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The First Evidence of a Host-to-Parasite Mitochondrial Gene Transfer in Orobanchaceae

Dagmara Kwolek[1], Magdalena Denysenko-Bennett[1*], Grzegorz Góralski[1], Magdalena Cygan[1], Patryk Mizia[1], Renata Piwowarczyk[2], Marek Szklarczyk[3] and Andrzej J. Joachimiak[1]

[1]*Department of Plant Cytology and Embryology, Institute of Botany, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland*

[2]*Department of Botany, Institute of Biology, Jan Kochanowski University, Świętokrzyska 15, 25-406 Kielce, Poland*

[3]*Unit of Genetics, Plant Breeding and Seed Science, Institute of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Cracow, Al. 29 Listopada 54, 31-425 Cracow, Poland*

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Several parasitic plants are known to have acquired mitochondrial genes via a horizontal transfer from their hosts. However, mitochondrial gene transfer in this direction has not yet been found in the parasite-rich family Orobanchaceae. Based on a phylogenetic analysis of the mitochondrial *atp6* gene in selected species of *Orobanche* s.l., we provide evidence of a host-to-parasite transfer of this gene in *O. coerulescens*, which is a Eurasiatic species that parasitises *Artemisia* (Asteraceae). We did not find the original *Orobanche atp6* gene in this species, which suggests that it has been replaced by a gene that was acquired from Asteraceae. In addition, our data suggest the occurrence of a second HGT event in the *atp6* sequence – from Asteraceae to *Phelipanche*. Our results support the view that the transfer of genetic material from hosts to parasites influences the mitochondrial genome evolution in the latter.

Keywords: *Orobanche*, HGT, horizontal gene transfer, *atp6*, mitochondrion

INTRODUCTION

Horizontal gene transfer (HGT) is the non-sexual DNA transmission between distantly related organisms that avoids the cross-species barriers. For prokaryotes and several unicellular phagotrophic protists, HGT is a common and important way of acquiring novel genes (Ochman et al., 2000; Koonin et al., 2001; Keeling and Palmer, 2008). A number of studies have shown that HGT is also not uncommon in land plants, especially those with a parasitic lifestyle (Richardson and Palmer, 2007; Sanchez-Puerta, 2014). A common way to detect HGT is to search for incongruences between gene trees and widely accepted species relationships (Keeling and Palmer, 2008; Renner and Bellot, 2012; Davis and Xi, 2015).

DNA-containing plant organelles do not participate in HGT equally. Mitochondria are very active in the DNA uptake and have been shown to acquire both single and multiple genes via HGT (Bergthorsson et al., 2004; Richardson and Palmer, 2007; Xi et al., 2013; Mower et al., 2010). Horizontal transfers of nuclear genes are significantly less frequent and there is no conclusive evidence of plastid-to-plastid HGT (Richardson and Palmer, 2007; Zhang et al., 2014; Sanchez-Puerta, 2014).

Horizontal transfer involving mitochondrial genes has been found in parasitic plants that belong to ten angiosperm families and in the majority of such cases, the genes were transferred from the host to the parasite (Davis and Xi, 2015 and references herein). Multiple host-to-parasite transfers of mitochondrial genes have been observed in the family Rafflesiaceae (Barkman et al., 2007; Xi et al., 2013). However, in another parasite-rich fam-

* Corresponding author, email: magdalena.denysenko@doctoral.uj.edu.pl

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ily, Orobanchaceae, the transfer of mitochondrial DNA in the opposite direction – from the parasitic *Bartsia* L. to two *Plantago* L. species – was described only once (Mower et al., 2004). According to Bennett and Mathews (2006), Orobanchaceae is the largest parasitic angiosperm family. It comprises 90 genera and includes 2060 species, most of which are hemi- or holoparasites of other plants (McNeal et al., 2013). The family has a worldwide distribution with species-rich centers in the Mediterranean, western and central Asia, northern Africa and North America. The most numerous holoparasitic group within Orobanchaceae is *Orobanche* s.l., which comprises ca. 200 *Orobanche* L. and *Phelipanche* Pomel species (Pusch and Günther, 2009).

