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ORIGINAL ARTICLE

Origin and systematic position of *Jacobaea vulgaris* (Asteraceae) octoploids: genetic and morphological evidence

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Abstract Five cytotypes have been reported for *Jacobaea vulgaris* (syn.: *Senecio jacobaea*); three of them with euploid (tetraploid, hexaploid, and octoploid; $2n = 40$, 60, and 80) and one with aneuploid ($2n = 32$) chromosome numbers. Among them, only tetra- and octoploid cytotypes are regularly found, the other two are very rare. In this study we re-evaluated the origin and systematic position of *J. vulgaris* octoploids. DNA ploidy levels, morphological, and genetic (AFLP, amplified fragment length polymorphism) data were generated for 38 populations of *J. vulgaris* from Central and Eastern Europe, and adjacent parts of North-Western Europe. Genetic dataset was supplemented with 16 populations of five closely related species: *J. alpina*, *J. aquatica*, *J. erratica*, *J. erucifolia*, and *J. subalpina*. The octoploid cytotype of *J. vulgaris*, known thus far only from Pannonia and Ukrainian Podillya regions, has also been found on two Baltic islands, Öland and Gotland. AFLP analyses showed clear genetic differences between tetra- and octoploid cytotypes and revealed that all octoploid plants are most likely of autopolyploid origin. The AFLP data also indicate that octoploids form two separate allopatric and monophyletic lineages, one represented by Pannonian and Öland populations, and the other represented by the populations from Podillya and Gotland. The octoploids from Gotland correspond to the previously recognized subspecies *J. vulgaris* subsp. *gotlandica*. The octoploids distributed in Pannonia are described here as a new subspecies, *J. vulgaris* subsp. *pannonica*.

Keywords AFLP fingerprinting · Flow cytometry · Multivariate morphometrics · Polyploidy · *Senecio jacobaea*

Introduction

During the past decade, there has been a tremendous increase of interest in polyploidy, to a large extent stimulated by the development of genetic and genomic tools (Soltis et al. 2010; Dufresne et al. 2014; Vanneste et al. 2014). It is now known that most lineages of angiosperms underwent at least one episode of whole-genome duplication during their evolutionary history, and polyploidization has been recognized as a major force of vascular plants speciation (Soltis et al. 2009; Madlung 2013). The importance of polyploidy is manifested mainly in sympatric speciation because polyploids can become genetically abruptly isolated from their lower ploid progenitors (Otto and Whitton 2000) and may also have evolutionary advantages due to novel physiological, ecological, and life history traits, that is, polyploids might be preadapted to new environments (e.g., Levin 1983; Segraves and Thompson 1999; Thompson et al. 2004).

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Two major types of polyploids are distinguished: auto- and allopolyploids (Clausen et al. 1945; Ramsey and Schemske 1998; Ramsey and Ramsey 2014). From these two processes of polyploid formation, allopolyploidy has been earlier recognized as an important speciation mechanism (Rieseberg and Willis 2007). Conversely, genome doubling within a taxon (autopolyploidy) and its importance in plant speciation have largely been neglected, since autopolyploidy often remains phenotypically hidden within their lower ploid progenitors; cf. Soltis et al. 2007). Nevertheless, Ramsey and Schemske (1998) estimated the rate of autopolyploid formation to be higher than that of allopolyploids, suggesting that autopolyploids are much more common than traditionally anticipated (cf. Soltis and Soltis 1999; Soltis et al. 2007). Despite the common occurrence of autopolyploids, it still remains an open question whether genome doubling meaningfully contributes to long-term evolution. Some authors believed that autopolyploids represent evolutionary deadends (e.g., Clausen et al. 1945; Stebbins 1971). On the other hand, successful range expansion demonstrated in various natural autopolyploids suggests that genome multiplication per se may represent an evolutionary advantage and autopolyploidy should be considered as one of the important mechanisms of speciation (cf. Soltis et al. 2007; Martin and Husband 2012).

Important mechanism, which can promote speciation in plants is hybridization (for recent examples see e.g., Marcussen et al. 2012; Zozomová-Lihová et al. 2014). Hybridization can lead to the origin of new species either via allopolyploidy (Soltis and Soltis 1993; Leitch and Bennett 1997) or homoploidy (i.e., without change in ploidy level; Rieseberg 1997). Hybridization may generate novel traits (Rieseberg et al. 1999; Abbott et al. 2003) allowing hybrid lineages to adapt to new environments and colonize spatially and ecologically isolated niches from their parents, which can be the first step in speciation (Rieseberg et al. 2003). In particular hybridization in connection with genome duplication (allopolyploidy) is important mechanism of speciation as it can instantly lead to formation of a fertile novel species that is reproductively isolated from its parents.

Tribe Senecioneae of the Asteraceae family is known to harbour large cytotype diversity (Nordenstam et al. 2009). In recent years, this tribe has undergone a major sectional and generic reorganization, stimulated by molecular phylogenetic studies, and several genera have become more narrowly defined (Pelser et al. 2007; Nordenstam et al. 2009). One such resurrected and narrowly circumscribed genus is *Jacobaea* Mill., in which ca 45 species have been included, most of which were formerly classified in *Senecio* sect. *Jacobaea* (Mill.) Dumort. (Pelser et al. 2006; Nordenstam 2006; Nordenstam and Greuter 2006). *Jacobaea* species are known to hybridize in nature (e.g., Holub 1972; Lowe and Abbott 2004; Kirk et al. 2004) and there is evidence that hybridization may contribute to the evolutionary history of the genus (Pelser et al. 2003). Several *Jacobaea* taxa are karyologically heterogeneous, having two or more ploidy levels (cf. Bolkhovskikh et al. 1969; Dobeš and Vitek 2000; Hodálová et al. 2010; Sonnleitner et al. 2010). One of them is *Jacobaea vulgaris* Gaertn. (syn. *Senecio jacobaea* L.; Hodálová et al. 2010), with three ploidy levels discovered (see below).

Jacobaea vulgaris is a biennial or short living perennial, cross-pollinated herb. It grows in lowland to mountain vegetation belt in different types of open habitats, such as primary or secondary grasslands, sands, roadsides, and abandoned man-made habitats. The species is native to Eurasia and was introduced into North and South America, South Africa, Australia, and New Zealand (Bain 1991). It is known for its high content of toxic pyrrolizidine alkaloids (e.g., Macel et al. 2004; Kirk et al. 2004; Pelser et al. 2005). Aggressive range expansion of this species, especially in regions where it was introduced by the human activities, has recently been reported. This also has a significant impact on the economy of affected regions because the toxicity of this species negatively impacts livestock production, and crop and forage yields (Bain 1991).

Jacobaea vulgaris is a taxonomically heterogeneous taxon, in which three subspecies have widely been recognized (Nordenstam and Greuter 2006; Wysk et al. 2009). A nominate subspecies (*J. vulgaris* subsp. *vulgaris*; syn. *Senecio jacobaea* subsp. *jacobaea*) has been described from the pastures of Europe ("*Habitat in Europae pascuis*"; Linnaeus 1753: 870). It is distributed from the Iberian Peninsula in the west, throughout submediterranean and temperate Europe (Fig. 1a), and eastwards to the river Lena in Russia (Meusel and Jäger 1992). The taxonomic status of the invasive populations of *J. vulgaris* introduced to different parts of the world (see above) has not been studied yet. The second subspecies, *J. vulgaris* subsp. *gotlandica* (Neuman) B.Nord. [syn. *Senecio jacobaea* var. *gotlandicus* Neuman, *S. jacobaea* subsp. *gotlandicus* (Neuman) Sterner], has been described from the island of Gotland (Neuman and Ahlfvengren 1901; based on our lectotypification in this paper, the type locality is Klinteberget hill) and, for a long time, was considered to be an endemic to the Swedish islands of Öland and Gotland. However, based on ITS nuclear data and morphology, Wysk et al. (2009) argued that this subspecies also occurs in Austria, Greece, and Russia. Based on morphological evidence, Conti et al. (2012) also reported this subspecies from Italy and Montenegro (see Fig. 1a). The most important morphological diagnostic character that should delimit *J. vulgaris* subsp. *gotlandica* from the other two subspecies is the shape of the lower and middle cauline

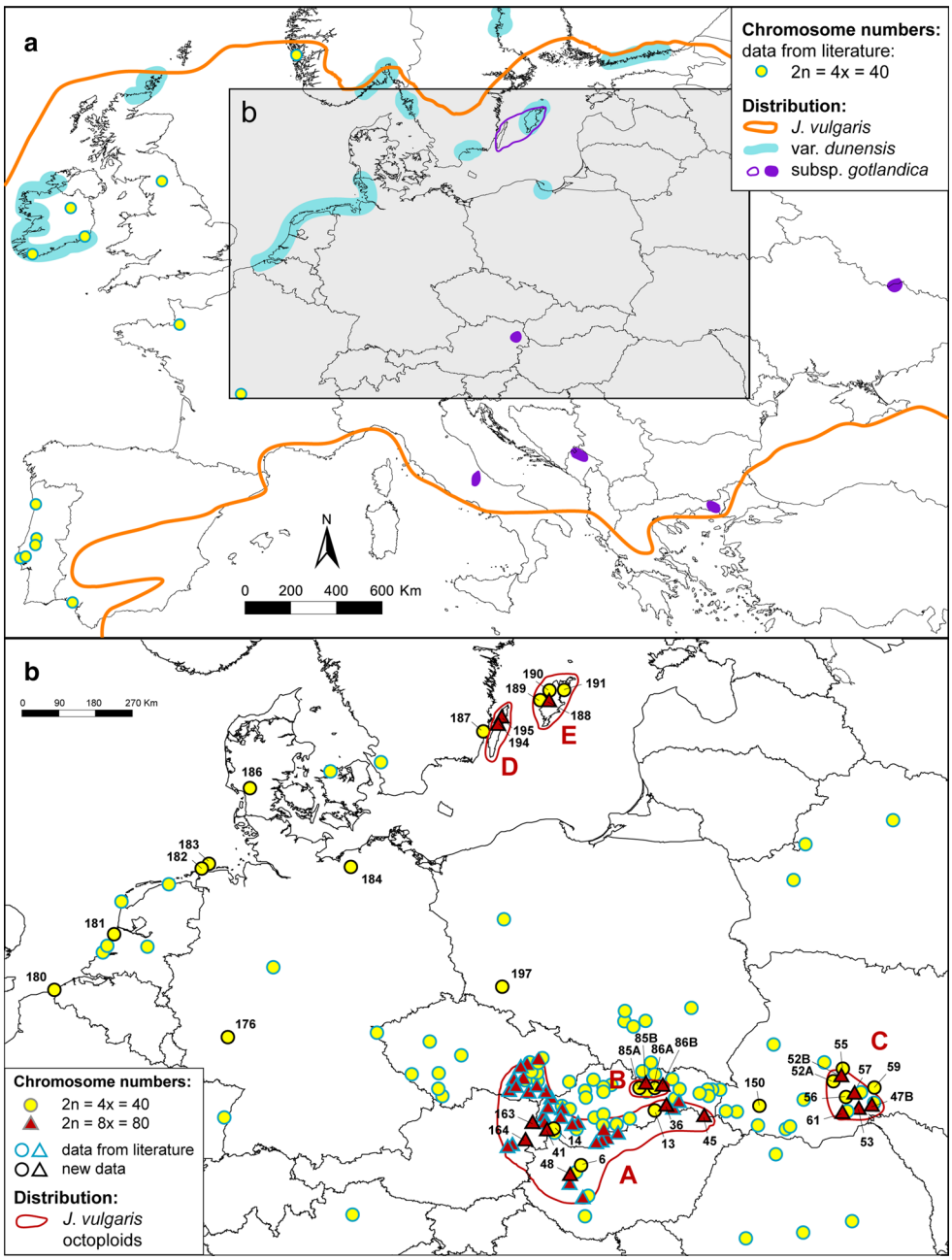

Fig. 1 Distribution of tetraploid and octoploid cytotypes of *Jacobaea vulgaris* in Europe based on data from literature (see Hodálová et al. 2007a, 2010) and the present study (see Table 1). *Symbols* referring to the literature data are marked by *blue edges*, those referring to the data from the present study are marked by *black edges*. Overview of the distribution range of *J. vulgaris* in Europe: *orange lines* indicate the southern and northern boundary of the assumed native distribution range of *J. vulgaris* (following Meusel and Jäger 1992); distribution range of *J. vulgaris* var. *dunensis* according to Kadereit and Sell (1986), Andersson (2001a), and herbarium data (BP, LINN, LD, W, WU) is marked by *blue patches* (inland localities were not marked); the *violet line* indicates the distribution of *J. vulgaris* subsp. *gotlandica* known up to 2009 (see Wysk et al. 2009), while *violet patches* indicate new occurrences reported by Wysk et al. (2009) and Conti et al. (2012). **b** Detailed view of the area studied. *Red line* indicates the distribution of octoploid cytotype based on the present study (A–Pannonia, B–Spiš region, C–Podillya, D–Öland, E–Gotland); *numbers* indicate populations used in molecular and morphometric studies (for population numbers see Table 1)

leaves. In particular, the lower cauline leaves should be almost entire with enlarged apical leaf segment while those in the other two subspecies should be deeply pinnatisect without enlarged apical leaf segment (Wysk et al. 2009). The third subspecies, *J. vulgaris* subsp. *dunensis* (Dumort.) Pelser & Meijden [syn. *Senecio dunensis* Dumort., *S. jacobaea* subsp. *dunensis* (Dumort.) Kadereit & P.D.Sell], has been described in the dunes on the Belgian sea coast (Dumortier 1827). Recently, this taxon was reported mainly from the coastal areas of Ireland to southern Finland (Fig. 1a). It is distinguished from the other two subspecies mainly by its lost or reduced ray florets (Kadereit and Sell 1986; Andersson 2001a, b).

Concerning cytotype variation in *J. vulgaris*, with the exception of the single aneuploid chromosome count of $2n = 32$, only tetraploids ($2n = 4x = 40$) were reported for this species until the 1960s (for details see Hodálová et al. 2007a). In 1970, the first octoploid count was reported for this species from Slovenský kras Karst in the Pannonian region of Slovakia (Murín and Váchová 1970). Subsequently, octoploid plants have been found in a number of localities in the Pannonian and Podillyan (Podolian)

biogeographic regions (Slovakia, Czech Republic, Austria, Hungary, and Ukraine; Murín and Májovsky 1987; Murín et al. 1999; Grulich 2005; Hodálová et al. 2007a, b; Vinikarová 2009). DNA sequence data and AFLPs revealed that the closest allied congeners of *J. vulgaris* occurring in Central Europe, which could have potentially contributed to the origin of octoploids are: *J. alpina* (L.) Moench, *J. aquatica* (Hill) G.Gaertn. et al., *J. erratica* (Bertol.) Fourr., *J. erucifolia* (L.) G.Gaertn. et al., and *J. subalpina* (W.D.J.Koch) Pelser & Veldk. (cf. Pelser et al. 2003).

Detailed cytotype screening also revealed the rare occurrence of hexaploids ($2n = 6x = 60$) of *J. vulgaris* in both Pannonia and Podillya (Hodálová et al. 2010). However, the distribution area of *J. vulgaris* is unequally explored regarding cytotype distribution. Thus far, the cytotype variation found in *J. vulgaris* almost exclusively concerns the nominate subspecies and the European populations. In the remaining two *J. vulgaris* subspecies, only three tetraploid chromosome records were reported; two for *J. vulgaris* subsp. *dunensis* [by van den Brand et al. (1979) as *Senecio jacobaea* var. *nudus* West., and by Kockx-van Roon and Wieffering (1982) as *S. jacobaea* var. *flosculosus* Lam. & DC.] and one for *J. vulgaris* subsp. *gotlandica* (Wysk et al. 2009). Outside Europe, chromosome numbers are reported for this species only from North America, where only the tetraploid cytotype was detected (Bain 1991). The single diploid chromosome number ($2n = 2x = 20$) reported for *J. vulgaris* from Bulgaria is erroneous and refers to *J. aquatica* or *J. erratica* (cf. Hodálová et al. 2010).

Despite the fact that few studies dealing with morphological and/or genetic variation of particular cytotypes/taxa of *J. vulgaris* have been published (Hodálová et al. 2007a; Wysk et al. 2009), none of them is comprehensive with respect to the employed methods and sampling strategies. Hodálová et al. (2007a) investigated morphological variation in tetra- and octoploid cytotypes of *J. vulgaris* from Pannonia and adjacent parts of the Carpathians. They showed that both cytotypes exhibited a slight tendency for mutual morphological differentiation; however, only floral traits have been considered. Because no genetic data were available, no taxonomical classification of the central European cytotypes of *J. vulgaris* has been proposed. In contrast, Wysk et al. (2009), who investigated the genetic variation of *J. vulgaris* using nuclear and plastid sequences, reported the presence of two *J. vulgaris* subspecies in Central Europe, *J. vulgaris* subsp. *vulgaris* and *J. vulgaris* subsp. *gotlandica*. These authors also studied the morphology of both subspecies, but they considered only a limited number of vegetative traits (leaf morphology); moreover, they did not consider the cytotype variation of the studied taxa.

From the above-mentioned evidence it was apparent that the taxonomy and systematic position of Central European populations of *J. vulgaris* and especially the octoploid plants remained unclear. This encouraged us to re-evaluate the origin and systematic position of *J. vulgaris* octoploids from Pannonia and Ukrainian Podillya regions, using a combination of flow cytometry, and genetic and morphological methods, considering both vegetative and generative morphological characters. Particular questions addressed by this study are as follows: (1) Are the *J. vulgaris* octoploids of auto- or allopolyploid origin? (2) Did octoploids evolve only once or did they arise repeatedly (polytopically) from sympatric tetraploid populations? (3) Is it possible to infer some taxonomic conclusions from the obtained ploidy level, genetic, and morphological data?

Hexaploid individuals of *J. vulgaris* were found in Pannonia, Podillya (Hodálová et al. 2010), and on Gotland (I. Hodálová and P. Mered'a unpublished data). They likely arose through the fusion of reduced and unreduced gametes of tetraploids and through hybridization between tetra- and octoploids (Hodálová et al. 2010, I. Hodálová and P. Mered'a unpublished data). They are partly or completely sterile (Hodálová et al. 2010) and do not form separate populations, therefore, their occurrence on particular localities is probably only temporary. These were the reasons why they were not included in this study.

In a study of genetic variation, we used AFLP data, which have already been successfully used to resolve genetic and evolutionary relationships within numerous polyploid complexes (e.g., Hedrén et al. 2001; Španiel et al. 2011; Kuzmanović et al. 2013). Sequence markers such as the nuclear ribosomal ITS region and/or various plastid markers were not used in our study because they were shown to be insufficiently variable within *J. vulgaris* and among closely related taxa (see Wysk et al. 2009) and thus not suitable for addressing the proposed aims of the current study.

