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Bewildering biogeography: Waves of dispersal and diversification across southern Wallacea by bent-toed geckos (genus: *Cyrtodactylus*)

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ABSTRACT

Bent-toed Geckos, genus *Cyrtodactylus*, are one of the most diverse terrestrial vertebrate groups, and their range extends from South Asia into Australo-Papua and adjacent Pacific islands. Given the generally high faunal endemism on Wallacean islands, it is rather paradoxical that the diversity in these geckos appears to be so low (21 species in Wallacea, 15 in the Philippines) compared with continental shelf assemblages (>300 species on Sunda + Sahul Shelves + adjacent islands). To determine whether this shortfall was real or an artifact of historical undersampling, we analyzed mitochondrial DNA sequences of hundreds of southern Wallacean samples (Lesser Sundas + southern Maluku). After screening to guide sample selection for target capture data collection, we obtained a 1150-locus genomic dataset (1,476,505 bp) for 119 samples of southern Wallacean and closely related lineages. The results suggest that species diversity of *Cyrtodactylus* in southern Wallacea is vastly underestimated, with phylogenomic and clustering analyses suggesting as many as 25 candidate species, in contrast to the 8 currently described. Gene exchange between adjacent candidate species is absent or minimal across the archipelago with only one case of > 0.5 migrants per generation. Biogeographical analysis suggests that the hitherto unrecognized diversity is the result of at least three independent dispersals from Sulawesi or its offshore islands into southern Wallacea between 6 and 14 Ma, with one invasion producing small-bodied geckos and the other two or three producing larger-bodied geckos. The smaller-bodied *laevigatus* group appears to be able to coexist with members of either larger-bodied clade, but we have yet to find members of the two larger-bodied clades occurring in sympatry, suggesting that ecological partitioning or competitive exclusion may be shaping individual island assemblages.

1. Introduction

Bent-toed geckos of the genus *Cyrtodactylus* are the third-most species-diverse genus of terrestrial vertebrates, with 346 recognized species (Uetz et al., 2023). The genus has a massive range extending from South Asia and Southeast Asia across Wallacea into northern Australia, New Guinea, and many smaller islands of Melanesia. Amazingly, 76% of current species diversity (260/341 species) has been described in the

past 20 years (Grismer et al., 2021a). These new species have included many insular species from the Indo-Australian archipelago as well as narrow-range habitat specialists on the Sunda and Sahul Shelves (Brennan et al., 2017; Grismer et al., 2018; Davis et al., 2021; Grismer et al., 2021a). Ecological divergence and niche partitioning have allowed sympatry among *Cyrtodactylus* species on the continental shelves and mid-to-large size islands, demonstrating the remarkable potential for local adaptation and diversification within the genus

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(Grismer et al., 2021b).

Wallacea remains an understudied area for *Cyrtodactylus* diversity due to the sheer number of oceanic islands and the logistical challenge of conducting field surveys across the region. Most of the currently described Wallacean species of *Cyrtodactylus* are considered to represent one (the “Sunda” clade of Wood et al., 2012) or two (the “*darmandvillei*” and “*marmoratus*” clades of Grismer et al., 2021a) dispersal events into Wallacea, an assemblage that currently contains 21 described species (Grismer et al., 2022; Uetz et al. 2023). Previous studies have shown morphological clines in *Cyrtodactylus* clades as one moves from west to east through Wallacea (Oliver et al., 2014), with extensive radiations on the Sunda Shelf Islands (O’Connell et al., 2019; Davis et al., 2020, 2021, 2023), in the Philippines (Siler et al., 2010; Oaks et al., 2019), and on the Sahul Shelf and Melanesian Islands (Oliver et al., 2018; Tallowin et al., 2018).

The southern portion of Wallacea is a geologically and biologically distinct series of adjacent geomorphological features, including the eastern portion of the Sunda Arc islands, the continental plate fragment of Sumba Island, the southern portions of the parallel Inner and Outer Banda Arc islands, and a number of small islands between Sulawesi and the Sunda Arc (Fig. 1). These islands range in age from ~ 12 myr to <1 myr, with the Sunda Arc islands being the oldest (5–12 myr), the Inner Banda Arc Islands about 5 myr old, and Sumba and the Outer Banda Arc Islands the youngest (1–4 myr) (Fortuin et al., 1997; Hall, 2009, 2011; Roomawati and Harris, 2009; Harris, 2011; Nguyen et al., 2013; Tate et al., 2017).

Due to the clustered and nearly linear arrangement and biological

cohesiveness of the eastern Sunda Arc, Sumba, and western Banda Arcs, these islands are often collectively referred to as the Lesser Sunda Archipelago. Within the Lesser Sundas, *Cyrtodactylus* occupies nearly every island excluding very small islets, often with two or more species on the larger islands. One of the relatively large-bodied species in the region is *C. darmandvillei* (Weber, 1890), which occurs across the Sunda Arc (Sumbawa, Moyo, Sangeang, Komodo, Rinca, Flores, Adonara, and Lembata) and into the western portion of the Inner Banda Arc (Pantar, Alor, and Atauro), and which has long been thought to comprise a species complex based on both morphology (see Fig. 2A–D) and genetics (Wood et al., 2012; Grismer et al., 2021a). We call this group the *darmandvillei* complex. Spread across the Lesser Sundas and even present on a small island off northwestern Australia is a group of small-bodied *Cyrtodactylus* species that we call the *laevigatus* complex, which includes *C. laevigatus* Darevsky, 1964 from Komodo, Rinca, and Flores Islands (Auffenberg, 1980), *C. tambora* Riyanto et al., 2017 from Sumbawa Island, *C. celatus* Kathriner et al., 2014 from western Timor Island, and *C. kimberleyensis* Bauer & Doughty, 2012 from Montalivet Island, Australia, which lies to the south of Timor. A number of unassigned populations in this complex occur throughout the archipelago (Fig. 2E–F). Other relatively large-bodied species in the region include *C. gordongekko* (Das, 1994) from Lombok Island (Fig. 2G), *C. wetariensis* (Dunn, 1927) from Wetar Island (Fig. 2H), and the recently described *C. santana* Chan et al., 2023 from the eastern end of Timor Island. We here recognize the latter two species as composing the *wetariensis* complex. *Cyrtodactylus gordongekko* is part of the *sadleiri* complex, of which *C. sadleiri* Wells & Wellington, 1985 is the earliest described

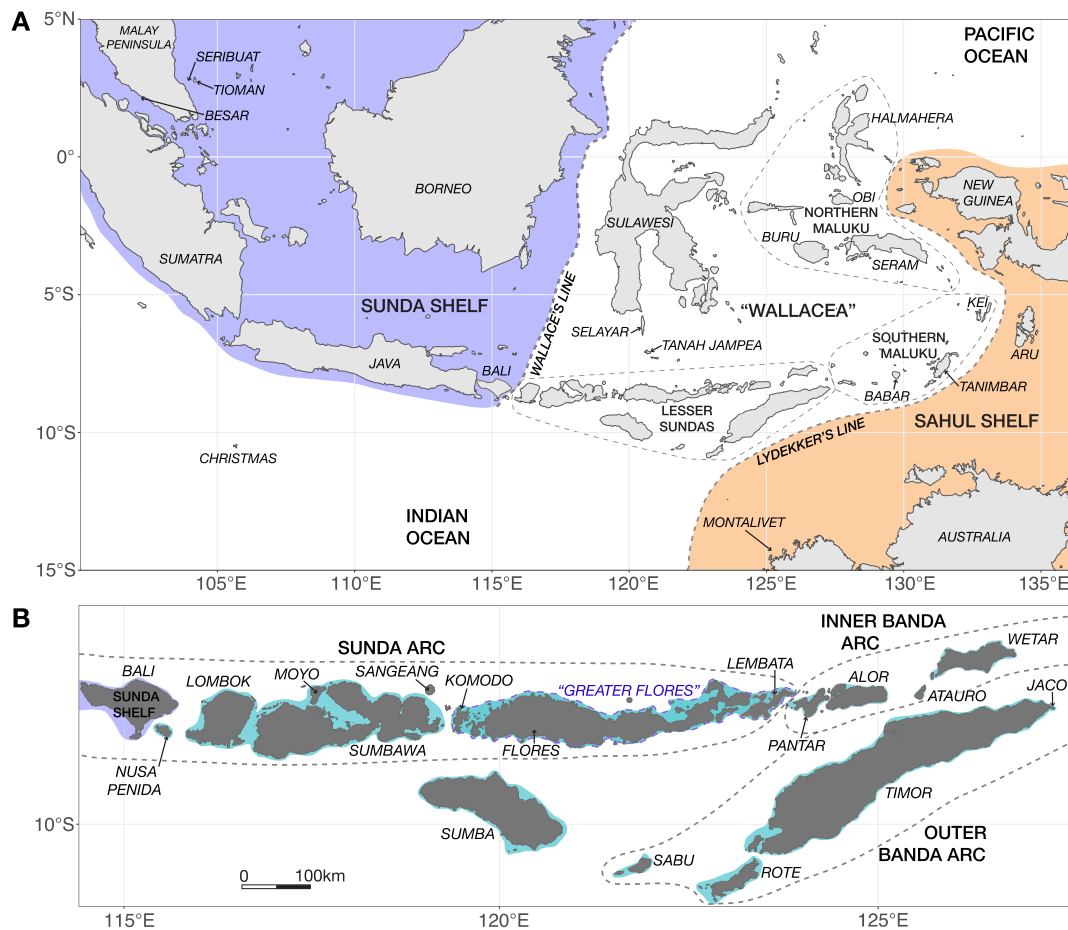


Fig. 1. A) Map of the transition zone between the Sunda and Sahul Shelves with relevant islands and boundaries identified. B) Map of Bali and the Lesser Sunda Archipelago with the 120 m ocean depth contours around each island shaded purple for the Sunda Shelf and light blue for the Lesser Sundas. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Fig. 2. A-D) Members of the *Cyrtodactylus darmandvillei* complex from A) Bajawa, West Flores (MVZ 293372), B) Larantuka, East Flores (MVZ 293374), C) West Sumbawa (MVZ 293453), and D) Alor (MVZ 293361). E-F) Members of the *C. laevigatus* complex from E) Wetar (MVZ 293500) and F) Sabu (MVZ 293508). G) *C. gordongekkoi* from Lombok (JAM 3176). H) *C. wetariensis* from Wetar (MVZ 293535). (Photos: A = SB Reilly; B–H = JA McGuire).

species. The *sadleiri* complex also includes the Sunda shelf species *C. petani* Riyanto et al., 2015, *C. jatnai* Amarasinghe et al., 2020, *C. seribuatensis* Youmans & Grismer, 2006, and *C. batucolus* Grismer et al., 2008.

The true ranges of these southern Wallacean *Cyrtodactylus* species or species complexes are poorly understood, though recent surveys have expanded our knowledge of their range limits (Western Australian Museum surveys 1987–1993; Kaiser et al., 2011, 2013; Sanchez et al., 2012; McKay and Lilley, 2012; O’Shea et al., 2015; Reilly et al., 2020). Additionally, while some species of lizards in southern Wallacea recently dispersed throughout the archipelago, such as *Gekko gecko* and *Eutropis multifasciata* (Reilly et al., 2019b), other lizard taxa have been shown to be nearly as old as the islands themselves and represent

undescribed species complexes (Blom et al., 2019; Reilly et al., 2022a, 2022c). Analyses based on mitochondrial genes and a small number of nuclear loci suggest that *Cyrtodactylus* represent one of the older groups to have inhabited southern Wallacea (Wood et al., 2012; Grismer et al. 2022).

In addition to the described species listed above, a number of *Cyrtodactylus* populations across southern Wallacea have not yet been described, assigned to a particular species, or remain of unknown relationship to described species (Grismer et al., 2021a). In this study, we include the most complete sampling of *Cyrtodactylus* from across the islands of southern Wallacea in a phylogenomic framework to infer the relationships among species and complexes, the species status of genetically distinct populations, and historical dispersal routes through

the archipelago. We utilize the general lineage concept of species (de Queiroz, 1998) such that we are attempting to identify independently evolving lineages on their own evolutionary trajectories. To assess lineage status, we employ a variety of methods including phylogenetic and population clustering analyses aimed at identifying putatively distinct genetic clusters, and then utilize migration analyses to assess lineage independence (using a threshold of ≤ 0.5 migrants per generation; Shaffer and Thompson, 2007; Burbrink and Ruane 2021; Reilly et al. 2022a; McGuire et al. 2023).