There are several examples of non-mitochondrial sequences that have been acquired by Orobanchaceae from other (distantly related) plant lineages, for instance a nuclear gene with an unknown function from *Sorghum bicolor* (L.) Moench in *Striga hermonthica* (Delile) Benth. and a chloroplast sequence of *Haloxylon ammodendron* (C.A.Mey.) Bunge ex Fenzl in the nuclear genome of *Cistanche deserticola* Ma (Li et al., 2013). HGT events can also be observed between members of the same plant family, i.e., in Orobanchaceae plastid genes *rbcL* and *rps2* of *Orobanche* were found in *Phelipanche* (Manen et al., 2004; Park et al., 2007).

In a preliminary phylogenetic study on mitochondrial *atp1*, *atp6*, *cob*, *cox3*, *nad6* and *matR* sequences in *Orobanche* and selected non-parasitic plants, we observed an unexpected placement of *O. coerulescens* Stephan ex Willd. in the *atp6* gene tree (closer to *Artemisia* L. than to other *Orobanche* species). Because the horizontal transfer of the *atp6* gene has been reported in at least three angiosperm species – *Plantago coronopus* L., *P. macrorhiza* Poir. and *Amborella trichopoda* Baill. (Mower et al., 2010; Bergthorsson et al., 2004), we suspected that the unusual placement of *O. coerulescens* in the *atp6* tree might have resulted from HGT. *Orobanche coerulescens* is a species with a Eurasian distribution (Pusch and Günther, 2009) and a narrow host range. It parasitises *Artemisia* spp., mainly *A. campestris* L., and grows in the habitats of the host (steppes, rocky grasslands and sandy areas). This broomrape is very rare or extinct in most of its original localities at the western limit of its distribution (Piwowarczyk and Przemyski, 2009).

This study presents a molecular phylogenetic analysis of the mitochondrial *atp6* and chloroplast *trnL-trnF* sequences from some representatives of *Orobanche* s.l. (including *O. coerulescens* plants that originated from three different localities), *Artemisia campestris* and some other more and less related species. The *atp6* gene codes for the 6th subunit of F-type ATP synthase, which plays a crucial role in the process of oxidative phosphorylation. The gene is considered to be very conservative within the core of its coding region, whereas its 5' pre-sequences show variations in both closely and distantly related species (Soltani et al., 2014). We used the chloroplast sequence in conjunction with the mitochondrial gene because plastids are considered to be immune to HGT (Richardson and Palmer, 2007). Among them, the *trnL-trnF* sequence is relatively short and easy to amplify even from a suboptimal starting material. It has been successfully used for phylogenetic studies and barcoding in plants (e.g., Gielly and Taberlet 1994; Drábková et al., 2004; Hao et al., 2009). Also we successfully used this sequence for phylogenetic analysis of *Orobanche* and *Phelipanche* (unpublished data).

MATERIALS AND METHODS

PLANT MATERIAL

The specimens of *Orobanche coerulescens* that had *Artemisia* as their host species were collected during a field study in Poland (central Europe) in 2013 and in Georgia (Caucasus, western Asia) in 2014. The analyzed samples, collected in the years 2009–2014, include selected sections of *Orobanche* and *Phelipanche*, which originated from Poland, Austria and Georgia (Table 1). Specimens were deposited in the herbarium of the Jan Kochanowski University in Kielce (KTC). In addition, one specimen of *Lindenbergia sinaica* (Decne.) Benth. was analyzed as it is an autotrophic, non-parasitising sister taxon to all parasitic Orobanchaceae. In a few instances, additional sequences were taken from GenBank in order to confirm the position of species in the trees. The respective voucher information as well as the geographic origin and GenBank accession numbers are listed in Table 1.

MOLECULAR ANALYSES

Total cellular DNA was obtained from silica gel-dried material following Piwowarczyk et al. (2015). The partial sequences encoding the ATP synthase F_o subunit 6 (ATP6) were amplified using the atp6-r primer (Szklarczyk, 2016) and a newly designed atp6-f4 primer (5'-GGAAACTTUTATTTCTCATTCAC-3'). A plastid DNA fragment comprising the *trnL* intron, the *trnL* 3' exon and the intergenic spacer between the latter and the *trnF* gene (later described as *trnL-trnF*) was amplified using primers c and f (Taberlet et al., 1991). The PCR reactions (15 μl total volume) contained 1 × Dream Taq Buffer (Thermo Scientific), 0.25 mM dNTPs (Thermo Scientific), 0.25 μM each primer, 1.125 U of

TABLE 1. List of investigated species with GenBank accession numbers and voucher information or literature reference. Sequences obtained from the GenBank are indicated by an asterisk; those of an unknown origin are indicated by a question mark.

