Phylogeny and cryptic diversification in Southeast Asian flying geckos

Rafe M. Brown [a,b,*], Cameron D. Siler [a,b], L. Lee Grismer [c], Indraneil Das [d], Jimmy A. McGuire [e,f]

[a] Biodiversity Institute, University of Kansas, Lawrence, KS 66045-7561, USA
[b] Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045-7561, USA
[c] Department of Biology, La Sierra University, Riverside, CA 92515, USA
[d] Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[e] Museum of Vertebrate Zoology, University of California, Berkeley, CA 94720-3160, USA
[f] Department of Integrative Biology, University of California, Berkeley, CA 94720-3160, USA

ABSTRACT

The closed-canopy forests of Southeast Asia are home to an impressive number of vertebrates that have independently evolved morphologies that enhance directed aerial descent (gliding, parachuting). These assemblages include numerous mammal, frog, snake, and lizard clades. Several genera of gekkonid lizards, in particular, have evolved specialized structures such as cutaneous expansions, flaps, and midbody patagia, that enhance lift generation in the context of unique gliding and parachuting locomotion. The genus *Ptychozoon* represents arguably the most morphologically extreme, highly specialized clade of gliding geckos. Despite their notoriety and celebrated locomotor ability, members of the genus *Ptychozoon* have never been the subject of a species-level molecular phylogenetic analysis. In this paper, we utilize molecular sequence data from mitochondrial and nuclear gene fragments to estimate the evolutionary relationships of this unique group of flying geckos. Capitalizing on the recent availability of genetic samples for even the rarest of known species, we include the majority of known taxa and use model-based phylogenetic methods to reconstruct their evolutionary history. Because one species, *P. kuhli*, exhibits an unusually wide distribution coupled with an impressive range of morphological variation, we additionally use intensive phylogeographic/population genetic sampling, phylogenetic network analyses, and Bayesian species delimitation procedures to evaluate this taxon for the possible presence of cryptic evolutionary lineages. Our results suggest that *P. kuhli* may consist of between five and nine unrecognized, distinct species. Although we do not elevate these lineages to species status here, our findings suggest that lineage diversity in *Ptychozoon* is likely dramatically underestimated.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Among Southeast Asia's myriad of highly specialized gliding vertebrates (Colbert, 1967; Russell, 1979a; Emerson and Koehl, 1990; Goldingay and Scheibe, 2000; Dudley et al., 2007), flying geckos of the genus *Ptychozoon* have inspired more awe[1] and speculation than perhaps any other group of Asian geckos (Annandale, 1904, 1905; Barbour, 1912; de Rooij, 1915; Tweedie, 1954; Tiwari, 1961; Pong, 1974; Tho, 1974; Taylor, 1975; Russell, 1979a,b). Most species in this small genus (seven species) are rarely encountered by biologists and, as a consequence, are poorly represented in natural history collections (Brown et al., 1997). The majority of species are known from the Southeast Asian mainland and adjacent Sundaland island archipelagos (Taylor, 1915, 1922a,b; Brown et al., 1997; Das and Vijayakumar, 2009), but three taxa, *P. kuhli*, *P. lionotum*, and *P. trinotaterra*, possess geographic ranges extending northward well into Indochina (Taylor, 1963; Inger and Colwell, 1977; Biswas and Sanyal, 1980; Das, 1994a,b; Brown, 1999; Pauwels et al., 2000; Stuart and Emmett, 2006; Grismer et al., 2008; Grismer, 2011), and a single species (*P. lionotum*) has been recorded from the Indian subcontinent (Pawar and Biswas, 2001; Venugopal, 2010).

Until recent taxonomic work clarified species boundaries (Brown et al., 1997; Brown, 1999; Das and Vijayakumar, 2009), specimen identification in museum collections was often tenuous (RMB, pers. obs), with numerous conflicting literature accounts resulting in general confusion regarding species identifications and geographic distributions (Cantor, 1847; Günther, 1864, 1885; Boulenger, 1885; Flower, 1896; Stejneger, 1907; Smith, 1930, 1935; Taylor, 1928, 1963; Wermuth, 1965; Dring, 1979; Bobrov,

---

* Corresponding author at: Biodiversity Institute, 1345 Jayhawk Blvd., Lawrence, KS 66045, USA. Fax: +1 785 864 5335.

E-mail addresses: rafe@ku.edu (R.M. Brown), camsiler@ku.edu (C.D. Siler), lgrismer@lasierra.edu (L. Lee Grismer), idas@ibec.unimas.my (I. Das), mcguirej@berkeley.edu (J.A. McGuire).

[1] "Tiny wing like flaps were on either side of the face, other broad winglike expansions were along the side of its body, a similar flap bordered the back of the thigh. The long tail had a series of frills on each side, as if scalloped lace had been sewn on the sides for decoration" (Taylor, 1975, p. 47).

1995; Nabhitabhata et al., 2000; Cox et al., 1998; Chan-ard et al., 1999). Only recently have naturalists developed a reliable understanding of the taxonomy, ranges of morphological variation within, and geographic distributions of each species (Manthey and Grossmann, 1997; Das, 2004; Malkmus et al., 2002; Nguyen et al., 2009; Grismer, 2011).

Flying geckos possess highly derived morphological specializations, which are present in only a few other groups of gekkonid lizards (Southeast Asian genus *Hemidactylus* and possibly the Malagasy genus *Uroplatus*). They are characterized by highly distinctive and elaborate cutaneous expansions bordering the nuchal region and anterior and posterior margins of the limbs, a broadly expanded cutaneous flap bordering both sides of the body (the midbody patagium), and a series of serrated, denticulate lobes extending the length of the tail (terminating in an ornate distal tail flap in some species; Manthey, 1985; Brown et al., 1997; Brown, 1999). Lizards of the genus *Ptychozoon* are so morphologically distinctive, that it has come as a surprise to herpetologists that recent molecular phylogenetic studies have found this genus to be nested within the morphologically generalized widespread Eurasian genus *Gekko* (Brown et al., 2012).

The hypothesized flight structures (the midbody patagia and other extensive cutaneous flaps throughout lateral surfaces of the body) of species in the genus actually have been the subject of historical debate. Although some authors have maintained that they serve no purpose[2] or contribute to camouflage ability by breaking up the body's outline against similarly-colored substrates (Gadow, 1901; Barbour, 1912; Pong, 1974; Medway, 1975; Vetter and Brodie, 1977; Kiew, 1987; Russell, 1979a), the ability of *Ptychozoon* species to glide or parachute has been well documented (Tweedie, 1954; Heyer and Pongsapipatana, 1970; Marcellini and Keefer, 1976; Young et al., 2002). More recent studies, however, have acknowledged that lift-generating surfaces other than just the patagia may contribute to directed aerial descent (Brown et al., 1997; Russell et al., 2001; Young et al., 2002; Dudley et al., 2007); these may include the ventrally flattened surfaces of the body, limbs, and tail, as well as extensive interdigital webbing of the hands and feet. In the absence of underlying skeletal elements or striated muscle, the patagia are not under voluntary muscle control (Russell, 1972, 1979a; Russell et al., 2001). Instead they curl under the body at rest, and only extend passively in response to air resistance during gliding or parachuting (Marcellini and Keefer, 1976; Brown et al., 1997; Young et al., 2002). Additionally, Brown et al. (1997) argued that the enlarged, imbricate scales on the dorsal surfaces of the midbody cutaneous expansion may assist in supporting the expanded patagia during flight by preventing inversion (see also Russell, 1979a; Russell et al., 2001).

To date, exemplars of only a few species have ever been included as outgroups in phylogenetic studies of presumably related genera (e.g., *Luperosaurus*, Brown et al., 2000a,b) or in higher-level gekkonid phylogenies (Brown et al., 2012). No species-level hypothesis of relationships within *Ptychozoon* has ever been attempted, although the relevant taxonomic literature contains a variety of predictions with regard to species phenotypic similarity and implied systematic affinities (Taylor, 1963; Russell, 1972, 1979a,b; Brown and Alcala, 1978; Dring, 1979; Manthey, 1985; Brown et al., 1997; Brown, 1999; Manthey and Grossmann, 1997).

We undertook the present study to estimate phylogenetic relationships of the members of the genus *Ptychozoon* using a multilocus dataset of mitochondrial and nuclear DNA sequences. Although some studies have hinted at the presence of geographic variation in various morphological characters that might eventually prove useful for diagnosing additional evolutionary lineages (Taylor, 1963; Brown et al., 1997, 1999; Das and Vijayakumar, 2009), no comprehensive analysis of morphology or molecular variation has ever been performed across the geographical range of the most widespread species, *P. kuhli* (Fig. 1). At present, an analysis of potential diagnostic morphological characters is beyond the scope of this paper and may be prevented by a lack of adequate numbers of specimens in museum collections. However the aim of this study was to use genetic data, phylogenetic network analysis, and a recently developed Bayesian lineage delimitation method (Yang and Rannala, 2010) to screen the widespread species *P. kuhli* for the presence of genetic partitions that might identify highly divergent lineages corresponding to putative taxonomic entities warranting additional scrutiny by taxonomists.

In this study we provide the first estimate of phylogenetic relationships for members of the genus *Ptychozoon*. Our results additionally suggest that the taxon currently recognized as *P. kuhli* consists of at least five (and possibly as many as nine) divergent lineages (possible new species), and this taxon should be the subject of a future comprehensive taxonomic review aimed at exploring patterns of morphological variation in this group and its correlation with patterns of genetic variation identified here.

2. Materials and methods

2.1. Taxon sampling and data collection

Ingroup sampling included 32 individuals collected from numerous localities throughout Sundaland (the land-bridge islands adjacent to the Malay Peninsula and the Asian mainland) and the island archipelagos of Indonesia and the Philippines. We collected or gained access to genetic samples of six of the seven currently recognized species of *Ptychozoon* (Fig. 1; Table 1); to the best of our knowledge, no tissues have ever been collected for *Ptychozoon nicobarensis* (Das and Vijayakumar, 2009). To assess the monophyly of the genus and investigate appropriate outgroup taxa, a broad sampling from the family Gekkonidae was included, as well as a single outgroup sample from the Gekkotan family Phyllodactylidae (Table 1).

