















Research Article



Shedding light on taxonomic chaos: Diversity and distribution of South Asian skipper frogs (Anura, Dicroglossidae, *Euphlyctis*)

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A known haven of amphibian diversity, South Asia is also a hotspot of taxonomic confusions. Vastly distributed from Saudi Arabia to Myanmar, the dicroglossid genus *Euphlyctis* (“skittering” or “skipper” frogs) is a representative example. Combining phylogenetic analyses with 16S barcoding and genome size variation of 403 frogs from 136 localities, we examined genetic diversity and distributions across the whole range of *Euphlyctis*, with a particular focus on taxonomic and nomenclatural issues. We recovered two deeply divergent mitochondrial clades totalling ten lineages that we considered as species, and eight could be attributed valid taxonomic names and junior synonyms. The first clade (subgenus *Phrynoderma*) is confirmed in South India, Bangladesh and Sri Lanka, and comprises six species: *E. karaavali*, *E. hexadactyla*, *E. aloysii*, *E. kerala* and two undescribed taxa. Five are endemic to the Western Ghats biodiversity hotspot and four of them form the *E. aloysii* species complex. The second clade (subgenus *Euphlyctis*) extends across South Asia and neighbouring regions, and comprises four species: *E. ehrenbergii*, *E. jaladhara*, and two widespread lineages erroneously called “*E. mudigere*” and “*E. kalasgramensis*” in recent literature, while their oldest valid names appear to be *E. cyanophlyctis* and *E. adolfi*, respectively. Additional analyses on this pair of taxa highlighted strong phenotypic resemblance, notable intraspecific phylogeographic structure, and an extensive contact zone along the southern slopes of the Himalaya, with putative signs of genetic introgression. Through an independent investigation of the historical literature, we identified overlooked issues and misconceptions regarding the status of many old and recent taxa, and proposed solutions, such as transferring “*E. ghoshii*” to the genus *Limnonectes*. Our study illustrates how range-wide genetic barcoding can clarify taxonomic confusions, and we call to solve remaining issues prior to the description of new taxa.

Key words: *Euphlyctis adolfi*, *Euphlyctis cyanophlyctis*, genetic barcoding, genome size, phylogeography, systematics, *Phrynoderma*, taxonomy, Western Ghats biodiversity hotspot

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Introduction

Assessing the geographic extent of newly discovered species is necessary to provide first-hand evaluations of their conservation status and ascertain their taxonomic identity. As genuine evolutionary lineages are increasingly considered to be valid species by taxonomists, including when external differences are not obvious (“cryptic taxa”), species identification using molecular methods (“genetic barcoding”) has become an important part of biodiversity surveys (Borkin *et al.*, 2004; Mahony *et al.*, 2020; Padial *et al.*, 2010; Vences *et al.*, 2005a). In turn, reliable knowledge of lineage distributions and divergence can provide key insights onto the biogeography of species and regions.

In this respect, the diversity and evolution of a substantial part of the herpetofauna of South Asia (India and neighbouring countries), which encompasses several of Earth’s biodiversity hotspots (Myers *et al.*, 2000; Meegaskumbura *et al.*, 2002; Roelants *et al.*, 2004; Das *et al.*, 2006), remains poorly understood. In particular, amphibian taxonomy has been studied by naturalists since colonial times (Chanda *et al.*, 2001; Günther, 1860, 1864), but genetic studies have highlighted strong discordances with previous taxonomic arrangements and assumptions regarding the distributions of many species (e.g. Chen *et al.*, 2005). This “taxonomic chaos” stems from various causes. First, as the phylogeographic literature increasingly suggests, the genetic diversity of South Asian amphibians remains insufficiently documented (e.g. Vijayakumar *et al.*, 2016). Second, amphibian surveys in South Asia still essentially rely on phenotypic identification in the field. However, some species appear more polymorphic than previously assumed, in turn causing frequent misidentification and thus erroneous conclusions on distribution ranges (Dinesh *et al.*, 2021). Third, when genetic barcoding is implemented, it often targets amphibian communities of a given region (e.g. Akram *et al.*, 2021; Hasan *et al.*, 2012, 2014), not entire species ranges, and relies only on mitochondrial DNA (mtDNA). Yet, clarifying the taxonomic identity of species sometimes requires range-wide sampling that encompasses the type localities of all available nomina. In addition, due to the high propensity of cyto-nuclear discordance among amphibian species complexes (e.g. ghost mitochondrial lineages or super-cryptic species; Dufresnes *et al.*, 2019, 2020), barcoding analyses are more informative when combining mitochondrial with nuclear-encoded markers.

In respect to the above issues, we aimed to clarify lineage distribution and taxonomy in a complex group of South Asian anurans, the microglossid genus *Euphlyctis* Fitzinger, 1843, also known as “skittering frogs” or “skipper frogs.” Widespread from the Arabian

Peninsula, across desert areas of Iran and Afghanistan, to tropical Myanmar and Sri Lanka (Akram *et al.*, 2021; Dinesh *et al.*, 2021; Khatiwada *et al.*, 2021; Wagner *et al.*, 2016; Zug, 2022), most populations have long been attributed to two species groups, namely the *Euphlyctis cyanophlyctis* (Schneider, 1799) group and the *Euphlyctis hexadactyla* (Lesson, 1834) group. Both groups have been reported to differ externally, form monophyla that diverged since the Mid- or Early-Miocene, and occur in sympatry over large parts of their ranges (Alam *et al.*, 2008; Dinesh *et al.*, 2021; Dubois *et al.*, 2021; Dufresnes & Litvinchuk, 2022; Howlader *et al.*, 2015). In their attempt to name every supraspecific amphibian clade, Dubois *et al.* (2021) raised the two groups as separate genera, respectively *Euphlyctis* (*E. cyanophlyctis* group) and *Phrynoderma* Fitzinger, 1843 (*E. hexadactyla* group). Using molecular approaches, especially 16S ribosomal DNA sequences, a myriad of lineages was revealed within both groups (Akram *et al.*, 2021; Alam *et al.*, 2008; Dinesh *et al.*, 2021; Khajeh *et al.*, 2014) and new allopatric species were formally described from Bangladesh and India (Dinesh *et al.*, 2021, 2022; Howlader *et al.*, 2015; Joshy *et al.*, 2009). Taxonomists now list nine species, more than half reported within the last 15 years (Frost, 2022).

An inherent challenge in *Euphlyctis* taxonomic and conservation research is that allopatric species are morphologically very similar (Joshy *et al.*, 2009), especially for sexually immature individuals, which complicates species identification without genetic barcoding. As a consequence, the respective distributions of taxa remain largely elusive, which casts doubts on the identity of type populations. For instance, several cases of synonymy were suspected in the *E. cyanophlyctis* group, such as *Euphlyctis mudigere* Joshy, Alam, Kurabayashi, Sumida, and Kuramoto, 2009 with *E. cyanophlyctis* (Dinesh *et al.*, 2021); *Euphlyctis kalasgramensis* Howlader, Nair, Gopalan, and Merilä, 2015 with *Euphlyctis cyanophlyctis seistanica* (Nikolskii, 1899) (Dinesh *et al.*, 2021, as “*Euphlyctis seistanica*”) or with *Euphlyctis adolfi* (Günther, 1860) (Borkin *et al.*, 2020; Dufresnes & Litvinchuk, 2022; Litvinchuk *et al.*, 2017). Some presumed species are yet to receive a molecular assessment, like *Euphlyctis ghoshi* (Chanda, 1991), while the strong mitochondrial divergence documented within others are perhaps indicative of species complexes in need of taxonomic attention, like *Euphlyctis aloysii* Joshy, Alam, Kurabayashi, Sumida, and Kuramoto, 2009 (Dinesh *et al.*, 2021). In parallel, the status of historical taxa such as *Rana bengalensis* Gray, 1830 and *Rana leschenaultii* Duméril and Bibron, 1841 remains unsolved (Dinesh *et al.*, 2021), and these may

be found to have priority over several of the recently discovered and described lineages.

To get a more comprehensive picture of the *Euphlyctis* diversity, distribution and taxonomy across South Asia, here we integrate new and available molecular barcoding data (16S) from the ranges of all known lineages. We further conduct phylogeographic and morphometric analyses of the two most widespread species, which have been the sources of much confusion in recent publications. Finally, we provide an independent examination of the historical literature in order to identify and propose solutions to overlooked taxonomic issues that are presently threatening the stability of several species.

Methods

16s rRNA barcoding, phylogeny and taxonomy

For mtDNA barcoding, 81 tissue samples from 39 localities across India, Nepal, Iran, Pakistan and Saudi Arabia, were obtained from voucher specimens (thigh muscles) or live individuals (toe clips, blood, buccal swabs), preserved in 70–96% ethanol. DNA was isolated using DNAeasy Blood and Tissue kits (Qiagen). For 74 samples, a ~550bp fragment of the 16S rRNA gene was amplified using universal primers 16SA–L and 16SB–H (Palumbi et al., 2002; Vences et al., 2005b), and sequenced in one direction (16SA–L). For seven samples (series “RGK” in Supplemental material - File S1a), a ~1500–2000 bp fragment encompassing 12S-tVal–16S was obtained using the primers pairs LX12SN1(f) and LX16S1R(r), and 12SAL(f) and 16S2000H(r) (Zhang et al., 2008), and sequenced in both directions. Sequences were quality-checked in MEGA X (Kumar et al., 2018) and uploaded in GenBank under accession numbers ON818920–ON818993 (16S) and ON814172–ON814178 (12S-tVal–16S). We then aligned and trimmed the sequences manually in Seaview 5 (Gouy et al., 2010), which involved adding 1–3 bp gaps at a few positions.

To complement this dataset, we searched for *Euphlyctis* 16S sequences available on GenBank (Sayers et al. 2022). A total of 182 sequences were retrieved as of March 2022 (Supplemental material - File S1a), originating from 35 sources – 19 associated to published studies (Akram et al., 2021; Alam et al., 2008, 2010; Ali et al., 2020; Al-Qahtani & Amer, 2019; Anoop et al., 2017; Bossuyt & Milinkovitch, 2000; Dinesh et al., 2021; Hasan et al., 2012, 2014; Howlader et al., 2015; Joshy et al., 2009; Khajeh et al., 2014; Khatiwada et al., 2021; Kosuch et al., 2001; Kotaki et al., 2010; Kurabayashi et al., 2005; Priti et al., 2016; Singh &

Prakash, 2006). Among these, 165 sequences could be accurately or approximately georeferenced. The remaining 17 were also included in order to consider all the genetic variation documented in *Euphlyctis*, and to crosscheck their taxonomic identity. The final alignment (480 bp) thus consisted of 263 *Euphlyctis* sequences, including 246 georeferenced sequences from 104 localities (Supplemental material - File S2). We first identified the main lineages from a preliminary tree estimated in PhyML 3.2 (Guindon et al., 2010) and collapsed identical sequences into unique haplotypes. Net pairwise divergences between and within lineages were computed in MEGA, with default settings. Sampled localities were then mapped in QGIS 3.4 (QGIS Development Team, 2018) and visualized according to their 16S lineages.

