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CHROMOSOME NUMBERS AND STOMATAL CELL LENGTH IN *TARAXACUM* SECT. *PALUSTRIA* FROM POLAND

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Chromosome numbers are given for the following species of *Taraxacum* sect. *Palustria* from Poland: *T. paucilobum* Hudziok (2n = 24, 25), *T. belorussicum* Val. N. Tikhom. (2n = 24), *T. subdolum* Kirschner & Štěpánek (2n = 24), *T. udum* Jordan (2n = 24), *T. trilobifolium* Hudziok (2n = 24), *T. bavaricum* Soest (2n = 24), *T. portentosum* Kirschner & Štěpánek (2n = 32), *T. vindobonense* Soest (2n = 32), and *T. brandenburgicum* Hudziok (2n = 32). The chromosome numbers of *T. belorussicum* and *T. portentosum* are published for the first time, and for *T. subdolum*, *T. bavaricum* and *T. brandenburgicum* for the first time from Poland. The analyzed group of taxa is heterogenous in respect of stomatal size, and after pooling of data the tetraploids show bigger stomata than the triploids.

Key words: Asteraceae, *Taraxacum*, *Palustria*, chromosome number, stomata size, Poland

Taraxacum Wigg. (dandelion) is one of the most common plant genera in the temperate zone of the Northern Hemisphere, found native on five continents (Richards, 1970). The basic chromosome number in *Taraxacum* is eight and the genus reveals considerable variation of chromosome number, from 2n=2x=16 to 2n=12x=96 (Kirschner and Štěpánek, 1996). The frequency of diploid taxa within this genus was estimated at 14% (Richards, 1970). In Europe it is dominated by triploid (2n=3x=24) and tetraploid (2n=4x=32) microspecies belonging to the advanced *Taraxacum* sections, growing mainly on disturbed ground, meadows and woods.

Taraxacum has been widely studied as a model for analysis of apomixis and breeding systems (Richards, 1970; van Dijk, 2003; Mártonfiová et al., 2007) in which ploidy level is a good indicator of the mode of reproduction. Sexuals are diploid, whereas polyploids (excluding three tetraploid taxa of sect. Piesis) are obligate or facultative agamosperms producing seeds asexually and endosperm autonomously (van Dijk, 2003; Záveská Drábková et al., 2009).

There are 373 *Taraxacum* species in Poland, among which only three diploid sexuals have been identified: *T. pieninicum* Pawł., *T. bessarabicum* (Hornem.) Hand.-Mazz., and *T. erythrospermum* Andrz. ex Besser. The Polish *Taraxacum* flora is strongly dominated by weedy species belonging to *T.* sect. *Ruderalia* (292 taxa). Also relatively well-represented are *T.* sect. *Erythrosperma* (24 species) and *T.* sect. *Palustria* (21 species). The plants of the latter section deserve special attention, because they are endangered in Poland due to the loss of their natural habitat (extensively used wet meadows and bogs).

There are reliable data on the chromosome numbers of Polish dandelions for only 22 species, including five from sect. Palustria (data not shown). Some previously published chromosome-reports from this area (http://www.binoz.uj.edu.pl:8080/chromosomes/) were for species that probably were too broadly treated or erroneously determined (Grzesiuk et al., 2008).

Here we examined the chromosome numbers of nine microspecies belonging to *Taraxacum* sect. *Palustria* from Poland: *T. bavaricum, T. belorussicum, T. brandenburgicum, T. paucilobum, T. portentosum, T. subdolum, T. trilobifolium, T. udum*

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TABLE 1. *Taraxacum* species studied, with locality, geographic coordinates, chromosome number (2n) and stomatal length (μm)