We extracted genomic DNA from liver tissues stored in 95–100% ethanol using the guanidine thiocyanate method of Esselstyn et al. (2008). We sequenced the mitochondrial gene NADH Dehydrogenase Subunit 2 (ND2) and components of three flanking transfer RNA genes (tRNA^{trp}, tRNA^{ala}, tRNA^{asn}) using the primers and protocols of Brown et al. (2009a, 2012) for 31 vouchered specimens (Table 1). For the same specimens, plus one additional vouchered species, we also sequenced the nuclear Phosducin (PDC) gene using the primers and protocols of Gamble et al. (2008, 2011). Thermal profiles and PCR and sequencing protocols followed Siler et al. (2012). Amplified products were visualized on 1.5% agarose gels. PCR products were purified with 1 μL of a 20% dilution of ExoSAP-IT (US78201, Amersham Biosciences, Piscataway, NJ). Cycle sequencing reactions were run using ABI Prism BigDye Terminator chemistry (Ver. 3.1; Applied Biosystems, Foster City, CA), and purified with Sephadex (NC9406038, Amersham Biosciences, Piscataway, NJ) in Centri-Sep 96 spin plates (CS-961, Princeton Separations, Princeton, NJ). Purified products were analyzed with an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems). Continuous gene sequences were assembled and edited using Sequencher 4.8 (Gene Codes Corp., Ann Arbor, MI). To these

---

[2] Barbour (1912), on the topic of *Ptychozoon* cutaneous expansions, stated "They may possibly assist in rendering the creature less conspicuous at certain rare momentary crises. I believe it is far more probable that these developments serve at present no purpose whatever. They may be taken to represent, perhaps, the result of an inherent tendency to vary in a definite direction, coupled with what Cope has called superabundant growth force."
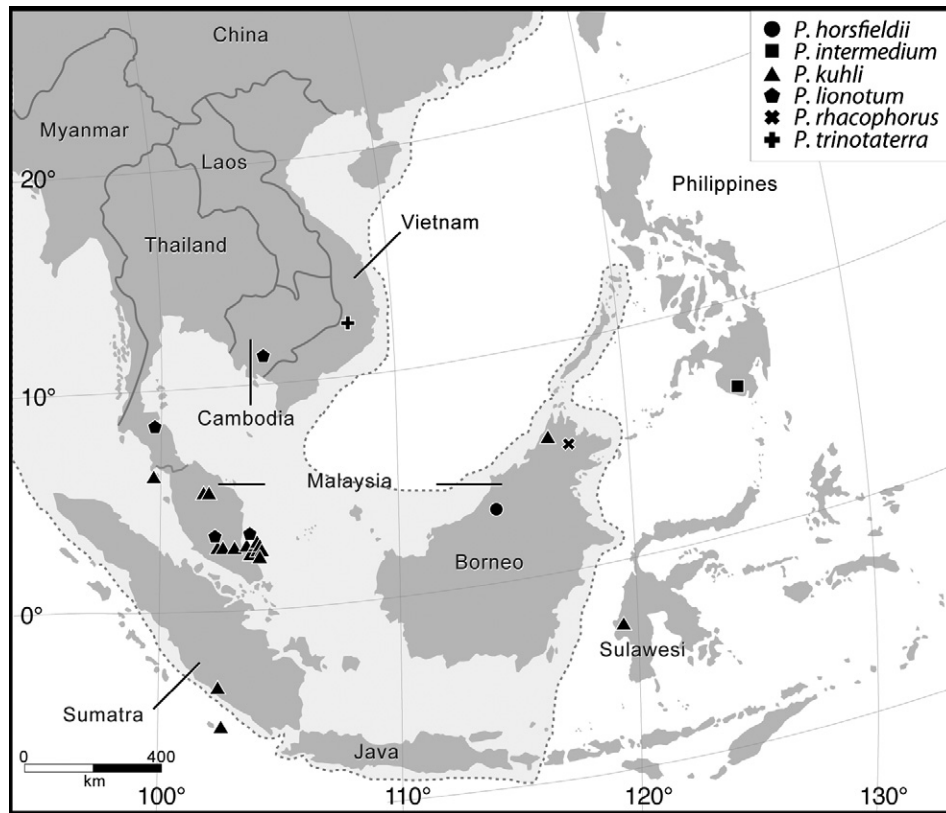
Fig. 1. Sampling for this study, with various symbols representing the species included (key).

data, we added 29 published sequences of other gekkonid taxa as outgroup samples (Bauer et al., 2008; Albert et al., 2009; Siler et al., 2012). All sequences were deposited in GenBank (Table 1).

2.2. Sequence alignment and phylogenetic analyses

Initial alignments were produced in Muscle (Edgar, 2004) with minimal manual adjustments. To assess phylogenetic congruence between the mitochondrial and nuclear data, we inferred the phylogeny for each gene independently using maximum likelihood and Bayesian analyses and assessed all strongly supported nodes for differences in relationships between mitochondrial and nuclear gene partitions. After observing no statistically significant incongruence between datasets, we concatenated our data for subsequent analyses. Exploratory analyses of the combined dataset of 61 individuals (including outgroup taxa with missing data for PDC) and a reduced dataset of individuals with no missing data exhibited identical relationships; we therefore chose to include all available data for subsequent analyses of the concatenated dataset.

Partitioned Bayesian analyses were conducted in MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). Both datasets (ND2, PDC) were partitioned by codon position and the three flanking tRNAs were analyzed as a single subset. The Akaike Information Criterion (AIC), as implemented in jModeltest v0.1.1 (Posada, 2008), was used to select the best model of nucleotide substitution for each partition (Table 2). A rate multiplier model was used to allow substitution rates to vary among subsets, and default priors were used for all model parameters. We ran eight independent MCMC analyses, each with four Metropolis-coupled chains, an incremental heating temperature of 0.02, and an exponential distribution with a rate parameter of 25 as the prior on branch lengths (Marshall, 2010). All analyses were run for 20 million generations,

with parameters and topologies sampled every 5000 generations. We assessed stationarity with Tracer v1.4 (Rambaut and Drummond, 2007) and confirmed convergence with AWTY (Wilgenbusch et al., 2004). Stationarity was achieved after 3 million generations (i.e., the first 15%), and we conservatively discarded the first 20% of samples as burn-in.

Partitioned maximum likelihood (ML) analyses were conducted in RAxMLHPC v7.0 (Stamatakis, 2006) on the concatenated dataset with the same partitioning strategy as for the Bayesian analysis. The more complex model (GTR + I + Γ) was used for all subsets (Table 2), and 100 replicate ML inferences were performed for each analysis. Each inference was initiated with a random starting tree and nodal support was assessed with 100 bootstrap pseudoreplicates (Stamatakis et al., 2008).

2.3. Population structure

In order to visualize population genetic structure and possible reticulating relationships within the widespread (Figs. 1 and 2) species, *P. kuhli*, the NeighborNet algorithm (Bryant and Moulton, 2004) was implemented in the program SplitsTree version 4.10 (Huson, 1998; Huson and Bryant, 2006) to generate phylogenetic networks for the ND2 and PDC datasets, independently. To assess the support for the observed structure, a bootstrap analysis was conducted with 1000 replicates. Finally, the pairwise homoplasy index (PHI) statistic (Bruen et al., 2006) was calculated in SplitsTree 4.10 to test for recombination within the mitochondrial and nuclear loci.

2.4. Bayesian delimitation of putative species

With no *a priori* hypotheses concerning species diversity within the *Ptychozoon kuhli* Complex, and low numbers of individuals

Table 1

Summary of gekkonid specimens corresponding and genetic samples included in the study. ACD = Arvin C. Diesmos field number, uncataloged specimen deposited at the National Museum of the Philippines; AMB = Aaron M. Bauer field series; AMS = Australian Museum, Sydney, Australia; CAS = California Academy of Sciences Herpetological Collections; CMNH = Cincinnati Museum of Natural History (Cincinnati Museum Center); DSM = David McLeod field series, specimen deposited Chulalongkorn University (Thailand) reference collection; HOFH = Hidetoshi Ota genetic samples deposited in the Museum of Nature and Human Activities, University of Hyogo, Japan; JAM = Jim McGuire field series, specimen deposited in the Forest Research Institute of Malaysia reference collection); JB = Jon Boone captive collection; JFBM = James Ford Bell Museum of Natural History; KU = University of Kansas Natural History Museum; LSUHC = La Sierra University Herpetological Collections; UNIMAS P = Pui Yong Min field series, deposited at Universiti Malaysia Sarawak, Kota Samarahan (UNIMAS); PNM = National Museum of the Philippines (Herpetology collection); RMB = Rafe Brown field number, uncataloged specimen deposited at the National Museum of the Philippines; TNHC = Texas Natural History Collections of the Texas Memorial Museum, University of Texas at Austin; USNM = United States National Museum; ZRC = Zoological Reference Collection of the Raffles Museum Collections at the National University of Singapore; ∗ = no voucher/locality information provided by source publication.

