

## *Orthotrichum pamiricum* (Bryophyta), a new epiphytic moss species from Pamir Mountains in Central Asia

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**Abstract:** *Orthotrichum pamiricum* Plášek & Sawicki is described as a new species from the western foothills of the Pamir Mountains of the Tajik–Afghan border in Central Asia. It is placed in *Orthotrichum* Hedw. subg. *Pulchella* (Schimp.) Vitt. The species is characterized by obtuse leaf apices, cryptoporous stomata, an exostome composed of 8 pairs of the teeth that are recurved when dry, and an endostome with 16 thin and incurved segments. Molecular data indicating the phylogenetic placement of *O. pamiricum* are also presented.

**Key words:** Afghanistan, Tajikistan, molecular phylogenetics, new species, Orthotrichaceae, taxonomy

### 1. Introduction

Since 2008 the senior author has carried out field investigations in Tajikistan, a Central Asian state that is relatively poorly studied in terms of bryology. Special attention was paid to the genus *Orthotrichum* Hedw., which is known to be represented in this country by 14 species (Mamatkulov et al., 1998; Ignatov et al., 2006). Four species have been added recently to the moss flora of Tajikistan by the present authors, namely *Orthotrichum sordidum* Sull. & Lesq. (Blockeel et al., 2009), *Orthotrichum crenulatum* Mitt. (Ellis et al., 2011), *Orthotrichum moravicum* Plášek & Sawicki (Ellis et al., 2012), and *Orthotrichum urnigerum* Myrin (Ellis et al., 2014). During a bryological survey of the Pamir in southern Tajikistan along the Tajik–Afghan border, several specimens of a puzzling *Orthotrichum* were collected. Superficially, they resembled *O. crenulatum*, but closer examination of the material revealed that they represented a species new to science, described in the present account.

The nomenclature of the moss taxa in the text below follows Hill et al. (2006).

### 2. Materials and methods

The material was collected during a bryological expedition into Tajikistan in 2011. It was compared with collections of similar species (mainly with *Orthotrichum crenulatum*, *O. sprucei* Mont., and *O. pumilum* Sw.) deposited at KRAM, LE, OP, OSTR, S, H, and W and in the private herbarium of Alfons Schäfer-Verwimp. Detailed morphological

examination was carried out using light and scanning electron microscopy.

Total genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN). A single stem was ground with silica in a Mini-Beadbeater-1 tissue disruptor for 40 s and subsequently treated with kit reagents following the manufacturer's protocol. Extracted DNA samples were stored at –20 °C. For amplification and sequencing of the internal transcribed spacer (ITS), we used the primers of Fiedorow et al. (1998), which were successfully used in previous studies on the genus *Orthotrichum* (Sawicki et al., 2009a, 2009b; Plášek and Sawicki, 2010). The ITS was amplified in a volume of 25 µL containing 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 50 mM Tris-HCl (pH 9.0 at 25 °C); 1.5 mM MgCl<sub>2</sub>; 1 µL of bovine serum albumin; 200 mM each dATP, dGTP, dCTP, and dTTP; 1.0 mM of each primer; 1 unit of Taq polymerase (Novazym); and 60 ng of the DNA. The reaction was carried out at 94 °C for 1 min followed by 30 cycles of 94 °C for 1 min, 59 °C for 1 min, and 72 °C for 1.5 min, with a final extension step of 72 °C for 5 min. Finally, 5 µL of the amplification products was visualized on 1.5% agarose gel with GelView staining (Novazym). Purified PCR products were sequenced in both directions using the ABI BigDye 3.1 Terminator Cycle Kit (Applied Biosystems) and then visualized using an ABI Prism 3130 Automated DNA Sequencer (Applied Biosystems).

The maximum parsimony tree was obtained using the close-neighbor-interchange algorithm with search level 1, in which the initial trees were obtained with the random

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addition of sequences (10 replicates) as implemented in MEGA 5 (Tamura et al., 2011). The statistical significance of clades within the inferred trees was evaluated using the bootstrap method (Felsenstein, 1985) with 2000 replicates.

