Title

A phylogenomic approach to disentangling the evolution of the large and diverse daisy tribe Anthemideae (Asteraceae)

Short running title

Phylogenomics of of Anthemideae

Authors

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Abstract

The daisy tribe Anthemideae is one of the largest and most diverse tribes within Asteraceae. We analysed a dataset including 61 out of 111 Anthemideae genera, and all but four of the 19 currently recognized subtribes (Inulantherinae, Lapidophorinae, Lonadinae, and Vogtiinae) using a targeted high-throughput sequencing approach, the first focused on the tribe. We followed different phylogenomic approaches, using nuclear and plastid data, as well as additional analytical methods to estimate divergence times and diversification rates, to unravel the evolutionary history and classification of this tribe. Our results reinforce the phylogenetic backbone of the Anthemideae advanced in previous studies, and further reveal the possible occurrence of ancient hybridization events, plastid capture, and/or incomplete lineage sorting, suggesting that complex evolutionary processes have played an important role in the evolution of this tribe. The results also support the merging of subtribe Physmasperminae into Athanasiinae and subtribe Matricariinae into Anthemidinae, and clarify previously unresolved relationships. Furthermore, the study provides additional insights into the biogeographic patterns within the tribe by identifying three main groups: Southern African Grade, Asian Clade, and circum-Mediterranean Clade. These groups partially coincide with previously identified ones. Overall, this research provides a more detailed understanding of the Anthemideae tribe, and improves its classification. The study also emphasises the importance of phylogenomic approaches for deciphering the evolutionary dynamics of large and diverse plant lineages.

Key Words

Anthemideae, Cyto-nuclear discordances, Divergence Times, Hybridization, Phylogenomics.

With over 1,800 species distributed across 111 genera (Oberprieler et al. 2022), Anthemideae, i.e., the daisy tribe, is one of the largest tribes in the sunflower family (Asteraceae) (Watson et al. 2020). Mainly distributed in the Old World, Anthemideae species exhibit a wide environmental tolerance and harbor most plant growth forms (annual, biennial, perennial herbs, subshrubs, and shrubs) (Malik et al. 2017). A large number of species occur in environments with a dry climate, with the Mediterranean basin being a diversification centre for several of its lineages (e.g, *Anacyclus* L., *Anthemis* L., *Tanacetum* L., *Tripleurospermum* Sch.Bip.), followed by Asia (e.g, *Ajania* Poljakov, *Arctanthemum* (Tzvelev) Tzvelev, *Artemisia* L., *Cancrinia* Kar. & Kir., *Chrysanthemum* L., *Pseudohandelia* Tzvelev) and southern Africa (*Hippia* L., *Inezia* E.Phillips, *Lasiospermum* Lag., *Oncosiphon* Källersjö, *Osmitopsis* Cass.) (Oberprieler, 2005). Many Anthemideae taxa play key ecological roles, e.g, dominating and shaping arid and steppe landscapes (species of shrubby *Artemisia*; Kapustina et al. 2021; Ahmadian et al. 2022). Others have been used for different purposes (Molina-Venegas et al. 2021), in particular as medicines (*Achillea* L., *Artemisia*, source of the antimalarial artemisinin; Bora & Sharma, 2011; Vallès et al. 2011; Garcia-Oliveira et al. 2021; Kachura & Harris, 2022) and as ornamentals (e.g, marguerite daisies, *Argyranthemum* Webb ex Sch.Bip.; oxeye and Shasta daisies, *Leucanthemum* Mill.; or mums, *Chrysanthemum* L., also used as a tea and in traditional Chinese medicine; Khuroo et al. 2010; Shahrajabian et al. 2019; Hadizadeh et al. 2022; Xu et al. 2023).

The taxonomy and classification of the tribe was initially based on morphological characters primarily associated with inflorescence (e.g., involucral and receptacular bracts) and fruit (cypsela) structures (Cassini, 1819; Bremer & Humphries, 1993). However, in the last two decades, the classification of Anthemideae has been the subject of numerous studies relying on molecular phylogenetics (Watson et al. 2000;

Oberprieler et al. 2007, 2019, 2022; Himmelreich et al. 2008; Sanz et al. 2008; Vitales et al. 2018). These studies demonstrated a high level of homoplasy with regard to traditional morphological characters, which are incongruent with molecular phylogenies, affecting the circumscription of several subtribes and genera (e.g., Vitales et al. 2018). A major revised classification by Oberprieler et al. (2007) based on morphological, genetic, chromosomal, phytochemical, and histological characters, included 14 subtribes, with 20 genera that remained unplaced. Subsequent studies have assigned some of these genera to existing subtribes, e.g, *Tridactylina* Sch.Bip. and *Opisthopappus* C.Shih to Artemisiinae (Masuda et al. 2009; Gu et al. 2019; Kim & Kim, 2020; Shen et al. 2021), and *Allardia* Decne., *Cancriniella* Tzvelev, *Richteria* Kar. & Kir., *Trichanthemis* Regel & Schmalhausen, and *Sclerorhachis* Rech.f. to Handeliinae (Oberprieler et al. 2019). In the latest classification by Oberprieler et al. (2022), remaining genera were placed into subtribes: *Cancrinia* Kar. & Kir., *Polychrysum* Kovalevsk. and *Ugamia* Pavlov into Handeliinae; *Phalacrocarpum* Willk. into Leucanthemopsidinae; *Daveaua* Willk. ex Mariz, *Heteromera* Pomel, and *Otospermum* Willk. into Leucantheminae; and *Endopappus* Sch.Bip., *Nivellea* B.H.Wilcox & al. and *Otoglyphis* Pomel into Glebionidinae. In addition, *Brocchia* Vis., *Inulanthera* Källersjö, *Lepidophorum* Necker ex DC., *Lonas* Adans. and *Vogtia* Oberpr. & Sonboli have each been assigned to their own newly-defined subtribes (Brocchiinae, Inulantherinae, Lepidophorinae, Lonadinae, and Vogtiinae, respectively). However, this study still shows poor resolution within some subtribes (i.e, Artemisiinae, Handeliinae, Pentziinae, and Phymasperminae), as well as uncertainties in the circumscription of others (such as Anthemidinae and Matricariinae).

The use of a relatively limited number of nuclear and plastid markers (i.e, nuclear ITS, ETS, *DAP*, *VIP5*, and *NPF3.1* and plastid *ndhF*, *psbA-trnN*, and *trnL-trnF*; Watson et

al. 2000; Oberprieler et al. 2007, 2019, 2022; Himmelreich et al. 2008; Sanz et al. 2008; Vitales et al. 2018) limited these researchers' ability to fully resolve the phylogeny of the Anthemideae or to interpret putative biological processes involved, such as hybrid speciation. To date, no phylogenomic study has specifically focused on the breadth and depth of Tribe Anthemideae (Mandel et al. 2019: 13 species and genera; Watson et al. 2020: 12 species and genera; Pascual-Díaz et al. 2021: 7 species and genera; Zhang et al. 2021: 16 species, 12 genera; Lu et al. 2022: 16 species, 4 genera; Jiao et al. 2023: 228 species, 19 genera) to fully resolve its evolutionary relationships, step required to interpret complex evolutionary patterns.

Large and diverse lineages, many of which are represented in various tribes in Asteraceae, have been considered models to address key questions in evolution (Watson et al. 2020; Palazzesi et al. 2022a). Anthemideae is one such lineage, with a particularly interesting and complex evolutionary history. Indeed, evolutionary and biological processes such as adaptive radiation (White et al. 2020, 2021), hybridization (Abd El-Twab & Kondo, 2001; Tang et al. 2009, 2010; Kim & Kim, 2020; Criado Ruiz et al. 2021), polyploidization (Pellicer et al. 2011; Tomasello & Oberprieler, 2017; Shen et al. 2021; Zhang et al. 2021; Oberprieler et al. 2023), and genome size expansions and contractions (resulting from mechanisms such as whole-genome multiplication, the activation of transposable elements, and genome restructuring.; Vitales et al. 2020; Pellicer & Fernández 2023) have been inferred in the recent and ancient evolutionary history of the tribe (Vallès et al. 2012; Vitales et al. 2020), in addition to uncommon genomic structural variation (Olanj et al. 2015; Rosato et al. 2017, 2018). Thus, sequences from a few loci may not provide the phylogenetic signal necessary for understanding diversification patterns in such a large and complex tribe (Bravo et al. 2019).

Phylogenomic approaches have proved useful in resolving previously-intractable relationships, at least using Sanger sequencing alone, shedding light on deep phylogeny and the origins of some lineages (OTPTI, 2019; Li et al. 2019; Yang et al. 2020; Baker et al. 2022), even in cases involving reticulation (Morales-Briones et al. 2018, 2021), incomplete lineage sorting (García et al. 2017), and/or rapid radiation (Carlsen et al. 2018; Larridon et al. 2020; Shee et al. 2020; Valderrama et al. 2020). Hyb-Seq is a high-throughput sequencing (HTS) approach combining target capture sequencing (TCS), that provides the opportunity to sequence hundreds of low-copy nuclear genes, with genome skimming, a low-coverage shotgun sequencing to assemble high-copy genomic regions (Straub et al. 2012; Weitemier et al. 2014; Dodsworth et al. 2019). This approach has provided insightful results in reconstructing evolutionary history both at the population level and above (Villaverde et al. 2018; Slimp et al. 2021). Further, Hyb-Seq is useful for obtaining sequence data from degraded DNA from century-old herbarium specimens (Brewer et al. 2019; Shee et al. 2020). The development of several bait kits or probe sets (Mandel et al. 2014; Johnson et al. 2019; Siniscalchi et al. 2020) and the availability of optimised molecular protocols (Hale et al. 2020) for target capture sequencing, as well as bioinformatic pipelines for data post-processing (Johnson et al. 2016; Herrando-Moraira et al. 2018), have made Hyb-Seq more accessible and cost-effective (Dodsworth et al. 2019; Hale et al. 2020) to generate large datasets to address a diversity of systematic and evolutionary questions (Villaverde et al. 2020; Ma et al. 2021; Ogutcen et al. 2021; Simmonds et al. 2021). Mandel et al. (2014) designed a specific probe set for Asteraceae. However, due to the good performance of the Angiosperms353 probe set in phylogenomic studies of several flowering plant groups (Villaverde et al. 2020; Larridon et al. 2021; Maurin et al. 2021; Elliott et al. 2023; Pérez‐Escobar et al. 2023; Nicol et al. 2024), reports of paralogy problems in Mandel et

al.'s probe set (Siniscalchi et al. 2021), and funding limitations, we chose to use sequences generated within the PAFTOL project with the Angiosperms353 probe set that were already available. Recently, a new probe set has been designed by Mandel's team (Moore-Pollard et al. 2024) that addresses the detected paralogy issues of targeted nuclear genes in the Compositae-specific probe set (Siniscalchi et al. 2021).