In a second step, we reconstructed and dated phylogenetic divergences among 16S haplotypes, adding a sequence of the closely related dicroglossid species *Fejervarya cancrivora* (Gravenhorst, 1829) as the out-group (AB070738, Sumida et al., 2002). We performed a Bayesian analysis with BEAST 2.5 (Bouckaert et al., 2019), using a strict clock and a birth-death tree prior. Although our phylogenetic dataset is limited to a single gene, we implemented a time calibration prior in order to get general insights about divergence times in *Euphlyctis*, and to retrieve a rough estimation of the 16S clock rate for downstream demographic analyses. Specifically, we used a normally distributed prior for the split between the *E. hexadactyla* and *E. cyanophlyctis* groups, estimated at $\sim 20 \pm 3$ million years (My) in the dicroglossid phylogeny of Alam et al. (2008). The chain was run for 10 million iterations, which was enough to reach stationarity and independently sampled trees, as confirmed by inspection of the log file in Tracer 1.7 (Rambaut et al., 2018).

In addition, to explore the relevance of the identified lineages in species delimitation, we applied the partitioning algorithm of ASAP (Puillandre et al., 2021) included in the iTaxoTools software collection (Vences et al., 2021). We explored the solutions obtained with (Jukes-Cantor, Kimura K80) or without substitution models to compute genetic distances, and retained the ones with the lowest ASAP score.

Finally, we provisionally assigned 16S lineages to taxonomic names based on an investigation of the taxonomic literature, especially the historical publications, museum records (notably online registries), and on the examination of relevant scientific collections and curated specimens by one of us (SM). Type localities and nomenclatural validity of older and junior synonyms were then critically assessed to update and discuss taxonomic arrangements. As part of this effort, the following museum catalogue number prefixes were used. BMNH:

Natural History Museum, London; BNHS: Bombay Natural History Society, Mumbai; CAS: California Academy of Sciences; MASB: Museum of the Asiatic Society of Bengal; MNHNP, MNHN and NMNHP: Museum National d'Histoire Naturelle, Paris; NMSL: Sri Lanka National Museum; ZMB: Universität Humboldt, Zoologisches Museum, Berlin; ZSI: Zoological Survey of India, Kolkata.

Ranges and diversity of widespread taxa

Additional analyses were performed to get preliminary insights into the phylogeography of the two most widespread taxa, identified as *E. adolfi* and *E. cyanophlyctis* (see Results). First, to complement the mtDNA barcoding, we assessed range limits based on genome sizes. To this end, 151 individuals from 37 localities were anaesthetized (immersion 1% MS222) and sampled for blood. Genome size (nuclear DNA content) was then measured by DNA flow cytometry following the procedures in Borkin *et al.* (2001). All the sampled specimens are curated at the Institute of Cytology of the Russian Academy of Science (INCRAS). Their origins and taxonomic identity (based on 16S barcoding for several samples) are detailed in [Supplemental material - File S1a](#). Variation in genome size, expressed in picograms (pg), was compared between species, averaged over populations, and mapped using a diagnostic threshold (see Results).

Second, the 16S dataset was used to build haplotype networks for the species identified as *E. adolfi* ($n = 154$ sequences) and *E. cyanophlyctis* ($n = 51$ sequences) with the R package *pegas*, under default settings (Paradis, 2010). For each species, haplogroups were identified, and mapped across geographic ranges with QGIS. In addition, we inferred coalescent Bayesian Skyline Plots (BSP) in BEAST (Drummond *et al.*, 2005) to model demographic fluctuations, in which we applied the clock rate (0.005 substitution per site per My) obtained from the time-calibrated phylogeny (see above). The chain was run for 10 million iterations, and skyline plots were produced with Tracer.

Finally, building up on the newly inferred ranges of *E. adolfi* and *E. cyanophlyctis*, we compared their morphology based on 11 characters typically used in the *Euphlyctis* literature (Joshy *et al.*, 2009). To this end, one of us (SNL) measured 48 specimens (representative of 17 localities) from the Zoological Institute of St. Petersburg (ZISP) and INCRAS collections, using a digital caliper to the nearest 0.1 mm. Sexual maturity and sex were determined by direct examination of gonads. Measurements comprised snout-vent length (SVL), head length, from the tip of the snout until the posterior edge of the mandibles (HL), head width,

between the posterior edges of the mandibles (HW), snout to nostril distance, from the snout tip to the anterior edge of the nostril (SN), inter-nostril distance (NN), nostril to eye distance, from the posterior edge of the nostril to the anterior bony orbital border (NE), eye diameter, measured horizontally between the bony orbital borders (ED), inter-orbital distance, as the minimum distance between orbits (EE), tympanum diameter (TD), femur length, from mid cloacal opening to the knee (FEL) and shank length (TIL). We then computed body ratios for the latter ten characters (character/SVL), and included five additional ratios, namely HL/HW, SN/NE, TD/ED, NN/EE and TIL/FEL (as in Joshy *et al.*, 2009). A PCA and a MANOVA were performed on these 15 values in R 3.6, with the specific aims of assessing how the morphology varied with respect to sex and species, identifying the contributing variables, and eventually designing diagnostic criteria. Furthermore, we combined the SVL measured for the 48 ZISP/INCRAS specimens with an additional 32 adults of known origin and sex (16 newly measured by SLN and RGK, 16 taken from Howlader *et al.*, 2015; Joshy *et al.*, 2009; Prasad *et al.*, 2020), and performed ANOVAs to assess the effect of sex and species. Raw measurements and specimen origins are detailed in [Supplemental material - File S1b](#).

Results

Genetic barcoding and taxonomic arrangements

Across the 263 *Euphlyctis* 16S sequences analyzed (480 bp), we identified 60 unique haplotypes forming 10 clear lineages, which we mapped across 104 localities (Fig. 1; [Supplemental material - Files S1a](#)). The haplotype alignment and full tree with terminal branches are available in [Supplemental material - Files S2–S3](#), respectively. The best species delimitation partition obtained with ASAP distinguished eight species (Fig. 1) with an ASAP score of 3.0 (ranked 2nd and 4th for the P-val and W indices, respectively), while the second best partition (10 species) received a score of 5.0. The same results were obtained with different models of sequence evolution.

Combining lineage distributions (circles in Fig. 1) with georeferencing of known type localities (stars in Fig. 1) and an examination of the historical literature, we critically assigned available taxonomic names to most of the lineages by applying the International Code of Zoological Nomenclature's principle of priority rule (ICZN, 1999) (Table 1). As developed in the following, we uncovered a multitude of taxonomic and

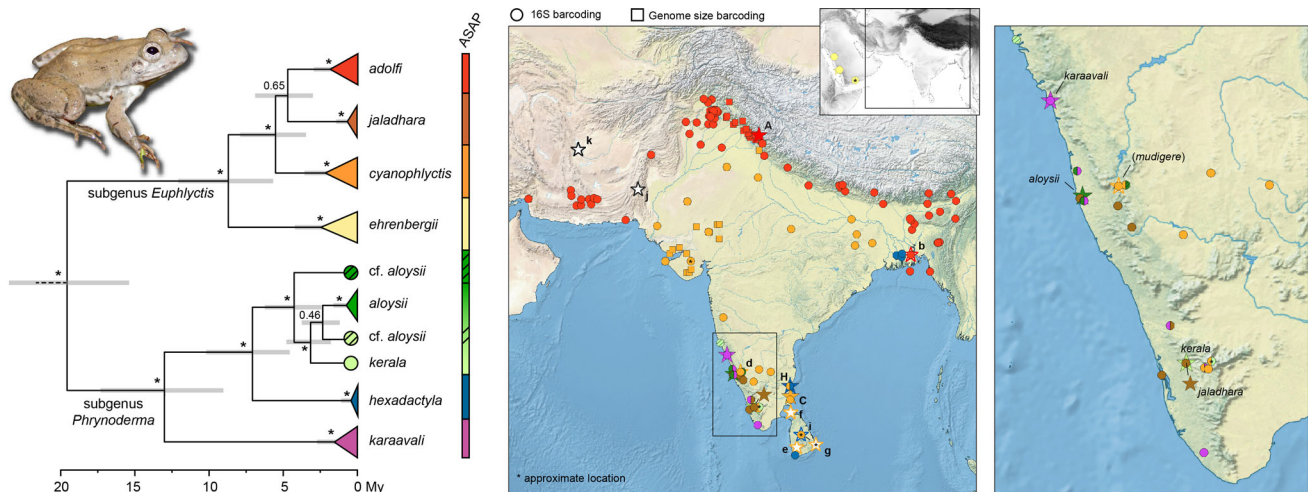


Fig. 1. Diversity and distribution of *Euphlyctis*. The tree shows phylogenetic relationships among 16S lineages, with statistical support (posterior probability) for internal branches (*: > 0.95) and 95% highest probability density of divergence times (grey bars). Species delimitation with ASAP are shown, based on the best partition (eight species). The maps illustrate lineage distribution, as inferred from 16S barcoding (circles) and genome size (squares). Note that the Arabian *E. ehrenbergii* is displayed on the small top inset (yellow circles). Type localities of taxa are represented by stars: filled stars labelled with upper case letters for valid names; empty stars labelled with lower case letters for junior synonyms, as follows. *Euphlyctis adolfi* (A), with junior synonym *E. kalasgramensis* (b); *E. cyanophlyctis* (C, presumed location), with junior synonym *E. c. flavens* (e), *E. c. fulvus* (f) and *E. c. typicus* (g); *E. hexadactyla* (H), with junior synonym *Rana robusta* (i). The affiliation of subspecies *E. c. microspinulata* (j) and *E. c. seistanica* (k), as well as the type localities (and the corresponding species) of the nomina *R. leschenaultii* and *R. bengalensis* remain unsettled (see text). See [Supplemental material - File S1a](#) for locality and haplotype information, and [Supplemental material - File S3](#) for the full 16S tree. Photo credit: SNL.

nomenclatural issues that appear to have not been previously reported in the *Euphlyctis* literature.

Gender of *Euphlyctis* Fitzinger, 1843. *Euphlyctis* taxa alternatively appear feminine (e.g. *Euphlyctis cyanophlyctis seistanica*) or masculine (e.g. *Euphlyctis hexadactylus*) in the literature, and it seems that the etymology and gender of the name have never been clarified. Among the first modern uses of *Euphlyctis* as a genus, Dubois (1992) listed *Euphlyctis hexadactylus*, thus assuming that it was masculine, a conjugation subsequently followed until nowadays.

In Brown's (1954) "Composition of Scientific Words," the prefix "eu" and suffix "phlyctis" are seemingly derived from Greek, and are defined as follows:

"eu- < Gr. *eu*, good, well, agreeable, easy, very, true, original, primitive" (p. 309).

"*L. phlyctaena* (Gr. *phlyktaina*; *phlyktis*, -idos), f. blister, pustule; *phlyzakion*, n. dim." (p. 169).

Fitzinger (1843) did not provide any indication of an etymology for *Euphlyctis*, and his description may have largely been based on the description of the type species *Rana leschenaultii* Duméril and Bibron, 1841. Although this taxon (considered as a synonym of *E. cyanophlyctis*,

but see below) can hardly be considered a very tuberculated frog, Duméril & Bibron (1841) did mention small warts/tubercles in the description and elsewhere, for instance "Corps semé de petites éminences coniques, et percé de pores disposés en lignes parcourant le cou, les côtés du dos et le ventre [Body strewn with small conical eminences, and pierced with pores arranged in lines running along the neck, the sides of the back and the belly]" (p. 342). Based on these observations, and from the variety of meanings given above for the prefix "eu," we thus interpret that the Greek name *Euphlyctis* was probably intended as a translation for "very pustular." Moreover, as indicated by the abbreviation "f." in Brown (1954)'s definition, the suffix "phlyctis" is feminine.