### 3. Results

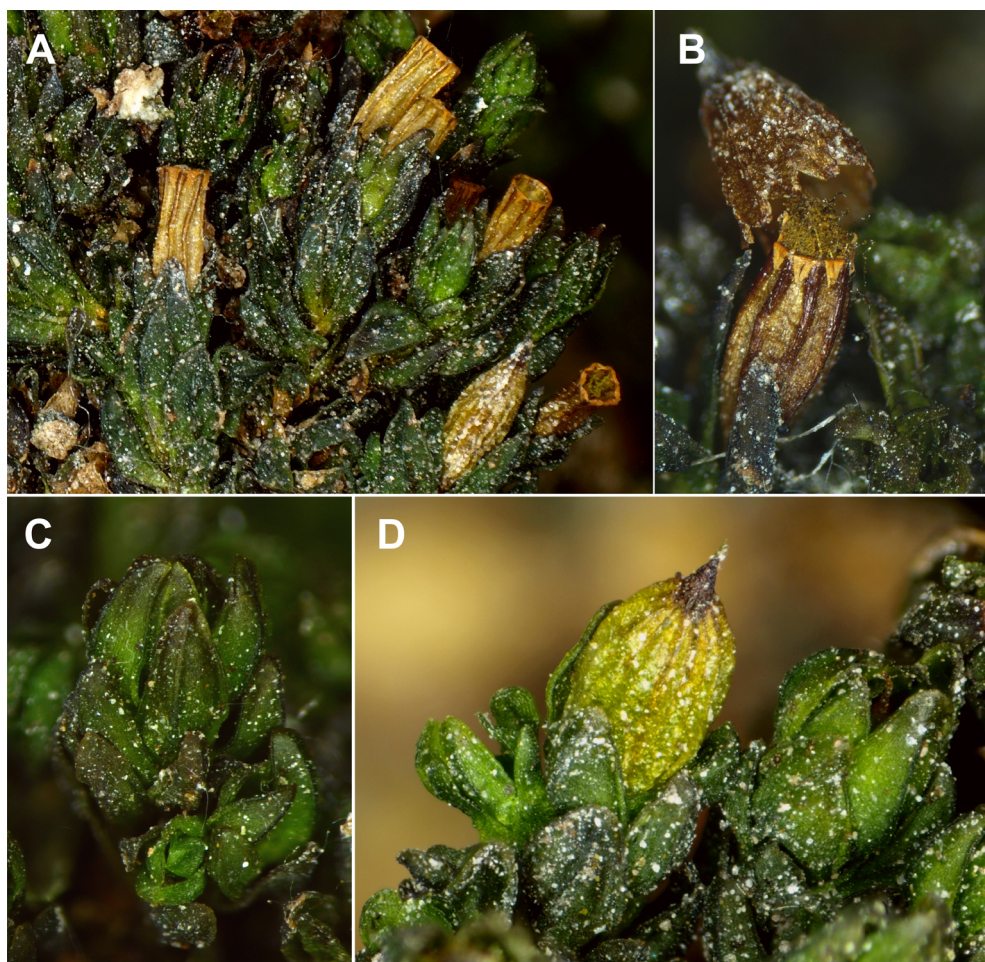
*Orthotrichum pamiricum* Plášek & Sawicki sp. nov. (Figures 1–3).

Type: Tajikistan. Tajik–Afghan border, 150 km SE of Dushanbe capital city, 35 km E of Kulob town, valley of Panj river, edge of Kisht village, 840 m, coordinates (WGS84): 37°56.055'N, 070°10.264'E, 01.06.2011, V. Plášek s.n. (holotype: OSTR, isotype: KRAM).

