Clarifying Recent Adaptive Diversification of the Chrysanthemum-Group on the Basis of an Updated Multilocus Phylogeny of Subtribe Artemisiinae (Asteraceae: Anthemideae)

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Understanding the roles played by geography and ecology in driving species diversification and in the maintenance of species cohesion is the central objective of evolutionary and ecological studies. The multi-phased orogenesis of Qinghai-Tibetan Plateau (QTP) and global climate changes over late-Miocene has profoundly influenced the environments and evolution of organisms in this region and the vast areas of Asia. In this study, we investigate the lineage diversification of Chrysanthemum-group in subtribe Artemisiinae (tribe Anthemideae, Asteraceae) likely under the effects of climate changes during this period. Using DNA sequences of seven low-copy nuclear loci and nrITS and the coalescent analytical methods, a time-calibrated phylogeny of subtribe Artemisiinae was reconstructed with emphasis on Chrysanthemum-group. The monophyletic Chrysanthemum-group was well resolved into two major clades corresponding to Chrysanthemum and Ajania, two genera which can be well identified by capitulum morphology but have been intermingled in previous plastid and ITS trees. Within Chrysanthemum, a later divergence between Ch. indicum-complex and Ch. zawadskii-complex can be recognized. The time frames of these sequential diversifications coincide with the late Cenozoic uplift of the Northern QTP and the concomitant climatic heterogeneity between eastern and inland Asia. Reconstruction of historical biogeography suggested the origin of Chrysanthemum-group in Central Asia, followed by eastward migration of Chrysanthemum and in situ diversification of Ajania. Within Chrysanthemum, Ch. indicum-complex and Ch. zawadskii-complex exhibited contemporary distributional division, the former in more southern and the latter in more northern regions. The geographic structure of the three lineages in Chrysanthemum-group have been associated with the niche differentiation, and environmental heterogenization in Asia interior.

Keywords: Chrysanthemum, Ajania, coalescence, phylogeny, niche differentiation
INTRODUCTION

Speciation is usually associated with morphological innovation or modification that occurs for internal molecular and/or external environmental reasons. Geographic isolation and ecological segregation are both important external forces for speciation (Coyne and Orr, 2004). Understanding the relative roles of geographic and ecological factors in the increase of species diversity and maintenance of species coherence is one of the central tasks of evolutionary biology (Wiens and Graham, 2005; Rundle and Nosis, 2010, 2012; Anacker and Strauss, 2014; Ebersbach et al., 2018).

In East Asia, the process of species diversification has been greatly influenced by the topographic activities of the Northern Qinghai-Tibet Plateau (QTP) and the aridification in Asian interior (Zheng et al., 2000; An et al., 2001; Guo et al., 2004; Miao et al., 2012; Ge et al., 2013; Li et al., 2015; Shi et al., 2015; Spicer, 2017; Shi et al., 2019; Ding et al., 2020). To what extent have ecogeographical gradients and diverse macrohabitats in this region promoted speciation (He et al., 2010; Qin et al., 2013; Yan et al., 2013)? To gain insight into this issue, comparative phylogeographic analyses of closely related species are required as such studies may help us trace trajectories of lineage splitting and reuniting (if these events happened) and infer evolutionary forces behind rapid speciation (Anacker and Strauss, 2014; Ortiz-Rodriguez et al., 2018; Wang Z. M. et al., 2019; Knope et al., 2020).

Chrysanthemum-group, one of the youngest branches of the largest angiosperm family, Asteraeeae, is a proper system for studying the aforementioned problem. This group belongs to subtribe Artemisiiinae, tribe Anthemideae of Asteraeeae (Bremer and Humphries, 1993; Watson et al., 2002; Kondo et al., 2003; Sanz et al., 2008; Masuda et al., 2009). According to their cladistic analysis of morphological characteristics, Bremer and Humphries (1993) defined 18 genera in subtribe Artemisiiinae. This circumscription has been widely accepted with the later incorporation of Hippolytia, Opisthopappus, and Tanacetum tatischense, which formerly belonged to subtribe Tanacetinae, as well as Leucanthemella and Nipponanthemum, which were formerly in subtribe Leucantheminae. Despite of its uncertain circumscription, the subtribe Artemisiiinae is mainly composed of species belonging to two groups, Chrysanthemum-group and Artemisia-group (Bremer and Humphries, 1993; Oberprieler et al., 2007; Sanz et al., 2008; Oberprieler et al., 2009; Sonboli et al., 2012).

Chrysanthemum-group was historically recognized mainly by solitary flower heads or corymbose synflorescences (in contrast to Artemisia-group with paniculate synflorescences), radiate capitula (Chrysanthemum, Arctanthemum, and Brachanthemum) or disciform capitula (Ajania and Phacostigma), and echinate Anthemis-type pollen grains (except Phacostigma with microechinate Artemisia-type) (Bremer and Humphries, 1993; Sanz et al., 2008; Pellicer et al., 2010). However, the circumscription and monophyly of the two groups have remained questionable (Bremer and Humphries, 1993; Torrell et al., 1999; Oberprieler et al., 2007; Zhao et al., 2010a,b). These problems are probably due to ongoing speciation, including recent divergence and secondary contacts of lineages (Masuda et al., 2009; Miao et al., 2011; Liu et al., 2012b; Li et al., 2014; Chen et al., 2020).

Within Chrysanthemum-group, there are two major genera, Chrysanthemum and Ajania, both mainly distributed in East Asia and each consisting of 30–35 species (Shih and Fu, 1983; Oberprieler et al., 2006). Poljakov (1955) speculated that Ajania was closely related to Artemisia, but Tzvelev (1961) considered Ajania and Chrysanthemum to be sister lineages derived from a most recent common ancestor that had radiate capitula (Muldashev, 1983; Bremer and Humphries, 1993). Ajania was even once treated as a section under Chrysanthemum (Kitamura, 1978; Kishimoto et al., 2003; Ohashi and Yonekura, 2004). Considering their distinct capitulum morphologies, we have postulated that these two genera must have experienced adaptive divergence associated with differential environmental conditions (Chen et al., 2020). Our recent developmental genetic study revealed that a disciform capitulum may have evolved from a radiated type owing to the dysfunction of a key ray-flower regulator, CYCLOIDEA2g (Chen et al., 2018; Shen et al., 2021). To date, however, no molecular phylogeny has resolved Chrysanthemum and Ajania each as monophyletic (Kondo et al., 2003; Masuda et al., 2009; Zhao et al., 2010a,b; Liu et al., 2012b; Huang et al., 2017). In another aspect, the small genus Phacostigma (established by Muldashev in 1981) was once described under Ajania (Muldashev, 1981), but was clarified by our previous analysis as a monophyletic group that is probably closer to Artemisia than to Ajania despite its corymbose synflorescence similar to that of the latter (Huang et al., 2017). Brachanthemum, which was supposed to be closely related to Chrysanthemum due to its thin-walled and pappus-lacking achenes (Bremer and Humphries, 1993), was suggested by molecular phylogeny to be closer to Nipponanthemum, Leucanthemella, or Kaschgarhia than to Chrysanthemum (Vallès et al., 2003; Sanz et al., 2008; Masuda et al., 2009; Zhao et al., 2010a). As to the monotypic genus Arctanthemum, it has been incorporated into Chrysanthemum in the floristic work of China (Shih and Fu, 1983) thus the name Chrysanthemum arcticum was used instead of Arctanthemum arcticum.