Figure code	Genus	Species	Voucher number	Country	Island/landmass	General locality	Specific locality	ND2	PDC
N/A	*Ptychozoon*	*horsfieldii*	ZRC 2.5339	Malaysia	Borneo	Sarawak	Lambir Hills	JQ437907	JQ437949
N/A	*Ptychozoon*	*intermedium*	PNM 2501	Philippines	Mindanao Island	Davao City Province	Municipality of Calinan, Barangay Malagos	JQ437908	JQ437950
N/A	*Ptychozoon*	*intermedium*	CMNH 4747	Philippines	Mindanao Island	Davao City Province	Municipality of Calinan, Barangay Malagos	JQ437909	JQ437951
N/A	*Ptychozoon*	*intermedium*	TNHC 56144	Philippines	Mindanao Island	Davao City Province	Municipality of Calinan, Barangay Malagos	JQ437911	JQ437953
16	*Ptychozoon*	*kuhli*	LSUHC 3835	Malaysia	Malay Peninsula	Pahang	Pulau Tioman, Tekek-Juara Trail	JQ437935	JQ437977
14	*Ptychozoon*	*kuhli*	LSUHC 4679	Malaysia	Malay Peninsula	Pahang	Pulau Tioman, Tekek-Juara Trail	JQ437918	JQ437960
15	*Ptychozoon*	*kuhli*	LSUHC 6433	Malaysia	Malay Peninsula	Pahang	Pulau Tioman, Tekek-Juara Trail	JQ437931	JQ437973
18	*Ptychozoon*	*kuhli*	LSUHC 5042	Malaysia	Malay Peninsula	Pahang	Pulau Tioman, Sungai Mentawak	JQ437930	JQ437972
8	*Ptychozoon*	*kuhli*	LSUHC 7141	Malaysia	Malay Peninsula	Kedah	Pulau Langkawi, Air Terjun below Telaga Tuju	JQ437924	JQ437966
9	*Ptychozoon*	*kuhli*	LSUHC 4819	Malaysia	Malay Peninsula	Selangor	Kepong	JQ437920	JQ437962
10	*Ptychozoon*	*kuhli*	LSUHC 3518	Malaysia	Malay Peninsula	Pahang	Pulau Tioman	JQ437936	JQ437978
22	*Ptychozoon*	*kuhli*	LSUHC 5587	Malaysia	Malay Peninsula	Johor	Pulau Babi, Besar	JQ437939	JQ43798
11	*Ptychozoon*	*kuhli*	LSUHC 5055	Malaysia	Malay Peninsula	Pahang	Pulau Tulai	JQ437929	JQ437971
12	*Ptychozoon*	*kuhli*	LSUHC 6273	Malaysia	Malay Peninsula	Johor	Pulau Tulai	JQ437938	JQ437980
17	*Ptychozoon*	*kuhli*	LSUHC 5199	Malaysia	Malay Peninsula	Johor	Pulau Sembilan	JQ437925	JQ437967
21	*Ptychozoon*	*kuhli*	LSUHC 6321	Malaysia	Malay Peninsula	Johor	Pulau Tinggi, Pasir Panjang	JQ437934	JQ437976
5	*Ptychozoon*	*kuhli*	ZRC 2.5332	Malaysia	Borneo Island	Sarawak	Kapit. Kelep, Asap	JQ437928	JQ437970
6	*Ptychozoon*	*kuhli*	RMB T-1134	"Malaysia"	"Malay Peninsula"	"Pahang"	Pet trade sample: reportedly "Gua Musang"	JQ437919	JQ437961
7	*Ptychozoon*	*kuhli*	RMB T-1139	"Malaysia"	"Malay Peninsula"	"Pahang"	Pet trade sample: reportedly "Gua Musang"	JQ437937	JQ437979
1	*Ptychozoon*	*kuhli*	RMB T-0001	"Indonesia"	"Java Island"	Unknown	Pet trade sample: reportedly "Java"	JQ437927	JQ437969
20	*Ptychozoon*	*kuhli*	LSUHC 5708	Malaysia	Malay Peninsula	Pahang	Pulau Aceh	JQ437932	JQ437974
13	*Ptychozoon*	*kuhli*	LSUHC 8024	Malaysia	Malay Peninsula	Johor	Pulau Pemanggil	JQ437933	JQ437975
19	*Ptychozoon*	*kuhli*	LSUHC 7640	Malaysia	Malay Peninsula	Johor	Endau-Rompin	JQ437926	JQ437968
3	*Ptychozoon*	*kuhli*	MVZ 239588	Indonesia	Sumatra Island	Kabupaten Bengkulu	Kecematan Kepahiang, 46 km E of Bengkulu, Cagar Alam Tabapenangjung vicinity Malakoni village	JQ437922	JQ437964
2	*Ptychozoon*	*kuhli*	MVZ 239358	Indonesia	Enggano Island	Kecematan Enggano		JQ437921	JQ437963
4	*Ptychozoon*	*kuhli*	JAM 6445	Indonesia	Sulawesi Island	Sulawesi Barat Province, Kabupaten Mumuju	Kecamatan Tapalang, Desa Takandeang,	JQ437923	JQ437965
N/A	*Ptychozoon*	*lionotum*	JAM 1426	Malaysia	Malay Peninsula	Selangor	Ulu Gombak Field Studies Centre, km 30N of Kuala Lumpur via Rt. 68	JQ437916	JQ437958
N/A	*Ptychozoon*	*lionotum*	LSUHC 6437	Malaysia	Malay Peninsula	Pahang		JQ437917	JQ437959
N/A	*Ptychozoon*	*lionotum*	DSM 798	Thailand	Southern Malay Peninsula	Nakhon Si Thammarat Province	Khao Luang National Park	JQ437914	JQ437956
N/A	*Ptychozoon*	*lionotum*	FMNH 261852	Cambodia	Asian mainland	Kampong Speu Province	Phnom Sruoch District	JQ437915	JQ437957

Table 1 (continued)

Figure code	Genus	Species	Voucher number	Country	Island/ landmass	General locality	Specific locality	ND2	PDC
N/A	*Ptychozoon*	*rhacophorus*	UNIMAS P-0501	Malaysia	Borneo	Sarawak	Gunung Penrissen	JQ437913	JQ437955
N/A	*Ptychozoon*	*trinotaterra*	ROM 31912	Vietnam	Asian mainland	Yok Don Province	Yok Don National Park	JQ437912	JQ437954
N/A	*Gehyra*	*australis*	AMS 139934	Australia	Australia	Western Australia, Nimalawa	El Questro Station, Jackeroos Waterhole,	JN019081	JN019113
N/A	*Gehyra*	*mutilata*	AMB 7515	Sri Lanka	Sri Lanka			JN019082	JN019114
N/A	*Hemidactylus*	*aquilonius*	CAS 206649	Myanmar	Asian mainland	Sagaing Division	Alaungdaw Kathapa National Park	EU268373	–
N/A	*Cyrtodactylus*	*annulatus*	KU 314944	Philippines	Mindanao Island	Agusan del Sur Province	Municipality of San Francisco, Barangay Kaimpugan	GU366088	–
N/A	*Cyrtodactylus*	*philippinicus*	KU 304784	Philippines	Babuyan Claro Island	Cagayan Province	Municipality of Calayan, Barangay Babuyan Claro	GU550900	–
N/A	*Luperosaurus*	*joloensis*	KU 314947	Philippines	Mindanao Island	Zamboanga City	Barangay Pasanonca	JQ437900	See Dryad Submission
N/A	*Luperosaurus*	*cumingii*	TNHC 61910	Philippines	Luzon Island	Albay Province	Municipality of Tiwi, Mt. Malinao	JQ437902	–
N/A	*Luperosaurus*	*angliit*	KU 322189	Philippines	Luzon Island	Aurora Province	Municipality of Baler, Barangay Zabali	JQ437903	JQ439744
N/A	*Pseudogekko*	*smaragdinus*	KU 302819	Philippines	Polillo Island	Quezon Province	Municipality of Polillo, Barangay Pinaglubayan	JQ437898	JQ437941
N/A	*Pseudogekko*	*compressicorpus*	KU 324426	Philippines	Bohol Island	Bohol Province	Municipality of Sierra Bullones, Barangay Danicop	JQ437897	JQ437940
N/A	*Lepidodactylus*	*herrei*	RMB 4330	Philippines	Leyte Island	Leyte Province	Municipality of Baybay	JQ173539	–
N/A	*Lepidodactylus*	*moestus*	USNM 521730	Palau	Ngerur Island			JN019079	JN019111
N/A	*Gekko*	*athymus*	KU 314944	Philippines	Palawan Island	Palawan Province	Municipality of Brooke's Point, Barangay Mainit	JQ173403	JQ173559
N/A	*Gekko*	*crombota*	KU 304825	Philippines	Babuyan Claro Island	Cagayan Province	Municipality of Calayan, Barangay Babuyan Claro	JQ173410	JQ173549
N/A	*Gekko*	*romblon*	KU 315348	Philippines	Tablas Island	Romblon Province	Municipality of Calatrava, Barangay Balogo	JN710497	JN710506
N/A	*Gekko*	*mindorensis*	KU 302668	Philippines	Mindoro Island	Oriental Mindoro Province	Municipality of Bongabong, Barangay Formon	JN710490	JN710501
N/A	*Gekko*	*monarchus*	ACD 1278	Philippines	Palawan Island	Palawan Province	Municipality of Brooke's Point, Mt. Mantalingajan	JQ173501	JQ173594
N/A	*Gekko*	*smithii*	LSUHC 6095	Malaysia	Malay Peninsula	Pahang	Pekan	JQ173534	JQ173616
N/A	*Gekko*	*gecko*	CAS 204952	Myanmar	Asian mainland	Ayeyarwady Division	Myaungmya District	JQ173416	–
N/A	*Gekko*	*chinensis*	LSUHC 4210	China	Hainan Island	Wuzhi Shan		JQ173409	JQ173547
N/A	*Gekko*	*japonicus*	HOFH 10061402	Japan	Ryukyu Islands			JQ173424	JQ173558
N/A	*Gekko*	*swinhonis*	NNU Z 20051124.001	China	Asian mainland	Szechuan	Chengdu	JN019061	JN019095
N/A	*Gekko*	*subpalmatus*	AMB 6567	China	Asian mainland	Szechuan	Chengdu	JN019063	JN019097
N/A	*Gekko*	*hokouensis*	HOFH 89053103	China	Orchid Island	Lanyu Township		JQ173422	JQ173556
N/A	*Gekko*	*vittatus*	USNM 533255	Solomon Islands	Santa Cruz Island	Temotu	Luesalo	JN019073	JN019106
N/A	*Gekko*	*petricolus*	JB 70	Unknown*	Pet trade			JN019066	JN019100
N/A	*Gekko*	*badenii*	JB 13	Unknown*	Pet trade			JN019065	JN019099
N/A	*Gekko*	*grossmanni*	JFBM 9	Unknown*	Pet trade			JN019064	JN019098

sampled per population, we approached the question of species-level diversity from the most liberal perspective; we treated each of the nine sampled localities as separate populations. We then explored support for hypothesized species boundaries using the program Bayesian Phylogenetics and Phylogeography (BPP v.2.0; Yang and Rannala, 2010). In order to provide an objective starting topology for BPP, we used the multi-species coalescent model implemented in the program *BEAST (v1.6.2; Drummond and Rambaut, 2007; Heled and Drummond, 2010) to estimate relationships among the nine divergent populations observed in phylogenetic

Table 2

Models of evolution selected by AIC and applied for partitioned, model-based phylogenetic analyses.

