Morphological and molecular diversity of some populations of *Gagea* (Liliaceae) in Southwest Germany

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Abstract

Three unidentified populations of *Gagea* recorded during the floristic mapping of Baden-Württemberg (Southwest-Germany) were analysed using molecular and morphological methods. One of them proved to be a form of *Gagea villosa*. The second population was primarily classified as *G. pomeranica* based on morphological characters. The molecular results, however, revealed a relationship to *G. pratensis*. A third population, not identified by its morphological characters, turned out to be another hybrid in the *Gagea lutea/pomeranica/pratensis/megapolitana* complex and is likely a part of a hybrid swarm resulting from a recent radiation of *Gagea* in Central Europe.

Keywords: Liliaceae, *Gagea*, hybrid complex, floristic mapping, Baden-Württemberg.

Zusammenfassung

Während der Arbeiten zur Floristischen Kartierung von Baden-Württemberg wurden drei Populationen der Gattung *Gagea* gefunden, die sich zunächst keiner Art eindeutig zuordnen ließen. Diese wurden mit morphologischen und molekularen Methoden untersucht. Eine davon erwies sich als *Gagea villosa*, die zweite wurde auf Grund morphologischer Merkmale als *G. pomeranica* bestimmt. Die molekularen Ergebnisse deuten jedoch auf eine enge Verwandtschaft zu *G. pratensis* hin. Eine dritte, an Hand der morphologischen Merkmale nicht eindeutig bestimmbare Sippe erwies sich als Hybrid im *Gagea lutea/pomeranica/pratensis/megapolitana*-Komplex und ist ein Teil dieses aus einer jungen Radiation in Mitteleuropa entstandenen Hybridschwarmes.

Contents

1 Introduction

Many monocotyledons show broad ranges in their morphological characters hampering their taxonomic classification. This is exemplified in the genus *Gagea* Salisb., the Yellow Star-of-Bethlehem. Depending on the taxonomic concept *Gagea* comprises between 90 and more than 280 species (e. g. Uphof 1958–1960, Levichev 2008, Peterson et al. 2008, Zarrei et al. 2009). The species delimitations are quite difficult due to the lack of differential characters. These mostly concern quantitative characters like the numbers of the bulbs or of the basal leaves.

The centres of diversity of *Gagea* are located in Central and SW Asia (Meusel et al. 1965: 91). Early revisions of the genus were presented by Pascher (1904, 1907), Stroh (1937), and Uphof (1958–1960), for Central Europe by Ascherson & Graebner (1905), who listed 11 species. For NE-Germany (Mecklenburg-Vorpommern) Henker (2005) published an interesting and thorough revision. A recent infrageneric classification was presented by Levichev (1990) and supported by molecular studies (Peterson et al. 2008, 2009). The confusing variability and diversity and the relative scarcity of consistent morphological characters draw the attention on several aspects of the genus: Levichev (1999) studied the subterranean organs (bulbs and bulbils) and Schnittler et al. (2009) examined the reproductive advantages of bulbils and seeds. Chromosome counts revealed a widespread polyploidy (for overviews see Peruzzi 2003, 2008) and recent molecular studies combined with morphological data resulted in the inclusion of *Lloydia* Salisb. ex Reichenb. in *Gagea* (Peterson et al. 2008, Peruzzi et al. 2008).

For Central Europe, the molecular results suggest a classification of the genus in four sections (Peterson et al. 2004, John et al. 2004, Peterson et al. 2008, Zarrei et al. 2009), consisting of Sect. *Gagea*, Sect. *Didymobolbos*

Fig. 1. *Gagea pomeranica* from "Eichert" near Trochtelfingen/Ries. – Scale: 2 cm.