With the overarching goal of reconstructing the evolutionary history of the Anthemideae, we present the results of our Hyb-Seq implementation, using the Angiosperms353 enrichment panel (Johnson et al. 2019), to tackle the systematic problems that have resisted deep inquiry using other sources of data (i.e, Sanger sequencing). Specifically, this study aims to address (1) the monophyly of currently accepted subtribes; (2) the extent to which the use of genome-wide data improves the resolution of relationships, from the backbone (among subtribes) to the tips (genera, and even species); (3) whether previously detected plastid-nuclear incongruence persists when using genome-wide data (and explore potential events of hybridization and/or incomplete lineage sorting, ILS). In addition, (4) we aim to estimate divergence times and a timeline for the diversification of this lineage to further explore the possible association between diversification and palaeoclimatic conditions.

Materials and Methods

Sampling and vouchering

Sampling and laboratory protocols were carried out following the Plant and Fungal Trees of Life (PAFTOL) programme workflow (Baker et al. 2022). One specimen per genus was selected, from a list standardised according to the World Checklist of Vascular Plants (Govaerts et al. 2021), prioritising genera that are not represented by published transcriptomic/genomic data in public repositories (e.g, GenBank) or already sampled under the umbrella of other genomics consortia (such as the One Thousand

Plant Transcriptomes Initiative; OTPTI, 2019). Fifty-four species across 50 genera were sampled for DNA, representing about half of all known Anthemideae genera and all subtribes considered in previous studies with the exception of four: Inulantherinae, Lapidophorinae, Lonadinae, and Vogtiinae. Thirteen species from eight different tribes were included as outgroups. Additionally, we completed our Anthemideae sampling by including 77 additional taxa available from public open access repositories (e.g, National Center for Biotechnology Information; NCBI), with emphasis on species-rich genera (Supporting Table S1) and bringing the total genus count up to 61.

Plant material was obtained from (1) collections at Royal Botanic Gardens, Kew (K herbarium, DNA and tissue bank, and living collection; https://www.kew.org/science/collections-and-resources/collections) and (2) material provided by specialists through collaborative networks (Supporting Table S2).

DNA extraction, library preparation, hybridization, and sequencing

DNA was extracted following a modified CTAB protocol (Doyle & Doyle, 1987) and purified with MagBind TotalPure NGS magnetic beads (Omega Biotek, Norcross, GA, USA). Concentration and degree of fragmentation were checked by electrophoresis with 1.5× agarose gel and with a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), respectively. Samples with fragment sizes >350 bp were sonicated using a M220 Focused-ultrasonicator with microTUBEs AFA Fiber PreSlit SnapCap (Covaris, Woburn, MA, USA), following the manufacturer's protocol. Dual-index libraries were prepared using the DNA NEBNext Ultra™ II Library PrepKit, at half the recommended volume, with dual index primers, NEBNext Multiplex Oligos for Illumina Set 1 (New England BioLabs). Library quality and quantity were assessed with a 4200 TapeStation System with standard D1000 tapes (Agilent Technologies, Santa Clara, CA, USA) and a Qubit fluorometer, respectively. Equimolar pools of 20 to 25 DNA libraries, for a total

of 1 µg of DNA, were hybridised with biotinylated probes using the myBaits Expert Angiosperms353 enrichment panel (v.1, Daicel Arbor Biosciences, Ann Arbor, MI, USA) following the manufacturer's protocol. Hybridizations were performed at 65°C for 28 to 32 h in a Hybex Microsample Incubator (SciGene, Sunnyvale, CA, USA), using an equal volume of red Chill-out Liquid Wax (Bio-Rad, Hercules, CA, USA) to prevent evaporation. Probe-bound library fragments were captured with streptavidin-coated magnetic beads. These target-enriched pools were then amplified using KAPA HiFi (2×) HotStart ReadyMix PCR Kit (Roche, Basel, Switzerland) for a total of 10 cycles. PCR products were cleaned with the QIAquick PCR purification kit (Qiagen, Germantown, MD, USA) and quantified with a Qubit fluorometer. Final products were run on a 4200 TapeStation System using the High Sensitivity D1000 ScreenTape (Agilent Technologies) to assess quality and average fragment size. Lastly, several target enriched library pools were further multiplexed, and sequencing was performed on an Illumina HiSeq X (2 × 150 bp paired-end reads) at Genewiz (Takeley, UK).

Sequence Rescue and Assembly of Nuclear Data Matrices

Output raw reads (FASTQ files) were trimmed using Trimmomatic v0.39 (Bolger et al. 2014), to remove adapters and other low-quality begin/end base pairs (ILLUMINACLIP: TruSeq3-PE-2.fa:2:30:10 LEADING:20 TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:50), and quality checked before and after trimming with FastQC (Andrews, 2010). Only paired reads were used to rescue on-target nuclear sequences using HybPiper v2.0.1 (Johnson et al. 2016), using the "mega353" target file (McLay et al. 2021) as the mapping template, which is available at https://github.com/chrisjackson-pellicle/NewTargets, to optimise recovery of targets. Unaligned multi-FASTA files were generated for coding regions only for each one of

our targets (exon-only contigs). The R (R Core Team, 2020) script max_overlap (see https://github.com/keblat/bioinfo-utils/blob/master/docs/advice/scripts/max_overlap.R) was used to identify and discard suboptimal samples and/or genes, and to build balanced data matrices (Shee et al. 2020). This script outputs an overlap (coverage) score calculated from three summary statistics: representativeness, completeness, and evenness. When assembling our data matrices (Lozano-Fernández, 2022), this overlap (coverage) score, can help us avoid bias in the phylogenetic inference, e.g, driven by a handful of genes (Shen et al. 2017) or by an excess of unevenly distributed missing data (Roure et al. 2013), among other artefacts. Thus, samples and genes with overlap (coverage) score < ⅔ median were discarded. Furthermore, putative paralogs, as flagged by HybPiper (available at https://github.com/mossmatters/HybPiper/wiki/Paralogs) were eliminated from downstream analyses.

Resulting data matrices (multi-FASTA files) were aligned using MAFFT v7.508 (Katoh & Standley, 2013) with the accuracy-oriented algorithm (--genafpair). Aligned matrices were refined through an iterative process with check points (alignment summary statistics calculated with AMAS; Borowiec, 2016). Preliminary gene trees were generated from each alignment using FastTree v2.1.11 (Price et al. 2010), which relies on approximately-maximum-likelihood to infer phylogenies. These trees were then checked for outliers using TreeShrink (Mai & Mirarab, 2018), which identifies taxa that increase the diameter of each tree based on the false positive error rate (α) and removes them from the aligned matrices. After the exploration of different α (flag -q), we fixed it at -q 0.05. These shrunk data matrices were then re-aligned with MAFFT v7.508, checked with AMAS, and trimmed using trimAl v1.4.rev15 (Capella-Gutiérrez et al. 2009) to filter poorly aligned regions. We explored two different alignment filtering schemes: a lax one (gap threshold, -gt: 0.1; percent original data matrix conserved,

-cons: 35) and a more restrictive one (-gt 0.3; -cons 30). Summary statistics for these two trimming schemes were also calculated with AMAS.

Nuclear Phylogenomic Inference

Species trees were inferred using two approaches: a multispecies pseudo-coalescent approach (hereafter MSC-ASTRAL), which infers a species tree from gene trees with ASTRAL III (Zhang et al. 2018); and a maximum likelihood approach (ML-IQTREE) which infers a phylogeny from the data matrix resulting from concatenating all shrunk and trimmed re-aligned data matrices, using IQ-TREE 2 (Minh et al. 2020).

MSC-ASTRAL approach. - Gene trees were generated from polished data matrices using IQ-TREE multicore v 2.2.0.3 (Minh et al. 2020), choosing the substitution model with ModelFinder, -m MFP (Kalyaanamoorthy et al. 2017), and performing 1,000 replicates of ultrafast bootstrap (-B 1000 UFBoot2; Hoang et al. 2017). Low support branches were collapsed in each gene tree to improve accuracy (Zhang et al. 2018) using Newick Utilities v1.6 (Junier & Zdobnov, 2010) under different collapse thresholds (-bs; 5 to 75, each 5) to address their impact (sensitivity analysis) on species tree inference (Mirarab, 2019; Simmons & Gatesy, 2021). Coalescent analysis to infer the species tree was performed with these sets of gene trees in ASTRAL-III with extensive branch annotations (-t 2 flag), which include normalised quartet score (QS) values and local posterior probabilities (LPP).

ML-IQTREE approach. - A concatenated matrix was generated from the shrunk and trimmed alignments using AMAS (Borowiec, 2016). ML analyses were conducted using the IQ-TREE 2 partitioned analysis (-p) (Chernomor et al. 2016), specifying a substitution model for each gene partition. Partition files were also generated using AMAS (--part-format nexus). The best substitution model was chosen using

ModelFinder and 1,000 bootstrap replicates (UFBoot2) were done to estimate branch support values.

The R package *treespace* (Jombart et al. 2017) was used to explore and compare MSC topologies inferred throughout the phylogenomic pipeline. Upon inspection of the landscape of trees, and given the pairwise tree distances calculated, a data matrix was chosen that minimised these pairwise tree distances and maximised the collapse threshold (e.g, collapse when bs < 20%), for every alignment filtering scheme (two possible gap threshold and conservation combinations, see above). For the ML approach, with only two generated trees, we selected the tree with the greatest support values throughout the topology. Following this inspection, trees chosen from both MSC and ML approaches were visualised in FigTree 1.4.4 (Rambaut, 2018).

Plastome Rescue, Data Matrix Assembly, and Phylogenomic Analyses

Sequence rescue and data matrix assembly was conducted following a workflow similar to the one used for the nuclear targets. Off-target plastid gene recovery was also done with the HybPiper pipeline using a custom plastid target file, which included coding sequences for a set of representative angiosperm plastomes (Pokorny et al. 2024; https://github.com/mossmatters/plastidTargets). Unlike the workflow followed for nuclear analyses, here we fixed the false positive error rate (α) for TreeShrink at -q 1.0. For the inference of species trees, only the ML-IQTREE approach was implemented, following Doyle (2022) in that plastid genes are c-genes that should not be analysed with MSC approaches. Because our study encompasses a large group spanning a considerable phylogenetic depth, in addition to partitioning our plastome data matrices by gene, a second partition was generated by codon (--codons 123) in AMAS, and a third partition was produced modifying the codon partition to exclude the third position. Programs *treespace* and FigTree 1.4.4 were also used for the exploration and

visualisation of the resulting topologies. The final plastome tree was obtained by selecting the most represented topology in the *treespace* landscape with the best support values.

