AN ADAPTIVE RADIATION OF FROGS IN A SOUTHEAST ASIAN ISLAND ARCHIPELAGO

David C. Blackburn, Cameron D. Siler, Arvin C. Diesmos, Jimmy A. McGuire, David C. Cannatella, and Rafe M. Brown

SUPPLEMENTARY TEXT

Materials and Methods

TAXON SAMPLING

Fieldwork was conducted by RMB, CDS, ACD, JAM and colleagues throughout Southeast Asia between 1992 and 2008. Tissue samples were flash-frozen in liquid nitrogen, or immersed in \geq 90% ethanol or in a tissue buffer, then stored at -80°C. Most specimens are deposited in the Natural History Museum of the University of Kansas (KU), the National Museum of the Philippines (PNM), the Texas Memorial Museum of University of Texas at Austin (TNHC), or the Museum of Vertebrate Zoology of the University of California at Berkeley (MVZ; Supplementary Table 1). To avoid issues regarding uncertainty in interpreting results of phylogenetic inference because of including DNA sequence data from tissue samples lacking voucher specimens (Yates 1985; Winker et al. 1996; Ruedas et al. 2000; Lehn et al. 2007), we examined voucher specimens corresponding to 98% of all in-group specimens sampled and employed a strict method of species identification based on external morphology, microhabitat, and advertisement call (Diesmos et al. 2002; Brown et al. 2006a,b), which is consistent with widely accepted lineage-based species concepts (Wiley, 1978; de Queiroz, 1998, 1999).

Our DNA dataset is based on sequences from 172 specimens of frogs of the family Microhylidae, including members of 17 outgroup genera. Because our goals were to estimate the phylogeny of the Southeast Asian genus *Kaloula* and previous phylogenetic analyses have not definitively established the sister taxon to this genus (e.g., Bossuyt et al. 2006; Frost et al. 2006; van Bocxlaer et al. 2006; van der Meijden et al. 2007; Matsui et al. 2011; Trueb et al. 2011), we sampled broadly across Asian microhylid genera, including eight of nine recognized genera of the Microhylinae (sensu van der Meijden et al. 2007; no genetic samples were available for *Uperodon*), as well as members of several New World genera; the brevicipitid *Callulina* was used as an outgroup. The ingroup sampling included 140 specimens of the genus *Kaloula*, assigned to eleven of the fifteen formally recognized species (Inger 1954; Alcala and Brown 1998; Brown 2007). Genetic resources were unavailable for several currently recognized species (*K. assamensis*, *K. borealis*, and *K. rugifera*); one of these (*K. assamensis*) remains poorly known and was described on the basis of only a few specimens (Das et al. 2005). Because the taxonomic status of two additional species, *K. aureata* and *K. macrocephala*, remains unclear, we do not include them in our study (Ohler 2003; Pauwels and Chérot 2006; IUCN 2012). Our sampling is especially dense in the Philippines (106 samples) and includes all known allopatric island populations of each species, seven of which are found naturally in the Philippines (*K. pulchra* has been introduced recently; Diesmos et al. 2005, 2006; Siler et al. 2011). This sampling includes the widespread Philippine endemic *Kaloula picta* from throughout the archipelago as well as all four subspecies of the Philippine species *K. conjuncta* (*K. c. conjuncta, K. c. meridionalis, K. c. negrosensis*, and *K. c. stickeli*; Inger 1954). Additionally, we include

nearly all known allopatric populations of the widespread *Kaloula baleata*, including samples from Java (the type locality), Bali, Sulawesi, the Togian Islands, Peninsular Malaysia, Vietnam, Borneo, and Palawan Island (Philippines). Finally, we include samples of the widespread species *Kaloula pulchra* from throughout the entirety of its native range (China, Peninsular Malaysia, Laos, Thailand, Vietnam, Sumatra, and Sulawesi). However, our sampling also includes a number of undescribed species. Further details on specimens and localities can be found in the Supplementary Table 1.