2. Materials and Methods

2.1. Sample collection and screening

Cyrtodactylus specimens were collected during several surveys throughout southern Wallacea. Liver tissues from sacrificed lizards were stored in 95% ethanol or RNALater, and/or flash-frozen in liquid nitrogen. Specimens and tissues have been deposited in the Museum of Vertebrate Zoology (MVZ) at the University of California at Berkeley, USA, the Museum Zoologicum Bogoriense (MZB) in Cibinong, Indonesia, the United States National Museum of Natural History (USNM), Smithsonian Institution, Washington, D.C. (Timor-Leste material), and the Museum of Comparative Zoology (MCZ-R) at Harvard University, Massachusetts, USA.

DNA was extracted following standard salt extraction methods or using DNeasy Blood and Tissue kits (Qiagen). PCR-amplification and sequencing of approximately 919 bp of the *ND2* gene and ~ 60 bp of tRNAs (Trp, Ala, partial Asx) were performed under standard conditions using primers *CyrtND2L* (5'- CCGCTGGGCTAGGATGTTTAG -3') and *CyrtND2R* (5'- AAGCRTCAAACGCATGAC -3') (this study). Forward and reverse sequence reads were combined in GENEIOUS PRIME v2019.2 (<https://www.geneious.com>) and aligned along with relevant sequences previously deposited in GenBank (Table S1) using MUSCLE (Edgar, 2004) with default settings. The final *ND2* sequence alignment (216 samples, 1231 bp) was imported into IQTREE v2.1.3 (Minh et al., 2020) where model selection of the alignment as a single partition (TN + F + R4 according to BIC scores) and Maximum Likelihood (ML) phylogeny estimation are coupled (Kalyaanamoorthy et al., 2017). Node support was assessed by 1000 ultrafast bootstrap replicates (UFBoot, Hoang et al., 2018) where UFBoot probabilities $\geq 95\%$ indicate strong clade support. Levels of sequence divergence between mitochondrial lineages were estimated using the web server DIVEIN (Deng et al. 2010) with the built-in program PhyML v3.0 (Guindon et al., 2010). Mean divergence levels were calculated based on pairwise distances under a GTR substitution model with 4 substitution rate categories where both the proportion of invariant sites and the gamma distribution parameters are estimated.

2.2. Target-Capture experiment

Genome-wide sequence data were collected following the transcriptome-based exon-capture protocol of Bi et al. (2012). Transcriptomes of four samples were sequenced, including representatives from the *Cyrtodactylus darmandvillei*, *C. laevigatus*, and *C. wetariensis* species complexes (Table S2). Orthologous exon loci target regions were derived from aligned contigs, and after a number of filtering steps 1200 loci (target size ~ 1.06 MB) from three of the transcripts were used to design 120-bp, 2.5X-tiled RNA library baits (57,552 unique probes) for an in-solution targeted capture experiment (Mybaits, Mycarray). Genomic library preparation included 31 samples from the *darmandvillei* complex, 25 from the *laevigatus* complex, 16 from the *wetariensis* complex, 28 from the *sadleiri* complex, and 19 outgroup samples or samples believed to be closely related to our focal clades (Table S1). Genomic library pools of 5–6 samples were hybridized to the probes for an incubation period of 24 h, and target sequences were isolated with magnetic streptavidin beads. The target sequences were then PCR-amplified,

pooled, and sequenced on an Illumina HiSeq 2500 with 100 bp paired-end reads. More detailed methods describing locus discovery, sequencing library preparation, the exon-capture experiment, and bioinformatics are in the Supplementary Methods.

2.3. Phylogenomics

Maximum Likelihood (ML) phylogenetic analysis of the concatenated target-capture alignment (1150 loci after filtering, totaling 1,476,505 bp) was performed in IQTREE v2.1.2 under the GTR + I + G mutation model (Minh et al., 2020). Node support was assessed by 5000 ultrafast bootstrap replicates and by 5000 bootstrap replicates for SH-like approximate likelihood ratio tests (SH-aLRT) where UFBoot probabilities $\geq 95\%$ and SH-aLRT values $\geq 80\%$ indicate strong clade support (Hoang et al., 2018). A summary coalescent species tree was estimated with ASTRAL3 (Zhang et al., 2018), for which the input consisted of ML gene trees estimated for each of the 1150 nuclear loci in IQTREE v2.1.3 with automatic model selection (Kalyaanamoorthy et al., 2017). Both ML and summary coalescent trees were rooted using *C. elok* Dring, 1979 based on other genus-wide multilocus phylogenetic studies (Wood et al., 2012; Grismer et al., 2021a).

A time-calibrated phylogeny was estimated using the Bayesian software BEAST v2.4.8 (Boukaert et al., 2014). Due to the large size of the dataset one sample per lineage was chosen by selecting the sample with the most complete data. Two independent runs were performed under the JC69 mutation model, a strict molecular clock with a clock rate of 0.001 (Brandley et al., 2011; Blom et al., 2016; Allio et al., 2017; Reilly et al., 2022a), a Yule birth death prior, and 50 million generations sampling every 5000 generations. A maximum clade credibility tree was produced following removal of burn-in samples (10–20%), with stationarity assessed via visual inspection of parameter trace plots and confirmation that effective sample sizes (ESS) for all parameters were > 200 . This time-calibrated tree was used in biogeographical analyses described below.

2.4. Population structure

Genetic structure was visualized within each focal group in four ways: 1) as an unrooted genetic network, 2) as a population structure bar graph, 3) as a principal component analysis (PCA) of genetic covariance, and 4) as scatterplots of genetic versus geographic distance. For both the mitochondrial *ND2* gene and the full genomic dataset, relationships among populations within a focal group were visualized as a genetic network using the NeighborNet algorithm in SPLITSTREE v4.6 (Bryant and Moulton, 2004; Hudson and Bryant, 2006).

Individual samples were assigned to genetic clusters with the Bayesian clustering software STRUCTURE v2.3.2 (Pritchard et al., 2000). Input files contained one informative single nucleotide polymorphism (SNP), which excluded singleton SNPs, per locus from within each species complex. SNPs were chosen by randomly selecting one bi-allelic nucleotide position that is sequenced for $> 90\%$ of all samples with $> 10X$ coverage for each sample. No prior location information was supplied, and analyses were run under the admixture model with correlated allele frequencies. Results from the phylogenetic trees and PCA analyses guided the maximum number of populations (K) tested within each focal group with $K = 1$ to $K = 10$ tested for the *darmandvillei*, *laevigatus*, and *sadleiri* groups and $K = 1$ to $K = 5$ tested for the *wetariensis* group. From $K = 1$ to $K = 5$ the program was run with 250,000 burn-in generations and 250,000 retained generations, and for $K = 6$ and higher the program was run with 350,000 burn-in generations and 350,000 retained generations. Ten replicates were run for each K setting with the optimal number of K determined by the delta-K method (Evanno et al., 2005) in STRUCTURE HARVESTER (Earl and vonHoldt, 2012). Population assignment bar graphs were estimated for each optimal K and the largest K that returned biogeographically meaningful clusters with a 1,000,000 generation burn-in and 1,000,000 retained generations.

Genetic covariance was visualized with PCA for each of four focal groups with the ADEGENET package (Jombart, 2008) in R (R Core Team, 2022). All informative SNPs from each locus, which exclude singleton SNPs, served as the input for each group with 48,248 SNPs from the *darmandvillei* complex, 17,426 SNPs from the *laevigatus* complex, and 38,498 SNPs from the *wetariensis* and *sadleiri* complexes. The top two components were then plotted against each other to highlight biogeographically cohesive groups of samples.

Mantel tests were performed on matrices of Euclidean geographic distances (in km) and Edwards' genetic distances to test for evidence of isolation-by-distance (IBD) using the mantel.randtest function in ADEGENET with statistical significance assessed with 100,000 permutations. Genetic and geographic distance were plotted against each other for each sample pair, and two-dimensional kernel density estimation was used to produce a colored cloud representing local point density with the R package MASS (Ripley and Venables, 2002). This method was undertaken for the four species complexes, with the distant Sunda Shelf samples from the *sadleiri* complex removed to visualize southern Wallacean patterns.

2.5. Biogeography

While we are missing some key samples, such as *Cyrtodactylus sadleiri*, *C. celatus*, *C. petani*, and *C. kimberleyensis*, and the phylogeny has a few poorly supported nodes, we nonetheless estimated ancestral ranges to identify possible historical dispersal pathways. Ancestral range reconstruction was performed on the time-calibrated BEAST phylogeny using the R program BIOGEOBEARS (Matzke, 2013, 2014). Because the program only handles a maximum of 12 defined regions, we were first tasked with defining the 12 most important regions given our sampling (focused on the Lesser Sundas), which was informed by the shared geological history and connectivity between islands. For example, oceanic islands that contain distinct lineages of *Cyrtodactylus* were considered to be separate regions, Pleistocene Aggregate Island Complexes (PAICs) that contain shared biogeographic patterns were considered to be single regions, and closely spaced oceanic islands with shared biogeographic patterns were considered to be single regions. Our definitions are as follows: 1) Sunda Shelf islands, 2) Sahul Shelf islands, 3) Sulawesi and its offshore islands including Selayar and Tanah Jampea, 4) northern Maluku islands, 5) southern Maluku islands, 6) Wetar Island, 7) Pantar and Alor islands, 8) Timor and its offshore islands including Jaco, Atauro, and Sabu, 9) Sumba Island, 10) "Greater Flores" which includes Flores and Lembata, 11) Sumbawa Island, and 12) the nearest islands to the Sunda Shelf, which are Lombok and Nusa Penida. We utilized the distance model (+X) where the minimum distance in km between regions (estimated using Google Earth, 2022) scales the dispersal probability between those regions (Van Dam and Matzke, 2016). Six different biogeographical models were compared (DEC, DEC + J, DIVALIKE, DIVALIKE + J, BAYAREALIKE, BAYAREALIKE + J) using log likelihood scores, and AICc values plus weights.

2.6. Gene flow and introgression

Signatures of introgression between lineages were evaluated using pruned asymmetric four-taxon tree hypotheses (the "ABBA-BABA" test; Green et al., 2010; Durand et al., 2011) and symmetric five-taxon tree hypotheses (the "Dfoil" test; Pease and Hahn, 2015) based on our phylogenomic results. These tests can effectively infer introgression by detecting bi-allelic site patterns that are discordant with the relationships of the lineages (Martin et al., 2013; Eaton and Ree, 2013; Pease and Hahn, 2015; Lambert et al., 2019; Musmann et al., 2020). We converted the concatenated alignment of 1,476,505 bp to bi-allelic site data, including heterozygous sites (Eaton et al., 2015), to serve as the input. Tests were performed with the software COMPD (Musmann et al., 2020) on four- and five-taxon tree hypotheses where we suspected introgression based on geographic proximity of the lineages or from

other genetic analyses. Tests were performed with one individual per lineage, and the results of all possible combinations of samples were summarized as a population-wide Z-score and *p*-value with 1000 bootstrap replicates for each test.

Migration rates across putative lineage boundaries were estimated with the coalescent-based program G-PHOCs (Gronau et al., 2011) for lineage pairs that are either parapatrically distributed or that are on adjacent islands. Intronic flanking sequence regions were used because these regions are less likely to be under selection, and after filtering 1145/1150 loci were retained for analysis. The program provides posterior distribution estimates of extant and ancestral effective population sizes (θ), population divergence times (τ), and migration rates (*m*). The priors for θ and τ were set at 1 (alpha) and 10,000 (beta) and the migration priors were set at 0.002 (alpha) and 0.00001 (beta). Each analysis was run for 1,000,000 generations after the removal of 100,000 burn-in generations. Log files for each run were visualized in TRACER v1.7 (Rambaut and Drummond, 2009) to ensure parameter convergence by examining ESS values (>200) and individual parameter trace plots. To convert parameter estimates to biologically meaningful migration values (migrants per generation) a nuclear DNA mutation rate (μ) of 1×10^{-9} mutations/site/year was used (Brandley et al., 2011; Blom et al., 2016; Allio et al., 2017) following the equations from the supplementary materials of Gronau et al. (2011). Migration rates were estimated assuming both one- and two-year generation times.