	Species	Locality	Geographic coordinates	2n	Length of stomata
A	*T. tenuifolium* (Hoppe & Hornsch) W.D.J. Koch	Croatia (leg. Ingo Uhlemann)	–	16#	26.60 (1.90)
B	*T. paucilobum* Hudziok	Czuchów, Tocznia River valley	N: 52°16′36″; E: 22°46′57″	24, 25	26.07 (3.12)
C	*T. belorussicum* Val. N. Tikhom.	Mścichy, weir in Biebrza National Park	N: 52°25′46″; E: 22°30′55″	24	27.81 (2.32)
D	*T. subdolum* Kirschner & Štěpánek	Czuchów, Tocznia River valley	N: 52°16′36″; E: 22°46′57″	24	28.50 (2.48)
E	*T. udum* Jordan	Pomiechowo, near enbranchment of Wkra and Narew rivers	N: 52°27′18″; E: 20°43′47″	24	30.24 (2.93)
F	*T. trilobifolium* Hudziok	Krześlin, the Liwiec river valley	N: 52°13′21″; E: 22°21′34″	24	30.70 (2.54)
G	*T. bavaricum* Soest	Czuchów, Tocznia River valley	N: 52°16′36,68″; E: 22°46′57,36″	24	31.11 (1.71)
H	*T. portentosum* Kirschner & Štěpánek	Wilczonek near Kotuń	N: 52°10′35″; E: 22°01′24″	32	31.54 (1.94)
I	*T. vindobonense* Soest	Górka Kocka Drewnik	N: 51°38′22″; E: 22°30′15″ N: 51°35′20″; E: 22°16′36″	32	31.55 (2.30)
J	*T. brandenburgicum* Hudziok	Pyzdry near Poznań	N: 52°10′04″; E: 17°42′40″	32	32.24 (2.83)

according to Záveský et al. 2005; ( ) standard deviation

and *T. vindobonense* (Tab. 1). We also analyzed stomatal cell length in these species and in diploid *T. tenuifolium* (the only diploid species in sect. *Palustria* not found in Poland) to see whether this feature can be helpful in determining ploidy level. The size of stomatal guard cells, a nucleotypic character influenced by nuclear DNA amount (Bory et al., 2008; Hodgson et al., 2010), is correlated with ploidy level in a range of natural and commercial cytotypes/varieties of different plant species (Speckman et al., 1965; Przywara et al., 1988; Cohen and Yao, 1996; Mishra, 1997; Joachimiak and Grabowska-Joachimiak, 2000). Chromosome counting is time-consuming and not suited for routine screening of ploidy level in *Taraxacum* microspecies. A simple, quick and inexpensive method for determining the degree of ploidy would be very useful in studies of this large genus.

We incubated root tips of young seedlings in a saturated water solution of α-bromonaphtalene overnight at 4°C and fixed them in 1:3 acetic alcohol. For chromosome counts the root tips were stained in acetic orcein (according to Golczyk and Joachimiak, 2003) or else hydrolyzed with 1N HCl at 60°C and stained with toluidine blue (according to Grabowska-Joachimiak and Joachimiak, 2002). For stomatal length measurements, pieces of bottom epidermis removed from the central part of the leaf were analyzed under a Nikon microscope equipped with a Moticam 2000 video camera (Motic China Group, China). Image grabbing and stomatal length measurements used Motic Images Plus software (Motic China Group, China). In each specimen the length of at least 30 stomata were measured. One-way ANOVA was applied to stomatal length measurements (http://faculty.vassar.edu/lowry/VassarStats.html, http://www.physics.csbsju.edu/stats/anova_pnp_NGROUP_form.html). Significance of differences was checked with the F-test and Tukey test.