DNA DATA COLLECTION

We used either Qiagen DNeasy Tissue Kit (Cat. No. 69506) or a non-commercial guanidine thiocyanate method (Esselstyn et al. 2008) to extract genomic DNA from liver (or occasionally muscle) samples. Four primer pairs (MVZ59–Val, 12L1–16Sh, 12Sm– 16Sa, and 16Sc–16Sd; Moriarty and Cannatella 2004; Darst and Cannatella 2004) were used to amplify via polymerase chain reaction (PCR) an approximately 2400 bp region of the mitochondrial genome spanning most of the 12S and 16S ribosomal RNA (rRNA) genes and the intervening transfer RNA (tRNA) for Valine. We used the now standard reaction mixtures and thermal cycle profiles for both PCR and sequencing reactions, using the same primers and following the methods of Moriarty and Cannatella (2004) and Evans et al. (2003). Samples were purified using Qiagen gel preps or Exosap purification protocols (USB Corp., Cleveland, OH, USA; Esselstyn et al. 2008). Sequencing reactions were conducted with identical undiluted PCR primers, using ABI Big Dye terminator chemistry (Perkin-Elmer, Boston, MA, USA) and Sephadex clean-ups (GE Healthcare, Uppsala, Sweden). Sequencing was performed on an ABI 3130*xl* automated PRISM

sequencer (Applied Biosystems, Foster, CA, USA). All sequences are deposited in GenBank (Supplementary Table 1); a multiple alignment file is deposited in Dryad (doi:10.5061/dryad.dj342).

Because PCR products were amplified with varying success, the full length of the target sequence (approx. 2400 bp) could not be obtained for some specimens. In most cases this resulted from failure to amplify the 5' end of our target region, namely the MCZ59–Val region; the resulting sequences are approx. 1900 bp in length. Although missing data can bias or mislead phylogenetic inference, especially when concentrated in particular taxa or certain clades, taxa with missing data still add information to phylogenetic inference and missing data will not necessarily mislead phylogenetic analyses (Wiens 2006; Wiens and Moen 2008). Our goal in including specimens with incomplete data is to place particular samples with statistical support into the phylogeny to facilitate species delimitation and identification; in this way, we increased the number of specimens represented in both our molecular and morphological datasets. We included a given sample if we obtained sequence data for at least one of the four overlapping targeted gene regions; in most cases, these incomplete sequences derived from degraded tissue samples for which only 600–700 bp of the 16Sc–16Sd region could be amplified and sequenced. Taxa with incomplete gene representation were included in our analyses to aid in the confident identification of all specimens; analyses excluding taxa with missing data provided largely similar results (data not shown).

All fragments were sequenced in both directions. Sequences were assembled and manually vetted in Sequencher 4.5 (Genecodes, Ann Arbor, MI, USA), and then initially aligned using default parameter values in Clustal X 1.83.1 (Thompson et al. 1997). Non-

overlapping DNA fragments from a single specimen were aligned separately and then merged in Mesquite 2.5 (Maddison and Maddison, 2008) to form a single terminal taxon for phylogenetic analyses. Alignments were examined exhaustively by eye and manually adjusted to minimize the number of parsimony informative change across sites (Moriarty and Cannatella 2003). Because excluding ambiguously aligned regions and autapomorphic insertion-deletions ("indels") from phylogenetic analyses resulted in minimal differences in topology and bootstrap values in exploratory parsimony searches (data not shown), we chose to maintain these sites in our analysis. The resulting alignment of 2567 bp corresponds to positions 2062–4620 of the *Xenopus laevis* mitochondrial genome (GenBank NC-001573). Uncorrected pairwise distances (*p*distances) were calculated using MEGA 4.0.1 (Tamura et al. 2007).