Divergence Time and Diversification Rate Estimation

Divergence times were estimated with MEGA version 11 (Tamura et al. 2021) using the rapid relaxed-clock method RelTime (Tao et al. 2020a). Since we used the RelTime-Branch Lengths option, which requires a phylogenetic tree with previously estimated branch length (in substitutions per site), we optimised branch lengths for the selected MSC-ASTRAL tree (where branch lengths are instead in coalescent units) in RAxML-NG (using --evaluate, --brlen scaled flags) (Kozlov et al. 2019). *Reichardia picroides* in tribe Cichorieae was set as the outgroup taxon for both the MSC and the ML nuclear species trees, but was excluded from the time tree. Calibration densities were lognormally distributed, with MEAN = 0.0 and STD DEVIATION = 0.5 (Tao et al. 2020b). Four time constraints, mainly pollen grains (Palazzesi et al. 2022a), were imposed on our MSC-ASTRAL and ML-IQTREE nuclear phylogenies: (1) Asteraceae stem node excluding Barnadesioideae and Famatinanthoideae (age ~56 Mya), (2) Cichorioideae crown node (age ~28 Mya), (3) Asteroideae crown node (age ~28 Mya), and (4) Anthemideae crown node (age ~12 Mya). We follow the “safe but late” option for calibrations given by Sauquet et al. (2011), instead of the "early but risky” option, largely because some modern pollen grains –especially those of the Artemisiinae, and in particular, *Artemisia*, which are anemophilous and produce large amounts of pollen (Piotrowska, 2008; Grewling et al. 2012)– are usually contaminants in fossil palynological slides. The maximum relative rate ratio was set to 20. The resulting chronograms were visualised in FigTree 1.4.4.

Diversification rate estimates were performed with R v4.1.3 (R Core Team, 2022) in RStudio v2022.02.0+461 (RStudio Team, 2022), using both the MSC and the ML nuclear time-trees. These topologies were pruned to remove all outgroup taxa, with function drop.tip from the R package *ape* (Paradis et al. 2004). Pruned chronograms had to be restored as ultrametric trees with function force.ultrametric from the R package *phytools* (Revell, 2012). Function ltt.plot, also from the R package *ape,* was used to inspect the diversification trajectory, by plotting the number of sampled lineages through time (LTT). We used the CoMET model (Compound Poisson Process of Mass Extinction; May et al. 2015), implemented in the R package TESS (Höhna, 2013), to estimate the timing and magnitude of changes in diversification rates and mass extinction events. The analysis was performed following the recommendations in López-Estrada et al. (2019): two chains of 10 million iterations, a sampling frequency of 100, and a minimum effective sample size (ESS) of 1,000. The shape of the prior distributions for speciation and extinction rates was estimated from the data using the argument "empiricalHyperPriors = TRUE". We set the sampling probability (sampling fraction) to 0.04, given the partial representation of Anthemideae in this study, and used default values in TESS for the initial speciation rate (2.0), initial extinction rate (1.0), and number of expected rate changes and mass extinction events (MEEs; 2). Mixing and convergence of the two chains were assessed by estimating MCMC diagnostics in TESS (Höhna, 2013), i.e, the Rubin-Gelman statistic and ESS values (> 500), and by comparing posterior density plots between chains.

Results

Capture success and data matrix assembly

A total of 145 taxa were analysed: 67 accessions sequenced under the umbrella of the PAFTOL research programme and 78 obtained from the NCBI Sequence Read Archive

(SRA). Thirteen of the SRA accessions corresponded to target capture sequencing (TCS), 14 to whole genome sequencing (WGS), 23 to genome skimming, one to expressed sequenced tags (ESTs), one to whole genome amplification (WGA), and 26 to RNA-seq experiments (Supporting Table S1). After quality filtering with Trimmomatic, on average we obtained 545,258 on-target reads (±582,890 SD; 2841 min; 2,628,136 max) mapping to nuclear targets (~16% recovery), and 38,867 off-target reads (±130,202 SD; 9 min; 1,431,580 max) mapping to plastid regions (~0.18% plastid recovery), regardless of data provenance in either case. From these mapped reads, a median 172 nuclear genes (158 avg, ±130 SD; 0 min; 347 max) and 31 plastid genes (36 avg, ±29 SD; 0 min; 72 max) were recovered with HybPiper at 50% length. For our PAFTOL accessions, we obtained on average 539,517 on-target reads (±479,064 SD; 8,407 min; 2,587,499 max) mapping to nuclear targets (~31% nuclear target recovery), and 1,972 off-target reads (±3,730 SD; 9 min; 27,078 max) mapping to plastid regions (~0.1% plastid recovery). NCBI accessions recovered on average 550,189 on-target reads (±662,290 SD; 2,841 min; 2,628,136 max) mapping to nuclear targets (~3% nuclear target recovery), and 70,560 off-target reads (±171,725 SD; 522 min; 1,431,580 max) mapping to plastid regions (~0.3% plastid recovery). From these mapped reads, a median of 230 nuclear genes (199 avg, ±78 SD; 5 min; 313 max) and 10 plastid genes were recovered (17 avg, ±18 SD; 0 min; 72 max) with HybPiper at 50% length for PAFTOL accessions, while 17 nuclear genes (123 avg, ±154 SD; 0 min; 347 max) and 68 plastid genes were recovered (52 avg, ±25 SD; 0 min; 72 max) with HybPiper at 50% length for NCBI accessions (Appendix 1). Given their low overlap scores (< ⅔ median coverage score; see max_overlap script in Methods above), 18 genes and 53 accessions (4 from PAFTOL) were discarded from the (on-target) nuclear dataset, while 62 accessions (48 from PAFTOL) were discarded from the (off-target) plastid dataset.

Another 91 nuclear and 6 plastid regions were flagged as having potential paralogs (with ≥ 3 paralogs) and excluded from downstream analyses (Appendix 1). Of the total 19 subtribes recognized by Oberprieler et al. (2022), 15 and 13 are represented in the nuclear and plastid datasets, respectively (Supporting Table S1). As a consequence, there is limited overlap between the nuclear and plastid datasets. Following automated outlier removal (with TreeShrink) and alignment trimming (using trimAl), we generated four datasets comprising on average (1) 907 bp and (2) 945 bp per locus, across 244 nuclear coding regions, and (3) 748 bp and (4) 750 bp per locus, across 66 plastid coding regions. The proportion of parsimony-informative characters (P_{PIC}) was low for most off-target plastid genes. Missing data accounted for (1) 22% and (2) 24% of on-target nuclear markers, and (3) 6% and (4) 7% of off-target plastid regions (Table 1, Appendix 2).

Our MSC-ASTRAL approach resulted in 30 possible nuclear species trees, depending on the combination of collapse thresholds and the two different alignment filtering schemes. Based on the results of treespace, and following the criteria described above (see Nuclear Phylogenomic Inference in Material and Methods) we chose the bs20gt0.1cons35 data matrix to infer the final MSC-ASTRAL nuclear species tree (Supporting Fig. S1A). This data matrix results from alignments filtered in trimAl with a 0.1 gap and a 35 conservation thresholds (any given column was filtered out if it presented gaps in over 90% of samples, as long as < 65% of the total data matrix is not lost), with bipartitions in the resulting topology collapsed when bs < 20% (UFBoot values), since collapsing more aggressively could substantially reduce topological accuracy (Mirarab, 2019; Simmons et al. 2021).

For the ML-IQTREE approach, we obtained two nuclear species trees and six plastid topologies. In both nuclear and plastid trees, we chose the gt0.3cons30 data matrices,

where the gap and conservation thresholds implemented in trimAl were 0.3 and 30, respectively (collapsing does not apply). In either case, partitioning by marker gene resulted in better supported topologies, which in the plastome happens to also be the most represented topology in the *treespace* landscape (Supporting Fig. S1B).

Hereafter, we consider that a topology has strong or full support when LPP = 1.0 or BS = 100%, high support when 1.0 > LPP ≥ 0.9 or 100% > BS ≥ 95%, moderate support when 0.9 > LPP ≥ 0.7 or 95% > BS ≥ 75% and weak or low support when LPP < 0.7 or BS < 75%.

Nuclear Phylogenomic Relationships in Anthemideae

Analyses of the nuclear dataset under the two approaches (MSC and ML) fully support the monophyly of Anthemideae (clade C.A, Figs. 1, 2). The MSC-ASTRAL phylogeny (Fig. 1) strongly supports the monophyly of most studied subtribes, except for Anthemidinae, Athanasiinae, and Matricariinae. The subtribe Osmitopsidinae (*Osmitopsis* Cass. genus) is sister to the remainder of the tribe (clade C.2) with strong support. All analyzed members of subtribe Cotulinae (*Cotula* L., *Hippia* L., *Inezia* E.Phillips, *Leptinella* Cass., *Schistostephium* Less., and *Soliva* Ruiz & Pav.) group together in the fully supported clade C.9, sister to clade C.3. Clade C.3 includes *Ursinia* Gaertn. (subtribe Ursiniinae) and the remaining Anthemideae. Clade C.4 splits into clade C.10, containing Phymasperminae and Athanasiinae taxa and the sister clade C.5. As currently circumscribed, Athanasiinae (*Athanasia* L., *Eriocephalus* L., *Hymenolepis* Cass., and *Lasiospermum* Lag.) is paraphyletic, since the sampled Phymasperminae (*Eumorphia* DC. and *Phymaspermum* Less.) are embedded within it. Clade C.5 splits into a fully suported Pentziinae (clade C.11 encompassing *Cymbopappus* B.Nord., *Foveolina* Källersjö, *Marasmodes* DC., *Oncosiphon* Källersjö, and *Pentzia* Thunb.) and the remainder of the tribe. Clade C.6 is divided into clade C.12, which contains subtribe

Handeliinae (only represented by *Cancrinia* Kar. & Kir.) sister to the Artemisiinae, and clade C.7. The Artemisiinae clade presents four fully/highly supported monophyletic groups: A1 (*Brachanthemum* DC. and *Hippolytia* Poljakov), A2 (*Filifolium* Kitam. and *Kaschgaria* Poljakov), A3 (*Ajania* Poljakov, *Chrysanthemum* L., *Opisthopappus* C. Shih, and *Stilpnolepis* Krasch.), and A4 (*Artemisia* L. and *Crossostephium* Less.). Clade C.7 splits into subtribe Brocchiinae (*Brocchia* Vis.) and a clade (C.8) comprising the remaining sampled subtribes: Anthemidinae and Matricariinae, mingling in the fully supported clade C.13, and Glebionidinae, Leucanthemopsidinae, Leucantheminae, and Santolininae, grouped in the weakly supported clade C.14. Clade C.13 encompasses two clades, one including the fully supported monophyletic genera *Achillea* L. and *Matricaria* L., Matricariinae) sister to *Tanacetum coccineum* (Anthemidinae), and another one also including genera in both Anthemidinae (*Anthemis* L., *Cota* J. Gay*, Gonospermum* Less., *Tanacetum* L. and *Tripleurospermum* Sch.Bip.) and Matricariinae (*Anacyclus* L., and *Heliocauta* Humphries). In clade C.14, subtribe Glebionidinae (*Argyranthemum* Webb ex Sch.Bip. and *Glebionis* Cass.) is sister to a clade that includes three monophyletic subtribes: Leucanthemopsidinae (*Castrilanthemum* Vogt & Oberpr., *Hymenostemma* Kunze ex Willk., *Leucanthemopsis* (Giroux) Heywood*, Phalacrocarpum* Willk. and *Prolongoa* Boiss.), Leucantheminae (*Coleostephus* Cass., *Leucanthemum* Mill. and *Rhodanthemum* B.H.Wilcox & al.), and Santolininae (*Chamaemelum* Mill., *Cladanthus* Cass., and *Santolina* L.). Leucantheminae and Santolininae are sister clades, although with low support.