In conclusion, *Euphlyctis* should be considered feminine, and we hereby implement the proper spelling to the relevant taxa.

Genus-level split within *Euphlyctis*. The 16S phylogeny recovered the *E. cyanophlyctis* and *E. hexadactyla* groups as two strongly supported clades, estimated to be ~20 My old in Alam et al. (2008), an estimation re-used here for the molecular calibration (Fig. 1). Dinesh et al. (2021) provided a morphological diagnosis of the two groups that comprised broadly overlapping size ranges and the presence or absence of longitudinal

Table 1. Summary of the taxonomy and distribution of *Euphlyctis* species.

Lineage	Taxon	Synonyms* or subspecies**	Distribution
<u>Subgenus <i>Euphlyctis</i></u>			
yellow	<i>Rana ehrenbergii</i> Peters, 1863	none	Yemen and Saudi Arabia
brown	<i>Euphlyctis jaladhara</i> Dinesh, Channakeshavamurthy, Deepak, Shabnam, Ghosh, and Deuti, 2022	none	Western Ghats
red	<i>Dicroglossus adolfi</i> , 1860	<i>Euphlyctis kalasgramensis</i> Howlader, Nair, Gopalan, and Merilä, 2015*	Iran, southern Afghanistan, Pakistan, Nepal, northern India, southern Bhutan, Bangladesh, Western Myanmar
orange	<i>Rana cyanophlyctis</i> Schneider, 1799	<i>Rana cyanophlyctis</i> var. <i>typicus</i> De Silva, 1958** <i>Rana cyanophlyctis</i> var. <i>fulvus</i> De Silva, 1958** <i>Rana cyanophlyctis</i> var. <i>flavens</i> De Silva, 1958** <i>Euphlyctis mudigere</i> Joshy, Alam, Kurabayashi, Sumida, and Kuramoto, 2009*	most of India, Sri Lanka, Eastern Pakistan
<u>Subgenus <i>Phrynoderma</i></u>			
purple	<i>Euphlyctis karaavali</i> Priti, Naik, Seshadri, Singal, Vidisha, Ravikanth, and Gururaja, 2016	none	Western Ghats
blue	<i>Rana hexadactyla</i> Lesson, 1834	<i>Dactylethra bengalensis</i> Lesson, 1835* <i>Rana cutipora</i> Duméril & Bibron, 1841* <i>Rana robusta</i> Blyth, 1855*	eastern India, Sri Lanka, Bangladesh
dark green	<i>Euphlyctis aloysii</i> Joshy, Alam, Kurabayashi, Sumida, and Kuramoto, 2009	none	Western Ghats (Karnataka)
pale green	<i>Euphlyctis kerala</i> Dinesh, Channakeshavamurthy, Deepak, Ghosh, and Deuti, 2021	none	Western Ghats (Kerala)
dashed dark green	none (cf. <i>alloysii</i>)	–	Western Ghats (Karnataka)
dashed pale green	none (cf. <i>alloysii</i>)	–	Western Ghats (Goa)
<u>Unsettled taxa</u>			
red / orange / purple / dark green / pale green / dashed dark green / dashed pale green	–	<i>Rana leschenaultii</i> Duméril and Bibron, 1841	Bengal and perhaps Pondicherry and/or Malabar Coast
red / orange	–	<i>Rana bengalensis</i> Gray, 1830 <i>Rana cyanophlyctis</i> var. <i>seistanica</i> Nikolskii, 1899** <i>Euphlyctis cyanophlyctis microspinulata</i> Khan, 1997**	Bengal Neizar in Seistano, Iran southwest of Khuzdar in Pakistanese Balochistan

Color codes refers to the Fig. 1.

stripes on the dorsum and flanks. We find the presence or absence of stripes unreliable based on observations of variation within several species, e.g. *E. hexadactyla* and *E. adolfi* individuals with no visible stripes, and examples of all species with stripes on the flanks (SM pers.

obs.). Recently, Dubois *et al.* (2021) recognized these two groups as separate genera, *Euphlyctis* (= *E. cyanophlyctis* group) and *Phrynoderma* (= *E. hexadactyla* group), solely on the basis that their sister relationship was poorly supported among other closely related

dicroglossids. However, their phylogeny did not provide evidence of paraphyly, included only five *Euphlyctis* and *Phrynoderma* species, and the authors warned that “our terminals are ‘composite’, often consisting of gene sequences from more than one specimen” and “While we have attempted to minimise incorrectly labelled sequences from Genbank (see 4.3.1.2 below), there is no doubt that not all our ‘species’ are holophyletic, as a few of them are likely to be hybrid populations or clusters of cryptic species, and that some specimens have been misidentified.” (p. 294). Due to the widespread misidentification of GenBank sequences in *Euphlyctis* (see below) and the lack of resolution of this part of Dubois et al.’s (2021) tree, the proposed taxonomic rearrangements appear based on little evidence, which we consider potentially highly destabilizing. Accordingly, their split does not meet two out of the three priority taxon naming criteria (TNCs) formalized by Vences et al. (2013), namely phenotypic diagnosability and clade stability. For the latter, at least four subcriteria are violated: robust clade support (ii), absence of evidence for non-monophyly (iii), dense taxon sampling (iv) and support by independent data sets (v). We therefore do not recognize the two genera within the long-established genus *Euphlyctis*, but instead suggest that *Euphlyctis* and *Phrynoderma* may be used as subgenera for the simplification of discussion in the scientific literature, as implemented below.

Subgenus *Euphlyctis* (*E. cyanophlyctis* group). The subgenus *Euphlyctis* forms a strongly supported clade composed of four notable lineages, all partitioned as different species in the ASAP analysis (Fig. 1, Table 1):

1. An Arabian lineage barcoded in Yemen and Saudi Arabia (yellow-coded in Fig. 1). It corresponds to *Rana ehrenbergii* Peters, 1863, the only name available in the region, now *Euphlyctis ehrenbergii* (Peters, 1863).
2. A southern lineage restricted to the southwestern coastal plains of India (brown-coded in Fig. 1). This lineage has been associated with the recently described *Euphlyctis jaladhara* Dinesh, Channakeshavamurthy, Deepak, Shabnam, Ghosh, and Deuti, 2022, which shares the same type locality (“Thattekad Bird Sanctuary”) as *Euphlyctis kerala* Dinesh, Channakeshavamurthy, Deepak, Ghosh, and Deuti, 2021, a more distantly related lineage from subgenus *Phrynoderma* (see below). The brown-coded lineage was accordingly barcoded in Thattekad (Dinesh et al., 2021), but given the information provided in the GenBank records and the description of *E. jaladhara* (Dinesh et al. 2022), it is unclear whether these sequences correspond to the type specimens.
3. A northern lineage extending from Iran to Bangladesh along a narrow corridor flanking the Himalayas (red-coded in Fig. 1), which requires taxonomic revisions. As shown by the barcoding of three topotypes (Supplemental material - File S1a), this lineage inhabits the type locality of *Dicroglossus adolfi* Günther, 1860 (“Kulu and Simla, Himalaya (2400–4200 feet above the level of the sea),” now situated in Himachal Pradesh State, India). The original description (Günther, 1860) mentioned considerable variation within the species, based on a type series that contained multiple specimens, of which four syntypes are currently available (BMNH 1947.2.28.46 [previously BMNH 60.3.19.1365], BMNH 1947.2.28.47 [previously BMNH 60.3.19.1367], BMNH 1947.2.4.60 and 1947.2.4.61 [both previously BMNH 60.11.6.5]; SM pers. obs.). Boulenger (1882) has been credited with synonymizing this name under *Rana cyanophlyctis* Schneider, 1799 (e.g. Frost, 2022), although he provided no explanation for this action. The name was subsequently forgotten or considered invalid by many authors (e.g. Baig et al., 2008; Howlader et al., 2015; Masroor, 2011), and the same biological species was recently described again as *Euphlyctis kalasgramensis* Howlader, Nair, Gopalan, and Merilä, 2015, from a type series collected in Bangladesh (Howlader et al., 2015).
As previously proposed (Borkin et al., 2020; Dufresnes & Litvinchuk 2022; Litvinchuk et al., 2017), the oldest available nomen that can be assigned with certainty to the red-coded lineage is *Euphlyctis adolfi* (Günther, 1860). Given the lack of genetic differentiation between *E. adolfi* topotypes and the *E. kalasgramensis* type series, we formally consider *Euphlyctis kalasgramensis* Howlader, Nair, Gopalan, and Merilä, 2015 as a junior subjective synonym of *E. adolfi*.
4. A widespread lineage distributed in India, Sri Lanka and Western Pakistan (orange-coded in Fig. 1), which occurs at the currently accepted type localities of *Rana cyanophlyctis* Schneider, 1799, *Rana bengalensis* Gray, 1830, *Rana leschenaultii* Duméril and Bibron, 1841 and *Euphlyctis mudigere* Joshy, Alam, Kurabayashi, Sumida, and Kuramoto, 2009. Several nomenclatural and taxonomic ambiguities require clarification because recently described species have largely relied on questionable assumptions associated with these names.
First, the type locality of *R. cyanophlyctis* was given by Bauer (1998) as “Syntypes: ZMB

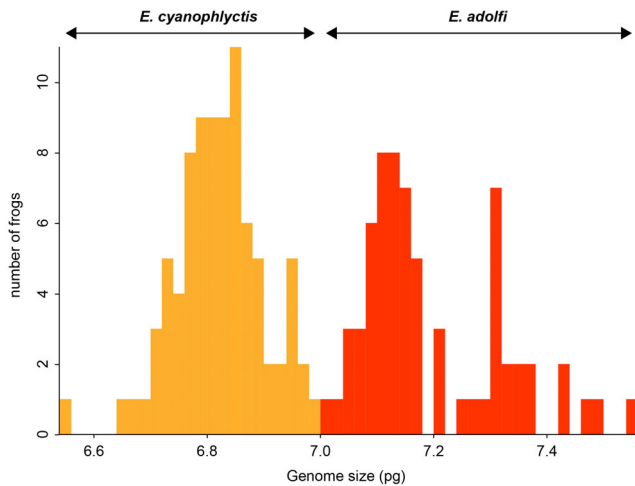


Fig. 2. Genome size variation in *Euphlyctis adolfi* and *E. cyanophlyctis* among 159 individuals. See [Supplemental material - File S1a](#) for the raw data.

3197–98*, “ex. India orientali,” two specimens sent by [Christoph Samuel] John, probably from Tranquebar.” Though Bauer (1998) provided a reasonable hypothesis for why John’s collections “probably” came from the historical region of Tranquebar (now Tharangambad in Tamil Nadu State), he did not make an explicit statement restricting the type locality. The type specimens must be re-examined in order to verify whether they actually correspond to the orange-coded lineage now widely regarded as *E. cyanophlyctis*. Until this is verified, the type locality for the species remains “ex. India orientali,” which in 1799 included most of South and Southeast Asia (and not “Kerala, India,” as stated in Howlader *et al.*, 2015).

