

A new subspecies of *Saxifraga artvinensis* V.A.Matthews (Saxifragaceae) from northeastern Anatolia

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Abstract: *Saxifraga artvinensis* V.A.Matthews, which is endemic to Turkey, was discovered on Tiryal Mountain (A8 Artvin, northeastern Turkey). Approximately 175 km from its type locality, a new population of the species was discovered, in the *Picea orientalis* forest in the Altındere Valley National Park, in A7 Trabzon (northeastern Turkey). A comparison with the known population of *S. artvinensis* confirmed that this new population represented a new taxon and is described as *S. artvinensis* subsp. *meryemii* Terzioğlu & Coşkunç. An identification key and comparison with closely related taxa, based on both morphological and molecular properties, are also provided. The studied samples, belonging to subsp. *artvinensis* and subsp. *meryemii*, had identical sequences in the entire internal transcribed spacer region. On the other hand, subsp. *meryemii* was easily distinguishable from subsp. *artvinensis* by its hairy flowering stem, 3–8 flowers, subsessile pedicels, and sepals hairy at base.

Key words: Biodiversity, endemic, ITS, *Saxifraga*, taxonomy, Turkey

1. Introduction

Saxifraga L., the most species-rich genus of *Saxifragaceae*, is dominantly distributed in montane to alpine vegetation belts in mountain ranges of the Northern Hemisphere (Soltis, 2007; Tkach et al., 2015). The genus comprises more than 440 species worldwide (Tkach et al., 2015) and 21 species in Turkey (Matthews, 1972; Aslan, 2012; Firat, 2016). The Caucasus region is recognized as one of the world's 35 biodiversity hotspots (Marchese, 2015), and the plant biodiversity of this region is facing significant threats (Solomon et al., 2013). The Turkish part of this hotspot holds approximately 3210 vascular plant taxa, of which 465 are endemic to Turkey (Terzioğlu et al., 2015). The mountainous part of the Caucasus, including the northeastern part of Turkey, is among the centers of biodiversity for *Saxifraga* (Tkach et al., 2015). The Altındere Valley National Park, in northeastern Turkey, is home to many endemic species. This national park was studied floristically by Anşin (1979) for the first time, after which 2 further surveys focused on the subalpine-alpine (Palabaş-Uzun and Anşin, 2006) and forest ecosystems of the valley (Uzun and Terzioğlu, 2008). In the latter study, concentrating on the type location of the present new taxon, 383 vascular taxa were reported, including 16

endemics, and 2 *Saxifraga* species: *S. paniculata* Mill. and *S. rotundifolia* L.

During an excursion in the Altındere Valley in April 2013, the authors found dwarf *Saxifraga* on a damp and shady rock below the Sumela Monastery in the valley (A7 Trabzon). Literature and herbarium surveys revealed that the plants did not belong to any of the taxa recorded from the national park; instead, they represented *Saxifraga artvinensis* V.A.Matthews, which is endemic to Turkey, and thus only known from the type location (A8 Artvin) (Mathews, 1972). Further comparisons showed that the specimens represented a new subspecies of *S. artvinensis*.

2. Materials and methods

Morphological characters were noted and measured from the authors' collection from the type locality. To determine the morphological limits of the new and related taxa, additional plant material for morphological and molecular studies was also collected during the field trips (Table 1). All of the morphological measurements were done using at least 3 specimens. An identification key of the new subspecies and related species is therefore given, together with images of both the subspecies and line drawings of useful distinguishing characteristics. Specimens of 2 sub-

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species of *S. artvinensis* were deposited in the Herbarium of the Karadeniz Technical University, Faculty of Forestry (KATO) and Department of Biology (KTUB).

The total genomic DNA was extracted from healthy leaves dried with silica gel or the herbarium materials following the modified cetyl trimethylammonium bromide extraction procedure of Doyle and Doyle (1987). Primers internal transcribed spacer ITS4 and ITS5 (White et al., 1990) were used to amplify the nrDNA ITS region. The polymerase chain reaction (PCR) protocol for the amplification of the region followed that of Gültepe et al. (2010). The PCR products were sequenced by Macrogen Inc. (Seoul, Korea) using the ITS4 and ITS5 primers. For the phylogenetic analysis, 9 sequences belonging to *Saxifraga* were newly generated (Table 1) and 15 more were downloaded from GenBank, comprising 13 of *Saxifraga* and 2 selected as outgroups (Table 1). *Chrysosplenium alternifolium* L. and *Micranthes nivalis* (L.) Small were the

