Habrosia (Caryophyllaceae) a monotypic genus endemic to Western Asia: morphological and molecular remarks

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Abstract – *Habrosia* (Sagineae, Caryophyllaceae) is a genus that includes only *H. spinuliflora*, a species occurring in Iran, Iraq, Syria, Lebanon, and Turkey (Irano-Turanian floristic chorological element). Based on the available molecular data published in 2011, *Habrosia* appears to be nested in a *Minuartia*-clade, which includes taxa currently recognized under the genus *Sabulina*. Consequently, *Habrosia* should be treated as a genus to be included in *Sabulina*. However, the molecular tree published in 2011 considered only 9 *Sabulina* members whereas, according to the current concept, *Sabulina* is a genus comprising about 65 species. Unfortunately, the molecular phylogeny including a larger *Sabulina* sample published in 2014 did not include *H. spinuliflora* and the taxonomic position of *Habrosia* remains, therefore, uncertain. With the aim of verifying the correct position of *Habrosia* in the tribe Sagineae with respect to its relationship to *Sabulina*, a comprehensive molecular investigation based on ITS sequences, linked to detailed morphological data, is presented. The results obtained revealed that *Habrosia* is not part of *Sabulina*. A detailed description of *H. spinuliflora*, its ecological preference, and a distribution map are provided. Eventually, the name *Arenaria spinulifolia* (basionym of *H. spinuliflora*) is lectotypified on a specimen preserved at G (barcode G00212963).

Keywords: Arenaria spinulifolia, Iraq, ITS, Lebanon, Syria, Turkey, typification.

Introduction

The family Caryophyllaceae Juss. comprises ca. 100 genera and 3000 species, occurring mainly in the northern hemisphere (Hernández-Ledesma et al. 2015). Caryophyllaceae is monophyletic as circumscribed by Bittrich (1993), but the traditional recognition of three subfamilies (Alsinoideae Fenzl, Caryophylloideae Arnott, and Paronychioideae Meisner; see e.g., Bittrich 1993) based on features of stipules, petals, sepals, and fruits does not provide monophyletic groups and should be replaced with the tribe-based scheme as reported by Harbaugh et al. (2010) and confirmed by subsequent studies (e.g. Greenberg and Donoghue 2011). At genus rank, several studies have been carried out on Arenaria L., Minuartia L., Dianthus L., Gypsophila L., Polycarpon L., Silene L., etc. (see e.g., Kool et al. 2007, Iamonico 2013, 2014, 2015, 2016, 2018, Dillenberger and Kadereit 2014, Iamonico and Domina 2015, Iamonico et al. 2015, Sadeghian et al. 2015, Dillenberger and Rabeler 2018, Madhani et al. 2018), but various questions are still open.

As part of the ongoing studies on Caryophyllaceae (e.g., Iamonico 2013, 2014, 2015, 2018, 2019, 2020, Iamonico and Domina, 2015), I here present a note about the monotypic genus *Habrosia* Fenzl [including the species *H. spinuliflora* (Ser. ex DC.) Fenzl], since some issues about its position in the tribe Sagineae J.Presl still need clarification. The aims of the research are: 1) to verify the correct position of *Habrosia* in the tribe Sagineae with special regards to its relationship to *Sabulina*, 2) to consider the morphology of *H. spinuliflora* in comparison with its position in the molecular tree, 3) to clarify the identity of the name of *Arenaria spinuliflora* Ser. ex DC. (basionym of *H. spinuliflora*).

Materials and methods

The present research is based on both the analysis of the relevant literature and the examination of the specimens preserved at BAG, G, MO, P, SAV, and W (codes according to Thiers 2021-onward).

The ITS sequences, used for the alignment and phylogenetic reconstruction, were publicly available in GenBank (see Smissen et al. 2003) and refer to 65 members of *Sabulina* Rchb., *Colobanthus* Bartl., *Drypis* L., *Facchinia* Rchb., *Habrosia*, *Minuartia*, *McNeillia* Dillenb. & Kadereit,

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Sagina L., and (outgroups) *Bufonia tenuifolia* L. and *Cherleria garckeana* (Asch. and Sint. ex Boiss.) A.J. Moore and Dillenb. RAxML v8.2.12 (Stamatakis 2014) was run under the GTRGAMMA model (bootstrapping was stopped automatically) for phylogenetic reconstruction.

The distribution map was prepared using Google Earth Pro (2021). Data derive from both herbarium specimens and literature.