PHYLOGENETIC ANALYSES

We used maximum-likelihood and Bayesian approaches to estimate phylogenetic relationships. A maximum-likelihood tree was obtained using RAxML v.7.0.4 (Stamatakis 2006) using a random starting tree, the faster rapid hill-climbing algorithm of (Stamatakis et al. 2007), and a GTR + I + Γ model of evolution (with four discrete categories for Γ); the data were not partitioned into subsets. Three hundred replicate searches were conducted to avoid basing our phylogenetic inference on a local search optimum; the tree with the best –ln likelihood from these replicate searches was selected as our maximum likelihood estimate of the phylogeny. Support for the preferred ML topology was estimated using 1000 independent nonparametric bootstrap replicates (ML BS), using the same model of sequence evolution, a random starting tree, and one search

replicate per bootstrap replicate. Split-support for clades was summarized using SumTrees in DendroPy (Sukumaran and Holder 2010) and clades present in $\geq 70\%$ of the bootstrap trees were considered well-supported (Hillis and Bull 1993).

A Bayesian estimate of phylogeny was obtained with MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) using the GTR $+ I + \Gamma$ model and unpartitioned dataset. Four replicates of four MCMC chains were run for 20 million generations, sampled every 2000 generations, using a temperature of 0.2 and default priors. We examined trends and distributions of log-likelihoods and parameter values using Tracer 1.5 (Rambaut and Drummond 2009). We assessed convergence by examining correlations of split frequencies and chain variability among independent runs using Are We There Yet? (AWTY; Nylander et al. 2008). Based on examination of trends in Tracer and correlations of split frequencies, stationarity and convergence was achieved after one million generations. However, because of patterns of chain variability during the first few million generations, we took a conservative approach and discarded trees sampled during the first ten million generations as burn-in. Based on the cumulative set of post-burn-in trees from each run, all effective samples sizes (ESS) were greater than 400. The topology and posterior probabilities (PP) were then summarized based on the post-burnin trees using SumTrees and Tracer. We considered topologies with posterior probabilities ≥ 0.95 to be well supported (Huelsenbeck et al. 2001).

We generated a time-calibrated estimate of phylogenetic relationships to test patterns of lineage diversification and morphological evolution. Analyses were conducted using the uncorrelated relaxed clock method (Drummond et al. 2006) implemented in BEAST v.1.6.2 (Drummond et al. 2010). Phylogenetic relationships were estimated using

the Yule pure-birth speciation prior for the tree shape and a $GTR + I + \Gamma$ model of sequence evolution. For comparative analyses, we generated chronograms with relative divergence times by setting a normal prior of 100 (mean: 100; standard deviation: 0.001) on the most recent common ancestor (MRCA) of all tips in the tree; the distant outgroup *Callulina* was excluded from these analyses. MCMC analyses were run for 200 million generations with MCMC steps and divergence times recorded every 5000 generations; to avoid autocorrelation between MCMC steps, the sampling was further thinned to every 10,000 generations. We then assessed stationarity of parameter estimates using Tracer and thus discarded the first 5 million generations as burn-in; all ESS values were > 190. The 95% highest posterior density interval and maximum clade credibility topology (MCCT) were calculated using TreeAnnotator (Rambaut and Drummond 2011).

While the absolute timing of divergences are of interest, there are no internal fossil calibration points for the family Microhylidae. To provide a rough estimate of divergence times, we conducted another BEAST analysis using two secondary calibration priors based on van der Meijden et al. (2007): (1) MRCA of Microhylidae at 66.0 ± 11.0 million years ago (mya); and (2) MRCA of Microhylinae at 52.0 ± 9.0 mya. A normal distribution was used for each calibration point with mean and standard deviation of the prior based on the estimates provided by van der Meijden et al. (2007); we used a normal distribution for these priors because of the bidirectionality of uncertainty in these estimates (Ho and Phillips 2009).

ECOTYPES AND MORPHOLOGICAL VARIATION

Our characterization of ecotype summarizes data on ecology and natural history, including reproductive and larval biology, and microhabitat preferences. These assessments utilize a half-century of accumulated information on the life history and biology of the frogs of interest (Inger 1954, 1966; Inger and Stuebing 1989, 1997; Zhao and Adler 1993; Dutta and Manamendra-Arachichi 1996; Manthey and Grossman 1997; Fei 1999; Inger et al. 1999; Fei and Ye 2000; Diesmos et al. 2002; Schleich and Kastle 2002; Malkmus et al. 2002), as well as our collective personal experience with many of these species.

