

Evidence for multiple species of Sunda colugo

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Flying lemurs, or colugos, are among the most enigmatic mammals. They have the most extensive gliding membrane of any volant mammal (Figure 1A), allowing them to traverse distances up to 136 meters [1]. Current taxonomy recognizes just two colugo species, each assigned to a unique genus: the Sunda colugo (*Galeopterus variegatus*) and the Philippine colugo (*Cynocephalus volans*) [2]. Because the evolutionary history of colugos is poorly known we undertook the first study to examine genetic variation in the broadly distributed Sunda colugo. Our results, combined with morphological data, provide compelling evidence that mainland, Javan, and Bornean colugo subspecies may be better recognized as distinct species, potentially tripling species diversity in the genus *Galeopterus*.

Colugos are members of the mammalian order Dermoptera and are the closest living relatives of Primates, having diverged in the Late Cretaceous about 86 million years ago (Mya) [3]. Despite our increasing understanding of the taxonomic position of colugos within mammals, very little is known about these two species [1,3]. The Sunda colugo is endemic to Indochina and Sundaland, an area of the Asian continental shelf that incorporates the Malay Peninsula and the large islands of Borneo, Sumatra, and Java, as well many smaller islands (Figure 1B) [1]. Although this species has been divided into many subspecies in the past [4], only four are currently recognized [5]. Despite extensive size and pelage variation between populations from different geographically isolated regions [1,4,5], the Sunda colugo has never been the subject of a detailed taxonomic review assessing morphological and genetic variation across its range.

To investigate genetic diversity within Sunda colugos, we amplified and sequenced two fragments of the mitochondrial genome (mtDNA:

cytochrome b and *12S rRNA*, totaling 1,442 base pairs [bp]) and eight nuclear gene introns (*BTK*, *CHRNA1*, *CYP1A1*, *FAH*, *FES*, *GHR*, *HK1*, *MAOA*, totaling 4,291 bp; Supplemental Data). Our phylogenetic analysis included specimens from the Malay Peninsula (*G. v. peninsulæ*), Borneo (*G. v. borneanus*), and Java (*G. v. variegatus*, subspecies designations follow [5]). Our results reveal very large, unexpected genetic distances between these three subspecies at both mtDNA and nuclear loci (Supplemental Data). Using the established divergence between Philippine and Sunda colugos *circa* 20 million years ago (Mya) (95% credibility interval = 14.0–27.0 Mya) [3] based on independent molecular data as a surrogate clock calibration, we estimate the Javan subspecies diverged from the mainland subspecies 5.4 (3.8–7.3) Mya based on mtDNA, and 3.9 (2.8–5.3) Mya based on nuclear DNA (Figure 1C,D). The estimated mtDNA divergence of the Bornean subspecies from the mainland subspecies was 4.3 (3.0–5.8) Mya; no nuclear segments were available from Bornean colugos. Within the Malay Peninsula, the mtDNA and nuclear divergence between the Thailand and mainland Singapore samples was 0.7 (0.5–1.0) Mya and 2.7 (1.9–3.7) Mya, respectively.

Sundaland is a hotspot of biodiversity [6], as a consequence of allopatric speciation processes that occurred during repeated isolation events in the Pliocene and Pleistocene as sea levels, river systems, and forest communities fluctuated [7–9]. Several recent molecular studies have illuminated species-level differences between mammal populations from mainland Indochina and Borneo previously thought to be conspecific (e.g., clouded leopards) [10], as well as in the Sundaland archipelago (e.g., orangutans) [8]. Our subspecies divergence estimates for colugos exceed those of many mammalian sister-species in the region (Supplemental Data), including primates and felids (e.g., 1.1–2.6 My for *Macaca*, *Pongo*, *Prionailurus*, and *Panthera* sister-species) [8–11] and are within the range of basal radiations of many modern genera (e.g., *Prionailurus*, 4.6 My [11], *Panthera*, 3.7 My [11], *Macaca*, 5.5 My [9]). Furthermore, the nuclear DNA divergence between mainland and Javan colugos exceeds values observed between described canid sister-species at four of the genes used in our study [12] (Supplemental Data).

