Phylogenetic Relationships Within *Orobanche* and *Phelipanche* (Orobanchaceae) From Central Europe, Focused on Problematic Aggregates, Taxonomy, and Host Ranges

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Holoparasitic genera within the family Orobanchaceae are characterized by greatly reduced vegetative organs; therefore, molecular analysis has proved to be a useful tool in solving taxonomic problems in this family. For this purpose, we studied all species of the genera *Orobanche* and *Phelipanche* occurring in Central Europe, specifically in Poland, the Czech Republic, Slovakia, and Austria, supplemented by samples mainly from Spain, France, Germany, and Ukraine. They were investigated using nuclear sequences (ITS region) and a plastid *trnL-trnF* region. The aim of this study was to examine phylogenetic relationships within *Orobanche* and *Phelipanche* from Central Europe; we focused on problematic species and aggregates, recent taxonomic changes in these (rank and secondary ranks), and host ranges. The most interesting results concern the exlusion of *O. mayeri* from *O. alsatica* aggr. Additionally, following the rules of traditional taxonomy, the correct names and types of some secondary ranks are given and, as a result of this, a new combination below the *Phelipanche* genus is made (*P.* sect. *Trionychon*). The host ranges of the investigated species in Central Europe include 102 species from 12 families, most often from Asteraceae. For this purpose, ca. 400 localities were examined in the field. Moreover, data acquired from the literature and European and Asian herbaria were used.

Keywords: Orobanchaceae, *Orobanche*, *Phelipanche*, phylogeny, host, ITS, *trnL-trnF*, holoparasitic plants

INTRODUCTION

Only about one percent of all known angiosperm species lead a parasitic lifestyle. They belong to different taxonomic groups and are present in most ecosystems from equatorial and tropical rainforests to Arctic areas (Press, 1998). They exert an influence on the structure and dynamics of many ecosystems and can have a positive effect on nutrient circulation and biodiversity (Press and Phoenix, 2005). According to their capacity or lack

of ability to photosynthesize, parasitic plants are divided into photosynthetic hemiparasites and non-photosynthetic holoparasites (Nickrent, 2002). Using special structures known as haustoria, they connect to the host's shoots (shoot parasites) or roots (root parasites).

The family Orobanchaceae Vent., according to its current circumscription, is the largest parasitic plant family, containing 90 genera and 2060 species (McNeal et al., 2013). It remains the only family among plants that includes non-parasitic

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autotrophs as well as root hemiparasites and holoparasites, thus presenting a valuable model for understanding the patterns and pathways of evolution of parasitism (Westwood et al., 2010). Generally, it is believed that hemiparasitism in Orobanchaceae evolved once and was followed by several independent origins of holoparasitism (dePamphilis et al., 1997; Young et al., 1999).

Moreover, some members belonging to the genera *Striga*, *Alectra*, *Aeginetia* or *Orobanche* s.l. are noxious agricultural pests, mostly of sunflower, nightshades, grass and legume crops, so the study of this family is not only scientifically important but also has practical and economic impact (Joel et al., 2013).

Until now, several sequences have been used for reconstructing phylogenetic patterns in Orobanchaceae: plastid *matK* (Hilu and Liang, 1997; dePamphilis et al., 1997; Young et al., 1999), *rps2* (Young et al., 1999), *rbcL* (Manen et al., 2004), nuclear internal transcribed spacer region (ITS) (Schneeweiss et al., 2004a), phytochrome A (PHYA) (Bennett and Mathews, 2006), and phytochrome B (PHYB) (McNeal et al., 2013).

The first molecular approach to Oroban-chaceae phylogeny included the sequence of plastid gene *matK* commonly used in plant taxonomy (Hilu and Liang, 1997; dePamphilis et al., 1997; Young et al., 1999). The results showed that Orobanchaceae s. str. is not a monophyletic group and thus its taxonomical circumscription should be re-evaluated (dePamphilis et al., 1997). The combination of two plastid sequences (*rps2* and *matK*) and an increase of species sampling led to a re-circumscription of the family Orobanchaceae that included incorporation of several genera formerly placed in Scrophulariaceae (Bremer et al., 1997; Young et al., 1999). The newly outlined Orobanchaceae includes Orobanchaceae s. str., hemiparasites traditionally included in Scrophulariaceae (tribes *Buchnereae* and *Rhinantheae*) and autotrophic *Lindenbergia* (subfam. *Antirrhinoideae*) (Nickrent et al., 1998; Young et al., 1999; Young and dePamphilis, 2000; Bremer et al., 2001; Olmstead et al., 2001; McNeal et al., 2013).

The genus *Orobanche* s.l., following the comprehensive monograph of Beck (1930), has traditionally been divided into four sections, namely *Trionychon*, *Osproleon*, *Gymnocaulis*, and *Myzorrhiza*. However, in recent taxonomic, molecular, carpological and palynological approaches, members of these sections have been recognized again and placed in separate genera: *Orobanche* L. (syn. *Orobanche* sect. *Osproleon* Wallr., p.p. max.), *Phelipanche* Pomel (syn. *Orobanche* sect. *Trionychon* Wallr.), monotypic *Boulardia* F.W. Schultz (syn. *O.* sect. *Osproleon* Wallr., p.p. min. [comprising *Orobanche*

latisquama (F.W. Schultz) Batt.]) and *Aphyllon* Mitch. (*Orobanche* sects. *Gymnocaulis* [*Aphyllon*] and *Nothaphyllon* [*Myzorrhiza*]) (e.g. Holub, 1990; Teryokhin et al., 1993; Schneeweiss et al., 2004a, 2004b; Manen et al., 2004; Park et al., 2007a, 2007b; Schneeweiss, 2007; Carlón et al., 2008; Joel, 2009; Joel et al., 2013; Schneider, 2016; Domina, 2017). Some authors still recognize the genus *Orobanche* s.l. in the wider sense used previously (Chater and Webb, 1972; Kreutz, 1995; Velasco et al., 2000; Pujadas Salvà, 2007; Pusch, 2009).

In Orobanchaceae, *Orobanche* L. and *Phelipanche* Pomel are the largest holoparasitic genera that comprise ca. 150–200 species (Pusch and Günther, 2009; Schneider, 2016) which are parasitic on roots of other vascular plants. The family is characterized by worldwide distribution, but the main centers of its diversity are the Mediterranean region, western and central parts of Asia, and North America (Pusch and Günther, 2009). Both the Caucasus and the mountains of Central Asia are possible centers of origin of the genus *Orobanche* s.l., with presence of many endemic species (e.g., Piwowarczyk, 2015a; Piwowarczyk et al., 2015a, 2017a, b, c). In the central and northern parts of Europe, *Orobanche* and *Phelipanche* comprise ~30 species, in many cases rare, endangered or declining ones (e.g., Zázvorka, 1997, 2000; Pusch and Günther, 2009; Piwowarczyk and Przemyski, 2009, 2010; Piwowarczyk et al., 2010, 2011; Piwowarczyk, 2011, 2012a-h, 2014a, b; Piwowarczyk and Krajewski, 2014, 2015).

Orobanche s.l. is difficult for taxonomical and phylogenetic analysis due to the lack or strong reduction of leaves, absence of roots (functionally replaced by haustoria) and intraspecific variability of such features as color or shape of the corolla. Additional problems, such as uniformed darkening of specimens during desiccation, increase the difficulty in labelling or re-evaluating herbal specimens (Kreutz, 1995; Schneeweiss et al., 2009). Otherwise, seed and pollen micromorphology (e.g., Plaza et al., 2004; Tsymbalyuk and Mosyakin 2013; Zare et al., 2014; Piwowarczyk et al., 2015b; Piwowarczyk, 2013, 2015b, c), petal micromorphology (Piwowarczyk and Kasińska, 2017) and floral volatile organic compounds (VOC) (Tóth et al., 2016) proved to be useful as complementary sources of taxonomic data.

Phylogenetic relationships of geographically dispersed species from *Orobanche* and *Phelipanche* have been presented in several works using DNA sequences (Manen et al., 2004; Schneeweiss et al., 2004a; Carlón et al., 2005, 2008; Park et al., 2007b, 2008; Thorogood et

al., 2009), ISSR (Benharrat et al., 2001) as well as RAPD analysis (Román et al., 2003) or proteomic data (Castillejo et al., 2009). The mentioned studies have shown that the results of molecular analyses do not always correspond to the frequently used taxonomy (e.g., Beck, 1930; Novopokrovskii and Tzvelev, 1958; Teryokhin et al., 1993).