We selected measurements of morphological features likely related to habitat utilization in anurans. For instance, we expect the relative size of limbs, toe pads, and metatarsal tubercles to be directly related to behaviors of habitat specialists such as climbing or burrowing. These measurements are standard in Asian anuran taxonomy (Inger 1954, 1966; Taylor 1962; Matsui 1984, 1994; Inger and Stuebing 1989, 1997; Zhao and Adler 1993; Dutta et al. 1996; Fei and Ye 2000; Brown and Guttman 2002; Malkmus et al. 2002) with the exception of our measure of webbing (see below). The measurements used in our analyses are as follows: snout–vent length; head width (measured at the widest point of the head, near the jaw articulation); snout length (measured from the most anterior margin of the eye to the tip of the rostrum); forearm length (measured from the most proximal margin of the elbow to the proximal margin of the most proximal palmar tubercle); third finger length (measured from the proximal margin of the most proximal subarticular tubercle to the distal digit tip); third finger width (measured at the level of the most distal subarticular tubercle); third finger-tip width (maximal width of the distalmost finger); thigh length (measured from the cloaca

to the distalmost knee); crus length (measured from the most proximal knee to the most distal tibiotarsal joint); inner metatarsal tubercle length (maximum length); outer metatarsal tubercle length (maximum length); third toe length (measured from the proximal margin of the most proximal subarticular tubercle to the distal digit tip); third toe width (measured at the level of the most distal subarticular tubercle); third toe-tip width (maximal width of the distalmost toe); and extent of pedal webbing (measured from the distal tip of the third toe to the most proximal lateral margin of the toe where intradigital webbing is attached). Typically, intradigital webbing is described using the formula developed by Savage and Heyer (1967, 1997). However, this description does not lend itself to analyses using multivariate statistics. We instead opted for a novel approach by taking a measurement in which greater values indicate less webbing; thus, for example, if a principal components axis loads strongly and positively on this variable it means that species with large positive scores have less webbing than those with negative scores. For some taxa (e.g., *K. kalingensis*), the outer metatarsal tubercle is essentially absent and thus results in a measurement of nil. Because all mean scores were subsequently natural log-transformed, we added the value of 1 to all mean scores before further calculations. We were thus able to include information from all species for all measurements even though the outer metatarsal tubercle is absent in some species. All measurement data used for these analyses are deposited in Dryad (doi:10.5061/dryad.dj342).

RECONSTRUCTION OF ANCESTRAL ECOTYPES

We evaluated patterns of change in ecotype class across the phylogeny and determine the ecotype class of the MRCA of the Philippines clade. Because of uncertainties in the phylogenetic estimate, we used Bayesian methods for reconstructing ancestral ecotype states across *Kaloula* phylogeny. In diversification analyses, we used the pruned specieslevel time-calibrated post-burn-in trees from our BEAST analysis to reconstruct ancestral states and analyze patterns of morphological evolution. We integrated over uncertainties in topologies using Bayesian mutational mapping as implemented in SIMMAP 1.5.2 (Bollback 2006), which employs a stochastic model of character change. SIMMAP analyses treated character states as unordered and utilized an equal prior for the bias parameter, a gamma distribution for the rate parameter prior ($\alpha = 1.25$, $\beta = 0.25$, $k = 60$), 10 sample replicates, and 10,000 draws from the prior distribution in order to have sufficient sampling of the posterior distribution for bias and rates. We evaluated the robustness of our inference to priors for the gamma distribution by conducting similar analyses in which we varied α , k, and θ ; because the results of these various analyses were qualitatively similar (data not shown), we interpret our inference as robust.

Results

PHYLOGENETIC RELATIONSHIPS

Of the 2567 sites included in the phylogenetic analyses, 1469 are variable and 1145 are parsimony informative. Below, we discuss patterns of relationships based on maximum likelihood (ML) and Bayesian analyses (including the maximum clade credibility tree [MCCT] estimated from MrBayes analyses); divergence times are derived based on BEAST analyses using secondary calibration points.