Taxon	Accession number		Origin	Voucher information or reference
	atp6	*trnL–trnF*		
Artemisia campestris	KU180471	–	Poland	Pasturka, Piwowarczyk 2013 (KTC)
Artemisia campestris	–	JX073793*	United Kingdom	Hobbs and Baldwin, 2013
Centaurea scabiosa	KU180476	KU238878	Poland	Sławków, Piwowarczyk 2013 (KTC)
Helianthus annuus ssp. *texanus*	X82388*	–	?	Spassova et al., 1994
Helianthus tuberosus	–	GU818008*	USA	Pelser et al., 2010
Lindenbergia sinaica	KX524674	KX524675	Israel	Eliat, Gabrielyan et al. 1998 (ERE)
Magnolia kobus	–	AY743457*	Japan	Pirie et al., 2005
Magnolia stellata	KC879653*	–	USA	Richardson et al., 2013
Mimulus guttatus	–	AY575533*	USA	Beardsley et al., 2004
Mimulus guttatus	JN098455*	–	?	Mower et al., 2012
Orobanche alba ssp. *alba*	KU180469	KU238873	Poland	Bieszczady Mts, Połonina Caryńska, Piwowarczyk 2009 (KTC)
Orobanche caryophyllacea	KU180465	KU238869	Poland	Chomentówek, Piwowarczyk 2013 (KTC)
Orobanche cernua	KU180472	KU238874	Georgia	Between Rustavi and Idumala, Piwowarczyk 2014 (KTC)
Orobanche coerulescens	KU180462	KU238865	Poland	Dobrowoda, Piwowarczyk 2013 (KTC)
Orobanche coerulescens	KU180461	KU238864	Poland	Pasturka, Piwowarczyk 2013 (KTC)
Orobanche coerulescens	KU180473	KU238875	Georgia	Between Rustavi and Idumala, Piwowarczyk 2014 (KTC)
Orobanche elatior	KU180470	KU238866	Poland	Sławków, Piwowarczyk 2013 (KTC)
Orobanche gracilis	KU180467	KU238871	Austria	Hundsheim, Piwowarczyk 2014 (KTC)
Orobanche grenieri	KU180474	KU238876	Georgia	Kortaneti, Piwowarczyk 2014 (KTC)
Orobanche picridis	KU180463	KU238867	Poland	Pęczelice, Piwowarczyk 2013 (KTC)
Peucedanum cervaria	KU180475	KU238877	Poland	Długoszyn, Piwowarczyk 2013 (KTC)
Phelipanche arenaria	KU180464	KU238868	Poland	Zwierzyniec, Piwowarczyk 2013 (KTC)
Phelipanche bohemica	KU180468	KU238872	Poland	Zawiercie, Piwowarczyk 2010 (KTC)
Phelipanche ramosa	KU180466	KU238870	Poland	Szewce, Piwowarczyk 2013 (KTC)
Solanum tuberosum	AF095276*	–	Germany	Lössl et al., 1999
Solanum villosum	–	GU323356*	?	unpublished

Dream Taq DNA Polymerase (Thermo Scientific) and 30 ng of a DNA template.

Amplifications were performed using a T-100 Thermal Cycler (Bio-Rad) and a PCR program that consisted of an initial denaturation for 5 min at 94°C, followed by 35 cycles of denaturation for 45 s at 92°C, annealing for 45 s at 57°C, an extension for 2 min at 72°C and a final extension of 10 min at 72°C. The same program was used for both primer pairs. The products that were obtained were sequenced following the procedure previously described in Piwowarczyk et al. (2015).

SEQUENCE ANALYSIS

DNA sequences were aligned using ClustalW 2.1 (Larkin et al., 2007) for *atp6* and Probcons 1.12 (Do et al., 2005) for *trnL-trnF* and then manually corrected and trimmed. The final alignment of the *atp6* region was 642 bp long (no indels were present), whereas the *trnL-trnF* alignment was 1046 bp long.

Phylogenetic trees were generated using the maximum likelihood and Bayesian methods. We used the *atp6* sequence of *Magnolia stellata* (Siebold & Zucc.) Maxim. and the *trnL-trnF* sequence of *M. kobus* DC. as the outgroups. Substitution models were determined using the jModelTest 2 (Guindon and Gascuel, 2003; Darriba et al., 2012) for three schemes (JC/F81, K80/HKY, SYM/GTR) using AIC.