Although molecular data frequently allow similar taxa to be distinguished morphologically, in some cases they do not indicate differences between species that are distinguishable morphologically and ecologically, or between potential host specific parasite taxa like in the case of *O. minor* aggr. (e.g., Manen et al., 2004; Thorogood et al., 2008, 2009). There are many similar examples of problematic, variously recognized and closely related species, particularly in areas where species diversity is high but insufficiently known, for example, *Orobanche cernua* s.l. (including *O. australiana*, *O. cumana* or *O. grenieri*), *O. crenata / O. owerinii*, *Phelipanche mutelii / P. nana / P. ramosa* or *P. aegyptiaca / P. brassicae* (e.g., Schneeweiss et al., 2004a; Carlón et al., 2005, 2008; Piwowarczyk et al., 2015a). Sometimes it is not even known how many species can be distinguished in a given taxon, which makes description of phylogenetic relationships between them even harder. In these and many other cases, there is a need for further research (taxonomic, morphological and ecological) in conjunction with molecular analysis, including also novel methods like next-generation sequencing (Schneeweiss, 2013).

The host range within Orobanchaceae is still poorly understood and may be of little use if primary hosts are not distinguished from occasional hosts (Thorogood et al., 2009). Moreover, earlier data and records are regarded as incorrect and still need to be updated (see e.g., Uhlich et al., 1995; Sánchez Pedraja et al., 2016+). Holoparasites tend towards a narrow host range, except for a few invasive species. This ecological specialization in *Orobanche* and *Phelipanche* shows a significant association of host range and life form (e.g., perennial or annual) (Schneeweiss, 2007), and may be an important driver of speciation, similar to the case of animal parasites (Huyse et al., 2005). Furthermore, a wide host range may be an important parasite preadaptation for becoming weedy. It is also interesting that such changes are believed to be epigenetic modifications and alternative mRNA splicing may enable new host species to be parasitized (Schneeweiss, 2013). In addition, parasite-host relationship may be so unique that a correctly identified host in many cases facilitates or even enables the species of parasite to be verified. Molecular evidence supports the claim that the ancestor of all parasitic lineages in this family had a narrowed host range, while a wide host range evolved independently at least twice in *O. minor* and *O. cumana* (Manen et al., 2004).

The aim of this study was to assess phylogenetic relationships of Central European *Orobanche* and *Phelipanche* and address unresolved issues of their phylogeny. In our study, nuclear sequences (ITS region) and a plastid *trnL-trnF* region were used. We focused on problematic species and aggregates, taxonomy, the correct names and types of traditional morphology-based sections and subsections (McNeill et al., 2012: Art. 4.1), with also recent changes, and relationship with host ranges.

MATERIALS AND METHODS

PLANT MATERIAL

We studied samples of *Orobanche* and *Phelipanche* of Central European species mainly from Poland, the Czech Republic, Slovakia, and Austria. For some species, specimens were collected in other countries, e.g., *O. mayeri* in Germany, *O. leptantha* and *O. loscosii* in Spain, *O. hederae* in Montenegro, and *P. caesia* in Ukraine, or from Asia, e.g., *O. cernua* from Georgia (see supplementary Table S1). *Lindenbergia sinaica* from Israel was used as an outgroup. In each case, the host was precisely identified. In the case of most of these species, more than one individual was investigated, often from different localities and host species, in order to assess possible intraspecific variation. These specimens were mostly collected in the field in 2006–2016. Voucher information, as well as geographic origin or GenBank accession numbers are listed in supplementary Table S1.

Plant materials newly collected are deposited in KTC (herbarium codes according to Thiers, 2017).

Systematic division was adopted according to Beck (1930) and Teryokhin et al. (1993), the scheme followed, explicitly or implicitly, by most researchers, and some recent taxonomic contributions made conforming to McNeill et al. (2012).

HOST ANALYSIS

The study was conducted in 2006–2016 in Central Europe, mainly Poland, the Czech Republic, Slovakia, Austria, with supplementary work done in western and southern Europe. Using a gardening shovel, the soil was exposed to the level of the attachment of the haustoria to the root of the host. In the case of host species with a delicate root system, such as *Galium*, the soil and then roots

were rinsed in water to observe for the presence of haustoria. A total of ca. 400 localities were analyzed in the field. Additionally, validated literature data were used (e.g., Uhlich et al., 1995; Zázvorka 1997, 2000; Dakskobler et al., 2010; Pusch, 2009), and observations were made in herbaria in Poland (CHRZ, KRA, KRAM, KTC, KTU, LBL, LOD, POZ, TRN, WA, WRSL), the Czech Republic (PR, PRC), Slovakia (SAV), Germany (B, GLM), Austria (W), and comparatively: Russia (LE), Georgia (BATU, TBI), Armenia (ERE, ERCB), and in private herbaria.

MOLECULAR METHODS

DNA was extracted primarily from freshly collected and silica gel-dried material, but in a few instances the material was obtained from herbarium vouchers. Total cellular DNA was isolated according to the manufacturer's protocol using a Plant & Fungi DNA Purification Kit (EURx).

The entire nuclear ribosomal DNA ITS region (internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2), later described as ITS, was amplified using primers ITS7A (Fuertes Aguilar et al., 1999) and ITS4 (White et al., 1990). The tRNA-Leu (*trnL*) intron, the partial *trnL* gene, and the intergenic spacer between the *trnL* 3' exon and tRNA-Phe (*trnF*) gene region's plastid DNA (later described as *trnL-trnF*) were amplified using c and f primers (Taberlet et al., 1991). Both, nuclear and plastid, sequences were amplified as described in Kwolek et al. (2017).

Special steps were applied in the case of degraded DNA samples. The approach described in Piwowarczyk et al. (2015a) with the additional pairs of primers was used for the amplification of the ITS regions. For the plastid *trnL-trnF* region new primers were designed and used: trn-F1 (5'-RAYGAGAATAAAGATAGAGTCC-3') and trn-R1 (5'-ATAGAGGGACTTGAACCCTC-3'). The primer trn-R1 was used with c and trn-F1 was paired with f. The final sequence was a combination of the products of these two pairs of primers. The PCR reaction composition and the program used for obtaining the nuclear and plastid sequences from the degraded DNA samples were as described in Piwowarczyk et al. (2015a).

All the obtained products were sequenced following the procedure previously described in Piwowarczyk et al. (2015a).

PHYLOGENETIC METHODS

Information about sequences used in phylogenetic analysis is presented in supplementary Table S1. Most of them were obtained by us; however, a few sequences were found in GenBank. DNA sequences were aligned using Muscle version 3.8.425 (Edgar, 2004) and then manually corrected and trimmed. The final alignment of the 65 sequences of ITS was 629 positions, whereas the *trnL-trnF* alignment (62 sequences) was 1005 positions long. For these sequences, Bayesian and Maximum Likelihood phylogenetic trees were generated. As the outgroup, sequences of *Lindenbergia sinaica* were used.

Bayesian phylogenetic trees were generated using mrBayes v. 3.2.3 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) with the following main settings: ngen=10000000, samplefreq=1000, nchains=4, checkfreq=100000, diagnfreq=5000, stopval=0.01, stoprule=yes. Substitution models (GTR+I+G for ITS and GTR+G for *trnL-trnF*) were determined by the jModelTest 2 (Guindon and Gascuel, 2003; Darriba et al., 2012) for three schemes (JC/F81, K80/HKY, SYM/GTR) using AIC.

For Maximum Likelihood (ML) trees, IQ-TREE multicore version 1.5.3 (Nguyen et al., 2015) was used with ultrafast bootstrap approximation (2000 bootstrap replicates). Substitution models (TIM3e+G4 for ITS and K3Pu+G4 for *trnL-trnF*) were auto-determined according to BIC by IQ-TREE.

The trees that were generated were visualized using iTOL tool (Letunic and Bork, 2016) and manually adjusted. ML trees contain bootstrap values, Bayesian trees show Bayesian posterior probabilities. These values are also mentioned in the text when support values are described, usually in parentheses. One value represents Bayesian posterior probability (if not mentioned else), when two values are presented the first one is Bayesian posterior probability, the second is bootstrap.

