

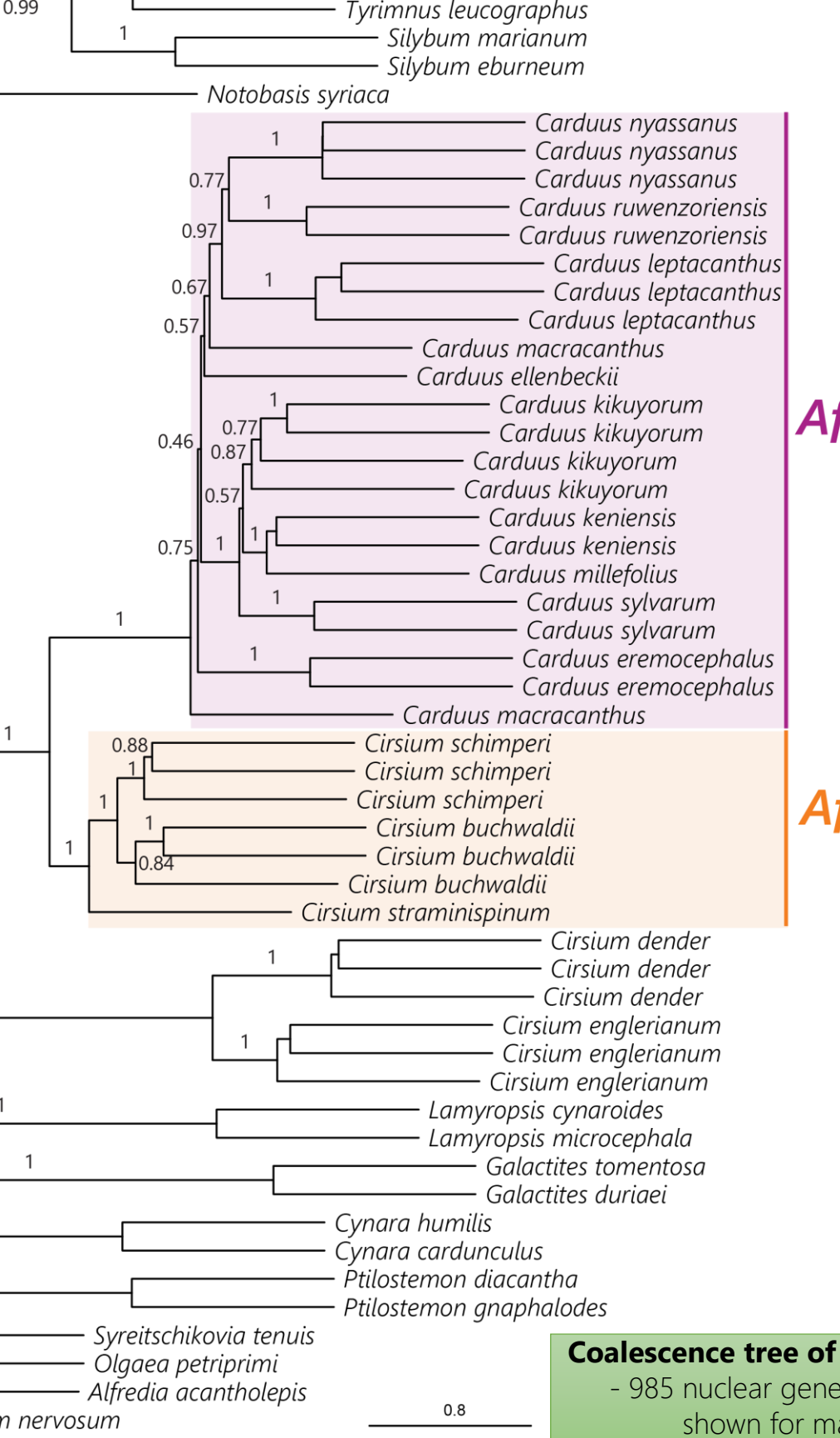
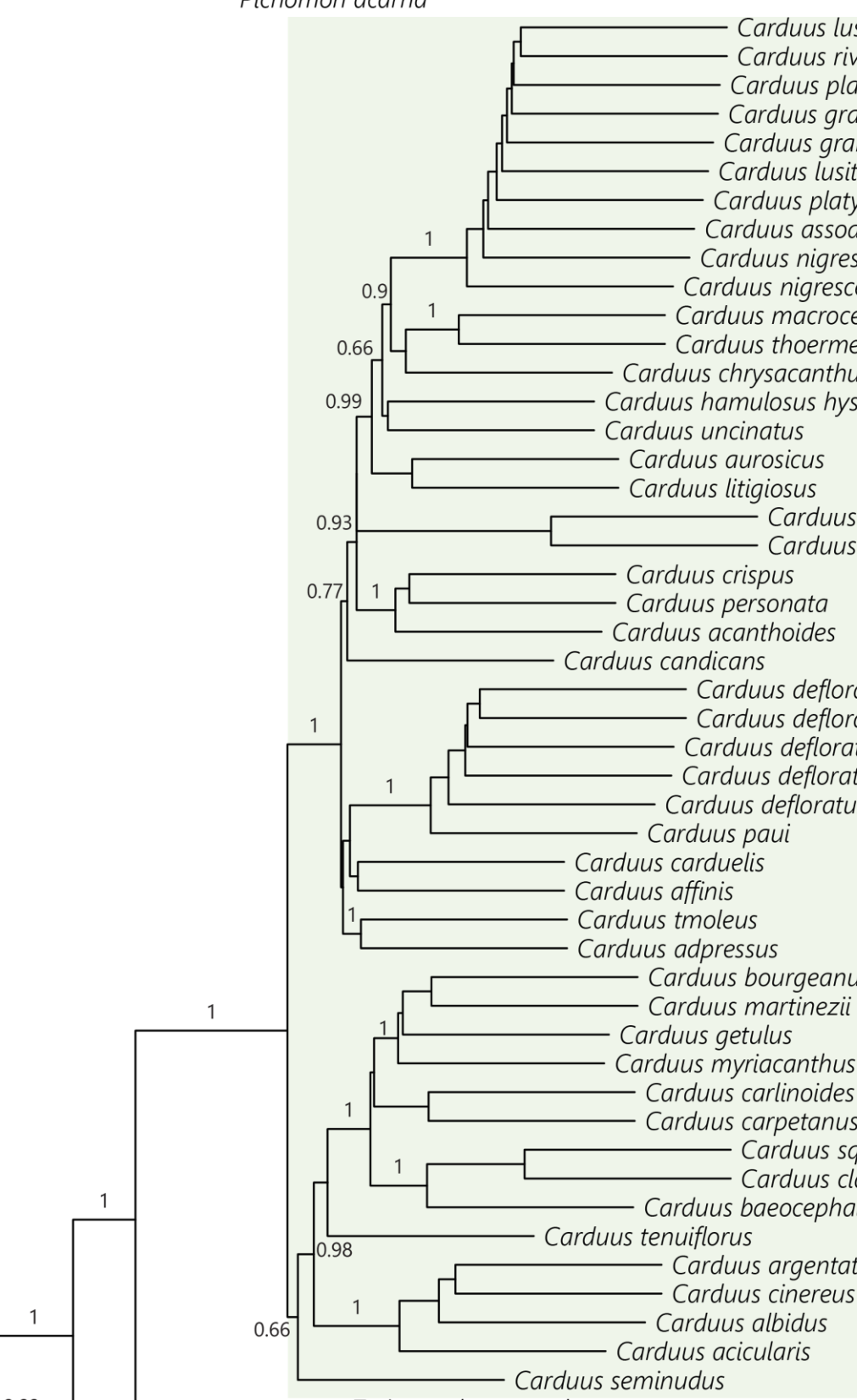
