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OCCURRENCE OF ONEROW YELLOWCRESS *NASTURTIUM MICROPHYLLUM* (BOENN.) RCHB. IN THE ILANKA RIVER (LUBUSZ LAND)

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ABSTRACT. The paper presents three new localities of *Nasturtium microphyllum* reported on the Ilanka River near Rzepin. In one of the localities onerow yellowcress specimens were in the flowering and fruiting stages, while in the two other localities plants were flowering, but they were not bearing fruits. Fruiting specimens were identified on the basis of seed sculpture traits, while flowering plants – by flow cytometry, based on the genome size.

KEY WORDS: *Nasturtium microphyllum*, protected plants, the Ilanka, distribution, SEM, genome size

INTRODUCTION

In Poland onerow yellowcress *Nasturtium microphyllum* (Boenn.) Rchb. 1832 (Brassicaceae) is covered by strict species protection (Rozporządzenie Ministra... 2012) and it has been included in the Polish Red Book of Plants, where it is classified as a vulnerable taxon, VU (Smoczyk 2001). To date a total of ten localities have been reported in Poland, while at present only seven localities of this species exist, i.e. Mydlniki near Kraków (Tacik 1985), Makrosice near Gubin (Czarna and Morozowska 2009), two localities near Kraków (Kruk and Szymańska 2009), Bielinek, the Odra river valley near Chlewice (Smoczyk 2001), Zieleniewo near Kołobrzeg and Babimost near Zielona Góra (Czarna and Morozowska 2013). The distribution of *N. microphyllum* in Poland is still relatively little known due to the recent introduction of this species into Polish keys for the identification of species (Tacik 1985, Rutkowski 1998) and the great morphological similarity to common watercress *Nasturtium officinale* R. Br. 1812, as well as brown watercress *Nasturtium* ×*sterile* (Airy Shaw) Oefelein 1958.

Onerow yellowcress has practical and medicinal uses similar to those of watercress (http://www.pfaf.org). The species mentioned above as well as their hybrid are also occasionally cultivated. In the 17th century watercress was commercially grown in Europe. At present leaves of these species are used as an ingredient of salads, while seeds as a substitute of mustard seeds are used to season meat (Podgórska i Podgórski 2004).

IDENTIFICATION METHODS

Onerow yellowcress may be most reliably identified on the basis of the arrangement of seeds distributed in a single row on both sides of the fruit septum, due to which fruits are narrow. In watercress seeds are found in two rows and fruits are wider, while the third, hybrid species forms rudimental siliques, typically with a limited number of sterile seeds (Czarna et al. 2012). A valuable diagnostic tool is also provided by the reticullate seed sculpture, visible under a scanning electron microscope (SEM). On seeds of onerow yellowcress the sculpture is finely reticulate with over 130 cells on one side of the seed (Figs 1 A, B), on seeds of watercress seed surface cells are large, ranging in number from 25 to 60, while on seeds of the hybrid the number of seed sculpture cells ranges from 60 to 120 (Bleeker et al. 1997, 1999, Naquinezhad 2006, Haeupleur and Muer 2007).

Identification of species from the genus *Nasturtium* in the vegetative state or during flowering is very difficult. It may prove helpful to compare leaf outline, which in all examined cress species is pinnate. Their morphological variation is manifested first of all in the shape of individual leaflets, although it does not ensure an unambiguous taxonomic diagnosis (Fig. 2). Flow cytometry, which was originally used only in medical research, is a reliable method that provides a correct identification of species in the *Nasturium* genus when the fruits and seeds are absent. Nowadays this method is applied in plant science by botanists, genetics, biotechnologists, plant breeders and seed producers. Flow cytometry has been used mostly to determine genome size in various plant species, to analyse ploidy level, cell cycle and endoreduplication in different plant organs.