RESULTS

HOST RANGE

The hosts of *Orobanche* and *Phelipanche* in Central Europe included 102 species from 12 families. The most abundant families are Asteraceae (39 species), Fabaceae (18), Lamiaceae (11), Rubiaceae (11), and Apiaceae (8). Other families contain only 1–5 host species each, such as Solanaceae, Dipsacaceae, Brassicaceae, Berberidaceae, Araliaceae, Cannabaceae, and Geraniaceae (Fig. 1). The most numerous host species are from six genera: *Artemisia*, *Cirsium*, *Centaurea*, *Achillea*, *Salvia*, and *Galium*. Some species of unrelated parasites may have, here, the same host, such as *O. coerulescens*, *O. artemisiae-campestris*, *P. arenaria*, *P. bohemica*, which parasitize *Artemisia campestris*, or *O. elatior* and *O. kochii* most often parasitizing the same host
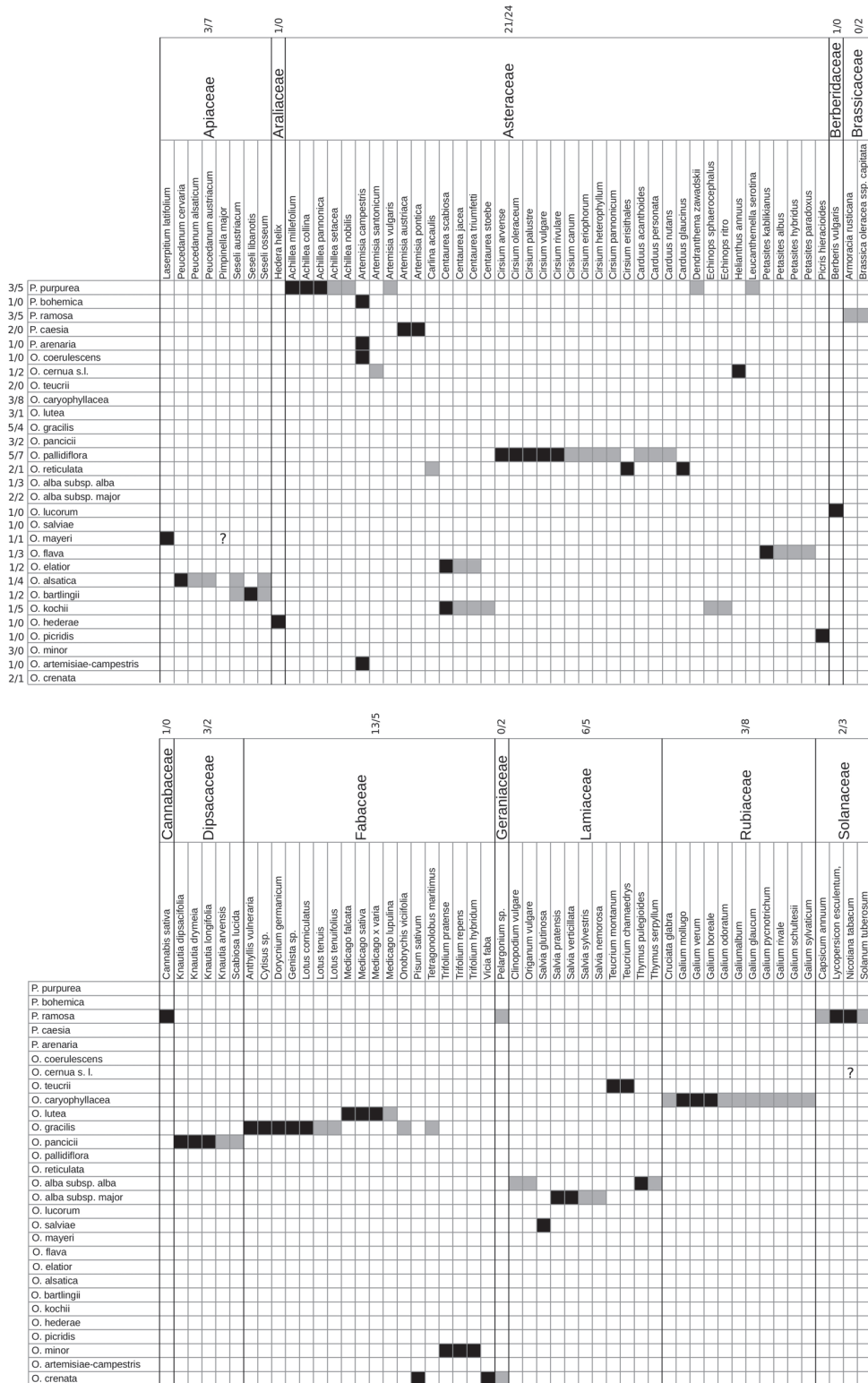
Fig. 1. Parasites and host species. Black squares indicate preferred, gray squares occasional hosts for a given species of parasite. Parasite species are ordered analogously to their presence in the ITS tree. Numbers near parasite species show the total number of preferred/occasional hosts, numbers near hosts families show for how many parasites they are preferred/occasional hosts.

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species, *Centaurea scabiosa*. The number of hosts ranges from 1 to 12 species per parasite, but the preferred number of hosts is significantly lower and generally does not exceed 5 species (Fig. 1). Our research has shown that with respect to host range tolerance, parasitic species are divided into three groups:

– monophagous (single host species, 9 parasites): *O. artemisiae-campestris*, *O. coerulescens*, *O. hederae*, *O. lucorum*, *O. mayeri*, *O. picridis*, *O. salviae*, *P. arenaria*, *P. bohemica*;

– oligophagous (hosts from one genus, rarely one family, 16 parasites): *O. alba* subsp. *alba*, *O. alba* subsp. *major*, *O. alsatica*, *O. bartlingii*, *O. caryophyllacea*, *O. elatior*, *O. flava*, *O. gracilis*, *O. kochii*, *O. lutea*, *O. minor*, *O. pancicii*, *O. pallidiflora*, *O. reticulata*, *O. teucrii*, *P. caesia*, *P. purpurea*;

– polyphagous (hosts from various families, 3 parasites): *O. crenata*, *O. cernua s.l.*, *P. ramosa*.

On the other hand, a given host species in most cases is parasitized by only one species of parasite. There are some exceptions, among which, previously mentioned *Artemisia campestris*, which has four parasites, is the most striking one.

PHYLOGENY

Phylogenetic trees obtained for Bayesian and Maximum Likelihood methods for a given sequence type were mostly the same, and the differences did not significantly influence taxonomical relationships. For this reason, we present here Bayesian trees (Figs. 2, 3); however, some remarks to ML trees (that are available in supplementary Figures S1 and S2) are mentioned below.

In the studied species of *Phelipanche* and *Orobanche*, clearly separated clades (1.0) are formed (Figs. 2, 3) which are described below.

PHELIPANCHE POMEL

Phelipanche purpurea and *P. bohemica* have ITS sequences divergent enough to clearly distinguish these species (1.00). However, the *trnL-trnF* region is much less variable. Both, together with *P. arenaria* and *P. caesia,* are included in the section *Arenariae* by Teryokhin et al. (1993), which is not separated from the section *Phelipanche* represented here by *P. ramosa*. Although in the ITS tree *P. ramosa* seems to be closer to *P. caesia* and *P. arenaria* (0.90) than these two species to other members of the section *Arenariae*, the *trnL-trnF* tree shows a closer relationship to *P. purpurea* and *P. bohemica* (0.98). On both trees, the series *Purpureae* of the section *Arenariae* (*P. purpurea* and *P. bohemica*) is grouped in one clade (1.00). The series *Arenariae* (*P. arenaria*, *P. caesia*) and series *Purpureae*

(*P. purpurea*, *P. bohemica*) are separated on the ITS tree. However, on the *trnL-trnF* tree *P. caesia* is located closer to the species of the series *Purpureae* and *Phelipanche* (*P. ramosa*) than to ser. *Arenariae* (*P. arenaria*), but with low support (0.73).

OROBANCHE L.