The ML-IQTREE topology (Fig. 2) is very similar to the MSC-ASTRAL, but presents higher support values for the backbone and elsewhere, e.g., clade C.14. Most clades identified in the MSC-ASTRAL tree are found, with a few exceptions. Clades A1 and A2 (Fig. 1) are instead grouped in a weakly-supported clade B1. Clade B2, formed by

Chrysanthemum sp., *Ajania fruticulosa* (Ledeb.) Poljakov, *Chrysanthemum zawadzkii* Herbich, and *Stilpnolepis intricata* (Franch.) C. Shih (Fig. 2), is sister to the remainder of Artemisiinae, composed of clades B3 (*Chrysanthemum* spp. and *Opisthopappus*) and B4 (*Artemisia* spp. and *Crossostephium*) (Fig. 2). In the MSC-ASTRAL phylogeny, B2 is sister only to the clade containing *Chrysanthemum* spp. and *Opisthopappus* (Fig. 1, Supporting Fig. S2). In both topologies *Chrysanthemum* and *Artemisia* are paraphyletic as currently circumscribed. Relationships within strongly-supported Clade C.14 differ since Glebionidinae is sister to Santolininae, instead of Leucanthemopsidinae, Leucantheminae, and Santolininae.

Other differences between the MSC-ASTRAL and ML-IQTREE topologies include the position of *Achillea distans* subsp. *tanacetifolia* (All.) Janch. and *Achillea maritima* (L.) Ehrend. & Y.P.Guo, which are grouped with *Achillea* in the MSC tree and with *Heliocauta*, *Anacyclus*, and Anthemidinae in the ML tree. The clade including *Anthemis arvensis* L., *Cota tinctoria* (L.) J. Gay ex Guss., and *Tripleurospermum inodorum* (L.) Sch.Bip., sister with low support to *Anacyclus clavatus* (Desf.) Pers. and *Heliocauta atlantica* (Litard. & Maire) Humphries in the MSC tree (Fig. 1), is highly supported as the sister group of a clade that includes *Gonospermum ferulaceum* (Webb) Febles and *Tanacetum vulgare* L. (Anthemidinae), in addition to *Achillea distans* subsp. *tanacetifolia*, *Achillea maritima, Anacyclus clavatus,* and *Heliocauta atlantica* (Matricariinae), in the ML tree (Fig. 2, Supporting Fig. S1). Genus *Achillea* also presents differences between topologies, since it is monophyletic in the MSC tree but not in the ML tree. Furthermore, *Achillea acuminata* Sch.Bip. is sister to *A. alpina* L. and *A. wilsoniana* (Heimerl) Hand.-Mazz, with weak support, in the MSC tree (Fig. 1); whereas in the ML tree, *A. alpina* is sister with high support to the other two species (Fig. 2, Supporting Fig. S1). Altogether, these results suggest that neither *Tanacetum*

nor *Achillea* should be considered monophyletic. The last discordance occurs in Leucantheminae: *Coleostephus myconis* (L.) Rchb.f. is sister to *Leucanthemum graminifolium* (L.) Lam. plus *Rhodanthemum gayanum* (Coss. & Durieu) B.H.Wilcox & al. in the MSC tree; whereas in the ML tree, *Rhodanthemum gayanum* is sister to the other two species (Supporting Fig. S1).

Despite these differences, the MSC and ML topologies convey a clear biogeographic signal (Figs. 1, 2). The earliest six subtribes that subsequently diverged within Anthemideae, viz. Athanasiinae, Cotulinae, Osmitopsidinae, Pentziinae, Phymasperminae, and Ursiniinae, are primarily though not exclusively distributed in southern Africa. They will therefore be referred to as the Southern African Grade. The subtribes Handeliinae and Artemisiinae (C.12) form a monophyletic group distributed primarily in Central and Eastern Asia, respectively, and are hereafter referred to as the Asian Clade. The remaining subtribes Anthemidinae, Brocchiinae, Glebionidinae, Leucantheminae, Leucanthemopsidinae, Matricariinae, and Santolininae (C.7) also form a monophyletic group that is largely circum-Mediterranean and is hereafter referred to as the Circum-Mediterranean Clade.

Plastome Phylogenomic Inference

The plastid tree based on off-target regions presented some incongruences compared to the nuclear trees (Fig. 3). *Eumorphia davyi* (Phymasperminae) is unexpectedly sister to the large clade comprising Anthemidinae, Glebionidinae, Leucantheminae, Leucanthemopsidinae, Matricariinae, and Santolininae (D.6), instead of grouping with putatively more closely-related taxa (i.e., *Lasiospermum pedunculare*) in a clade sister to most Anthemideae tribes (D.3). The two Leucanthemopsidinae samples from which we recaptured plastid markers, *Phalacrocarpum oppositifolium* and *Prolongoa hispanica*, are sister to all Anthemidinae, Glebionidinae, Leucantheminae,

Matricariinae, and Santolininae (D.7), instead of sister to Leucantheminae in the ML tree or to Leucantheminae plus Santolininae in the MSC tree (Figs. 1, 2). Contrasting with the results of our nuclear analyses in which Matricariinae and Anthemidinae are intermingled, in the plastid topology they remain distinct. Changes are also observed within these subtribes. For example, *Solvia mexicana* and *S. sessilis* do not come out as sisters. *Kaschgaria brachanthemoides* and *Filifolium sibiricum* are not sister to most Artemisiinae but well-nested within it. Notably also in Artemisiinae, *Ajania*, *Artemisia*, and *Chrysanthemum* are markedly non-monophyletic; partly, but not only, due to the placements of *Neopallasia pectinata*, *Opisthopappus taihangensis*, and *Crossostephium chinense*, well nested within this subtribe. All sampled *Achillea* species for the plastid tree, except for *A. maritima*, form a weakly supported clade and *Matricaria* is non-monophyletic due to the placement of *A. maritima*; whereas in the MSC tree, but not in the ML tree, these genera are monophyletic for the currently sampled species.

Divergence Times in Anthemideae

Mean divergence time estimates (Fig. 4 and Table 2) inferred with RelTime are presented for the MSC and ML nuclear topologies (Fig. 4 A & B, respectively). The most recent common ancestor (MRCA) of Anthemideae (C.A) dates back to the mid Miocene (13.8_{MSC} and 15_{ML} Mya), which is also the age of the subtribe Osmitopsidinae stem node. The stem node age of Cotulinae (C.2) dates back to the Upper Miocene (9.9_{MSC} and 11_{ML} Mya). The Ursiniinae (C.3) stem node age is also inferred as Upper Miocene (7.2_{MSC} and 8_{ML} Mya), as is the Athanasiinae s.l. (including Phymasperminae; C.4) stem node age (5.2_{MSC} and 6_{ML} Mya). Four subtribes subsequently diverged during the Pliocene (stem node ages provided in parentheses): (C.5) Pentziinae (4_{MSC} and 4.5_{ML} Mya); (C.6) Handeliinae plus Artemisiinae (3.2_{MSC} and 3.7 Mya; these two diverging from each other at 2.8_{MSC} and 3.2_{ML} Mya; C.12); and (C.7) Brocchiinae (3_{MSC} and 3.5_{ML}

Mya). The remaining subtribes diverged during a relatively short period of time, spanning the Early Pliocene. (C.8) Both the divergence of Anthemidinae s.l. (including Matricariinae, C.13) from the remaining tribes (2.4_{MSC} and 2.9_{ML} Mya) and the diversification within Anthemidinae s.l. (2.1_{MSC} and 2.4_{ML} Mya) date back to the Early Pliocene. So do the divergences of Leucanthemopsidinae (2_{MSC} and 2.4_{ML} Mya), Santolininae (1.6_{MSC} and 2.1_{ML} Mya), Leucantheminae (1.6_{MSC} and 1.9_{ML} Mya), and even Glebionidinae (1.4_{MSC} and 1.6_{ML} Mya), which is the most recently diverged subtribe in this clade.

Diversification Patterns in Anthemideae

The LTT plots (Fig. 4 A3 & B3) showed a progressive exponential increase in the accumulation of lineages up to ~4 Mya, time at which the increase is accentuated up to ~2 Mya, when a transition to a stationary phase begins. Results from CoMET analyses mostly agree with the LTT plots. CoMET estimated a very low net diversification rate (r = speciation rate, λ − extinction rate, μ), until ~10 Mya, followed by a gradual increase towards the present, with a steep increase starting ~4 Mya ($r \sim 0.5$) and peaking at ~2 Mya ($r \sim 1.5$) (Fig. 4 A1 & B1). This is coincident with an initial high relative-extinction rate ($\varepsilon = \mu / \lambda$) ~14 Mya ($\varepsilon \sim 2$), which decreases until stabilising ($\varepsilon \sim 1$) over time, except for a slight decrease at ~ 2 Mya (Fig. 4, A2 & B2). The other parameters estimated by CoMET, i.e., speciation and extinction rates, rate shifts, time estimates of potential mass extinction events (MEEs), and the Bayes Factor comparisons for the timing of MEE events, are shown in Supporting Fig. S3.

Discussion

Our study represents the first in-depth targeted HTS study focused on the large and diverse Anthemideae tribe. The results, based on 244 low-copy nuclear genes and 66 plastid genes, were largely congruent with previous evolutionary studies conducted and

further resolve the phylogenetic backbone of this tribe. However, the discordance that affects some groups, detected when comparing topologies within (MSC vs. ML nuclear trees; Figs. 1, 2) and between (nuclear vs. plastid trees) genomic compartments, suggests hybridization events and/or ILS may be at play. Certainly, some of these incongruences could be associated with polyploidization events. In this sense, transcriptomic analyses evidenced a whole-genome multiplication (WGM) event along the backbone of tribe Anthemideae, which could have taken place at some point during the Plio-Pleistocene (Fig. 4) in an ancestor shared by the Circum-Mediterranean and Asian Clades, following the Miocene divergence of Cotulineae and Ursiniinae (Zhang et al. 2021). Unfortunately, this transcriptomic study did not include representatives of the Athanasiinae, Pentziinae, and Phymasperminae, which prevents a more precise determination of the timing of this WGM event.

