SEM differences in sporophyte micromorphology of *Plagiothecium nemorale* and *P. longisetum* (Plagiotheciaceae, Bryophyta)

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Abstract: *Plagiothecium nemorale sensu lato* is described as a variable Eurasian taxon. Recent studies indicate that this variability is reflected in both qualitative and quantitative features of its gametophyte and its genetic variation. This taxon comprises at least two separate species: *P. nemorale* (Mitt.) A. Jaeger and *P. longisetum* Lindb. The present paper examines whether the gametophyte variability of these species is reflected in the micromorphological variation of the sporophyte. The analysis revealed that the seta of *P. nemorale* is shorter than that of *P. longisetum*. In addition, regarding the exostome teeth, the lower portion of the outer surface is characterised by loosely-arranged, moniliform cristae in *P. nemorale*, with conical papillae on the upper side; however, in *P. longisetum* it possesses tightly arranged, epapillose cristae, with clearly flattened papillae on the upper side. In addition, the inner surface is more gently papillose in *P. nemorale* than in the latter. These findings not only describe hitherto unknown features of the sporophyte of these species, but also justify the resurrection of *P. longisetum* as separate from *P. nemorale*.

Keywords: Scanning Electron Microscope, sporophyte morphology, taxonomy, *Plagiothecium*

INTRODUCTION

As most species of the genus *Plagiothecium* Schimp. are sterile when collected (Greene, 1957), their taxonomic features tend to concern the morphological characteristics of their vegetative organs, particularly the qualitative features of the stem leaves (Wolski, 2018). Nevertheless, although previous works have focused mainly on the characteristics of the gametophyte, some also provide information on the sporophytes of these species (Lindberg, 1872; Jedlička, 1948, 1950; Nyholm, 1965; Ireland, 1969; Iwatsuki, 1970; Buck & Ireland, 1989; Noguchi, 1994; Ochyra et al., 2008). However, these data refer only to features that are observable with the use of a light microscope. Only Ireland (1987, 2001) and Ignatov et al. (1996) have described the species of this genus using a Scanning Electron Microscope (SEM). Even so, their studies are fragmentary, referring only to a few selected species and sporophytic structures of these taxa.

One species belonging to the genus *Plagiothecium* is *P. nemorale* (Mitt.) A. Jaeger, which was described by Mitten (1859) as *Stereodon nemoralis* Mitt. Over the past 50 years, studies have found that *P. nemorale* was a very variable species, which has resulted in significant taxonomic problems (Greene, 1957; Nyholm, 1965; Ireland, 1969; Iwatsuki, 1970; Lewinsky, 1974); however, the causes of this variation were not identified. Eventually, a detailed qualitative and quantitative analysis of the intraspecific variability of the gametophyte of *P. nemorale sensu lato* distinguished at least two groups of specimens. The most important features supporting this division was found to be the length and width of the cells from the upper, middle, and lower parts of the stem leaves. This confirmed the hypothesis that *P. nemorale* had previously been too broadly described, and that it should be considered a complex (Wolski, 2017, 2018). These observations were later confirmed by further taxonomic and molecular research (Wolski & Nowicka-Krawczyk, 2020), which found that two groups of specimens distinguished by Wolski (2017, 2018) are in fact two separate species: *P. nemorale sensu stricto* and *P. longisetum* Lindb.

Unfortunately, there remains a lack of detailed data on the sporophyte micromorphology of *P. nemorale* and *P. longisetum*. The present study was performed to determine whether the gametophyte variability demonstrated by these species is reflected in the micromorphology of
the sporophyte.

**MATERIALS AND METHODS**

Sporophytes were separated into two groups depending on the qualitative and quantitative features of the gametophyte. The material was obtained from two separate species proposed by Wolski and Nowicka-Krawczyk (2020) – *P. nemorale sensu stricto* and *P. longisetum*. Specimens with symmetrical leaves, a denticulate leaf apex, as well as hexagonal and narrowly hexagonal leaf cells no longer than 100 µm in the central part of the leaf were considered *P. nemorale sensu stricto*. Material with asymmetrical leaves, an entire leaf apex, and elongated hexagonal leaf cells longer than 100 µm but not exceeding 150 µm in the central part of the leaf was classified as *P. longisetum* (Wolski, 2017, 2018; Wolski & Nowicka-Krawczyk, 2020).

