**Microchemical and molecular investigations reveal *Pseudephebe* species as cryptic with an environmentally modified morphology**

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**Supplementary Material S1.** *Additional specimens examined, morphology and chemistry studied, organized according to the original identifications*.

As *Bryoria mariensis:* **Falkland Islands:** *West Falkland*: Port Howard, feldmark and outcrops on summit ridge of Mt. María, UTM 21F UC 2078-2079, 2000-2150 ft [610-655 m], 28 January 1968, *H. A. Imshaug* (41393) *& R. C. Harris* (MSC 80871); Mt Maria, slope above Castle Rock, 51º37’S, 59º35’W, 500 ft. [150 m], on short dry grassland and *Empetrum,* 11 January 1992, *R. I. Lewis Smith* *8506* (AAS, as “*Bryoria falklandica”* ad int.).

As *Pseudephebe minuscula* or its synonyms (*cf.* Hillman 1936, Lamb 1964, 1948, Hawksworth 1972, Brodo & Hawksworth 1977). **Antarctica:** *Livingston Island*: Hurd Peninsula, moraine along the Reina Sofía glacier, 150 m, 14-February-1990, *L. G. Sancho* (MAF-Lich. 6824); Falen Bay, 80 m, on granite, 09-February-1991, on granite, *L. G. Sancho* (MAF-Lich. 4130); South Bay: False Burdick, 400 m, 26 January 1991, *L. G. Sancho* (MAF-Lich. 4129); 80 m, on granite, 25 February 1990, *L. G. Sancho* (MAF-Lich. 4131). *Antarctic Peninsula*:W coast of Horseshoe Island, Marguerite Bay, 23 February 1965, *R. E. Longton* 1270(BM 1089100, as *Alectoria minuscula*; TLC: norstictic acid).―**Canada:** *Yucon Territory:* Slopes above W shore of Kusawa Lake, 18 km S of Alaska Hwy (Yucon 1), on road to Kusawa Lake Campground, 60º36’14”N, 136º08’05”W, 700 m, exposed volcanic rock outcrops with soil communities on E-facing slope, on rock, 07 June 2011, *J. C. Lendemer* 29275 (MAF-Lich. 18338). ⎯**Greenland:** *Angmagssalik area*: Quigertieraq, 40 m, on stone in moraine, 18 July 1969, *F. J. A. Daniëls* (D525) *& J. G. Molenaar* (K(M) IMI 145918, as *A. minuscula*; TLC: no substances detected). *Central-West Greenland*: Qeqertaq, 70º00’N, 51º19’W, on gneissic rock, 31 July 2003, *E. S. Hansen* (MAF-Lich. 19401).―**Norway:** *Hordaland*: Hardangervida Natural Park, 23 July 1988, *L. G. Sancho & A. Belio* (MAF-Lich. 11313).―**Spain:** *Huesca*: Aristas de Brazato, 2550 m, July 1994, *L. G. Sancho & al.* (MAF-Lich. 6130). *Salamanca*: Sierra de Béjar, Circo del Calvitero, 2100 m, 24 October 1980, *L. G. Sancho* (MAF-Lich. 11314). *Segovia*: La Granja de San Ildefonso, *c.* Puerto de Navacerrada to Puerto de Cotos road, brook of Los Puentes, towards the Loma del Noruego, 40º47'36''N, 03º57'12''W, 1900 m, 7 March 2014, on granite, *V. J. Rico* *4614* (MAF-Lich. 20109).―**USA:** *Arizona*: Coconino Co., Coconino National Forest, San Francisco Peaks, 3500 m, on basalt, 12 June 1998, *T. H. Nash III* 42036 [Anonymous, *Lich. Exs. Arizona State Univ.* no 329] (MAF-Lich. 6750). *Idaho*: Lemhi Co., Salmon-Challis National Forest, Lemhi Mountains, Meadow Lake, 44.4324ºN, 113.3249ºW, 2869 m, on quartzite in alpine ridge, 4 June 2013, *N. Noell* (1561) *& J. Hollinger* (Hb. N. Noell).―**Switzerland:** *Canton Valais*:Zermatt, below Schwartzsee, 2450 m, 24 September 1972, *A. M. Burnet* 451 (BM s. n.; TLC: norstictic acid). *Uri*: c. Wassen, 46º45’08”N, 08º29’01”'E, 2210 m, alpine siliceous rocky outcrops, 15-June-2014, *C. G. Boluda & Ch. Scheidegger* (MAF-Lich. 19477) ⎯**USA:** *Colorado*:Boulder Co., West of Diamond Lake, 11600 ft. [3535 m], 21 August 1964, *S. Shushan* 4814(BM 1089099, as *A. minuscula*; TLC: unidentified substance 1).

