

2. *Ambrosia tenuifolia*, instead of *A. psilostachya*, in Romania

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Abstract: In this paper, *Ambrosia tenuifolia* (Asteraceae) is reported for the first time in the alien flora of Romania. Data resulting from the revision of herbarium specimens of the perennial ragweed previously collected from the locality of C. A. Rosetti (Danube Delta), as well as the results of our recent field work, revealed that *A. psilostachya* (a native species from North America) has been erroneously reported from Romania, and the correct identity of this plant is actually *A. tenuifolia* (which originates from South America). The two species are similar in some respects (e.g. by habitus, morphology of roots and inflorescences etc.), but they are easily distinguishable from each other mainly by leaf morphology. Both species are currently naturalized worldwide, and considered harmful weeds, especially because of their high production of allergenic pollen.

Keywords: *Ambrosiinae*, identification key, neophytes, ragweed, vascular flora

INTRODUCTION

Ambrosia L. (ragweed) is a genus of the subtribe *Ambrosiinae* Less. (tribe *Heliantheae* Cass., family *Asteraceae* Mart.) (Robinson, 1981; Strother, 2006). It consists of about 45 species (León de la Luz & Rebman, 2010; Rojas-Sandoval, 2018) representing annual or perennial herbs and shrubs, native (with one exception) to the New World, showing highest species diversity in arid and semi-arid habitats of North America (Payne, 1964; Strother, 2006; Tomasello *et al.*, 2019).

Eleven taxa of *Ambrosia* have been reported so far in Europe (see partial lists to Hansen, 1976; Greuter, 2006; Rich, 1994; DAISIE, 2009; Amor Morales *et al.*, 2012; Karrer *et al.*, 2016). Among them, *A. maritima* L. is probably indigenous species (native to the Mediterranean region and tropical Africa) (Hansen, 1976; Orsenigo *et al.*, 2017). *A. peruviana* Willd. and *A. tenuifolia* Spreng. are native to South America (Arechavalleta, 1906; Thellung, 1912; Payne, 1966), and the other seven species (i.e. *A. acanthicarpa* Hook., *A. ambrosioides* (Delp.) Payne, *A. artemisiifolia* L., *A. confertiflora* DC., *A. psilostachya* DC., *A. tomentosa* Nutt., and *A. trifida* L. (including *A. aptera* DC.)) - originate to North America (Strother, 2006).

Of the nine neophyte taxa of *Ambrosia* accepted at the species level and listed so far in Europe, five are very rare and not established, i.e. *A. acanthicarpa*, *A. peruviana*, *A. ambrosioides* - in Britain (Rich, 1994), *A. tomentosa* - in Spain (Amor Morales *et al.*, 2012) and *A. confertiflora* - without precise location and possibly erroneous (DAISIE, 2009). The remaining four introduced species are naturalized, being widespread, either rather regionally (*A. psilostachya*, *A. tenuifolia* and *A. trifida*), or throughout the continent (*A. artemisiifolia*) (Greuter, 2006; Karrer *et al.*, 2016).

Three of the alien species of *Ambrosia* from Europe have also been reported in Romania so far. Two of these (*A. artemisiifolia* L. and *A. trifida* L.) are annuals and one (*A. psilostachya* DC.) is perennial.

Ambrosia artemisiifolia L., which was first collected between the years 1907 and 1912, in the Banat province (Orșova and Băile Herculane) (Jávorka, 1925; Csontos *et al.*, 2010), is now widespread throughout the Romania, being considered one of the most invasive neophytes in the country (Anastasiu & Negrean, 2009; Sîrbu & Oprea, 2011; Sârbu *et al.*, 2013; Țușnia *et al.*, 2020).

Ambrosia trifida L. was first recorded in Romania, as a neophyte, in 1976, in the city of Constanța (Dobrogea) (Vițalariu *et al.*, 1977), and its distribution is currently limited to the south-eastern provinces (Dobrogea and Muntenia) (Sîrbu & Oprea, 2011). *Ambrosia trifida* L. was first recorded in Romania, as a neophyte, in 1976, in the city of Constanța (Dobrogea) (Vițalariu *et al.*, 1977), and its distribution is currently limited to the south-eastern provinces (Dobrogea and Muntenia) (Sîrbu & Oprea, 2011).