Materials and methods

Plant material

The sampling design was focused on the two regions where octoploid populations of *J. vulgaris* have been reported (Hodálová et al. 2010): (1) Pannonia and adjacent parts of the (Western and Eastern) Carpathians (Pannonia, hereafter), (2) the Podillya Upland in western Ukraine (Podillya, hereafter). To verify the taxonomic position of *J. vulgaris* populations from these two regions, we also included material from regions from where currently accepted subspecies of *J. vulgaris* (*J. vulgaris* subsp. *dunensis* and *J. vulgaris* subsp. *gotlandica*) have been described, namely, (3) the coast of North-Western Europe from Belgium to Gotland Island, and adjacent parts of Germany and Poland

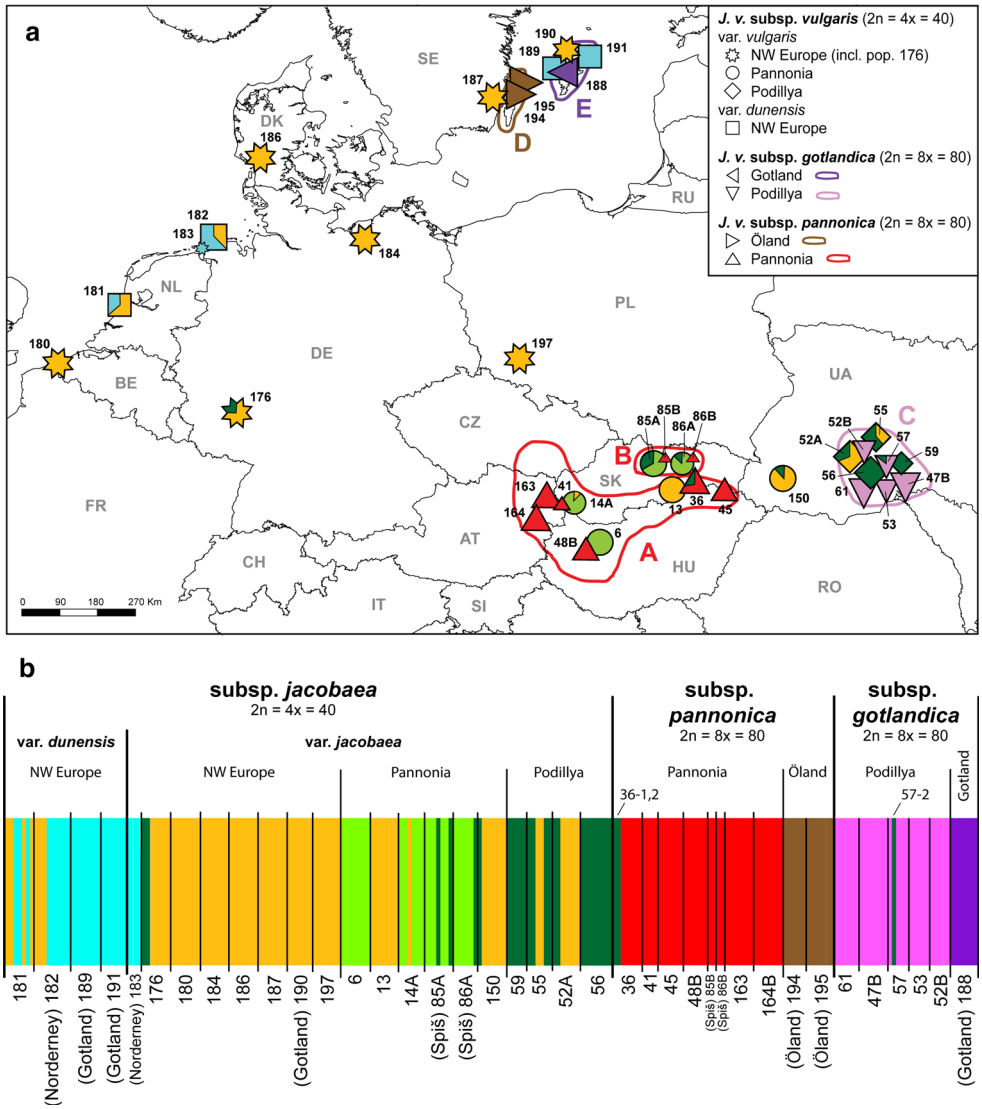

Fig. 2 **a** Distribution map of the *Jacobaea vulgaris* samples used in molecular and morphometric analyses. The *size of symbols* is related to the number of individuals used in genetic analyses (see Table 1). *Colour proportions within symbols* indicate membership in the eight genetic clusters identified by the Bayesian analysis of the genetic population structure. *Continuous colour lines* indicate the boundaries of the geographic distribution of octoploids according to the present study: A–Pannonia, B–Spiš region, C–Podillya, D–Öland, E–Gotland. **b** Clusters of *Jacobaea vulgaris* samples (236 individuals from 38 populations), as resolved by the Bayesian analysis of genetic population structure (BAPS software) based on AFLP genotypes. Each individual is represented by a *vertical bar*, *coloured* according to its cluster assignment. The population numbers follow those in Table 1

(NW Europe hereafter; Fig. 1). The studied areas of NW Europe also involved the type locality from where *J. vulgaris* subsp. *gotlandica* was described (population no. 188). However, we were not able to find *J. vulgaris* subsp. *dunensis* at the type locality of the dunes on the Belgian coast ["Belgium, in arenosis maritimis", Dumortier (1827: 66)].

Altogether, 38 populations of *J. vulgaris* were sampled for ploidy level, genetic, and morphometric analyses (Fig. 2a; Table 1): 14 populations in Pannonia [six tetraploid and eight octoploid, including two populations (no. 163 and 164) identified by Wysk et al. (2009) as *J. vulgaris* subsp. *gotlandica*], nine populations in Podillya (four tetraploid and five octoploid), and 15 populations in NW Europe [eight representing nominate subspecies, four *J. vulgaris* subsp. *dunensis*, and three *J. vulgaris* subsp. *gotlandica*, including the type locality of *J. vulgaris* subsp. *gotlandica* (pop. no. 188)]. This includes both material collected as part of this study and material collected for studies by Hodálová et al. (2007b, 2010; 171 plants with known DNA ploidy level and available silica gel-dried material, see Table 1). In cases of co-occurrence of tetra- and octoploid plants of *J. vulgaris* on the same locality (sampling site), the individuals of two ploidy levels were treated here as separate populations.

The populations of *J. vulgaris* were genetically compared with those of closely allied congeners (cf. Pelser et al. 2003; Wysk et al. 2009), which could have potentially contributed to the allopolypoid origin of the studied *J. vulgaris* octoploids. The following five species occurring in Central and NW Europe were selected: *J. alpina* (four populations), *J. aquatica*, *J. erratica*, *J. erucifolia*, and *J. subalpina* (all represented by three populations). According to the literature data, all these species are known as exclusively (or almost exclusively) tetraploid, with

Table 1 Plant material of the *Jacobaea* species used in the combined ploidy level, morphometric, and/or molecular analyses

Taxon, pop. no.	Locality description: country, closest village or town (unless stated otherwise), altitude, voucher information	Coordinates (WGS84)	2*n*	No. of plants analysed		
				FCM	AFLP	Morph
J. alpina (L.) Moench						
205	Germany, Bergen, ca 1590 m, 18 Aug 2012, IH	47°45′45″N, 12°33′39″E	–	–	7	–
214	Austria, Mühlbachl, ca 1210 m, 9 Sep 2012, IH & PM	47°07′59″N, 11°26′07″E	–	–	7	–
215	Austria, St. Christoph am Arlberg, 1795 m, 9 Sep 2012, IH & PM	47°07′49″N, 10°12′38″E	–	–	6	–
216	Italy, Calice, 1560 m, 9 Sep 2012, IH & PM	46°51′50″N, 11°21′44″E	–	–	7	–
J. aquatica (Hill.) G.Gaertn. et al.						
200	Czech Republic, Kosov, ca 480 m, 16 Aug 2012, IH & PM	48°52′31″N, 14°25′03″E	–	–	7	–
201	Czech Republic, Pištín, ca 390 m, 16 Aug 2012, IH & PM	49°02′37″N, 14°20′10″E	–	–	7	–
202	Austria, Steinbach, ca 540 m, 16 Aug 2012, IH & PM	48°49′26″N, 15°00′11″E	–	–	6	–
J. erratica (Bertol.) Fourr.						
155	Hungary, Püspökladány, 84 m, 2 Aug 2010, JK & MS	47°18′41″N, 21°12′01″E	–	–	7	–
158	Romania, Crucea, 679 m, 10 Aug 2010, JK & MS	47°19′51″N, 25°37′13″E	–	–	7	–
203	Slovakia, Búč, ca 105 m, 17 Aug 2012, PM	47°47′47″N, 18°26′33″E	–	–	7	–
J. erucifolia (L.) G.Gaertn. et al.						
157	Romania, Doiceşti, 340 m, 7 Aug 2010, JK & MS	45°00′08″N, 25°24′04″E	–	–	7	–
198	Slovakia, Palárikovo, ca 110 m, 15 Aug 2012, PM	48°03′53″N, 18°04′39″E	–	–	5	–
199	Hungary, Aggtelek, ca 330 m, 15 Aug 2012, PM	48°28′18″N, 20°29′21″E	–	–	5	–
J. subalpina (W.D.J.Koch) Pelser & Veldk.						
156	Romania, Urdele, 1405 m, 4 Aug 2010, JK & MS	45°29′08″N, 23°37′37″E	–	–	7	–
206	Slovakia, Mengusovská dolina valley, ca 1650 m, 25 Aug 2012, IH	49°09′55″N, 20°04′03″E	–	–	6	–
207	Slovakia, Štrbské Pleso, ca 1680 m, 27 Aug 2012, IH	49°09′01″N, 20°02′50″E	–	–	7	–
J. vulgaris subsp. *gotlandica* (Neum.) B.Nord.						
47B	Ukraine, Demshyn, 270 m, 14 Aug 2005 IH & PM; 26 Jul 2007, IH & PM	48°36′52″N, 26°46′52″E	=8x = 80	25*	7	25
52B	Ukraine, Vikno, 320 m, 13 Aug 2005, IH & PM; 24 Jul 2007, IH & PM	49°21′24″ N, 26°04′25″E	~8x~80	13* +5**	5	18
53	Ukraine, Smotrych, 180 m, 14 Aug 2005, IH & PM; 25 Jul 2007, IH & PM	48°39′03″N, 26°35′05″E	~8x~80	7* +1**	5	8
57	Ukraine, Ivakhnivtsi, 320 m, 25 Jul 2007, IH & PM	49°06′02″N, 26°20′58″E	~8x~80	7*	5	7
61	Ukraine, Babyntsi, 240 m, 27 Jul 2007, IH & PM	48°41′21″N, 26°04′06″E	~8x~80	8* +5**	7	13

Table 1 continued

Taxon, pop. no.	Locality description: country, closest village or town (unless stated otherwise), altitude, voucher information	Coordinates (WGS84)	2*n*	No. of plants analysed		
				FCM	AFLP	Morph
188	Sweden, Gotland Island, Klinte, ca 50 m (type locality of the subspecies), 3 Aug 2012, IH, PM & DRL	57°22′30″N, 18°14′14″E	~8x~80	10	7	10
J. vulgaris subsp. *pannonica* Hodálová & Mered'a						
36	Slovakia, Krásnohorské Podhradie, 440 m, 15 Jul 2009, IH & PM	48°39′20″N, 20°35′42″E	~8x~80	8	7	7
41	Slovakia, Sandberg hill, 220 m (type locality of the subspecies), 16 Jul 2008, IH	48°12′02″N, 16°59′28″E	~8x~80	18**	4	18
45	Slovakia, Tarbucka hill, 243 m, 16 Jul 2009, IH & PM	48°21′35″N, 21°47′21″E	~8x~80	23	6	13
48B	Hungary, Hárskút, 370-420 m, 21 Jul 2008, IH, PM & AV	47°11′49″N, 17°50′20″E	~8x~80	21**	6	21
85B	Slovakia, Hôrka-Primovce, 600-650 m, 11 Aug 2009, IH & PM; 20 Jul 2012, PM	49°01′05″N, 20°22′50″E	~8x~80	1 + 1	2	2
86B	Slovakia, Sivá brada hill, 470-480 m, 11 Aug 2009, IH & PM	49°00′21″N, 20°43′16″E	~8x~80	2	2	2
163	Austria, Oberweiden, ca 160 m, 19 Jul 2012, IH & PM	48°17′03″N, 16°49′55″E	~8x~80	15	7	15
164	Austria, Sollenau, ca 150 m, 19 Jul 2012, IH & PM	47°55′12″N, 16°16′43″E	~8x~80	12	7	12
194	Sweden, Öland Island, Bruddesta, 16 m, 6 Aug 2012, IH, PM & DRL	56°59′22″N, 16°46′59″E	~8x~80	15	6	14
195	Sweden, Öland Island, Grönvik, 13 m, 7 Aug 2012, IH, PM & DRL	57°00′29″N, 16°48′29″E	~8x~80	15	7	15
J. vulgaris subsp. *vulgaris* var. *dunensis* (Dumort.) Hodálová & Mered'a						
181	Netherlands, Katwijk–Katwijk aan Zee, 15 m, 26 Jul 2012, PM & DRL	52°11′36″N, 04°23′29″E	~4x~40	15	7	15
182	Germany, Norderney Island, ca 5 m, 27 Jul 2012, PM & DRL	53°42′48″N, 07°12′02″E	~4x~40	15	9	15
189	Sweden, Gotland Island, Klintehamn, 6 m, 3 Aug 2012, IH, PM & DRL	57°22′48″N, 18°12′35″E	~4x~40	15	7	15
191	Sweden, Gotland Island, Slite, 2 m, 5 Aug 2012, IH, PM & DRL	57°40′59″N, 18°48′18″E	~4x~40	15	7	15
J. vulgaris Gaertn. subsp. *vulgaris* var. *vulgaris*						
6	Hungary, Zirc, 435 m, 6 Jul 2008, IH & PM	47°18′34″N, 17°53′07″E	~4x~40	8**	7	8
13	Slovakia, Plešivec, 210 m, 15 Jul 2009, IH & PM	48°34′24″N, 20°25′13″E	~4x~40	13	7	10
14	Slovakia, Štokeravská vápenka quarry, 160 m, 16 Jul 2008, IH	48°12′14″N, 17°00′44″E	=4x = 40	22**	6	22
52A	Ukraine, Vikno, 320 m, 13 Aug 2005, IH & PM; 24 Jul 2007, IH & PM	49°21′24″N, 26°04′25″E	~4x~40	8*	7	8
55	Ukraine, Ostap"e, 390 m, 25 Jul 2007, IH & PM	49°23′51″N, 26°05′00″E	~4x~40	7*	6	7
56	Ukraine, Vil'khivtsi, 325 m, 25 Jul 2007, IH & PM	49°05′26″N, 26°18′20″E	~4x~40	9*	7	9
59	Ukraine, Adamovka, 270 m, 27 Jul 2007, IH & PM	49°06′11″N, 27°03′04″E	~4x~40	5* +2**	5	7
85A	Slovakia, Hôrka-Primovce, 600-650 m, 11 Aug 2009, IH & PM; 20 Jul 2012, PM	49°01′05″N, 20°22′50″E	~4x~40	4 + 31	7	31

Table 1 continued

Taxon, pop. no.	Locality description: country, closest village or town (unless stated otherwise), altitude, voucher information	Coordinates (WGS84)	2*n*	No. of plants analysed		
				FCM	AFLP	Morph
86A	Slovakia, Sivá brada hill, 470-480 m, 11 Aug 2009, IH & PM; 28 Jul 2012, IH	49°00′21″N, 20°43′16″E	~4x~40	3 + 11	6	10
150	Ukraine, Verb'yazh, 683 m, 7 Jul 2010, JS	48°48′13″N, 23°09′45″E	~4x~40	10	7	10
176	Germany, Sankt Goarshausen-Heide, 240 m, 24 Jul 2012, PM & DRL	50°09′03″N, 07°43′57″E	~4x~40	16	7	16
180	Belgium, De Panne, 4 m, 25 Jul 2012, PM & DRL	51°04′54″N, 02°33′06″E	~4x~40	15	7	15
183	(Same as 182)	(Same as 182)	~4x~40	3	3	3
184	Germany, Vorder Bollhagen, 22 m, 28 Jul 2012, PM & DRL	54°07′31″N, 11°51′11″E	~4x~40	15	7	15
186	Denmark, Esbjerg, ca 25 m, 30 Jul 2012, PM & DRL	55°30′59″N, 08°28′37″E	~4x~40	15	7	15
187	Sweden, Kalmar, 3 m, 2 Aug 2012, IH, PM & DRL	56°40′28″N, 16°19′31″E	~4x~40	15	7	15
190	Sweden, Gotland Island, Sjonhem-Bjärby, ca 30 m, 4 Aug 2012, IH, PM & DRL	57°29′24″N, 18°31′01″E	~4x~40	8	6	8
197	Poland, Rościce, 143 m, 9 Aug 2012, IH & PM	51°35′37″N, 15°00′06″E	~4x~40	15	7	15

Each record is given as taxon name; population (voucher) number; geographic origin; coordinates; 2*n*-DNA ploidy level (by flow cytometric assesment or direct chromosome counting); the number of individuals analysed for flow cytometry (FCM), AFLP, and morphometric analyses (Morph). Data marked with asterisks were taken from the literature: * Hodálová et al. (2007b), ** Hodálová et al. (2010); all other data represent new records

Abbreviations of the collectors: *AV* A. Vinikarová, *DRL* D. R. Letz, *EM* E. Michalková, *IH* I. Hodálová, *JK* J. Kučera, *JS* J. Smatanová, *MS* M. Slovák, *PM* P. Mered'a Jr

$2n = 4x = 40$ (e.g., Májovský et al. 1987; Dobeš and Vitek 2000; Grulich 2005).

Details on the origin of the materials used are given in Table 1 (see also Fig. 1). Voucher specimens were deposited in the herbarium SAV.

Ploidy level analyses

In total, 336 *J. vulgaris* individuals from 25 populations were screened for DNA ploidy levels as part of this study.

DNA ploidy levels were estimated from silica gel-dried leaf tissues using flow cytometry (FCM). First, samples of reference plants with known chromosome numbers ($2n = 40$ and 80, cf. Hodálová et al. 2010) were analysed simultaneously with the most appropriate internal DNA reference standard (*Glycine max* 'Polanka', 2C DNA = 2.50 pg; Doležel et al. 1994 or *Bellis perennis* L., 2C DNA = 3.38 pg; Schönswetter et al. 2007) and the ratio of their G_0/G_1 peak positions was recorded. Then, the DNA ploidy levels of the analysed plants (of unknown chromosome number) were assessed by their peak position relative to the DNA reference standard peak. Sample preparation and the FCM procedure followed Hodálová et al. (2010).