Second, *R. bengalensis* was described from “Bengal” in 1830, which at that time comprised modern-day Bangladesh and several states in neighbouring India (West Bengal, Jharkhand, Bihar and the Cachar region of Assam). The type specimen is the animal depicted in Gray (1830: fig. 2), the whereabouts of which is currently unknown: it is present in neither the BMNH (SM *pers obs.*) nor the ZSI collections (Sclater, 1892). Thus, this specimen might have been lost, Gray (1830) may have provided an inaccurate drawing, or he described the species based on an uncollected animal. Third, *R. leschenaultii* was described from a syntype series involving at least four collectors and two localities, “Pondichéry” (collector Leschenault) and “Bengale” (collectors M. Duvaucel, M. Dussumier and M. Roux) (Duméril & Bibron, 1841). By 1841, Bengal had further expanded to include modern day Assam, Nagaland State and Meghalaya State. Boulenger (1920) stated to have examined nine type

specimens in MNHNP, and according to Frost (2022), Guibé (1950) identified MNHNP 4366–70 as syntypes. However, the online collection database for MNHNP lists as many as 20 specimens as syntypes: MNHNP 0.4366–0.4368, 0.4370, 0.6431, 0.768, 1999.7922–7924, 1999.7927–7934 collected by “Roux” from “Bengale,” and MNHNP 0.4369, 1999.7925 and 1999.7926 collected by “Jean-Jacques Dussumier” from “Côte de Malabar” (Malabar Coast). This conflicts with historical literature (including the description) with respect to the size, collectors and locality details of the syntype series. Importantly, if the MNHNP details are correct, the lectotypification of a Malabar Coast type would likely threaten the status of one of the species recently described from the Western Ghats.

Cantor (1847) suggested that *R. bengalensis* and *R. leschenaultii* might represent the same species but did not propose a synonymy. Peters (1863) formally synonymized *R. leschenaultii* with *R. cyanophlyctis*, stating that the former matches the description and the two type specimens of the latter. Both *R. bengalensis* and *R. leschenaultii* were later included in the synonymy of *R. cyanophlyctis* (although without explanations) by Günther (1864) and Steindachner (1867), where they have remained until today. However, based on lineage distributions (Fig. 1), *R. bengalensis* and *R. leschenaultii* could each represent either senior subjective synonyms of *E. adolfi* (red-coded lineage), or junior subjective synonyms of *E. cyanophlyctis* (orange-coded lineage), even more so because these species lack clear morphological distinction (see below), especially in former Bengal (SM *pers. obs.*). Hence, *R. bengalensis* requires neotypification and *R. leschenaultii* requires lectotypification (after verifying its actual syntype series), in order to resolve their nomenclatural and taxonomic status.

Fourth, De Silva (1958) provided brief descriptions for three colour “varieties” of *Rana cyanophlyctis* from Sri Lanka, given as “Variety *typicus* (Schneider)”, “Variety *fulvus* (De Silva)” and “Variety *flavens*”, the first one having been completely ignored in subsequent literature. De Silva’s taxonomic intentions are somewhat ambiguous because he did not provide the new names as trinomials, hence the need for clarification. Of particular confusion is that an authorship was assigned to two of the names proposed, although they may not necessarily convey taxonomic authorities. First, the “Variety *fulvus* (De Silva)” makes reference to De Silva (1957), but this publication reports on zoological observations (involving *Rana cyanophlyctis*) on the northern Sri Lankan coastal islands and lacks any new nomenclatural

content. Second, “Variety *typicus* (Schneider)” makes reference and actually copies parts of Boulenger’s (1890) account of *Rana cyanophlyctis* Schneider, 1799 in Sri Lanka (p. 442), which tends to indicate that this variety is meant as the nominal form, i.e. “*Rana cyanophlyctis cyanophlyctis*.” Because it is impossible to disentangle whether these citations correspond to authorships of the names or simply mentions of authors who had previously discussed the colour varieties, we refer to Article 45.6 of the Code (ICZN, 1999) to settle on their status:

"The rank denoted by a species-group name following a binomen is subspecific, except that [...]

45.6.4. it is subspecific if first published before 1961 and its author expressly used one of the terms "variety" or "form" (including use of the terms "var.," "forma," "v." and "f."), [...]."

All three varieties of De Silva (1958) are nomenclaturally available subspecies names, given that they are explicitly referred to as varieties, “following” a binomen (*Rana cyanophlyctis*), and published before 1961. We must thus consider *Euphlyctis cyanophlyctis typicus* (De Silva, 1958), with type locality “Ceylon [= Sri Lanka],” and unstated type specimens, which may include specimens available to De Silva in the National Museum of Colombo (now NMSL), or specimens examined by Boulenger (1890); *Euphlyctis cyanophlyctis fulvus* (De Silva, 1958), with type locality “from brackish water pools on the islands of Karaitivu, Leyden [= Velenai] and Pungkudutiva [= Pungudutivu] off Jaffna (N.P.)” in Jaffna District, Northern Province, Sri Lanka, and unstated syntypes collected in 1956, presumably deposited in NMSL (De Silva, 1957); *Euphlyctis cyanophlyctis flavens* (De Silva, 1958), with type locality “from clear pools from Ratnapura, ... Ceylon” in Ratnapura City, Ratnapura District, Sabaragamuwa Province, Sri Lanka, and unstated syntypes, two collected in 1952 and one in 1957 or 1958, presumably deposited in NMSL (De Silva, 1958). Attempts to locate and examine the putative type specimens would help clarify the taxonomic status of these three nomina. Given the variable coloration of *E. cyanophlyctis* throughout its range, and because our phylogeographic analyses suggested little genetic differentiation between mainland and Sri Lankan populations (Fig. 1, Supplemental material - File S3), these taxa most likely represent junior synonyms of *E. cyanophlyctis*. However, these three names have not been formally synonymized, and without

direct evidence of the contrary, they remain valid subspecies of *E. cyanophlyctis*.

According to current knowledge, we thus tentatively maintain application of the oldest nomen *Euphlyctis cyanophlyctis* (Schneider, 1799) to the orange-coded lineage and provisionally retain the other nomina as synonyms or subspecies.

In the *E. cyanophlyctis* group, we could further infer the ranges of *E. adolfi* and *E. cyanophlyctis* using genome-size estimates of 151 individuals. Genome sizes varied from 6.5 to 7.6 pg, and the variation was mostly diagnostic between the two taxa (Fig. 2). The species means differed by nearly 6% (7.2 pg in *E. adolfi* and 6.8 pg in *E. cyanophlyctis*), and frogs assigned to *E. adolfi* and *E. cyanophlyctis* had genome sizes above and below 7.0 pg, respectively. Genome size could thus serve as a nuclear barcoding tool to distinguish these species. Specifically, it confirmed the respective distributions of *E. adolfi* and *E. cyanophlyctis* in northern India and Nepal. We did however notice some regional discordances: in parapatric ranges, genome sizes typical of *E. cyanophlyctis* were measured near populations that featured only *E. adolfi* mtDNA haplotypes, which could indicate genetic introgression.

Subgenus *Phrynoderma* (*E. hexadactyla* group). The subgenus *Phrynoderma* forms a strongly supported clade composed of six notable lineages, assembled into four species in the ASAP analysis (Fig. 1, Table 1):

1. A southwestern Indian lineage (purple-coded in Fig. 1) that corresponds to *Euphlyctis karaavali* Priti, Naik, Seshadri, Singal, Vidisha, Ravikanth, and Gururaja, 2016, as confirmed by barcoding of the holotype. Furthermore, the purple-coded lineage is the only one detected at the type locality, and no other nomina seem to correspond to this taxon.
2. A widespread coastal lineage found in Sri Lanka, eastern India and neighbouring Bangladesh (blue-coded in Fig. 1). The oldest nomen for this lineage has long been considered to be *Rana hexadactyla* Lesson, 1834, for which topotypes were accordingly barcoded.

The nomenclatural priority of *E. hexadactyla* requires some clarification because Lesson published a second name for the species, *Dactylethra bengalensis*, which has been the source of a lot of confusion. Although *D. bengalensis* is sometimes credited to Dumeril & Bibron (1841), these authors clearly referred to Lesson’s “Illustrations de zoologie [= *Illustrations de zoologie, ou, Recueil de figures d’animaux peintes d’après nature*]” and not Lesson (1834) (as suggested by Frost, 2022). Various, usually reliable online sources (e.g. British Library, Biodiversity Heritage Library) give a variety of

publication dates for this book (e.g. “1831–1835?,” “1832,” “1832–1835,” “1835”), thus it is necessary to determine which one is correct to infer whether “*hexadactyla*” or “*bengalensis*” has priority. Lesson’s “Illustrations de zoologie” comprises 60 plates and was issued in 20 livraisons of 3 plates each. The date “Juin 1831 [= June 1831]” appears at the end of the text description of the species; however, the published prospectus for the books stated that the first livraison was due to be published on 1st March 1832. Mathews (1911) obtained the publication dates and noted that the first livraison was actually published on 14th July 1832, while the 16th to 20th livraisons were all issued some time between 17th January 1835 and December 1835. The figure and associated description of *Dactylethra bengalensis* was published in “plate XLVII [= plate 47]” and thus was published in 16th livraison. Therefore, the correct year of publication for *Dactylethra bengalensis* is 1835.

Moreover, Lesson (1835) erroneously mention *R. hexadactyla* as a synonym of *D. bengalensis*. The original descriptions of these taxa are identical, suggesting that both are referring to the same specimen(s), collected by Belanger from “Pondichéry [Puducherry, southeastern India].” Hence, we consider *Dactylethra bengalensis* Lesson, 1835 to be a junior objective synonym of *Rana hexadactyla* Lesson, 1834. The “paratype” specimen reported by Guibé (1950), NMNHP 4636 (now catalogued as MNHN-RA-0.4363) from “Bengale” appears to have a blotched dorsal pattern with no midvertebral stripe, which does not resemble the frog illustrated in Lesson (1835) — a mostly plain brown frog with a pale vertebral stripe. Since there is no mention of any specimens from “Bengale” in either of Lesson’s descriptions (Lesson, 1834, 1835), MNHN-RA-0.4363 should not be regarded as a type (contra Frost, 2022). Instead, the lack of holotype designation for *R. hexadactyla* (Lesson 1834) suggests a syntype series; it is conceivable that historical specimens in the MNHNP collection from “Pondichéry” (e.g. MNHN-RA-0.4361, 0.4364) and “Indes Orientales” (e.g. MNHN-RA-0.4365) could have been a part of this series, providing that they were made available to Lesson.

Other junior synonyms include the names *Rana cutipora* Duméril & Bibron, 1841 and *Rana saparoua* Duméril & Bibron, 1841, which have both been treated as substitute names (objective synonyms) for *R. hexadactyla* (e.g. Dinesh *et al.*, 2021; Frost, 2022). Though Duméril & Bibron (1841) clearly created *R. cutipora* to apply to *R. hexadactyla*, they made no indication as to why they rejected Lesson’s

older valid name. *Rana cutipora* was even adopted for the species until Günther (1859), who as far as we are aware of was the first to relegate it as a synonym, where it remains today. In contrast, *R. saparoua* seems to have been listed in the synonymy of *E. hexadactyla* only recently — the earliest mention we have noticed is Dinesh *et al.* (2009), apparently following Frost’s website. This name thus requires some clarification.