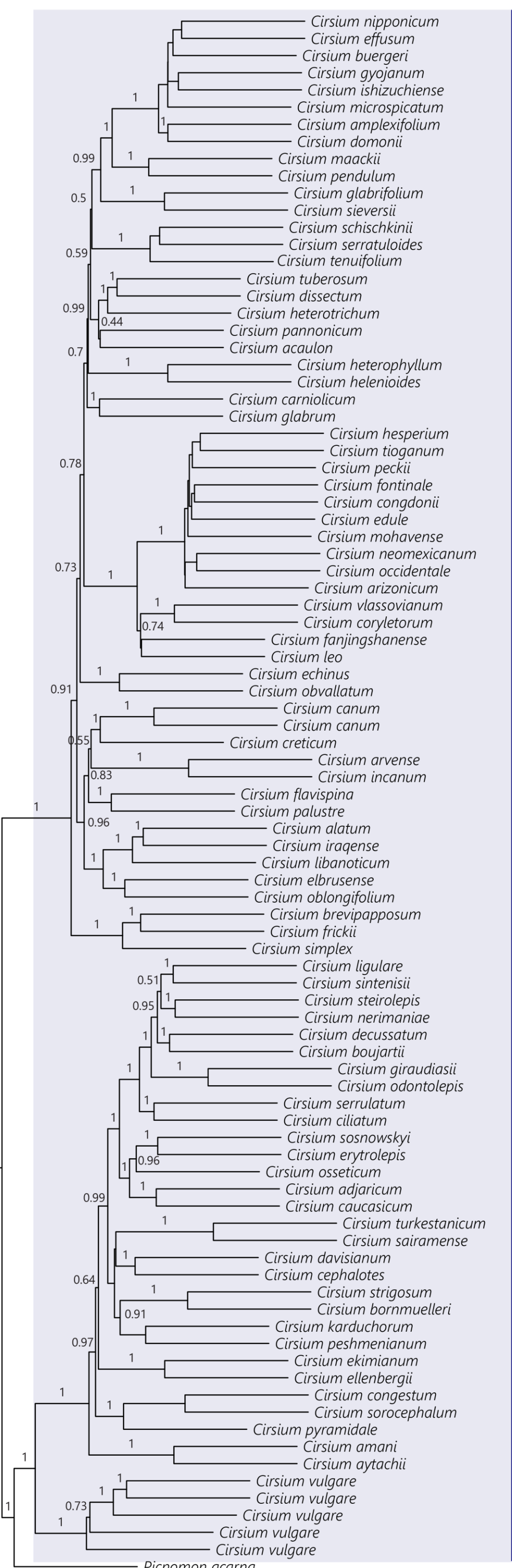


