

# Molecular phylogeny and species definition within the species rich *Astragalus/Oxytropis* complex (Fabaceae)

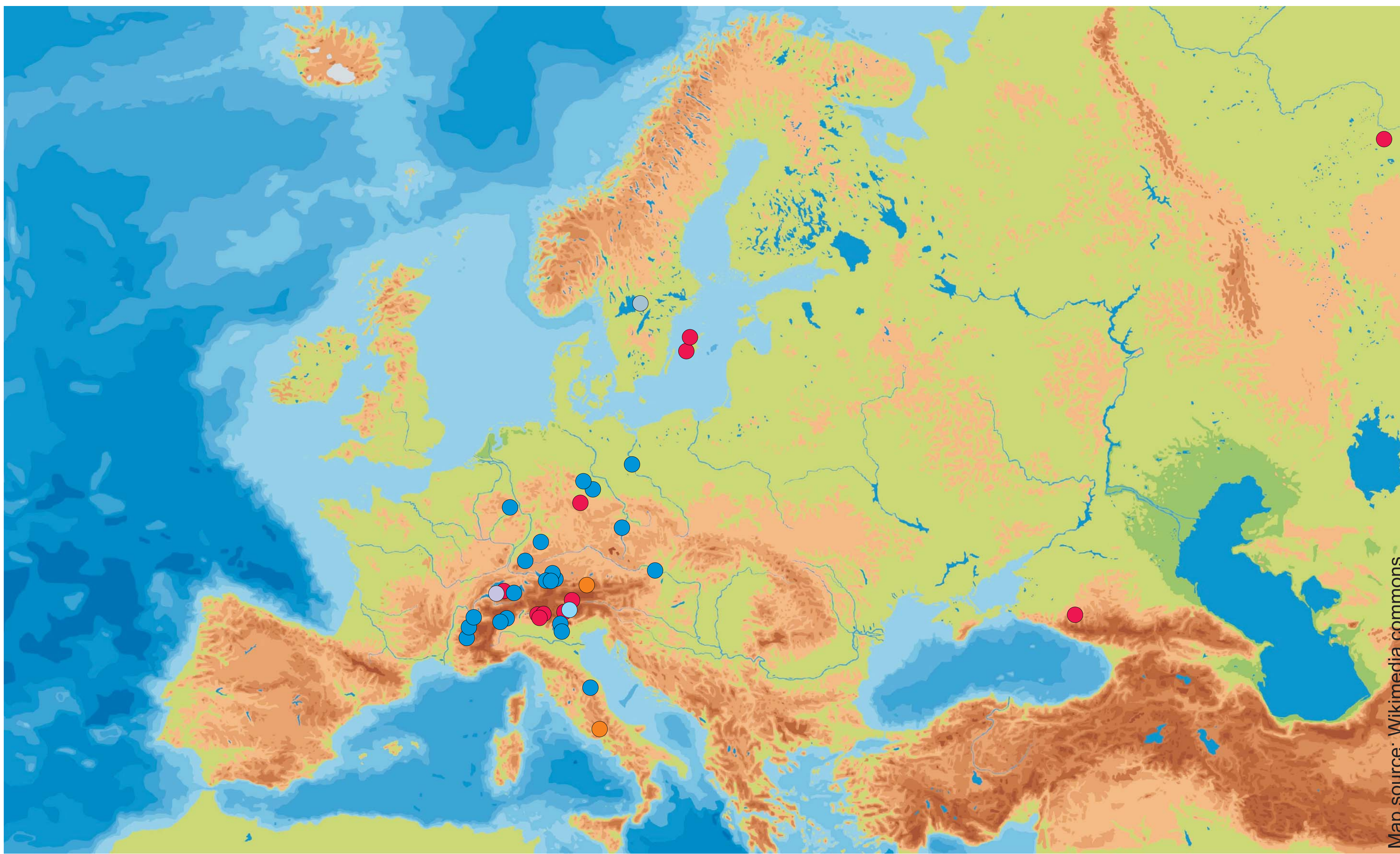
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## Introduction

*Oxytropis pilosa* is a widespread but highly disjunctive relict species that originated in the steppes of the Altai, Siberia, from where it spread to the West (Fig. 1). Having left the steppe-like areas where its distribution is facilitated by the tumbleweed-like behaviour of the infructescences, the species is now restricted to extreme xeric habitats such as gypsum or disturbed stony banks (Figs. 2 a-b).



