Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 33 (2004) 482–500
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Phylogeny and intraspecific variability of holoparasitic *Orobanche* (Orobanchaceae) inferred from plastid *rbcL* sequences

Jean-François Manen[a,*], Christine Habashi[a], Daniel Jeanmonod[a], Jeong-Mi Park[b], Gerald M. Schneeweiss[b,c]

[a] *Conservatoire et Jardin Botaniques, Impératrice 1, CH-1292 Chambésy/Genéve, Switzerland*
[b] *Department of Higher Plant Systematics and Evolution, Institute of Botany, University of Vienna, A-1030 Vienna, Austria*
[c] *Department of Plant Biogeography, Institute of Botany, University of Vienna, A-1030 Vienna, Austria*

Received 30 March 2004; revised 19 June 2004
Available online 3 August 2004

Abstract

The *rbcL* sequences of 106 specimens representing 28 species of the four recognized sections of *Orobanche* were analyzed and compared. Most sequences represent pseudogenes with premature stop codons. This study confirms that the American lineage (sects. *Gymnocaulis* and *Myzorrhiza*) contains potentially functional *rbcL*-copies with intact open reading frames and low rates of non-synonymous substitutions. For the first time, this is also shown for a member of the Eurasian lineage, *O. coerulescens* of sect. *Orobanche*, while all other investigated species of sects. *Orobanche* and *Trionychon* contain pseudogenes with distorted reading frames and significantly higher rates of non-synonymous substitutions. Phylogenetic analyses of the *rbcL* sequences give equivocal results concerning the monophyly of *Orobanche*, and the American lineage might be more closely related to *Boschniakia* and *Cistanche* than to the other sections of *Orobanche*. Additionally, species of sect. *Trionychon* phylogenetically nest in sect. *Orobanche*. This is in concordance with results from other plastid markers (*rps*2 and *mat*K), but in disagreement with other molecular (nuclear ITS), morphological, and karyological data. This might indicate that the ancestor of sect. *Trionychon* has captured the plastid genome, or parts of it, of a member of sect. *Orobanche*. Apart from the phylogenetically problematic position of sect. *Trionychon*, the phylogenetic relationships within sect. *Orobanche* are similar to those inferred from nuclear ITS data and are close to the traditional groupings traditionally recognized based on morphology. The intraspecific variation of *rbcL* is low and is neither correlated with intraspecific morphological variability nor with host range. Ancestral character reconstruction using parsimony suggests that the ancestor of *O.* sect. *Orobanche* had a narrow host range.

Keywords: *rbcL*; Orobanchaceae; *Orobanche*; Phylogeny; Pseudogene; Evolution of host range

1. Introduction

Orobanchaceae in its current circumscription (sensu Young et al., 1999) is monophyletic. This includes species formerly grouped in two tribes of Scrophulariaceae (sensu Wettstein, 1891) with predominantly hemiparasitic species, Buchnereae and Rhinantheae, and all species of the exclusively holoparasitic Orobanchaceae s. str. (sensu Beck-Mannagetta, 1890, 1930). Together, these are sister to *Lindenbergia*, a non-parasitic traditional Scrophulariaceae (Olmstead et al., 2001; Young et al., 1999). The holoparasitic (non-photosynthetic) Orobanchaceae s. str. is not monophyletic and holoparasitism is suggested to have arisen several times from different hemiparasitic ancestors (dePamphilis et al., 1997; Olmstead et al., 2001; Young et al., 1999).

The genus *Orobanche* comprises ca. 170 species (Uhlich et al., 1995), traditionally grouped in four sections

* Corresponding author.
E-mail address: manen@cjb.ville-ge.ch (J.-F. Manen).

1055-7903/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2004.06.010

(Beck-Mannagetta, 1930): sect. *Orobanche* (=sect. *Osproleon* Wallr.), sect. *Trionychon* Wallr., sect. *Gymnocaulis* Nutt., and sect. *Myzorrhiza* (Philippi) Beck. These are treated by some authors as separate genera *Orobanche* L., *Phelipanche* Pomel, *Aphyllon* Mitchell (=*Thalesia* Britton), and *Myzorrhiza* Philippi, respectively (Holub, 1977, 1990; Soják, 1972; Teryokhin et al., 1993). These sections (or genera) are unequal in species number and distribution (Uhlich et al., 1995). The main section *Orobanche* has about 100 species in Eurasia, while sect. *Trionychon* has ca. 45 species in Eurasia, mainly Southwest Asia. Section *Gymnocaulis* has only two or three species in North America, and sect. *Myzorrhiza* comprises ca. 10 species in North and South America. Morphological differences between these sections concern mostly the presence or absence of bracteoles and the structure of the calyx. Both sect. *Orobanche* and sect. *Trionychon* have zygomorphic calyces (with four teeth), but only sect. *Trionychon* has bracteoles. Similarly, sect. *Gymnocaulis* and sect. *Myzorrhiza* are identical in having actinomorphic calyces (with five equal teeth), but differ by bracteoles being present only in sect. *Myzorrhiza*.

The holoparasitic life-form often results in a dramatic reduction of morphological characters traditionally used in taxonomic identification. This is also the case for the genus *Orobanche*, which is known among taxonomists and field botanists for difficulties in species identification. Few morphological characters exist to discriminate between species: strongly reduced leaves with little variation; little variation in size, shape, and color of the corolla; uniform fruits; and microscopic seeds without any peculiar characters. In addition, dried specimens lose colors and become blackish, making herbarium specimens difficult to identify. Consequently, taxonomic literature on *Orobanche* is scarce, and identification keys differ strongly from one author to another who often proposes his or her own discriminating characters, sometimes applicable only in a restricted geographic area. Therefore, identifications are often based on information about the host, but this is problematic for two reasons. First, although some species are restricted to one or few host species, most of them parasitize several host species. Second, the host is often simply assumed to be the nearest plant, but frequently this is not the case. Above ground parts of the host can be 1 m or more away from its parasite. This also impedes digging out *Orobanche* with its host without destroying the connection. For these reasons, the application of molecular methods is not only interesting for studying phylogeny, phylogeography or evolutionary trends in *Orobanche*, but also for providing tools for species identification, particularly for dried herbarium material.

The first molecular phylogenetic studies of *Orobanche* are based on few species but use several plastid sequences (*rbcL*, *rps*2, and *mat*K; Wolfe and dePamphilis, 1997; Young et al., 1999). Among those, *rbcL* and *mat*K suggest that *Orobanche* is not monophyletic, because the American lineage is more closely related to the genus *Boschniakia* than to the Eurasian *Orobanche* lineages (Wolfe and dePamphilis, 1997; Young et al., 1999). Results of a recent study by Schneeweiss et al. (2004a) based on a much wider sampling using nuclear ITS sequences also indicate that *Orobanche* is not monophyletic. Instead, *Orobanche* falls into two clearly separated lineages. One includes species of *O.* sect. *Orobanche* and the small Southwest Asian genus *Diphelypaea*, while the second comprises *O.* sects. *Trionychon*, *Gymnocaulis*, and *Myzorrhiza*. In that study, *Boschniakia* was found to belong to a clade that also includes *Epifagus* and *Conopholis*. The relationship of this clade to any of the *Orobanche* lineages, however, was unresolved.

Holoparasitic plants are not self-reliant for photosynthates, therefore their plastid genome is under relaxed functional constraints (dePamphilis and Palmer, 1990; Wolfe and dePamphilis, 1998). Depending on the lineage, the consequences of this relaxation are variable. Plastid genes involved in photosynthesis are either intact, modified as pseudogenes or missing (Colwell, 1994; dePamphilis and Palmer, 1990; Morden et al., 1991; Wimpee et al., 1992; Wolfe and dePamphilis, 1997; Wolfe et al., 1992a,b). For instance, the *rbcL*-gene has an intact open reading frame in *Orobanche fasciculata* (Wolfe and dePamphilis, 1997), is a pseudogene in *Epifagus* (dePamphilis and Palmer, 1990), and is suspected to be absent in *Conopholis americana* (Colwell, 1994). These authors suggested that this situation depends on the time since acquisition of holoparasitism and subsequent relaxation of functional constraints. Alternatively, the activity of some genes (particularly *rbcL*) may still be required in some lineages, but not in others (Wolfe and dePamphilis, 1997). The relaxed constraints and subsequent high rate of substitution and indel formation allows to use *rbcL* sequences in phylogenetic studies on the inter- and intraspecific level (Benharrat et al., 2000; Wolfe and dePamphilis, 1998).

In this study, for the first time, the evolution of *rbcL* sequences was investigated in a large sample of 106 specimens covering 28 species of *Orobanche*. Phylogenetic analyses were undertaken to address the suitability of *rbcL* sequences as phylogenetic markers in *Orobanche*. Specifically, we want to answer (1) if indels are reasonable phylogenetic markers in *Orobanche*; (2) if the phylogenetic relationships inferred from *rbcL* sequences are congruent with previous hypotheses; (3) if *rbcL* exhibits intraspecific variability and, if so, to what extent; and (4) if *rbcL* sequences can be used to identify species that are difficult to determine based on morphology alone. In a second part, we investigate the relationship between host range and sequence variability and try to answer (5) whether host range is correlated with variability of *rbcL* sequences and, finally, (6) whether there

is an evolutionary trend from wide to narrow host range or vice versa.

2. Materials and methods

2.1. Plant material

The list of *Orobanche* specimens studied with voucher indications, locality, and DNA accession numbers is provided in Table 1. In Fig. 4 the host plant is indicated in those cases where the physical connection between the host and the parasite has been demonstrated. The *rbcL* sequences for the outgroup taxa were obtained from GenBank with the exceptions of all sequences of *Cistanche phelypaea* and one sequence of *Boschniakia*, which were newly obtained in the course of this study. From several species more than one individual was investigated in order to assess possible intraspecific variation.

2.2. Molecular methods

Genomic DNA was extracted from silica gel dried material with a modified DTAB/CTAB method of Gustincich et al. (1991). Briefly, 20–40 mg of liquid nitrogen ground tissue was rapidly mixed in 700 µl of hot extraction buffer (5.5% DTAB, 1 M NaCl, 70 mM Tris–HCl, and 30 mM EDTA, pH 8.0) and incubated at 65 °C for 30 min. After chloroform extraction, the DNA was precipitated by adding 1.7 vol. of precipitation buffer (0.5% CTAB, 40 mM NaCl) for 10 min at room temperature. The pellet was dissolved in 100 µl of 1.2 M NaCl, precipitated with 2.5 vol. of 100% ethanol, washed in 5 vol. of 70% ethanol, dried, and dissolved in 40 µl of TE8 (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). Alternatively, total genomic DNA was extracted as described in Schneeweiss et al. (2004a).

Most *rbcL* sequences were amplified with primers 1F (5′-ATGTCACCACAAACAGAAAC) and 1352R (5′-CAGCAACTAGTTCAGGRCTCC) designed on the basis of the *rbcL* alignment of Wolfe and dePamphilis (1997). For *O. nana*, *O. mutelii*, and *O. gracilis*, a new reverse primer 1294R (5′-AGCAAGATYACRTCCT TC), designed on the basis of our preliminary alignment, was used in order to obtain a PCR product. PCR conditions were either 35 cycles of 1 min at 94 °C, 0.5 min at 55 °C, and 1 min at 72 °C or 35 cycles of 1 min at 94 °C, 1 min at 59 °C, and 3 min at 72 °C. These conditions yielded strong amplification signals with the exception of a few species, such as *O. purpurea* and *O. arenaria* (sect. *Trionychon*) or *O. crinita* (sect. *Orobanche*), for which PCR was not successful despite several trials. PCR fragments were purified with Prep-A-Gene (Bio-Rad, Richmond, CA, USA) or the GFX PCR DNA & Gel Band Purification Kit (Amersham Biosciences Europe, Vienna, Austria) according to the manufacturer's instructions. Purified fragments were directly sequenced with the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster, CA, USA) using the same primers as for PCR plus additional internal sequencing primers 520F (5′-GAAYAAGTAT GGTCGTCC), 624F (5′-CAGCCATTTATGCGTTG GA-3′), 682R (5′-CTCCARCGCATAGGCTG) or 813R (5′-GTGAATCCTCCWGTTAAGTA-3′) and finally analyzed on the ABI PRISM 377 DNA Sequencer.