Molecular analyses

AFLP data were generated from 341 individuals sampled within this study and within studies by Hodálová et al. (2007b, 2010) from 38 populations of *J. vulgaris* (236 individuals of known ploidy level) and 16 populations (105 individuals) of the related species. Mostly, five to seven individuals per population were analysed, with exception of three populations where fewer individuals were available at particular localities (no. 85B, 86B, 183; see Table 1).

DNA extraction

DNA was extracted from intact, silica gel-dried leaf tissues using the DNeasy Plant Mini Kit (Qiagen). The DNA quality was controlled both on agarose gels and using a spectrophotometer.

AFLP fingerprinting and data analyses

The AFLP procedure was conducted following the original protocol (Vos et al. 1995) with modifications described by Mered'a et al. (2008). Forty-one selective primer combinations were tested on four *J. vulgaris* individuals,

including tetra- and octoploid accessions originating from different geographic regions. Four primer combinations, *Eco*RI-ATC-(6-FAM)/*Mse*I-CTA, *Eco*RI-AGG-(VIC)/*Mse*I-CAG, *Eco*RI-ACC-(NED)/*Mse*I-CAC, and *Eco*RI-AGC-(PET)/*Mse*I-CTC (Applied Biosystems), which provided the clearest and best reproducible AFLP profiles, were selected for final analyses. The reproducibility of the AFLP profiles was proved by replicates of 34 randomly chosen samples (9.9 % of the final dataset) encompassing different populations. Error rate (Bonin et al. 2004) expressed as the ratio of mismatches (scoring 1 vs. 0) over matches (1 vs. 1) was calculated for replicated samples. Fragment analysis of the AFLP products was done with the internal size standard GeneScan–500 LIZ® (Applied Biosystems) in the BITCET Consortium, Department of Molecular Biology, Comenius University, Bratislava (ABI 3130xl).

The DAx software (Van Mierlo Software Consultancy, The Netherlands) was used for reading and analysing AFLP trace files. Only markers ranging between 50 and 500 bp, and those that could be scored unambiguously were recorded, and coded as present (1) or absent (0) and used for the generation of two binary data matrices: (1) the '*Jacobaea* matrix' comprised all analysed taxa and populations (341 individuals), and (2) the '*Jacobaea vulgaris* matrix' included *J. vulgaris* populations only (236 individuals).

The overall genetic structure in studied datasets was also analysed using a neighbour-joining algorithm including 5,000 replicates for bootstrap support (NJ; Saitou and Nei 1987) and principal coordinate analysis (PCoA; Krzanowski 1990). The latter two were computed in the FAMD 1.108 beta software (Schlüter and Harris 2006). Split network analysis, particularly neighbour-net (NN) analysis, which enables the identification of not only congruent but also conflicting signals was conducted using SplitsTree 4 (Huson and Bryant 2006). Both the NJ tree and the NN analysis were based on Sørensen's similarities transformed into a distance matrix ($d = 1-s$), while pairwise genetic similarities in PCoA were computed using Jaccard's coefficient (Jaccard 1908).

To assign analysed individuals to genetically homogeneous clusters, the Bayesian multilocus assignment method based on stochastic optimisation with module "clustering of individuals" was conducted in the BAPS 5.2 software (Corander et al. 2006). The analysis of molecular variance (AMOVA) using Euclidean pairwise distances and a significance test with 10,000 permutations, carried out in Arlequin 3.11 (Excoffier et al. 2005), was used to explore the variance partitioning in two steps: (1) within and among populations and (2) within and among populations as well as among clusters detected by the Bayesian multilocus assignment method.

To study the genetic diversity within and among the studied taxa/genetic groups, the following statistical parameters were recorded: (1) the total number of AFLP multilocus genotypes, (2) the average number of AFLP fragments (±SD), (3) the average proportion of pairwise differences between individuals (Nei's gene diversity, D_{Nei}, Nei and Li 1979) using the R-script AFLPdat (Ehrich 2006), and (4) the percentage of polymorphic markers (P %) using the Pop-Gene 1.32 software (Yeh et al. 1997). Genetic divergence among analysed taxa/genetic groups was estimated by calculating the following: (5) private fixed fragments (i.e., diagnostic, those restricted to a certain taxon/genetic group and present in all of its individuals), (6) private fragments (those restricted to a certain taxon/genetic group, but not necessarily present in all of its individuals), (7) the number of rare fragments (those present at a frequency <10 % of the investigated individuals), using FAMD 1.108 beta (Schlüter and Harris 2006).

We also tried to analyse the *J. vulgaris* octoploids in respect to time and mode (allo- vs autopolyploidization) of their origin. In particular, we studied whether octoploids evolved in the past in one (or a few) polyploidization events or, alternatively, recently from broadly sympatric tetraploids. For this purpose we used virtually generated AFLP octoploid profiles, so called in silico octoploids (see also Greiner et al. 2013). Generation of in silico octoploid AFLP profiles simulates recent duplication of complete genomes and thus, logically, such in silico profiles are placed in genetic space together with parental tetraploids. Consequently, the shared position of natural octoploids, potential parental tetraploids and in silico octoploids would indicate recent autopolyploid origin of natural octoploids. In contrast, appearance of natural octoploids in genetic space in a position separated from both tetraploids and in silico octoploids may reflect either autopolyploid evolution of natural octoploids from tetraploids in a more distant past, and their subsequent long-term genetic isolation, or allopolyploid origin of natural octoploids.

As natural octoploid groups were detected to be restricted to particular geographic regions (see Fig. 1b), we decided to model in silico octoploids for each region separately. We used in silico combinations of AFLP profiles from each geographic group of natural *J. vulgaris* tetraploids from NW Europe, Pannonia, and Podillya. Additionally, because two tetraploid morphological types occur within NW Europe, a morph with absent or rudimentary ray florets (corresponding to *J. vulgaris* subsp. *dunensis*) and a morph with well-developed ray florets (corresponding to *J. vulgaris* subsp. *vulgaris*), in silico octoploids were generated for each of them separately. Thus, in total four in silico matrices were created: (1) the 'IS NW Europe *dunensis* matrix', (2) the 'IS NW Europe *vulgaris* matrix', (3) the 'IS Pannonia *vulgaris* matrix', and (4) the 'IS Podillya *vulgaris* matrix'. From each in silico auto-octoploid dataset, forty profiles (amount comparable with the number of

available natural octoploid AFLP profiles) were randomly chosen and analysed together with primary '*Jacobaea vulgaris* matrix'. This procedure was repeated three times to test whether random selection of forty profiles included a representative proportion of overall variation of generated in silico auto-octoploids. All datasets were analysed separately using PCoA analyses (for details, see above).

The Tukey–Kramer multiple comparison analysis at the probability level $P < 0.001$ [the Tukey test for unequal sample sizes (Zar 2010); performed using SAS version 9.3 software (SAS Institute Inc. 2011)], was calculated to determine the possible differences in the number of AFLP bands per individual among natural and in silico cytotypes.

Morphometric analyses

The aim of the morphometric analyses was to test whether it is possible to distinguish tetraploids and octoploids of *J. vulgaris* as well as population groups, defined by molecular markers within this study, using some combination of morphological characters.

The morphometric analyses included only *J. vulgaris* samples with known ploidy levels (484 individuals, 38 populations) collected within this study and within studies by Hodálová et al. (2007b, 2010). All populations selected for morphometric analyses were also used in the molecular analyses. Typically, 10–15 flowering plants were collected per population (Table 1), depending on the population size and the number of plants with well-preserved lower leaves. In particular, a preservation of lower leaves was a limiting factor for population sampling because the lower leaves in *J. vulgaris* often wither already at anthesis, and after withering, they are not suitable for morphometric analyses.

A total of 14 morphological characters (eight vegetative and six generative) were measured or scored for each individual (Table 2, Online Resource 1), except for *J. vulgaris* subsp. *dunensis*, where only 13 characters were examined (without character OAI, i.e. quality of indument of outer achenes). Character OAI was not scored for *J. vulgaris* subsp. *dunensis* because the outer achenes were absent for most of the studied individuals of this taxon. Ray florets were absent for most of the individuals of this taxon as well; in their absence, we scored their length and width (characters RFL and RFW) by the dummy value of 0 mm. The characters used involved those reported as diagnostic for the studied taxa in the literature (e.g., Wysk et al. 2009), or those found useful based on our previous studies of the group (Hodálová et al. 2007a) or during our field sampling.

Prior to multivariate analyses, non-parametric Spearman correlation coefficients (Legendre and Legendre 1998) based on the matrix including all of the studied plants were computed to eliminate pairs of highly correlated characters from further analyses. Both canonical discriminant analyses (CDAs; Klecka 1980) and principal component analysis (PCA; Sneath and Sokal 1973) were employed.

Table 2 The list of the characters used in the morphometric analyses

Characters		Character explanation
Lower cauline leaf[1]		
LLP	Relative length of the petiole [=(length of the petiole/length of the whole leaf) × 100]	%
LLS	Relative length of the segmented part of the leaf [=(length of the segmented part of leaf/length of the whole leaf) × 100]	%
LLA	Relative length of the apical leaf segment [=(length of the apical leaf segment/length of the whole leaf) × 100]	%
LLL[2]	Relative width of lateral lobe on apical segment [=(width of lateral lobe on apical segment/length of the apical leaf segment) × 100]	%
LLNS	Number of lateral leaf segments (on one side of leaf)	–
LLNT[3]	Number of teeth per lateral leaf segment	–
Middle cauline leaf		
MLNS	Number of lateral leaf segments (on one side of leaf)	–
MLNT[3]	Number of teeth per lateral leaf segment	–
Generative characters		
IBL	Length of involucral bracts	mm
RFL	Length of ray florets	mm
RFW	Width of ray florets	mm
TFN	Number of tubular florets	–
TFL	Length of tubular florets	mm
OAI	Indument of outer achenes	0: absent or 1–3 hairs 1: ≥4 hairs

For character illustrations, see Fig. 3

[1] Measured on the first or second cauline leaf from the bottom, not on the rosette leaves

[2] Measured on the deepest lobe or tooth, respectively

[3] Counted on the most divided segment of the scored leaf

To reveal the degree of morphological separation of tetra- and octoploids of *J. vulgaris*, two CDAs were computed. CDA 1, based on 13 morphological characters (without character OAI), was performed on the populations of the entire dataset (38 populations as OTUs characterized by mean values; '*J. vulgaris* populations matrix'). CDA 2, based on 13 morphological characters (without character OAI), was computed for the individuals of the whole dataset (484 individuals as OTUs; '*J. vulgaris* individuals matrix'). To reveal the degree of morphological separation

of two main groups of octoploids recognized within *J. vulgaris* by genetic analyses (see "Results"), two other CDAs based on all 14 morphological characters were computed. CDA 3 was performed on the dataset of octoploid populations (16 populations as OTUs and characterized by mean values of morphological characters; '*J. vulgaris* 8*x* populations matrix'); CDA 4 was computed on the dataset of individual plants (200 plants as OTUs; '*J. vulgaris* 8*x* individuals matrix').

Principal component analyses based on populations or individuals as OTUs and correlation matrices between the characters were used to study the morphological homogeneity of the three main groups (tetraploids and two groups of octoploids) revealed by the FCM and AFLP analyses. Three different subsets were assembled and subjected to four PCAs. PCA 1 and PCA 2 were computed on all tetraploid populations from the whole area studied (22 populations; '*J. vulgaris* 4*x* populations matrix'). PCA 1 was based on 13 morphological characters (without character OAI). To test whether *J. vulgaris* subsp. *dunensis* differs from the rest of tetraploid plants of *J. vulgaris* also in other characters except of lost or reduction of ray florets, PCA 2 based on 11 morphological characters (without length and width of ray florets, and indument of outer achenes) was used. PCA 3, based on 14 morphological characters, was performed on octoploid individuals from Pannonia and Öland (119 individuals; 'Pannonia+Öland 8*x* individuals matrix'). Finally, PCA 4, based on 14 morphological characters, was performed on octoploid individuals from Podillya and Gotland (81 individuals; 'Podillya+Gotland 8*x* individuals matrix').

The Tukey–Kramer multiple comparison analysis, at the probability level $P \leq 0.05$, was calculated to determine which morphological characters show significant differences among homogeneous groups resulting from the FCM, AFLP, and morphometric analyses (see "Results"). Descriptive statistics (Tukey 1977), including mean, standard deviation, minimum, maximum, and 10th and 90th percentiles were computed for all quantitative characters. Finally, boxplots of selected quantitative morphological characters were generated.

Morphometric data analyses were performed using the SAS version 9.3 software (SAS Institute Inc. 2011). The data matrices are available from the first author upon request.

Results

Ploidy level analyses

Results of the analyses of DNA ploidy levels are presented in Table 1 (cf. also Fig. 1). The localities (sampling sites) of *J. vulgaris* analysed by flow cytometry within this study were heterogeneous; 14 were tetraploid, seven were octoploid, and two (no. 85 and 86) consisted of mixed-ploidies levels (Table 1). Individuals from the two mixed-sampling sites were subsequently divided into separate populations, 85A, 85B, 86A, and 86B, for further analyses.

Plants belonging to *J. vulgaris* subsp. *dunensis* were shown to be exclusively tetraploid. Octoploid plants were confirmed for the Pannonian and Podillyan regions (cf. Hodálová et al. 2010). For the first time, octoploidy was also determined in a population (no. 188) from the type locality of *J. vulgaris* subsp. *gotlandica* from Gotland, and two populations (no. 194, 195) from Öland, traditionally considered to be *J. vulgaris* subsp. *gotlandica*. Two Austrian populations from Pannonia (no. 163 and 164) assigned by Wysk et al. (2009) to *J. vulgaris* subsp. *gotlandica* were found to be octoploid as well.

Molecular analyses

The error rate estimated from replicated AFLP profiles did not exceed 2.4 % and thus indicated the high reproductibility of our AFLP data. The AFLP dataset included 341 profiles, out of which 236 belonged to *J. vulgaris*, and the remaining 105 individuals corresponded to closely related *Jacobaea* taxa (Table 1). Four primer combinations yielded 305 clearly scorable fragments sized from 55 to 476 bp, with 99.4 % of them being polymorphic. The population means of the AFLP markers scored, ranged from 70 ± 2.88 SD (*J. erucifolia*, no. 157) to 98 ± 4.63 SD (*J. alpina*, no. 215). Altogether, 339 AFLP genotypes were individual specific, indicating that very few individuals (1.7 %) shared the same genetic profile (Online Resource 2).

The results of the AFLP analyses based on the entire dataset ('*Jacobaea* matrix')—NJ tree (Fig. 3), NN diagram, and PCoA (figures not shown)—unequivocally showed that *J. alpina*, *J. aquatica*, *J. erratica*, *J. erucifolia*, and *J. subalpina* are clearly distinct from the *J. vulgaris* group as well as from each other. *J. vulgaris*, being monophyletic, is heterogeneous with several tetraploid and octoploid groups.

All ten repeats of the Bayesian multilocus assignment analysis conducted in BAPS software and performed on the '*Jacobaea vulgaris* matrix' were identical and resulted in eight genetic clusters (Fig. 2b), all reflecting geographic patterns (Fig. 2a): (1) tetraploids from a coastal part of NW Europe, represented especially by *J. vulgaris* subsp. *dunensis* (blue group); (2) tetraploids from the entire sampled area (yellow group); (3) tetraploids from Pannonia (light green group); (4) tetraploids from Podillya, Pannonia, and Germany, two octoploid individuals from Pannonia (individuals no. 36-1, 36-2) and a single octoploid plant from Podillya (no. 57-2; dark-green group); (5) octoploids from Pannonia (red group); (6) octoploids from Podillya (pink

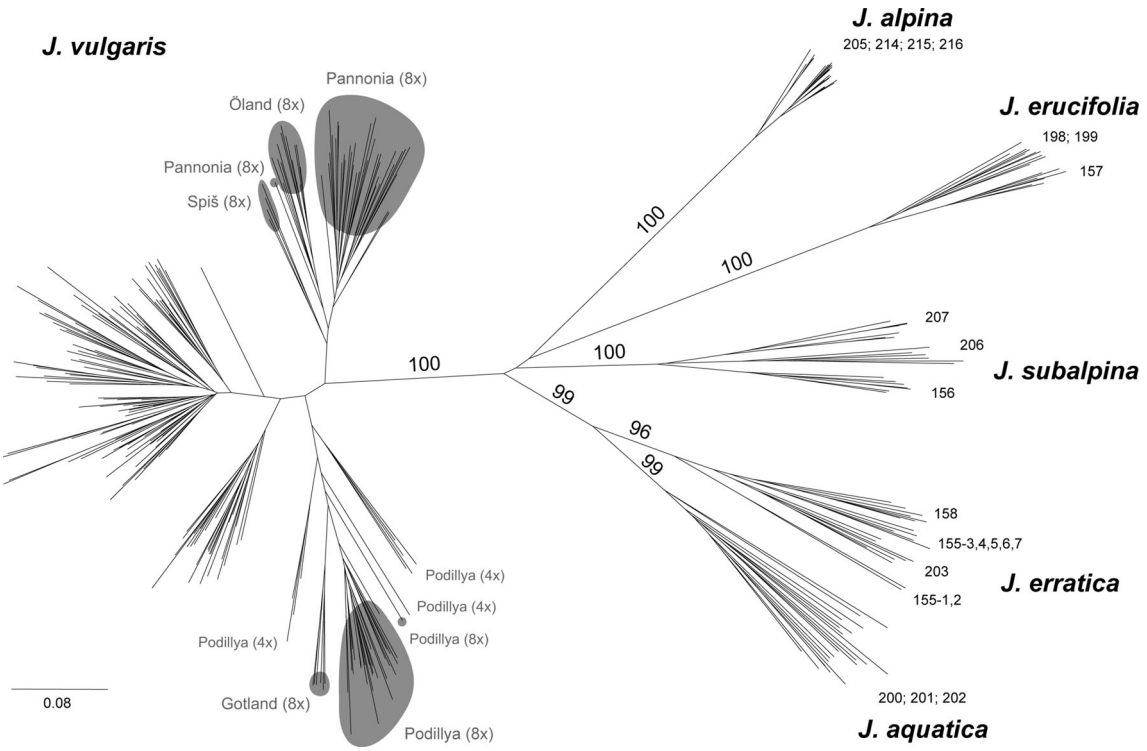

Fig. 3 Unrooted neighbour-joining tree based on the AFLP data from 341 individuals of *Jacobaea* taxa. The *numbers* above the basal branches indicate bootstrap support. The population numbers follow those in Table 1 (in the case of population no. 155 also individual plant numbers within this population are given). Octoploid individuals of *Jacobaea vulgaris* are highlighted in *grey*

group); (7) octoploids from Öland (brown group); and (8) octoploids from the type population of *J. vulgaris* subsp. *gotlandica* from Gotland (violet group).

