

## Appendix - Supplementary Figures and Tables

SAJB-D-25-02682

### DNA barcoding and anti-tyrosinase activities of three species-representative populations of the genus *Greyia* Hook & Harv.

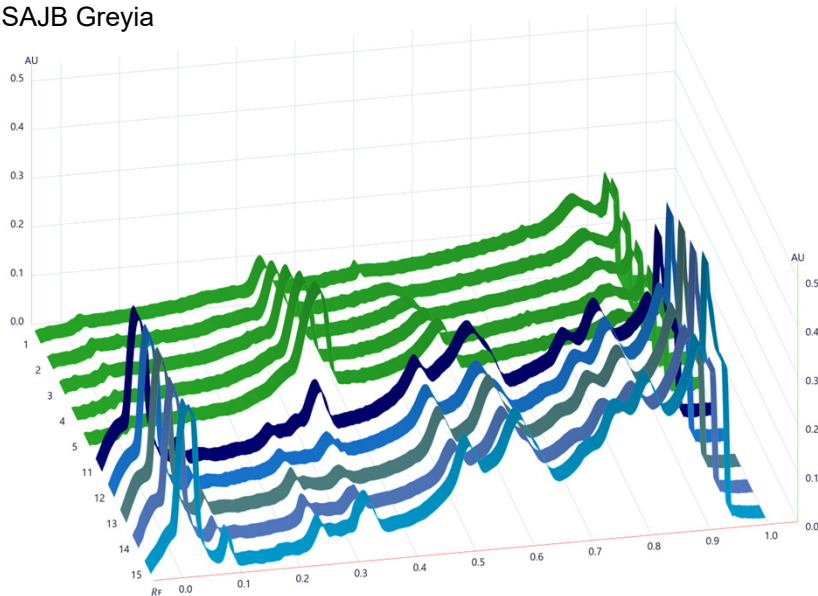
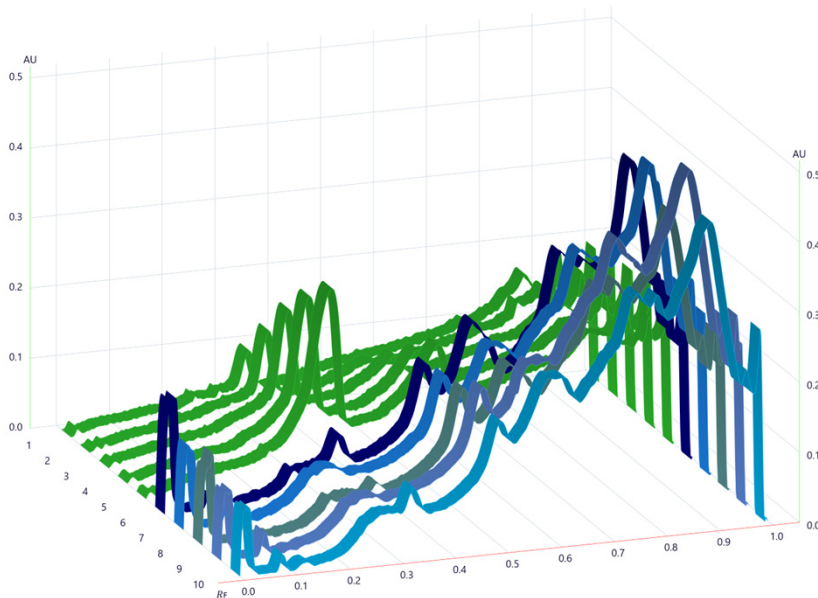
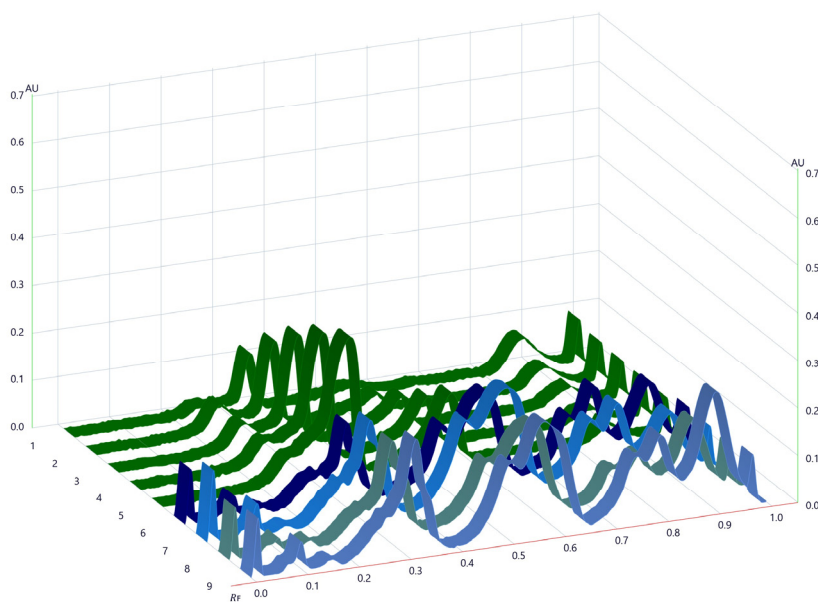
Iné Botha, Marco N. De Canha, Kenneth Oberlander, Jana Botes, Namrita Lall,