The maximum likelihood trees were generated using PhyML software version 20131022 (Guindon et al., 2010) with the following main options: no. of bootstrap replications = 1000; general time reversible model with invariable sites and discrete gamma rate heterogeneity (GTR+I+G) substitutions model (Posada, 2003); ML estimation of the proportion of invariable sites and gamma shape parameter, optimization of the tree topology, branch length and substitution rate parameters.

For the Bayesian trees, mrBayes v. 3.2.2 software (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) was used with the following main settings: ngen=10000000, samplefreq=1000, nchains=4, temp=0.2, checkfreq=50000, diagnfreq=1000, stopval=0.01, stoprule=yes and substitution model GTR+G+I.

The trees that were generated were visualized and adjusted using Mesquite 3.04 software (Maddison and Maddison, 2015).

Also, we generated phylogenetic trees for sequences of the ITS region (ITS1-5.8S-ITS2). However, because the results were generally similar to those that were obtained from the *trnL-trnF* data, the respective procedures and results are not included in this paper.

RESULTS

All of the PCR products of the *atp6* sequences were approximately 670 bp long. The lengths of the *trnL-trnF* products varied and they were approximately 990 bp long for *Orobanche* sp., 870 bp for *Phelipanche* sp., 950 bp for *Peucedanum cervaria* (L.) Lapeyr. and 920 bp for *Centaurea scabiosa* L.

For further analyses, the *atp6* sequences were trimmed to the length of 642 bp. No stop codons or indels were observed within the aligned sequences, which suggests that the investigated sequences did not represent pseudogenes. Species with identical sequences were distinguished – a) *O. cernua* Loefl. and *O. grenieri* F.W. Schultz, b) *O. coerulescens* and c) *P. arenaria* Borkh. and *P. ramosa* (L.) Pomel.

The *trnL-trnF* sequences were much more divergent and had many indels (up to 162 bp in length). Only two sequences of *O. coerulescens* (*GenBank*: KU238864; *GenBank*: KU238865) were identical. The aligned and trimmed sequences had 1062 sites but the number of nucleotides ranged from 627 in *P. arenaria* (three *Phelipanche* species had the shortest sequences in the set) to 858 in *O. coerulescens* (KU238875).

Figures 1 and 2 present the phylogenetic trees that were obtained using Bayesian analysis. The ML trees were very similar, so they are not shown. There were only small differences regarding *O. gracilis* Sm. relationship to other *Orobanche* species. The comparisons of the *trnL-trnF* and *atp6* sequences are presented in Fig. 1. The *O. coerulescens* specimens on both of the trees that were generated for *trnL-trnF* are grouped together with other *Orobanche* species and close to the *Phelipanche* species. They are sister clades to *Mimulus guttatus* DC. (Phrymaceae) and *Lindenbergia sinaica*, which are non-parasitic species that belong to the same order (Lamiales); the latter one belongs to Orobanchaceae and its genus is regarded to be sister to the holoparasites and hemiparasites in the family (Bennett and Mathews, 2006). The species of the Asteraceae family (*Helianthus annuus* L., *Centaurea scabiosa*, *Artemisia campestris*) that were tested are grouped in a clearly separate clade (further referred to as the host group). This arrangement is in accordance with the generally accepted relationships among the investigated species. Conversely, the three *O. coerulescens* samples in both of the *atp6* trees form one clearly separate branch with all of the investigated taxa of Asteraceae. Such results suggest a horizontal gene transfer between the host and parasite *O. coerulescens*. The postulated HGT is shown in Figures 1 and 2.

