

Genome size confirms the presence of *Artemisia verlotiorum* (Asteraceae) in Lebanon

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Abstract: The Far Eastern weed *Artemisia verlotiorum* was formerly reported from Lebanon. However, for a number of reasons this record was questionable. In 2019, the genuine presence of this species was confirmed based on macromorphology and strengthened by assessing the somatic nuclear DNA content (genome size or 2C-value) of the Lebanese populations.

Key words: *Artemisia verlotiorum*, genome size, Lebanon, weed

1. Introduction

Although initially described based on specimens collected near Clermont-Ferrand in France in the second half of the 19th century (Lamotte, 1877), *Artemisia verlotiorum* Lamotte (Asteraceae) is native to China (Ling et al., 2011). It is a rhizomatous, weedy species that has naturalized widely in other areas across the world. It is reportedly known from Australia and New Zealand, many countries in Europe and Southeastern Asia, and in North and South America (Ling et al., 2011; Thompson, 2015). However, these claims are in part erroneous, and result from the ongoing confusion of *A. verlotiorum* with other representatives of *Artemisia* L. sect. *Artemisia* (Verloove, 2013¹; Mosyakin et al., 2018; Mosyakin et al., 2019).

Artemisia verlotiorum was formerly reported from Lebanon by Mouterde (1983) as follows: “Mct. Çà et là dans la plaine de Beqâa, Ksara dès 1932 (Mt) (reste de cultures antérieures?), vers Chtaura (Mt), Haouch-el-‘Oumara (Mt, Np)” [translation (FV): Mct. Here and there in the valley of river Beqâa, Ksara since 1932 (Mt) (remnants of previous cultures?), towards Chtaura (Mt), Haouch-el-‘Oumara (Mt, Np)]. The species had not been mentioned by Bouloumoy (1930) and Thiébaud (1936–1953). The presence of *A. verlotiorum* in Lebanon required confirmation for several reasons; first, because it was considered a probable relic of cultivation. Indeed, *A. verlotiorum* is sometimes cultivated for its medicinal

properties, but to our knowledge, only in China (Ling et al., 2011). Moreover, it is unclear whether the medicinal plant corresponds with genuine *A. verlotiorum*. Secondly, Lebanon—and more precisely the inland areas where *A. verlotiorum* was reported by Mouterde (1983)—has a predominantly hot and dry climate with an annual average temperature above 18 °C (BSh: ‘hot semiarid’ climate type, Köppen-Geiger; Kottek et al., 2006). *Artemisia verlotiorum* is slightly thermophilous, but it is normally found in wetter and cooler climate types than the area in Lebanon where it was reportedly discovered. The populations known to be naturalized in Algeria in Northern Africa are a notable exception (Battandier, 1904). Finally, a phylogenetic analysis of accessions of *Artemisia* section *Artemisia* from another region in the Middle East (Ordu Province in Northeastern Turkey) demonstrated that there were more and different species from section *Artemisia* in the area than previously assumed, i.e. *A. vulgaris* L. and *A. verlotiorum* (Cullen, 1975; Davis, 1988). Four distinct haplotypes were resolved, and these appeared in the same lineage with *A. sylvatica* Maxim., *A. argyi* H. Lév. & Vaniot, and *A. verlotiorum* (Eker & Koloren, 2016; Koloren et al., 2016).

Mouterde apparently made several collections, and according to Stafleu & Cowan (1981), these are mostly preserved in the Geneva herbarium (G). Indeed, at least 5 collections are present in G, all collected between 1932

¹ Verloove F (2013). Not every Far Eastern mugwort is *Artemisia verlotiorum*! In: Verloove F. Manual of the Alien Plants of Belgium. Botanic Garden of Meise, Belgium. Website: <http://alienplantsbelgium.be/content/not-every-far-eastern-mugwort-artemisia-verlotiorum> [accessed 29 November 2019].

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and 1957². All collections except one refer to nonflowering individuals. A collection from Ksara from September 1932 (Figure 1) represents a flowering specimen; however, this does not allow us to determine the identity unambiguously.

In May 2019 2 of the authors (AM and MB) found nonflowering *Artemisia* individuals in Ouyoun el Samak (Btormaz) in Lebanon. Some roots were collected in order to obtain ex situ flowering material and assess genome size. Genome size can help in discriminating critical taxa in *Artemisia* (Torrell & Vallès, 2001; Garcia et al., 2008; Pellicer et al., 2010).

The identity of this species was assessed based on the morphology of these plants as well as their genome size.