Dave K. Berger

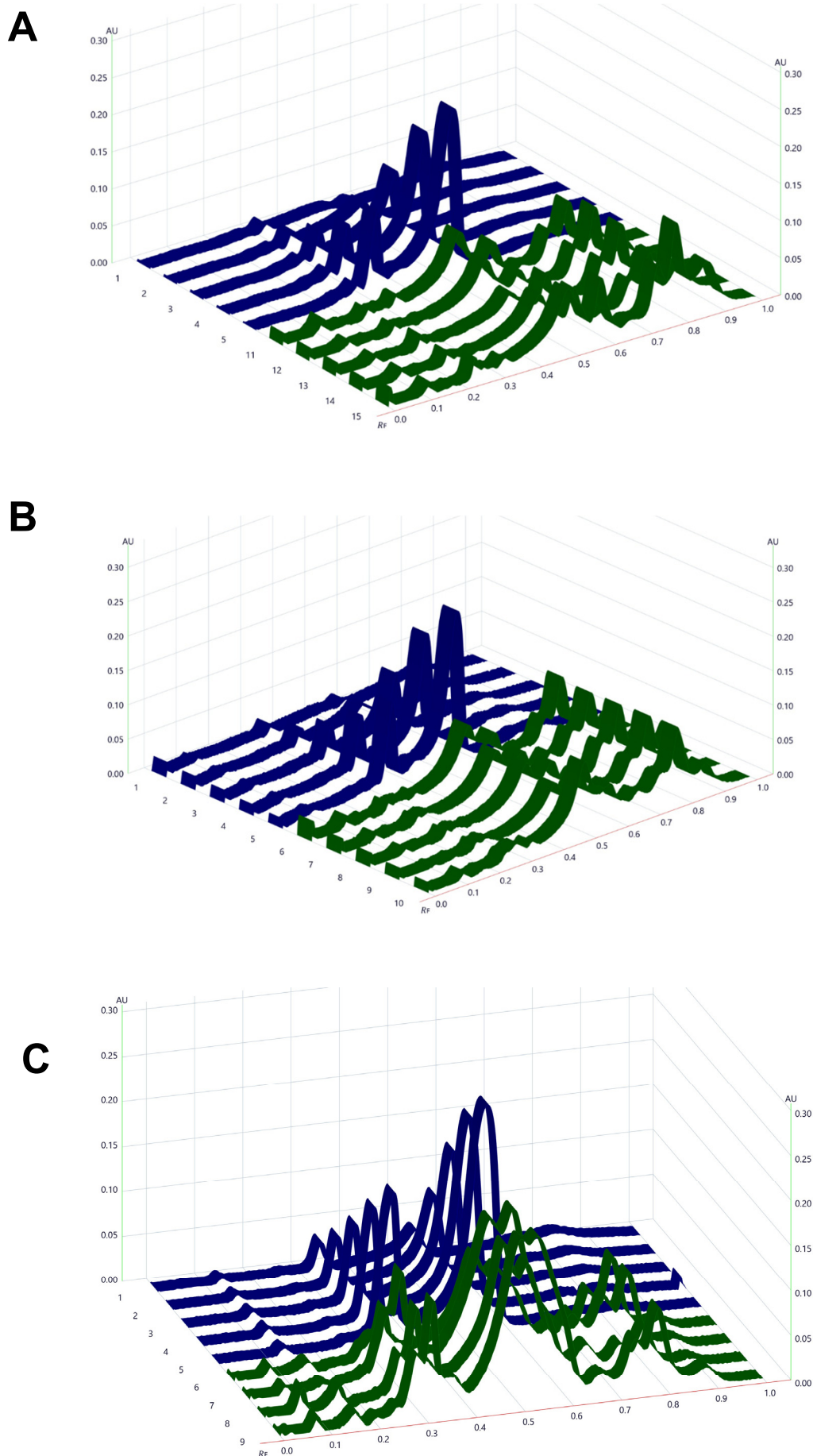
Table of Contents	Page
<b>Fig. S1.</b> Representative images of <i>Greyia</i> species core populations – trees in habitat, inflorescences, and leaves used for ethanolic extracts.....	<b>2</b>
<b>Fig. S2.</b> Profiles generated from Fig.3 A-C - HPTLC plates derivatized with NP reagent and <i>p</i> -anisaldehyde (2',4',6,-trihydroxydihydrochalcone).....	<b>3</b>
<b>Fig. S3.</b> Profiles generated from Fig.3 D-F - HPTLC plates visualized under UV254 nm (galangin).....	<b>4</b>
<b>Fig. S4.</b> HPTLC of <i>Greyia</i> extracts derivatized with natural product and visualized with either RT light, or UV 366 nm.....	<b>5</b>
<b>Fig. S5a-d.</b> Geraniales single gene phylogenies (complete trees).....	<b>6</b>
<b>Table S1.</b> Anti-tyrosinase assays - goodness-of-fit values ( $R^2$ ) for individual trees of each species and for each independent replicate.....	<b>10</b>
<b>Table S2.</b> Barcode gap analysis reporting median intra- and interspecific pairwise distances under a K2P model for four barcoding markers of <i>Greyia</i> spp.....	<b>10</b>