This pattern was, in general, also visible in the results obtained by the NJ tree (figure not shown), NN diagram (Fig. 4), and PCoA (Fig. 5) analyses based on the same '*Jacobaea vulgaris* matrix'. Whereas octoploid groups formed rather homogeneous clusters, tetraploids were highly heterogeneous. However, besides the basic pattern detected in Bayesian analysis, more detailed relationships were revealed: octoploids from Öland are clustered with Pannonian octoploids, while octoploids from Gotland appeared in close proximity to Podillyan octoploids. Additionally, geographically outlying octoploids from northern Slovakia (Spiš region) displayed a slight trend toward separation from the main Pannonian octoploid group. Finally, tetraploids from Pannonia were clustered into two subgroups, A and B (Figs. 4, 5).

The results of non-hierarchical AMOVA revealed that 50.56 % ($F_{ST} = 0.51$, $df = 36$, $P < 0.001$) of the total variation was accounted for by the among-population variation, although a high level of overall variation (49.44 %) was also found within populations. The other AMOVA run based on eight genetic groups as revealed by Bayesian analysis showed that 36.5 % ($F_{CT} = 0.36$, $df = 6$, $P < 0.001$) of variation was assigned to among-group variation; only 16.43 % was accounted for by variation among populations within groups and 47.11 % of overall variation was shared within populations.

Private fixed fragments occurred only in taxa related to *J. vulgaris* (seven in *J. erucifolia*, three in *J. aquatica*, and one in *J. erratica*, respectively; Online Resource 2). Private fragments were found both in non-*J. vulgaris* taxa (*J. alpina*—ten, *J. erratica*—five, *J. aquatica*—four, *J. subalpina*—three, *J. erucifolia*—two) as well as in *J. vulgaris*: six in tetraploids from the entire area (the yellow group) and one in tetraploids from Pannonia (the light-green group). Concerning the overall pattern of genetic diversity in studied taxa, there were no apparent differences in Nei's gene diversity among studied taxa or genetic groups. Somewhat higher variation occurred in the percentage of polymorphic markers, where the highest values were found in two genetic groups of *J. vulgaris* tetraploids (the yellow and dark-green groups). In contrast, the lowest value was found in *J. alpina* (Online Resource 2).

Series of PCoA's (Fig. 6a–d) based on datasets comprising also in silico *J. vulgaris* octoploids consistently revealed that in silico octoploids were expectedly intermingled with the tetraploid accessions from which they were generated. In contrast, natural *J. vulgaris* octoploids

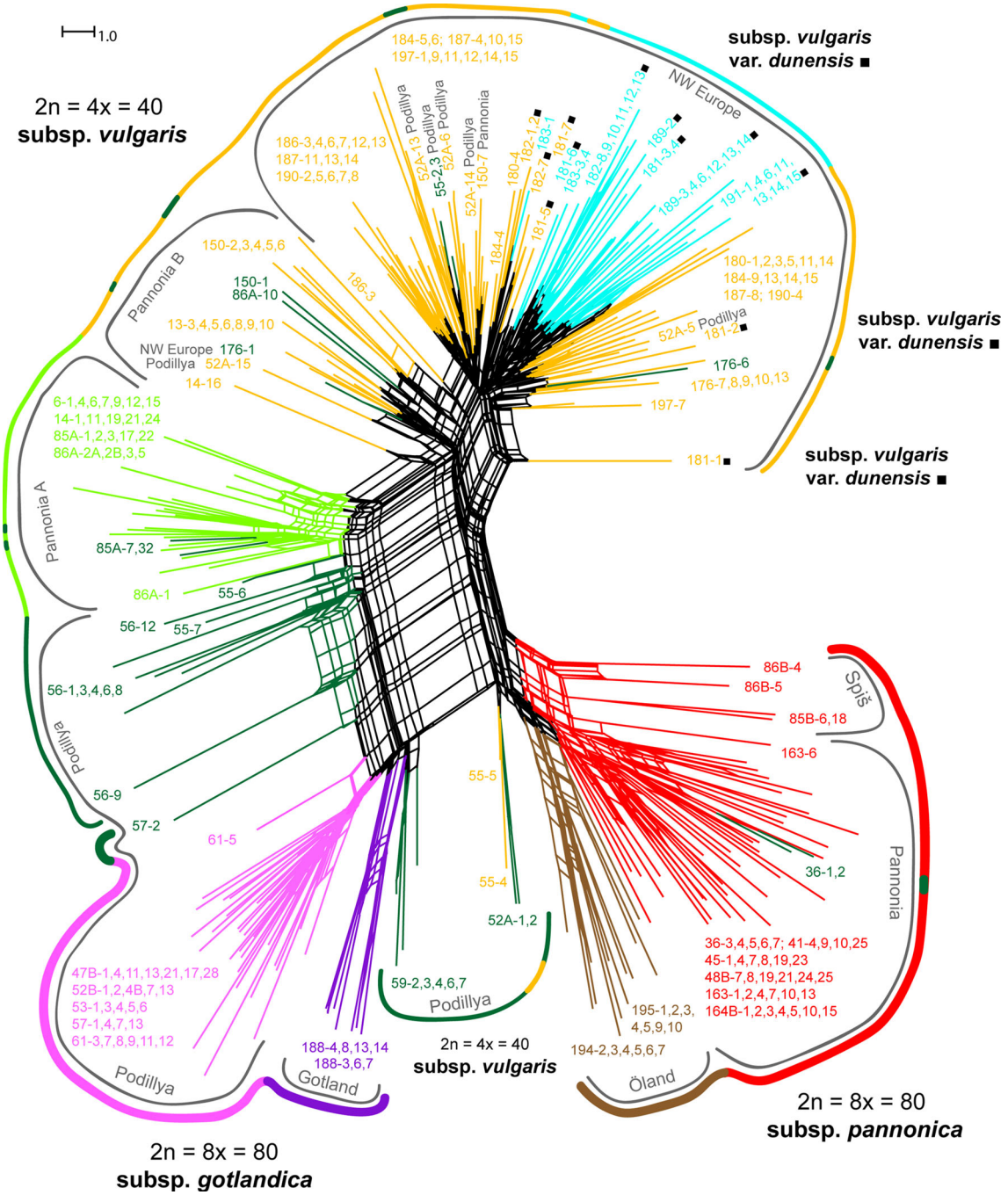

Fig. 4 Neighbour-Net diagram based on the AFLP data from 236 *Jacobaea vulgaris* individuals. *Colours* indicate the genetic grouping corresponding to the Bayesian analysis of genetic population structure (see Fig. 2b). *Jacobaea vulgaris* var. *dunensis* individuals are indicated with *black squares*. The population numbers (see Table 1) are followed by hyphen and individual plant numbers within a given population

appeared in genetic space clearly separated from both tetraploids and in silico octoploids. All three repetitions performed for each in silico octoploid group resulted in essentially identical patterns, confirming that the selection of forty randomly chosen individuals included representative proportions of the variation harboured in in silico octoploids.

Interestingly, the number of AFLP fragments recovered per individual showed significant (at $P < 0.001$) differences between tetraploids and natural octoploids. In silico octoploids had significantly higher amounts of fragments compared with natural octoploids. The lowest number of AFLP bands per individual was found in tetraploids from NW Europe.

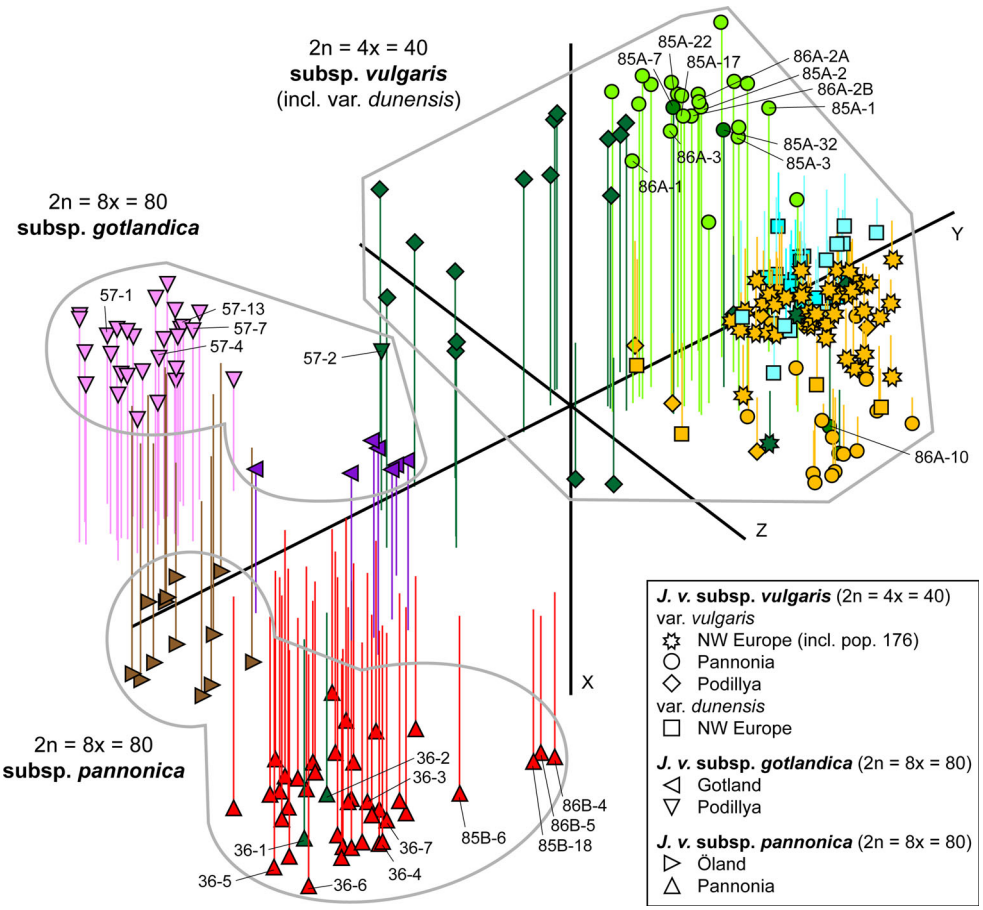

Fig. 5 Principal coordinates analysis of 236 individuals of *Jacobaea vulgaris*, based on AFLP markers. *Colours* indicate the genetic groupings corresponding to the Bayesian analysis of genetic population structure (see Fig. 2b). The members of populations 36, 57, 85, 86 (see Table 1) are marked, including individual plant numbers within a given population. The first three axes explain 49.09 % of the total variation ($x = 32.04$ %, $y = 13.31$ %, and $z = 3.74$ %)

Morphometric analyses

The distribution of most of the measured characters departed from the normal distribution, which is why a non-parametric correlation coefficient was used.

Spearman correlation coefficients did not reveal the presence of any highly correlated pairs of characters (exceeding the value 0.95). The highest values obtained were 0.894 (between LLS and LLNS) and 0.883 (between characters LLS and LLNT, for abbreviations see Table 2); therefore, all characters could be used in further analyses.

CDA 1 and 3 (based on '*J. vulgaris* populations matrix' and '*J. vulgaris* 8x populations matrix', respectively) largely supported the hypothesis of morphological separation of the tetra- and octoploid cytotypes (Fig. 7a) detected by the FCM analyses (Table 1) and the possibility of morphological separation of the two main groups of *J. vulgaris* octoploids recognized by the AFLP analyses (Figs. 3, 4 and 5): (1) octoploids from Pannonia and Öland, and (2) octoploids from Podillya and Gotland (represented by the type population of *J. vulgaris* subsp. *gotlandica*; Fig. 7b). The histograms of CDA 2 and 4 (based on '*J. vulgaris* individuals matrix' and '*J. vulgaris* 8*x* individuals matrix', respectively) affirmed the phenetic distinctness of the tested groups (tetra- vs. octoploids—Online Resource 3a, and Pannonian and Öland vs. Podillyan and Gotland octoploids—Online Resource 3b) at the individual level, although they partly overlap morphologically. The characters that best separated tetra- and octoploids were related to the (a) shape (segmentation) of leaves (LLL, MLNT, LLS, LLNS, LLA), (b) the size of the capitula (IBL, TFL, RFW, RFL), and (c) the indument of outer achenes (OAI) (Online Resource 4). Characters important for the separation of the two main groups of octoploids (Pannonian and Öland octoploids, and Podillyan and Gotland octoploids) were mainly related to the size of the capitula (RFL, TFN, TFL, IBL; Online Resource 4).

To test the morphological homogeneity of the three main groups (tetraploids, Pannonian and Öland octoploids, and Podillyan and Gotland octoploids) revealed by the FCM and AFLP analyses, a series of four PCAs based on populations or individuals of different data matrices were performed. PCA 1 (without character OAI) and PCA 2 (without RFL, RFW, OAI), based on '*J. vulgaris* 4*x* populations matrix', were used to test the morphological homogeneity of the *J. vulgaris* tetraploids from the entire

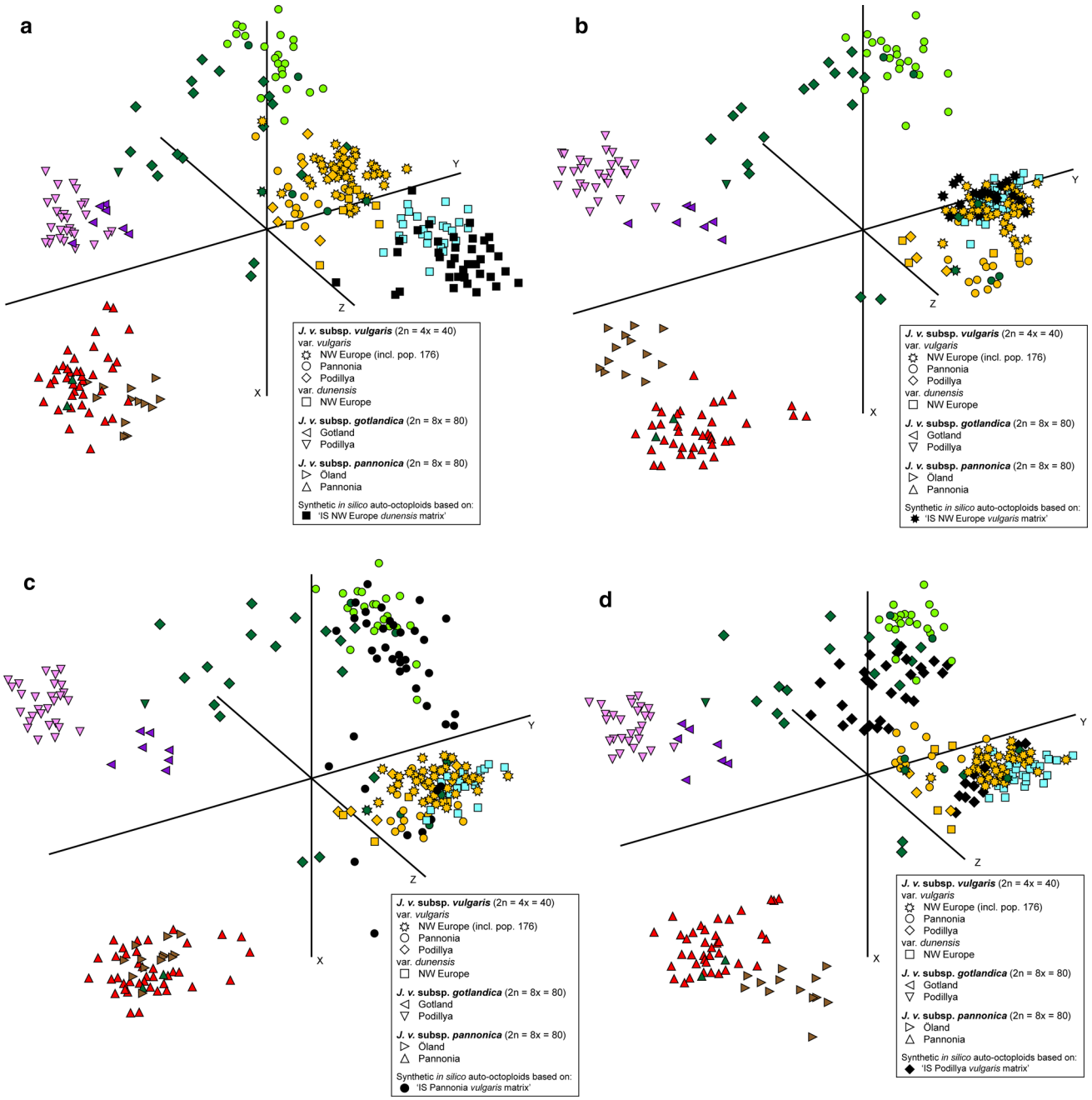

Fig. 6 Principal coordinate analyses of four in silico generated octoploid groups of *Jacobaea vulgaris*. *Colours* indicate the genetic grouping of natural AFLP profiles of *J. vulgaris* as inferred by the Bayesian analysis of genetic population structure (see Fig. 2b), *black symbols* indicate in silico octoploids. **a** In silico octoploids generated from *J. vulgaris* var. *dunensis* tetraploids (*black squares*). The first three axes explain 49.24 % of the total variation ($x = 32.66$ %, $y = 11.67$ %, and $z = 4.91$ %). **b** In silico octoploids generated from *J. vulgaris* var. *vulgaris* tetraploids from NW Europe (*black stars*). The first three axes explain 48.55 % of the total variation ($x = 33.21$ %, $y = 11.97$ %, and $z = 3.37$ %). **c** In silico octoploids generated from *J. vulgaris* var. *vulgaris* tetraploids from Pannonia (*black circles*). The first three axes explain 48.23 % of the total variation ($x = 28.61$ %, $y = 15.18$ %, and $z = 4.44$ %). **d** In silico octoploids generated from *J. vulgaris* var. *vulgaris* tetraploids from Podillya (*black diamonds*). The first three axes explain 47.94 % of the total variation ($x = 29.49$ %, $y = 14.57$ %, and $z = 3.88$ %)

area studied. Both analyses showed the separation of populations into two morphological groups (Fig. 8, Online Resource 5). The populations placed in the left part of the diagram represent the plants from the continental part of Europe (i.e., those from Pannonia, Podillya, and population no. 176 from central Germany), whereas the populations from the coastal or Atlantic part of Europe (i.e., populations from NW Europe, incl. *J. vulgaris* subsp. *dunensis*)

