

Research Article

Molecular and morphological evidence for a new grass genus, *Dupontiopsis* (Poaceae tribe Poeae subtribe Poinae s.l.), endemic to alpine Japan, and implications for the reticulate origin of *Dupontia* and *Arctophila* within Poinae s.l.Robert J. Soreng^{1*}, Lynn J. Gillespie², Hidehisa Koba³, Ekaterina Boudko², and Roger D. Bull²¹Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington DC 20013-7012, USA²Research and Collections, Canadian Museum of Nature, Ottawa 3443, ON, Canada³Division of Natural Sciences, J.F. Oberlin University, Machida, Tokyo 1940294, Japan

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Abstract Phylogenetic analyses within Poaceae tribe Poeae subtribes Puccinellinae (=Coleanthinae), Phleinae, Poinae s.l. (including Alopecurinae), and Miliinae (PPAM clade), revealed that one species formerly placed in *Poa* represents a new monotypic genus belonging to subtribe Poinae s.l., *Dupontiopsis* gen. nov., *D. hayachinensis* comb. nov. (based on *Poa hayachinensis*), endemic to wet, gravelly, serpentine, alpine habitats in northern Japan. This genus forms a strongly supported clade (DAD) with two circumarctic Poinae genera, *Arctophila* and *Dupontia*, in phylogenetic analyses of plastid and nuclear ribosomal DNA sequence data. Both morphology and DNA sequence analyses provide support for *D. hayachinensis* as a lineage distinct from either *Arctophila* or *Dupontia*, with moderate DNA support for a position as sister to these two genera. *Dupontiopsis* resembles these other monotypic genera in its several-flowered spikelets, lemmas usually 3-nerved, with frequently awned attenuate scarious apices (as in *Dupontia*) and calluses with a crown of hairs around the base of the lemma, but differs in its keeled lemmas, scabrous palea keels, glumes shorter than the first lemma. Our investigation suggests that the most recent shared ancestor of the DAD clade evolved from a single hybridization event, as a hexaploid, probably in western Beringia. The probable parentage of the ancestor is considered to be within the Poinae–Alopecurinae clade excluding *Poa*. We provide evidence for possible secondary hybridization and introgression of duodecaploid *Dupontia fisheri* with *Puccinellia*. A key to perennial genera of PPAM with hairy calluses, and a supplemental table of morphological characters in the genera accepted in PPAM are provided.

Key words: *Arctophila*, DNA, *Dupontia*, Japan, leaf anatomy, phylogeny, *Poa*, polyploidy, reticulate evolution, taxonomy.

Phylogenetic analyses of *Poa* L. and its relatives have been informative in defining major clades, refining their classification, and identifying or confirming misplaced taxa. Three new genera have recently been described for species and taxa misplaced in *Poa* (Soreng & Gillespie, 2007; Gillespie et al., 2009; Soreng et al., 2009). Here we provide molecular and morphological evidence for a fourth new genus based on a species of *Poa* endemic to Japan.

Phylogenetic analyses of plastid and nuclear ribosomal (nr) DNA sequences of tribe Poeae strongly support a major clade comprising subtribes Alopecurinae, Miliinae, Phleinae, Poinae, and Puccinellinae (=Coleanthinae; *Coleanthus* Seidl being sister to *Phippisia* (Trin.) R. Br.; Soreng et al., 2014 based on Gillespie et al., unpublished plastid and nrDNA data) (PPAM clade of Gillespie et al., 2008, 2010) within the tribe (Soreng & Davis, 2000; Gillespie et al., 2007, 2008, 2009, 2010; Döring,

2009; Kim et al., 2009; Schneider et al., 2009, 2012; Hoffmann et al., 2013). There are ca. 40 genera accepted within the PPAM clade, ten in Coleanthinae, one each in Miliinae and Phleinae, with the remaining 28 genera placed in Poinae s.l. (including Alopecurinae and Cinninae) (Soreng et al., 2014). Morphologically PPAM genera are highly diverse with no clear synapomorphies and many features that are homoplasious across the tribe (Soreng et al., 2007). They are widely adapted to cool temperate regions with winter precipitation, and alpine and arctic habitats. Five or six monophyletic lineages are strongly supported: *Poa*; remaining Poinae genera plus Alopecurinae; *Milium* L.; *Phleum* L.; Coleanthinae; *Zingeria* P.A. Smirn., and *Colpodium* Trin. (the latter two are probably best placed in Coleanthinae). However, relationships among these lineages are mostly not well supported (except for strong support for subtribe Coleanthinae [at least in the