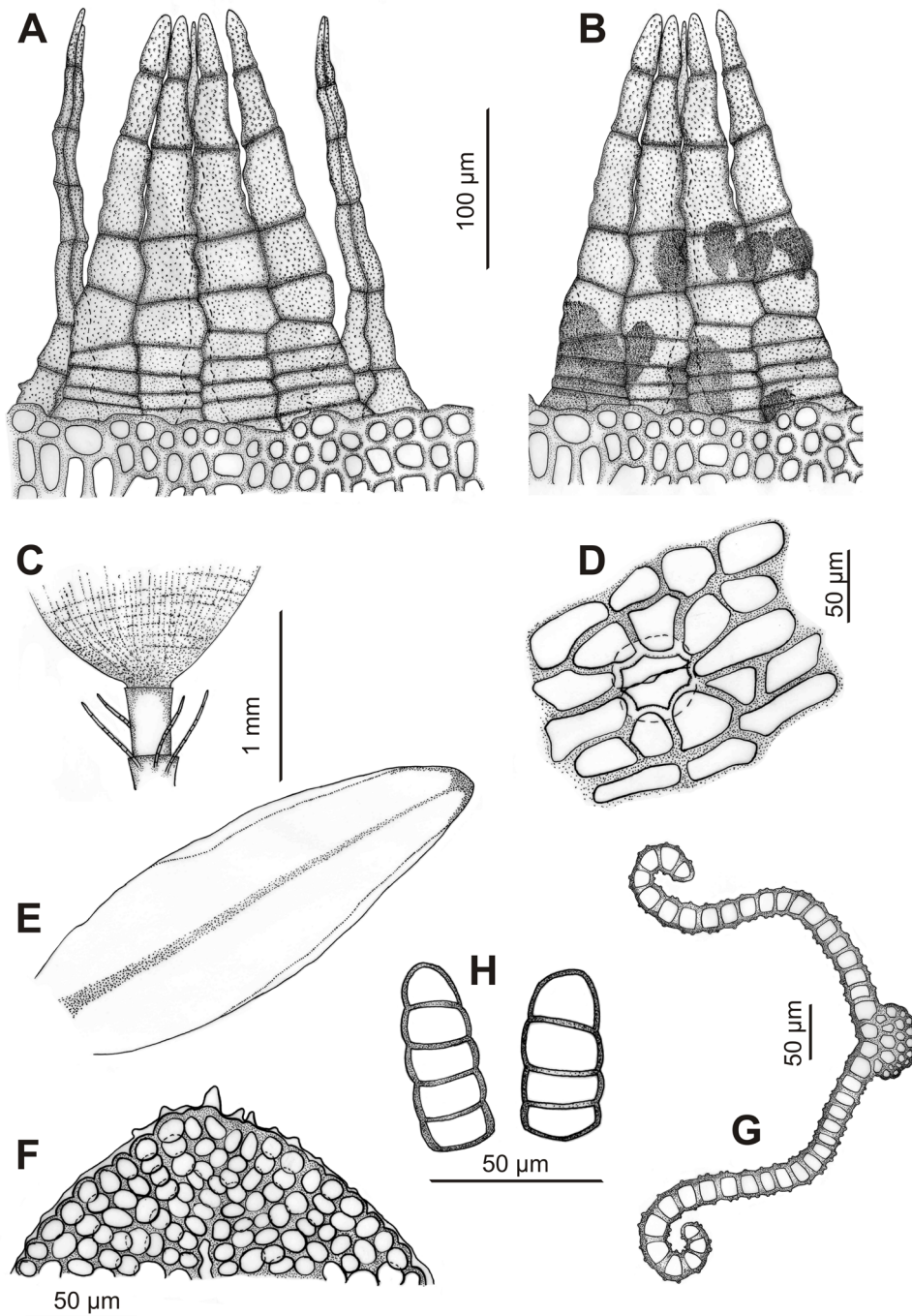
**Diagnosis:** Plantae usque ad 4.5 mm altae, foliis erecto-adpressis, oblongo-lanceolatis, apicibus obtusis. Cellulae superiores cum (1)–2 papillis humilis. Capsulae magis quam dimidium emergentes, oblongae vel oblongo-cylindricae. Stomata cryptopora. Peristomium duplex, prostomium interdum praesens. Exostomia 8 segmentis, in sicco recurvatis, in basi subtiliter papillois, in dimidio superiore

grosse papillois; endostomia tam-quam exostomia longum, 16 segmentis incurvatis, papillois, in basi gracilibus et brevi appendicis. Calyptra campanulata, nuda.

