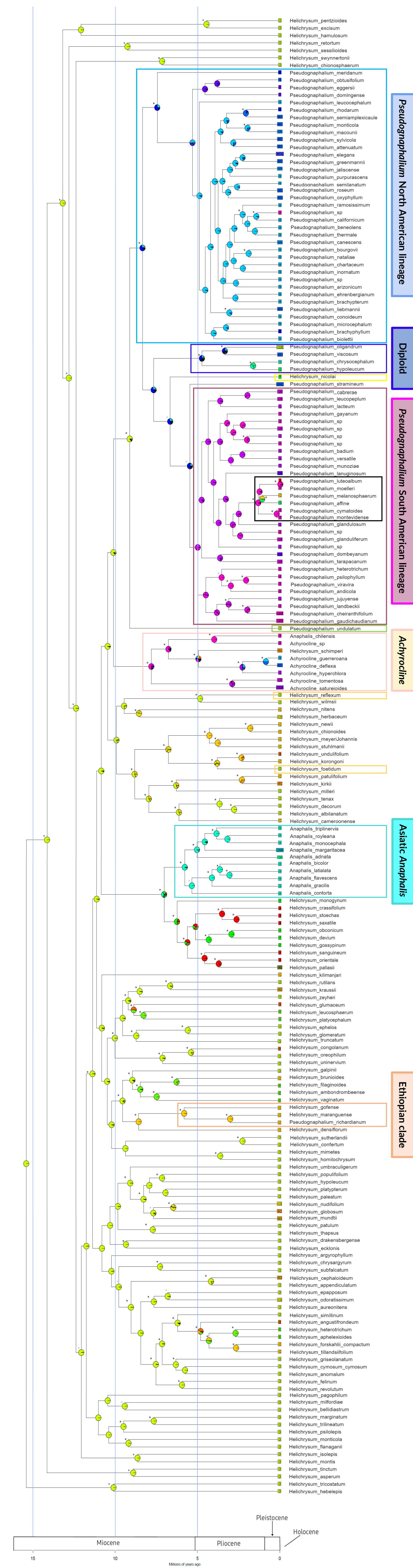


# Evolutionary and biogeographic study of the genus *Pseudognaphalium* (Compositae) based on Hyb-Seq

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## Introduction

The systematics of the **Gnaphalieae** tribe (Compositae) has always been problematic, especially regarding the genera of the **HAP clade** (*Helichrysum-Anaphalis-Pseudognaphalium*). The phylogenetic position and relationships within *Pseudognaphalium* are troublesome, since in previous works the genus appears embedded in a paraphyletic *Helichrysum* and its monophyly is not clear due to low phylogenetic resolution and different results depending on taxon sampling.

The presence of polyploid taxa, combined with phylogenetic incongruence between nuclear and plastid markers, lead to hypothesize one or several **allopolyploid** origins for *Pseudognaphalium*. Also, its wide geographic range and disjunct distribution make it an excellent case for exploring the role of **long-distance dispersal (LDD)** and polyploidy in plant biogeography.

