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Original article

Polymorphism of nuclear DNA in selected species of *Taraxacum* sect. *Palustria*

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ABSTRACT

This paper presents the results of research on nuclear DNA polymorphism in six apomictic species of marsh dandelions *(Taraxacum* sect. *Palustria): Taraxacum bavaricum, T. belorussicum, T. brandenburgicum, T. paucilobum, T. subdolum and T. vindobonense.* The studies demonstrated the existence of clear genetic differences between species and the existence of nuclear DNA polymorphism within each of the studied species.

1. Introduction

Taraxacum genus belongs to the largest and most taxonomically complicated apomictic complexes. So far, more than 3000 species have been described and traditionally classified into about 60 sections (Kirschner and Štěpánek, 1997, 2004; Uhlemann et al., 2004, Kirschner et al., 2015). Most of the *Taraxacum* sections encompass either exclusively apomictic taxa or apomicts with a small contribution of sexual species. Only small, relic, mainly Asiatic sections are characterized by prevalence of diploid and tetraploid sexual species (Kirschner and Štěpánek, 1996). Such high morphological differentiation in the genus of relatively small sexuality and of geographic range limited to middle Asia and southern Europe is explained by several episodes of intensive hybridization between sexual plants from the south and apomicts retreating from the north between subsequent glacial periods (Richards, 1973; Kirschner and Štěpánek, 1996). Formation of new taxa through hybridization is also taking place now in mixed populations composed of apomictic polyploids and sexual diploids (Hughes and Richards, 1988; van Baarlen et al., 2000; Meirmans et al., 2003; Mitsuyuki et al., 2014; Matsuyama et al., 2018). However, neither historic nor current hybridization between sexual plants and apomicts does not explain the surprisingly high genetic polymorphism found in apomictic dandelions growing in regions separated by hundreds of kilometres from the nearest populations of sexual species (King and Schaal, 1990; Van Der Hulst et al., 2003). Moreover, genetic differences were observed not only among well-defined apomictic species but also among the progeny of a single mother plant (King and Schaal, 1990). Probable reasons for the appearance of so many apomictic clones in polyploid *Taraxacum* are point and chromosome mutations and incidental sexual reproduction (Kirschner and Štěpánek, 1994; Richards, 1996).

In our pilot study we aimed at checking how genetically distinct are the apomictic microspecies of *Taraxacum,* which belong to a large and kariologically differentiated *Palustria* section with prevailing asexual mode of reproduction. This section groups 133 species of dandelions among which two are sexual diploids (*T. tenuifolium* and *T. raii* of the range limited to a small area in southern Europe), relatively numerous triploids and tetraploids and less frequent pentaploids and hexaploids (Kirschner and Štěpánek, 1998; Marciniuk et al., 2018). The second aim was to find out if and how large polymorphism of nuclear DNA within an apomictic species is. The number of chromosomes in the studied taxa was reported earlier (Marciniuk et al., 2010).

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Table 1
Species of the genus *Taraxacum* used in this study.

Taxon	Section	Location and the date of fruit collection	The number of individuals from which seeds were collected	The number of samples
T. brandenburgicum	*Palustria*	Pyzdry near Poznań, 16.05.2007	14	10
T. vindobonense	*Palustria*	Stasin near Siedlce, 18.05.2008	2	10
T. subdolum	*Palustria*	Czuchów, 20.05. 2009	2	10
T. bavaricum	*Palustria*	Czuchów, 17.05.2008	1	10
T. belorussicum	*Palustria*	Mścichy - Biebrza National Park, 23.05.2008	4	9
		Sulicha, Orzyc River Valley, 10.05.2008, 19.05.2009	4	1
T. paucilobum	*Palustria*	Czuchów, 20.05.2007, 17.05.2008	ca. 20	10
T. pieninicum	*Erythrocarpa*	Pieniny National Park, 19.05.2008	1	1
T. sp.	*Ruderalia*	Kraków, 17.05.2009	–	10

2. Material and methods

2.1. Plants material

For this study we selected 6 apomictic species of *Taraxacum* (Table 1) from the section *Palustria* growing in Poland. Two of them are tetraploids (*T. brandenburgicum* and *T. vindobonense*), while the others are triploids. We chose species, classified by Kirschner and Štěpánek (1998) into various morphological groups that can pose serious identification problems, especially for atypical specimens.