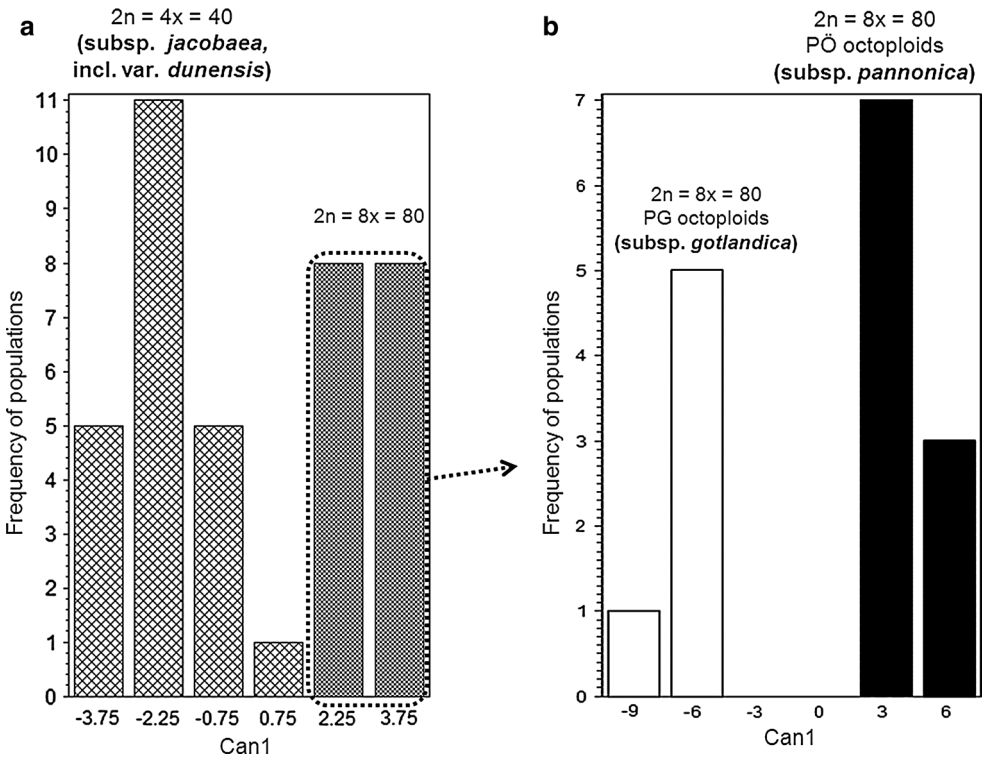

Fig. 7 **a** Canonical discriminant analysis of 38 *Jacobaea vulgaris* populations (22 tetraploid populations and 16 octoploid ones as OTUs, and two groups defined by ploidy levels) based on mean values of 13 morphological characters (except character indument of outer achenes, OAI) (CDA 1). **b** Canonical discriminant analysis of 16 *J. vulgaris* octoploid populations and two groups representing PÖ (Pannonian and Öland) and PG (Podillyan and Gotland) octoploids, based on mean values of 14 morphological characters (including character OAI; CDA 3)

were placed on the right of the diagram. Moreover, PCA 2 analysis (Online Resource 5) showed that *J. vulgaris* subsp. *dunensis* shares the majority of morphological characters (except for loss or reduction of rays) with the Atlantic morph of *J. vulgaris* subsp. *vulgaris*. In both PCAs, the characters showing the highest correlations with the first component axis, and thus, the best separation of these two groups/morphs, were related to the segmentation of leaves (LLNT, LLS, LLA, LLNS, LLL, MLNT, MLNS; Online Resource 4). PCA 3, based on octoploid individuals from Pannonia and Öland ('Pannonia+Öland 8*x* individuals matrix'), showed little (or neglected) tendency for separation of plants from Pannonia and Öland along the first two component axes, placing the Öland plants into the upper left corner of the diagram (Online Resource 6). The last PCA 4, based on octoploid individuals from Podillya and Gotland ('Podillya+Gotland 8*x* individuals matrix'), resulted in two partly separated groups/morphs corresponding to geographic regions. Their slight separation was visible along the first component axis (Online Resource 7). The greatest influence on the division of these two morphotypes was explained by characters corresponding to the segmentation of lower cauline leaves (LLS, LLNS, LLNT; Online Resource 4).

The Tukey–Kramer multiple comparison analysis and descriptive statistics were based on the comparison of five homogeneous groups resulting from the FCM, AFLP (two main octoploid groups—Pannonian and Öland octoploids, and Podillyan and Gotland octoploids resolved in NN diagram and PCoA) and morphometric (two tetraploid groups resolved in PCA 1 and one morphologically clear non-ray "*dunensis*" morph) analyses: (1) the tetraploid "*dunensis*" morph, (2) the tetraploid Atlantic morph, (3) the tetraploid continental morph, (4) the Pannonian and Öland octoploids, and (5) the Podillyan and Gotland octoploids. The results are presented in Online Resource 8 and in Fig. 9. Several common features were detected in both main groups of octoploids differentiating them from tetraploids: less segmented leaves, larger flower heads, and more hairy outer achenes. Specifically, octoploids significantly differ from tetraploids by shorter segmented part of lower cauline leaf (LLS), longer apical leaf segment of lower cauline leaf (LLA), narrower lateral lobe on apical segment of lower cauline leaf (LLL), fewer lateral leaf segments of lower and middle cauline leaf (LLNS, MLNS), fewer teeth per lateral segment of lower and middle cauline leaf (LLNT, MLNT), longer involucral bracts (IBL), longer and wider ray florets (RFL, RFW), longer tubular florets (TFL), and more hairy outer achenes (OAI). Within tetraploid morphotypes, the "*dunensis*" morph is in whole morphology (except for the loss or reduction of rays and small differences in shape of the middle leaves) ± identical with the Atlantic morphotype, i.e., with tetraploid plants with well-developed ray florets from NW Europe. Both morphs significantly differ from the continental morph in seven leaf characters: LLS, LLA, LLL, LLNS, LLNT, MLNS, and MLNT. In all the mentioned characters, the continental morph has an intermediate position

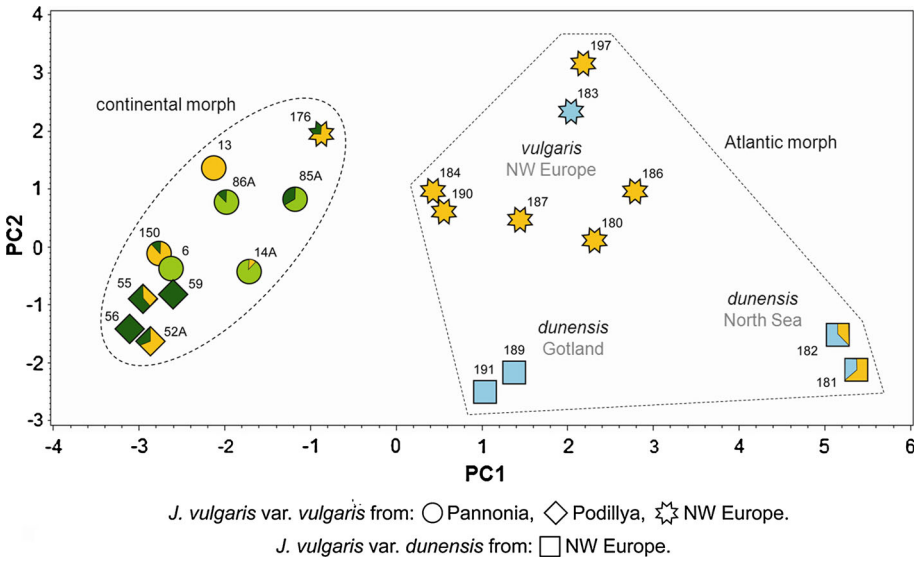

Fig. 8 Principal component analysis (PCA 1) of 22 *Jacobaea vulgaris* tetraploid populations (=*J. vulgaris* subsp. *vulgaris*, including var. *dunensis*) from the whole area studied based on 13 morphological characters (except character indument of outer achenes, OAI). *Colour proportions within the symbols* indicate membership in the eight genetic clusters identified by the Bayesian analysis of genetic population structure (see Fig. 2). The population numbers follow those in Table 1. The first two axes explain 53.01 and 17.97 % of the variation

between the tetraploid Atlantic and "*dunensis*" morphs on the one side and the octoploid plants on the other side. Between the Pannonian and Öland vs. Podillyan and Gotland octoploids, significant differences were found in five characters: IBL, RFL, TFN, TFL, and OAI.

Discussion

Ploidy levels in *Jacobaea vulgaris*

In accordance with the previous reports for *J. vulgaris* (see Hodálová et al. 2007a, 2010), we confirmed both the tetra- and octoploid cytotypes in Pannonia and Podillya, as well as the tetraploids in western Europe.

All examined plants belonging to *J. vulgaris* subsp. *dunensis* were found to be exclusively tetraploid (Table 1), confirming two chromosome counts reported for this taxon by van den Brand et al. (1979; as *Senecio jacobaea* var. *nudus*) and Kockx-van Roon and Wieffering (1982; as *S. jacobaea* var. *flosculosus*).

In contrast and fairly unexpectedly, *J. vulgaris* subsp. *gotlandica* from the type locality (population no. 188 from the Gotland Island; Table 1) was found to be octoploid. We did not confirm the tetraploid chromosome number reported for this taxon from Gotland Island by Wysk et al. (2009). Most likely, instead of *J. vulgaris* subsp. *gotlandica*, these authors analysed plants of the nominate subspecies *J. vulgaris* subsp. *vulgaris*, which also occurs on Gotland Island and is tetraploid. Octoploidy has also been found in populations from Öland (populations no. 194, 195), traditionally considered to be *J. vulgaris* subsp. *gotlandica*, as well as in two Austrian populations from Pannonia (populations no. 163 and 164) assigned by Wysk et al. (2009) to *J. vulgaris* subsp. *gotlandica* (Table 1). It is evident that *J. vulgaris* subsp. *gotlandica* as defined thus far is exclusively an octoploid taxon. The taxonomic identity of octoploid populations from Öland and Austria is discussed further below.

Modes of origin of *Jacobaea vulgaris* octoploids

Our AFLP data revealed two main genetically separated groups of octoploids within *J. vulgaris*; one group represented by the Pannonian and Öland populations, and the other group by the populations from Podillya and Gotland (Figs. 3, 4 and 5). The close genetic relationship of octoploid plants to *J. vulgaris* tetraploids (Fig. 3), and the absence of fragments shared between octoploids and closely related congeners indicate that both main octoploid groups are of autopolyploid origin.

Autopolyploid origin is clearly evident especially in the case of the Podillyan and Gotland octoploids, the AFLP profiles of which are at least partially mixed with those of the Podillyan tetraploids (Figs. 3, 4 and 5). Somewhat contrastingly, the octoploids from Pannonia and Öland formed a more discrete genetic group distinct from all the *J. vulgaris* tetraploids, although some affinity to the Podillyan tetraploids was apparent. Thus, the identity of the presumably parental populations of octoploids from Pannonia and Öland is unclear. Several hypothetical scenarios regarding the origin of the Pannonian and Öland octoploids can be considered: (1) they could have evolved in different time horizons, i.e., they are older in origin than the Podillyan octoploids and, therefore, more differentiated from parental tetraploids; or (2) they originated from populations

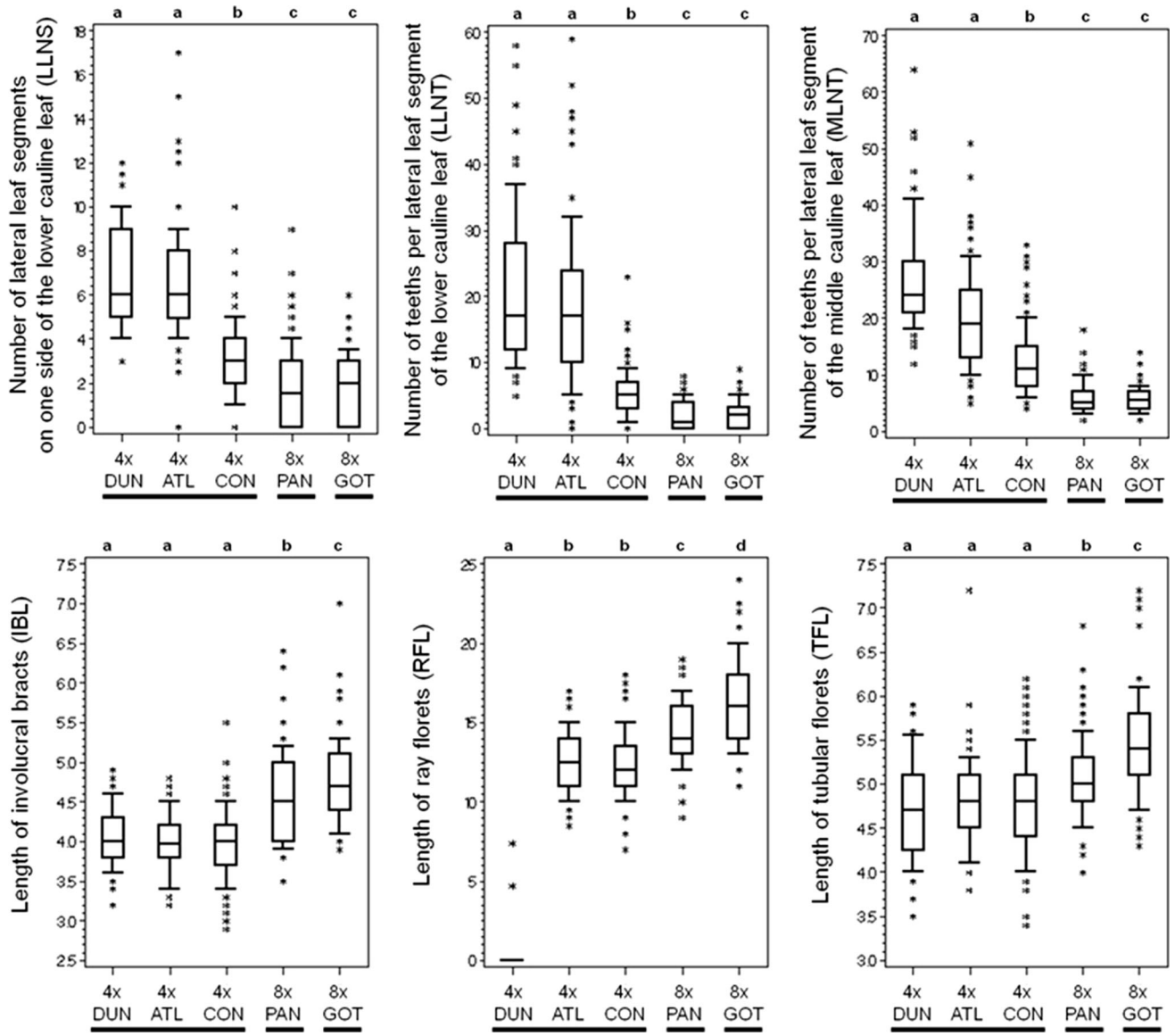

Fig. 9 Variation in selected morphological characters in *Jacobaea vulgaris*: 4x DUN—*J. vulgaris* var. *dunensis*, 4x ATL—*J. vulgaris* var. *vulgaris* Atlantic morph, 4x CON—*J. vulgaris* var. *vulgaris* continental morph, 8x PAN—*J. vulgaris* subsp. *pannonica*, 8x GOT—*J. vulgaris* subsp. *gotlandica*; 4x = tetraploids, 8x = octoploids. *Rectangles* define the 25th and 75th percentiles; *horizontal lines* show the median; *whiskers* are from the 10th to 90th percentiles; *asterisks* show extreme values. Labels **a**–**d** given above the *boxplots* indicate homogeneous groups according to post hoc comparisons following one-way ANOVA (Tukey–Kramer multiple comparison analysis at a probability level of $P \leq 0.05$)

growing in areas not covered by our study; (3) finally, it cannot be completely excluded that the Pannonian and Öland octoploids diverged from some Eastern European or Asian species closely related to *J. vulgaris* that were not included in our analyses. This third possibility is less likely due to the considerable morphological similarity of the Pannonian and Öland octoploids to the Podillyan and Pannonian tetraploids of *J. vulgaris*.

Previously, *J. vulgaris* subsp. *gotlandica* was often confused with *J. aquatica* (see for example specimens deposited in the LD herbarium). The morphological similarities of this subspecies (represented in our analyses by the Podillyan and Gotland octoploids; see below) with *J. aquatica* on the one side (both characterized by subentire basal leaves with large terminal lobe) and with *J. vulgaris* subsp. *vulgaris* on the other (characterized by densely pubescent disc floret achenes) raises the possibility of a hybrid origin of *J. vulgaris* subsp. *gotlandica* due to crossing of these two taxa. This possibility was also mentioned by Wysk et al. (2009). However, such an allopolyploid scenario is not supported by our genetic data. As mentioned above, individuals from the type locality of

J. vulgaris subsp. *gotlandica* are clearly of autopolyploid origin, most likely evolving (together with the Podillyan octoploids) from the Podillyan tetraploids of *J. vulgaris* subsp. *vulgaris* (Fig. 3).

Recurrent and polytopic autopolyploid origins have been reported recently in several species groups of the Asteraceae family (e.g., Mráz et al. 2008; Grubbs et al. 2009; Richardson et al. 2012). However, the number of polyploid events leading to the formation of *J. vulgaris* octoploids was most likely not high. Our AFLP data indicate, that octoploids arose independently twice in the evolutionary history of *J. vulgaris*. One polyploid event is represented by Pannonian and Öland octoploids and the other by Podillyan and Gotland octoploids. However, it cannot be excluded that octoploids from Öland and Gotland evolved independently from those from Pannonia and Podillya, and that grouping of these four genotypes into two lineages in our AFLP analyses is only a result of the close genetic relatedness of tetraploid progenitors of Pannonian and Öland octoploids on the one hand, and Podillyan and Gotland octoploids on the other.

In any case, the formation of *J. vulgaris* octoploids is apparently not caused by recurrent and recent polytopic origin from sympatric tetraploid populations. This is supported also by the separation of natural octoploids from the grouping of in silico octoploids and parental tetraploids on ordination diagrams of PCoA (Fig. 6). Furthermore, in both studied mixed-ploidy sampling sites (populations no. 85A, 85B, 86A, and 86B), there is apparent genetic differentiation between sympatric cytotypes, supporting the hypothesis that detected octoploids are not of in situ origin (Figs. 2b, 3, 4 and 5). The two studied mixed-ploidy sampling sites, as well as other studied European localities of *J. vulgaris* thus clearly belong to the secondary and not primary contact zones (cf. Petit et al. 1999). Perhaps the only exception might be the Podillyan octoploid individual no. 57-2, which was in the intermediate position based on the AFLP profile, placed between the Podillyan octo- and tetraploids (Figs. 4, 5), indicating a recent in situ polyploidization event.