chosen outgroup for the phylogenetic reconstruction. All of the sequences (24 accessions) were aligned using Muscle v.3.8.31 (Edgar, 2004) and edited in PhyDE v.0.9971 (Müller et al., 2010). Indels were coded as informative characters according to the Simple Indel Coding method (Simmons and Ochoterena, 2000), as implemented in the program SeqState v.1.40 (Müller, 2005), and added at the end of the sequence data set before being subjected to the maximum parsimony (MP) analysis. The MP analysis was conducted using the parsimony ratchet (Nixon, 1999) with PRAP v.2.0 (Müller, 2004) in combination with PAUP v.4.0b10 (Swofford, 2002). Standard ratchet settings were used (200 ratchet iterations, with 25% of the positions randomly upweighted (weight = 2) during each replicate, and 10 random addition cycles). The generated command files, also including the nexus data matrix, were run in PAUP using the heuristic search option with the following parameters: all of the characters had equal

Table 1. Voucher data used in the phylogenetic analysis based on the nrDNA ITS data.

Taxa	Voucher/reference	¹ Code/ ² GenBank accession number in the MP tree
<i>S. adscendens</i> L.	M. A. Chapman and John M. Burke, Evolution, 2007, 61-7: 1773–1780	² EF028688
<i>S. artvinensis</i> subsp. <i>artvinensis</i>	Turkey, <i>Coşkunçelebi</i> 1278 (KTUB)	¹ C1278/ ² MN059938
<i>S. artvinensis</i> subsp. <i>artvinensis</i>	Turkey, <i>Coşkunçelebi</i> 1279 (KTUB)	¹ C1279/ ² MN059939
<i>S. artvinensis</i> subsp. <i>artvinensis</i>	Turkey, <i>Coşkunçelebi</i> 1280 (KTUB)	¹ C1280/ ² MN059940
<i>S. artvinensis</i> subsp. <i>meryemii</i>	Turkey, <i>Terzioğlu & Coşkunçelebi</i> 1246 (KTUB)	¹ T&C1246/ ² MN059941
<i>S. bulbifera</i> L.	Italy, Fiuminata, Conti (MA)	² AF261166
<i>S. exarata</i> Vill.	Austria, Glockturm, Plats D. Gomez 1994 (JACA)	² AJ233861
<i>S. cymbalaria</i> L.	Ferguson 1994-04	² AF087599
<i>S. flagellaris</i> Willd. subsp. <i>stenophylla</i> (Royle) Hulten	Tajikistan, <i>B. Dickore</i> , 18385 (MSB)	² KU524187
<i>S. graeca</i> Boiss. & Heldr.	Greece, Olimbos, Stauros, Richards (RNG)	² AF261179
<i>S. hederacea</i> L.	Italy, Sicily, Trapani	² KU524139
<i>S. hirculus</i> L.	Canada, Yukon Territory	² MG237161
<i>S. juniperifolia</i> Adams	Turkey, <i>Coşkunçelebi</i> 1284 (KTUB)	¹ C1284/ ² MN059943
<i>S. juniperifolia</i> Adams	Turkey, <i>Coşkunçelebi</i> 1244 (KTUB)	¹ C1244/ ² MN059944
<i>S. moschata</i> Wulfen	Turkey, <i>Coşkunçelebi</i> 1282 (KTUB)	¹ C1282/ ² MN059942
<i>S. paniculata</i> Mill.	Turkey, <i>Coşkunçelebi</i> 1281 (KTUB)	¹ C1281/ ² MN059937
<i>S. rotundifolia</i> L.	Turkey, <i>Coşkunçelebi</i> 1283 (KTUB)	¹ C1283/ ² MN059936
<i>S. rotundifolia</i> L.	BBG 267-94-80-10	² AF087598
<i>S. sancta</i> Gris.	Greece: <i>M. Roeser</i> 2700 (HAL)	² LN812523
<i>S. sempervivum</i> K.Koch	Kew 1984-3743	² AF087590
<i>S. tridactylites</i> L.	M. A. Chapman and John M. Burke, Evolution, 2007, 61-7: 1773–1780	² EF028687
<i>S. yezhiensis</i> C.Y.Wu	China: <i>C.H. Wang</i> , 68389 (HUH)	² KU524203
<i>Micranthes nivalis</i> (L.) Small	Denmark, Groenland	² LM654384
<i>Chrysosplenium alternifolium</i> L.	Poland, PE: 01505547	² N375572