Duméril & Bibron (1841) published the following synonymy in their species account for “*Rana cutipora*. Nobis,” where a “*Rana saparoua*” is first mentioned:

“SYNONYMIE. *Rana saparoua*. Mus. Lugd. Bat.

Rana hexadactyla Less. Voy. Indes orient. Bel. zool. Rept. pag. 331, tom. VI.

Dactylethra Bengalensis. Id. Illustrat. zool. Pl. XLVII.

Rana hexadactyla. Tschudi, Classif. Batrach. Mém. Sociét. scienc. nat. Neuchât. tom. II, pag. 80.”

The name “*saparoua*” appears nowhere else in Duméril & Bibron (1841), and this single mention assigned neither an author citation for the name, nor was the name followed by “nobis,” so they were not intentionally creating a new name. In addition, the abbreviation “Mus. Lugd. Bat.” that follows the name corresponds to the Musei Lugduno-Batavae, Leiden, so it probably indicated a specimen label. The word “*saparoua*” refers to the Indonesian island known today as Saparua, which in the 19th century had various spelling (e.g. “Ile Saparoua,” “Saparna,” “Saparnae,” “Saparoea”; Duméril & Duméril, 1851; Balfour, 1885; Tschudi, 1838). In his discussion of “*Rana hydromedusa* Kuhl,” Tschudi (1838) made a similar reference to the Saparua specimen(s), using one of the alternative spellings of the island:

“*Hyla histrionica* Boje ist das junge, und *Rana Saparnae* Mus. Lugd. das ganz alte Their davon.”

In what appears to be the earliest mention of a “*Rana Saparnae*,” Tschudi (1838) also did not associate an author to the name, and neither did he indicate that he was coining a new name. While “*Rana Saparnae*” reminds of a binomen, it could also simply be a reference to a specimen or specimens identified as *Rana* from “Saparnae” Island, in which case it would not have any nomenclatural status. If

it was actually meant as a binomen, it would then have first appeared as a synonym of *R. hydromedusa* Tschudi (1838), and thus be nomenclaturally unavailable (Article 11.6 of the Code). In the case of *R. saparoua*, the use of italics by Duméril and Bibron (1841) suggests that they intended it as a binomen, but because it was likewise first published in a synonymy, *R. saparoua* is also nomenclaturally unavailable. Duméril and Bibron's (1841) inclusion of this name in the synonymy of their *R. hexadactyla* (as *R. cutipora*) appears to have been accidental because: (1) they cited the distribution of *R. cutipora* as eastern India, and specifically as "Bengale" and "Pondichéry" (not Indonesia); (2) they made no reference to Tschudi's (1838) synonymization (or updated identification) of the Saparua population under "Rana hydromedusa Kuhl"; (3) they did treat *Rana grunniens* (Latreille, 1801) as a valid species (now *Limnonectes grunniens*), under which they included "Rana hydromedusa Kuhl" in the synonymy (where it remains today); and (4) they recognized that the type series of *R. grunniens* comprised two specimens (now in MNHNP), one of which represented *R. hexadactyla* and the other *R. grunniens* sensu stricto (noted earlier by Tschudi, 1838, see also Stejneger, 1940). *Limnonectes grunniens* has accordingly been reported from Saparna Island (Evans et al., 2003). In conclusion, both *Rana Saparnae* Tschudi 1838 and *Rana saparoua* Duméril & Bibron 1841 should be placed in the synonymy of *L. grunniens* (rather than *E. hexadactyla*) and be considered nomina nuda.

The Sri Lankan population was described as *Rana robusta* Blyth, 1855, based on two male syntypes currently reported as ZSI 9123 and ZSI 9124 (Chanda et al., 2001; Sclater, 1892). *Rana robusta* was then synonymized shortly after by its descriptor in a brief footnote (Blyth, 1856).

"*R. robusta*, nobis, J. A.S. XXIII, 298, is (we are now satisfied) a phase of *R. CUTIPORA*, D. and B."

The original description of the two specimens generally conforms with *Euphlyctis* morphology, and the size provided for the species (SVL 3 inches = 76.2 mm) places it within the adult size range of *E. hexadactyla*. Theobald (1868), however, referred to two specimens of *Rana cutipora* collected by Kelaart from Ceylon in MASB (where the types were originally deposited), as an "adult and young." Because these two specimens were the only ones presented by Kelaart in the collection (Sclater, 1892), they are likely to be the *R. robusta* types, in which case Blyth (1855) may have reported only the size for the larger

male (the one identified as an adult by Theobald, 1868). The young specimen mentioned by Theobald (1868) particularly requires re-examination because MASB scientists (e.g. Blyth, Theobald, Anderson) usually determined the sex and maturity of frogs by the observation of obvious sexually dimorphic characters (e.g. external vocal sacs) and relative size differences between individuals thought to represent the same species, rather than dissection and gonad identification (SM pers obs.). This practice led to considerable taxonomic confusions when those authors erroneously synonymized superficially similar species despite size differences (Mahony et al., 2018). Hence, the syntype of *R. robusta*, alternatively mentioned as a male (Blyth, 1855) or a young (Theobald, 1868) could as likely be an adult male *E. cyanophlyctis* instead of a young *R. robusta*.

Given these ambiguities, the two syntypes of *R. robusta* must be re-examined to verify that they do not represent different species. Without further information, here we provisionally maintain *R. robusta* as a synonym of *E. hexadactyla*, also because our barcoding data confirmed that Sri Lankan and mainland populations lack notable divergence (Fig. 1, Supplemental material - File S3).

In summary, the oldest valid nomen available for the blue-coded lineage is *Euphlyctis hexadactyla* (Lesson, 1834). Junior synonyms include the objective synonyms *Dactylethra bengalensis* Lesson, 1835, *Rana cutipora* Duméril & Bibron, 1841, and *Rana robusta* Blyth, 1855 as a subjective synonym.

3–6. Four closely related lineages found in southwestern India, allied to *E. aloysii* (Fig. 1).

One unambiguously represents *Euphlyctis aloysii* Joshy, Alam, Kurabayashi, Sumida, and Kuramoto, 2009 sensu stricto (s. s.), given barcoding of the holotype (dark green-coded in Fig. 1).

The second one putatively corresponds to *Euphlyctis kerala* Dinesh, Channakeshavamurthy, Deepak, Ghosh, and Deuti, 2021 (pale green-coded in Fig. 1). This association is not straightforward, however, because Dinesh et al. (2021, 2022) remained vague about the origin of the three sequences attributed to *E. kerala*: GU136102 ("India," but labelled as being part of a Western Ghats unpublished barcoding project), MH423736 and MH423738 (both "NA") (Supplemental material - File S1a). According to the metadata submitted to GenBank, the latter two were obtained from vouchers (labeled "EU1S" and "EU2S"), but which cannot be related to the type series of the species (holotype ZSI/WGRC/A/973, paratypes ZSI/WGRC/A/974 and ZSI/WGRC/A/975). It thus

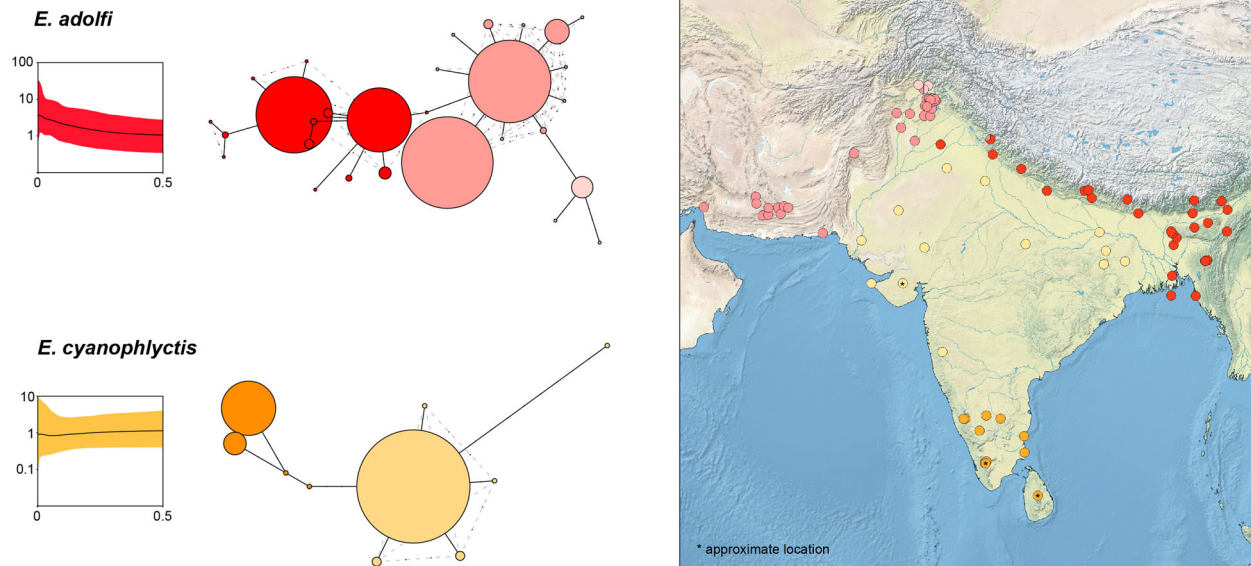


Fig. 3. Mitochondrial phylogeography of the two most widespread *Euphlyctis* lineages, *E. adolfi* and *E. cyanophlyctis*, illustrated by 16S haplotype networks, Bayesian skyline plots and sampling localities. Both lineages are genetically structured and show signals of population expansion for *E. adolfi*, but not for *E. cyanophlyctis*. See [Supplemental material - File S3](#) for a phylogenetic representation.

remains to be clarified whether the type series was actually barcoded.

The two remaining lineages do not seem to correspond to any known taxon, and we refer to them as *Euphlyctis* cf. *aloyisii*. One was detected only in Mudigere, Karnataka (in syntopy with *E. cyanophlyctis*) and appears to be splitting from the most basal node of the complex (dashed dark green-coded in Fig. 1). The last one was discovered with our sample from Baga in Goa State (dashed pale green-coded in Fig. 1), which groups with *E. aloyisii* s. s. in our 16S tree.

The *E. aloyisii* complex is the only *Euphlyctis* phylogroup where some currently recognized species are not delimited as such in our 16S-based ASAP analysis (Fig. 1). The best partition only distinguished two species for the complex: *E. cf. aloyisii* from Karnataka and a set of lineages including *E. aloyisii*, *E. kerala* and *E. cf. aloyisii* from Goa (Fig. 1).

Additional taxa. From our barcoding data, we could not definitely settle on which lineage the subspecies *Euphlyctis cyanophlyctis microspinulata* Khan, 1997 is affiliated with. This taxon was described from Balochistan in western Pakistan (type locality in southwest of Khuzdar, southeast Kalat Division; Khan, 1997), where *E. adolfi* (present in northern and southern Balochistan) or *E. cyanophlyctis* (present in eastern Pakistan) could potentially occur (Fig. 1). The type locality is located above 1,200 m a.s.l., and because only

E. adolfi has been recorded at such elevations (*E. cyanophlyctis* is always found below 1,100 m, SNL pers. obs.), it would a priori make a more plausible candidate. In addition, it remains to be confirmed that Balochistan skipper frogs actually represent a distinct taxon: no additional lineage was discovered among our samples from southern and northern parts of this mountain range (Figs. 1, 3). Without more decisive information (barcoding of the type locality), we must maintain this taxon where it was originally described, as the subspecies *Euphlyctis cyanophlyctis microspinulata* Khan, 1997.

