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Hidden on the frontiers: *Calotes vultuosus* (Harlan, 1825) (Squamata, Agamidae), a species new for the reptile list of Pakistan

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Abstract. We document the first confirmed record of *Calotes vultuosus* (Harlan, 1825) from Pakistan, based on three specimens from Keamari, Sindh. DNA barcodes (16S, COI) and morphological traits place the studied material firmly within *C. vultuosus*, clearly distinct from related congeners. This finding extends the species' distribution westward from India into coastal Pakistan and highlights the biogeographic significance of southern Pakistan and integrative approaches for resolving the distribution of the *C. versicolor* complex in South Asia.

Key words. DNA barcoding, first record, Sindh, South Asia, taxonomy

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Introduction

The reptile diversity of South Asia, particularly Pakistan, is high but remains greatly underestimated (Khan 2006; Masroor 2012). The country spans a vast territory encompassing habitats from extreme deserts to high-elevation mountains and lies at the intersection of two (Khan 2006) or three (Holt et al. 2013) major terrestrial zoogeographical realms: the Palearctic, Saharo-Arabian, and Oriental realms. This unique position has allowed faunal elements from these realms to meet, interact, and evolve, making the region a natural laboratory for studying ongoing speciation and colonization processes. However, given the limited resources available for biodiversity research, reliable estimates of reptile diversity remain challenging.

In recent years, DNA data from local amphibian and reptile populations have substantially improved our understanding of vertebrate diversity between the Hindu Kush, Himalaya, and the Arabian Sea (e.g. Jablonski et al. 2020, 2025; Masroor et al. 2022; Amarasinghe et al. 2023; Hofmann et al. 2023). These advances also highlight several reptile groups in need of further taxonomic and biogeographic attention, including the Agamidae, one of the most diverse families in the region. Pakistan hosts multiple agamid genera that display notable ecological and phenotypic variation. Among these genera, for example, *Laudakia* Gray, 1845 comprises large-bodied lizards with high species diversity across both montane habitats and the arid landscapes, yet their genetic structure and distribution patterns remain insufficiently explored. A similarly incomplete picture is evident in *Calotes* Cuvier, 1817, which reaches its westernmost distribution along the eastern foothills of the Hindu Kush and the Iranian Plateau, but whose regional diversity and phylogeographic history are still poorly known.

Traditionally, most populations of *Calotes* in the Indian subcontinent were identified as *C. versicolor* (Daudin, 1802). However, an integrative study combining morphological and genetic data (Gowande et al. 2021) demonstrated that this taxon represents a complex of four distinct species distributed across South Asia. Of these, two occur within Pakistan or in its proximity in India. The first, *C. farooqi* Auffenberg & Rehman, 1993, inhabits hilly areas of northern Pakistan and eastern Afghanistan (and was surprisingly detected in one Sindh population), while *C. vultuosus* (Harlan, 1825) is distributed widely in central and northern India, including Gujarat and Rajasthan, near the Pakistani border. Conversely, *C. versicolor* sensu stricto, which is restricted to southern and eastern India and introduced to

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the Maldives, does not occur in Pakistan (Gowande et al. 2021). Despite these advances, the distribution and diversity of *Calotes* in Pakistan remain poorly understood, and it is unclear whether one or more species are present. To address this question, we collected and analysed specimens from Sindh Province using both morphological and genetic data.

Methods

During fieldwork in the coastal areas of Sindh and Balochistan, Pakistan, in 2021, we collected three specimens of the genus *Calotes* near Keamari village (Karachi Division) for further examination (Figure 1A). Geographic coordinates were recorded using the WGS84 datum. The collection comprised two adults and one subadult. Specimens were euthanized and preserved in 70% ethanol for long-term storage in the personal collection of the first author (Comenius University Herpetological Collection, CUHC), with tissue samples for DNA analysis preserved separately in 96% ethanol. In addition, we recorded 37 morphological characters (Table 1), as defined by Zug et al. (2007), from newly available specimens included in this study.

To visualize the distribution of the species and update its known range, we prepared a distribution map using QGIS v. 3.44 Solothurn (QGIS Development Team 2025) and morphologically and genetically verified records from Gowande et al. (2021). Based on previous research and the availability of comparative datasets, we generated new mitochondrial sequences of 16S rRNA (16S) and cytochrome oxidase subunit I (COI) using primers and PCR conditions described by Gowande et al. (2021). Total genomic DNA was extracted with the E.Z.N.A.® Plasmid DNA Maxi Kit, following the manufacturer's protocol. Sequences were obtained both from the newly collected material from Keamari village and from two specimens of *C. farooqi* from northern Pakistan (CUHC 10228, 10247), which had previously been sequenced only for 16S (Gowande et al. 2021).