Seeds used in this study were collected directly in natural sites or from plants which were transferred from natural sites to the culture (Table 1). As external groups, we used leaves from 10 individuals of unidentified apomictic species *T.* sect. *Ruderalia* (material collected near Kraków) and one seedling of *T. pieninicum* sect. *Erythrocarpa* - a diploid endemic species of the Pieniny Mountains (seeds collected with acceptance of the Director of the Pieniny National Park).

2.2. Random amplification of polymorphic DNA (RAPD-PCR)

Seeds were sown in a medium composed of agar and water. The obtained seedlings were used for isolation of nuclear DNA. The amount of DNA in the studied samples was determined with NanoDrop ND1000. Samples of the highest, for a given species, concentration of DNA were selected for further studies so as to obtain the total number of 10 samples for one species. The exception was *T. pieninicum* for which one sample was prepared.

Eleven primers (out of 15 preliminarily tested) giving clearly distinguishable bands in all analysed specimens were selected for amplification. The names of the primers and their sequence are given in Table 2. Amplified DNA was diluted in sterile water in such a proportion as to obtain 10 ng in each case. The composition of reaction mixture is set up in Table 3.

Amplification of DNA with RAPD primers was performed in a thermocycler Mastercycler made by Eppendorf and programmed as follows: initial denaturation – 94 °C/1min; 39 cycles composed of denaturation 93 °C/30 s, annealing of the primers 34 °C/1min, elongation 68 °C/2.5 min; final elongation 72 °C/5min. PCR products after adding the buffer (40% saccharose, xylene green 0.25%, methylene blue 0.25%) were separated in 1% agar gel in the TBE buffer for 2 h at a voltage of 3.5 V/cm. A size marker of 100 bp DNA Ladder plus (MBI Fermentas) was used. The gels were photographed with VilberLourmat 05 10766 type CN-3000 using the BioCapt ver. 12.3 software. Photographs of sample gels are given in Fig. 1.

WPGMA trees were made with the PragmaTax software (Moraczewski, 2009). Statistical tests (one-way ANOVA) of the differences in the number of bands per primer were performed using the VassarStats computation web site (http://vassarstats.net/).

3. Results

An RAPD PCR analysis of nuclear DNA was made for 6 species of the *Taraxacum* sect. *Palustria* and for two external groups - *T. pieninicum* and an unidentified taxon of the *T.* sect. *Ruderalia*. In total 72 individuals were analyzed. The lengths of the obtained PCR products ranged between 5320 and 110 bp but due to only sporadic occurrence of very long and very short bands, the analyses covered PCR products ranging from 3940 to 250 bp. The total number of the analyzed PCR products was 1652 which made 319 bands of various lengths.

The studied species differed in the number and length of products generated in the presence of the applied primers (Table S1 in supplementary materials). The number of bands was clearly higher in the polyploid taxa than in diploid *T. pieninicum,* where 176 bands were counted. In the *Palustria* section it amounted to 234 bands on average and variations between the species were small (219–249 bands). Only a slight difference was also noted between triploids and tetraploids (231.5 vs. 239 bands on average). The same applies to the average number of bands per primer, which ranges from19.91 to 22.64. In statistical terms, the difference between tri- and tetraploids in this respect was not significant ($P = 0.445$). On the other hand, both the specimen of *T.* sect. *Ruderalia* (26 ± 3.0 bands) and *T. pieninicum* (16 ± 3.5 bands) differed significantly from both tetraploid and triploid representatives of the *Palustria* section (in all instances $P < 0.01$).

Molecular similarity between taxa depends on the general combination of all obtained PCR products and on the presence of products exclusive for a given taxon. Products which were present in all studied species of *Taraxacum* sect. *Palustria* and at the same time

Table 2
Primers used for DNA amplification.

Primer name	Sequence (5′-3′)
RAPD1	GCAAGTAGCT
RAPD2	TGGCTCAAAG
RAPD3*	CAGTGTGTGG
RAPD4*	GTGTCAGGCA
RAPD5*	ATACCATCCC
RAPD6	GATCCCCTGA
RAPD7*	GATAACCGCA
RAPD8*	ATCCGCGTTC
RAPD9*	CCAGTGGTTC
RAPD10*	TGACGATGCA
RAPD11*	ACGGCATATG
RAPD12*	TAACCATCCC
RAPD13*	ATGTCCGCAC
RAPD14*	GTGTGGATGG
RAPD15	GTCGTTACGA

* The primers used for detailed research.