Regardless of its status and affiliation, the type series of *E. c. microspinulata* is a matter of issue. It putatively comprised the holotype, “BMNH 1990.8,” and multiple paratypes: CAS 170531, four specimens grouped under “BMNH 1990.9,” and 16 or 17 specimens with only MSK numbers (the author’s private collection; Khan, 1997). Wagner et al. (2016) confirmed that the holotype was never inventoried in the BMNH, being “probably still in a private collection or lost,” and although the BMNH specimen accessions register does include the lot 1990.1–1990.17 (presented by M.S. Khan), the numbers 1990.8, 1990.9 and 1990.10 are indeed marked as “vacant” (= no specimen). In addition, one of us (SM) was unable to find *Euphlyctis* specimens in the BMNH with collection data that would match the missing types. Hence, the holotype and presumed BMNH paratypes must be considered lost, and the only extant type specimen known to be accessioned into a museum collection is the paratype CAS 170531, which would be a potential candidate for neotypification.

Table 2. Net pairwise distances (% of sequence divergence) between the 16S lineages of *Euphlyctis* species.

	1	2	3	4	5	6	7	8	9	10
1. <i>E. adolfi</i>	<i>1.2</i>									
2. <i>E. jaladhara</i>	4.0	<i>0.4</i>								
3. <i>E. cyanophlyctis</i>	3.3	3.0	<i>1.0</i>							
4. <i>E. ehrenbergii</i>	6.7	6.1	4.8	2.3						
5. <i>E. karaavali</i>	11.7	12.0	12.0	13.0	<i>1.1</i>					
6. <i>E. hexadactyla</i>	12.2	13.3	12.3	12.7	9.3	<i>0.3</i>				
7. <i>E. aloysii</i> s. s.	12.3	13.6	11.9	13.2	9.0	5.5	<i>0.7</i>			
8. <i>E. kerala</i>	13.1	15.9	14.1	15.6	11.4	6.7	4.6	–		
9. <i>E. cf. aloysii</i> (Karnataka)	11.9	13.3	11.5	12.4	9.6	4.5	2.7	4.0	–	
10. <i>E. cf. aloysii</i> (Goa)	11.0	12.3	11.0	12.7	9.8	5.9	2.3	3.5	3.1	–

Diagonal values (italics) show mean 16S haplotype distance within each species (not computed for the last three, for which only a single haplotype was identified).

Rana cyanophlyctis var. *seistanica* Nikolskii, 1899 was described from eastern Iran (type locality: “Neizar in Seistano, Iran”), from where we also lack barcoding data (Fig. 1). This taxon was recently thought to have been a synonym of *E. cyanophlyctis* by Dinesh et al. (2021), who hypothesized that it required “resurrection.” With no taxonomic justification, the authors consequently published the new combination *Euphlyctis seistanica*. We are unaware of a reference that formally synonymized *E. c. seistanica*, so the name would already have been technically valid as a variety or subspecies. Only the red-coded lineage was confirmed in Iran (Fig. 1), so it is likely that *seistanica* corresponds to a junior synonym or a subspecies of *E. adolfi*. Before this is formally demonstrated however, the present status of this taxon remains *Euphlyctis cyanophlyctis seistanica* (Nikolskii, 1899).

Finally, *Rana ghoshi* Chanda, 1991 was transferred to *Euphlyctis* by Dutta (1997), referring to a comment in the original description that the species “appears to be closely allied to *Rana cyanophlyctis*.” This taxon was described based on a single specimen collected from “Khugairk Reserve Forest, Manipur, India (alt. ca 925 m)” (Chanda, 1991) and was never included in a phylogenetic study. *Euphlyctis adolfi* is the only *Euphlyctis* lineage that we detected in northeast India (Fig. 1), yet several authors ruled out the possibility that *R. ghoshi* and *E. adolfi* were conspecific due to unusual characters such as a rounded snout and the presence of rounded finger-tips (e.g. Howlader et al., 2015; Joshy et al., 2009). One of us (SM) has examined the holotype of *R. ghoshi*, and based on morphology (including the aforementioned characteristics), concluded that it should be assigned to the genus *Limnnectes*, namely a member of the *Limnnectes kuhlii* (Tschudi, 1838) species group. Prior to additional work to refine its exact taxonomic nature, *R. ghoshi* should thus be referred to as *Limnnectes ghoshi* (Chanda, 1991) comb. nov., and removed from *Euphlyctis* species lists.

Phylogeographic analyses

Our time-calibrated 16S phylogeny suggested that most extant taxa appeared during the Late Miocene and the Pliocene. 16S sequence divergences were higher between taxa (2.3–11.4% in each subgenus) than within taxa ($\leq 2.3\%$) (Table 2). In the subgenus *Euphlyctis*, Middle Eastern (*E. ehrenbergii*) and Indian populations diverged first (~ 9 million years ago, Mya), and the later diversified around the Mio-Pliocene transition into *E. cyanophlyctis*, *E. jaladhara* and *E. adolfi*.

Intraspecific analyses of the widespread taxa *E. adolfi* and *E. cyanophlyctis* revealed some genetic structure (Fig. 3, Supplemental material - File S3). In *E. adolfi*, a western haplogroup is restricted to Iran, Pakistan, and northwestern India; a northern haplogroup was identified in the three northernmost localities; an eastern haplogroup extends along the Shiwalik Hills from Bangladesh to northeastern Pakistan. Overall, these haplogroups show $\leq 1.2\%$ of 16S divergence. Bayesian Skyline Plots (BSPs) suggested that populations of *E. adolfi* experienced a demographic expansion within the last tens of thousands of years, which is consistent with the star-like haplotype network (Fig. 3). In *E. cyanophlyctis*, two distinct haplogroups were sampled between the northern and southern ranges (1.0% of 16S divergence), but we did not retrieve a signal of population increase with the BSP (Fig. 3). The Sri Lankan samples of *E. cyanophlyctis* bear private haplotypes from the southern haplogroup (Supplemental material - Files S1a, S3).

In the subgenus *Phrynoderma*, the Western Ghats *E. karaavali* lineage first appeared in the Mid-Miocene, *E. hexadactyla* diverged from the *E. aloysii* complex in the Late-Miocene, and the latter then diversified during the Pliocene (Fig. 1). In Sri Lanka, the available sample of *E. hexadactyla* bears a private haplotype (Supplemental material - Files S1a, S3).

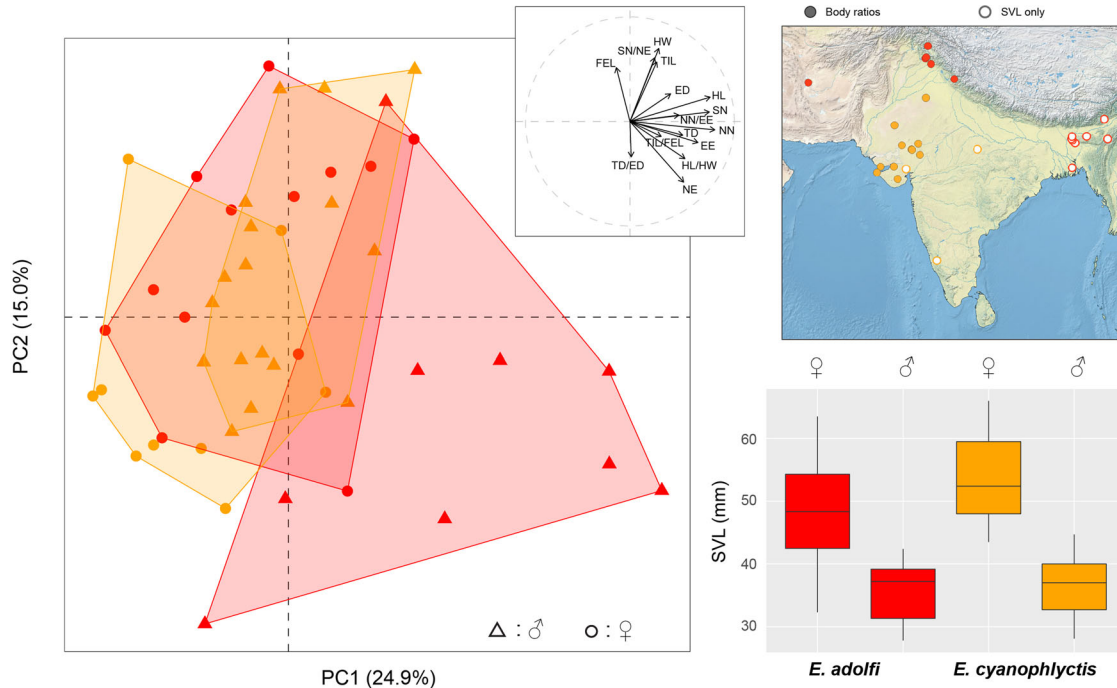


Fig. 4. Morphometric comparison of *Euphlyctis adolfi* (red) and *E. cyanophlyctis* (orange). The left panel shows the first axes of a PCA based on 15 body ratios, distinguishing males (triangles) from females (circles); variable contributions are highlighted by arrows in the adjacent circular diagram. The bottom right panel compares body sizes (SVL). The top right panel shows sampling locations of specimens, distinguishing those measured for SVL and body ratios (filled circles), from those measured only for SVL (empty circles).

Morphometric comparison of *E. adolfi* and *E. cyanophlyctis*

Comparison of body sizes (SVL) between *E. adolfi* ($n=16$ males and 22 females) and *E. cyanophlyctis* ($n=29$ males and 13 females) found that females were larger than males ($F=87.9$, $P=2.6 \times 10^{-14}$), yet suggested no differences between species ($F=0.28$, $P=0.60$) nor a significant interaction between sex and species ($F=3.2$, $P=0.08$).

Body proportions in *E. adolfi* ($n=9$ males and 13 females) and *E. cyanophlyctis* ($n=17$ males and 9 females), computed from 15 standard morphometric ratios (e.g. Joshy *et al.*, 2009) further indicated some fine differences between species and sexes (Fig. 4). On the PCA, the respective males and females of each species differ only slightly, but the overlap was less pronounced for males, which featured distinctive scores on the first axis (Fig. 4). Accordingly, the MANOVA retrieved a significant effect of species ($F=2.93$, $P=0.006$), sex ($F=2.54$, $P=0.01$), and their interaction ($F=3.40$, $P=0.002$). Six body ratio variables significantly contributed to the species effect, seven to the sex effect, and six to their interaction (Supplemental material - File S4).

Among the most significant variables, a few featured almost non-overlapping distributions and can be used to

obtain diagnostic thresholds (Supplemental material - Files S5–S6). Specifically, the relative inter-nostril distance (NN/SVL) was shorter in both sexes for *E. cyanophlyctis* than for *E. adolfi*. The relative snout-nostril distance (SN/SVL) also differed between males of the two species. Moreover, the statistical differences highlighted for the femur length (FEL) and the inter-nostril distance (NN) (Supplemental material - File S4) can be combined into an additional ratio (FEL/NN) that shows almost no overlap between the males of each species (Supplemental material - Files S5–S6).