● 1a putative stem type ● 1b variant  
● 2a second main type replacing the stem type  
● 2b ● 2c ● 2d variants

Fig. 1: Distribution map of the obtained types inferred by UPGMA analysis for *O. pilosa* (cf. Fig. 4).

## A deep glance into *Oxytropis pilosa*

The morphologically very homogeneous *O. pilosa* was studied by phytosociological and molecular methods (Schlee et al. 2003). We obtained more than 200 cloned ITS rDNA sequences which enabled us to evaluate the intra- and interindividual variability (Fig. 3). Genetic diversity within individuals is comparable to that within populations. The populations can be grouped into two main types using the MOD transformation (Göker & Grimm 2008; Fig. 4) and principal coordinate analysis of uncorrected distances between the clones (Fig. 5). One type is characteristic for the putative stem populations of Russia (Fig. 3). The second main type gradually replaces the first type throughout Europe, the stem type is completely lost within the most remote populations in the French Sea Alps (Fig. 4). Mixtures of both types occur within Central Europe and Northern Europe. The data indicate repeated (post-)glacial exchange between temporally disjunct populations. Unique mutations (Hegau mountains, SW Germany; Bormio, Italian Alps; Östergötland, Sweden; Fig. 3) could possibly mirror such nunatak-associated relicts.

country	no. of clones	ITS1	ITS2
RUS (E)	23	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
RUS (S)	14	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
A	16	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
D (S)	16	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
D (SW)	17	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
D (E)	15	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
S (G1)	16	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
S (G2)	13	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
S	16	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
CH	15	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
F	15	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
I (N1)	13	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
I (N2)	321	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
I (C1)	12	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
I (C2)	12	ACCTGTCACCGGGGATAAATGCTGTCGATGATTCACACGTCGTCGATGATTCATCT	TTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT

Fig. 3: Part of the alignment of ITS1, 5.8S rDNA, and ITS2 sequences for *Oxytropis pilosa* (extract only shows variable positions anywhere in the total alignment). Data inferred via cloning technique.

Tab. 1: Relevés inferred via Braun-Blanquet approach (extract).

No.	S84	S83	S85	R14	R4	R5
Relieve dimension	1	1.5	1	4	0.4	1
Inclination	20	45	45	15	45	5
Exposition	SW	SW	S	SW	SW	SW
Coverage altogether	60	15	50	75	60	70

Column 1-3: near Tübingen, 4: Abruza, 5-6: Lower Austria. *Oxytropis pilosa* secondary stands. Increase of species numbers and abundances related to the fringing communities leads to overgrowing of the whilom open stands (decrease of *O. pilosa*). Only disturbances keep the habitats open and favour the rare plants.