Table 3
Composition of the mixture for standard PCR reaction.

Component	Initial concentration	Volume of the component	Final concentration
MQH_2O	————————	11,7µl	————————
Taq Buffer - $MgCl_2$ (MBI Fermentas)	10×	2,0 µl	1 ×
$MgCl_2$	25 mM	1,6 µl	2 mM
dNTP'y (dATP, dCTP, dGTP, dTTP)	10 mM (each)	0,5 µl	0,25 mM
Primer	5 µM	2,0 µl	0,5 µM
Taq Polymerase (recombinant MBI Fermentas)	5 µ/µl	0,2 µl	1 µ/reaction
DNA	10 ng/µl	2,0 µl	20 ng/reaction
Sum	————————	20 µl	————————

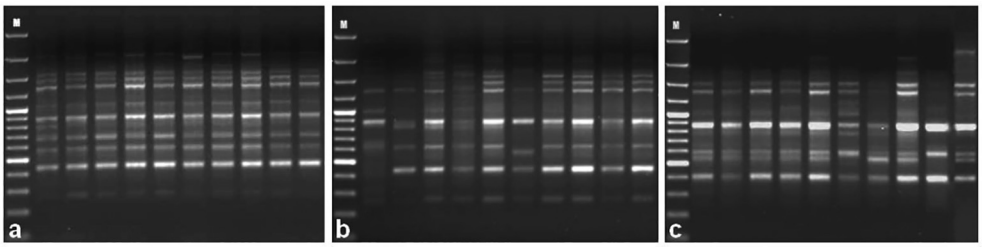

Fig. 1. Agarose gel electrophoresis of sequences amplified with RAPD3 primer. a - *T. brandenburgicum*, b - *T. subdolum*, c - *T. bavaricum*. M - molecular weight marker.

absent in external groups of *T.* sect. *Ruderalia* and *T. pieninicum* (sect. *Erythrocarpa*) were the bands of a length of: 450 bp for RAPD4, 500 bp for RAPD7, 560 bp for RAPD12 and 1310 bp for RAPD11. Products unique for *T.* sect. *Ruderalia* and *T. pieninicum* are presented in Table S2 in supplementary materials. Molecular differences between the studied species of *T.* sect. *Palustria* are distinct, which is evidenced by a high number of PCR products unique for a given species (Fig. 2, Table 4). Moreover, all studied apomictic species of *T.* sect. *Palustria* showed modest but distinct internal genetic differentiation. The presence of polymorphic products (detected in 5–6 individuals) and of highly polymorphic products (detected in 1–4 individuals out of 10 analyzed) decided upon this differentiation (Figs. 3 and 4). The percentage of polymorphic and highly polymorphic products in particular species of the section *Palustria* was as follows: *T. subdolum* 10.5%; *T. bavaricum* 15.5%; *T. belorussicum* 10.5%; *T. paucilobum* 12.5%; *T. brandenburgicum* 18%; *T. vindobonense* 13%. The average proportion of polymorphic bands in triploids and tetraploids was the same (15.5%). No relationship was observed between the number of polymorphic bands and the total number of bands observed in a given species.

Although the molecular differences between the studied species are noticeable, five of out six *Palustria* species showed mutual similarity of RAPD profiles. Interestingly, tetraploid *T. brandenburgicum* seems to be more different from them (Fig. 5).

4. Discussion

Our analysis of nuclear DNA shows clear genetic differences among the analyzed apomictic taxa from the section *Palustria* as well as a significant difference between the former and *Taraxacum pieninicum* (diploid species of the section *Erythrocarpa*) and an unidentified representative of the section *Ruderalia* (Fig. 4). The results fully confirm the commonly accepted concept of the division of the *Taraxacum* genus into sections and of distinguishing small apomictic species (Kirschner and Štěpánek, 1996, 1997). Genetic similarity of the studied species of *T.* sect. *Palustria* (Fig. 5) indicates a clear identity of *T. brandenburgicum*, which markedly differs also in the morphological aspect. Other species form a rather coherent group with *T. paucilobum* occupying the most separate place. This species is morphologically closest to

