Species composition of Saxifraga sect. Saxifraga subsect. Arachnoideae (Saxifragaceae) based on DNA sequence evidence

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Species composition of *Saxifraga* sect. *Saxifraga* subsect. *Arachnoideae* (*Saxifragaceae*) based on DNA sequence evidence

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Abstract: We investigated the species composition of *Saxifraga* sect. *Saxifraga* subsect. *Arachnoideae*, a recently recognized taxon, using DNA sequence data (ITS, *trnL–trnF*, *rpl32–trnL*$_{(UAG)}$). We conclude that the subsection contains 12 species, i.e. *S. aphylla*, *S. arachnoidea*, *S. berica*, *S. facchinii*, *S. hohenwartii*, *S. muscoides*, *S. paradoxa*, *S. petraea*, *S. prenja*, *S. presolanensis*, *S. sedoides* and *S. tenella*. Of these, we provide the first molecular evidence for the membership of *S. muscoides* and *S. prenja* in *S.* subsect. *Arachnoideae*. We provide an extended morphological characterization of the subsection and an identification key to its species.

Key words: ecology, homoplasy, ITS, morphological characters, *rpl32–trnL*$_{(UAG)}$, *Saxifraga*, *Saxifraga* subsect. *Arachnoideae*, *Saxifragaceae*, *trnL–trnF*

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Introduction

The history of the infrageneric classification of *Saxifraga* L. (*Saxifragaceae*), a genus of between 440 to 500 species in its current circumscription (Tkach & al. 2015), has been described in detail by Webb & Gornall (1989). Probably the most important contributor to the knowledge of the genus was Adolf Engler as author of a monograph (Engler 1872) and of all treatments in both editions of *Die natürlichen Pflanzenfamilien* and in *Das Pflanzenreich* (Engler 1891; Engler & Irmscher 1916, 1919; Engler 1930). The Engler & Irmscher (1916, 1919) classification was later modified by Gornall (1987), who provided the basis for the classification used by Webb & Gornall (1989) in their account of *Saxifraga* in Europe. The use of DNA sequence data for the study of *Saxifraga* soon revealed that the genus is monophyletic only after exclusion of *Micranthes* Haw. (Soltis & al. 1993; Fernández Prieto & al. 2013), and non-monophyly of several infrageneric taxa had been demonstrated in other early DNA studies (e.g. Conti & al. 1999; Vargas 2000). In their analysis of *Saxifraga* using DNA sequence data of 254 species (since expanded by Ebersbach & al. 2017), Tkach & al. (2015) identified several further instances of non-monophyly of infrageneric taxa recognized by Engler & Irmscher (1916, 1919) or Gornall (1987).

Tkach & al. (2015) also introduced *Saxifraga* sect. *Saxifraga* subsect. *Arachnoideae* (Engl. & Irmsch.) Tkach & al., based on *S.* sect. *Nephrophyllum* Gaud. grex *Arachnoideae* Engl. & Irmsch. Tkach & al. (2015) identified *S. aphylla* Sternb., *S. arachnoidea* Sternb., *S. berica* (Bég.) D. A. Webb, *S. paradoxa* Sternb., *S. petraea* L., *S. presolanensis* Engl., *S. sedoides* L. (of which only ssp. *hohenwartii* (Vest ex Sternb.) Schwarz was sequenced by

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these authors) and *S. tenella* Wulfen as members of *S.* subsect. *Arachnoideae*. They described the group as follows: "The biennial to perennial plants are characterized by a diffuse habit, fragile, decumbent to ascending stems, soft leaves, frequently have long hairs, and pale white to yellowish or greenish petals. The species occur in shadowy, rocky places, frequently at high altitudes. They are distributed in the Alps and extend to the Apennines and the Balkan Peninsula." The group was expanded by *S. sedoides* subsp. *sedoides* by Ebersbach & al. (2017), who also treated *S. sedoides* subsp. *hohenwartii* at specific rank as *S. hohenwartii* Vest ex Sternb., and by *S. facchinii* W. D. J. Koch by Tkach & al. (2019). *Saxifraga facchinii* had earlier been identified as closely related to *S. aphylla* by Vargas (2000). *Saxifraga* subsect. *Arachnoideae* in this circumscription contained elements of Engler & Irmscher's (1916, 1919) *S.* sect. *Tridactylites* Haw., *S.* sect. *Dactyloides* Tausch and *S.* sect. *Nephrophyllum* and of the genus *Zahlbrucknera* Rchb., which surprisingly was used to accommodate *S. paradoxa* (as *Z. paradoxa* (Sternb.) Rchb.) even in the last of Engler's treatments of *Saxifraga* (Engler 1930). Of the taxa recognized by Gornall (1987) and Webb & Gornall (1989), members of their *S.* sect. *Saxifraga* subsect. *Triplinervium* (Gaudin) Gornall and *S.* sect. *Saxifraga* subsect. *Holophyllae* Engl. & Irmsch were found in *S.* subsect. *Arachnoideae*.