The articles cited throughout the text follow the International Code of Nomenclature for algae, fungi, and plants (Turland et al. 2018, hereafter as ICN).

Results

Literature data

Arenaria spinuliflora Ser. ex DC., after its original description in the 1st volume of Candolle's *Prodromus* (Candolle 1824: 406), was treated under the genus *Arenaria* up until 1833, when Fenzl (1833: 57) proposed transferring this taxon to the genus *Alsine* L. (note that neither morphological information nor a general reason about this nomenclatural choice was given by Fenzl 1833). No subsequent papers or monographs, in which Fenzl's *Al. spinuliflora* was accepted, have been traced. Ten year later, Fenzl (1843: 323–324) validly described the new genus *Habrosia* to accomodate Candolle's species, correcting his previous choice under *Alsine*. A detailed generic description was provided, as well as a diagnosis and description of his *H. spinuliflora* (Ser. ex DC.) Fenzl. The genus *Habrosia* has been accepted until to-day (see e.g., Hernández-Ledesma et al. 2015, Rabeler 2020).

At suprageneric rank, Habrosia spinuliflora is currently to be included in the tribe Sagineae, together with Sagina, Colobanthus, Sabulina, and Bufonia Sauvage. Fenzl (1843: 326) proposed to treat his new genus Habrosia as belonging to a new subtribe (named "Habrosieae") of the tribe Sclerantheae Link. In the same year, Endlicher (1843), proposed transferring Fenzl's subtribe into tribe rank [Habrosieae (Fenzl) Endl.]. More recently, Novosel (1982) published "Habrosieae Novosel, tribus nov." which would be morphologically similar to the tribe Pollichieae DC. (including the genus Pollichia Aiton) from which it would differ by the indehiscent fruit, absence of sterile branches, occurrence of petals in flowers, and 1-4 ovules [see the diagnostic key (steps 1-3) provided by Novosel 1982: 222]. According to Art. 6.3 (Note 2) of ICN, Novosel's name "Habrosieae" is an isonym (and therefore invalid) being based on the same type (H. spinuliflora) of Endlicher's name.

The first authors to have included *Habrosia* in a molecular analysis were Smissen et al. (2003) in their study on the genus *Scleranthus* L. where Fenzl's genus resulted to be sister of *Drypis*.

A more detailed study was carried out by Greenberg and Donoghue (2011) who investigated many samples of Caryophyllaceae members (630 accessions) and revealed that *Drypis* (a monotypic genus with *Drypis spinosa* L.) was basal to a well-supported clade (bootstrap value: 91) including species of *Sagina*, *Colobanthus*, *Minuartia*, *Habrosia*, and *Bufonia*, plus *Arenaria fontinalis* (Short and R.Peter) Shinners. This clade corresponds to the tribe Sagineae.

Dillenberger and Kadereit (2014), who investigated in detail the genus *Minuartia*, did not consider the genus *Habrosia* in their analysis. However, the species of *Minuartia*, included in the tribe Sagineae by Greenberg and Donoghue (2011), were treated by Dillenberger and Kadereit (2014) as belonging to the resurrected genus *Sabulina* Rchb. In contrast to *Habrosia*, Dillenberger and Kadereit (2014) included *Arenaria fontinalis* in their analysis, which was also investigated by Greenberg and Donoghue (2011), and confirmed that it is to be treated as a member of *Sabulina* and, in fact, a new combination, *S. fontinalis* (Short and R. Peter) Dillenb. and Kadereit, was proposed.

Based on Greenberg and Donoghue (2011), *Habrosia* should be a genus to be included in *Sabulina*.

Molecular data

Greenberg and Donoghue (2011: 1642, Fig. 2) considered nine members of Sabulina (sub Minuartia spp.). However, according to the current concept, Sabulina is a genus comprising ca. 65 species (Hernández-Ledesma et al. 2015, Rabeler 2020). As a consequence, the position of Habrosia in the ITS tree (clade Sagineae) published by Greenberg and Donoghue (2011) cannot be considered as conclusive. As discussed above (see paragraph "Literature data"), Dillenberger and Kadereit (2014) studied the majority of the Minuartia s.l. taxa, and included in their analyses the Sabulina clade by Greenberg and Donoghue (2011, sub Minuartia), reaching the conclusion that these taxa are to be transferred to a different genus, i.e. the resurrected Sabulina. However, Habrosia was not included in the study by Dillenberger and Kadereit (2014), and an indirect inclusion of Habrosia into Sabulina would represent a risk from the taxonomical point of view.