Boiss., Sect. Minimae (Pascher) Davlianidze, and Sect. Spathaceae Levichev. For Baden-Württemberg (SW-Germany) four taxa are recorded (Wörz et al. 2010): *Gagea lutea*, *G. pratensis* agg. (incl. *G. pratensis* and *G. pomeranica* as supported by herbarium specimens in STU), both species belonging to sect. *Gagea*, *G. spathacea* (Sect. Spathaceae), and *G. villosa* (Sect. *Didymobolbos*). During the works of the floristic mapping, three remarkable taxa were recorded of which a clear classification based on morphological characters was difficult. One of those collected at "Eichert" near Trochtelfingen/Ries (*Gagea* cf. *pomeranica* I, Fig. 1) may likely be attributed to *G. pomer-*

Fig. 2. *Gagea* cf. *villosa* from "Karzberg" near Kirchheim/Ries. – Scale: 2 cm.

anica Ruthe which is new to the region. A form of *G. villosa* (M. Bieb) Sweet was found on the Karzberg close to Kirchheim/Ries (*Gagea* cf. *villosa*, Fig. 2) and a hitherto unidentifiable taxon from the Ipf near Bopfingen (*Gagea* cf. *pomeranica* II, Fig. 3), which may be an intermediate of *G. pratensis* and *G. pomeranica*.

These identification problems prompted the present study. Thus, the aim is to verify these preliminary classifications by using detailed morphological and molecular characters and to evaluate the results in the context of the flora of Baden-Württemberg and the position of these local taxa in the *Gagea* complex of Central Europe. It is furthermore intended to demonstrate how molecular methods may solve problems occurring during practical floristic work.

Acknowledgements

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2 Materials and Methods

Taxon Sampling

From the three studied populations with uncertain identity, between one and nine specimens were collected depending on the size of the populations. For the purpose of comparison, further specimens of recent herbarium material were investigated originating from various regions in Central Europe (*G. pratensis*, *G. pomeranica*, *G. lutea*, *G. saxatilis*, *G. villosa*, *G. minima*, and *G. megapolitana*, see Tab. 2). All specimens mounted on a herbarium sheet were examined. For the numbers of individuals see Tab. 1 . All were in flower.

Molecular analysis

For molecular analyses, one individual of these three populations collected in the state of Baden-Württemberg was used (Tab. 1). As basis for the molecular study we used the data sets of *Gagea* by Peterson et al. (2008) and Zarrei et al. (2009). To test phylogenetic relationships of the three accessions within *Gagea* we aimed at sampling all major clades of the genus, largely corresponding to its sections (Levichev 1999, Zarrei et al. 2009).

Fig. 3. *Gagea* "Ipf-taxon" from the Ipf mountain.

To reduce the number of taxa we selected a few species of each major lineage. Some putative close relatives, in special of Central European species, which partly share similar characters with the three populations of Baden-Württemberg were collected and added to the data set. By comparing the phylogenetic patterns between markers of biparental and maternal inheritance possible hybrids may be detected. Therefore, we produced two data sets of 1) the nrITS and 2) the cp trnL IGS regions with an almost identical species and accession composition (cf. appendix in ZARREI et al. 2009). The EMBL accession numbers are after the species names in Figs. 4 and 5.

Laboratory work

For DNA extraction from silica dried leaf material, the DNeasy plant extraction kit (Qiagen) was used according to the manufacturer's protocol. Amplifications were performed using 1.5 mM buffer, 0.625 mM $MgCl_2$, 0.2 mM dNTPs, 0.05 U/µL Taq DNA polymerase (Amersham Biosciences, Freiburg, Germany), 0.325 µ M primer, and 5 ng/µL DNA template. PCR profiles included 33 cycles of 94 °C for 1 min, 50–55 °C for 1 min, and 72 °C for 2–3 min. For amplifications and sequencing, the following primers were used. IT S nrDNA: ITS-A 5′-GGAAGGAGAAGTCGTAACAAGG-3′, ITS-B 5′-CTTTTCCTCCGCTTATTGATATG-3′ (BLATTNER 1999). The trnL-F intergenic spacer (IGS) region trnL-E 5′-GGTTCAAGTCCCTCTATCCC-3′ and trnL-F 5′-ATTTGAACTGGTGACACGAG-3′ (TABERLET et al. 1991). PCR products were cleaned using the PCR purification kit (Qiagen, Hilden, Germany). Cycle sequencing using the same primers was conducted using ABI PRISM BigDye 2.1 to obtain sequences of each of the two strands. Resulting products were analysed using automated sequencing systems ABI PRISM 3100 (PE Biosystems, Darmstadt, Germany).