**Description:** Plants dark green, 1.1–2.8 mm tall, up to 4.5 mm in the central part of sparse cushions. Stems not or rarely branched. Rhizoids pale brown, branched, smooth, occurring only at base of the stem. Stem leaves loosely erect to erect-appressed when dry, erect when moist, ovate to ovate-lanceolate, 1.8–2.6 mm, slightly keeled, obtuse; margins recurved from 1/5 to near apex, entire. Upper laminal cells rounded in the apex (9–)11–13(–15)  $\mu\text{m}$ , oval to quadrate-oval in mid-leaf, (17–)19–23(–25)  $\times$  14–16  $\mu\text{m}$ , 1-stratose, with (1)–2 short conical papillae (2–3  $\mu\text{m}$ ) on both abaxial and adaxial sides; basal laminal cells long-rectangular, 16–20  $\times$  32–40  $\mu\text{m}$ . Leaf apex entire or crenulate by bulging walls of marginal cells (papillae 5–10  $\mu\text{m}$  high). Costa percurrent, (70–)75(–80)  $\mu\text{m}$  wide at base, gradually tapering upwards, (25–)30(–33)  $\mu\text{m}$  wide near apex. Sexual condition gonautoicous. Seta short 0.3–0.5 mm, ochrea up to 3/4 of the seta. Vaginula naked



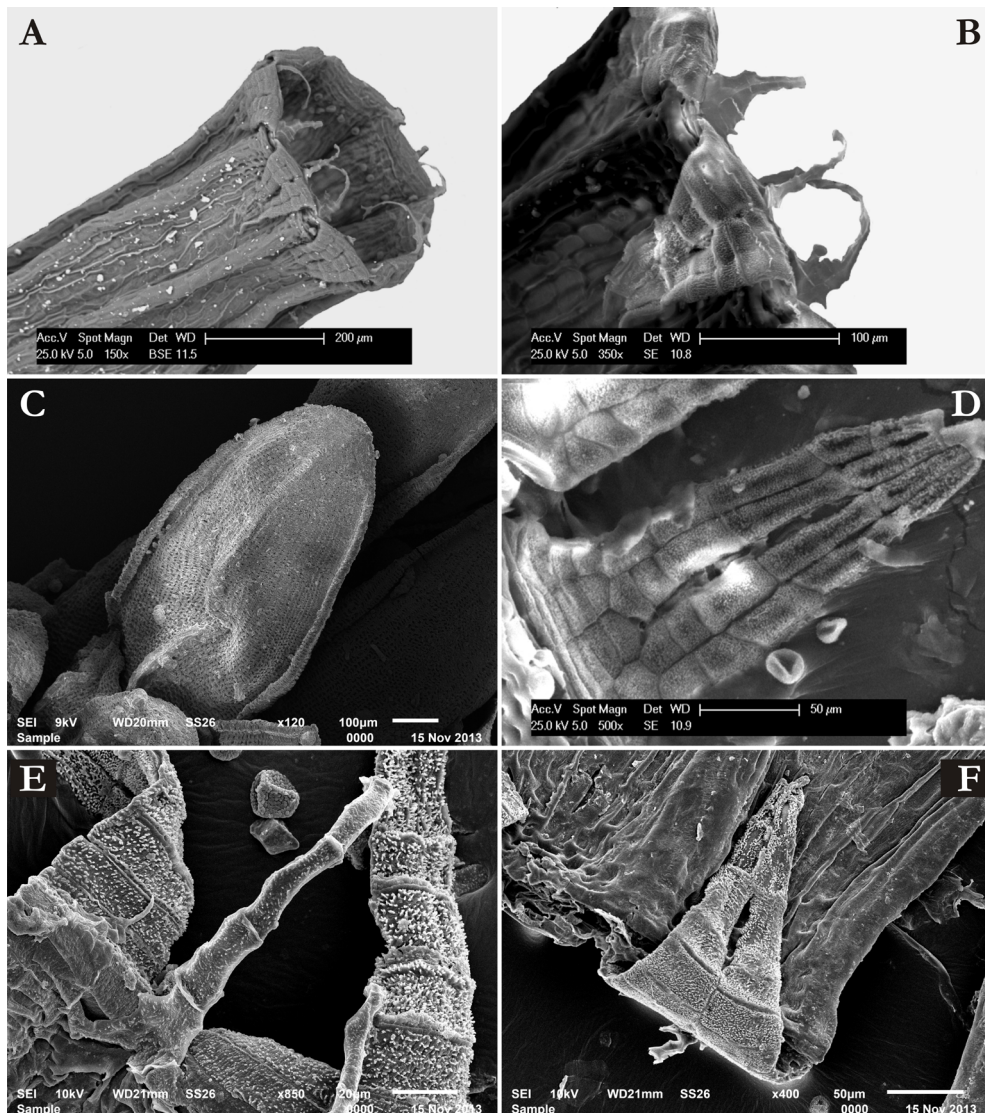
**Figure 1.** Microphotographs of *Orthotrichum pamiricum*. A- Habit, B- mature capsule with calyptra, C- sterile plants, D- capsule covered by calyptra. Photo of holotype taken by V Plášek.