Regarding the problematic complex of *Orobanche alsatica* aggr., we chose to study material from Poland, Slovakia, the Czech Republic, and Germany (supplementary Table S1), of *O. alsatica* (four localities: three on *Peucedanum cervaria* [*Cervaria rivini*] and one on *Seseli osseum*), *O. bartlingii* (five localities: four on *Seseli libanotis*, one on *S. osseum*), and *O. mayeri* (three localities on *Laserpitium latifolium*). It turned out that *O. mayeri* is clearly distinct in relation to *O. alsatica* and *O. bartlingii*, and is much closer to species like *O. flava*. Sequences of *O. mayeri* from Germany (*locus classicus*) and Poland are the same. The analyzed samples of *O. alsatica* and *O. bartlingii* are in a very close relationship and only on the ITS tree these species are separated (1.00).

The results of analysis of four (ITS) or three (*trnL-trnF*) localities of *O. pallidiflora* parasitizing different hosts (*Cirsium arvense*, *C. oleraceum* and *Carduus personata*) and two localities of *O. reticulata* (on *Carduus glaucinus*) showed the close relationship of these two species (1.00). On ITS tree the separation of these two species is rather weak (0.62), in the case of the *trnL-trnF* tree, one specimen of *O. pallidiflora* (KY484497) on *C. personata* seemed to be closer to *O. reticulata* (0.99) than to other specimens of its own species.

Orobanche elatior and *O. kochii* were placed on separate branches, which clearly confirms that they can be treated as separated species. Moreover, on the ITS tree, the latter species seems to be closer (0.90) to the members of the subsect. *Minores* – *O. picridis*, *O. artemisiae-campestris* and subsect. *Speciosae* – *O. crenata*, than to the first one (Fig. 2). On the other hand, molecular results show that *O. leptantha* (from Spain) is very closely related to *O. elatior* (1.00). The phylogenetic position of *O. ritro* in *trnL-trnF* sequences displayed a similarity to *O. kochii* (1.00) (Fig. 3).

Two subspecies of *O. alba* (two localities of subsp. *alba* parasitizing *Thymus pulegioides* and one of subsp. *major* on *Salvia verticillata*) show some differentiation in their sequences. However, whereas on the ITS tree one specimen of subsp. *alba* is closer to subsp. *major* than the others of subsp. *alba*, on the *trnL-trnF* tree subsp. *alba* is separated from subsp. *major* on a distinct branch (0.98).

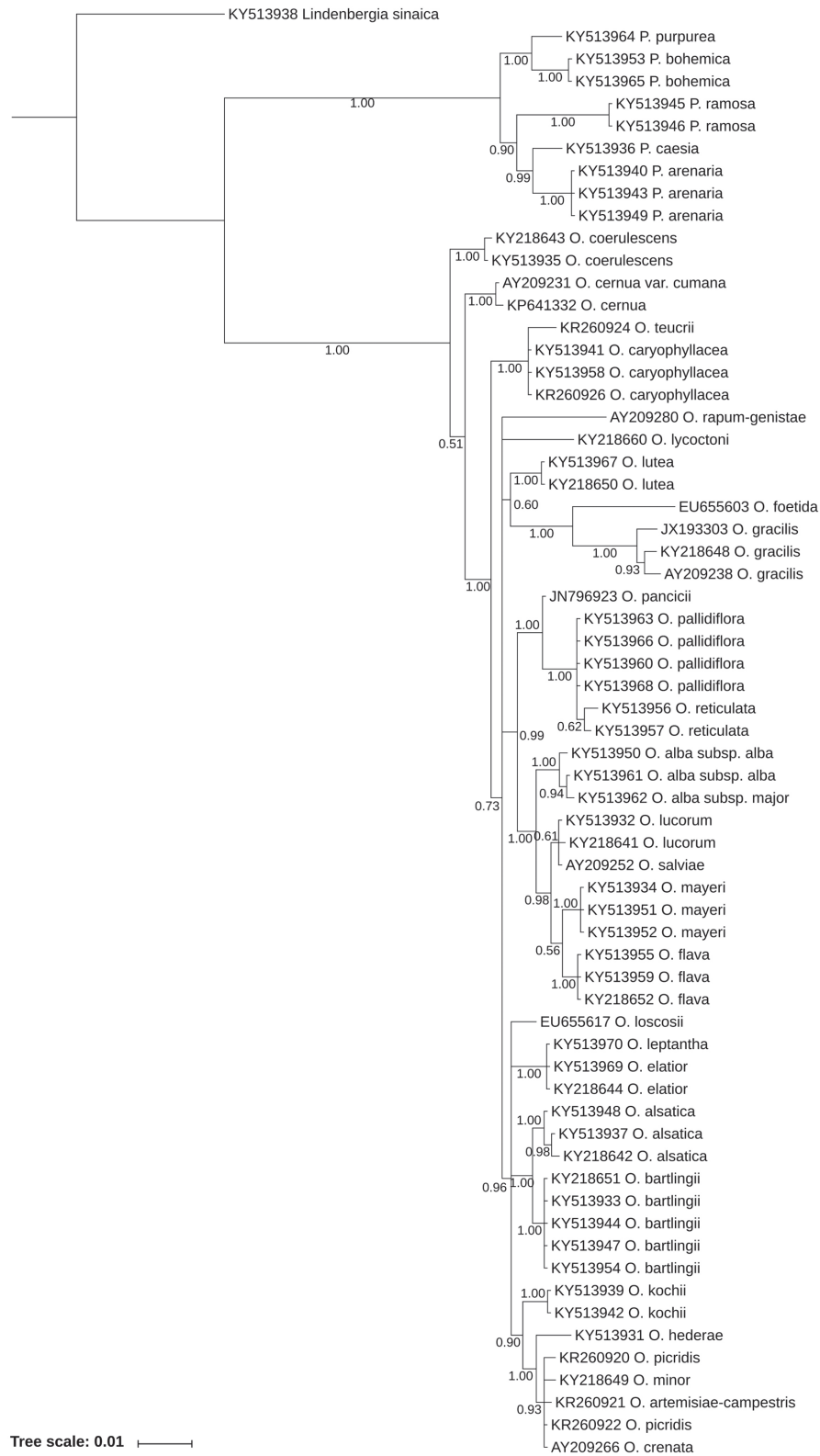We also analyzed some examples of other poly-/oligophagous species, parasitizing different

Fig. 2. Rooted Bayesian phylogenetic trees constructed for ITS sequences. As an outgroup, *Lindenbergia sinaica* was used. Numbers near branches show Bayesian posterior probabilities. The bar represents the amount of genetic change (nucleotide substitutions per site).

Fig. 3. Rooted Bayesian phylogenetic trees constructed for *trnL-trnF* sequences. As an outgroup, *Lindenbergia sinaica* was used. Numbers near branches show Bayesian posterior probabilities. The bar represents the amount of genetic change (nucleotide substitutions per site).

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hosts, like *O. caryophyllacea* (*Galium mollugo*, *G. odoratum*, *G. boreale*), or *P. ramosa* (*Nicotiana tabacum*, *Lycopersicon esculentum* [*Solanum lycopersicum*]). Generally, there was not a clear differentiation between samples from different plants of the same species that parasitize different hosts.

The phylogenetic position of *O. gracilis* (sect. *Orobanche* subsect. *Cruentae*) on the presented trees seems to be rather unclear. On the *trnL-trnF* Bayesian tree, it is, as well as *O. cernua* and *O. coerulescens* which belong to the section *Inflatae*, outside the branch containing the rest of *Orobanche* species. On the ML tree the situation is similar, but these three species are grouped on a common branch (76) with *O. gracilis* placed closer to *O. coerulescens* (83). ITS trees suggest that *O. gracilis* is closest to *O. foetida* (1.00, 100) and these two species form a common branch with *O. lutea* but with rather weak support (0.60, 71).

Although the sequences used in the studies do not allow all taxonomic problems to be resolved in the subsection *Minores* (*O. minor*, *O. picridis*, *O. artemisiae-campestris*), some interesting conclusions can be made: i) *trnL-trnF* tree allows *O. picridis* to be distinguished from the rest of species, however with weak support (0.89), ii) the ITS tree shows that *O. crenata* (subsect. *Speciosae*) is clustered with the subsect. *Minores* (0.93), iii) *O. hederae* (sect. *Inflatae*, subsect. *Hederae*) is grouped on both trees with the subsection *Minores* (1.00) in accordance with Beck's ideas (1890: 228 "trib."; 1930: 167 "Grex") and not with other species of the section *Inflatae*, where it is placed erroneously in the authors' opinion, according to Teryokhin et al. (1993).