## Methods

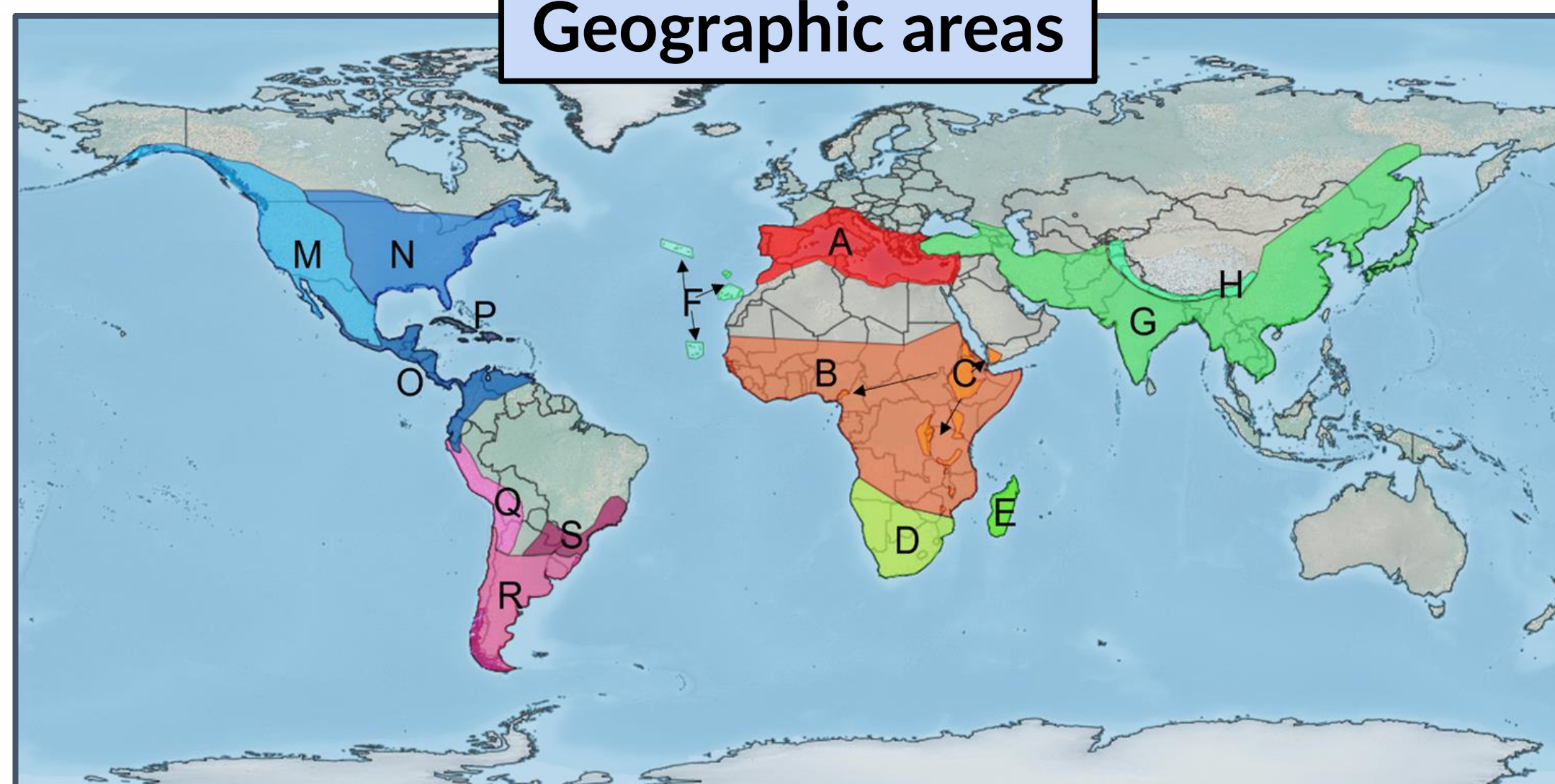
We employed a Hyb-Seq technique that targets 1061 nuclear conserved ortholog loci designed for Compositae and we recovered 853 loci. We constructed a phylogeny under the **concatenation approach** with the maximum likelihood method implemented in **RAXML-NG** after automatic nucleotide substitution model selection for each locus using **ModelTest-NG**. Then, we performed a rapid bootstrap analysis with 100 replicates with **RAXML v.8** providing a partitioning scheme in which each locus corresponded to a unit. The divergence time analysis was performed on the best-scoring ML phylogenetic tree obtained. This tree was **time-calibrated** using the penalized likelihood method implemented in the software **treePL v.1.0**. We defined 15 geographic areas based on species distributions and previous **biogeographic** works and conducted the maximum likelihood method implemented in the R package **BioGeoBEARS v.1.1.1** to estimate geographic range evolution.

## Origins of *Pseudognaphalium*

Our results recover *Pseudognaphalium* as polyphyletic as most of its species are grouped in a clade that is sister to *Achyrocline*, but this clade also contains an *Helichrysum* species, and *P. richardianum* is placed in a different clade. Within the main *Pseudognaphalium* clade, two greatly diversified lineages arose, one composed of mainly North American species and a second of South American taxa. Also, a less diversified clade, with maximum BS support but of unresolved affinities, appears sister to the South American clade + *H. nicolai*, and includes some diploid species. Other diploid species show an uncertain position within the South American clade.

The existence and position of several polyploid *Pseudognaphalium* clades suggest at least **two independent ancient hybridization events**. The results suggest ancestors of diploid *Pseudognaphalium* and ancestors of South African *Helichrysum* from *H. reflexum* and *H. foetidum* groups as possible parental species. This scenario would imply two independent **LDD** to America around 7.5 and 5.5 Mya followed by northwards and southwards migrations respectively. It should be noted that clades formed by diploid species are poor in species but distributed in great geographic ranges (involving multiple dispersals) while polyploid clades have notably diversified but only in the **New World**.

## Geographic areas



## Diploid *Pseudognaphalium*

Clade of doubtful affinities that includes diploid species. Even though the ancestral area reconstruction suggests an American ancestry, the most plausible explanation given the diploid condition of some species and the inclusion of an African species would be an African origin followed by dispersals to America and Asia.