For accurate species assignment based on mitochondrial sequences, we included material originating from or near the type localities of related South Asian congeners (Figure 1A), as reported by Gowande et al. (2021). The comparative dataset comprised DNA material of *C. farooqi* (CUHC 10228, 10247), *C. irawadi* (AG855, CAS 204862), *C. versicolor* (NCBS AT102), and *C. vultuosus* (CESL 1063, 1081). The following sequences were used for comparison: MW901313–14 (*C. farooqi*), MW901300–01 (*C.*

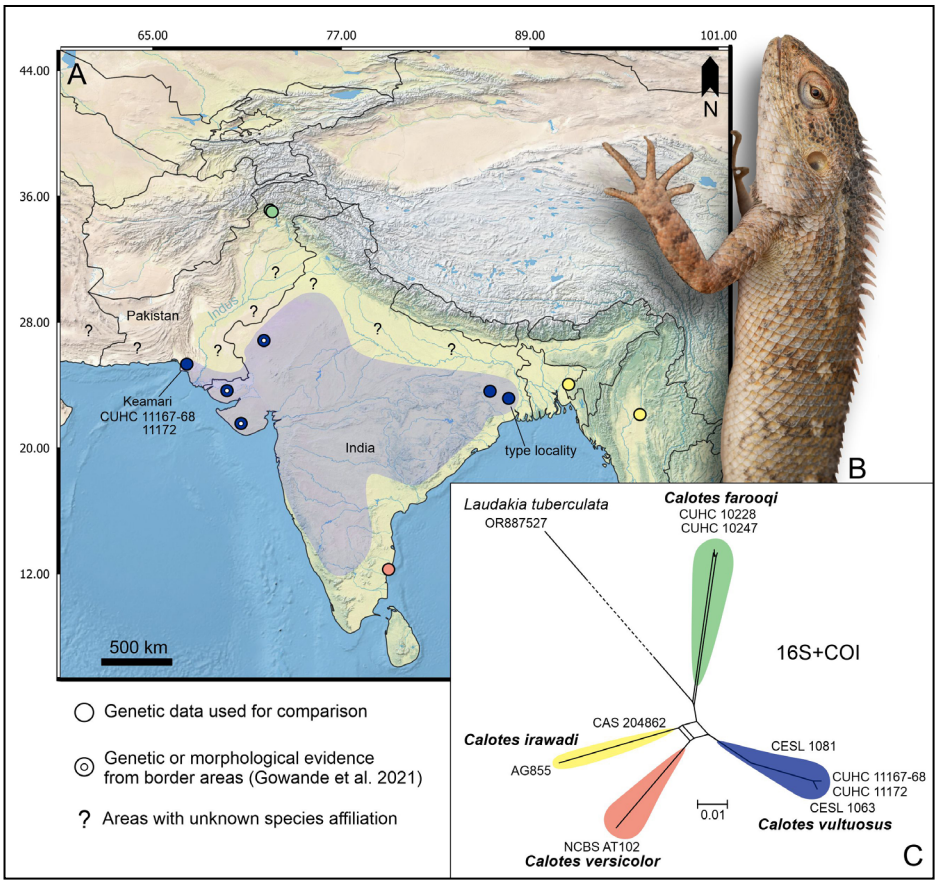

Figure 1. Distribution and first confirmation of *Calotes vultuosus* for Pakistan. **A.** Map showing the new locality record from Keamari, Karachi Division, Sindh Province, Pakistan (blue circle), with additional comparative localities and type locality of the species (data from Gowande et al. 2021). Question marks indicate areas with uncertain species affiliation. **B.** Adult female *Calotes vultuosus* (CUHC 11172) from Keamari, Karachi Division, Sindh Province, Pakistan. **C**. Phylogenetic network based on concatenated mitochondrial 16S rRNA (524 bp) and COI (608 bp) sequences, generated in SplitsTree, showing the placement of Pakistani specimens (CUHC 11167, CUHC 11168, CUHC 11172) within *C. vultuosus* (blue), distinct from *C. farooqi* (green), *C. irawadi* (yellow), and *C. versicolor* (red). Scale bar indicates genetic distance.

