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ORIGINAL ARTICLE

Mitoplastomic discordance in Brassicaceae phylogenomics confirms the complex evolutionary history of the family

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• **Background and Aims** The phylogeny of the Brassicaceae family has traditionally been inferred from plastid and nuclear DNA. However, early studies were limited by the availability of genetic markers and incomplete taxon sampling. Recent phylogenomic studies, leveraging more densely sampled nuclear and plastid datasets, have resolved many taxonomic uncertainties. These studies either targeted complete plastomes or provided extensive representation of the nuclear genome. Nevertheless, substantial cytonuclear discordance, poorly resolved backbone relationships and challenges in placing 'rogue taxa' have left unresolved questions about deeper relationships, notably of the five supertribes of the family. In this context, we performed the first phylogenomic analysis of the slower-evolving, maternally inherited mitogenome, which presents a promising avenue for resolving deeper phylogenetic nodes.
• **Methods** Using published mitogenomes from nine Brassicaceae species, we generated a mitogenomic reference file to recover mitogenomic sequencing read data from Hendriks *et al*. (2023. Global Brassicaceae phylogeny based on filtering of 1000-gene dataset. *Current Biology: CB* **33**: 4052–4068.e6). Subsequently, we reconstructed a codon-aware mitogenomic supermatrix, alongside updated nuclear (281 genes) and plastome (76 genes) supermatrices and inferred family-wide maximum likelihood phylogenies from each of these three genomes. Congruence among the resulting phylogenies was assessed thoroughly.
• **Key Results** We present the first densely sampled family-wide mitogenomic Brassicaceae phylogeny, including 167 species, 145 genera (40% of the family) and 40 tribes (69% of the family), and the first family-wide phylogenomic comparison based on all three plant genomes. Although cytonuclear discordance was evident, we also uncovered strong phylogenomic discordance between the two organellar genomes (mitogenome and plastome), coined here as 'mitoplastomic discordance'. Our findings offer new insights into the placement of several rogue and previously unplaced taxa.
• **Conclusions** Phylogenomic discordance in Brassicaceae was more pervasive than expected. Although bifurcating phylogenies offer clear evolutionary hypotheses, they do not fully capture evolutionary complexities. Our results have implications for understanding Brassicaceae evolution, taxonomy and systematics, shedding light on processes such as hybridization and genome duplication, commonly resulting in evolutionary reticulation.

Key words: Brassicaceae, Cruciferae, mustard family, phylogenomics, mitogenomics, cytonuclear discordance, mitoplastomic discordance, rogue taxa.

INTRODUCTION

The Brassicaceae Tree of Life (BrassiToL) has traditionally been inferred from plastid (Beilstein *et al.*, 2010; Liu *et al.*, 2011, 2021; Guo *et al.*, 2017) or nuclear (Bailey *et al.*, 2006; Beilstein *et al.*, 2008; German *et al.*, 2009; Warwick *et al.*, 2010; Liu *et al.*, 2011, 2021; Huang *et al.*, 2016; Nikolov *et al.*, 2019) DNA sequences. Until recently, such phylogenies were hampered by limited marker sampling (generally fewer than five markers) and/or limited taxon sampling. The first issue was tackled by Nikolov *et al.* (2019), who designed a 764 single-copy nuclear gene set to allow accurate inference of the nuclear Brassicaceae

phylogeny. The second issue was tackled by Hendriks *et al.* (2023),; https://treeoflife.naturalis.nl/brassicaceae) who generated the most densely sampled nuclear and plastid Brassicaceae phylogenies to date, including representatives of 92% of the genera and all 58 tribes, based on the analysis of 1081 nuclear and 76 plastid genes, respectively. Together, their work resolved long-standing issues regarding the position of many tribes and genera and laid the foundation for describing several new tribes (Arabidopsideae, Asperuginoideae, Hemilophieae and Schrenkielleae) and supertribes (Arabodae, Camelinodae, Heliophilodae and Hesperodae) and the formal description of subfamily Aethionemoideae as opposed to Brassicoideae (German *et al.*, 2023; Givnish, 2023). Despite these substantial advancements, the relationships among many deeper nodes (the 'family backbone') and the placement of various so-called 'rogue taxa' remain unresolved. These rogue taxa (defined as those that shift positions in the phylogeny depending on the subset of data or methodology used) include tribes such as Anastaticeae, Biscutelleae, Cochlearieae, Iberideae and Megacarpaeeae (Walden *et al.*, 2020; German *et al.*, 2023; Hendriks *et al.*, 2023).

Many factors have been suggested to contribute to the challenges in clarifying the (deeper) phylogenetic relationships within the Brassicaceae family. First, artefacts might introduce errors, such as substitution saturation or incorrect phylogenetic inference owing to compositional heterogeneity (differences in the nucleotide composition attributable to mutational bias or evolutionary rate variation, for example; Liu *et al.*, 2014). Second, biological factors such as chloroplast capture (Kawabe *et al.*, 2018), polyploidy resulting from whole-genome duplication or hybridization (Franzke *et al.*, 2011; Huang *et al.*, 2020), incomplete lineage sorting and introgression can lead to phylogenetic incongruences. Polyploidy is well known to be present widely in the Brassicaceae (Mandáková *et al.*, 2017*a*), triggering rapid radiation (Schranz *et al.*, 2012), adding a further challenge in phylogenetic reconstructions (Hohmann *et al.*, 2015; Huang *et al.*, 2020).

A promising addition for resolving the deep-time Brassicaceae relationships might come from the mitochondrial genome, which has not been studied thoroughly in Brassicaceae at the family level before. First, the mitogenomic mutation rate is much lower than that of either the plastome or nuclear genome (Wolfe *et al.*, 1987; Palmer and Herbon, 1988), with rates in seed plants at a ratio of 1:3:10, respectively (Drouin *et al.*, 2008), potentially mitigating the risk of substitution saturation. Second, mitochondria, like chloroplasts (Palmer, 1985; Daniell *et al.*, 2016), are predominantly inherited maternally, with only rare cases of biparental (Corriveau and Coleman, 1988; Duminil, 2014) or paternal inheritance (Duminil, 2014; Shen *et al.*, 2015; Chybicki *et al.*, 2016), potentially alleviating the challenge of a distorted allopolyploid signal (albeit at the cost of a partial loss of parental information). Third, although mitochondrial genomes in plants vary widely in size (often 200–400 kb, with the largest flowering plant mitogenome recorded being 11 300 kb in *Silene conica*, Caryophyllaceae; Sloan *et al.*, 2012), and the structural complexity of the mitogenome evolves rapidly (Palmer and Herbon, 1988; Sloan *et al.*, 2012), the number of mitogenomic genes remained relatively constant (Palmer *et al.*, 2000).

Using the slower-evolving mitogenome to resolve deeper evolutionary relationships in land plants is not a novel concept, yet its inclusion in phylogenetic analyses remains relatively uncommon. Beyond the Brassicaceae, several phylogenetic studies have specifically targeted the mitogenome, e.g. focusing on land plants (Liu *et al.*, 2014; Sousa *et al.*, 2020), angiosperms (Qiu *et al.*, 2000; Xue *et al.*, 2022), mycoheterotrophic lineages (Lin *et al.*, 2022), order Pandanales (Soto Gomez *et al.*, 2020), the Rubiaceae family (Rydin *et al.*, 2017), genus *Pelargonium* (Bakker *et al.*, 2000) and genus *Potentilla* (Xue *et al.*, 2024). Many of these studies have identified incongruences between phylogenies derived from mitogenomes and plastomes. Nonetheless, mitochondrial genomes have frequently demonstrated their value in resolving deep evolutionary relationships (Van de Paer *et al.*, 2016; Lin *et al.*, 2022; Xue *et al.*, 2022).

Mitogenomic phylogenetic studies within the Brassicaceae have focused primarily on single genes. Franzke *et al.* (2009) analysed the NADH subunit 4 gene in 49 Brassicaceae species spanning much of the diversity of the family, but could not resolve most deep polytomies. Likewise, Couvreur *et al.* (2010) examined the *nad4* intron 1 and integrated these data with nuclear and plastome gene sequences in a supermatrix analysis of 226 Brassicaceae species. Despite the extended dataset, deeper branches remained poorly supported, probably owing to cytonuclear discordance (i.e. evolutionary differences between the nuclear genome and cytoplasmic genomes, such as the plastome and mitogenome). Likewise, Liu *et al.* (2011) investigated the *nad7* intron 2 in 71 Chinese Brassicaceae taxa, but their results also showed relatively low support for backbone nodes and poor resolution of more recent intertribal clades. Interestingly, both Franzke *et al.* (2009) and Liu *et al.* (2011) found discrepancies between mitochondrial and plastid-derived phylogenies in Brassicaceae. Qiao *et al.* (2020) presented a comparison between plastome and mitogenomic phylogenies (with a focus towards genus *Brassica*), but the first comparative phylogenomic analysis comparing all three plant genomes (nuclear, plastid and mitochondrial) in Brassicaceae was applied to unravel the reticulate evolutionary history of tribe Cochleariae at the species level (Wolf *et al.*, 2021). However, these studies do not entail the complete family.

