

Orthotrichum affine var. *bohemicum* (Orthotrichaceae), a new variety of epiphytic moss from the Czech Republic

Vítězslav Plášek^{1*}, Jakub Sawicki², Ivana Marková³, Sylwia Wierzcholska⁴

¹ Department of Biology & Ecology, University of Ostrava, Chittussiho 10, 710 00 Ostrava, Czech Republic

² Department of Botany and Nature Protection, University of Warmia and Mazury in Olsztyn, 10-728 Olsztyn, Poland

³ Bohemian Switzerland National Park Administration, Pražská 52, 407 46 Krásná Lípa, Czech Republic

⁴ Department of Biodiversity and Plant Cover Protection, Wrocław University, Kanonia 6/8, 50-328 Wrocław, Poland

Abstract

Orthotrichum affine var. *bohemicum* Plášek & Sawicki, var. *nova*, is a confirmed record of a new variety from the Czech Republic. The new taxon is described and illustrated by photos and drawing. It is characterized by having superficial stomata and a peristome with 16 endostome segments (practically as long as exostome teeth). Molecular data indicating the phylogenetic placement of the species are presented.

Keywords: *Orthotrichum affine* var. *bohemicum*, *Orthotrichum affine*, *Orthotrichum pallens*, mosses, new variety, taxonomy, Czech Republic

Introduction

The genus *Orthotrichum* is a widespread moss group, which includes approximately 155 species [1]. Taxa belonging to this genus are found throughout the world from the Arctic to the Antarctic, avoiding only deserts and wet tropical forests. Species of the genus *Orthotrichum* grow on trees and rocks to a height of ca. 5000 m a.s.l. [2].

In the Czech Republic there were until recently 20 known taxa [3]. Subsequently in 2009, new species of *O. moravicum* have been added [4].

Material and methods

During a bryofloristic survey in the Protected Landscape Area Lužické hory Mts. (NW part of the Czech Republic) in October 2006 a noteworthy epiphytic moss from the genus *Orthotrichum* was collected (Fig. 1). Upon closer examination the specimen proved to represent a variety new to science.

Type localization

Europe, Czech Republic, Prov. Bohemia, PLA Lužické hory

* Corresponding author. Email: vitezslav.plasek@osu.cz

This is an Open Access digital version of the article distributed under the terms of the Creative Commons Attribution 3.0 License (creativecommons.org/licenses/by/3.0/), which permits redistribution, commercial and non-commercial, provided that the article is properly cited.

Mts., 0.9 km NNW center of the village Doubice, a small group of *Fraxinus excelsior* in a man-made spruce forest by the trail called “Vápenná cesta” (green marked tourist trail), 100 m from the road from Kyjov towards the village Doubice, 414 m a.s.l., coordinates (WGS 84): N = 50° 53' 44" and E = 14° 27' 21", leg. I. Marková 2006.10.18 (herb. OP, KRAM & priv. herb. I. Marková #142/2006).

We are pleased to name the variety after the province Bohemia of the Czech Republic, where the type material has been found.

The nomenclature of the moss taxa in the text below follows Hill et al. [5], hepatics are dealt with Grolle and Long [6] and vascular plants follow Kubát et al. [7].

Results and discussion

Orthotrichum affine var. *bohemicum* Plášek & Sawicki, var. *nova*

Plantae usque ad 1.6 cm altae, cum foliis erecto-adpressis, lanceolatis, apicis acutis. Cellulae superiores cum 1-2 papillis altis, bifurcatis sive conicis. Capsulae fere cunctae emergentes, cylindricae vel oblongo-cylindricae. Stomata phaneropora, in media parte capsulae locata. Peristomium duplex; exostomium 8 dentium (plerumque senectis in 16 fassis), in sicco recurvatum, in basi papilloso-striatum, in dimidio superiore papillosum; endostomium tam-quam exostoma longum, 16 segmentis glabris (planum externum) et papillois (planum internum), uniseriatis et biseriatis segmentis alternis. Calyptra oblongo-conica, in dimidio superiore dense hirsuta.