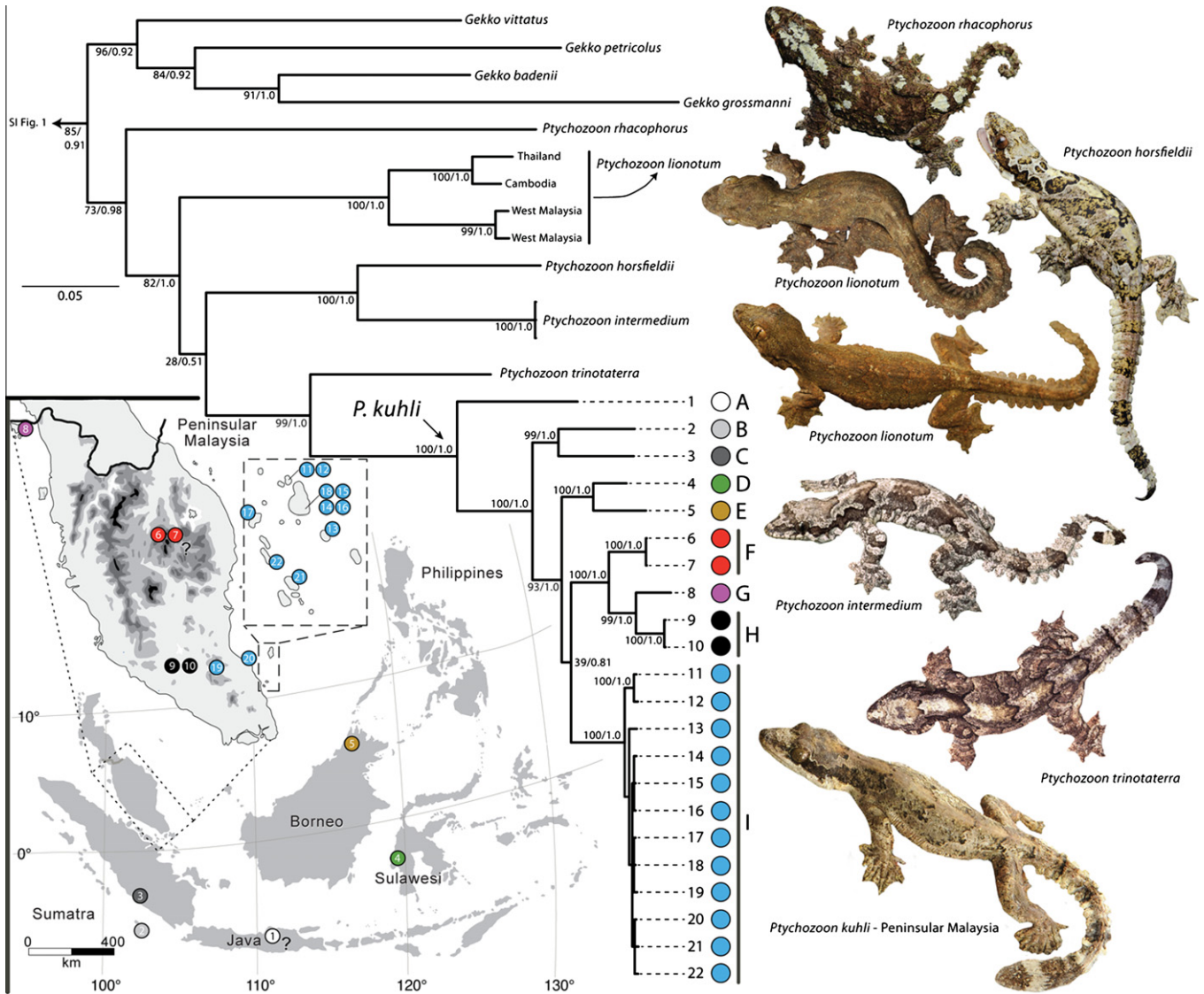| Partition	AIC model	Number of characters
NADH 2, 1st codon position	HKY + I + Γ	346
NADH 2, 2nd codon position	HKY + Γ	346
NADH 2, 3rd codon position	GTR + Γ	346
All tRNAs (Trp, Ala, Asn)	HKY + Γ	223
Phosducin, 1st codon position	HKY + I	137
Phosducin, 2nd codon position	HKY + I	136
Phosducin, 3rd codon position	GTR + I	136

Fig. 2. Hypothesized species level relationships of the genus *Ptychozoon*, illustrated by the maximum clade credibility tree resulting from Bayesian analyses. Nodes supported by ⩾0.95 Bayesian posterior probabilities and ⩾70% ML bootstrap support were considered significantly supported. Numbered and differently colored terminals within *P. kuhli* correspond to numbered localities on the map and the same color scheme is utilized in Fig. 3. Letters refer to inferred genetic lineages (hypothesized species) from the BPP analyses (Fig. 3).

analyses. Individual sequences were assigned to lineages on the basis of sampling locality, which also corresponded to well-supported lineages observed in phylogenetic analyses (Fig. 2).

For *BEAST analyses, we applied the following settings: (1) separate GTR + Γ nucleotide substitution models and lognormal-distributed relaxed clock models to nuclear and mitochondrial subsets, (2) a mean rate of the mitochondrial relaxed clock hyper-parameter to 1.0, (3) estimates of the rate of the nuclear relaxed clock relative to the mitochondrial clock, (4) a uniform prior (U(0, 2.0)) on the mean of the lognormal-distributed nuclear relaxed-clock hyper-parameter, (5) an exponentially distributed prior (Exp(20)) on the standard deviation of both lognormal-distributed relaxed-clock hyper-parameters, and (6) default priors for the parameters of both GTR models. For each of the two loci, we implemented appropriate ploidy levels and random starting trees to infer gene trees conditional on the species tree. For the species tree we implemented a Yule process prior, and constrained, constant effective population size (N_e) along each branch. We chose the prior on N_e in an effort to choose a model with fewer parameters. With only two loci, our goal was to keep the model

simple while still capturing the important aspects of the system. To investigate support for species relationships under a different tree prior, we reanalyzed the data under a piecewise linear prior, or unconstrained N_e across all branches on the tree.

We ran two independent analyses for 80 million generations, sampling every 15,000 generations. Using the program Tracer (Rambaut and Drummond, 2005), we assessed stationarity and convergence of each run by plotting all parameters and likelihood, prior, and posterior scores over generations. Both of our analyses showed patterns consistent with convergence, and we observed effective sample sizes >200 after conservatively removing the first 20 million generations as burn-in.

Using the topology observed in the resulting chronogram from *BEAST analyses, we evaluated the statistical support for our liberal hypothesis of putative species boundaries by employing the model-based program BPP. We phased the nuclear data, using the program PHASE v. 2.1.1 (Stephens and Donnelly, 2003), and retained haplotypes with the highest probabilities for subsequent analyses. For BPP analyses, we used the mitochondrial (ND2) data and nuclear (PDC) allelic data following the approach advocated by Setiadi

et al. (2011). We assigned samples to one of the nine inferred lineages. Following Setiadi et al. (2011), we accommodated expected differences between the effective population size (N_e) of mtDNA and autosomal DNA, and we incorporated two additional parameters into BPP analyses as follows: (1) heredity parameter with gamma prior G[1.39, 2.22], and (2) locusrate parameter calculated as the largest Jukes Cantor-corrected sequence divergence from the outgroup sequence.

Analyses were run for 500,000 generations, sampling every 50 generations, with a burn-in of 10,000. The "0" algorithm with the fine-tuning parameter $\varepsilon = 15$ was employed after preliminary runs employing lower (5, 10) and greater (20, 25) values of ε had no major impact on resulting inferences of species diversity. Following the methods of Leaché and Fujita (2010), we explored the impact of prior regime (ancestral population size [θ] and root age [τ]) on speciation probabilities. Three prior settings were employed: (1) a relatively large ancestral population with shallow divergences ($\theta = 1, 10$; $\tau = 2, 2000$; both prior means = 0.1 and variance = 0.01), (2) a relatively large ancestral population with deep divergences ($\theta = 1, 10$; $\tau = 1, 10$; both prior means = 0.001 and variance = 5×10^{-7}), and (3) a relatively small ancestral population and shallow divergences ($\theta = 2, 2000$; $\tau = 2, 2000$).

3. Results

3.1. Taxon sampling, data collection, and sequence alignment

The complete, aligned matrix contains 32 samples of *Ptychozoon*, representing six of the seven currently recognized species. Twenty-nine additional samples are included as outgroups from the families Gekkonidae and Phyllodactylidae, including representative taxa of the following genera: *Cyrtodactylus*, *Gehyra*, *Gekko*, *Hemidactylus*, *Lepidodactylus*, *Luperosaurus*, *Pseudogekko*, and *Tarentola*. Following initial unrooted analyses, and gekkonid phylogenetic analyses (Gamble et al., 2011, 2012; Brown et al., 2012) we rooted the tree using the representative sample of *Tarentola mauritanica* (Phyllodactylidae). Variable and parsimony-informative characters are: 908 and 824 of 1208 (ND2); 77 and 49 of 419 (PDC).