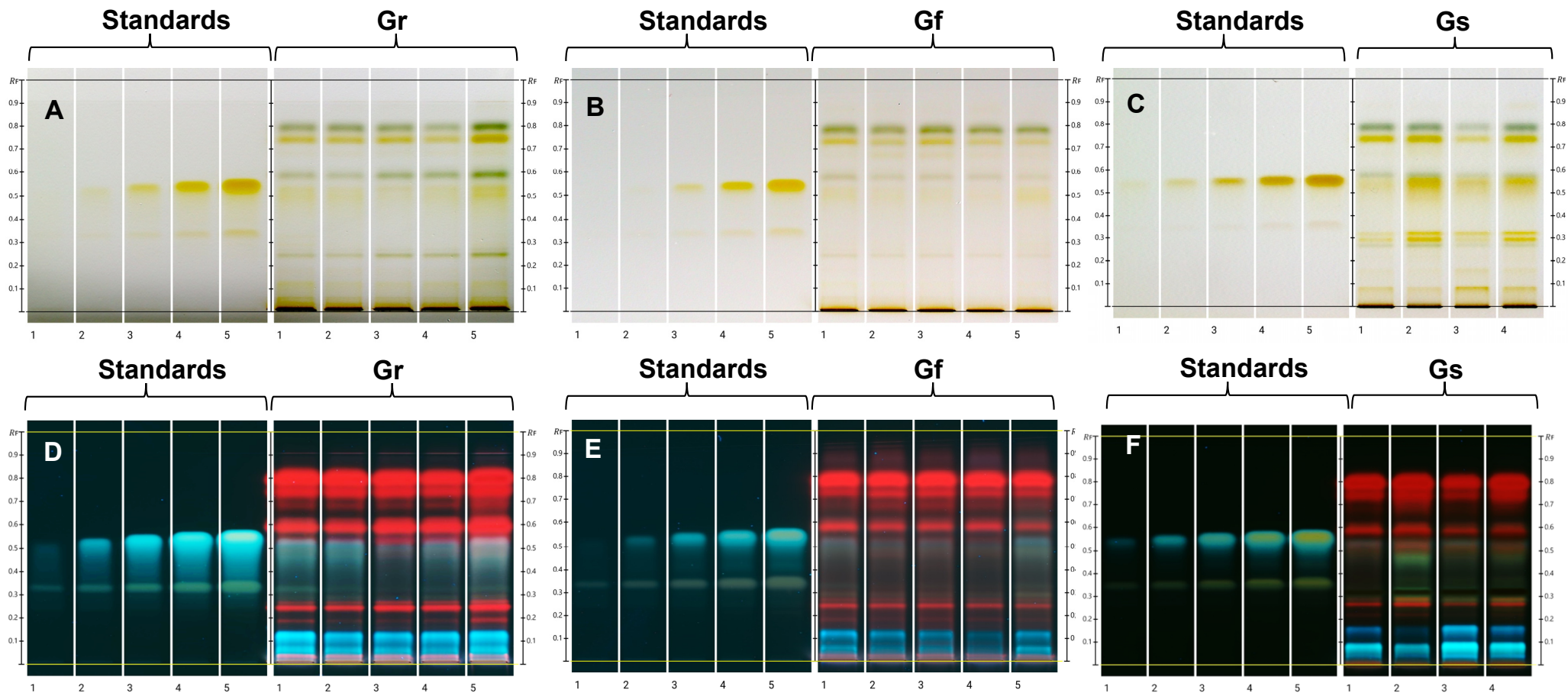
**Fig. S1.** Representative images of Greyia species core populations – trees in habitat, inflorescences, and leaves used for ethanolic extracts. **A:** *Greyia radlkoferi*, **B:** *Greyia sutherlandii* **C:** *Greyia flanagani*. White scale bars = 50 cm, Black scale bars = 2 cm. Photos: DK Berger.