Discussion

Taxonomic insights

Despite long-lasting disputes on species and taxonomic concepts, many herpetologists agree that genetic divergence is an important currency of evolutionary independence and by extension, the validity of taxa featured in biodiversity lists (Borkin *et al.*, 2004; de Queiroz, 1999, 2007; Dubois *et al.*, 2021; Frost, 2022; Hillis, 2019; Padial *et al.*, 2010; Speybroeck *et al.*, 2020), except for instances of reticulate speciation (Borkin & Darevsky, 1980; Litvinchuk *et al.*, 2016). In contrast,

external differentiation sometimes represents ephemeral processes such as phenotypic plasticity, drift, or local adaptation, rather than evolutionary independence, reproductive isolation, and speciation. Hence, deeply diverged lineages are best considered as single valid taxa albeit with potentially substantial phenotypic variation, and the younger names proposed to describe this variation thus become junior synonyms, or may eventually be kept in subspecies arrangements. These practices are important to acknowledge for Asian herpetology, where a non-negligible proportion of new species have been essentially described on the basis of external criteria (often limited to single populations, thus not accounting for the entire polymorphism and potential phenotypic overlap of species), and without conclusive evidence for evolutionary independence (Dufresnes & Litvinchuk, 2022).

With these general principles in mind, we attempted to improve the chaotic taxonomy of skipper frogs by clarifying lineage diversity and distribution in respect to the identity of described taxa (summarized in Table 2). In total, ten mitochondrial lineages could be identified, and in the wait of more powerful phylogenetic datasets (e.g. multilocus nuclear-encoded markers), we consider all of them as candidate species given the substantial 16S divergences (Table 1, see also Dinesh et al., 2021), which may be sufficient to warrant reproductive isolation and thus biological speciation (Dufresnes & Litvinchuk, 2022). This arrangement potentially overestimates species richness in the *E. aloysii* complex, where all four species are closely related and only two were distinguished by the best ASAP partition (Fig. 1). Yet, we choose to provisionally maintain these species, given that the 16S divergence is accompanied by morphological differences at least between *E. aloysii* and *E. kerala* (Dinesh et al., 2021). In contrast, the shallow mitochondrial divergences found in the two most widespread species (*E. cyanophlyctis* and *E. adolfi*) rather seem to correspond to intraspecific phylogeographic structure. It is worth emphasizing that mtDNA-based phylogenetic species delimitation approach (automated or not) should obviously be taken with extreme caution, especially for short 16S fragments (Chan et al., 2022). Monophyletic mitochondrial phylogroups cannot be considered as direct evidence for speciation without additional lines of support from nuclear-encoded sequences and/or phenotypic data (Padiál et al., 2010).

Our 16S tree is largely congruent with previous work (Dinesh et al., 2021; Howlader et al., 2015; Khajeh et al., 2014), and our denser, range-wide sampling yielded new insights into species distributions. Combined with a careful examination of the historical literature, we provide what appears to be the most

educated account of the names that should apply to each species and identified several issues that have been overlooked in the literature.

First, we agree with Dinesh et al. (2021) that the frogs called *E. mudigere* in the past decade (Joshy et al., 2009) most likely belong to the lineage attributed to *E. cyanophlyctis*. This taxon remains frequently misidentified. For instance, the recently described *E. jaladhara* is assumed to extend as far north as Gujarat State (Dinesh et al., 2022), although these populations were here barcoded as *E. cyanophlyctis* (Fig. 1).

Second, the most striking ambiguity pertains to the lineage recently called “*E. kalasgramensis*.” Initially described as a Bangladesh endemic by Howlader et al. (2015), this taxon was later reported by genetic barcoding in nearby India (Lalronunga et al., 2020), Nepal (Khatiwada et al., 2021) and Pakistan (Akram et al., 2021; Ali et al., 2020), where *E. cyanophlyctis* was historically thought to occur. By mapping the extensive distribution of this lineage from northeast India to Iran, we have provided decisive evidence (topotype barcoding) that *E. adolfi* is an older synonym and thus has priority (see also Borkin et al., 2020; Dufresnes & Litvinchuk, 2022; Litvinchuk et al., 2017). This case illustrates how external (especially color) polymorphism can be misleading in *Euphlyctis* taxonomy: Howlader et al. (2015) discarded *E. adolfi* as a possible name for their new species based on putative differences in ventral pigmentation, dorsal color patterns and body size, assessed from limited and unrepresentative sets of specimens. Our comparisons of specimens from multiple origins have instead showed that *E. cyanophlyctis* and *E. adolfi* share highly variable morphology and largely overlapping body sizes. Moreover, strong color polymorphism was noted among the individuals studied here, with a spectrum of blotched and plain morphs in both species (illustrated in Fig. 5). Hence, a few indirect measurements aside (Supplemental material - Files S5–S6), external criteria cannot be trusted as diagnostic among these closely related taxa.

Third, the *E. aloysii* complex exemplifies the need for herpetological surveys in southwestern India. Based only on a handful of samples, we retrieved no less than four allopatric lineages, two presently undescribed (identified as *A. cf. aloysii*). Of particular interest is our discovery of the lineage from Goa, which was initially suspected based on the unusually uniform bright green dorsum coloration of the female sample analyzed (SNL pers. obs.). Its description as a new taxon is pending further investigations.

In this respect, our study calls for a thorough review of historical type specimens prior to any new description. The haste of describing recently discovered lineages in the last decade, without first attempting to resolve the taxonomic status of long-available names,

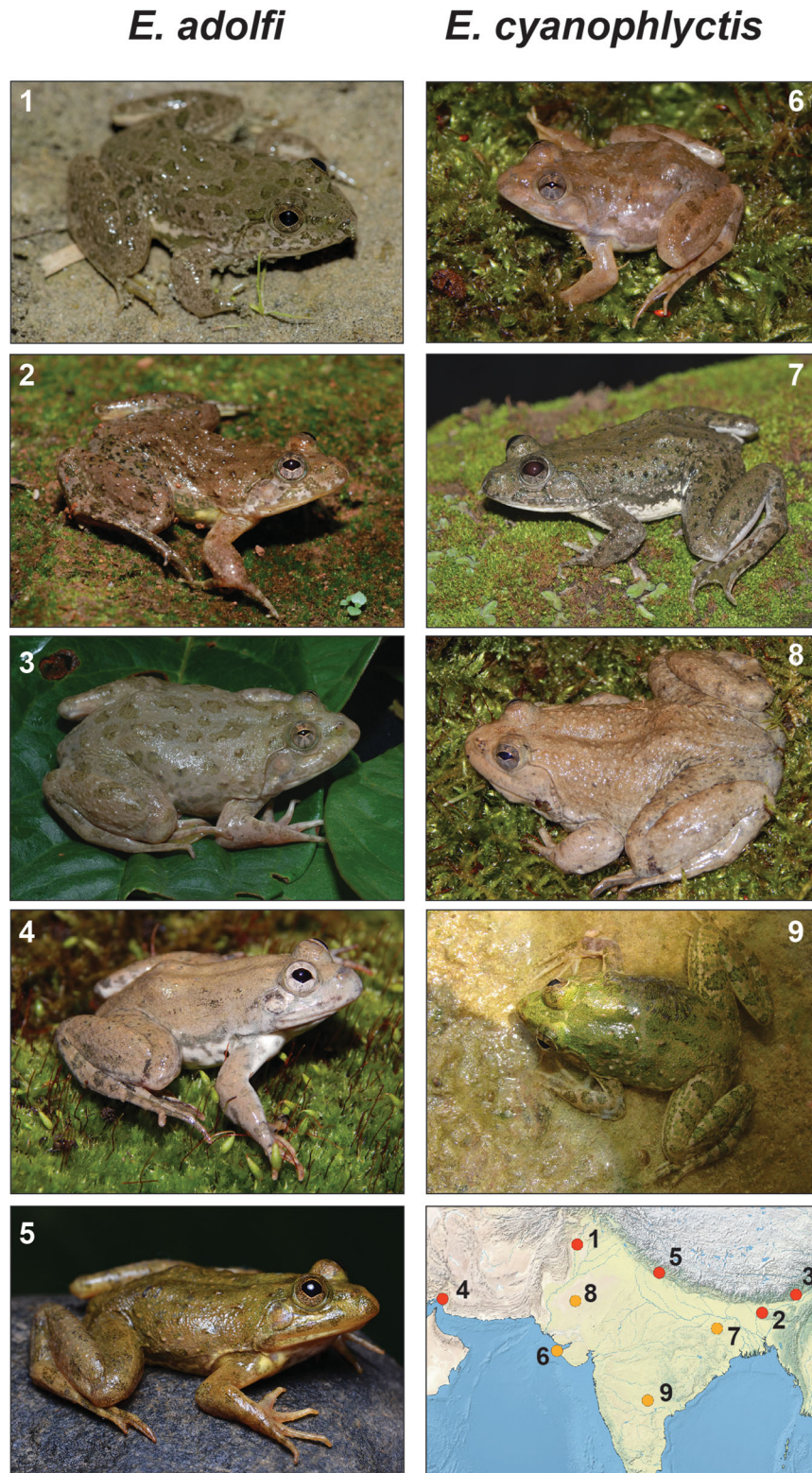


Fig. 5. Example of color variation in *Euphlyctis adolfi* and *E. cyanophlyctis* and geographic origin of photographed specimens (bottom-right map), most being included in our analyses ([Supplemental material - File S1](#)). Blotched and plain specimens frequently occur in both species, so these color patterns are not reliable for species recognition. 1: DJ.9502 (DJ); 2: BNHS 6150 (RGK 0229) (RGK); 3: BNHS 6140 (RGK 0839) (RGK); 4: INCRAS.2016-76 (SNL); 5: E.KOSI (VKP); 6: INCRAS.2014-115 (SNL); 7: ECJ2 (VKP); 8: INCRAS.2014-307 (SNL); 9: unsampled specimens from Golconda, Telangana, India (SM).