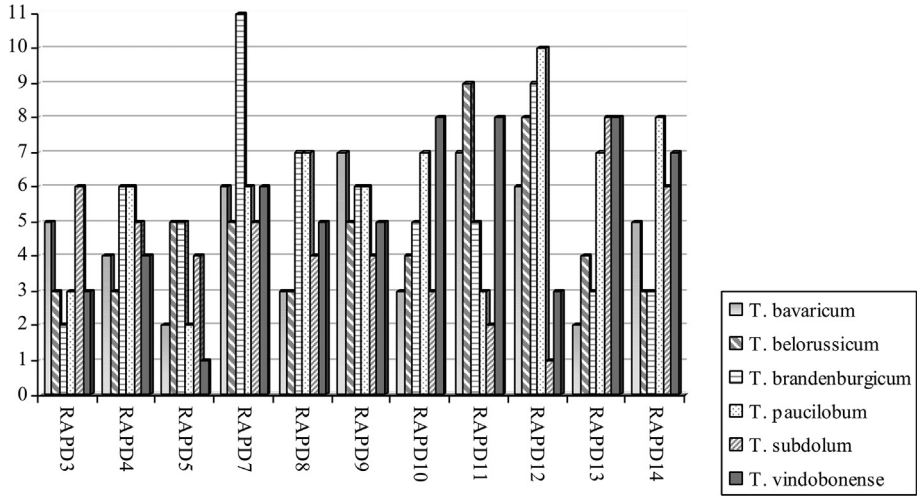

Fig. 2. The number of PCR products unique for the studied species of *Taraxacum* sect. *Palustria* generated in the presence of particular RAPD primers. y axis - number of PCR products.

Table 4
PCR products unique for *Taraxacum* brandenburgicum, *Taraxacum vindobonense, Taraxacum subdolum, Taraxacum bavaricum, Taraxacum belorussicum* and *Taraxacum paucilobum.*

Primer	Product length in bp		
	Taraxacum brandenburgicum	*Taraxacum vindobonense*	*Taraxacum subdolum*
RAPD3	2640, 2180	2660, 1580, 620,	3720, 3550, 3250, 2920, 2690, 2260, 1780,
RAPD4	3320, 3290, 2580, 2430, 2180, 670	2830, 2130, 1930, 630,	3030, 2030, 1520, 1300, 950, 380,
RAPD5	2880, 2420, 1100, 870, 540,	230, 170	1830, 1620, 650, 450,
RAPD7	3840, 3600, 3560, 3190, 3030, 2130, 2030, 1780, 1440, 1240, 250	3090, 2690, 2050, 930, 600, 290,	3710, 3140, 2390, 2170, 1600,
RAPD8	3670, 3080. 3060, 2590, 1960, 1220	3400, 2250, 2100, 990, 920,	3560, 3270, 2120, 1570,
RAPD9	3520, 3310, 3100, 2060, 1110, 760,	3000, 1880, 1440, 1080, 280	3710, 2580, 1530, 1190, 120
RAPD10	4500, 4130, 3200, 2960, 2360, 2140, 1500,	4940, 4610, 4100, 3940, 3390, 2860, 2320, 1430, 1120, 740, 440	5030. 4230, 4000, 3030, 2780, 2430,
RAPD11	3710, 3450, 3180, 2430, 830,	3550, 3300, 3150, 2320, 2270, 1500, 1020, 870,	4560, 3000, 1480,
RAPD12	3820, 3460, 2920, 2620, 2400, 2320, 1900, 1520, 590,	4070, 2790. 870, 450	4570, 1670
RAPD13	2660, 2180, 1240,	3300, 3100, 2640, 2470, 2240, 1930, 1810, 1520,	3740, 3190, 3030, 2260, 1780, 1320, 800, 330,
RAPD14	2810, 2150, 1230,	2660, 1440, 1270, 1040, 800, 600,	3260, 2940, 2020, 1890, 1650, 1470, 680,
	Taraxacum bavaricum	*Taraxacum belorussicum*	*Taraxacum paucilobum*
RAPD3	3780, 3370, 3340, 1830, 250	2770, 2520, 2240	3590, 2560,
RAPD4	2540, 490, 210	3700, 3390, 1780,	3480, 3260, 3100, 2790, 2050, 1710,
RAPD5	4420, 3910, 2930, 120	3890, 3750, 2360, 980, 860,	1870, 1650, 510,
RAPD7	4940, 2930, 2000, 890, 800,	3410, 1630, 700, 560, 200, 110	3530, 3070, 2890, 1330, 570
RAPD8	3460, 3310, 1290,	3580, 3390, 880,	4000, 3750, 3360, 2730, 2430, 2150, 1270, 580,
RAPD9	3540, 3260, 2100, 1900, 1680, 960,	3360, 2420, 1750, 1460, 240	2900, 2340, 2230, 1650, 1330, 1280, 980
RAPD10	5100, 4550, 4030, 3870, 3480, 2300, 200	3730, 3370, 2670, 1450,	4070, 3440, 2640, 2290, 1000, 340, 270,
RAPD11	3600, 3210, 2940, 2040, 1780, 1220, 1050,	2970, 2900, 2510, 2290, 2020, 1870, 1350, 940, 380,	1600, 850, 500, 200
RAPD12	4430, 3610, 2800, 1770, 1540, 1300,	3330, 2530, 2290, 2000, 1800, 1640, 1170, 660,	4900, 4480, 3860, 3480, 2850, 2440, 2340, 2030, 1790, 1610, 1060, 620
RAPD13	2000, 1570,	4230, 3230, 2200, 2030, 1880,	3160, 3060, 2760, 2500, 2310, 1600,
RAPD14	3340, 2900, 2700, 1500, 530, 220	3550, 1570, 950,	4000, 3070, 2740, 1960, 1860, 1520, 1290, 700, 620