Data analysis

Sequences of the selected *Gagea* species were manually aligned. For the molecular characterisation of our taxa we used a model based Maximum likelihood (ML; FELSENSTEIN 1981) approach which provides branch lengths. The two markers were analysed separately. For the ITS data set, the GTR+I+Γ model was used as indicated by the Akaike information criterion (AIC) for both DNA data sets in the program Modeltest 3.06 (POSADA & CRANDALL 1998). The corresponding settings were used in GARLI (ZWICKL 2006): ratehetmodel = gamma, numratecats = 4 and invariantsites = estimate. For trnL IGS the K81uf+I model was chosen by AIC. Therefore, the settings were ratehetmodel = none, numratecats = 1 and invariantsites = estimate. Ten search replicates were run using chains of 5×10^6 generations with a sample frequency of 10^2. The tree with the highest likelihood was chosen as optimal tree. The same corresponding options were applied to each data set for a 100 replicates bootstrap (BS) analysis.

3 Results

3.1 Molecular results (Figs. 4, 5)

The aligned sequence length for ITS1, 5.8 S rDNA and ITS2 was 663 bp with individual sequences ranging from 553–621 bp and for trnL IGS sequences 257 bp with unaligned lengths of 169–203 bp. The ML trees of both data sets are very similar results in respect of the three analysed taxa of interest. These accessions are placed in two relatively well supported clades. They belong to the *Gagea bohemica*/*villosa*-clade within section *Didymobolbos* (BS ITS: 82 %, trnL IGS < 50 %), and to a *G. lutea*/*pratensis* clade of section *Gagea* (BS ITS: 95 %, trnL IGS 61 %). Within the first clade, the position of *G.* cf. *villosa* is well supported within other accessions of *G. villosa*. This group is part of a clade with other Central European taxa as *Gagea bohemica* (Zauschn.) Schult. & Schult. f., *G. saxatilis* (Mert. & Koch) Schult. & Schult. f. (both synonymous according to PETERSON et al. 2010).

Gagea cf. *pomeranica* I groups together with *G. megapolitana*, closely related to *G. pratensis* in the trnL IGS analysis. For the same gene the sequence of *Gagea* cf. *pomeranica* II is identical to other accessions of *G. pratensis*. In the ITS study, both taxa of interest fall into a clade of *G. pratensis* and *G. pomeranica*.

Tab. 1. New material used for this study including voucher specimens.

