from a Latin grammar point of view that both species names in these cases represent not Latin adjectives but nouns (i.e., not "smooth-bellied" or "yellow-lipped" but "smooth belly" and "yellow lips") and therefore their gender should be invariable. Herein we correct the species names of these taxa as "*Diploderma laeviventris*" and "*Diploderma flavilabris*" following the rules of Latin grammar. The same is also true for the name "*Diploderma brevicauda*" where the species epithet actually represents a noun ("short tail") and therefore should not be adjusted as "*brevicaudum*" ("short-tailed") as proposed by Wang et al. (2021a).

Despite some recent progress in biodiversity assessments of Laotian herpetofauna, every year new country records and species descriptions are being made (e.g., Poyarkov et al., 2023; Nguyen et al., 2020). Interestingly, herpetological surveys have been made to the karst regions surrounding Khammouan Prov. for the last 15 years, yet earlier studies failed to record the presence of *Laodracon carsticola* **Gen. et sp. nov.** (e.g., Luu et al., 2013, 2016). However, the limestone massif in which it occurs was never surveyed by previous researchers, and we cannot rule out that *Laodracon carsticola* **Gen. et sp. nov.** represents a point-endemic species with a very narrow range. Secondly, the newly discovered agamid likely evaded earlier discovery due to its very specific habitat preferences of living high on hardly accessible steep karst pinnacles. The construction of an elevated walkway at the Rock Viewpoint tourism attraction site obviously facilitated the accessibility of these unique habitats and likely aided the chances of observing this unique agamid species. Our study provides further evidence that Indochinese karstic landscapes, forming numerous hardly accessible habitats such as isolated valleys, caves, underground rivers, and pinnacles still cradle unique and yet-to-be-discovered biodiversity. At the same time, due to human activities such as cement production, limestone massifs represent one of the most endangered habitats in Southeast Asia. A large limestone quarry is located ca. 8 km south of from the type locality of *Laodracon carsticola* **Gen. et sp. nov.**, representing a potential threat for the newly discovered genus and species. Unfortunately, the immense financial returns of mineral extraction from karsts largely outweighs concerns for biodiversity conservation, leaving approximately 99% of karstic landscapes with no legal protection (Grismer et al., 2021b). Our work further underscores the urgent need for the appropriate management and conservation of karst massifs in Laos and in Indochina in general so that species such as *Laodracon carsticola* **Gen. et sp. nov.** can be protected.

NOMENCLATURAL ACTS REGISTRATION

The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic versions are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved, and the associated information can be viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/.
Publication LSID: urn:lsid:zoobank.org:pub:ED70A497-9243-4405-9EAE-773B91914A15
Laodracon, LSID: urn:lsid:zoobank.org:act:87905AE2-DD99-49DD-B978-CC08C19B14A0
Laodracon carsticola, LSID: urn:lsid:zoobank.org:act: 4BDBE7D7-A9CC-4C02-8EE8-5C435003A63F

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Fieldwork in Laos was permitted by the letter from the National University of Laos, Lao PDR (permit no. 011 of 23 October 2022); sample exportation was permitted by the decision No. 3820/DoF of 10 August, 2023 by the Department of Forestry of the Ministry of Agriculture and Forestry Lao PDR.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

P.B., T.V.N. and N.A.P. designed the study. P.B., S.X. and S.S. collected

specimens in the field. N.A.P. and S.I. performed molecular experiments. P.B., T.V.N, S.S.I., N.M, P.P., K.W. and N.A.P. prepared figures and tables and performed data analyses. P.B., K.W., T.V.N. and N.A.P. wrote the manuscript. K.W., T.V.N. and N.A.P. revised the manuscript. All authors read and approved the final version of the manuscript.

REFERENCES

Ananjeva NB, Guo XG, Wang YZ. 2011. Taxonomic diversity of Agamid lizards (Reptilia, Sauria, Acrodonta, Agamidae) from China: a comparative analysis. *Asian Herpetological Research*, **2**(3): 117−128.

Ananjeva NB, Stuart BL. 2001. The agamid lizard *Ptyctolaemus phuwuanensis* manthey and nabhitabhata, 1991 from thailand and laos represents a new genus. *Russian Journal of Herpetology*, **8**(3): 165−170.

Bannikova AA, Lebedev VS, Abramov AV, et al. 2014. Contrasting evolutionary history of hedgehogs and gymnures (Mammalia: Erinaceomorpha) as inferred from a multigene study. *Biological Journal of the Linnean Society*, **112**(3): 499−519.