Description

Plants pale green, 11-16 mm tall, in the central part of

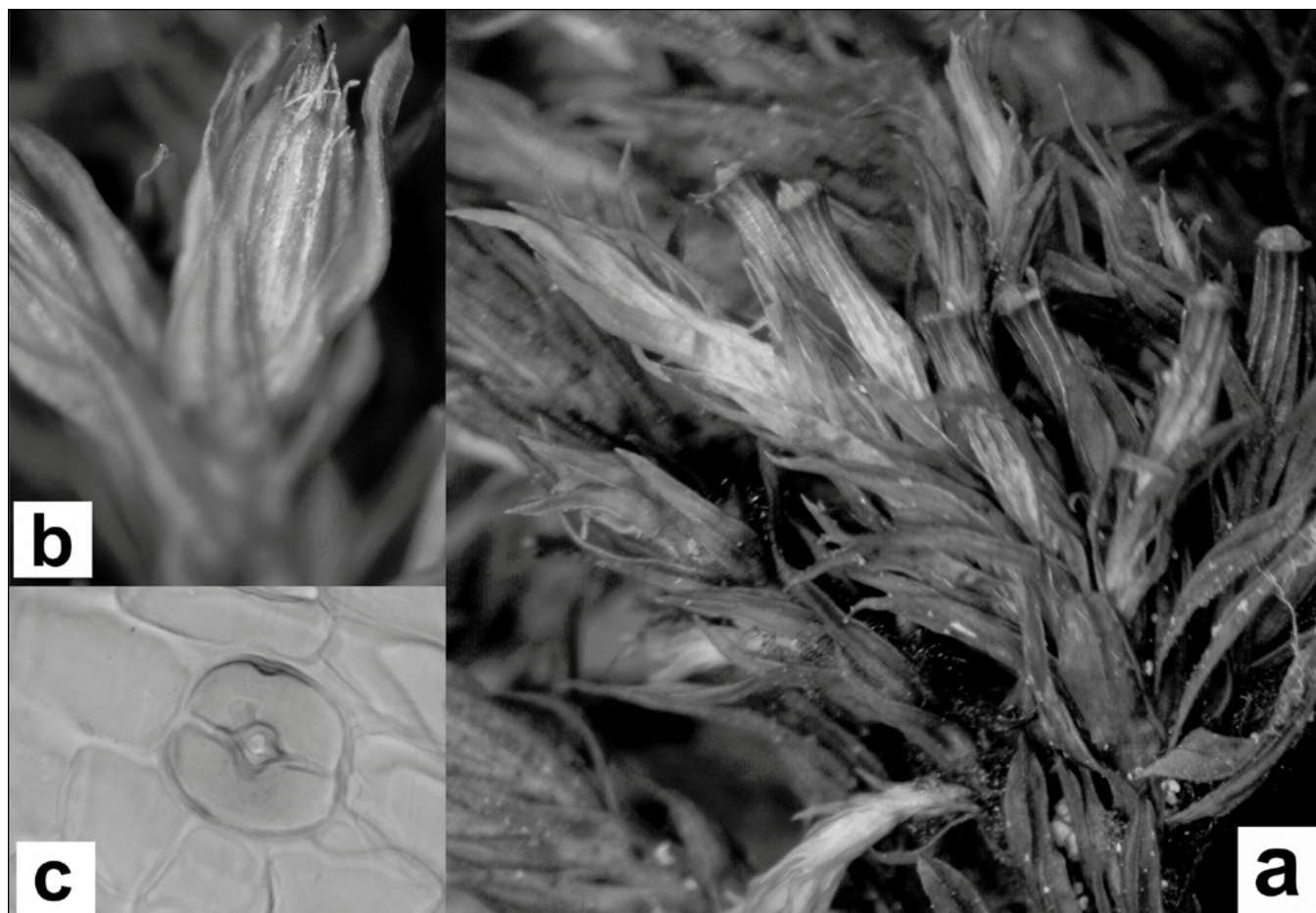


Fig. 1 Photomicrographs of *Orthotrichum affine* var. *bohemicum*. **a** Habit. **b** Hidden capsule with hairy calyptra. **c** Superficial stomata. Photos of the holotype specimen were made by V. Plášek.