As *Pseudephebe pubescens* or its synonyms (*cf.* Hillman 1936; Lamb 1964, 1948; Hawksworth 1972; Brodo & Hawksworth 1977). **Antarctica:** *Livingston Island*: South Bay, Spanish Antarctic Base, 30 m, 16 February 1995, *L. G. Sancho* (MAF-Lich. 10194); El Peñón line, 120 m, saxicolous, January-1990, *L. G. Sancho* (MAF-Lich. 4132); Bayers Peninsula, 15 m, 31 January 1990, *L. G. Sancho* (MAF-Lich. 4133); 60 m, 27 January 1990, on moraine at the edge of the glacier, *L. G. Sancho* (MAF-Lich. 4134); Reina Sofía, 270 m, February-1990, *L. G. Sancho* (MAF-Lich. 4135); Roca Partida: 110 m, 10 February 1990, *L. G. Sancho* (MAF-Lich. 4136); False Burdick, 400 m, 26 January 1991, *L. G. Sancho* (MAF-Lich. 4137); Caleta Argentina, 90 m, 14 February 1990, *L. G. Sancho* (MAF-Lich. 6829); Barnard Peninsula, False Bay, 300 m, top of the mount, 28 July 1995, *L. G. Sancho & A. Pintado* (MAF-Lich. 6816); Falen Bay, 80 m, on granite, 09 February 1991, on granite, *L. G. Sancho* (MAF-Lich.4130); 80 m, on granite, 25 February 1990, *L. G. Sancho* (MAF-Lich.4131).―**Australia:** *Australian Capital Territory*: Brindabella Range, summit of Mt. Franklin, 35º29’S, 148º47’E, 1644 m, on metamorphic rock outcrops with sparse vegetation, 03 November 1999, *S. H. J. J. Louwhoff, M. C. Molina* (350, 385) *& J. A. Elix* (MAF-Lich. 9697, 9725). *Tasmania*: Mt Field National Park, Mt Field West Plateau, 1400 m, on calcite boulders, 11 March 1980, *G. Kantvilas* 32/80(BM 1089102; TLC: norstictic acid).―**Austria:** *Tirol*: Grieskogel und Larstigkopf, 2700 m, October 1975, *G. Follmann* [Follmann, *Lich. Exs. Sel. Cassel*. no. 237] (MAF-Lich. 1109).―**Chile:** *Magallanes y Antártida Chilena (Región XII)*: Navarino Island, Bandera Hill, N slope, 54º57’37”S, 67º37’57”W, 550 m, alpine soil, 09 January 2005, *J. Etayo* (22271)*, A. Gómez-Bolea & L. G. Sancho* (MAF-Lich. 15691, 15925); Puerto Williams, Virgen de Lourdes track to Barranca Guarriaco, 54º56’46”S, 67º34’52”W, 90 m, 14 January 2005, on soil, *J. Etayo* (22495)*, A. Gómez-Bolea, U. Søchting & R. Vilches* (MAF-Lich. 15828). ⎯**France:** *Corsica*: Haute Corse, Corte, Gorges de la Restonica, a block of granite, 6 October 2011*, C. Gueidan* CG1982 (BM 980053; TLC: no substances detected).