narrow sense] as the basal PPAM lineage in plastid analyses), and often inconsistent among analyses (Gillespie et al., 2007, 2008, 2009, 2010; Döring et al., 2007; Döring, 2009; Kim et al., 2009; Schneider et al., 2009, 2012; Hoffmann et al., 2013). These studies have led to progressive realignments of the genera and subtribes (Soreng et al., 2003a, 2014; Gillespie et al., 2008, 2010). The genus *Poa* (ca. 530 species) is the most species rich group, comprising over three quarters of the species in the PPAM clade. Phylogenetic analyses of plastid restriction sites and plastid and nuclear ribosomal DNA sequences have also revealed significant structure within *Poa* (Soreng, 1990; Gillespie & Soreng, 2005; Gillespie et al., 2007, 2008, 2009; Soreng et al., 2010; Refulio-Rodríguez et al., 2012; Hoffmann et al., 2013), including strong support for the position of *P.* subgen. *Sylvestres* (V.L. Marsh ex Soreng) Soreng and L.G. Gillespie (Gillespie et al., 2007 [as a sect. in subgen. *Arctopoa* (Griseb.) Tzvelev based on plastid only], 2008, 2009) as sister to all other *Poa* lineages, and has led to revisions in the infrageneric classification of *Poa* (Soreng, 1998; Gillespie et al., 2007; Soreng et al., 2009).

Molecular data have been instrumental in identifying and/or confirming taxa that have been misplaced in *Poa* and genera that belong within the *Poa* clade. Armed with the above phylogenetic data and a broad taxonomic understanding of *Poa* (Tzvelev, 1976; Edmondson, 1980; Rajbhandari, 1991; Veldkamp, 1994; Soreng, 1998, 2007; Soreng et al., 2003b, 2009; Zhu et al., 2006; Giussani et al., 2012 etc.), several cryptic genera were recently segregated from the genus *Poa* based on our initial discernment of odd morphological character combinations, and corroboration of their independent origins based on DNA sequence data. The Poinae genus *Nicoraepoa* Soreng and L.J. Gillespie was based on *Poa* subgen. *Andinae* Nicora of southern South America (Soreng & Gillespie, 2007), and the monotypic Poinae genera *Saxipoa* Soreng, L.J. Gillespie and S.W.L. Jacobs and *Sylvipoa* Soreng, L.J. Gillespie and S.W.L. Jacobs were based on two Australian species placed in *Poa* (Gillespie et al., 2009; Soreng et al., 2009). Ancient intergeneric reticulation (between *Poa* and an unidentified Poinae genus) was determined to be the origin of *Poa* subgen. *Arctopoa* (Griseb.) Tzvelev (Gillespie et al., 2008, 2010), now accepted as the genus *Arctopoa* (Griseb.) Prob. (Probatova, 1974, 1985, 2003; Gillespie et al., 2008, 2010; Soreng et al., 2014). In contrast to this splitting, DNA data aided decisions to lump widely or occasionally accepted genera into *Poa*: *Anthochloa* Nees and Meyen, *Austrofestuca* (Tzvelev) E.B. Alexeev s.s. (excluding *Hookerochloa* E.B. Alexeev s.l. including *Festucella* E.B. Alexeev), *Eremopoa* Roshev., *Neuropoa* Clayton, *Parodiochloa* C.E. Hubb., *Tzvelevia* E.B. Alexeev, *Dissanthelium* Trin., and *Tovarochoa* T.D. Macfarl. and But (Gillespie & Soreng, 2005; Gillespie et al., 2007, 2009; Jacobs et al., 2008; Refulio-Rodríguez et al., 2012). More recently ITS data from Hoffmann et al. (2013; reconfirmed by our own preliminary analyses) supported the inclusion of *Libyella* Pamp. and *Lindbergella* Bor within *Poa* (where their type species were originally placed) (Soreng et al., 2014).