sparse cushions up to 2.5 cm. Stem densely branched (Fig. 1), branches up to 5 mm long. Rhizoids pale brown, branched, smooth densely distributed along the stem. Stem leaves erect-appressed and slightly incurved when dry, erect when moist, elongate to lanceolate in shape, 1.3-1.8 mm, keeled, acuminate, sharply acute; margins recurved (Fig. 2f, Fig. 3b) from base to near apex, entire. Upper laminal cells rounded in the apex (9-)10-12(-13) μm , oval to elliptic in the middle of the leaves, (9-)10-12(-13) \times 8-10 μm , 1-stratose, with 1(-2) fairly tall forked or conical papillae (3-6 μm); basal laminal cells long-rectangular to elongate, slightly nodose, 8-10 \times 30-50 μm wide. Costa ending near apex, in basal part (75-)80(-90) μm wide, near apex (28-)35(-40) μm wide. Sexual condition goniatocous. Seta 0.8-1.2 mm, ochrea up to 1/6 of the seta, vaginula naked. Capsule emergent, from 2/3 to 3/4 exerted (Fig. 1); urn-shaped cylindrical when dry, oblong-cylindrical when moist, 2.2-2.6 mm long, yellowish brown or pale brown with 8 strong ribs that do not reach the capsule base, contracted to 3/4 or along entire length when old. Exothecial bands 4 cells wide, formed by moderately differentiated exiguous thick-walled cells, extending from the mouth to 2/3 or 3/4 of capsule length. Neck concolorous with theca, gradually narrowed to the seta. Stomates superficial (Fig. 1c, Fig. 2e, Fig. 3i), situated in the central part of the capsule. Peristome double (Fig. 3a). Endostome formed by 16 segments, whitish to pale brown, incurved when dry, smooth on external side, coarsely papillose on internal side; principal segments biseriolate (at least at base), (176-)180-185(-195) μm long, intermediate ones as tall as principal ones or a little shorter, (170-)175-180(-186)

μm long, consisting of a single row of cells. Exostome teeth 8 (usually split into 16 when old), light brown, 191-200 μm long, strongly recurved when dry. Ornamentation (Fig. 3) of the external side (OPL): papillose – striate at base and coarsely papillose to reticulate-papillose in the upper part. The internal side (PPL) roughly ornamented with papillae. Exostome teeth often remarkably cleft or perforated in upper parts. Calyptra conic-oblong, light brown, in the upper part hairy with papillose hairs (Fig. 1b, Fig. 2c). Lid not seen. Spores 13-15 μm , densely papillose (Fig. 3h). Asexual propagules not seen.

Orthotrichum affine var. *bohemicum* is similar to type *O. affine* in terms of many gametophytic and sporophytic characters, but it can be easily distinguished based on 16 endostome segments (practically as long as exostome teeth) and smaller spore size of 13-15 μm (normal *O. affine* has spore size of 14-26 μm) in *O. affine* var. *bohemicum*. *Orthotrichum pallens*, which seems superficially related to the above species, can be differentiated from them primarily due to the cryptopore type of stomates and shorter and blunter ended leaves. In addition, *O. pallens* has endostome segments alternately longer and shorter, whereas in *O. affine* var. *bohemicum* all segments are equally long (practically as long as exostome teeth).

Ecology and distribution of *O. affine* var. *bohemicum*

The new variety was firstly observed growing on the bark of *Fraxinus excelsior*, in the lower vertical part of the trunk, on the southwestern side, at a height of 72 cm; the population size was 7.5 cm^2 , and it was accompanied by *Brachythecium salebrosum*, *B. velutinum* and *Chiloscyphus profundus*. The