To elucidate the deeper relationships within the entire Brassicaceae, we reconstructed the most comprehensive and robust mitogenomic family phylogeny to date. We compared it with the nuclear and plastome phylogenies. To this end, we reanalysed raw sequencing data from the study by Hendriks *et al.* (2023), used an improved codon-aware gene alignment strategy and applied a uniform phylogenomic approach to each of the three genomic datasets for fair comparison.

MATERIALS AND METHODS

Species sampling and the mitogenome

We filtered all mitogenomic DNA reads from the raw sequencing data (target capture sequencing with genome spiking) of Hendriks *et al.* (2023), available from NCBI SRA BioProject PRJNA806513 (340 samples; Supplementary Data Table S1). Like Hendriks *et al.* (2023), we added a subset of data from Nikolov *et al.* (2019; 37 samples; Supplementary Data Table S2; BioProject PRJNA518905). To filter out the mitogenomic reads, we created a new Brassicaceae-specific mitogenomic target genes file to map raw reads. This included sequence

TABLE 1. *Brassicaceae phylogenomic sampling overview. The focus in descriptions and comparisons is on the datasets in bold.*

Genome, sampling strategy	Threshold minimum number of genes per sample	Number of genes studied	Species	Genera	Tribes
Mitochondrial, relaxed	5	32	248	214	49
Mitochondrial, selective	**15**	**32**	**167**	**145**	**40**
Mitochondrial, stringent	25	32	115	101	35
Plastome	**5**	**76**	**338**	**291**	**57**
Nuclear genome	**–**	**281**	**360**	**311**	**57**

data from nine previously published and well-annotated mitogenomes (Supplementary Data Table S3) covering most of the taxonomic diversity of the family (supertribes Arabodae, Brassicodae and Camelinodae). The exons of 32 protein-coding genes, as defined for *Arabidopsis thaliana* (Unseld *et al.*, 1997) were selected from each mitogenome, and exons were concatenated by gene; 24 ribosomal RNA and transfer RNA genes were excluded, because they were either very short (<100 bp) or not consistently annotated across the reference mitogenomes (Supplementary Data Table S4). In cases where two exon copies were annotated in a mitogenome, we ignored the most dissimilar copy, most probably representing a paralogue (Supplementary Data Table S4). Finally, trailing stop codons were removed (Supplementary Data Table S5). Likewise, we used an unpublished reference file for 76 plastid genes for the plastome, based on 15 previously published chloroplast genomes from across the Brassicaceae (Supplementary Data Table S6; Hay *et al.*, 2023).

Sequence mapping and gene alignments. We used the bioinformatic pipeline of Hendriks *et al.* (2023) in a parallel computing environment using GNU Parallel (Tange, 2011), with the following improvements. For mapping raw sequencing data, we turned to HybPiper v.2.1.2 (Johnson *et al.*, 2016) instead of the original version, v.1.3.1. More importantly, we applied the codon-aware alignment algorithm of OMM-MACSE (Ranwez *et al.*, 2018), which greatly outperforms (in terms of alignment quality) alignment tools ignoring codon-position information. To be methodologically consistent when comparing species phylogenies from all three genomes (i.e. mitogenome, nuclear genome and plastome) and because our methods differed slightly from those of Hendriks *et al.* (2023), we reanalysed the same raw sequencing data using reference genomes for the nuclear genome (Supplementary Data Table S7) and plastome (Supplementary Data Table S8). For the nuclear genome, we used the gene reference file from the 'mixed baits' approach of Hendriks *et al.* (2021), designed for combined target capture sequencing of 764 Brassicaceae-specific genes (Nikolov *et al.*, 2019) and 353 Angiosperm-wide genes (Johnson *et al.*, 2019); a method successfully applied in several recent phylogenomic studies in Brassicaceae (Farhat *et al.*, 2023; Hay *et al.*, 2023; Hendriks *et al.*, 2023; Walden *et al.*, 2024).

Genetic variation. We focused on the mitogenome as a potential means to resolve the early evolutionary history of the Brassicaceae, because genetic variation is commonly much lower in the mitogenome than in the nuclear genome and plastome. To test explicitly for this in the Brassicaceae, we calculated genetic mean pairwise distance (MPD) for each gene from each of the three genomes studied using the function 'dist.dna' from R-package 'ape' (Paradis and Schliep, 2019).

Gene sampling. Not all 32 mitogenomic genes could be recovered for all samples, because the target capture sequencing approach of Hendriks *et al.* (2023) introduced a heavy bias towards selecting the 1081 nuclear genes. To allow assessment of the impact of incomplete gene sampling, we defined three gene sampling strategies to reconstruct three mitogenomic phylogenies (Table 1). In the 'relaxed' strategy, we aimed to keep as many samples (and therefore species, genera and tribes) as possible, setting a lower threshold of five genes per sample; any samples with fewer than five genes were excluded before phylogenetic reconstruction. The 'selective' (minimum of 15 genes per sample) and 'stringent' (minimum of 25 genes per sample) strategies were aimed at including more genes per sample, hence more genetic information, but at the cost of species sampling. Because plastid genes were on average more informative (more variation per gene) than the mitogenomic genes (see Results), we applied a single lower threshold of five genes per sample (Supplementary Data Table S9). Finally, we focused on the 297 nuclear genes identified by Hendriks *et al.* (2023) for the nuclear genome in their so-called 'strict' routine. This strategy represents a subset of the 1081 nuclear genes studied, refined by excluding all genes with a single nucleotide polymorphism fraction of >0.02 across all samples. Using our improved alignment strategy, 281 of the 297 genes passed our bioinformatic pipeline (Supplementary Data Table S10). All analyses had representatives from all supertribes, plus tribe Aethionemeae.

Phylogenetic reconstructions. The mitogenome and plastome are generally inherited as single non-recombining units; therefore, their phylogenies can best be inferred using a supermatrix approach. We reconstructed the species phylogenies using a maximum likelihood supermatrix approach in IQ-TREE v.2.2.0 (Minh *et al.*, 2020). Node support/informativeness was calculated using 1000 bootstrap (BS) replicates and site concordance factors (sCF; Lanfear and Hahn, 2024). Following Hendriks *et al.* (2023), all phylogenies were calibrated using treePL (Smith and O'Meara, 2012) with the *Dressiantha bicarpellata* fossil

(estimated at 93.6–89.3 Myr old; Gandolfo *et al.*, 1998) at the crown of order Brassicales. Node age ranges were calculated by running treePL for all 1000 BS trees, after which results were summarized in TreeAnnotator v.2.7.6 (Bouckaert *et al.*, 2014). To be consistent, we applied the same methods to all three genomes. Finally, phylogenies were visualized using R package 'ggtree' (Xu *et al.*, 2022), and tanglegrams of pairs of final species phylogenies were drawn using 'phytools' (Revell, 2012).

RESULTS

Genetic variation

Genetic variation was lowest in the mitogenome, followed by the plastome and the nuclear genome (Fig. 1), confirming our initial assumption. Mitogenomic gene MPD ranged from 0.5 % (*rps7* gene) to 3.8 % (*rps12* gene), with a mean of 1.4 % across all 32 genes studied ('relaxed' strategy; Supplementary Data Table S11). For the plastome, these values ranged from 0.4 % (*rps7* gene) to 5.9 % (*rps16* gene), with a mean of 2.7 % across all 76 genes studied (Supplementary Data Table S12). Finally, for the nuclear genome, values ranged from 3.9 % (B764-derived gene 2G38280) to 23.9 % (A353-derived gene 6559), with a mean of 8.8 % across all 1044 successfully aligned genes (8.9 % across the subset of 281 genes used in reconstructing the new nuclear phylogeny in this study; Supplementary Data Table S13).

Global mitogenomic Brassicaceae phylogeny

We generated three novel mitogenomic Brassicaceae phylogenies based on ever-stricter sampling, thereby improving data density, at the cost of a reduction in species (and, as a result, genera and tribes) included (Table 1). Topologies from all three mitogenomic strategies ('relaxed', 'selective' and 'stringent') are highly similar, with the same backbone topology and general topological position of the tribes, indicating that the overall pattern in the mitogenomic phylogeny is not very sensitive to data selection (Supplementary Data Figs S1–S3). Unless stated otherwise, details and numbers are given for results from the 'selective' strategy (Fig. 2; Supplementary Data Fig. S2), because it offers the most balanced option between species and gene sampling. This mitogenomic sampling strategy resulted in a representation of 167 species, 145 genera (~40 % of the family) and 40 tribes (69 % of the family).

