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Evolutionary diversification and historical biogeography of orchidaceae in Central America with emphasis on Costa Rica and Panama

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Summaries

Summary

Historically, the isthmus of Costa Rica and Panama has been a source of fascination for its strategic position linking North America to South America. The isthmus is one of the world's most biodiverse regions where Orchidaceae is the most species-rich plant group. The area harbors more than 2,010 orchid species; representing about 8% of all species in the family on just about 1% of the Earth's land surface. Three genera of orchids are among the six most speciose angiosperm groups surpassing 1,000 species: *Epidendrum* L. (1,459 species), *Lepanthes* Sw. (1,125) and *Stelis* Sw. (1,128). The origin of this extraordinary orchid diversity has been attributed to the epiphytic habitat, CAM photosynthesis, pollination mechanisms, orogenic processes, past climatic fluctuations, or key innovations such as colonizations (extrinsic) or trait evolution (intrinsic). However, the influence of these factors in the diversification of the most speciose Neotropical orchid lineages has not been evaluated due to the insufficient knowledge on these challenging complex groups. In this thesis, I targeted the hyperdiverse orchid genus *Lepanthes*, as a study model to investigate the evolutionary processes that promoted species diversifications. To test hypotheses about the main drivers behind the evolution of these miniature orchids, we improved the taxonomy of *Lepanthes* and allies by combining morphological characters with solid, densely sampled phylogenies using phylogenetic comparative methods, described a new pollination system in the group and identified morphological characters associated with similar pollination mechanisms combining field observations, microscopy and histochemistry and discussed the impact of orogenic processes (formation of the Andes and Central America) on the actual species richness of *Lepanthes*. This thesis provides new insights in the taxonomy and systematics, pollination systems, biogeography and evolutionary history of *Lepanthes* and allies to understand the complex evolution of one of the most species-rich angiosperm lineages in the Neotropics. *Lepanthes* contains more than 1,128 species and new species are constantly being discovered. I described two new species from Panama based on morphological observations, named *Lepanthes aures-ursinae* and *Lepanthes vertebrata*. Some species are easily diagnosable based on morphological characters, however, others belonging to species complexes are difficult to separate because of the morphological similarity, especially in floral traits. In addition, lineages derived from rapid diversifications are often challenging to resolve using morphology or few standard DNA barcoding markers. Therefore, I used the anchored hybrid enrichment approach (AHE) to obtain 446 markers from the three plant genomes in order to disclose species relationships in the Costa Rican-Panamanian endemic *Lepanthes horrida* species group. I obtained a fully resolved phylogeny inferred with coalescent-based species tree estimations and disclosed two undescribed species, named *L. amicitiae* and *L. genetopophantica*. In addition, I found high topological discordance among individual gene trees, suggesting that hybridization/polyploidy may have promoted speciation in the lineage via formation of new hybrid taxa. Similar to the poor understanding of inter-specific relationships, the inter-generic relationships in the *Lepanthes* clade have been unclear because of insufficient phylogenetic sampling and because of the convergent and variable nature of its phenotypic traits. To clarify these relationships, I used phylogenetic comparative methods to test the suitability of selected traits for generic delimitations in the *Lepanthes* clade, evaluating every generic name proposed in the group. Based on these findings, I proposed a new classification recognizing fourteen genera, including four novel generic concepts, and discussed the changes needed to reorganize the *Lepanthes* clade. From the

18 morphological traits evaluated, I identified 16 plesiomorphies, 12 homoplastic characters, and 7 synapomorphies, the latter of which are reproductive features, mostly related to pollination by pseudocopulation and possibly correlated with rapid diversifications within *Lepanthes*. Furthermore, the ancestral states of some reproductive characters suggest that these traits are associated with similar pollination mechanisms promoting homoplasy. The role of pollinators as drivers of species diversity in the *Lepanthes* clade is largely unknown because knowledge of pollination systems is scarce. The only known pollination system in the group is a pseudocopulatory strategy of *Lepanthes* involving male fungus gnats (Diptera, Sciaridae). I disclosed the pollination mechanism of the *Lepanthes*' closely related *Trichosalpinx* through the study of pollinator behavior and floral anatomy. I found that two *Trichosalpinx* spp. are pollinated exclusively by female biting midges of the genus *Forcipomyia* (Diptera, Ceratopogonidae). I detected secretion of carbohydrates and proteins on the lip with microscopy and histochemical techniques. These secretions might stimulate the protein collection instinct of female biting midges. These biting midges show well-developed mandibles and poorly developed laciniae, indicating that they mainly feed on invertebrate hosts from which they draw haemolymph. *Trichosalpinx* flowers offer small quantities of proteins and carbohydrates that may act as flavor teas as part of a complex deceptive system. Some other angiosperms such as *Bulbophyllum* (Orchidaceae), *Ceropegia* spp. (Asclepiadaceae) and *Theobroma cacao* (Malvaceae) that are also pollinated by biting midges possess similar dark purple flowers with ciliate ornamentation and use myophily, sapromyophily or kleptomyiophily as strategies to exploit different families of Diptera as pollinators. One *Forcipomyia* sp. (Euprojoannisia) is kleptoparasitic, suggesting that kleptomyiophily may have evolved in *Trichosalpinx*. The similar floral morphology among members of *Trichosalpinx* and some species of the closely related genera *Anathallis* and *Lankesteriana* suggests that they are all pollinated by biting midges. To further test this hypothesis, I studied the micromorphology and histochemistry of the flowers of *Trichosalpinx*, *Anathallis*, and *Lankesteriana* and found similar floral secretions such as carbohydrates and proteins on the lip and petals supporting a hypothesis of floral parallelism driven by pollinators.

