



Cryptic and morphologically recognizable species diversity within Scandinavian *Plagiopus oederianus* (Bryophyta: Bartramiaceae)

Author: Hedenäs, Lars

Source: Lindbergia, 2020(1)

Published By: Dutch Bryological and Lichenological Society and Nordic Bryological Society

URL: <https://doi.org/10.25227/linbg.01130>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Cryptic and morphologically recognizable species diversity within Scandinavian *Plagiopus oederianus* (Bryophyta: Bartramiaceae)

Lars Hedenäs

L. Hedenäs ✉ (lars.hedenas@nrm.se), Dept of Botany, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden.

Based on studies of the nuclear marker ITS, the plastid ones *atpB-rbcL* and *rpl16*, and morphology, five species are recognizable within Scandinavian *Plagiopus oederianus*. Four of these species are cryptic, whereas the fifth one, *Plagiopus alpinus* (Schwaegr.) Hedenäs, *comb. et stat. nov.*, can be distinguished from the other ones by morphology. *Plagiopus alpinus* is smaller than the four cryptic species within *P. oederianus* and has smaller spore capsules, smaller exothecial cells, shorter leaves and shorter lamina cells in the middle and base of the leaves. In Scandinavia, *P. alpinus* occurs in the mountain range, whereas the cryptic species within *P. oederianus* occur both in the mountains and in the lowlands. Outside Scandinavia, *P. alpinus* is known from the European Alps but it seems likely that it is widespread in mountains and northern regions of the northern hemisphere. The cryptic species within *P. oederianus* differ in several molecular bases.

Keywords: *comb. et stat. nov.*, integrative taxonomy, mountains, northern diversity, phylogenetic, *Plagiopus alpinus* (Schwaegr.) Hedenäs, principal component analysis

In Scandinavia and Svalbard, *Plagiopus oederianus* (Sw.) H.A. Crum & L.E. Anderson is currently the only accepted *Plagiopus* species (Frisvoll and Elvebakk 1996, Nyholm 1998, Hallingbäck et al. 2006, 2008). Small plants with small spore capsules that occur in the mountains and in Svalbard are sometimes distinguished as *Plagiopus oederianus* var. *alpinus* (Schwaegr.) Ochyra (Möller 1925, Jensen 1939, Hallingbäck et al. 2006). Plants called *Bartramia oederi* var. *microcarpa* Kindb. (Kindberg 1888), a *nomen nudum*, likely belong to this kind, and *Plagiopus oederi* var. *condensata* Brid. ex Limpr. (Hagen 1899–1904) is another name used for such plants in Scandinavia. The latter name is based on the illegitimate *Bartramia oederi* var. *condensata* Brid. (Bridel 1817), which included the older basionym of *P. oederianus* var. *alpinus*, i.e. *Bartramia oederi* var. *alpina* Schwaegr. in its description (Schwaegrichen 1816). However, whether such small plants should be recognized as a distinct taxon is frequently doubted because plants intermediate between var. *oederianus* and var. *alpinus* are thought to be relatively frequent (Möller 1925, Nyholm 1998) and some of the mentioned authors do not distinguish them or they consider the small plants as a mere form.

In a recent series of papers based on molecular evidence, sometimes in combination with morphological studies (Hedenäs 2017a, 2018b, 2019), it was shown that so-far hidden diversity exists in many Scandinavian moss species, especially in the north and mountains. Some of the found molecular variation corresponds with cryptic species only (Hedenäs 2020a), whereas in other cases some of the variation correlates with morphological differentiation and has revealed additional morphologically recognizable species (Hedenäs 2017a, 2018b, 2020b). The purpose of the present study is to investigate whether some of the morphological variation in Scandinavian *P. oederianus* deserves formal recognition, by placing the species' morphological variation in the context of its molecular variation.

Material and methods

Studied material

This study includes 42 samples of *Plagiopus oederianus* s.l. (Table 1). Thirty-five come from Sweden and five from Norway, representing its distribution in the Scandinavian peninsula except southernmost Sweden. Based on results by Virtanen (2003) and Damayanti et al. (2012), sequences for *Bartramia*, *Conostomum* and *Philonotis* species were downloaded from GenBank with the intention of using these taxa as outgroups. In some species of each potential outgroup genus, two of the three sequences employed in the present

This work is licensed under the terms of a Creative Commons Attribution 4.0 International License (CC-BY) <<http://creativecommons.org/licenses/by/4.0/>>. The license permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Table 1. Specimen data and GenBank accession numbers for the sequences. All specimens are in herbarium S, and except where noted their geographical origin is Sweden. The data are arranged as: sample no. [=leaf and selected sporophyte characters measured in the detailed morphological study; (A)–(E)=group according to Fig. 1]; locality; coll. year, collector (LH=L.Hedenäs) [collector's no.]; S herbarium registration no.; GenBank accession numbers for ITS, *atpB-rbcL*, *rpl16* (NA=Sequence not available).

Plagiopus oederianus* (Sw.) H.A. Crum & L.E. Anderson:** **P576 (A): Östergötland, Godegård; 2009, LH; B177547; MT006267, MT005106, MT005141. **P577** (A): Västmanland, Grythyttan; 1991, N.Hakelien; B281130; MT006268, MT005107, MT005142. **P578*** (B): Värmland, Järnskog; 1985, S.Fransson 1985/190; B106237; NA, MT005108, MT005143. **P579*** (B): Värmland, Järnskog; 1985, S.Fransson 1985/222; B106235; NA, MT005109, MT005144. **P580*** (B): Värmland, Filipstad; 2010, LH & G.Odelvik; B178132; MT006269, MT005110, MT005145. **P581*** (A): Södermanland, Utö; 2010, LH; B175296; MT006270, MT005111, MT005146. **P582*** (E): Södermanland, Utö; 2016, LH; B240778; MT006271, MT005112, MT005147. **P583** (A): Södermanland, Björkvik; 2016, A.Stansvik AS347; B270139; MT006272, MT005113, MT005148. **P584*** (A): Uppland, Djurö; 2014, LH; B208346; MT006273, MT005114, MT005149. **P585*** (A): Dalarna, Ore; 2018, LH; B288109; MT006274, MT005115, MT005150. **P586*** (B): Gästrikland, Torsåker; 2004, LH; B96463; MT006275, MT005116, MT005151. **P587*** (C): Dalarna, Hamra; 2000, LH; B37586; MT006276, MT005117, MT005152. **P589** (A): Ångermanland, Viksjö; 2013, LH et al.; B200859; MT006277, MT005118, MT005153. **P590*** (A): Ångermanland, Viksjö; 2013, LH et al.; B200856; MT006278, MT005119, MT005154. **P592*** (A): Härjedalen, Tännäs; 2007, LH; B122837; MT006279, MT005120, MT005155. **P593*** (C): Jämtland, Ragunda; 2014, LH; B205130; MT006280, MT005121, MT005156. **P594*** (C): Jämtland, Ragunda; 2014, LH; B204257; MT006281, MT005122, MT005157. **P595*** (A): Jämtland, Kall; 2006, LH & I.Draper; B112544; MT006282, NA, MT005158. **P596*** (A): Jämtland, Frostviken; 1988, LH J88–406; B33381; MT006283, NA, MT005159. **P597*** (C): Västerbotten, Jörn; 2016, LH & G.Odelvik; B240425; MT006284, MT005123, MT005160. **P598*** (B): Norrbotten, Tärändö; 1990, LH & M.Aronsson NT90–803; B33382; MT006285, MT005124, MT005161. **P599*** (C): Åsele Lappmark, Dorotea; 2004, LH; B93238; MT006286, MT005125, MT005162. **P600** (A): Lycksele Lappmark, Tärna; 2016, LH; B237379; MT006287, NA, MT005163. **P601*** (A): Lycksele Lappmark, Lycksele; 1987, LH; B281129; MT006288, MT005126, MT005164. **P602*** (A): Pite Lappmark, Arjeplog; 2015, LH et al.; B228141; MT006289, MT005127, MT005165. **P603*** (B): Pite lappmark, Arjeplog; 2017, LH et al.; B258199; MT006290, MT005128, MT005166. **P604*** (C): Pite Lappmark, Arjeplog; 2015, LH et al.; B227721; MT006291, MT005129, MT005167. **P606*** (B): Torne Lappmark, Jukkasjärvi; 2017, LH; B254924; MT006292, MT005130, MT005168. **P612*** (E): Norway. Troms, Lyngen; 2003, LH; B83045; MT006293, MT005131, MT005169. **P614*** (C): Norway. Oppland, Dovre; 2012, LH; B193273; MT006294, MT005132, MT005170. **P615** (A): Norway. Nordland, Saltdal; 2013, LH; B197540; MT006295, MT005133, MT005171. **P618** (A): Switzerland. Ct. Vaud, Tévenon; 2017, LH; B262946; MT006296, MT005134, MT005172. **P619** (A): Switzerland. Kt. Bern, Sonvilier; 2017, LH; B262617; MT006297, MT005135, MT005173. ***Plagiopus alpinus* (Brid.) Hedenäs:** **P591*** (D): Härjedalen, Storsjö; 2007, LH; B122919; MT006298, MT005136, MT005174. **P605*** (D): Lule Lappmark, Jokkmokk; 1998, T.-B.Engelmark; B63796; MT006299, NA, MT005175. **P607*** (D): Torne Lappmark, Jukkasjärvi; 1994, LH; B53420; MT006300, MT005137, MT005176. **P608*** (D): Torne Lappmark, Jukkasjärvi; 2017, LH; B256561; MT006301, MT005138, MT005177. **P609*** (D): Torne Lappmark, Jukkasjärvi; 2017, LH; B256689; MT006302, MT005139, MT005178. **P610*** (D): Torne Lappmark, Jukkasjärvi; 2017, LH; B256538; MT006303, MT005140, MT005179. **P611*** (D): Torne Lappmark, Jukkasjärvi; 2017, LH; B254948; MT006304, NA, MT005180. **P613*** (D): Norway. Oppland, Lom; 1972, N.Hakelien; B281131; MT006305, NA, MT005181. **P616*** (D): Norway. Troms, Bardu; 2008, LH; B138428; MT006306, NA, MT005182.

study were available. However, to align these reliably with those of *Plagiopus* turned out to be impossible since they differed too strongly. As a reference for molecular identity outside Scandinavia, two specimens from montane habitats in Switzerland were included to compare with the Scandinavian material.