Our work provides clues to address several remaining questions following the latest phylogenetic analyses of Oberprieler et al. (2022): (1) the phylogenetic placement of *Osmitopsis*, here sister to the remaining Anthemideae subtribes; (2) the monophyly of Cotulinae, which we recovered in all analyses; (3) the phylogenetic affinities of Glebionidinae and Santolininae, which are strongly supported as sister groups here; and (4) the circumscription of paraphyletic subtribes Matricariinae and Anthemidinae, which could be solved by merging them into a single subtribe.

Regarding divergence times, our estimates are more recent than those given in four previous studies (Tomasello et al. 2015; Panero & Crozier, 2016; Mandel et al. 2019; Lu et al. 2022). Estimated divergence times outside Anthemideae in Mandel et al. (2019) are not markedly different from ours (e.g., Anthemideae vs. Senecioneae ~35 Mya compared to our ~30 Mya; Fig. 5). However, differences within Anthemideae are significant (e.g., the stem node of Athanasiinae ~20 Mya compared to our 5.2_{MSC} and

6_{ML} Mya). Three other studies focused on Anthemideae also estimated older divergence times (Tomasello et al. 2015; Panero & Crozier, 2016; Lu et al. 2022). For instance, stem nodes of Ursiniinae and Leucanthemopsinidae were dated to ~33.78 Mya and ~20.47 Mya in Tomasello et al. (2015), compared to our 7.2_{MSC} and 8_{ML} Mya for Ursiniinae and 2.2_{MSC} and 2.6_{ML} Mya, for Leucanthemopsinidae. Whether some contentious pollen records are used to calibrate nodes does explain this apparent temporal discordance. We follow the review by Palazzesi et al (2022a) and specifically avoid using *Artemisia* records from the Middle and Upper Oligocene (in Wang, 2004), due to concerns that their attribution to these periods may be due to potential contamination from recent individuals (see Materials and Methods). Interestingly, the same author (Wang, 2006) states that *Artemisia* first appears in a late Middle Miocene site. Conversely, Mandel et al. (2019), Tomasello et al. (2015), Panero and Crozier (2016) and Lu et al. (2022) used the pollen record by Wang (2004), attributed to the Oligocene, to calibrate the *Artemisia* stem node. Instead, following Wang (2006) and Palazzesi et al (2022a), we used *Artemisia*-like pollen records (*Artemisiapollenites*) dated to 12 Mya (late Middle Miocene) to calibrate Anthemideae crown node for our divergence time estimates. It is worth noting that the time node estimates obtained with or without the use of this calibration point (results not shown) remain consistent (e.g., 13.05 Mya versus 13.8 Mya for the MSC trees), and markedly different from those reported in the three aforementioned studies.

In addition, the phylogenomic analyses presented here also strengthen the biogeographic signal previously reported in the phylogenetic reconstructions of Anthemideae based on a much smaller number of nuclear ribosomal and plastid markers (Watson et al. 2000; Oberprieler et al. 2005, 2007, 2009, 2022). These previous studies identified four biogeographic groups: (1) the Southern Hemisphere Grade, (2) the

Asian-Southern African Grade, (3) the Eurasian Grade, and (4) the Mediterranean Clade (the only monophyletic group).

Our Southern African Grade, which we so name because most of its species occur in southern Africa (Table S3), corresponds to the Southern Hemisphere Grade first described Watson et al. (2000) and Oberprieler et al. (2005, 2007, 2009, 2022), although in our study it also includes subtribe Pentziinae (Figs. 1, 2). In previous studies, this subtribe appeared more closely related to Artemisiinae and Handeliinae, although often without statistical support. It was additionally argued that some *Pentzia* species are distributed outside southern Africa (i.e., Somalia, Morocco, Algeria, and Yemen), which according to Oberprieler et al. (2007, 2009, 2022) provided additional evidence for excluding Pentziinae from their Southern Hemisphere Grade. However, given that not all genera of the Southern Hemisphere Grade are restricted to the southern hemisphere (e.g., *Cotula* contains species occurring in north and southern Africa, southern and eastern Asia, Australia, South America, Mexico, New Zealand, and sub-Antarctic islands), this argument is not entirely convincing, especially in light of our results (Figs. 1–3). It makes more sense from a biogeographic perspective to place these early diverging subtribes as part of the Southern African Grade. The majority of genera and species in subtribes Athanasiinae, Cotulinae, Pentziinae, Physmasperminae, Osmitopsidinae, and Ursiniinae are found in southern Africa, even though some species of *Lasisospermum* occur in Egypt (Sinai Peninsula), while *Leptinella* species can be found in New Guinea, Australia, New Zealand, South America, and the Falkland Islands, as well as other sub-Antarctic islands (apart from the already mentioned *Cotula* and *Pentzia* species). As mentioned above, rather than strictly referring to the distribution of the species that make this grade, the name of this Southern African Grade corresponds to the area where most of its contemporary diversity is concentrated. More

importantly, Mandel et al. (2019) inferred this region as the ancestral area for tribe Anthemideae. The current distribution of genera such as *Leptinella*, *Cotula,* and Pentzia, which diverged no earlier than the mid Miocene and yet include widely distributed species, particularly in the Southern Hemisphere, suggests repeated dispersal events outside of the South African region. However, ancestral area reconstruction analysis with a more comprehensive species sampling should provide explicit biogeographic hypotheses.

The inclusion of Pentziinae in the Southern African Grade leaves the subtribes Artemisiinae and Handeliinae as a monophyletic group largely restricted to Asia, our Asian Clade. Some species reach other regions beyond this continent (e.g., *Arctanthemum*, Arctic Eurasia and North America; *Artemisia*, the entire Holarctic kingdom; *Chrysanthemum* and *Leucanthemella*, eastern Europe) (Table S3). However, these subtribes have their centres of diversity in Asia, specifically Handeliinae in Central Asia extending into southwestern Asia, and Artemisiinae in East Asia.

Our results also support the merging of the Eurasian Grade and Mediterranean Clade (Oberprieler et al. 2005, 2007, 2009, 2022) into a single monophyletic group, the Circum-Mediterranean Clade. Most genera and species in this clade are distributed in the Mediterranean basin and adjacent regions (Table S3). The few exceptions include: (i) *Achillea, Matricaria, Tanacetum*, and *Tripleurospermum*, with some species extending into northern Europe and temperate regions of Asia and North America; (ii) *Anacyclus*, extending into northern Europe and southwestern Asia; and (iii) *Anthemis* and *Cota*, also extending into southwestern Asia. As noted above, the previous recognition of two separate biogeographic groups, one of them not monophyletic, was mainly due to lack of resolution (Oberprieler et al. 2005, 2007, 2009, 2022).

The implications for the subtribal classification are discussed in more detail below for each subtribe. Inconsistencies with previous studies stem from the increased phylogenetic signal of our genomic level data matrices, different sampling regimes (e.g., to test the monophyly of large genera we sampled several species per genus), and the use of combined plastid-nuclear datasets in previous studies, which we purposefully avoided to explore cyto-nuclear incongruence. Since the strong conflict detected between our nuclear and plastid topologies hints toward hybridization and plastid capture, concatenating datasets would have buried whichever phylogenetic signal was weaker (minority ancestry; Bravo et al. 2019), thereby precluding an accurate reconstruction of the evolutionary history of tribe Anthemideae.

I. Southern African Grade

Osmitopsidinae

Our results corroborate the segregation and early divergence of Osmitopsidinae (Figs. 1, 2) from the rest of Anthemideae in the Early Miocene (stem node age: 13.8_{MSC} and 15_{ML} Mya), preceding the divergence (stem node age: 9.9_{MSC} and 11_{ML} Mya) and diversification (crown node age: 6.4_{MSC} and 7.2_{ML} Mya) of the Cotulinae (Fig. 4). The segregation of *Osmitopsis* from the rest of subtribe Cotulinae was formally proposed by Bremer and Humphries (1993), although they refer to this latter subtribe as Thaminophyllinae, and mentioned in previous works (Bremer, 1972; Nordenstam, 1987). Ultimately, *Osmitopsis* was included in an unigeneric subtribe, Osmitopsidinae, although its relationship with regard to Cotulinae remained uncertain (Oberprieler et al. 2007, 2022; Himmelreich et al. 2008) (Fig. 5).

Cotulinae

The monophyly of this subtribe was supported by Oberprieler et al. (2022), even though their MSC topology recovered *Hippia* nested in Pentziinae, albeit with low support. In

our study, Cotulinae appears as a fully supported clade in all trees (Figs. 1–3, 5) that diverged earlier than Pentziinae (stem node age: 4_{MSC} and 4.5_{ML} Mya), which is also recovered as strongly monophyletic (see Pentziinae-specific section below).

Consistent with previous studies, we found a close relationship between the widespread genus *Cotula*, the South American genus *Soliva*, and the Australasian genus *Leptinella* (Lloyd & Webb, 1987; Bremer & Humphries, 1993; Oberprieler et al. 2007; Powell et al. 2014; Jakoet et al. 2022), as well as the African genera *Schistostephium* and *Inezia* (Oberprieler et al. 2022) (Figs. 1, 2). However, a cyto-nuclear discordance is recovered for *Solvia*, an inconsistency that could result from biological processes such as ILS or introgression driven by rapid radiation and/or hybridization, respectively (Fig. 4). However, methodological artefacts, such as long branch attraction (LBA), missing data, or batch effects (Leek et al. 2010), could also explain this apparent incongruence. Despite our frequent quality checks and careful data filtering, exploratory analyses indicate that our current plastome data matrices may suffer from these artefacts (e.g, presence of long branches, similarly processed samples grouping together), thus casting doubts on the reliability of the signal recovered from plastid data. Additional plastome sequencing is needed to address this problem.

Ursiniinae

This subtribe, which just comprises genus *Ursinia*, diverged early in the history of the Anthemideae tribe (stem node age: 7.2_{MSC} and 8_{ML} Mya). However, unlike in Oberprieler et al. (2022), in our nuclear trees Ursiniinae is not sister to all other Anthemideae subtribes (Figs. 1, 2, 5). Unfortunately, we could not get off-target plastid data for *Ursinia*, and so, we have been unable to address whether this alternative phylogenetic placement is due to cyto-nuclear discordance.

Athanasiinae and Phymasperminae

The paraphyly of Athanasiinae in our study is consistent with previous studies(Figs. 1, 2). Its close relationship with Phymasperminae has been described in several studies (Watson et al. 2000; Oberprieler et al. 2007; Himmelreich et al. 2008; Oberprieler et al. 2022). Akimana et al. (2020) performed a phylogenetic study on an extended sampling of these subtribes using two nuclear ribosomal (ITS and ETS) and two plastid (*rpl32-trnL* and *3'rps16-5'trnK*) regions. They expanded Athanasiinae to include Phymasperminae and synonymized *Eumorphia* with *Phymaspermum*, a consideration also supported by morphological data. Our results are partly congruent with this study, since the Phymasperminae clade was recovered nested within a broadly defined monophyletic Athanasiinae with full/high support in our nuclear analyses (Figs. 1, 2).