The perennial *A. psilostachya* DC. has been reported in Romania (as *A. coronopifolia* Torrey et *A. Gray*) by Ciocârlan & Constantin (1992), from the Danube Delta. Seven voucher specimens (no. 20961 to 20967) collected by Prof. V. Ciocârlan from the village C. A. Rosetti have been deposited at the Herbarium of the "Nicolae Bălcescu" University of Agronomical Sciences and Veterinary Medicine (UASVM), from Bucharest: "roadsides, ruderalised sandy places", alt. ca. 2 m a.s.l.; leg. et det. V. Ciocârlan, 13.IX.1991.

Subsequently, in the *Flora of Danube Delta*, Ciocârlan (1994) changed the locality C. A. Rosetti, with a neighbouring village, Sfiștofca, while Oprea (2005) added two more localities (Cardon and Letea) to the distribution of this species in the Danube Delta. Since the above-mentioned specimens, collected in 1991 from the locality of C. A. Rosetti, are the only ones deposited in a herbarium from Romania, the chorological data added after 1992 must be considered as uncertain. As a result, the only place in the Danube Delta where a perennial species of *Ambrosia* has been certainly documented is C. A. Rosetti, as mentioned by Ciocârlan & Constantin (1992), and the location was confirmed by more recent botanic references (Ciocârlan, 2009).

However, data resulting from the revision carried out by the first author of the present paper on the specimens deposited by Prof. V. Ciocârlan at the Herbarium of the UASVM Bucharest, as well as the results of our recent field work on the perennial ragweed from the locality of C. A. Rosetti, revealed that *A. psilostachya* DC. has been erroneously reported from Romania, and the correct identity of this plant is actually *A. tenuifolia* Spreng.

Therefore, the aim of this paper is to argue the presence of *A. tenuifolia*, instead of *A. psilostachya*, in the Danube Delta, Romania.

MATERIAL AND METHODS

The paper is a result of revision carried out by the first author, on perennial ragweed specimens held by the Herbarium of the University of Agricultural Sciences and Veterinary Medicine from Bucharest, as well as of our recent field works (2020), in the Danube Delta, Romania.

The geographic coordinates were recorded in the field using the offline navigation application OsmAnd, available at <https://osmand.net/>.

Voucher specimens collected during our field work were deposited at the Herbarium of the University of Life Sciences Iași (IASI) and the Institute of Botany, University of Natural Resources and Life Sciences Vienna (WHB).

For species identification we used various keys, descriptions and iconography published by Cosson & Kralik (1849-1850), Godron (1852), Baker (1882), Arechavaleta (1906), Pignatti (1982), Aizpuru *et al.* (1999), Strother (2006), Amor Morales *et al.* (2012), Orchard (2015), Karrer *et al.* (2016), Montagnani *et al.* (2017), Karrer (2018, 2019) and many other references as indicate below. The plant nomenclature follows Strother (2006).

RESULTS AND DISCUSSIONS

Ambrosia tenuifolia Spreng., *Syst. Veg.* 3: 851. 1826 (silver ragweed), unknown in the flora of Romania, until now, has been identified in the locality of C. A. Rosetti, the Danube Delta.

Herbarium specimens: UASVM Bucharest Herbarium, no. 20961 to 20967 (C. A. Rosetti, Tulcea County, roadsides, ruderalised sandy places; ca. 2 m a.s.l.; leg. et det. V. Ciocârlan, 13.IX.1991, as *A.*

coronopifolia Torrey et A. Gray; reviewed by G. Karrer, Vienna, 2019, as *A. tenuifolia* Spreng.); IASI (Herbarium of the University of Life Sciences Iași), no. 17983 to 17988 (C. A. Rosetti, Tulcea County, ruderalized grassland and abandoned garden, on sandy soil; ca. 2 m a.s.l., 45,295127°N, 29,568970°E; leg. C. Sîrbu, A. Oprea, M. Doroftei & S. Covaliov, 07.VIII.2020; det. C. Sîrbu & A. Oprea; confirmed by G. Karrer, Vienna, 2020); WHB, no. 78842 (duplum in the private Herbarium Gerhard Karrer).

Description of the examined specimens (Fig. 1; Fig. 2). Perennial herb, with bud-bearing roots. **Stems** erect, branched, up to 90 cm tall, ± sulcate, ± greyish-green, with multicellular hairs (appressed or curved upwards), mixed with sparse short glands.

Leaves compound, 2(3)-pinnate (only the uppermost ones 1-pinnate), opposite to alternate (the uppermost), with ± dense multicellular appressed stiff hairs (strigose) and sparse short glands, somewhat greyish-green, especially beneath; petiole up to 3 cm long, thin, wingless; blade ± deltoid, 5-7 × 4-6 cm, with about 4 pairs of primary segments; ultimate segments linear, one-ribbed, 1-1.3 mm wide.