Table 1. Morphological data for 37 characters collected from *Calotes vultuosus* from Keamari, Sindh Province, Pakistan. Measurements in mm.

Character	Voucher		
	CUHC 11167	**CUHC 11168**	**CUHC 11172**
Sex	Female	?	Female
Eye–ear length	5.8	4.2	7.3
Head height	19.9	17.5	21.8
Head length	26.3	23.0	35.3
Head width	22.4	14.6	26.0
Interorbital width	11.8	9.8	13.3
Jaw width	16.3	13.2	22.6
Naris–eye length	3.8	3.4	5.0
Snout–eye length	9.0	7.1	10.9
Snout width	4.5	4.4	5.1
4th finger	10.1	10.0	12.1
4th toe	16.4	14.7	19.6
Crus length	20.0	17.5	26.1
Forefoot length	17.9	15.4	19.9
Hindfoot length	27.3	23.7	29.3
Lower arm length	14.4	14.1	18.5
Pectoral width	15.8	13.3	21.9
Pelvic width	12.7	7.9	16.1
Snout–vent length	87.7	78.5	115.6
Snout–forelimb length	35.9	26.8	39.0
Tail height	8.4	7.0	15.3
Tail length	225	155	161 (incomplete)
Tail width	11.8	7.4	14.1
Trunk length	45.9	42.5	67.5
Upper arm length	17.9	14.9	23.8
Upper leg length	21.7	19.2	24.3
Canthus rostralis	8	8	8
Dorsal eyelid scales	12	10	13
Dorsal head scales	14	13	14
Head scales	12	11	13
Infralabials	12/13	11/11	11/11
Snout scales	6	6	6
Supralabials	12/11	12/11	12/12
Temporal spines	2	2	2
4th finger lamellae	18	17	18
4th toe lamellae	25	24	25
Dorsal scales	43	42	46
Mid-body scale rows	42	42	43

irawadi), MW901307 (*C. versicolor*), and MW901298–99 (*C. vultuosus*) for 16S; MZ489212 (*C. irawadi*), MZ489209 (*C. versicolor*), and MZ489206 (*C. vultuosus*) for COI. Two COI sequences of *C. farooqi* (CUHC 10228, 10247) were newly produced in this study. As an outgroup, we used 16S and COI genes extracted from the mitogenome sequence of *Laudakia tuberculata* (OR887527). Newly generated sequences have been deposited in GenBank under the accession numbers PX418822–24 (16S) and PX418913–17 (COI).

Sequences of 16S (524 bp) and COI (608 bp) were trimmed in Seaview (Gouy et al. 2021), concatenated, and analyzed using a phylogenetic network constructed in SplitsTree v. 4 (Huson and Bryant 2006) based on uncorrected *p*-distances.

Results

Order Squamata
Family Agamidae
Genus *Calotes* Cuvier, 1817

***Calotes vultuosus* (Harlan, 1825)**

New records. PAKISTAN — **Sindh Province** • Karachi Division, Keamari District, Keamari; 24.8842°N, 66.8159°E; alt. 29 m; 14.IX.2021; Jablonski D, Masroor R. leg.; semi-arid lowland with sandy–loamy soil, scattered *Acacia* trees, grass patches, and an open cultivated–natural ecotone near low rocky hills; 2 adult ♀ CUHC 11167, 11172 (Figure 1B), GenBank: PX418822, PX418824 (16S), PX418915, PX418917 (COI), 1 sex indet., subadult, CUHC 11168, GenBank: PX418823 (16S), PX418916 (COI).

Identification. Molecular analyses of mitochondrial markers (concatenated 16S rRNA, COI) place the Pakistani specimens firmly within *C. vultuosus*, clearly distinct from *C. farooqi*, *C. irawadi*, and *C. versicolor* (Figure 1C). The specimens from Keamari form a well-supported clade with Indian sequences of *C. vultuosus* (*p*-distance < 1%). This represents the first confirmed record of *C. vultuosus* from Pakistan, extending the known distribution of the species westward from India to the coastal Sindh region. Metric and meristic characteristics obtained (Table 1) support the species identification. Morphological examination of the three specimens from Keamari (CUHC 11167, 11168, 11172) corresponds well with the diagnosis of *C. vultuosus* as presented by Gowande et al. (2021). Key characters such as midbody scale rows (42–43), dorsal scales (42–46), supralabials (11–12), infralabials (11–13), and two temporal spines are fully consistent with the published ranges. Subdigital lamellae on the fourth finger (17–18) and fourth toe (24–25) fall at the lower end of the reported variation (19–22 and 23–28, respectively) but still overlap with the species' limits. Snout–vent length of the two females (87.7–115.6 mm) also matches the values known for adult *C. vultuosus*, with the largest specimen slightly exceeding the mean size reported for females. Taken together, both meristic and morphometric data clearly support the identification of the Pakistani specimens as *C. vultuosus*.