3. Results

3.1. Mitochondrial screening

Maximum Likelihood analysis of the ND2 mitochondrial gene recovered four major clades from southern Wallacea (Fig. S1a): 1) the *Cyrtodactylus laevigatus* clade ($n = 49$; Fig. S1b), 2) the *C. darmandvillei* clade ($n = 62$; Fig. S2), 3) the *C. sadleiri* clade ($n = 39$; Fig. S3), and 4) the *C. wetariensis* clade ($n = 49$), with the latter two identified as sister clades that together have a sister-group relationship with the *C. laevigatus* clade. These four clades form the sister group to a *C. jellesmae* (Boulenger, 1897) complex, which is then rooted by a clade consisting of *Cyrtodactylus* samples from the *C. marmoratus* Gray, 1831 and *C. papuensis* (Brongersma, 1934) complexes.

The most basal split within the *C. laevigatus* clade separates *C. kimberleyensis* from a common ancestor of the remaining species, and each island except Timor has a lineage representing this clade (Timor has multiple lineages; Fig. S1b). The *C. laevigatus* lineages range in uncorrected mean divergence from ~ 3 –8% (Table S3). The *C. darmandvillei* complex is split into the sister groups of Sumbawa + Moyo + Lembata, and Flores + Pantar + Alor + Atauro (Fig. S2). Notable relationships include Lembata recovered as the sister lineage to eastern Sumbawa (Sumbawa and Lembata are separated by Flores), and Atauro recovered as the sister lineage to eastern Flores (Atauro and Flores are separated by Lembata, Pantar, and Alor). The uncorrected mean divergence among *darmandvillei* lineages ranges from ~ 3 –19% (Table S4). The *C. wetariensis* and *C. sadleiri* clades are sister groups, with the *wetariensis* clade occurring on three of the Lesser Sunda Islands and the *sadleiri* clade on far-flung islands extending from the Sunda Shelf east to the Kei Islands (Fig. S3). Within the *C. wetariensis* complex, a population from eastern Sumba is more closely related to populations on Wetar and Timor than it is to those of neighboring southwestern Sumba. The recently described *C. santana* is the sister lineage to *C. wetariensis*. The lineages within the *C. wetariensis* group range from ~ 8 –11% in uncorrected mean ND2 divergence while the *sadleiri* lineages range in uncorrected mean divergence from <1% to ~ 12 % (Table S5).

3.2. Target-Capture experiment

After the filtering stages (outlined in the supplemental methods) 1150 of the original 1200 loci were retained for analysis, and these loci

totaled 1,476,505 bp when concatenated, with an average locus size of 1284 bp. The average sequence depth, or coverage, was >50X for all but 14 samples, and all samples had >10X average coverage (Fig. S4). The lowest coverage was obtained from the pooled Bali samples reaction (15.2X) and the pooled Alor *C. laevigatus* samples (26.6X).

3.3. Phylogenomics

The concatenated ML phylogeny was well supported for nearly every node relating to intra-population and intra-specific relationships with bootstrap and SH-aLRT values of 100 (Fig. 3). In contrast to the mitochondrial phylogeny, the *C. darmandvillei* complex is the sister group to *C. wallacei* Hayden et al., 2008 of Sulawesi rather than the *laevigatus* + *sadleiri* + *wetariensis* complexes. The *sadleiri* and *wetariensis* clades are sister groups, and relationships within *wetariensis* recover each island as monophyletic. Within the *sadleiri* clade, a pectinate-like topology shows samples from Selayar Island (off the Southwest Peninsula of Sulawesi), *C. batucolus* (Besar Island off the Malay Peninsula), and the Kei Islands (eastern Maluku) representing early separations from the other species

(Fig. 3a). The remainder of the tree includes sister-group relationships between Babar + Tanimbar and between Bali (*C. jatnai*) and Lombok (*C. gordongekkoi*). Within the *laevigatus* complex, the deepest splits separate the sample from Flores (the type locality of *C. laevigatus*) followed by *C. tambora* from Sumbawa (Fig. 3b) from the remaining species. Samples from Timor form two non-sister lineages, with Central Timor samples forming the sister group to the rest of the Banda Arc samples, and the more eastern Timor samples forming the sister group to Wetar samples. A possible widespread northern Wallacean clade groups the *C. jellesmae* complex from Sulawesi and its offshore islands and *C. papuensis* complex samples from Halmahera, Buru, and Aru (Fig. 3c). Relationships among lineages within the *darmandvillei* complex match the mitochondrial tree with respect to the positions of the major phylogeographic lineages (Fig. 3d). Notable relationships include Lembata being nested within East Sumbawa, and Atauro recovered as nested within Flores.

The ASTRAL summary coalescent species tree recovered nearly the same topology as the ML tree with two major exceptions: 1) within the *sadleiri* clade, Bali is the sister group to a clade containing Babar,

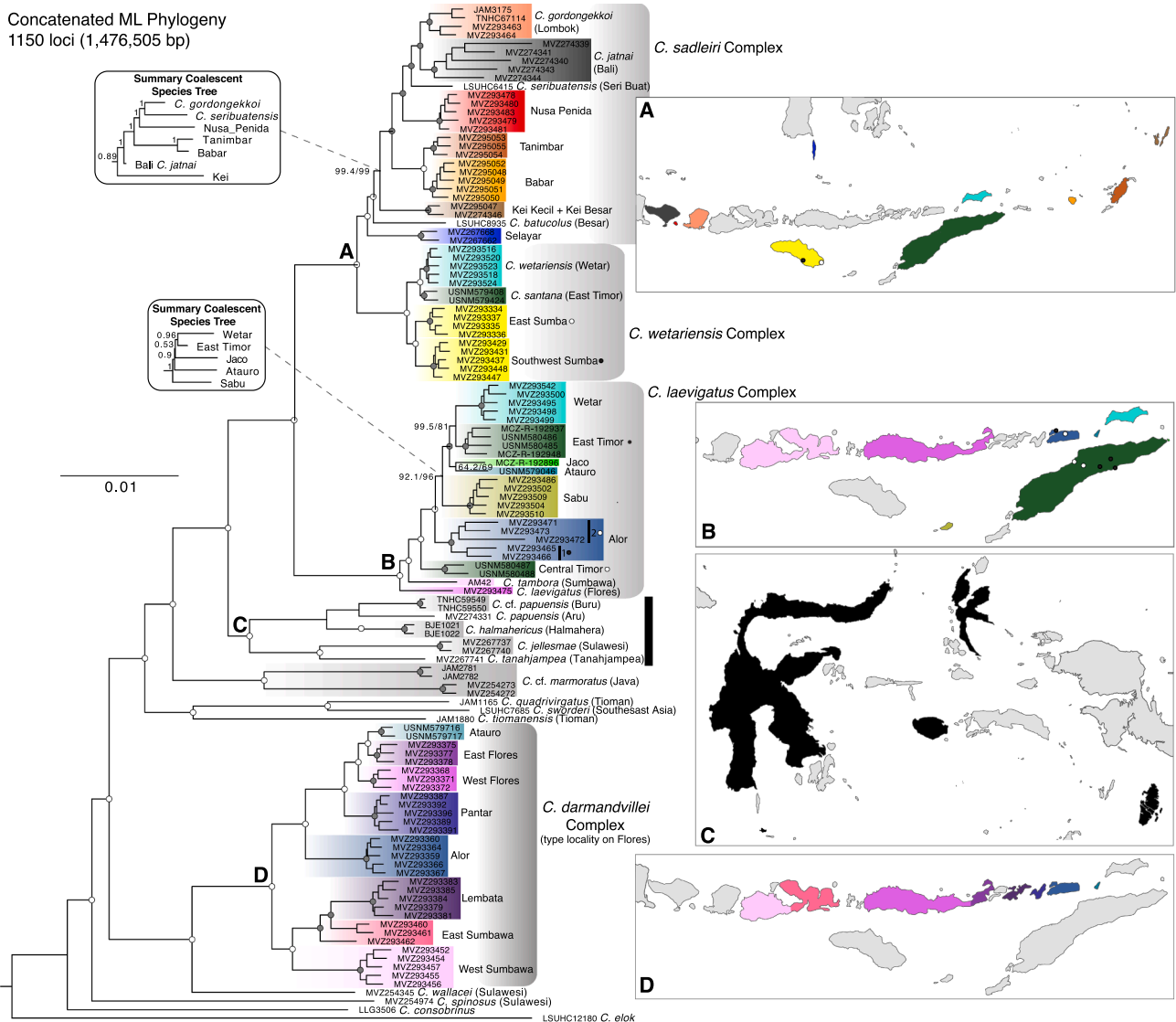


Fig. 3. Maximum Likelihood phylogeny of the concatenated 1150 loci dataset. White circles at nodes represent ultrafast bootstrap (UFB) support of 100, SH-aLRT support of 100, and local posterior probability (LPP) support of 1 (from ASTRAL-III species tree analysis). Gray circles at nodes represent UFB support of 100 and SH-aLRT support of 100. Insets highlight topological differences with the ASTRAL species tree topology, where numbers at nodes represent LPP. Focal clade distributions (A–D) are shown in the maps to the right of the phylogeny. The distributions of *C. batucolus* and *C. seribuensis* are omitted from map A to visualize southern Wallacean lineage distributions.

Tanimbar, Nusa Penida, *C. seribuatensis*, and *C. gordongekko*, (rather than being closest to Lombok) and 2) within the *laevigatus* clade, Atauro is the sister lineage to a group containing Jaco, East Timor, and Wetar (rather than being sister to Jaco) (Fig. 3; Fig. S5).

The time-calibrated Bayesian phylogeny generally had high node support (posterior probability = 1 at all but three nodes) but recovered a slightly different topology than the ML and summary coalescent trees with respect to the *laevigatus* and *sadleiri* clades (Fig. S6). The 95% posterior distributions for node ages were very narrow—no >0.5 million years more or less than the mean estimate. The estimate of the most recent common ancestor (MRCA) of the *darmandvillei*, *laevigatus*, *sadleiri*, and *wetariensis* clades is ~ 23.6 Ma. The *laevigatus* and *sadleiri/wetariensis* clades diverged ~ 11.5 Ma. The estimates of the age of the MRCA for the *darmandvillei* clade is ~ 7.9 Ma, for the *laevigatus* clade is ~ 4.8 Ma, for the *sadleiri* + *wetariensis* clade is ~ 6.6 Ma, for the *sadleiri* clade is ~ 5.8 Ma, and for the *wetariensis* clade is ~ 2.4 Ma. The age estimates from this analysis are similar to those obtained in other multilocus studies that use fossil calibrations. For example, 1) our study finds the split between *C. spinosus* Linkem et al., 2008 and *C. consobrinus* (Peters, 1871) to be ~ 26 Ma while Grismer et al. (2022) estimated ~ 28 Ma, and

2) our study finds the MRCA of the *laevigatus* clade to be ~ 4.8 Ma while Wood et al. (2010) estimated ~ 3 Ma and Grismer et al. (2022) estimated ~ 5.3 Ma.