Notes on the biogeography of *Jacobaea vulgaris* octoploids on islands of Gotland and Öland

The occurrence of thermophilous *J. vulgaris* octoploids within Scandinavia, particularly on the islands of Gotland and Öland, is not surprising. Since the first visit of Carl von Linné in 1741, these Baltic islands are well known for their xerothermophilous vegetation, which has among other a close connections with the steppe vegetation of South-Eastern and Central Europe (e.g., Pettersson 1965; Ekstam et al. 1984; Sterner and Lundqvist 1986; Löbel and Dengler 2007). Several thermophilous plant species, with a centre of distribution in temperate and submediterranean parts of Europe occur as native within Scandinavia only on these islands and reach the northern limit of their (native) distribution here (e.g., *Adonis vernalis* L., *Galatella linosyris* (L.) Rchb.f.; Meusel and Jäger 1992; Karlsson 2001). This is due to calcareous (limstone) warm bedrock forming world famous karst alvar habitats and due to a mild maritime climate of the islands (Pettersson 1965).

However, the unexpected finding of our study is that octoploids from Öland and Gotland are apparently of separate origin. No less surprising is that the Gotland octoploids are closely related to the Podillyan octoploids, and the Öland octoploids are closely related to the Pannonian octoploids (Figs. 3, 4 and 5). From both islands, we have analysed only a limited number of octoploid plants (10 individuals from one locality in Gotland, and 29 individuals from two Öland populations located close to each other), and therefore, it remains unclear whether octoploids on both islands are represented only by one or more genetic lineages. The floristic difference between Gotland and Öland is a well-known phenomenon (e.g., Pettersson 1965). Several recent studies using molecular markers revealed, that populations of the same taxon occurring on these islands (or on different parts within one island) could have originated from different source populations (e.g., Runyeon and Prentice 1997; Rosquist and Prentice 2000; Ahlgren 2011).

Octoploid *J. vulgaris* populations growing on Gotland and Öland are found hundreds of kilometres away from their nearest-known genetically related octoploids in continental Europe. This disjunctive occurrence of octoploids on the two Baltic islands provides some intriguing biogeographic insights regarding its place of origin, colonisation mode, and time.

Obtained genetic results do not indicate that Gotland or Öland octoploids arose on these islands (or in the adjacent part of the Swedish mainland) from the local tetraploid populations. Since the Gotland and Öland were completely covered by ice during the last glacial period it is obvious, that octoploid cytotypes reached the islands sometimes after 12,000 years BP, when the ice cover had retreated (Björck 1995). Octoploids on both Baltic islands thus might represent (1) relicts or remnants of postglacial colonisation from the continental Europe (by land bridges between continent and Sweden, Swedish mainland and Öland, and probably also between Swedish mainland and Gotland; cf. Björck 1995; Ahlgren 2011), (2) they could have arisen from long-distance dispersal events from continental Europe, or (3) they may have been (unintentionally) introduced (also from continental Europe) by human activity.

Genetic differences found between the island and continental octoploids within two main genetic groups of

octoploids detected in our study (i.e. between Podillyan vs. Gotland octoploids and between Pannonian vs. Öland octoploids) can be attributed to one of the given reasons: (1) island octoploids has been for a long time isolated from their continental octoploid progenitors, (2) island and continental octoploids arose independently, but from genetically related tetraploids, or (3) island genotypes represent marginal (but in our sampling overlooked) variation, which could be found by more detailed sampling of island or continental populations (for example in Poland or in Baltic states). In the present study, however, the available data did not provide reliable information to distinguish among these hypotheses. The fact that no octoploids are known in Poland, Belarus, Latvia, Lithuania or Estonia might have various causes. The octoploids in these areas may have been overlooked, gone extinct, or never occurred.

Morphological and ecological differentiation of *Jacobaea vulgaris* cytotypes

The octoploid populations included in our study, although not of monophyletic origin, exhibit several common morphological and ecological features. Octoploid plants morphologically differ from tetraploids by having: less segmented lower and middle cauline leaves, longer involucral bracts, longer and wider ray and tubular florets, greater number of tubular florets, more hairy outer achenes (Fig. 9; Online Resource 8, 9–11; cf. Hodálová et al. 2007a), larger pollen grains (Hodálová et al. 2010), loose corymbiform inflorescence with fewer heads (I. Hodálová and P. Mered'a unpublished data), and sometimes fleshier lower cauline leaves with different texture and surface [pointed already by Nordenstam in Wysk et al. (2009) referring to *J. vulgaris* subsp. *gotlandica*].

Nevertheless, the identification of *J. vulgaris* subsp. *gotlandica* (or octoploids in general) could not be based solely on the morphology of basal leaves as reported by Wysk et al. (2009) and also used by Conti et al. (2012). The shape of the lower and middle cauline leaves is rather variable within *J. vulgaris* throughout tetraploid and octoploid populations. Plants with less segmented leaves and large terminal lobes, the shape of which match that of *J. vulgaris* subsp. *gotlandica* (or octoploids generally), are occasionally found in populations of *J. vulgaris* subsp. *vulgaris* (or tetraploids generally), especially in Central and Eastern Europe (Online Resource 9–11).

The most pronounced morphological differences within tetraploids have been found in flower morphology, namely, between discoid flower populations of *J. vulgaris* subsp. *dunensis* and rayed flower populations of *J. vulgaris* subsp. *vulgaris*. Although the "*dunensis*" morph genetically does not belong to a single monophyletic lineage (Fig. 4), our data do not support the hypothesis that the "*dunensis*" morph has evolved polytopically within each or within a majority of populations of the rayed phenotype. It seems more likely that the discoid phenotype has evolved only at a limited number of sites and/or within limited geographical areas. Such an assumption is in accordance with the suggestion by Andersson (2001b) that most of the discoid populations in the Baltic regions can be attributed to introduction through ship ballast in the 1800s. Additionally, Andersson (2001b) assumed that a single allelic substitution could be responsible for the transition from rayed to discoid heads in *J. vulgaris* subsp. *dunensis*. Similar evolutionary "lability" of ray morphology has also been referred to in several other Asteraceae genera (for references, see Andersson 2001b). Due to the likely simple genetic basis of the discoid phenotype, its specific ecological niche and restricted geographic area, the rank of variety, *J. vulgaris* var. *dunensis* (Dumort.) Hodálová & Mered'a (see below), seems to be the most appropriate for this morph.

The above-mentioned morphological differences withstanding, multivariate morphometrics revealed two distinct morphotypes in tetraploids, one occurring in the Atlantic coast region and the second one in the continental part of the studied area. The populations from the Atlantic coastal part of Europe, harbouring both discoid and rayed flower phenotypes, differed from those from the continental area with more segmented leaves (Fig. 9; Online Resource 8, 9, 10), more robust habit (with longer stems and leaves) and more branched stem from the base (I. Hodálová and P. Mered'a unpublished data). However, in all given characters, there is considerable variation, even among individuals within the same population, and individuals of different morphotypes can sometimes be found within a single population.

Because the variation in all morphological characters of cytotypes of *J. vulgaris* overlaps to a certain extent, for their reliable recognition, a number of characters should be taken into account, both in leaf and floral morphology. Nevertheless, in certain cases, only chromosome counting or ploidy level analysis provide a tool for their reliable and unambiguous identification. The same differentiation pattern is also found among infraspecific cytotypes of other plant species; for example, in the Asteraceae family, in *Aster amellus* agg. (Mandáková and Münzbergová 2008), *Centaurea phrygia* agg. (Koutecký et al. 2012) or *Centaurea stoebe* agg. (Španiel et al. 2008).

Although not yet investigated in detail, the habitat preferences of *J. vulgaris* cytotypes are also quite different. Our field observations indicate that octoploids, compared to tetraploids, possess narrower ecological amplitude and are less competitive. They occur mostly in xerothermic habitats, with natural or semi-natural vegetation, while the

tetraploids occupy both xerothermic and relatively mesophilous sites of natural, semi-natural, and man-made habitats (I. Hodálová and P. Mered'a unpublished data). Recently, tetraploids have massively spread into various anthropogenically disturbed habitats such as roadsides, pastures, abandoned vineyards, and waste areas, where octoploids are unable to survive (I. Hodálová and P. Mered'a unpublished data).

Taxonomic status of *Jacobaea vulgaris* octoploids

The taxonomic status of polyploid derivatives vary considerably in plant systematics. In cases of no morphological differences and reproductive barriers between polyploids and their lower ploid progenitors, the polyploid derivatives are usually not recognized as separate taxa [e.g., polyploids of *Pilosella rhodopaea* (Griseb.) Szeląg; Šingliarová et al. 2011]. However, this is not the case for *J. vulgaris* octoploids, which differ from tetraploids genetically, morphologically, and ecologically. Additionally, it seems that there is little if any gene flow between the tetra- and octoploids of *J. vulgaris* (Hodálová et al. 2010; see also the note on *J. vulgaris* hexaploids above). Therefore, the *J. vulgaris* octoploids should have their own infraspecific taxonomic status within *J. vulgaris*.

Our AFLP data clearly showed that there are at least two octoploid lineages of *J. vulgaris*, which evolved independently and they are recently (at least in the part of their distribution area) allopatric and partially morphologically different. Although octoploid lineages grow in similar habitats and are morphologically very similar (see Fig. 9, Online Resource 8, 11) we are convinced that the most appropriate taxonomic solution is to treat these two lineages as separate taxa at the subspecies level. Thus, two names for them have to be selected. While the name *J. vulgaris* subsp. *gotlandica* is applicable to the Gotland and perhaps also to Podillyan octoploids (to which this name is tentatively coined here as well), there is no name available for the second lineage represented primarily by Pannonian octoploids. They are, therefore, described here as a new subspecies, *J. vulgaris* subsp. *pannonica* Hodálová & Mered'a (see below). Öland octoploids are tentatively classified here within *J. vulgaris* subsp. *pannonica* as well, nevertheless such treatment requires more substantial support than provided by our study.

However, it should be clarified, whether the two main octoploid genetic lineages are truly monophyletic or consist of genetically similar but independently arosen groups (see "Discussion" above). Thus, before any definitive taxonomic decision on the taxonomic identity of Podillyan and Öland octoploids is drawn, analyses based on a more detailed sampling on Baltic islands, Poland, Belarus, and Baltic states (Latvia, Lithuania, and Estonia) are necessary. In any case, it does not seem likely, that additional sampling would merge the two main octoploid lineages into one group. More likely such sampling would either fill the gaps between the island and continental octoploid genotypes of one or both subspecies, or split up the two main octoploid lineages into further groups (lineages).

Taxonomic treatment

Character values represent 10th and 90th percentiles, and those in brackets represent minima and maxima.

Key to the infraspecific taxa of *Jacobaea vulgaris*

1a Ray florets absent, rarely in some individuals within a discoid population rudimentary (ca up to 7 mm long)… *J. vulgaris* var. *dunensis*.

1b Ray florets well developed, more than 10 mm long, rarely in some individuals within a rayed population very small (ca 7 mm long)… 2.

2a Number of lateral segments on one side of the lower cauline leaf 1–9; number of teeth per lateral segment of the lower cauline leaf 1–32; involucral bracts 3.4–4.5 mm long; ray florets 10–15 mm long; outer achenes often glabrous, rarely hairy (hairs present in ca 25 % of individuals). Plants growing in xero- to mesothermophilous natural to ruderal communities, from lowlands to mountain vegetation belt. Plants tetraploid… *J. vulgaris* var. *vulgaris*.

2b Number of lateral segments on one side of the lower cauline leaf 0–4.5; number of teeth per lateral segment of the lower cauline leaf 0–6; involucral bracts 4–5.2 mm long; ray florets 11–19 mm long; outer achenes often hairy (hairs present in ca 65 % of individuals), rarely glabrous. Plants growing in xerothermophilous natural or semi-natural communities, from lowlands to hilly areas. Plants octoploid… 4

3a Ray florets 13–20 mm long; tubular florets 4.7–6.1 mm long. Plants growing in Podillya and on Gotland… *J. vulgaris* subsp. *gotlandica*.

3b Ray florets 12–17 mm long; tubular florets 4.5–5.5 mm long. Plants growing in Pannonia and adjacent parts of the Eastern Alps, the Bohemian Massif, and the Western Carpathians, and on Öland… *J. vulgaris* subsp. *pannonica*.

Jacobaea vulgaris Gaertn. Fruct. Sem. Pl. 2: 445. 1791.

Replaced name: *Senecio jacobaea* L. Sp. Pl. 2: 870. 1753. Ind. loc.: "Habitat in Europae pascuis". Lectotype (Kadereit and Sell 1986): LINN 996.44 (LINN!).

Jacobaea vulgaris Gaertn. **subsp**. ***vulgaris***

Jacobaea vulgaris Gaertn. **subsp**. ***vulgaris*** **var**. ***vulgaris***

Jacobaea vulgaris **subsp**. ***vulgaris*** **var**. ***dunensis*** (Dumort.) Hodálová & Mered'a, **comb. nov.**

Basionym: *Senecio dunensis* Dumort. Fl. Belg.: 66. 1827. Ind. loc.: "[Belgium], in arenosis maritimis!". Type unknown.

Jacobaea vulgaris **subsp. *gotlandica*** (Neuman) B.Nord. Compositae Newslett. 44: 12. 2006.

Basionym: *Senecio jacobaea* var. *gotlandicus* Neuman Sver. Fl.: 26. 1901. Ind. loc.: "Gotland". Lectotype (designated here): Gotland, Klinteberget, 2 Aug 1890, F. Ahlfvengren s.n. (LD 1173500!).

Jacobaea vulgaris **subsp. *pannonica*** Hodálová & Mered'a, **subsp. nov.**

Holotype: Slovakia, Devínska Kobyla Hills, Bratislava-Devínska Nová Ves, south of the city quarter, Sandberg hill, 220 m a. s. l., 48°12′02″ N, 16°59′28″ E, 16 Jul 2008, I. Hodálová s.n. (SAV).

Etymology: The subspecific epithet "*pannonica*" refers to the geographical region of Pannonia, where this taxon was discovered.

Plants annual or biennial. Stem erect, 60–120 cm, branched only in inflorescence. Lower cauline leaves lyrate-pinnatifid, usually withering at anthesis, with petiole reaching (0–)20–52(–72) % of the whole leaf length and with segmented part reaching 0–37(–67) % of the whole leaf length; number of lateral segments on one side of leaf 0–4(–9), irregularly toothed, with 0–5(–8) teeth per segment, apical leaf segment reaching (25–)35–62(–73) % of the whole leaf length, width of the largest lobe reaching (2.6–)5.4–15.8(–21.9) % of length of the apical leaf segment. Middle cauline leaves 1- to 2-pinnatifid, sessile, with (2–)4–7.2(–11) lateral segments on one side of leaf, irregularly toothed, with (2–)3–9.2(–18) teeth per segment, apical leaf segment linear or lanceolate. Heads aggregated to loose corymbiform inflorescence, usually with fewer heads. Involucre densely or sparsely covered by arachnoid hairs, (3.5–)3.9–5.2(–6.4) mm long, with (1–)2–6 outer involucral bracts. Ray florets 13, (9–)12–17(–19) mm long and (1.8–)2.1–3.2(–4.4) mm wide. Tubular florets (34–)49–71(–85) per one head, (4–)4.5–5.5(–6.8) mm long. Peripheral achenes often hairy (hairs present in ca 60 % of individuals) or rarely glabrous, the inner achenes densely hairy. (Online Resource 11).

Chromosome number: $2n = 8x = 80$.

Habitats: Open (sub)xerothermophilous natural or semi-natural communities, such as dry or sand grasslands, shrubs, and rocky karst places; on sands, shallow skeleton-rich or deeper soils, mainly on basic substrata, such as sands, loesses, limstones, dolomites, travertines, andesites, and rarely quertzites; from lowlands to hilly (colline) vegetation belts.

Distribution area: Pannonia and adjacent parts of the Eastern Alps, the Bohemian Massif, the Western Carpathians (Hungary, eastern Austria, southern Moravia, Slovakia); an isolated occurrence probably (see discussion above) on the Swedish island of Öland (Fig. 2a).

Phenology: Flowering from the end of June to August.

Morphological affinities: The new subspecies is morphologically very close to the octoploid *J. vulgaris* subsp. *gotlandica*, mainly due to (a) less segmented leaves (with shorter segmented part and longer apical segment of lower cauline leaves, narrower lowermost lateral lobe on apical segment of lower cauline leaves, fewer lateral segments and teeth per lateral segment of lower and middle cauline leaves), (b) loose inflorescence composed of fewer heads, (c) larger flower heads (with longer involucral bracts, larger ray florets and longer tubular florets), and (d) more hairiness of outer achenes. Concurrently, these characters distinguish the new subspecies from the nominate (tetraploid) subspecies *J. vulgaris* subsp. *vulgaris*. The new subspecies differs from the *J. vulgaris* subsp. *gotlandica* mainly by shorter rays and tubular florets.