Ecologically, *Saxifraga* subsect. *Arachnoideae* as understood by Tkach & al. (2015) and Ebersbach & al. (2017) is a highly diverse lineage. Whereas some species (*S. berica*, *S. paradoxa*) grow exclusively at very low altitudes mostly in shady recesses of calcareous or non-calcareous rocks, others (*S. aphylla*, *S. hohenwartii*, *S. sedoides*) are distributed at mostly subalpine to alpine altitudes in the Alps, where they mostly grow on calcareous gravel, scree or rocks, while a third group of species (*S. arachnoidea*, *S. petraea*, *S. presolanensis*, *S. tenella*) shows various combinations of the ecological properties of the preceding two groups (Webb 1993; Kaplan 1995; Aeschimann & al. 2004). Supported topological incongruencies between phylogenies reconstructed from nuclear (ITS) and plastid sequences as found by Tkach & al. (2015) and ourselves (see Results) raise the suspicion that interspecific transfer of adaptive traits by hybridization may play an important role in the evolution of *S.* subsect. *Arachnoideae*.

In preparation of a detailed analysis of this hypothesis, we here make an effort to identify all possible members of the subsection. Obvious candidates for such membership are *Saxifraga prenja* Beck from the Dinaric Alps, part of the *S. sedoides* group (Hörandl 1993) and treated at either specific (Hörandl 1993) or subspecific (Webb & Gornall 1989) rank, and *S. muscoides* All., which had been considered closely related to *S. facchinii* by Webb & Gornall (1989) and Kaplan (1995).

As evident from the above comparison of lineages identified by DNA sequence data and non-molecular classifications, morphology alone clearly is no reliable basis for identifying the complete species composition of *Saxifraga* subsect. *Arachnoideae*. However, all species of *S.* subsect. *Arachnoideae* as understood to date (Tkach & al. 2015; Ebersbach & al. 2017) belong to *S.* subsect. *Triplinervium* and *S.* subsect. *Holophyllae* as understood by Webb & Gornall (1989). Therefore we obtained, in addition to using DNA sequences of *Saxifraga* available at GenBank, DNA sequences (ITS, *trnL–trnF*, *rpl32–trnL*$_{(UAG)}$) of those species of *S.* subsect. *Triplinervium* and *S.* subsect. *Holophyllae* as understood by Webb & Gornall (1989) that were available to us and had not been sequenced before. We also considered the possibility of a close relationship to *S.* subsect. *Arachnoideae* of species of *S.* sect. *Saxifraga* for which no DNA sequences were available.

Material and methods

Sample collection and DNA extraction

Samples of species of *Saxifraga* subsect. *Arachnoideae*, *S.* subsect. *Androsaceae* (Engl. & Irmsch.) Tkach & al. and *S.* subsect. *Tridactylites* (Haw.) Gornall sensu Tkach & al. (2015), corresponding to *S.* subsect. *Holophyllae*, *S.* subsect. *Triplinervium* and *S.* subsect. *Tridactylites* sensu Webb & Gornall (1989), were collected in 2016 from wild populations in the European Alps, the Apennines and the Dinaric Alps. Fresh leaves were collected and dried on silica gel. Vouchers were deposited at MJG (herbarium codes according to *Index herbariorum*; http://sweetgum.nybg.org/science/ih/). Additional samples from *S.* subsect. *Holophyllae* and *S.* subsect. *Triplinervium* sensu Webb & Gornall (1989) were obtained from BC, GJO, LZ, MA and WU (see Supplementary Table 1 in Supplemental content online). Correct species identification was checked for all herbarium specimens prior to sampling, particularly in order to avoid inclusion of hybrid individuals. Herbarium specimens were used only when all morphological traits necessary for species identification were visible. DNA was extracted with a Macherey-Nagel NucleoSpin Plant II kit (Machery-Nagel GmbH & Co. KG, Düren, Germany) using the SDS-based Lysis Buffer PL2. We mostly followed the manufacturer's instructions, but extended the duration of the cell lysis step to 60 min. DNA was quantified with a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) using the dsDNA HS Assay. DNA was purified using ethanol precipitation to remove plant compounds that inhibited PCR (Sambrook & Russell 2001).