Molecular methods

Total DNA was extracted using the NucleoMag Plant kit for DNA isolation from plant tissue (Macherey-Nagel) with the KingFisher Duo magnetic particle processor. Double stranded DNA templates were prepared by polymerase chain reaction (PCR). PCR was performed using Illustra™ Hot Start Mix RTG (GE Healthcare) in a 25 µl reaction volume according to the manufacturer's instructions.

In all cases, the specified PCR programs were initiated by a denaturation step of 5 min at 95°C and followed by a final extension period of 10 min at 72°C. The PCR programs were, for the nuclear internal transcribed spacers 1 and 2 (ITS), 4 cycles of 30 s at 95°C, 40 s at 55°C, and 1 min at 72°C, 4 cycles of 30 s at 95°C, 30 s at 53°C, and 1 min at 72°C, 35 cycles of 30 s at 95°C, 30 s at 50°C, and 1 min at 72°C, with the primers 'ITS4bryo' (Stech 1999) and 'ITSbryoR' (Hedenäs 2014). For the plastid *atpB-rbcL* spacer (*atpB-rbcL*), 4 cycles of 30 s at 95°C, 40 s at 57°C, and 1 min at 72°C, 4 cycles of 30 s at 95°C, 30 s at 55°C, and 1 min at 72°C, 35 cycles of 30 s at 95°C, 30 s at 52°C, and 1 min at 72°C, with the primers 'ATPB-1' and 'RBCL-1' (Chiang et al. 1998), and for the plastid *rpl16* G2 intron

(*rpl16*), 43 cycles of 30 s at 95°C, 30 s at 58°C, and 1 min 15 s at 72°C, with the primers 'F71' (Jordan et al. 1996) and 'rpl16-antR2' (Hedenäs 2008).

The amplified PCR products were purified from excess primers and nucleotides using ExoSap-IT (Applied Biosystems). For all samples, 5 µl ExoSap-IT were added to 20 µl PCR product and incubated at 37°C for 30 min followed by an enzyme inactivation step at 80°C for 15 min. The purified PCR products, together with the same primers used for PCR amplification, were subsequently sent to MacroGen Europe B.V for single-stranded sequencing on an Applied Biosystems 3730XL sequencer.

Sequence editing and analysis

Nucleotide sequence fragments were edited and assembled for each DNA region using PhyDE 0.9971 (<www.phyde.de/index.html>; accessed 14 November 2019). The assembled sequences were aligned manually in PhyDE. Regions of partially incomplete data in the beginning and end of the sequences were identified and were excluded from subsequent analyses. Gaps were coded using the simple indel coding of Simmons and Ochoterena (2000) in SeqState (Müller 2005). Gaps provided additional information and this was included in the analyses. The sequence alignments used in the analyses are available on request. GenBank accession numbers are listed in Table 1.

ITS paralogues are occasionally encountered in bryophytes (for examples see Košnar et al. 2012, Hedenäs et al. 2019). The ITS chromatograms included in this study did

not show ‘messy’ patterns or noise that could suggest paralogy, and the 5.8S gene was invariable among all samples (cf. Shaw et al. 2002, Feliner and Rosselló 2007). Therefore, the revealed ITS variation was interpreted as being among homologous haplotypes.

Reticulation was revealed using TCS (Clement et al. 2000), and relationships among specimens were therefore evaluated in a network context. The relationships were evaluated in NeighborNet (NN) split networks, produced in SplitsTree ver. 4.12.6 (Huson and Bryant 2006) and in TCS networks, and potential support for lineages in a tree context was tested by jackknife analyses (1000 replications) performed with the program TNT (Goloboff et al. 2003). Because visual inspection of jackknife results and NN split networks revealed no conflicts between well-supported structures in the nuclear and plastid NN split networks, the data sets were combined. The final analyses were based on those specimens having complete data for all three markers, with the approximate relationships of other specimens estimated from either ITS or plastid information. When only one of the plastid markers, *rp16*, amplified, this was used for comparing sequence lengths, but for such specimens plastid data was not included for the network construction.

Morphological study and analysis of measurements

After the molecular relationships among the studied *P. oederianus* s.l. specimens had been clarified, the morphology of ten selected specimens from one of the lineages/grades (from here onwards informally called ‘groups’), with >10 specimens, and all specimens from the other groups were studied in detail. Since the authors cited in the introduction had failed to distinguish well-circumscribed groups within *P. oederianus* s.l., both standard comparisons of qualitative and quantitative characters and detailed measurements of selected gametophyte and sporophyte features were performed, employing dissecting and compound microscopes.

Specimens for which selected gametophyte and sporophyte features were measured in detail are indicated with an asterisk (*) in Table 1. For each of these specimens, a first set of measurements were taken from each of three vegetative leaves, sampled from two stems (2 leaves from one stem and 1 from the other, to avoid sampling all leaves from an untypical shoot for the specimen). (a) Length and maximal leaf width (mm), (b) costa width near base (μm) and (c) length (μm), width (μm) and length to width ratio of 20 cells in the upper 25%, middle 25% and basal portions of the lamina. In a second set, (a) shoot length (mm) and (b) range in seta length (mm) in the specimen, (c) length and width of five (three in P581) dehiscent, dry capsules (mm), (d) length (μm), width (μm) and length to width ratio of 20 exothelial cells on the dorsal side of one arbitrarily selected capsule, and, when available, (e) diameters of 25 spores (μm) were measured. An Olympus SC50 digital camera and the Olympus cellSens Standard 1.13 software for automatic and continuous image stacking were used to produce temporary images of leaves and cells. Measurements were taken from these leaf and cell images, using the Olympus cellSens Standard 1.13 software.

Comparisons of the detailed measurements among the groups within *P. oederianus* s.l. are based on two approaches.

First, measurements were compared between the molecularly identified groups. Potential influence of leaf size on lamina cell size was evaluated by adjusting cell sizes to a standard leaf length of 2.5 mm and a width of 0.5 mm, by dividing the actual leaf lengths or widths with these values and multiplying the resulting values with the cell lengths and widths, respectively. The mean values of the 20 leaf cells or exothelial cells measured at the respective positions were used in the statistical comparisons. Shapiro Wilks W-test (normality) was statistically significant for most measurements, indicating that the data do not meet the criterion of normality. Thus, the nonparametric Kruskal–Wallis ANOVA by ranks for multiple comparisons was used to compare the measurements among or between the groups, respectively. Second, the leaf and leaf cell measurements on the one hand (in total 105 measured leaves), and overall shoot size plus sporophyte measurements on the other (in total 35 specimen measurements) were subjected to separate principal component analyses (PCA) to see whether the combined information within each data set corresponds with the molecularly identified groups. For the leaves, leaf length and width, costa width near base and the mean lamina cell length, width and cell length to width ratio in the upper, middle and basal leaf, in total twelve parameters, were included. For the second PCA, shoot length, median seta length, mean capsule length and width, and the mean exothelial cell length, width and cell length to width ratio, in total seven parameters, were included. Since spore size was not available for all specimens, this was excluded from the second PCA. All statistical calculations were made in STATISTICA 12 (StatSoft 2013). Bonferroni corrections were applied in cases of multiple statistical comparisons.

Results

Molecular relationships

The total number of aligned ITS sites in the 40 studied *Plagiopus* specimens for which ITS sequences could be generated, after deletion of regions at the beginnings and ends that were incomplete for some specimens, was 751. Of these, 57 sites were variable, with 45 of the variable ones parsimony-informative; 12 indels were present, with 12 informative. For the 35 specimens for which *atpB-rbcL* sequences could be generated, the length was 623, 5 sites were variable, and all were parsimony-informative; 4 indels with 2 informative. For the 35 specimens which *rp16* sequences could be generated, the length was 672, 15 sites were variable, and 10 of these were parsimony-informative; 4 indels with 3 informative.