In line with previous findings (e.g. Nikolov *et al.*, 2019; Walden *et al.*, 2020; Hendriks *et al.*, 2023), we recovered the Brassicaceae family as a well-supported monophyletic group sister to family Cleomaceae (node support BS/sCF: 98/63.8; reported in this order in the remaining Results), with subfamily Aethionemoideae strongly supported as sister to subfamily Brassicoideae (the 'core Brassicaceae'; 98/88.3). Surprisingly, we recovered a crown age of 77.0 Mya for the 'selective' strategy [95 % highest posterior density (HPD): 84.0–64.6 Mya; Supplementary Data Table S14], much older than any previous estimate (see Discussion). For the 'relaxed' and 'stringent' strategies, the crown age of the family was 75.5 Mya (HPD: 77.2–69.8 Mya) and 54.9 Mya (HPD: 60.0–46.7 Mya), respectively. In all three mitogenomic phylogenies, subfamily Brassicoideae was divided into two main clades, although this division was poorly supported (93/36.4), with extremely short branch lengths. The first of these main Brassicoideae clades was composed of supertribes Hesperodae (lineage III) and Camelinodae (lineage I) as poorly supported sisters (84/24.7), each non-monophyletic. The second Brassicoideae clade was a mix of subclades from supertribes Arabodae (lineage IV), Heliophilodae (lineage V) and Brassicodae (lineage II), with only the last a monophyletic group (except for two outliers; see 'Rogue taxa and phylogenomic outliers' below). Focusing on more recent clades, we found nearly all tribes (for exceptions, see 'Rogue taxa and phylogenomic outliers' below) recovered as strongly supported monophyletic groups (BS > 95 and sCF 50–80), supporting the taxonomic usage of the tribe as a valid taxonomic entity.

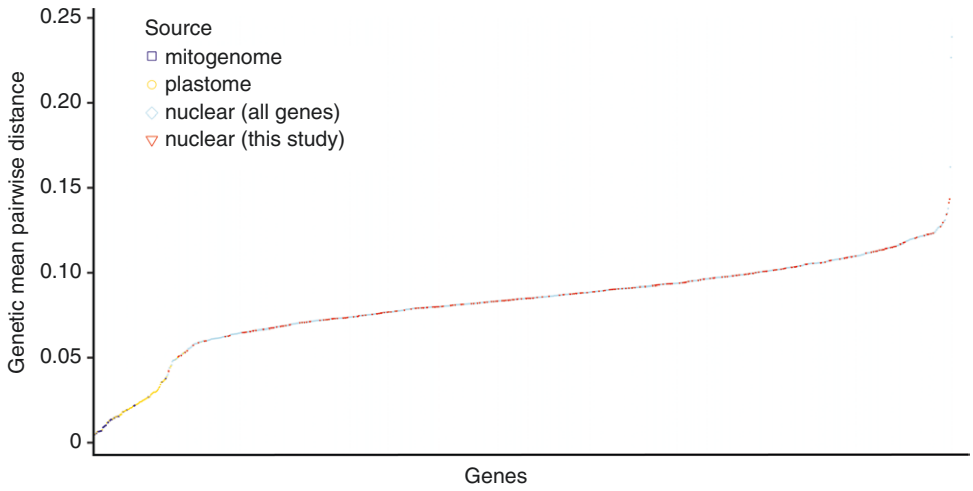

FIG. 1. Genetic mean pairwise distance (MPD) across all studied genes. The colours represent the source genome: purple for mitochondrial (mitogenome), yellow for chloroplast (plastome), and blue (all genes) and red (genes used in this study) for nuclear genome. Genetic variation is lowest in mitochondrial genes, highest in nuclear genes, and intermediate in plastid genes.

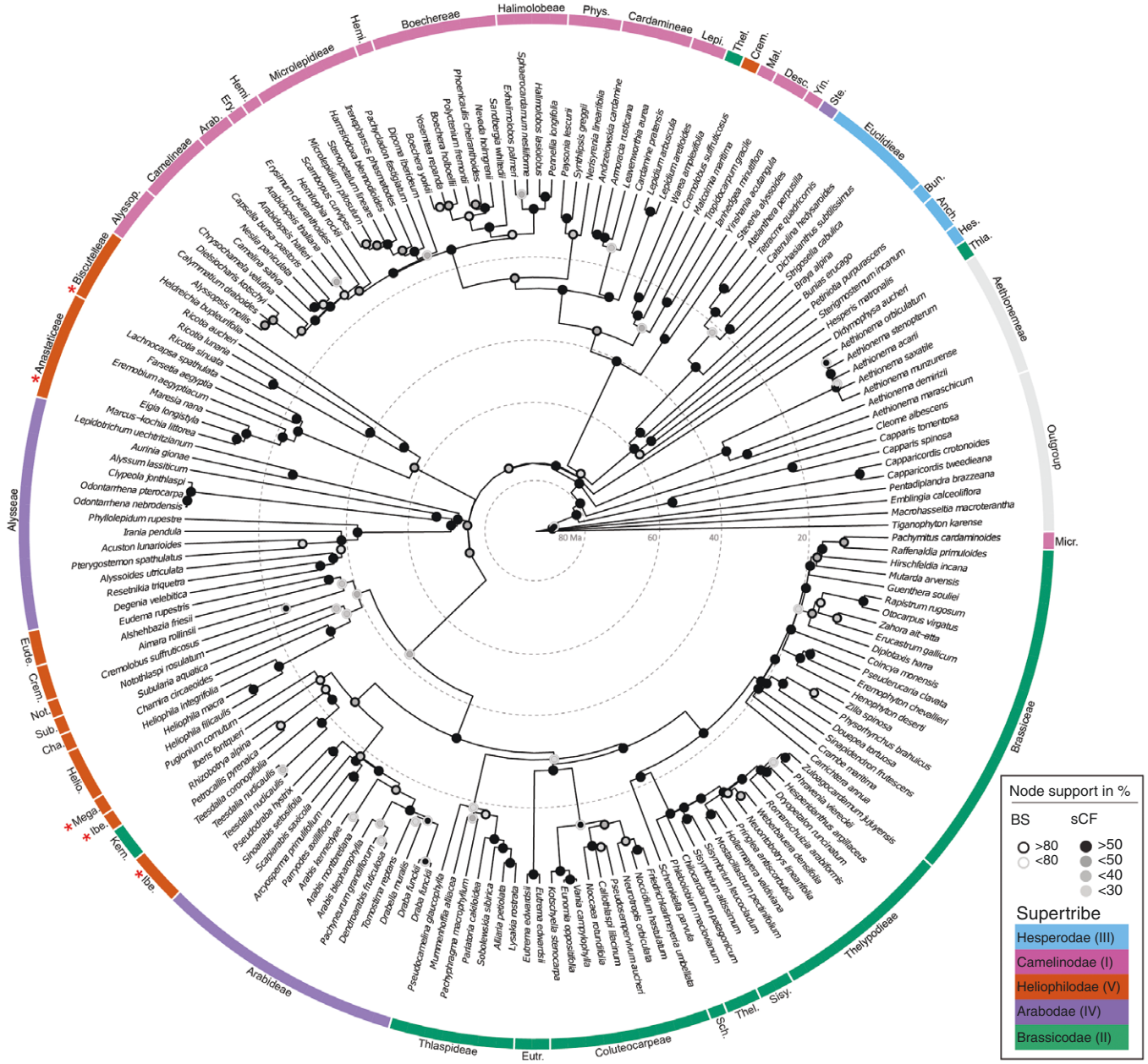

FIG. 2. Time-calibrated genus-level mitogenomic Brassicaceae phylogeny based on the 'selective' strategy, from a maximum likelihood approach analysis of 32 mitochondrial genetic markers. Node support is indicated by circles, with outer circles representing bootstrap (BS) values and inner circles representing site concordance factors (sCF). Supertribes are colour-coded, and red asterisks mark rogue taxa (as defined by German *et al.*, 2023; Hendriks *et al.*, 2023; see also Table 2). Tribe names are given along the outer edge of the phylogeny. For a richly annoated and detailed version, see Supplementary Data Fig. S2. Abbreviations of tribes are as follows: Anch., Anchonieae; Arab., Arabidopsideae; Bun., Buniadeae; Cha., Chamireae; Crem., Cremolobeae; Desc., Descurainieae; Ery., Erysimeae; Eude., Eudemeae; Eutr., Eutremeae; Helio., Heliophileae; Hemi., Hemilophieae; Hes., Hesperideae; Ibe., Iberideae; Lepi., Lepidieae; Mal., Malcolmieae; Mega., Megacarpaeeae; Micr., Microlepidieae; Not., Notothlaspideae; Phys., Physarieae; Ste., Stevenieae; Sub., Subularieae; Thel., Thelypodieae; Thla., Thlaspideae; Yin., Yinshanieae.

Updated nuclear and plastome Brassicaceae phylogenies

In addition to our new global mitogenomic Brassicaceae phylogenies, we revisited the data of Hendriks *et al.* (2023) to generate updated nuclear and plastome Brassicaceae phylogenies using the same methodology as applied in our mitogenomic analysis, thereby avoiding methodologically derived discrepancies among the three datasets (Fig. 3). In our updated nuclear phylogeny (Supplementary Data Fig. S4), we recovered subfamilies Aethionemoideae and Brassicoideae as well-supported sisters (100/72.8) and all supertribes as defined by German *et al.* (2023) as monophyletic groups, with the main topology ('backbone') in line with that of the nuclear phylogeny of Hendriks *et al.* (2023). The crown age of the family was 39.5 Mya (HPD: 40.6–39.2 Mya), again older than the 24.5

Mya found by Hendriks *et al.* (2023) based on the same nuclear genomic dataset.