DNA sequencing and genome skimming

The nuclear Internal Transcribed Spacer (ITS), the plastid *trnL–trnF* intergenic spacer (IGS) and the *rpl32–trnL*$_{(UAG)}$ IGS were chosen for Sanger sequencing. PCR amplification of the ITS region was performed using primers ITS 17SE_m and ITS 26SE_m (Grudinski & al. 2014). The *trnL–trnF* IGS was amplified using primers trnLF c and trnLF f (Taberlet & al. 1991). The *rpl32–trnL*$_{(UAG)}$ IGS

was amplified using primers rpl32-F and trnL$_{(UAG)}$ (Shaw & al. 2007). PCRs were carried out using the following programs: 120 s at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 59 °C for ITS, 51 °C for *trnL–trnF* IGS, and 48 °C for *rpl32–trnL*$_{(UAG)}$ IGS, 120 s at 72 °C and a post-treatment of 600 s at 72 °C. PCR products were cleaned using a NucleoSpin Gel and PCR Clean-up kit (Machery-Nagel GmbH & Co. KG, Düren, Germany). Cycle Sequencing was carried out by StarSEQ GmbH, Mainz, Germany. We obtained an ITS sequence for *Saxifraga coarctata* W. W. Sm. by mapping the reads from NCBI short read archive SRR7901466 (deposited under the synonym *S. humilis* Engl. & Irmsch.) against the ITS sequence of *S. arachnoidea* MG16072311 using BBMAP v.38.58 (Bushnell 2019) with a minimum identity of 80 percent. The consensus sequence of the 946 mapped reads was called in Geneious v9.0.5 (https://www.geneious.com/). All new sequences obtained in this study were submitted to GenBank and accession numbers for new and existing sequences are given in Supplementary Table 1 (Supplemental content online).

Sanger sequence alignment and phylogenetic analyses

Sanger sequencing raw data were manually edited in PortableSequencer 4.1.4 (Gene Codes Corporation, Ann Arbor, MI) and aligned in MEGA 10.0.5 (Kumar & al. 2018). We added additional samples from GenBank (Supplementary Table 1) for all subsections of *Saxifraga* sect. *Saxifraga* sensu Tkach & al. (2015). To take into account the possibility that one or more of the species sampled belong to other sections of *Saxifraga*, we also sampled more distantly related outgroups. These were *S.* sect. *Cotylea* Tausch, *S.* sect. *Cymbalaria* Griseb., *S.* sect. *Gymnopera* D. Don, *S.* sect. *Ligulatae* Haw., *S.* sect. *Mesogyne* Sternberg, *S.* sect. *Porphyrion* Tausch and *S.* sect. *Trachyphyllum* (Gaudin) W. D. J. Koch. Alignments were trimmed at both ends to ensure that at least 50 percent of all samples of each alignment had sequence data at both ends of the alignment. Sequences of plastid (cp) markers *trnL–trnF* and *rpl32–trnL*$_{(UAG)}$ IGS were combined. For samples of *S.* subsect. *Arachnoideae*, only plastid sequences of the same individual sample or from the same sampling locations were combined. For species of all other subsections of *S.* sect. *Saxifraga* and of all outgroup sections, plastid sequences were combined. Sequence alignments were annotated and alignments were inspected in Partitionfinder2 v2.1.1 (Lanfear & al. 2017) on the CIPRES Science Gateway (Miller & al. 2010). Maximum likelihood (ML) analyses were carried out in RAxML v8.2.12 (Stamatakis 2014) under an unpartitioned GTR + Γ substitution model. Bootstrapping was conducted under GTR + Γ with automated bootstrapping halt under the extended majority-rule consensus tree criterion (autoMRE). Bayesian inferences (BI) were carried out in MrBayes 3.2.6 (Ronquist & al. 2012) under substitution models SYM+I+G for ITS and GTR + Γ for the plastid markers. Markov chains were run for 1M generations with the first 250K generations discarded as burn-in to ensure that minimum estimated sample sizes for all parameters were greater than 200. Two independent runs were conducted, each sampling 7501 trees. Trees were rooted with *S.* sect. *Cymbalaria*. All alignments and trees are available in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S25221).

Results

Sample collection and DNA sequencing

We added 90 new DNA sequences to the existing record of DNA sequences of *Saxifraga*, including the first sequences for *S. glabella* Bertol., *S. maireana* Luizet, *S. maweana* Baker, *S. muscoides* and *S. prenja*. Therefore, of the 86 accepted species (Gornall 1987; Webb & Gornall 1989; Tkach & al. 2015; Ebersbach & al. 2017) of *S.* sect. *Saxifraga*, DNA sequences of 77 species were included in the present study.

Sanger sequence alignment and phylogenetic analyses

The phylogenetic trees obtained using *trnL–trnF* and *rpl32–trnL*$_{(UAG)}$ IGS were topologically congruent. Accordingly, these two markers were combined. Total alignment lengths for ITS and the combined cpDNA sequences were 812 bp and 1888 bp, respectively.

