

Temporal origins and diversification of *Artemisia* and allies (Anthemideae, Asteraceae)

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Editor: M. Galbany Casals

Received 14 January 2011; Accepted 15 March 2011

Abstract

Temporal origins and diversification of *Artemisia* and allies (Anthemideae, Asteraceae).- To assess temporal origins and diversification of lineages within subtribe Artemisiinae and *Artemisia* group a penalized likelihood analysis was applied on nrDNA ITS and ETS of 63 representatives. The tree was calibrated at the stem node of the *Kaschgaria/Artemisia* lineage with the most reliable early *Artemisia* fossil pollen record from Late Oligocene (23 Ma). The results from this study suggest that the origin of the subtribe goes back to the Late Oligocene (24.6 ± 2.6 Ma) whilst the onset of differentiation of the genus *Artemisia* and most closely related genera is dated to the Early Miocene (19.8 ± 2.3 Ma). Divergence ages for lineages within the *Artemisia* group are often between the Early and Middle Miocene, whereas their radiations mostly occurred in the Late Miocene and Pliocene. The temporal context was also used to examine biogeographic and morphological (capitula and pollen type) evolution. Within the *Artemisia* group all lineages except the North American endemic have colonized the Mediterranean Basin at different epochs from Asian ancestors. Our analyses suggest the divergence of the North American endemic group from Asian ancestors (10.8 ± 1.5 Ma) in the Late Miocene. Homogamous-discoid capitula, characteristic of subgenera *Seriphidium* and *Tridentatae*, evolved not only in different geographic regions, but also at different times (2.0 ± 0.8 Ma and 7.9 ± 0.9 Ma respectively) within the *Artemisia* group. The loss of fertility of central flowers of disciform capitula should be considered as an ancient event in the genus since subgenus *Dracunculus* is one of the first groups that diverged (17.6 ± 2.1 Ma).

Key words: Anthemideae; *Artemisia*; Artemisiinae; biogeography; Compositae; molecular dating; morphological evolution.

Resumen

Evolución temporal y diversificación en *Artemisia* y géneros relacionados (Anthemideae, Asteraceae).- Con el objetivo de estimar el tiempo de diversificación de los linajes de la subtribu Artemisiinae y el grupo *Artemisia* se aplicó un análisis de verosimilitud penalizado en base a las secuencias de ADNrn, ITS y ETS, de 63 representantes. El árbol se calibró en el nodo del linaje de *Kaschgaria/Artemisia* con el polen fósil de *Artemisia* que se consideró más fiablemente datado, siendo esta datación del Oligoceno Superior (23 Ma). Los resultados de este estudio datan el origen de la subtribu en el Oligoceno Superior ($24,6 \pm 2,6$ Ma), mientras que la diferenciación del género *Artemisia* y los géneros relacionados es datada en el Mioceno Inferior ($19,8 \pm 2,3$ Ma). El tiempo de divergencia de los linajes dentro del grupo *Artemisia* está situado entre el Mioceno Inferior y el Medio, mientras que la mayoría de sus radiaciones tuvieron lugar en el Mioceno Superior y en el Plioceno. El contexto temporal también se usó para examinar la evolución biogeográfica y morfológica (tipo de capítulo y polen). Todos los linajes dentro del grupo de *Artemisia*, excepto el complejo endémico norteamericano, han colonizado la cuenca mediterránea en diferentes épocas. Nuestros análisis sugieren la divergencia del grupo endémico norteamericano de antepasados asiáticos ($10,8 \pm 1,5$ Ma) en el Mioceno Superior. El capítulo homogamo-discoides, característico de los subgéneros *Seriphidium* y *Tridentatae*, evolucionó no sólo en diferentes regiones geográficas, sino también en distintas épocas ($2,0 \pm 0,8$ Ma y $7,9 \pm 0,9$ Ma respectivamente) dentro del grupo *Artemisia*. La pérdida de fertilidad de las flores centrales del capítulo disciforme se debería considerar como un evento antiguo en el género, puesto que el subgénero *Dracunculus* es uno de los primeros grupos que divergió ($17,6 \pm 2,1$ Ma).