In terms of geography, Chrysanthemum-group is widely distributed in Middle to East Asia, with a few members extending to Central Europe and North America (Shih and Fu, 1983). Two major genera, Ajania and Chrysanthemum, cover most of its full range of distribution, with Ajania in China (from northwestern to northeastern and southwestern parts), Korea, Japan, and the Far East, and Chrysanthemum in the more eastern part of China, Korea, Japan, and Russia (Shih and Fu, 1983; Zhao et al., 2009). Within Chrysanthemum, there are two species complexes corresponding to morphological characteristics and weakly supported by the chloroplast phylogeny—Ch. zawadskii-complex, with relatively larger flower heads and white to purple ray flowers, and Ch. indicum-complex, with smaller flower heads and white or yellow rays (Shimizu, 1961; Lee, 1969; Liu et al., 2012b; Li et al., 2013, Li et al., 2014; Kim et al., 2014; Meng et al., 2020). Considering geographic distribution, Ch. zawadskii-complex is distributed mainly in the northern region of East Asia,
while *Ch. indicum*-complex is relatively in the more southeastern part (Shih and Fu, 1983; Shishkin and Bobrov, 1995; Zhao et al., 2009).

Untangling recent speciation events usually requires a reliable phylogenetic framework. To date, all deep phylogenetic relationships within subtribe Artemisiinae inferred from plastid and nrITS markers have provided limited information (Watson et al., 2000, 2002; Sanz et al., 2008; Pellicer et al., 2010; Zhao et al., 2010a; Liu et al., 2012b). Here, we utilized multilocus nuclear DNA sequences and a coalescent analytical method to update the tree of this subtribe with emphasis on *Chrysanthemum*-group. Subsequently, we estimated the optimal ancestral distribution and biogeographical history of the major clades in this subtribe. Furthermore, we conducted ecological niche modeling and niche overlap tests to verify the ecological differentiation of lineages within *Chrysanthemum*-group and to see whether patterns of geographic distribution were linked to environmental conditions. With all these analytical results, we attempted to resolve possible rapid species divergence under macrohabitat differentiation in interior East Asia.

**MATERIALS AND METHODS**

**Taxon Sampling**

Taxon sampling for this study covered all major branches of subtribe Artemisiinae. In total, 101 accessions of 96 species were sampled. Of the 96 species, 53 were of *Chrysanthemum* subtribe Artemisiinae. In total, 101 accessions of 96 species. Taxon sampling for this study covered all major branches of Taxon Sampling.

**MATERIALS AND METHODS**

**DNA Extraction and Gene Isolation**

Genomic DNA was extracted from silica gel-dried or fresh leaves with a Plant Genome Extraction Kit (Tiangen Biotech, China) following the manufacturer’s protocol. For better phylogenetic resolution, we utilized nrITS and seven low-copy nuclear genes, *AGO1* (*ARGONAUTE 1*; Zhang et al., 2015), BRC1 (*BRANCHED1*; Zhou et al., 2012; Wang M. et al., 2019), CDS (*chrysanthemyl diphosphate synthase gene*; Rivera et al., 2001; Liu et al., 2012a), F3′H (*flavonoid 3′-hydroxylase gene*; Zhao et al., 2013), LFY (*LEAFY*; Ma et al., 2016), NAM (*No Apical Meristem*; Sha et al., 2017) and UEPI (*gene of ubiquitin extension protein*; Annadana et al., 2002). Polymorphic regions mostly covering introns and 5′UTRs of five of the six genes were amplified using conserved primer pairs that were developed according to sequences acquired from GenBank (Table 1). To isolate *AGO1* orthologs from the species of interest, we downloaded the *AGO1* sequence of *Helianthus tuberosus* and then ran a local BLAST in the genome data of *Ch. nankingense* (Song et al., 2018). The primer pair was designed based on the orthologous sequence of *Ch. nankingense* (Table 1). All PCR products were ligated into pGEM-T vectors (Promega, United States) and cloned. At least 4–6 positive clones were randomly taken for sequencing. DNA sequences obtained by this study are deposited in GenBank with accession numbers MW344433–MW344631 (*AGO1*), MW195142–MW195312 (*BRC1*), MW543604–MW543703 (*CDS*), MW543450–MW543603 (*F3′H*), MW011041–MW011206 (*LFY*), MW195313–MW195497 (*NAM*), MW344310–MW344432 (*UEPI*), and MW545598–MW545801 (nrITS).
TABLE 1 | Primers for the amplification of nuclear genes in this study.

| Locus | Primer name | Primer sequence | Developed by | Accession No. of the ref. sequence |
|-------|-------------|-----------------|--------------|-----------------------------------|
| AGO1  | AGO1_CKR    | AAAAGGGAGAGGCCGCCAGCTAT | This study    | MG710521.1                        |
|       | AGO1_utR    | AGCCACAGCAAGAAGGCTAT | This study    |                                   |
| BRC1  | BRC1aR      | AATCTCAAACACCCCGACACT | This study    | JX870411.1                        |
|       | BRC1aF      | CCATATTTCCTCATTTCGGTT | Liu et al., 2012a |                                 |
| CDS   | CDS II      | CTGTTTCTGTCATATGTGCC | Liu et al., 2012a |                                 |
|       | CDS Vb      | TGGCTCTTCAATCTGTTCCG | Liu et al., 2012a |                                 |
|       | CDS Ila     | ATGTTATGTCCATGTGATG  | Liu et al., 2012a |                                 |
|       | CDS Vs      | CAGATGGTGGAGATGAATT  | Liu et al., 2012a |                                 |
| F3’H  | F3’H_int2F  | GCTGATATTGAAGGTGGGAAGCT | This study    | MF663713.1 and AB523844.1        |
|       | F3’H_int2R  | AATGAGTGGCTATTGGCCATT | This study    |                                   |
| ITS   | ITS1        | AGAAACTGCAAAGGTTTCCCAG | Li et al., 2014 |                                   |
|       | ITS4        | TCTCGTCCTATTGATATGC  | Li et al., 2014 |                                   |
| LFY   | LFY_int2F   | TGTTCTGAGCTTTGCTCAAGT | This study    | KF151334.1                       |
|       | LFY_int2R   | TGGTTGTGAGGACATACCAT | This study    |                                   |
| NAM   | NAM_int2R   | CTCTTCCTGACACACAGTGAAT | This study    | KX722453.1                       |
|       | NAM_int2F   | TGGTTATGCGATATGTCCTT | This study    |                                   |
| UEP1  | UEP1-R      | AGATCCATCAATTGGGTCCCAAT | This study    | EU862325                         |
|       | UEP1-F      | GCCCACACCATATAAAGCCCATT | This study    |                                   |