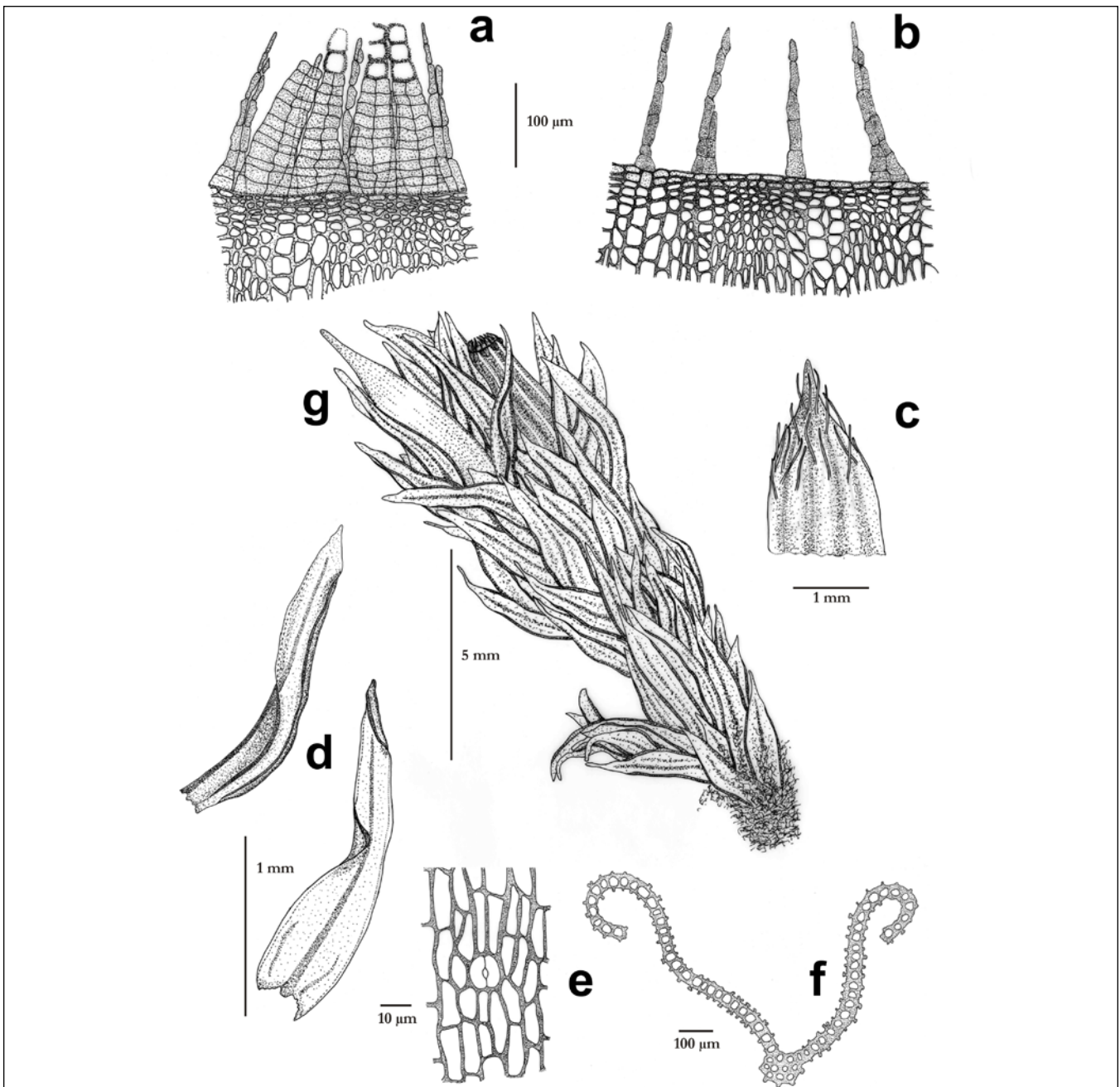


Fig. 2 *Orthotrichum affine* var. *bohemicum*. **a** Peristome. **b** Detail of endostome segments after exostome teeth were cut off. **c** Calyptra. **d** Leaves. **e** Superficial stomata. **f** Transverse section of leaf. **g** Habit. Scale bars are given for every picture. Drawings of holotype by Sylwia Wierzcholska.

tree was growing within a small group of *Fraxinus excelsior* in a man-made spruce forest, by the road (Fig. 4). After the specimen had been identified and confirmed as a new species, a detailed survey of the locality was carried out. In July 2009, two new populations of *O. affine* var. *bohemicum* were found on another *Fraxinus excelsior* tree in the proximity of the first one. Both populations were growing at heights of 100 and 120 cm above ground, together with *Brachythecium salebrosum*. The size of the lower-growing population was 4.5 cm², and that of the upper-growing one was 3 cm².