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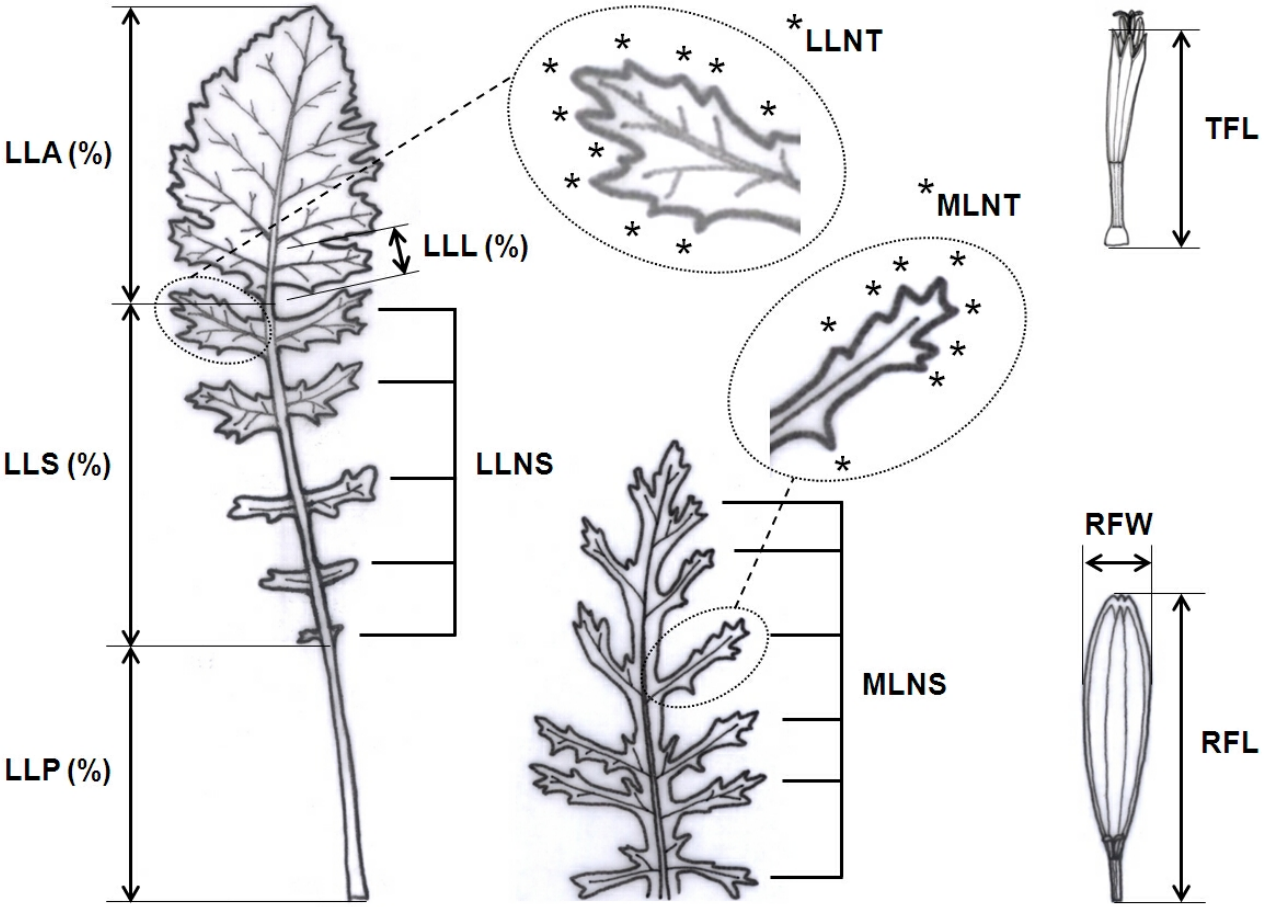
Online Resource 1 Morphological characters scored and measured for morphometric analyses. Characters IBL (length of involucral bracts), TFN (number of tubular florets), and OAI (indument of outer achenes) are not illustrated. For character explanations, see Table 2. Drawings by P. Mered'a Jr.

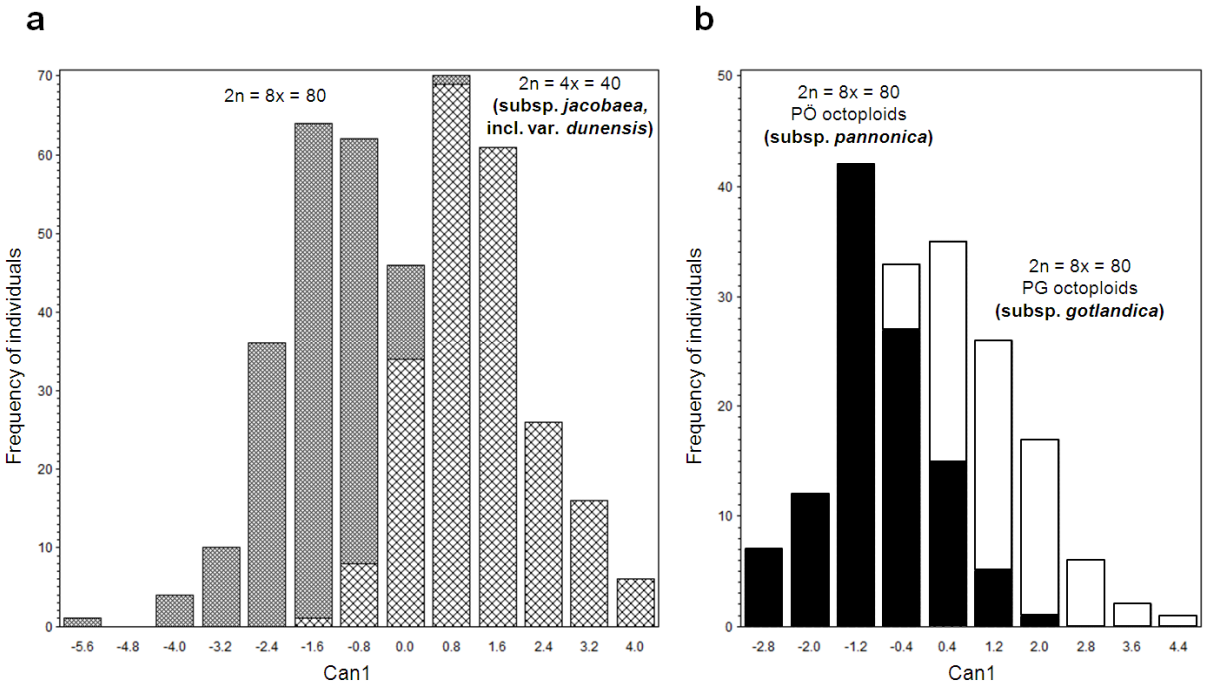


Online Resource 2 Distribution of AFLP fragments across the investigated *Jacobaea* taxa and *J. vulgaris* groups corresponds to the BAPS results (see Fig. 2a). The columns show taxon name; number of analysed individuals (N_{ind}); number of AFLP multilocus phenotypes (N_{phen}) resolved in a particular taxon/group; average number of AFLP fragments (N_{fragm}) per individual ± standard deviation; average proportion of pairwise differences between individuals (Nei's gene diversity); percentage of polymorphic markers (P%); number of private fixed (diagnostic) fragments (N_{dg}); number of private (exclusive) fragments (N_{pr}); number of rare fragments (present at a frequency < 10% of the investigated individuals) (N_{rare}). 4x = tetraploids, 8x = octoploids.

Taxa/BAPS groups	**N_{ind}**	**N_{phen}**	**N_{fragm}**	**N_{pr}**	**D_{Nei}**	**P %**	**N_{dg}**	**N_{rare}**
J. alpina	27	24	94±7.56	0	1.05	13.77	4	9
J. aquatica	20	20	83±5.43	3	1.15	37.05	10	38
J. erratica	21	21	79±4.63	1	1.15	30.82	5	22
J. erucifolia	17	17	74±3.94	7	1.10	21.31	2	47
J. subalpina	20	20	82±11.7	0	1.19	31.08	3	17
J. vulgaris subsp. *gotlandica*								
Pink group (8x from Podillya)[1]	29	29	87±3.47	0	1.07	26.56	0	9
Violet group (8x from Gotland)	7	7	96±1.68	0	1.11	18.03	0	4
J. vulgaris subsp. *pannonica*								
Red group (8x from Pannonia)[2]	41	41	84±3.69	0	1.11	30.49	0	15
Brown group (8x from Öland)	13	13	91±4.86	0	1.09	19.67	0	6
J. vulgaris subsp. *vulgaris*								
Blue group (4x from a coastal part of NW Europe)	26	25	84±2.45	0	1.09	23.93	0	27
Yellow group (4x from entire sampled area)	74	74	78±3.93	0	1.08	40.66	6	21
Light green group (4x from Pannonia)	22	22	89±4.35	0	1.08	23.61	0	13
Dark green group (4x from Podillya, Pannonia and Germany)[3]	23	23	86±8.03	0	1.15	40.33	1	7

[1] Including one individual (no. 57-2) from the dark green group.
[2] Including two individuals (no. 36-1, 36-2) from the dark green group.
[3] Except for two octoploid individuals from Pannonia (no. 36-1, 36-2), which are included in *J. vulgaris* subsp. *pannonica* (red group) and single octoploid plant from Podillya (no. 57-2), which is included in *J. vulgaris* subsp. *gotlandica* (violet group).

Online Resource 3 a Canonical discriminant analysis of 484 *Jacobaea vulgaris* individuals, based on 13 morphological characters (without character indument of outer achenes, OAI) and two groups defined by ploidy levels, tetraploids and octoploids (CDA 2). **b** Canonical discriminant analysis of 200 *J. vulgaris* octoploid individuals based on 14 morphological characters (CDA 4) and two groups representing PÖ (Pannonian and Öland) and PG (Podillyan and Gotland) octoploids.



Online Resource 4 The results of canonical discriminant analyses (CDAs) and principal component analyses (PCAs).

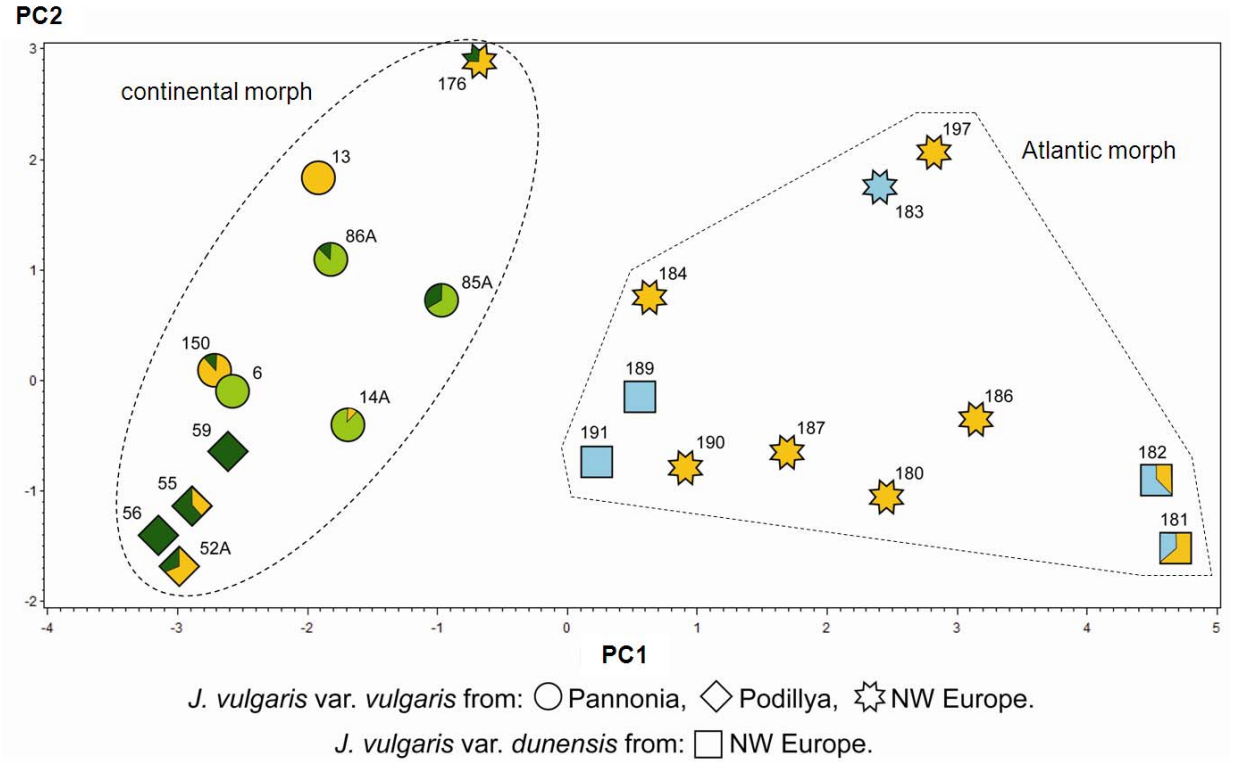
In CDAs, the values of total canonical structure express the correlation of characters with canonical axis Can1. The values were retrieved from the following: CDA 1 of all 38 *Jacobaea vulgaris* populations based on mean values of 13 morphological characters (without character indument of outer achenes, OAI) (Fig. 7a); CDA 2 of all 484 *J. vulgaris* individuals, based on 13 morphological characters (without character OAI) (Online Resource 3a); CDA 3 of 16 *J. vulgaris* octoploid populations based on mean values of 14 morphological characters (Fig. 7b); CDA 4 of 200 *J. vulgaris* octoploid individuals based on 14 morphological characters (Online Resource 3b).

In PCA1–4 component loadings show the contribution of the characters to the principal components PC1 and PC2. The values were retrieved from PCAs performed on populations or individual samples as OTUs: PCA 1 of 22 *J. vulgaris* tetraploid populations (= *J. vulgaris* subsp. *vulgaris*, including var. *dunensis*) from the whole area studied based on 13 morphological characters (without character OAI) (Fig. 8); PCA 2 of 22 *J. vulgaris* tetraploid populations (= *J. vulgaris* subsp. *vulgaris*, including var. *dunensis*) from the whole area studied based on 11 morphological characters characters [without characters length of ray florets (RFL), width of ray florets (RFW), and OAI] (Online Resource 5); PCA 3 of 119 *J. vulgaris* octoploid individuals from Pannonia and Öland based on 14 morphological characters (Online Resource 6); PCA 4 of 81 *J. vulgaris* octoploid individuals from Podillya and Gotland based on 14 morphological characters (Online Resource 7).

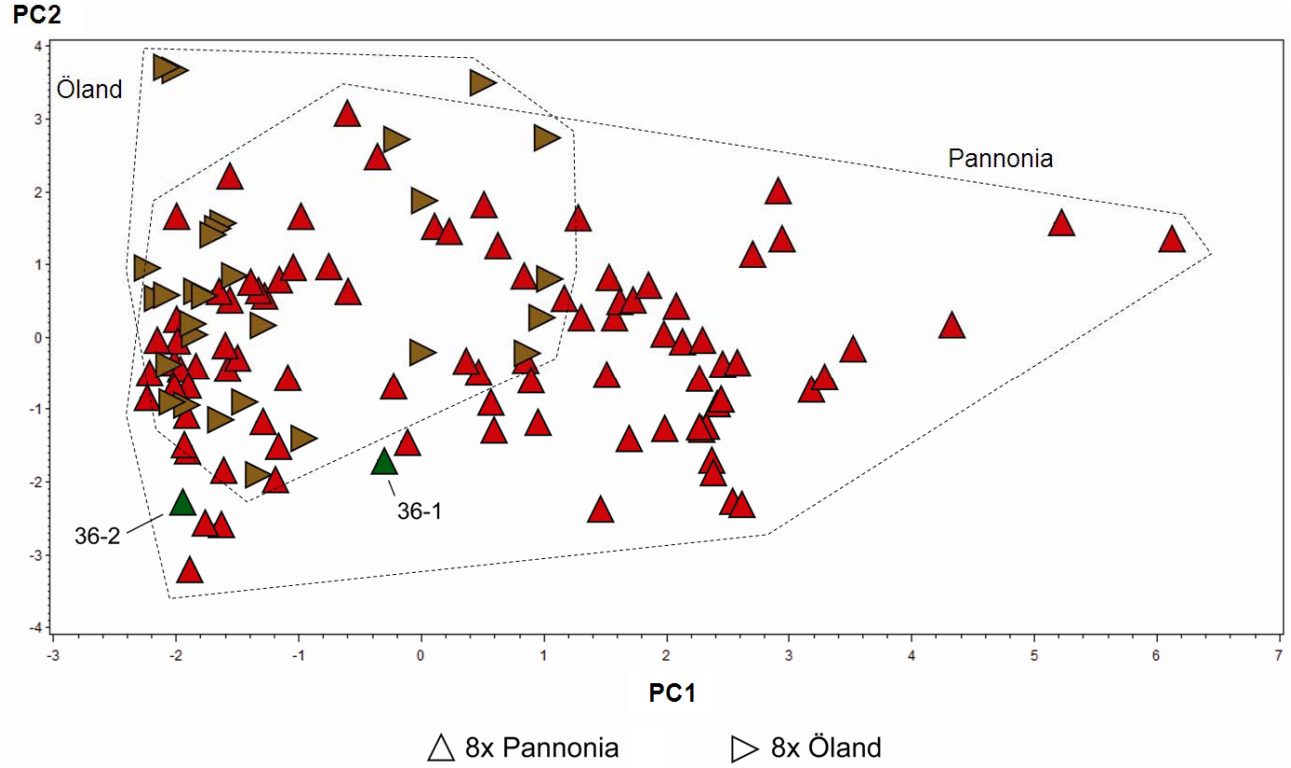
The three highest values in each column are printed in bold. For character illustrations, see Online Resource 1.