**Figure 2.** *Orthotrichum pamiricum*. A- Peristome, B- view of prostome, C- vaginula with short hairs, D- cryptopore stomata, E- midstem leaf, F- leaf apex, G- cross-section of middle of leaf, H- gemmae. Drawings from the holotype by L. Čihal.

or sparsely hairy with 3–4 smooth hairs (0.2 mm long, of 12–15 cells, reaching up to 4/5 of seta). Capsule from 1/2 to 3/4 emergent; urn oblong to oblong-cylindrical when dry, cylindrical-ovoid when moist, 1.8–2.1 mm long, not constricted below the mouth, yellowish-brown to pale brown with 8 strong ribs reaching the capsule base.

Exothecial bands 4 cells wide, formed by moderately differentiated exiguous thick-walled cells, extending from the mouth up to 2/3 of capsule length. Neck concolorous with theca, gradually narrowed to the seta. Stomata cryptoporous, arranged in 2 rows in the upper half of the capsule, partly (1/4–1/3, rarely more) covered by



**Figure 3.** Scanning electron microscopy images of *Orthotrichum pamiricum*. A- Capsule with peristome, B- peristome in detail, C- leaf, D- external view of peristome showing OPL ornamentation of the exostome and presence of the prostome, E- PPL ornamentation of the exostome and endostome segment showing IPL ornamentation, F- view of PPL ornamentation of the exostome tooth. Photo from the holotype taken by V Plášek and R Vojtkovská.

well differentiated and moderately protruding subsidiary cells. Peristome double. Prostome occasionally present. Exostome teeth 8, occasionally split into 16 prongs when old, often remarkably cleft or perforate in upper half, light brown, 250–310  $\mu\text{m}$  long, strongly recurved when dry, densely and finely papillate at base and coarsely papillate in the upper part of the external side, roughly papillate-striate ornamented on the internal side. Endostome formed by 16 segments, almost as long as the exostome teeth, whitish, narrowly lanceolate, with broader bases, sometimes united by a low basal membrane, incurved when dry, sparsely papillate on external side, finely

papillate on internal side; principal segments biseriate, (200–)215–235(–250)  $\mu\text{m}$  long, with short appendages on the base; intermediate ones as tall as principal ones or a little shorter, (170–)190–210(–240)  $\mu\text{m}$  long, consisting of a single row of cells. Calyptra campanulate, light brown with dark brown top, plicate, glabrous. Operculum flat conic with of long beak 200  $\mu\text{m}$ . Spores (15)–17–(20)  $\mu\text{m}$ , densely papillate. Gemmae club-shaped, abundant on the leaf surfaces, 4–6-celled, 60–70  $\mu\text{m}$  long.

**Ecology and distribution:** *Orthotrichum pamiricum* was found to grow on the bark of *Morus* sp., on both the trunk and inclined branches, with southern exposure, at a

height of between 80 and 150 cm. The tree was growing at the margin of a sparse mulberry grove, situated between the Panj River and a public road. The population size was around 5 cm<sup>2</sup>. The following associated species were recorded at the locality: *Orthotrichum affine* Schrad. ex Brid. and *O. anomalum* Hedw. The species was also collected along the Panj River valley, in a section of 50–60 km, NE of the type locality (Figure 4).