CHROMOSOME NUMBERS

Taraxacum paucilobum Hudziok, 2n = 24, 25.
This most common representative of sect. *Palustria* in Poland has been studied cytologically by several authors. Eutriploid chromosome number 2n = 3x = 24 in *T. paucilobum* was reported (as *T. austrinum*) by Małecka (1970, 1972, 1973) from Poland and by Kirschner and Štěpánek (1985) from the Czech Republic. Grzesiuk et al. (2008) found hypertriploid number 2n=26 in representatives of *T. paucilobum* from two stands in Poland, and Małecka (l.c.) 2n=25 for plants described by her as *T. austrinum* f. *minor*. The occurrence of hypertriploidal forms in *T. paucilobum* is interesting and deserves further study, particularly since that phenomenon has also

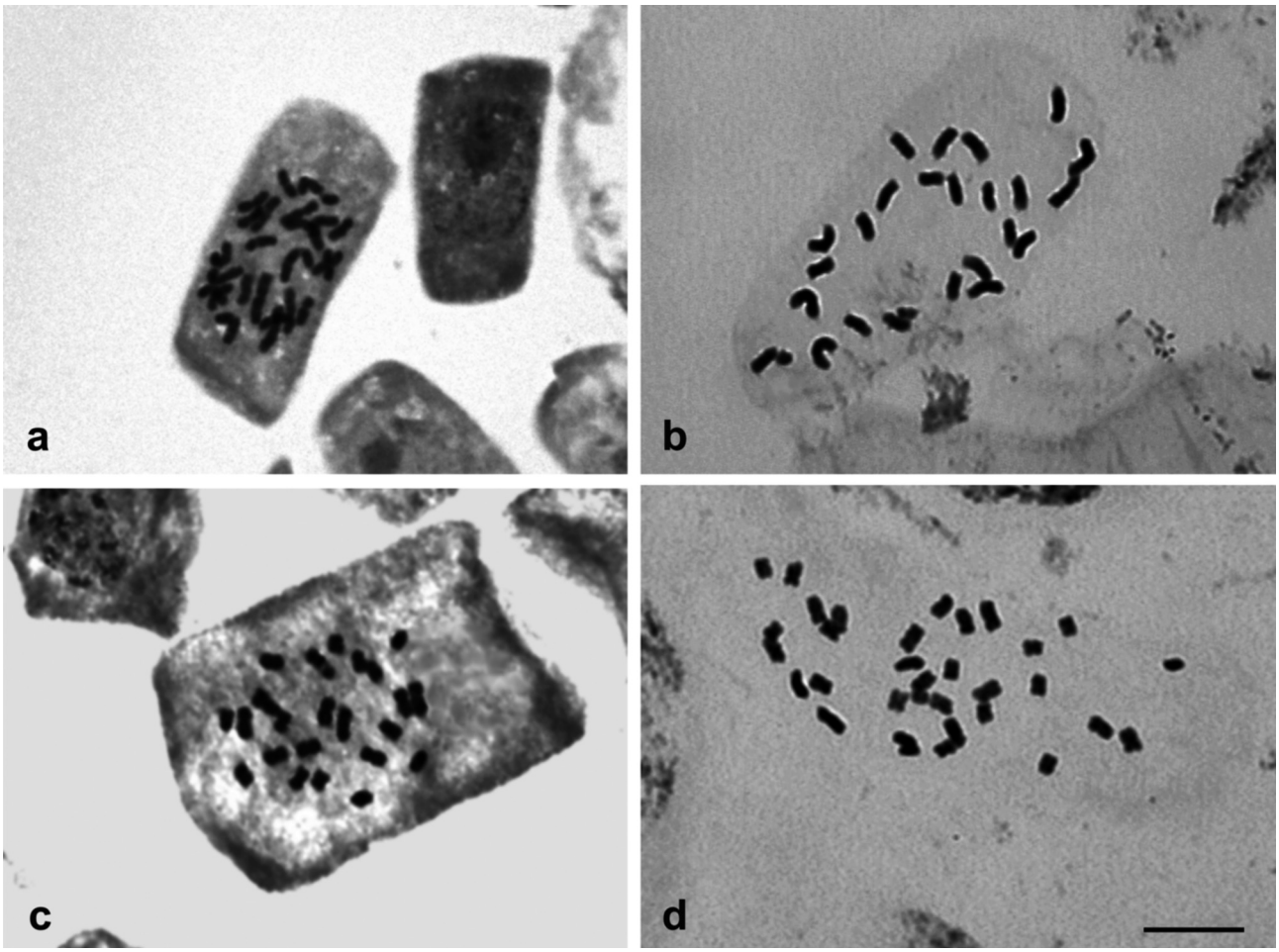

Fig. 1. Mitotic chromosomes of *T. belorussicum*, 2n=24 **(a)**, *T. udum*, 2n=24 **(b)**, *T. trilobifolium*, 2n=24 **(c)** and *T. portentosum*, 2n=32 **(d)**. a,c – acetic orcein, b,d – toluidine blue. Bar = 5 μm.

been observed in some other *Taraxacum* species (http://www.chromosomes.sav.sk/, http://www.binoz.uj.edu.pl:8080/chromosomes/).

Taraxacum belorussicum Val. N. Tikhom., 2n = 24 (Fig. 1a).