Molecular analysis

A list of species used in the molecular analysis, details concerning voucher data, and GenBank accession numbers are given in the Appendix S1. Total genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen). Single stems were 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 ITS we used the primers of Fiedorow et al. [8], which were successfully used in previous studies on genus *Orthotrichum* [4,9-12]. The ITS were amplified in a volume of 25 µl containing 20 mM (NH₄) SO₄, 50 mM Tris-HCl (pH 9.0 at 25°C), 1.5 mM MgCl₂, 1 µl BSA, 200 µM each dATP, dGTP, dCTP, dTTP, 1.0 µM of each primer, one unit of Taq polymerase (Novazym) and 1 µl of the DNA solution. The reaction was processed at 94°C for 1 min followed by 30 cycles at 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 were visualized on 1.5% agarose gel with ethidium bromide staining. Purified PCR products were sequenced in both directions using ABI BigDye 3.1 Terminator Cycle Kit (Applied Biosystems) and then visualized using an ABI Prism 3130 Automated DNA Sequencer

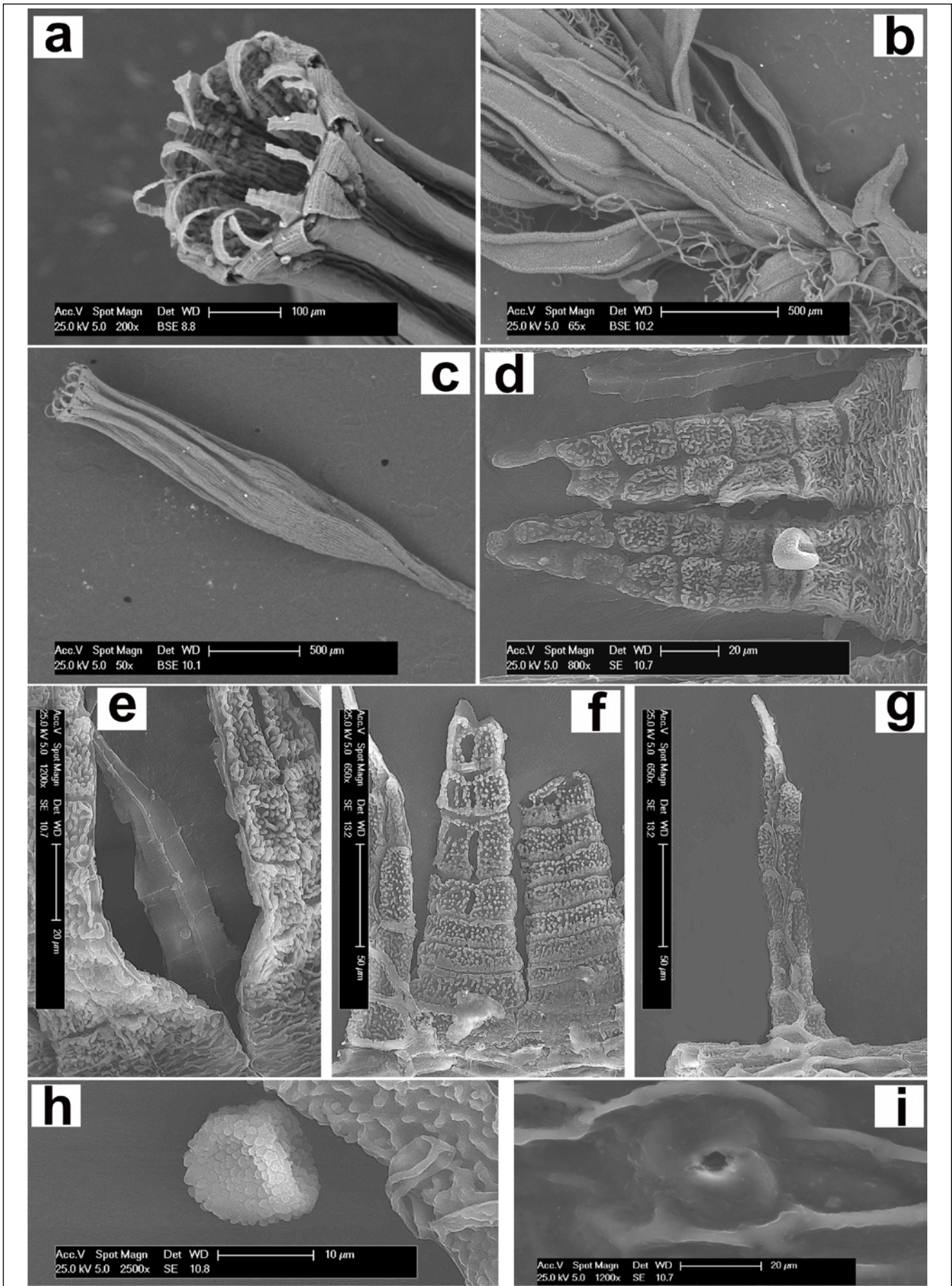


Fig. 3 SEM micrographs of *Orthotrichum affine* var. *bohemicum*. **a** Peristome. **b** Leaves (with recurved margins). **c** Capsule shape. **d,e**. External view of peristome showing OPL ornamentation of the exostome and PPL ornamentation of the endostome. **f** PPL ornamentation of the exostome. **g** Endostome segment showing IPL ornamentation. **h** Spore. **i** Detail of pore. Photos of the holotype specimen were made by V. Plášek and G. Kratošová.