The main topology of our updated plastome phylogeny (Supplementary Data Fig. S5) is also in line with that of the plastome phylogeny of Hendriks *et al.* (2023), again with subfamilies Aethionemoideae and Brassicoideae moderately supported sisters (100/52.5) and only supertribes Camelinodae (sister to the rest of the Brassicoideae; 100/73.6) and Hesperodae (again sister to the rest of the Brassicoideae; 100/65.8) recovered as well-supported monophyletic groups (but with some outliers; see 'Rogue taxa and phylogenomic outliers' below). The crown age of the family was 35.7 Mya (HPD: 39.0–31.7 Mya) and therefore very close to that based on the nuclear genome, but again much older than the 20.2 Mya found from plastome data by Hendriks *et al.* (2023). In line with the plastome phylogeny of Hendriks *et al.* (2023), several supertribes were polyphyletic: Arabodae (tribe Alysseae not the direct sister to tribe Arabideae, as in the nuclear phylogeny); Brassicodae (tribes Brassiceae, Sisymbrieae and Thelypodieae sisters within this supertribe, but tribes Coluteocarpeae and Conringieae within supertribe Heliophilodae); and Heliophilodae (some tribes sister to tribes from supertribe Arabodae or Brassicodae; for results of rogue Heliophilodae taxa, see 'Rogue taxa and phylogenomic outliers' below). In nearly all these deep-node cases, support was (very) low (sCF generally < 50) and branch lengths short, suggesting strong conflict in the data. Importantly, as in the mitogenomic phylogeny, we recovered nearly all tribes as well-supported monophyletic groups in the nuclear and plastome phylogenies (BS > 95 and sCF 50–80; Supplementary Data Figs S1–S3), again confirming the delimitation of most tribes as valid taxonomic entities (for exceptions, see 'Rogue taxa and phylogenomic outliers' below).

Rogue taxa and phylogenomic outliers

Cytonuclear discordance in the Brassicaceae based on plastome and nuclear genome sequence data has been well documented, and our results corroborate previous findings (Supplementary Data Fig. S6). We find cytonuclear discordance to be equally strong when comparing the mitogenomic results against the nuclear genomic results (Fig. 4). Surprisingly, our data also show strong discordance between the two (generally) maternally inherited organellar genomes, i.e. the mitogenome and the plastome (Fig. 5), a phenomenon we coin 'mitoplastomic discordance'.

Although monophyly and strong support for the tribes within Brassicaceae were observed consistently across the phylogenomic analyses in this study, the placement of several tribes remained problematic (Table 2), echoing the findings of Nikolov *et al.* (2019) and Hendriks *et al.* (2023). Specifically, there was no consensus among the three genomic datasets to resolve unequivocally the positions of Anastaticeae, Biscutelleae, Iberideae and Megacarpaeeae (all belonging to supertribe Heliophilodae). Although a close relationship with tribe Heliophileae was evident, their relative positions varied across datasets, and these tribes frequently appeared sister to lineages from supertribes other than Heliophileae. For the previously unplaced tribe Cochlearieae (German *et al.*, 2023), the plastome phylogeny indicated a relationship with other rogue Heliophilodae tribes. In contrast, the nuclear phylogeny supported an association with tribes from supertribe Brassicodae; the latter relationship being significantly more robust based on node support.

Our analyses shed light on the placement of three previously unplaced monospecific genera (Table 2). Both nuclear and plastome phylogenies associated *Atacama* with other genera in the South American CES clade (composed of tribes Cremolobeae, Eutremeae and Schizopetaleae) within supertribe Heliophilodae. In addition, both phylogenies positioned the Argentinian endemic *Delpinophytum* as a sister to the large radiation of the genus *Lepidium* within supertribe Camelinodae. For *Chrysochamela*, the nuclear and mitogenomic analyses recovered the genus as sister to tribe Alyssopsideae, whereas plastome data suggested a sister relationship with tribe Turritideae. However, there is consensus among the results from all three genomes that *Chrysochamela* should be placed within supertribe Camelinodae.

We identified 15 'phylogenomic outliers'; specimens or species recovered in unexpected or doubtful positions in at least one of our three phylogenies (Table 2). In six cases (tribes Hemilophieae, Kernereae and Stevenieae, in addition to the genera *Bivonaea*, *Orychophragmus* and *Sinalliaria*), topological

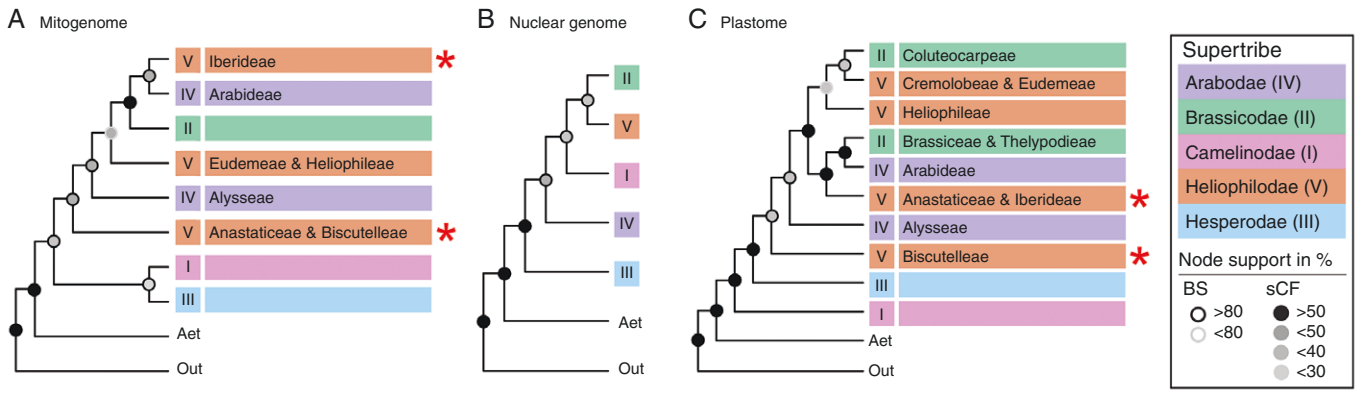

FIG. 3. Comparison of Brassicaceae family-level phylogenetic topologies based on: (A) mitogenome ('selective' strategy); (B) nuclear genome; and (C) plastome sequence data. Tip label colours correspond to supertribes, with prime constituent tribes listed. Supertribal numbers follow those of Nikolov *et al.* (2019). Abbreviations: Aet., tribe Aethionemeae; Out., outgroup families. Rogue tribes are marked with a red asterisk (*).