**A****B****C**

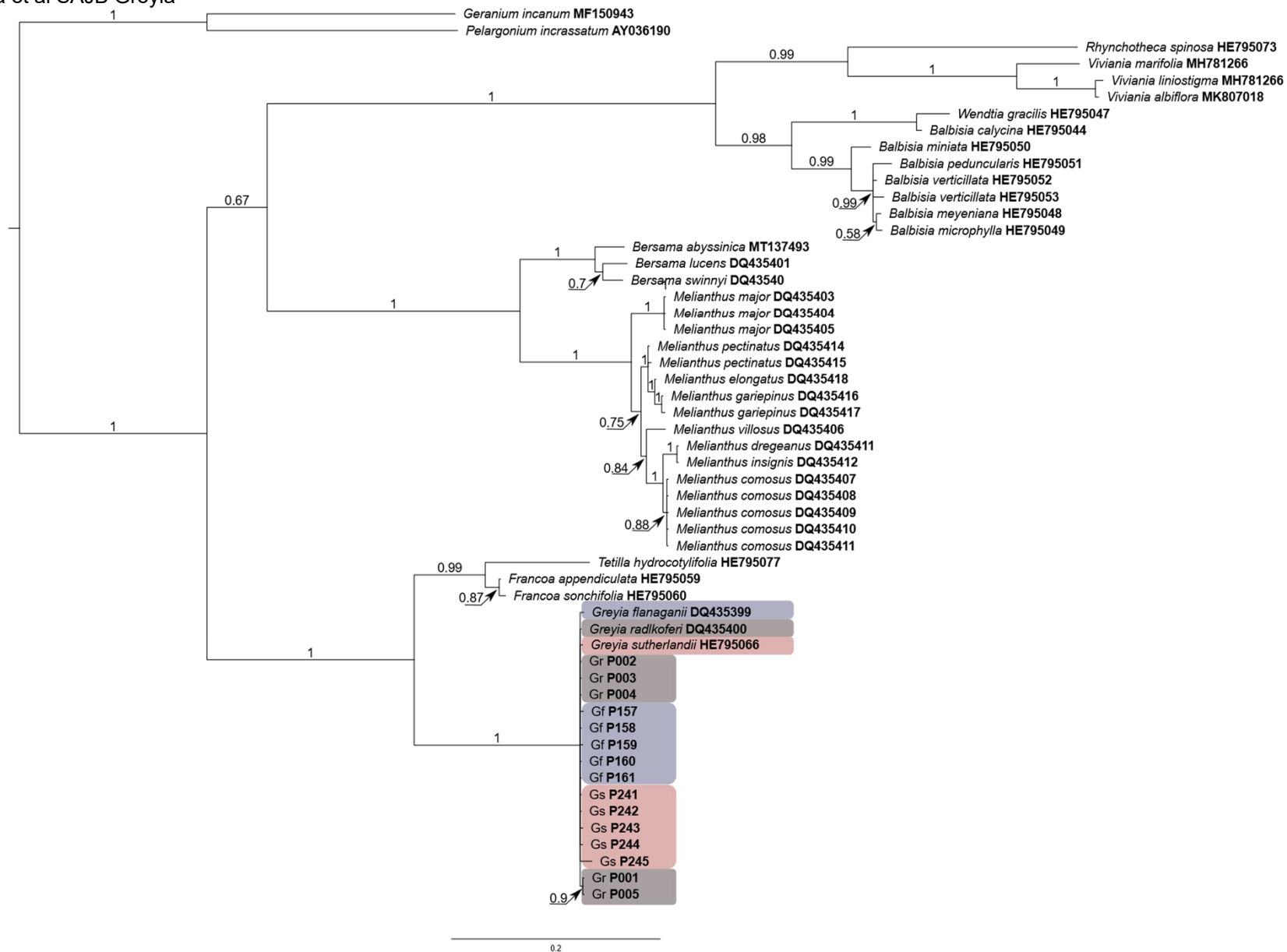
**Fig. S2.** Profiles generated from Fig.3 A-C - HPTLC plates derivatized with NP reagent and *p*-anisaldehyde (2',4',6,-trihydroxydihydrochalcone). **(A)** 2',4',6,-trihydroxydihydrochalcone (Track 1-5 in green) and *Greyia radlkoferi* extracts (Tracks 11-15: P001-P005 in blue), **(B)** 2',4',6,-trihydroxydihydrochalcone (Track 1-5: green) and *Greyia flanaganii* extracts (Tracks 6-10: P157 – P161 in blue) and **(C)** 2',4',6,-trihydroxydihydrochalcone (Track 1-5 in green) and *Greyia sutherlandii* extracts (Tracks 6-9: P241 – P244 in blue).