We recovered strong support for a clade consisting of Southeast Asian *Kaloula*, *Kaloula taprobanica* (Sri Lanka), *Ramanella obscura* (Sri Lanka) and the two species of the Southeast Asian genus *Metaphrynella* (PP = 1.00; ML NBS = 94%). Our divergence time analyses suggest that the MRCA of this clade occurred in the Late Paleogene (median: 31.4 mya; 95% HPD: 17.0–48.0 mya). In the ML and MCCT trees, sister to this clade is a weakly supported ($PP = 0.47$; ML NBS = 34%) clade of south Asian genera (*Micryletta, Glyphoglossus, Caluella, Chaperina,* and *Microhyla*) that comprise the remainder of the subfamily Microhylinae. This sister relationship between these two clades supports the monophyly of the Microhylinae albeit with low support ($PP = 0.61$; ML NBS = 34%). In the ML and MCCT trees, sister to the clade comprising the Microhylinae, is a clade containing the Papuan genera *Oreophryne, Cophixalus,* and *Australochaperina* plus the south Asian genus *Kalophrynus* (Supplementary Fig. 1).

We do not recover *Kaloula* as monophyletic (Supplementary Figure 1). Instead, in both the ML and MCCT trees, *K. taprobanica* (from eastern India, Bangladesh, and Sri Lanka) is recovered as the sister of a clade comprising *Metaphrynella* and *Ramanella* (recovered as sister taxa; PP = 0.94; ML NBS = 62%) and all other *Kaloula*. Based on these analyses, there is low support for alternative placements of *K. taprobanica*, including a monophyletic *Kaloula* (PP = 0.04; ML NBS = 29%) and clade containing *K. taprobanica*, *Ramanella*, and *Metaphrynella* (PP = 0.01; ML NBS = 2%). In contrast, the Bayesian analysis placed the *Ramanella* + *Metaphrynella* clade sister to the Southeast Asian members of the genus *Kaloula* (PP = 0.90; ML NBS = 38%) to the exclusion of *K. taprobanica*. Excluding *K. taprobanica*, there is unequivocal support for the monophyly of other species of *Kaloula* (PP = 1.0; ML NBS = 96%), and the MRCA of these species

probably occurred in the Late Oligocene to Early Miocene (median: 22.3 mya; 95% HPD: 11.4–33.9 mya).

There is strong support ($PP = 1.00$; ML NBS = 96%) for a clade containing divergent and geographically circumscribed populations typically referred to *K. baleata*, but which we consider a complex of species, some of which are obviously morphologically distinct; we refer to this as the "*baleata* clade." A new morphologically distinct species within the *baleata* clade that occurs on Palawan is the only Philippine species of *Kaloula* not derived from the primary Philippine radiation and, thus, represents a second invasion of the archipelago. Relationships of the *baleata* clade remain unclear. In the ML tree, the *baleata* clade is sister to the endemic Philippines clade ($PP = 0.57$; ML NBS = 44%) whereas in the MCCT tree, it is sister to the mainland species *K. mediolineata* (PP = 0.16; ML NBS = 24%). The clade containing *K. mediolineata*, the *baleata* clade, and the endemic Philippine clade receives high support (PP = 1.00; ML NBS = 96%). There is also strong support for the relationship of *K. verrucosa* (China) and *K. pulchra* (a widespread Asian species) forming successively branching taxa sister to the remaining members of *Kaloula* (Fig. 1).

Most species, subspecies, and candidate species are recovered as monophyletic with high support (Table 2). The relationships of one specimen of *K. rigida* from the type locality remains unclear, but otherwise *K. ridiga* and *K. walteri* are each recovered as monophyletic with strong support. A number of well-supported clades exist within the Philippines, though relationships between these clades remain unclear. *Kaloula rigida* and *K. walteri* are resolved as sister taxa (PP = 1.00; ML NBS = 100%). There is strong support for the monophyly of an undescribed species from Samar and Leyte islands.

Kaloula kalingensis, *K. kokacii*, and two undescribed species are recovered as a strongly supported clade (PP = 1.00; ML BS = 100%), which we refer to as the "*kalingensis*" clade.