3.4. Population structure

The genetic networks group the samples into their candidate species clusters similar to the ML phylogeny, yet they illuminate some non-bifurcating relationships within the *laevigatus* and *sadleiri* clades (Fig. 4). Within *laevigatus*, a disagreement between the *ND2* and genomic networks shows the Central Timor samples separated by *ND2* but clustered together by the genomic data. The relationships of some island populations that could not be included in the genomic dataset can be inferred using the *ND2* dataset including: 1) within *darmandvillei*, the Moyo Island population clusters with West Sumbawa, 2) within the *laevigatus* complex, *C. kimberleyensis* from an island off northern Australia are most closely related to samples from eastern Timor and Jaco, 3) within the *sadleiri* complex, *C. sadleiri* from Christmas Island groups with Nusa Penida and *C. jatnai* from Bali, 4) another unidentified sample from Bali groups closest with *C. gordongekko* from Lombok, and

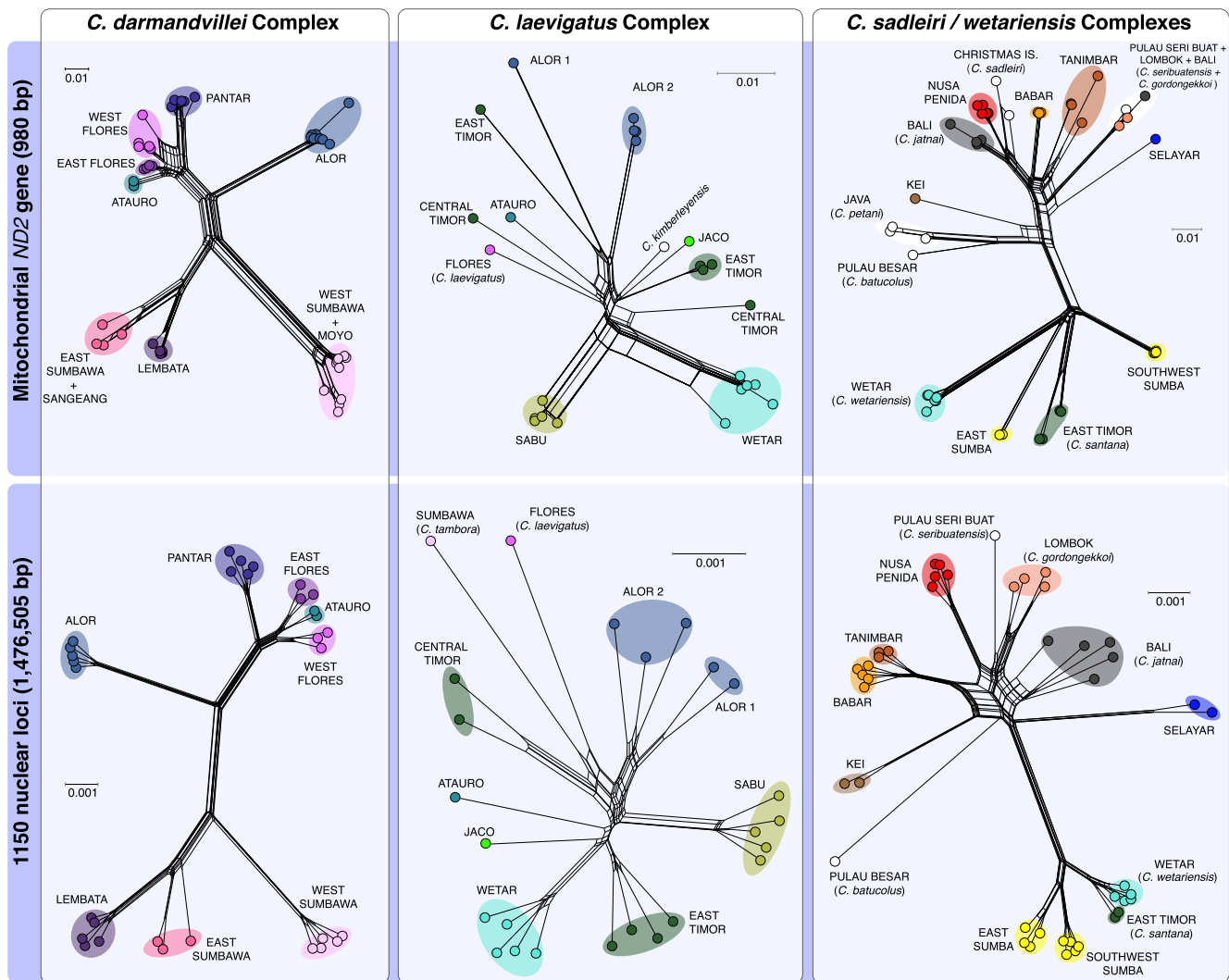


Fig. 4. Unrooted parsimony networks of the mitochondrial *ND2* gene (top row) and full genomic dataset (bottom row) for the *darmandvillei* complex, *laevigatus* complex, and the *gordongekko* + *wetariensis* complexes. These networks allow direct comparison of branching patterns and relative levels of divergence between the mitochondrial and nuclear datasets as well as uncertainty in the lineage relationships. For example, the branching patterns of *darmandvillei* are identical for mtDNA and nDNA, with the level of mtDNA divergence being ~ 10X greater than nDNA divergence. In contrast, the *laevigatus* networks are more star-like with many different branching patterns indicating a more rapid sequence of divergence events.

5) *C. petani* from eastern Java is most closely related to *C. batucolus* from Besar Island (off Peninsular Malaysia).

Population structuring within *darmandvillei* suggests that K2 is optimal, which corresponds to Sumbawa + Lembata and Flores + Pantar + Alor + Atauro, with no admixture between them (Fig. 5a; Table S6). The highest K with meaningful clusters is K = 5, which separates West and East Sumbawa, Lembata, and Alor as individual clusters with Flores + Pantar + Atauro as the fifth cluster. Some Lembata ancestry is found within East Sumbawa samples and only one East Sumbawa sample has any West Sumbawa ancestry. Population structure within the *laevigatus* complex suggests that K = 3 is optimal, with Sabu and Wetar each forming distinct non-admixed groups, Sumbawa, Flores, Alor, and Central Timor forming another non-admixed group, and samples from East Timor, Jaco, and Atauro having limited mixed ancestry with Wetar and Sabu (Fig. 5a; Table S6). The highest K to produce meaningful clusters is K = 10 where each region is a distinct group and with an unexpected splitting of two Alor populations, each of which occurs on a distinct section of the island connected by a narrow isthmus. The optimal K for the *sadleiri* complex is 2 (Table S6), corresponding to Babar + Tanimbar versus all other populations, and the highest meaningfully supported K of 8 divides the *sadleiri* clade into regional populations (Fig. 5a). The optimal K for the *wetariensis* complex is K = 3, which corresponds to *C. wetariensis* + *C. santana*, southwestern Sumba, and eastern Sumba, though using K = 4 the *wetariensis* and *santana* samples are distinct.

The PCA for *darmandvillei* returns the same five clusters of samples as the STRUCTURE analysis when K = 5 (Fig. 5b). The PCA for the *laevigatus* complex returns five clusters of samples where Wetar, Sabu, and Alor each form distinct clusters, and two multi-regional clusters are formed

with samples from 1) eastern Timor + Atauro + Jaco, and 2) Sumbawa + Flores + central Timor. Seven clusters of samples are returned from the PCA of the *sadleiri* complex corresponding to 1) Bali, 2) Kei, 3) Tanimbar + Babar, 4) Selayar, 5) Lombok + *C. seribuatensis*, 6) Nusa Penida, and 7) *C. batucolus*. Three groups are returned from the *C. wetariensis* complex: eastern Sumba, southwestern Sumba, and Wetar + eastern Timor. Signatures of IBD were detected within all four major species complexes, though the distinct separate clusters of points suggest divergent, geographically separated populations rather than clinal variation typical of recent range expansions (Fig. S7).

3.5. Biogeography

The preferred BIOGEOBEARS model according to the likelihood score and AICc score and weight was DEC + J (Table S7), which estimated an identical best joint history as the DIVALIKE + J and BAYAREALIKE + J models but with minor differences in the probabilities at each node. Under the DEC + J model the ancestral range estimate for the MRCA of all included samples occurred on the Sunda Shelf, from which two independent dispersal events occur into Sulawesi (Fig. 6). One of these dispersals gives rise to a clade including *C. spinosus* and *C. wallacei*, and the other gives rise to a clade containing *C. marmoratus*, *C. quadrivirgatus* Taylor, 1962, *C. sworderi* (Smith, 1925), and *C. tiomanensis* Das & Lim, 2000. A dispersal event from the *spinosus/wallacei* clade into the Lesser Sundas gives rise to the *darmandvillei* complex. A dispersal event from the “*marmoratus*” clade into Sulawesi gives rise to the ancestor of the *jellesmae* complex and *C. sp.* “*Selayar*”. One dispersal event from the “*Selayar*” lineage into the Lesser Sundas gives rise to the *laevigatus* complex, a second dispersal event from the sp. “*Selayar*” lineage gives

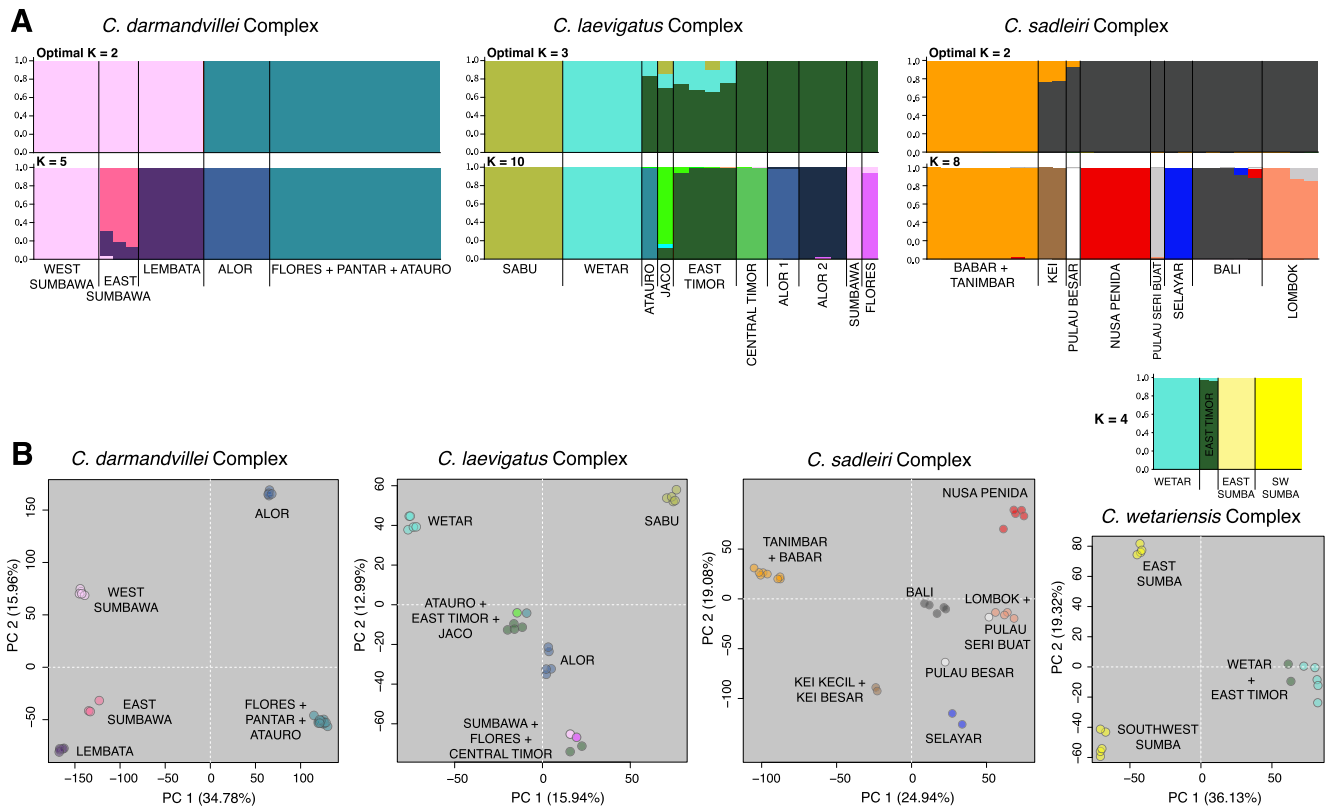


Fig. 5. A) Population structure clustering results are presented for the optimal K as determined by the delta-K method and the highest K that returned biogeographically meaningful results. The highest biogeographically meaningful K values for the *darmandvillei*, *laevigatus*, *sadleiri*, and *wetariensis* complexes are 5, 10, 8, and 4, respectively, which collectively corresponds to 27 candidate species. B) PCA plots of genetic covariance for the *darmandvillei*, *laevigatus*, *sadleiri*, and *wetariensis* complexes. Both the structure and PCA results mostly reflect the geographic discontinuity of the individual islands, except for a few cases like the clustering of multiple non-adjacent island populations (e.g. Flores + Pantar + Atauro *darmandvillei* populations) and the separation of regional populations on the same island (e.g. East and West Sumbawa populations of *darmandvillei*).