2.3. Phylogenetic methods

Sequences were aligned manually by the insertion of gaps and no ambiguous regions were found. Deletions regarding functional *rbcL* sequences coded as 0. Insertions regarding functional *rbcL* sequences were removed (bracketed according to the Nexus format) and coded as 1. This complete matrix comprising the alignment and indels coded as 0/1 is available at http://www.cjb.unige.ch/ and deposited at TreeBASE (Study No. SN1831; http://www.treebase.org).

Maximum parsimony analysis was conducted using PAUP 4.0b10 (Swofford, 2003) employing a heuristic search with the following options: random addition sequence with 1000 replicates, TBR branch swapping, MulTrees on, swap on best trees only, and collapse branches if minimum length is zero. For each replicate, no more than 1000 trees were saved. Analysis of a data set including sequences from all accessions of *Orobanche* to assess intraspecific variation (see Section 3) was conducted using the same settings with the exceptions of the number of replicates (100) and the number of trees retained at each step (100). Characters were treated as unordered and of equal weight. Clade support was assessed using bootstrap, employing 100 bootstrap replicates and heuristic search options as above, but only 10 addition-sequence replicates and no more than 100 trees saved per replicate. In order to compare the pattern of nucleotide substitution and the pattern of indel formation, additional maximum parsimony analyses were conducted on a nucleotide matrix consisting exclusively of nucleotide positions and an indel matrix consisting exclusively of indels (coded as 1/0). The settings for these analyses were the same as above with the exception of the number of addition-sequence replicates being only 400 for the indel matrix.

The nucleotide matrix was also subjected to maximum likelihood and Bayesian analysis conducted with PAUP 4.0b10 (Swofford, 2003) and with MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003), respectively. The best-fit substitution model was selected using the hierarchical likelihood ratio test as implemented in ModelTest 3.06 (Posada and Crandall, 1998). Accordingly, the general time reversible model (nst = 6) plus site-specific rate variation drawn from a gamma distribution (rates = gamma) was chosen with the following

Table 1
List of *Orobanche* specimens and outgroup species analyzed

Species name (Accession No.)	Locality	Collectors (voucher information) or reference	Accession No.
Alectra orobanchoides Benth.		Wolfe and dePamphilis (1998)	AF026819*
Alectra sessiliflora Kuntze		Wolfe and dePamphilis (1998)	AF026820*
Bartsia alpina L.		Olmstead et al. (2001)	AF190903*
Boschniakia hookeri Walp. (1)		Wolfe and dePamphilis (1998)	AF026817*
Boschniakia hookeri Walp. (2)	Washington, USA	WTU: A. Colwell and A. Denton (Colwell 96 WA-CB)	AY582175
Boschniakia strobilacea A. Gray		Wolfe and dePamphilis (1998)	AF026818*
Buchnera floridana Gand.		Wolfe and dePamphilis (1998)	AF026822*
Castilleja linariifolia Benth.		Wolfe and dePamphilis (1998)	AF026823*
Cistanche phelypaea (L.) Coutinho ssp. *lutea* (Desj.) Fernandez-Casas & M. Lainz (1)	Spain	WU: GS & HW (S&T 7656)	AY582176
Cistanche phelypaea (L.) Coutinho ssp. *lutea* (Desj.) Fernandez-Casas & M. Lainz (2)	Spain	WU: GS & HW (S&T 7652)	AY582177
Cycnium racemosum Benth.		Wolfe and dePamphilis (1998)	AF026826*
Harveya purpurea Hook.		Wolfe and dePamphilis (1998)	AF026830*
Harveya squamosa Steud.		Wolfe and Randle (2001)	AF245021*
Hyobanche atropurpurea Bolus		Wolfe and Randle (2001)	AF245023*
Hyobanche sanguinea N. E. Br.		Wolfe and dePamphilis (1998)	AF026832*
Lathraea clandestina L.		Delavault et al. (1995)	X83719*
Lindenbergia philippinensis Benth.		Olmstead et al. (2001)	AF123664*
Lindenbergia sp.		Oxelman et al. (1999)	AJ001768*
Melampyrum lineare Lam.		Wolfe and dePamphilis (1998)	AF026834*
Pedicularis foliosa L.		Wolfe and dePamphilis (1998)	AF026836*
Seymeria pectinata Pursh.		Wolfe and dePamphilis (1998)	AF026837*
Striga asiatica Kuntze		Wolfe and dePamphilis (1998)	AF026838*
Striga gesnerioides Vadke		Wolfe and dePamphilis (1998)	AF026839*
Tozzia alpina L.		Wolfe and dePamphilis (1998)	AF026843*
Orobanche			
sect. *Gymnocaulis*			
O. uniflora L.	California, USA	PENN: C.W. dePamphilis (dePamphilis, Heckard & Chuang 94.15)	AY582180
O. fasciculata Nutt.		Wolfe and dePamphilis (1997)	U73970*
sect. *Myzorrhiza*			
O. californica Cham. & Schlecht.	California, USA	No voucher	AY582178
O. corymbosa (Rydb.) Ferris (1)		Wolfe and dePamphilis (1997)	U73969*
O. corymbosa (Rydb.) Ferris (2)	California, USA	PENN: C. Hedham (8.8.1997)	AY582179
sect. *Orobanche*			
O. aconoti-lycoctoni Moreno Moral & al.	Spain	G. Gomez Casares and G. Moreno Moral (2.8.2003)	AY582181
O. alsatica Kirschl.	Austria	WU: GS & PS (S&T 7609)	AY582182
O. amethystea Thuill. (1)		Unpublished	AF161798*
O. amethystea Thuill. (2)	Spain	WU: GS & HW (S&T 7565)	AY582183
O. artemisiae-campestris Gaudin (1)	Switzerland	CH (no voucher)	AY582184
O. artemisiae-campestris Gaudin (2)	Austria	WU: PS (S&T 7674)	AY582185
O. bartlingii Griseb.	Croatia	WU: GS, PS & AT (S&T 6250)	AY582186
O. caryophyllacea Sm.	Georgia	WU: GS & AT (S&T 6653)	AY582187
O. cernua Loefl. var. *cernua*	Spain	WU: GS & HW (S&T 7584)	AY582189
O. cernua Loefl. var. *australiana* (F.Muell.) Beck	Australia	WU: s. coll. (AD110996)	AY582188
O. cernua Loefl.		Wolfe and dePamphilis (1997)	U73968*
O. coerulescens Stephan	Austria	WU: T. Habeler (S&T 7669)	AY582190
O. crenata Forssk. (1)	Corsica, France	G: DJ, AS & CH (J6298)	AY582191
O. crenata Forssk. (2)	Corsica, France	G: DJ, AS & CH (J6492)	AY582192
O. crenata Forssk. (3)	Greece	WU: WG (Gu 34899)	AY582193
O. crenata Forssk. (4)	Corsica, France	G: DJ, AS & CH (J6491)	AY582194
O. crenata Forssk. (5)	Corsica, France	G: DJ, AS & CH (J6561)	AY582195
O. crenata Forssk. (6)	Greece	WU: WG (Gu 34176)	AY582196
O. cumana Wallr.		Delavault and Thalouran (2002)	AF090349*
O. gracilis Sm. (1)	Spain	G. Moreno Moral (Moreno Moral 0159/03)	AY582197

(*continued on next page*)

Table 1 (*continued*)

Species name (Accession No.)	Locality	Collectors (voucher information) or reference	Accession No.
O. gracilis Sm. (2)	Spain	WU: GS & HW (S&T 8746)	AY582198
O. cf. *gracilis* Sm.	Italy	WU: GS, PS & AT (S&T 6368)	AY582199
O. hederae Duby (1)	Corsica, France	G: DJ, AS & CH (J6294)	AY582201
O. hederae Duby (2)	Corsica, France	G: DJ, AS & CH (J6386)	AY582204
O. hederae Duby (3)		Unpublished	AF078682*
O. hederae Duby (4)	Corsica, France	G: DJ, AS & CH (J6315)	AY582200
O. hederae Duby (5)	Corsica, France	G: DJ, AS & CH (J6295)	AY582202
O. hederae Duby (6)	Corsica, France	G: DJ, AS & CH (J6317)	AY582203
O. hederae Duby (7)	Corsica, France	G: DJ, AS & CH (J6348)	AY582205
O. lutea Baumg.	Georgia	WU: GS & AT (S&T 6966)	AY582206
O. minor Sm. (1)	Greece	WU: WG (Gu 34184)	AY582208
O. minor Sm. (2)	Corsica, France	G: DJ, AS & CH (J6304)	AY582228
O. minor Sm. (3)	Corsica, France	G: DJ, AS & CH (J6309)	AY582231
O. minor Sm. (4)	Corsica, France	G: DJ, AS & CH (J6516)	AY582240
O. minor Sm. (5)		Unpublished	AF130336*
O. minor Sm. (6)	Corsica, France	G: DJ, AS & CH (J6313)	AY582207
O. minor Sm. (7)	Corsica, France	G: DJ, AS & CH (J6559)	AY582209
O. minor Sm. (8)	Corsica, France	G: DJ, AS & CH (J6558)	AY582210
O. minor Sm. (9)	Corsica, France	G: DJ, AS & CH (J6314)	AY582211
O. minor Sm. (10)	Corsica, France	G: DJ, AS & CH (J6316)	AY582212
O. minor Sm. (11)	Corsica, France	G: DJ, AS & CH (J6331)	AY582213
O. minor Sm. (12)	Corsica, France	G: DJ, AS & CH (J6344)	AY582214
O. minor Sm. (13)	Corsica, France	G: DJ, AS & CH (J6338)	AY582215
O. minor Sm. (14)	Corsica, France	G: DJ, AS & CH (J6338b)	AY582216
O. minor Sm. (15)	Corsica, France	G: DJ, AS & CH (J6338)	AY582217
O. minor Sm. (16)	Corsica, France	G: DJ, AS & CH (J6342)	AY582218
O. minor Sm. (17)	Corsica, France	G: DJ, AS & CH (J6351)	AY582219
O. minor Sm. (18)	Corsica, France	G: DJ, AS & CH (J6350)	AY582220
O. minor Sm. (19)	Corsica, France	G: DJ, AS & CH (J6357)	AY582221
O. minor Sm. (20)	Corsica, France	G: DJ, AS & CH (J6354)	AY582222
O. minor Sm. (21)	Corsica, France	G: DJ, AS & CH (J6378)	AY582223
O. minor Sm. (22)	Corsica, France	G: DJ, AS & CH (J6400)	AY582224
O. minor Sm. (23)	Corsica, France	G: DJ, AS & CH (J6417)	AY582225
O. minor Sm. (24)	Corsica, France	G: DJ, AS & CH (J6419)	AY582226
O. minor Sm. (25)	Corsica, France	G: DJ, AS & CH (J6427)	AY582227
O. minor Sm. (26)	Corsica, France	G: DJ, AS & CH (J6472)	AY582229
O. minor Sm. (27)	Corsica, France	G: DJ, AS & CH (J6308)	AY582230
O. minor Sm. (28)	Corsica, France	G: DJ, AS & CH (J6494)	AY582232
O. minor Sm. (29)	Corsica, France	G: DJ, AS & CH (J6497)	AY582233
O. minor Sm. (30)	Corsica, France	G: DJ, AS & CH (J6503)	AY582234
O. minor Sm. (31)	Corsica, France	G: DJ, AS & CH (J6506)	AY582235
O. minor Sm. (32)	Corsica, France	G: DJ, AS & CH (J6310)	AY582236
O. minor Sm. (33)	Corsica, France	G: DJ, AS & CH (J6510)	AY582237
O. minor Sm. (34)	Corsica, France	G: DJ, AS & CH (J6514)	AY582238
O. minor Sm. (35)	Corsica, France	G: DJ, AS & CH (J6515)	AY582239
O. minor Sm. (36)	Corsica, France	G: DJ, AS & CH (J6526)	AY582241
O. minor Sm. (37)	Corsica, France	G: DJ, AS & CH (J6527)	AY582242
O. minor Sm. (38)	Corsica, France	G: DJ, AS & CH (J6534)	AY582243
O. minor Sm. (39)	Corsica, France	G: DJ, AS & CH (J6537)	AY582244
O. minor Sm. (40)	Corsica, France	G: DJ, AS & CH (J6545)	AY582245
O. minor Sm. (41)	Corsica, France	G: DJ, AS & CH (J6549)	AY582246
O. spec. nov.	Corsica, France	G: DJ, AS & CH (J6439)	AY582250
O. pubescens D'Urv. (1)	Greece	WU: WG (Gu 34153)	AY582275
O. pubescens D'Urv. (2)	Corsica, France	G: Gilles Dutartre (s. n.)	AY582276
O. rapum-genistae Thuill. (1)	Corsica, France	G: DJ, AS & CH (J6390)	AY582254
O. rapum-genistae Thuill. (2)	Corsica, France	G: DJ, AS & CH (J6407)	AY582255
O. rapum-genistae Thuill. (3)	France	WU: GS, PS & AT (S&T 6404)	AY582256
O. rapum-genistae Thuill. (4)	Corsica, France	G: DJ, AS & CH (J6434)	AY582253
O. rigens Loisel. (1)	Corsica, France	G: DJ, AS & CH (J6318)	AY582257
O. rigens Loisel. (2)	Corsica, France	G: DJ, AS & CH (J6327)	AY582260
O. rigens Loisel. (3)	Corsica, France	G: DJ, AS & CH (J6329)	AY582258
O. rigens Loisel. (4)	Corsica, France	G: DJ, AS & CH (J6328)	AY582259