TABLE 2 | Sequence information of the present phylogenetic reconstruction.

| Dataset | Locus | Num. of taxa | Num. of sequences | Aligned length | Variable sites | Parsimony informative sites | Model selected by BIC |
|---------|-------|--------------|-------------------|----------------|----------------|----------------------------|-----------------------|
| Subtribe Artemisiinae | AGO1 | 101 | 198 | 805 | 459 (57.0%) | 291 (36.1%) | TN + G |
|         | BRC1 | 101 | 170 | 1229 | 790 (64.3%) | 534 (43.4%) | TN + G + I |
|         | F3’H | 101 | 153 | 289 | 189 (65.4%) | 154 (53.3%) | HKY + G + I |
|         | ITS  | 101 | 101 | 403 | 191 (47.4%) | 124 (30.7%) | TN + G |
|         | LFY  | 101 | 164 | 1464 | 937 (64.0%) | 664 (45.4%) | HKY + G + I |
|         | NAM  | 101 | 161 | 751 | 543 (72.9%) | 369 (49.1%) | GTR + G |
| Chrysanthemum-group | AGO1 | 57 | 120 | 808 | 271 (33.5%) | 126 (15.6%) | TN + G |
|         | BRC1 | 57 | 103 | 1210 | 437 (36.1%) | 219 (18.1%) | TN + G + I |
|         | CDS  | 57 | 101 | 356 | 157 (44.1%) | 69 (19.4%) | HKY + G + I |
|         | F3’H | 57 | 83 | 557 | 238 (42.7%) | 159 (28.5%) | HKY + G + I |
|         | ITS  | 57 | 57 | 403 | 81 (20%) | 30 (7.4%) | TN + G |
|         | LFY  | 57 | 98 | 1290 | 600 (46.5%) | 369 (28.5%) | HKY + G |
|         | NAM  | 57 | 107 | 751 | 361 (48.1%) | 183 (24.4%) | GTR + G |
|         | UEP1 | 57 | 124 | 503 | 290 (57.6%) | 164 (32.5%) | HKY + G |

Uncorrelated relaxed clock by setting two secondary calibration points with normal distributions: The first was the divergence time of the Eurasian-Mediterranean clade and the Asian-southern African clade (17.3 Ma ± 1 SD), and the second was the age of the crown clade of Artemisiinae (9.7 Ma ± 1 SD) according to Oberprieler (2005). To test the influence of different time-point settings on molecular dating, the secondary calibration point was set according to Tomasello et al. (2015) (the divergence time of Artemisiinae and Santolininae as 18 Ma ± 1 SD; or the divergence time of Artemisiinae-Santolininae-Glebionidinae and other Eurasian lineages as 22.5 Ma ± 1 SD). Moreover, the fossil records of Artemisia-like pollens were also considered for calibration. We set tmrca prior at 13 Ma ± 1 SD for the crown lineage of Artemisiinae (the stem node of lineages sharing Artemisia-type pollens, e.g., Artemisia, Elychanchemum, and Phaeostigma) according to the records of the commonly occurred Artemisia-like pollen fossils (Wang and Zhang, 1990; Ma et al., 2005). Trees were sampled every 10000 generations for a total of 400 million generations. We checked for topological convergence and adequate ESS (>200) using Tracer v.1.7.1 (Rambaut et al., 2018). The consensus tree was exported using TreeAnnotator v.1.8.4, discarding the first 30% of trees as burn-in.

Then, all eight loci were applied to construct a tree of Chrysanthemum-group to better resolve relationships within this particular group following the same analytical method specified above.

Estimation of Diversification Dynamics

Subsequently, 5000 trees from the *BEAST analysis were resampled to construct a lineage-through-time (LTT) plot using
the R package ape v. 5.0 (Paradis and Schliep, 2019). Then, 1000 random trees were simulated under birth-death (BD) and pure-birth (PB) models using the R package geiger to avoid errors due to incomplete sampling (Pennell et al., 2014). These simulations were used to establish 95% confidence intervals on the LTTs for each model for comparison with the empirical dataset. The γ statistic for the optimal *BEAST* phylogeny was calculated using ape v. 5.0. Speciation rates were compared with the null hypothesis that a clad diversified at a constant rate using 2∗[1−pnorm(abs(gammaStat(tree)))] provided by the R package ape v. 5.0 for a two-tailed test (Paradis and Schliep, 2019). A significantly positive or negative γ value meant an accelerated/decelerated rate of speciation toward the present.

**Reconstruction of Ancestral Distributions and Character States**

To infer the biogeographic history of lineages in subtribe Artemisiinae, dispersal and vicariance analysis (s-DIVA), Bayesian binary MCMC analysis (BBM) and dispersal-extinction-cladogenesis (DEC, s-DEC) were conducted to estimate the optimized geographical distributions of internal nodes using RASP 4.0 (Yu et al., 2015). The distribution information of all samples was obtained from floristic works (Shih and Fu, 1983; Shishkin and Bobrov, 1995; Zhao et al., 2009) and herbarium specimens. Based on this information and major biogeographic boundaries, eight geographic units were defined: (A) Europe and the Mediterranean coast; (B) Middle Asia; (C) Central Asia; (D) the QTP region; (E) the southern part of East Asia; (F) the northern part of East Asia; (G) Korea-Japan; and (H) the pan-Arctic region (Spencer and Robinson, 1968; Xie et al., 2002; Cowan, 2007; Lu et al., 2018). The ranges of geographic units are as shown in Supplementary Figure 2. The current distribution of each species is marked by a particular color before the species name. For s-DIVA, s-DEC and DEC, 10000 trees that were resampled from the *BEAST* phylogenies after burn-in and the consensus tree were loaded into RASP to estimate the likelihoods of ancestral states at each internal node of the consensus tree. BBM was run with F81 state frequencies using gamma variation for 1,000,000 iterations. The reconstructed state was sampled every 1000 generations, and the first 10% was discarded as burn-in.

To estimate the ancestral states of flowerhead architectures, character states were mapped onto terminal branches of the consensus tree resulting from the *BEAST* analysis, and then searches for optimized states at the internal nodes were run with the likelihood model in Mesquite v. 3.5.2 and with the continuous-time Markov model using the phytools v. 0.7-70 package in R (Maddison and Maddison, 2009; Revell, 2012).