Discussion

Our study provides the first confirmed record of *Calotes vultuosus* in Pakistan, extending the known range of the species westward from India into the Sindh coastal region and likely further into Pakistan through mainland Sindh and Balochistan, where it appears to be restricted to oases and watercourse margins (Minton 1966; Mertens 1969; Sindaco and Jeremcenko 2008). The specimens from Keamari are morphologically and genetically consistent with *C. vultuosus* as defined by Gowande et al. (2021). Meristic traits (e.g. midbody scale rows, dorsal scales, temporal spines), mitochondrial DNA data (16S and COI), and geographical context clearly distinguish the Pakistani specimens from *C. farooqi* (northern Pakistan, eastern Afghanistan), *C. irawadi* (Myanmar and Indochina), and *C. versicolor* (southern India and the Maldives).

The presence of *C. vultuosus* in southern Pakistan indicates that the species has a broader distribution than previously recognized (Gowande et al. 2021) and underscores the importance of the semi-arid plains and coastal areas of southern Pakistan as both colonization corridors and contact zones between different zoogeographic realms (Agarwal et al. 2022; Jablonski et al. 2025). The record from Keamari village highlights such biogeographic significance of this region, suggesting that similar cases of past colonization from South Asia into the Palearctic (west of the Indus River) may be revealed in other widely dispersing reptile species once genetic data are applied.

In this context, a key question concerns the species identity of the regionally westernmost *Calotes* populations reported from Iran's Sistan and Balochistan Province (Mobaraki et al. 2013). Based on morphological traits visible in available photographs (see also iNaturalist 2023), this part of Iran is likely inhabited by *C. vultuosus* rather than *C. versicolor*. Although this would not be unexpected from a biogeographic perspective (see distribution of *Euphlyctis adolfi*, *Varanus bengalensis* or *Bungarus persicus*; Kamali 2020; Safaei-Mahroo et al. 2023; Arianejad et al. 2025) genetic confirmation is still required.

Our discovery also underscores the need for continued, rigorous herpetological exploration in Pakistan, where several lineages of the *C. versicolor* complex occur in proximity (Gowande et al. 2021). The recently elevated *C. farooqi* is restricted to the hilly regions of northern Pakistan and eastern Afghanistan, whereas *C. vultuosus* now appears to occupy the lowland coastal areas of Sindh and likely Balochistan Provinces. We also expect its presence in the lowland areas of Punjab Province, although this has not yet been genetically confirmed. Photographic records on iNaturalist (2021) and the species documented presence on the Indian side of the border (Figure 1A; Gowande et al. 2021), however, suggest that its distribution in Pakistan might be broader than currently recognized, highlighting the need for further field surveys and genetic sampling. More broadly, the distribution of *Calotes* from the foothills of Hindu Kush and Himalaya to the Indian plains remains unresolved, and the extent of contact zones or possible hybridization between species in this complex has yet to be studied. In this context, the genetic affiliation of the population from Mithi, Sindh (loc. 79 in Gowande et al. 2021), remains unclear. Although it unexpectedly clusters with the lineage related to *C. farooqi*, its geographic position suggests (cf. Figure 1A) that it should fall within the expected range of *C. vultuosus*. This discrepancy warrants further investigations.

In conclusion, the documentation of *C. vultuosus* in Pakistan not only fills a distributional gap for this taxon but also emphasizes the significance of integrative taxonomy combining morphological and molecular evidence for correctly identifying cryptic members of the *C. versicolor* complex and

their accurate distribution in South Asia. Further surveys across Balochistan and adjoining regions are expected to clarify whether *C. vultuosus* is restricted or more widespread species of southwestern Pakistan.

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Additional information

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Author contributions

Conceptualization: DJ. Data curation: all authors. Formal analysis: DJ, MK. Funding acquisition: DJ. Investigation: all authors. Methodology: DJ. Resources: DJ. Visualization: DJ. Writing – original draft: DJ. Writing – review and editing: all authors.

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Data availability

All data that support the findings of this study are available in the main text or GenBank depository.

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