Our recent molecular phylogenetic studies have identified yet another species described in *Poa* that resolves outside of the *Poa* clade (Soreng et al., 2011). *Poa hayachinensis* Koidz. is a rhizomatous perennial species endemic to alpine areas of northern Japan. Restricted to one mountain on northern

Honshu Island and several mountains on Hokkaido Island, and with a preference for serpentine habitats, the species is currently listed as Endangered on the Red List of Threatened plants of Japan (available online http://www.biodic.go.jp/english/rdb/rdb_e.html [accessed 30 January 2015]). Although the species is morphologically unusual in *Poa*, particularly in its crown of callus hairs, and 3-nerved lemmas, we initially thought it might be related to *P.* sect. *Secundae* V. L. Marsh ex Soreng. However, preliminary DNA sequence data suggested a close relationship with the circumarctic Poinae genera *Dupontia* R.Br. and *Arctophila* (Rupr.) Rupr. ex Andersson, and prompted us to reexamine the morphology of *P. hayachinensis*.

Here we use phylogenetic analyses of plastid (*trnT-trnL-trnF*, *matK*, *rpoB-trnC*) and nuclear ribosomal (ITS and ETS) DNA sequences to support the status of *Poa hayachinensis* as a distinct lineage worthy of recognition as a new genus and to investigate its relationships. We investigate its morphology and compare this with *Poa* and other Poinae genera. Based on this evidence we describe a new monotypic genus, *Dupontiopsis*, in subtribe Poinae and provide a key to morphologically similar PPAM genera. We also consider implications of the new genus for the hypothetical reticulate origin of *Dupontia* (Tzvelev, 1976; Soreng & Davis, 2000; Brysting et al., 2004) and their relationships to *Arctophila*.

Material and methods

Molecular sampling

A total of 110 samples of 100 species was chosen: 101 samples of 91 species belonging to 26 of 40 genera accepted in the PPAM clade including two *P. hayachinensis* samples (in-group) and nine out-group samples (Elect. Suppl.: Appendix S1). Most voucher specimens are at CAN and US (herbarium acronyms follow Thiers, continuously updated). The PPAM samples represent all major lineages and subtribes of the PPAM clade of tribe Poeae (Gillespie et al., 2008): subtribes Alopecurinae (*Alopecurus* L., *Limnas* Trin.; 18 species sampled), Coleanthinae (*Catabrosa* P. Beauv., *Catabrosella* (Tzvelev) Tzvelev, *Hyalopoa* (Tzvelev) Tzvelev, *Paracolpodium* (Tzvelev) Tzvelev, *Phippsia* R. Br., *Puccinellia* Parl., *Sclerochloa* P. Beauv.; 21 spp.), Miliinae (*Milium*, 3 spp.), Phleinae (*Phleum*, 6 spp.), and the Colpodium-Zingeria lineage (*Colpodium*, 1 spp.). Subtribe Poinae (including Cinninae, but as in the traditional sense excluding Alopecurinae) was represented by 16 genera (*Aniselytron* Merr., *Apera* Adans., *Arctagrostis* Griseb., *Arctophila*, *Arctopoa*, *Bellardiochloa* Chiov., *Cinna* L., *Dupontia*, *Gaudinopsis* (Boiss.) Eig, *Hookerochloa* E.B. Alexeev, *Nephelochloa* Boiss., *Nicoraepoa*, *Poa*, *Sylvipoa*, *Saxipoa*, *Ventenata* Koeler) and 41 species. In addition to *Poa hayachinensis*, 15 species were chosen to represent a broad sampling of the large genus *Poa*, including three belonging to subgenus *Sylvestres* (the well-supported sister clade to the rest of *Poa*; Gillespie et al., 2007, 2008, 2009; Soreng et al., 2010), five diploid species (*P. chaixii*, *P. dolosa*, *P. sibirica*, *P. thessala*, *P. ursina*), and seven other species that are polyploid or of unknown ploidy. Nine out-group species were chosen to represent a diversity of lineages in tribe Poeae outside of the PPAM clade.