	Taxon	Origin	Date	Collector, no. (herbarium)	EMBL accession no. ITS	EMBL accession no. trnL IGS
G1	*Gagea minima* (L.) Ker-Gawl.	Germany, Bad Doberan	20.IV.2010	Wörz 10.04.20.01. (STU)	FR874901	FR874917
G2	*Gagea pomeranica* Ruthe	Germany, Insel Poel	18.IV.2010	Wörz 10.04.18.01. (STU)	FR874902	FR874918
G3	*Gagea megapolitana* Henker	Germany, Wismar	17.IV.2010	Wörz 10.04.17.01. (STU)	FR874903	FR874919
G4	*Gagea pratensis* (Pers.) Dumort.	Germany, Wismar	17.IV.2010	Wörz 10.04.17.02. (STU)	FR874904	FR874920
G5	*Gagea lutea* Ker Gawl.	Germany, Lübeck	16.IV.2010	Wörz 10.04.16.01. (STU)	FR874905	FR874921
G6	*Gagea pratensis* (Pers.) Dumort.	Czech Republic, Pardubice	10.IV.2008	Danihelka 500246 N (STU)	–	FR874922
G7	*Gagea bohemica* (Zauschner) Schultes & Schultes f.	Czech Republic, Kamenište	III.2008	Danihelka s. n. (STU)	FR874906	FR874923
G8	*Gagea bohemica* (Zauschner) Schultes & Schultes f.	Czech Republic, Kamenište	III.2008	Danihelka s. n. (STU)	FR874907	FR874924
G9	*Gagea bohemica* (Zauschner) Schultes & Schultes f.	Germany, Kirchheimbolanden	19.III.2008	Wörz & Engelhardt 28.03.19.01. (STU)	FR874908	FR874925
G10	*Gagea bohemica* (Zauschner) Schultes & Schultes f.	Germany, Kirchheimbolanden	19.III.2008	Wörz & Engelhardt 28.03.19.03. (STU)	FR874909	FR874926
G11	*Gagea bohemica* (Zauschner) Schultes & Schultes f.	Germany, Bad Münster	19.III.2008	Wörz & Engelhardt 28.03.19.09. (STU)	FR874910	FR874927
G12	*Gagea bohemica* (Zauschner) Schultes & Schultes f.	Germany, Sieversheim	19.III.2008	Wörz & Engelhardt 28.03.19.08. (STU)	FR874911	FR874928
G14	*Gagea pomeranica* Ruthe	Germany, Eichert N Trochtelfingen/Ries	21.III.2007	Wörz & Engelhardt 27.03.21.05 (STU)	FR874912	FR874929
G15	*Gagea pratensis* (Pers.) Dumort.	Germany, Ipf/ Bopfingen	21.III.2007	Wörz & Engelhardt 27.03.21.02. (STU)	FR874913	FR874930
G16	*Gagea* cf. *villosa* (Bieb.) Duby	Germany, Karzberg	21.III.2007	Wörz & Engelhardt 27.03.21.04. (STU)	FR874914	FR874931
G17	*Gagea pratensis* (Pers.) Dumort.	Germany, Nördlingen	21.III.2007	Wörz & Engelhardt 27.03.21.07. (STU)	FR874915	FR874932
G18	*Gagea lutea* Ker-Gawl.	Germany, Tübingen	?	Engelhardt s. n. (STU)	FR874916	FR874933

3.2 Morphological results

Tab. 2 presents the most important morphological characters of the examined populations with uncertain identity and some related *Gagea* species. Important diagnostic features are the bulbs and bulbils, the number, form and breadth of the basal leaves, the form of the bract of the lower inflorescence branch, and the indumentum (Henker 2005). Bulbs and bulbils are considered as important characters and were reviewed by Levichev (1999). Apart from the maternal bulb, occasionally a secondary replacement bulb and one or sometimes more subterranean bulbils are present.

Gagea pratensis and *G. pomeranica* mostly bear two bulbs (maternal and replacement), occasionally only one. Bulbils are extant in several cases; in the others they may have been lost during the conservation of the herbarium specimens. As suggested by Levichev (1999: 381), the tunica of the subterranean organs of these species covers only the stem base and the rest of the maternal bulb and not the replacement bulbs and the bulbils. Levichev recorded this character exclusively for *G. transversalis* and *G. pratensis* but not for other species. It is, however, also present in *G. pomeranica* and in *G.* cf. *pomeranica* II.

The number of the basal leaves is one, rarely two in *G. pratensis*, *G. pomeranica*, *G.* cf. *pomeranica* II, *G. lutea*, *G. megapolitana*, and *G. pusilla*, two or more in all other species. The basal leaves are mostly slightly broader in *G. pomeranica* compared to *G. pratensis* and *G.* cf. *pomeranica* II.

A relatively constant diagnostic character of *G. pomeranica* is the basally broadened and sheathed cauline leaf inserted at the base of the inflorescence. Apart from *G. pomeranica*, it is also present in *G. minima* and *G. megapolitana*. It is similar, though not identical, to the relatively broad cauline leaves of *G. lutea*. The leaves are flat and more or less keeled in *G. pratensis* and *G. pomeranica*, flat and not keeled in *G. lutea*, *G. megapolitana* and *G. minima*, and more or less grooved in the other species.

The indumentum of the inflorescence is considered as an important character for the identification of *Gagea*. The hairs are usually at margins of the bracts (cauline leaves) or

Tab. 2. Morphological characters of the *Gagea* taxa examined.