―**Greenland:** *Angmagssalik area*:SW Great Blomsterdalen, 80 m, 16 June 1969, *F. J. A. Daniëls* (D522) *& J. G. Molenaar* (K(M) IMI 145921, as *Alectoria pubescens*; TLC: norstictic acid). *South Greenland:* Nanortalik, 60º09’N, 45º15’W, on gneissic gravel with *Alectoria sarmentosa* subsp. *vexillifera*, *Sphaerophorus globosus*, *Umbilicaria hyperborea* and *U. torrefacta*, 29 July 2004, *E. S. Hansen* (S L65904). *South West Greenland*: Alluitsup Paa (Sydprøven), 60º28’N, 45º35’W, on stone together with *Parmelia saxatilis, Rhizocarpon geographicum* and *Umbilicaria hyperborea*, 31 July 2008, *E. S. Hansen* [*Hansen, Lich. Groenl. Exs.* no 1049] (S F169137).⎯**Ireland:** *Galway Co.*: Benchoona, 1850 ft. [560 m], 27 June 1966, *D. L. Hawksworth* 506(BM 1089094;TLC: no substances detected); near Leckavrea Mt., rocks near loch, 2000 ft. [610 m], 25 June 1966, *D. L. Hawksworth* 485(BM 1089095; TLC: no substances detected).⎯**Japan:** *Hokkaido*, *Prov. Ishikari*:[Kamikawa District] Mt. Daisetsu National Park, Mt Antaromadeke, 2200 m, 23 August 1971, *I. Yoshimura* 12425[*Yoshimura, Lich. Japon. Exs.* no. 2, as *Alectoria pubescens*] (K(M) IMI 172381; TLC: norstictic acid).―**Norway:** *Lofoten Island*: 100 m, 31 June 1988, *L. G. Sancho & A. Belio* (MAF-Lich. 1134).**―Spain:** *Asturias*: Leitariegos, Laguna de Arvás, 06 September 1980, *A. Crespo & al.* (MAF-Lich. 1605). *Ávila*: Sierra de Gredos, Circo de Gredos, 2040 m, 15 July 1982, *L. G. Sancho* (MAF-Lich. 11305). *Lérida*: between Viella and Puerto de la Bonaigua, 2250 m, on rock, 22 July 1967, *H. Sipman* [*Stud. Biol. Rheno-Trai in itinere D 211*] (MAF-Lich. 982). *Logroño*: Ezcaray, track to San Lorenzo Pike, 30TWM0276, 1780 m, screes, 07 September 2004, *A. Argüello* (MAF-Lich. 12522). *Madrid*: Sierra de Guadarrama, 30TVL198233, 2340 m, 15 November 1988, *F. Valladares* (286) *& L. G. Sancho* (MAF-Lich. 13124); Cabezas de Hierro, 30TVL221172, 2240m, 21 November 1987, *F. Valladares* (288) *& L. Ramírez* (MAF-Lich. 11831); Cuerda Larga, 30TVL196166, 2150 m, 29 October 1988, *F. Valladares* 287 (MAF-Lich. 13125); Guarramillas, 30TVL180160, 2200 m, 21 November 1987, *F. Valladares* (288) *& L. Ramírez* (MAF-Lich. 13126); El Nevero, 30TVL302378, 2080 m, 15 January 1989, *F. Valladares* (290) *& L. Ramírez* (MAF-Lich. 13128); Cabeza Lijar, 30TVL019053, 1780 m, 01 November 1988, *F. Valladares* (291) *& L. Ramírez* (MAF-Lich. 13129); Valdemartín, 30TVL196166, 2150 m, 29 October 1988, *F. Valladares* 163 (MAF-Lich. 13345). Somosierra, 30TVL531545, 1750 m, 19 December 1988, *F. Valladares* 283 (MAF-Lich. 13121); 2000 m, *F. Valladares* 284 (MAF-Lich. 13122). Sierra de Camorritos, 30 June 1991, *A. Pintado* (MAF-Lich. 14204). Sierra de Abantos, 30TVL012043, 1780 m, 01 November 1988, *F. Valladares* 289 *& L. Ramírez* (MAF-Lich. 13127). Cercedilla, 30TVL090150, 1780 m, 16 December 1988, *F. Valladares* 285 (MAF-Lich. 13123). *Zaragoza*: Moncayo, August-1898, on siliceous rocks (MAF-Lich.12764, as *Alectoria lanata*). *Zamora*: Lago de Sanabria Natural Park, Lagunas del Padornelo, 29TPG7860, 1700 m, 09 September 1998, *A. Crespo* (MAF-Lic.6774); on *Erica* sp., *M. Pugh Jones* 1521 (MAF-Lich. 7206).**―Sweden:** *Åsele Lappmark*: Vilhelmina sn: 65º06’N, 15º02’E, top of hill, on boulder, 01 July 2004, *G. Oldevik* 4508, 4315, 4307, 4304 (S L64662, L63058, L63068, L63070); 65º06’N, 14º27’E, top of hill, on *Betula nana* fallen death twig, 30 June 2004, *G. Odelvik* 4405, 4825 (S L63640, F57499); 65º07’N, 14º31’E, on pebbles on the ground, 30 June 2004, *G. Odelvik* 4401, 4527, 4850 (S L63644, L64642, F57452). *Hälsingland*: Järvsö sn, top of hill, on boulder, 14 October 2005, *G. Oldevik* 5781 (S F52360). *Härjedalen*: Tännäs sn, 62º20’24.2”N, 12º47’12.1E, sparse mixed forests, on rocks, 27 June 2007, *G. Oldevik* (7335) *& M. Myrdal* (S F79205); Vemdalen sn, Vemdalsskalet, Varggransfjället, on rocks, 03 July 2005, *G. Oldevik* 5424(S L70225). *Pite Lappmark*: Arjeplog sn, top of hill, on gravel, 22 August 2006, *G. Odelvik* 6399, 6108 (S F60992, F60239); on boulder with *Melanelia hepatizon*, 22 August 2006, *G. Odelvik* 6678 (S F192894). *Södermanland*: Vårdinge sn, 59º05’08.2”N, 17º20’20.1”E, pine rocky ground, on cliff, 15 May 2012, *G. Odelvik* 12119 (S F235654).⎯**UK:** *S.**Aberdeenshire*: Braemar, summit of Morrone, [undated], *J. M. Crombie* [*Crombie, Lich. Brit. Exs.* no. 20, as *Alectoria lanata* var. *parmelioides*] (K(M) IMI 109522 also as *Pseudephebe pubescens*; TLC: norstictic acid, unidentified substance 2). *E. Inverness*: Cairngorm Mountains, Cairn Gorm, 4000 ft. [1220 m], 2 August 1968, *D. L. Hawksworth* 1347(BM 1089096; TLC: norstictic acid). *Shetland Islands:* Mainland, Ronas Hill, on granite boulders, 22 July 1966, *D. L. Hawksworth* 538 (BM 1089098; TLC: no substances detected). *Wales*:Caernarvonshire, Cader Idris, 1877, *J. Carroll* (BM 1089097; TLC: no substances detected).⎯**Canada:** *Nunavut*:BaffinIsland, 31 km SE of Nanisivik, on granite stones, 13 July 1999, *P. K. Wong* 4679(BM 1089101; TLC: norstictic acid).