However, in the off-target plastid tree (Fig. 3), the sample representing Phymasperminae (*Eumorphia davyi*) was consistently recovered as sister to D6 (Anthemidinae, Glebionidinae, Leucantheminae, Leucanthemopsidinae, Matricariinae, and Santolininae; i.e., the Circum-Mediterranean Clade), nowhere near the representative of Athanasiinae (*Lasiospermum pedunculare*), which was sister to all Anthemideae subtribes with the exception of Cotulinae. Himmelreich et al. (2008) already noted a plastid-nuclear inconsistent placement for three Phymasperminae genera, and proposed two hypotheses to explain it: (1) a putative hybrid origin or (2) an intermediate position of Phymasperminae, between the earliest-diverged S African Anthemideae and the later diverged crown clades. Our results are better explained by a putative ancient hybridization of a Phymasperminae taxon with a member of the Circum-Mediterranean Clade. However, as previously mentioned, the position of these two subtribes in our plastome tree should be taken with caution, since we failed to recover plastid loci from

most of our TCS samples. A more thorough plastome sampling is needed to test this hypothesis.

Pentziinae

Previous works did not obtain a strong signal for the monophyly of Pentziinae (Oberprieler et al. 2022), and pointed to its close relationship with Artemisiinae (Oberprieler et al. 2007, 2022). However, our analyses consistently show Artemisiinae more closely related to Handeliinae, rather than to Pentziinae (Figs. 1–3, 5), in addition to recovering Pentziinae as monophyletic in all trees, regardless of data provenance. Within Pentziinae, our single *Foveolina* accession is nested within *Oncosiphon* (Figs. 1, 2); both genera were first described by Källersjö (1988). Based on morphological characters, several authors indicate *Foveolina* consists of two groups. From a phylogenetic perspective, one of these groups is more closely related to *Oncosiphon*, while the other is closer to *Myxopappus* (Källersjö 1988, Magee et al. 2015). However, these authors refrained from making nomenclatural changes, arguing that there are clear morphological synapomorphies uniting *Foveolina*. We understand these two genera should be synonymized.

II. Asian Clade

Artemisiinae and Handeliinae

Our study strongly supports the monophyly of these subtribes based on both nuclear and plastid data, as reported in previous works (Obreprieler et al. 2007, 2022). It also confirms they are sister lineages, in spite of our limited sampling, particularly with regards to Handeliinae. Although the delimitation of genera is out of the scope of this paper, our results allow us to discuss some interesting implications within Artemisiinae. Consistent with previous works (Zhao et al. 2010; Liu et al. 2012; Hao et al. 2022), navigating our nuclear topologies (Figs. 1, 2) from the root towards the tips, we find a

clade with *Brachanthemum* and *Hippolytia* as sister to two clades, one composed of *Filifolium* and *Kaschgaria*, and the other one comprising all other genera of the subtribe (hereafter, core Artemisiinae). In our plastid tree (Fig. 3), *Filifolium* is instead nested within a clade composed mostly of *Artemisia* species, and *Kaschgaria* is nested within core Artemisiinae (both *Brachanthemum* and *Hippolytia* are missing from this plastid dataset). Pertaining to core Artemisiinae, genera *Chrysanthemum* and *Artemisia* are not intermingled in the nuclear trees; they are, however, associated with other genera. *Chrysanthemum* is associated with *Opisthopappus*, *Ajania*, and *Stilpnolepis* in our MSC tree (Fig. 1, clade A3) or divided in two separate clades in our ML tree (Fig. 2, clades B2 and B3), while *Artemisia* is associated with *Crossostephium* in either nuclear tree (Fig. 1, clade A4; Fig. 2, clade B4).

The relationship between *Chrysanthemum* and the aforementioned associated genera has been previously described (Zhao et al. 2010; Hong et al. 2015; Tyagi et al. 2020; Masuda et al. 2022). The two clades in which *Chrysanthemum* species are found (Fig. 1, clade A3; Fig. 2, clades B2 and B3) are consistent with two species complexes (*C. zawadzkii* and *C. indicum*, respectively) identified in recent phylogenetic studies (Liu et al. 2012; Li et al.2014; Shen et al. 2021; Lu et al. 2022). Morphological differences between *Ajania* and *Chrysanthemum* are limited to their capitula (Bremer and Humphries, 1993), whereas their delimitations in molecular studies have yet to be clarified. Indeed, some authors have argued that *Chrysanthemum* may be paraphyletic (Hao et al. 2022). The close relationship between *Opisthopappus* and *Chrysanthemum* has also been previously reported (Zhao et al. 2010; Liu et al. 2012; Shen et al. 2021; Hao et al. 2022; Lu et al. 2022).

Regarding the *Artemisia* group (Bremer and Humphries, 1993), systematic and phylogenetic studies over the last decades point to a complex history. On one hand,

several genera have been segregated: *Brachanthemum*, *Elachanthemum*, *Hippolytia*, *Kaschgaria*, and *Stilpnolepis* (Watson et al. 2002; Vallès et al. 2003, 2011). On the other, several authors have only inferred a monophyletic *Artemisia* when including other Artemisiinae genera: *Artemisiastrum*, *Crossostephium, Filifolium, Mausolea, Neopallasia, Sphaeromeria*, *Picrothamnus*, and *Turaniphytum* (Watson et al. 2002; Vallès et al. 2003, Sanz et al. 2008; Garcia et al. 2011, Pellicer et al. 2011; Hobbs & Baldwin, 2013). Concerning *Crossostephium,* all our analyses place this genus deeply nested within the *Artemisia* group*,* lending additional support to its already proposed synonymization (Hobbs & Baldwin, 2013; Jiao et al. 2023). *Neopallasia* could only be included in our off-target plastid data matrices (Fig. 3) and appears to be sister to the *Chrysanthemum indicum* group, which is deeply nested within the *Artemisia* group in our plastid topology. There are discrepancies concerning the integration of *Filifolium* in the *Artemisia* group. Sanz et al. (2008) supported its inclusion, whereas Zhao et al. (2010) and our own results (Figs. 1, 2) do not support this relationship and instead recommend removal of this genus from the *Artemisia* group. In the case of *Kaschgaria*, Jiao et al. (2023) argued that this genus is nested within the *Artemisia* group, whereas our own results (Figs. 1, 2) do not lend support to their claim. This difference could be due to the nature of the data used in their phylogenomic analysis vs. ours. These authors relied on nuclear single nucleotide polymorphisms (SNPs) obtained from low-coverage whole genome sequencing (lcWGS) reads, using the complete coding sequence (CDS) datasets of *Artemisia annua* and *Chrysanthemum seticuspe* as references separately. SNP-calling reference-based approaches are sensitive to the reference used as well as the bioinformatic pipeline implemented, including the chosen algorithm and parameter settings (Altmann et al, 2012; Shaffer et al, 2016). In highly diverse species groups, mapping reads accurately and identifying variants remains quite difficult, since

available reference assemblies might be considerably different from the study organisms. A valuable alternative in these cases is a reference-free assembly-based variant calling strategy (Pfeifer, 2017). Benjelloun et al. (2019) also found a clear effect of coverage and argued that at least 5× coverage seemed to be necessary for accurate assessment of genomic variants. We argue that the approach taken by Jiao et al. (2023), albeit powerful, still lacks the resolution needed to place *Kaschgaria* outside of *Artemisia*, resolution that HTS techniques such as Hyb-Seq provide (with the Angiosperms353 probe set).

Incongruences between nuclear topologies (MSC and ML), and between nuclear and plastid trees, in which most of the sampled Artemisiinae genera are paraphyletic (Fig. 3), are likely to be explained by rapid diversification and subsequent hybridization that may have resulted in plastid capture (Fig. 4). Hybridization, polyploidization, and gene flow (i.e., introgression and plastid capture) between Artemisiinae taxa has been reported to occur frequently in its evolutionary history (Yang et al. 2006; Liu et al. 2012; Li et al. 2014; Lu et al. 2022).

III. Circum-Mediterranean Clade

Anthemidinae and Matricariinae

As previously suggested (Oberprieler et al. 2007, 2022), our results confirm the paraphyly of both subtribes as currently circumscribed (Figs. 1, 2, 5). Taxa in Anthemidinae (sensu Oberprieler et al. 2007, 2022) share a tetrasporic embryo sac, an apomorphic character that has been used to support the monophyly of this tribe. However, our nuclear topologies present Anthemidinae and Matricariinae taxa interspersed, with Anthemidinae split into two or three groups (Figs. 1, 2). Only in our plastid tree (Fig. 3), which includes a low number of specimens that do not fully coincide with those in the nuclear dataset, is Anthemidinae monophyletic and sister to

Matricariinae. Our results thus suggest that the tetrasporic embryo sac is homoplasious and that these two subtribes should be merged together into a single one, whose name, according to the principles of priority in taxonomy, should be Anthemidinae (hereafter, Anthemidinae s.l.).

It should also be noted that the overall placement of this broader Anthemidinae s.l. is incongruent between our nuclear and plastid trees. With our current dataset, we cannot address the causes underlying this conflict. A possible ancient hybridization event (followed by plastid capture) in the origin of the group, probably with ancestors of the other members of the Circum-Mediterranean clade (specifically Glebionidinae, Leucantheminae, and Santolininae), could partly underpin such incongruence. However, this would not explain why Leucanthemopsidiinae is sister to the remaining four subtribes in the plastid tree (Fig. 3), and not in the nuclear trees. A more comprehensive taxonomic and molecular sampling is needed to tackle this challenge.

As within Artemisiinae (Asian Clade), our results allow comments on the circumscription of some Anthemidinae s.l. genera. In particular, *Tanacetum* and *Achillea* are clearly not monophyletic. *Tanacetum*, one of the largest genera in the subtribe, is considered to be one of the most problematic, mainly due to its generic delimitation and infrageneric classification (Sonboli et al. 2012). As currently circumscribed, its species form a paraphyletic assemblage that should be rearranged along several genera. In *Achillea*, paraphyly is due to the position of *A. maritima* (also known as *Otanthus maritimus* Hoffmanns. & Link.) in the nuclear MSC and plastid trees, and *A. distans* subsp. *tanacetifolia* in the nuclear MSC tree (Figs. 2, 3). Some authors had found cyto-nuclear incongruence in *Achillea*, suggesting hybridization and ILS as the most likely explanations (Guo et al. 2005). Thus, further work with a more extensive sampling for these genera is needed to revise their circumscription.