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African mountain thistles: generic delimitation problems analyzed with NGS

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Cirsium

Carduus

Afrocarduus

Afrocircium

Coalescence tree of subtribe Carduinae
- 985 nuclear genes - LPP values are shown for main branches

Introduction

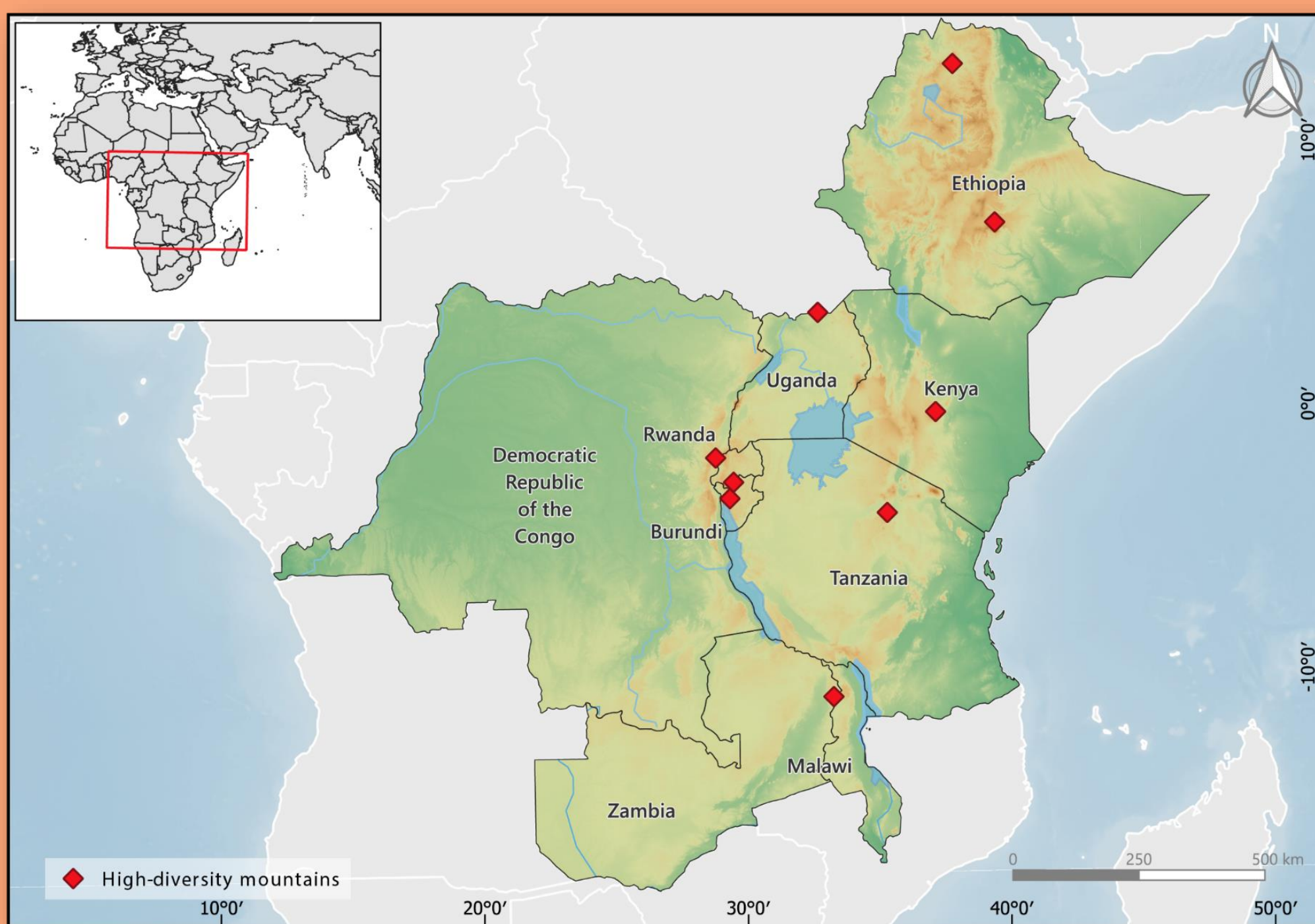
The Afrotropical region comprises humid and temperate isolated refuges in the highest African mountains. A question of major interest is understanding the origins and evolution of their diverse and singular flora (Brochmann et al., 2021 and references therein). The **complex *Carduus-Cirsium*** (Cardueae-Carduinae, Compositae) has some species endemic to this region, with a distribution restricted mainly to **Tropical East Africa**. These species show a combination of morphological characters that resemble *Carduus* and *Cirsium*; however, they do not fit properly with the definition of any of the two genera. Likewise, molecular evidence has suggested a **high divergence between the Afrotropical species and the species of the complex *Carduus-Cirsium*** (Häffner and Hellwig, 1999; Barres et al., 2013; Ackerfield et al., 2020). Thus, a thorough study including a complete representation of the species of the subtribe and a morphological evaluation are needed to understand properly the evolution of isolated and highly adapted mountain species.