Species	Locality	No. of specimens	Replacement bulb outside tunica	Bulbils	No. of basal leaves	Broadth of the basal leaves	Sheath of the upper cauline leaf	Leaf form	Indumentum
pratensis	BW, Riegel	2	y	0–1	1	4–5 mm	absent	flat, keeled	bracts weakly hairy
pratensis	BW, Karlsruhe	6	y	1	2	3–5 mm	absent	flat, keeled	bracts weakly hairy
pratensis	MV, Wismar	5	y	1	1–2	3–5 mm	absent	flat, keeled	bracts weakly hairy
pratensis	MV, Bad Doberan	3	y	0	1	3–4 mm	absent	flat, keeled	bracts weakly hairy
pratensis	RP, Rotenfels	1	y	0	1	3 mm	absent	flat, keeled	bracts hairy
pratensis	BY, Aufhausen	1	y	0	1	4–5 mm	absent	flat, keeled	bracts hairy
pratensis	BY, Nördlingen	2	y	0	1	6 mm	absent	flat, keeled	bracts weakly hairy
pomeranica	MV, Dargast	7	y	1	1	2–4 mm	present	flat	bracts arachnoid
pomeranica	MV, Poel	9	y	1	1	2–4 mm	present	flat	bracts arachnoid
pomeranica	BY, Möhrendorf	2	y	0	1	2–4 mm	present	flat, keeled	bracts weakly hairy
pomeranica	BY, Oberringingen	1	y	1	1	5 mm	present	flat, keeled	bracts arachnoid
cf. *pomeranica* I	**BW, Eichert**	**3**	**y**	**0–1**	**1–2**	**5 mm**	**present**	**flat**	**bracts arachnoid**
pomeranica	BW, Waldmannshofen	4	y	0	1	2–4 mm	present	flat	bracts arachnoid
pomeranica	BW, Reinsbronn	4	y	0	1	2–3 mm	present	flat	bracts arachnoid
cf. *pomeranica* II	**BW, Ipf**	**4**	**y**	**0–1**	**1**	**2–5 mm**	**absent**	**flat, in part canaliculate**	**bracts arachnoid**
cf. *pomeranica* II	**BW, Ipf**	**3**	**y**	**1**	**1**	**2–3 mm**	**absent**	**canaliculate**	**bracts arachnoid**
cf. *pomeranica* II	**BW, Ipf**	**2**	**y**	**1**	**1**	**2–3 mm**	**absent**	**flat**	**bracts arachnoid**
lutea	BW, Jettenberg	2	n	0	1	7–8 mm	absent	flat	bracts arachnoid
lutea	BW, Kloster Wald	4	n	0	1	6–8 mm	absent	flat	bracts arachnoid
bohemica	RP, Kirchheimbolanden	3	n	0	2	1 mm	absent	canaliculate	bracts and peduncles pubescent
bohemica	RP, Kirchheimbolanden	1	n	0	2	1 mm	absent	canaliculate	bracts and peduncles pubescent
villosa	BW, Mettenberg	1	n	1	2	2–3 mm	absent	canaliculate	Infl. pubescent
villosa	BW, Gammertingen	4	n	0–1	2	2 mm	absent	canaliculate	Infl. pubescent
villosa	BW, Eichert	1	n	1	2	2 mm	absent	canaliculate	Infl. pubescent
cf. *villosa*	**BW, Karzberg**	**1**	**n**	**0**	**2**	**1–2 mm**	**absent**	**canaliculate**	**pubescent**
minima	MV, Bad Doberan	7	n	0–1	1–2	1–2 mm	present	flat	Infl. weakly hairy
megapolitana	MV, Wismar	7	y	several	1	6–10 mm	present	flat	bracts arachnoid

on the peduncles. The marginal indumentum of the bracts is arachnoid in *G. pomeranica*. It is relatively faint and dispersed in *G. pratensis*. The differences are, however, weak and transitions are common. These arachnoid indumentums are also present in *G. lutea* and *G. megapolitana*. In *G. villosa*, the whole inflorescence, peduncles, bracts and even the petals are pubescent (not arachnoid). *G. saxatilis/bohemica* is similar, whereas *G. minima* is faintly hairy.