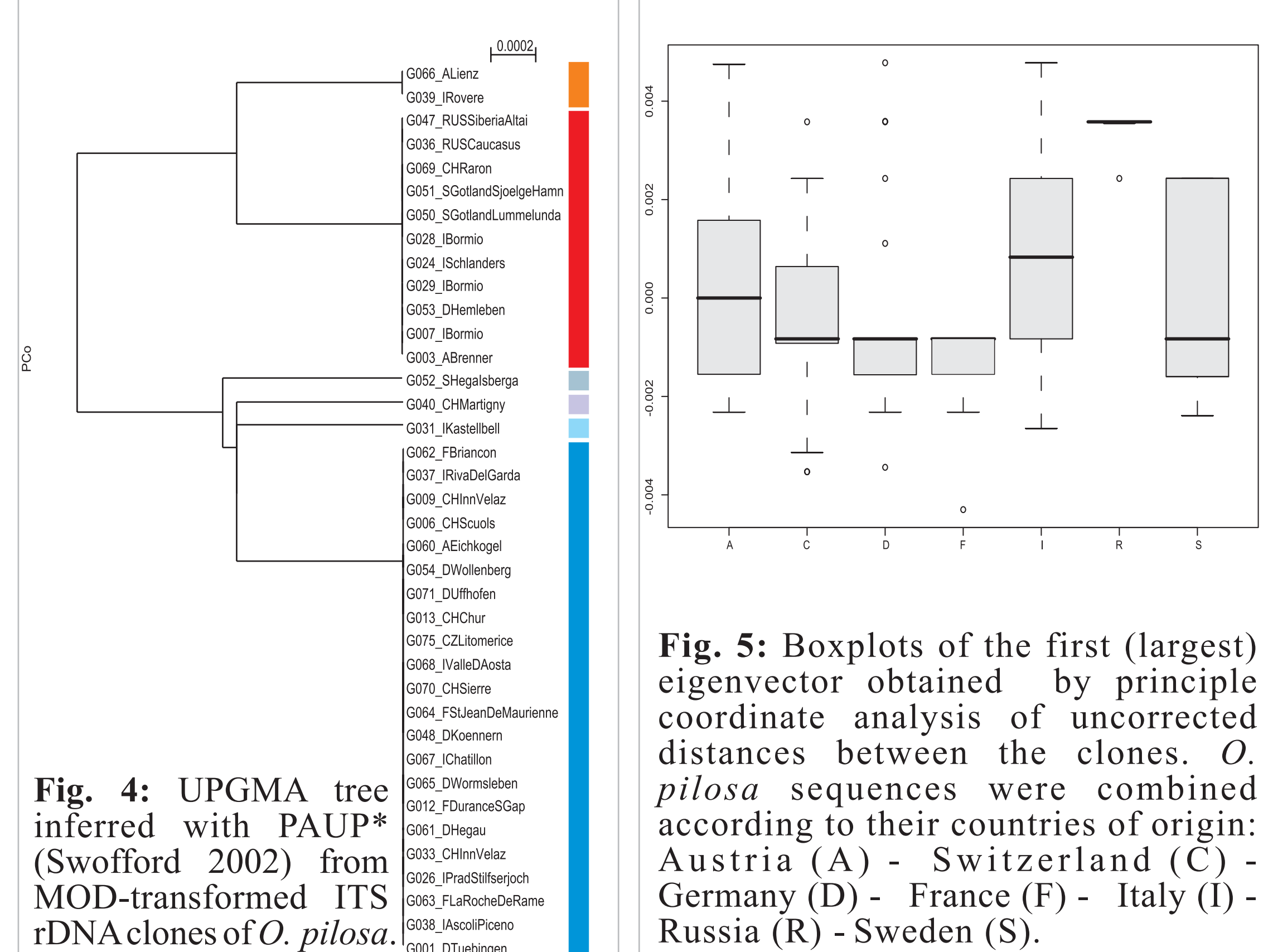


Fig. 4: UPGMA tree inferred with PAUP\* (Swofford 2002) from MOD-transformed ITS rDNA clones of *O. pilosa*.

## Outlook: Identifying genetic resources beyond species concepts

The outlined methods provide objective means to identify populations which represent evolutionary hot spots. Also, relict populations within assumed homogeneous ranges can be detected easily. These populations should considerably benefit from protection efforts. Habitats of *O. pilosa* show dramatic secondary successions due to improper management regimes, highlighting the need for improved protection efforts (cf. phytosociological relevés, Tab. 1).

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Fig. 2a: *Oxytropis pilosa*, Italy, Marche Fig. 2b: *O. pilosa*, Switzerland, Valais

## Divergence in species of *Oxytropis* and *Astragalus*

Additionally, the genetic divergence within *O. pilosa* was compared to other taxa within the *Astragalus/Oxytropis* complex (2.500/300 species) – one of the largest Angiosperm genus complexes altogether (Fig. 6). Species clusters were defined by non-hierarchical single-linkage clustering (Göker et al. 2009); as threshold the smallest possible distance was chosen that resulted in all *O. pilosa* sequences being assigned to the same cluster. The ENT transformation of Göker & Grimm (2008) was applied to detect statistically significant differences between the genetic diversity of the resulting clusters. Molecular divergence within *O. pilosa* was insignificantly different from that observed in clusters comprising numerous accepted *Astragalus* and *Oxytropis* species (Schlee et al., in press).