The structure of the NN split network received high jackknife support (95–100) for the recognition of five groups (lineages/grades) within *Plagiopus oederianus* (Fig. 1A). The support for the different groups comes either from ITS (groups C, E and a portion of D) or from the plastid markers (group D, and the distinction of A and B). Sequence differences that unambiguously differentiate the five *P. oederianus* s.l. groups are indicated in Appendix 1. From the TCS network, it is evident that each molecular group differs from the most similar other group by at least nine mutational changes

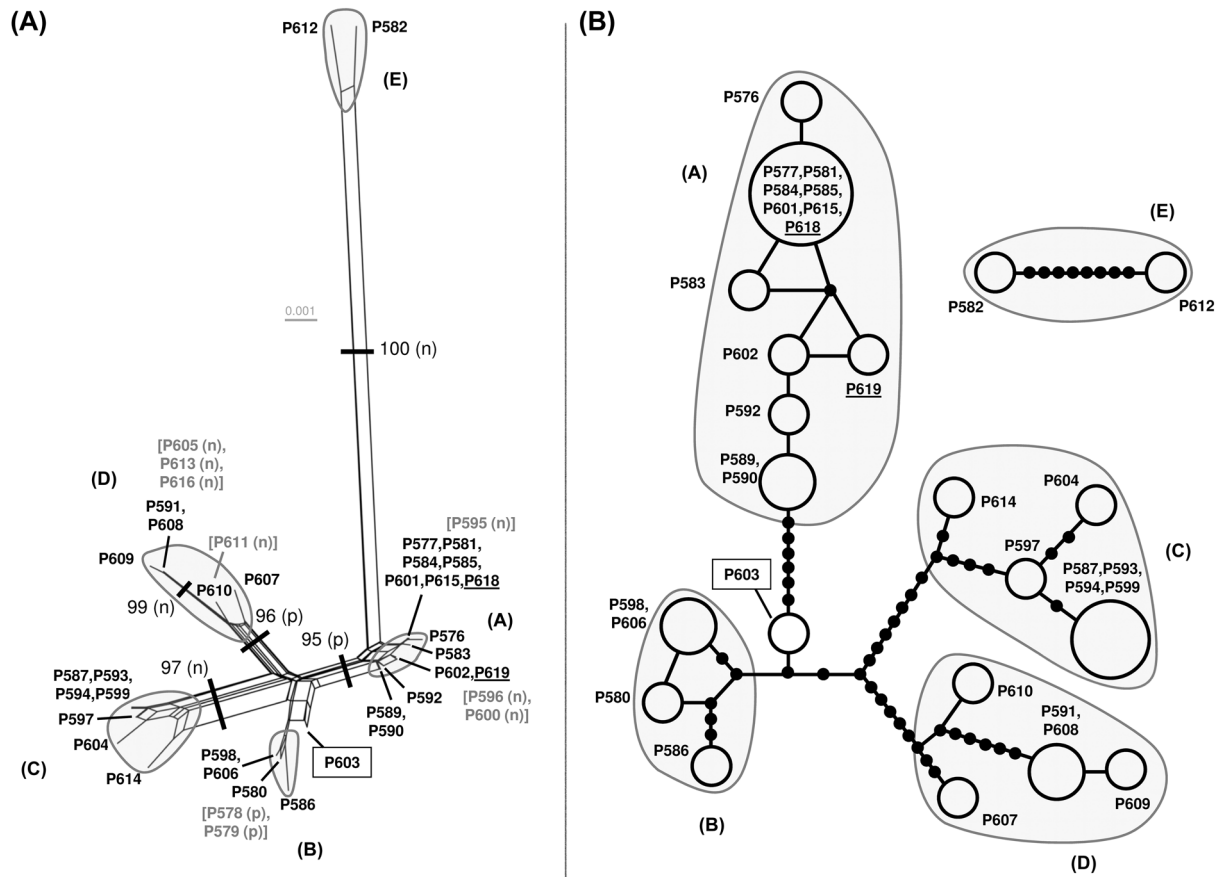


Figure 1. NeighborNet split network (A) and TCS haplotype network (B) for 29 specimens of *Plagiopus oederianus* s.l. from Scandinavia plus two reference specimens from Switzerland (P618, P619; underlined) based on the nuclear ITS and the plastid *atpB-rbcL* and *rpl16* regions, for specimens that had complete information for all three. Thick black lines and support values indicate Jackknife support of at least 95. Specimen P 603, in a frame, has ITS of group B and plastid markers of group A. In Fig. A, specimen numbers in brackets and grey indicate the approximate positions of nine specimens with incomplete information. The 'n' and 'p' following in parentheses after jackknife values and specimen numbers, for the nine specimens with incomplete information, indicate whether the support comes mainly from the nuclear (n) or plastid markers (p), respectively.

(Fig. 1B). Specimen P603, which appears between groups A and B has ITS of A and plastid markers from B. The two montane Swiss specimens belong to group A. The *atpB-rbcL* sequences are longer and the *rpl16* ones shorter for A and E than for the other groups (Table 2).

Morphological evaluation

The PCAs based on the detailed measurements of 1) selected leaf features and 2) other, mainly sporophyte, features of *P. oederianus* s.l. suggest that group D samples, collected in the mountain range, are possible to distinguish in most cases (Fig. 2A–B, 3A–B). In the PCA for leaf features (Fig. 2B),

Table 2. Sequence lengths for the five groups (lineages/grades) recognized by the molecular data (Fig. 1). Number of samples are given in parentheses after the sequence lengths.

Group	ITS	<i>atpB-rbcL</i>	<i>rpl16</i>
A	744–748 (17)	622–623 (14)	662 (17)
B	743–748 (6)	612 (7)	665 (7)
C	742–743 (6)	612–616 (7)	669 (7)
D	742–747 (9)	612 (5)	668–671 (9)
E	746 (2)	622 (2)	662 (2)

a partial overlap with the other groups along the first and second axes is present, whereas for the other features (Fig. 3B) the situation appears clearer along the first axis. For the leaves, upper cell width and to some degree mid-leaf cell length, width and length to width ratio correlate with the second axis, whereas the other characters correlate mainly or almost entirely with the first axis (Fig. 2C). Among the characters in the second PCA, shoot length and exothecial cell length to width ratio correlate to some degree with axis 2, and the other characters correlate mainly or almost entirely with the first axis (Fig. 3C).

Group D samples differ from several of the other groups in some of the measured features (Fig. 4). However, D specimens do not differ significantly from all the other groups in these characters and in some cases, the states overlap significantly. The characters where the differences between group D and the other groups are most distinct are listed in Table 3.

Habitat and geographical distribution

All Scandinavian *Plagiopus* species grow on base-rich to calcareous substrates. Groups A–C and E specimens were almost exclusively collected on rocks or in rock-crevices and

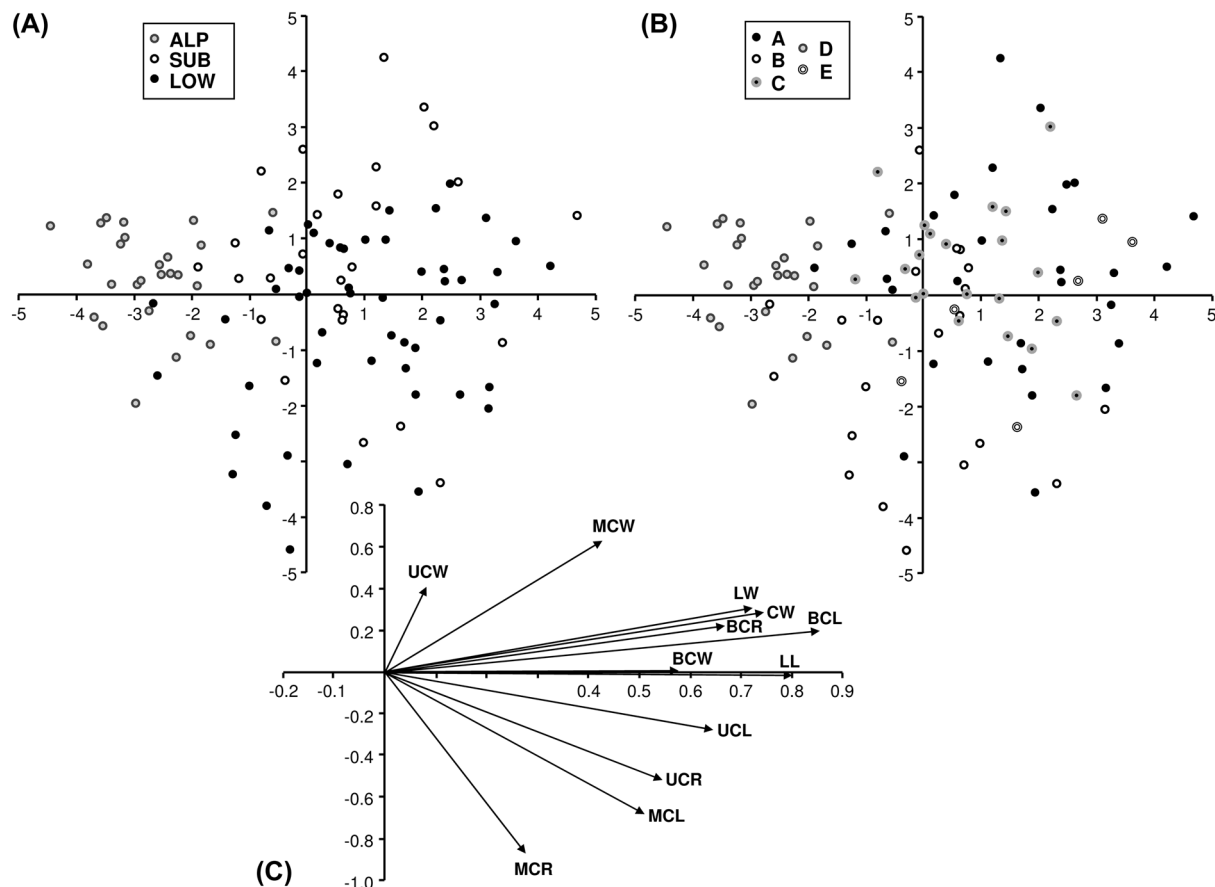


Figure 2. The positions of three leaves from each of ten specimens of group A, seven of group B, seven of C, nine of D and two of group E (cf., Fig. 1), along the first two axes in a PCA. Factors 1 and 2 explain 37.24% and 20.19% of the variation. This PCA is based on each leaf's length (LL), width (LW), costa width at base (CW), upper leaf lamina cell length, width and length/width ratio (UCL, UCW, UCR), mid-leaf lamina cell length, width and length/width ratio (MCL, MCW, MCR) and basal leaf lamina cell length, width and length/width ratio (BCL, BCW, BCR). Cell sizes and length/width ratios are the mean values of 20 measured cells in each leaf. (A) Leaves classified according to their specimens' origin in alpine (ALP), subalpine (SUB) or lowland (LOW) environments. (B) Leaves classified according to which of the lineages/grades A–E its specimen belongs to (Fig. 1). (C) Explanatory factors in the plane of factors 1 and 2.

frequently in escarpments and only rarely on soil (soil or ground was mentioned on labels in 1 out of 33 cases). Group D specimens, on the other hand, were collected on soil in five out of eight cases.