Fig. 4 Map of Central Europe marking the type locality of *Orthotrichum affine* var. *bohemicum* – black star [coordinates (WGS 84): N = 50°53'44" and E = 14°27'21"] and another two known localities – white stars (for description see the text in "Conclusion").

(Applied Biosystems). The assembled sequences were aligned using Muscle 3.6 [13] and manually adjusted with BioEdit 7 [14]. Phylogenetic analyses were conducted using Minimum Evolution (ME) analysis [15] as implemented in MEGA 4 [16]. The pairwise distances were estimated with the Kimura 2-parameter method [17] and initial trees were generated using the neighbor-joining (NJ) method. The ME tree was searched using the Close Neighbor Interchange (CNI) algorithm [18] at a search level of 2, and the maximum number of trees retained at each step was set to 100. Bootstrap analysis [19] was carried out with 1000 replicates.

A combined dataset included 984 characters, of which 849 were constant, 33 variable but parsimony-uninformative and 102 potentially phylogenetically informative. The tree based on ME analysis revealed three well-supported clades (Fig. 5). Clade A (BS 100%) is formed by *O. pulchellum* and *O. stramineum*, i.e. species with immersed stomata, which were used as an outgroup based on previous studies [9,11]. The specimens of the dioecious *O. lyellii* formed clade B (BS 100%), which suggests its distinctiveness from monoecious members of the subgenus *Gymnoporos*, which were included in clade C. Within this group, an analysis of ITS revealed the specimens of *O. affine* var. *bohemicum* to represent a sister group to *O. affine*, but clearly separate (BS 90%) from the species, which