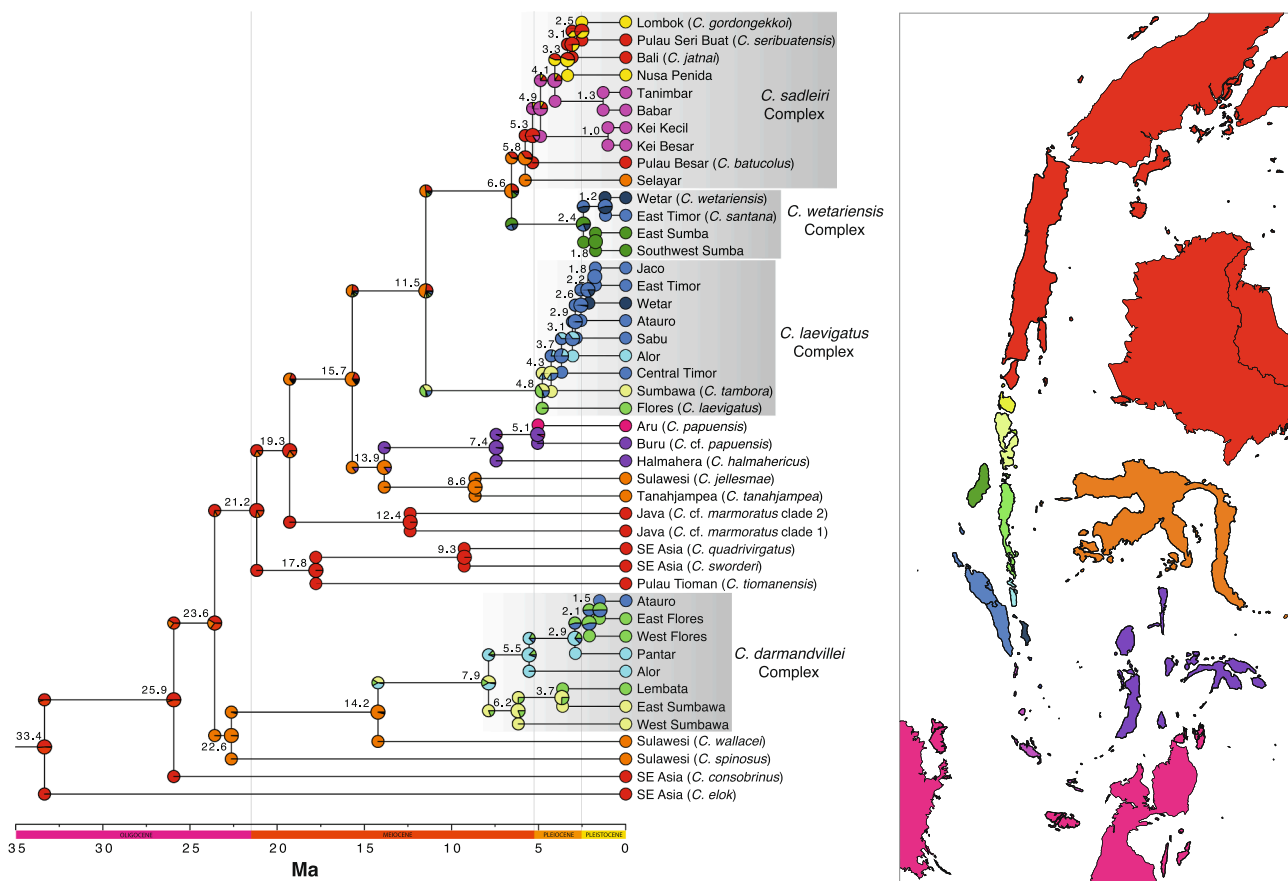


Fig. 6. Ancestral range estimation overlaid on a time-calibrated Bayesian phylogeny. Numbers at nodes represent mean divergence time estimates, and colors within pie charts at nodes represent the probability of that node occurring at a specific region. Regions are colored in the map below as: red = Sunda Shelf, orange = Sulawesi + offshore islands, purple = northern Maluku, light pink = Sahul Shelf, purplish pink = southern Maluku, dark blue = Wetar, light blue = Timor + offshore islands, cyan = Pantar + Alor, green1 = greater Flores, green2 = Sumba, green3 = Sumbawa, yellow = Lombok + Nusa Penida. Note that this color scheme differs from that used in Figs. 3–5 and Fig. 7. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

rise to the *wetariensis* complex, and then a third dispersal event from the sp. “*Selayar*” lineage (presumably back to the Sunda Shelf) eventually gives rise to the *sadleiri* complex. It should be noted that the probability of this joint history is low given the uncertainty of ancestral range estimation at each node, and other possibilities (too many to list here) can be inferred by examining the pie charts at the nodes of the phylogeny (Fig. 6).

3.6. Gene flow and introgression

Four-taxon tests detected significant signals of introgression between the *laevigatus* Jaco lineage and both the central ($Z = 3.889$, $P < 0.001$) and eastern Timor ($Z = 4.417$, $P < 0.001$) lineages (Table S8). All other tests were non-significant, with the next closest near-significant value indicating a low possibility of introgression between the *darmandvillei* western and eastern Sumbawa lineages ($Z = -1.090$, $P = 0.276$). None of the five-taxon tests returned significant signatures of introgression (Table S9).

Converted G-PHOCS estimates of divergence time are generally younger than the BEAST phylogeny estimates and range from ~1–5 Ma (Table S10). We will consider estimates of ≥ 0.5 migrants per generation (M) to be significant, $0.1 \leq M < 0.5$ to be moderate, $M < 0.1$ to be negligible, and cases where the upper 95% posterior density estimate is < 0.05 to represent the absence of gene flow (Reilly et al., 2022a; McGuire et al., 2023). We follow Reilly et al. (2022a) for our definitions of “unidirectional” migration and “bidirectional asymmetric” migration.

Estimates reported are based on a 1-year generation time (estimates based on a 2-year generation time are found in Table S10). Within the *sadleiri* complex, significant unidirectional migration was detected from Lombok to Bali (0.67) and moderate bidirectional migration was detected between Tanimbar and Babar (0.26 and 0.16). Moderate asymmetric migration was detected from Nusa Penida to Bali (0.15). Migration estimates for the *laevigatus* complex ranged from absent to moderate with moderate asymmetric migration from Sabu to Alor (0.14), central Timor to Alor (0.11), and eastern Timor to Alor (0.14). Migration is absent among *wetariensis* populations (all below < 0.02). Estimates of migrants per generation within the *darmandvillei* complex were mostly absent with moderate asymmetric migration between western and eastern Flores ($M = 0.14$ from eastern to western Flores).

4. Discussion

Wallacea has been considered species-poor for bent-toed geckos, but this study shows that *Cyrtodactylus* species diversity in the region is substantially underestimated. Indeed, our results suggest that *Cyrtodactylus* species diversity within southern Wallacea includes at least 25 species in the region between Bali and Kei rather than the 8 currently described. Most islands have highly divergent, endemic lineages that warrant species description, and at least three of the larger islands contain multiple candidate species within the same species complex. Below we discuss the conservation policy and land management implications of this diversity, the incredibly complex historical biogeography

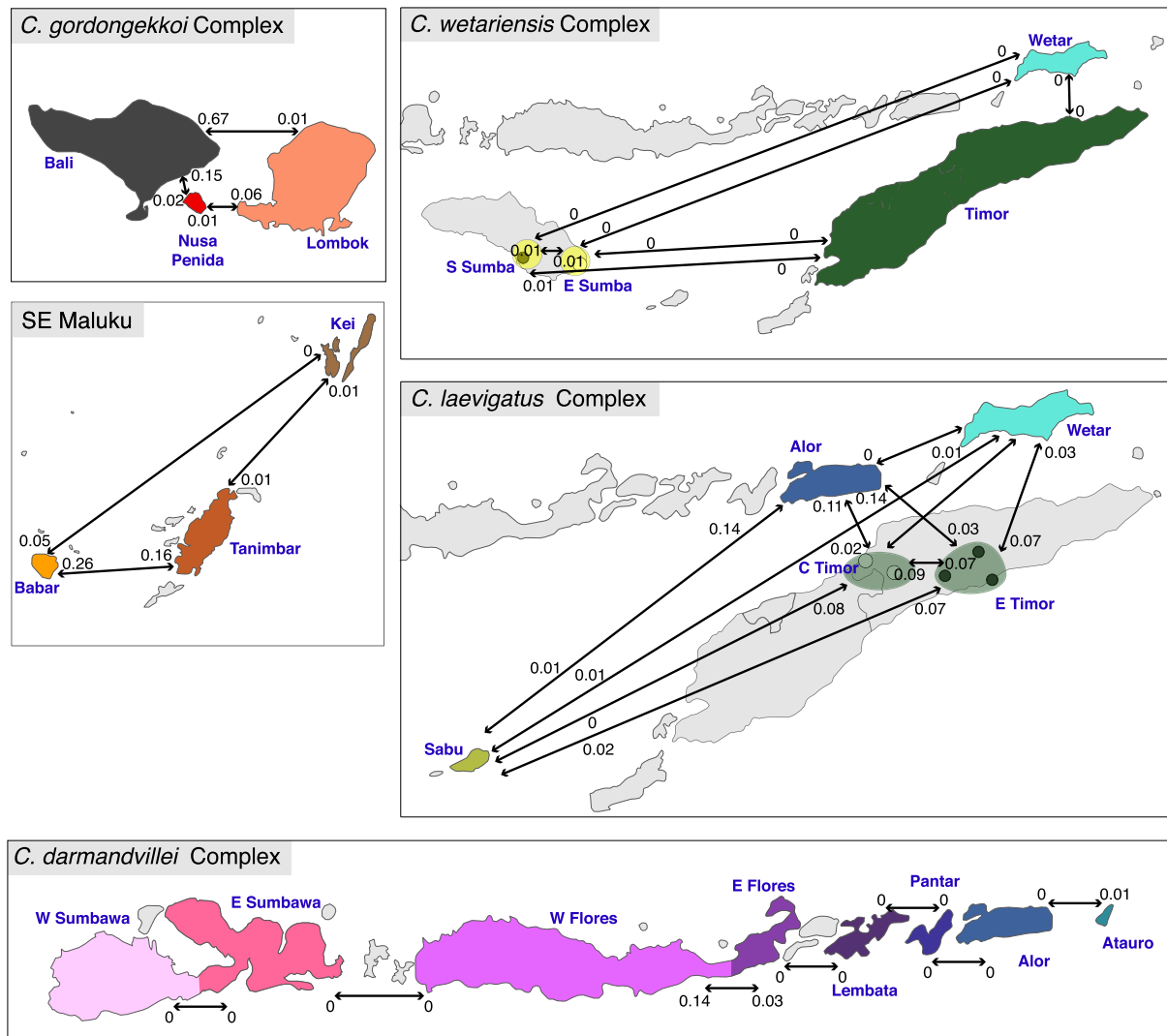


Fig. 7. Estimates of migrants per generation between geographically clustered lineages. Estimates below 0.5 generally indicate genetic isolation.

of these southern Wallacean *Cyrtodactylus* lineages, and some of our ecological and natural history observations from the field in light of the phylogenomic relationships of these lineages.

4.1. Unrecognized Species-level diversity

There is evidence for four major *Cyrtodactylus* clades within southern Wallacea, including the *darmandvillei*, *laevigatus*, *wetariensis*, and *sadlei* species complexes. Phylogenomic, mitochondrial, and population genetic analyses all suggest that each of these four clades contains undescribed species. However, while these results provide some measure of phylogenetic clarity and a biogeographic context, the taxonomic consequences turn out to be rather convoluted.