Most of the identified groups do not show particular geographic distribution patterns in the Scandinavian peninsula (Fig. 5). Groups A–C were recorded from the lowlands to the low-alpine region of the mountains, whereas the two group E samples were from lowlands. Group D, on the other hand, is clearly restricted to the low- and middle-alpine regions of the Scandinavian mountain range.

Discussion

Plagiopus oederianus s.l. includes five molecular groups, which may be lineages or grades. The latter can only be evaluated when suitable outgroups are found. The groups differ from each other by nine or more mutational changes in the three studied markers and their distinction receives high (95–100) jackknife support (Fig. 1). In addition, the sequence lengths for the plastid markers differ between groups A and E on the one hand, and the other groups on the other. The

molecular differences are comparable to those between well-known and morphologically distinguishable species (Draper and Hedenäs 2009, Cezón et al. 2010, Hedenäs 2011, 2017a), and suggest that the five *P. oederianus* groups A–E correspond with species. In terms of mutational changes, the differences are much larger than between the two well-established cryptic species of *Hamatocaulis vernicosus* (Mitt.) Hedenäs (Hedenäs 2018a, Manukjanová et al. 2019). The analysis of the morphological variation shows that four of the *P. oederianus* s.l. species are cryptic, whereas specimens of species D differ from the other ones in quantitative morphological features. The morphologically different species D is molecularly as different from the four cryptic species as these are from each other, showing that morphological evolution is not correlated with molecular evolution in this complex. Similar situations are found in *Oncophorus* (Hedenäs 2018b) and the *Racomitrium lanuginosum* (Hedw.) Brid. complex (Hedenäs 2020a), where cryptic species are as well differentiated molecularly as are morphologically distinct species.

A formal naming of truly cryptic moss species, and the so far unnamed semi-cryptic species within *Neckera pen-nata* Hedw., would immediately add eight species names only in Scandinavia (this paper, Appelgren and Cronberg

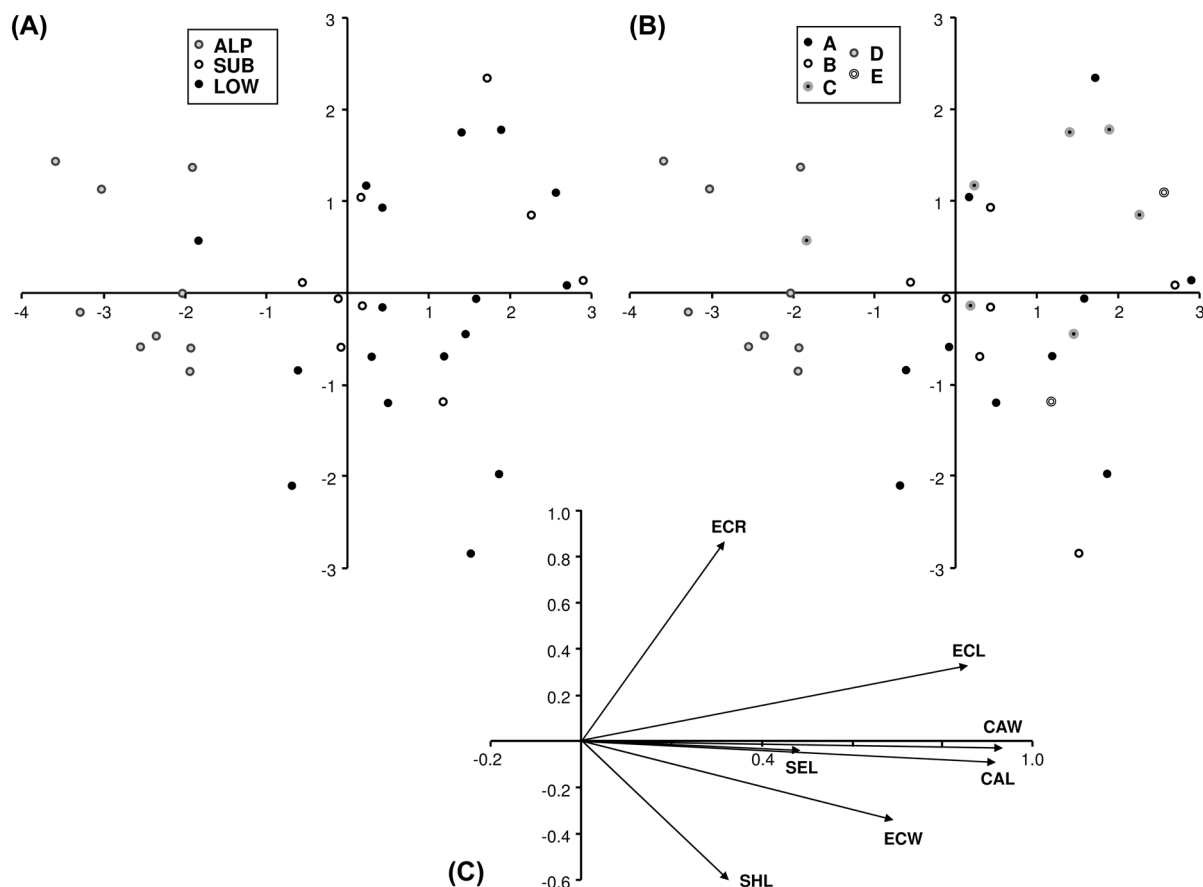


Figure 3. The positions of ten specimens of group A, seven of group B, seven of C, nine of D and two of group E (cf., Fig. 1), along the first two axes in a PCA. Factors 1 and 2 explain 48.08% and 19.19% of the variation. This PCA is based on the shoot length (SHL), seta length (SEL), capsule length (CAL) and width (CAW) and exothelial cell length, width and length/width ratio (ECL, ECW, ECR). Median values are used for seta length, and capsule sizes based on (3)5 measured sporophytes/per specimen, and exothelial cell sizes and length/width ratios are the mean values of 20 measured cells in an arbitrarily selected urn. (A) Specimens classified according to their origin in alpine (ALP), subalpine (SUB) or lowland (LOW) environments. (B) Specimens classified according to which of the lineages/grades A–E they belong (Fig. 1). (C) Explanatory factors in the plane of factors 1 and 2.

1999, Hedenäs 2018a, b, 2020a, Manukjanová et al. 2019). This corresponds with c. 0.9% of the currently recognized Scandinavian moss species, but this figure is based on the approximately 5–10% of the mosses of the area that have been sufficiently investigated molecularly to reveal potential cryptic species. Since it is impossible to predict which morpho-species include cryptic ones (Hedenäs 2019), a very conservative guess on the true number of cryptic moss species therefore suggests that in Scandinavia this is in the range of 80–100. When cryptic bryophyte species are likely frequent, an important issue is if and how these should be recognized or even formally named based on molecular evidence only. Whether cryptic species are acknowledged or not has important implications on how we understand the magnitude of our total species diversity as well as on how this should be conserved (Crespo and Lumbsch 2010, Gwi-azdowski et al. 2011, DeliĆ et al. 2017, Scott et al. 2018, Cerca et al. 2020). Cryptic species are necessarily rarer than the morpho-species to which they are referred, but some are still relatively widespread and frequent whereas other ones display narrow geographical distributions and/or are much less common. Further, not recognizing cryptic species will mislead research on the biology and ecology of species, when

incorrectly circumscribed biological species entities are investigated. Some authors have advocated that truly cryptic species should not be recognized by formal names (Wagner and Wagner Jr. 1989), and this is very much the current practice among bryologists. In an earlier paper (Hedenäs 2020a), some of the problems with formally recognizing cryptic species, including how to know which of the cryptic species is represented by old type material, were discussed. However, like researchers in other fields (Crespo and Lumbsch 2010, Pérez-Ponce de León and Nadler 2010), bryologists need to reach a consensus regarding their recognition, formal or not, or we shall neither succeed in conserving as much as possible of bryophyte species diversity or to fully understand how the biologically relevant species entities evolve and function. Leaving out an estimated 10% of the bryophyte species stock in studies or conservation of biodiversity in Scandinavia should not be an option.

Specimen P603, from Pite Lappmark in Sweden, has ITS from cryptic species A and plastid markers from cryptic species B. Comparable circumstances, with nuclear and plastid markers from different closely related species, are frequent among mosses (Hedenäs 2015a, 2017b, Hedenäs et al. 2019), and could have different explanations that are dif-