**Supplementary Material S2.** *Data on apothecial morphological characters in* Pseudephebe *studied specimens.*

Specimens with apothecia: S-F149958, hb. N. Noell1442 and MAF-Lich. 20102 in Clade A; MAF-Lich. 20100 in Clade B.

Ciliate apothecia were observed in both clades, and spore measurements were not discriminatory.

The mean values for 30 spores in the specimens with apothecia were:

* S F149958 – 8.93 × 5.92 µm, σ = 0.74 × 0.72 µm, Clade A
* hb. N. Noell1442 – 8.12 × 5.96 µm, σ = 0.87 × 0.90 µm, Clade A
* MAF-Lich. 20102 – 8.50 × 6.80 µm, σ = 1.00 × 0.65 µm, Clade A
* MAF-Lich. 20100 – 9.30 × 5.90 µm, σ = 0.57 × 0.96 µm, Clade B.

We did not, however, compare length/width ratios, undertake further metric studies on the spores, or examine any pycnidia in view of the small numbers present in our material.

**Supplementary Material S3.** *Node ages estimation.*

**Materials and Methods**

The molecular dating of tree-nodes was performed with BEAST v1.8.2 (Drummond *et al*. 2012) using a relaxed clock model (uncorrelated lognormal) with a birth-death model prior for the node heights and unlinked substitution models across the loci. The nucleotide-substitution model and parameters for all nucleotide-sites across each marker were selected using the Akaike information criterion (AIC) as implemented in jModelTest (Posada 2008). Mutation rates used were 2.43 x 10­-9 s∙s-1∙yr-1 for ITS region, estimated from the parmelioid genus *Melanelixia* (Leavitt *et al.* 2012b), and 2.57 x 10­-9 s∙s-1∙yr-1 for *RPB1* and 1.73 x 10­-9 s∙s-1∙yr-1 for *MCM7* estimated from the family *Parmeliaceae* excluding the basal genus *Protoparmelia* (Amo de Paz *et al*. 2011; Divakar *et al.* 2015). The analyses were run with 50 million generations and parameter values were sampled every 1000th generation. We plotted the log-likelihood scores of sample points against generations using TRACER v 1.5 (Rambaut et al. 2014) and determined that stationarity had been achieved when the log-likelihood values of the sample points reached an equilibrium and ESS values exceeded 200 (Huelsenbeck & Ronquist 2001). In the absence of *Pseudephebe* fossils, a candidate alectorioidfossil (Kaasalainen *et al*. 2015) of 24-35 Mya was used as a calibration point for the group in a second run with the same parameters as above but with an estimation of the loci mutation rates.

**Results**

A calibrated maximum clade credibility chronogram analysis using predefined substitution rates is shown in Fig. S1. Analysis using a candidate alectorioid fossil as calibrator (Kaasalainen *et al*. 2015) and calculating the loci mutation rates, resulted in the next estimation: (a) 35.8 Mya (95 % HPD = 23.9−45.8 Mya) for the origin of *Pseudephebe*. (b) 11.6 Mya (95 % HPD = 5.5−18.5 Mya) for the split of clades A and B. (c) 4.2 Mya (95 % HPD = 2.4−6.3 Mya) for clade A diversification. (d) 1.1 Mya (95 % HPD = 0.5−2.0 Mya) for clade B diversification. (e) 42.5 Mya (95 % HPD = 37.3−49.8 Mya) for the origin of the Alectorioid clade. The fossil used as calibration point, however, could be an *Oropogon* and not an alectorioid species(Kaasalainen *et al*. 2015)*.* The analysis using defined mutation rates (Fig. S1) suggest that *Pseudephebe* as a genus could have arisen *c*. 26.6 Mya in the Oligocene (95 % HPD = 20.6−31.8 Mya). In the absence of data from *Nodobryoria* species, this estimate needs to be treated with caution. If *Nodobryoria* proved to be a sister group to *Pseudephebe* (Divakar *et al.* 2015), a younger date might be supported. Divergence between the two *Pseudephebe* species was estimated at 9.5 Mya (95 % HPD = 5.5−14.0 Mya), with 4.3 Mya (95 % HPD = 2.9−5.9 Mya) for the diversification of Clade A, and 1.2 Mya (95 % HPD = 0.6−2.2 Mya) for Clade B. As could be expected in Clade A, the high genetic variability resulted in older intraspecific clades. These results should be interpreted with caution, given the confusing fossil as well as the non-clock like topology of the phylogenetic tree in which they are based.



Figure S1. Dated BEAST maximum clade credibility tree estimated from three-loci concatenated data. Grey bars indicating the 95% highest posterior density interval for the estimated divergence times. Posterior probabilities of interesting nodes and its divergence time as the mean posterior estimate of their age in Mya. Clades A, A’ and B indicated as in Fig. 1.

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