weight, simple addition of sequences, tree bisection and reconnection branching swapping, maxtrees setting to 100 and autoincreased by 100, one nonbinary starting tree arbitrarily dichotomized before branch swapping, and only 1 tree saved. A majority-rule consensus tree was calculated from the most parsimonious trees. Jackknife (JK) support values for the nodes found by the MP analysis were calculated in PAUP by applying the optimal JK parameters according to the method of Farris et al. (1996) with 10,000 JK replicates.

3. Results and discussion

Saxifraga artvinensis V.A.Matthews subsp. **meryemii** Terzioğlu & Coşkunç. subsp. nov. (Figure 1)

S. artvinensis subsp. *meryemii* is easily distinguished from subsp. *artvinensis* by its pilose flowering stems (not glabrous), flowers 3–8 (not 3–5), pedicels subsessile (not longer than flowers) and sepals hairy at base (not glabrous), and its distribution at altitudes of 1100–1150 m in forest (not in alpine zone higher than 2135 m) (Figures 2 and 3).

Type: —Turkey. A7 Trabzon: Altındere Vadisi National Park, around the Sumela Monastery, 1126 m, 31.03.2013, Terzioğlu & Coşkunçelebi 1246 (KATO 9843), holo: KATO; iso: KTUB.

Description: —Perennial, caespitose forming cushions up to 10 cm in diameter. Cauline shoots erect, imbricate-leaved, previous years’ dead leaves light brown and remaining in dense rosettes. Leaves lanceolate to linear-lanceolate, glabrous, not lime-encrusted, margin ciliate in lower half only. Flowering stem 1–4 cm long,

pilose. Inflorescence racemose with 3–8 flowers, pedicel pubescent, up to 2.5 mm long, shorter than flower. Sepals 2–3.5 mm long, oblong-oval, glabrous, ciliate-margined. Petals 3–5 mm long, linear-spathulate or oblong-spathulate, white, shorter than stamens. Fl. 3–4. Damp rocks under oriental spruce forest, 1100–1150 m.

Distribution, habitat, and conservation status: —*S. artvinensis* subsp. *meryemii* is endemic to Turkey. It belongs to the Euxinian element, and grows on a damp and shady rock in the *Picea orientalis* (L.) Peterm. forest between 1100 and 1150 m, together with *Pachyphragma macrophyllum* (Hoffm.) Busch, *Geranium robertianum* L., *Impatiens noli-tangere* L., *Fragaria vesca* L., *Saxifraga paniculata* Mill., *Sanicula europaea* L., *Telekia speciosa* (Schreb.) Baumg., *Petasites albus* (L.) Gaertn., *Cyclamen parviflorum* Pobed., *Brachypodium sylvaticum* (Hudson) P. Beauv. and *Festuca drymeja* Mert. & Koch.

This is very different from the habitat of subsp. *artvinensis*, which occurs at alpine elevations between 2135 and 2410 m (Figure 4). This new subspecies is a narrow local endemic and is only known from the type locality. There are approximately 175 km between the 2 subspecies of *S. artvinensis*. Currently, the habitat quality is high, but it is under the negative effects of the religious tourism related to the Sumela Monastery. The entire known population comprises less than 20 individuals, covers a single large rock, and has an area of occupancy smaller than 100 m². Consequently, the threat category of this new subspecies has been assessed as critically endangered [CR B1ab(ii,iii,iv)+2ab(i,ii)] according to the International Union for Conservation of Nature (2014).