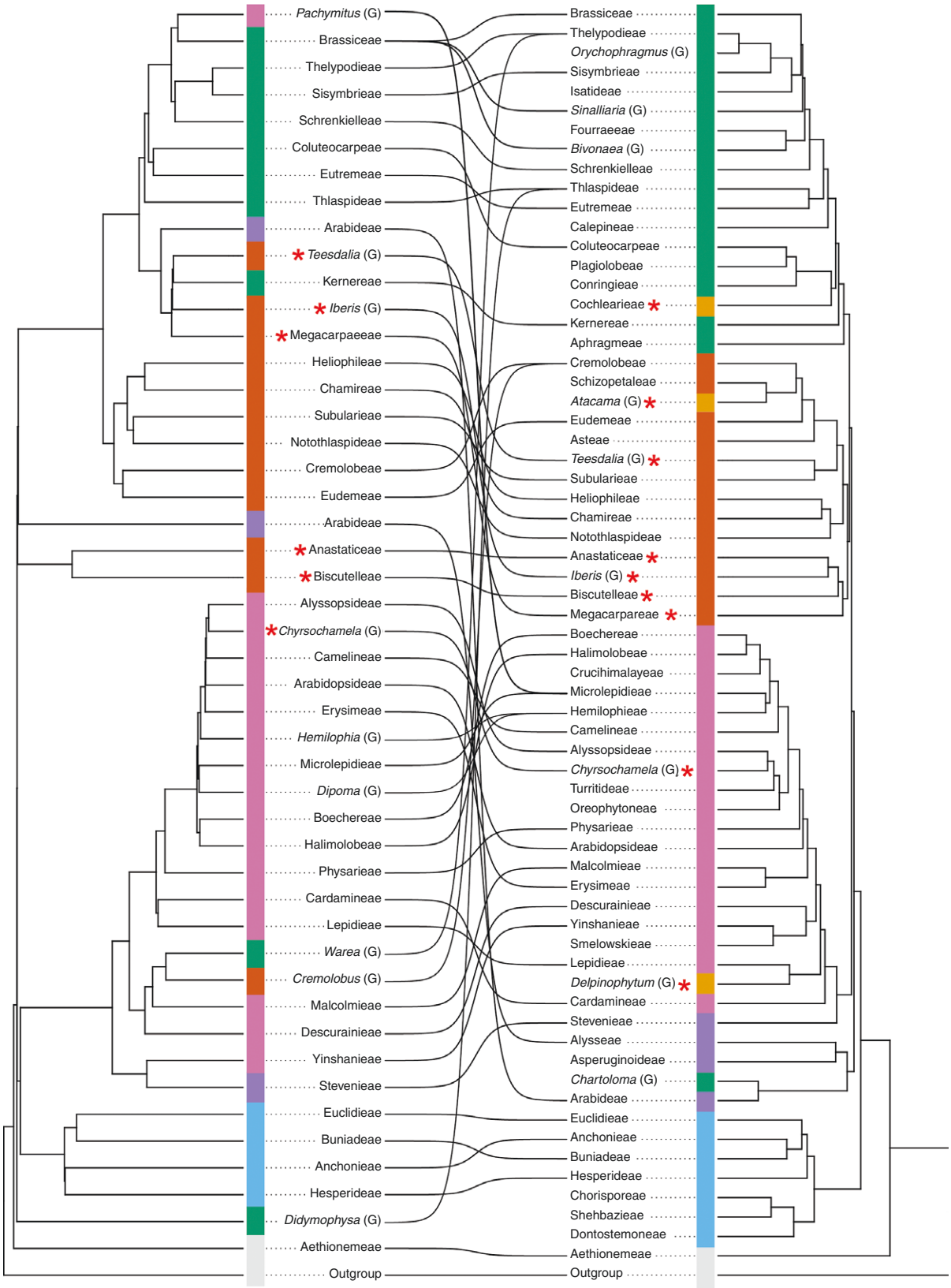

FIG. 4. Tanglegram comparing Brassicaceae tribe-level phylogenies based on mitogenome (left) and nuclear genome (right) data. Colours represent supertribes as in Fig. 1, and red asterisks mark rogue taxa. All genera (labelled 'G') recovered as polyphyletic to their respective tribe are included. Note that more tribes were included in the nuclear phylogeny owing to higher data recovery; taxa without connecting lines indicate those missing from the mitogenomic phylogeny.

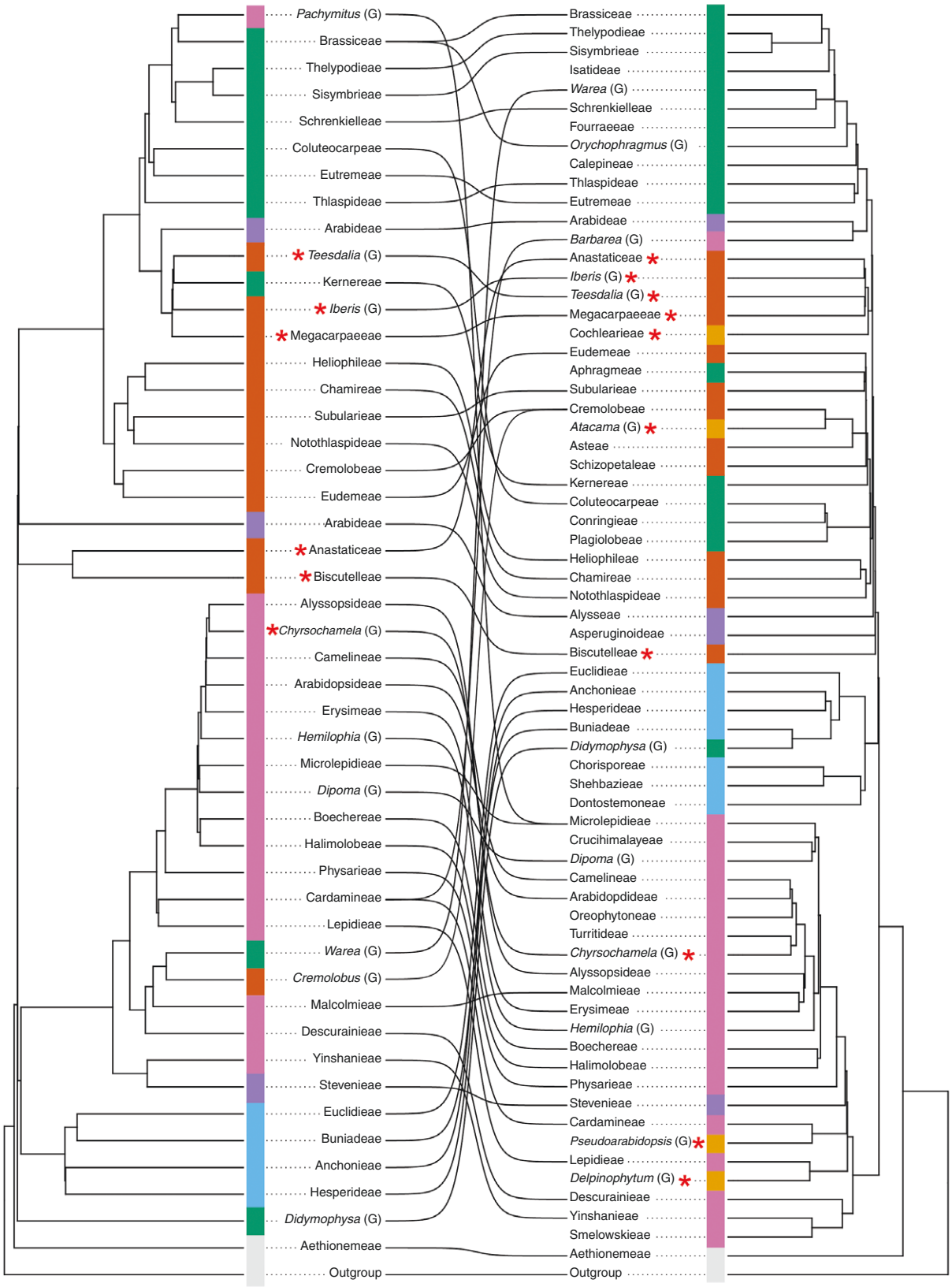

FIG. 5. Tanglegram comparing Brassicaceae tribe-level phylogenies based on mitogenome (left) and plastome (right) data. Colours represent supertribes as in Fig. 1, and red asterisks mark rogue taxa. All genera (labelled 'G') recovered as polyphyletic to their respective tribe are included. Note that more tribes were included in the plastome phylogeny owing to higher data recovery; taxa without connecting lines indicate those missing from the mitogenomic phylogeny.

TABLE 2. *Brassicaceae family rogue taxa and phylogenomic outliers. Taxonomic classifications (first two columns) are based on tribe and supertribe assignments following German et al. (2023). Phylogenomic corroboration is scored as follows: '−' (different tribe, different supertribe), '0' (same tribe, different supertribe), and '+' (same tribe, same supertribe). Likely phylogenomic outliers are indicated by an asterisk (*). Mitogenomic results from 'selective' strategy; when taxon only recovered in 'relaxed' strategy (when missing from 'selective' strategy) results are in square brackets.*