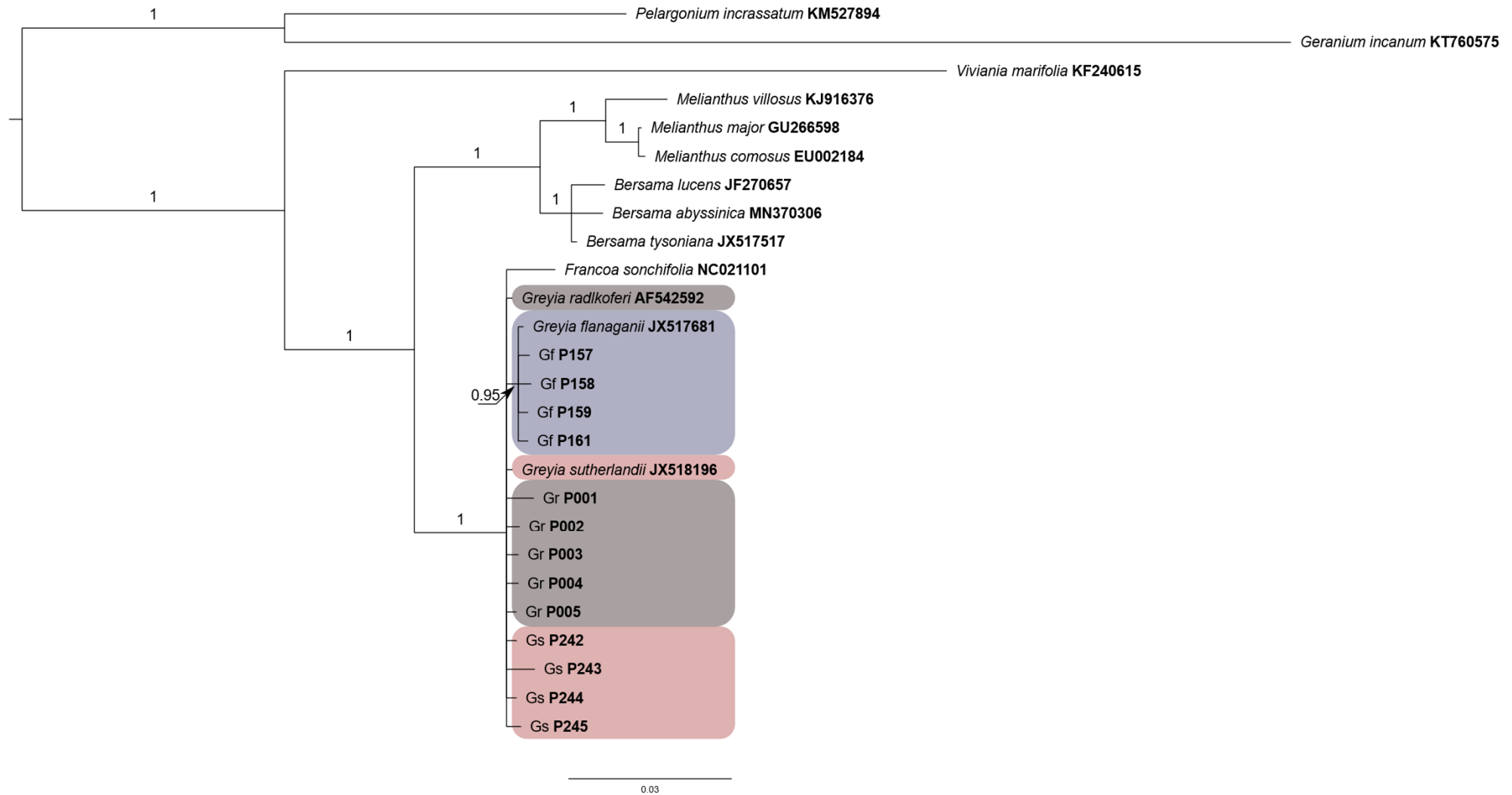
**Fig. S3.** Profiles generated from Fig.3 D-F - HPTLC plates visualized under UV254 nm (galangin). Profiles generated from developed HPTLC plate images under UV 254 nm for **(A)** galangin (Tracks 1-5 in blue) and *Greyia radlkoferi* extracts (Tracks 11-15: P001-P005 in green); **(B)** galangin (Tracks 1-5 in blue) and *Greyia flanaganii* extracts (Tracks 6-10: P157 – P161 in green); and **(C)** galangin (Tracks 1-5 in blue) and *Greyia sutherlandii* extracts (Tracks 6-9: P241 – P244 in green).