Glebionidinae, Leucantheminae, Leucanthemopsidinae, and Santolininae

The monophyly of these subtribes is well supported both in nuclear and plastid analyses. However, relationships among the four subtribes remain controversial, in light of their incongruent placement in our nuclear (MSC and ML) vs. plastid trees, as well as the weak support values for internal nodes in the MSC tree (Fig. 1). The placement of Leucanthemopsidinae is particularly problematic, i.e., its position in our plastid tree (Fig. 3) suggests plastid capture may have taken place. In this plastid tree Leucanthemopsidinae is sister to a large group that, in addition to Glebionidinae, Leucantheminae, and Santolinineae, includes the newly circumscribed Anthemidinae s.l. (including Matricariinae) (Figs. 1–3). Until a more thorough sampling of these four subtribes is available, caution should be exercised.

The three topologies here obtained do not match the tree in Oberprieler et al. (2022) based on concatenated plastid and nuclear data, in which Leucanthemopsidinae is sister to a large group that includes Glebionidinae, Leucantheminae, and Santolininae but not Anthemidinae s.l. (Fig. 5). Previous works did not find a close relationship between Leucantheminae and Leucanthemopsidinae (Oberprieler & Vogt, 2000; Oberprieler, 2005; Oberprieler et al. 2007). However, in our ML nuclear tree they are sister subtribes. Glebionidinae and Santolininae were recovered as sister with full and high support in the ML nuclear and plastid trees, respectively, consistent with other studies (Oberprieler & Vogt, 2000; Oberprieler, 2005; Oberprieler et al. 2007, 2022).

The observed incongruence suggests a rapid radiation taking place between the Lower Pliocene (~5 Mya) and the Early Pleistocene (1.7 Mya) (Fig. 4), which could have favoured ILS or introgression events, as mentioned above. Previous works have demonstrated that rapidly diversifying groups may have weak reproductive barriers that facilitate hybridization (Fritz et al. 2006; Martin et al. 2007) and introgression, which

can lead to cyto-nuclear topological incongruence (Rieseberg & Soltis, 1991; Moore, 1995; Hardig et al. 2000; Xu et al. 2012; Lee‐Yaw et al. 2019). Plastid and mitochondrial genomes have the potential to be fixed rapidly because of their low effective population sizes (Moore, 1995), which makes organellar genomes less prone to ILS (Vargas et al. 2017). Several groups that underwent rapid radiations in their evolutionary history have shown to be prone to hybridization (Mallet et al. 2007; Bremer & Salzburger, 2015; Vargas et al. 2017; Nge et al. 2021), and even in some cases giving rise to a hybrid swarm (Meier et al. 2017).

Overall, our study suggests that the explosive radiations inferred, for the circum-Mediterranean subtribes and Asian Artemisiinae, are concordant with those inferred in many other plant groups for the late Miocene-Pleistocene (Valente et al. 2010; Fior et al. 2013; Azani et al. 2019), as well as with the increased diversification rate inferred for the whole family for this period (Palazzesi et al. 2022b). Important climatic changes occurred during that time span, such as the initial opening of the Bering Strait at the beginning of the Pliocene (~ 5.3 Mya) (Marincovich JR & Gladenkov, 1999), the Messinian salinity crisis (~ 5.9–5.5 Mya) (Hsü et al. 1977; Rouchy et al. 2006; Ryan 2023), and even the advent of the Mediterranean climate ~ 3.2 Mya (Suc, 1984). These resulted in the intensification of aridity and rainfall seasonality, forest cover decline, and the spread of arid and open grassland habitats in Northern America and Eurasia (Janis, 1993; Fortelius et al. 2006). In addition, several glacial-interglacial cycles during Pliocene-Pleistocene triggered intense species range dynamism, which may have induced geographic isolation, environmental variation, and changes in resource availability, providing new niches and fostering diversification (Janssens et al. 2009; Testolin et al. 2021).

Overall, our results show that the Hyb-Seq approach using the Angiosperms353 probe set is efficient in evaluating relationships in Anthemideae and has shed light at different taxonomic levels. MSC and ML nuclear topologies are largely congruent in the reconstruction of the Anthemideae backbone with high support. In addition, many of the uncertainties concerning the relationships of several subtribes, highlighted in previous studies based on a few genes, are resolved with our target capture sequencing approach. However, incongruent placements are observed for the most recently evolved groups. Topological conflict is especially evident when comparing nuclear species trees with the plastid tree. As stated above, these outcomes may be linked to introgression with plastid capture (Stull et al. 2020; Zhang et al. 2020; Maurin et al. 2021) and ancient hybridization (Stull et al. 2023) following explosive radiation events (Seehausen, 2004, 2013; Genner & Turner, 2011). Specifically, we point out two possible episodes of plastid capture, one between Phymasperminae and the circum-Mediterranean subtribes (i.e, Anthemidinae s.l., Glebionidinae, Leucantheminae, Leucanthemopsidinae, and Santolininae), and another one at the origin of the Circum-Mediterranean Clade. Our divergence time and diversification rate estimation analyses are consistent with rapid radiation events driven by climatic oscillations during the Plio-Pleistocene, which would explain the diversification of Artemisiinae s.l. and the circum-Mediterranean subtribes accompanied by hybridization events. A broader sampling, covering a higher representation of species diversity across the Anthemideae, is needed to further elaborate a fine-scale reconstruction of the complex evolutionary history of this tribe. A comparison between the results obtained with the Angiosperms353 probe set and the custom Compositae-ParaLoss-1272 probe set (Moore‐Pollard et al. 2024) would be of great interest.

Taxonomic implications

Based on the results from our phylogenomic analyses, at least two strongly-supported changes are needed pertaining the subtribal classification of Anthemideae. Accordingly, we here propose merging subtribes Athanasiinae and Phymasperminae into a single one, i.e., Athanasiinae. Likewise, we propose merging Anthemidinae and Matricariinae into a single subtribe, i.e., Anthemidinae. A description, genera included, and geographic distribution of the newly circumscribed subtribes is given below, partly based on Oberprieler et al. (2022).

1. Athanasiinae Pfeiff, Nomencl. Bot. 1: 323. 1872. Type — *Athanasia* L. [*Athanasia crithmifolia* (L.) L.].

= Phymasperminae Oberpr. & Himmelr. in Willdenowia 37: 99. 2007. – Type: *Phymaspermum* Less. [*Phymaspermum junceum* Less.].

Distribution — Botswana, Egypt (Sinai Peninsula), Jordan, Lesotho, Namibia, Palestina, South Africa, Swaziland, Zimbabwe.

Members — *Adenoglossa* B. Nord. (1), *Athanasia* L. (41), *Eriocephalus* L. (32), *Eumorphia* DC. (6), *Gymnopentzia* Benth. (1), *Hymenolepis* Cass. (7), *Lasiospermum* Lag. (4), *Leucoptera* B. Nord. (3), *Phymaspermum* Less. (17).

Description — Shrubs or shrublets, rarely perennial to annual herbs (*Adenoglossa*, *Lasiospermum*). Indumentum absent or composed of basifixed or stellate (*Athanasia*, *Hymenolepis*) hairs. Leaves alternate or opposite, entire or lobed to pinnatifid or 1- or 2-pinnatisect. Capitula solitary or in lax to dense corymbs, radiate, disciform or discoid. Involucre hemispheric, spheric to urceolate, rarely cylindric to obconic. Phyllaries in 2–5 rows, without or with scarious margins, sometimes ciliate, without or with central resin canals or sacs. Receptacle flat, hemispheric to conic, paleate or epaleate; paleae

flat or canaliculate, rarely villous (*Eriocephalus*). Ray florets female; limb yellow, white or reddish. Disc florets hermaphrodite (male in *Eriocephalus*); corolla 5-lobed; tube sometimes with long stalked hairs (*Athanasia*); anthers with polarised endothecial tissue, rarely non-polarized (*Eriocephalus*), and a slender filament collar. Achenes cylindric to obovate, even ellipsoid, either terete and with 5–12(–18) ribs or dorsoventrally flattened and laterally winged (*Adenoglossa*, *Leucoptera*); apex truncated or with an entire to dentate, thickened rim or with a corona or scales; pericarp glabrous, papillose (*Eumorphia, Gymnopentzia, Phymaspermum*) or densely hairy (*Eriocephalus, Lasiospermum*), with or without myxogenic cells and/or resin sacs (in *Phymaspermum*) with ovoid myxogenic trichomes and resin sacs in some of ribs. Embryo sac development only known for *Lasiospermum* (monosporic).

2. Anthemidinae Dumort, Fl. Belg.: 69. 1827. Type — *Anthemis* L. [*Anthemis arvensis* L.].

= Pyrethrinae Horan., Char. Ess. Fam.: 90. 1847. – Type: *Pyrethrum* Zinn. [*Pyrethrum corymbosum* (L.) Willd.].

= Matricariinae Willk. in Willk. & Lange, Prodr. Fl. Hispan. 2: 92. 1870. – Type: *Matricaria* L. [*Matricaria recutita* L., typ. cons.].

= Achilleinae K. Bremer & Humphries in Bull. Nat. Hist. Mus. London, Bot. 23: 126. 1993. – Type: *Achillea* L. [*Achillea millefolium* L.].

= Gonosperminae K. Bremer & Humphries in Bull. Nat. Hist. Mus. London, Bot. 23: 106. 1993. – Type: *Gonospermum* Less.[*Gonospermum fruticosum* Less.].

= Tanacetinae K. Bremer & Humphries in Bull. Nat. Hist. Mus. London, Bot. 23: 99. 1993. – Type: *Tanacetum* L. [*Tanacetum vulgare* L.].

Distribution — Europe, Asia, N and E Africa (including Canary Islands), North America.

Members — *Achillea* L. (115), [incl. *Leucocyclus* Boiss. (1), *Otanthus* Hoffmanns. & Link (1), see Guo et al. (2004), Ehrendorfer & Guo (2005, 2006)], *Anacyclus* L.(9), *Anthemis* L. (175), *Archanthemis* Lo Presti & Oberpr. (4), *Cota* J. Gay (43), *Heliocauta* Humphries (1), *Matricaria* L. (6), *Nananthea* DC. (1), *Tanacetum* L. (154) [incl. *Gonospermum* Less. (4), *Lugoa* DC. (1), see Sonboli & al. 2012)], *Tripleurospermum* Sch. Bip. (40), *Xylanthemum* Tzvelev (8).