2. Materials and methods

2.1. Plant material

Field work in Lebanon was undertaken in May 2019 by 2 of the authors (Attila Mesterházy and Marc El Beyrouthy). Rhizomes were collected and were further grown ex situ in the garden of the first author in Belgium. A pressed specimen was deposited in the herbarium of Meise Botanic Garden, Belgium (BR; acronym following Thiers 2019)³

2.2. Flow cytometry

Fresh leaf fragments were harvested and used by Ben JM Zonneveld to assess the somatic nuclear DNA content (genome size or 2C-value). For the isolation of nuclei, c. 0.5 cm² of a young leaf was chopped together with a piece of *Agave americana* L. 'Aureomarginata' as an internal standard (Galbraith et al., 1983). A nuclear DNA content (2C-value) of 15.9 picograms (pg) per nucleus was determined with human leukocytes (2C = 7 pg) as the standard (Tiersch et al., 1989). The 2C-value of *Artemisia verlotiorum* is very close to the 2C-value of *Agave americana*; *Agave attenuata* Salm-Dyck (7.9 pg) was used as a standard as well. Based on the published male human genome size of 6.294×10^9 base pairs, the nucleus was calculated to contain 6.436 pg (Doležel et al., 2003). However, this is based on a human sequence; as the size of the very large repeat sequences could not be accurately determined, the genome size could be closer to 7 pg than currently envisioned. The Animal Genome Size database (version 2.0; <http://www.genomesize.com>) gives a haploid size (1C-value) of 3.5 pg.

Leaflets were chopped at room temperature with a new razor blade in a petri dish in 0.30 mL of nuclei isolation buffer [per liter: MgCl₂ × 6H₂O, 9.15 g; tri-

² personal comm. C. Chatelain, April 2020

³ Thiers B (2019) onward (continuously updated). Index Herbariorum. A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Website: <http://sweetgum.nybg.org/science/ih> [accessed 29 November 2019].

⁴ Verloove F, Janssens SB, Andeweg R, Zonneveld BJM (2020). Morphological, genome-size and molecular evidence for the presence of another invasive East Asian *Artemisia* (Asteraceae) in Western Europe. *BioInvasions Records* (in press).

natrium citrate, 8.8 g; MOPS, 4.15 g; Triton X-100, 1 mL; polyvinylpyrrolidone 10,000, 25 g; dithiothreitol, 1.55 g; and 0.01 % RNase, pH 7 (c. 1.12 g KOH)] (changed after Bharathan et al., 1994). After adding 1.40 mL of propidium iodide solution (50 mg PI/L) in isolation buffer, the suspension containing the nuclei was filtered through a 20 µm nylon filter. Fluorescence of the nuclei of individual plants was measured twice, at 30 and 60 min after the addition of PI using a BD Accuri C6 flow cytometer. The average of these 2 measurements for each sample is given in Table. The more DNA present in a nucleus, the higher the intensity of fluorescence. The 2C-value or DNA content per nucleus was calculated as the sample peak mean, divided by the *Agave* peak mean, and multiplied by the DNA amount of the *Agave* standard. For each clone, 2 to 4 different runs (determinations with around 2000–5000 nuclei) were measured.

3. Results and discussion

A population sampled in Ouyoun el Samak (Btormaz) in Lebanon in May 2019 agrees well morphologically with the initial circumscription of *Artemisia verlotiorum* (Lamotte, 1877). The plants are clearly long-rhizomatous, strikingly aromatic, and upper cauline leaves have a long, entire terminal leaf lobe. However, in order to assure identification based on morphological grounds, genome size of the Lebanese plant was also measured by flow cytometry and compared with our previous measurements for *A. verlotiorum* and similar weedy species such as *A. princeps* Pamp. and *A. vulgaris* (Verloove et al., 2020). Protocols, standards, and fluorochromes that were used in the latter study were exactly the same as those applied here.

The genome size of *Artemisia verlotiorum* from Lebanon was 2C = 13.5 picograms per nucleus. This is exactly in line with our own measurements for that species, from other origins, where values for *A. verlotiorum* varied between 13.3 and 14.2 pg (Table; Verloove et al., 2020)⁴. A previous assessment by Garcia et al. (2013) produced a similar value (2C = 12.54 pg). In our study, *A. vulgaris* and *A. princeps* have a genome size that ranges between 6.45–6.75 pg and 9.99–10.4 pg, respectively (Verloove et al., 2020)⁴.

We found *A. verlotiorum* in the northernmost part of Lebanon, relatively close to the Syrian border. It is fully naturalized there in relatively cooler inland valleys, which explains the naturalization of this species in an



Figure 1. Historical herbarium collection of *Artemisia verlotiorum* from Lebanon, collected in September 1932 by Paul Mouterde and preserved in the Geneva herbarium.