Staminate heads nodding, with pedicel up to 3 mm long, bractless, in long (15-20 cm) racemiform arrays; involucre 3-4 mm diameter, wide-cup shaped, with uniseriate bracts, united for most of their length (margins sub-entire), with ± dense multicellular appressed stiff hairs; receptacular paleae narrowly linear, membranous, glandular, up to 2 mm long.

Male florets: 15-20 per head; corolla pale yellow, funnelform, 2-2.3 mm long, glandular; anthers yellowish-whitish, coherent, with a triangular and mucronulate appendage, bent inward (a rudimentary, filiform gynoeceum is also present).

Pistillate heads solitary or few in the axils of the upper leaves, just beneath male inflorescence, sessile, each with 1 floret; involucre in flower 2 mm long, slightly higher at fruiting (ca. 3.5 mm long), obpyramidal, gamophyllous, sparsely pilose, eglandular or with sparse glands, with a central thick rostrum up to 1 mm long, and 4-6 lateral conical teeth, of 0,5-1 mm long.

Pistillate florets without corolla or pappus; receptacle obovoid, ca. 1 mm long; style and stigma lobes filiform, the latter longer.

Cypselas (= achene) ± obovoid, enclosed within the hardened fruiting involucre.

These features of plants examined by us fully correspond to the descriptions and iconography published for *A. tenuifolia* by Arechavaleta (1906), in *Flora Uruguaya*, vol. 3, pp. 304-306, Estampa 60. Similar descriptions of *A. tenuifolia* has been published, in Europe, by Cosson & Kralik (1849-1850), Godron (1852), Pignatti (1982), Gallego & Valdés (1984), Amor Morales *et al.* (2012), Karrer *et al.* (2016), Montagnani *et al.* (2017), as well as, in Australia, by Orchard (2015).

The distinguishing traits of this species indicated in the short original diagnosis of Sprenger (1826) are also identifiable at our plants. Unfortunately, Sprenger (see also De Candolle, 1836) did not refer to the underground system of *A. tenuifolia*.

Baker (1882) published a very good picture of *A. tenuifolia* (*Flora Brasiliensis*, vol. 6(3), Tab. 49), which very well corresponds to our plants. However, this picture contains only the above-ground parts of the plant, and, on the other hand, the cited author described this species as annual. *A. tenuifolia* has also been described erroneously as an annual plant in other botanical references (Lawalree, 1947; Hansen, 1976; Behçet, 2004).

According to the recent literature (Aizpuru *et al.*, 1999; Verloove, 2005; Insausti & Grimoldi, 2006; Fuentes *et al.*, 2010; Amor Morales *et al.*, 2012; Orchard, 2015; Karrer *et al.*, 2016; Montagnani *et al.*, 2017; Luebert & García, 2020; Yair *et al.*, 2020), but see also Cosson & Kralik (1849-1850) or Godron (1852) - which published the earliest detailed diagnoses of this species - *A. tenuifolia* is, no doubt, a perennial species.

Identification key. In the literature, there is no identification keys including all species of the genus *Ambrosia*. However, there are publications that contain such useful keys, as well as detailed descriptions for species identified in different regions of the world, or synthetic tables that compare species with one another, such as: **North America:** Payne (1970), Basset & Crompton (1975), Strother (2006); **Central America:** León de la Luz & Rebman (2010); **South America:** Baker (1882), Payne (1966), Luebert & García (2020); **Europe:** Lawalree (1947), Hansen (1976) (but see comments elsewhere), Pignatti (1982), Aizpuru *et al.* (1999), Amor Morales *et al.* (2012), Karrer *et al.* (2016), Montagnani *et al.* (2017), Karrer (2018, 2019); **Eurasia:** Smoljaninova (1999/1959); **Asia:** Chen & Hind (2011); **Australia:** Orchard (2015).



Fig. 1. *Ambrosia tenuifolia*. C. A. Rosetti (Danube Delta, Romania)

The key below, based on the literature published by the authors cited above, can be used to distinguish only the four species that have been published from Romania, so far:

- 1a.** Leaf blade palmately 3(-5)-lobed, sometime entire. All leaves opposite. Annual ***A. trifida* L.**
- 1b.** Leaf blade pinnatipartite or 1-3 pinnate compound. At least the distal leaves alternate, the proximal opposite. Annual or perennial **2**
- 2a.** Annual, with tap root. Leaves sparsely hairy (subglabrous), green ***A. artemisiifolia* L.**
- 2b.** Perennial, with bud-bearing creeping roots (stolon-like). Leaves more densely hairy, somewhat greyish-green **3**
- 3a.** Leaves 2(3)-pinnate compound (only the uppermost ones 1-pinnate), with long and wingless petioles; ultimate segments linear, c. 1 mm wide. Pistillate heads solitary or few in the axils of the upper leaves; lateral teeth of the fruiting involucre 4-6, conical ***A. tenuifolia* Spreng.**
- 3b.** Leaves 1-pinnate compound or pinnatipartite, subsessile or occasionally on short-winged petioles; ultimate segments wider (2-3 mm). Pistillate heads clustered in the axils of the upper leaves; lateral teeth of the fruiting involucre 1-6, sometimes lacking, blunt ***A. psilostachya* DC.** (*Ambrosia coronopifolia* Torr. & A. Gray.).



Fig. 2. *Ambrosia tenuifolia*: foliar leaf, (a), leaf lacinia (b), staminate heads (c), rudimentary gynoceium of male flower (d), female heads (e, f) and pistil (g). Scale bar: a - 10 mm; b, e, f, g - 1 mm; c - 5 mm; d - 0.3 mm

Useful iconography for distinguishing between the last two species within the key is available in literature as follows: *A. tenuifolia* - Baker (1882), Arechavaleta (1906), Fuentes *et al.* (2010), Campagna (2014); Verloove & Aymerich (2020); *A. psilostachya* - Stinchfield Ferris (1960), Payne (1970), Reed & Hughes (1970), Basset & Crompton (1975), Montagnani *et al.* (2017); both species - Pignatti (1982), Aizpuru *et al.* (1999), Amor Morales *et al.* (2012), Karrer *et al.* (2016), Karrer (2018, 2019), Yair *et al.* (2019).

Possible reason of misidentifiy. As shown above, the perennial ragweed from Romania has previously been misidentified as *A. coronopifolia* (today a synonym of *A. psilostachya*). This is not a singular case. As Payne (1966) pointed out, "specimens of *A. tenuifolia* are occasionally misidentified as *A. psilostachya* DC., probably because of similarity of fruiting involucre morphology, or as *A. confertiflora* DC., which may have similar leaves".

In the paper published by Ciocârlan & Constantin (1992) the morphological description and taxonomic treatment of *A. psilostachya* (in the paper: *Ambrosia coronopifolia* Torrey et A. Gray, syn. *A. psilostachya* auct., non DC.) are in line with the *Flora Europaea*, vol. 4, pp. 143 (Hansen, 1976), while the iconography was taken over from the book *Selected weeds of the United States*, pp. 367, Fig. 180, published by Reed & Hughes (1970). In the figure published by Reed & Hughes (1970), the morphological features of *A. psilostachya* are indeed expressed very accurately. But, while some features in this figure are also found in plants collected from the Danube Delta (e.g. the habitus, the morphology of roots and inflorescences), concerning the leaves morphology (i.e. pinnatifid, subsessile or on short-winged petioles, with wide laciniae) and, to some extent, the fruiting involucre (i.e. with very short, blunt teeth), the differences are quite obvious. However, in the *Flora Europaea*, the species *A. tenuifolia* has been described (Hansen, 1976) as an annual plant, the only perennial ragweed species there indicated being *A. coronopifolia*. This can explain the identification of the perennial ragweed from Romania as *A. coronopifolia*, by Ciocârlan & Constantin (1992), the authors following in their paper this standard *Flora*, as a main reference.

Origin and general distribution. Unlike the other *Ambrosia* species reported so far in Romania, which are native to North America (Strother, 2006), *A. tenuifolia* is of South American origin (Baker, 1882; Arechavalleta, 1906; Thellung, 1912; Montagnani *et al.*, 2017). In Europe, it was first identified in France, in 1839, on sea sands, possibly introduced with ships ballast (Planchon, 1864; Thellung, 1912), and subsequently it spread to other regions of the continent, being up to now reported from France (Cosson & Kralik, 1849-1850; Godron, 1852; Planchon, 1864; Thellung, 1912), Germany (Thellung, 1912), Spain (Montserrat, 1954), and Italy (Vignolo-Lutati, 1935). According to Montagnani *et al.* (2017), excepting Germany (casual), in other European countries this neophyte is fully naturalized, however, only locally invasive. This is also the case of the populations from the Danube Delta, Romania.