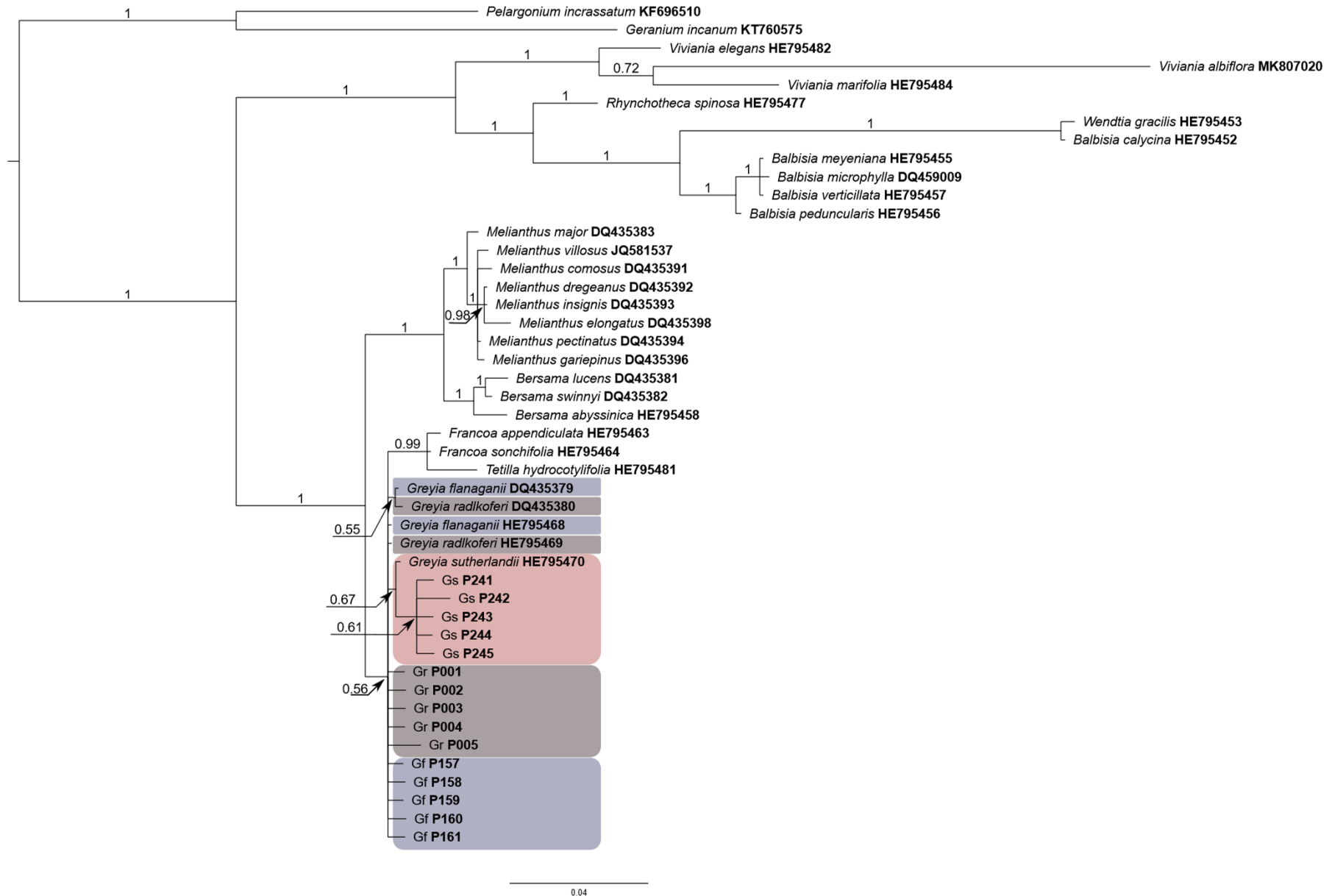
**Figure S4.** High Performance Thin Layer Chromatography (HPTLC) of *Greyia* leaf extracts derivatized with natural product reagent and visualized with either RT light, or UV 366 nm. **A, B, C:** Developed HPTLC plates derivatized with natural product reagent and illuminated under Remission-Transmission light. **D, E, F:** Developed HPTLC plates derivatized with natural product reagent and illuminated under UV light at 254 nm. For all panels **A-F**, dilution series of a mixture of 2',4',6'-trihydroxydihydrochalcone and galagin were loaded in a volume of 10  $\mu$ l per track with each compound applied from stock concentrations of 31.25, 62.5, 125, 250 and 500  $\mu$ g/ml (tracks 1-5 on the left), and the ethanolic leaf extracts of *Greyia radlkoferi* (P001-P005) (panel **A** and **D**), *G. flanaganii* (P157 – P161) (panel **B** and **E**) and *Greyia sutherlandii* (P241 – P244) (panel **C** and **F**) were loaded in volumes of 20  $\mu$ l of 10 mg/ml stock concentrations (tracks 1-5 or 1-4 on the right).