Taxon	Tribe (supertribe)	Phylogenomic position						Comments
		Mitogenome		Plastome		Nuclear genome		
Rogue tribes and previously unplaced taxa								
Rogue tribe composed of *Cochlearia* and *Ionopsidium*	Cochlearieae (unplaced)	−	[Sister to *Teesdalia* (tribe Iberideae); with tribes Kernereae and Megacarpa each sister to tribe Arabideae]	−	Sister to clade of rogue tribes Anastaticeae, Iberideae and Megacarpaeeae, together within supertribe Heliophilodae	+	Sister to clade of tribes Coluteocarpeae, Conringieae and Plagiolobeae, within supertribe Brassicodae	Sister node support much higher in nuclear (sCF 96) than plastome (sCF 36.7) and very low (sCF 3) in mitogenomic phylogeny
Rogue tribe including *Diceratella*, *Farsetia*, *Lobularia* and *Parolinia*	Anastaticeae (Heliophilodae)	0	Tribe monophyletic and sister to tribe Biscutelleae, together making supertribe Heliophilodae polyphyletic	0	Tribe monophyletic and sister to *Iberis*, together sister to a clade including Cochlearieae, Megacarpaeeae and *Teesdalia*	+	Tribe monophyletic and sister to *Iberis*, together sister to tribe Biscutelleae	Most data confirm close relationship between tribes Anastaticeae and Biscutelleae, but the exact relationship remains unresolved
Rogue tribe including *Biscutella*, *Lunaria* and *Ricotia*	Biscutelleae (Heliophilodae)	0	Tribe monophyletic and sister to tribe Anastaticeae, together making supertribe Heliophilodae polyphyletic	−	Tribe monophyletic and sister to large clade of supertribes Arabodae, Brassicodae and remaining Heliophilodae	+	Tribe monophyletic and sister to clade of Anastaticeae and *Iberis*	
Rogue tribe composed of *Iberis* and *Teesdalia*	Iberideae (Heliophilodae)	−	*Iberis* sister to clade of *Teesdalia* and Kernereae	−	*Iberis* sister to Anastaticeae, with *Teesdalia* sister to tribe Megacarpaeeae	+	*Iberis* sister to Anastaticeae, with *Teesdalia* sister to *Subularia aquatica*	None of the genomes supports the combination of *Iberis* and *Teesdalia* into a single tribe, Iberideae
Rogue tribe composed of *Megacarpaea* and *Pugionium*	Megacarpaeeae (Heliophilodae)	−/?	*Pugionium* sister to clade of Iberideae and Kernereae (*Megacarpaea* not recovered)	−	Tribe monophyletic and sister to *Teesdalia*	+	Tribe monophyletic and sister to clade of Anastaticeae, Biscutelleae and *Iberis*	Close relationship to Iberideae follows from all three genomes
Atacama nivea	Unplaced (unplaced)	−	[Sister to *Didymophysa aucheri*, together sister to clade of CES tribes and tribe Heliophileae]	0	Sister to tribe Cremolobeae, within supertribe Heliophilodae	+	Sister to tribe Schizopetaleae, within supertribe Heliophilodae	Clearly associated with or within CES clade; nuclear results appear most solid
Chrysochamela velutina	Unplaced (Camelinodae)	0	Sister to tribe Alyssopsideae, together sister to tribe Camelineae, within supertribe Camelinodae	0	Sister to tribe Turritideae, together sister to clade including tribe Camelineae, within supertribe Camelinodae	+	Sister to tribe Alyssopsideae, together sister to tribe Turritideae, within supertribe Camelinodae	German *et al*. (2023) removed *Chrysochamela* from tribe Camelineae, which our results corroborate, however, our results suggest that it is closely related to Camelineae
Delpinophytum patagonicum	Unplaced (unplaced)	?	(Not recovered)	+	Sister to tribe Lepidieae, within supertribe Camelinodae	+	Sister to tribe Lepidieae, within supertribe Camelinodae	Available data confirm this species as sister to tribe Lepidieae
Phylogenomic outlier taxa in one or more phylogenies in this study								
Aphragmus obscurus	Aphragmeae (Brassicodae)	?	(Not recovered)	−	Within tribe Subularieae, within supertribe Heliophilodae*	+	Part of supertribe Brassicodae and sister to the rest of that supertribe	Position from plastome phylogeny likely false
Barbarea vulgaris	Cardamineae (Camelinodae)	?	(Not recovered)	−	Within tribe Arabideae, within supertribe Arabodae*	?	(Not recovered)	Raw data from Hendriks *et al*. (2023) did not pass the improved bioinformatic pipeline for mitogenome and nuclear genome analysis in the present study, suggesting poor-quality data for this sample

TABLE 2. *Continued*

Taxon	Tribe (supertribe)	Phylogenomic position						Comments
		Mitogenome		Plastome		Nuclear genome		
Bivonaea lutea	Brassiceae (Brassicodae)	0	[Within tribe Thelypodieae, within supertribe Brassicodae]	+	Sister to large clade containing most genera within tribe Brassiceae, within supertribe Brassicodae	0	Sister to tribe Fourraeeae, together sister to large clade containing tribes Isatideae, Sisymbrieae and Thelypodieae, within supertribe Brassicodae	Our results corroborate those of Hendriks *et al.* (2023), who also recovered *Bivonaea* well outside tribe Brassiceae
Chartoloma platycarpum	Isatideae (Brassicodae)	?	(Not recovered)	+	Within tribe Isatideae, within supertribe Brassicodae	–	Within genus *Draba*, within supertribe Arabodae*	Most nuclear data did not pass our improved bioinformatic pipeline, possibly explaining odd position
Cremolobus suffruticosus (two specimens)	Cremolobeae (Heliophilodae)	–/+	One specimen within supertribe Camelinodae*, the other sister to *Aimara rollinsii* in supertribe Heliophilodae	+	Both specimens in tribe Cremolobeae, within supertribe Heliophilodae	+	Both specimens in tribe Cremolobeae, within supertribe Heliophilodae	Two specimens from this species were included; unexpected position recovered for only one of these two
Didymophysa aucheri	Thlaspideae (Brassicodae)	–	Sister to subfamily Brassicoideae*	0	Sister to *Thlaspi arvense*, within tribe Thlaspideae, within supertribe Brassicodae	+	Sister to *D. fenestrata* in supertribe Brassicodeae	Position from mitogenomic phylogeny likely to be false
Didymophysa fenestrata	Thlaspideae (Brassicodae)	?	(Not recovered)	–	Within supertribe Hesperodae*	+	Sister to *D. aucheri* in supertribe Brassicodeae	Position from plastome phylogeny likely to be false
Dipoma iberideum and *Hemilophia rockii*	Hemilophieae (Camelinodae)	0	*Dipoma* sister to tribe Microlepidieae; *Hemilophia* sister to large clade of several tribes, all within supertribe Camelinodae	0	*Dipoma* sister to tribe Crucihimalayeae, together sister to tribe Microlepidieae; *Hemilophia* sister to large clade of several tribes; all within supertribe Camelinodae	+	Sister genera within tribe Hemilophieae, and together sister to tribe Microlepidieae, all within supertribe Camelinodae	The position of genus *Dipoma* might need revision based on the organellar genomes
Macropodium nivale and *Stevenia alyssoides*	Stevenieae (Arabodae)	–/?	*Stevenia alyssoides* within supertribe Camelinodae (also in 'stringent' strategy) (*Macropodium nivale* not recovered)	0	Together form tribe Stevenieae, within supertribe Camelinodae	0	Together form tribe Stevenieae, sister to supertribe Camelinodae	As in Walden *et al.* (2020), but in contrast to Hendriks *et al.* (2023), a close relationship to supertribe Camelinodae is inferred from all three genomes
Orychophragmus violaceus	Brassiceae (Brassicodae)	?	(Not recovered)	0	Sister to large clade containing tribes Brassiceae, Fourraeeae, Isatideae, Schrenkielleae, Sisymbrieae and Thelypodieae, within supertribe Brassicodae	0	Sister to genus *Raphanorhyncha*, within tribe Thelypodieae, within supertribe Brassicodae	Our results do not corroborate those of Hendriks *et al.* (2023), who recovered *Orychophragmus* as sister to the rest of tribe Brassiceae
Pachymitus cardaminoides	Microlepidieae (Camelinodae)	–	Sister to *Raffenaldia*, within supertribe Brassicodae*	+	Within tribe Microlepidieae, within supertribe Camelinodae	+	Within tribe Microlepidieae, within supertribe Camelinodae	Position from mitogenomic phylogeny likely to be false
Kernera saxatilis, Petrocallis pyrenaica and *Rhizobotrya alpina*	Kernereae (Brassicodae)	0	Within tribe Kernereae, but tribe within supertribe Heliophilodae (genus *Kernera* not recovered)	0	Within tribe Kernereae, but tribe sister to large clade of supertribe Heliophilodae representatives	+	Within tribe, within supertribe	Organellar genomes suggest relationship with supertribe Heliophilodae
Pseudoarabidopsis toxophylla	Unplaced (unplaced)	?	(not recovered)	–	Within tribe Cardamineae*	?	(Not recovered)	Position from plastome phylogeny likely to be false

TABLE 2. *Continued*

Taxon	Tribe (supertribe)	Phylogenomic position			Comments
		Mitogenome	Plastome	Nuclear genome	
Sinalliaria limprichtiana	Brassiceae (Brassicodae)	– [Sister to Turritis, within supertribe Camelinodae]	+ Sister to rest of tribe Brassiceae, within supertribe Brassicodae	0 Sister to large clade of tribes Brassiceae, Isatideae, Sisymbrieae and Thelypodieae, within supertribe Brassicodae	Our results do not corroborate those of Hendriks *et al.* (2023), who recovered *Sinalliaria* within tribe Brassiceae
Warea amplexifolia	Thelypodieae (Brassicodae)	– Within supertribe Camelinodae (also in 'stringent' strategy)*	– Sister to tribe Isatideae, not within tribe Thelypodieae, but within supertribe Brassicodae	+ Within tribe Thelypodieae, within supertribe Brassicodae	Position from mitogenomic phylogeny likely to be false

positions might have taxonomic significance and warrant further investigation (see Discussion). In contrast, the remaining nine outliers (representatives of *Aphragmus*, *Barbarea*, *Chartoloma*, *Cremolobus*, *Didymophysa*, *Pachymitus*, *Pseudoarabidopsis* and *Warea*) show clear topological mismatching in one of the datasets, appearing well outside their expected clades (see Discussion).

Phylogenomic placement of *Arabidopsis*

Our sampling included the scientifically important model species *Arabidopsis thaliana* and *Arabidopsis halleri* (tribe Arabidopsideae), consistently recovered as sister species. However, phylogenomic discordance was evident in their topological placement: *Arabidopsis* was sister to tribes Alyssopsideae and Camelineae in the mitogenomic phylogeny (Fig. 2); to a larger clade including tribes Alyssopsideae, Boechereae, Camelineae, Microlepidieae and Physarieae in the nuclear phylogeny (Supplementary Data Fig. S4); and to tribes Camelineae, Crucihimalayeae, Hemilophieae and Microlepidieae in the plastome phylogeny (Supplementary Data Fig. S5).