Table. Genome size of accessions of *Artemisia verlotiorum*, *A. vulgaris* and *A. princeps* from various countries (Verloove et al., 2020 and Garcia et al., 2013). An accession from Lebanon is in line with our previous measurements for *A. verlotiorum*.

Species name	Provenance	pg/2C (average/individual)	pg/2C (average/species)	Standard deviation
<i>Artemisia vulgaris</i>	Belgium, port of Antwerp, Kanaaldock	6.45	6.63	0.117
<i>Artemisia vulgaris</i>	The Netherlands, Leiden, Apollolaan	6.52		
<i>Artemisia vulgaris</i>	The Netherlands, Leiden, Amphoraweg	6.66		
<i>Artemisia vulgaris</i>	The Netherlands, Leiden	6.66		
<i>Artemisia vulgaris</i>	Ukraine, Kiev	6.72		
<i>Artemisia vulgaris</i>	Ukraine, Kiev	6.75		
<i>Artemisia princeps</i>	Belgium, port of Antwerp, Kruisweg	9.99	10.12	0.155
<i>Artemisia princeps</i>	Belgium, Wilrijk, A12 motorway	10		
<i>Artemisia princeps</i>	Belgium, port of Antwerp, E of Churchilldock	10		
<i>Artemisia princeps</i>	The Netherlands, Rotterdam, A15 motorway	10		
<i>Artemisia princeps</i>	Belgium, port of Zeebrugge, Koffieweg	10		
<i>Artemisia princeps</i>	Belgium, Gent, R4 motorway	10.1		
<i>Artemisia princeps</i>	Belgium, port of Antwerp, Ekerse Dijk	10.1		
<i>Artemisia princeps</i>	Belgium, Lokeren, E17 motorway	10.1		
<i>Artemisia princeps</i> (subsp. <i>momiyaamae</i>)	Great Britain, Sidmouth, Twibell Artemisia collection	10.2		
<i>Artemisia princeps</i>	Great Britain, Sidmouth, Twibell Artemisia collection	10.4		
<i>Artemisia princeps</i>	Belgium, port of Antwerp, Berendrecht	10.4		
<i>Artemisia verlotiorum</i>	Unknown	12.54 (Garcia et al., 2013)	13.75	0.29
<i>Artemisia verlotiorum</i>	Belgium, Kortrijk (Marke)	13.3		
<i>Artemisia verlotiorum</i>	Belgium, Gent, Burggravenlaan	13.4		
<i>Artemisia verlotiorum</i>	Belgium, Kortrijk (Marke)	13.5		
<i>Artemisia verlotiorum</i>	Lebanon	13.5		
<i>Artemisia verlotiorum</i>	The Netherlands, Leeuwarden	13.6		
<i>Artemisia verlotiorum</i>	Australia, Melbourne	13.6		
<i>Artemisia verlotiorum</i>	Belgium, Anderlecht, West Station	13.6		
<i>Artemisia verlotiorum</i>	Belgium, Kortrijk, river Leie	13.7		
<i>Artemisia verlotiorum</i>	Belgium, Strombeek-Bever	13.8		
<i>Artemisia verlotiorum</i>	The Netherlands, Haarlem, Jansstraat	13.8		
<i>Artemisia verlotiorum</i>	Spain, Granada	13.8		
<i>Artemisia verlotiorum</i>	Belgium, Brussels (Evere)	14.1		
<i>Artemisia verlotiorum</i>	The Netherlands, Rotterdam, De Punt	14.1		
<i>Artemisia verlotiorum</i>	The Netherlands, Rotterdam, Blijdorp	14.2		
<i>Artemisia verlotiorum</i>	Great Britain, Tottenham	14.2		

otherwise unfavorable climatologic area. Mouterde (1983) reported *A. verlotiorum* from the Ammiq swamps in the Bekaa Valley in Central Lebanon. This region underwent dramatic development in recent decades, and it is unknown whether *A. verlotiorum* is still present there. Recently, Dilabazian & Na'was (2019) reported *A. verlotiorum* from the Byblos region, which is located in

the Mount Lebanon Governorate. Upon request, these authors (who are not botanists) could not present photos or vouchers. Moreover, the plant material originated from Tyre in the South Governorate, not from Byblos. The plant material was bought as a dry sample from an herbalist in Southern Lebanon. The authors were told that the sample was an *Artemisia*, without further information⁵. Herbalists

⁵ pers. comm. H. Dilabazian and T. Na'was, January 2020.