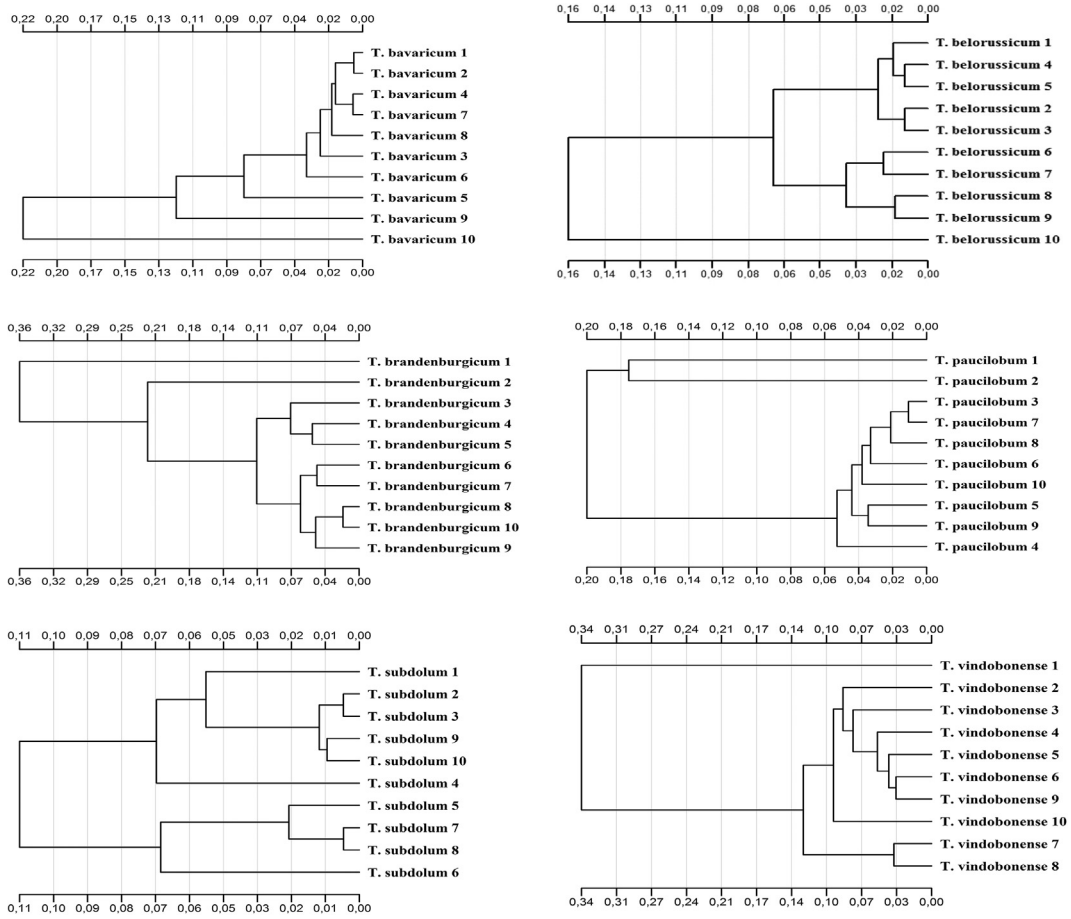

Fig. 3. Dendrograph of genetic similarity between individuals of the studied *Taraxacum* sect. *Palustria* specimens (WPGMA/Jaccard dissimilarity index).

