

Ancient divergence time estimates in *Eutropis rugifera* support the existence of Pleistocene barriers on the exposed Sunda Shelf

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ABSTRACT

Episodic sea level changes that repeatedly exposed and inundated the Sunda Shelf characterize the Pleistocene. Available evidence points to a more xeric central Sunda Shelf during periods of low sea levels, and despite the broad land connections that persisted during this time, some organisms are assumed to have faced barriers to dispersal between land-masses on the Sunda Shelf. *Eutropis rugifera* is a secretive, forest adapted scincid lizard that ranges across the Sunda Shelf. In this study, we sequenced one mitochondrial (*ND2*) and four nuclear (*BRCA1*, *BRCA2*, *RAG1*, and *MC1R*) markers and generated a time-calibrated phylogeny in BEAST to test whether divergence times between Sundaic populations of *E. rugifera* occurred during Pleistocene sea-level changes, or if they predate the Pleistocene. We find that *E. rugifera* shows pre-Pleistocene divergences between populations on different Sundaic land-masses. The earliest divergence within *E. rugifera* separates the Philippine samples from the Sundaic samples approximately 16 Ma; the Philippine populations thus cannot be considered conspecific with Sundaic congeners. Sundaic populations diverged approximately 6 Ma, and populations within Borneo from Sabah and Sarawak separated approximately 4.5 Ma in the early Pliocene, followed by further cladogenesis in Sarawak through the Pleistocene. Divergence of peninsular Malaysian populations from the Mentawai Archipelago occurred approximately 5 Ma. Separation among island populations from the Mentawai Archipelago likely dates to the Pliocene/Pleistocene boundary approximately 3.5 Ma, and our samples from peninsular Malaysia appear to coalesce in the middle Pleistocene, about 1 Ma. Coupled with the monophyly of these populations, these divergence times suggest that despite consistent land-connections between these regions throughout the Pleistocene *E. rugifera* still faced barriers to dispersal, which may be a result of environmental shifts that accompanied the sea-level changes.

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