**Ecological Niche Modeling, Lineage Distribution Models and Niche Overlap Tests**

To analyze niche differentiation and to understand how the distributions of three major clades of *Chrysanthemum*-group changed during the last glacial maximum (LGM), niche modeling was conducted under recent climatic scenarios and LGM scenarios from the CCSM4 model (current climate at a 30 arcsec resolution and LGM at a 2.5 arcminute resolution). Ecological niche data including temperature and precipitation (19 bioclimatic variables) data that were drawn as climate layers were downloaded from WorldClim 1.4⁴. Coordinate information was collected from occurrence records in the Global Biodiversity Information Facility (GBIF⁵) and from herbarium specimen information acquired from NSII⁶. For every two occurrences, only one was kept if less than 1 km in distance. All of the coordinates used for inferring lineage distribution modes are listed in Supplementary Table 2. In total, the current environmental data of 1249 occurrence records, including 529 of *Ajania*, 529 of *Ch. indicum*-complex and 191 of *Ch. zawadskii*-complex were sampled. To avoid collinearity, Pearson pairwise correlation analysis of environmental factors was conducted. For each pair, one factor with a correlation value (|R|) higher than 0.75 was eliminated, and the result was visualized in SPSS 22 and Heml (Deng et al., 2014). We used lineage geographic distribution spots to estimate the environmental space of each of the three main subclades of *Chrysanthemum*-group with the PCA-env approach in SPSS 22 and R (R Core Team, 2013). Species distribution models (SDMs) were built under the maximum-entropy method implemented in MAXENT 3.3 following Papeš and Gaubert (2007) for parameter settings (25% of occurrence records were used as testing data) (Papeš and Gaubert, 2007; Phillips and Dudík, 2008). The area under the receiver operating characteristic curve (AUC) was used to evaluate the prediction performance of the models (Phillips et al., 2006). AUC values > 0.75 indicate good predictions, whereas values < 0.5 suggest poor predictions (not better than random). We also used the true skill statistics (TSS) to evaluate the accuracy of the resulting distribution models (Liu et al., 2005; Allouche et al., 2006), where TSS values ranging from 0.4 to 0.8 are indicative of good model performance (Landis and Koch, 1977; Fielding and Bell, 1997). The 10-percentile training presence threshold was used to generated binary map. Subsequently 10000 random geographic points were extracted to calculated TSS values for each niche model in R. Moreover, DIVA-GIS v. 7.5 was applied to model potential distributions under the current scenario to ensure the reliability of SDMs (Hijmans et al., 2001). To quantify climatic niche overlap, Schoener’s D-index (Schoener, 1970) and the modified Hellinger distance I-index (Van der Vaart, 2000) were calculated in R. Subsequently, niche equivalency and similarity tests (Evans et al., 2009; Warren et al., 2008) were performed between subclades using 50 permutations in the R packages phyloclim v. 0.9.5 and ENMTools v. 1.0.2 (Warren et al., 2010; Heibl and Calenge, 2013). To analyze pairwise differentiation in detail on every explanatory bioclimatic variable resulting from analysis by PCA-env, the profiling niche occupancy (PNO) of each climatic factor was computed, and then the D-index and the modified I-index were calculated using the phyloclim v. 0.9.5 package in R (Heibl and Calenge, 2013).

⁴https://www.worldclim.org
⁵https://www.gbif.org
⁶http://www.nsii.org.cn/2017/home.php
RESULTS

Sequence Characteristics
Despite repeated amplifications, we failed to isolate the target CDS fragment from some species, e.g., Artemisia frigida and Seriphidium finitum, and we failed to isolate its complete open reading frame in Nipponanthemum nipponicum, Hippolytia spp. We were not able to obtain UEP1 from Stilpnolepis centiflora and the outgroup taxa. Therefore, the analyses were conducted separately with two datasets, with one excluding CDS and UEP1 for all the sampled taxa of subtribe Artemisiinae and the other including all eight loci but limited to Chrysanthemum-group. For each of the datasets, the number of species/taxa included, the number of haplotypes obtained at each locus and other information related to phylogenetic analyses are given in Table 2.

Phylogeny of Subtribe Artemisiinae and the Estimation of Diversification Dynamics
The coalescent species tree of Artemisiinae overall was inferred from six of the eight markers (Figure 1). Rooted by outgroup species from the Eurasian-Mediterranean clade of tribe Anthemideae (Oberprieler et al., 2007), the tree showed subtribe Artemisiinae as monophyletic. Except for the basal monotypic genus Stilpnolepis, two main clades, Clades I and II, were found (Figure 1: posterior probabilities PP = 0.78 and 1, respectively). These two clades roughly corresponded to but differed slightly in circumscriptions from the Artemisia-group and Chrysanthemum-group traditionally defined by morphological characteristics. The species of Artemisia and its allies, e.g., Filifolium and Crossostepheum, were found in Clade I. However, the broadly defined Artemisia was not supported as monophyletic. Several genera with radiate capitula but uncertain phylogenetic positions, such as Leucanthemella, Nipponanthemum, Hippolytia, Brachanthemum, and Tanacetum tatsuianense, fell into this clade. Two subclades, Ia and Ib, were further recognized with high posterior probability (PP = 1). Notably, the present data showed that species of Phaeostigma were clustered in subclade Ia, supporting the proposal that they were independent of Ajania but were a lineage within Artemisia (Figure 1). Subclade Ib contained species formerly described under Artemisia subgenus Dracunculus, as well as Kaschgaria and Brachanthemum (with radiate capitula) (Figure 1).

Clade II included Chrysanthemum and Ajania and two small genera, Elachanthemum and Opisthopappus. Two sister branches, Ia and Iib were also found in Clade II: Elachanthemum, the only member of Iib, is different from Chrysanthemum and Ajania by having a discoid capitulum and an annual life form. Interestingly, Opisthopappus, which was formerly treated in subtribe Tanacetinae and then were incorporated into Artemisiinae (Bremer and Humphries, 1993; Oberprieler et al., 2009), was clustered in Iia; morphologically, this small genus is indeed similar to Chrysanthemum by having radiate capitulum and myxogenic achenes (Figures 1, 2).