This is the first chromosome count for this species. *Taraxacum belorussicum* is of uncertain taxonomical position. Herbarium specimens of the species collected in Poland had been assigned to the *T. belorussicum* by the author of the species description, Valery Tikhomirov (Belarus). Jan Štěpánek, who saw the specimens, believes that they probably represent an unknown male-sterile line of *T. dentatum* Kirschner & Štěpánek. The taxonomic differentiation of *T. belorussicum* and *T. dentatum* requires further studies.

Taraxacum subdolum Kirschner & Štěpánek, 2n = 24.
This is the first chromosome count for this species from Poland. The triploid chromosome number of the examined seedlings was in accordance with previous results given for this species (Kirschner and Štěpánek, 1992). The complicated range of the species involves the Czech Republic and Slovakia, and a dispersed distribution in eastern Germany, northern Austria and southern Poland. In Czuchów the species *T. subdolum* reaches its northern range limit.

Taraxacum udum Jordan, 2n = 24 (Fig. 1b).
In Poland the species is present in a single and very isolated stand located ~1000 km from the closest stands in western Germany (Schmid, 2002). Małecka (1981) reported chromosome number 2n=24 for *T. udum* but the description of the herbarium specimen placed by the author differs significantly from the original description of *T. udum* and seems to be a mistaken determination.