3.3 Chorological/ecological results

The distribution ranges of *G. minima* and *G. pratensis* cover Central and Eastern Europe, and *G. lutea* occurs in Europe with disjunct ranges in the Himalaya and SE-Asia (Meusel et al. 1965: 92). *Gagea megapolitana* is a local endemic of NE-Germany (Henker 2005). *Gagea villosa* is widespread in Central and Southern Europe, North Africa and West Asia. For *G. pomeranica*, Henker (2005: 47) indicates a distribution in Germany, Southern Sweden and in the Czech Republic. The distribution of the *Gagea*-species in Baden-Württemberg is presented in Wörz et al. (2010). *G.* cf. *pomeranica* II is a local endemic not found elsewhere.

The habitats of *G. lutea* are wet forests, mostly along rivers, where geophytes have an evolutionary advantage. These habitats are near-natural even in the region of great human impacts like Central Europe.

In contrast, the habitats of *G. pratensis*, *G. pomeranica*, *G. megapolitana* and *G.* cf. *pomeranica* II are all intensively influenced by human activities. They are located in parks, meadows, fields, vineyards and disturbed places, which could not exist without man. Natural habitats are not recorded for these species from SW-Germany. The same is true for *G. villosa*, which often occurs on cemeteries; mostly at the bases of old trees (see for example Hügin & Hügin 1998).

Gagea bohemica grows on various rocky soils and on dry grassland, with little human impacts (Oberdorfer 1994: 125, Peterson et al. 2010). These habitats are, however, not completely natural.

4 Discussion

The three taxa studied in this paper are dispersed in the two detected main clades which represent the sections *Gagea* and *Didymobolbos*. *G.* cf. *villosa* is arranged in the *G. villosa* clade in both markers and, thus, proved to belong to this species. Compared to the other populations of *G. villosa*, the basal leaves are extremely narrow and the stems are relatively slender.

The arrangement of *G.* cf. *pomeranica* I and *G.* cf. *pomeranica* II in clades together with *G. pratensis* and in part with *G. pomeranica* indicates a close relationship between these taxa. Morphologically, *G.* cf. *pomeranica* I can be included in *G. pomeranica* by its sheathy bracts. The molecular results support this classification only in part, as *G.* cf. *pomeranica* I is included in a clade with *G. pratensis* and a population of *G. pomeranica* from the island of "Poel" (Mecklenburg-Vorpommern, North Germany), whereas other *G. pomeranica* accessions are arranged outside of this clade. This may result from the hybrid nature of *G. pomeranica*. The discrepancy of its position in our trnL IGS tree as sister to *G. megapolitana* and in our ITS tree with *G. pratensis* and *G. pomeranica* may support such hypothesis. Still, a maternal parenthood of *G. megapolitana* as indicated by our chloroplast data seems unlikely to us as this species has a different area of distribution. Thus, we rather speculate that *G. pomeranica* may be a hybrid in the *G. pratensis* group.

Gagea cf. *pomeranica* II is placed in the molecular trees within the *G. pratensis/pomeranica* clade. Morphologically intermediate between these two species it differs from *G. pomeranica* by the absence of sheaths of the upper cauline leaf, from *G. pratensis* by the nearly constant presence of bulbils. It has an extraordinary high chromosome number (2n = 86, 88; Henker 2005: 66) with an odd number of sets (considered as heptaploid with an aneuploid chromosome content by Henker 2005: 66; Tab. 3). Odd-numbered sets of chromosomes are common in *G. pratensis* (3x = 36, 5x = 60) and in *G. bohemica* (Peruzzi 2003: 120, 122). Octoploid populations are unknown in this group. A possible explanation for the evolution of this taxon may therefore be hybridisation, polyploidisation and backcross of *G. pomeranica* and *G. pratensis*. This is, of course, conjectured and requires cytological studies.