The chronograms achieved using relaxed molecular clock analyses showed the time frames were largely similar among four different calibration settings (Supplementary Figure 3). According the chronogram inferred from setting two secondary calibration time points suggested that subtribe Artemisiinae began to diverge at approximately 10 Ma ago, and most of its major lineages diversified during the late Miocene, particularly densely at approximately 7-9 Ma ago (Figure 1). Artemisia-group split from Chrysanthemum-group at approximately 9 Ma ago and then diversified immediately, while the latter group diversified much later. Within Chrysanthemum-group, the monotypic genus Elachanthemum diverged much earlier during the mid-Pliocene (Figure 1, median age 4.90 Ma, 95% HPD 4.10-5.70 Ma), while other species seemed to have undergone evolutionary radiation since the boundary between the Tertiary and Quaternary (median age 2.22 Ma, 95% HPD 1.76-2.58 Ma). The LTT plots highlighted a significant speedup of diversification of subtribe Artemisiinae (γ = 4.50, p < 0.001), which was largely correlated with the cooling climate estimated by the δ18O content (Figure 1; Hansen et al., 2013). The time frame of evolution of Chrysanthemum-group was in concordance with the intensive aridification of Middle to Central Asia that was probably due to late-Miocene to Pliocene geographic activity of the northern region of QTP and the global climatic changes (Figure 1).

Internal Relationships Within the Chrysanthemum-Group
To better resolve relationships within Chrysanthemum-group, all eight nuclear gene sequences were used. As a result, two major genera, Chrysanthemum and Ajania were well separated into two clades (PP > 0.99) with a few exceptions (Figure 2), which provided important support for traditional taxonomy that treats them as independent genera mainly according to capitulum morphology. In Chrysanthemum, two Chinese endemic species with white ray flowers, Ch. rhombifolium and Ch. vestitum, formed a small clade sister to Ch. indicum-complex with yellow or white ray flowers (Figure 2). In the clade of Ch. indicum-complex, two branches, a and b, were recognized—species in ‘b’ basically all had island distributions, while species in ‘a’ were mostly distributed in mainland China (Figure 2). Another relatively large group is the so-called Ch. zawadskii-complex (purple-colored in the tree, PP = 0.99, Figure 2). This group is characterized by white to purple ray flowers with continuous color variation. Species of Opisthopappus, which were shown at the basal position of Chrysanthemum-group in the ITS and plastid DNA tree, fell into this clade. In contrast to Chrysanthemum, relationships within Ajania were still ambiguous, with most branches being poorly supported.

Reconstructions of Ancestral Distributions of Chrysanthemum-Group
To explore the historical process of diversification of Chrysanthemum-group, the ancestral distribution state of each of the major nodes was estimated based on their recent distributions acquired from floristic works and specimen information. The Bayesian BBM analysis, s-DIVA analysis and DECs produced similar results regarding the ancestral area of Artemisiinae (Table 3 and Supplementary Table 3). Eight
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FIGURE 1 | Coalescent species tree inferred from six nuclear gene sequences and the lineage through time (LTT) plot of subtribe Artemisiinae. The tree was constructed in *BEAST and rooted by four Eurasian-Mediterranean (E.-M.) species of tribe Anthemideae. Bayesian posterior probabilities (>0.5) are indicated above branches. Two secondary calibration time points used for estimating divergence times are marked with gray squares. Five major clades are highlighted by blue circled numbers, and their estimated divergence times are noted in the upper-left box. Blue, green, and yellow blocks indicate different geologic ages. The dotted square with N1–N8 inside indicates the nodes analyzed for biogeographic history, as shown in Table 3. Abbreviations of genus names: Ch., Chrysanthemum; Op., Opisthopappus; Aj., Ajania; El., Elachanthemum; Ar., Artemisia; Ph., Phaeostigma; Cr., Crossostephium; Se., Seriphidium; Le., Leucanthemella; Nm., Mauranthemum; Sa., Santolina; Ar., Argyranthemum; Ch., Chrysanthemum; Op., Opisthopappus; Aj., Ajania; El., Elachanthemum; Ar., Artemisia; Ph., Phaeostigma; Cr., Crossostephium; Se., Seriphidium; Le., Leucanthemella; Nm., Mauranthemum; Sa., Santolina; Ar., Argyranthemum. The LTT plot using the uncorrected relaxed clock model in *BEAST is shown in the upper-left corner in gray, where the red line indicates the LTT plot of the maximum credibility tree for the *BEAST analysis. Lineages accumulated over time under the pure-birth model are marked in blue, and those under birth-death are marked in pink. The thin dotted line represents the decrease in the environmental temperature during the time frame of diversification of subtribe Artemisiinae, referring to Hansen et al. (2013). Statistic γ takes on positive values when there are accelerated speciation rates toward the present; p indicates the significance value (<0.001). Two brown columns mark the periods of uplift of the Northern QTP during the late Miocene.
FIGURE 2 | Coalescent tree of the Chrysanthemum-group inferred from eight nuclear gene sequences. Bayesian posterior probabilities higher than 0.5 are indicated on branches. States of important morphological characters (represented by small squares in different colors) are mapped at the right side of terminal nodes. In this tree, two subclades, Ch. indicum-complex and Ch. zawadskii-complex, can be recognized within Chrysanthemum. Naming of the two Chrysanthemum subclades follows Liu et al. (2012b).

geographic units and optimal area reconstruction at each major node are summarized in Supplementary Figure 2. In RASP, both s-DIVA and BBM favored ancestral distributions of most of the major clades of Artemisiinae (N1-N8) in Central Asia (region C; > 50%) (Table 3 and Supplementary Figure 2). Subsequent vicariance between Central Asia and other regions of Asia was reconstructed by s-DIVA for node N3 and N5, and dispersal from Central Asia to the QTP and eastern parts of Asia was reconstructed for nodes N4, N6-N8 and Chrysanthemum-group

TABLE 3 | Biogeographic history of subtribe Artemisiinae inferred by reconstructing ancestral distributions with BBM and s-DIVA.

| Node | s-DIVA Event | BBM Event |
|------|--------------|------------|
| N1   | C (94.19%)   | C (91.41%) |
| N2   | C (100%)     | C (96.12%) |
| N3   | C (91.87%)   | Vicariance |
| N4   | C (63.17%)   | Dispersal  |
| N5   | C (65.52%)   | Vicariance |
| N6   | C (85.95%)   | Dispersal/Vicariance |
| N7   | C (22.17%)   | Dispersal/Vicariance |
| N8   | C (56.61%)   | Dispersal  |

N1-N8 are indicated in both Figure 1 and Supplementary Figure 2 near the corresponding nodes.

The time-effect curve reconstructed with BBM showed that the dispersal curve had extremely high peaks over the last 3 Ma (Supplementary Figure 2).

SDMs and Niche Differentiation Among Lineages Within Chrysanthemum-Group

To reduce multicollinearity, one factor of each pair of environmental factors was eliminated when the correlation coefficient of that pair was larger than 0.75. Thus, six factors were finally selected: isothermality (bio3), temperature seasonality (bio4), mean temperature of the wettest quarter (bio8), mean temperature of the driest quarter (bio9), annual precipitation (bio12) and the precipitation of the driest quarter (bio17)
(Figure 3 and Supplementary Figure 4A). The variable loadings for PCA-env are shown in Table 4. The first two PCs explained 81.8% of the niche variation among subclades (51.1 and 30.7%, respectively). PC1 is dominated by the absolute temperature and precipitation variables (bio9, bio12, bio17) while PC2 by the two variables describing oscillations of temperature (bio3, bio4) (Table 4). The PCA-env assay suggested great variability in the environmental space inhabited by the different subclades within Chrysanthemum-group, especially between Ajania and Ch. indicum-complex (Figure 3 and Supplementary Figure 4).