According to population divergence timing estimates, the *darmandvillei* complex appears to be the oldest of the four clades, containing the most within-clade divergence (i.e., the initial split within *darmandvillei* occurred ~ 8 Ma). Population clustering results along with estimates of gene flow suggest that there are at least five species within *darmandvillei*. For the purposes of finding sound taxonomic decisions in the future we note that the type locality of *C. darmandvillei* is recorded as “Sikka” by Weber (1890), and a close reading of Weber (1890) and the activities of Father Le Coq d’Armandville reveal this locality to be at approximately 8.7495°S, 122.1968°E in central Flores. This locality is ~ 125 km by air distant from our Bajawa samples in western Flores, ~ 100 km by air

distant from our Larantuka eastern Flores samples, and ~ 50 km by air distant from another eastern Flores sample for which we have only mitochondrial data (WAMR:105415). It has been documented and discussed (e.g., Muraoka et al., 2005; Reilly et al., 2022a, 2022c) that emerging volcanoes in this archipelago may have arisen as separate paleo-islands before eventually amalgamating into composite islands. Flores appears to have once existed as a string of five separate paleo islands that began to coalesce as the entire region uplifted by over 800 m in the last 2.5 Ma, though the exact timing of coalescence is not known (Muraoka et al., 2005). Our estimate of the divergence time between West and East Flores *darmandvillei* populations is ~ 1.4 –2 Ma suggesting that Flores may have still been disconnected at that time.

There is no signal of gene exchange between the western and eastern Sumbawa *darmandvillei* lineages and they are estimated to have diverged ~ 4.5 –6 Ma indicating that they are distinct species. The narrow isthmus connecting the two halves of Sumbawa is estimated to have been submerged until ~ 0.2 Ma based on the rate of uplift in the region (Borel-Best et al., 1985) implying that any contact zone would have formed relatively recently compared to the divergence time of these two lineages. Two other lizard taxa that have species-level phylogeographic breaks between the western and eastern halves of Sumbawa include *Draco* flying lizards, estimated to have diverged ~ 2.5 Ma (Reilly et al., 2022a), and *Sphenomorphus* forest skinks, estimated to have diverged ~ 1 –2 Ma (Reilly et al., 2022c). Further sampling of Sumbawan

Cyrtodactylus will be useful to understand their distributions and if, or how, they contact one another.

The *laevigatus* complex, as we have termed it in this paper, contains several described species including *C. kimberleyensis*, *C. tambora*, and likely *C. celatus* (based on morphology), as well as many insular populations in the Lesser Sundas currently recognized as *C. laevigatus*. The phylogenomic and network analyses show that the group began diversifying approximately 5 Ma with many divergence events occurring in rapid succession. The lineages that compose the *laevigatus* complex, excluding those from the type locality of Flores, are more closely related to *C. tambora* than they are to true *C. laevigatus*. According to genetic clustering and divergence time analyses there could be as many as 8 undescribed species in the complex. Surprisingly, islands such as Timor and Alor contain multiple candidate species highlighting the complex biogeographical history of this group. The two Alor candidate species are particularly unexpected due to the small size of the island, with one lineage occurring on the main portion of the island and the other on a small peninsula on the northwest coast. Given rates of uplift on Alor of 1 m per 1000 years (Hantoro et al., 1994) the peninsula, connected by a low-lying isthmus no >40 m in elevation, would have been continuously disconnected from the main part of the island until ~ 160,000 years ago, and periodically disconnected between ~ 160,000 and ~ 40,000 years ago as Pleistocene sea level fluctuations occurred lowering sea level as much as 120 m.

The *wetariensis* and *sadleiri* complexes are sister clades, with the former being known from only three islands, and the latter occurring across a broad geographical range that extends from small islands off Peninsular Malaysia to the Moluccan islands of Kei Kecil and Kei Besar. Within the *wetariensis* clade, there are four candidate species supported by the data, with Sumba lineages from southwest and eastern Sumba that split ~ 1.4–2 Ma and that are completely genetically isolated from one another (<0.02 migrants per generation in either direction). *Sphenomorphus* forest skinks sampled from these same localities on Sumba also revealed two distinct species with the same biogeographic pattern (Reilly et al., 2022c), strongly implying that the tectonic history of Sumba is more complicated than currently understood and in a manner that may have driven species divergences in these two lizard groups on Sumba. The *sadleiri* complex contains at least four undescribed species in southern Wallacea from 1) Selayar, 2) the Kei Islands, 3) Nusa Penida, and 4) Babar + Tanimbar.

4.2. Dispersal pathways

Accurate reconstructions of ancestral geographic ranges and actual dispersal events are highly dependent upon adequate sampling of all relevant lineages involved. Given these limitations, one must cautiously interpret our historical biogeographical analyses and divergence estimates. According to our ancestral range reconstructions, an initial dispersal event occurred from the Sunda Shelf to Sulawesi approximately 20 Ma, followed by a dispersal event into the Sunda Arc islands of southern Wallacea ~ 14 Ma (~2 myr older than the age of the oldest islands but in the range of when they are estimated to have first become emergent). Ancestral *darmandvillei* complex lineages then dispersed from Sumbawa eastward into the neighboring islands reaching as far east as Ataúro. This is not a stepping-stone dispersal scenario; rather, many leap-frog dispersal events spanning multiple islands are required such as from eastern Sumbawa to Lembata (~450 km apart and separated by at least four major islands, including the very elongated Flores), from Alor to western Flores, and from eastern Flores to Ataúro. These dispersal scenarios are unexpected given the current arrangement of the islands, suggesting that either the past configuration of the archipelago was dramatically different, wind direction or oceanic currents played a role, or both (as suggested by Reilly et al., 2019a, 2022a, 2022c).

Another dispersal event from the Sunda Shelf to Sulawesi is estimated to have occurred approximately 19 Ma, and it is from this Sulawesi clade (which includes *C. jellesmae*) that a dispersal event into

eastern Wallacea occurred that eventually reached the Sahul Shelf, possibly giving rise to the *C. papuensis* clade. Though it is inferred from a weakly supported topology, it appears that a propagul from within this same *jellesmae* complex clade may have dispersed south into the Lesser Sundas ~ 11 Ma, giving rise to the *laevigatus* species group. Between 5 Ma and 1 Ma this *laevigatus* group ancestor dispersed throughout the Lesser Sundas and even southward to Montalivet Island off northern Australia.

The *sadleiri* and *wetariensis* species groups split ~ 6.5 Ma. The *wetariensis* complex ancestor is estimated to have first occurred on Sumba (though Sumba is currently estimated to have become emergent no later than ~ 4 Ma) before dispersing eastward to Timor and Wetar. Although Sumba is widely separated from Timor and Wetar now, the close biogeographical connection between Sumba and the Banda Arc islands is also seen in other taxa (Reilly et al., 2022a, 2022b, 2022c), suggesting that Sumba may have once been geographically closer to the Banda Arcs in the past. The remainder of the dispersal events within the *sadleiri* complex involve either relatively small distances (e.g., the 12 km separating Nusa Penida and Bali), or extremely large distances (e.g., the 3500 km that separate Pulau Besar off the Malay Peninsula and the Kei Islands in eastern Maluku). Further sampling of populations/species in this clade (such as *C. sadleiri* and *C. petani*) will be needed to reveal the polarized biogeographical history of this complex.

4.3. Conservation of southern Wallacean biodiversity

Fine-scale studies of southern Wallacean herpetofaunal diversity are beginning to show repeated phylogeographic patterns, with taxa that dispersed into the Lesser Sunda Archipelago >2 Ma typically being composed of multiple species-level (or near species-level) lineages (Blom et al., 2019; Reilly et al., 2019a, 2021, 2022a, 2022b, 2022c). Few major taxa in the Lesser Sundas have received the same level of scientific scrutiny as have amphibians and reptiles, and recently published phylogenomic studies (e.g. Blom et al., 2019; Reilly et al. 2019b, 2022a, 2022c) strongly suggest that species diversity in the Lesser Sundas is substantially underestimated, with many more small-range endemics than previously appreciated, and larger islands being divisible into multiple discrete areas of endemism. This pattern is repeated by *Cyrtodactylus*, in which four separate groups dispersed into southern Wallacea between 5 and 14 Ma, each of which has diversified *in situ*, sometimes extensively. Importantly, this study has identified the first endemic reptile species for the small islands of Nusa Penida, Sabu, Lembata, and Babar, as well as new endemics for the small islands Pantar and Wetar. These studies point to the need for a reassessment of conservation priorities in the Lesser Sundas, because small islands such as Nusa Penida and Sabu without conservation protections are home to at-risk endemic species, and even larger islands such as Sumbawa, Flores, Sumba, and Timor may not have conservation protections in place for all areas of endemism.

4.4. Sympatry of *Cyrtodactylus* lineages

Most of the larger islands in the Lesser Sundas contain representatives of two different *Cyrtodactylus* lineages. However, the shared pattern appears to be that members of the smaller-bodied *laevigatus* complex can coexist with members of the larger-bodied *darmandvillei*, *sadleiri*, or *wetariensis* lineages, but that larger bodied lineages cannot occur in sympatry. This is supported by the fact that we have not yet found any two large-bodied lineages in sympatry or, indeed, even inhabiting the same island. A possible case of island co-occurrence among large-bodied species involves a reported but unconfirmed population of *C. darmandvillei* on Lombok (McKay and Lilley, 2012) where *C. gordongekkoi* occurs, though *C. gordongekkoi* is known only from the mountainous area of Mount Rinjani while the *C. darmandvillei* population was reported from near sea level along the southern coast of the island.

5. Conclusions

This study has uncovered at least three independent dispersals from Sulawesi and its offshore islands into southern Wallacea followed by independent *in situ* diversification of each lineage. Some lineages have remained somewhat localized by dispersing among adjacent islands while other lineages have incredibly disjunct distributions with the *sadlei* complex spanning thousands of kilometers and involving four over-water dispersal events of > 1500 km and one dispersal of > 3500 km (Pulau Besar to Kei). Species-level diversity is massively underestimated in the region with at least 15–18 cryptic species requiring formal description. These candidate species are supported by the apparent lack of gene flow between lineages of the same species group that occur on the same island, with notable examples including the two *darmandvillei* lineages on Sumbawa and the two *wetariensis* lineages on Sumba. Of ecological interest is the apparent inability of large-bodied species groups to co-occur on a single island, while the smaller-bodied *laevigatus* group appears able to co-occur with any of the larger bodied species. The biogeographic patterns of the distribution of diversity across the Lesser Sundas are being refined with each new study and should be used to help identify potential protected areas that will conserve their endemic terrestrial faunas.

CRedit authorship contribution statement

Sean B. Reilly: Conceptualization, Data curation, Funding acquisition, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. **Alexander L. Stubbs:** Conceptualization, Investigation, Writing - review and editing. **Benjamin R. Karin:** Investigation, Writing - review and editing. **Evy Arida:** Investigation, Writing - review and editing. **Umilaela Arifin:** Investigation, Writing - review and editing. **Amir Hamidy:** Investigation, Writing - review and editing. **Hinrich Kaiser:** Funding acquisition, Investigation, Resources, Supervision, Writing - review and editing. **Ke Bi:** Methodology, Software, Writing - review and editing. **Awal Riyanto:** Investigation, Writing - review and editing. **Djoko T. Iskandar:** Investigation, Supervision, Writing - review and editing. **Jimmy A. McGuire:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing - review and editing.

Declaration of Competing Interest

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

Data availability

Sequence data for the *ND2* mitochondrial gene have been submitted to the GenBank database under accession numbers OP356255–OP356432. Sequencing data from the exon-capture experiment have been submitted to the NCBI sequence read archive under accession number PRJNA880575. Software input files, exon-capture probe design, and annotated transcriptomes are available from the Dryad Digital Repository at <https://doi.org/10.7291/D1WQ2P>.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2023.107853>.