3.2. Phylogenetic analyses

Analyses of the combined data result in topologies with high ML bootstrap support and posterior probabilities between species within and between the majority of clades in the inferred phylogeny, with general topological patterns congruent across these analyses (Fig. 2). The focal taxa from the genus *Ptychozoon* were supported to be monophyletic (Fig. 2). As observed in a recent study (Brown et al., 2012), the genus *Ptychozoon* is supported to be sister to a clade consisting of some members of the genus *Gekko* (*G. badenii*, *G. grossmanni*, *G. petricolus*, and *G. vittatus*). As observed by Brown et al. (2012) the enigmatic species, *Ptychozoon rhacophorus*, is strongly supported as the sister species to all other sampled

taxa (Fig. 2). The four sampled populations of *P. lionotum* were recovered as part of two divergent clades, with one clade consisting of populations from Peninsular Malaysia and the other clade consisting of populations from Thailand and Laos (Fig. 2). Members of these two highly divergent clades were separated by 15.2–16.0% uncorrected pairwise ND2 sequence divergence.

A strongly supported clade consisting of *P. horsfieldii* + *P. intermedium* was observed to be sister to a well supported clade of *P. trinotaterra* + *P. kuhli* (Fig. 2), albeit with weak support (likelihood bootstrap proportion = 28%; Bayesian poster probability 0.51). The widespread species *P. kuhli* was recovered as a highly structured, clade, consisting of nine well supported, divergent (Table 3) lineages (Fig. 2). Finally, our results confirm recent findings (Brown et al., 2012) that the genus *Ptychozoon*, and some species of the genus *Luperosaurus* are nested within *Gekko* (SI Fig. 1).

3.3. Phylogenetic networks of Ptychozoon kuhli

Tests of recombination within the mitochondrial or nuclear locus were not significant (ND2 PHI value = 0.746, PDC PHI value = 1.0), and we therefore felt justified in exploring phylogenetic network analysis for each locus. The analysis of the nuclear locus (PDC) revealed little structure (Fig. 3A); however, the analysis of the mitochondrial locus (ND2) revealed nine highly divergent, well-supported, groups and a high degree of structure (Fig. 3A) corresponding to divergent lineages (Table 3) identified in the phylogenetic analysis of concatenated mtDNA + nDNA data (Fig. 2).

3.4. Bayesian inference of potential species boundaries within Ptychozoon kuhli

Relationships inferred in our *BEAST analyses mirror those of concatenated gene tree phylogenetic analyses, with support for the same topological relationships among the nine well-supported lineages of the *P. kuhli* Complex (Figs. 2 and 3B); we took this topology as our user-specified guide tree for subsequent BPP analyses. The choice of prior on *Ne* had no impact on the resulting relationships between species (results not shown). Taking an initial liberal approach to identifying species boundaries, and starting with the assumption that all nine genetically divergent (Table 3) lineages could conceivably be unique species, Bayesian species delimitation results for *P. kuhli* support five species with speciation probabilities of 1.0 on the species guide tree (Fig. 3B). The remaining three putative "speciation events" on the species guide tree receive low to moderate support (Fig. 3B). These results were not affected by varying prior distributions for θ or τ (Fig. 3B). The five putative entities supported in the PB&B analysis correspond to a lineage sampled from the pet trade (reportedly from Java Island, Indonesia), a clade consisting of populations from Enggano and Sumatra islands, a clade consisting of samples from Sulawesi and Borneo islands, a lineage consisting of populations from southeast Peninsular Malaysia and the surrounding coastal islands of the

Table 3
Uncorrected pairwise sequence divergence (%) for mitochondrial data for the nine supported lineages within the *Ptychozoon kuhli* Complex (Fig. 1). Percentages on the diagonal represent intraspecific genetic diversity, when sampling permits (bolded for emphasis).

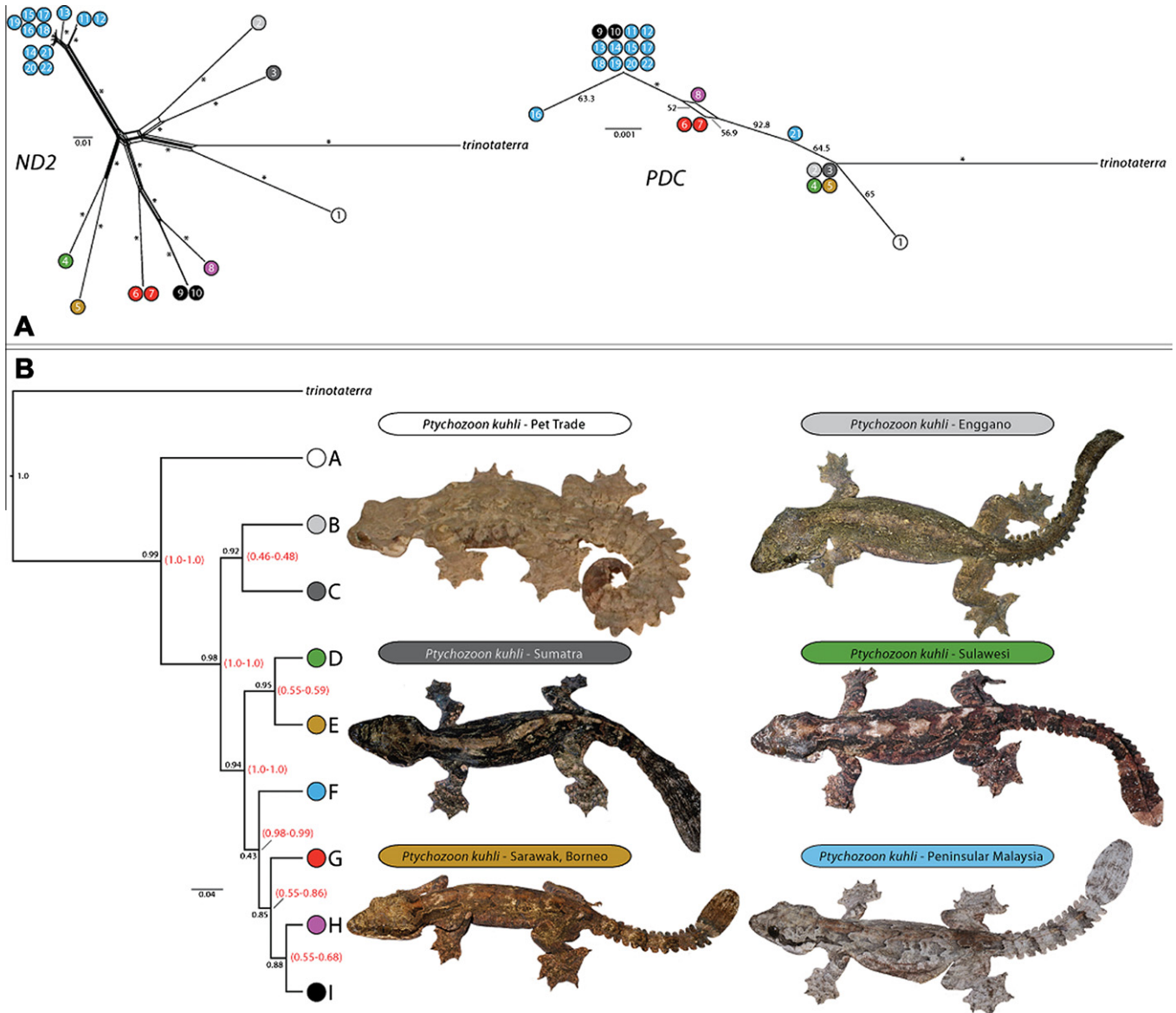|	A	B	C	D	E	F	G	H	I
A	–								
B	17.2	–							
C	17.8	11.6	–						
D	16.4	13.4	13.1	–					
E	16.9	14.7	14.1	7.5	–				
F	16.6	12.9	12.3	10.3	11.2	**0.0**			
G	17.1	14.1	13.5	10.9	11.6	7.8	–		
H	16.7	13.9–14.0	13.9–14.0	10.6	11.5	7.3–7.4	5.4	**0.0**	
I	16.2–17.0	12.6–12.9	12.6–13.3	9.4–10.0	10.7–11.0	10.1–10.5	11.2–11.6	11.0–11.5	**0.1–1.8**

Fig. 3. SplitsTree networks (Huson and Bryant, 2006) (A) for two loci (same number and color scheme as that presented in Fig. 2) and *BEAST (v1.6.2; Heled and Drummond, 2010) topology (B) with results of Bayesian lineage delimitation analyses inferred by BPP. Posterior probabilities of inferred splits are provided at each node, with ranges representing split probabilities produced by variance in prior settings for ancestral population size and relative divergence times.

Seribuat Archipelago, and a lineage consisting of trade samples reportedly from north-central Peninsular Malaysia, together with samples from southwestern Peninsular Malaysia and the Langkawi Islands adjacent to northwest Peninsular Malaysia (Fig. 3B).