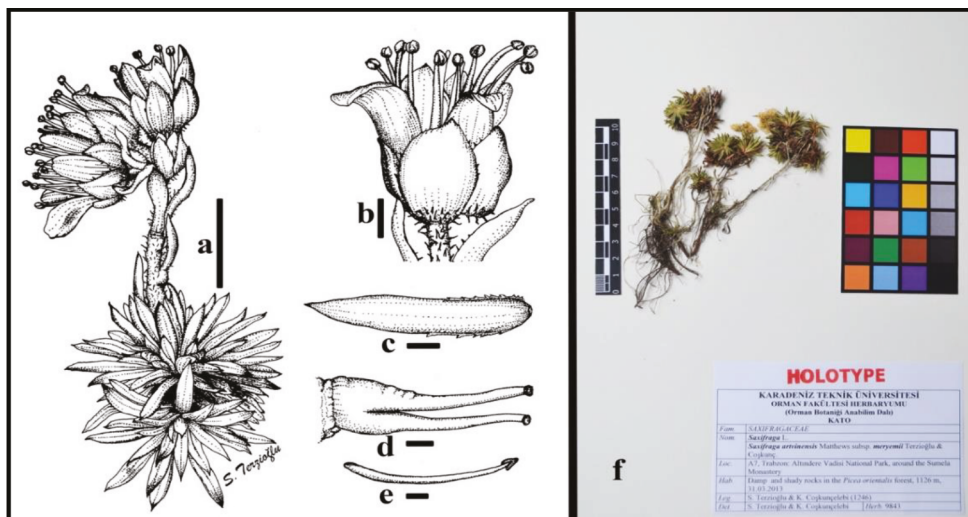


Figure 1. *Saxifraga artvinensis* subsp. *meryemii*. a- General view (scale bar: 0.5 cm), b- flower (scale bar: 1 mm), c- leaf (scale bar: 1 mm), d- gynoecium (scale bar: 0.5 mm), e- stamen (scale bar: 0.5 mm), f- holotype (Terzioğlu & Coşkunçelebi 1246, KATO 9843).

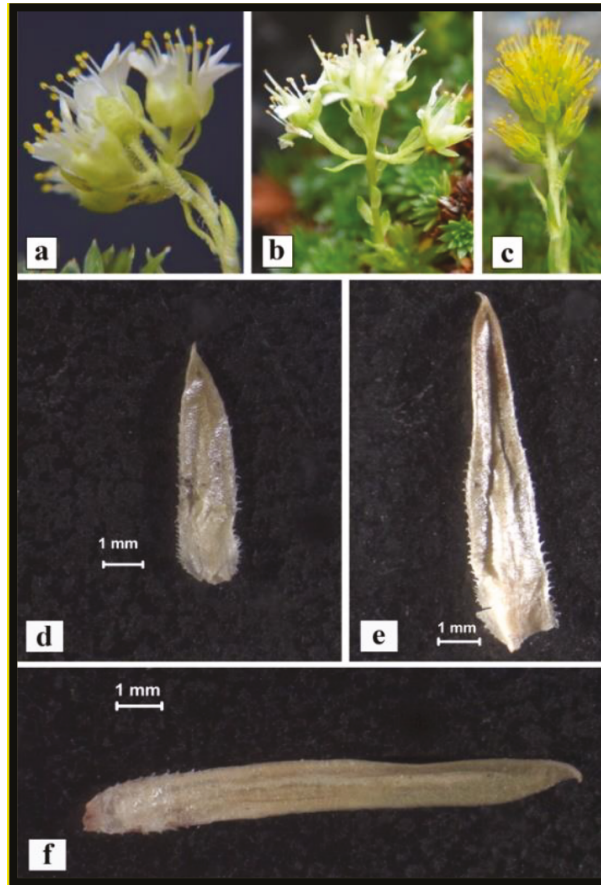


Figure 2. Inflorescence and pedicels: **a-** *S. artvinensis* subsp. *meryemii*, **b-** *S. artvinensis* subsp. *artvinensis*, **c-** *S. juniperifolia*. Leaves: **d-** *S. artvinensis* subsp. *artvinensis*, **e-** *S. juniperifolia*, **f-** *S. artvinensis* subsp. *meryemii*.