Furthermore, the potential distribution of subclades within Chrysanthemum-group was predicted through MAXENT and DIVA-GIS. The results from both approaches were, in principle, similar, with the area predicted by DIVA-GIS being slightly restricted (Figure 4 and Supplementary Figures 5A-C). The AUC values for the replicate runs in MAXENT were 0.905 ± 0.003, 0.930 ± 0.003, and 0.907 ± 0.009 for Ajania, Ch. indicum-complex and Ch. zawadskii-complex, respectively. Establishing the threshold probability for niche models using TSS of each of three subclades resulted in 0.72 ± 0.055, 0.77 ± 0.047, and 0.70 ± 0.092. Lineage distribution models indicated that each lineage occurred over different geographical areas with more or less contact areas at margins (Figure 4). The suitability map reconstructed based only on the occurrences of three aberrant Ajania species of Korea-Japan distribution was congruent with those of other Ch. indicum-complex species (Figures 2, 4 and Supplementary Figure 5D).

Considering the predicted distribution of the modeled lineages (Figure 4), the comprehensive pairwise D-values among lineages ranged from 0.34 to 0.4 (Table 5), suggesting ecological differentiation (Figure 4 and Table 5). The background niche similarity tests indicated that the observed overlaps were greater than 95% of the simulated values, suggesting that lineages occupy an ecological space that is more similar to each other than expected by chance (Table 5). However, the null hypothesis of niche equivalency was rejected for all comparisons between subclades. The profiling niche occupancy (PNO) of each ecological factor was computed for the detailed adaptation patterns (Supplementary Table 4). As a result, Ajania had the lowest niche overlap with two subclades of Chrysanthemum in precipitation in the driest quarter and isothermality (D-values of 0.4 and 0.46) (Figure 5 and Supplementary Table 4). Two Chrysanthemum subclades greatly diverged in temperature seasonality and mean temperature in the driest quarter (Figure 5 and Supplementary Table 4, D-value of ca. 0.46).

DISCUSSION

Clarifying Phylogenetic Positions of Some Taxa in Subtribe Artemisiinae

Despite a series of cladistical studies based on morphological and molecular information, there remain quite a number of taxa whose systematic positions are unclear in subtribe Artemisiinae (Bremer and Humphries, 1993; Torrell et al., 1999; Martin et al., 2001; Vallès and McArthur, 2001; Vallès et al., 2003; Oberprieler et al., 2007; Sanz et al., 2008; Zhao et al., 2010a,b). Phaeostigma is one such taxon. It was separated from Ajania as an independent genus by Muldashev (1981). However, neither morphological data nor molecular phylogenies could resolve its relationships with Ajania, Artemisia, and Chrysanthemum (see Huang et al., 2017 and references therein). By integrating morphological analyses of 20 characters, and palynological and molecular data, Huang et al. (2017) circumscribed a monophyletic Phaeostigma by including three species formerly named under Ajania, which was in line with the proposal of Bremer and Humphries (1993) that the circumscription of Phaeostigma should be extended to some members of Ajania. Moreover, Huang et al. (2017) supposed that Phaeostigma might be phylogenetically closer to Artemisia
FIGURE 4 | Potential distribution area of Ajania and two subclades of Chrysanthemum predicted by the maximum-entropy model. (A–C) Represent the predicted distributions of Ajania, Ch. indicum-complex and Ch. zawadskii-complex, respectively, during the current period (left) and during the Last Glacial Maximum (LGM) (right). Shadings in color show the probability of geographic occurrences. The prediction for the Korea–Japan distributed Ajania species is shown in Supplementary Figure 5.

than to Ajania (Huang et al., 2017; Chen et al., 2020), which was strongly supported by the present analysis, that is, Phaeostigma was nested in the clade of Artemisia-group (Figure 1). Nevertheless, the similarity between Ajania and Phaeostigma in terms of characteristics such as synflorescence and capitulum, as well as in diversification ages and distribution patterns, implies that they may have experienced convergent evolution under similar habitats (Figures 1, 2 and Supplementary Figure 2). More detailed studies are required to determine the adaptive significance of these traits.

Opisthopappus is another taxon worth discussion. It was placed in subtribe Tanacetinae by Bremer and Humphries (1993) mainly due to the presence of pappi or coronas on achenes but was different from other members of Tanacetinae according to its myxogenic achenes. Its systematic position in subtribe Tanacetinae was called into question by the present (Figure 2) and previous (Zhao et al., 2010a,b) molecular data that suggested a closer relationship to Chrysanthemum and Ajania. Besides, several studies reported crossability between Opisthopappus and species of Chrysanthemum-group, e.g., Ch. lavandulifolium, Ch. dichrum, A. pallasiana, and Elachanthemum intricatum and A. pacifica x Ch. vestitum (Hu and Zhao, 2008; Yang et al., 2010; Zheng et al., 2013). Combining all the evidence, we suggest a taxonomic treatment to incorporate Opisthopappus into Chrysanthemum-group. Nevertheless, to clarify its phylogenetic position, more data of morphological and ecological traits, reproductive biology and phylogenomics are required.
Comparisons Niche similarity P-values and equivalency via randomization test: **The airy and equivalency of subclade a with subclade b in terms of Schoener’s D and pairwise niche overlap values are presented for the comparison of niche similarity $D_{A b}$ ($D$-index) ($D$-index) a vs. b b vs. a equivalency

| Comparisons | Niche overlap | Niche overlap | Niche similarity | Niche |
|-------------|---------------|---------------|------------------|-------|
| A b         | 0.3389        | 0.5312        | Similar**        | Similar** |
| A j Cj      | 0.3948        | 0.5827        | Similar**        | Similar** |
| A j Cz      | 0.4101        | 0.6017        | Similar**        | Similar** |

Pairwise niche overlap values are presented for the comparison of niche similarity and equivalency of subclade a with subclade b in terms of Schoener’s D and modified Hellinger distances (j-indices).

** Aj, Ajania Cj, Ch. indicum-complex Cz, Ch. zawadski-complex.

Niche similarity P-values and equivalency via randomization test: **The comprehensive niche is significantly ($P < 0.01$) more similar or different than expected due to randomness. Pairwise niche overlap values for each essential niche are also shown.