Material and Methods

A total of **198 samples** representing all the genera of subtribe Carduinae were selected for the phylogenetic analyses according to Herrando-Moraira et al. (2019). Eleven raw sequences were obtained from this study and the rest of the samples were obtained from herbarium specimens or fresh material collected and preserved in silica-gel. DNA extraction, library, capture and sequencing were carried out in our facilities at the Botanic Institute of Barcelona. In some cases, DNA extractions were sent to Daicel Arbor Biosciences (Ann Arbor, Michigan, USA).

Phylogenetic analyses were carried out using HybPhyloMaker pipeline (Fér & Schmickl, 2018) and Paralog Wizard (Ufimov et al., 2022). For coalescence analysis, gene trees were calculated using RAXML v.8.2.12 and the analysis was done using ASTRAL-III v.5.7.8, both implemented in the HybPhyloMaker pipeline.

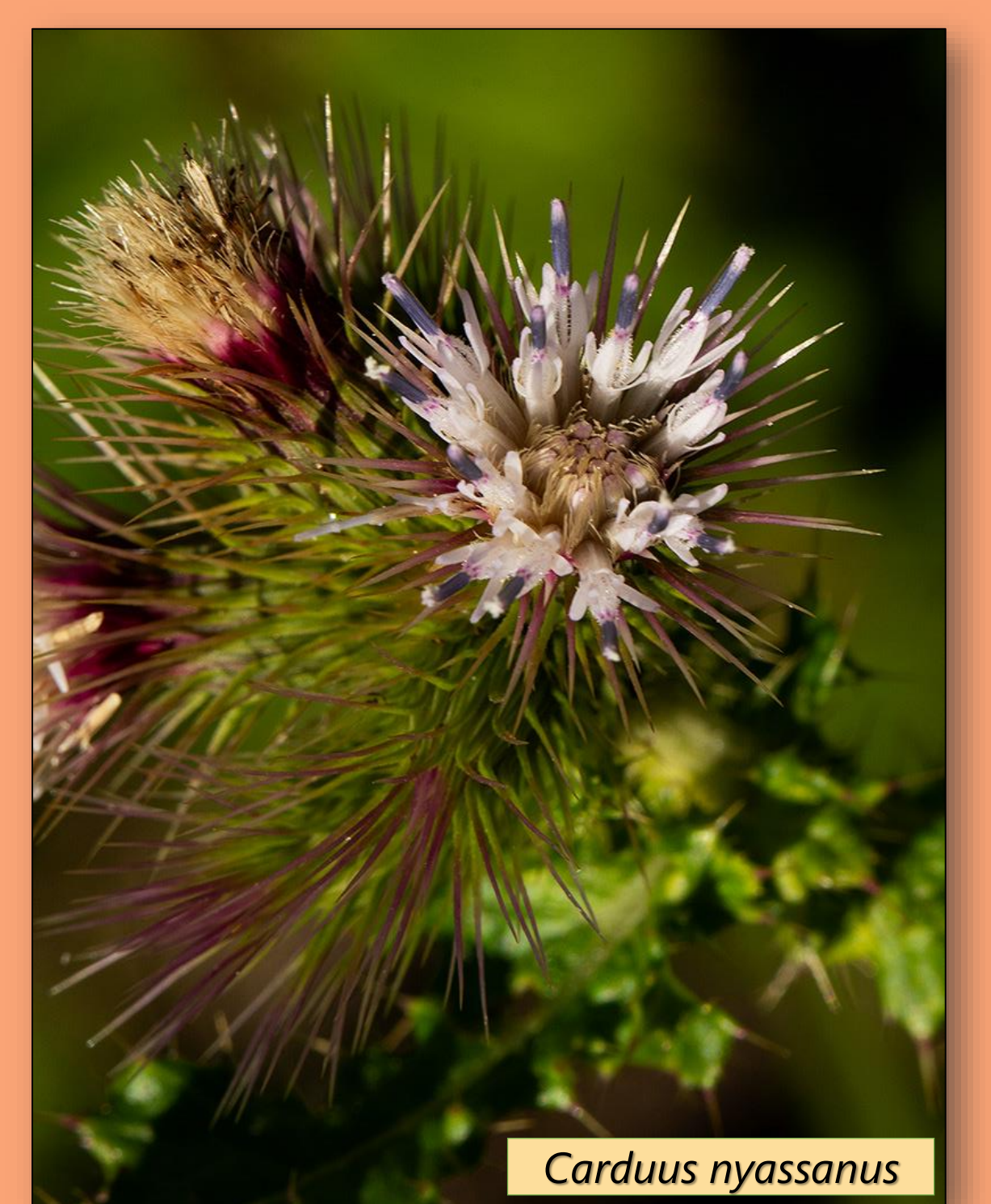


Results and Discussion

As in Herrando-Moraira et al. (2019), subtribe Carduinae was recovered as monophyletic with high resolution by the Hyb-Seq data analyses. Within the *Carduus-Cirsium* complex, *Picnomon*, *Galactites*, *Silybum* and *Tyrinnus* are natural genera. On the other hand, *Carduus* and *Cirsium* are both monophyletic only if the African species are excluded. This result was partly expected from previous evidence both morphological and molecular provided by Häffner and Hellwig (1999), Barres et al. (2013) and Ackerfield et al. (2020). **Species of *Carduus* subg. *Afrocarduus* and *Cirsium* from Tropical East Africa are nested at the base of the complex *Carduus-Cirsium* with high support (LPP=1), except for two species of *Cirsium* that were recovered out of the complex *Carduus-Cirsium* and appear closely related to *Galactites* and *Lamyropsis*.**