The MRCA of the *conjuncta* (median: 4.4 mya; 95% HPD: 1.9–7.6 mya) and *rigida* + *walteri* (median: 4.8 mya; 95% HPD: 1.7–8.5 mya) clades likely both occurred in the Early Pliocene, whereas the MCRA of the *kalingensis* clade may be slightly older (median: 9.1 mya; 95% HPD: 4.5–14.4 mya). Notably, because of the overlap in 95% HPD for the ages of these clades we cannot reject the hypothesis that these clades diversified contemporaneously.

INTRASPECIFIC PATTERNS OF GEOGRAPHIC DIVERSIFICATION

Our intraspecific sampling within several species allows for a characterization of divergence within these taxa (Table 2). For reference to the values reported for other species, the mean *p*-distance across *Kaloula* (excluding *K. taprobanica*) is 7.5%. Two widespread species, *K. pulchra* (throughout Southeast Asia) and *K. picta* (throughout the Philippines) exhibit nearly identical sequences across their respective broad geographical distributions (*K. pulchra*: 0.5%; *K. picta*: 0.2%). *Kaloula baleata* consists of a highly divergent and morphologically distinct Vietnam population (> 7% divergent from other species in the "*baleata*" clade) that is sister to a clade comprising moderately divergent lineages from Borneo, the Malay Peninsula, Borneo, Java + Bali, Sulawesi, and Palawan $(< 3\%$ from one another).

Divergences among species in the *K. kalingensis* clade are an order of magnitude greater than those observed for other species of *Kaloula* (Table 2). The *kalingensis* clade

consists of four strongly supported and highly divergent clades: *K. kalingensis* from northern Luzon (including samples from close to the type locality in Kalinga-Apayao Province), *K. kokacii* from the Bicol Peninsula of southern Luzon Island (adjacent to the type locality on Catañduanes Island), and then two undescribed species from Panay Island and east Luzon Island, respectively.

Within populations assigned to *K. conjuncta*, moderate levels of divergence were detected and not all described subspecies corresponded to monophyletic entities. *Kaloula conjuncta conjuncta* from numerous localities on Luzon Island was paraphyletic with respect to a population on Mindoro Island that is morphologically and acoustically distinct (Brown et al. unpubl. data). Similarly, populations diagnosable morphologically and acoustically as *K. conjuncta negrosensis* from Panay and Negros Islands did not form a clade exclusive of other subspecies; in the ML estimate, the Panay population of *K. c. negrosensis* is most closely related to a new form from Sibuyan Island that is morphologically and acoustically distinct from all other taxa (Brown et al. unpubl. data). Specimens identified as *K. c. stickeli* were genetically identical to those identified as *K. c. meridionalis*.

Moderate levels of both geographic structure and genetic divergence were also detected within allopatric populations of the species *K. rigida* and *K. walteri*.

ECOTYPE EVOLUTION

Analysis using Bayesian mutational mapping provides an ambiguous perspective on patterns of ecotype evolution. For example, posterior probabilities of each ecotype for the

MRCA of the endemic Philippines radiation are all very similar (tree hole frog: 0.33; ground frog: 0.36; scansorial shrub frog: 0.31).

Discussion

RELATIONSHIPS, DIVERGENCES, AND TAXONOMIC IMPLICATIONS

Our analysis suggests that *Kaloula taprobanica*, a species with a range extending from Sri Lanka to Bangladesh, does not form a clade with other species of *Kaloula*. While *K. taprobanica* clearly forms a clade with *Metaphrynella*, *Ramanella*, and other species of the genus *Kaloula*, the more precise relationships of *K. taprobanica* remain unclear. Similarly, in analysis of microhylid relationships that included *K. pulchra* and *K. taprobanica*, van Bocxlaer et al. (2006) did not resolve *Kaloula* as monophyletic. Similar results were obtained by Matsui et al. (2011) and Trueb et al. (2011). Because of differences in the topology between ours and other studies, we refrain from taking taxonomic action on the generic status of *K. taprobanica*. Our findings indicate a need for further study employing greater sampling of loci and taxa within South Asian microhylid frogs.