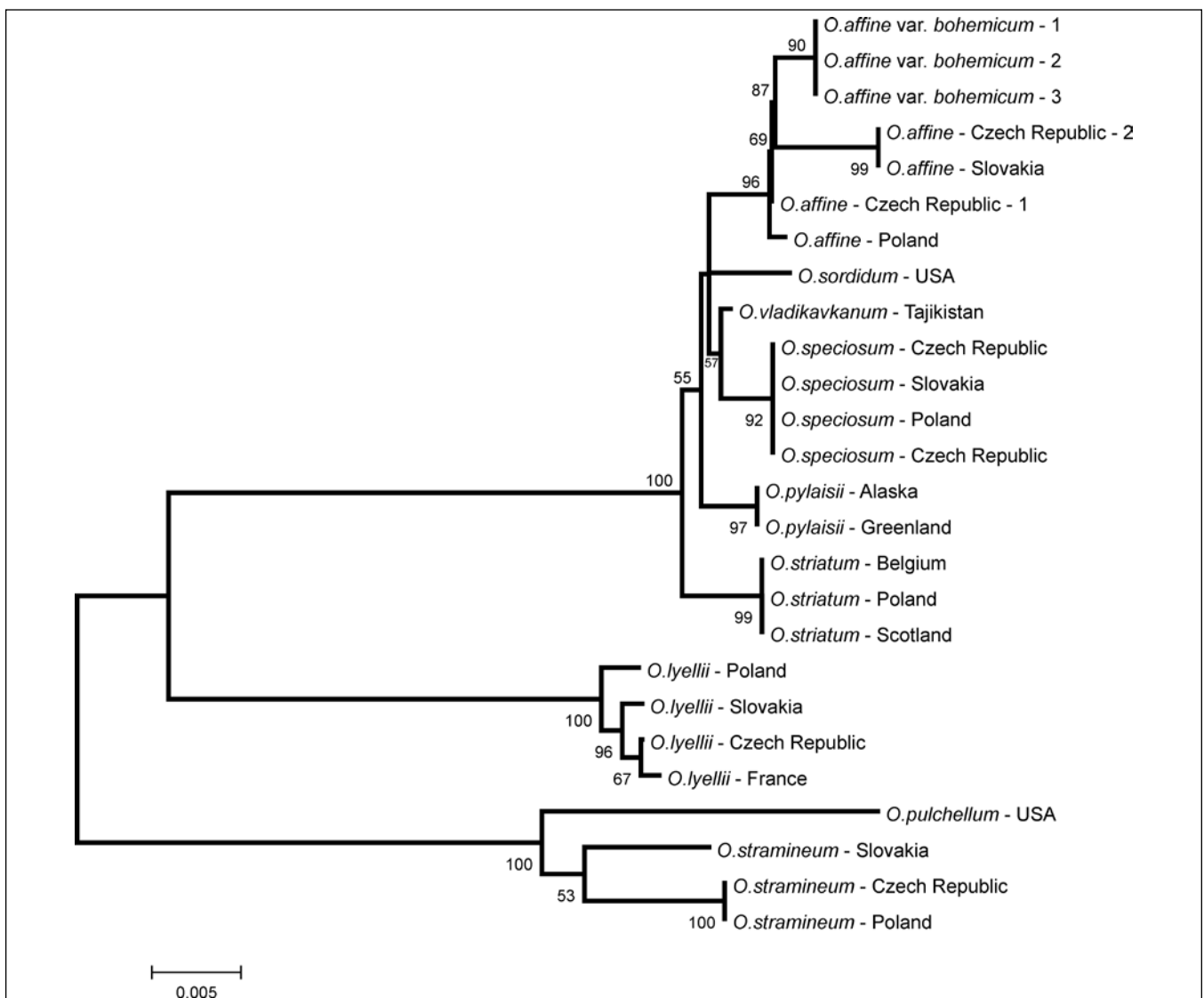


Fig. 5 Minimum evolution tree based on ITS sequences. Bootstrap values above 50% are given above branches.

is consistent with morphological data.

The samples of *O. affine* var. *bohemicum* differ from the specimens of *O. affine* from Slovakia and the Czech Republic (specimen 2) by one deletion and two substitutions, and from other specimens of *O. affine* (Poland and the Czech Republic 1) by additional three substitutions.

Conclusion

According to Lewinsky [2], the morphological features of *Orthotrichum affine* var. *bohemicum*, which were supported by the results of our molecular analysis [10], suggest its inclusion within the subgenus *Gymnopus*, sect. *Affinia*.

The new taxon is closely related to type *O. affine*. These two taxa are morphologically similar with respect to the size of plants, leaf shape, and the type of stomates as well as the hairiness of calyptra. However, they differ significantly in the number of endostome segments (*O. affine* = 8, *O. affine* var. *bohemicum* = 16) and in the shape of leaf cell papillae. Whereas *O. affine* exceptionally rarely has forked branched papillae, *O. affine* var. *bohemicum* almost always develops forked papillae and rarely conical ones.

Subsequently, after we had distinguished the new species from the type-material, we also made a revision of selected Czech herbarium collections. As a result, two more specimens of *O. affine* var. *bohemicum*, reported from the territory of the Czech Republic, were found (Fig. 4). The first specimen was collected in the Bílé Karpaty Mts. in a locality near the village Velká nad Veličkou, 513 m a.s.l. (loc. No. 1 in the map, Fig. 4), on the bark of *Tilia cordata*, leg. V. Plášek 2006.04.21 (OP #185624). The other one was found in the Hrubý Jeseník foothills, near the town of Rýmařov, in the Moravice river valley, 537 m a.s.l. (loc. No. 2 in the map, Fig. 4), on the bark of *Salix fragilis*, leg. V. Plášek 2006.10.15 (OP #187354).