Gagea cf. *pomeranica* II may have evolved by a similar pattern as found in *G. spathacea* by Schnittler et al. (2009) and Pfeiffer et al. (2011), which has an odd number of chromosome sets (like *G. pomeranica*) and completely lost its generative reproduction but still retained a partial fertility of the pollen. Therefore, *G. spathacea* is able to cross-pollinate and to produce hybrid offspring. Studies are required to prove, whether this pattern may also apply to *G. pomeranica*.

The dispersion of *G. pomeranica* in the *G. lutea* and the *G. pratensis* clade in the ITS tree (Fig. 4) and morphological results (Tab. 2) indicate a hybrid origin of *G.* cf. *pomeranica* II. This hybrid nature was proved by molecular data (Peterson et al. 2004, Peterson et al. 2009, John et al. 2004). Peterson et al. (2004) propose *G. lutea* as pollen donator. The hybrid origin is supported by the chromosome numbers which are both 6x = 72 (as in *G. lutea*) and 5x = 60 (as in *G. pratensis*) in *G. pomerancia*. Nevertheless, *G. pomeranica* is rarely seen growing together with *G. lutea* and *G. pratensis*. One case is reported by John et al. (2004), but by far the most populations grow allopatrically. It is therefore not a hybrid "inter parentes" and may be considered as a species (or subspecies) of hybrid origin as it is not rare in higher plants. In this context, *G.* cf. *pomeranica* II may be considered as another hybrid in the complex of *G. lutea*, *G. pratensis* and *G. pomeranica*.

As all species except for *G. lutea* grow exclusively in man-made habitats which are extremely common in the Central European landscape with its intensive human impact, the *G. pratensis*/*pomeranica* complex with *G.* cf. *pomeranica* I and *G.* cf. *pomeranica* II is most probably a result of a recent radiation, which occurred during hu-

Tab. 3. Chromosome numbers in *Gagea*. Data from Central Europe.

Species	**2n** =	**Selected references**
G. villosa	69, 72, mostly 48	Henker (2005: 62), Peruzzi (2003: 122)
G. lutea	72	Henker (2005: 63), Peruzzi (2003: 121)
G. pratensis	36, 48, 72, mostly 60	Henker (2005: 63–65), Peruzzi (2003: 122)
G. pomeranica	60, 72	Henker (2005: 65)
G. megapolitana	mostly 72, 1 count 84	Henker (2005: 65–66)
G. cf. *pomeranica* II	86, 88	Henker (2005: 66)
G. minima	24, rarely 32	Henker (2005: 62), Peruzzi (2003: 121)
G. pusilla	24	Peruzzi (2003: 122)

Fig. 4. ML tree of nrITS sequences of selected *Gagea* taxa. – Bootstrap values are at the branches. EMBL accession numbers after the species names. The three taxa from SW Germany are indicated in bold. Dotted lines indicate that branch lengths were longer in the analysis.