Two contrasting patterns of genetic diversity were unexpected. Two species demonstrated almost no geographic-based genetic variation, despite extensive morphological variation and wide geographical ranges. Both *K. pulchra* (throughout Southeast Asia) and *K. picta* (throughout the Philippines) exhibit nearly identical sequences across their respective broad distributions and yet both have been the subject of much discussion by taxonomists who anticipated impending taxonomic subdivision of these species (Parker 1934; Inger 1954, 1966). Populations historically referred to

Kaloula baleata (type locality: Java) include several putative new species, including a highly divergent and morphologically distinct Vietnam population ($> 7\%$ divergent from other species in the *baleata* clade) and morphologically similar populations from Peninsular Malaysia, Java + Bali, and Sulawesi (< 3% from one another). In addition, the Philippine exemplar of the *baleata* clade (*Kaloula* sp. nov. from Palawan) is a highly distinctive, small-sized, tree-hole breeding species. In accordance with their status as distinct and diagnosable allopatric evolutionary lineages (Wiley 1978; de Queiroz 1998,1999), we consider these allopatric populations of the *baleata* clade to be distinct species that require formal description.

Divergences among species in the *kalingensis* clade are much greater than those observed between other species of *Kaloula* (Table 2). The *kalingensis* clade contains two described species (*K. kalingensis* from northern Luzon, including the type locality in Kalinga Province, and *K. kokacii* from the Bicol Peninsula of southern Luzon Island, adjacent to the type locality on Catañduanes Island), and two undescribed species from Panay Island and east Luzon Island, respectively.

Kaloula conjuncta exhibits moderate levels of divergence between clades, not all of which correspond to existing taxonomy. *Kaloula c. conjuncta* from numerous localities on Luzon Island was paraphyletic with respect to a population on Mindoro Island that is morphologically and acoustically distinct (Brown et al. unpubl. data). Similarly, populations that are morphologically and acoustically diagnosable as *K. conjuncta negrosensis* from Panay and Negros Islands did not form a clade exclusive of other subspecies; in the ML estimate, the Panay population of *K. c. negrosensis* is most

closely related to a putative new species from Sibuyan Island, the latter of which is morphologically and acoustically distinct from all other taxa (Brown et al. unpubl. data).

Mindanao specimens phenotypically similar to the type series of *K. c. stickeli* (holotype FMNH 60786) are nested within *K. c. meridionalis* and are genetically identical to this morphologically distinct taxon. *Kaloula c. stickeli* is morphologically intermediate between *K. c. meridionalis* (a smaller scansorial species with widely expanded digital disks) and *K. picta* (larger terrestrial species with non-expanded digital disks) and was originally described from northern portions of the Mindanao aggregate island complex (Samar and Leyte Islands; Inger 1954). The possibility of a hybrid origin for *K. c. stickeli* seems likely. Specimens included in our analysis were collected on Mindanao Island from a large mixed chorus of individuals of *K. c. meridionalis*, *K. picta* and those similar to *K. c. stickeli*. Interspecific amplexus was observed between *K. c. meridionalis* and *K. picta* (RMB and JAM, personal observation). Taken together, the field observations and genetic similarity between specimens identified phenotypically as *K. c. stickeli* and *K. c. meridionalis* suggest that *K. c. stickeli* is a morphologically intermediate phenotype that results from natural hybridization between these *K. c. meridionalis* and *K. picta*. As a result of these findings, and for the purposes of our comparative analyses, *K. c. stickeli* was not treated here as a valid taxon. We anticipate that future taxonomic work will place *K. c. stickeli* in the synonymy of *K. c. meridionalis.* In fact, this hybridization among species in the Philippines radiation is not surprising and fits an emerging pattern highlighting hybridization as a common and important part of radiations (Grant and Grant 2002; Seehausen 2004; Wiens et al. 2006; Cristecu et al. 2010).