The examined sporophytes were collected from 50 specimens deposited in nine herbaria (BIL, BM, C, E, H, LOD, NY, POZG, S). In good condition and mature sporophytes were gently removed under a binocular microscope from homogeneous turf. The fragments of sporophytes were mounted onto aluminium stubs and double-sided adhesive tape and directly coated with a layer of gold in Quorum Sputter Coater SC7620. The coated sporophytes were observed, and relevant parts were photographed under a Carl Zeiss EVO LS 10 scanning electron microscope (15 kV accelerating voltage was used). Qualitative and quantitative analysis was performed on selected characteristics of the sporophyte samples: the setae (surface, color and length), capsules (orientation, shape, color, length, width), exothecial cells (shape, length, width), opercula (shape, length), exostome and endostome teeth (shape, color, length, width), cilia (length, surface).

**Specimens examined**

*Plagiothecium nemorale* (Mitt.) A. Jaeger, Ber. S. Gall. Naturw. Ges. 1876–1877: 451 (1878) = *Stereodon nemoralis* Mitt., Journ. Linn. Soc. Bot. Suppl. 1: 104 (1859) = *P. silvaticum* var. *nemorale* (Mitt.) Paris, Index Bryol.: 967 (1898). Type citation: Hab. In Himalayaе orient. reg. temp., Sikkim, in monte Tonglo (ad radicem filicis cujusdam), J. D. Hooker ! Lectotype: “Herb. Ind Or Hook. Fil. & Thomson Stereodon nemorale* m. Hab. Sikkim, Tonglo Regio temp. Alt. – J. D. H.” – BM 1030713!: isolectotype: NY 913349!

**AUSTRIA**, Salzburg, am Dürenberg bei Hallein, 26 Jun. 1869, *L. Béroger*, S B281704. CHINA, Liaoning Prov., Kuanidian County, Bai-shi-la-ji National Natural Reserve, trail from fire tower to Xiang-shui-gou at Hu-li Mt., 40°50’N, 124°52’E, alt. 900–1000 m, moist hardwood forest with numerous granitic boulders, 21 Aug 1993, *W. R. Buck* 23747, NY 3103644. DENMARK, Nyborg, 6 Dec. 1971, C-20156. FRANCE, Aveyron, Abbaye Notre-Dame de Bonnecombe, 29 Aug. 1959, G. Een, F258, S B30110. GERMANY, Bavaria, an Granit in Falkensteiner Park, in Bachufern in der Klamme, alt. 360–580, May 1903, *I. Faimiller*, S B281858; Berchtesgaden district, Schleching, on the ground in a wood, 6 Jun 1962, *S. S. Hooper*, E905518; Baden-Württemberg, Headwaters of the Elz River near Korallenhaus, NW from Schönwald, Naturpark, Südswarowald, alt. 927 m, on rock in stream, 24 Apr. 2010, J. T. Wynns & M. Lüth 3044, C10632. HUNGARY, Comit Komárom, pr. Oroszlány, alt. 250 m, 8 Sep. 1937, *A. Boros*, E905534; Vertes Gebirge, in Erlenäuldern längs des Baches im Walde „Mocsárberek“ genannt, neben dem Dorfe Oroszlány, alt. 200–250 m, 6 Dec. 1957, *A. Boros*, C-20084. IRAN, Mazandaran province, Nowshahr country, lower part of Veisar forest, *Fagus-Carpinus* forest type, 890 m, 2009, *H. Zare*, S B171134. ITALY, Campello-Monti, prov. Novara. Pedemontii, in silva Valdo prope cataractam „die Dannaï“, alt. 1250 m, 27 Aug. 1906, *E. Levier*, S B281867; Milano, Artaria, 16 Dec. 1896, S B281882. JAPAN, Hokkaido, Prov. Sōya, Isl. Rishiri, Mt. Rishiri, alt. 100–500 m, 8 Aug. 1954, *H. Hasegawa*, Z. Iwatsuki 15944, S Hasegawa 15783, S1131590; 500 m, 8 Aug 1954, *H. Hasegawa*, S1131589; Honysy, Mt. Kiso-Ontake, on rocks in conifer forest, alt. 1250 m, 24 Aug 1953, *N. Takaki*, S1131623; Kagohgima Pref., Yukushima Island, alt. 420 m, on moist vertical face of boulder in full shade, 24 Sep. 1975, *Z. Iwatsuki*, G. L. Smith J-936, NY 3103681; Hiroshima Pref., Mt. Hibayama, alt. 1200 m, on rock, 10 Nov 1973, *H. Ando*, S B281902; Ibazi Prov., 27 Oct 1921, *R. Jakeneke*, H1311587. NORWAY, Rogaland, Vindafjord, Opsal, Vikedal, Edelauvsvog, alt. 60 m, 59,5025’N 5,8746’E, 17 Oct 2015, *A. H. Øygarden et al.*, TRH B-12753. POLAND, Białystok nadl. Doliłdy oddz. 120, łg. 26 May 1988, *A. Sokolowski*, BIL 8671; Nadleśnictwo Balinka, Puszcza Augustowska, łg. 5 Sep. 1963, *D. Godawska*, BIL 1437. RUSSIA, Caucaus, Karachaeko-Cherkessian Republic, Teberda Natural Reserve, Shumka Creek, near waterfall, *Abies-Pagus* forest, on soil on steep slope, 43°24’N, 44°11’E, alt. 1500 m, 16 Sep. 2005, *M. Ignatov*, E. Ignatov, S B114218. SWEDEN, Södermanland, Ägetaberget, 27 Oct 1959, *G. Een*, S B55593. SLOVAKIA, Ungern, Jul. 1913, *J. A. Báumkin*, S B281872. SWITZERLAND, Kt. Zürich, Hirzel, W of Rüebgarten, tree base, 9 Mar. 1996, *L. Hedenäs*, S B281691. UNITED KINGDOM, Creinch, Loch, Lomond, on rock in oak wood, 24 May 1980, *A. C. Crundwell*, E905487; on rock in wood, 17 May 1953, *A. C. Crundwell*, E905494; Scotland, above the Sleat Peninsula, N. Ebudes, on sandy soil in the shade from a rock, alt. 104. 23 Aug. 1968, *C. C. Townsend*, E905483.
**RESULTS**

