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Pollinator-driven divergence and speciation in three *Aeschynanthus* species

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How does pollinator-driven divergence lead to plant speciation? We examined the role of pollinators in an intriguing case of incipient species in the SE Asian sunbird-pollinated genus *Aeschynanthus*. Previous findings suggested an expansion of pollinator niche breadth led to a shift to mixed vertebrate pollination in the origin of *A. acuminatus*. Its two putatively closest relatives, *A. moningerae* and *A. pedunculatus*, share identical vegetative traits but differ only in key floral traits indicative of sunbird pollination, i.e. their longer corolla tubes and longer peduncles. We first confirmed their sunbird specialized pollination systems, then tested between two specific phylogenetic hypotheses: (1) Reciprocal monophyly - where the two sunbird specialists form a clade sister to *A. acuminatus* (2) Genetic nestness - where the two geographically restricted endemics reflected a budding history with reversal to sunbird pollination from a widespread *A. acuminatus*. Phylogenetic inference based on genome-wide SNPs revealed a combination of two, with *A. moningerae* nested within *A. acuminatus* while *A. pedunculatus* form a sister lineage with *A. bracteatus*. Morphological analyses on floral traits demonstrated convergence of the two sunbird specialists despite their independent origins. Progenitor-derivative speciation led to a localized reversal to sunbird specialization in *A. moningerae* on geographically isolated Hainan Island. In contrast, sympatric populations of *A. pedunculatus* and *A. acuminatus* in southern Vietnam still partially overlap in flowering phenology and pollinator niche even with pollinator-adapted floral divergence. We detected introgression from *A. pedunculatus* into *A. acuminatus*, resulting in unusually wide floral variation. Together, we demonstrated multiple expansion and contraction of pollinator niche breadths concurrent with the series of speciation events in focal *Aeschynanthus* species. However, pollinator shifts alone did not generate complete reproductive isolation unless complemented with geographic or phenological isolation.

Keywords: Speciation; Sunbirds; Pollinator shift; Floral trait evolution