Table 1 (*continued*)

Species name (Accession No.)	Locality	Collectors (voucher information) or reference	Accession No.
O. rigens Loisel. (5)	Corsica, France	G: DJ, AS & CH (J6404)	AY582261
O. rigens Loisel. (6)	Corsica, France	G: DJ, AS & CH (J6406)	AY582262
O. rigens Loisel. (7)	Corsica, France	G: DJ, AS & CH (J6475)	AY582263
O. rigens Loisel. (8)	Corsica, France	G: DJ, AS & CH (J6478)	AY582264
O. rigens Loisel. (9)	Corsica, France	G: DJ, AS & CH (J6479)	AY582265
O. rigens Loisel. (10)	Corsica, France	CH (no voucher)	AY582266
O. rigens Loisel. (11)	Corsica, France	G: DJ, AS & CH (J6520)	AY582267
O. rigens Loisel. (12)	Corsica, France	G: DJ, AS & CH (J6556)	AY582268
O. teucrii Holandre (1)	Corsica, France	G: DJ, AS & CH (J6435)	AY582269
O. teucrii Holandre (2)	Corsica, France	G: DJ, AS & CH (J6442)	AY582270
O. teucrii Holandre (3)	Switzerland	CH (no voucher)	AY582271
O. transcaucasica Tsvelev	Georgia	WU: GS, AT, M. Staudinger & PS (S&T 7890)	AY582272
sect. *Trionychon*			
O. mutelii F. W. Schultz	Corsica, France	G: DJ, AS & CH (J6402)	AY582247
O. nana (Reut.) Beck (1)	Corsica, France	G: DJ, AS & CH (J6508)	AY582248
O. nana (Reut.) Beck (2)	Corsica, France	G: DJ, AS & CH (J6536)	AY582249
O. ramosa L. (1)	Spain	G: F. B. Navarro (Navarro 18.8.03)	AY582251
O. ramosa L. (2)	Georgia	WU: GS & AT (S&T 6880)	AY582252
O. tunetana Beck (1)	Spain	WU: GS & HW (S&T 7558)	AY582273
O. tunetana Beck (2)	Spain	WU: GS & HW (S&T 8745)	AY582274

Abbreviations of the collectors: AS, A. Schlüssel; AT, A. Tribsch; CH, C. Habashi; DJ, D. Jeanmonod; GC, G. Gomez Casares; GD, Gilles Dutartre; GS, G.M. Schneeweiss; HE, L.R. Heckard; HW, Hanna Weiss; PS, P. Schönswetter; and WG, W. Gutermann.

[*] Sequences obtained from GenBank.

parameters as suggested by ModelTest: basefreq = (0.2926 0.1688 0.2154) Rmat = (1.4853 2.3642 0.2025 0.5086 3.6253) Shape = 0.7602. A heuristic search was conducted in PAUP using a neighbor joining tree as starting tree followed by TBR branch swapping. Clade support was assessed using bootstrap employing 100 replicates with the same settings as above, but only five trees saved per replicate. For Bayesian analysis, the values of these parameters were estimated during the analysis. Because some of the included *rbcL* sequences are functional or at least have an intact open reading frame, substitution rates at the third codon positions could differ strongly from those at first and second position. Therefore, in addition to an analysis with the same substitution model for all positions (hereinafter the complete model) a mixed model approach in a Bayesian framework was applied, in which the two partitions, first plus second position and third position, are allowed to have different substitution models and parameters. For both partitions, the transversion model plus site-specific rate variation drawn from a gamma distribution was chosen as suggested by ModelTest. The settings for the Markov chain Monte Carlo process were: four chains were run simultaneously for 2×10^6 generations, with trees being sampled every 100th generation. The stationarity of the maximum likelihood scores of two runs starting from different random starting trees was checked by comparing means and variances of the model likelihood after the burnin period of 2000 trees. The posterior probability (PP) of the phylogeny and its branches was thus determined from 18,000 trees. The topologies of those trees were used to generate a 50% majority-rule consensus tree, with the percentage of samples recovering any particular clade representing that clade's posterior probability.

Some phylogenetic relationships inferred from these analyses do not agree with previous phylogenetic hypotheses of *Orobanche*. Therefore, alternative topologies (see Section 3 for details) were tested using the nucleotide matrix in a maximum parsimony framework employing the Templeton (Wilcoxon signed-rank) test as implemented in PAUP 4.0b10 (Swofford, 2003). This test should properly be applied to pairs of a priori hypotheses, and therefore the significance values are interpreted cautiously (see Steppan et al., 2004). Similarly, in a maximum likelihood framework we used the Shimodaira–Hasegawa test as implemented in PAUP 4.0b10 employing 100,000 bootstrap replicates to generate a test distribution by the resampling estimated log-likelihood (RELL) method.

The synonymous (K_s) and non-synonymous (K_a) substitution rates for all *rbcL* sequences relative to *Lindenbergia* sp. (AJ001768) were calculated after exclusion of 16 stop codons using the method of Yang and Nielsen (2000) as implemented in PAML 3.13d (Yang, 2003).

Evolution of host range was analyzed only in species of sect. *Orobanche*. Host-range was coded as binary character and considered narrow (0) if the parasite is family-specific, and wide (1), if more than one host-family can be successfully attacked. The evolution of this life trait was analyzed using equally weighted unordered parsimony as implemented in Mesquite 1.0 (Maddison and Maddison, 2003).