Palabras clave: Anthemideae; *Artemisia*; Artemisiinae; biogeografía; Compositae; datación molecular; evolución morfológica.

INTRODUCTION

Artemisia L. is the largest and most diverse genus of the tribe Anthemideae (Asteraceae) comprising more than 500 taxa at the specific or subspecific level (Vallès & Garnatje, 2005 and references therein). It is a cosmopolitan genus, mainly distributed in temperate areas of the northern hemisphere colonizing mainly arid and semiarid environments, from sea level to high altitudes, with only a few representatives in the southern hemisphere (Pellicer *et al.*, 2010). Central Asia is the main centre of diversification, secondary ones being the Mediterranean region and Northwest America (McArthur & Plummer, 1978; Vallès & McArthur, 2001). Currently, five subgenera are distinguished, mostly based on floral characters: *Absinthium* DC., *Artemisia*, *Dracunculus* Besser, *Seriphidium* Besser and *Tridentatae* (Rydb.) McArthur (see Table 1 in Torrell *et al.*, 1999).

Bremer & Humphries (1993) included *Artemisia* in subtribe Artemisiinae, which additionally comprises 17 allied or segregated genera. The latest phylogenetic studies (Oberprieler *et al.*, 2007, 2009; Sanz *et al.*, 2008; Tkach *et al.*, 2008a) prove that the genus *Artemisia* in the circumscription of Bremer & Humphries (1993) is paraphyletic because *Crossostephium* Less., *Filifolium* Kitam., *Mausolea* Bunge, *Neopallasia* Poljakov, *Picrothamnus* Nutt., *Sphaeromeria* Nutt. and *Turaniphytum* Poljakov are nested within *Artemisia*. These studies also prove that the subtribe, in order to be monophyletic, should also include some genera placed by Bremer & Humphries (1993) in other subtribes: *Hippolytia* Poljakov (Tanacetinae), *Leucanthemella* Tzvelev and *Nipponanthemum* Kitam. (both Leucantheminae).

Traditionally, pollen type (see Martín *et al.*, 2001, 2003 for references) and capitula (Besser, 1829, 1832, 1834, 1835; Candolle, 1837) have been considered as important characters for generic and infrageneric circumscription of *Artemisia*, respectively. Pollen morphological data (Martín *et al.*, 2001, 2003; Pellicer *et al.*, 2009) confirm the coexistence of two pollen patterns in subtribe Artemisiinae: the *Anthemis*-type with conspicuous spines (echinate pollen), and the *Artemisia*-type, with spinules (microechinate pollen). Martín *et al.* (2001, 2003) determined that ornamentation with short spinules is a good taxonomic marker for *Artemisia* and closely

related genera. According to Sanz *et al.* (2008), the ancestral condition in the subtribe was discoid capitula arranged solitarily or in laxly corymbose synflorescences with *Anthemis* pollen type, whereas the ancestor of the *Artemisia-Kaschgaria* clade had disciform capitula (with central hermaphrodite florets) arranged in densely corymbose synflorescences, with *Artemisia* pollen type.

The first appearance of *Artemisia* has been widely discussed (Miao *et al.*, 2011 and references therein). Pollen fossil records suggest that *Artemisia* originated in temperate Asia during the Late Eocene (Miao *et al.*, 2011) or the Mid-Tertiary (Wang, 2004). According to Wang (2004), there are two reliable fossil pollen records of *Artemisia* in China, one from the Early Oligocene in Xinjiang province (Song, 1965), the second from the Late Oligocene in the Qinghai province (Zhu *et al.*, 1985; Miao *et al.*, 2011). The age of the rocks for both places was originally determined by biostratigraphic evidence, but that of the Qinghai province was later adjusted by paleomagnetic data (CGTRP, 1992). The most ideal data are the fossil pollen sites with reliable paleomagnetic dating and high percentages of *Artemisia* pollen (Miao *et al.*, 2011), therefore we considered the Late Oligocene date (Zhu *et al.*, 1985) the most reliable early *Artemisia* fossil pollen record. Following Ling (1991a, 1991b, 1994), the putative ancestor of *Artemisia* existed in Northern Asia and the migrations from this place supposedly occurred along three lines: (1) westward into Europe, Western Asia, Asia Minor, the Mediterranean Basin, and Africa; (2) eastward into Siberia and into Western North America, and (3) further South into Asia.