Because our results showed possible HGT, we performed closer analysis of the *atp6* sequences

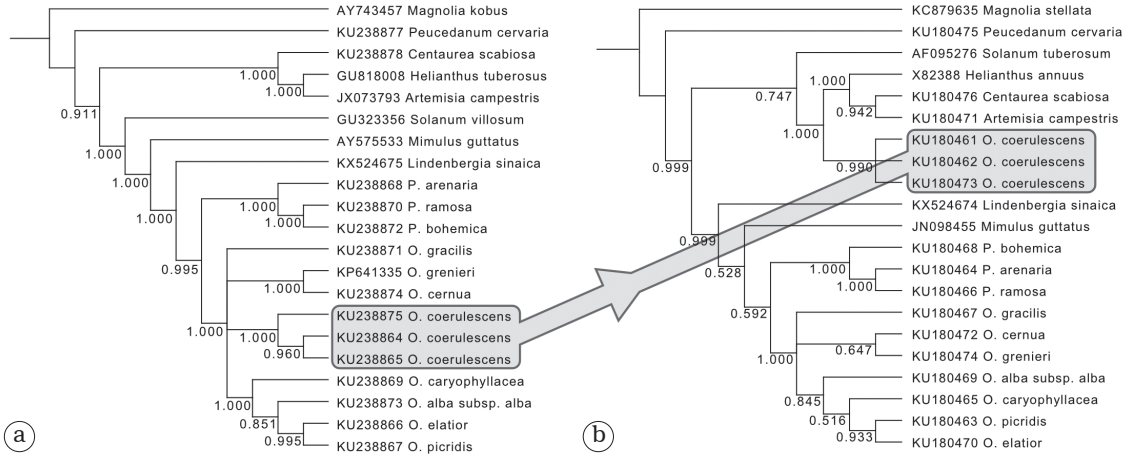

Fig. 1. Rooted Bayesian phylogenetic trees constructed for the investigated *trnL-trnF* (**a**) and *atp6* (**b**) regions. The phylogenetic tree for the *atp6* regions shows a conflicting phylogenetic signal for placement of *O. coerulescens* caused by the supposed HGT event(s). The postulated HGT is marked by boxes and an arrow. Numbers near nodes show Bayesian posterior probabilities.

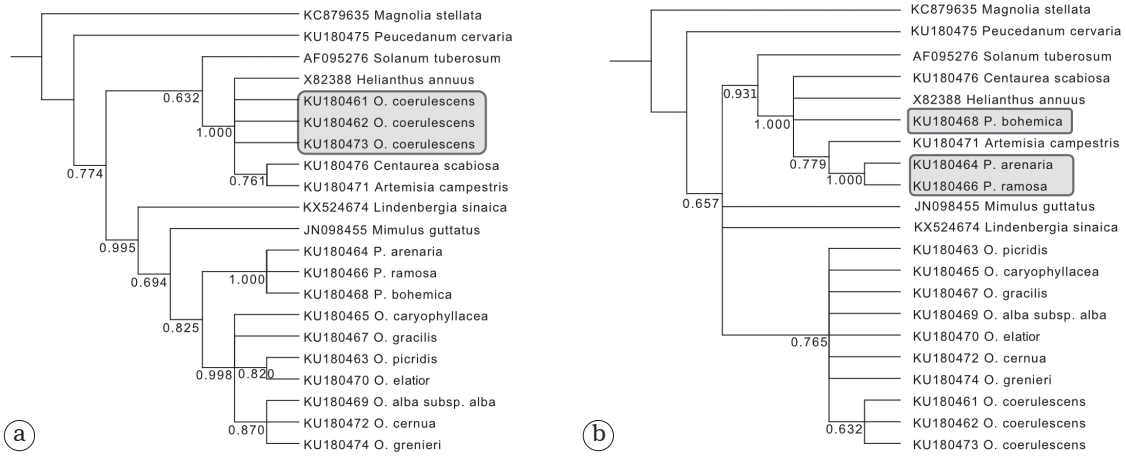

Fig. 2. Rooted Bayesian phylogenetic trees constructed from partial sequences of the investigated *atp6* region corresponding to the supposed transferred segments: HGT1 (**a**), HGT2 (**b**). The postulated HGT is marked by boxes. Numbers near nodes show Bayesian posterior probabilities.

(Fig. S1 in the supplementary material). The three Asteraceae species that were analyzed possessed almost identical *atp6* sequences that differed from the typical *Orobanche* sequence (occurring in *O. picridis* F. W. Schultz, *O. caryophylalcea* Sm., *O. gracilis*, *O. alba* Stephan ex Willd., *O. elatior* Sutton, *O. cernua* and *O. grenieri*) in 36 (5.6%) nucleotide positions. In 28 of these sites, *O. coerulescens* had the same nucleotides as in the Asteraceae that were investigated (Tab. 2). The single-nucleotide polymorphisms between this species and other representatives of *Orobanche* were estimated to be 4.4%. Interestingly, in 13 out of the 36 sites discussed, *Phelipanche* had nucleotides of the 'Asteraceae' type.