**Sporophyte description of *Plagiothecium nemorale* and *P. longisetum***

The reddish-brown seta is smooth and straight in both species. The capsule is inclined to horizontal and is usually cylindrical (Fig. 1). Even when dry, the capsule is smooth and usually dark brown, 2.6–3.5 × 1–1.2 mm. The exothecal cells are thin-walled, hexagonal to long-hexagonal, 34–68 × 21–51 µm. The operculum is usually straight, 0.8–1.8 mm in length, long-conic to rostrate, and the annulus is composed of 2–3 rows of cells, 50–64 × 21–35 µm. The double peristome is well developed (Fig. 1). The exostome teeth are lanceolate, narrowly triangular, and bright orange to yellowish, 400–600 × 80–119 µm. The outer surface of the exostome teeth in the lower part is striolate, and clearly papillose in the upper part. The exostome teeth themselves are trabeculated at the back, 10–26 µm (Figs 2-3). The endostome teeth are yellowish clearly segmented and form a triangular prism shape, 360–600 µm. At the base, the segments are sparsely papillose, much more densely so in the upper part, and clearly and intensively papillose inside (Fig. 4). The cilia, 300 µm in length, are clearly nodose with large swellings at cell boundaries, and clearly papillose along their entire length. The spores are spherical, 9–12 µm in diameter, and quite strongly papillose.

No explicit variation was observed for the shape, arrangement, length, or width of the capsule; the shape or dimensions of the exothecial cells, the dimensions or micromorphology of the endostome teeth, or the spores. However, in the group of studied specimens, some variability was noted in the length of the seta, and in the micromorphology of the surface of the exostome teeth. In addition, even the same trabeculated areas can be differentially papillose, both quantitatively and qualitatively, with their ornamentation varying with regard to density (sparsely or dense), diameter (thinner, thicker) and shape (straight, curved); sometimes even bifid papilae are present. The surface is heterogeneously papillose, varies according to the specimen being tested, which can be densely or rarely spiculose. It also depends on the position of the trabecula,
Fig. 1. The shape and arrangement of the capsule and double peristome of *Plagiothecium nemorale* (A. Sokołowski, BIL 8671); scale bar in µm.

Fig. 2. Rarely spiculose trabeculae of the exostome teeth of *Plagiothecium nemorale* (A – H. Hasegawa, H3113589; B – Eggers & Frahm, NY 3103761; C – A. C. Crundwell, E905487; D – A. H. Øygarden et al. TRH B-12753). The gray arrows indicate selected examples of places where the described feature is located; scale bars in µm.
with the lower positions much more weakly and loosely papillose than the higher locations (Figs 2-3).