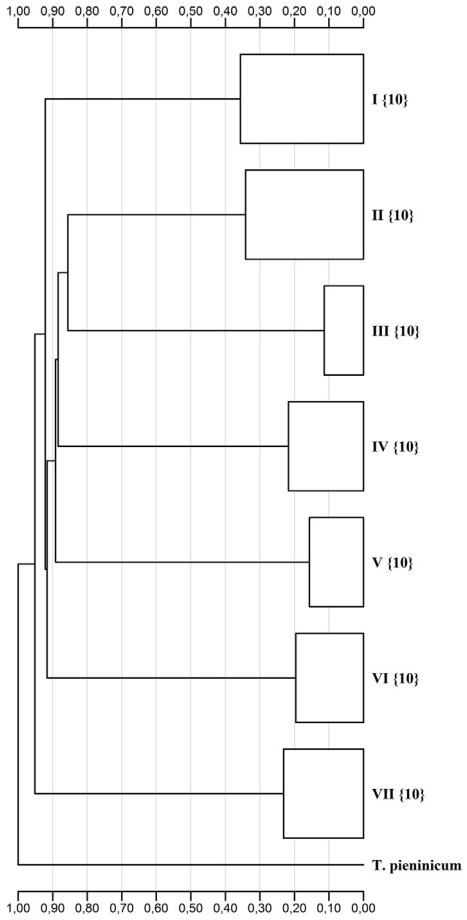

Fig. 4. Dendrograph of genetic similarity of studied *Taraxacum* species (WPGMA/Jaccard dissimilarity index). I - *T. brandenburgicum*; II - *T. vindobonense*; III - *T. subdolum*; IV - *T. bavaricum*; V - *T. belorussicum*; VI - *T. paucilobum*; VII - *T.* sect. *Ruderalia.*

T. vindobonense (Kirschner and Štěpánek, 1998) but genetically it is definitely less similar to it than the two species from other morphological groups (acc. to Kirschner and Štěpánek, 1998): *T. subdolum* and *T. belorussicum* - the species of uncertain taxonomic status which might be a pollen-free form of *T. dentatum* (compare

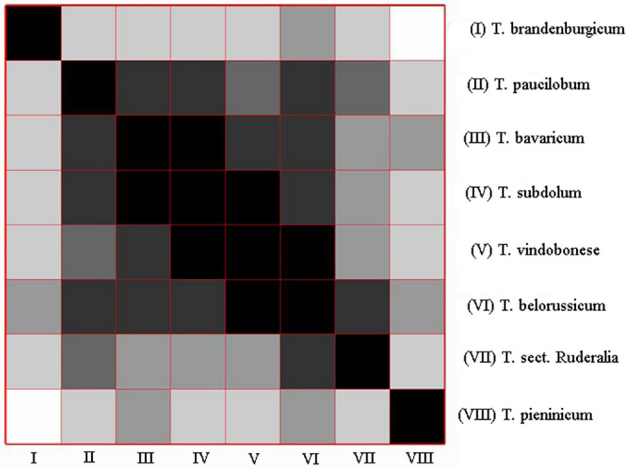

Fig. 5. Czekanowski's diagramme of genetic similarity of studied *Taraxacum* taxa (based on the Jaccard dissimilarity index). Darker color denotes higher similarity between species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Marciniuk et al., 2010). It is possible that morphological similarity does not coincide with the kinship among species of the *T.* sect. *Palustria*. This issue requires further studies on more representative material.