Phylogenetic trees from both datasets, ITS and cpDNA, support a monophyletic *Saxifraga* subsect. *Arachnoideae* comprising *S. aphylla*, *S. arachnoidea*, *S. berica*, *S. facchinii*, *S. hohenwartii*, *S. muscoides*, *S. paradoxa*, *S. petraea*, *S. prenja*, *S. presolanensis*, *S. sedoides* and *S. tenella* (Fig. 1, 2). In the ITS trees (Fig. 1), *S. aphylla*, *S. arachnoidea*, *S. berica*, *S. facchinii*, *S. hohenwartii*, *S. paradoxa*, *S. petraea* and *S. tenella* were reconstructed as monophyletic (all ML Bootstrap support ≥ 99/ BI posterior probability = 1.00). Accessions of *S. prenja* and *S. sedoides* constitute a well-supported clade (96/1.00), with *S. prenja* inferred as monophyletic under BI and ML but with low bootstrap support in the ML tree (63/0.96). Accessions of *S. muscoides* and *S. presolanensis* together formed a well-supported clade (86/1.00), but the two species were not inferred as monophyletic. These 12 species form the monophyletic *Arachnoideae* clade in both ML and BI reconstructions (99/1.00). In contrast to the ITS tree, the cpDNA tree is less well resolved (Fig. 2). *Saxifraga paradoxa* was reconstructed as monophyletic (100/1.00) and placed as sister to a clade with all other species of the *Arachnoideae* clade (99/0.89). This relationship, however, is supported only in the ML tree (99/0.94). Of all other species of which more than one accession was sequenced, only *S. arachnoidea* was reconstructed as monophyletic under ML and BI, but with low support in ML and no support in BI (72/0.71).