We identified some characteristic sites/nucleotides as: common for the host group and some taxa of Orobanchaceae (*O. coerulescens*, *Phelipanche*) and which were typical of the genus *Orobanche* except *O. coerulescens*. The sequences of *O. coerulescens* were similar to their host counterparts in two segments that were separated and were followed by sequences that were quite similar to the rest of Orobanchaceae. It was not possible to indicate the strict borders between the native and transferred segments but using the first and last

TABLE 2. Nucleotide sites of the *atp6* region differentiating Asteraceae from "typical" sequences of *Orobanche* species. NC – nucleotide-site, Cs – *Centaurea scabiosa*, Ac – *Artemisia campestris*, Ha – *Helianthus annuus*, Oc – *O. coerulescens*, Or – other *Orobanche* species, Ph – *Phelipanche* species, x – nucleotides common for Asteraceae and *O. coerulescens*/*Phelipanche* and different from other *Orobanche* s.l. A – nucleotides indicating HGT to *O. coerulescens*, B – nucleotides indicating HGT to *Phelipanche*, * – sites that are common for *O. coerulescens*/*Phelipanche* and *Asteraceae* but also for *Lindenbergia* and/or *Mimulus*, so not informative for HGT, N_A – sites that indicate absence of HGT1, N_B – sites that indicate absence of HGT2.

NC	Asteraceae			Orobanchaceae			HGT
	Cs	Ac	Ha	Oc	Or	Ph	
3	x	x	x	x	–	–	A
6	x	x	x	x	–	–	A
28	x	x	x	x	–	–	A
31	x	x	x	x	–	–	A
51	x	x	x	–	–	–	N_A
65	x	x	x	–	–	–	N_A
80	x	x	x	x	–	x	*
81	x	x	x	x	–	x	*
88	x	x	x	–	–	x	N_A
91	x	x	x	x	–	x	*
111	x	x	x	x	–	x	*
116	x	x	x	x	–	–	A
141	x	–	x	x	–	–	A
185	x	x	x	x	–	–	A
189	x	x	x	x	–	–	A
203	x	x	x	x	–	–	A
211	x	x	x	x	–	–	A
219	x	x	x	x	–	–	*
249	x	x	x	x	–	–	*
262	x	x	x	x	–	x	*
265	x	x	x	x	–	x	*
273	x	x	x	x	–	–	A
323	x	x	x	x	–	–	A
350	x	x	x	x	–	–	A
375	–	–	x	x	–	–	A
408	x	x	x	x	–	–	A
409	x	x	x	x	–	–	A
412	x	x	x	x	–	–	A
531	–	x	x	x	–	–	A
537	x	x	x	x	–	–	A/ N_B
548	x	x	x	x	–	x	A, B
585	x	x	x	–	–	x	N_A/*
605	x	x	x	–	–	x	B
613	x	x	x	–	–	x	B
620	x	x	x	–	–	x	B
629	x	x	x	–	–	x	B

nucleotides that are the same in the host group but are different from *O. coerulescens* ('core' regions of HGTs), it was possible to distinguish two transferred parts that spanned nucleotides 1–31 and 116–548 (further referred to as HGT1). Moreover, we observed a second possible HGT (HGT2) between *Phelipanche* and its host. This was found near the end of the tested sequences – starting from nucleotide 548 and probably extending further downstream from the studied *atp6* region.

The nucleotide sites that indicate HGTs are presented in Table 2. These nucleotides, which are common between 'Asteraceae' and the target taxa, are different from the sequences of *Mimulus* and/or *Lindenbergia* that are close taxons to *Orobanche* and *Phelipanche*. It indicates that similarities between Asteraceae and the parasites with HGTs are not inherited from a common ancestor. The aligned *atp6* sequences with the characteristic sites indicated are shown in Supplementary Fig. S1.

Next, we constructed phylogenetic trees, which were based on the sequences corresponding only to the HGT1 and HGT2 parts. The results, which are presented in Fig. 2, support the scenario of the two postulated horizontal gene transfers. The *atp6* sequence that was gained in HGT1 showed the highest similarity to the sequence of *Helianthus*. In the case of HGT2, the species are not so clearly grouped but the genus *Artemisia* seems to be the most probable source of the transferred sequence. The pattern of the HGTs is presented in Fig. 3.