Characters		CDA 1	CDA 2	CDA 3	CDA 4	PCA 1		PCA 2		PCA 3		PCA 4	
		Can1	Can1	Can1	Can1	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Lower cauline leaf													
LLP	relative length of the petiole	0.584	-0.278	0.370	-0.179	-0.256	0.294	-0.246	**0.482**	-0.343	-0.089	-0.284	0.270
LLS	relative length of the segmented part of the leaf	-0.744	0.626	0.053	-0.082	**0.352**	0.060	**0.371**	-0.123	**0.495**	0.003	**0.418**	0.157
LLA	relative length of the apical leaf segment	0.720	-0.601	-0.392	0.334	**-0.343**	-0.066	**-0.360**	0.087	-0.299	0.112	-0.113	**-0.512**
LLL	relative width of lateral lobe on apical segment	**-0.774**	**0.673**	0.093	-0.096	0.327	-0.020	0.337	-0.156	0.245	0.128	0.194	0.323
LLNS	number of lateral leaf segments (on one side of leaf)	-0.737	0.558	-0.200	-0.077	0.337	0.046	0.349	-0.062	**0.480**	0.037	**0.415**	0.162
LLNT	number of teeths per lateral leaf segment	-0.628	0.550	-0.070	-0.010	**0.361**	0.057	**0.375**	-0.054	**0.475**	0.029	**0.407**	0.157
Middle cauline leaf													
MLNS	number of lateral leaf segments (on one side of leaf)	-0.619	0.534	0.351	**-0.344**	0.291	0.313	0.323	0.267	0.018	**0.481**	0.278	-0.015
MLNT	number of teeths per lateral leaf segment	**-0.756**	**0.717**	0.105	-0.040	0.330	-0.062	0.327	-0.007	0.015	**0.402**	0.212	-0.174
Generative characters													
IBL	length of involucral bracts	**0.767**	**-0.671**	-0.386	0.343	0.191	0.349	0.211	**0.571**	0.004	**0.500**	-0.259	0.265
RFL	length of ray florets	0.567	-0.591	**-0.491**	**0.568**	-0.205	**0.462**	–	–	-0.151	0.335	-0.190	**0.404**
RFW	width of ray florets	0.584	-0.639	0.098	0.075	-0.198	**0.491**	–	–	-0.042	0.165	0.042	0.209
TFN	number of tubular florets	0.065	-0.062	**-0.491**	0.341	0.099	-0.108	0.094	-0.131	0.046	0.137	0.207	0.043
TFL	length of tubular florets	0.609	-0.432	**-0.429**	**0.608**	0.146	**0.459**	0.180	**0.547**	-0.054	0.363	-0.267	0.084
OAI	indument of outer achenes	–	–	-0.133	0.291	–	–	–	–	0.042	-0.166	0.121	**-0.411**

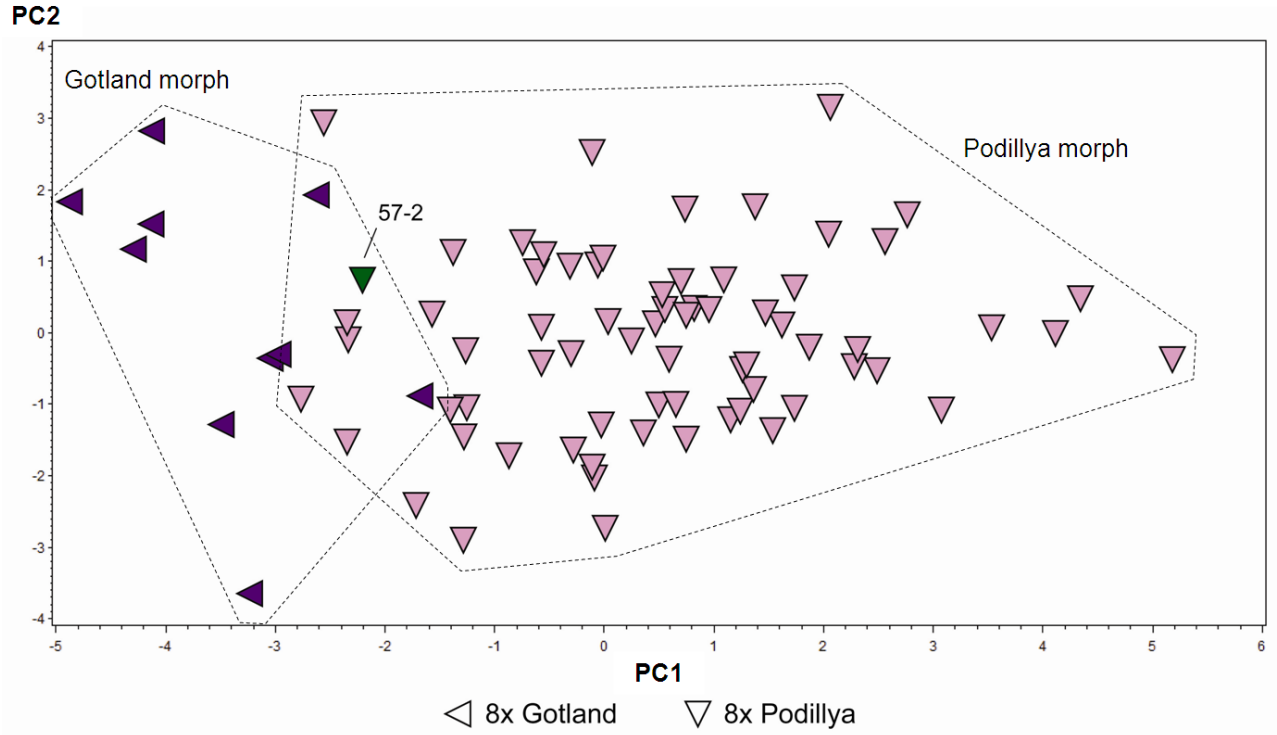
Online Resource 5 Principal component analysis (PCA 2) of 22 *Jacobaea vulgaris* tetraploid populations (= *J. vulgaris* subsp. *vulgaris*, including var. *dunensis*) from the whole area studied based on 11 morphological characters [without characters length of ray florets (RFL), width of ray florets (RFW), and indument of outer achenes (OAI)]. Colour proportions within the symbols indicate membership in the eight genetic clusters identified by the Bayesian analysis of genetic population structure (see Fig. 2). The population numbers follow those in Table 1. The first two axes explain 41.84 % and 13.82 % of the variation.



Online Resource 6 Principal component analysis of 119 octoploid *Jacobaea vulgaris* individuals (from 10 populations) from Pannonia and Öland (= *J. vulgaris* subsp. *pannonica*) based on 14 morphological characters (including character indument of outer achenes, OAI; PCA 3). Colours indicate the genetic grouping corresponding to the Bayesian analysis of genetic population structure (see Fig. 2b). Two individuals of population 36 are marked (see comments in the text). The first two axes explain 26.42 % and 13.88 % of the variation.



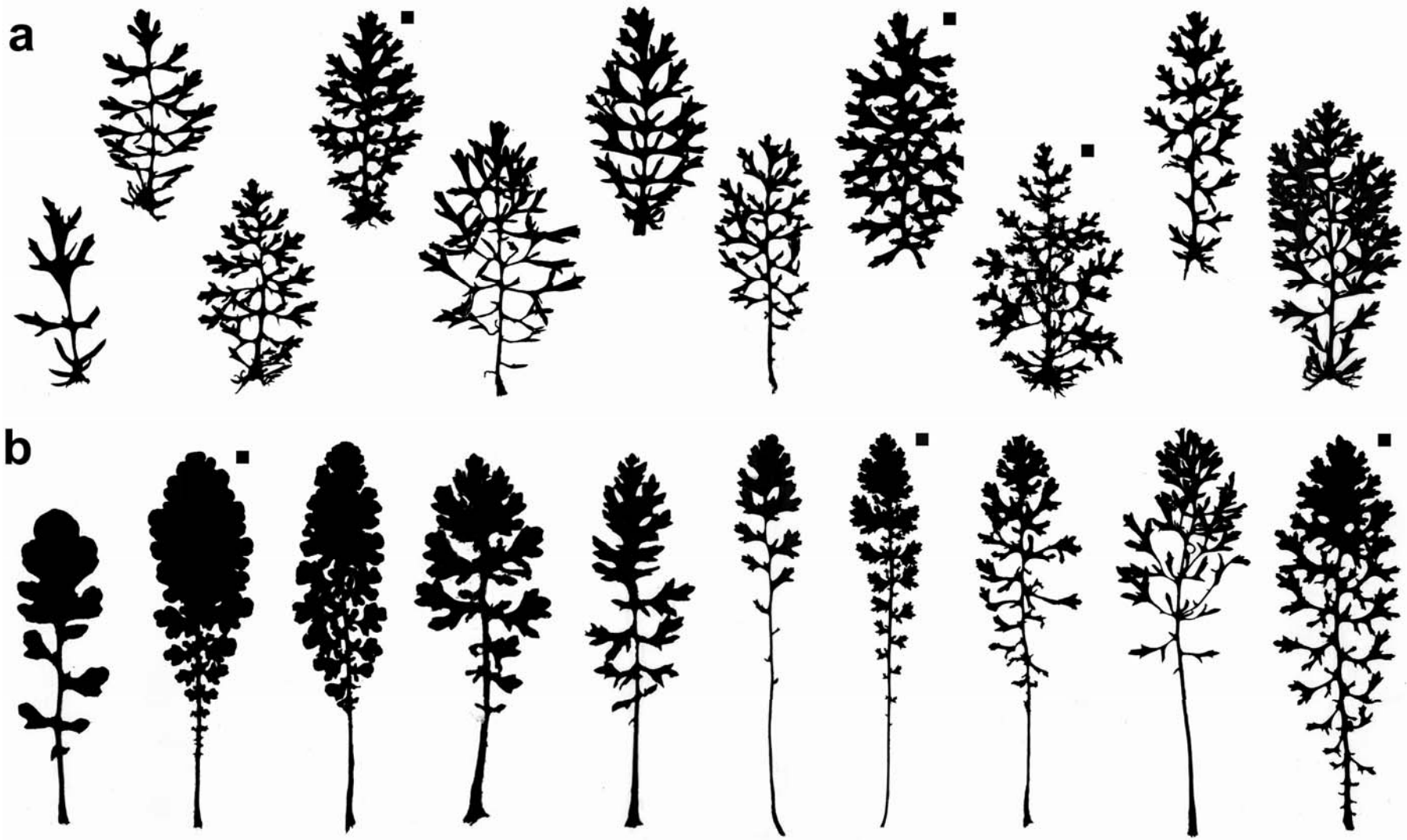
Online Resource 7 Principal component analysis of 81 octoploid *Jacobaea vulgaris* individuals (from 6 populations) from Podillya and Gotland (= *J. vulgaris* subsp. *gotlandica*) based on 14 morphological characters (PCA 4). Colours indicate the genetic grouping corresponding to the Bayesian analysis of genetic population structure (see Fig. 2b). One individual of population 57 is marked (see comments in the text). The first two axes explain 30.10 % and 12.67 % of the variation.



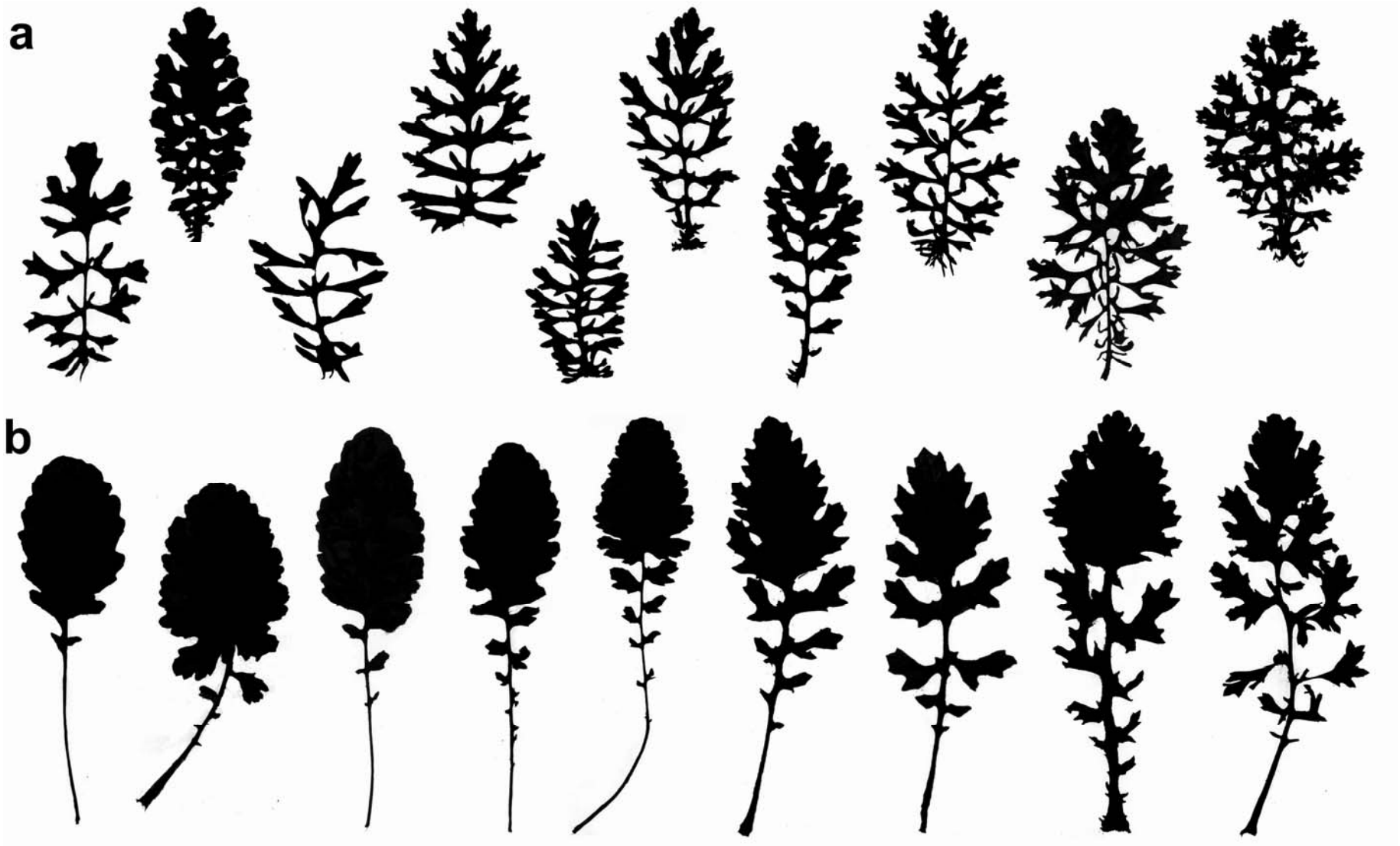
Online Resource 8 Mean±standard deviation, (minimum–) 10th and 90th percentiles (–maximum) of quantitative characters and character state frequencies of qualitative character of the morphological groups/taxa of *Jacobaea vulgaris*. Labels **a**–**e** indicate homogeneous groups according to post-hoc comparisons following one-way ANOVA (Tukey-Kramer multiple comparison analysis at a probability level of $P \leq 0.05$). For character explanations, see Table 2.

Character/taxon		*J. v.* subsp. *vulgaris* (2n = 4x = 40)			*J. v.* subsp. *pannonica* (2n = 8x = 80) N = 119	*J. v.* subsp. *gotlandica* (2n = 8x = 80) N = 81
		var. *dunensis* N = 60	var. *jacobaea* – Atlantic morph N = 86	var. *jacobaea* – continental morph N = 138		
LLP	relative length of the lower cauline leaf petiole [%]	22±14 **a** (0–)4–41(–48)	27±12 **ab** (0–)12–38(–68)	31±12 **bc** (2–)17–44(–66)	37±13 **d** (0–)20–52(–72)	34±13 **cd** (0–)21–49(–83)
LLS	relative length of the segmented part of the lower cauline leaf [%]	52±19 **a** (18–)28–77(–89)	49±16 **a** (0–)26–67(–88)	26±13 **b** (0–)8–41(–59)	15±16 **c** 0–37(–67)	13±12 **c** 0–29(–41)
LLA	relative length of the apical leaf segment of the lower cauline leaf [%]	27±10 **a** (9–)15–40(–48)	24±10 **a** (7–)13–38(–52)	43±10 **b** (18–)31–56(–72)	49±10 **c** (25–)35–62(–73)	53±10 **c** (17–)32–66(–93)
LLL	relative width of lateral lobe on apical segment of the lower cauline leaf [%]	29.9±10 **a** (11.3–)19–42.6(–55)	27.7±10.6 **a** (10–)16.1–41.4(–66.7)	16.7±6.3 **b** (2.9–)10–25.5(–39.1)	9.7±4.3 **c** (2.6–)5.4–15.8(–21.9)	9.2±2.9 **c** (3.2–)6–12.8(–20)
LLNS	number of lateral leaf segments on one side of the lower cauline leaf	6.6±2.5 **a** (3–)4–10(–12)	6.5±2.7 **a** (0–)4–9(–17)	3±1.8 **b** (0–)1–5(–10)	1.7±1.9 **c** 0–4(–9)	1.9±1.6 **c** 0–3.5(–6)
LLNT	number of teeths per lateral leaf segment of the lower cauline leaf	21±12.3 **a** (5–)9–37.3(–58)	18.8±11.9 **a** (0–)5.5–32(–59)	5.3±3.5 **b** (0–)1–9(–23)	2.1±2.4 **c** 0–5(–8)	2.2±2.1 **c** 0–5(–9)
MLNS	number of lateral leaf segments on one side of the middle cauline leaf	7.4±1.6 **a** (4–)5–9(–12)	8.1±1.2 **b** (5–)6.5–10	6.4±1.4 **c** 4–8(–9)	5.7±1.4 **d** (2–)4–7.2(–11)	5.2±1.1 **d** (3–)3.5–7(–8)
MLNT	number of teeths per lateral leaf segment of the middle cauline leaf	27.1±9.9 **a** (12–)18.9–41.2(–64)	19.9±9.1 **b** (5–)10.5–31(–51)	12.5±5.8 **c** (4–)6–10(–33)	5.7±2.5 **d** (2–)3–9.2(–18)	5.6±2.1 **d** (2–)3–8(–14)
IBL	length of involucral bracts [mm]	4.1±0.4 **a** (3.2–)3.6–4.6(–4.9)	4±0.4 **a** (3.2–)3.5–4.5(–4.8)	3.9±0.4 **a** (2.9–)3.4–4.5(–5.5)	4.5±0.6 **b** (3.5–)3.9–5.2(–6.4)	4.8±0.6 **c** (3.9–)4.1–5.3(–7)
RFL	length of ray florets [mm]	0.2±1.1 **a** 0(–7.4)	12.4±1.9 **b** (8.5–)10–15(–17)	12.1±2.2 **b** (6.5–)10–15(–18)	14.2±2.1 **c** (9–)12–17(–19)	16.2±2.9 **d** (11–)13–20(–24)
RFW	width of ray florets [mm]	0.1±0.4 **a** 0(–2.8)	2.2±0.4 **b** (1.5–)1.8–2.7(–3.4)	2.2±0.4 **b** (1.5–)1.7–2.6(–3.1)	2.7±0.5 **c** (1.8–)2.1–3.2(–4.4)	2.8±0.4 **c** (1.9–)2.2–3.2(–3.7)
TFN	number of tubular florets	62.6±8.4 **ab** (45–)52.9–75.1(–84)	58.2±7.1 **a** (45–)49.5–67(–79)	59.8±8.9 **a** (40–)47–72(–82)	59±8.9 **a** (34–)49–71(–85)	63.6±10.3 **b** (42–)52–76(–94)
TFL	length of tubular florets [mm]	4.7±0.6 **a** (3.5–)4–5.5(–5.9)	4.8±0.5 **a** (3.8–)4.2–5.4(–7.2)	4.8±0.6 **a** (3.4–)4–5.5(–6.2)	5.1±0.5 **b** (4–)4.5–5.5(–6.8)	5.5±0.6 **c** (4.3–)4.7–6.1(–7.2)
OAI	indument of outer achenes [%]	-	present: 29% **a**	present: 20% **b**	present: 60% **c**	present: 72% **d**

Online Resource 9 Middle (**a**) and lower (**b**) cauline leaf variation in the tetraploid Atlantic morph of *J. vulgaris* var. *vulgaris* and the "dunensis" morph (*J. vulgaris* var. *dunensis*). Leaves are arranged from left to right in order to their decreasing segmentation. *J. vulgaris* var. *dunensis* individuals are indicated with black squares.



Online Resource 10 Middle (**a**) and lower (**b**) cauline leaf variation in the tetraploid continental morph of *J. vulgaris* var. *vulgaris*. Leaves are arranged from left to right in order of their decreasing segmentation.



Online Resource 11 Middle (**a**) and lower (**b**) cauline leaf variation in *J. vulgaris* octoploids. Leaves are arranged from left to right in order of their decreasing segmentation.