References

- Allio, R., Donega, S., Galtier, N., Nabholz, B., 2017. Large variation in the ratio of mitochondrial to nuclear mutation rate across animals: implications for genetic diversity and the use of mitochondrial DNA as a molecular marker. *Mol. Biol. Evol.* 34, 2762–2772.
- Amarasinghe, A.T., Riyanto, A., Mumpuni, M., Grismer, L.L., 2020. A new bent-toed gecko species of the genus *Cyrtodactylus* Gray, 1827 (Squamata: Gekkonidae) from The West Bali National Park, Bali, Indonesia. *Taprobanica* 9, 59–70.
- Auffenberg, W., 1980. The herpetofauna of Komodo, with notes on adjacent areas. *Bull. Florida State Mus. Biol. Sci.* 25, 39–156.
- Bauer, A.M., Doughty, P., 2012. A new bent-toed gecko (Squamata: Gekkonidae: *Cyrtodactylus*) from the Kimberley region, Western Australia. *Zootaxa* 3187, 33–42.
- Bi, K., Vanderpool, D., Singhal, S., Linderth, T., Moritz, C., Good, J.M., 2012. Transcriptome-based exon capture enables highly cost-effective comparative genomic data collection at moderate evolutionary scales. *BMC Genomics* 13, 403.
- Blom, M.P., Horner, P., Moritz, C., 2016. Convergence across a continent: adaptive diversification in a recent radiation of Australian lizards. *Proc. R. Soc. B Biol. Sci.* 283, 20160181.
- Blom, M.P., Matzke, N.J., Bragg, J.G., Arida, E., Austin, C.C., Backlin, A.R., Carretero, M. A., Fisher, R.N., Glaw, F., Hathaway, S.A., Iskandar, D.T., McGuire, J.A., Karin, B.R., Reilly, S.B., Rittmeyer, E.N., Rocha, S., Sanchez, M., Stubbs, A.L., Vences, M., Moritz, C., 2019. Habitat preference modulates trans-oceanic dispersal in a terrestrial vertebrate. *Proc. R. Soc. B Biol. Sci.* 286, 20182575.
- Borel-Best, M., Moll, H., Boekschoten, G.J., 1985. Investigations of recent and fossil coral reefs in eastern Indonesia (Snellius-II Expedition): a preliminary report. *Proc. Fifth Int. Coral Reef Cong.* 6, 311–316.
- Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLOS Comput. Biol.* 10, e1003537.
- Boulenger, G.A., 1897. A catalogue of the reptiles and batrachians of Celebes with special reference to the collections made by Drs P & F Sarasin in 1893–1896. *Proc. Zool. Soc. Lond.* 1897, 193–237.
- Brandley, M.C., Wang, Y., Guo, X., Montes de Oca, A.N., Feria-Ortiz, M., Hikida, T., Ota, H., 2011. Accommodating heterogeneous rates of evolution in molecular divergence dating methods: an example using intercontinental dispersal of *Plestiodon* (*Eumeces*) lizards. *Syst. Biol.* 60, 3–15.
- Brennan, I.G., Bauer, A.M., Van Tri, N., Wang, Y.Y., Wang, W.Z., Zhang, Y.P., Murphy, R. W., 2017. Barcoding utility in a mega-diverse, cross-continental genus: keeping pace with *Cyrtodactylus* geckos. *Sci. Rep.* 7, 5592.
- Brongersma, L.D., 1934. Contributions to Indo-Australian herpetology. *Zool. Mededelingen Leiden* 17, 161–251.
- Bryant, D., Moulton, V., 2004. Neighbor-net: an agglomerative method for the construction of phylogenetic networks. *Mol. Biol. Evol.* 21, 255–265.
- Burbrink, F.T., Ruane, S., 2021. Contemporary philosophy and methods for studying speciation and delimiting species. *Ichthyol. Herpetol.* 109, 874–894.
- Chan, K.O., Grismer, L.L., Santana, F., Pinto, P., Loke, F.W., Conaboy, N., 2023. Scratching the surface: a new species of Bent-toed gecko (Squamata, Gekkonidae, *Cyrtodactylus*) from Timor-Leste of the *darmandvillei* group marks the potential for future discoveries. *ZooKeys*, 1139, 107–126. <https://doi.org/10.3897/zookeys.1139.96508>.
- Darevsky, I.S., 1964. Two new species of gekkonid lizards from the Komodo island in Lesser Sundas Archipelago. *Zool. Anz.* 173, 169–174.

- Das, I., 1994. *Cnemaspis gordongekko*, a new gecko from Lombok, Indonesia, and the biogeography of oriental species of *Cnemaspis* (Squamata: Sauria: Gekkonidae). *Hamadryad* 18, 1–9.
- Das, I., Lim, L.J., 2000. A new species of *Cyrtodactylus* (Sauria: Gekkonidae) from Pulau Tioman. *Raffles Bull. Zool.* 48, 223–231.
- Davis, H.R., Chan, K.O., Das, I., Brennan, I.G., Karin, B.R., Jackman, T.R., Brown, R.M., Iskandar, D.T., Nashriq, I., Grismer, L.L., Bauer, A.M., 2020. Multilocus phylogeny of Bornean Bent-toed geckos (Gekkonidae: *Cyrtodactylus*) reveals hidden diversity, taxonomic disarray, and novel biogeographic patterns. *Mol. Phylogenet. Evol.* 147, 106785.
- Davis, H.R., Das, I., Leaché, A.D., Karin, B.R., Brennan, I.G., Jackman, T.R., Nashriq, I., Chan, K.O., Bauer, A.M., 2021. Genetically diverse yet morphologically conserved: hidden diversity revealed among Bornean geckos (Gekkonidae: *Cyrtodactylus*). *J. Zool. Syst. Evol. Res.* 59, 1113–1135.
- Davis, H.R., Nashriq, I., Woytek, K.S., Wikramanayake, S.A., Bauer, A.M., Karin, B.R., Brennan, I.G., Iskandar, D.T., Das, I., 2023. Genomic analysis of Bornean geckos (Gekkonidae: *Cyrtodactylus*) reveals need for updated taxonomy. *Zool. Scr.* 2023, 1–15.
- De Queiroz, K., 1998. The general lineage concept of species, species criteria, and the process of speciation. In: Howard, D.J., Berlocher, S.H. (Eds.), *Endless Forms: Species and Speciation*. Oxford Univ. Press, Oxford, United Kingdom, pp. 57–75.
- Deng, W., Maust, B.S., Nickle, D.C., Learn, G.H., Liu, Y., Heath, L., Pond, S.L.K., Mullins, J.I., 2010. *divine*: a web server to analyze phylogenies, sequence divergence, diversity, and informative sites. *Biotechniques* 48, 405–408.
- Dring, J.C.M., 1979. Amphibians and reptiles from northern Trengganu, Malaysia, with descriptions of two new geckos; *Cnemaspis* and *Cyrtodactylus*. *Bull. Br. Mus. Nat. Hist. Zool.* 34, 181–241.
- Dunn, E.R., 1927. Results of the Douglas Burden Expedition to the Island of Komodo. III. Lizards from the East Indies. *American Museum Novitates* 288, 1–13.
- Durand, E.Y., Patterson, N., Reich, D., Slatkin, M., 2011. Testing for ancient admixture between closely related populations. *Mol. Biol. Evol.* 28, 2239–2252.
- Earl, D.A., vonHoldt, B.M., 2012. *structure harvester*: a website and program for visualizing structure output and implementing the Evanno method. *Conserv. Genet. Res.* 4, 359–361.
- Eaton, D.A., Ree, R.H., 2013. Inferring phylogeny and introgression using RADseq data: an example from flowering plants (Pedicularis: Orobanchaceae). *Syst. Biol.* 62, 689–706.
- Eaton, D.A., Hipp, A.L., González-Rodríguez, A., Cavender-Bares, J., 2015. Historical introgression among the American live oaks and the comparative nature of tests for introgression. *Evolution* 69, 2587–2601.
- Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797.
- Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* 14, 2611–2620.
- Fortuin, A.R., Van der Werff, W., Wensink, H., 1997. Neogene basin history and paleomagnetism of a rifted and inverted forearc region, on- and offshore Sumba, Eastern Indonesia. *J. Asian Earth Sci.* 15, 61–88.
- Gray, J.E., 1831. A synopsis of the species of Class Reptilia. In: Griffith, E., Pidgeon, E. (Eds.), *The Animal Kingdom Arranged in Conformity With Its Organisation by the Baron Cuvier With Additional Descriptions of All the Species Hither Named, and of Many Before Noticed*. Whittaker, Treacher and Co., London, pp. 1–110.
- Green, R.E., Krause, J., Briggs, A.W., Maricic, T., Stenzel, U., Kircher, M., Pääbo, S., 2010. A draft sequence of the Neandertal genome. *Science* 328, 710–722.
- Grismer, L.L., Chan, K.O., Grismer, J.L., Wood Jr., P.L., Belabut, D., 2008. Three new species of *Cyrtodactylus* (Squamata: Gekkonidae) from Peninsular Malaysia. *Zootaxa* 1921, 1–23.
- Grismer, L.L., Wood Jr., P.L., Thura, M.K., Zin, T., Quah, E.S., Murdoch, M.L., Grismer, M.S., Lin, A., Kyaw, H., Lwin, N., 2018. Twelve new species of *Cyrtodactylus* Gray (Squamata: Gekkonidae) from isolated limestone habitats in east-central and southern Myanmar demonstrate high localized diversity and unprecedented microendemism. *Zool. J. Linn. Soc.* 182, 862–959.
- Grismer, L.L., Wood Jr., P.L., Poyarkov, N.A., Le, M.D., Kraus, F., Agarwal, I., Oliver, P.M., Nguyen, S.N., Nguyen, T.Q., Karunarathna, S., Welton, L.J., Stuart, B.L., Luu, V. Q., Bauer, A.M., O'Connell, K.A., Quah, E.S.H., Chan, K.O., Ziegler, T., Ngo, H., Nazarov, R.A., Aowphol, A., Chomdej, S., Suwannapoom, C., Siler, C.D., Anuar, S., Tri, N.V., Grismer, J.L., 2021a. Phylogenetic partitioning of the third-largest vertebrate genus in the world, *Cyrtodactylus* Gray, 1827 (Reptilia; Squamata; Gekkonidae) and its relevance to taxonomy and conservation. *Vert. Zool.* 71, 101–154.
- Grismer, L.L., Wood Jr., P.L., Poyarkov, N.A., Le, M.D., Karunarathna, S., Chomdej, S., Suwannapoom, C., Qi, S., Liu, S., Che, J., Quah, E.S., Kraus, F., Oliver, P.M., Riyanto, A., Pauwels, O.S.G., Grismer, J.L., 2021b. Karstic landscapes are foci of species diversity in the world's third-largest vertebrate genus *Cyrtodactylus* Gray, 1827 (Reptilia: Squamata; Gekkonidae). *Diversity* 13, 183.
- Grismer, L.L., Poyarkov, N.A., Quah, E.S.H., Grismer, J.L., Wood Jr., P.L., 2022. The biogeography of bent-toed geckos, *Cyrtodactylus* (Squamata: Gekkonidae). *PeerJ* 10, e13153.
- Gronau, I., Hubisz, M.J., Gulko, B., Danko, C.G., Siepel, A., 2011. Bayesian inference of ancient human demography from individual genome sequences. *Nat. Genet.* 43, 1031–1034.
- Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* 59, 307–321.
- Hall, R., 2009. Southeast Asia's changing paleogeography. *Blumea* 54, 148–161.
- Hall, R., 2011. Australia-SE Asia collision: plate tectonics and crustal flow. *Geol. Soc. Spec. Publ.* 355, 75–109.
- Hantoro, W.S., Pirazzoli, P.A., Jouannic, C., Faure, H., Hoang, C.T., Radtke, U., Causse, C., Borel-Best, M., Lafont, R., Bieda, S., Lambeck, K., 1994. Quaternary uplifted coral reef terraces on Alor Island, East Indonesia. *Coral Reefs* 13, 215–223.
- Harris, R., 2011. The nature of the Banda Arc-continent collision in the Timor region. In: Brown, D., Ryan, P.D. (Eds.), *Arc Continent Collision*. Springer, Berlin, Heidelberg, pp. 163–211.
- Hayden, C.J., Brown, R.M., Gillespie, G., Setiadi, M.I., Linkem, C.W., Iskandar, D.T., Bickford, D.P., Riyanto, A., McGuire, J.A., 2008. A new species of bent-toed gecko *Cyrtodactylus* Gray, 1827, (Squamata: Gekkonidae) from the island of Sulawesi, Indonesia. *Herpetologica* 64, 109–120.
- Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* 35, 518–522.
- Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* 23, 254–267.
- Jombart, T., 2008. *adeigenet*: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24, 1403–1405.
- Kaiser, H., Carvalho, V.L., Ceballos, J., Freed, P., Heacox, S., Lester, B., Richards, S.J., Trainor, C.T., Sanchez, S.C., O'Shea, M., 2011. The herpetofauna of Timor-Leste: a first report. *ZooKeys* 109, 19–86.
- Kaiser, H., Sanchez, C., Heacox, S., Kathriner, A., Ribeiro, A.V., Soares, Z.A., de Araujo, L. L., Mecke, S., O'Shea, M., 2013. First report on the herpetofauna of Ataúro Island, Timor-Leste. *Check List* 9, 752–762.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermin, L.S., 2017. *ModelFinder*: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589.
- Kathriner, A., Bauer, A.M., O'Shea, M., Sanchez, C., Kaiser, H., 2014. Hiding in plain sight: a new species of bent-toed gecko (Squamata: Gekkonidae: *Cyrtodactylus*) from West Timor, collected by Malcolm Smith in 1924. *Zootaxa* 3900, 555–568.
- Lambert, S.M., Streicher, J.W., Fisher-Reid, M.C., Méndez de la Cruz, F.R., Martínez-Méndez, N., García-Vázquez, U.O., Montes, N., de Oca, A., Wiens, J.J., 2019. Inferring introgression using RADseq and DFOIL: power and pitfalls revealed in a case study of spiny lizards (*Sceloporus*). *Mol. Ecol. Resour.* 19, 818–837.
- Linkem, C.W., McGuire, J.A., Hayden, C.J., Setiadi, M.I., Bickford, D.P., Brown, R.M., 2008. A new species of bent-toe gecko (Gekkonidae: *Cyrtodactylus*) from Sulawesi Island, Eastern Indonesia. *Herpetologica* 64, 224–234.
- Martin, S.H., Dasmahapatra, K.K., Nadeau, N.J., Salazar, C., Walters, J.R., Simpson, F., Blaxter, M., Manica, A., Mallet, J., Jiggins, C.D., 2013. Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. *Genome Res.* 23, 1817–1828.
- Matzke, N.J., 2013. *BioGeoBEARS*: BioGeography with Bayesian (and likelihood) evolutionary analysis in R Scripts. R package, version (2), 1.
- Matzke, N.J., 2014. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Syst. Biol.* 63, 951–970.
- McGuire, J.A., Huang, X., Reilly, S.B., Iskandar, D.T., Wang-Claypool, C., Werning, S., Chong, R.M., Lawalata, S.Z.S., Stubbs, A.L., Frederick, J.H., Brown, R.M., Evans, B.J., Arifin, U., Riyanto, A., Hamidy, A., Arida, E., Koo, M.S., Supriatna, J., Andayani, N., Hall, R., 2023. Species delimitation, phylogenomics, and biogeography of Sulawesi flying lizards: a diversification history complicated by ancient hybridization, cryptic species, and arrested speciation. *Syst. Biol.* syad020 <https://doi.org/10.1093/sysbio/syad020>.
- McKay, J.L., Liley, R., 2012. New distributional records from the Lesser Sunda. Indonesia. *Herpetol. Rev.* 43, 109–111.
- Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* 37, 1530–1534.
- Muraoka, H., Nasution, A., Simanjuntak, J., Dwipa, S., Takahashi, M., Takahashi, H., et al., 2005. Geology and geothermal systems in the Bajawa volcanic rift zone, Flores, Eastern Indonesia. *Proc. World Geotherm. Congr.* 2005, 1–13.
- Musmann, S.M., Douglas, M.R., Bangs, M.R., Douglas, M.E., 2020. *Comp-D*: a program for comprehensive computation of D-statistics and population summaries of reticulated evolution. *Conserv. Genet. Resour.* 12, 263–267.
- Nguyen, N., Duffy, B., Shulmeister, J., Quigley, M., 2013. Rapid Pliocene uplift of Timor. *Geology* 41, 179–182.
- O'Shea, M., Sanchez, C., Kathriner, A., Mecke, S., Carvalho, V.L., Ribeiro, A.V., Soares, Z. A., de Araujo, L.L., Kaiser, H., 2015. Herpetological diversity of Timor-Leste: updates and a review of species distributions. *Asian Herpetol. Res.* 6, 73–131.
- Oaks, J.R., Siler, C.D., Brown, R.M., 2019. The comparative biogeography of Philippine geckos challenges predictions from a paradigm of climate-driven vicariant diversification across an island archipelago. *Evolution* 73, 1151–1167.
- O'Connell, K.A., Smart, U., Sidik, I., Riyanto, A., Kurniawan, N., Smith, E.N., 2019. Diversification of bent-toed geckos (*Cyrtodactylus*) on Sumatra and West Java. *Mol. Phylogenet. Evol.* 134, 1–11.
- Oliver, P.M., Skipwith, P., Lee, M.S.Y., 2014. Crossing the line: increasing body size in a trans-Wallacean lizard radiation (*Cyrtodactylus*, Gekkota). *Biol. Lett.* 10, 20140479.
- Oliver, P.M., Travers, S.L., Richmond, J.Q., Pikacha, P., Fisher, R.N., 2018. At the end of the line: independent overwater colonizations of the Solomon Islands by a hyperdiverse trans-Wallacean lizard lineage (*Cyrtodactylus*: Gekkota: Squamata). *Zool. J. Linn. Soc.* 182, 681–694.
- Pease, J.B., Hahn, M.W., 2015. Detection and polarization of introgression in a five-taxon phylogeny. *Syst. Biol.* 64, 651–662.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.
- R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rambaut, A., Drummond, A.J., 2009. TRACER version 1.7.1. URL <http://tree.bio.ed.ac.uk/software/tracer/> [accessed 5 October 2021].