DISCUSSION

The phylogenetic tree obtained from the analysis of the *trnL-trnF* sequences presented in Fig. 1a shows the actual evolutionary relationships among the investigated species (e.g., Manen et al., 2004; Oxelman et al., 2005; Bennett and Mathews, 2006; Park et al., 2007). However, the analysis of the *atp6* sequences resulted in quite a different outcome. The gene tree in Fig. 1b shows that the sequences that were found in *O. coerulescens* are more similar to those found in representatives of the Asteraceae family (*A. campestris*, *C. scabiosa*, *H. annuus*), especially to *Helianthus*. This position of *O. coerulescens* is in contrast with the *trnL-trnF* tree, which reflects the widely accepted relationships between the analyzed taxa.

These results indicate that the *atp6* gene was transferred from a member of Asteraceae to *O. coerulescens* or to one of its ancestors, apparently via HGT. The fact that *Artemisia campestris* is currently a host for *O. coerulescens* supports this assumption; however, it is worth noting that the *atp6* sequences of *A. campestris* are more similar to those of the rest of the analyzed Asteraceae species than to that of *O. coerulescens*. The most similar sequence to *O. coerulescens* belongs to *Helianthus*, which may suggest that this genus might be a donor *atp6*, especially due to the fact that it was also found to be a possible host of *O. coerulescens* (Liu and Li, 1988). However, *Helianthus* is a rather improbable source of the transferred sequence, because it is a New World plant, so was imported to Eurasia not earlier that c.a. 500 years ago and the transfer is relatively old (see below). Consequently, the donor was an unidentified taxon of Asteraceae, possibly extinct. However, the investigation of a wider spectrum of hosts and their relatives may shed more light on that problem.

A more thorough analysis showed that the *atp6* sequence of *O. coerulescens* consists of segments that have different affinities – two of them corresponded to the respective fragments in Asteraceae and the other two were similar to the corresponding fragments in all of the other *Orobanche* species studied (Fig. 3). This probably indicates a recombination between the original and acquired sequences. During our analysis, we were not able to find the original copy of the *atp6* gene in *O. coerulescens*. The sequence of the hybrid *atp6* gene that was found in this species showed no indels or stop codons, which suggests that it might be active.

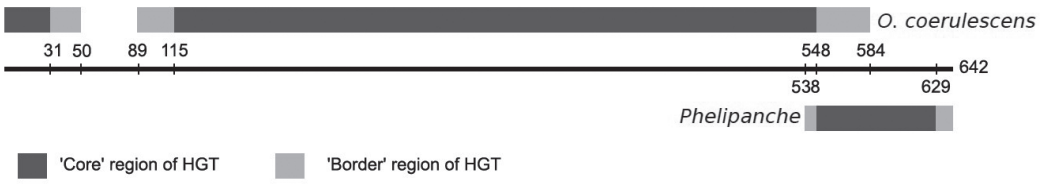

Fig. 3. Extent of the proposed HGT events in the *atp6* sequence studied. The line indicates the entire investigated *atp6* sequence with critical positions annotated. The proposed HGT regions are marked above (HGT1 in *O. coerulescens*) and below (HGT2 in *Phelipanche*). The 'core' HGT regions encompass nucleotides that are common to the 'Asteraceae' group and the proposed recipient species. 'Border' regions are not informative for HGT – they extend between nucleotides that indicate HGT and those that indicate the absence of HGT.

It is also interesting that the two closest studied *O. coerulescens* relatives (*O. grenieri* and *O. cernua* – sect. *Inflatae*) showed *atp6* similar to the rest of the Orobanchaceae and that the altered gene was found in very distant *O. coerulescens* populations. This suggests that the gene transfer occurred in a direct ancestor of *O. coerulescens* rather than in a common ancestor of the sect. *Inflatae*. Because the samples used in our study did not cover the entire range of *O. coerulescens*, sequences for samples of this species from East Asia and a wider spectrum of the host species would be worth investigating.