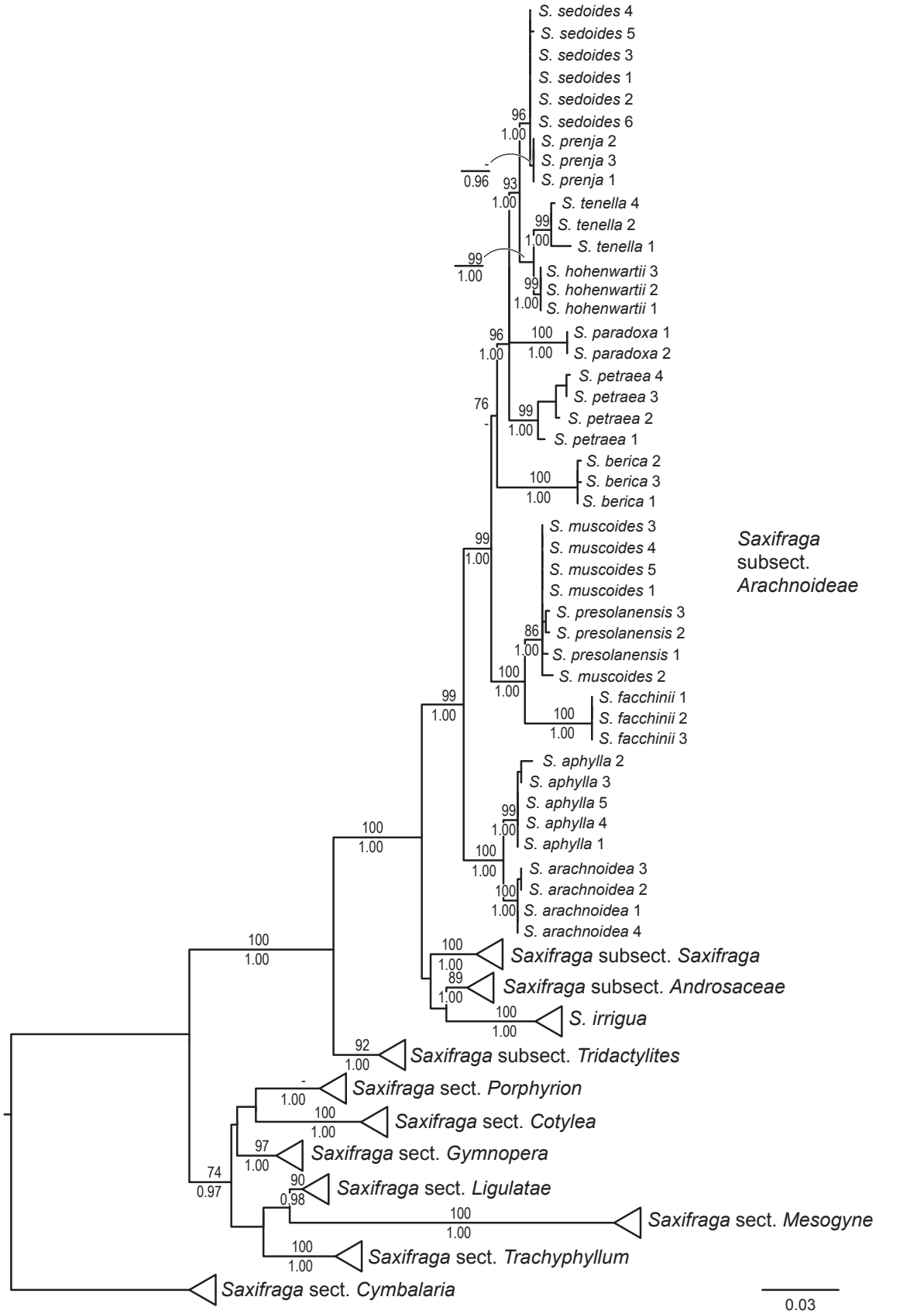

Fig. 1. Maximum likelihood phylogeny of *Saxifraga* sect. *Saxifraga* based on ITS. Triangles represent collapsed clades, *S.* subsect. *Arachnoideae* is shown uncollapsed. Values above branches are maximum likelihood bootstrap values, values below branches are posterior probability values. Only bootstrap values ≥ 70 and posterior probabilities ≥ 0.95 are shown. Intraspecific support values are not shown. Scale bar: no. of substitutions per site.

