Appendix S2 Supplemental text

SUPPLEMENTAL MATERIALS AND METHODS

Population genetic summary statistics
We focused historical demographic analyses on two focal clades, one spanning the mainland and land-bridge islands of the Sunda Shelf (H. picturata group) and another situated entirely in the oceanic portions of the adjacent Philippines (H. grandocula–H. similis group).

To assess general patterns of genetic diversity in our two focal clades (Fig. 2, Clades 6, 13), we calculated the numbers of mtDNA haplotypes ($N_h$), numbers of polymorphic sites ($P_N$), mean number of pairwise nucleotide differences ($k$), haplotype diversity ($h$; Nei, 1987) and nucleotide diversity ($\pi$; Nei & Tajima, 1981) using DnaSP v5.0 (Rozas et al., 2003) and Arlequin v3.5.1.3 (Excoffier et al., 2005) for Hylarana picturata and H. similis/grandocula complexes.

Demographic inferences
We compared these same two focal lineages by assessing both for evidence of recent change in effective population size. We calculated mismatch distributions in Arlequin v3.5.1.3 (Excoffier et al., 2005), assuming an infinite sites model of selectively neutral nucleotide substitutions and assessing significance via coalescent simulations of a large, neutrally evolving population of constant size (Slatkin & Hudson, 1992; Rogers & Harpending, 1992). This approach involved the assessment of a mismatch distribution for ragged and/or multimodal distributions, which could stem from a structured population, versus smooth or unimodal, which is indicative of possible recent population expansion or sudden panmixia (Harpending et al., 1998). We also employed Fu’s $F_S$ neutrality test (Fu, 1997) as an assessment of possible population expansion. This assumes neutrality and potentially diagnoses a recent population expansion via a highly negative value of $F_S$. Finally, we calculated Tajima’s $D$ (implemented as a test for selective neutrality; Tajima, 1989), and Ramos-Onsins & Rozas’ $R_2$ statistic (Ramos-Onsins & Rozas, 2002) as additional indicators of potential population expansion (based on distributions of haplotypes and numbers of segregating sites).
SUPPLEMENTAL RESULTS

NEW PHYLGENETIC RELATIONSHIPS

Our preferred phylogenetic estimate (Figs 2 & 3) provides a new perspective on systematic relationships of Southeast Asian spotted stream frogs (Brown & Guttman, 2002). *Hylarana banjarana*, a high elevation forest stream species from Peninsular Malaysia (Leong & Lim, 2003) was recovered as the sister species to all remaining *H. signata* Complex taxa, and analyses reveal considerable intraspecific genetic structure among populations (Fig. 2, Clade 3) from montane localities of western Malaysia. The next diverging lineage consists of three Sundaic species, the Siberut Island (off the coast of southern Sumatra) endemic *Hylarana siberu* (Dring et al., 1989), an undescribed species from the Malay Peninsula (Leong & Lim, 2004), and an undescribed Sumatran highland species *Hylarana siberu* cf. *siberu* (Van Kampen, 1910; Iskandar & Colijn, 2000; Manthey & Grossman, 1997). The remaining eight taxa fall into a large clade distributed geographically in the island archipelagos of the Sunda Shelf and the Philippines. Within this clade, one group consisting of four highly divergent lineages from the Malay Peninsula, Thailand, Sumatra, and Borneo islands is currently identified as *Hylarana picturata*, and is sister to the remaining taxa (Figs 2 & 3). In contrast to the topology presented by Brown & Guttman (2002), our data support *Hylarana signata* as the sister species of the western Philippine island arc endemics *H. mangyanum* (Mindoro Island) and *H. moellendorffi* (Palawan Island). The eastern Philippine island arc species *H. similis* (Luzon faunal region) and *H. grandocula* (Mindanao faunal region) appear most closely related to *H. melanomenta* (Tawi-tawi Island of the Sulu Archipelago) and an undescribed species (*Hylarana* sp.) from Mindanao Island. As in Brown & Guttman (2002), we observed strong support for sister-species relationships between the Philippine endemics of the eastern island arc (*H. grandocula* and *H. similis*) and those of the western island arc (*H. moellendorffi* and *H. mangyanum*). Although the apparent phylogenetic affinity of each species pair is inferred differently in this study (Fig. 2), and two additional species (*H. melanomenta* of Tawi-tawi Island and *H. sp.* from southeast Mindanao Island) are inferred to be related to the *H. grandocula–H. similis* clade. As observed in phylogenetic estimates, the *H. picturata* clade consisted of four, highly divergent, well supported lineages (Fig. 3C), whereas analyses did not support the reciprocal monophyly of the two currently recognized species in the the geographically structured *H. similis–H. grandocula* clade. However, reticulating relationships among three shallowly divergent clusters of populations were inferred (Fig. 4c), with the northern Mindanao faunal region islands of Samar, Leyte, Dinagat and Bohol forming a separate, genetically distinct but weakly supported clade.