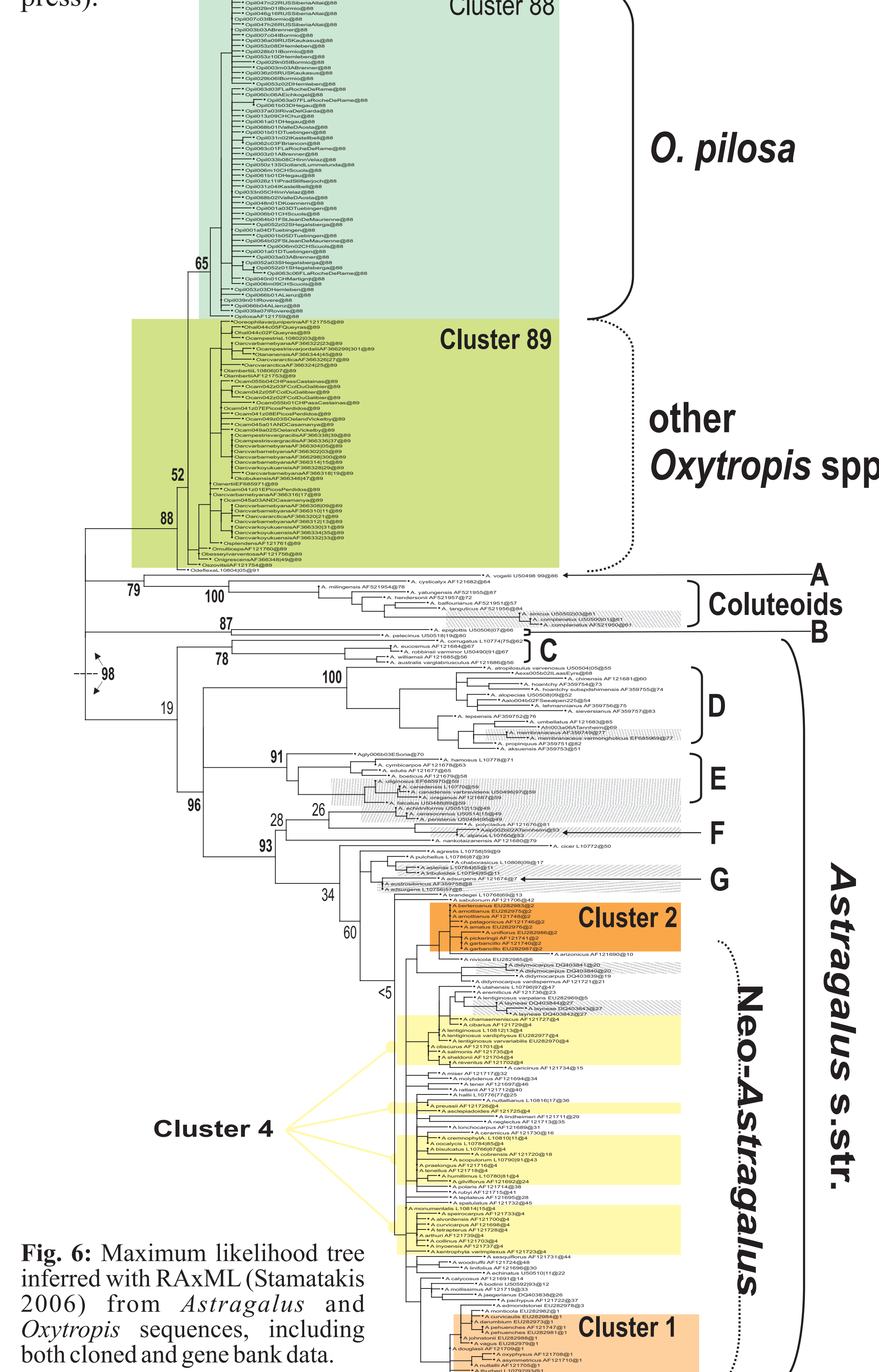


Fig. 6: Maximum likelihood tree inferred with RAXML (Stamatakis 2006) from *Astragalus* and *Oxytropis* sequences, including both cloned and gene bank data.