Two plastid spacer (*rpoB-trnC*, *trnT-trnL-trnF*), one plastid coding (*matK*), and two nuclear ribosomal (nr) DNA regions, internal transcribed spacer (ITS) and external transcribed spacer (ETS), were utilized. *MatK* sequences are partial gene sequences corresponding to the official plant barcoding region (CBOL, 2009). *TrnT-trnL-trnF* (*TLF*) sequences comprise two spacer regions and the *trnL* intron (Taberlet et al., 1991). ETS sequences are partial sequences of approximately 500 base pairs at the 3' end (Gillespie et al., 2009).

For this paper 61 samples were newly sequenced for all five DNA regions, the remaining samples were newly sequenced for one to four regions. All *matK* and *rpoB-trnC* sequences are new to this paper (except for the *Festuca matK* sequence). 101 ITS, ETS, and *TLF* sequences are GenBank samples from our previously published papers (Gillespie et al., 2007, 2008, 2009, 2010; Soreng et al., 2010). Anne Brysting shared two cloned ITS sequences of *Dupontia* (CAN2 c4 KP794861, SVA5 c13 KP794862) that resolved outside the *Dupontia-Arctophila* clade in her study (Brysting et al., 2004; figure 4).

DNA sequencing

DNA was extracted from silica-gel dried or herbarium leaf material using a silica-based column method (Alexander et al., 2007) similar to commercially available DNA extraction kits. Amplification primers and protocols for *TLF*, ITS, and ETS are outlined in Gillespie et al. (2007, 2008, 2009, 2010). The plastid spacer *rpoB-trnC* was amplified using primers *rpoB* and *trnCgcaR* (Shaw et al., 2005). The barcoding part of the *matK* gene, approximately 500 bases long mid-gene, was amplified using primers 390f and 1326r (Cuénoud et al., 2002). Amplification of *rpoB-trnC* was performed in a 10 μ L volume with a final concentration of 1 \times buffer, 1.5 mmol/L MgCl₂, 0.2 mmol/L dNTP, 0.25 μ mol/L of each primer, 0.4 units of Taq, and 1 μ L of 1:10 dilutions of extracted DNA. Amplification of *matK* was performed in a 10 μ L volume with a final concentration of 1 \times buffer, 2.25 mmol/L MgCl₂, 0.3 mmol/L dNTP, 0.375 μ mol/L of each primer, 0.6 units of Taq, and 1 μ L of 1:10 dilutions of extracted DNA. For both regions, undiluted DNA was used for difficult samples. The following touchdown cycling conditions were used for *rpoB*: 3 min at 94 $^{\circ}$ C, followed by 15 cycles of 94 $^{\circ}$ C for 45 s, 60–48 $^{\circ}$ C for 60 s, and 72 $^{\circ}$ C for 2 min, followed by 20 cycles of 94 $^{\circ}$ C for 45 s, 48 $^{\circ}$ C for 60 s, and 72 $^{\circ}$ C for 2 min. The following cycling conditions were used for *matK*: 60 s at 94 $^{\circ}$ C, followed by 30 cycles of 94 $^{\circ}$ C for 45 s, 50 $^{\circ}$ C for 45 s, and 72 $^{\circ}$ C for 90 s. For both regions, a final elongation step of 72 $^{\circ}$ C for 5 min completed the amplification reactions. Amplification products were purified using the enzymes Exonuclease I and shrimp alkaline phosphatase. Big Dye version 3.1 (Life Technologies Corporation, Carlsbad, Calif.) was used for sequencing reactions using 0.6 μ L of Big Dye in a 10 μ L reaction. Sequencing reaction products were purified via ethanol—EDTA—sodium acetate precipitation. Nucleotide sequences were generated using automated cycle-sequencing on an Applied Biosystems 3130 \times 1 automated sequencer.

DNA sequence alignment and phylogenetic analysis

For each DNA region sequences were assembled, checked, and manually edited using either Sequencher ver. 4.7 (Gene Codes Corp., <http://genecodes.com>) or Geneious vers. 5.4 or 6.1.5 (Biomatters Ltd., <http://www.geneious.com>). Obvious base polymorphisms (i.e., two peaks of similar height in

otherwise clean data) were coded using standard ambiguity codes. Sequences were aligned in Geneious using the MAFFT ver. 7.017 plugin (Katoh et al., 2002; Katoh & Standley, 2013) and then manually adjusted. The two nrDNA alignments and the three plastid alignments were concatenated using Geneious v.6.1.5. The concatenated nrDNA alignment has 1.1% missing data, the plastid alignment 1.4% missing data. All samples are complete for all regions (apart from minor missing data at the ends), except for the single *Limnas* sample for which only ITS and ETS were successfully sequenced and thus included only in the nrDNA analysis and as a nrDNA sequence in the combined plastid-nrDNA analysis. Four taxa (described in the Results) were found to have conflicting phylogenetic signal between plastid and nuclear analyses. For the combined plastid-nrDNA analysis, plastid and nuclear sequences of these taxa were not concatenated, but instead were treated as separate partitions. Insertion-deletion gaps were not coded as separate characters.