All things considered, I decided to merge the molecular data of Dillenberger and Kadereit (2014) and Greenberg and Donoghue (2011) in a single matrix and run a new comprehensive tree to verify if *Habrosia* is actually nested in the *Sabulina* clade or not. The results obtained (Fig. 1) reveal that *Habrosia* is not nested in the clade comprising the members belonging to *Sabulina*, but is in an unresolved position outside of *Sabulina*.

Morphological data

Starting from important works by McNeill (1962, 1967), the genus *Minuartia* (at that time morphologically related to *Arenaria*), was later accepted by most authors until the molecular studies by Dillenberger and Kadereit (2014). These latter authors demonstrated that *Minuartia* is highly polyphyletic, and 11 different genera were recognized and later accepted by many authors (e.g., Iamonico 2014, Legler and Dillenberger 2017, Moore and Dillenberger 2017, Dillenberger and Rabeler 2018).



Fig. 1. Maximum likelihood phylogeny based on ITS sequences obtained with RAxML under the GTRGAMMA model. Bootstraps values are shown. Arrow indicates the position of *Habrosia spinuliflora* (Ser. ex DC.) Fenzl.

Among the resurrected genera, there is *Sabulina* which comprises McNeill's sects. *Acutiflorae* (Fenzl) Hayek, *Alsinanthe* (Fenzl) Graebn., *Greniera* (Gay) Mattf., *Sabulina* (Rchb.) Graebn., *Sclerophylla* Mattf., and *Tryphane* (Fenzl) Hayek, as well as *Stellaria fontinalis* Short & R.Peter (Dillenberger and Kadereit 2014, Hernández-Ledesma et al. 2015: 330). *Sabulina* can be morphologically distinguished in having the following characters (see Dillenberger and Kadereit 2014: 80-81): stems neither spiny nor quadrangular, leaves linear to subulate, flowers white without episepalous staminoids, nectary glands not cup-shaped, calyx not hardened at the base, sepals acute and 1–3-veined, petals usually not exceeding the sepals, styles 3 and free, fruit dehiscent (capsule) 3-toothed.

According to my examination of herbarium specimens, *Habrosia* differs from *Sabulina* by the following characters, which have a high taxonomic value in Caryophyllaceae (see e.g., Dillenberger and Kadereit 2014, Hernández-Ledesma et al. 2015): apex of the sepals [awned (awns about 2/3 as long as the membranous part of the sepals) in *Habrosia vs.* not awned in *Sabulina*], number of styles (2 *vs.* 3), and fruit [indehiscent (utricule) in *Habrosia vs.* dehiscent (capsule) in *Sabulina*].

Typification of Arenaria spinuliflora

Arenaria spinuliflora Ser. ex DC. [basionym of Habrosia spinuliflora (Ser. ex DC.) Fenzl] was validly published by Candolle (1824: 406) who provided a short diagnosis and the provenance ("in Oriente") and cited a syntype ("v. s. [vidi sicco] comm. à cl. Rosseau"). Candolle (1824) reported "Ser. mss." just after the binomial, so referring to an unpublished Seringe's manuscript.

Tropicos (2021) does not list the name Arenaria spinuliflora, erroneously reporting "Habrosia spinuliflora Fenzl" and citing, as syntypes, a specimen at MO (barcode MO256214, available at http://legacy.tropicos.org/Image/59784) collected by T. Kotschy (no. 120) in Aleppo (Syria) in April 20, 1841. However, not only did Fenzl (1843: 323-324) not propose a new species (rather a new combination of Candolle's name), but the cited specimen of Kotschy (MO256214) cannot be regarded as syntype (or included in the original material) since it was not cited by Candolle (1824: 406) but only by Fenzl (1843). Also The Plant List (2013) and IPNI (2021-onward) accepted the citation "Habrosia spinuliflora Fenzl" [note that The Plant List (2013) reported, as synonym of "Habrosia spinuliflora Fenzl", the name Arenaria spinuliflora which is therefore (and wrongly) considered as heterotypic synonym]. Among the main online database of plant names, only POWO (2021-onward) correctly cited the name by Fenzl (1843) which is considered a new combination of Candolle's name (basionym).