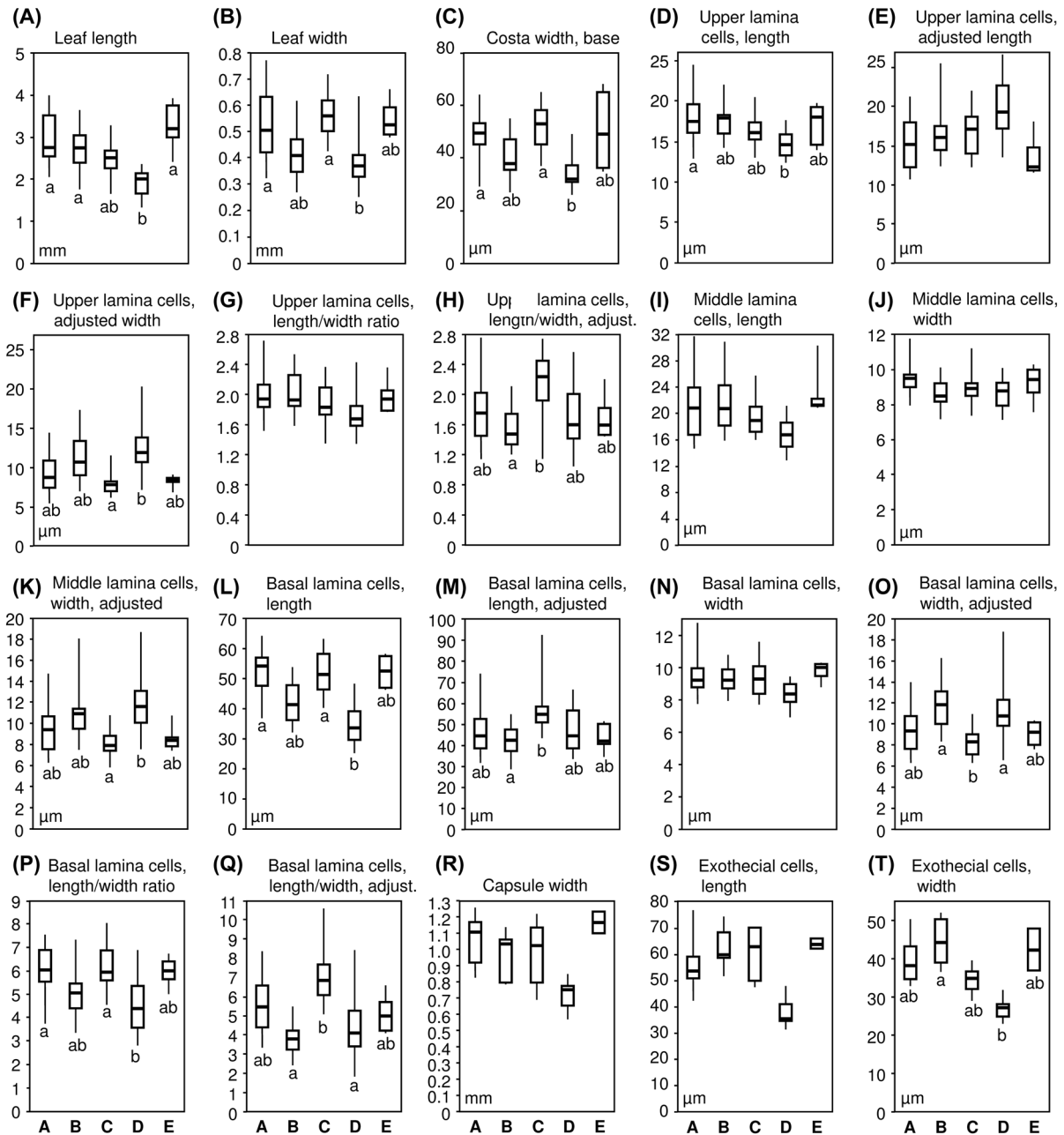


Figure 4. Boxplots with median values, quartiles and whiskers from maximum to minimum values, for measured characters in *Plagiopus oederianus* s.l. groups A–E (cf. Fig. 1). Only characters where overall significant differences were found among the groups are included. When significant differences were in addition found in pairwise comparisons (Kruskal–Wallis ANOVA by ranks) these are indicated by different letters under the lower whiskers. The compared measurements are the same as those used for the PCA's in Fig. 2, 3, respectively. For characters A–Q, n was for A: 30, for B and C: 21; for D: 27, and for E: 6. For characters R–T, n was for A: 10, for B and C: 7; for D: 9, and for E: 2.

difficult to distinguish without further evidence (Wendel and Doyle 1998). It could be a result of a number of processes, such as hybridisation, incomplete lineage sorting or horizontal gene or plastid transfer (Wendel and Doyle 1998, Harris 2008, Stegemann et al. 2012, Twyford and Ennos 2012, Gao et al. 2014).

The morphologically differentiated species D appears to be most distinct in its sporophyte features, where the PCA suggests lack of overlap towards the other cryptic species. However, for these features, the number of measurements

from molecularly identified specimens is relatively small. To confirm this sharp PCA limit between species D and the other ones requires a larger sample than the present one. Common garden experiments or finds of mixed occurrences could potentially cast additional light on this issue.

This study adds to several earlier ones (Hedenäs 2015b, 2019, 2020a, b), which show that bryophyte diversity, at the morpho-species, cryptic species and intraspecific levels in northern and mountain areas of Scandinavia are underestimated and deserve further study.

Table 3. Characters that differentiate *Plagiopus oederianus* from *P. alpinus* (group D), based on molecularly identified specimens. The 5–95% ranges are provided for lamina and exothelial cell sizes, based on the originally measured 1560/520 and 540/180 lamina/exothelial cells for *P. oederianus* and *P. alpinus*, respectively. Values in parentheses indicate extreme values.

Character	<i>P. oederianus</i>	<i>P. alpinus</i>
Shoot height (mm)	25–95	15–50
Leaf length (mm)	1.7–4.0	1.3–2.4
Length of mid-leaf lamina cells (µm)	(9.5)12.0–33.0(52.0)	(8.0)11.0–25.0(30.5)
Length of basal leaf lamina cells (µm)	(20.5)30.0–82.5(132.0)	(12.5)21.5–56.5(78.5)
Dry spore capsule length (mm)	1.1–2.1	0.7–1.4
Dry spore capsule width (mm)	0.6–1.4	0.5–0.9
Exothelial cells, dorsal capsule, length (µm)	(26.5)38.5–87.0(108.0)	(21.0)25.5–51.5(60.0)
Exothelial cells, dorsal capsule, width (µm)	(19.5)25.5–58.0(76.0)	(14.5)18.5–36.5(45.5)

Taxonomy

Despite the found overlap in morphological features between species D and the cryptic species within *Plagiopus oederianus* s.l., most leaves from species D and the type material of *Bartramia oederi* var. *alpina* Schwaegr. can unambiguously be placed in this species (Table 3). When spore capsules are present, the recognition of species D is relatively easy and, fortunately, spore capsules are common in *Plagiopus* in Scandinavia. Therefore, species D is here formally recognized, as *Plagiopus alpinus* (Schwaegr.) Hedenäs.

1. *Plagiopus oederianus* (Sw.) H.A. Crum & L.E. Anderson Fig. 6A–F

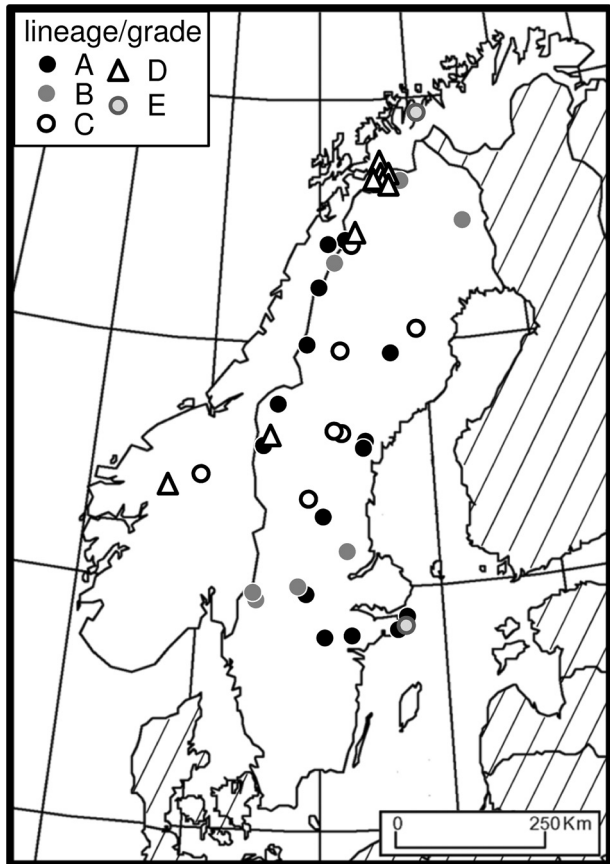


Figure 5. The Scandinavian distributions of specimens belonging to groups A–E (Fig. 1). The two included Swiss specimens belong to group A.

Mosses of eastern North America 1: 636. 1981. *Bartramia oederiana* Sw., J. Bot. (Schrader) 1800(2): 180. 1800 [1801] – Type locality (Swartz 1800): ‘Germanica’ and ‘Suecica’ (n.v.).

Shoots 25–95 mm tall, green. Stem in transverse section triangular, with hyalodermis, central strand narrow or absent; rhizoids brown, much branched and forming tomentum, densely papillose with tall papillae; axillary hairs consisting of 1–3 upper long and narrow, hyaline cells and 1 basal rectangular, elongate-rectangular or shortly linear, pale brownish cell. Leaves 1.7–4.0 × 0.3–0.8 mm, when moist strongly recurved or slightly recurved-spreading and often somewhat screwed, keeled above; margin broadly recurved except in upper dentate portion, in upper 15–20% margin coarsely and irregularly dentate, at least partly with geminate teeth, partly bistratose below apical region; costa single, percurrent or excurrent, 27–68 µm wide near base, back of upper costa with strongly prorate distal cell ends. Leaf lamina cells incrassate, eporose or basal cells sometimes slightly porose, weakly or strongly longitudinally papillose-striate (often most distinct in mid-leaf), unistratose or rarely partly bistratose above; cells in upper 25% of leaf 7.5–37.0 × 5.0–14.5 µm, 0.7–5.0 times as long as wide, sometimes with distally prorate cell ends; cells in middle 25% of leaf 9.5–52.0 × 5.0–14.0 µm, 0.9–8.4 times as long as wide; basal cells 20.5–132.0 × 5.0–17.0 µm, 1.5–16.0 times as long as wide, cells near insertion often brownish; alar cells more or less inflated, narrowly but longly decurrent. Synoicous. Calyptra cucullate, smooth, naked. Seta reddish, 5–15 mm; capsule subglobose, 1.1–2.1 mm long, 0.6–1.4 mm wide, furrowed when dry; operculum lowly conical-domed. Exothelial cells on dorsal side of capsule 26.5–108.0 × 19.5–76.0 µm, 0.6–3.6 times as long as wide; stomata long-pored, numerous near base of capsule. Exostome 184–395 µm long, red, in recently deoperculate capsules with finely reticulate ornamentation on outside; endostome shorter than exostome, yellowish, with well-developed basal membrane, reduced segments and cilia rudimentary or absent. Spores 14.5–32.0 µm, mostly slightly elongate, strongly warty-papillose.