The use of DNA sequences to estimate the timing of evolutionary events is increasingly popular. In plants, molecular dating has been used in numerous studies to investigate the timeframe of evolutionary events, e.g. for testing biogeographical hypotheses or to investigate the causes of recent radiations (reviewed in Sanderson *et al.*, 2004). Dating techniques have been applied on taxa from very different taxonomic levels, e.g. angiosperms (Magallón & Sanderson, 2001; Wikström *et al.*, 2001; Bell *et al.*, 2005), Compositae (Funk *et al.*, 2009), genera or species level (Park *et al.*, 2006; Smith *et al.*, 2008; Goodall-Copestake *et al.*, 2009). Oberprieler (2005) estimated temporal evolution of the tribe Anthemideae based on ITS sequences. In

Artemisia, Tkach *et al.* (2008b) dated the origins of Arctic lineages of the genus using a Bayesian approach with an uncorrelated lognormal clock on molecular ETS and ITS data. However, none of them have dealt with temporal evolution of lineages within the subtribe Artemisiinae and/or within the *Artemisia* group.

Therefore, the main goals of this study are (1) to assess temporal origins and diversification of the group, and (2) to examine biogeographic and morphological (capitula and pollen type) evolution of the group using this temporal context.

MATERIALS AND METHODS

Data set

For the reconstruction of the temporal evolution of the group presented here we use the same data set as in our previous study (Sanz *et al.*, 2008) that comprises sequence information for nrDNA ITS and ETS for 63 representatives of the subtribe. The data set consists of 45 species of *Artemisia* (46 populations) representing all five traditional subgenera and the geographical range of the genus, and 14 species from 13 genera belonging to subtribe Artemisiinae (*Ajania* Poljakov, *Brachanthemum* DC., *Dendranthema* (DC.) Des Moul., *Elachanthemum* Y. Ling & Y.R. Ling, *Filifolium*, *Kaschgaria* Poljakov, *Mausolea*, *Neopallasia*, *Picrothamnus*, *Sphaeromeria*, *Turaniphytum*, *Nipponanthemum nipponicum* Kitam. and *Hippolytia megacephala* (Rupr.) Poljakov.

Divergence time estimation

We used the most accurately dated and thus most reliable early *Artemisia* fossil pollen found in the Late Oligocene in the Qinghai province (Zhu *et al.*, 1985) as calibration point. The age of this fossil cannot be assigned to any of the species within the *Artemisia* group and, hence, it can only be used to suggest a minimum age for *Artemisia* and closely related genera with *Artemisia* pollen type. Considering the appearance of microechinate pollen in the lineage leading to *Elachanthemum intricatum* (Franch.) Y. Ling & Y.R. Ling as a convergence, the most suitable calibration point (Magallón, 2004) would be the stem node of *Kaschgaria/Artemisia*

lineage. Consequently, we fixed the age of the stem node of this clade at 23 million years ago (Ma), *i.e.* the Oligocene-Miocene boundary.

Previous maximum likelihood (ML) analysis to infer tree topologies and branch lengths from the combined dataset was employed (Sanz *et al.*, 2008). Likelihood ratio test (LRT; Felsenstein, 1988) for rate constancy indicated that neither ITS nor ETS evolved in a clock-like manner. Hence, to obtain ultrametric trees we applied penalized likelihood (PL; Sanderson, 2002) as implemented in r8s 1.7 (Sanderson, 2003) to estimate minimum ages of the most recent common ancestor (MRCA) of selected nodes. PL is a semiparametric method that allows substitution rates to vary among lineages according to a smoothing parameter. The optimal smoothing parameter was chosen on the basis of the data by cross-validation (Sanderson, 2003). Confidence intervals were calculated by data bootstrapped 100 times. We generated 100 phylograms topologically identical, but with different branch length and then was summarized age distribution for each particular node. The central 95% of the age distribution provides an interval of confidence (Sanderson & Doyle, 2001). Bootstrap data matrices were generated using the Seqboot program in Phylip package (Felsenstein, 1999). First, we obtained relative ages by arbitrarily setting the root age to an age of 100, which were subsequently translated into absolute ages by calibrating the stem node of the *Kaschgaria/Artemisia* lineage to 23 Ma based on fossil pollen record. All divergence time estimates are given in Ma and were placed into the current timescale for the Cenozoic based on the International Union of Geological Sciences 2009 (IUGS) (Gibbard *et al.*, 2009). The ultrametric tree was visualized in the program FigTree ver. 1.1.2 (Rambaut & Drummond, 2007).