As in the findings of Brown & Guttman (2002), we find sister relationships to be well supported between the species pairs *H. moellendorffi* and *H. mangyanum* (the western Philippine arc species) and *H. grandocula* and *H. similis* (the eastern arc species). Interestingly, whereas Brown & Guttman (2002) inferred a close relationship between *H. picturata* and the western arc species, our data clearly indicate that *H. signata* is mostly closely related to *H. moellendorffi* and *H. mangyanum* (Fig. 5). Additionally, the Brown & Guttman (2002) study suggested that the *H. grandocula–H. similis* species couplet might be most closely related to *H. signata*—our results indicate that these eastern arc
species are part of a clade containing other Sulu and Mindanao faunal region species and that this clade is reciprocally monophyletic with the \((H.\ signata),(H.\ moellendorffi, H.\ mangyanum)\) clade.

SUPPLEMENTAL DISCUSSION

PREVALENCE OF THE DUAL-INVASION PATTERN?

Additional lineages have been shown in properly sampled molecular phylogenetic studies to exhibit the same ‘dual-invasion’ pattern elucidated here. These include litter toads of the genus *Leptobrachium* (Brown *et al.*, 2009), splash frogs of the genus *Staurois* (Arifin *et al.*, 2011), fanged frogs of the genus *Limnonectes* (Evans *et al.*, 2003), shrub frogs of the genus *Philautus* (Hertwig *et al.*, 2011), Old World tree frogs of the genus *Polypedates* (Brown *et al.*, 2010), and narrow mouth frogs of the genus *Kaloula* (Blackburn *et al.*, 2013). Additional groups that have not yet been included in phylogenetic analyses, but which nonetheless exhibit distributions (Borneo, Palawan and Mindanao) suggesting dual invasions, include frogs of the genera *Chaperina*, *Megophrys*, *Pelophryne*, and *Rhacophorus* (Inger, 1954, 1999; Brown, 2007).

Geographic variation in the degree to which these and other groups extend into the paired Philippine island arcs have led early biogeographers to conceive of the Philippines as a ‘fringing archipelago’ (Dickerson, 1928; Delacour & Mayr, 1946; Darlington, 1957; Myers, 1962; Leviton, 1963; Brown & Alcala, 1970) with a typical ‘immigrant pattern’ of nested faunal distributions (Taylor, 1928; Leviton, 1963; Brown & Alcala, 1970; Siler *et al.*, 2012). Although the dual-invasion scenario suggested by our results does not refute this general view of the Philippines as an island archipelago continually receiving colonists from an adjacent continental source, the fringing archipelago view is clearly an oversimplification of the mechanisms driving colonization and subsequent diversification of Philippine terrestrial vertebrates (Brown & Diesmos, 2009). First, *in situ* evolutionary processes of diversification, species interactions, adaptive radiation, and sea-level fluctuations undoubtedly have contributed to speciation within the oceanic portions of the archipelago (Heaney *et al.*, 2005, 2011; Esselstyn & Brown, 2009; Esselstyn *et al.*, 2009, 2011; Linkem *et al.*, 2011; Siler *et al.*, 2011; Siler & Brown, 2011). Second, it is clear that elevational and atmospheric gradients associated with the steep volcanic topography of many of the islands have either contributed to the process of diversification or allowed species accumulation via ecological processes, filtering, and complex processes of community assembly (Catibog-Sinha & Heaney, 2006; Siler *et al.*, 2011; Brown & Diesmos, 2009; Brown *et al.*, 2012, 2013). Lastly, as our understanding of the geological history of the region has greatly expanded and new studies have inferred deep phylogenetic lineage divergences, it is clear that ancient colonization of the archipelago (Jansa *et al.*, 2006; Blackburn *et al.*, 2010; Esselstyn *et al.*, 2009; Heaney *et al.*, 2011; Siler *et al.*, 2012), coupled with major land movements, subduction zone orogeny, and movements along major fault lines (Hamilton, 1979; Hall, 1996, 1998; Zamoros *et al.*, 2008; Yumul *et al.*, 2003, 2009a,b) have provided ample opportunities for within-archipelago diversification over the last 15–20 millions of years (Holloway, 1982; Jansa
et al., 2006; Brown & Diesmos, 2009; Esselstyn et al., 2009; Linkem et al., 2011; Siler et al., 2012; Brown et al., 2013).

REFERENCES