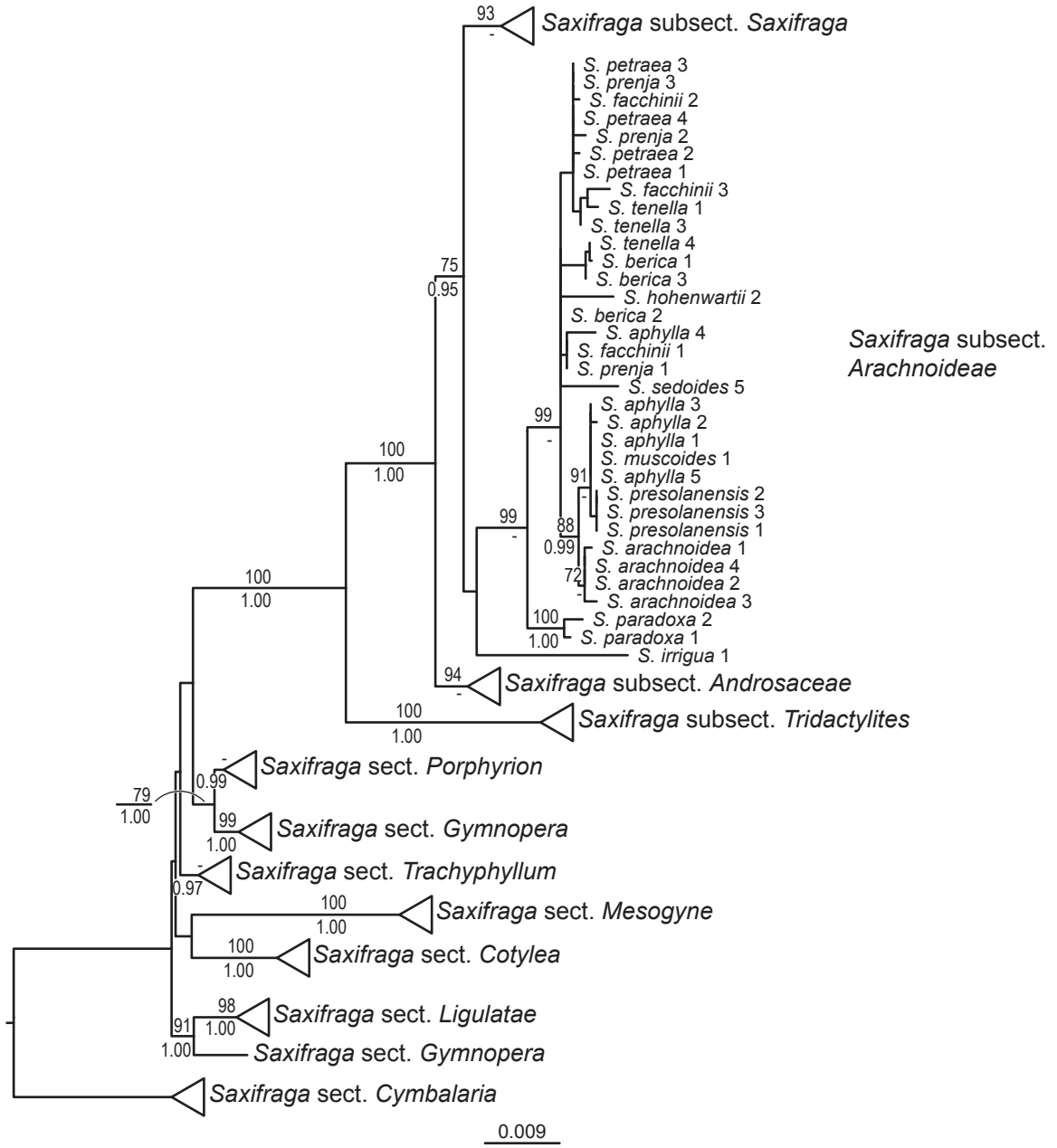

Fig. 2. Maximum likelihood phylogeny of *Saxifraga* sect. *Saxifraga* based on plastid *rpl32–trnL*$_{(UAG)}$ and *trnL–trnF* IGS. Triangles represent collapsed clades, *S.* subsect. *Arachnoideae* is shown uncollapsed. Values above branches are maximum likelihood bootstrap values, values below branches are posterior probability values. Only bootstrap values ≥ 70 and posterior probabilities ≥ 0.95 are shown. Intraspecific support values are not shown. Scale bar: no. of substitutions per site.

Discussion

As indicated in the introduction, and as found earlier by Tkach & al. (2015), the phylogenies based on ITS and cpDNA sequences respectively contain supported topological incongruencies. Whereas *Saxifraga paradoxa* is supported sister (ML only) to the remainder of the subsection in the cpDNA tree (Fig. 2), it is part of a supported trichotomy with *S. petraea* and *S. hohenwartii*/*S. prenja*/*S. sedoides*/*S. tenella* in the ITS tree (Fig. 1). Also, *S. aphylla*, *S. arachnoidea*, *S. muscoides* and *S. presolanensis* form one of only three clades supported under ML in the cpDNA tree. However, in the ITS tree *S. muscoides* and *S. presolanensis* as well as *S. aphylla* and *S. arachnoidea* are found in two supported clades, which cannot be closest relative to each other and which partly contain species from outside the cpDNA clade just described. In view of these incongruencies, which we suspect are the result of interspecific hybridization (but also may reflect incomplete lineage sorting), we refrain from discussing possible interspecific relationships. Instead, we will discuss the species composition of *S.* subsect. *Arachnoideae*.

Based on our data, *Saxifraga* subsect. *Arachnoideae* contains twelve species (for further discussion of species

status see below), i.e. *S. aphylla*, *S. arachnoidea*, *S. berica*, *S. facchinii*, *S. hohenwartii*, *S. muscoides*, *S. paradoxa*, *S. petraea*, *S. prenja*, *S. presolanensis*, *S. sedoides* and *S. tenella*. The nine species of *S.* sect. *Saxifraga* not included in the present analysis are *S. adenodes* Poepp. ex Sternb. and *S. boussingaultii* Brongn. (both distributed in the Andes), *S. embergeri* Maire, *S. luizetiana* Emb. & Maire, *S. numidica* Maire, *S. tricrenata* Pau & Font Quer and *S. werneri* Font Quer & Pau. (all Atlas Mountains) and *S. trautvetteri* Manden. and *S. verticillata* Losinsk. (both Caucasus). We believe to have good reason to assume that none of these nine species is part of *S.* subsect. *Arachnoideae*. First, all these species belong to former *S.* ser. *Gemmifereae* (Willk.) Pawłowska, *S.* ser. *Cespitosae* (Rchb.) Pawłowska and *S.* ser. *Ceratophyllae* (Haw.) Pawłowska. The vast majority of species of these three series were included in our (and earlier) analyses and all fell into *S.* subsect. *Saxifraga*. This finding makes it likely that the unsampled species of the three series also belong to *S.* subsect. *Saxifraga*. Second, whereas all species now identified as belonging to *S.* subsect. *Arachnoideae* are distributed in or near the S and SE Alps (and in the Apennines and Balkan Peninsula in the case of *S. prenja*), all unsampled species have an extra-European distribution. Considering the continuous geographical range of *S.* subsect. *Arachnoideae* as circumscribed using DNA sequence data, it seems unlikely to us that extra-European species are part of this lineage. However, given discrepancies between morphological classification and phylogenetic evidence, and given the existence of unusual geographical disjunctions, we cannot completely rule out that DNA sequences may show that unsampled species of *Saxifraga* fall into *S.* subsect. *Arachnoideae*.

In comparison to previous molecular studies by Vargas (2000), Tkach & al. (2015; 2019) and Ebersbach & al. (2017), *Saxifraga muscoides* and *S. prenja* are new additions to *S.* subsect. *Arachnoideae*. This is not surprising at all for *S. prenja*. This species had been treated as one of three species of the *S. sedoides* group by Hörandl (1993), and as one of three subspecies of *S. sedoides* by Webb & Gornall (1989). Following Hörandl (1993), this group of three taxa is characterized by mucronate to apiculate leaf tips and rather small yellowish white petals. As regards *S. muscoides*, Engler (1872) had treated *S. facchinii* as a variety of *S. muscoides*, implying close similarity between the two. Morphological similarities between *S. facchinii* and *S. muscoides* and particularly *S. presolanensis* had been pointed out earlier. Webb & Gornall (1989) emphasized that only these three species (*S. facchinii*, *S. muscoides*, *S. presolanensis*, with the exception of some variants of *S. moschata* Wulfen) share obtuse, entire and glandular hairy leaves and a compact, cushion-like habit. Kaplan (1995) used the firm cushions formed by *S. facchinii* and *S. muscoides* and the silvery grey colour of recently withered leaves as characters linking them again to *S. presolanensis*. From a morphological point of view, it is therefore perfectly plausible that *S. muscoides* is part of *S.* subsect. *Arachnoideae*.