Plagiopus oederianus includes four cryptic species, with geographic distributions that overlap to a high degree (Fig. 5). Cryptic species C may have a more northern distribution than A and B, but the present sampling is not dense enough for a firm conclusion regarding this. The four cryptic species are molecularly as distinct as *P. alpinus*, which in addition differs from the four *P. oederianus* cryptic species in morphology (Table 3) and in having a more restricted geographi-

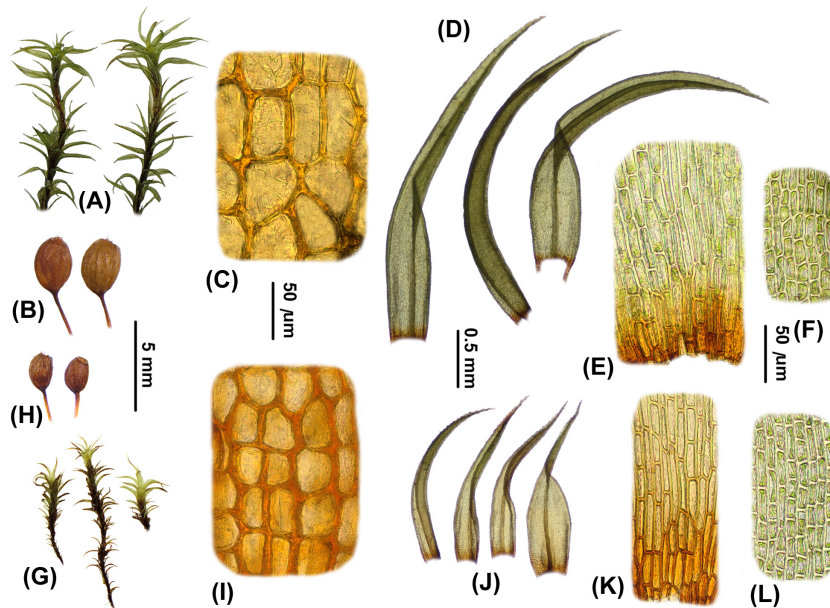


Figure 6. Illustrations of salient features of *Plagiopus oederianus* (A–F; P615: Norway. Nordland, Saltdal, 2013, *L. Hedenäs*, S, reg. no. B197540) and *P. alpinus* (G–L; P608: Sweden. Torne Lappmark, Jukkasjärvi, 2017, *L. Hedenäs*, S, reg. no. B256561), from specimens included in the molecular analysis. (A), (G) Habit. (B), (H) Spore capsules. (C), (I) Exothelial cells between ridges, from dorsal side of spore capsule. (D), (J) Stem leaves. (E), (K) Lamina cells from base of stem leaf. (F), (L) Lamina cells from middle of stem leaf.

cal distribution. *Plagiopus oederianus* grows on base-rich to calcareous rocks or in rock crevices, only rarely on soil.

2. *Plagiopus alpinus* (Schwaegr.) Hedenäs, comb. et stat. nov. Fig. 6G–L

Basionym: *Bartramia oederi* var. *alpina* Schwaegr., Sp. Musc. Frond., Suppl. 1, 2: 50. pl. 59. 1816 \equiv *Plagiopus oederi* var. *alpinus* (Schwaegr.) Dalla Torre & Sarnth., Fl. Tirol. 5: 405. 1902 \equiv *Plagiopus oederianus* var. *alpinus* (Schwaegr.) Ochrya, J. Hattori Bot. Lab. 64: 341. 1988 – Type: [Switzerland] Plant ‘d, var. *alpina* Sw. a Schleich’, on a sheet with *Bartramia oederi* in herbarium Hedwig-Schwaegrichen [G-00048944! Lectotype, designated here; isotype in G-00048947!].

Plagiopus oederi var. *condensata* Brid. ex Limpr., Laubm. Deutschl. 2: 550. 1895 (*Bartramia oederi* var. *condensata* Brid., Muscol. Recent. Suppl. 3: 87. 1817, nom. illeg, included *B. oederi* var. *alpina* Schwaegr., 1816) – Type locality (Bridel 1817): ‘In Alpihus Helveticus’ (n.v.).

? *Bartramia oederi* var. *microcarpa* Kindb., Skr. Vidensk.-Selsk. Christiania, Math.-Naturvidensk. Kl. 1888(6): 28. 1888, nom. nud. – Original material (Kindberg 1888): [Norway] Knudshø (n.v.).

Shoots 15–50(80) mm tall, green. Stem in transverse section triangular, with hyalodermis, central strand narrow or absent; rhizoids brown, much branched and forming tomentum, densely papillose with tall papillae; axillary hairs consisting of 1–2 upper long and narrow, hyaline cells and 1 basal rectangular or elongate-rectangular, pale brownish cell. Leaves 1.3–2.4(2.6) \times 0.3–0.6 mm, when moist recurved or strongly so, and often somewhat screwed, keeled above; margin broadly recurved except in upper dentate portion, in upper 15–20% margin coarsely and irregularly dentate, at least partly with geminate teeth, partly bistratose below apical region; costa single, percurrent or excurrent, 26–49 μ m wide near base, back of upper costa with strongly prorate

distal cell ends. Leaf lamina cells incrassate, eporose, weakly or strongly longitudinally papillose-striate (most distinct in mid-leaf), unistratose; cells in upper 25% of leaf 8.0–32.0 \times 5.5–13.0 μ m, 0.8–4.9 times as long as wide, often with distally prorate cell ends; cells in middle 25% of leaf (7.0)8.0–30.5 \times 5.0–14.0 μ m, 0.8–4.9 times as long as wide; basal cells 12.5–78.5 \times 4.5–15.5 μ m, 1.4–12.7 times as long as wide, cells near insertion often brownish; alar cells slightly inflated, narrowly decurrent. Synoicous. Calyptra cucullate, smooth, naked. Seta reddish, 4–12 mm; capsule subglobose, 0.7–1.4 mm long, 0.5–0.9 mm wide, furrowed when dry; operculum lowly conical-domed. Exothelial cells on dorsal side of capsule 21.0–60.0 \times (11.0)14.5–45.5 μ m, 0.6–2.6 times as long as wide; stomata long-pored, numerous near base of capsule. Exostome 164–251(301) μ m long, red, in recently deoperculate capsules with finely reticulate ornamentation on outside; endostome shorter than exostome, yellowish, with well-developed basal membrane, reduced segments and cilia rudimentary or absent. Spores 17.5–28.0 μ m, rounded or slightly elongate, strongly warty-papillose.

Plagiopus alpinus specimens can usually be distinguished from the four cryptic species of *P. oederianus* without problems, especially when sporophytes are present (Table 3). The sporophytes are distinctly smaller in *P. alpinus* than in the cryptic species of *P. oederianus*, with smaller exothelial cells, the plants usually grow in smaller and more compact tufts, and the leaves and leaf cells are smaller.

In Scandinavia, *P. alpinus* occurs only in the mountain range. Outside Scandinavia, *P. alpinus* is known to occur in the European Alps, but even if it is not reported or recognized for northern Asia (Ignatov et al. 2006) or North America (Griffin III 2014), it seems likely that it occurs in mountains of the northern temperate region, and further north also at lower elevations. *Plagiopus alpinus* grows on base-rich to calcareous substrates, both in rock crevices and on soil.

Note

In herbarium Hedwig-Schwaegrichen in G there are two syntypes that are likely portions of the original collection of *Bartramia oederi* var. *alpina* Schwaegr. One is specimen 'd' on the sheet with number G00048944, with the annotation 'd, var. alpina Sw. a Schleich.' and the other one is specimen G00048947, under the name *Bartramia oederi* var. *condensata* Brid. An added label on the latter has 'Ad rupes in planitie Vallesiae' (on rocks of the plains of Valais) printed and 'Bartr. Oederiana v. alpina Sw. Schl 812' written by Hedwig (according to a note by G. Colomb-Duplan, who also states 'Schleicher leg.'). Both these specimens fit well with the description of var. *alpina* in the protologue (Schwaegrichen 1816), 'uncia parum altior et magis stricta et compacta' [(an) inch deep and a little more tight and compact (than *Bartramia oederi*)]. A third specimen, G00048945 from herb. Schleicher, with 'Bartramia Oederi [...] alpina Schw.' written on the label, belongs to the same species as the first two specimens. However, this specimen disagrees with the protologue in that the tufts are ca 8 cm, or around three inches deep. Further, whereas the first two specimens have both capsules with their opercula remaining and deoperculate

capsules despite that only 4–5 capsules are present in each, the herb. Schleicher specimen has only deoperculate capsules even if there are well over 100 capsules present. Whereas the third specimen clearly deviates from the protologue, and is unlikely part of the same collection as the first two specimens, there is no significant difference between the first two specimens. G00048944 (Fig. 7) is here designated as lectotype, with G00048947 thus becoming an isolectotype.

Both the type and specimen G00048945 from Schleicher's herbarium fit into the range of measurements for *P. alpinus* based on molecularly identified specimens (Table 3), except that the tuft of G00048945 is ca 8 cm deep and one measured leaf from the lectotype was 2.6 mm long. The leaf lengths (three leaves from two stems in the Schleicher specimen, three leaves from a single stem in the lectotype) vary between 1.6–2.6 mm, the mid-leaf lamina cells (60 from each specimen = 120) vary between 7.0 and 30.5 µm long, and the basal cells (120) between 18.5 and 61.5 µm long. The spore capsules vary between 0.8–1.2 mm long and 0.6–0.8 mm wide (20), and the exothecial cells (20 cells from dorsal side of one capsule in G00048945) are 29.5–49.0 × 11.0–30.0 µm.