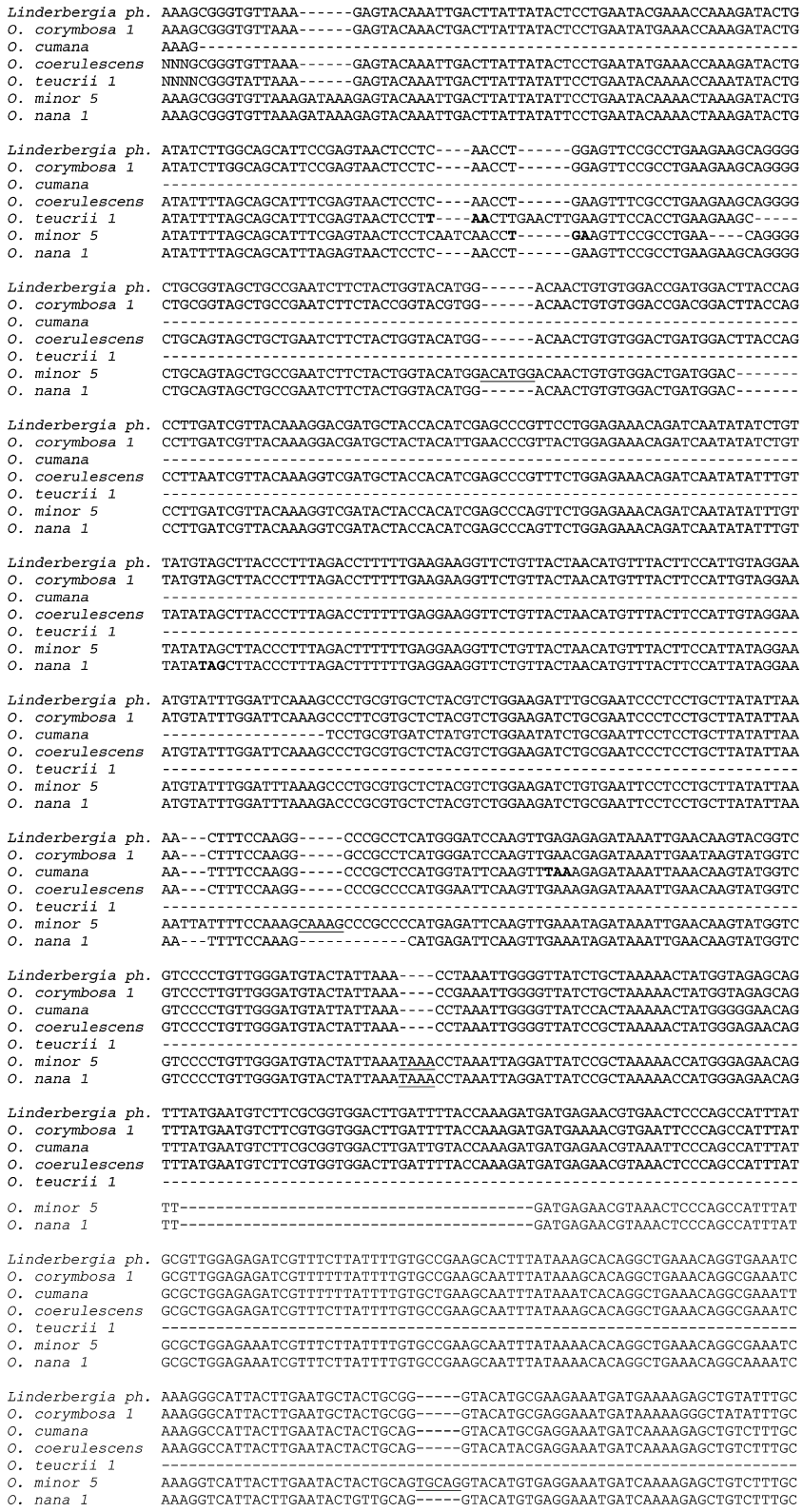

Fig. 1. Alignment of selected sequences showing insertions and deletions observed in *rbcL* pseudogenes of the genus *Orobanche*. Insertions resulting from duplications of adjacent sequences are underlined. In pseudogene sequences, bold letters indicate positions of the first premature stop codon.

```
Linderbergia ph.  TAGAGAATTGGGAGTTCCTATCGTAA---TGCATGACTACTTAACAGGAGGATTCACTGCAAATACTAGC
O. corymbosa 1    TAAAGAATTAGGAGTTCCTATCATAA---TGCATGACTACTTAACAGGAGGATTCACTGCAAATACTAGC
O. cumana         TAGAGAATTGGGAGTTCCTATTATAA--ATGTACGACTACTTAACAGGAGGATTCACTTCAAATACTAGC
O. coerulescens   CAGAGAATTGGGAGTTCCTATTATAA---TGCACGACTACTTAACAGGAGGATTCACTGCAAATACTAGC
O. teucrii 1      ----------------------------------------------------------------------
O. minor 5        CAGAGAATTGGGAGTTCCTATTATAATAATGCACGACTACTTAACAGGAGGATTCACTTCAAATACTAGC
O. nana 1         CAGAGAATTGGGAGTTCCTATTATAA---TGCACGACTACTTAACAGGAGGATTCACTTCAAATACTAGC

Linderbergia ph.  TTGGCTCATTATTGCCGAGATAA-----CGGCCTACTTCTTCACATTCACCGTGCAATGCATGCAGTTAT
O. corymbosa 1    TTGGCTCATTATTGCCGAGATAA-----TGGCCTACTTCTTCACATTCACCGTGCAATGCATGCAGTTAT
O. cumana         TTGGCTCATTATTGCCGTAATAA-----TGTCCTACTTC-------TCACCGTGCAATGCATGTAGTTAT
O. coerulescens   TTGGCTCATTATTGCCGTAATAA-----TGGCTTACTTCTTCACATTCACCGTGCAATGCATGCAGTTAT
O. teucrii 1      ----------------------------------------------------------------------AT
O. minor 5        TTGGCTCATTATTGCCATAATAATGGCCTGGCCTACTTC------TTCACC-TGCAATGCATGCAGTTAT
O. nana 1         TTGGCTCATTATTGCCATAATAATGGTCTGGCCTACTTC------TTCACC-TGCAATGCATGCAGTTAT

Linderbergia ph.  TGATAGACAGAAGAACCATGGGATACACTTCCGT-GTACTAGCTAAAGCGTTACGTATGTCTGGTGGGGA
O. corymbosa 1    TGATAGACAGAAGAACCATGGTATACACTTCCGT-GTACTAGCTAAAGCGTTGCGTATGTCTGGTGGGGA
O. cumana         TGATAGACAGAAGAACCATAGTATACATTTCCGTTGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGA
O. coerulescens   TGATAGACAGAAGAACCATGGTATACATTTCCGT-GTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGA
O. teucrii 1      TGATAGACAGAAGAACCATGGTATACATTTCCGT-GTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGA
O. minor 5        TGATAGACAGAAGAACCATGGTATACATTTCCGT-GTACTAGCTAAAGCATTACGTATGTCTGGTGGAGA
O. nana 1         TGATAGACAGAAGAACCATGGTATACATTTCCGT-GTACTAGCTAAAGCATTACGTATGTCTGGTGGAGA

Linderbergia ph.  TCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTTTGTTGAT
O. corymbosa 1    TCATATTCACTCGGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTTTGTTGAT
O. cumana         TCATATTCACTCTAGGACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATTACTTTGGGCTTTGTTGAT
O. coerulescens   TCATATTCACTCTGGGACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATTACTTTGGGCTTTGTTGAT
O. teucrii 1      TCATATTCACTTGGGGACCGTAGTAGGTAAACTTGAAGGAGAAAGAGGCATTACTTTTGGCTTTGTTGAT
O. minor 5        TCATATTCACTTGGGGACTGCAGTAGGTAAACTTGAAGGAGAAAGAGACATTACTTTTGACTTTGTTGAT
O. nana 1         TCATATTCACTTGGGGACTGTAGTAGGTAAACTTGAAGGAGAAAGAGACATTACTTTTGACTTTGTTGAT

Linderbergia ph.  TTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTTACTCAAGATTGGGTCTCTC
O. corymbosa 1    TTATTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACCCAAGATTGGGTCTCTC
O. cumana         TTATTGCGTGATGATTTTATTAAAAAAGATCGAAGTCACGGTATTTATTTTACTCAAGATTGGGTTTCTT
O. coerulescens   TTATTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGTTTCTC
O. teucrii 1      TTATTGCGTGATGATTTTATTGAAAAATATCGAAGTCGCGATATTTATTTCACTCAAGATTGGGTTTATC
O. minor 5        TTATTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGATATTTATTTCACCCAAGATTGGGTTTCTC
O. nana 1         TTATTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCTATATTTATTTCACCCAAGATTGGGTTTCTC

Linderbergia ph.  TACCAGGTGTTATTCCCGTGGCTTCGGGGGGTATTCACGTTTGGCATATGCCTG-CTCTGACT--GAGAT
O. corymbosa 1    TACCGGGTGTGATTCCCGTGGCTTCTGGTGGTATTCACGTTTGGCATATGCCTG-CTCTGACC--GAGAT
O. cumana         TACCAGGTGTTTTTACTGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTTCATCTGACG--G-GAT
O. coerulescens   TACCAGGTGTTATTCCTGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTT-CCCTGACC--GAGAT
O. teucrii 1      TACCAGGTGTTATTCCTGTGGCTTCTGGGAGTATTCACGTTTGGCATATGCCTT-CCCTGACC--GAGAT
O. minor 5        TACCAGGTGTTATTCCTGTGGCTTCAGGGGGTATTCACGTTTGGAATATGCCTT-CCCTGACCGCGAGAT
O. nana 1         TACCAGGTGTTATTCCTGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTT-CCCTGACC--GAGAT

Linderbergia ph.  CTTTGGGG----ACGATTCCGTACTACAGTTTGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCC
O. corymbosa 1    CTTTGGGG----ATGATTCTGTACTGCAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCC
O. cumana         CTTTGGGG----ATGATTCCGTACTACAGTTTGGTGGAGGAACTTTAGGACATCCTTAGCGTAATGCACC
O. coerulescens   CTTTGGGG----ATGATTCCGTACTACAGTTTGGCGGAGGAACTTTAGGACATCCTTGGGGTAATGCACC
O. teucrii 1      CTTTGGGGATGGATGATTCCGTACTACAGTTTGGTGGAGTAACTTTAGGACATCCTTGGGGTAATGCACC
O. minor 5        CTTTGGGG----ATGATTCCATACTACAGTTTGGCGGAGGAACTTTAGGACATCCTTGGGGTAATGCACC
O. nana 1         CTTTGGGG----ATGATTCCATACTACCGTTTGGCGGAGGAACTTTAGGACATCCTTGGGGTAATGCACC

Linderbergia ph.  AGGTGC--CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTG
O. corymbosa 1    CGGTGC--CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTG
O. cumana         AGGCGCTCTGTAGCTAATCGAGTAGCTATAGAAGCATGTGTACAAGCTCGTAATGAAGGATGTAATCTTG
O. coerulescens   AGGTGC--TGTAGCTAATCGAGTAGCTATAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTG
O. teucrii 1      AGATGC--TGTAGCTAATCGAGTAGCTATATAAGCATGTGTACAAGTTCGTAATGAAGGACGTGATCTTG
O. minor 5        AGGTGC--TGTAGCTAATCGAGTAGCTATAGAAGCATATGTACAAGCTCGTAATGAAGGACGTGATCTTG
O. nana 1         AGGTGC--TGTAGCTAATCGAATAACTATAGAAGCATATGTACNNNNNNNNNNNNNNNNNNNNNNNNN

Linderbergia ph.  CTGCTGAGGGTAA
O. corymbosa 1    CTGATGAGGGTAA
O. cumana         CTACTGAGGGGAA
O. coerulescens   CTTCTGAGGGTAA
O. teucrii 1      CTGCTGAGGGTAA
O. minor 5        CTGCTGAGGGTAA
O. nana 1         NNNNNNNNNNNNN
```

Fig 1. (*continued*)

3. Results

3.1. Pseudogenes, indels, and substitution patterns of rbcL in Orobanche

The *rbcL* sequences of most *Orobanche* species are pseudogenes with several insertions and deletions that distort the open reading frame by insertion of premature stop codons, thus making this gene not functional (Fig. 1). As already shown by Wolfe and dePamphilis (1997, 1998), *rbcL* of species of the American sections *Myzorrhiza* and *Gymnocaulis* is potentially functional. This is confirmed here for two new accessions from sect. *Myzorrhiza*. Additionally, the present study shows for the first time a potentially functional *rbcL* sequence in a Eurasian species (*O. coerulescens* of sect. *Orobanche*; Fig. 1), whereas all other investigated species of the Eurasian sects. *Orobanche* and *Trionychon* have *rbcL* pseudogenes.

Pseudogene sequences of *rbcL* are characterized by the presence of several insertions and deletions. Most indels are relatively short fragments (from 1 to 20 nucleotides), causing a change in sequence length of less than 5% of the length of functional genes (Table 2). In most