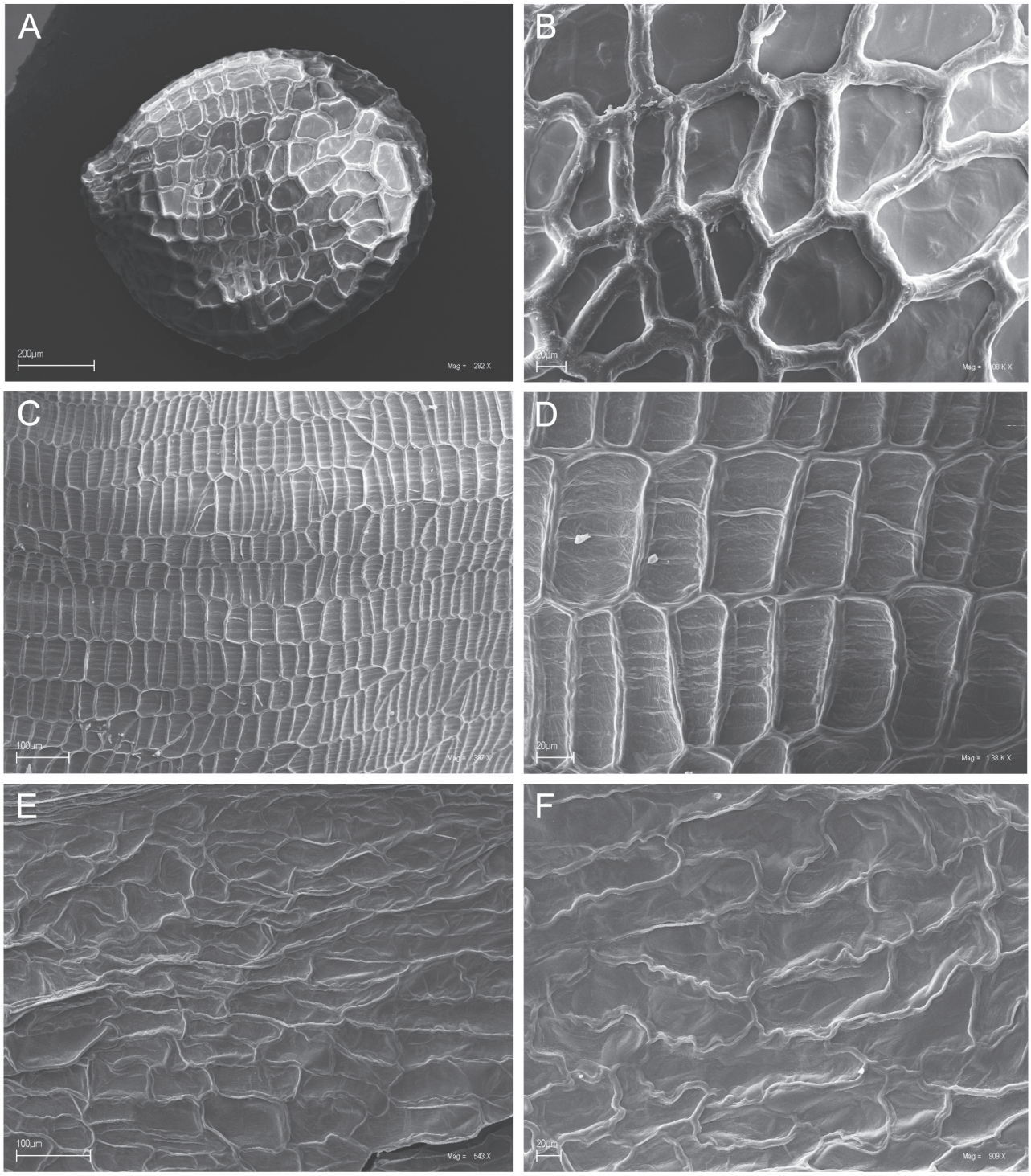

Fig. 1. Carpological diagnostic features of *Nasturtium microphyllum* (SEM): A, B – seed sculpture; C, D – ultrastructure of internal surface of fruit valve; E, F – microornamentation pattern of fruit septum

The measurement is based on the linear relationship between fluorescence of stained nuclei and DNA content. Estimation of plant nuclear DNA content is usually performed using young and fresh leaves (Contim et al. 2005, Hanson et al. 2005). However, plant material such as roots, hypocotyls, flowers, pollen grains or seed can be used for FCM as well (Suda et al. 2003, Śliwińska et al. 2005). Flow cytometry analysis is performed on a nuclear suspension, after chopping plant material in a nuclear isolation buffer supplemented with a fluorescent dye. Total nuclear DNA content in the sample is calculated by the comparison of the sample G1 peak positions and the internal standard on the histogram. Plant or animal cells with accurately estimated DNA contents should be used as a standard. The amount of 2C DNA is expressed in picograms (pg) or the number of base pairs (bp; 1 pg DNA = 0.978 × 109 bp; Doležel et al. 2003).