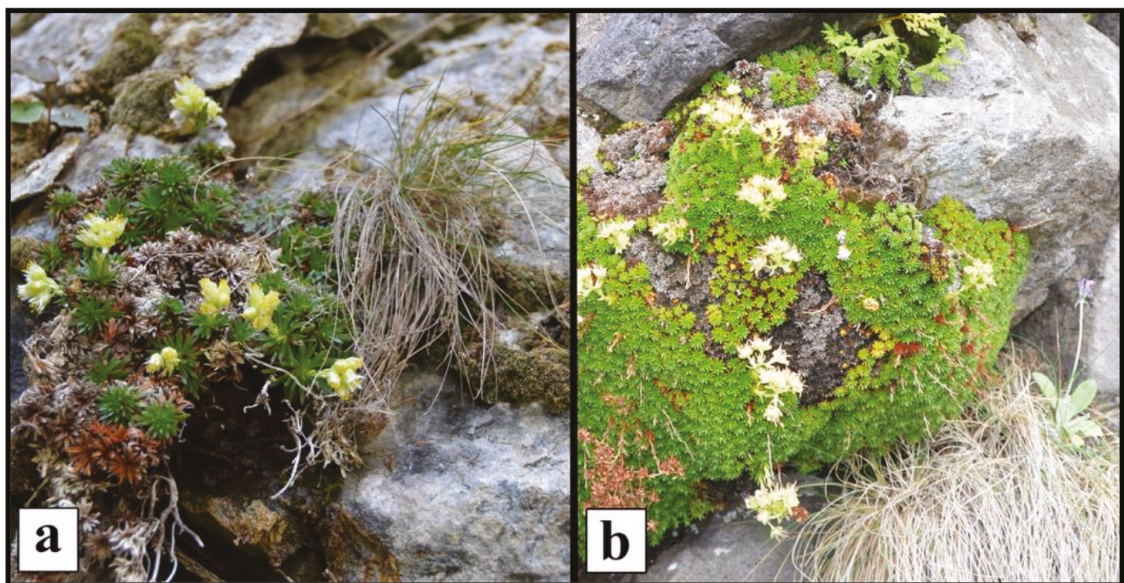


Figure 3. Habitat and habits. **a-** *Saxifraga artvinensis* subsp. *meryemii*, **b-** *S. artvinensis* subsp. *artvinensis*.



Figure 4. The distribution in northeastern Turkey and habitat of *S. artvinensis* subsp. *meryemii*; a- Conifer forest at 1125 m in Trabzon. *S. artvinensis* subsp. *artvinensis*; b- Alpine rocky slopes at 2410 m in Artvin.

Additional specimens examined: —*S. artvinensis*: A8 Artvin (Çoruh): Tiryal Dağı, above Murgul, 2300 m, shady vertical igneous rocks, 23.06.1957, Davis & Hedge, D. 29957 (Holotype: E photo); Murgul, Tiryal Dağı, 2420 m, damp rocks, 19.06.2018, Terzioğlu & Coşkunçelebi 1278, 1279, 1280 (KTUB). *S. juniperifolia* Adams A8 Artvin (Çoruh): Şavval Tepe above Murgul, 2800 m, D.32290 (E photo). Trabzon: Çaykara, Uzungöl, Demirkapı Dağı, rocky places, nival zone, 3102 m, 19.09.2013, Coşkunçelebi 1244 (KTUB). A8 Trabzon: Demirkapı Dağı, above Aygır Gölü, 3208 m, cliffs, 20.06.2018, Coşkunçelebi 1284 (KTUB). *S. moschata* 1781: 128): A8 Trabzon: Demirkapı Dağı, above Aygır Gölü, 3000 m, cliffs, 20.06.2018, Coşkunçelebi 1282 (KTUB). *S. paniculata*: A8 Artvin: Murgul, Tiryal Dağı, 2306 m, 19.06.2018, Coşkunçelebi 1281 (KTUB). *S. rotundifolia*: A8 Artvin: Murgul, Tiryal Dağı, 2306 m, 19.06.2018, Coşkunçelebi 1283 (KTUB). *S. sancta*: B1 Balıkesir: Edremit, Kazdağ, Sarıkız site, limestone, 1600 m, 08.05.2004, F. Satıl 1316 (TTUB).

Etymology: —This new subspecies is named in honor of the first author's mother, who died in 2008.

Key to the subspecies of *S. artvinensis*:

—Flowering stem and base of sepal glabrous, pedicels longer than flowers, flowers produced from June–July; alpine plant, at altitudes of 2135–2410 m.

subsp. *artvinensis*

—Flowering stem and base of sepal pilose, pedicels as long as or shorter than flowers, flowers produced from March–April; forest plant, at altitudes of 1100–1150 m.

subsp. *meryemii*

Relationship and molecular phylogeny: The *Saxifraga* species distributed in Turkey were not divided into sections by Matthews (1972). However, according to Gornall (1987), *S. artvinensis* should be classified under the sect. *Porphyron* Tausch subsect. *Kabschia* (Engler) Rouy & Camus. Depending on both the morphological (Table 2) and molecular studies (Figure 5), this new subspecies clearly belongs to *S. artvinensis*. A comparison of the diagnostic morphological characters of the 2 subspecies of *S. artvinensis* is given in Table 2.