The systematic positions of Hippolytia, Nipponanthemum, Leucanthemella, Brachanthemum, and Tanacetum tatisienese have also been the subjects of much debate. Hippolytia and Tanacetum were formerly described in subtribe Tanacetinae, while Nipponanthemum and Leucanthemella were classified into subtribe Leucantheminae, but all were later shown to belong to subtribe Artemisiinae according to molecular phylogenetic data (Vallés and McArthur, 2001; Watson et al., 2002; Vallés et al., 2003; Oberprieler, 2005; Oberprieler et al., 2007, 2009; Masuda et al., 2009). Using multiple nuclear loci with a coalescent analytical method, we found that they belonged to Artemisiinae, which was supported by our analysis. Meanwhile, our analysis showed that Hippolytia, Nipponanthemum, Leucanthemella, Tanacetum tatisienese, and Brachanthemum were nested into different branches of Artemisia-group (Figure 1) rather than being basal grades of Artemisiinae, as suggested by previous ITS-ETS data (Watson et al., 2002; Sanz et al., 2008; Zhao et al., 2010a).

**Evolution of the Capitulum Architecture in Subtribe Artemisiinae**

Subtribe Artemisiinae is one of the youngest lineages in the daisy family, and it appears as an assemblage of taxa of plesiomorphic features, especially with regard to pollination syndrome, including capitulum architectures and affiliated features (Harris, 1999; Martin et al., 2001; Sanz et al., 2008; Pellicer et al., 2010; Huang et al., 2017). In terms of capitulum architecture, three types can be observed in wild Artemisiinae species: radiate, disciform and discoid. The radiate capitula are composed of many central disk florets and peripheral conspicuous ray flowers, whereas, the discoid consist of bisexual disk flowers only, and the disciform are discoid-like but with a few tubular female marginal florets). The evolutionary orientation of these capitulum types has remained a key question in teasing apart historical patterns of lineage divergence and/or hybridization within Artemisiinae. Bremer and Humphries (1993) regarded the radiate capitulum as the plesiomorphy of Artemisiinae and the discoid as a derivative of the disciform, rather than evolving directly from the radiate. However, ancestral state reconstruction based on the ITS-ETS tree suggested that the discoid was the plesiomorphy of Artemisiinae (Sanz et al., 2008).

In the present study, using a similar strategy, we tried to detect the direction of capitulum evolution in Artemisiinae. The results also showed discoid or disciform flowerheads as ancestral states (Supplementary Figure 6). However, model-based ancestral character reconstruction of a particular trait may not provide really accurate information without mechanism evidences, because missing data of extinct taxa or bias in models for state transition might influence the accuracy of the inference (Omland, 1999; Griffith et al., 2015). A clear answer to the problem should rely on evidence from evolutionary developmental genetic studies. Trow (1912), as well as Ford and Gottlieb (1990) examined the inheritance of rayed and rayless heads in Senecio and Layia, respectively, and suggested that the switch between the presence and absence of ray flowers may be governed by simple genetic rules. Later molecular genetic studies indicated that the CYC2-mediated module participates in inflorescence repatterning (Broholm et al., 2008; Chapman et al., 2008, 2012; Kim et al., 2008; Tähtiharju et al., 2012; Chen et al., 2018), and the rise of rayless capitula is always linked to a reduction in the expression or gene loss of one or more CYC2 members (Chen et al., 2018). Our recent analyses demonstrated that dysfunction of CYC2g led to a shift from the radiate flowerhead to the disciform head in Chrysanthemum-group (Shen et al., 2021). Therefore, the radiate should be the ancestral state of the capitulum in subtribe Artemisiinae, despite Stilpnolepis centiflora at its basal position possessing discoid flowerheads (probably an autoapomorphic state). The shifts from radiate to disciform capitula may have happened repeatedly during the diversification of this group. Early on, in 1961, Tzvelev considered that Ajania and Artemisia were convergent lineages evolved independently from Chrysanthemum-like ancestors. Similarly, our phylogenetic tree suggested that convergent capitula evolution may also have occurred within the Artemisia-group (Figure 1). For example, the ‘disciform’ capitula in Artemisia look similar with, but may have different developmental underpinnings from that of Ajania. Therefore, the contradiction between the reconstructed ancestral state of capitulum of subtribe Artemisiinae (Sanz et al., 2008) and the evo-devo insights is probably due to the morphological convergence.

To date, there have been no data demonstrating whether there is a transition from the discoid to the disciform capitula. During our previous morphogenetic study (Ren and Guo, 2015), a uniformly acropetal developmental sequence was found on the homogamous discoid capitulum, but basipetal development probably occurred in the marginal florets of the heterogamous radiate/disciform flowerhead. This was also postulated by Harris (1995). Although functional assays of chrysanthemum CICYG2c and sunflower $HaCYC2c$ showed that downregulating the expression of these genes led to the appearance of dorsal petals and stamens in ray florets, we assume that the developmental process of the discoid capitulum might be completely different from that of the radiate/disciform due to lacking a module responsible for the development of zygomorphic or asymmetric marginal female flowers, namely, a marginality identity module (Pozner et al., 2012; Zhao et al., 2016; Elomaa et al., 2018; Zoulias et al., 2019; Shen et al., 2021). The finding of our previous study...

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**Table 5 | Ecological niche comparison among three subclades of the Chrysanthemum-group.**

| Comparisons | Niche overlap | Niche overlap | Niche similarity | Niche |
|-------------|---------------|---------------|------------------|-------|
| A b         | 0.3389        | 0.5312        | Similar**        | Similar** |
| A j Cj      | 0.3948        | 0.5827        | Similar**        | Similar** |
| A j Cz      | 0.4101        | 0.6017        | Similar**        | Similar** |

Pairwise niche overlap values are presented for the comparison of niche similarity and equivalency of subclade a with subclade b in terms of Schoener’s D and modified Hellinger distances (j-indices).

** Aj, Ajania Cj, Ch. indicum-complex Cz, Ch. zawadski-complex.

Niche similarity P-values and equivalency via randomization test: **The comprehensive niche is significantly ($P < 0.01$) more similar or different than expected due to randomness. Pairwise niche overlap values for each essential niche are also shown.
that the \textit{CYC2g} gene was lost in the discoid species \textit{Stilpnolepis centiflora} (Chen et al., 2018; Shen et al., 2021) may be a hint for this hypothesis. Certainly, to test this hypothesis, more detailed molecular developmental studies are required.

### Flowerhead Morphology Associated With Climatic Niche Divergence as a Driver of Species Diversification of \textit{Chrysanthemum}-Group

As mentioned above, the relationship between \textit{Chrysanthemum} and \textit{Ajania} and their patterns of diversification and evolution have remained highly controversial. Considering their distinct capitulum morphology and geographic distribution, with members of \textit{Ajania} being more western than \textit{Chrysanthemum}, and members of \textit{Ch. indicum}-complex being more southeastern than \textit{Ch. zawadskii}-complex (Figure 4 and Supplementary Figure 5), we postulate that the three main taxa must have experienced morphological differentiation under niche divergence.