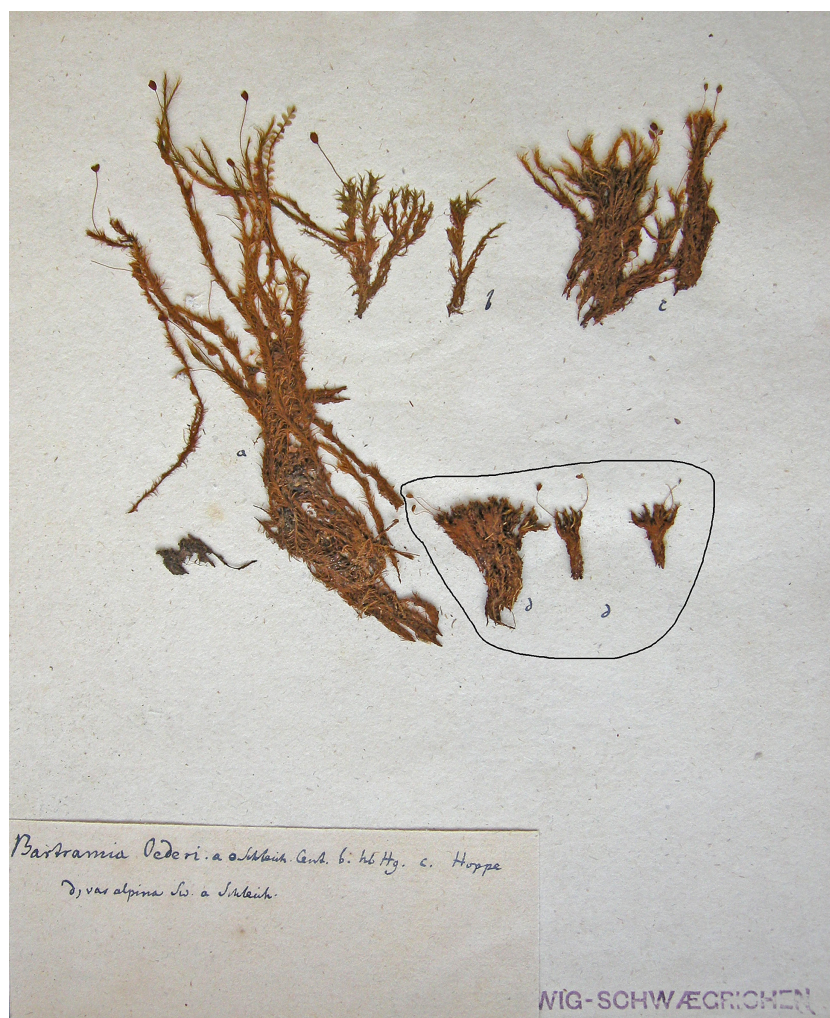


Figure 7. Herbarium sheet no. G00048944 from herbarium Hedwig-Schwaegrichen, with the lectotype of *Bartramia oederi* var. *alpina* Schwaegr. encircled by a thin line (added on the image).

Acknowledgements – I thank Petter Larsson and Rodrigo Esparza-Salas for their efficient laboratory work, and Michelle Price in G for help with locating and sending potential type material of *Bartramia oederi* var. *alpina* on loan. Comments by two reviewers helped improving the text. Internal institutional sources funded the molecular portion of the study. All studied specimens were collected in Sweden or Switzerland, where genetic resources are free for non-commercial research, or were collected before 12 October 2014 (i.e. before the Nagoya protocol or the EU ABS regulation 511/2014 were applicable).

References

- Appelgren, L. and Cronberg, N. 1999. Genetic and morphological variation in the rare epiphytic moss *Neckera pennata* Hedw. – *J. Bryol.* 21: 97–107.
- Bridel, S. E. 1817. *Muscologia recentiorum supplementum* 3. – Officina Libraria Ettingeriana, Gotha.
- Cerca, J., Meyer, C., Purschke, G. et al. 2020. Delimitation of cryptic species drastically reduces the geographical ranges of marine interstitial ghost-worms (*Strygocapitella*; Annelida, Sedentaria). – *Mol. Phylog. Evol.* 143: 106663.
- Cezón, K., Muñoz, J., Hedenäs, L. et al. 2010. *Rhynchostegium confusum*, a new species from the Iberian Peninsula and its relation to *R. confertum* based on morphological and molecular data. – *J. Bryol.* 32: 1–8.
- Chiang, T. I., Schaal, B. A. and Peng, C.-I. 1998. Universal primers for amplification and sequencing a noncoding spacer between the *atpB* and *rbcL* genes of chloroplast DNA. – *Bot. Bull. Acad. Sin.* 39: 245–250.
- Clement, M., Posada, D. and Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. – *Mol. Ecol.* 9: 1657–1659.
- Crespo, A. and Lumbsch, H. T. 2010. Cryptic species in lichen-forming fungi. – *IMA Fungus* 1: 167–170.
- Damayanti, L., Muñoz, J., Wicke, S. et al. 2012. Common but new: *Bartramia rosamrosiae*, a ‘new’ widespread species of apple mosses (Bartramiaceae, Bryophyta) from the Mediterranean and western North America. – *Phytotaxa* 73: 37–59.
- Delić, T., Trontelj, P., Rendoš, M. et al. 2017. The importance of naming cryptic species and the conservation of endemic subterranean amphipods. – *Sci. Rep.* 7: 1–12.
- Draper, I. and Hedenäs, L. 2009. Circumscription of European taxa within the *Sciuro-hypnum reflexum* complex (Brachytheciaceae, Bryophyta), based on molecular and morphological data. – *Taxon* 58: 572–584.
- Feliner, G. N. and Rosselló, J. A. 2007. Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. – *Mol. Phylog. Evol.* 44: 911–919.
- Frisvoll, A. A. and Elvebakk, A. 1996. A catalogue of Svalbard plants, fungi, algae and cyanobacteria. Part 2. Bryophytes. – *Norsk Polarinstitutt Skrifter* 198: 57–172.
- Gao, C., Ren, X., Mason, A. S. et al. 2014. Horizontal gene transfer in plants. – *Funct. Integr. Genomics* 14: 23–29.
- Goloboff, P., Farris, J. and Nixon, K. 2003. Tree analysis using new technology. – <www.lillo.org.ar/phylogeny/tnt/>, accessed 3 May 2017.
- Griffin III, D. G. 2014. 40. Bartramiaceae Schwägrichen. – In: *Flora-of-North-America-Editorial-Committee* (ed.), *Flora of North America North of Mexico*. Vol. 28. Bryophyta, part 2, pp. 97–112.
- Gwiazdowski, R. A., Veal, I. M., Andersen, J. C. et al. 2011. Discovery of cryptic species among North American pine-feeding *Chionaspis* scale insects (Hemiptera: Diaspididae). – *Biol. J. Linn. Soc.* 104: 47–62.
- Hagen, I. 1899–1904. Musci norvegiae borealis. – *Tromsø Museums Aarshefter* 2122: 1–112.
- Hallingbäck, T., Hedenäs, L. and Weibull, H. 2006. Ny checklista för Sveriges mossor. – *Sv. Bot. Tidskr.* 100: 96–148.
- Hallingbäck, T., Lönnell, N., Weibull, H. et al. 2008. Nationalnyckeln till Sveriges flora och fauna. Bladmossor: Kompaktmossor-kapmossor. Bryophyta: *Anoetangium–Orthodontium*. – ArtDatabanken, SLU, Uppsala.
- Harris, E. S. J. 2008. Paraphyly and multiple causes of phylogenetic incongruence in the moss genus *Plagiomnium* (Mniaceae). – *Taxon* 57: 417–433.
- Hedenäs, L. 2008. Molecular variation and speciation in *Antitrichia curtipendula* s.l. (Leucodontaceae, Bryophyta). – *Bot. J. Linn. Soc.* 156: 341–354.
- Hedenäs, L. 2011. Incongruence among morphological species circumscriptions and two molecular data sets in *Sarmentypnum* (Bryophyta: Calliergonaceae). – *Taxon* 60: 1596–1606.
- Hedenäs, L. 2014. Intraspecific genetic variation in selected mosses of Scandinavian interglacial refugia suggests contrasting distribution history patterns. – *Bot. J. Linn. Soc.* 176: 295–310.
- Hedenäs, L. 2015a. Molecular and morphological incongruence among the genera around *Sarmentypnum* (Bryophyta: Calliergonaceae). – *Nova Hedwig.* 100: 279–292.
- Hedenäs, L. 2015b. *Rhytidium rugosum* (Bryophyta) colonized Scandinavia from at least two glacial refugial source populations. – *Bot. J. Linn. Soc.* 179: 635–657.
- Hedenäs, L. 2017a. Scandinavian *Oncophorus* (Bryopsida, Oncophoraceae): species, cryptic species and intraspecific variation. – *Eur. J. Taxon.* 315: 1–34.
- Hedenäs, L. 2017b. Three molecular markers suggest different relationships among three *Drepanocladus* species (Bryophyta: Amblystegiaceae). – *Plant Syst. Evol.* 303: 521–529.
- Hedenäs, L. 2018a. Conservation status of the two cryptic species of *Hamatocaulis vernicosus* (Bryophyta) in Sweden. – *J. Bryol.* 40: 307–315.
- Hedenäs, L. 2018b. *Oncophorus demetrii*, a fifth Scandinavian species of *Oncophorus* (Musci) possible to recognize by morphology. – *Lindbergia* 41: linbg.01098.
- Hedenäs, L. 2019. On the frequency of northern and mountain genetic variants of widespread species: essential biodiversity information in a warmer world. – *Bot. J. Linn. Soc.* 191: 440–474.
- Hedenäs, L. 2020a. Cryptic speciation revealed in Scandinavian *Racomitrium lanuginosum* (Hedw.) Brid. (Grimmiaceae). – *J. Bryol.* 42: 117–127.
- Hedenäs, L. 2020b. Disentangling Scandinavian species hidden within *Meesia uliginosa* Hedw. s.l. (Bryophyta, Meesiaceae). – *Lindbergia* 42: linbg.01125.
- Hedenäs, L., Heinrichs, J. and Gallego, M. T. 2019. The Scandinavian *Syntrichia ruralis* complex (Musci, Pottiaceae): a chaos of diversification. – *Plant Syst. Evol.* 305: 639–661.
- Huson, D. H. and Bryant, D. 2006. Application of phylogenetic networks in evolutionary studies. – *Mol. Biol. Evol.* 23: 254–267.
- Ignatov, M. S., Afonina, O. M. and Ignatova, E. A. 2006. Checklist of mosses of East Europe and North Asia. – *Arctoa* 15: 1–130.
- Jensen, C. 1939. Skandinaviens bladmossflora. – Ejnar Munksgaard, København.
- Jordan, W. C., Courtney, M. W. and Neigel, J. E. 1996. Low levels of infraspecific genetic variation at a rapidly evolving chloroplast DNA locus in North American duckweeds (Lemnaceae). – *Am. J. Bot.* 83: 430–439.
- Kindberg, N. C. 1888. Enumeratio Bryinearum Dovrensium. – Christiania Videnskabs-Selskabs Forhandling 1888: 1–30.
- Košnar, J., Herbstová, M., Kolář, F. et al. 2012. A case of intragenomic ITS variation in bryophytes: assessment of gene flow and role of ploidy in the origin of European taxa of the *Tortula muralis* (Musci: Pottiaceae) complex. – *Taxon* 61: 709–720.
- Manukjanová, A., Štechová, T. and Kučera, J. 2019. Expressed sex ratio in populations of the moss *Hamatocaulis vernicosus* (Mitt.)

