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Pilosella bauhinii (Schult.) Arv.-Touv. and *P. cymosa* subsp. *vaillantii* (Tausch) S. Bräut. & Greuter (Asteraceae) from new localities in north-central Poland

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Abstract

We provide information about two species, *Pilosella bauhinii* and *P. cymosa* subsp. *vaillantii* (Asteraceae), from new localities in north-central Poland (ATPOL DC18). All individuals of both species grew on a railway embankment in Górzno-Lidzbark Landscape Park. The ecological preferences for *P. cymosa* and *P. bauhinii* estimated according to average Ellenberg values nearly coincided with the original description of Ellenberg. We also sequenced the barcode marker, plastid *trnH-psbA* intergenic spacer, for two individuals of *P. bauhinii*, three specimens of *P. cymosa* subsp. *vaillantii*, and additionally two individuals of *P. officinarum* L. and one *H. murorum* L. growing in close proximity. A pairwise comparison of *trnH-psbA* sequences showed that each species has a unique haplotype. Taking into account their morphological coherence, it is possible that both *P. bauhinii* and *P. cymosa* have not hybridized yet, at least locally, with the more abundant species (e.g., *P. officinarum*). A search for reference sequences did not provide additional information because of the low quality of the reference database for this group in GenBank. Only 14 sequences of *trnH-psbA* were available with some apparently being misidentified or of low quality. None were identical to sequences of *P. cymosa* subsp. *vaillantii* and *P. bauhinii* found in this study.

Keywords: *trnH-psbA* intergenic spacer; GenBank; Górzno-Lidzbark Landscape Park; hawkweed; railway

Introduction

The genus *Pilosella* Vaill. [formerly *Hieracium* L. subgenus *Pilosella* (Hill) S.F. Gray] is considered one of the most complicated genera in European flora [1]. This is due to hybridization, polyploidization and a complicated reproductive system including apomixis [1,2]. Plants from this genus prefer open habitats such as grassland, rocky outcrops and heathlands. Many species, moreover, successfully colonize man-made habitats, for example, railways and roadsides [3]. Especially interesting is their occurrence on railways, because these linear landscape elements are considered as migration avenues for many alien as well as rare and endangered species [4–6]. In Poland, species of *Pilosella* are often locally rare, and some are considered endangered [7–10]. It is therefore important to document their occurrence to estimate local richness and distribution.

The correct identification of plant material is crucial as mistakes confound interpretation of the biogeographical pattern and preclude correct estimation of local species richness. One useful technique complementary to traditional morphological determination is plant barcoding [11]. However, in difficult taxa such as *Pilosella*, hybridization, polyploidy and speciation via breeding system transition can impede species discrimination. Nonetheless, documenting genetic variation in isolated populations using rapidly evolving markers such as plastid *trnH-psbA* intergenic spacer [11] can provide information about their origin and putative hybridization when compared to a reference database. A robust database with an extensive taxonomic coverage may also facilitate the identification process providing information on which haplotype should be expected in collected specimens if morphological determination is correct.

In this paper, we provide information about two rare species from the genus *Pilosella*, *P. cymosa* subsp. *vaillantii* (L.) F.W. Schultz & Sch. Bip. and *P. bauhinii* (Schult.) Arv.-Touv. from new localities in north-central Poland. The population of the former species was discovered in 2003 and of the latter in 2010 during floristic work at Górzno-Lidzbark Landscape Park (ATPOL DC18). We briefly characterize habitat properties and provide *trnH-psbA* sequences for the discovered species and two other species from the genus *Pilosella* and *Hieracium* growing in close proximity to reveal putative hybridization and complement morphological identification.

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Material and methods

The *Pilosella* specimens were initially determined by the first and third author as well as by Dr. Tomasz Załuski (Nicolaus Copernicus University, Poland) as *P. cymosa* and *P. bauhinii*. The final determination was performed by Prof. Zbigniew Szeląg (Jagiellonian University) in the case of *P. bauhinii* and Dr. Franz Schuhwerk (Botanische Staatssammlung München) in the case of *P. cymosa* ssp. *vaillantii*. All plant material was deposited in the TRN herbarium (Fig. S1) and high-resolution scans of specimens are available from the first author on request.

We estimated average Ellenberg values for each stand to characterize the ecological requirements of studied species.

Fresh leaves for molecular analyses were sampled from two specimens of *Pilosella bauhinii*, three specimens of *P. cymosa*, two individuals of *P. officinarum* F. Schultz & Schultz-Bip. and one *H. murorum* L. growing nearby. Total genomic DNA was isolated from ca. 50–100 mg of fresh leaf tissue using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). The entire intergenic spacer *trnH-psbA* was amplified using primers psbAF and trnH2 [12]. PCR reactions and thermal cycling was set up according to Fazekas et al. [12]. The PCR products were sequenced using forward and reverse primers with the BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies) and resolved using a DNA sequencer. All newly obtained sequences were deposited in GenBank (accession numbers: KP890072–KP890079).

BLAST searches were performed against the GenBank database at NCBI for each new DNA sequence to determine its similarity with deposited sequences.