Table 2
Parameters of *rbcL* sequences of Orobanchaceae

Taxon[a]	Sequence length in bp (%) of *Nicotiana tabacum*[b,c]	K_a	K_s	K_a/K_s
Lindenbergia sp.*	1287 (100.00)	—	—	
*Lindenbergia philippinensis**	1287 (100.00)	0.005	0.118	0.046
*Lathraea clandestina***	1287 (100.00)	0.005	0.151	0.035
*Melampyrum lineare**	1287 (100.00)	0.005	0.104	0.052
*Seymeria pectinata**	1287 (100.00)	0.011	0.143	0.076
*Castilleja linariifolia**	1287 (100.00)	0.005	0.147	0.037
*Pedicularis foliosa**	1287 (100.00)	0.005	0.149	0.036
*Buchnera floridana**[d]	1287 (100.00)	0.005	0.184	0.029
*Bartsia alpina**	1287 (100.00)	0.005	0.127	0.042
*Harveya squamosa***	1287 (100.00)	0.005	0.170	0.031
*Harveya purpurea***	1287 (100.00)	0.005	0.166	0.033
*Tozzia alpina**	1287 (100.00)	0.011	0.263	0.042
Boschniakia hookeri 1	1324 (102.88 [102.04])	0.016	0.123	0.133
Boschniakia hookeri 2	1329 (103.26 [102.89])	0.016	0.106	0.153
Boschniakia strobilacea	1324 (102.88)	0.016	0.106	0.153
Cistanche phelypaea 1	1294 (100.54 [100.55])	0.039	0.271	0.143
Cistanche phelypaea 2	1291 (100.31 [100.31])	0.039	0.271	0.143
*Striga asiatica**	1287 (100.00)	0.005	0.169	0.032
*Striga gesnerioides**	1287 (100.00)	0.005	0.169	0.032
*Alectra orobanchoides***	1287 (100.00)	0.011	0.152	0.070
*Alectra sessiliflora**	1287 (100.00)	0.011	0.183	0.057
*Cycnium racemosum**	1287 (100.00)	0.005	0.169	0.032
*Hyobanche atropurpurea***[d]	1287 (100.00)	0.016	0.174	0.093
Hyobanche sanguinea	1279 (99.38)	0.016	0.174	0.093
*Orobanche fasciculata***	1287 (100.00)	0.016	0.327	0.050
*Orobanche uniflora***	1287 (100.00 [100.00])	0.011	0.317	0.035
Orobanche corymbosa 1**	1287 (100.00 [100.00])	0.011	0.244	0.045
Orobanche corymbosa 2**	1287 (100.00)	0.006	0.245	0.022
*Orobanche californica***	1287 (100.00 [100.00])	0.011	0.244	0.045
Orobanche cernua 1	943 (73.27)	0.048	0.248	0.195
Orobanche cernua 2	931 (72.34)	0.065	0.256	0.253
Orobanche cernua 3	942 (73.19)	0.054	0.250	0.215
Orobanche cumana	942 (73.19)	0.048	0.228	0.209
*Orobanche coerulescens***	1287 (100.00 [100.00])	0.011	0.288	0.037
Orobanche rigens 1	1286 (99.92)	0.029	0.299	0.098
Orobanche rigens 2	1286 (99.92)	0.029	0.299	0.098
Orobanche rapum-genistae 1	1278 (99.30)	0.035	0.298	0.117
Orobanche rapum-genistae 2	1278 (99.30)	0.035	0.298	0.117
Orobanche cf. *gracilis*	1278 (99.30 [99.29])	0.035	0.298	0.117
Orobanche lutea	1232 (95.73 [95.66])	0.071	0.303	0.234
Orobanche teucrii	555 (43.12 [42.81])	0.046	0.278	0.165
Orobanche versicolor	1278 (99.30)	0.052	0.333	0.155
Orobanche amethystea 1	1247 (96.89)	0.051	0.316	0.162
Orobanche amethystea 2	1243 (96.58 [96.53])	0.046	0.335	0.137
Orobanche hederae 1	1244 (96.66)	0.046	0.335	0.137
Orobanche hederae 2	1243 (96.58)	0.046	0.335	0.137
Orobanche hederae 3	1244 (96.66)	0.046	0.314	0.145
Orobanche crenata	1258 (97.75)	0.048	0.304	0.159
Orobanche minor 1	1264 (98.21 [98.18])	0.046	0.310	0.147
Orobanche minor 2	1274 (98.99)	0.046	0.310	0.147
Orobanche minor 3	1273 (98.91)	0.046	0.310	0.147
Orobanche minor 4	1269 (98.60 [98.59])	0.046	0.310	0.147
Orobanche minor 5	1273 (98.91)	0.045	0.290	0.156
Orobanche artemisiae-campestris	1269 (98.60)	0.046	0.310	0.147
Orobanche nova spec.	1245 (96.74 [96.46])	0.032	0.327	0.098
Orobanche mutelii	1242 (96.50 [96.41])	0.051	0.308	0.167
Orobanche nana 1	1242 (96.50 [96.40])	0.051	0.308	0.167
Orobanche nana 2	1242 (96.50 [96.40])	0.051	0.308	0.167
Orobanche gracilis	1271 (98.76)	0.040	0.341	0.118
Orobanche ramosa	1242 (96.50 [96.47])	0.052	0.332	0.155
Orobanche aconiti-lycoctoni	1292 (100.39 [100.43])	0.027	0.287	0.093
Orobanche caryophyllacea	1287 (100 [100])	0.062	0.321	0.192

Table 2 (*continued*)

Taxon[a]	Sequence length in bp (%) of *Nicotiana tabacum*[b,c]	K_a	K_s	K_a/K_s
Orobanche bartlingii	1253 (97.36 [97.34])	0.048	0.299	0.162
Orobanche alsatica	1232 (95.73 [95.72])	0.043	0.300	0.143
Orobanche transcaucasica	1245 (96.74 [96.73])	0.046	0.310	0.147
Orobanche tunetana	1242 (96.50 [96.50])	0.057	0.329	0.174

[a] Taxa with functional or potentially functional *rbcL* are marked with an asterisk, non-photosynthetic taxa among those are marked with a second asterisk.

[b] GenBank Accession No. NC_001879, positions 37–1323 (of *rbcL*).

[c] In cases where, at the beginning and/or end, stretches of missing data are present (ranging in total from 3 to 101 bp), length was estimated by assuming the presence of nucleotides at these positions when present in >50% of taxa; values in square brackets are percentages of length after exclusion of stretches with missing data.

[d] Sequence includes premature stop codons, but the open reading frame is intact.

species, pseudogene sequences are shorter than functional copies in other taxa, but some sequences are longer, e.g., *O. aconiti-lycoctoni* or the genus *Cistanche* (see Table 2). A few species, however, experienced large deletions. *Orobanche cernua* and *O. cumana* have a 342 bp deletion (Delavault and Thalouran, 2002; Wolfe and dePamphilis, 1997) and all three accessions of *O. teucrii* investigated here have a 742 bp deletion, resulting together with other deletions in reductions in sequence length of 28 and 57%, respectively. Sizes of insertions range from 1 to 31 bp, but most of them are shorter than 10 bp. Insertions are often repetitions of an immediately adjacent fragment (Fig. 1) as is also the case for the 31 bp insertion in *O. pubescens*.

In *Orobanche*, the proportion of synonymous substitutions (K_s) is similar for all sequences ranging from 0.244 to 0.327 in potentially functional genes and from 0.228 to 0.341 in pseudogenes (Table 2). The proportion of non-synonymous substitutions (K_a), however, is higher for pseudogenes (0.027–0.071) than in potentially functional genes (0.006–0.016). This was shown so far for four species (Wolfe and dePamphilis, 1997) and is confirmed here for 28 species. Accordingly, the ratio of non-synonymous to synonymous substitutions (K_a/K_s) as indicator for selective constraints compared to *Lindenbergia* sp. is generally lower in potentially functional genes (0.022–0.050 compared to 0.031–0.093 in non-photosynthetic parasites other than *Orobanche* and 0.029–0.076 in photosynthetic species) than in pseudogenes (0.093–0.253 compared to 0.133–0.153 in *Boschniakia* and *Cistanche*; Table 2).

3.2. Phylogeny of Orobanche

Parameters of the different data sets and tree statistics are summarized in Table 3. Phylogenetic relationships inferred from maximum parsimony analysis of the nucleotide and the complete matrix are virtually identical (Fig. 2). Analysis of the indel matrix resulted in a much less resolved tree, but all retained clades are also present in the most parsimonious trees from analysis of the nucleotide matrix (Fig. 2). Additionally, the indel matrix has a low homoplasy index (0.158, excluding uninformative sites) when applied on trees based on the nucleotide matrix. Bayesian analyses employing the more parameter-rich mixed model consistently resulted in lower likelihood scores than those from the simpler complete model, irrespective of whether branch lengths were estimated for each partition separately or not (see Table 2). Additionally, several runs with the mixed model (two runs with 2×10^6 and one run with 5×10^6 generations, branch lengths estimated for both partitions combined ["linked" in the MrBayes terminology]) starting from different starting points reached stationarity at different likelihood levels (likelihood scores of 7129.1932, 7194.0863, and 7192.9095, respectively). Under Bayesian model selection, however, the Bayes Factor (B_{10}) of 1.0232 between the two most distinct runs with a complete and a mixed model, respectively, do not favor the simpler model over the more complex one (Nylander et al., 2004). More problematic than model selection is that different runs converge on different levels. This might be solved by employing longer runs than used here. Phylogenetic relationships inferred from different runs of the mixed model were identical not only with each other, but also with those inferred from the complete model analysis (data not shown), therefore we did not further investigate this problem. In the following, we therefore only refer to results from Bayesian analysis using the complete model.

Maximum parsimony analysis suggests that *Orobanche*, in its current circumscription, is not monophyletic but exists as two lineages (Fig. 2). One comprises the two Old World sections *Orobanche* and *Trionychon* (bootstrap support [BS] of 100), and the second comprises the two New World sections *Gymnocaulis* and *Myzorrhiza* (BS 100). A clade including *Hyobanche*, *Striga*, *Alectra*, and some other genera (BS 96) is suggested as sister to the Old World *Orobanche* sections, while *Cistanche* and *Boschniakia* are proposed as sister to the New World *Orobanche* sections, rendering *Orobanche* polyphyletic. However, none of these relationships is sufficiently supported statistically and if *Orobanche* is constrained to be monophyletic the trees are only one step longer and not significantly different from the unconstrained tree (Templeton test, $P = 1.0$).

Table 3
Parameters of different data sets and tree statistics

Data set	Number of characters			Maximum parsimony					Maximum likelihood ln score	Bayesian analysis	
	Total	Variable	Parsimony-informative	Number of trees	Tree length	Consistency index[a]	Retention index	Rescaled consistency index		Complete model[b] ln score (SD)	Mixed model[c] ln score[d] (SD)
Nucleotide	1290	491	327	114	845	0.6117	0.9071	0.6291	−6940.3007	−7030.9345 (10.1372)	−7125.6804 (15.1320)
Indel	76	76	53	76,002	83	0.8833	0.9766	0.8942			
Complete	1366	567	380	1204	929	0.6332	0.9135	0.6510			

[a] Excluding uninformative characters.
[b] Same substitution model for all positions.
[c] Different models for the two partitions first plus second codon position and third codon position, respectively.
[d] From an Bayesian analysis with 2×10^6 generations and all parameters (including branch lengths) estimated for each partition separately.

Like maximum parsimony, maximum likelihood and Bayesian analysis detect an Old World and a New World clade within *Orobanche* (BS 100, posterior probability [PP] 1.00 for both of them; Fig. 3). However, neither maximum likelihood nor Bayesian analysis refutes a monophyletic *Orobanche*. Instead, they suggest that the two clades of *Orobanche* form a clade with *Cistanche* and *Boschniakia*, if only weakly supported (BS 54, PP 0.92). The relationships among these groups are either unresolved or only weakly supported, e.g., a clade consisting of *Cistanche* and *Boschniakia* (BS 51, PP 0.81).

All three analyses suggest congruent internal relationships for the two *Orobanche* clades (Figs. 2 and 3). While the two American groups are sister taxa (each BS 100/100 [from maximum parsimony/maximum likelihood analysis], PP 1.00), species of sect. *Trionychon* nest within sect. *Orobanche*, rendering the latter paraphyletic. The alternative hypothesis of a clade including the American sections and sect. *Trionychon* being sister taxa, as suggested by other data (see Section 4), results in significantly different trees both in a maximum parsimony and a maximum likelihood framework (tree length 913, $P < 0.001$; likelihood score of −7180.36638, $P < 0.001$). In detail, species of sect. *Trionychon* (BS 100/100, PP 1.00) are sister to a clade including *O. minor*, *O. crenata*, and related species (BS 94/51, PP 0.75). This is also supported by the presence of a 39 bp deletion in species of sect. *Trionychon*, which they share with the above-mentioned species of sect. *Orobanche* plus some other taxa (Fig. 1). Two further clades are observed: one including *O. lutea*, *O. caryophyllacea*, and *O. teucrii* (BS 95/90, PP 1.00) and a second including *O. gracilis* and *O. rapum-genistae* s. l. (BS 100/100, PP 1.00).

3.3. Intraspecific variation and evolution of host range

Several individuals (from 2 to 41) have been analyzed for 15 species (Table 4). No intraspecific variability was found in several of those species where only two individuals were analyzed, e.g., *O. artemisiae-campestris* and *O. gracilis*, but also in *O. crenata*, where six accessions from distant localities (Corsica and Greece) had identical *rbcL* sequences. Intraspecific variation was not only found in species with a higher number of individuals investigated, such as *O. minor* (five haplotypes in 41 individuals) or *O. rigens* (two haplotypes in 12 individuals), but also in less densely sampled species, e.g., *O. cernua* (three haplotypes in three individuals) or *O. nana* (two haplotypes in two individuals). Species are generally phylogenetically well delimited and different intraspecific haplotypes, when present, are monophyletic (Fig. 4A). Exceptions concern mostly species which appear paraphyletic, e.g., *O. corymbosa* and *O. rigens*, but *O. hederae* and *O. amethystea* are found to be polyphyletic (Fig. 4A).