- Hedenäs (Scorpidiaceae) in the Czech Republic with consideration of its cryptic species. – *Cryptogam. Bryol.* 40: 41–58.
- Müller, K. 2005. SeqState. – *Appl. Bioinf.* 4: 65–69.
- Möller, H. 1925. Lövmossornas utbredning i Sverige. IX. Bartramiaceae. – *Ark. Bot.* 19: 1–147.
- Nyholm, E. 1998. Illustrated flora of Nordic mosses. Fasc. 4. Aulacomniaceae – Meesiaceae – Catosciaceae – Bartramiaceae – Timmiaceae – Encalyptaceae – Grimmiaceae – Ptychomitriaceae – Hedwigiaceae – Orthotrichaceae. – Nordic Bryological Society, Copenhagen and Lund.
- Pérez-Ponce de León, G. and Nadler, S. A. 2010. What we don't recognize can hurt us: a plea for awareness about cryptic species. – *J. Parasitol.* 96: 453–464.
- Schwaegrichen, F. 1816. Ioannis Hedwig species Muscorum Frondosorum descriptae et tabulis aenis coloratis illustratae. Opus posthumum. Suppl. 1. – *Sumptus Ioannis Ambrosii Barth, Lipsiae.*
- Scott, R. J., Willink, P. W. and Norton, B. M. 2018. Biogeography and distribution of the cryptic species rosyface shiner *Notropis rubellus* and carmine shiner *Notropis percobromus* in Illinois. – *Copeia* 106: 524–531.
- Shaw, A. J., McDaniel, S. F., Werner, O. et al. 2002. New frontiers in bryology and lichenology. Phylogeography and phylodemography. – *Bryologist* 105: 373–383.
- Simmons, M. P. and Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analyses. – *Syst. Biol.* 49: 369–381.
- StatSoft, I. 2013. STATISTICA (data analysis software system), ver. 12. – <www.statsoft.com>.
- Stech, M. 1999. Molekulare Systematik haplolepidier Laubmoose (Dicranaceae, Bryopsida). – PhD thesis, Freie Universität Berlin, Berlin.
- Stegemann, S., Keuthe, M., Greiner, S. et al. 2012. Horizontal transfer of chloroplast genomes between plant species. – *Proc. Natl Acad. Sci. USA* 109: 2434–2438.
- Swartz, O. 1800. [1801] Observationes species nonnullas Muscorum minus cognitae illustrantes. – *J. Bot.* 1800: 171–185.
- Twyford, A. D. and Ennos, R. A. 2012. Next-generation hybridization and introgression. – *Heredity* 108: 179–189.
- Wagner, F. S. and Wagner Jr., W. H. 1989. Cryptic species, species delimitation and taxonomic practice in homosporous ferns. – *Am. Fern J.* 79: 46–54.
- Wendel, J. F. and Doyle, J. J. 1998. Phylogenetic incongruence: window into genome history and molecular evolution. – In: Soltis, D. E. et al. (eds), *Molecular systematics of plants II. DNA sequencing*. Chapman and Hall, pp. 265–296.
- Virtanen, V. 2003. Phylogeny of the Bartramiaceae (Bryopsida) based on morphology and on *rbcL*, *rps4* and *trnL-trnF* sequence data. – *Bryologist* 106: 280–296.

Appendix 1. Sequence alignment portions of ITS, *atpB-rbcL* and *rp16* that display variation between the four cryptic *Plagiopus oederianus* or between these and the non-cryptic *P. alpinus*. Numbers above the alignment portions indicate the positions within the entire alignments. The column to the left of a set of alignment portions indicates a cryptic species within *P. oederianus* (A–C, E) or *P. alpinus* (D), with the sample number of the shown sequence indicated. Shaded boxes indicate variable portions that characterize one of the five species.

ITS									
	25	45	85	115	135	155	175		
A (P619)	TGAACT	AACTT	TT C GCT	TT C GG	GGG-AG	TAGCGG	T C CGCACCCGGCGA		
B (P586)	TGAACT	AACTT	TT C GCT	TT C GG	GGG-AG	TNNCGG	TTCCGCACCCGGCGA		
C (P587)	TGAACT	AACTT	TT C GCT	TT C GG	GGG-AG	TAGCGG	TTCCGCACCCGGCGA		
D (P610)	TGAACT	AACTT	TT C GCT	TT C GG	GGG-AG	TAGCGG	TTCCGCACCCGGCGA		
E (P612)	TGA A TT	AA T TT	TT T GCT	TT T GG	G A G-AG	TAG G GG	TT C CGCACCCGG C GA		
	215	235	265	275	295	375			
A (P619)	GATGGC	GCTTTGACCCAT	TGGGAGGAGCCGGCGTCTCG	CCNN--GAG	CGATAC				
B (P586)	GATGGC	GCTTTGACCCAT	TGGGAGGAGCCGGCGTCTCG	CCC---GAG	CGATAC				
C (P587)	GATGGC	GCTTTGACCCAT	TGGGAGGAGTCGGCGTCTCG	CCC---GAG	CGATAC				
D (P610)	GATGGC	GCTTTGACCCAT	TGGGAGGAGCCGGCGTCTCG	CCCCNNAG	CGATAC				
E (P612)	GAT T GC	G T TTGAC C AT	T A GGAG A GCCGGCG A CG	C TC--- A AG	CG T AC				
	405	435	485	505	515	545			
A (P619)	CGAATCATCG	TGCGC	CCCCACCGAT	AGTGT T TT-CGCGTGGA	GCGGC				
B (P586)	CGAATCATCG	TGCGC	CCCCACCGAT	AGTGT T TT-CGCGTGGA	GCGGC				
C (P587)	CGAATCATCG	TGCGC	CCCCACCGAT	AGTGT T TTTCGCGTGGA	GCGGC				
D (P610)	CGAATCATCG	TGCGC	CCCCACCGAT	AGTGT T TT-CGCGTGGA	GCGGC				
E (P612)	C GAATCAT T CG	TG C GC	C TTCCAC C AT	AGT T TTTTTCGCGT A GA	G TTGGC				
	585	605	615	675	695	715	735		
A (P619)	AGAGAA	CGACGGAGCCCGCGAT	GCCCAGTTGG	GCAGGT	CGTCCTTCGGGCG	CGT			
B (P586)	AGAGAA	CGA T GGAGCCCGCGAT	GCCCAGTTGG	GCAGGT	CGTCCTTCGGGCG	CGT			
C (P587)	AGAGAA	CGACGGAGCCCGCGAT	GCCCAGTTGG	GCAGGT	CGTCCTTCGGG T G	CGT			
D (P610)	AGAGAA	CGACGGAGCCCGCGAT	GCCCAGTTGG	GCAGGT	CGTCCTTCGGGCG	CGT			
E (P612)	AGA A AA	CGACGGAGCCCG A AT	G TTCCA T TTAG	GCA A GT	C ATCCTTCGGGCG	C AT			
<i>atpB-rbcL</i>									
	145	385							
AE (P589)	AAA A	ATT T GA							
B (P580)	AAATA	ATT T GA							
C (P614)	AAATA	ATT T GA							
D (P591)	AAATA	AT T GA							
<i>rp16</i>									
	25	45	125	215	245	395	565	645	
AE (P576)	A T TCCAATGT	TTAAT	TAAAA	AGAAAAA	TTTGAT	AAATA	T A ATA A	AATTAA	
B (P580)	ATTCCAAT T T	TTAAT	TAAAA	AGAAAAA	TTTGAT	AAATA	TTATAATAA	AAT A AA	
C (P587)	ATTCCAATGT	TT T AT	TAAAA	AGAAAAA	TT T AT	AA A A	TTATTATAA	AATTAA	
D (P591)	ATTCCAATGT	TTAAT	T T AAA	A A AAAAA	TTTGAT	AAATA	TTATTATAA	AATTAA	