DISCUSSION

We present the most comprehensive mitogenomic phylogeny of the Brassicaceae to date, encompassing 167 species, 145 genera (representing 40 % of the family) and 40 tribes (69 % of the family; 'selective' strategy). We also reconstructed updated nuclear and plastome phylogenies to allow direct comparisons using the same raw sequencing data files (i.e. same specimens) and bioinformatic pipeline. Contrary to expectations, the slower-evolving mitogenome did not produce a well-supported phylogenetic backbone. Instead, we observed rampant cytonuclear and mitoplastomic discordance, particularly at the supertribal level, with each genomic dataset producing a different backbone topology. Nonetheless, our combined phylogenomic analyses support the taxonomic delimitation of 55 of 58 tribes (exceptions being tribes Iberideae, Hemilophieae and Subularieae), confirm the classification into two subfamilies (cf. German *et al.*, 2023) and shed more light on the evolutionary histories of several previously unplaced or taxonomically challenging lineages. Supertribal definitions as defined by German *et al.* (2023) are fully supported by our nuclear results, but only in part by the mitogenomic and plastomic results, suggesting a complex (early) evolutionary history of the family. Furthermore, our improved codon-aware gene alignments yielded significantly older crown age estimates for the Brassicaceae family (mitogenome 'relaxed' strategy: 75.5 Mya; 'selective' strategy: 77.0 Mya; 'stringent' strategy: 54.9 Mya; nuclear genome: 39.5 Mya; plastome: 35.7 Mya) in comparison to the 24.5 Mya estimate based on the nuclear genome reported by Hendriks *et al.* (2023). This highlights the strong sensitivity of calibration results to the choice of data and data handling, underscoring the urgent need for further research in this area.

The backbone boggle

We aimed to reconstruct a robust Brassicaceae phylogenetic backbone based on conserved mitogenomic coding genes, a method shown to be effective in resolving deeper relationships in other plant families (Liu *et al.*, 2014; Rydin *et al.*, 2017; Soto Gomez *et al.*, 2020; Lin *et al.*, 2022; Xue *et al.*, 2022). Our analyses confirmed that mitochondrial gene exons have the lowest genetic variation among the three Brassicaceae genomes (Fig. 1). However, backbone topologies varied across the three genome phylogenies (Fig. 3), with none showing consistently higher node support across the deeper nodes (sCF ranges: mitogenome 24–88; nuclear 39–89; plastome 35–83). This suggests that substitution saturation and compositional heterogeneity are less significant than biological factors such as incomplete lineage sorting (Som, 2015), horizontal gene transfer (Filip and Skuza, 2021) and organelle capture (Stegemann *et al.*, 2012). Further research is necessary, because historical genome reshuffling presents a significant obstacle to resolving the Brassicaceae backbone fully (Degnan and Rosenberg, 2009), while at the same time, a hard polytomy (i.e. a rapid radiation following the origin of the Brassicaceae family) should be considered, as recently suggested for Fabaceae (Koenen *et al.*, 2021) and Poaceae (Grass Phylogeny Working Group III, 2025).

Rampant mitoplastomic discordance

Surprisingly, we found strong incongruences between the mitogenome and plastome phylogenies (Fig. 5), a phenomenon

we coin 'mitoplastomic discordance'. Although differences between nuclear and organellar phylogenies (i.e. cytonuclear discordance) are common owing to distinct inheritance patterns (Larson *et al.*, 2024), it was expected that mitochondrial and plastid genomes would show strong phylogenetic concordance owing to: (1) their vertical transfer as (generally) non-recombinant genetic units; and (2) their joint uniparental inheritance (Birky, 2001; Tyszka *et al.*, 2023). On the contrary, recent studies have highlighted such mitoplastomic or 'interorganellar discordance' before, for example in algae (Kao *et al.*, 2022), Rubiaceae (Rydin *et al.*, 2017), Orchidaceae (Li *et al.*, 2019) and *Potentilla* (Xue *et al.*, 2024). One explanation could be that maternal inheritance of mitochondria and plastids is not as strict as previously thought, and instances of both paternal and biparental inheritance in plants have been documented in both (Corriveau and Coleman, 1988; Shen *et al.*, 2015; Chybicki *et al.*, 2016; Van de Paer *et al.*, 2016). Additionally, lateral gene transfer (including organelle capture) can introduce foreign or chimeric sequences, leading to divergent evolutionary histories (Hao *et al.*, 2010; Rice *et al.*, 2013; Xian *et al.*, 2025).

Taxonomic insights into rogue and unplaced taxa

In line with the work of Nikolov *et al.* (2019) and Hendriks *et al.* (2023), we faced several difficult-to-place taxa (mostly tribes; Table 2). These rogue taxa have the tendency to change position within the phylogeny, depending on the gene (sub)set analysed and the choice of phylogenetic inference tool (for further discussion, see Hendriks *et al.*, 2023) and are likely to be the result of inter-tribal or even inter-supertribal hybridizations (Hohmann *et al.*, 2015; Mandáková *et al.*, 2017*b*; Guo *et al.*, 2021; Huang *et al.*, 2023).

As in the study by Hendriks *et al.* (2023), we recovered the complex polyploid tribe Cochlearieae (Koch, 2012; Wolf *et al.*, 2021) as a monophyletic group sister to either a clade of tribes Coluteocarpeae, Conringieae and Plagiolobeae, within supertribe Brassicodae (nuclear phylogeny; sCF 96) or a clade of the rogue tribes Anastaticeae, Iberideae and Megacarpaeeae, within supertribe Heliophilodae (plastome phylogeny; sCF 37; conform Walden *et al.*, 2020). No representatives of this tribe were recovered in the 'selective' mitogenomic phylogeny, whereas results from the 'relaxed' mitogenomic phylogeny suggest a possible close relationship with *Teesdalia*. Notably, the nuclear phylogeny provided much stronger node support, highlighting higher topological variation in the plastome. Interestingly, Wolf *et al.* (2021) found no incongruences between the two 'materal phylogenies' when studying 19 *Cochlearia* taxa. This might suggest that mitoplastomic discordance is mostly an intertribal issue, but more research in other Brassicaceae tribes is needed. Finally, the changing phylogenetic placement of *Cochlearia* might be attributable to its hexaploid origin (Mandáková *et al.*, 2017*a*), which arose probably through hybridization between distantly related lineages (possibly from different tribes).

A close relationship among tribes Anastaticeae (mesotetraploid origin; Mandáková *et al.*, 2017*a*), Biscutelleae (mesopolyploid origin; Guo *et al.*, 2021), Iberideae (with at least *Iberis* of mesotetraploid origin; Mandáková *et al.*, 2017*a*) and Megacarpaeeae (polyploid origin; Yang *et al.*, 2020) was supported by all three genome phylogenies. Interestingly, all three genomes refute uniting *Iberis* and *Teesdalia* into a single tribe, as was traditionally accepted following the seminal work of Warwick *et al.* (2010). Instead, we found *Teesdalia* to be moderately supported (sCF 50) as a sister to tribe Kernereae (supertribe Brassicodae) in the mitogenomic phylogeny, moderately supported (sCF 55) as a sister to tribe Megacarpaeeae in the plastome phylogeny, and weakly supported (sCF 42) as a sister to *Subularia aquatica* (tribe Subularieae) in the nuclear phylogeny. At this point, the most practical solution to classify *Iberis* and *Teesdalia* is to establish two monogeneric tribes within supertribe Heliophilodae.

Our findings further unravel the intricate evolutionary relationships within the South American CES clade (composed of tribes Cremolobeae, Eudemeae and Schizopetaleae; Salariato *et al.*, 2016) and its close relatives, *Atacama nivea* and tribe Asteae. Each tribe was well supported as monophyletic in each of the three phylogenies, except for polyphyletic Cremolobeae in the plastome phylogeny (Supplementary Data Fig. S5). *Atacama nivea*, previously considered part of tribe Schizopetaleae but recently listed as unplaced (German *et al.*, 2023), grouped as sister to tribe Schizopetaleae in the nuclear phylogeny (sCF 48) and to Cremolobeae (sCF 63) in the plastome phylogeny and remains difficult to assign unequivocally to a tribe (cf. Toro-Núñez *et al.*, 2015). Likewise, tribe Asteae was sister to the CES clade in the nuclear phylogeny but nested within it in the plastome phylogeny. Tribe Subularieae, shifting affiliations across analyses and surprisingly recovered within the CES clade in the plastome phylogeny, is perhaps best treated as yet another 'rogue tribe', requiring further study. Moreover, in none of the phylogenetic results were its constituent genera, *Idahoa* and *Subularia*, recovered as sisters, meaning that the combination of these two genera within tribe Subularieae (*sensu* German *et al.*, 2023) needs further study.