A close analysis of the *atp6* sequences also showed a possible HGT trace in the three *Phelipanche* species that were analyzed (Fig. 2b, Fig. 3, Fig. S1, Tab. 2). Unfortunately, the HGT2 segment that did not fit to the other Orobanchaceae was found just at the end of the DNA fragment that was analyzed and it was relatively short. It is very likely that a longer sequence was transferred, but it is not possible to verify this without sequencing the downstream mtDNA segments. Interestingly, in all of the indicative sites (Fig. S1, pos. 548 marked in violet, and pos. 505, 513, 520, 529 marked in red), the HGT2 segment of the Asteraceae and *Phelipanche* that were analyzed contained Ts instead of Cs, which were present in most of the other species that were analyzed. There are two exceptions that had also T in the indicative sites: 1) *O. coerulescens*, *P. cervaria* and *Solanum tuberosum* L. in the first indicative site (Fig. S1, pos. 548, marked in violet) but this is a common site for HGT1 and HGT2; 2) *S. tuberosum* that had also T in the last indicative site (Fig. S1, pos. 629, marked in red). This suggests that prior to the transfer to *Phelipanche*, the respective mtDNA fragment of Asteraceae was substituted with its cDNA copy carrying the footprints of C to U RNA editing. This hypothesis is supported by the fact that all of these sites corresponded to the RNA editing sites that are known from other plant species.

There are several suggested mechanisms of plant-to-plant HGT that include illegitimate pollination, vector-mediated DNA transfer and direct contact between a donor and a recipient. A close physical association between a parasite and its host appears to be the major source of HGTs among flowering plants (Sanchez-Puerta, 2014; Davis and Xi, 2015).

As yet it is unclear how genes are transferred between physically linked organisms, but there is an evidence of a massive macromolecule flow (involving mRNA, siRNA, proteins, viruses and possibly DNA) through the haustorium of *Cuscuta* L., which is a widely known parasite of many plants (Kim et al., 2014; Kim and Westwood, 2015). A similar situation is observed in Orobanchaceae although no mRNA flow has yet been observed. Surprisingly, molecular relics such as the poly(A) tail at the end of a gene with an unknown function that was acquired by *Striga hermonthica* from *Sorghum* Moench suggest that HGT might be an mRNA-mediated process (Yoshida et al., 2010). On the other hand, many of the horizontally transferred DNA fragments are genes with introns or donor-specific introns that are considered to be footprints of HGT events (Renner and Bellot, 2012; Sanchez-Puerta, 2014). This may be explained by the direct uptake of genomic DNA without any cDNA intermediates. Unfortunately, the direct flow of DNA strands between two physically linked plants has only been observed in artificial conditions to date, i.e., during grafting (Stegemann and Bock, 2009).

The importance of HGT in land plants remains unclear because the majority of the genes that are acquired appear to be non-functional in the recipient (Richardson and Palmer, 2007; Rice et al., 2013). However, the cases of horizontal transfer involving fully expressed genes suggest a beneficial influence of HGT on plant evolution. An example would be the albumin 1 KNOTTIN-like protein gene that was acquired from a legume host by a common ancestor of all *Phelipanche* and *Orobanche* species and that was conserved during the evolution of these two parasitic genera (Zhang et al., 2013). Another case of the successful "domestication" of a gene that was acquired from a host involves the Brassicaceae-specific *strictosidine synthase-like* (SSL) gene that is expressed in the root parasite *Orobanche aegyptiaca* Pers. and the shoot parasite *Cuscuta australis* R.Br. (Zhang et al., 2014). It cannot be excluded that host-like gene products help a parasite to avoid host's defenses and to tune in to its metabolites.

Taking into account that at least two independent horizontal gene transfers occurred at the single mitochondrial locus that was analyzed, it seems to be highly probable that HGT may be a frequent process in the mitochondrial genomes of parasitic Orobanchaceae. Further studies that would examine more mitochondrial loci, or even complete mitochondrial genomes, and that would include more Orobanchaceae species should verify this hypothesis.

AUTHOR'S CONTRIBUTIONS

DK – study design, laboratory analysis, analysis and interpretation of data, drafting manuscript; MD-B – laboratory analysis, drafting manuscript, data collection; GG – study design, bioinformatic analysis, analysis and interpretation of data, drafting manuscript; MC – laboratory analysis; PM – drafting manuscript; RP – originator of the research topic, field studies, provision of plant material, critical revision of manuscript; MS – analysis and interpretation of data, critical revision of manuscript; AJJ – original idea, analysis and interpretation of data, critical revision of manuscript.

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