Results and discussion

All individuals of both species grew on the railway embankment approximately 150 m from one another. The railway was surrounded by deciduous forest, wet meadows and willow thickets. Railway embankments are often considered as good migration corridors for thermophilic plants [4] such as members of the genus *Pilosella*. We observed dozens of individuals of *P. cymosa*, but only several specimens of *P. bauhinii*. According to Ellenberg values, individuals of both species occurred in sunlit, dry, warm and more or less infertile and weakly acidic sites in the study area (Tab. 1). This characteristic nearly coincided with the original description of ecological preferences for *P. cymosa* and *P. bauhinii* [13] (Tab. 1).

Both species of *Pilosella* considered in this study are rare in Poland. *Pilosella bauhinii* was recorded mainly in the north, south and west of Poland. The newly described stand occur close to three previously reported localities – two historical ones recorded by Abromeit et al. [14] in the vicinity of Olsztyn and Brodnica and a third found near Kwidzyn by Rutkowski [15]. According to the literature, this species prefers dry and open places including grasslands, ruderal and pioneering communities [13,16]. Similar preferences were encountered in our study (Tab. 1).

Pilosella cymosa is rarer than *P. bauhinii* and has been found only in a few localities in Poland, mainly in Dolny Śląsk [17]. It is therefore listed as an endangered species in Poland [10], and included in local red lists for several regions such as Kujawsko-Pomorskie [9], Wielkopolska [8] and Pomorze Gdańskie [7]. Our new locality in Górzno-Lidzbark Landscape Park is isolated, although several occurrences of this species in the region were recorded in the 19th century [14,18]. Kępczyński and Rutkowski [19], moreover, observed this species near Kwidzyn (ATPOL DB52). However, there is no known record of subsp. *vaillantii* in Poland [18].

The length of the *trnH-psbA* sequences varied from 393 bp (*H. murorum*) to 426 bp (*P. bauhinii*). All sampled individuals within *P. bauhinii*, *P. cymosa* subsp. *vaillantii* and *H. murorum* had the same *trnH-psbA* haplotype. In contrast, no identical haplotypes were identified among species (Tab. 2). Hence, our sample included separated entities at both molecular and morphological levels. It is possible that both species have not hybridized yet or served only as a pollen donor for more abundant species (e.g., *P. officinarum*). Search for reference sequences did not provide additional information because of the low quality of the reference

Tab. 1 The average Ellenberg values for phytosociological relevés for light (L), temperature (T), moisture (M), soil reaction (R) and nitrogen content (N). The original Ellenberg values for *Pilosella bauhinii* and *P. cymosa* s. l. are also provided.

Relevé No.	No. of species	L	T	M	R	N
P. bauhinii						
1	15	7.2 ±2.6	6.1 ±2.0	3.8 ±1.5	6.6 ±2.4	4.6 ±1.9
Ellenberg values for *P. bauhinii*		8	5	3	5	3
P. cymosa* subsp. *vaillantii						
2	39	6.7 ±1.8	5.6 ±1.4	4.6 ±1.8	6.9 ±1.6	4.3 ±1.9
3	11	6.8 ±2.6	6.0 ±2.2	3.8 ±1.6	7.0 ±2.9	4.6 ±2.3
Ellenberg values for *P. cymosa* s. l.		7	6	3	8	2

Tab. 2 A pairwise comparison of *trnH-psbA* sequences for *Pilosella* and *Hieracium* species sampled in the study. Number of nucleotide differences, number and length of indels and poly(A) motifs are provided.

Species	No. of nucleotide differences	Indels	Poly(A)
P.b. vs. *P.c.*	1	2 (-/13, 18/-)	2 (10/8, 8/4)
P.b. vs. *H.m.*	6	2 (13/-, 18/-)	3 (10/11, 8/4, 7/8)
P.b. vs. *P.o.*	0	2 (-/13, 18/-)	2 (10/14, 8/4)
P.c. vs. *H.m.*	7	2 (13/-, 13/-)	2 (8/11, 7/8)
P.c. vs. *P.o.*	1	0	1 (8/14)
H.m. vs. *P.o.*	6	2 (-/13, -/13)	2 (11/14, 8/7)

P.b. – *Pilosella bauhinii*; *P.c.* – *P. cymosa* subsp. *vaillantii*; *P.o.* – *P. officinarum*; *H.m.* – *Hieracium murorum*.

database for genera *Pilosella* and *Hieracium* in GenBank. There were only 14 sequences of *trnH-psbA* and no identical matches to haplotypes of *P. bauhinii* and *P. cymosa* subsp. *vaillantii*. Moreover, a detailed examination of GenBank resources revealed that the sequences were in some cases misidentified (e.g., *H. murorum* FR865084 likely confused with *Picris* L.) or were of low quality (e.g., *P. officinarum* HE966661 sequence shows multiple 1 bp insertions at the end). Ironically, both examples come from barcoding studies [20,21] which provides yet another example pointing to the need for careful taxonomic assessment and documentation in barcoding projects. In summary, future research in *Hieracium*/*Pilosella* barcoding should focus on generating a robust reference database to provide complementary information for morphological identification.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: performed floristic surveys: RP, LR; analyzed the molecular data: MP; performed molecular laboratory work: DW; wrote the text: MP, RP.

Competing interests

No competing interests have been declared.

Supplementary material

The following supplementary material for this article is available online at http://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.2015.043/0:

1. Fig. S1: digital images of *Pilosella* deposited in TRN herbarium.

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