Fig. 2. One of the most parsimonious trees (length = 929) obtained from analysis of the complete matrix including several sequences per species only if these are not identical (branch lengths obtained via ACCTRAN optimization). Numbers at nodes are bootstrap values. Arrowheads indicate branches collapsed in the strict consensus tree, which is identical to that from analysis of the nucleotide matrix (see text for details) with the exception of *O. crenata* and *O. transcaucasica*, which constitute a clade (asterisk). Thick branches indicate clades obtained with the indel matrix alone. Large deletions are indicated by a vertical bar on the corresponding branches, numbers indicating their sizes (in bp). Taxa in bold possess two bracteoles. Sections of the genus *Orobanche* (according to Beck-Mannagetta, 1930) and intrasectional structuring in sect. *Orobanche* proposed by Beck-Mannagetta (1930; left column) and Teryokhin et al. (1993; right column) are indicated. Abbreviations: AM, *Amoenae*; AR, *Arcuatae*; CO, *Coerulescentes*; CR, *Cruentae*; CU, *Curvatae*; GA, *Galeatae*; HE, *Hederae*; MI, *Minores*; and SP, *Speciosae*.

Ancestral character state reconstruction using maximum parsimony suggests that the ancestor of sect. *Orobanche* had a narrow host range (Fig. 4B). Wide host range evolved independently at least twice: once in *O. cumana*, which is nested within *O. cernua*, the latter being restricted to Asteraceae, and a second time at the base of the clade comprising *O. minor* and related species. A reversal from wide to narrow host range is suggested for *O. artemisiae-campestris*, which is restricted to *Artemisia* spp. (Asteraceae) as hosts.

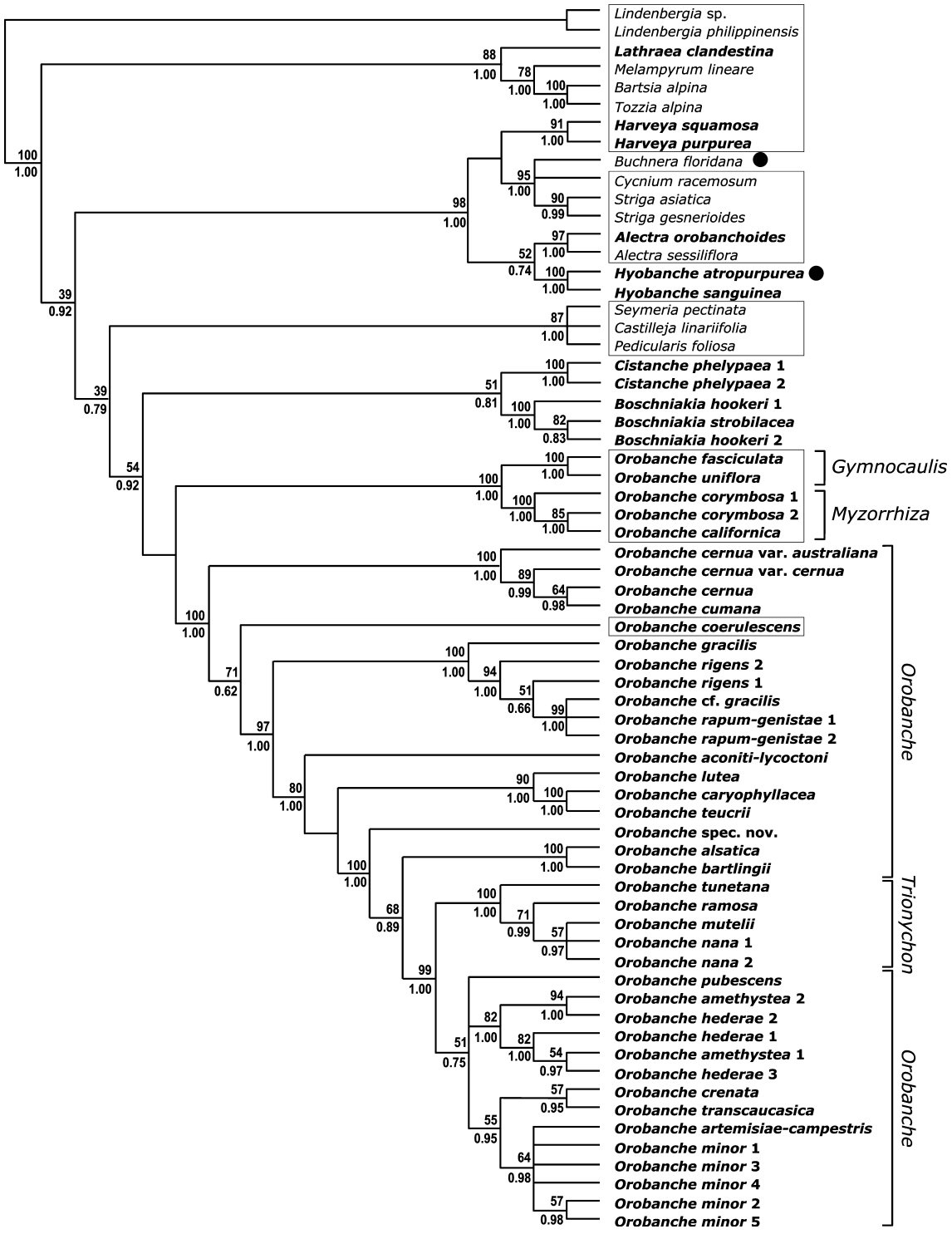

Fig. 3. One of the two most likely trees, which is topologically identical to the 50% majority-rule consensus tree from Bayesian analysis. Numbers above branches are bootstrap values, those below branches are posterior probabilities. Taxa with a potentially functional *rbcL* sequence are boxed, taxa with a stop codon in the *rbcL* sequence, but otherwise unaltered reading frames, are marked with a dot. Taxa in bold are holoparasitic plants (according to http://www.science.siu.edu/parasitic-plants/). Indications of sections of *Orobanche* as in Fig. 2.

4. Discussion

As the *rbcL* sequence in holoparasitic plants could be highly degenerated, its PCR amplification might be problematic. Universal primers do not work for all *Orobanche* species, and even the design of universal *Orobanche rbcL* primers is problematic. The use of different combinations of several primers is the only way to pro-

Table 4
Numbers of individuals and haplotypes found in species, where more than one individual was investigated

Taxon	Number of individuals	Number of haplotypes
sect. *Orobanche*		
amethystea	2	2
artemisiae-campestris	2	1
cernua	3	3
crenata	6	1
gracilis	2	1
hederae	7	3
minor	41	5
pubescens	2	1
rapum-genistae	4	2
rigens	12	2
teucrii	3	1
sect. *Myzorrhiza*		
corymbosa	2	2
sect. *Trionychon*		
nana	2	2
ramosa	2	1
tunetana	2	1

duce an *rbcL* PCR product for some species, but this random strategy does not always guarantee success. For instance, we were unable to amplify *rbcL* sequences from *O. crinita*, *O. arenaria* or *O. purpurea*. In those cases, the *rbcL* sequence might be too highly degenerated like in the unalignable remnant *rbcL* sequence of *Epifagus* (dePamphilis and Palmer, 1990), or simply no appropriate primer pairs were found. In such situations a false positive signal due to contamination could arise and precaution should be taken as in PCR amplification of low copy templates. We believe that the results obtained in this study are not affected by the problem of contamination for two reasons. First, non-*Orobanche* *rbcL* sequences could be easily identified using BLAST search and always belonged to plant groups investigated by other people in the same lab. Second, independent amplifications in two different labs (Geneva and Vienna) of different specimens of the same species yielded identical or nearly identical *rbcL* sequences.

4.1. Potentially functional copies of rbcL in Orobanche

Potentially functional copies of *rbcL* are present in species of the American sections *Myzorrhiza* and *Gymnocaulis* and in *O. coerulescens* of sect. *Orobanche*, while all other species of the Old World sections *Orobanche* and *Trionychon* have pseudogenes (Wolfe and dePamphilis, 1997, 1998; this study). This could indicate that pseudogene formation has occurred twice independently in *Orobanche* (in the *O. cernua*/*cumana* clade and in the clade including the majority of *Orobanche* species; Figs. 2 and 3). The phylogenetic position of *O. coerulescens*, however, is statistically only moderately supported (Schneeweiss et al., 2004a; this study), and further data are necessary to corroborate it. Leebens-Mack and dePamphilis (2002) have shown that potentially functional *rbcL* sequences can be under selective constraint (e.g., in *O. fasciculata*) or not (e.g., in *O. corymbosa*). The presence of a potentially functional *rbcL* gene in *O. coerulescens* provides an additional opportunity to study such questions using methods taking phylogeny in account (e.g., Leebens-Mack and dePamphilis, 2002; Yang and Bielawski, 2000), which goes beyond the scope of this paper.

4.2. Suitability of indels as phylogenetic markers

Assuming that the formation of indels follows cladogenesis, they may represent a valuable complementary phylogenetic marker. However, as the mechanism of deletions and insertions could be the result of particular sequence structure and pattern (Kelchner, 2000) this could happen independently in different lineages. This has been suggested for *Orobanche*, where the majority of indels are believed to have arisen independently (Wolfe and dePamphilis, 1997). Deletions are suggested to be the result of a strand-slippage replication error involving sequence repeats found along the gene. This is the case for the large deletion found in *O. cernua* and *O. cumana* (Delavault and Thalouran, 2002; Wolfe and dePamphilis, 1997). This is apparently not true for other large deletions, for instance the 39 bp deletion in *O. minor* and relatives or the 742 bp deletion in *O. teucrii* (see Fig. 1) and for other smaller deletions. Wolfe and dePamphilis (1997) based their conclusions on only four *Orobanche* species and sequences. Our study, based on 106 specimens of 28 *Orobanche* species, clearly indicates that the phylogenetic signals in the indel matrix and the nucleotide matrix are congruent (Fig. 2). Thus, the formation of indels does not introduce homoplasy but follows cladogenesis and accordingly indels are valuable phylogenetic markers.

4.3. Phylogeny of Orobanche

In *rbcL* sequences of holoparasitic species, the rate of non-synonymous substitutions is increased compared with that of photosynthetic non-parasitic and parasitic species (Table 2). This is expected in holoparasitic plants because genes involved in photosynthesis are no longer under functional constraints. Although *rbcL* sequences of some species, e.g., *O. corymbosa* or *O. coerulescens*, still have an intact open reading frame and thus might still be functional, most *Orobanche* species have pseudogenes with high substitution rates. Therefore, a bias in the phylogenetic signal cannot be ruled out (Wolfe and dePamphilis, 1998). Those clades including species with *rbcL* pseudogenes are supported by long branches

Fig. 4. Intraspecific variability (A) and host range evolution (B) in *Orobanche* sect. *Orobanche*. (A) One of 506 most parsimonious trees based on all specimens analyzed in sect. *Orobanche* using the American species as outgroup (length = 463, consistency index excluding uninformative characters = 0.7702, retention index = 0.9314, and rescaled consistency index = 0.7826). If known, host plants are indicated. Numbers at nodes are bootstrap values. (B) Evolution of host range based on one of the most parsimonious trees (the same as in Fig. 2). Black branches indicate wide host range, white branches indicate narrow host range, and mixed branches indicate ambiguous character reconstruction.