The seta of *Plagiothecium nemorale* is short (3-3.5 cm), the lower portion of the outer surface of the exostome teeth is characterised by loosely-arranged, moniliform cristae, while the upper side has conical papillae, and the inner surface is much more gently papillose (Figs 2, 5, 6). In contrast, the seta of *P. longisetum* is markedly longer (i.e. up to 5.5 cm). The lower portion of the outside of the exostome teeth of *P. longisetum* has tightly arranged, epapillose cristae, while the upper side has clearly flattened papillae, and their inner surface is much more strongly papillose (Figs. 3, 7, 8).

**DISCUSSION**

Many species of *Plagiothecium* have never been the object of SEM research. Although three studies have examined the micromorphology of species of *Plagiothecium* (Ireland, 1987; Ignatov et al., 1996; Ireland, 2001), the cited articles did not address the sporophytes of *P. nemorale*.

Our present findings confirm the presence of known qualitative and quantitative features of the sporophyte visible through the light microscope (Lindberg, 1872; Jedlička, 1948; Nyholm, 1965; Ivatsuki, 1970; Lewinsky, 1974; Noguchi, 1994; Smith, 2001). However, Noguchi (1994) gives much higher values for the length of the capsules (4.5 mm) than observed here. In addi-

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**Fig. 3.** Densely spiculose trabeculae of the exostome teeth of *Plagiothecium longisetum* (A – J.-P. Frahm, C-20098; B – R. A. Boniface, BM1103855; C – J. A. Paton & R. Fisk, 2821, E905435; D – H. Zare, S B171128); scale bars in µm.
**Fig. 4.** The endostome of *Plagiothecium nemorale*: segmented and densely papillose in the upper part (A), and sparsely papillose in the lower part (B) *(G. J. Wolski, LOD 14926)*; scale bars in µm.

**Fig. 5.** The moniliform cristae of the bottom part of the outer surface of the exostome teeth of *Plagiothecium nemorale* *(A – R. Jakenekin, H3113587; B, E – C. C. Townsend, E905483; C – A. Boros, E905534; D – W. R. Buck, 23747, NY 3103644).* The gray arrows indicate selected examples of places where the described feature is located; scale bars in µm.
In sporophyte micromorphology *P. nemorale* and *P. longisetum* differ significantly from other species. The outer and inner surfaces of the exostome teeth of *P. cavifolium*, *P. laetum* and *P. denticulatum* are quite similar, with both surfaces having small, sparse papillae (Ignatov et al., 1996). The structure of the sporophyte has been associated with the taxonomic status of Plagiotheciaceae species (Ignatov et al., 1996; Ignatov et al., 1998). According to these authors, the deviating sporophyte features of *P. latebricola* and *P. piliferum* justify their exclusion from the genus *Plagiothecium* (Ignatov et al., 1996).

Our findings indicate that the structures of the exostome teeth of the tested samples are variable. The lower part of the outer surface of the exostome is epapillose or moniliform. In the examined specimens, noticeable quantitative and qualitative differences were observed regarding the upper part of the outer surface of the exostome, as well as the papillose trabeculae of the inner surface of the exostome teeth.

A combination of our present findings with those of previous studies of the gametophyte of *P. nemorale* and *P. longisetum* (Wolski, 2017, 2018; Wolski & Nowicka-Krawczyk, 2020) suggest that the following characteristics are indicative of *P. nemorale sensu stricto*: symmetrical leaves, a denticulate leaf apex, hexagonal or narrowly hexagonal leaf cells not longer than 100 µm in the central part of the leaf, a short seta, a sporophyte with loosely-arranged, moniliform cristae on the lower portion of the outer surface of the exostome teeth, the presence of conical papillae in the upper portion, as well as a gently papillose
inner surface. In contrast, for *P. longisetum*, the gametophyte specimens are characterised by asymmetrical leaves, an entire leaf apex, the cells in the central part of the leaf elongated, hexagonal longer than 100 µm but not exceeding 150 µm, a long seta, tightly arranged epapillose cristae on the bottom side of the outside of the exostome teeth with clearly flattened papillae on the upper side, and a much more strongly papillose inner surface. These associations between the respective states remain constant, despite the variation in other features observed for the studied species.

Our findings provide new information on the micromorphology of the exostome and endostome teeth in the studied species. These data not only confirm the hypothesis of Wolski (2017, 2018) that currently *P. nemorale* is too widely described, but also – as indicated by Wolski &
Nowicka-Krawczyk (2020) – that two distinct species can be distinguished within *P. nemorale* sensu lato. Furthermore, our study confirms that gametophyte variability is clearly reflected in the micromorphology of the sporophyte.

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