Of the 12 species belonging to *Saxifraga* subsect. *Arachnoideae*, eight were supported as monophyletic in the ITS tree. These were *S. aphylla*, *S. arachnoidea*, *S. berica*, *S. facchinii*, *S. hohenwartii*, *S. paradoxa*, *S. petraea* and *S. tenella*. The accessions of *S. muscoides* and *S. presolanensis* together fell into one polytomy, and the six accessions of *S. sedoides* formed a polytomy with the three accessions of *S. prenja*, which formed a supported clade in the BI but not in the ML analysis. While ITS variation is clearly not sufficient to resolve these four taxa as monophyletic, the ITS topology does not contradict their taxonomic recognition provided they can be distinguished morphologically. This clearly is the case for *S. muscoides* and *S. presolanensis*. For example, flowering stems of *S. muscoides* have one or rarely up to three flowers with ovate, light lemon-yellow or pale yellow petals. In contrast, flowering stems of *S. presolanensis* have two to eight flowers with oblong-cuneate and pale greenish yellow petals (Webb & Gornall 1989; Kaplan 1995).

Although, as stated at the beginning of this discussion, our main aim is not to discuss interspecific relationships, it is evident that the three species of the *Saxifraga sedoides* group do not form a monophylum. Instead, *S. hohenwartii* groups apart from *S. prenja* and *S. sedoides* and is sister to *S. tenella* in our ITS tree. Following the descriptions provided by Hörandl (1993), a possibly closer relationship between *S. prenja* and *S. sedoides* might be supported by the shared possession of short glandular hairs (longer in *S. hohenwartii*) and bright yellow (orange-red in *S. hohenwartii*) fresh anthers. The same assessment of similarities among the three species was given by Hörandl (1993). The ITS topology found by us (Fig. 1) clearly implies that *S. hohenwartii*, treated as a subspecies (Webb & Gornall 1989) or species (Hörandl 1993) in the past, should be treated at specific rank because *S. hohenwartii* and *S. tenella* can be easily distinguished (Webb & Gornall 1989; Hörandl 1993; Kaplan 1995). Following Hörandl (1993), we prefer specific rank also for *S. prenja* and *S. sedoides*, which are rather distinct morphologically (see below), geographically and ecologically (Hörandl 1993). Interestingly, one sample from the Apennines falls into *S. prenja* (*S. prenja* 1; Supplementary Table 1 in Supplemental content online), which extends the known distribution range of the species and makes it an example of an amphi-Adriatic distribution (Trotter 1912).

Tkach & al. (2015) stated that *Saxifraga* subsect. *Arachnoideae* is characterized by, among other traits, a diffuse habit and fragile, decumbent to ascending stems. However, this characterization does not cover the compact, cushion-like habit of *S. facchinii*, *S. muscoides* and, to some extent, *S. presolanensis*. As now understood, it is not possible in our opinion to reliably distinguish *S.* subsect. *Arachnoideae* from all other species of *S.* subsect. *Saxifraga* using morphological characters alone because *S.* subsect. *Saxifraga* shows substantial morphological and ecological diversity (Webb & Gornall 1989; Kaplan 1995; Aeschimann & al. 2004) with high levels of homoplastic

character evolution according to Tkach & al. (2015). Nevertheless, below we provide an improved description of the group that is sufficient to distinguish *S.* subsect. *Arachnoideae* from almost all species of *S.* subsect. *Saxifraga*.

Using existing keys (Webb & Gornall 1989; Webb 1993), all species of *Saxifraga* subsect. *Arachnoideae* except *S. hohenwartii*, *S. prenja* and *S. sedoides* can be identified when flowering. Special attention is required in the identification of *S. muscoides*. Of the c. 30 herbarium specimens labelled as *S. muscoides* and examined by us, about half belonged to *S. moschata*. A reliable character to distinguish these two species is the silvery grey colour of recently withered leaves in *S. muscoides*, which are brownish in *S. moschata. Saxifraga hohenwartii*, *S. prenja* and *S. sedoides* together can be identified as *S. sedoides* using the keys by Webb & Gornall (1989) and Webb (1993). Based on the extensive descriptions provided by Webb & Gornall (1989), Webb (1993) and Hörandl (1993), and on our own observations, we provide an identification key that, together with our description of the group, is sufficient to identify members of *S.* subsect. *Arachnoideae* and to avoid misidentification with members of *S.* subsect. *Saxifraga*.