RESULTS

Likelihood ratio test for rate constancy rejected the assumption of a molecular clock for both data sets ($p < 0.001$). Cross-validation analyses identified the optimal smoothing parameter for the combined data set as 2. Average divergence times plus standard deviations for selected nodes in the ML combined tree are given in Table 1 and the chronogram obtained is shown in Fig. 1.

Table 1. Average divergence times (\pm standard deviation) in Ma of selected nodes (their designation by letters used in Fig. 1 is given in parentheses).

Most recent common ancestor of...	Divergence times Ma \pm SD
Artemisiinae (A)	24.6 \pm 2.6
<i>Nipponanthemum</i> clade (B)	12.1 \pm 3.2
<i>Dendranthema</i> group (C)	4.3 \pm 1.2
<i>Kaschgaria</i> / <i>Artemisia</i> lineage (D)	21.6 \pm 2.4
<i>Artemisia</i> group (E)	19.8 \pm 2.3
Subgenus <i>Dracunculus</i> (F)	17.6 \pm 2.1
Alpine/Mediterranean split (G)	6.8 \pm 0.8
<i>Artemisia absinthium</i> complex (H)	5.9 \pm 1.5
Alpine group (I)	5.8 \pm 0.8
<i>Artemisia afra</i> clade (J)	6.6 \pm 1.6
Subgenus <i>Seriphidium</i> (K)	2.0 \pm 0.8
North American group/ <i>A. vulgaris</i> complex (L)	10.8 \pm 1.5
North American group (M)	7.9 \pm 0.9
<i>Artemisia vulgaris</i> complex (N)	7.8 \pm 1.3

Molecular clock analyses suggest that subtribe Artemisiinae diversified at least 24.6 ± 2.6 Ma (node A), whereas the *Artemisia* group diverged from its sister *Kaschgaria* group at 21.6 ± 2.4 Ma (node D). In the basal groups, the diversification of the *Dendranthema* group and of the *Nipponanthemum* clade was estimated at 4.3 ± 1.2 Ma (node C) and 12.1 ± 3.2 Ma (node B), respectively. Within the *Artemisia* group, the oldest lineage was subgenus *Dracunculus* (node F: 17.6 ± 2.1 Ma) and the youngest subgenus *Seriphidium* (node K: 2.0 ± 0.8 Ma). Other noteworthy divergence ages are the split of the North American endemic group from the Eurasian species (node L: 10.8 ± 1.5 Ma), the diversification of the alpine group (node I: 5.8 ± 0.8 Ma), the diversification of the *A. absinthium* L. complex (node H: 5.9 ± 1.5 Ma) and the split of *A. afra* Jacq. from *A. santolinifolia* Turcz. ex Besser (node J: 6.6 ± 1.6 Ma).