(Fig. 2), which might cause long-branch attraction. Phylogenetic relationships among long-branch clades inferred from maximum likelihood and Bayesian analysis, however, result in largely congruent results and the few alternative hypotheses have insufficient statistical support. We are therefore confident, that long-branch attraction artifacts, if present, do not affect the phylogenetic interpretations.

Orobanche in its current circumscription is not monophyletic, but falls into two clades. One includes the two American sections *Gymnocaulis* and *Myzorrhiza*, and the second includes the two Old World sections *Orobanche* and *Trionychon*. This is also supported by previous studies using plastid *mat*K and *rps*2 sequences (Young et al., 1999). The relationships of these two clades to each other and to *Boschniakia* and *Cistanche* are unclear. While analyses of *rbcL* data do not refute monophyletic *Orobanche* (maximum likelihood, Bayesian inference) or at least do not result in significantly different trees (maximum parsimony; see Section 3), both *mat*K and *rps*2 suggest a diphyletic *Orobanche* (Young et al., 1999). These genes showed the American sections of *Orobanche* to be sister to *Boschniakia*, a hypothesis supported by the most parsimonious tree from analysis of the *rbcL* data. Nuclear ITS data support a diphyletic *Orobanche*, but are equally unclear regarding the phylogenetic relationships of the two *Orobanche* lineages to *Boschniakia* and *Cistanche*, which group in a polytomy (Schneeweiss et al., 2004a). Evidently, further molecular studies are necessary to elucidate the exact phylogenetic relationships among those lineages.

The most surprising result, however, is the phylogenetic position of sect. *Trionychon* being nested in sect. *Orobanche* (Figs. 2 and 3). A bias in the phylogenetic signal due to different substitution rates as discussed above cannot explain this unexpected position of sect. *Trionychon* because both *Orobanche* sections include *rbcL* pseudogenes. Moreover, the clade including species of sect. *Trionychon* is statistically well supported and has a synapomorphic deletion of 39 bp (see Section 3). Evidence from other data clearly contradicts this phylogenetic position. Phylogenetic analyses of nuclear ITS suggest that sect. *Trionychon* is more closely related to the American sections (Schneeweiss et al., 2004a). Such a relationship is strongly supported by the distribution of chromosome base numbers, because sect. *Trionychon* shares $x = 12$ with the American sections, while sect. *Orobanche* has $x = 19$ (Schneeweiss et al., 2004b). Morphologically, sect. *Trionychon* differs from sect. *Orobanche* by actinomorphic calyces and the presence of two bracteoles. These characters connect sect. *Trionychon* rather with the American sections, especially sect. *Myzorrhiza*, as opposed to sect. *Orobanche*.

This unexpected phylogenetic position could also result from the fact that the amplified *rbcL* sequences are from sequences transferred to the nuclear or the mitochondrial genome, as has been shown for *O. cumana* (Delavault and Thalouran, 2002). Indeed, it could be argued that the plastid *rbcL* sequence of sect. *Trionychon* could not be amplified by the primers used, which would also explain the failure to amplify *rbcL* from several species of sect. *Trionychon*, such as *O. purpurea* and *O. arenaria*. Instead, another *rbcL* sequence, which possibly escaped from the plastid genome (Martin and Herrmann, 1998; Stegemann et al., 2003; Thorness and Weber, 1996), could have been amplified. This is expected to result in a spurious phylogenetic position of sect. *Trionychon*. In order to examine this possibility, a complete matrix including the nuclear *rbcL* sequence of *O. cumana* (GenBank AF090350) has been analyzed. This sequence falls in the *O. cumana* and *O. cernua* clade with high support (BS 98; data not shown), suggesting that the nuclear *rbcL* sequence has retained characteristics of the plastid *rbcL* sequence of *O. cumana*. Therefore, spurious attraction of *rbcL* sequences of sect. *Trionychon* to those of sect. *Orobanche* by this mechanism is unlikely.

Another explanation for the nested position of sect. *Trionychon* in sect. *Orobanche* might be that species of sect. *Trionychon* have captured the plastid genome of a member of sect. *Orobanche*. If so, then all plastid genes should follow this trend. Data are available for two other plastid sequences, *rps*2 and *mat*K (Young et al., 1999), for three species of sect. *Orobanche* (*O. hederae*, *O. cernua*, and *O. caryophyllacea*), and one species of sect. *Trionychon* (*O. ramosa*). Phylogenetic analyses of both genes show a highly supported sister relationship between *O. ramosa* and *O. hederae*, thus rendering sect. *Trionychon* nested in sect. *Orobanche*. If three different plastid genes indicate the same phylogenetic behavior it is more likely that the whole plastid genome of sect. *Trionychon* resembles that of some members of sect. *Orobanche*. We have examined *rps*2 sequences in our specimens of *O. nana*, *O. mutelii*, and *O. ramosa* (sect. *Trionychon*) and found that in phylogenetic analyses they indeed group with *rps*2 sequences of sect. *Orobanche* (*O. minor* and *O. hederae*; data not shown). However, based on other evidence described above, it is very unlikely that sect. *Trionychon* is phylogenetically nested in sect. *Orobanche*. Transfer of the plastid genome from sect. *Orobanche* to sect. *Trionychon* might be invoked as an explanation. This could have happened via hybridization (e.g., Cronn and Wendel, 2004; Rieseberg, 1995; Rieseberg et al., 1996), although the lack of any hybrids between species of sect. *Orobanche* and sect. *Trionychon* makes this hypothesis less likely. A second possibility is via horizontal gene transfer by vectors such as viruses, fungi, or insects. This mode of gene transfer is found in an increasing number of plants, albeit unequivocally shown only for mitochondrial genes (Bergthorsson et al., 2003; Städler and Delph, 2002; Won and Renner, 2003). Further studies are currently underway to investigate this phenomenon in more detail.

4.4. Phylogenetic relationships within sect. Orobanche

Apart from the nested position of sect. *Trinychon* in sect. *Orobanche* discussed above, *rbcL* sequences are in agreement with those from nuclear ITS data (Schneeweiss et al., 2004a). The sampling in this study is smaller than in the study of Schneeweiss et al. (2004a), and for some groups, such as "grex" *Cruentae* and "grex" *Glandulosae*, no conclusions can be drawn. Nevertheless, several aspects are worth mentioning. Both *rbcL* and ITS suggest that *O. cernua* (including *O. cumana*) and *O. coerulescens* are not monophyletic. Thus, they support the taxonomic view of Teryokhin et al. (1993), who classified them into two subsections *Amoenae* and *Coerulescentes*, respectively, rather than that of Beck-Mannagetta (1930), who united them in "grex" *Coerulescentes*. Both markers also agree that "grex" (subsect.) *Speciosae*, comprising the economically important *O. crenata* and related species, is nested in "grex" (subsect.) *Minores* including *O. minor*, *O. hederae*, and *O. amethystea* among others. In a few cases, however, the phylogenetic relationships inferred from *rbcL* sequences do not agree with those from nuclear ITS. For instance, an accession of *O.* cf. *gracilis* of "grex" *Cruentae* groups with *O. rapum-genistae* and *O. rigens* of "grex" *Arcuatae* in the *rbcL*-tree, while it groups with other species of "grex" *Cruentae* in the ITS-tree. Based on the shape of the corolla, Beck-Mannagetta (1930) unites *O. caryophyllacea*, *O. lutea*, and *O. teucrii* in "grex" *Galeatae*, which is supported by *rbcL*, but not by ITS.

Two species included here were not sampled for nuclear ITS. The first is a new species from Corsica, which combines morphological characters of "grex" *Arcuatae* and "grex" *Minores*, but according to the molecular data it does not belong to any of these groups. *Orobanche aconiti-lycoctoni* has been recently described from Spain (Carlón et al., 2003) and is thought to be closely related or even identical to *O. flava* of "grex" *Curvatae* (Pujadas Salvà, 2003). Although *O. aconiti-lycoctoni* does not group with the other species of "grex" *Curvatae* included in our study, *O. alsatica* and *O. bartlingii*, a close relationship to *O. flava* cannot be ruled out, because *O. flava* belongs to a different clade than *O. bartlingii* (Schneeweiss et al., 2004a), but was not sampled for this study.

4.5. Intraspecific variability

This is the first study where *rbcL* sequences were sampled from several individuals (2–41) across several species. Because of the relaxation of functional constraints, increased intraspecific variation might be expected, but this appears to be rare. In most cases, intraspecific haplotypes group together, exceptions concerning species of the taxonomically difficult *O. minor* aggregate (*O. artemisiae-campestris*, *crenata*, *minor*, and *transcaucasica*; see Schneeweiss et al., 2004a). This is particularly pronounced in *O. hederae* and *O. amethystea* (Fig. 4A), which might have several reasons: (1) both species actually are polyphyletic, (2) the two species are in fact conspecific; and (3) some of the accessions have been misidentified. In general, however, different haplotypes appear as monophyletic groups, and *rbcL* gene trees can be expected to mirror the species trees to an extent comparable to other markers.

4.6. Potential tool for species identification

Orobanche species are generally well differentiated by their *rbcL* sequences and different haplotypes, if present, are monophyletic. This confirms their utility for testing existing identifications based on morphological features or to directly determine morphologically very similar species as has been shown for *O. minor* and related species (Benharrat et al., 2000). However, in other cases species-level identification using *rbcL* sequences is not possible, e.g., for *O. minor* and *O. artemisiae-campestris*. This and the lack of monophyly of some species (see above) indicate that *rbcL* sequences can aid in species delimitation and specimen determination, but only when augmented with other markers.

4.7. The evolution of host range

Most *Orobanche* species exhibit narrow host ranges, often being restricted to members of only one family or even genus (e.g., *O. teucrii* on several species of *Teucrium*, Lamiaceae). In the most extreme case, the parasite grows exclusively on one particular host, e.g., *O. aconiti-lycoctoni* on *Aconitum lycoctonum* (Ranunculaceae). A few species, however, have very wide host ranges growing on many different species often belonging to different families. For instance, accessions of *O. minor* investigated in this study were found on more than 20 different host species belonging to Apiaceae, Asphodelaceae, Asteraceae, Fabaceae, Lamiaceae, Plantaginaceae, and Rubiaceae. If intraspecific variability on the genetic level is present, this might be correlated with the presence of different parasite races as defined by their hosts. However, this does not seem to be the case in *Orobanche*, because identical haplotypes can grow on many different hosts and the same host can be utilized by different haplotypes (Fig. 4A).

Maximum parsimony reconstruction suggests that the ancestor of *O.* sect. *Orobanche* had a narrow host range. Holoparasitic plants are completely dependent on their hosts, therefore they must be well adapted to them. Such adaptations include efficient recognition of putative host plants via chemical signals (see review by Bouwmeester et al., 2003) or mechanisms to promote a successful establishment on the host by, e.g., overcoming of host resistances. These adaptations also imply that a holoparasitic plant will be specialized to a few

hosts, to which it is well adapted. Although this specialization decreases the number of putative hosts, at the same time it increases the chance of a successful establishment on the host plants to which the parasite is adapted. It can be speculated that such an increase in the chance of successful establishment is particularly important in the transitional phase from facultative hemiparasitism to obligate hemiparasitism and eventually holoparasitism, when the parasite must successfully attack a host in order to survive. This hypothesis also predicts that holoparasitic plants in general should have narrower host ranges than (facultative) hemiparasitic plants, which seems to be the case in Orobanchaceae (Estabrook and Yoder, 1998; Press and Graves, 1995). Further investigations are necessary to better understand the evolutionary significance of changes in host range and the exact mechanisms causing such changes.