Finally, moderate levels of both geographic structure and genetic divergence were also detected within allopatric populations of the species *K. rigida* and *K. walteri*. Our one sample of *K. rigida* from the type locality (Baguio City, Luzon Island) is highly divergent from the remaining *K. rigida* from the forested mountains of northern Luzon and falls out sister to *K. walteri* plus the remaining *K. rigida*. Future studies, involving targeted geographical sampling and additional sampling near Baguio City will be necessary to resolve uncertainty in the status of *K. rigida*.

In summary, several discrepancies between existing taxonomy and observed distribution of phylogenetic diversity will require an eventual comprehensive taxonomic review of species diversity—an undertaking beyond the scope of this paper. We note that the recognition of the above undescribed species will result in \sim 40% increase in known diversity in *Kaloula* (see Brown and Diesmos 2002, Brown 2007, and Brown et al. 2008 for review of rapidly changing taxonomy in Philippine anurans). This increase in diversity is unsurprising given recent similar studies of taxonomic diversity and cryptic species in Southeast Asian amphibians (Brown et al. 2008; Stuart and Bain 2008; Brown and Stuart 2012).

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- Supplementary Figure 1. Maximum-likelihood phylogram estimated from mitochondrial DNA sequences (12S and 16S ribosomal RNA genes) depicting the phylogenetic relationships of other microhylid taxa in relation to *Kaloula*. Note that *K. taprobanica* does not form a clade with other species of *Kaloula.* The outgroup *Callulina* is not shown.
- Supplementary Figure 2. Maximum-likelihood phylogram estimated from mitochondrial DNA sequences (12S and 16S ribosomal RNA genes) depicting the phylogenetic relationships of *Kaloula* (Anura: Microhylidae; figure complements Fig. 1). Species groups discussed in the text are highlighted in gray boxes.
- Supplementary Figure 3. Uncertainty in disparification through time in *Kaloula*. Panels show disparity-through-time (DTT) plots for $PC1_{\text{phylo}}$, $PC2_{\text{phylo}}$, $PC3_{\text{phylo}}$, and all three axes together $(PC1-3_{phylo})$. Solid black line on DTT plots represtent observed disparity based on MCCT; gray polygons represent 95% confidence interval for observed disparity based on post-burnin trees from BEAST analysis.

Supplementary Table 1.—Summary of specimens corresponding to genetic samples included in the study. ABTC = South Australian Museum genetic resources collection; BS-FS = Biotic Survey (David Bickford) field series, deposited at the University of Papuan New Guinea Reference Collection; ACD = Arvin Diesmos field series, specimen deposited at the National Museum of the Philippines; CAS = California Academy of Sciences Herpetological Collections; DCC = David Cannatella field series; DLSUD = deposited in De La Salle (Cavite, Philippines) University Reference collection; DWNP = deposited in University of Malaya Reference Collection; Franky Bossyut personal collection; FMNH = Field Museum of Natural History Herpetological Collections; FRIM = Forest Research Institute of Malaysia Reference Collection; GVAG = Genevieve V. A. Gee field series, deposited at the National Museum of the Philippines; ID = Indraniel Das field number, specimen deposited at Raffles Museum; JS = Jeet Sukumaran field number, specimen deposited at FRIM; KU = University of Kansas Natural History Museum; LSUHC = La Sierra University Herpetological Collections; LSUMZ = Louisiana State University Herpetological Collection; $MF = Mike Forstner (Texas State University) frozen tissue collection; MG = Marcn Gaulke field series (voucher specimens lost); MM = Madhava Meegaskumbura$ field series; MZB = Museum Zoologicum Bogoriense, Java, Indonesia; NMNS = National Museum of Natural Science, Taiwan; PNM/CMNH-H = uncatalogued material, deposited in the Cincinnati Museum of Natural History; RMB = Rafe Brown field number, uncataloged specimen deposited at the National Museum of the Philippines; ROM = Royal Ontario Museum; SP = Sabah Parks Collection, Sabah, Borneo, Malaysia; TNHC = Texas Natural History Collections, University of Texas at Austin; TZ = Thomas Ziegler, personal collection; USNM FS = United States National Museum Field Series; ZUEC = Museu de História Natural, Universidade Estadual de Campinas, Brazil.