- Reilly, S.B., Stubbs, A.L., Karin, B.R., Bi, K., Arida, E., Iskandar, D.T., McGuire, J.A., 2019a. Leap-frog dispersal and mitochondrial introgression: phylogenomics and biogeography of *Limnonectes* fanged frogs in the Lesser Sundas Archipelago of Wallacea. *J. Biogeogr.* 46, 757–769.
- Reilly, S.B., Stubbs, A.L., Karin, B.R., Arida, E., Iskandar, D.T., McGuire, J.A., 2019b. Recent colonization and expansion through the Lesser Sundas by seven amphibian and reptile species. *Zool. Scr.* 48, 614–626.
- Reilly, S.B., Stubbs, A.L., Arida, E., Arifin, U., Bloch, L., Hamidy, A., Harmon, K., Hykin, S., Karin, B.R., Ramadhan, G., Iskandar, D.T., McGuire, J.A., 2020. New island records for anurans and squamates from the Lesser Sunda Archipelago. *Herpetol. Rev.* 51, 785–789.
- Reilly, S.B., Stubbs, A.L., Karin, B.R., Arifin, U., Arida, E., Iskandar, D.T., McGuire, J.A., 2021. Genetic divergence of the Sunda ratsnake (*Coelognathus subradiatus*) across the Lesser Sunda Islands (Squamata: Colubridae). *Amphib.-Reptil.* 42, 269–273.
- Reilly, S.B., Stubbs, A.L., Arida, E., Karin, B.R., Arifin, U., Kaiser, H., Bi, K., Iskandar, D.T., McGuire, J.A., 2022a. Phylogenomic analysis reveals dispersal-driven speciation and divergence with gene flow in Lesser Sunda flying lizards (genus *Draco*). *Syst. Biol.* 71, 221–241. <https://doi.org/10.1093/sysbio/syab043>.
- Reilly, S.B., Arifin, U., Stubbs, A.L., Karin, B.R., Kaiser, H., Frederick, J.H., Arida, E., Iskandar, D.T., McGuire, J.A., 2022b. Phylogenetic relationships of southern Wallacean ranid frogs (Anura: Ranidae: *Hylarana*). *Zootaxa* 5150, 591–599.
- Reilly, S.B., Karin, B.R., Stubbs, A.L., Arida, E., Arifin, U., Kaiser, H., Bi, K., Hamidy, A., Iskandar, D.T., McGuire, J.A., 2022c. Diverge and conquer: phylogenomics of southern Wallacean forest skinks (genus *Sphenomorphus*) and their colonization of the Lesser Sunda Archipelago. *Evolution* 76, 2281–2301.
- Ripley, B.D., Venables, W.N., 2002. *Modern Applied Statistics with S*. Springer, New York, NY.
- Riyanto, A., Grismer, L.L., Wood Jr., P.L., 2015. The fourth bent-toed gecko of the genus *Cyrtodactylus* (Squamata: Gekkonidae) from Java, Indonesia. *Zootaxa* 4059, 351–363.
- Riyanto, A., Mulyadi, McGuire, J.A., Kusri, M.D., Febylasmia, F., Basyir, I.H., Kaiser, H., 2017. A new small bent-toed gecko of the genus *Cyrtodactylus* (Squamata: Gekkonidae) from the lower slopes of Mount Tambora, Sumbawa Island, Indonesia. *Zootaxa* 4242, 517–528. <https://doi.org/10.11646/zootaxa.4242.3.5>.
- Roosmawati, N., Harris, R., 2009. Surface uplift history of the incipient Banda arc-continent collision: geology and synorogenic foraminifera of Rote and Savu Islands, Indonesia. *Tectonophysics* 479, 95–110.
- Sanchez, C., Carvalho, V.L., Kathriner, A., O'Shea, M., Kaiser, H., 2012. First report on the herpetofauna of the Oecusse District, an exclave of Timor-Leste. *Herpetol. Notes* 5, 137–149.
- Shaffer, H.B., Thompson, R.C., 2007. Delimiting species in recent radiations. *Syst. Biol.* 56, 896–906.
- Siler, C.D., Oaks, J.R., Esselstyn, J.A., Diesmos, A.C., Brown, R.M., 2010. Phylogeny and biogeography of Philippine bent-toed geckos (Gekkonidae: *Cyrtodactylus*) contradict a prevailing model of Pleistocene diversification. *Mol. Phylogenet. Evol.* 55, 699–710.
- Smith, M.A., 1925. A new ground-gecko (*Gymnodactylus*) from the Malay Peninsula. *J. Malays. Branch R. Asiat. Soc.* 3, 87.
- Tallowin, O.J., Tamar, K., Meiri, S., Allison, A., Kraus, F., Richards, S.J., Oliver, P.M., 2018. Early insularity and subsequent mountain uplift were complementary drivers of diversification in a Melanesian lizard radiation (Gekkonidae: *Cyrtodactylus*). *Mol. Phylogenet. Evol.* 125, 29–39.
- Tate, G.W., McQuarrie, N., Tiranda, H., van Hinsbergen, D.J., Harris, R., Zachariasse, W. J., Fellin, M.G., Reiners, P.W., Willett, S.D., 2017. Reconciling regional continuity with local variability in structure, uplift and exhumation of the Timor orogen. *Gondwana Res.* 49, 364–386.
- Taylor, E.H., 1962. New oriental reptiles. *Univ. Kansas Sci. Bull.* 43, 209–263.
- Uetz, P., Freed, P., Aguilar, R., Hošek, J., 2023. The Reptile Database, <http://www.reptile-database.org>, accessed 13 May 2023.
- Van Dam, M.H., Matzke, N.J., 2016. Evaluating the influence of connectivity and distance on biogeographical patterns in the southwestern deserts of North America. *J. Biogeogr.* 43, 1514–1532.
- Weber, M. 1890. Reptilia from the Malay Archipelago. 1. Sauria, Crocodylidae, Chelonia. In: *Zoologische Ergebnisse einer Reise in Niederländisch Ost-Indien* 1: 158–177. M. Weber (Ed.). E. J. Brill, Leiden.
- Wells, R.W., Wellington, C.R., 1985. A classification of the Amphibia and Reptilia of Australia. *Aust. J. Herpetol. (Supplement Series)* 1, 1–61.
- Wood Jr., P.L., Heinicke, M.P., Jackman, T.R., Bauer, A.M., 2012. Phylogeny of bent-toed geckos (*Cyrtodactylus*) reveals a west to east pattern of diversification. *Mol. Phylogenet. Evol.* 65, 992–1003. <https://doi.org/10.1016/j.ympev.2012.08.025>.
- Youmans, T.M., Grismer, L.L., 2006. A new species of *Cyrtodactylus* (Reptilia: Squamata: Gekkonidae) from the Seribu Archipelago, West Malaysia. *Herpetol. Nat. Hist.* 10, 61–70.
- Zhang, C., Rabiee, M., Sayyari, E., Mirarab, S., 2018. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinform.* 19, 153.