Maximum parsimony (MP) analyses on the separate nrDNA and plastid data matrices and on the combined plastid-nrDNA matrix were performed in PAUP* 4.0b10 (Swofford, 2002). In order to find the most parsimonious trees two heuristic search strategies (both with tree-bisection-reconnection branch-swapping [TBR] and multiple most parsimonious trees retained [multrees = yes]) were used: 1) maximum number of trees retained (max trees) set at 100 000; 2) 1000 replications with random taxon addition sequence (add seq = random) saving ten most parsimonious trees per replication. Strict consensus trees from the PAUP MP analyses were prepared using FigTree v1.4.0 (Rambaut, 2006–2012). Bootstrap analyses were performed using a heuristic search strategy with 1000 bootstrap replicates, each with 5 random addition sequence replicates, saving 10 most parsimonious trees per replicate. Bootstrap support values (BS) are considered to represent the following support: <50%, unsupported; 50%–69%, weak; 70%–89%, moderate; and 90%–100%, strong. Heuristic MP analyses for nuclear and plastid data sets were also run in WinClada (WC) (Nixon, 2002) (hold = 400, mult*N = 20, hold/=20, [Multiple TBR + TBR] mult*max*) to examine nucleotide changes and polymorphisms at critical nodes, and to study distributions of ancillary characters not coded in the sequence datasets (indels and morphological features).

To investigate the relationships of the two divergent ITS sequences cloned from two individuals of *Dupontia* (Brysting et al., 2004: CAN2 c4 KP794861, SVA5 c13 KP794862), separate MP analyses were performed on the present ITS data matrix with these two clones plus other sequences cloned from the same individuals of *Dupontia* (Brysting et al., 2004: AY237867, AY237868, AY237869, AY237876, AY237877, AY237878, AY237879, AY237880). These ten *Dupontia* clones were also included in a larger ITS dataset for PPAM taxa (323 PPAM samples, 87 from subtribe Coleanthinae including 58 from *Puccinellia*; LJG, KB, and A. Alonso, unpubl.).

Bayesian posterior probabilities (PP) were estimated using MrBayes v.3.01 (Huelsenbeck & Ronquist, 2001; Ronquist et al., 2011) using default parameters (GTR = I + G model) for all three datasets. No alternative models were tested. The BI analyses were initiated with random starting trees and initially run until the value of standard deviation of split sequences

dropped below 0.01 as the convergence diagnostic value (Huelsenbeck & Ronquist, 2001). We ran the BI analyses for 1.6 to 4 million generations, sampling once per 100 generations. Burn-in was set at 0.25. As the BI and MP analyses yielded trees with nearly identical clades we placed the PP values on the MP strict consensus trees. PP support values of greater than 0.94 are considered significant.

Morphological study

Specimens at US or on loan (BM, K, TI) were used for morphological and (US) anatomical work. Much data were compiled from the literature, but specimens of every PPAM genus, including multiple specimens within variable species as needed and available, were examined for the gross morphological details reported. For anatomical work we sampled one specimen per species, using what appeared to be a leaf of average form and good health among herbarium specimens. Vouchers for taxa photographed are given in the figure legends. Leaf surfaces of *Arctophila*, *Dupontia*, and *Poa hayachinensis* were examined and photographed using a PHILIPS XL30 ESEM LaB6 scanning electron microscope at the National Museum of Natural History, Smithsonian Institution. Sections of blades were taken from about 1 cm above the collar of lower leaves of herbarium specimens, attached to stubs, and sputter coated with 12–18 nm of 60:40 gold/palladium alloys in a Cressington Scientific 108 Auto/SE. Both leaf blade abaxial and adaxial surfaces were examined. Leaf trans-sectional anatomy was examined by cutting rough sections with a hand-held razor blade. These and fine measurements and observations of various plant parts were made at 6× to 50×, using a Wild M5-46875 dissecting microscope fitted with an ocular micrometer. Anatomical preparations were observed under an Olympus BH-2 compound microscope (at 50× and 100×). Light micrographs were taken using a combination: Nikon SMZ-74ST dissecting scope; Nikon Digital Sight DS-U2/L2USB camera; NIS-Elements D Imaging Software (Laboratory Imaging Inc.) ver. 3.20.0. Anatomical terminology, as far as possible, follows Ellis (1976, 1979).