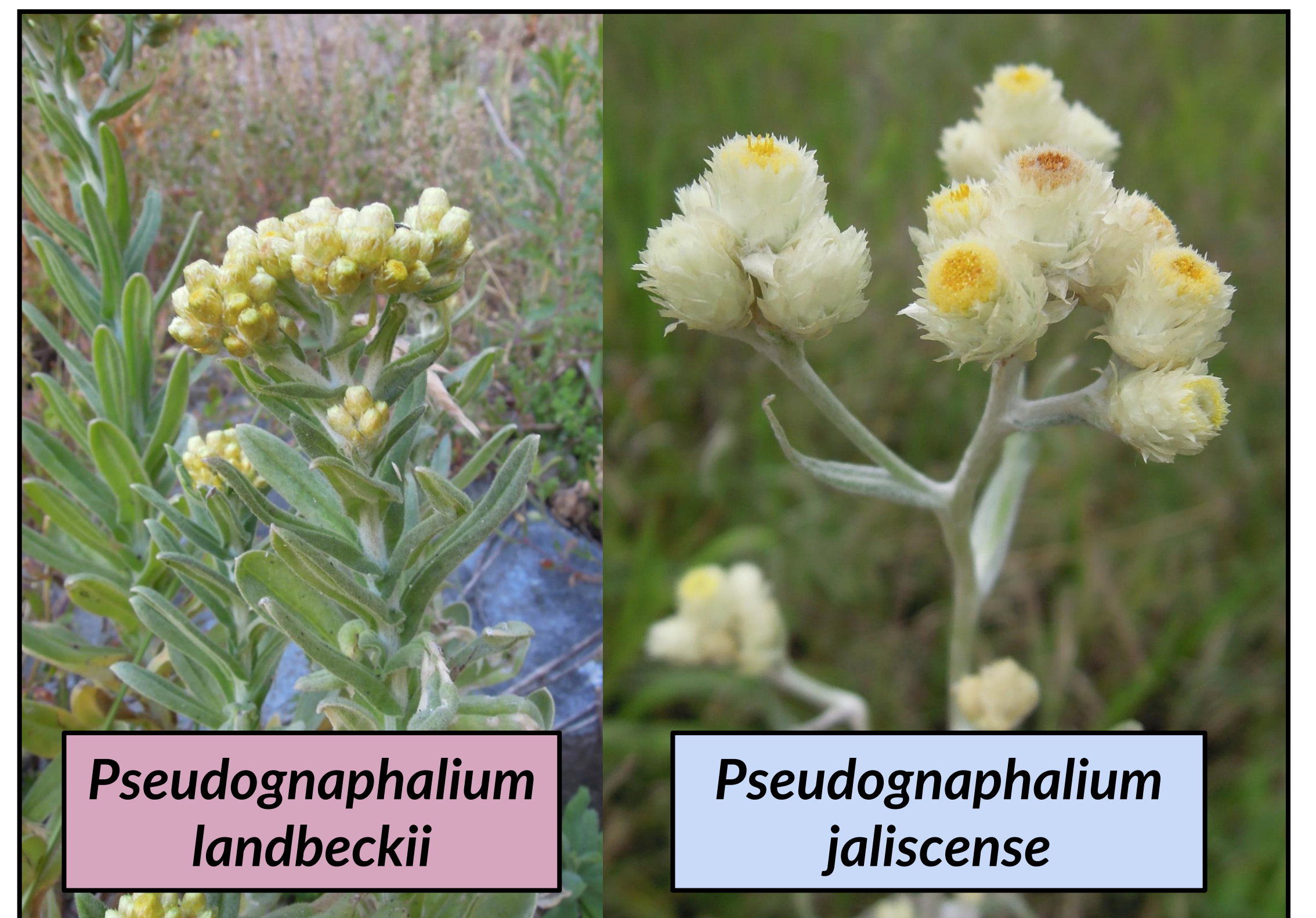
## *Pseudognaphalium* North American lineage

The West Indies (P) were colonized from the Madrean region (M), and there was a subsequent dispersal back to North America from the archipelago.

**Radiation in Sierra Madre:** Most of the North American species are found on these Mexican mountains that are considered a biodiversity hot-spot. *Pseudognaphalium* species grow on pine-oak forest clearings.

## *Pseudognaphalium* South American lineage

The genus radiated in the **Andes**, which also would have acted as a corridor to the Southern tip of South America for some species.



## Sierra Nevada, México, North America



## Chilean Andes, South America



Time-calibrated phylogeny of *Pseudognaphalium* and representatives of the other genera in the HAP clade. The pie charts at the nodes represents the most likely ancestral areas estimated by BioGeoBEARS under the DEC+j model. The symbol \* indicates high bootstrap (BS >70%), whereas \* indicates moderate support (BS=60–69%).