DISCUSSION

Temporal diversification

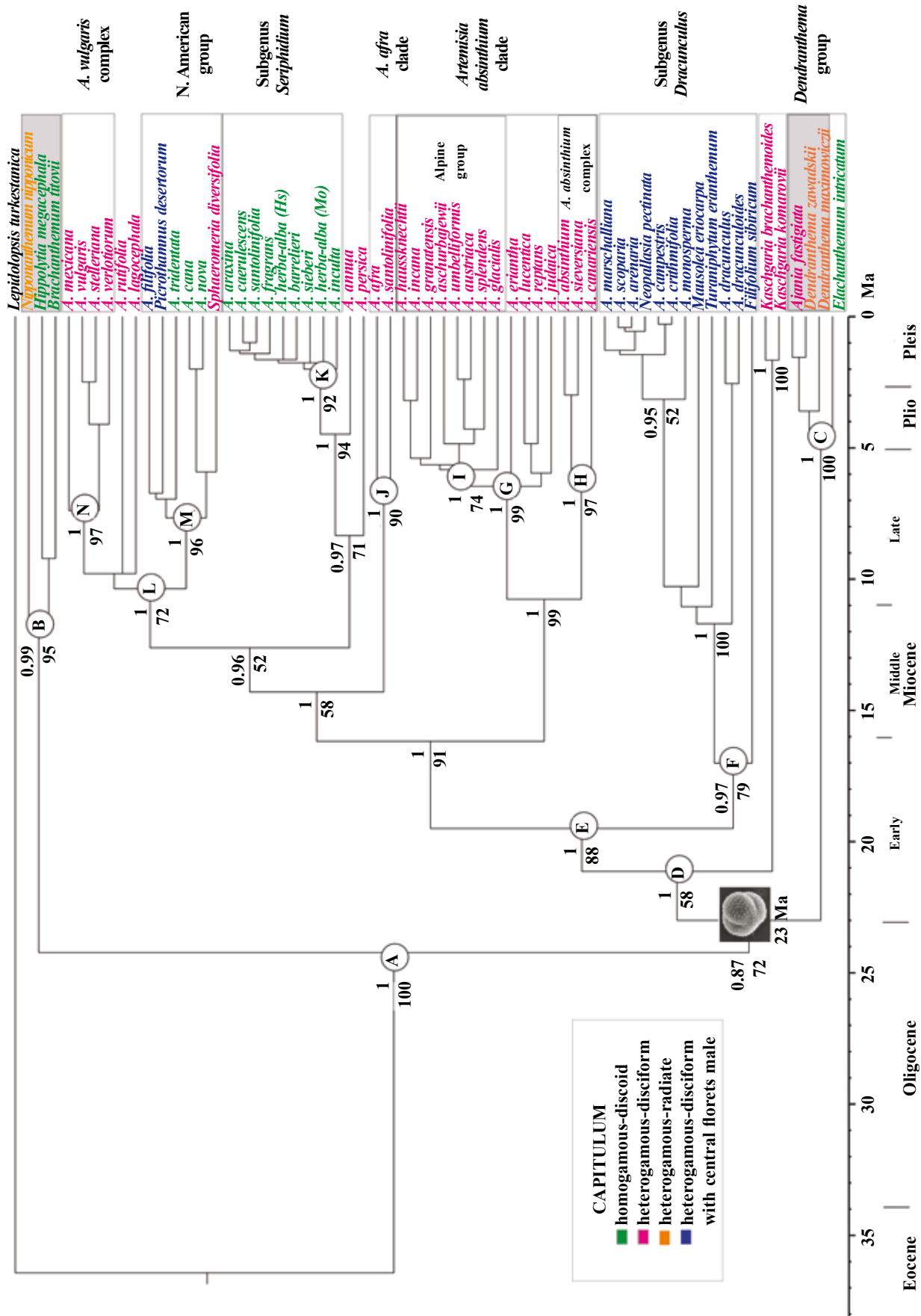
Following the calibration of the present tree (Fig. 1), the origin of the subtribe in Asia goes back to the Late Oligocene (node A: 24.6 ± 2.6 Ma) whilst the onset of differentiation of the genus *Artemisia* and most closely related genera is dated to the Early Miocene (node E: 19.8 ± 2.3 Ma). Divergence ages for the main lineages within the *Artemisia* group correspond mainly to the Early or Middle Miocene, whereas their radiations mostly occurred in the Late Miocene and Pliocene.