4. Discussion

4.1. Phylogeny of Ptychozoon and implications for morphological novelty in flying geckos

Species level relationships within the genus *Ptychozoon*, inferred from concatenated Bayesian and Maximum Likelihood gene trees (Fig. 2) confirm several earlier character-based predictions, but also contain a few surprises. The finding that *P. rhacophorus* is phylogenetically distinct from (but sister to) the remaining known taxa is not surprising given its distinctive morphology (Russell, 1972; Manthey, 1985; Brown et al., 1997; Brown and Diesmos, 2000). This species is smaller than all other members of the genus, possesses a greater

density of irregular, ornate, tuberculate scalation on dorsal surfaces of the body, and lacks multiple conspicuous characteristics (the nuchal/cephalic cutaneous expansion, expanded tail terminus, and dorsal enlarged imbricate support scales of the patagial cutaneous expansion) shared by all other species in the genus (Russell, 1972; Manthey, 1985; Brown et al., 1997). We are not surprised by the close relationship between Bornean *P. horsfieldii* and southern Philippine *P. intermedium*. Not only are these two species endemic to geographically very proximate landmasses (Fig. 1), but both uniquely share numerous distinctive characters (posterior angling of denticulate tail lobes, decreased distal tail lobe size, tail terminus ending in a minute flap, absence of caudal lobe fusion on proximate margin of tail terminus, and separation between the femoral and precloacal pore-bearing scales; Brown et al., 1997; Brown, 1999). In contrast, we were surprised to find the species couplet *P. horsfieldii* + *P. intermedium* recovered between *P. lionotum* and the clade consisting of *P. trinotaterra* + *P. kuhli* (albeit with low nodal support; Fig. 2). Previous authors (Boulenger, 1885; de Rooij, 1915; Smith 1930, 1935; Taylor, 1963; Brown et al., 1997) have noted the close

morphological similarity between *P. lionotum* and *P. kuhli* (and, by implication, *P. trinotaterra*; Brown, 1999), and we would find it surprising if the suite of morphological character states seemingly uniting these two species and *P. trinotaterra* were to have evolved convergently. Shared character states include denticulate tail lobes that do not diminish in size posteriorly, presence of a greatly expanded terminal tail flap, lobe fusion at the proximate margin of the tail terminus, and continuous precloacofemoral pore bearing scales (Brown et al., 1997; Brown, 1999). The nodal support for the placement of the *P. horsfieldii* + *P. intermedium* clade (Fig. 2) suggests to us that a conservative interpretation might be to consider these well supported clades (1: *P. lionotum*; 2: *P. horsfieldii* + *P. intermedium*; 3: *P. trinotaterra* + *P. kuhli*) to be an unresolved trichotomy. We would not be surprised to find with future analyses (preferably involving additional unlinked loci) that actual relationships among species of *Ptychozoon* are (*P. rhacophorus*, ((*P. horsfieldii* + *P. intermedium*), (*P. lionotum*, (*P. trinotaterra* + *P. kuhli*)))). The unsampled species *P. nicobarensis* is most likely closely related to *P. kuhli*, from which it is only slightly distinguishable on the basis of phenotypic traits discussed by Das and Vijayakumar (2009).

4.2. Taxonomic implications of genetic divergences, phylogenetic network analyses and Bayesian lineage delimitation in P. kuhli

The results of our Bayesian species delimitation analysis revealed strong support for subdivision of the widespread, morphologically variable *Ptychozoon kuhli* into five genetically distinct units, or putative species, with high "speciation" probabilities, some of which contain additional highly divergent gene lineages. Although the same nine genetically distinct lineages were identified in phylogenetic analyses (Fig. 2), phylogenetic networks (Fig. 3A), and examination of genetic distances (Table 3), Bayesian species delimitation analyses inferred low to moderate speciation probabilities between four pairs of terminals (Fig. 3B). Although we would not advocate the recognition of new putative species on the basis of genetic divergences, we do note that the nine genetic lineages identified by our analyses differ by substantially more mitochondrial sequence divergence (Table 3) than most divergences identified in numerous recent studies of cryptic gekkonid lizard diversification (see Gamble et al., 2012, for discussion) and, thus, may warrant further taxonomic scrutiny with other sources of data.

Five highly significant putative species splits were favored, primarily involving geographically circumscribed or isolated populations. These correspond to (letters referring to those in Figs. 2, 3A): A: a sample of unknown provenance (pet trade, reportedly shipped to the US from Jakarta, Indonesia and therefore, possibly from Java Island); Clade B + C: two divergent lineages, together most likely representing a single putative taxonomic entity (with a weakly supported split between them) from Sumatra and nearby Enggano islands, respectively; Clade D + E: two additional divergent lineages (possibly representing a single putative taxonomic entity) with a weakly supported split between them, from Borneo and adjacent Sulawesi islands, respectively; F: a possibly distinct species from central Peninsular Malaysia (two pet trade samples, reportedly from the vicinity of Gua Musang, central Peninsular Malaysia) and Langkawi Island (G), northwestern Peninsular Malaysia and of south-central Peninsular Malaysia (H) and I: a hypothetically distinct species from the lowlands of southern Peninsular Malaysia and nearby islands of the Seribuat Archipelago.

In general, our varied approaches to inferring the genealogical relationships among populations of *Ptychozoon kuhli* provided very similar results. Bayesian and Maximum Likelihood concatenated gene tree analyses generated the same topology inferred in our *BEAST species tree estimation procedures, which were again mirrored in Splits Tree phylogenetic networks (with strong

bootstrap support supporting all the same groupings; Fig. 3A). Interestingly, however, our results varied with respect to the conclusions derived from the BPP analyses vs. more traditional tree based species delimitation approaches.

NeighborNet bootstrapping analysis implemented in SplitsTree phylogenetic networks provided significant support for deep divergences between nine *Ptychozoon kuhli* lineages, even between those not supported with high speciation probabilities in BPP analyses. Thus, the genetic distinctiveness of each highly divergent lineage apparent in our concatenated gene tree (Fig. 2), examination of uncorrected *p*-distances between taxa, (Table 3) and confirmed by phylogenetic networks (Fig. 3A) is not sufficient for our Bayesian species delimitation analysis to infer significant splits between some terminals (B + C; D + E; F + G + H) included in our study. Confirmation or refutation of the hypothesis of taxonomic distinctiveness of the relevant populations (Sumatra vs. Enggano islands; Borneo vs. Sulawesi islands; and Langkawi vs. central Malaysian Peninsula) will necessarily involve examination of other sources of data (additional loci, morphology, ecology, behavior) before firm conclusions can be drawn.

The presence of substantial genetic structure and numerous unrecognized putative species masquerading within the widespread and morphologically variable (Fig. 3B) *P. kuhli* is not surprising. *Ptychozoon kuhli*, as currently recognized, has a geographic range encompassing nearly that of all remaining *Ptychozoon* species combined, and we find it extremely unlikely that many single native terrestrial vertebrate species have actual ranges this broad (Dickerson, 1928; Corbet and Hill, 1992; Inger, 1999; Inger and Voris, 2001; Brown and Diesmos, 2009; Brown and Stuart, 2012), given the ecologically heterogeneous and geographically partitioned nature of the Southeast Asian and Indo-Australian island archipelagos (Whitmore and Sayer, 1992; Whitmore, 1975, 1987; Woodruff, 2010; Lomolino et al., 2010). Previous workers have noted considerable morphological variation in this species (Brown et al., 1997), and recently, the Nicobar Island population, previously referred to this species, was described as a distinct species of *Ptychozoon* on the basis of morphological characters and color pattern (Das and Vijayakumar, 2009). Thus, we find the results of phylogenetic networks and BPP analyses compelling evidence for the possibility of additional species diversity, albeit with the caveat that before species can be formally recognized, a comprehensive review of the taxon *P. kuhli* must be performed such that character-based diagnoses can be formulated to identify distinct evolutionary lineage segments and define these as formal, lineage-based species (Simpson, 1961; Wiley, 1978; Frost and Hillis, 1990; de Queiroz, 1998; Brown and Diesmos, 2001; Bauer et al., 2010), hopefully using a variety of different types of evidence.

4.3. Conservation implications

Although its formal conservation status currently remains unassessed (IUCN, 2011), herpetologists have long considered *Ptychozoon kuhli* to be the most widespread, commonly encountered species of *Ptychozoon* (Manthey and Grossmann, 1997; Das, 2004; Malkmus et al., 2002; Nguyen et al., 2009; Grismer, 2011). Our study demonstrates that this single species is composed of at least nine distinct, genetically divergent lineages (Fig. 2; Table 3), some of which are endemic or range-restricted to one or two nearby landmasses (islands) or habitat types (lowland coastal areas, highland forests, tree canopies), and several of which might soon be defined as distinct evolutionary species. Although we did not perform Bayesian species delimitation analyses involving *P. lionotum*, we suspect the same taxonomic implications may apply to the highly divergent (15.2–16.0% uncorrected pairwise sequence divergence) north–south split detected in this species, roughly spanning the Isthmus

of Kra and the Kangar-Pattani Line, a recognized, and well characterized, biogeographic boundaries (Whitmore, 1987; Voris, 2000; Woodruff, 2010). Thus, the general results of this study suggest that, in addition to a taxonomic review, conservation status assessments (*sensu* IUCN, 2011) should be performed to the best ability of the available field-based data, soon after formal taxonomic revision of *P. kuhli* becomes available. As increasing numbers of "widespread" Southeast Asian species turn out to be complexes of range-restricted microendemics (Stuart et al., 2006; Brown et al., 2009b; Grismer et al., 2010; McLeod, 2010; Brown and Stuart, 2012), it becomes increasingly difficult—yet essential—to protect this diversity by mitigating habitat destruction and forest loss throughout the Asian mainland and adjacent archipelagos (Sodhi et al., 2004).

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.06.009.

References

Albert, E.M., San Mauro, D., Garcia-Paris, M., Ruber, L., Zardoya, R., 2009. Effect of taxon sampling on recovering the phylogeny of squamate reptiles based on complete mitochondrial genome and nuclear gene sequence data. Gene 441, 12–21.

Annandale, N., 1904. Contribution to Oriental Herpetology I: the lizards of the Andamans, with the description of a gecko and a note on the reproduced tail in *Ptychozoon homalocephalum*. J. Asiatic Soc. Bengal 73, 12–22.

Annandale, N., 1905. Notes on some oriental geckos in the Indian Museum, Calcutta, with descriptions of new forms. Ann. Mag. Nat. Hist., Lond. 15, 26–32.