FIG. 2. The outline and morphological differentiation of *Nasturtium microphyllum* leaves from sites A, B and C localized in the Ilanka River

NEW LOCALITIES

An inspiration for the undertaking of field studies along the entire course of the Ilanka River (approx. 32 km) was provided by the discovery, in August 2012, of a new locality of *N. microphyllum* on section of the river in Rzepin (A). In the course of further studies conducted in the same vegetation season at a distance of approx. 2 km from locality A two further localities of the investigated species (B and C) were found, at a distance of approx. 500 m from each other (Fig. 3). Watercress specimens growing there were in the flowering stage and for this reason cytometric analyses were performed to provide accurate identification of species. Material for measurements of nuclear DNA content comprised upper, fully developed leaves of *N. microphyllum* from individual localities. At each locality they were collected from 10 plants growing at a distance of at least 5 m from one another. Plant material for cytometric analyses was prepared following the procedure developed for species from the genus of *Nasturtium* (MOROZOWSKA et AL. 2010).

Herbarium data sheets, one from each locality, and a fruit with seeds collected at locality A were deposited at the herbarium of the Department of Botany, the Poznań University of Life Sciences (POZNB).

A – locality 'Rzepin 1'

In the course of field studies onerow yellowcress was found in the Ilanka River in the section in the area of Rzepin, the Rzepin commune, the Słubice county, the Lubuskie province. The occurrence of the investigated species was limited to the river segment from the dam at the road running in the north-westerly direction up to

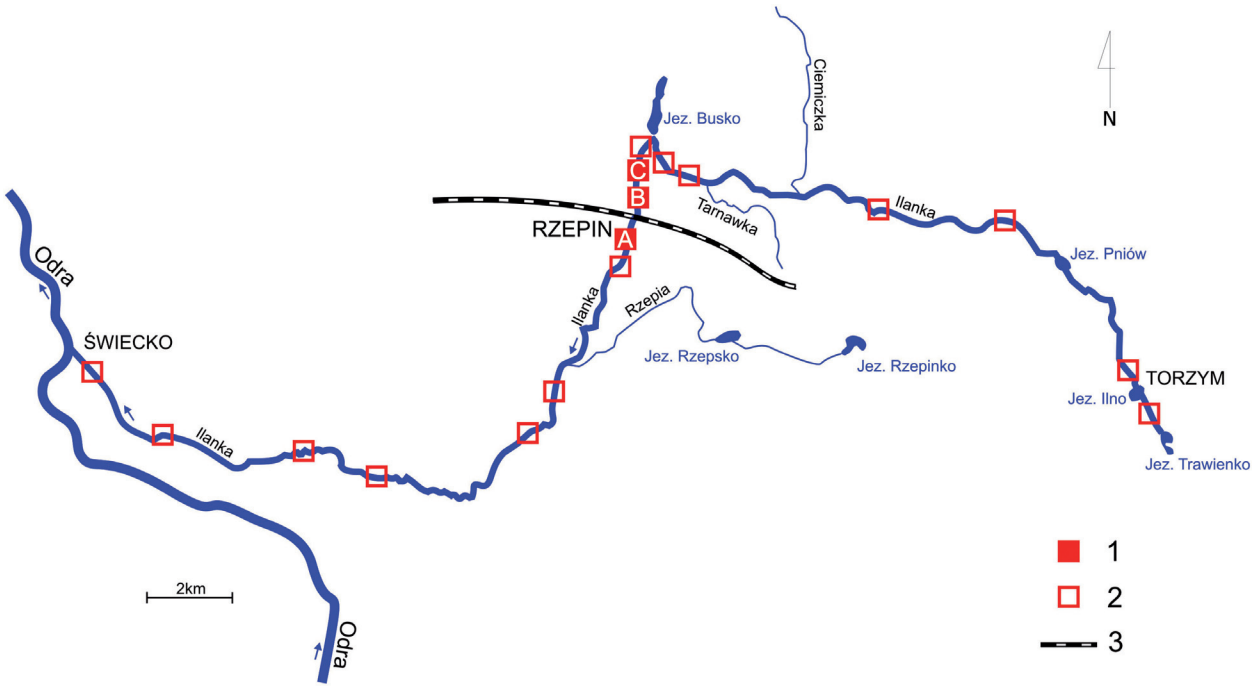

FIG. 3. Distribution of *Nasturtium microphyllum* occurrence along the Ilanka River: 1 – localities with the presence of *Nasturtium microphyllum*: A – 'Rzepin 1', B – 'Rzepin 2', C – 'Starościn'; 2 – localities without the presence of *Nasturtium microphyllum*; 3 – railway track