From the environmental aspect, different landscape features and climate patterns can drive directional selection (Sobel et al., 2010; Rundle and Nosil, 2012). According to *BEAST analysis, separation between \textit{Chrysanthemum}-group and \textit{Artemisia}-group
occurred around the late Miocene (ca. 9 Ma, Figure 1), and the radiation within Chrysanthemum-group was dated to ca. 2 Ma. The time frame largely corresponded to the period of the profound climatic change in Asia interior, i.e., extremely intensive aridification in Middle to Central Asia demonstrated by paleoenvironmental data on loess and clay deposits (An et al., 2001; Ma et al., 2005; Nie et al., 2014; Wang X. et al., 2016), which may be due to the synergy of recently geological activity of Northern QTP (Miao et al., 2012; deposits (An et al., 2001; Ma et al., 2005; Nie et al., 2014; demonstrated by paleoenvironmental data on loess and clay deposits). The time frame largely corresponded to the Chrysanthemum occurrence around the late Miocene (ca. 9 Ma, Figure 1), and the radiation within Chrysanthemum-group was dated to ca. 2 Ma. The time frame largely corresponded to the period of the profound climatic change in Asia interior, i.e., extremely intensive aridification in Middle to Central Asia demonstrated by paleoenvironmental data on loess and clay deposits (An et al., 2001; Ma et al., 2005; Nie et al., 2014; Wang X. et al., 2016), which may be due to the synergy of recently geological activity of Northern QTP (Miao et al., 2012; Favre et al., 2015; Shi et al., 2019; Yang et al., 2019). Our DIVA analysis also indicated the origin of Chrysanthemum-group in Central Asia, followed by an eastward dispersal of the Chrysanthemum lineage, and in situ diversification or colonization of the Ajania lineage along with tectonic activities of QTP (Table 3 and Supplementary Figure 2; Chen et al., 2020). Thus, the late Miocene origin and evolution of Chrysanthemum-group was strongly influenced by the establishment and development of climatic heterogeneity in Central-East Asia.

In Asteraceae, alterations of capitulum forms are closely associated with shifts in reproductive strategies, and therefore, variations in capitulum architecture should be consequences of trade-offs between energy consumption and adaptation in response to environmental stresses (Stuessy et al., 1986; Berry and Calvo, 1989; Andersson, 1999; Nielsen et al., 2002; Chen et al., 2020). Our previous analyses have demonstrated that the radiate flowerhead with white ligules (of Chrysanthemum zawadskii-complex) represents an earlier evolutionary status, and the disciform flowerhead of Ajania evolved from the radiate at approximately 8 Ma, somewhat overlapping with the time when Chrysanthemum-group arose but occurring much earlier than the divergence time of Chrysanthemum and Ajania (Figure 1 and Supplementary Figure 7). This suggests that capitulum polymorphism had long existed in the ancestral populations of the Chrysanthemum-group. Comparing the present multilocus coalescent tree and the tree based on CYC2g sequences of Chrysanthemum-group (Shen et al., 2021), we found a high consensus of topologies, implying that the evolution of this group is linked to the alteration of flowerhead morphology (Supplementary Figure 7). Within Chrysanthemum-group, low pairwise niche overlaps were found between subclades, although they diverged rather recently (Figures 4, 5). The ecological suitability diagrams based on the current climate scenario suggested that the Ajania lineage preferred environments with lower yearly mean temperatures and precipitation levels, together with higher climatic instability, compared to the two Chrysanthemum lineages, which is consistent with the habitat shift between the plants being related to anemophily and entomophily (Culley et al., 2002) and is concordant with the good differentiation of their capitulum morphologies (Figure 5).

Shown in Figure 2, three Korea-Japan distributed Ajania species, A. pallasiana_JJ, A. pacifica, and A. shiwogiku, fell into the clade of Ch. indicum-complex. This could be due to incomplete lineage sorting of genes analyzed here. We found that the sequences of their CYC2g genes were highly similar to those of A. parviflora (Shen et al., 2021; Supplementary Figure 7) which is a member distributed in the eastern part of the range of Ajania. However, the mix-up of three Ajania species with Chrysanthemum is more likely due to secondary contacts as we found all three species are polyploids with high ploidy levels from 2n = 6x to 8x and to 10x, suggesting complicated origins probably involving parentages from Ch. indicum-complex. The present SDM analysis suggested a broader geological overlap between Ajania and Chrysanthemum during the LGM (Figure 4), implying possible introgression between the two recently diverging lineages because morphological differentiation and reproductive barriers between them might still be incomplete (Comes and Kadereit, 1998; Potts et al., 2003; Hewitt, 2004; Li et al., 2013; Chen et al., 2020).

Overall, considering the divergence time, ancestral distribution, and niche differentiation data, we propose that the divergence of three major groups in Chrysanthemum-group, Ajania, Ch. indicum-complex, and Ch. zawadskii-complex, probably occurred in a rather short time span in response to environmental heterogeneity in interior East Asia, which might be related to divergence of capitulum types.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

C-ZS conceived the study, performed the most of the experiments, and data analyses. C-JZ and JC participated in the experiments. C-ZS and Y-PG wrote the manuscript. All the authors have read and approved the manuscript.

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Bayesian trees of the single-copy nuclear genes.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.648026/full#supplementary-material

Supplementary Figure 1 | Bayesian trees of the single-copy nuclear genes.

Supplementary Figure 2 | Historical biogeographical analyses of subtribe Artemisiinae.

Supplementary Figure 3 | Time frame comparison among four different calibration settings.

Supplementary Figure 4 | Principal component analysis (PCA) of environmental factors of the Chrysanthemum-group.

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Supplementary Figure 5 | Potential suitability distribution of climate conditions of major lineages within the Chrysanthemum-group.

Supplementary Figure 6 | Ancestral state reconstruction of capitulum architectures based on the phylogenetic framework under likelihood model (right) and continuous-time Markov model (left).

Supplementary Figure 7 | Comparison of the gene tree of CYC2g that regulates capitulum architectures in subtribe Artemisiinae and the present multilocus phylogeny (left, redrawn from Figures 1, 2 in the main text).

Supplementary Table 1 | Species used in the present study and the sampling information.

Supplementary Table 2 | GPS coordinates used for niche modeling in this study.

Supplementary Table 3 | Biogeographic history of subtribe Artemisiinae reconstituted with DEC and s-DEC.

Supplementary Table 4 | Parwise niche overlap among three subclasses of the Chrysanthemum-group under predicted niche occupancy profiles (PNO).

Supplementary Data Sheet 1 | Datasets of the sequence alignments.

SUPPLEMENTARY MATERIAL

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