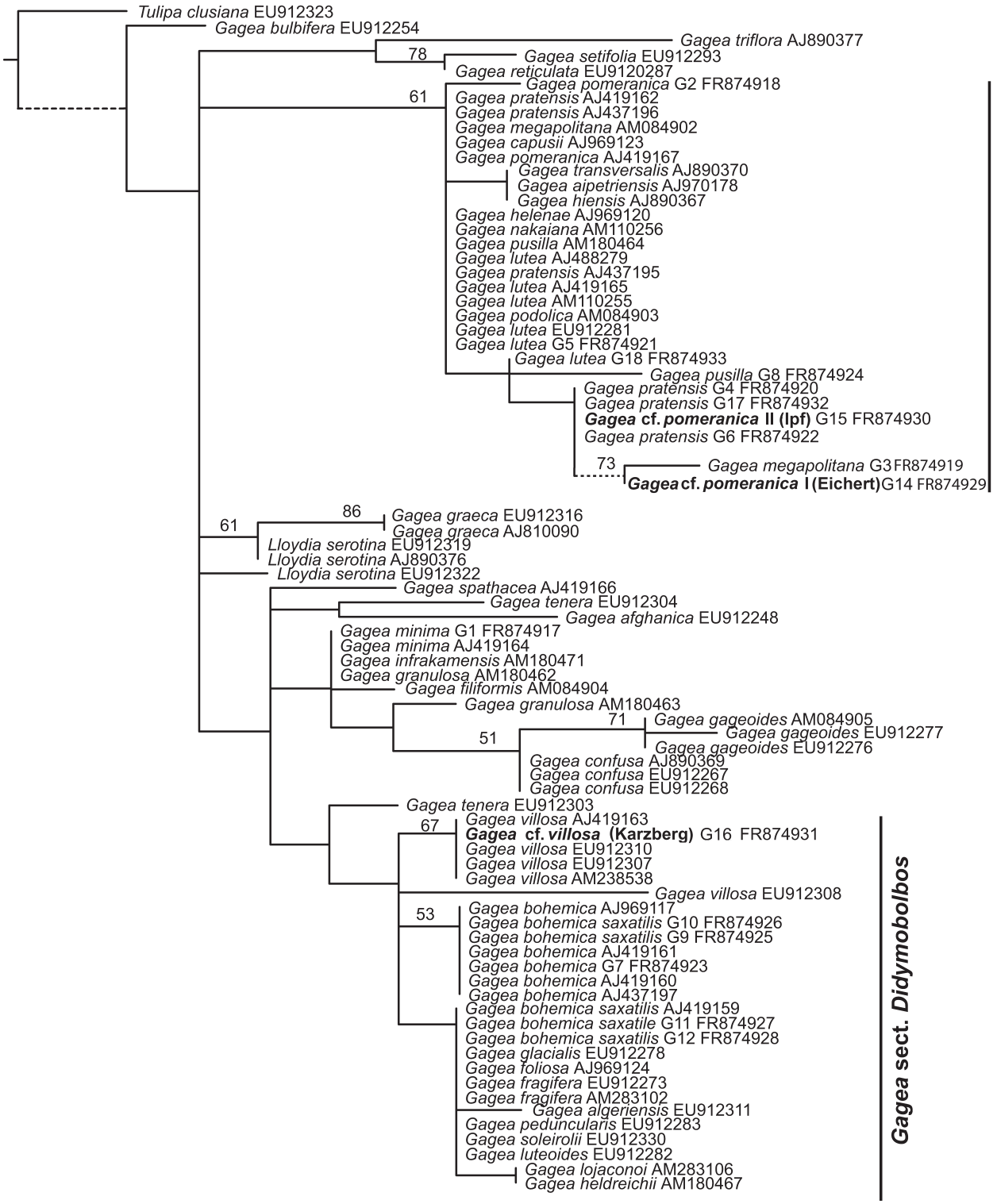

Fig. 5. ML tree of trnL IGS sequences of selected *Gagea* taxa. – Details see legend to Fig. 4.

man settlement and the creation of man-made habitats (see also PETERSON et al. 2009). Similar cases of evolution following human activities in Central Europe are for example *Bromus grossus* (SMITH 1973: 321, SMITH 1981: 505) and *Rhinanthus alectorolophus* (PLEINES et al. in prep.).

In a similar way the hexaploid *G. lutea* may have evolved by polyploidisation and probably hybridisation. Contrary to *G. pratensis*, it is a species indigenous to Central Europe occurring in natural habitats and which existed before the intensive human impact on the Central European flora. It produces fertile seeds (SCHNITTLER et al. 2009) and seems to be a well-stabilised species. The large disjunct distribution range may even suggest a pre-glacial evolution.

These hybrid complexes require the discussion of the species concept in *Gagea*: does every hybrid need a name and need to be treated as a separate species? This is to be rejected beyond doubt for *G.* cf. *pomeranica* II with its intermediate position and its only minute differences to *G. pratensis* and *G. pomeranica*. For *G. pomeranica*, doubts of its species rank raise from the small morphological differences and from the position in different clades in our molecular trees and in the tree in JOHN et al. (2004: 23). In any case, these populations are a part of the Central European plant diversity and require attention and protection where necessary.

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