the bridge on the river (Fig. 3). Onerow yellowcress was growing here on both sides of the river bed of approx. 2 m in width, filled with clean, slow-flowing and relatively shallow water. The rushes zone on both sides of the river was well-developed. Onerow yellowcress in this locality was in the flowering and fruiting stages. Based on SEM analyses it was found that the reticulate cell pattern on the inner surface of the fruit valve provides good diagnostic traits for the identification of onerow yellowcress. Cells are narrow, strongly elongated with acute or rounded endings and with a well-visible secondary sculpture in the form of transverse striations, on average 6-7 per cell, and accompanying small wrinkles (Figs 1 C, D). Microstructure of the fruit's septum surface, which is also of reticulate pattern, is characterised by strongly elongated cells with slightly rounded, straight or acute endings. Anticlinal walls of cells are straight or frequently sinuate. The described characteristics of fruit septum micromorphology are typical of *N. microphyllum* and also make it possible to distinguish this species from other representatives of the genus *Nasturtium* (Fig. 1 E, F).

In the discussed locality at an area of 50 × 2 m a relevé was prepared on 28 August 2012 according to Braun-Blanquet using a seven-point cover scale and a five-point abundance scale. All plants growing both along and in the river itself were found in layer C (total cover 70%) and they included *Stratiotes aloides* 3.3, ***Nasturtium microphyllum* 2.2**, *Glyceria maxima* 1.1, *Lemna minor* 1.1, *Phragmites australis* 1.2, *Berula erecta* +, *Callitriche stagnalis* +, *Cirsium oleraceum* +, *Epilobium hirsutum* +, *Hydrocharis morsus-ranae* +, *Myosotis palustris* +, *Potamogeton natans* +, *Sium latifolia* +, *Spirodela polyrrhiza* +, *Urtica dioica* +, *Veronica anagallis-aquatica* +, *Calystegia sepium* r, *Epilobium obscurum* r, *Polygonum persicaria* r. The analysed species is growing in the described locality in the *Nasturtietum microphylli* community classified to the *Sparganio-Glycerion fluviatili* association, in the *Phragmitetea* class.

B – locality 'Rzepin 2'

In locality B onerow yellowcress was found in the Ilanka River on both sides of its 2 m wide bed, over a 200 m long segment, in the form of distinct patches floating on the water surface near the rush vegetation (Fig. 3). Only flowering plants were observed in this locality. The relevé for that locality was prepared using the Braun-Blanquet on 1 October 2012 on a 50 × 2 m plot. Plants growing along the river bed were found only in layer C (total cover 70%) and included ***Nasturtium microphyllum* 3.3**, *Phragmites australis* 3.3, *Ceratophyllum demersum* 2.2, *Phalaris arundinacea* 2.2, *Acorus calamus* 1.1, *Carex acutiformis* 1.1, *Lemna minor* 1.1, *Myosostis palustris* 1.1, *Potamogeton natans* 1.1, *Sagittaria sagittifolia* 1.1, *Sparganium ramosum* 1.1, *Glycera maxima* +, *Sium latifolium* +, *Solanum dulcamara* +, *Nuphar luteum* r, *Equisetum limosum* r, *Filipendula ulmaria* r, *Iris pseudacorus* r, *Rumex hydrolapatum* r. The discussed *Nasturtium* species is growing in locality B also in the *Nasturtietum microphylli* community.