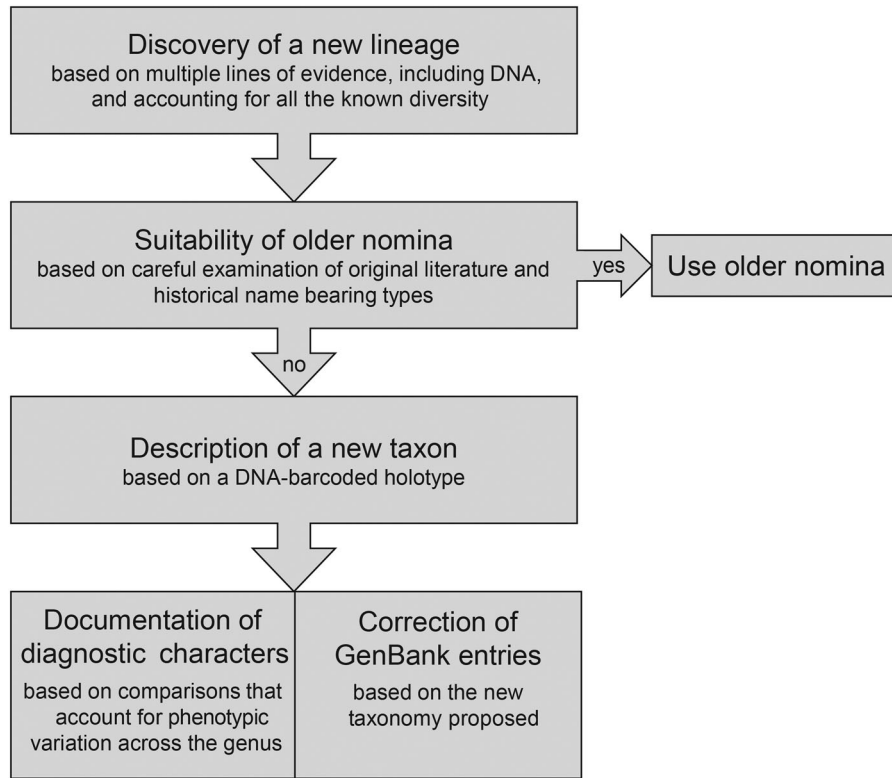


Fig. 6. General guidelines for future taxonomic revisions in *Euphlyctis*, which also apply in any problematic organismal groups. Our [Supplemental material - Files S1–2](#) can be used as a starting point to account for all known genetic diversity.

has proven problematic. As a consequence, the nomenclature of several recognized species might be threatened in the near future, especially if older nomina (*leschenaultii* and *bengalensis*) were to be attributed to lineages other than *E. cyanophlyctis*, or if *E. cyanophlyctis* types do not represent the (orange-coded) species it has long been assigned to. This Damocles sword potentially hangs above all the Western Ghats endemic taxa described so far (*E. kerala*, *E. jaladhara*, *E. aloysii*, *E. karaavali*), but also on the resurrected *E. adolfi*. Moreover, overreliance on Frost's (2022) accounts without independent examinations of the original literature has resulted in numerous misconceptions. Frost's online database is an invaluable resource for amphibian taxonomists, which documents most important taxonomic and nomenclatural changes reported in the literature. However, its purpose is not to assess the reliability of those changes, so all citations are provided with full references to allow and encourage taxonomists to read the literature themselves, a concept once considered standard practice when working on the taxonomy of any group. In addition, given the sympatric occurrence of several lineages (*E. aloysii* complex, *E. karaavali*, *E. cyanophlyctis/jaladhara* in the Western Ghats; *E. hexadactyla*, *E. cyanophlyctis* in eastern India and Sri Lanka), we stress the need for comprehensive barcoding

information of type specimens, and thus avoid ambiguities when attributing names to lineages (as e.g. for *E. kerala* and *E. jaladhara*). On the podium of the “taxonomic haste,” we retrieved a 16S sequence labeled “*Euphlyctis jalpari*” in GenBank, a name that does not (yet) exist in the literature. While this taxon obviously remains a *nomen nudum* for the time being, we advise its authors to reconsider its upcoming description, the “*jalpari*” sequence does not noticeably differ from other *E. cyanophlyctis* haplotypes ([Supplemental material - Files S1, S3](#)), and would thus become a junior synonym. Due to misidentifications, taxonomic changes and nomenclatural confusions, already 80% of the 16S *Euphlyctis* sequences available on GenBank are mislabeled ([Supplemental material - File S1a](#)). It is the authors' responsibility to update the information associated with previously published sequences (Mahony & Kamei, 2022). Hence, we strongly advocate that GenBank metadata are revised in the light of our results ([Supplemental material - File S1a](#)), in order to reduce the propagation of errors and confusion in the literature. In the meanwhile, we recommend using our 16S alignment (which combines all haplotypes reported as of March 2022) rather than randomly chosen subsets of GenBank sequences when designing barcoding or taxonomic studies on *Euphlyctis*. To assist future revisions,

the good practices preconized here are summarized by bullet-points in Fig. 6.

Phylogeographic insights

Previous molecular work on *Euphlyctis* primarily focused on new species descriptions (e.g. Dinesh *et al.*, 2021; Howlader *et al.*, 2015), yet the diversity and distributions of these frogs are also worth discussing in the light of phylogeographic patterns inferred in other South Asian herpetofauna. Seven out of the ten *Euphlyctis* species are found in southern India (including six endemics), especially in the Western Ghats, one of Earth's hottest biodiversity hotspots (Daniels, 2001; Myers *et al.*, 2000). This region is famously known as a haven of unique amphibians (Biju & Bossuyt, 2003; Bossuyt *et al.*, 2004; Das *et al.*, 2006). Nearly 90% of species are endemic (Aravind & Gururaja, 2011), which has been explained by the prolonged isolation of the mountain range since the Jurassic (Dutta *et al.*, 2004) and the ensuing history of stable humid tropical to subtropical climates that have remained favorable for ectotherms (Bossuyt *et al.*, 2004). From our time-calibrated phylogeny, South Indian *Euphlyctis* ancestors accordingly diversified in situ within the last 15 million years, following Earth's climate cooling and aridification (Burke *et al.*, 2018), which probably isolated the Western Ghats from neighboring regions (Deccan Plateau, Eastern Ghats), and caused range fragmentation within the Ghats. Similar processes are invoked to explain the deep divergences recovered in other South Indian amphibians, leading to the high rate of genus and even family endemism (e.g. Biju *et al.*, 2009, 2011; Biju & Bossuyt, 2003; Roelants *et al.*, 2004). Interestingly in *Euphlyctis*, micro-endemic lineages are only found in the *E. aloysii* complex, while other Western Ghats taxa are genetically homogenous across wider ranges (*E. karavaali*, *E. jaladhara*). These contrasting patterns might reflect distinct life histories and ecologies, hence different responses to the environmental changes that potentially disconnected many parts of the Western Ghats escarpment within the last millions of years (e.g. Gower *et al.*, 2011; Vijayakumar *et al.*, 2016).

In northern parts of the distribution, vast areas were colonized by single species of the *E. cyanophlyctis* group (*E. adolfi*, *E. cyanophlyctis*), suggesting high historical connectivity in the last thousands of years. In particular, the Indus and Gangetic plains feature massive wetlands in Pakistan, northern India and Bangladesh, which probably favored the spread of other amphibian lineages, such as *Duttaphrynus* Frost *et al.*, 2006, *Microhyla* Tschudi, 1838 and *Sphaerotheca* Günther, 1859 (Bisht *et al.*, 2021; Jablonski *et al.*, 2020, 2021).

In contrast, reptile species show much deeper phylogenetic differentiation between the northwestern, central and northeastern parts of the Indian subcontinent (e.g. Agarwal *et al.*, 2021, 2022; Gowande *et al.*, 2021).

Nevertheless, we did discover some mtDNA structure within these two widespread *Euphlyctis* species, which could be signatures of multiple independent refugia during the Quaternary climatic oscillations. These dynamics may have had lesser effects on the demography of *E. cyanophlyctis* compared to *E. adolfi*, which inhabits more northern ranges. In *E. cyanophlyctis* (and *E. hexadactyla*), glacial ranges probably extended across the Palk Strait, as India and Sri Lanka were connected by a land bridge (the "First Planated Surface") due to sea level drops down to ~150 m below current levels (Katupotha, 2016), hence little genetic divergence between the island and mainland populations. In *E. adolfi*, haplotype distributions suggest a bidirectional east-west recolonization route along the Shivalik Hills range (Himalayan foothills), perhaps following the Ganges River as a southern boundary. Screening for hotspots of genetic diversity, together with projections of past distributions, could shed light on the location of its refugia. Nowadays, the Himalayan foothills and the Indus drainage system seem to mediate range limits: *E. cyanophlyctis* is restricted east of the Indus valley, while the upper Indus River network segregates the three *E. adolfi* haplogroups (Fig. 3). Accordingly, most Oriental anurans do not penetrate far west of the Indus, and Central Asia is mostly inhabited by Palearctic species (Dufresnes & Litvinchuk, 2022).

The deep divergence and geographic remoteness of the Arabian *E. ehrenbergii* is also noteworthy. With vast deserts that isolate the few coastal areas and inland oases offering suitable environmental conditions, the Arabian Peninsula hosts only a handful of amphibian species. In turn, many of them are endemic, with origins as diverse as the Palearctic, like *Hyla felixarabica*, Gvoždík, Kotlík & Moravec, 2010 (Gvoždík *et al.*, 2010), the African, like *Sclerophrys tihamicus* (Balletto & Cherchi, 1973) and *S. arabicus* (Heyden, 1827) (Portik & Papenfuss, 2015), or the Oriental realms, like *Duttaphrynus dhufarensis* (Parker, 1931) (Portik & Papenfuss, 2015) and *E. ehrenbergii* (this study). These taxa are thus witnesses of faunal exchanges with the three continents at various points in time: ~17–20 Mya for *Sclerophrys* Tschudi, 1838 (Portik & Papenfuss, 2015), ~8–9 Mya for *Euphlyctis* (this study) and *Hyla* Laurenti, 1768 (Gvoždík *et al.*, 2010), a few hundred thousand years during the last glacial stages for the arid-tolerant *Bufo* *sitibundus* (Pallas, 1771) (Dufresnes *et al.*, 2019). The variety of connections is

also well illustrated by diversification patterns in reptiles, with a Miocene origin in several Arabian lineages (e.g. Šmíd et al., 2013; Tamar et al., 2016) but a Pleistocene origin in others (e.g. Barlow et al., 2013; Tamar et al., 2021). Some of the oldest taxa further diversified in situ, e.g. between the Red Sea and Persian coasts in *S. arabicus* (Portik & Papenfuss, 2015). Here, *E. ehrenbergii* showed the highest intraspecific 16S divergences among *Euphlyctis* (Table 2, see also Al-Qahtani & Amer, 2019), so a more extensive phylogeographic analysis would clarify the diversity and structure of this species across its barren range.

Finally, our overview of distributions and divergences in *Euphlyctis* taxa is consistent with the growing understanding that in amphibians, sympatry generally evolves after species have first differentiated in allopatry (Dufresnes et al., 2021; Dufresnes & Crochet, 2022; Rasolonjatovo et al., 2020; Wollenberg Valero et al., 2019). In theory, related species can coexist only if reproductive and ecological isolation efficiently prevent introgressive hybridization (that leads to lineage fusion and despeciation) and niche overlap (that leads to competitive exclusion), respectively. Here, phylogeographic patterns suggest that evolving such isolation requires long-term evolutionary independence: all the taxa living in sympatry have diverged at least 13 Mya (the split between *E. karaavali* and the *E. aloysii* complex), while younger lineages exclusively feature allopatric or parapatric distributions (e.g. all members of subgenus *Euphlyctis*, and of the *E. aloysii* complex). Accordingly, the discordances between 16S and genome size barcoding of samples from near the distributional transition of *E. adolfi* and *E. cyanophlyctis* could indicate genetic introgression: these relatively young species (~5–6 My) may still potentially hybridize along their secondary contact, and hence cannot overlap their ranges further. Our observations are thus largely congruent with numerous reports on anuran amphibians, where substantial divergence is the hallmark of sympatry (generally >10 My, but as low as 7 My in some speciose radiations, e.g. Mahony et al., 2017), and that conversely, evidence of sympatric speciation remains rare (Dufresnes & Crochet, 2022). This pattern is obviously not an absolute rule, as some exceptional phenomena are known to sometimes promote rapid speciation in sympatry (e.g. reinforcement, hybrid speciation). Because they feature a continuum of species spanning sympatric and allo-parapatric distributions across ecologically diverse regions, skipper frogs would constitute an interesting system to relate genetic and phenotypic divergence to the mode of speciation.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental material

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