**Additional specimens examined.** *Orthotrichum pamiricum* (paratypes): **Tajikistan:** 70 km NE of Kulob town, Panj river valley, edge of Khostav village, 1080 m a.s.l., bark of *Morus* sp., coordinates (WGS84): 38°11.929'N, 070°31.390'E, 02.06.2011, V. Plášek s.n. (OSTR). **Afghanistan:** 80 km N of Fayzabad town, Panj River Valley, edge of Kaji village, 950 m a.s.l., bark of *Morus* sp., coordinates (WGS84): 37°55.091'N, 070°12.978'E, 02.06.2011, V. Plášek s.n. (OSTR); 10 km NNE of Ab Darrah town, Panj River Valley, edge of Arzishk village, 1100 m a.s.l., bark of *Morus* sp., coordinates (WGS84): 38°12.251'N, 070°32.113'E, 02.06.2011, V. Plášek s.n. (OSTR).

**Etymology:** The species is named after the mountain range, Pamir, where it has been found.

**Phylogenetic position and molecular delimitation of *Orthotrichum pamiricum*:** The obtained sequences of *Orthotrichum pamiricum* were added to the dataset used

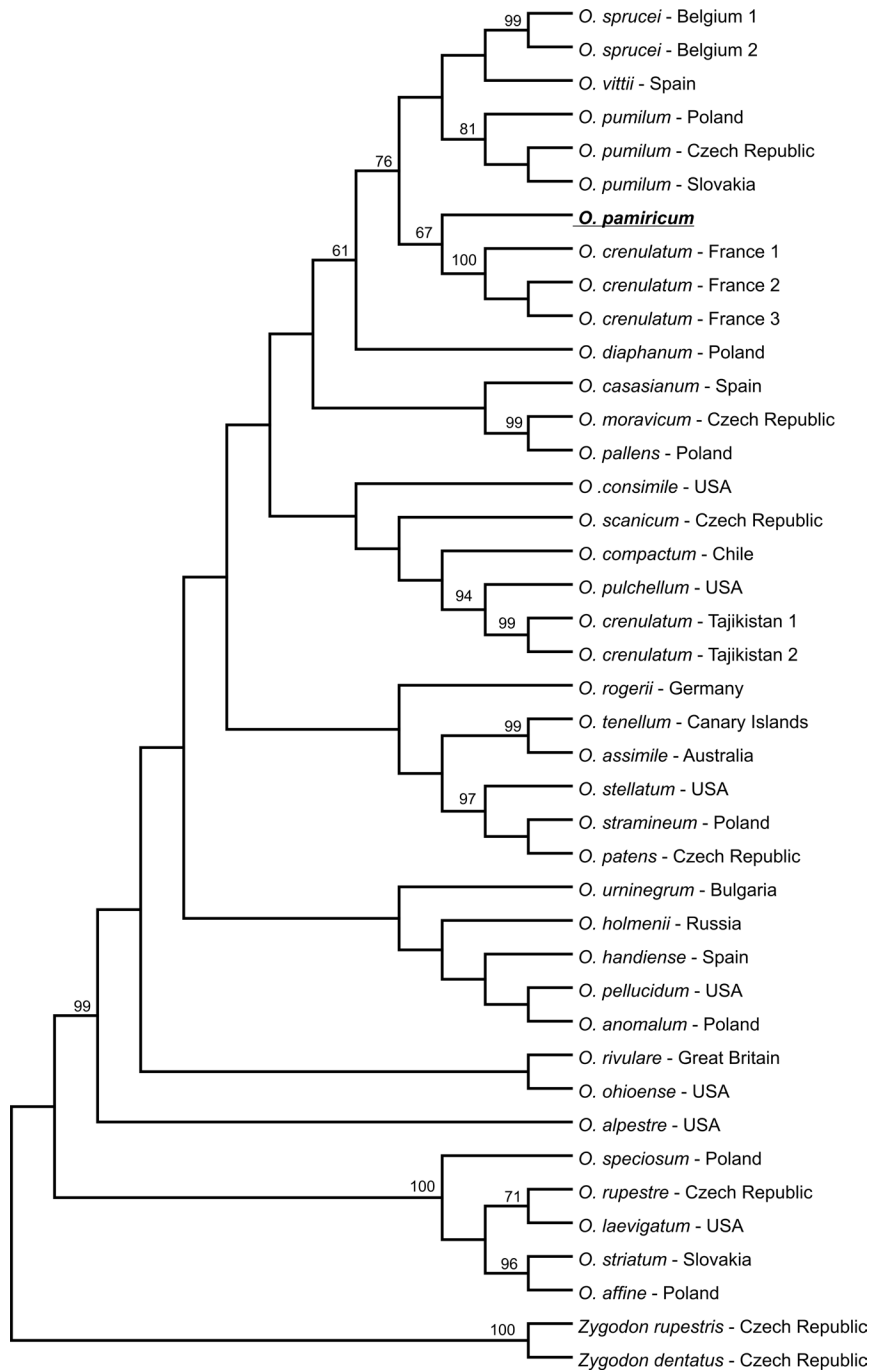
in our previous study (Sawicki et al., 2012), where *Zygodon* Hook. & Taylor and *Orthotrichum* with phaneropore stomata served as outgroups.