To understand the role of abiotic factors in the diversification of *Lepanthes* such as the impact of the Andean orogeny and the influence of neighboring regions such as the Amazon, Central America and the Antilles in extant species composition, we inferred the biogeographical history and dynamics of speciation, extinction and migration of the two largest Neotropical orchid groups Cymbidieae and Pleurothallidinae, using two unparallelled, densely sampled orchid phylogenies. We found that the majority of these orchid lineages only originated in the last 20–15 Ma. Andean lineages are derived from lowland Amazonian ancestors, with additional contributions from Central America and the Antilles. Species diversification is correlated with Andean orogeny, and multiple migrations and recolonizations across the Andes indicate that mountains do not constrain orchid dispersal over long timescales. This suggests that mountain uplift promoted species diversification across all elevational zones. Derived from this study, we also found three rate shifts in the Pleurothallidinae, with the highest diversification rates in *Lepanthes*. To further investigate these diversification rates and also the biogeographical history of *Lepanthes*, I increased the species sampling to 25%. I found that *Lepanthes* likely originated in the Central Andes (CA) and diversified between 7–8 Ma during the Miocene. The genus reached Southern Central America (SCA) from the Andean region twice and the extant lineages from Northern

Central America (NCA) and the West Indies (WI) are likely derived from SCA ancestors, suggesting that the isthmus of Panama served as a land bridge for lineages derived from Andean ancestors. As found previously for the Pleurothallidinae, cladogenesis by within-area speciation was the most common biogeographical model for *Lepanthes*. The most frequent dispersal routes were SCA with NCA and NA and NA with CA. Two of the most recent clades of *Lepanthes* containing species from SCA experienced shifts in species diversification with an acceleration around 2.5 Ma. This acceleration did not strictly correlate with mountain orogeny. Paleoclimatic evidence indicates that cooling periods started before 2.7 Ma and this partially correlates with species diversifications of *Lepanthes* in SCA. Botanical explorations, basic morphological documentation, and alpha-taxonomic work are the starting points to infer solid, densely sampled phylogenies in order to test evolutionary hypotheses on species diversifications in hyperdiverse orchid lineages.

In addition, new techniques such as high-throughput sequencing coupled with coalescence-based methods are a powerful tool to solve complicated phylogenetic relationships in lineages derived from recent, rapid diversifications. Selection of the most informative phylogenetic markers detected in phylogenomic datasets would be an adequate strategy to further increase the sampling because analyzing large datasets of hundreds of species and markers might be computationally arduous. Furthermore, phylogenomic datasets provide additional information on biological phenomena such as incomplete lineage sorting, hybridization or polyploidy that might cause discordance among individual gene trees.

Problematic inter-generic delimitations can be improved by assessing suitable morphological traits with phylogenetic comparative methods to detect synapomorphies and homoplastic characters. I am confident that this strategy will further improve the systematics of the Pleurothallidinae as a whole. This subtribe challenged systematists and taxonomists for centuries due to the floral homoplasy untangled here, which is possibly resulting from similar pollination systems. However, despite the new discoveries made on the pollination of *Lepanthes* and *Trichosalpinx* during my Ph.D project, many more observations on other species and genera are needed in order to fully understand the influence of pollinators on the diversification and evolution of floral traits. These systems should be disclosed not only by describing the pollination system but also linking behavior and natural history of pollinators to the strategy of attraction by the flowers. Based on two extensively sampled orchid phylogenies, combined with statistically robust diversification models, our results reveal that Andean orchid diversification has closely tracked the Andean-Central American orogeny. Further, the rise of some Neotropical mountains had little effect on restricting orchid biotic dispersal suggesting that they are semi-permeable barriers to lowland organisms, whose dispersal ability is more probably related to intrinsic traits (e.g. seed size, dispersal mechanism, mutualisms). Finally, *Lepanthes* showed the highest speciation rates across the Pleurothallidinae. The genus is estimated to have diversified recently, between 5-10 Ma. Future research should focus on increasing species sampling and adding new multiple markers to resolve recalcitrant nodes in its phylogeny. Likewise, the role of extant important biomes (i.e montane forests) in the diversification of this most diverse orchid groups within the Pleurothallidinae should be further studied to make sure that not only the orchid species but also the associated pollinators and hosts survive ongoing global warming at the cold, high- elevation areas where they are most diverse.