DISCUSSION

This study is the first one to present phylogenetic relationships between all *Orobanche* and *Phelipanche* species that are found in Central Europe. Moreover, in most cases we examined more than one locality of a species to study possible intraspecific genetic differentiation in relationships to their hosts and origin. Most of the specimens used in the study were newly collected in central European countries. However, in some cases, when they were not accessible for us, samples from other countries, herbaria specimens or data from GenBank were used. Also some sequences of species from other regions were added to clarify phylogenetic relationships.

Most of the molecular studies involving genetic sequences (dePamphilis, 1997; Young et al., 1999; Schneeweiss et al., 2004a), chromosomes (Schneeweiss et al., 2004b), and protein data (Castillejo et al., 2009) support the division between *Orobanche* and *Phelipanche*. In contrast, analyses of *rbcL* (Manen et al., 2004) and volatile organic compounds (Tóth et al., 2016) suggest that *Phelipanche* and *Orobanche* should not be divided into separate clades.

In the present study, two different sequences (ITS and *trnL-trnF*) were used to resolve some taxonomical and phylogenetic problems of Central European *Orobanche* and *Phelipanche*. Phylogenetic trees gave similar results in most cases, although in some instances only one of them could shed light on some of the problems and in others they gave inconsistent results for some Central European species (e.g., *O. gracilis*).

Phylogenetic trees show two clearly separated clades, in terms of the number of species: large *Orobanche* and smaller *Phelipanche*. Due to this, the *trnL-trnF* tree seems not to support, at least for the species studied, the hypothesis of Park et al. (2007a) that this sequence was transferred together with a fragment of (or complete) plastid genome by the horizontal gene transfer process.

The genus *Phelipanche* in Central Europe, in contrast to, e.g., the western and central Asia and the Mediterranean, contains only 5 species from the section *Phelipanche* and two traditionally recognized subsections (*Phelipanche* and *Trionychon* [*Arenariae*]). The genus *Orobanche*, according to Teryokhin et al. (1993), represents the largest genus with about 23 representatives of two sections and several subsections: section *Inflatae* with subsection *Coerulescentes* and *Hederae*, and section *Orobanche* with six subsections: *Curvatae* (Piwowarczyk et al., 2017c), *Minores*, *Orobanche*, *Glandulosae*, *Speciosae* and *Cruentae*.

We focused on phylogenetic relationships regarding problematic species and aggregates, recent taxonomic changes, and the impact of host range. The most important ambiguities are discussed below.

Phelipanche Pomel in Bull. Soc. Sci. Phys. Algérie 11: 102 (1874)
Type: *Orobanche ramosa* L. (syn. *Phelipanche ramosa* (L.) Pomel) (Tzvelev 1981: 325)

According the classical taxonomy based on morphological characters, the genus we now call *Phelipanche* was usually divided into two parts on the basis that the stem was either simple or branched (e. g., Reuter, 1847; Beck, 1890; Beck, 1930), later Novopokrovskii and Tzvelev (1958) treated these parts as subsect. *Holoclada* ("holo-", stem simple) and *Pleioclada* ("Pleio-", stem branched). Likewise, Andary (1994), according to his morphological, cytological and biochemical studies, also divides it into two parts (subsections *Ramosae* and *Arenariae*), although previously,

Teryokhin et al. (1993) had established new sections using different morphological characters. We now give the correct names of these sections conforming to McNeill et al. (2012), additionally, we take care especially of the problem of some aggregates indicated above.

Phelipanche sect. *Trionychon* (Wallr.) Piwow. & Ó. Sánchez, comb. nov.
Basionym: *Orobanche* sect. *Trionychon* Wallr., Sched. Crit.: 314 (1822).
Type (designated here): *Orobanche comosa* Wallr. [lectotype PR 11757, syn. *Phelipanche arenaria* (Borkh.) Pomel] (Skočdopolová & Chrtek 2008: 26) Syn. *Phelypaea* sect. ["I"] *Trionychon* (Wallr.) C.A. Mey. in Ledeb., Fl. Altaica 2: 460 (1830); *Orobanche* sect. *Holoclada* Novopokr. in Bot. Mat. Gerb. Bot. Inst. AN SSSR 13(1): 305 (1950); *Phelipanche* sect. *Arenariae* Teryokhin in Opred. Zarazikhovykh Fl. SSSR: 43 (1993), nom. superfl.; *Phelipanche* sect. *Holoclada* (Novopokr.) Tzvelev in Novosti Sist. Vyssh. Rast. 46: 200 (2015) (Tzvelev 2015: 200)

Note: We prefer to avoid displacing this traditional well-established name for the section because it is the widely used and earliest available name in that rank (McNeill et al. 2012: Art. 11). Wallroth based his new section on two main taxa (*O. comosa* Wallr. and *O. ramosa* L.), one of them was new and described by Wallroth as well and corresponds better with the original description of Wallroth section (e.g., "Antherae pilosae" (Wallroth, 1822) and "Scapus simpliciusculus" (Wallroth, 1825)). Consequently, the Wallroth taxon is chosen by us as the type of his new section. In additttion, the Linnean species is the type of genus *Phelipanche* Pomel (see above) and its autonym *P.* sect. *Phelipanche*, the other traditional section which has been used by the authors for the branched species of this genus following the ideas of Reuter (1847), Beck (1890), Beck (1930), Novopokrovskii and Tzvelev (1958).

Phelipanche ser. *Purpurea* Teryokhin in Opred. Zarazikhovykh Fl. SSSR: 43 (1993)
Type: *Phelipanche purpurea* (Jacq.) Soják

Phelipanche purpurea / *P. bohemica*
Phelipanche bohemica (Čelak.) Holub was described from the Czech Republic as a separate species by Čelakovský (1879). However, it was later classified at different taxonomic ranks within *P. purpurea*, e.g., as a variety by Hayek (1914), Beck (1930), a subspecies by Zázvorka (2000), Cárlon et al. (2008), and a species by Holub (1979), Holub and Zázvorka (1999), Pusch (2006), Pusch and Günther (2009), Piwowarczyk (2012b).

Previous morphological, ecological (e.g., Pusch, 2006; Piwowarczyk, 2012b) and molecular (Carlón et al., 2005; Schneeweiss et al., 2004a) differences suggest that *P. bohemica* is a separate species. It parasitizes only *Artemisia campestris*, unlike *P. purpurea* s. str., which infects mainly *Achillea* sp. In the Czech Republic, the stability of host choice by these two taxa was reported (Zázvorka, 1989). Furthermore, it is also confirmed by laboratory culture (Pusch, 2006) and observations from Poland (Piwowarczyk, 2012b). Moreover, the seeds of *P. bohemica* are characterized by smaller and thinner cell walls and by different sculpture of the fibrilla than for *P. purpurea* (Piwowarczyk, 2015c). Additionally, palynological analysis also showed auxiliary micromorphological differences between these taxa (Piwowarczyk et al., 2015). Our study shows that whereas the *trnL-trnF* tree does not separate these species, the ITS tree shows a clear difference between *P. bohemica* and *P. purpurea*. Moreover, in this case differences between sequences are visible in 14 positions. Due to this, we suggest that these two taxa should be rather regarded separate species.

Orobanche L., Sp. Pl.: 632 (1753)
Type: *Orobanche major* L. [lectotype BM 000646202, syn. *O. caryophyllacea* Sm.] (Brummitt 2000: 263)
Orobanche sect. *Orobanche*

Orobanche subsect. *Glandulosae* Teryokhin in Opred. Zarazikhovykh Fl. SSSR: 38 (1993) (McNeill et al., 2012: Art. 46.4). Syn. *Orobanche* trib. *Glandulosae* Beck in Biblioth. Bot. 19: 135 [208] (1890), nom. inval. (McNeill et al., 2012: Art. 37.6)
Type: *Orobanche alba* Stephan ex Willd.