Bauer, A.M., Parham, J.F., Brown, R.M., Stuart, B.L., Grismer, L.L., Papenfuss, T.J., Bohme, W., Savage, J., Carranza, S., Grismer, J., Wagner, P., Ananjeva, N., Inger, R.F., 2010. On the availability of new Bayesian-delimited gecko names and the importance of character based definitions of species. Proc. Roy. Soc. Lond. B 278, 490–492.

Barbour, T., 1912. A contribution to the zoogeography of the East Indian Islands. Mem. Mus. Comp. Zool. 44, 1–203.

Biswas, S., Sanyal, D.P., 1980. A report on the Reptilia fauna of Andaman and Nicobar Islands in the collection of Zoological Survey of India. Rec. Zool. Surv. India 77, 255–292.

Bobrov, V.V., 1995. Checklist and bibliography of the lizards of Vietnam. Smithsonian Herpetol. Infor. Ser. 105, 1–28.

Bauer, A.M., Giri, V.B., Greenbaum, E., Jackman, T.R., Dharne, M.S., Shouche, Y.S., 2008. On the systematics of the gekkonid genus *Teratolepis* Günther, 1869: another one bites the dust. Hamadryad 33, 13–27.

Boulenger, G.A., 1885. Catalogue of the Lizards in the British Museum (Nat. Hist.) I. Geckonidae, Eublepharidae, Uroplatidae, Pygopodidae, Agamidae. British Museum (Natural History), London.

Brown, R.M., 1999. New species of parachute gecko (Squamata; Gekkonidae; genus *Ptychozoon*) from northeastern Thailand and Central Vietnam. Copeia 1999, 990–1001.

Brown, R.M., Diesmos, A.C., 2000. The lizard genus *Luperosaurus*: taxonomy, history, and conservation prospects for some of the world's rarest lizards. Sylvatrop.: Tech. J. Philipp. Eco. Nat. Res. 10, 107–124.

Brown, R.M., Diesmos, A.C., 2001. Application of lineage-based species concepts to oceanic island frog populations: the effects of differing taxonomic philosophies on the estimation of Philippine biodiversity. Silliman J. 42, 133–162.

Brown, R.M., Diesmos, A.C., 2009. Philippines, biology. In: Gillespie, R., Clague, D. (Eds.), Encyclopedia of Islands. University of California Press, Berkeley, pp. 723–732.

Brown, R.M., Stuart, B.L., 2012. Patterns of biodiversity discovery through time: an historical analysis of amphibian species discoveries in the Southeast Asian mainland and island archipelagos. In: Gower, D.J., Johnson, K.G., Richardson, J.E., Rosen, B.R., Rüber, L., Williams, S.T. (Eds.), Biotic Evolution and Environmental Change in Southeast Asia. Cambridge University Press, pp. 348–389.

Brown, R.M., Ferner, J.W., Diesmos, A.C., 1997. Definition of the Philippine parachute gecko, *Ptychozoon intermedium* Taylor 1915 (Reptilia: Squamata: Gekkonidae): redescription, designation of a neotype, and comparisons with related species. Herpetologica 53, 357–373.

Brown, R.M., Oliveros, C., Siler, C.D., Diesmos, A.C., 2009a. Phylogeny of *Gekko* from the northern Philippines, and description of a new species from Calayan Island. J. Herpetol. 43, 620–635.

Brown, R.M., Siler, C.D., Das, I., Pui, Y.M., 2012. Testing the phylogenetic affinities of Southeast Asia's rarest geckos: flap-legged geckos (*Luperosaurus*), flying geckos (*Ptychozoon*) and their relationship to the pan-Asian genus *Gekko*. Mol. Phylogenet., Evol. 63, 915–921.

Brown, R.M., Siler, C.D., Diesmos, D.C., Alcala, A.C., 2009b. The Philippine frogs of the genus Leptobrachium (Anura; Megophryidae): phylogeny-based species delimitation, taxonomic revision, and descriptions of three new species. Herpetol. Monogr. 23, 1–44.

Brown, R.M., Supriatna, J., Ota, H., 2000b. Discovery of a new species of *Luperosaurus* (Squamata; Gekkonidae) from Sulawesi, with a phylogenetic analysis of the genus and comments on the status of *L. serraticaudus*. Copeia 2000, 191–209.

Brown, W.C., Alcala, A.C., 1978. Philippine Lizards of the family Gekkonidae. Silliman Univ. Nat. Sci. Monogr. Ser. 1, Dumaguete.

Bruen, T.C., Philippe, H., Bryant, D., 2006. A simple and robust statistical test for detecting the presence of recombination. Genetics 172, 2665–2681.

Bryant, D., Moulton, V., 2004. Neighbor-Net: an agglomerative method for the construction of phylogenetic networks. Mol. Biol. Evol. 21, 255–265.

Cantor, T., 1847. Catalogue of the reptiles inhabiting the Malayan Peninsula and islands, collected or observed by Theodore Cantor, Esqr., M. D. J. Asiat. Soc. Bengal 16, 607–656 (part).

Chan-ard, T., Grossmann, W., Gumprecht, A., Schulz, K.D., 1999. Amphibians and Reptiles of Peninsular Malaysia and Thailand – An Illustrated Checklist (bilingual English and German). Bushmaster Publications, Würselen.

Colbert, E.H., 1967. Adaptations for gliding in the lizard *Draco*. Am. Mus. Novitates 2283, 1–20.

Corbet, G.B., Hill, J.E., 1992. The Mammals of the Indomalayan Region. Oxford University Press, New York.

Cox, J., van Dijk, P., Nabhitabhata, J., Thirakhupt, K., 1998. A Photographic Guide to Snakes and Other Reptiles of Peninsular Malaysia, Singapore and Thailand. New Holland Publishers Ltd., UK, London.

Das, I., 1994a. A check-list of the amphibians and reptiles of Andaman and Nicobar Islands. J. Andaman Sci. Assoc. 10, 44–49.

Das, I., 1994b. The reptiles of south Asia: a checklist and distributional summary. Hamadryad 19, 15–40.

Das, I., 2004. Lizards of Borneo. Natural History Publications (Borneo) Sdn Bhd., Kota Kinabalu.

Das, I., Vijayakumar, S.P., 2009. New species of *Ptychozoon* (Sauria: Gekkonidae) from the Nicobar Archipelago, Indian Ocean. Zootaxa 2095, 8–20.

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 University Press, New York, pp. 57–75.

Dickerson, R.E., 1928. Distribution of Life in the Philippines. Philipp. Bur. Sci. Monogr. 21, Manila.

Dring, J.C.M., 1979. Amphibians and reptiles from northern Trengganu, Malaysia, with descriptions of two new geckos: *Cnemaspis* and *Cyrtodactylus*. Bull. British Mus. (Natur. Hist.) Zool. Ser. 34, 181–241.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Dudley, R., Byrnes, G., Yanoviak, S., Borrell, B., Brown, R.M., McGuire, J., 2007. Gliding and the functional origins of flight: biomechanical novelty or necessity? Ann. Rev. Ecol. Syst. 38, 179–201.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl. Acids Res. 32, 1792–1797.

Emerson, S.B., Koehl, M.A.R., 1990. The interaction of behavioral and morphological change in the evolution of a novel locomotor type—flying frogs. Evolution 44, 1931–1946.

Esselstyn, J.A., Garcia, H.J.D., Saulog, M.G., Heaney, L.R., 2008. A new species of Desmalopex (Pteropodidae) from the Philippines, with a phylogenetic analysis of the Pteropodini. J. Mammal. 89, 815–825.

Flower, S.S., 1896. Notes on a collection of reptiles and batrachians made in the Malay Peninsula in 1895–96; with a list of the species recorded from that region. Proc. Zool. Soc. Lond. 1896, 856–914.

Frost, D.R., Hillis, D.M., 1990. Species in concept and practice: herpetological applications. Herpetologica 46, 87–104.

Gadow, H., 1901. Amphibia and Reptiles. Macmillan and Co., London.

Gamble, T., Bauer, A.M., Greenbaum, E., Jackman, T.R., 2008. Evidence of Gondwanan vicariance in an ancient clade of gecko lizards. J. Biogeogr. 35, 88–104.

Gamble, T., Bauer, A.M., Colli, G.R., Greenbaum, E., Jackman, T.R., Vitt, L.J., Simons, A.M., 2011. Coming to America: multiple origins of New World geckos. J. Evol. 24, 231–244.

Gamble, T., Colli, G.R., Rodriques, M.T., Werneck, F.P., Simmons, A.M., 2012. Phylogeny and cryptic diversity in geckos (Phyllopezus; Phyllodactylidae; Gekkota) from South America's open biomes. Mol. Phylogenet. Evol. 62, 943–953.

Goldingay, R., Scheibe, J., 2000. Biology of Gliding Mammals. Filander Press, Fürth.

Grismer, L.L., 2011. Lizards of Peninsular Malaysia, Singapore and Their Adjacent Archipelagos. Edition Chimaira, Frankfurt.

Grismer, L.L., Sumontha, M., Cota, M., Grismer, J.L., Wood Jr., P.L., Pauwels, O.S.G., Kunya, K., 2010. A revision and redescription of the rock gecko Cnemaspis siamensis (Taylor 1925) (Squamata: Gekkonidae) from Peninsular Thailand with descriptions of six new species. Zootaxa 2576, 1–55.

Grismer, L.L., Thy, N., Thou, C., Grismer, J.L., 2008. Checklist of the amphibians and reptiles of southwestern Cambodia. Cambodian J. Nat. Hist. 2008, 12–28.

Günther, A.C.L.G., 1864. The Reptiles of British India. Ray Society, London. Reprinted 1982, Oxford and IBH Publishing Co., New Delhi.

Günther, A., 1885. Catalogue of Lizards in the British Museum. Geckonidae, Eublepharidae, Uroplatidae, Pygopodidae, Agamidae, vol. I, second ed. British Museum, London.

Heled, J., Drummond, A., 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27, 570–580.