C – locality 'Starościn'

Specimens of onerow yellowcress in the locality in Starościn were found on both sides of the Ilanka River bed of approx. 2 m in width, in the 100 m long segment, in the form of distinct patches floating on the water surface near the rush vegetation, on both sides of the bridge in the village (Fig. 3). Analogously as in the two former localities, on 1 October 2012 a relevé was prepared on a 10 × 2 m plot using the Braun-Blanquet method. Plants growing along the river were found only in layer C (total cover 35%) and included *Phragmites australis* 3.3, *Carex acutiformis* 2.2, ***Nasturtium microphyllum* 2.2**, *Sagittaria sagittifolia* 2.1, *Potamogeton natans* 1.1, *Lemna minor* 1.1, *Hydrocharis morsus-ranae* 1.1, *Typha latifolia* 1.1., *Berula erecta* +, *Glyceria maxima* +, *Myosotis palustris* +, *Nuphar luteum* +, *Filipendula ulmaria* r, *Iris pseudacorus* r, *Lycopus europaeus* r, *Mentha aquatica* r, *Padus avium* r, *Rumex hydrolapathum* r, *Salix triandra* r, *Scrophularia umbrosa* r, *Spirodela polyrrhiza* r, *Symphytum officinale* r. The discussed species in locality C is also growing in the *Nasturtietum microphylli* community.

The genome size of the studied plant material determined by flow cytometry was 1.443 pg/2C in octaploid *N. microphyllum* species collected from three localities. The highest nuclear DNA content was found in plants collected from locality B ('Rzepin 2'), which was 1.452 pg/2C. A similar 2C value was obtained for plants from locality C ('Starościn'). The lowest 2C DNA content was estimated for plants growing in locality A ('Rzepin 1') and it amounted to 1.432 pg (Table 1). The statistical analysis showed no significant differences between all these localities. The obtained histograms were of good quality (Fig. 4) with a low coefficient of variation (CV; Table 1).

Table 1. Nuclear DNA content of *Nasturtium microphyllum* leaves collected from three localities near Rzepin. Mean ±SD, n = 5

Locality	DNA content (pg, mean ±SD)	CV (%) of target species	CV (%) of internal standard
A ('Rzepin 1')	1.432 ±0.014^{NS}	3.4-4.5	3.2-4.2
B ('Rzepin 2')	1.452 ±0.019	3.1-4.2	2.8-3.6
C ('Starościn')	1.445 ±0.019	3.3-4.0	2.7-3.1

2C DNA values between the localities did not show statistically significant differences (NS) at P = 0.05 (Student's t-test).

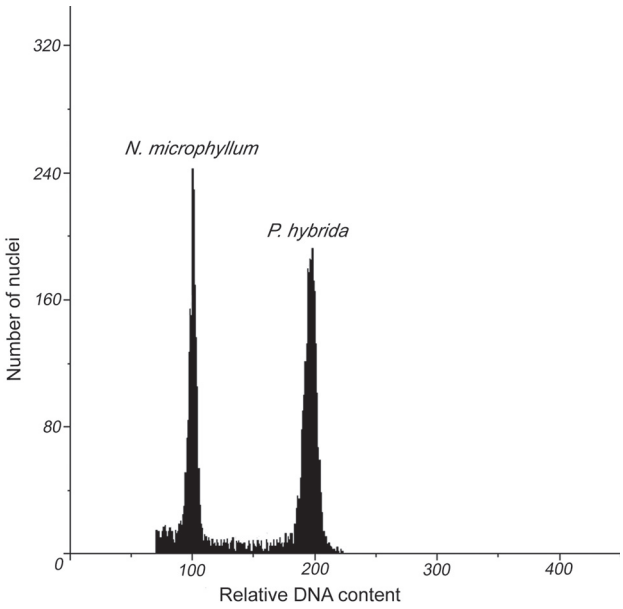

Fig. 4. Cytometric analysis of nuclear DNA content in leaves of *Nasturtium microphyllum* using Partec buffer with PI and RNase. Leaves of *Petunia hybrida* 'P × Pc6' (2C = 2.85pg) were used as an internal standard

Due to the abundance and regular occurrence of the investigated species in all the three localities and the fact that they are all situated in the vicinity of a large town, a question may arise whether onerow yellowcress was artificially introduced to be more extensively cultivated.

THREATS AND PROTECTION GUIDELINES

The greatest threat for all the discussed onerow yellowcress populations may be connected with floods, water pollution and maintenance of river banks and bed. In order to preserve these populations during river management works small patches with *N. microphyllum* would have to be transferred to a substitute water body and after the completion of river management works they would have to be placed back in the Ilanka River. The proposed protection measure is feasible and relatively easy to perform, since the Ilanka River Valley is a protected area within the Natura 2000 network (the Ilanka River Valley – Dolina Ilanki, area code: PLH 080009).