Our results indicate that the mainland, Javan, and Bornean colugo subspecies may be better recognized as distinct species (Figure 1), based

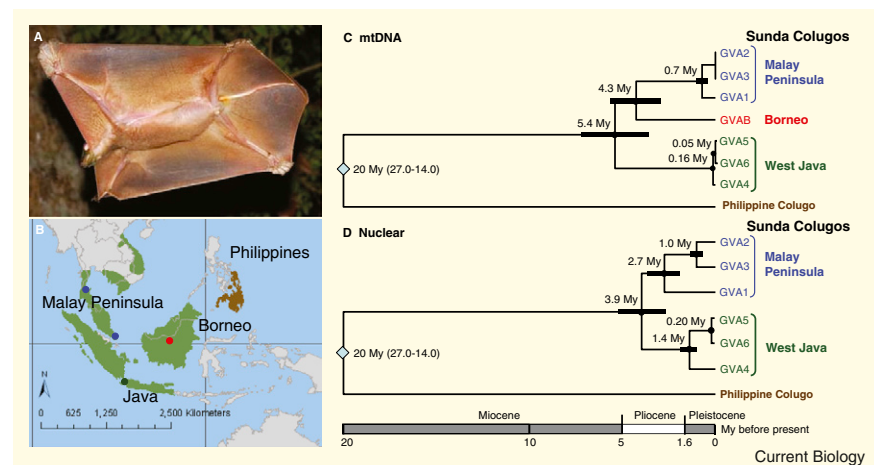


Figure 1. Appearance, distribution and phylogeny of Sunda colugos.

(A) Gliding colugo. (B) Geographic distribution of Sunda (green) and Philippine (brown) colugos in Southeast Asia, including locations sampled. (C,D) Maximum likelihood phylogenies reconstructed from mitochondrial (C) and nuclear (D) DNA segments for Sunda colugos, with the Philippine colugo as the outgroup. Branch lengths were estimated under a molecular clock in PAUP* and converted to time based upon an independent molecular calibration point [3] denoted with a blue diamond. The confidence intervals are derived from the upper and lower 95% credibility intervals of the Sunda/Philippine colugo divergence [3]. Taxon labels are colored to correspond with their respective geographic sampling locations in the map.

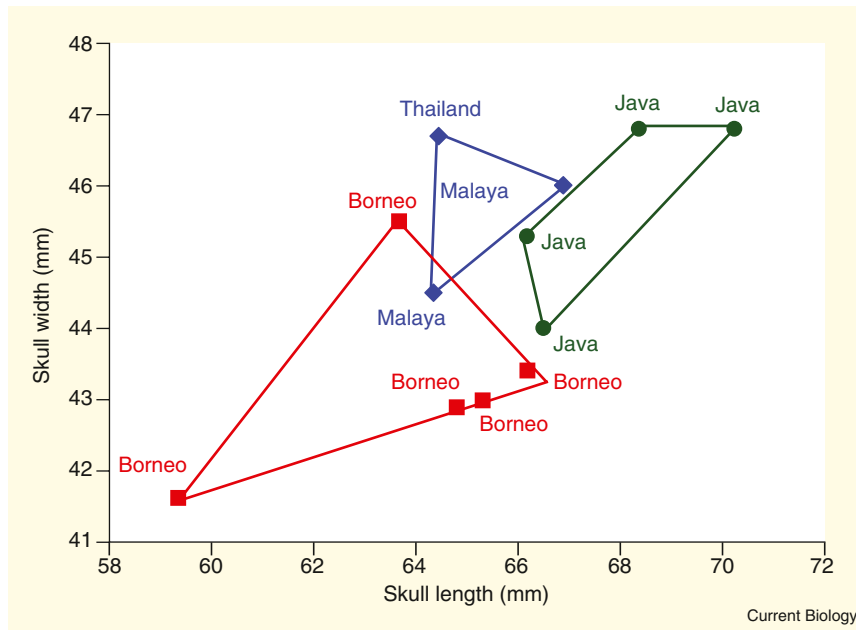


Figure 2. Skull size (as reflected by condylobasal length and zygomatic width) in colugos from Java, Borneo, and the Malay Peninsula (Malaya/Thailand).

Colors consistent with Figure 1. Plotted values are from adult males maintained by the National Museum of Natural History (Washington, D.C.) and the Naturalis Museum (Leiden, Netherlands). Bornean colugos are generally smaller than colugos from Java and the mainland; colugos from Malaya tend to have wider skulls relative to the length of the skull compared to specimens from Java. The significance of these, and other possible distinctions, requires further assessment in the context of a detailed genetic, morphological, and taxonomic overview for colugos.

on species concepts that incorporate geographic separation and genetic divergence [13,14], potentially tripling the diversity in *Galeopterus*. In addition, nuclear DNA evidence suggests the mainland populations may be further differentiated. Preliminary investigations identify distinctive morphological trends that may discriminate these taxa (Figure 2). Our findings underscore key outstanding questions in the biogeography of colugos, such as whether every island or region supports a distinct phylogenetic lineage, whether older splits within this genus are correlated with modern island boundaries, and whether the current recognition of a single *Galeopterus* species masks sympatric overlap between morphologically conservative, yet distinct species, on the Sunda Shelf.