The combined dataset included 970 characters, of which 343 were constant, 195 variable but parsimony-uninformative, and 432 potentially phylogenetically informative. A maximum parsimony (MP) analysis resulted in 13 most parsimonious trees [length 1049, consistency index (CI) 0.6854, retention index (RI) 0.8103].

The MP analysis revealed *Orthotrichum pamiricum* as a sister species to *O. crenulatum* from France (Figure 5), but their common clade is poorly supported (67% of bootstrap support). The higher ranking clade has better bootstrap support (78%) and comprises specimens of *O. pumilum*, *O. sprucei*, and *O. vittii* F.Lara, Garilleti & Mazimpaka. However, each of these species has a specific mutation in the ITS region, which enables molecular delimitation. *O. pamiricum* differs from the closest related *O. crenulatum* by 3 deletions and 16 substitutions. A similar number of fixed nucleotide differences was found for the other species compared above. *O. pamiricum* differs from *O. pumilum* by 1 deletion and 7 substitutions, from *O. sprucei* by 7 deletions and 17 substitutions, and from *O. vittii* by 3 deletions and 20 substitutions. In the ITS1 region of *O. pamiricum*, 3 substitution characteristics for this species were found.



**Figure 4.** Map of Central Asia marking the type locality of *Orthotrichum pamiricum* (black star) and other observed localities (striped rectangle) of the species.



**Figure 5.** Maximum parsimony consensus tree based on ITS sequences. Bootstrap values above 60% are indicated on branches.

#### 4. Discussion

*Orthotrichum pamiricum* is likely to be confused with a few congener species. A comparison of the characters among these species is shown in the Table.

The authors studied herbarium specimens of the closely related *Orthotrichum crenulatum* from Asia and Europe, including the type specimen from Tibet (NY No. 01060263), as well as specimens from, for example,

Pakistan (Schäfer-Verwimp herbarium No. 3193) and France (Boudier No. 3211 and Kučera CBFS No. 13539) to disprove the possibility that *O. pamiricum* is only a morphological variation of *O. crenulatum*. *Orthotrichum pamiricum* is similar to *O. crenulatum* in gametophytic characters, including an obtuse and crenulate leaf apex, but it can be easily distinguished from the latter by the 16 (papillate) endostome segments and a unistratose leaf