REFERENCES

Bleeker W., Hurka H., Koch M. (1997): Zum Vorkommen und zur Morphologie von *Nasturtium sterile* (Airy Shaw) Oef. in Südwestniedersachsen und angrenzenden Gebieten. Flor. Rundb. 31, 1: 1-8.

Bleeker W., Huthmann M., Hurka H. (1999): Evolution of hybrid taxa in *Nasturtium* R. Br. (Brassicaceae). Folia Geobot. 34: 421-433.

Contim L.A.S., de Carvalho C.R., Martins F.A., de Freitas D.V. (2005): Nuclear DNA content and karyotype of Rosewood (*Aniba rosaeodora*). Gen. Mol. Biol. 28: 754-757.

Czarna A., Morozowska M. (2009): Występowanie rukwi drobnolistnej *Nasturtium microphyllum* (Boenn.) Rchb. w Wielkopolsce. Chrońmy Przyr. Ojcz. 65, 6: 461-464.

Czarna A., Morozowska M. (2013): Nowe stanowiska rukwi drobnolistnej *Nasturtium microphyllum* (Boenn.) Rchb. w Polsce. Chrońmy Przyr. Ojcz. – in print.

Czarna A., Morozowska M., Nowińska R. (2012): Występowanie rukwi płonnej *Nasturtium* ×*sterile* w Polsce. Chrońmy Przyr. Ojcz. 68, 1: 55-58.

Doležel J., Bartoš J., Voglmayr H., Greilhuber J. (2003): Nuclear DNA content and genome size of trout and human. Cytometry 51A: 127-128.

Haeupler H., Muer T. (2007): Bildatlas der Farn- und Blütenpflanzen Deutschlands. Ulmer Verlag, Stuttgart.

Hanson L., Boyd A., Johnson M.T., Bennett M.D. (2005): First nuclear DNA C-values for 18 eudicot families. Ann. Bot. 96: 1315-1320.

Internet: http://www.pfaf.org (03.11.2008).

Kruk J., Szymańska R. (2009): Rukiew drobnolistna *Nasturtium microphyllum* (Boenn.) Rchb. – nowe stanowiska w okolicach Krakowa. Chrońmy Przyr. Ojcz. 65, 4: 279-286.

Morozowska M., Czarna A., Jędrzejczyk I. (2010): Estimation of nuclear DNA content in *Nasturtium* R. Br. by flow cytometry. Aquat. Bot. 93: 250-253.

Naquinezhad A. (2006): A short note on the genus *Nasturtium* R. Br. (Cruciferae) and a new hybrid state from this genus for Iran. Iran. J. Bot. 12, 1: 75-77.

Podgórska B., Podgórski A. (2004): Polski zielnik kulinarny. Krajowe zioła jadalne i przyprawowe. Leksykon i przepisy. Wydawnictwo Kurpisz, Poznań.

Rozporządzenie Ministra Środowiska z dnia 5 stycznia 2012 r. w sprawie gatunków dziko występujących roślin objętych ochroną. Dz.U. nr 168, poz. 1764 z dnia 28 lipca 2004 r.

Rutkowski L. (1998): Klucz do oznaczania roślin naczyniowych Polski Niżowej. Wyd. Nauk. PWN, Warszawa.

Smoczyk M. (2001): *Nasturtium microphyllum* (Boenn.) Rchb. In: Polska czerwona księga roślin. Eds R. Kaźmierczakowa, K. Zarzycki. Inst. Bot. im. W. Szafera PAN, Inst. Ochr. Przyr. PAN, Kraków: 158-160.

Suda J., Kyncl T., Freiova R. (2003): Nuclear DNA amounts in Macaronesian angiosperms. Ann. Bot. 92: 153-164.

Śliwińska E., Zielińska E., Jędrzejczyk I. (2005): Are seeds suitable for flow cytometric estimation of plant genome size? Cytometry 64A: 72-79.

Tacik T. (1985): *Nasturtium* R. Br., Rukiew [Water cress]. In: Flora Polski. Ed. A. Jasiewicz. Vol. 4. Rośliny naczyniowe. PWN, Warszawa–Kraków: 187-192.

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