Oberprieler (2005) reported the divergence time of subtribe Artemisiinae to be 7–10 Ma (Late Miocene) based on a molecular clock analyses of the tribe Anthemideae using ITS sequences. However, our dating estimates suggest that *Artemisia* fossil pollen was sparsely distributed during the Early Miocene, and widely distributed during the Late Miocene, being particularly abundant during the Pliocene and the Pleistocene (Wang, 2004; Song *et al.*, 2004; Miao *et al.*, 2011). Furthermore, the present results are consistent with geologic and ecological changes during the Late Tertiary in Asia (Axelrod *et al.*, 1996), specifically with the onset of increased aridity in Asian climate by at least 22 Ma (as evidenced by paleomagnetic data) due to regional tectonic changes and the uplift of the Tibetan-Himalayan complex (Guo *et al.*, 2002).

The dry conditions in Central Asia and the strong cooling of the Late Eocene, partly related to the above-mentioned tectonic events, probably favoured the *Artemisia* expansion (Miao *et al.*, 2011). A similar pattern of explosive radiation in the Qinghai-Tibetan plateau within the last 20 Ma has been reported for other Asteraceae groups (*Ligularia-Cremathodium-Parasenecio* complex; Liu *et al.*, 2006).

According to our reconstructions, within the *Artemisia* group all lineages except the North American endemic group have colonized the Mediterranean

Figure 1. Chronogram of *Artemisia* and allied genera based on penalized likelihood analysis of a maximum likelihood tree constructed from combined nrDNA ITS and ETS data. Posterior probability values (above) and bootstrap supports (below) derive from our previous study (Sanz *et al.*, 2008). To calibrate the tree, the stem node of the clade including *Artemisia* and *Kaschgaria* was fixed to 23 Ma. Nodes, for which ages were estimated, are indicated by capital letters. Geological epochs are abbreviated as follows: Plio, Pliocene; Pleis, Pleistocene. Capitula types are mapped in different colors and taxa with *Anthemis*-type pollen are shown in grey.



Basin at different epochs from Asian ancestors. The first colonization of this area took place in the Late Miocene during the Messinian salinity crisis (5.96–5.33 Ma), when the closure of the Mediterranean-Atlantic gateways caused the nearly completely evaporation of the Tethys sea (Hsü *et al.*, 1973; Bocquet *et al.*, 1978; Meulenkamp & Sissingh, 2003) and the progressive aridification of this region (Van Dam, 2006). Examples include Eastern and Western Mediterranean species (*A. judaica* L., *A. reptans* C. Sm. ex Link and *A. lucentica* O. Bolòs, Vallès-Xirau & Vigo) within the *Artemisia/Absinthium* clade (node G: 6.8 ± 0.8 Ma) and the ancestor leading to the *A. absinthium* complex (node H: 5.9 ± 1.5 Ma). Later on, at the end of Pliocene there was a secondary colonization of the Mediterranean region by members of the subgenera *Dracunculus* (*A. campestris* L., *A. monosperma* Delile) and *Seriphidium* (*A. barrelieri* Besser, *A. herba-alba* Asso, *A. inculta* Delile). The dramatic changes experienced in the Mediterranean climate from 3.5 Ma onwards, with the stabilization of summer drought (Suc, 1984; Thompson, 2005) could facilitate the latter colonizations.

A westward colonization of the Mediterranean Basin is a common trend in plants (Paun *et al.*, 2005; Mráz *et al.*, 2007) and has been also described in other Anthemideae groups (Lo Presti & Oberprieler, 2009).

Our dating results show that the divergence of *A. canariensis* Less. from its continental sister species (node H: 5.9 ± 1.5 Ma), and the split of Central Asian *A. santolinifolia* from the South African *A. afra* (node J: 6.6 ± 1.6 Ma) date back to Late Miocene. These estimates are consistent with the increase of land bridges in the Mediterranean during the Late Miocene (Meulenkamp & Sissingh, 2003) that also could have provided migration routes of the Eurasian flora into Africa and Macaronesia as described in several other plant groups (Oberprieler, 2005; Mansion *et al.*, 2008).