Acknowledgments

We thank Jarosław Proćków for help with developing the Latin diagnosis. Molecular work was supported by the Polish State Committee for Scientific Research (KBN grant N303 416237). SEM micrographs were made with help of Gabriela Kratošová. Article has been also done in connection with project Institute of environmental technologies, reg. No. CZ.1.05/2.1.00/03.0100 supported by Research and Development for Innovations Operational Programme financed by Structural Funds of Europe Union and from the means of state budget of the Czech Republic.

Supplementary material

The following supplementary material for this article is available online at <https://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.2011.030/0>:

1. Appendix S1. Accession data for plants included in the molecular analysis.

References

1. Goffinet BA, Buck WR, Wall MA. *Orthotrichum freyanum*

- (Orthotrichaceae), a new epiphytic moss from Chile. *Nova Hedw.* 2007;131:1-11.
2. Lewinsky J. A synopsis of the genus *Orthotrichum*. *Hedw.* (Musci, Orthotrichaceae). *Bryobrothera.* 1993;2:1-59.
3. Kučera J, Váňa J. Seznam a červený seznam mechorostů České republiky (2004). *Příroda.* 2005;23:1-104.
4. Plášek V, Sawicki J, Trávníčková V, Pasečná M. *Orthotrichum moravicum* (Orthotrichaceae), a new moss species from the Czech Republic. *Bryologist.* 2009;112(2):329-336. <http://dx.doi.org/10.1639/0007-2745-112.2.329>
5. Hill MO, Bell N, Bruggeman-Nannenga MA, Brugués M, Cano MJ, Enroth J, et al. An annotated checklist of the mosses of Europe and Macaronesia. *J Bryol.* 2006;28(3):198-267. <http://dx.doi.org/10.1179/174328206X119998>
6. Grolle R, Long DG. An annotated check-list of the Hepaticae and Anthocerotae of Europe and Macaronesia. *J Bryol.* 2000;22:103-140.
7. Kubát K, Hrouda L, Chrtek J, Kaplan Z, Kirschner J, Štěpánek J, editors. Klíč ke květeně České republiky. Praha: Academia; 2002.
8. Fiedorow P, Odrzykoski IJ, Szwejkowska-Kulińska Z. Phylogenetic studies of liverworts using molecular biology techniques. In: Małuszyńska J, editor. *Plant Cytogenetics.* Katowice: Silesian University Press; 1998. p. 244-249.
9. Sawicki J, Plášek V, Szczecińska M. Preliminary studies on the phylogeny of *Orthotrichum* (Bryophyta) inferred from nuclear ITS sequences. *Ann Bot Fennici.* 2009;46:507-515.
10. Sawicki J, Plášek V, Szczecińska M. Molecular evidence does not support the current division of *Orthotrichum* subgenus *Gymnopus*. *Plant Syst Evol.* 2009;279(1-4):125-137. <http://dx.doi.org/10.1007/s00606-009-0153-0>
11. Sawicki J, Plášek V, Szczecińska M. Molecular studies resolve *Nyholmiella* (Orthotrichaceae) as a separate genus. *J Syst Evol.* 2010;48(3):183-194. <http://dx.doi.org/10.1111/j.1759-6831.2010.00076.x>
12. Plášek V, Sawicki J. Is the hairy vaginula a diagnostic feature in the taxonomy of the genus *Orthotrichum*. *Acta Soc Bot Pol.* 2010;79(1):73-80.
13. Edgar RC. Muscle: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 2004;32(5):1792-1797. <http://dx.doi.org/10.1093/nar/gkh340>
14. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser.* 1999;41:95-98.
15. Rzhetsky A, Nei M. A simple method for estimating and testing minimum-evolution trees. *Mol Biol Evol.* 1992;9(5):945-967.
16. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol.* 2007;24(8):1596-1599. <http://dx.doi.org/10.1093/molbev/msm092>
17. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol.* 1980;16(2):111-120. <http://dx.doi.org/10.1007/BF01731581>
18. Nei M, Kumar S. *Molecular evolution and phylogenetics.* Oxford: Oxford University Press; 2000.
19. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution.* 1985;39(4):783-791. <http://dx.doi.org/10.2307/2408678>