Orobanche pallidiflora / *O. reticulata*
Orobanche pallidiflora Wimm. & Grab. (subsect. *Glandulosae*) was described from Poland in 1829 from Koberwitz /Kobierzyce/ (KIEL). *O. pallidiflora* is differently classified in various studies: as a separate species (e.g., Novopokrovskii and Tzvelev, 1958; Kreutz, 1995; Piwowarczyk et al., 2010; Tzvelev, 2015) or within *O. reticulata* Wallr., either as synonym or with different ranks (e.g., Chater and Webb, 1972; Zázvorka, 1997, 2000; Pusch, 2009). These oligophagous taxa prefer various species of the genera *Cirsium* and *Carduus* (supplementary Table S1), while *O. pallidiflora* has a broader lowland host range. However, *O. reticulata* and *O. pallidiflora* differ in morphology, habitat and altitudinal ranges as well as host preferences (Kreutz, 1995; Piwowarczyk et al., 2010). Furthermore, a recent study of pollen grains showed that *O. pallidiflora* has thinner exine and smaller equatorial and polar axes than

O. reticulata, along with differences in sculpture (Piwowarczyk et al., 2015b). Seed micromorphology analysis also showed significant differences between *O. pallidiflora* and *O. reticulata*; *O. reticulata* differs from *O. pallidiflora* by smaller seeds and cells, a distinctly thicker wall, and a greater perforation diameter by a factor of even 2 or 3. Additionally, comparison of seeds of *O. pallidiflora* parasitic on *Carduus personata* and *Cirsium arvense*, showed only subtle differences (Piwowarczyk, 2015c).

The altitudinal gradient can cause ecological variability in the morphology of *O. pallidiflora*, especially in color, and may result in the selection of different hosts that are also related to specific altitudinal ranges. The locality in the Western Bieszczady Mts. is the highest Polish site of the occurrence of *O. pallidiflora* (on *Carduus personata*) recorded so far (995-1205 m a.s.l.). The sample collected there, in the case of the *trnL-trnF* tree, seems to be closer to more mountainous *O. reticulata* (Fig. 3) than to the rest of the samples belonging to the same species.

Sequence analysis of *O. pallidiflora* parasitizing three different hosts (*Cirsium arvense*, *C. oleraceum* and *Carduus personata*) and of *O. reticulata* (on *Carduus glaucinus*) did not result in clear separation of these two species (Fig. 2, 3). Interspecific differentiation was comparable to differences between specimens from the same species (especially in the case of *O. reticulata*) and were visible only for a few nucleotides. Accordingly, we suggest that these two taxa should be considered ecological forms of a single species (Fig. 2, 3).

Orobanche alba
Orobanche alba Stephan ex Willd. (subsect. *Glandulosae*) is a very polymorphic species – almost 30 infraspecific taxa with different taxonomic value have been described, differentiated in morphometric traits, color, and preferred hosts (e.g., Beck, 1890, 1930).

In Central Europe, two subspecies are traditionally distinguished: subsp. *alba*, which here parasitizes *Thymus*, and subsp. *major* (Čelak.) Zázvorka, which parasitizes mainly *Salvia* (Kirschner and Zázvorka, 2000; Pusch, 2009; Piwowarczyk, 2012a, supplementary Table S1).

Orobanche alba subsp. *major* was described by Čelakovský (1871) as *O. epithymum* DC. var. *major* Čelak. Next, Beck (1890, 1930) listed it as *O. alba* f. *maxima*, and later Zázvorka (in Kirschner and Zázvorka, 2000; Zázvorka, 2000) classified it as a subspecies, *O. alba* subsp. *major* (Čelak.) Zázvorka.

Molecular results did not clearly explain the relationships between subspecies of *O. alba,* the differences were small and ambiguous.

Conversely, seed analysis showed some differences relating to perforation diameter, cell length and wall width, all of which were greater in subsp. *major* (Piwowarczyk, 2012a; Piwowarczyk, 2015c). Additionally, palynological analysis also revealed auxiliary micromorphological differences; subsp. *alba* has a thicker exine and larger equatorial and polar axes, with differences in sculpture (Piwowarczyk et al., 2015b). We suggest that these two taxa should be considered ecological forms of a single species.

Orobanche subsect. *Minores* Teryokhin in Opred. Zarazikhovykh Fl. SSSR: 39 (1993). Syn. *Orobanche* trib. *Minores* Beck in Biblioth. Bot. 19: 135 [228] (1890), nom. invalid. (McNeill et al., 2012: Art. 37.6)
Type: *Orobanche minor* Sm.
Representative species: *O. minor, O. picridis, O. artemisiae-campestris*
Species belonging to the central European representatives of subsection *Minores* (*O. artemisiae-campestris*, *O. minor*, *O. picridis*) are clearly distinguished from other sections on their morphological and also micromorphological basis, e.g., perforation diameter of seed epidermis, which is the smallest in that group (except subsection *Speciosae*), usually not more than 2 μm (Piwowarczyk, 2015c). However, molecular differences between species in this subsection, unfortunately, are not so clear.

In previous studies, neither nuclear ITS (Schneeweiss et al., 2004a) nor plastid *rbcL* sequences (Manen et al., 2004) revealed separation of *O. picridis* and *O. artemisiae-campestris* from *O. minor*, although RFLP analysis of the *rbcL* was able to distinguish between two groups of species: A) *O. amethystea*, *O. hederae* and B) *O. minor*, *O. artemisiae-campestris* (Benharrat et al., 2000). Additionally, the presence of host-specific molecular markers might suggest additional cryptic species or host-driven allopatric speciation in *O. minor* (Thorogood et al., 2008, 2009). It was suggested that *O. artemisiae-campestris* might be only one of many "races", within the morphologically very variable *O. minor*, which is apparently defined mainly by its host (Manen et al., 2004), likewise *O. picridis*. Although our results show that trnL-trnF sequences indicate some degree of separation of *O. picridis* (by three substitutions) from two other species of the subsection *Minores*, it is rather weakly supported. Moreover, *O. hederae* (which is surprisingly regarded a member of the *Inflatae* section, following Teryokhin et al., 1993) is clustered on ITS and *trnL-trnF* trees with species belonging to *Minores*, supporting the supposition that these species are relatives. Similar results were obtained by Benharrat et al. (2000) using RFLP on the *rbcL*. Generally, *O. crenata*, which is

usually placed in the subsection *Speciosae*, was not separated from the subsection *Minores* (ITS) where it should be included. Additional studies are needed to better understand this group of traditional species.

Orobanche subsect. *Orobanche*
Orobanche ser. *Orobanche*
Orobanche caryophyllacea
Orobanche caryophyllacea is oligophagous and parasitizes species of the family Rubiaceae. In Central Europe, it mostly occurs on *G. mollugo* aggr., rarely on other species of *Galium* (incl. *Asperula*) and *Cruciata* (Piwowarczyk, 2012c, 2014a; Piwowarczyk et al., 2011, supplementary Table S1). *O. caryophyllacea* Sm. [= *O. major* L.] is the type of the genus *Orobanche* L. (Brummitt, 2000: 263) and subsection *Orobanche* according to McNeill et al. (2012: Art. 22), but it was placed in the subsection *Galeatae* by Teryokhin (1993), partially following the ideas of Beck (1890, 1930), with other central European representatives like *O. teucrii* and *O. lutea*. The results obtained from phylogenetic analysis show that *O. caryophyllacea* and *O. teucrii* are more closely related than both these species to *O. lutea* (Figs. 2, 3). Based on this, it might be more appropriate to reserve this series only for *O. caryophyllacea* and *O. teucrii*, leaving out other species placed here by Teryokhin (1993), who had already placed outside two other species (*O. gamosepala* Reut. [*O.* sect. *Gamosepalae* Teryokhin] and *O. macrolepis* Coss. [*Boulardia latisquama* F.W. Schultz]) included by Beck (1890) in his "trib." *Galeatae*.

The plants of *O. caryophyllacea* parasitizing *Galium mollugo*, *G. boreale* and *G. odoratum* did not show any differences in either ITS or *trnL-trnF* sequences (Figs. 2, 3).