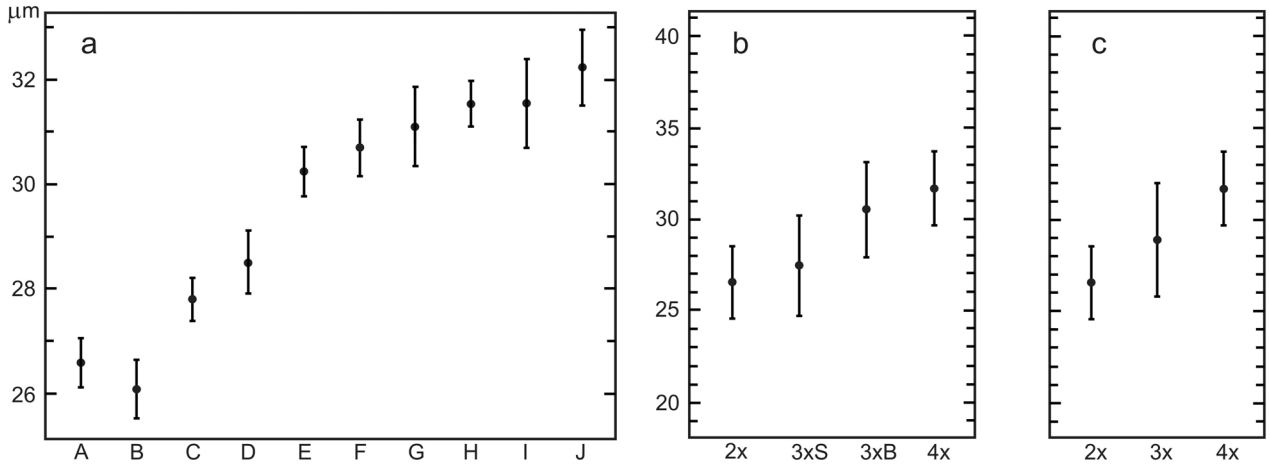

Fig. 2. Stomatal lengths in analyzed species **(a)** and different ploidy levels **(b, c). a** – group means with 95% confidence intervals: A – *T. tenuifolium* (2x), B – *T. paucilobum* (3x), C – *T. belorussicum* (3x), D – *T. subdolum* (3x), E – *T. udum* (3x), F – *T. trilobifolium* (3x), G – *T. bavaricum* (3x), H – *T. portentosum* (4x), I – *T. vindobonense* (4x), J – *T. brandenburgicum* (4x); 2x – diploid (A), 3x – triploids (B-G), 4x – tetraploids (H-J), 3xS – triploids with small stomata (B-D), 3xB – triploids with large stomata (E-G).

Taraxacum trilobifolium Hudziok, 2n = 24 (Fig. 1c).
This is the first report of a triploid chromosome number for this species. Kirschner and Štěpánek (1985) gave a tetraploid count for plants from Germany. *T. trilobifolium* occurs in two morphological forms previously described as separate species: *T. trilobifolium* (Hudziok, 1967) and *T. hemiparabolicum* (Hudziok, 1969). Possibly they differ in chromosome number. Different ploidy levels have only exceptionally been noted in *Taraxacum* microspecies (*T. freticola*, 2n=16 and 24; Grzesiuk et al., 2008). In some other apomicts, co-occurring sexual and apomictic biotypes commonly form hybrid swarms and populations of mixed ploidy levels (Krahulcová et al., 2009).

Taraxacum bavaricum Soest, 2n = 24.
This is the first chromosome count for this species from Poland. Kirschner and Štěpánek (1985) reported the same triploid chromosome number for this species for plants from the Czech Republic. *T. bavaricum* is a species from Central Europe; its compact range includes eastern Germany, the Czech Republic, Austria and Slovakia (Kirschner and Štěpánek, 1998; Schmid, 2002). In Poland it reaches its eastern range limit. The species developed two forms: one that produces pollen and one that does not. The one analyzed here is the latter form.

Taraxacum portentosum Kirschner & Štěpánek, 2n = 32 (Fig. 1d).
This is the first chromosome count for this very rare species. It is known from single sites in the Czech Republic and Slovakia (Kirschner and Štěpánek, 1998) and from a few stands in eastern Poland (Marciniuk and Marciniuk, 2006).

Taraxacum vindobonense Soest, 2n = 32.
The chromosome number of the examined seedlings is in accord with previous results given for this species (Małecka, 1972; Kirschner and Štěpánek, 1998; Grzesiuk et al., 2008).

Taraxacum brandenburgicum Hudziok, 2n = 32.
This is the first chromosome count for this species from Poland. The tetraploid chromosome number of the examined seedlings matches a previous result given for this species (Kirschner and Štěpánek, 1985).

STOMATA SIZE

The analyzed group of taxa was heterogenous in respect of stomatal size (Tab. 1, Fig. 2a) (F-test, $P<0.0001$), and the triploid species formed a group with smaller (26.07-28.50 µm) and larger (30.24–31.11 µm) stomata. Pairwise comparisons between the four distinguished groups (2x, 3x with smaller stomata, 3x with larger stomata, 4x) (Fig. 2b) showed significant differences between them (Tukey test, $P<0.01$). Although pooling of data according to the three ploidy levels showed a gradual increase in stomata size (Fig. 2c), caution is needed in using stomata measurements for determining ploidy in the analyzed group. In pairwise comparisons of species, the differences between *T. bavaricum* and two tetraploid species, *T. portentosum* and *T. vindobonense*, and between *T. paucilobum* (3x) and *T. tenuifolium* (2x), were not significant (Tukey test).

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