Barjadze S, Murvanidze M, Arabuli T, et al. 2015. Annotated List of Invertebrates of the Georgian Karst Caves. Tbilisi: Georgian Academic Book, 1–120.

Cai B, Zhang MH, Li J, et al. 2022. Three new species of *Diploderma* hallowell, 1861 (Reptilia: Squamata: Agamidae) from the Shaluli Mountains in western Sichuan, China. *Asian Herpetological Research*, **13**(4): 205−223.

Chen WH, Zhang YM, Li ZY, et al. 2018. *Hemiboea crystallina*, a new species of gesneriaceae from karst regions of China and vietnam. *Phytotaxa*, **336**(1): 95−99.

Chin SC. 1977. The Limestone Hill Flora of Malaya1. Singapore: The Gardens' Bulletin, 165–220.

Chung KF, Leong WC, Rubite RR, et al. 2014. Phylogenetic analyses of *Begonia* sect. *Coelocentrum* and allied limestone species of China shed light on the evolution of Sino-Vietnamese karst flora. *Botanical Studies*, **55**(1): 1.

Clements R, Ng PKL, Lu XX, et al. 2008. Using biogeographical patterns of endemic land snails to improve conservation planning for limestone karsts. *Biological Conservation*, **141**(11): 2751−2764.

Clements R, Sodhi NS, Schilthuizen M, et al. 2006. Limestone karsts of Southeast Asia: Imperiled arks of biodiversity. *BioScience*, **56**(9): 733−742.

Denzer W, Manthey U, Mahlow K, et al. 2015. The systematic status of *Gonocephalus robinsonii* Boulenger, 1908 (Squamata: Agamidae: Draconinae). *Zootaxa*, **4039**(1): 129−144.

Fuchs J, Pasquet E, Stuart BL, et al. 2018. Phylogenetic affinities of the enigmatic bare-faced bulbul *Pycnonotus hualon* with description of a new genus. *Ibis*, **160**(3): 659−665.

Gowande GG, Bhosale HS, Phansalkar PU, et al. 2021. On the systematics and the phylogenetic position of the poorly known, montane dragon-lizard species *Pseudocalotes austeniana* (Annandale, 1908) (Squamata, Agamidae, Draconinae). *Evolutionary Systematics*, **5**(1): 141−150.

Grismer JL, Schulte JA, Alexander A, et al. 2016. The eurasian invasion: phylogenomic data reveal multiple Southeast Asian origins for Indian Dragon lizards. *BMC Evolutionary Biology*, **16**: 43.

Grismer L, Wood PL, Poyarkov NA, et al. 2021a. Karstic landscapes are foci of species diversity in the World's third-largest vertebrate genus *Cyrtodactylus* Gray, 1827 (Reptilia: Squamata; Gekkonidae). *Diversity*, **13**(5): 183.

Grismer LL, Poyarkov NA, Quah ESH, et al. 2022. The biogeography of bent-toed geckos, *Cyrtodactylus* (Squamata: Gekkonidae). *PeerJ*, **10**: e13153.

Grismer LL, Wood PL, Poyarkov NA, et al. 2021b. Phylogenetic partitioning of the third-largest vertebrate genus in the world, *Cyrtodactylus* Gray, 1827 (Reptilia; Squamata; Gekkonidae) and its relevance to taxonomy and conservation. *Vertebrate Zoology*, **71**: 101−154.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**(2): 95−98.

Hillis DM, Moritz CM, Mable BK. 1996. Molecular systematics. 2nd ed. Massachusetts: Sinauer Sunderland, 655.

Hoang DT, Chernomor O, Von Haeseler A, et al. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, **35**(2): 518−522.

Huelsenbeck JP, Ronquist F, Nielsen R, et al. 2001. Bayesian Inference of phylogeny and its impact on evolutionary biology. *Science*, **294**(5550): 2310−2314.

IUCN Standards and Petitions Committee (2019). Guidelines for using the IUCN red list categories and criteria. ver. 14. Prepared by the standards and petitions committee. http://www.iucnredlist.org/documents/RedList-Guidelines.pdf. (accessed 30 January 2023).

Jenkins PD, Kilpatrick CW, Robinson MF, et al. 2005. Morphological and molecular investigations of a new family, genus and species of rodent (Mammalia: Rodentia: Hystricognatha) from Lao PDR. *Systematics and Biodiversity*, **2**(4): 419−454.

Jobb G. 2011[2021-01-30]. Treefinder. http://www.treefinder.de/.