I traced one sheet at G (barcode G00212963) bearing two plants which are clearly part of the same gathering. In fact, both the plants were collected by M. Rousseau in "*Orient*" in 1818. G00212963 is part of the original material for *Arenaria spinuliflora*, matches Candolle's diagnosis (1824: 406), and it is here designated as the lectotype (Arts. 9.3 and 9.4 of ICN). In addition, an original label by Seringe ("A. [Arenaria] *spinuliflora Ser.*") occurs on the right-corner of G00212963, supporting the choice of this specimen as lectotype.

Taxonomic treatment

Habrosia Fenzl., Bot. Zeitung (Berlin) 1: 323. 1843. Original type: *Habrosia spinuliflora* (Ser. ex DC.) Fenzl.

Habrosia spinuliflora (Ser. ex DC.) Fenzl, Bot. Zeitung (Berlin) 1: 323-324. 1843 (as "Habrosias-pinuliflora" which is a typographic error) \equiv *Arenaria spinuliflora* Ser. ex DC., Prodr. [DC.], 1: 406 (1824) \equiv *Alsine spinuliflora* (Ser. ex DC.) Fenzl, Vers. Darstell. Alsin.: 57 (1833), fig. 2).

Lectotype (designated here) – Unknown country, Orient, 1818, M. Rousseau s.n. (G00212963!).

Description – *Annual herb*, erect, 4–15 cm tall. *Stems* filiform, dichotomously branched, slightly purple in colour, glabrous to sparsely pubescent [trichomes short, uniseriate, multicellular, eglandular, see Chandra et al. 2019]. *Leaves* opposite, setaceous, 0.5–2.0(–2.5) cm long, up to 0.5 mm width, connate at the base, glabrous to sparsely pubescent, sessile, margins entire, apex obtuse. *Stipules* adnate to the margins at the base of the leaf, with membranous borders.



Fig. 2. *Habrosia spinuliflora* (Ser. ex DC.) Fenzl from Mardin, SE-Turkey. A – habit, B – detailed of the inflorescence (photo by Musa Geçit, @MusaGeçit).



Fig. 3. Distribution map of *Habrosia spinuliflora* (Ser. ex DC.) Fenzl. Scale bar: 200 km.

Inflorescences 4–10-flowered, terminal, lax; bracts leaf-like, shorter than leaves (ca. 3 mm long). *Flowers* peryginous, on pedicels up to 6 mm long; *sepals* 5, glabrous, ovate-sublanceolate, 1.5–2.5(–3.0) mm long, 1(–3)-veined, membranous at the margins, apex awned, 2/3–1 as long as the membranous part of the sepals; *petals* 5, ovate-rounded, 1.0–1.5 mm long, shorter than sepals, white, entire; *stamens* 5, alternate to the sepals, with distinct glands attached to the adaxial surface extending in front of petal bases; *styles* 2, free, very short; *ovary* small, up to 1 mm long, subsessile, each ovary including 2 ovules. *Fruit* indehiscent nutlet, ovoid, ca. 1 mm long; seed 1 per fruit, globose.

Pollen grain spheroidal, diameter about 27 μ m, polyporate with 12–13 pores, each pore circular, in diameter about 4 μ m, granulate; interpolar distance 5–8 μ m. Exine ca. 3 μ m thick, tectate. Sexine ca. 2.5 μ m thick, punctulate. Tegillum < 0.5 μ m thick with minute spines. Bacula broader at the apex than the base (Chanda 1962: 73–74, and Pl. 3, Figs. 10–12).

Etymology – From the Greek *Habros* ($\chi \alpha \beta \rho o \varsigma$) which means "delicate, graceful", presumably referring to the thinness of the plant, especially of stem and leaves. Vernacular names – *Çöl kumotu* (Turkish; Ekim 2012), Western Asian sandwort (English common name, here proposed).

Habitat and phenology – Rocky limestone slopes, gullies, scrubs, calcareous steppe, arable field, open banks, 300–1500 m a.s.l. Flowering and fruiting times March to June.

Distribution and chorology – NE Iran (see also Rechinger et al. 1988), N Iraq (see also Blakelock 1957, Ghazanfar and Edmandson 2016), C- and NW Syria (see also Boissier 1867, Post 1932), NE Lebanon (see also Musselman 2011), SE Turkey (see also Davis 1988, Kaya and Ketenoğlu 2010, Bakis et al. 2011, Ekim 2012) plus Mt Taurus (Fig. 3). According to Takhtajan (1986), the distribution area of *Habrosia spinuliflora* is included in the Irano-Turanian floristic region, Western Asiatic subregion, Mesopotamian Province.