Morphological analysis has shown that the species of *Carduus* from Africa have more characters shared with *Cirsium* than with *Carduus*. This evidence, along with our phylogenetic analyses, supports **subg. *Afrocarduus* as a monophyletic natural group independent from *Carduus* and *Cirsium***. Hence, after analyzing the morphological evidence and our molecular results, we propose a **new genus for the *Carduus* species of Africa**, using the name provided by Kazmi (1963) for the subgenus: ***Afrocarduus***.

As to the **African *Cirsium***, a close look at the morphology of these species has shown clear **unique characters**, such as phyllaries with well-developed appendages that are not present in *Cirsium*. This observation, along with the phylogenetic results, made us think of these species as a **genus independent of *Afrocarduus* and *Cirsium***. Here we propose a **second genus, *Afrocircium***, for the three African species of *Cirsium* that are nested in the base of *Afrocarduus* and constitute a monophyletic group.



Key to Complex *Carduus-Cirsium*

- a Achene pericarp with 10-15 longitudinal grooves.....***Carduus***
- a' Achene pericarp with 4 longitudinal lines.....**b**
- b Barbellate pappus.....***Afrocarduus***
- b' Plumose pappus.....**c**
- c Phyllaries without appendages.....***Cirsium***
- c' Phyllaries with well-developed appendages.....***Afrocircium***

* Key built only with the main genera of the complex; monotypic genera are excluded.

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