Karunarathna S, Poyarkov NA, Amarasinghe C, et al. 2020. A new species of the genus *Ceratophora* Gray, 1835 (Reptilia: Agamidae) from a lowland rainforest in Sri Lanka, with insights on rostral appendage evolution in Sri Lankan agamid lizards. *Amphibian & Reptile Conservation*, **14**(3): 103−126.

Katoh K, Misawa K, Kuma KI, et al. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**(14): 3059−3066.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**(7): 1870−1874.

Lanfear R, Calcott B, Ho SYW, et al. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, **29**(6): 1695−1701.

Liu S, Hou M, Rao DQ, et al. 2022. Three new species of *Diploderma* hallowell, 1861 (Squamata, Agamidae) from the Hengduan Mountain Region, south-western China. *ZooKeys*, **1131**: 1−30.

Liu S, Hou M, Wang J, et al. 2020. A new species of *Diploderma*

(Squamata: Sauria: Agamidae) from Yunnan Province, China. *Russian Journal of Herpetology*, **27**(3): 127−148.

Luu VQ, Bonkowski M, Nguyen TQ, et al. 2016. Evolution in karst massifs: Cryptic diversity among bent-toed geckos along the Truong Son Range with descriptions of three new species and one new country record from Laos. *Zootaxa*, **4107**(2): 101−140.

Luu VQ, Nguyen TQ, Calame T, et al. 2013. New country records of reptiles from Laos. *Biodiversity Data Journal*, (1): e1015.

Macey JR, Schulte II JA, Larson A, et al. 2000. Evaluating trans-tethys migration: an example using acrodont lizard phylogenetics. *Systematic Biology*, **49**(2): 233−256.

Mahony S. 2010. Systematic and taxomonic revaluation of four little known Asian agamid species, *Calotes kingdonwardi* Smith, 1935, *Japalura kaulbacki* Smith, 1937, *Salea kakhienensis* anderson, 1879 and the monotypic genus *Mictopholis* Smith, 1935 (Reptilia: Agamidae). *Zootaxa*, **2514**(1): 1−23.

Milto KD, Poyarkov NA Jr, Orlov NL, et al. 2013. Two new rhacophorid frogs from Cat Ba Island, Gulf of Tonkin, Vietnam. *Russian Journal of Herpetology*, **20**(4): 287−300.

Minh BQ, Nguyen MAT, Von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, **30**(5): 1188−1195.

Musser GG, Smith AL, Robinson MF, et al. 2005. Description of a new genus and species of Rodent (Murinae, Muridae, Rodentia) from the khammouan limestone national biodiversity conservation area in Lao PDR. *American Museum Novitates*, (3497): 1−31.

Nguyen LT, Schmidt HA, Von Haeseler A, et al. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**(1): 268−274.

Nguyen TV, Brakels P, Maury N, et al. 2020. New herpetofaunal observations from Laos based on photo records. *Amphibian & Reptile Conservation*, **14**(2): 218−249.

Nguyen TQ, Hendrix R, Böhme W, et al. 2008. A new species of the genus *Philautus* (Amphibia: Anura: Rhacophoridae) from the Truong Son Range, Quang Binh Province, central Vietnam. *Zootaxa*, **1925**(1): 1−13.

Orlov NL, Gnophanxay S, Phimminith T, et al. 2009. A new species of *Rhacophorus* genus (Amphibia: Anura: Rhacophoridae: Rhacophorinae) from Khammouan Province, Lao PDR. *Russian Journal of Herpetology*, **16**(4): 295−303.

Orlov NL, Ryabov SA, Bui TN, et al. 2004. A new species of *Trimeresurus* (Ophidia: Viperidae: Crotalinae) from Karst region in central vietnam. *Russian Journal of Herpetology*, **11**(2): 139−149.

Pal S, Vijayakumar SP, Shanker K, et al. 2018. A systematic revision of *Calotes* Cuvier, 1817 (Squamata: Agamidae) from the western Ghats adds two genera and reveals two new species. *Zootaxa*, **4482**(3): 401−450.

Poyarkov NA, Nguyen TV, Popov ES, et al. 2023. Recent progress in taxonomic studies, biogeographic analysis and revised checklist of Reptilians in Indochina. *Russian Journal of Herpetology*, **30**(5): 255−476.

Poyarkov NA Jr, Suwannapoom C, Pawangkhanant P, et al. 2018. A new genus and three new species of miniaturized microhylid frogs from Indochina (Amphibia: Anura: Microhylidae: Asterophryinae). *Zoological Research*, **39**(3): 130−157.

Rambaut A, Drummond AJ. 2007[2021-01-30]. Tracer. Version 1.5. http://beast.bio.ed.ac.uk/Tracer.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**(12): 1572−1574.