Additional specimens - Iran: Kurdistania Persica: montes supra pagum Režab dit. Kasr-i-Širin, in lapidosis, 05 May 1910, F. Nábělek 4114 (SAV0005141!). Iraq: in montis Kuh-Sefin reg. infer. Supra pagum Schaklava (ditionis Erbil), 1050 m, 15 May 1893, J. F. N. Bormüller 949 (P05380908!); ibidem (P05380911!); Mindan, 20 April 1947, Bradburne 44 (BAG); Khormal, Sulaimaniya liwa, 900 m, 21 April 1947, Rawi 8857 (BAG); Jabal Sinjar north of the town, Mosul liwa, 900 m, coppiced Quercus aegilops forest on limestone, localy abundant, 26 May 1948, C. C. Gillet 11091 (BAG); Acra, Mosul liwa, 30 May 1948, Rawi 11305 (BAG); Bikhair Mt., 900 m, 03 July 1957, Rawi 23144 (BAG); Jabal Maqlub, 550-750 m, 17 April 1958, Shahwani 25204 (BAG); Serkupkan Village c. 7 km NW of Rania, on hillside, Rawi 28533 (BAG); Dokan, near water, hillside, 15 May 1971, Omar and Karin 38029 (BAG); 20 km to Dahuk, clay soil wheat field, 310 m, 15 April 1980, Al Kaisi 52008 (BAG); Jebel Sinjan, racky clay mountain, 16 April 1980, Al Kaisi 52034 (BAG); bidem, 18 April 1980, Al Kaisi 52224 (BAG). Lebanon: ad Antilibani radices occidentales, in declibvitatibus supra Baalbek, 1150-1300 m, 20 May 1910, J. Bornmüller 11506 (P05380909!); Baalbek, 11 May 1933, M. R. Gombault 2233 (P05110463!); ibidem (P05110464!). Syria: In collibus lapidosis pr. Aleppum, 20 April 1841, T. Kotschy 120 (P05049676!, as "Habrosyne spinufiflora"); ibidem (P00712781!); ibidem (P00712782!); ibidem (P00712783!); ibidem (MO256214!); Aleppo, in graminis, 1330 ped. (= feet), 24 March 1865, C. Haussknecht s.n. (P05380913!, the nineteen plants on the botton-half of the sheet); Syria borealis, Aleppo, 1865, A. de Bunge s.n. (P00712788!); Dans Djebel Belas, 28 May 1895, coll. illeg. 2964 (P05380913!, the seven plants of the top-half of the sheet); Alep., 1834, A. Montbret s.n. (P00712786!); ibidem (P00712787!); Alep., s.d., s.coll. 590 (P00712785!); Alep., s.d., A. Montbret s.n. (P05380910!). Turkey: Mont Taurus, 1837, M. Aucher-Eloy 590 (P00712784!); Birecjik: Djebel Taken, 30 April 1888, O. Stapf 461 (P05380912!); ibidem (P05380915!).

Discussion

The available molecular data for *Habrosia* placed this genus as sister of *Drypis* (Smissen et al. 2003) or *Minuartia*

s.l. (tribe Sagineae; Greenberg and Donoghue 2011). More recently, Dillenberger and Kadereit (2014) resurrected the genus Sabulina in which to place the Minuartia species included in the tribe Sagineae by Greenberg and Donoghue (2011). Although Dillenberger and Kadereit (2014) did not consider the genus Habrosia in their analysis, Habrosia should be indirectly treated as a genus to be included in Sabulina based on published data. On the contrary, the phylogenetic tree obtained in the present study, which derives from a single matrix including the sequences by both Dillenberger and Kadereit (2014) and Greenberg and Donoghue (2011), shows that Habrosia cannot be merged with Sabulina and it should be left separate. This result highlights the relevance, in molecular analisys, of considering all the taxa involved in the investigated genus and the related ones, especially in taxonomically critical groups as Minuartia s.l. (specifically Sabulina in this case).

The morphological study confirms that *Habrosia* clearly differs from *Sabulina* in some characters (apex of the sepals, number of styles, and fruit dehiscence/indehiscence) which have a high taxonomic value in Caryophyllaceae (see e.g., McNeill 1962, Tutin et al. 1993, Rabeler and Hartman 2005, Greenberg and Donoghue 2011, Dillenberger and Kadereit 2014, Pignatti 2017) and that it has to be recognized at generic rank.

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