Wide host range evolved at least twice independently, once in *O. cumana* and a second time in the group including *O. minor* and related species (Fig. 4B). Although a wide host range seems to be advantageous because more plants could potentially be parasitized, this switch evidently has occurred only in a few lineages. Interestingly, these lineages also harbor the most noxious weed species of sect. *Orobanche*, *O. cumana*, *O. crenata*, and *O. minor*, which can cause severe damages to crop plants (Parker and Riches, 1993; Wegmann, 1998). A reversal to narrow host ranges is seen in *O. artemisiae-campestris* (Fig. 4B), a species restricted to *Artemisia* spp. (Asteraceae). However, neither nuclear ITS (Schneeweiss et al., 2004a) nor *rbcL* (this study) allow its separation from *O. minor*. Accordingly, *O. artemisiae-campestris* might be only one of many races within the morphologically very variable *O. minor* to be defined mostly by its host. More finely resolving molecular techniques should allow to address the question of whether populations growing on perennial *Artemisia* species form a monophyletic group distinct from closely related taxa, or whether the adaptation to these hosts has occurred several times independently.

Acknowledgments

This study has been financially supported by the Conservatoire et Jardin Botaniques de Genève, the Austrian Science Foundation (FWF Project P14352-BIO), and the University of Geneva.

References

Beck-Mannagetta, G., 1890. Monographie der Gattung *Orobanche*. Theodor Fischer, Cassel.

Beck-Mannagetta, G., 1930. IV. 261. Orobanchaceae. In: Engler, A. (Ed.), Das Pflanzenreich. Regni Vegetabilis Conspectus. Wilhelm Engelmann, Leipzig, pp. 1–348.

Benharrat, H., Delavault, P., Theodet, C., Figureau, C., Thalouarn, P., 2000. *rbcL* plastid pseudogene as a tool for *Orobanche* (Subsection *Minores*) identification. Plant Biol. 2, 34–39.

Bergthorsson, U., Adams, K.L., Thomason, B., Palmer, J.D., 2003. Widespread horizontal transfer of mitochondrial genes in flowering plants. Nature 424, 197–201.

Bouwmeester, H.J., Matusova, R., Zhongkui, S., Beale, M.H., 2003. Secondary metabolite signalling in host–parasitic plant interactions. Curr. Opin. Plant Biol. 6, 358–364.

Carlón, L., Gómez Casares, G., Laínz, M., Moreno Moral, G., Sánchez Pedraja, Ó., 2003. A propósito de algunas *Orobanche* (Orobanchaceae) del noroeste peninsular y de su tratamiento en Flora Iberica vol. XIV (2001). Doc. Jard. Bot. Atlantico 1, 1–44.

Colwell, A.E.L., 1994. Genome evolution in a non-photosynthetic plant. Ph.D. thesis, Washington University, St. Louis.

Cronn, R., Wendel, J., 2004. Cryptic trysts, genomic mergers, and plant speciation. New Phytol. 161, 133–142.

dePamphilis, C.W., Palmer, J.D., 1990. Loss of photosynthetic and chlororespiratory genes from the plastid genome of a parasitic flowering plant. Nature 349, 337–339.

dePamphilis, C.W., Young, N.D., Wolfe, A.D., 1997. Evolution of plastid gene *rps*2 in a lineage of hemiparasitic and holoparasitic plants: many losses of photosynthesis and complex patterns of rate variation. Proc. Natl. Acad. Sci. USA 94, 7367–7372.

Delavault, P., Thalouran, P., 2002. The obligate root parasite *Orobanche cumana* exhibits several *rbcL* sequences. Gene 297, 85–92.

Delavault, P., Sakanyan, V., Thalouarn, P., 1995. Divergent evolution of two plastid genes, *rbcL* and *atpB*, in a non-photosynthetic parasitic plant. Plant Mol. Biol. 29, 1071–1079.

Estabrook, E.M., Yoder, J.I., 1998. Plant–plant communications: rhizosphere signaling between parasitic angiosperms and their hosts. Plant Physiol. 116, 1–7.

Gustincich, S., Manfioletti, G., Del Sal, G., Schneider, C., Carninci, P., 1991. A fast method for high-quality genomic DNA extraction from whole human blood. Biotechniques 11, 298–302.

Holub, J., 1977. New names in Phanerogamae 6. Folia Geobot. Phytotax 12, 417–432.

Holub, J., 1990. Some taxonomic and nomenclatural changes within *Orobanche* s. l. (Orobanchaceae). Preslia 62, 193–198.

Kelchner, S.A., 2000. The evolution of non-coding chloroplast DNA and its implication in plant systematics. Ann. Mo. Bot. Gard. 87, 482–498.

Leebens-Mack, J., dePamphilis, C., 2002. Power analysis of tests for loss of selective constraint in cave grayfish and nonphotosynthetic plant lineages. Mol. Biol. Evol. 19, 1292–1302.

Maddison, W.P., Maddison, D.R., 2003. Mesquite: a modular system for evolutionary analysis. Version 1.0. Available from: <http://mesquiteproject.org>.

Martin, W., Herrmann, R.G., 1998. Gene transfer from the organelles to the nucleus: how much, what happens, and why?. Plant Physiol. 118, 9–17.

Morden, C.W., Wolfe, K.H., dePamphilis, C.W., Palmer, J.D., 1991. Plastid translation and transcription genes in a non-photosynthetic plant: intact, missing and pseudo gene. EMBO J. 10, 3281–3288.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47–67.

Olmstead, R.G., dePamphilis, C.W., Wolfe, A.D., Young, N.D., Elisons, W.J., Reeves, P.A., 2001. Disintegration of the Scrophulariaceae. Am. J. Bot. 88, 348–361.

Oxelman, B., Backlund, M., Bremer, B., 1999. Relationships of the Buddlejaceae s. l. investigated using parsimony jackknife and branch support analysis of chloroplast *ndhF* and *rbcL* sequence data. Syst. Bot. 24, 164–182.

Parker, C., Riches, C.R., 1993. Parasitic Weeds of the World: Biology and Control. CAB International, Wallingford.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Press, M.C., Graves, J.D., 1995. Parasitic Plants. Chapman & Hall, London.

Pujadas Salvà, A.J., 2003. *Orobanche flava* Mart. ex F. W. Schultz (Orobanchaceae) en la Península Ibérica. An. Jard. Bot. Madrid 60, 387–393.

Rieseberg, L.H., 1995. The role of hybridization in evolution: old wine in new skins. Am. J. Bot. 82, 944–953.

Rieseberg, L.H., Whitton, J., Linder, C.R., 1996. Molecular marker incongruence in plant hybrid zones and phylogenetic trees. Acta Bot. Neerl. 45, 243–262.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Schneeweiss, G.M., Colwell, A., Park, J.-M., Jang, C.-G., Stuessy, T.F., 2004a. Phylogeny of holoparasitic *Orobanche* (Orobanchaceae) inferred from nuclear ITS sequences. Mol. Phylogenet. Evol. 30, 465–478.

Schneeweiss, G.M., Palomeque, T., Colwell, A.E., Weiss-Schneeweiss, H., 2004b. Chromosome numbers and karyotype evolution in holoparasitic *Orobanche* (Orobanchaceae) and related genera. Am. J. Bot. 91, 439–448.

Soják, J., 1972. Nomenklatorické poznámky (Phanerogamae). Časopis Národniho Muzea, odd. přírodovědný 140, 127–134.

Städler, T., Delph, L.F., 2002. Ancient mitochondrial haplotypes and evidence for intragenic recombination in a gynodioecious plant. Proc. Natl. Acad. Sci. USA 99, 11731–11735.

Stegemann, S., Hartmann, S., Ruf, S., Bock, R., 2003. High-frequency gene transfer from the chloroplast genome to the nucleus. Proc. Natl. Acad. Sci. USA 100, 8828–8833.

Steppan, S.J., Storz, B.L., Hoffmann, R.S., 2004. Nuclear DNA phylogeny of the squirrels (Mammalia: Rodentia) and the evolution of arboreality from c-myc and RAG1. Mol. Phylogenet. Evol. 30, 703–719.

Swofford, D.L., 2003. PAUP∗. Phylogenetic Analysis Using Parsimony (∗and Other Methods). Version 4. Sinauer Associates, Sunderland, MA.

Teryokhin, E.S., Shibakina, G.V., Serafimovich, N.B., Kravtsova, T.I., 1993. Opredelitelj Sarasichovich Florii SSSR (Determinator of Broomrapes of the USSR Flora). Nauka, Leningrad.

Thorness, P.E., Weber, E.R., 1996. Escape and migration of nucleic acids between chloroplasts, mitochondria, and the nucleus. Int. Rev. Cytol. 165, 207–234.

Uhlich, H., Pusch, J., Barthel, K.-J., 1995. Die Sommerwurzarten Europas, Gattung *Orobanche*. Westarp Wissenschaften, Magdeburg, Germany.

Wegmann, K., 1998. The *Orobanche* problem in tobacco. In: Wegmann, K., Musselman, L.J., Joel, D.M. (Eds.), Proceedings of the 4th International *Orobanche* Workshop Albena, Bulgaria, 23–26 September 1998. Institute for Wheat and Sunflower, General Toshevo, Bulgaria, pp. 13–17.

Wettstein, R., 1891. Scrophulariaceae. In: Engler, A., Prantl, K. (Eds.), Die Natürlichen Pflanzenfamilien 4 (3b). Wilhelm Engelmann, Leipzig, pp. 39–107.

Wimpee, C.F., Morgan, R., Wrobel, R.L., 1992. Loss of transfer RNA from the plastid 16S-23S ribosomal RNA gene spacer in a parasitic plant. Curr. Genet. 21, 417–422.

Wolfe, A.D., dePamphilis, C.W., 1997. Alternate paths of evolution for the photosynthetic gene *rbcL* in four nonphotosynthetic species of *Orobanche*. Plant Mol. Biol. 33, 965–977.

Wolfe, A.D., dePamphilis, C.W., 1998. The effect of relaxed functional constraints on the photosynthetic gene *rbcL* in photosynthetic parasitic plants. Mol. Biol. Evol. 15, 1243–1248.

Wolfe, A.D., Randle, C.P., 2001. Relationships within and among species of the holoparasitic genus *Hyobanche* (Orobanchaceae) inferred from ISSR banding patterns and nucleotide sequences. Syst. Bot. 26, 120–130.

Wolfe, K.H., Morden, C.W., Ems, S.C., Palmer, J.D., 1992a. Rapid evolution of the plastid translational apparatus in a nonphotosynthetic plant: loss or accelerated sequence evolution of tRNA and ribosomal protein genes. J. Mol. Evol. 35, 304–317.

Wolfe, K.H., Morden, C.W., Palmer, J.D., 1992b. Function and evolution of a minimal plastid genome from a nonphotosynthetic parasitic plant. Proc. Natl. Acad. Sci. USA 89, 10648–10652.

Won, H., Renner, S.S., 2003. Horizontal gene transfer from flowering plants to *Gnetum*. Proc. Natl. Acad. Sci. USA 100, 10824–10829.

Yang, Z. 2003. Phylogenetic Analysis by Maximum Likelihood. Version 3.13d. Program downloadable from ftp://abacus.gene.ucl.ac.uk/pub/paml/.

Yang, Z., Bielawski, J.P., 2000. Statistical tests of adaptive molecular evolution. Trends Ecol. Evol. 15, 496–502.

Yang, Z., Nielsen, R., 2000. Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models. Mol. Biol. Evol. 17, 32–43.

Young, N.D., Steiner, K.E., dePamphilis, C.W., 1999. The evolution of parasitism in Scrophulariaceae/Orobanchaceae: plastid gene sequence refute an evolutionary transition series. Ann. Mo. Bot. Gard. 86, 876–893.