Orobanche subsect. *Curvatae* (Beck) Piwow., Ó. Sánchez & Moreno Mor. (Piwowarczyk et al., 2017c). Syn. *Orobanche* [unranked] *Curvatae* Beck in Halácsy & Braun, Nachtr. Fl. Nieder-Österr.: 128 (1882) (McNeill et al., 2012: Art. 21.4 and 37.3 Ex. 4; Piwowarczyk et al., 2017c)
Type: *Orobanche elatior* Sutton (Piwowarczyk et al., 2017c)

Orobanche ser. *Curvatae* Piwow., Ó. Sánchez & Moreno Mor. (Piwowarczyk et al., 2017c).
Syn. *Orobanche* ser. *Orobanche* sensu Teryokhin in Opred. Zarazikhovykh Fl. SSSR: 40 (1993), non *Orobanche* L. ser. *Orobanche*
Membrana cellularis exterior (cellula epidermatis pericarpi) cum dentibus ["Outer epidermis of pericarp consists of the cells with large tooth-like thickenings at the surface of outer tangential cell walls" (Teryokhin 1997: 18, sub "Seria 2. *Orobanche*")] (McNeill et al., 2012: Art. 39).
Type: type of subsection

Orobanche elatior aggr.
The species parasitizing mainly *Centaurea* L. in central Europe was previously considered as *O. elatior* s.l. [= *O. major* auct., non L.] but it is now recognized as two distinct species, *O. elatior* Sutton and *O. kochii* F.W. Schultz) (Zázvorka, 2010). *Centaurea scabiosa* is here the main host of *O. kochii*, which rarely parasites other *Centaurea* s.l. (incl. *Cyanus*, *Psephellus*, *Rhaponticoides*) or *Echinops* sp.; *O. elatior* mainly parasitizes *C. scabiosa* (Piwowarczyk and Krajewski, 2015 and cited references, supplementary Table S1).

However, this separation was quite clear in Central Europe, but more complicated in other areas, especially in the southwestern Europe. In the recent taxonomic treatment, it was preferred to consider other related to *O. elatior* Mediterranean taxa like *O. leptantha* Pomel (syn. *O. icterica* Pau) and *O. loscosii* L. Carlón & al. (syn. *O. ritro* auct. hisp.) as separate species, having different morphological characters and host plants (Sánchez Pedraja et al., 2016+). The molecular similarity of *O. elatior* and *O. leptantha* may support the view that they are very closely related, although they always parasitize different host plants and their habitat is very different.

Apart from this, *O. ritro* Gren. (= *O. major* f. *ritro* (Gren.) Beck, *O. major* var. *ritro* (Gren.) Willk., *O. major* subsp. *ritro* (Gren.) Bonnier), is a yellow form that was previously considered a separate species which parasitizes *Echinops* (Grenier, 1853). However, this was not the case of *O. echinopis* Pančić (*O. salviae* subsp. *echinopis* (Pančić) Nyman; *O. major* f. *typica* Beck), another typical color taxon described as parasitizing *Echinops* (Beck, 1890: 170, 1930: 250). It is known from scattered areas in western, central and southern Europe to the southwestern part of European Russia (Piwowarczyk in Nobis et al., 2014) and Crimea, and on the Crimean Peninsula it also seems to be parasitic on *Ptilostemon echinocephalus* [*Cnicus echinocephalus*], which is apparently a new host for this species. Some botanists treated *O. ritro* as a synonym for *O. major* or *O. elatior* (e.g., Chater and Webb, 1972). Recently, Pujadas Salvà (2012) reinstated its species rank as *O. ritro*. Carlón et al. (2011) used *O. loscosii* to name the Iberian plants, with reddish stigma, identified as *O. ritro*. However, Pujadas Salvà (2013) claims that this species should be included among the synonyms for *O. ritro*. Zázvorka (2010) or Sánchez Pedraja et al. (2016+) includes *O. ritro* and *O. echinopis* as a synonym of *O. kochii*, which seems to be more reasonable.

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Molecular analyses indicated that *O. elatior* is clearly separated from *O. kochii* and is closest to *O. leptantha* (ITS sequences of both species are identical).The phylogenetic position of *O. ritro* and *O. loscosii* remains unclear due to insufficient data.

Seed analysis also showed a significant difference in epidermis perforation diameter, which is three times larger in *O. elatior* s. str. than in *O. kochii* (Piwowarczyk and Krajewski, 2015; Piwowarczyk, 2015c). Furthermore, seed characters, especially epidermis perforation diameter, place *O. kochii* closer to the species of subsections *Minores* and *Speciosae* than to those of section *Curvatae* (Piwowarczyk, 2015c). This is supported by the ITS tree, but not by the *trnL-trnF* one, so genetic analysis cannot resolve the problem (Figs. 2, 3). Palynological analysis also showed less significant, subtle differences between these species (Piwowarczyk et al., 2015b).

Orobanche ser. *Alsaticae* Teryokhin in Opred. Zarazikhovykh Fl. SSSR: 39 (1993)
Orobanche alsatica aggr.
To the best of our knowledge, until now, no molecular phylogenetic analysis has been used for resolving taxonomical relationships in the problematic complex *O. alsatica* aggr. (Zázvorka, 1997).

Our research concerned the central European representatives of the aggregate, parasitizing Apiaceae species, including *O. alsatica* Kirschl., s. str. [= var. *typica* Beck, nom. illeg., subsp. *alsatica*; parasitizing mainly *Peucedanum cervaria*, rarely *P. alsaticum* or *Seseli osseum*], *O. bartlingii* Griseb. [= var. *libanotidis* (Ruprecht) Beck, subsp. *libanotidis* (Ruprecht) Tzvelev; parasitizing *Seseli libanotis* much more rarely other *Seseli* sp.] and *O. mayeri* (Suess. & Ronniger) Bertsch & F. Bertsch [= var. *mayeri* Suess. & Ronniger, subsp. *mayeri* (Suess & Ronniger) C.A.J. Kreutz; parasitising *Laserpitium latifolium*, in Slovakia from a single locality also on *Pimpinella major* subsp. *rhodochlamys*, but this needs confirmation]. These taxa were included in the various units, as varieties (Beck, 1930; Süssenguth and Ronniger, 1942; Gilli 1974), subspecies (Pusch, 2009), as a complex comprising *O. alsatica* (incl. *O. bartlingii*), with *O. mayeri* as a separate species (Zázvorka, 1997, 2000). Other authors described all of them as separate species (e.g., Bertsch and Bertsch, 1948; Kreutz, 1995; Pujadas Salvà and Gómez García, 2000; Carlón et al., 2009; Piwowarczyk, 2011, 2012e; Piwowarczyk et al., 2014; Tzvelev, 2015). However, to this day, there is disagreement among researchers regarding the status of all taxa belonging to this aggregate in the whole of its area, and sometimes they have been considered to be conspecific (e.g., Domina and Raab-Straube, 2010).

Orobanche mayeri was first reported from one locality in Germany (Süssenguth and Ronniger, 1942), and it was recognized as an endemic taxon, but later was recorded at over ten localities in Slovakia in the Carpathians: the Low Tatra Mts, Choč Mts and the Western Beskidy Mts in Orava (Zázvorka, 1997), and next, from two localities in the Pieniny Mts in Poland (Piwowarczyk, 2011). Although *O. mayeri*, cited by Zázvorka (1997) from localities from the Western Beskidy Mts (Červená skala, Biela skala) during fieldwork (Piwowarczyk unpublished 2013-2014, KTC, supplementary Table S1) was not found. However, *O. bartlingii* parasitizing *Seseli libanotis* was discovered. This is also confirmed by our molecular analysis (Fig. 2, 3).

In the course of our research, it turned out that *O. mayeri* is a distinct species, not related to *O. alsatica* aggr. and ser. *Alsaticae*, but much closer to the species from ser. *Curvatae*, like *O. flava* (Fig. 2, 3), as earlier suspected by Zázvorka (1997), in contrast to the previous opinion. The distinctiveness of *O. mayeri* from the complex of *O. alsatica* and *O. bartlingii* is also supported by detailed micromorphological analysis of seeds and pollen (Piwowarczyk et al., 2014, 2015b). Moreover, the latest investigations showed that VOC clearly separates *O. mayeri* from *O. alsatica* s.l. (Tóth et al., 2016).

Furthermore, on the ITS tree *O. lucorum* and *O. salviae* are very similar (Fig. 2), which suggests that these taxa diverged recently, which might be connected with adaptation to different hosts. Also, morphologically, especially in dry form, they are very difficult to distinguish, so the host-plant plays an important role in identifying these species. It seems that in the case of this related group of mostly mountain range habitats species (*O. lucorum*, *O. haenseleri*, *O. salviae*, as well as with *O. flava* and *O. mayeri* in herbarium materials), a taxonomic revision, comprehensive fieldwork, host analysis and further molecular study are required.