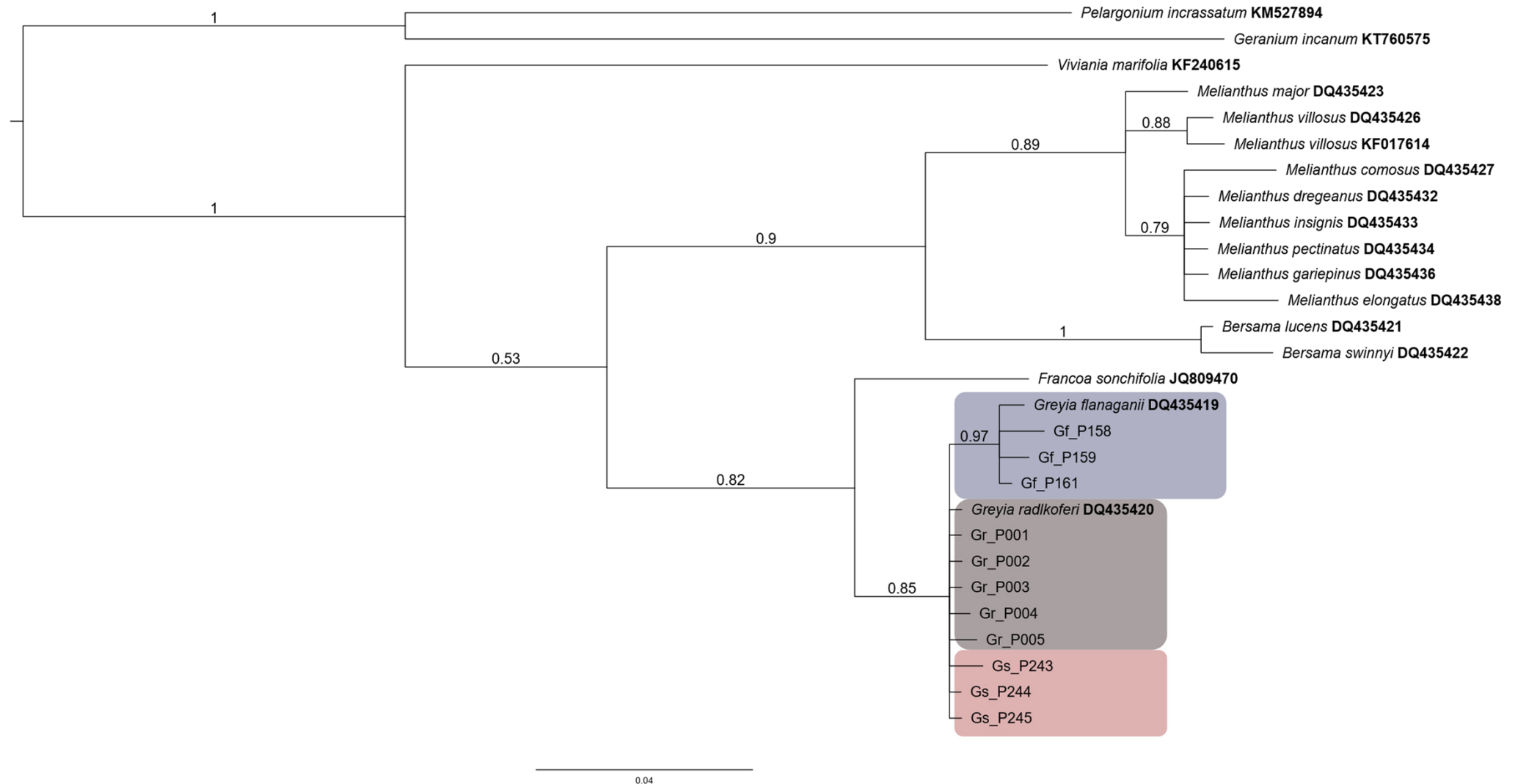
**Figure S5a:** ITS barcoding phylogeny of the Geraniales including *Greyia* spp. The complete Bayesian 50% majority-rule tree is shown. Numbers above branches/on arrows are Bayesian posterior probabilities. Scale bars are in number of substitutions per site. Genbank accessions used for each tip are in bold following the tip name. ITS sequences of five trees per core population of *Greyia radlkoferi* (P001-P005), *Greyia sutherlandii* (P241 – P245), and *G. flanaganii* (P157 – P161) are available from <https://portal.boldsystems.org> (search term = GREY and the qualifier [recordsetcode] from the dropdown menu). The ITS sequences for one tree per species have also been submitted to NCBI (P002: PX210468; P161: PX210469; P244: PX210470)