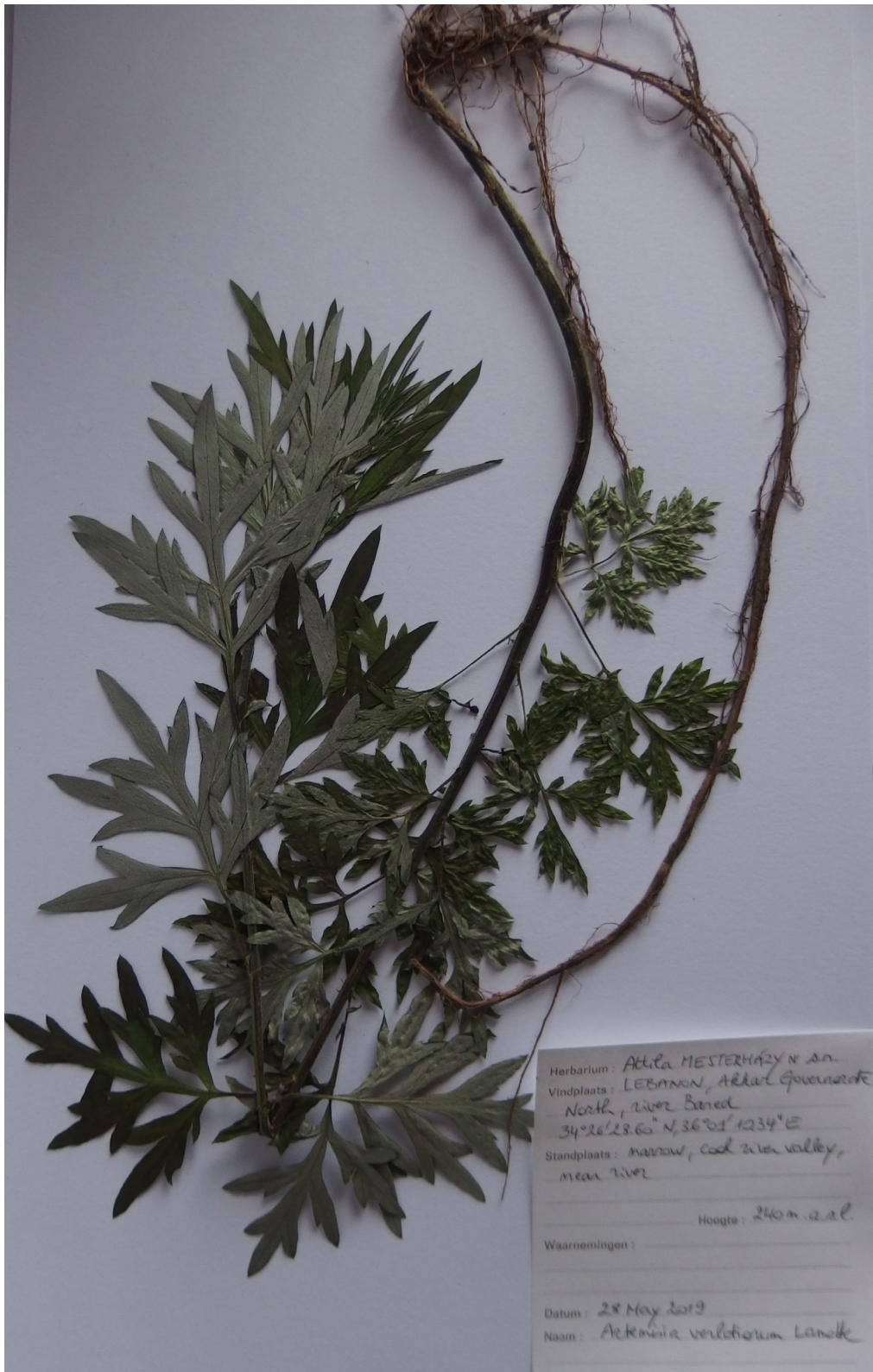


Figure 2. Herbarium specimen of *Artemisia verlotiorum* from a newly detected Lebanese population. The long rhizomes are characteristic and the identity of these plants was confirmed by assessing the genomic DNA content.

in Lebanon usually sell *Artemisia herba-alba* Asso (El Beyrouthy, 2009; El Beyrouthy et al., 2008). The presence of *A. verlotiorum* in that area still requires confirmation.

Herbarium:

LEBANON, Akkar Governorate North, River Bared, 34°26'28.60"N, 36°01'10.34"E, 240 m a.s.l., narrow, cool river valley, near river, 28 May 2019, *A. Mesterházy* s.n. (BR) (Figure 2).

4. Conclusion

The genuine presence of the Far Eastern weed *Artemisia verlotiorum* in Lebanon—considered doubtful for a number of reasons—was recently unequivocally

confirmed, based on macromorphological characters and genome size assessment. The species is naturalized in the valley of the Bared River in the Akkar Governorate North.

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