Likewise, our results further illuminate the phylogenetic position of two other enigmatic genera. First, we recovered the genus *Chrysochamela*, previously assigned to tribe Camelineae but recently reclassified as unplaced (German *et al.*, 2023), as sister to tribe Alyssopsideae in both mitogenomic and nuclear phylogenies. Second, aligning with the findings of Hendriks *et al.* (2023), we found the monospecific cushion-forming Argentinian endemic *Delpinophytum* [formerly placed in tribe Eudemeae (Salariato *et al.*, 2015*a*, *b*) but recently considered unplaced (German *et al.*, 2023)] as an early-diverging lineage sister to tribe Lepidieae. This relationship suggests that *Delpinophytum* might be best classified within tribe Lepidieae, as previously suggested by Al-Shehbaz *et al.* (2002).

Taxonomic insights into phylogenomic outliers

We identified 15 'phylogenomic outliers' that were retained for two reasons: (1) phylogenetic results from at least one genome align with prior findings, supporting the accuracy of specimen identification; and (2) we used raw sequencing data from the study by Hendriks *et al.* (2023), where these samples were phylogenetically recovered as sisters to their taxonomically expected relatives (based on the nuclear dataset). Given our strict thresholds for phylogenetic analysis (15 mitochondrial or five plastid genes, with plastid genes generally showing higher

variation; Fig. 1), data deficiency cannot explain these outliers in most cases. Given these considerations and to avoid the risk of subjective data cherry-picking, we chose not to discard these results solely based on taxonomic expectations but to retain them for future study by the scientific community and give a detailed description here.

Importantly, in at least six cases, outliers might offer valuable taxonomic insights. For instance, the monospecific Mediterranean genus *Bivonaea*, previously assigned to different tribes (Warwick *et al.*, 2010; Koch, 2012) and most recently to tribe Brassiceae (German *et al.*, 2023), was retrieved as sister to tribe Fourraeeae in the nuclear phylogeny (cf. Hendriks *et al.*, 2023) and sister all other Brassiceae members (except *Sinalliaria*) in the plastome phylogeny. This suggests that its previous placement in the monogeneric tribe Bivonaeeae (cf. Koch, 2012) might be more practical until further study tells otherwise.

The Central Asian monospecific genus *Dipoma* was found to be sister to another Central Asian genus, *Hemilophia*, based on nuclear target capture results from Nikolov *et al.* (2019) and Hendriks *et al.* (2023), which led to its inclusion in the new tribe Hemilophieae by German *et al.* (2023). However, our mitogenomic results suggest a sister relationship of *Dipoma* to the Australian tribe Microlepidieae, whereas our plastome results suggest a sister relationship to yet another Central Asian tribe, Crucihimalayeae (cf. Walden *et al.*, 2020), together sister to tribe Microlepidieae. These results imply that the combination of *Dipoma* and *Hemilophia* into a single tribe might have been premature and that *Dipoma* deserves its own monospecific tribe.

We recovered tribe Stevenieae, composed of the closely related genera *Stevenia* and *Macropodium* (German *et al.*, 2009; German and Al-Shehbaz, 2010), as sister to the supertribe Camelinodae in both mitogenome and nuclear genome analyses, or placed within Camelinodae in the plastome phylogeny (or former lineage I, cf. Walden *et al.*, 2020). Therefore, inclusion of tribe Stevenieae in supertribe Arabodae by German *et al.* (2023) based on results from Hendriks *et al.* (2023) warrants revision. Interestingly, Stevenieae was recovered as the earliest diverging lineage of the respective supertribes in this study and Hendriks *et al.* (2023), a pattern that often signals historical inter-supertribal genetic admixture (e.g. hybridization; Funk, 1985). A similar case involves the polyploid genus *Orychophragmus* (tribe Brassiceae), which we recovered within tribe Thelypodieae in the nuclear genome phylogeny or as sister to a large clade of tribes (Brassiceae, Fourraeeae, Isatideae, Schrenkielleae, Sisymbrieae and Thelypodieae) in the plastome phylogeny, aligning broadly with the findings of Zhang *et al.* (2023). Although the genus shares conduplicate cotyledons with members of tribe Brassiceae, our results contradict its sister-group placement to that tribe as previously suggested (Hu *et al.*, 2016; Hendriks *et al.*, 2023).

For the small Central European tribe Kernereae (represented here by the monospecific diploid genera *Kernera*, *Petrocallis* and *Rhizobotrya*), both mitogenomic and plastome phylogenies suggest a close relationship with supertribe Heliophilodae rather than supertribe Arabodae (German *et al.*, 2023). Results from the mitogenome indicate a relationship with the European tribe Iberideae, whereas the plastome links it to the South American CES clade, closely resembling Koch's (2012) finding of a relationship with tribe Buniadeae, the two together sister to a clade of several American tribes (Asteae, Cremolobeae and Schizopetaleae). Given the consistent clustering of the three genera of the tribe, data deficiency or methodological errors seem unlikely.

Lastly, the eastern China endemic monospecific genus *Sinalliaria* (tribe Brassiceae) was recovered in our nuclear phylogeny as sister to a clade comprising the tribes Brassiceae, Isatideae, Sisymbrieae and Thelypodieae, whereas in the plastome phylogeny, it was sister only to the remainder of tribe Brassiceae (cf. de Santana Lopes *et al.*, 2018; Hendriks *et al.*, 2023). Although its close relationship with tribe Brassiceae (supported also by shared conduplicate cotyledons) is evident, its precise phylogenetic placement requires further investigation.

CONCLUSION

Our phylogenomic study is the first to compare all three plant genomes (nuclear, plastid and mitochondrial) at the family level in Brassicaceae, a family with a complex evolutionary history. Although determining the 'best' phylogeny remains challenging, the nuclear phylogeny is the most robust owing to its large dataset (281 genes filtered from a 1081 nuclear gene dataset), broad taxonomic sampling and strong alignment with historical morphology-based taxonomic classifications (von Hayek, 1911; Schulz, 1936; Janchen, 1942; Beilstein *et al.*, 2006; Al-Shehbaz, 2012). A key outcome of our tri-genomic approach is a near-universal support across all genomes for tribal circumscriptions *sensu* German *et al.* (2023), emphasizing the validity and usefulness of the tribe as a taxonomic rank in Brassicaceae. Additionally, we confirm the monophyly of the two subfamilies [Aethionemoideae (comprising only monogeneric tribe Aethionemeae) and Brassicoideae (comprising the remaining 57 tribes)] and the early divergence of supertribes Hesperidae and Camelinodae within Brassicoideae. However, our family-wide mitogenomic phylogeny shows inconsistent supertribal relationships, and frequent shifts in rogue taxa (e.g. tribes Anastaticeae, Biscutelleae, Cochlearieae, Iberideae and Megacarpaeeae) among the three genome phylogenies remain, complicating our understanding of phylogenetic backbone. Until such conflicts are resolved, we advocate integration of the results from all three genomes to infer phylogenetic relationships and guide taxonomy.

Current phylogenomic methods relying on bifurcating trees cannot fully capture reticulate evolution, such as hybridization. Therefore, future studies should prioritize unravelling such complexities. Promising advances in Brassicaceae research, such as synteny analysis (Walden and Schranz, 2023) and 'Parlog PhyloGenomics' (Walden *et al.*, 2024), in addition to network-based methods, such as 'PhyloNetworks' (Solís-Lemus *et al.*, 2017), offer avenues to detect shared parentage in hybrid species. With increasing computational power, we anticipate that application of these algorithms to family-scale datasets will someday unveil the 'true' evolutionary history of the Brassicaceae.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Table S1: sample data from the study by Hendriks *et al.* (2023) reanalysed for this study. Table S2: sample data from the study by Nikolov *et al.* (2019) reanalysed for this study. Table S3: previously published mitogenomes used to create mitogenomic target file. Table S4: mitogenomic genes used in this study. Table S5: mitogenomic target genes reference file. Table S6: previously published plastomes used to create plastome target file. Table S7: nuclear genome target genes reference file. Table S8: plastome target genes reference file. Table S9: chloroplast gene sampling overview. Table S10: nuclear genome gene sampling overview. Table S11: gene mean pairwise distance (MPD) for the mitogenomic genes ('selective' strategy). Table S12: gene mean pairwise distance (MPD) for the chloroplast genes. Table S13: gene mean pairwise distance (MPD) for the nuclear genes. Table S14: node age estimates (in millions of years ago with 95 % confidence interval). Figure S1: time-calibrated genus-level mitogenomic Brassicaceae phylogeny from 'relaxed' strategy. Figure S2: time-calibrated genus-level mitogenomic Brassicaceae phylogeny from 'selective' strategy with gene sampling heatmap. Figure S3: time-calibrated genus-level mitogenomic Brassicaceae phylogeny from 'stringent' strategy. Figure S4: time-calibrated genus-level nuclear Brassicaceae phylogeny. Figure S5: time-calibrated genus-level plastome Brassicaceae phylogeny. Figure S6: tanglegram comparing Brassicaceae tribe-level phylogenies based on plastome (left) and nuclear genome (right) data.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest relevant to the content of this manuscript.

DATA AVAILABILITY STATEMENT

Dominicus_et_al_2025_gene_alignments.zip: this file contains all gene alignments used to calculate genetic mean pairwise distances and to infer species phylogenies using IQ-TREE 2. The file can be downloaded from Zenodo via doi 10.5281/zenodo.14770522.

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