Our analyses suggest two arrivals to North America in the Late Miocene. One concerns the divergence of the North American endemic group from Asian ancestors (node L: 10.8 ± 1.5 Ma) and the other the diversification of the American endemic *A. mexicana* Willd. from its Eurasian vicariants of the *A. vulgaris* L. complex (node N: 7.8 ± 1.3 Ma). Tkach *et al.* (2008b), based on uncorrelated log-normal clock method and calibrating the tree at the Eocene/Oligocene boundary (34 Ma), obtained

a similar divergence age of these groups (median age: 13.5 Ma and 7.5 Ma respectively). Floristic exchanges between Asia and North America were most likely across the Bering and the North Atlantic land bridges (Tiffney, 1985; Wen, 1999). The Beringian Land Bridge began to cool down from the Middle Miocene (15 Ma) onwards with an increasing cooling trend in the Late Miocene (Mosbrugger *et al.*, 2005; Milne, 2006) that would have favoured vicariance of Old and New world *Artemisia* species. In addition, our estimated age is consistent with a Middle Miocene (12 Ma) development of the sagebrush steppe in the Great Basin cold desert in North West America (Davis & Ellis, 2010).

Our age estimates suggest an origin of the alpine group in the Late Miocene (node I: 5.8 ± 0.8 Ma) and its diversification in the Pliocene. At that time, the old-tertiary Alpine orogeny had already formed the main European mountain belts (Smith *et al.*, 1994). This finding supports the origin of orophytic groups within the genus earlier than Quaternary glaciations. Similar conclusions have been drawn for other European alpine taxa (Comes & Kadereit, 2003; Paun *et al.*, 2005).

Regarding temporal evolution of morphological traits, in the basal groups the divergence of the monotypic genus *Nipponanthemum* (node B: 12.1 ± 3.2 Ma) is a relatively ancient event in the subtribe compared to that of the *Dendranthema* species (node C: 4.3 ± 1.2 Ma). Therefore, the origin of radiate capitula and *Anthemis* pollen-type (both characters only found together in these two genera among the studied ones) occurred in different periods of time during the evolution of the subtribe. The reconstruction of the capitula type evolution within the *Artemisia* group (Sanz *et al.*, 2008) suggests that all capitula types evolved from heterogamous-disciform capitula with central hermaphrodite and outer female, non radiate florets. Our age estimates indicate that *Dracunculus* (heterogamous-disciform capitula with central florets male) is one of the first groups that diverged (node F: 17.6 ± 2.1 Ma), therefore the loss of fertility of central florets of disciform capitula should be considered as an ancient event in the genus. In contrast, subgenus *Seriphidium* is the youngest group within *Artemisia* (node K: 2.0 ± 0.8 Ma) and it diverged independently of the North American subgenus *Tridentatae* that diverged approximately 6 million years earlier (node M: 7.9 ± 0.9). This implies that homogamous-

discoid capitula (with all florets hermaphrodite), characteristic for both subgenera, evolved not only in different geographic regions, but also at different times within the *Artemisia* group.

Similarly, the evolution of the heterogamous capitula with male central florets, found in the lineage that gave rise to subgenus *Dracunculus* from perfect heterogamous capitula (Sanz *et al.*, 2008) also arose in different periods in Asia and North America (*Artemisia filifolia* Torrey and the monotypic genus *Picrothamnus*).

CONCLUDING REMARKS

The results of this research suggest that the divergence ages for the main lineages within the *Artemisia* group chiefly correspond to the Early and Middle Miocene, whereas the radiation of the groups occurred mostly in the Late Miocene and Pliocene.

Our analyses suggest that all lineages, except the North American endemic group, have colonized the Mediterranean Basin at different epochs from Asian ancestors. The loss of fertility of central florets in *Dracunculus* from perfect heterogamous capitula is dated as an ancient event in the genus.

The scarce fossil data and the need of a broader sampling warrant caution in the interpretation of the molecular dating results, and further studies will be necessary to test our temporal hypotheses.

ACKNOWLEDGEMENTS

This study was subsidized by projects CGL2004-04563-C02-02/BOS of the Spanish government and the 2009/SGR/439 of the Generalitat de Catalunya. The first author received a predoctoral grant and support to perform a stay in the Botanical Garden of Vienna from the Spanish Government (FPI program). Víctor Suárez and an anonymous reviewer are thanked for their comments, which improved the manuscript.

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