The analyzed samples of *O. alsatica* and *O. bartlingii* are in a very close relationship, however, whereas the *trnL-trnF* tree does not indicate differences between them; on the ITS tree these species are clearly separated. Similar results were obtained by Manen et al. (2004) involving plastid *rbcL* sequence; also in that case *O. bartlingii* and *O. alsatica* were distinguished as two different species. Significant differences between *O. alsatica* and *O. bartlingii* were also shown in a work on the micromorphology of seeds and pollen (Piwowarczyk et al., 2014). An interesting taxon was considered by Zázvorka (1997) as a morphotype (fewer and smaller flowers and habit) of *O. alsatica* s.l. from Slovakia (e.g., Burda plateau, rocky habitats *Festucetalia*

valesiaceae, supplementary Table S1), that parasitize *Seseli osseum*, and which in the course of our studies was clearly closer to *O. bartlingii* (and we use that name) than to *O. alsatica* s.str. This is confirmed by morphological features, as well as micromorphological analysis of seeds and pollen, that clearly associate this morphotype with *O. bartlingii* (Piwowarczyk et al., 2014). This form (perhaps corresponding also to *O. cervariae* var. *seseli* Petitm. from Lorraine in France (Petitmengin, 1904), a parasite of *S. montanum*), may be provisorily referred to as an ecologic variant, host-related morphotype of *O. bartlingii*. However, *O. bartlingii* in France always parasitizes *S. libanotis* except this mention.

Recent surveys in Poland (Piwowarczyk, 2011, 2012c, e; Piwowarczyk et al., 2011), Slovakia and the Czech Republic (Piwowarczyk, unpublished), have shown that the three discussed species occur in different phytocoenoses, on distinct hosts, have different altitudinal distributions and differences in their general range. Separation of *O. alsatica* and *O. bartlingii* is difficult to accept in Moravia, because the lowland and mountain individuals of *O. alsatica* s.l. are morphologically variable, these are the especially mentioned above morphotypes parasitizing *Seseli osseum* (Zázvorka, 1997). However, it may be questioned to what extent this central European situation is representative for their entire distribution areas. This includes especially problematic and currently analyzed morphotypes on different hosts, such as *Heracleum*, *Prangos*, etc., especially in the Caucasus and Central Asia (Piwowarczyk unpublished). *Orobanche alsatica*, *O. bartlingii*, and *O. mayeri* are distinguished by macromorphology (Pujadas Salvà and Gómez García, 2000), seed and pollen micromorphology (Piwowarczyk et al., 2014), host and habitat preferences, and molecular differences; therefore, they should be considered separate species. *O. bartlingii* has probably two morphotypes in central Europe, one parasitizing *S. libanotis*, and the other on *S. osseum* (maybe other *Seseli* sp. too).

Orobanche sect. *Inflatae* (Beck) Rouy in Rouy & Foucaud, Fl. France 11: 167 (1909)
Syn. *Orobanche* sect. *Inflatae* (Beck) Tzvelev, Fl. Evropeiskoi Chasti SSSR 5: 328 (1981), comb. superfl.
Type: *Orobanche cernua* L. (Tzvelev 1981: 328 et 2015: 212, Teryokhin 1993: 37)

Orobanche subsect. *Inflatae* Beck in Halácsy & H. Braun, Nachtr. Fl. Nied.-Oest.: 124 (1882)
Representative species: *O. coerulescens*, *O. cernua*, *O. cumana*

Orobanche coerulescens (*O.* ser. *Coerulescentes* Novopokr. & Tzvelev (1958) (syn. *Orobanche* trib. *Coerulescentes* Beck in Biblioth. Bot. 19: 133 (1890) nom. invalid. [McNeill et al., 2012: Art. 37.6 and Art. 46.4]) and *O. cernua* s.l. (*O.* ser. *Cernua* Novopokr. (1930) (McNeill et al., 2012: Art. 22.6)) are placed on phylogenetic trees outside the rest of *Orobanche,* which correlates with some phenotypic features (such as violet color of flowers) or tricolpatae pollen (in *O. coerulescens*) (Piwowarczyk et al., 2015b) that make them similar to *Phelipanche* species. *O. cernua* s.l. (incl. *O. cernua* subsp. *parviflora* Kotov (1935) [*O. cernua* subsp. *cumana* (Wallr.) Soó (1972)] and subsp. *cernua*) with a wide range of distribution and hosts is a highly polymorphic species. However, in the studied area (as subsp. *cumana* [subsp. *parviflora*]), it is known only from Slovakia, and noticed on *Artemisia santonicum* and in cultivation of *Helianthus annuus* (Zázvorka, 1997). The differences between these two taxa/subsp. and their hosts cannot always be easily distinguished (Pujadas Salvà and Velasco, 2000; Piwowarczyk et al., 2015a).

HOST RANGE

The data on host species of *Orobanche* s.l. from Europe included over 800 species belonging to 280 genera and 54 families (Uhlich et al., 1995). However, many of these data require verification, and the currently created worldwide database of holoparasitic Orobanchaceae is constantly being updated (Sánchez Pedraja et al., 2016+). In some European countries, and especially in Asia, for most species of the genus *Orobanche* s.l., with a few exceptions, the hosts was mainly reported to the family or to the genus, sometimes not specified. Finding hosts of particular species of parasites may be very difficult. Frequently, they were misidentified, because often the researchers considered the host-species to be the species growing closest to the parasite. Moreover, plant configurations above ground often do not correspond to the root systems underground. This is mainly the case when the host species are characterized by a strongly branched root system that can be confused with the root system of neighbouring species. In practice, the shoot of a root-parasite above ground can be located even about a meter from the above ground shoot of the host. It has resulted in numerous errors in the identification of species of *Orobanche* and their host-plants. Precise identification of the host clearly helps to identify species, because they are usually in strict dependencies. Host specificity and host-switching are probably essential in

diversification and speciation, and may be an important driver of the evolutionary divergence of parasitic plants (Schneider et al., 2016).

Our results indicated that in Central Europe there are 102 host-species from 12 families, especially from Asteraceae, less frequently Fabaceae, Lamiaceae, Rubiaceae, and Apiaceae (Fig. 1), which corresponds to the most species-rich families in the world's flora. It seems that the *Orobanche* and *Phelipanche* in the whole range definitely most frequently parasitize species of the Asteraceae family, which is also confirmed by the present study, as well as by data from the western hemisphere (Schneider et al., 2016) and western Asia (Piwowarczyk unpublished). In addition, in our recent work (Kwolek et al., 2017), we demonstrated a host-to-parasite horizontal transfer (HGT) of the mitochondrial *atp6* gene in euroasiatic *O. coerulescens*, which parasitizes *Artemisia* sp. (Asteraceae). Moreover, our data suggest the occurrence of a second HGT event from Asteraceae to *Phelipanche*, and confirm that the HGT from hosts to parasites influences mitochondrial genome evolution in the latter (Kwolek et al., 2017).

In Central Europe, many Orobanchaceae parasitize peculiar host, or often common species from genera like *Artemisia*, *Cirsium*, *Centaurea*, *Achillea*, *Salvia* and *Galium*, and some species of unrelated parasites may parasitize the same host-species. In the studied area, oligophagous parasites are definitely dominant, there are far fewer monophagous parasites, meaning taxa with a narrow host range, and only sporadic polyphagous parasites. It is important to note that some species in Central Europe may be oligophagous, such as *O. minor* or *P. purpurea*, but in other parts of the world, with a warmer climate, e.g., in the Mediterranean Basin or Asia, they have a much broader range of hosts. *P. ramosa*, *O. crenata*, *O. minor* or *O. cumana* in the warmer regions of the world have a much larger and more diverse range of hosts and are also a significant threat of economic importance (Joel et al., 2013). In contrast, in central Europe they are currently not economically significant but the situation may be changed in the future, due to climate warming.

The molecular and host analysis of Central European species presented here, together with references to systematics, as well as earlier palynological (Piwowarczyk et al., 2015b) and carpological studies (Piwowarczyk, 2015c) from the same area, may help to resolve the unclear relationships between problematic taxa and set new questions that need future studies.

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