The order Dermoptera is an important conservation priority in the context of global biodiversity because of its phylogenetic, morphological and ecological uniqueness. Colugos' dependence on lowland forest habitats and limited dispersal abilities through open areas make them susceptible to local

population extinctions in a region with alarmingly high deforestation rates [6]. The discovery that at least three populations of Sunda colugos are genetically and morphologically [4,5] divergent lineages indicates a need for a reassessment of conservation priorities in tandem with revised taxonomy. The conservation status of the Sunda colugo is listed by IUCN as Lower Risk/Least Concern, yet as isolated populations are extirpated it is possible that some of these events may represent species-level extinctions. Firm understanding of colugo taxonomy will be necessary for developing conservation initiatives that more effectively safeguard dermopteran biodiversity.

Supplemental Data

Supplemental data including methods used in this study and supporting data are available at [http://www.current-biology.com/supplemental/S0960-9822\(08\)01190-1](http://www.current-biology.com/supplemental/S0960-9822(08)01190-1).

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References

- Lim, N. T.-L. (2007). *Colugo: The Flying Lemur of South-east Asia*, (Singapore: Draco Publishing and Distribution).
- Stafford, B.J. (2005). Order Dermoptera. In *Mammal Species of the World, Volume 1, 3rd Edition*, D.E. Wilson, and D.M. Reeder, eds. (Baltimore: The John Hopkins University Press), pp. 110.
- Janečka, J.E., Miller, W., Pringle, T.H., Wiens, F., Zitzmann, A., Helgen, K.M., Springer, M.S., and Murphy, W.J. (2007). Molecular and genomic data identify the closest living relative of primates. *Science* 318, 792-794.
- Chasen, F.N., and Kloss, C.B. (1929). Notes on the flying lemurs (*Galeopterus*). *Bulletin of the Raffles Museum* 2, 12-22.
- Stafford, B.J., and Szalay F.S. (2000). Craniodental functional morphology and taxonomy of Dermopterans. *J. Mammal.* 81, 360-385.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403, 853-858.
- Harrison, T., Krigbaum, J., and Manser, J. (2006). Primate biogeography and ecology on the Sunda Shelf Islands: A paleontological and zooarchaeological perspective. In *Primate Biogeography*, S.M. Lehman, and J.G. Fleagle, eds. (New York: Springer), pp. 331-372.
- Zhi, L., Karesh, W.B., Janczewski, D.N., Frazier-Taylor, H., Sajuthi, D., Gomberk, F., Andau, M., Martenson, J., and O'Brien, S.J. (1996). Genomic differentiation among natural populations of orang-utan (*Pongo pygmaeus*). *Curr. Biol.* 6, 1326-1336.
- Ziegler, T., Abegg, C., Meijaard, E., Perwitasari-Farajallah, D., Walter, L., Hodges, J. K., and Roos, C. (2007). Molecular phylogeny and evolutionary history of Southeast Asian macaques forming the *M. silenus* group. *Mol. Phylogenet. Evol.* 47, 807-816.
- Buckley-Beason, V.A., Johnson, W.E., Nash, W.G., Stanyon, R., Menninger, J.C., Driscoll, C.A., et al. (2006). Molecular evidence for species-level distinctions in clouded leopards. *Curr. Biol.* 16, 2371-2376.
- Johnson, W.E., Eizirik, E., Pecon-Slattery, J., Murphy, W.J., Antunes, A., Teeling, E., O'Brien, S.J. (2006). The Late Miocene radiation of modern Felidae: A genetic assessment. *Science* 311, 73-76.
- Beredeben, C., Moore, R.L., and Wayne, R.K. (2005). A molecular phylogeny of the Canidae based on 6 nuclear loci. *Mol. Phylogenet. Evol.* 37, 815-831.
- Coyne, J.A., and Orr, H.A. (2004). Speciation, (Sunderland; Sinauer Associates).
- Baker, R.J., and Bradley, R.D. (2006). Speciation in mammals and the Genetic Species Concept. *J. Mammal.* 87, 643-662.

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