Heyer, W.R., Pongsapipatana, S., 1970. Gliding speeds of Ptychozoon lionotum (Reptilia: Gekkonidae) and Chrysopelea ornata (Reptilia: Colubridae). Herpetologica 26, 317–319.

Huson, D., 1998. Splitstree—a program for analyzing and visualizing evolutionary data. Bioinformatics 14, 68.

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 245–267.

Inger, R.F., 1999. Distributions of amphibians in southern Asia and adjacent islands. In: Duellman, W.E. (Ed.), Patterns of Distribution of Amphibians: A Global Perspective. John Hopkins University Press, Baltimore, pp. 445–482.

Inger, R.F., Colwell, R.K., 1977. Organization of contiguous communities of amphibians and reptiles in Thailand. Ecol. Monogr. 47, 229–253.

Inger, R.F., Voris, H.K., 2001. The biogeographical relations of the frogs and snakes of Sundaland. J. Biogeogr 28, 863–891.

IUCN, 2011. IUCN Red List of Threatened Species. Version 2011.2. <http://www.iucnredlist.org> (downloaded 13.01.12).

Kiew, B.H., 1987. The flying gecko – Ptychozoon. Nat. Malaysiana 12, 18–19.

Leaché, A.D., Fujita, M.K., 2010. Bayesian species delimitation in West African forest geckos (Hemidactylus fasciatus). Proc. Roy. Soc. Lond. B 277, 3071–3077.

Lomolino, M.V., Riddle, B.R., Whittaker, R.J., Brown, J.H., 2010. Biogeography, fourth ed. Sinauer Associates, Sunderland.

Malkmus, R., Manthey, U., Vogel, G., Hoffmann, P., Kosuch, J., 2002. Amphibians and Reptiles of Mount Kinabalu (North Borneo). A.R.G. Ganter Verlag, K.G., Rugell.

Manthey, U., 1985. Die Gattung Ptychozoon Kuhl, 1822 (Faltengecko) Teil I mit einem Bestimmungsschlüssel für die fünf Arten. Sauria, Berlin 4, 5–12.

Manthey, U., Grossmann, W., 1997. Amphibien und Reptilien Südostasiens. Natur und Tier-Verlag, Münster.

Marcellini, D.L., Keefer, T.E., 1976. Analysis of the gliding behavior of Ptychozoon lionotum (Reptilia: Gekkonidae). Herpetologica 32, 362–366.

Marshall, D.C., 2010. Cryptic failure of partitioned Bayesian phylogenetic analyses: lost in the land of long trees. Syst. Biol. 59, 108–117.

McLeod, D.S., 2010. Of Least Concern? Systematics of a cryptic species complex: Limnonectes kuhlii (Amphiba; Anura: Dicroglossidae). Mol. Phylogenet. Evol. 56, 991–1000.

Medway, L., 1975. The function of the membranes of Ptychozoon spp. Malayan Natur. J. 29, 28–30.

Nabhitabhata, J., Chan-ard, T., Chuaynkern, Y., 2000. Checklist of Amphibians and Reptiles in Thailand. Office of Environmental Policy and Planning, Bankok.

Nguyen, V.S., Ho, Thu Cuc, Nguyen, Q.T., 2009. Herpetofauna of Vietnam. Chimaira, Frankfurt am Main.

Pauwels, O.S.G., Laohawat, O.-A., Naaktae, W., Puangjit, C., Wisutharom, T., Chimsunchart, C., David, P., 2000. Herpetological investigations in Phang-Nga Province, southern Peninsular Thailand, with a list of reptile species and notes on their biology. Dumerilia 4, 123–154.

Pawar, S., Biswas, S., 2001. First record of the Smooth-Backed Parachute Gecko Ptychozoon lionotum Annandale 1905 from the Indian mainland. Asiatic Herpetol. Res. 9, 101–106.

Pong, T.Y., 1974. Camouflage in the flying gecko, Ptychozoon kuhli Stj. Malayan Nat. J. 28, 36–37.

Posada, D., 2008. JModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.

Rambaut, A., Drummond, A.J., 2005. Tracer v1.4. <http://beast.bio.ed.ac.uk/Tracer>.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

de Rooij, N., 1915. The Reptiles of the Indo-Australian Archipelago, I: Lacertilia, Chelonia. Emydosauria. E. J. Brill, Ltd., Leiden.

Russell, A.P., 1972. The Foot of Gekkonid Lizards: A Study in Comparative and Functional Anatomy. Unpubl. Ph.D. Diss, Univ. of London, London.

Russell, A.P., 1979a. The origin of parachuting locomotion in gekkonid lizards (Reptilia: Gekkonidae). Zool. J. Linn. Soc. 65, 233–249.

Russell, A.P., 1979b. A new species of Luperosaurus (Gekkonidae) with comments on the genus. Herpetologica 35, 282–288.

Russell, A.P., Dijkstra, L.D., Powell, G.L., 2001. Structural characteristics of the patagium of Ptychozoon kuhli (Reptilia:Gekkonidae) in relation to parachuting locomotion. J. Morphol. 247, 252–263.

Setiadi, M.I., McGuire, J.A., Brown, R.M., Zubairi, M., Iskandar, D.T., Andayani, N., Supriatna, J., Evans, B.J., 2011. Adaptive radiation and ecological opportunity in Sulawesi and Philippine fanged frog (Limnonectes) communities. Amer. Natur. 178, 221–240.

Siler, C.D., Oaks, J.R., Linkem, C.W., Swab, J., Diesmos, A.C., Brown, R.M., 2012. Did geckos ride the Palawan raft to the Philippines? J. Biogeogr. 39, 1217–1234.

Simpson, G.G., 1961. Principles of Animal Taxonomy. Columbia University Press, New York.

Smith, M.A., 1930. The Reptilia and Amphibia of the Malay Peninsula. Bull. Raff. Mus. 3, 1–149.

Smith, M.A., 1935. The Fauna of British India, including Ceylon and Burma. Reptilia and Amphibia. II.-Sauria. Taylor and Francis, London.

Sodhi, N.S., Koh, L.P., Brook, B.W., Ng, P.K., 2004. Southeast Asian biodiversity: an impending disaster. Trends Ecol. Evol. 19, 654–660.

Stephens, M., Donnelly, P., 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Amer. J. Human Gen. 73, 1162–1169.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML Web Servers. Syst. Biol. 57, 758–771.

Stejneger, L.H., 1907. Herpetology of Japan and adjacent territory. Bull. US Natl. Mus. 58, 1–577.

Stuart, B.L., Emmett, D.A., 2006. A collection of amphibians and reptiles from the Cardamom Mountains, southwestern Cambodia. Fieldiana Zool. N.S. 109, 1–27.

Stuart, B.L., Inger, R.F., Voris, H.K., 2006. High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs. Biol. Lett. 2, 470–474.

Taylor, E.H., 1915. New species of Philippine lizards. Philipp. J. Sci. 10, 89–108.

Taylor, E.H., 1922a. The lizards of the Philippine Islands. Philipp. Bur. Sci., Monogr. 17, Manila.

Taylor, E.H., 1922b. Additions to the herpetological fauna of the Philippine Islands, I. Philipp. J. Sci. 21, 161–206.

Taylor, E.H., 1928. Amphibians, lizards, and snakes of the Philippines. In: Dickerson, R. (Ed.), Distribution of Life in the Philippines, pp. 214–241. Philippine Bur. Sci., Monogr. 21, Manila.

Taylor, E.H., 1963. The lizards of Thailand. Univ. Kansas Sci. Bull. 44, 687–1077.

Taylor, E.H., 1975. Philippine adventures: an autobiographical memoir. In: Taylor, E.H., Leonard, A.B., Smith, H.M., Pisani, G.R. (Eds.), Recollections of an Herpetologist. University of Kansas Museum of Natural History (Monogr. 4), Lawrence, pp. 1–105.

Tho, Y.P., 1974. Camouflage in the flying gecko, Ptychozoon kuhli Stejn. Malayan Nat. J. 28, 36.

Tiwari, K.K., 1961. The eggs and flight of the gecko Ptychozoon kuhli Stejneger from Car Nicobar. J. Bombay Nat. Hist. Soc. 58, 523–527.

Tweedie, M.W.F., 1954. Notes on Malayan reptiles, no. 3. Bull. Raff. Mus. 25, 107–117.

Venugopal, P.D., 2010. An updated and annotated list of Indian lizards (Reptilia: Sauria) based on a review of distribution records and checklists of Indian reptiles. J. Threatened Taxa 2, 725–738.

Vetter, R.S., Brodie, E.D., 1977. Background color selection and antipredator behavior of the flying gecko, Ptychozoon kuhli. Herpetologica 33, 464–467.

Voris, H., 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J. Biogeogr. 27, 1153–1167.

Wiley, E.O., 1978. The evolutionary species concept reconsidered. Syst. Zool. 21, 17–26.

Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2004. AWTY: A System for Graphical Exploration of MCMC Convergence in Bayesian Phylogenetic Inference. <http://ceb.csit.fsu.edu/awty>.

Wermuth, H., 1965. Gekkonidae, Pygopodidae, Xantusidae. In: Mertens, R., Hennig, W., Wermuth, H. (Eds.), Das Tierreich, vol. 80. Walter de Gruyter & Co., Berlin.

Whitmore, T.C., 1975. Tropical Rain Forests of the Far East. Clarendon Press, Oxford.

Whitmore, T.C. (Ed.), 1987. Biogeographical Evolution of the Malay Archipelago. Clarendon Press, Oxford.

Whitmore, T.C., Sayer, J.A., 1992. Tropical Deforestation and Species Extinction. Chapman and Hall, London.

Woodruff, D.S., 2010. Biogeography and conservation in Southeast Asia: how 2.7 million years of repeated environmental fluctuations affect today's patterns and the future of the remaining refugial-phase biodiversity. Biodivers. Conserv. 19, 919–941.

Yang, Z., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. Proc. Natl. Acad. Sci. 107, 9264–9269.