A much more interesting result of our studies is the finding of intraspecific polymorphism of nuclear DNA. Although the polymorphism is not high (percentage share of polymorphic PCR products in studied species of *T.* sect *Palustria* ranged from 10.5% in *T. subdolum* to 18% in *T. brandenburgicum*), it pertains to all studied species and all individuals (Fig. 3). In single similar research where the RAPD technique was used in molecular studies on six species of *T.* sect. *Erythrosperma* (Reisch, 2004), it was shown that the species of a range of variability similar to ours (maximum 15.8%) were accompanied by genetically uniform (polymorphism 0%) taxa. Such a complete lack of variability among apomictic plants seems to be rare (Richards, 1996). In the genus *Taraxacum* the species of very small genetic variability are probably phylogenetically young ones like *T. albidum* (Menken and Morita, 2005), most species of the *T.* sect. *Hamata* (Mogie, 1985) and taxa associated with specific habitats like *T. obliquum* (Van Oostrum et al., 1985) growing on seashore dunes, and alpine *T. alpestre* (Kirschner and Štěpánek, 1994). Very low variability was also found in widespread in Europe *T. hollandicum* (sect. *Palustria*) but *T. vindobonense* studied with the same methods showed high variability (Battjes et al., 1992; Kirschner and Štěpánek, 1998). Unfortunately, most studies on genetic variability in clonal plants are hardly comparable with our studies since the former are based on morphological data, on the variability of selected isoenzymatic systems (Lyman and Ellstrand, 1984; Ellstrand and Roose, 1987; Widén et al., 1994; Kashin et al., 2005) or on the polymorphism of chloroplast cpDNA (Kirschner et al., 2003; Mes et al., 2000; Shibaike et al., 2002; Van Der Hulst et al., 2003; Vijverberg et al., 2004; Wittzell, 1999). Studies on the variability of nuclear DNA were seldom and pertained mainly to the genera *Rubus* and *Hieracium* (Widén et al., 1994; Kashin et al., 2005). In *Taraxacum* these studies were limited to analyzing the polymorphism of ribosomal rDNA (King and Schaal, 1990; Záveská Drábková et al., 2009; Kirschner et al.,2015). However, in recent years nuclear simple sequence repeat (SSR, microsatellites) and amplified fragment length polymorphism (AFLP) markers were also used to characterize genotypic diversity of apomictic dandelion clones (Majeský et al., 2012, 2015; McAssey et al., 2016). Studies using different methods of DNA analysis were more often used in *Taraxacum* to solve various specific issues such as clone identification and gene flow between apomictic and sexual plants (Falque et al., 1998, and Brock, 2004 - microsatellite markers; Van Der Hulst et al., 2000 - AFLP; Shibaike et al., 2002 - cpDNA markers; Mitsuyuki et al., 2014 - PCR-RFLP of the rDNA) or the mutational variability among offspring of obligate apomicts (King and Schaal, 1990 - RFLP of the rDNA).

Particularly interesting is the finding of relatively high polymorphism in *T. bavaricum* (the share of polymorphic products was 15.5%) in which all studied individuals originated from one mother plant. This polymorphism was much higher than that in *T. belorussicum*, whose individuals were taken from two different populations and that in *T. subdolum*, whose seeds were collected from two plants of the same population. The reasons for the observed polymorphism are not clear but one may assume that they are partly an effect of an imperfect RAPD method, point mutations and sexual origin of some seeds. Some authors suggested that the additional source of genetic variation among *Taraxacum* offspring can be sporadic meiotic recombination during restitutional megasporogenesis (Małecka, 1973; van Baarlen et al., 2000; Archetti 2004).

The sexual production of some seeds seems to be probable in the case of an individual of *T. bavaricum* in which 19 polymorphic products were found in our RAPD studies (including 13 character-

istic only of itself) which markedly distinguished it from the rest of its siblings. However, this issue would require further investigation because apomictic mother plants in *Taraxacum* are generally considered incapable of producing seeds sexually in nature (Richards, 1973; Majeský et al., 2012). On the other hand, apomictic plants can produce viable, chromosomally balanced pollen grains, able to fertilize diploid sexuals (de Kovel and de Jong, 2000). Thus, apomicts can participate in gene exchange, although based on the existing data, this process is considered unidirectional.

Author contributions

All authors contributed equally to the study conception and design, material preparation, and data collection. The first draft of the manuscript was written by Jolanta Marciniuk and Paweł Marciniuk and all authors commented on previous versions of the manuscript. All authors read and approved the final version of manuscript.

Declaration of Competing Interest

None.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.07.025.

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