Description — Subshrubs, short- to long-lived perennial herbs, biennials or annuals; sometimes shrublets with basally woody, virgate and sometimes leafless stems (*Xylanthemum*). Indumentum absent or composed of medifixed and/or basifixed hairs. *Leaves* alternate or in a basal rosette (*Heliocauta*), rarely entire, dentate to lobed or 1–4-pinnatisect, sometimes vermiform. *Capitula* solitary or in lax to dense corymbs, radiate, disciform or discoid. Involucre hemispheric to cylindrical or obconic, sometimes umbonate. *Phyllaries* in 1–5 rows, with scarious margins. *Receptacle* flat, hemispheric or conic, paleate or epaleate; paleae flat, convex or canaliculate, sometimes subulate (*Anthemis*). *Ray florets* female or neuter; limb white, yellow or pink; tube ± flattened, sometimes hairy. *Disc florets* hermaphrodite; corolla (4 or)5-lobed, sometimes hairy (*Xylanthemum*); anthers with non-polarized endothecial tissue and a balusterform filament collar. *Achenes* obovoid to obconic, terete with 3–10(–15) ribs or dorsoventrally flattened with 2 lateral ribs or wings, and 3–10 ribs on each surface (*Cota*), sometimes triquetrous and with 3(–5) ribs (*Tripleurospermum*); apex with a corona or auricle, sometimes ecoronate and/or marginally rounded, sometimes (*Xylanthemum*) with 3–6 adaxial, elliptic scales shorter than achene body; pericarp with or without myxogenic cells, usually without resin sac or ducts, *Achillea*, *Heliocauta*

sometimes with longitudinal resin ducts, and in *Tripleurospermum* with (1 or)2(–5) abaxial-apical resin sacs. *Embryo sac development* monosporic or tetrasporic.

Author Contributions

DCR, GNF and OH: conceived the project and coordinated the study. OH, JV, RJB, LPa, JP, IPL and GNF: sampled the specimens. EF, SR: carried out laboratory work to generate phylogenomic data, with contributions from OM. DCR: performed the analyses under the supervision of LPo. WJB, FF and IJL: supervised the PAFTOL research programme. DCR and GNF: wrote the first draft. All authors reviewed and edited the draft and approved the published version of the manuscript.

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Tables

Table 1. Alignment statistics across retrieved regions. P_{PIC} stands for proportion of Parsimony-informative characters.

Genomic compartment	Region	Trimming parameters (trimAl)	Alignment length		P_{PIC}		% Missing data	
			Mean (bp)	SD	Mean (bp)	SD	Mean (bp)	SD
Nuclear	On-target	gt 0.1 cons 35	945	493	0.357	0.064	24	12
Nuclear	On-target	gt 0.3 cons 30	907	482	0.354	0.064	22	11
Plastid	Off-target	gt 0.1 cons 35	750	754	0.069	0.052	7	8
Plastid	Off-target	gt 0.3 cons 30	748	755	0.07	0.053	6	7

Table 2. Estimates of divergence times for Anthemideae tribe clades obtained from MSC and ML nuclear data matrices. C.16 and C.17 are exclusive to the MSC tree, while C.18 and C.19 are exclusive to the ML tree.

Node	MSC		ML	
	mean age (Mya)	95% credible intervals	mean age (Mya)	95% credible intervals
C.A	13.8	(13.9, 13.2)	15.0	(15.0, 14.0)
C.2	9.9	(11.2, 8.7)	11.0	(12.4, 9.8)
C.3	7.2	(9.8, 5.2)	8.0	(10.5, 6.1)
C.4	5.2	(7.5, 3.6)	6.0	(8.1, 4.4)
C.5	4.0	(5.9, 2.7)	4.5	(6.2, 3.2)
C.6	3.2	(5.2, 2.0)	3.7	(5.6, 2.5)
C.7	3.0	(5.2, 1.6)	3.5	(5.6, 2.1)
C.8	2.4	(4.6, 1.3)	2.9	(5.0, 1.7)
C.9	6.4	(9.1, 4.5)	7.2	(9.7, 5.3)
C.10	4.4	(7.5, 2.6)	5.0	(7.9, 3.2)
C.11	3.2	(5.2, 2.0)	3.7	(5.6, 2.5)
C.12	2.8	(5.2, 1.5)	3.2	(5.5, 1.9)
C.13	2.1	(4.0, 1.1)	2.4	(4.3, 1.4)
C.14	2.3	(4.6, 1.2)	2.8	(4.9, 1.6)
C.15	2.4	(4.8, 1.2)	2.8	(4.9, 1.6)
C.16	2.2	(4.4, 1.1)	NA	NA
C.17	2.1	(4.4, 1.0)	NA	NA
C.18	NA	NA	2.6	(4.8, 1.4)
C.19	NA	NA	2.6	(4.8, 1.4)
Leucantheminae	1.6	(3.8, 0.6)	1.9	(4.1, 0.9)
Santolininae	1.6	(4.0, 0.7)	2.1	(4.4, 1.0)
Leucanthemopsidinae	2.0	(4.4, 0.9)	2.4	(4.8, 1.2)
Glebionidinae	1.4	(3.2, 0.6)	1.6	(3.5, 0.8)

Figure legends

Fig. 1. (A) Cladogram showing relationships within Anthemideae and with the outgroup taxa, based on 244 low-copy on-target nuclear genes, inferred under the multispecies coalescent (MSC) with ASTRAL III. Annotations as pie charts display quartet score values (blue = species tree topology; orange = first alternative topology; beige = second alternative topology) and local posterior probability values (low support in red) are presented for each branch. The Anthemideae tribe clade is denoted as C.A, and successive clades are referred to as C.# (consecutive numbering). Coloured boxes encompass members studied for each Anthemideae subtribe. (B) Collapsed MSC nuclear phylogeny displayed with iTOL v5 (Letunic & Bork, 2021) showing subtribe relationships, branch lengths in coalescent units (see scale bar), and local posterior probability values. Collapsed clades are displayed as triangles with side lengths proportional to the distances of the closest and the furthest tips to the stem node. Terminals from genera that contain widely distributed species are marked with an asterisk.

Fig. 2. (A) Cladogram showing relationships within Anthemideae and with the outgroup taxa, based on 244 low-copy on-target nuclear genes, inferred using maximum likelihood (ML) in IQ-TREE. Bootstrap support values (low support in red) are presented for each branch. The Anthemideae tribe clade is denoted as C.A, and successive clades are referred to as C.# (consecutive numbering), as in Fig. 1. When a clade is absent from Fig.1 it is referred to as B.# (consecutive numbering). Coloured boxes encompass members studied for each Anthemideae subtribe. (B) Collapsed ML nuclear phylogeny displayed with iTOL v5 showing subtribe relationships, branch lengths in substitutions per site (see scale bar), and bootstrap support values. Collapsed

clades are displayed as triangles with side lengths proportional to the distances of the closest and the furthest tips to the stem node. Terminals from genera that contain widely distributed species are marked with an asterisk.

Fig. 3. (A) Cladogram showing relationships within Anthemideae and with the outgroup taxa, based on 66 off-target plastid genes, inferred using maximum likelihood (ML) in IQ-TREE. Bootstrap support values (low support in red) are presented for each branch. The Anthemideae clade is represented with C.A and successive clades are referred to as D.# (consecutive numbering). Coloured boxes include all members studied for each subtribe of Anthemideae. (B) Collapsed ML plastid phylogeny displayed with iTOL v5 showing subtribe relationships, branch lengths in substitutions per site (see scale bar), and bootstrap support values. Collapsed clades are displayed as triangles with side lengths proportional to the distances of the closest and the furthest tips to the stem node. Terminals from genera that contain widely distributed species are marked with an asterisk.

Fig. 4. RelTime time-trees, lineage-through time plots, and diversification rate estimation inferred from MSC (A) and ML (B) nuclear trees. Chronograms show mean ages for lineage divergences as estimated in MEGA using the rapid relaxed-clock method RelTime. Local posterior probability and bootstrap support values are presented for each branch, respectively. Mean node ages and 95% credible intervals are only indicated for the outgroup. Estimates of divergence times and credible intervals for Anthemideae are presented in Table 2. Left: (1) Variation in net-diversification, (2) relative extinction rates over time as estimated in CoMET using Bayesian episodic birth-death models, and (3) lineage-through-time trajectories inferred.

Fig. 5. Topological comparison of the Anthemideae backbone inferred in Oberprieler et al. (2022) vs. this study: (A) concatenated plastid and nuclear markers following a

bayesian approach (Oberprieler et al. 2022); (B) 244 on-target nuclear loci, MSC approach (this study); (C) 244 on-target nuclear loci, ML approach (this study); and (D) 66 off-target plastid loci, ML approach (this study).

Supporting Information

Fig. S1. Pairwise Euclidean distances between trees computed based on the two different trimAl parameter combinations (-gt & -cons), for each collapse threshold (-bs 5 to 75, every 5) for the (A) MSC on-target nuclear and the (C) ML off-target plastid (for each partitioning scheme) approaches. Multidimensional scaling representation of the tree space based on tip-tip path difference metric (Steel & Penny, 1993) and highlighting potential groups of similar trees for the (B) MSC on-target nuclear and the (D) ML off-target plastid approaches.

Fig. S2. Tanglegram comparing MSC (left) and ML (right) nuclear trees built with dendextend package (Galili, 2015) in R. Dashed lines represent "unique nodes" (nodes which contain a combination of labels/items not present in the other tree). Connecting lines and clades are coloured to highlight sub-trees which are present in either dendrogram.

Fig. S3. Speciation and extinction rates, rate shifts, potential mass extinction events (MEEs) time estimates, and Bayes Factor (BFs) comparisons for the timing of MEE events as estimated in CoMET using Bayesian episodic birth-death models in the (A) MSC and (B) ML nuclear chronograms.

Table S1. Voucher specimens and NCBI accessions sampled. Taxa highlighted in grey and marked with Y were finally included in our nuclear and/or plastid MSC/ML analyses.

Table S2. PAFTOL research programme sample metadata.

Table S3. Distribution of the genera recognized in the last classification by Oberprieler et al. (2022). The distribution ranges were sourced from the Kew Gardens' portal Plants of the World Online (POWO: https://powo.science.kew.org/., visited 26/03/2024).

Appendix 1. Recovery efficiency of HybPiper for the on-target nuclear genes (hybpiper_stats_nuc) and the off-target plastid genes (hybpiper_stats_pl) studied, max_overlap results (anthe_nuc_max_overlap and anthe_pl_max_overlap, respectively) and max_overlap summary by genes (summary_nuc_gene and summary_pl_gene) and taxa (summary_nuc_taxa and summary_pl_taxa) studied. In these summary sheets, highlighted samples and genes with overlap (coverage) score < ⅔ median were discarded from downstream analyses. Paralog genes are listed for both the on-target nuclear genes and the off-target plastid genes in nc_paralogs and pl_paralogs respectively. Genes with ≥3 paralogs were also discarded from downstream analyses.

Appendix 2. Statistics from AMAS for nuclear (summary_nuc_alignment_gt01con35 and summary_nuc_alignment_gt03con30) and plastid alignments (summary_pl_alignment_gt01cons35 and summary_pl_alignment_gt03cons30).