**Figure S5b:** *MatK* barcoding phylogeny of the Geraniales including *Greyia* spp. The complete Bayesian 50% majority-rule tree is shown. Numbers above branches/on arrows are Bayesian posterior probabilities. Scale bars are in number of substitutions per site. Genbank accessions used for each tip are in bold following the tip name. *MatK* sequences of five trees per core population of *Greyia radlkoferi* (P001–P005), *Greyia sutherlandii* (P241 – P245), and *G. flanaganii* (P157 – P161) are available from <https://portal.boldsystems.org> (search term = GREY and the qualifier [recordsetcode] from the dropdown menu). The *matK* sequences for one tree per species have also been submitted to NCBI (P001: PX393127; P161: PX393128; P245: PX393129).



**Figure S5c:** *TrnL-F* barcoding phylogeny of the Geraniales including *Greyia* spp. The complete Bayesian 50% majority-rule tree is shown. Numbers above branches/on arrows are Bayesian posterior probabilities. Scale bars are in number of substitutions per site. Genbank accessions used for each tip are in bold following the tip name. *TrnL-F* sequences of five trees per core population of *Greyia radlkoferi* (P001-P005), *Greyia sutherlandii* (P241 – P245), and *G. flanagani* (P157 – P161) are available from <https://portal.boldsystems.org> (search term = GREY and the qualifier [recordsetcode] from the dropdown menu). The *trnL-F* sequences for one tree per species have also been submitted to NCBI (P002: PX502302; P161: PX502303; P244: PX502304).



**Figure S5d:** *PsbA-TrnH* barcoding phylogeny of the Geraniales including *Greyia* spp. The complete Bayesian 50% majority-rule tree is shown. Numbers above branches/on arrows are Bayesian posterior probabilities. Scale bars are in number of substitutions per site. Genbank accessions used for each tip are in bold following the tip name. *PsbA-TrnH* sequences of five trees per core population of *Greyia radlkoferi* (P001-P005), *Greyia sutherlandii* (P241 – P245), and *G. flanaganii* (P157 – P161) are available from <https://portal.boldsystems.org> (search term = GREY and the qualifier [recordsetcode] from the dropdown menu). The *psbA-TrnH* sequences for one tree per species have also been submitted to NCBI (P002: PX430637; P161: PX430638; P244: PX430639).

**Table S1.** Goodness of fit for tyrosinase inhibition assay curves (R2 values)

Species	Tree Code	Trial 1	Trial 2	Trial 3
<i>G. radlkoferi</i>	P001	0.74	0.90	0.95
	P002	0.79	0.86	0.86
	P003	0.70	0.78	0.91
	P004	0.89	0.83	0.87
	P005	0.94	0.83	0.92
<i>G. sutherlandii</i>	P241	0.98	0.99	0.98
	P242	0.98	0.99	0.98
	P243	0.98	0.99	0.98
	P244	0.99	0.99	0.99
<i>G. flanaganii</i>	P157	0.95	0.91	0.95
	P158	0.68	0.89	0.88
	P159	0.75	0.87	0.89
	P160	0.79	0.87	0.91
	P161	0.92	0.92	0.92

**Table S2.** Barcode gap analysis reporting median intra- and interspecific pairwise distances under a K2P model for four barcoding markers of *Greyia* spp.

Marker*	Intraspecific distances**	Interspecific distances**
ITS	0.0018 (0 – 0.0108)	0.0018 (0 – 0.0144)
<i>matK</i>	0	0.0044 (0 – 0.0044)
<i>trnL-F</i>	0	0.0097 (0 – 0.0097)
<i>psbA-trnH</i>	0	0.0178 (0 – 0.0178)

\* DNA sequences for each marker used for this analysis were from five trees per core population of *Greyia radlkoferi* (P001-P005), *Greyia sutherlandii* (P241 – P245), and *G. flanaganii* (P157 – P161).

\*\* Ranges of median pairwise distances are presented in parentheses.