**Table.** Comparison of the features of *Orthotrichum pamiricum* with some similar species of the genus.

Features	<i>O. pamiricum</i>	<i>O. sprucei</i>	<i>O. pumilum</i>	<i>O. vittii</i>	<i>O. crenulatum</i>
Leaf apex	Obtuse, crenulate by bulging walls of marginal cells	Obtuse, mucronate	Slender acute, mucronate	Acute, with hyaline acumen	Obtuse to rounded acute, crenulate by bulging walls of marginal cells
Upper leaf cells	Papillate	Smooth	Smooth or with 1-2 papillae	Papillate	Smooth or slightly papillate
Lamina	Unistratose	Unistratose	Unistratose	Unistratose	Bistratose (in most specimens collected in Central Asia)
Stomata (cryptoporous)	Slightly covered	Almost completely covered	Almost completely covered	Half to completely covered	Slightly covered
Stomata position	2 rows in upper half of the capsule	Central part	Central part	Central part	Central part
Exostome teeth	8 pairs (sometimes split into 16 when old)	8 pairs	8 pairs	8 pairs (sometimes split into 16 when old)	8 pairs
OPL ornamentation	Densely and finely papillate at base and coarsely papillate in the upper part	Papillate to striate papillate toward apex	Finely papillate	Densely papillate	Densely papillate
PPL ornamentation	Roughly papillate-striate ornamented	Smooth	Finely papillate	Base with light striae, high simple papillae in remainder	Densely papillate
Endostome segments	16 with reduced appendages on base	8	8	16 with appendages	8 (without appendages)
PPL ornamentation	Finely papillate	Smooth	Smooth	Smooth (or with light longitudinal striae)	Smooth or finely scabrous
IPL ornamentation	Finely papillate	With scattered papillae	Smooth	Little dense, with thin and low papillae	Smooth
Prostome	Occasional	Lacking	Lacking	Occasional	Lacking
Vaginula	Naked or with 3–4 smooth hairs	Naked	Naked	Naked or sparsely hairy	Naked

lamina. In contrast, all studied Central Asian specimens of *O. crenulatum* have, at least partly, bistratose leaf lamina. In *O. pamiricum*, stomata are arranged in 2 rows in the upper part of the capsule, whereas in *O. crenulatum* they are concentrated in the median part. In addition, a prostome was observed in *O. pamiricum*, which is absent from *O. crenulatum*. *Orthotrichum crenulatum* is known from Central Asia, and it has similar ecological requirements to *O. pamiricum* (Lewinsky, 1992; Schäfer-Verwimp and Gruber, 2002), which is why both of them were found growing together in a few localities along the Tajik–Afghan border.

The sporophytic characters of *Orthotrichum pamiricum* are similar to those of *O. vittii*: 8 exostome teeth, sometimes split to 16; 16 endostome segments with appendages (although in *O. pamiricum* they are only rudimentary at the base); prostome occasionally present; and immersed stomata that are not strongly covered by subsidiary cells. A sparsely hairy vaginula sometimes occurs in both species. Nevertheless, *O. vittii* can be easily distinguished, mainly by acute leaves with hyaline awns. In any case, *O. vittii* is considered endemic to the Iberian Peninsula (Lara et al., 1999), and it has not been reported in Central Asia to date.

Significant differences in both the gametophytic and sporophytic characters can also be found between *Orthotrichum pamiricum* and *O. pumilum*. The latter has a slender acute leaf apex, usually ending in a 1-2-celled apiculus. Furthermore, the endostome of *O. pumilum* consists of 8 smooth segments.

*Orthotrichum sprucei* had long been considered a European endemic species, until it was found in Kazakhstan in 1991 (Goffinet, 2002). To date, it has not been recorded in other Central Asian countries, but it is likely that the species occurs there. *O. sprucei* can be distinguished from *O. pamiricum* mainly by a mucronate leaf apex. Other distinguishing features of *O. sprucei*

include almost completely covered stomata and smooth upper leaf cells.

Finally, based on a superficial view on the cushions, *O. pamiricum* can be confused with *Nyholmiella obtusifolia* (Schrad. ex Brid.) Holmen & E. Warncke. Although both species have ovate-obtuse leaves, *O. pamiricum* differs from *Nyholmiella* in having immersed stomata and an endostome of 16 segments.

The taxonomic character of the hairiness of the vaginula in *Orthotrichum* was discussed in detail by Plášek and Sawicki (2010). A hairy vaginula was observed in some specimens of *O. pamiricum*. The vaginula was usually sparsely covered by 3-4 smooth hairs, which reached up to 4/5 of seta length. A hairy vaginula was noted in approximately 40% of all studied specimens.

Similarly, the presence of a well-developed prostome was observed in around 60% of the studied capsules. The other examined diagnostic features (e.g., leaf shape, peristome character, seta length) appear to be stable.

Detailed bryological surveys in Central Asia can bring other interesting findings on bryophytes in the future, similarly as in the case of research in the southwestern part of the continent (Erdağ et al., 2013; Uyar and Ören, 2013).

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