It has also been introduced in other regions of the world, as: North America (Nelsson 1917); South Africa (Lalla, 2015); West Asia: Israel (Greuter & Raus, 1995; Yair *et al.*, 2019); Australia (Orchard, 2015). Global distribution of *A. tenuifolia* has been mapped by Montagnani *et al.* (2017) and Rojas-Sandoval (2018). A map of the European distribution of this species is also provided by Greuter (2006). The global distribution data published by Randal (2017) must be taken with some caution, as the cited author accepts the name *Franseria tenuifolia* Harv. & Gray as a synonym for *Ambrosia tenuifolia* Spreng., this synonymy being erroneous, as shown by Thellung (1912) (see also Montserrat, 1954, and Payne, 1964).

Biology, ecology and habitat preferences. The biology and ecology of *A. tenuifolia* were studied by many authors, and data from the literature has been synthesized by Montagnani *et al.* (2017) and Rojas-Sandoval (2018). Summarily, according to the literature, *A. tenuifolia* is a perennial herb (as already shown), monoecious, wind pollinated (Payne, 1970), with flowering time in February-March, in South America (Arechavalleta, 1906) or July-September, in the Northern Hemisphere.

It produces a great number of seeds, with long viability (Insausti & Grimoldi, 2006); however, outside the native area (in Israel), according to Yair *et al.* (2020), the number of viable seeds under field conditions was very low.

The 1-seeded fruits (cypselae=achenes), enclosed in lignified involucre, are not preadapted for being dispersed by a specific vector. Natural dispersion is done by wind, water and animals, starting at the end of summer (Insausti *et al.*, 1995); they can also be accidentally dispersed by man over long-distances, as contaminants of ship ballast (Planchon, 1864; Nelson, 1917) or of agricultural goods, machinery, etc. (Montagnani *et al.*, 2017).

Seed germination occurs mainly in spring, after the needed vernalisation (Insausti *et al.*, 1995), in light, on the soil surface or no more than 2 cm depth (Yair *et al.* 2020).

Ambrosia tenuifolia (just like *A. psilostachya*) has a great capacity to propagate clonally through ramets that sprout from adventitious root buds (Insausti & Grimoldi, 2006; Karrer *et al.* 2016).

It is a heliophilous species from warm (rather subtropical than temperate) climate, being drought tolerant, somewhat salt tolerant, and preferring well-aerated, \pm neutral soils, sometimes affected by floods (Insausti & Grimoldi, 2006).

Ambrosia tenuifolia is a pioneer species, typical of open habitats, which successfully colonizes vegetation gaps; however, taking advantage of clonal propagation (Insausti & Grimoldi, 2006), and allelopathic compounds (Mongelli *et al.* 1997), it also can persist in more evolved (yet disturbed) environments, where it can become co-dominant.

It prefers sand dunes, disturbed grasslands and ruderal habitats, on sandy grounds (Arechavalleta, 1906; Amor Morales *et al.*, 2012; Montagnani *et al.*, 2017; Rojas-Sandoval, 2018; Karrer, 2019), but has also been reported as a weed of cultivated fields (Campagna, 2014; Rojas-Sandoval, 2018).

Current status in Romania. In the locality of C. A. Rosetti (Danube Delta), the population of *A. tenuifolia* has conquered an area of approx. 100 sq.m, on sandy soil, near the crossroads of DC3 & DC4 (communal roads), just behind a local shop, north of the church (45,295127°N, 29,568970°E; ca. 2 m a.s.l.). Scattered individuals grew near the road and on the nearby ruderalized xerophilous grassland (which was dominated by *Cynodon dactylon*), but the population also expanded into a derelict neighbouring garden, where it was much denser, consisting of hundreds of shoots. From our preliminary data, it seems that the plants propagate locally slowly, only clonally, by root sprouting. Although it

produces morphologically normal fruits and seeds, to date there is no evidences that the seeds germinate in that place or in the surroundings. However, we consider that the species is naturalized in the Danube Delta, where it persists and flourishes for over three decades.

Potential impact Like other species of *Ambrosia* that are wide-spread outside their native range, *A. tenuifolia* is considered a harmful weed, mainly because it has a high production of allergenic pollen, that causes hay fever. It is also supposed (Montagnani *et al.*, 2017; Rojas-Sandoval, 2018) to potentially disrupt and outcompete native plant communities, especially during early successional stages.

CONCLUSIONS

Ambrosia tenuifolia Spreng. (Asteraceae), a neophyte native to the South America, is reported in this paper for the first time in the spontaneous flora of Romania. This species has been first found in Romania, in the village of C. A. Rosetti, from the Danube Delta, three decades ago, but up to now it has been known erroneously as *A. psilostachya* (*A. coronopifolia*).

Based on current data, although it seems to spread only locally by root sprouting, *A. tenuifolia* can be considered a naturalized neophyte in Romania.

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