Saxifraga subsect. ***Arachnoideae*** (Engl. & Irmsch.) Tkach & al. in Taxon 64: 1181. 2015 ≡ *Saxifraga* grex *Arachnoideae* Engl. & Irmsch. in Engler, Pflanzenr. 67: 233. 1919. – Type (designated by Gornall 1987: 288): *Saxifraga arachnoidea* Sternb.

Description — Biennial or evergreen perennial *herbs* with leafy stems. *Stems* straggling or ascending (habit diffuse), prostrate (forming loose mats) or erect (forming dense cushions). *Bulbils* and conspicuous summer-dormant *buds* absent. *Hairs* at least on margins or petioles of basal leaves; most species with conspicuous glandular hairs, at least on leaf margins. *Leaves* always without calcareous incrustations, hydathodes absent in almost all species; *leaf segments* flat on upper surface, never furrowed. *Petals* white or pale greenish-yellow in most species, ovate, obovate, oblong or linear, ± non-touching, apex often slightly to deeply notched. *Ovary* inferior or very nearly so.

Key to the species of Saxifraga subsect. *Arachnoideae*

1. Habit diffuse, plant with straggling or ascending leafy stems; leaves thin and soft, crenate or lobed; petiole usually longer than lamina **2**
– Leafy shoots erect or prostrate, forming cushions or mats; leaves entire or shortly 3-lobed at apex; petiole indistinct or very short **5**
2. Plant almost glabrous; leaves very thin, shiny and almost translucent; petals greenish . . . . . ***S. paradoxa***
– Leafy shoots with glandular hairs; leaves not very thin, shiny and almost translucent; petals pure white or yellowish white **3**
3. Glandular hairs on stems and petioles up to 10 mm long, viscid, tangled; petals yellowish white, apex truncate or slightly notched . . . . . ***S. arachnoidea***
– Glandular hairs shorter, not tangled; petals pure white, apex deeply notched **4**
4. Blades of basal leaves divided almost to base; petals 8–10 mm, in one flower of equal or slightly unequal size (when slightly unequal, smallest petal c. 4/5 size of largest petal), touching . . . . . ***S. petraea***
– Blades of basal leaves divided for not more than ½ their length; petals 4–8 mm, in one flower of markedly unequal size (smallest petal ½–¾ size of largest petal), non-touching . . . . . ***S. berica***
5. Leaves entire, linear, with a long, slender, translucent point, straw-coloured or silvery grey, with a single hydathode on upper surface near apex . . . . . ***S. tenella***
– Leaves entire or 3-lobed at apex, oblong-elliptic to oblong-oblanceolate, without hydathodes **6**
6. Leafy stems forming a dense cushion; recently withered leaves on living plants silvery grey, at least toward apex **7**
– Leafy stems prostrate or decumbent, forming an open mat or a loose cushion; recently withered leaves on living plants brown **9**
7. Petals 3.5–5 mm long, obovate, apex obtuse or slightly notched, overlapping . . . . . ***S. muscoides***
– Petals 1.5–2 mm long and obovate or 3–4 mm long and oblong-cuneate, apex truncate to slightly notched, non-overlapping **8**
8. Petals 1.5–2 mm long, obovate, dull yellow, ± strongly tinged purple or red, sometimes completely purple or red . . . . . ***S. facchinii***
– Petals 3–4 mm long, oblong-cuneate, translucent, dirty white, variably tinged pale greenish-yellow . . . . . ***S. presolanensis***
9. Leaves mostly shortly 3-lobed at apex, with obtuse lobes, but some leaves entire and oblanceolate, never apiculate; petals narrowly linear, much narrower than sepals . . . . . ***S. aphylla***
– Leaves mostly entire, oblanceolate to narrowly oblong, sometimes 3-toothed at apex or with a small lateral tooth, at least some leaves apiculate; petals linear to ovate, as wide as sepals or only slightly narrower **10**
10. Anthers orange-red in living plants, dark reddish-brown in older herbarium specimens; petals linear; glandular hairs on leaves 0.7–1.5 mm long . . . . . ***S. hohenwartii***
– Anthers bright yellow in fully opened flowers in living plants, pale yellow in older herbarium specimens; glandular hairs on leaves 0.1–0.7(–0.9) mm long **11**
11. Petals ovate-lanceolate or ovate, apex acuminate to apiculate . . . . . ***S. sedoides***
– Petals rectangular, apex truncate, retuse or emarginate, or petals obtuse . . . . . ***S. prenja***

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