Alignment of the entire nrDNA ITS sequence, including the outgroups, resulted in 731 characters. Identical sequences were observed in the entire ITS regions of samples belonging to subsp. *artvinensis* (3) and subsp. *meryemii* (1). In the MP tree, these 4 accessions of *S. artvinensis* were grouped with a high JK support value (93%) (Figure 5). The MP tree also revealed that *S. juniperifolia* is sister to *S. artvinensis*, with maximum JK support (99%) among the studied taxa. Because *S. juniperifolia* was treated under the sect. *Porphyron* by Gornall (1987), our data supported placing *S. artvinensis* under this section.

Table 2. Morphological comparison of the subspecies of *S. artvinensis* and *S. juniperifolia*.

Characters	<i>Saxifraga artvinensis</i> subsp. <i>artvinensis</i>	<i>S. artvinensis</i> subsp. <i>meryemii</i>	<i>S. juniperifolia</i>
Flower color	White	White	Yellow
Flower number	3–5	3–8	3–10
Sepal	Not ciliate	Ciliate	Ciliate
Pedicele	Longer than flower (5–15 mm)	Shorter than flower (1–2.5 mm)	Subsessile
Flowering stems	Glabrous	Pilose	Pilose
Flowering period	June–July	March–April	June–August
Habitat	Damp cliffs and rock in alpine places, 2135–2410 m	Damp shady rock in conifer forest, 1100–1150 m	Cliffs and rocks in nival zone, 2700–3300 m

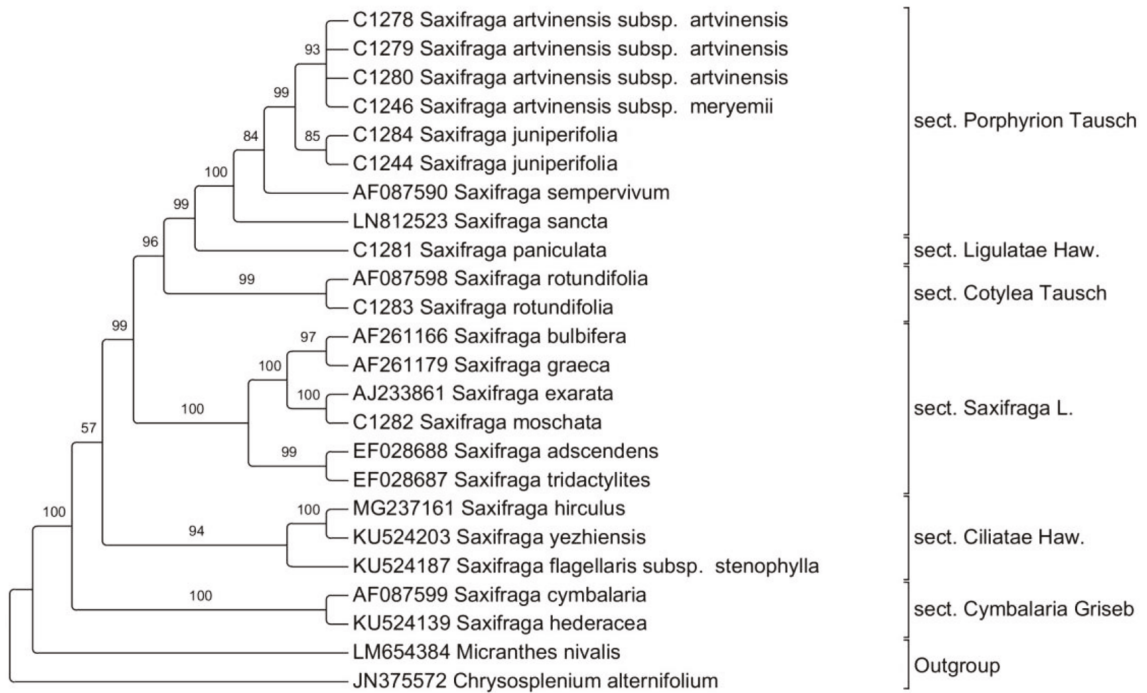


Figure 5. MP tree obtained using PAUP* v.4.0b10. Numbers above the branch are JK support values, in percentages, based on 1000 replicates.

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