Sambrook J, Russell RW. 2001. Molecular Cloning: A Laboratory Manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press.

Shaney KJ, Maldonado J, Smart U, et al. 2020. Phylogeography of montane dragons could shed light on the history of forests and diversification processes on Sumatra. *Molecular Phylogenetics and Evolution*, **149**: 106840.

Shimodaira H. 2002. An approximately unbiased test of phylogenetic tree selection. *Systematic Biology*, **51**(3): 492−508.

Sodhi NS, Koh LP, Brook BW, et al. 2014. Southeast Asian biodiversity: An impending disaster. *Trends in Ecology & Evolution*, **19**(12): 654−660.

Sodhi NS, Posa MRC, Lee TM, et al. 2010. The state and conservation of southeast Asian biodiversity. *Biodiversity and Conservation*, **19**(2): 317−328.

Suwannapoom C, Sumontha M, Tunprasert J, et al. 2018. A striking new genus and species of cave-dwelling frog (Amphibia: Anura: Microhylidae: Asterophryinae) from thailand. *PeerJ*, **6**: e4422.

Tolentino PJS, Navidad JRL, Angeles MD, et al. 2020. Review: biodiversity of forests over limestone in southeast Asia with emphasis on the philippines. *Biodiversitas Journal of Biological Diversity*, **21**(4): 1597−1613.

Trifinopoulos J, Nguyen TL, Von Haeseler A, et al. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research*, **44**(W1): W232−W235.

Uetz P, Freed P, Aguilar R, et al. 2023[2023-01-30]. The Reptile Database. http://reptile-database.org/.

Vences M, Guayasamin JM, Miralles A, et al. 2013. To name or not to name: criteria to promote economy of change in Linnaean classification schemes. *Zootaxa*, **3636**(2): 201−244.

Wake DB. 2013. The enigmatic history of the European, Asian and American plethodontid salamanders. *Amphibia-Reptilia*, **34**(3): 323−336.

Wang K, Che J, Lin SM, et al. 2019a. Multilocus phylogeny and revised classification for mountain dragons of the genus *Japalura s. l.* (Reptilia: Agamidae: Draconinae) from Asia. *Zoological Journal of the Linnean Society*, **185**(1): 246−267.

Wang K, Gao W, Wu JW, et al. 2021a. Two new species of *Diploderma* Hallowell, 1861 (Reptilia: Squamata: Agamidae) from the Hengduan Mountain Region in China and rediscovery of *D. brevicaudum* (Manthey, Wolfgang, Hou, Wang, 2012). *Zootaxa*, **4941**(1): 1−32.

Wang K, Jiang K, Wang YF, et al. 2018. Discovery of *Japalura chapaensis* Bourret, 1937 (Reptilia: Squamata: Agamidae) from Southeast Yunnan Province, China. *Zoological Research*, **39**(2): 105−113.

Wang K, Jiang K, Zou DH, et al. 2016. Two new species of *Japalura* (Squamata: Agamidae) from the Hengduan Mountain Range, China. *Zoological Research*, **37**(1): 41−56.

Wang K, Qi S, Wang J, et al. 2022. Revision of the *Diploderma fasciatum* (Mertens, 1926) Complex (Reptilia: Agamidae: Draconinae). *Ichthyology & Herpetology*, **110**(3): 511−525.

Wang K, Ren JL, Wu JW, et al. 2021b. Systematic revision of mountain dragons (Reptilia: Agamidae: *Diploderma*) in China, with descriptions of six new species and discussion on their conservation. *Journal of Zoological Systematics and Evolutionary Research*, **59**(1): 222−263.

Wang K, Wu JW, Jiang K, et al. 2019b. A new species of Mountain Dragon (Reptilia: Agamidae: *Diploderma*) from the *D. dymondi* complex in southern Sichuan Province, China. *Zoological Research*, **40**(5): 456−465.

Wilcox TP, Zwickl DJ, Heath TA, et al. 2002. Phylogenetic relationships of the Dwarf Boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molecular Phylogenetics and Evolution*, **25**(2): 361−371.

Woxvold IA, Duckworth JW, Timmins RJ. 2009. An Unusual New Bulbul (Passeriformes: Pycnonotidae) from the Limestone Karst of Lao PDR. Oxford: Forktail, 1−12.

Ziegler T, Herrmann HW, David P, et al. 2000. *Triceratolepidophis sieversorum*, a new genus and species of pitviper (Reptilia: Serpentes: Viperidae: Crotalinae) from Vietnam. *Russian Journal of Herpetology*, **7**(3): 199−214.