Results

Phylogenetic analyses

Strict consensus trees (SCT) resulting from PAUP MP analysis of plastid, nrDNA and combined plastid-nrDNA alignments are shown in Figs. 1–3, respectively. For all three data matrices the two PAUP MP heuristic search strategies used resulted in the same strict consensus trees and tree statistics. MP statistics of the PAUP analyses are provided in Table 1. BI and MP consensus trees were congruent for the PPAM clade (in-group) in the plastid analysis (Fig. 1). The BI tree was locally incongruent with the MP consensus tree at five in-group nodes in the nuclear analysis (Fig. 2, indicated by diamonds) and at one in-group node in the combined analysis (Fig. 3); however, these incongruences had little or no support.

Results given below focus mainly on the relationships of *Poa hayachinensis*. In the plastid analyses (SCT shown in Fig. 1), *P. hayachinensis*, *Arctophila*, and *Dupontia* form a strongly supported clade (DAD clade, BS = 99, PP = 1). Within DAD

P. hayachinensis (BS = 87, PP = 1) is sister to a moderately supported *Arctophila*–*Dupontia* clade (BS = 80, PP = 1). Two indels (not included in the analysis) provide additional support for *Arctophila* and *Dupontia* as sister taxa. A unique 22 bp insertion in *rpoB*–*trnC* is shared by all samples except for one *Dupontia* (Go286). A unique 33 bp insertion in *trnT*–*trnL* is shared by all *Arctophila* and one *Dupontia* sample; the remaining three *Dupontia* samples have shorter (9 and 14 bp) but otherwise identical indels. DAD is resolved in the moderately supported PA-P (read as “Poinae + Alopecurinae minus *Poa*”) clade (BS = 84, PP = 1), as one of five lineages in a polytomy in the SCT, but as sister to *Cinna* in both the Bootstrap (BS = 56) and BI (PP = 0.84) analyses, these again as sister to *Aniselytron* (PP = 0.83) in the BI analysis. *Alopecurus* (BS = 86, PP = 1) and the *Bellardiochloa* clade (BS = 99, PP = 1) are strongly supported as sisters (BS = 90, PP = 1). The PA-P clade and three strongly supported lineages—*Milium*, *Phleum*, and *Poa* (BS = 100, 100, and 98, respectively, and PP = 1)—form a strongly supported clade (BS = 100, PP = 1; clade called PAM by Gillespie et al., 2008, 2010) that is sister to the strongly supported Coleanthinae clade (BS = 100, PP = 1). The collective PPAM clade had strong support (BS = 90, PP = 1).

In the strict consensus tree resulting from the nrDNA analysis (Fig. 2), the DAD clade was resolved with moderate bootstrap support (BS = 84, PP = 1); *P. hayachinensis* (BS = 98, PP = 1) and *Arctophila* (BS = 62, PP = 1) formed a clade with no BS support (PP = 0.97). All DAD samples exhibited some evidence of base polymorphisms (mostly T-C, and A-G) in their nrDNA sequence trace files. DAD was sister to *Limnas* with moderate support (BS = 75, PP = 0.69). *Limnas* shares no non-homoplasious synapomorphies with any combination of *P. hayachinensis*, *Arctophila* and *Dupontia*. DAD, *Alopecurus*, *Limnas*, and all sampled Poinae genera except *Poa* form the moderately supported PA-P clade (BS = 86, PP = 1). The PA-P clade comprises two subclades, here PA-P (1) (BS = 92, PP = 1) and PA-P (2) (subclades 1 and 2, respectively, in Gillespie et al., 2010). Within the larger moderately supported PA-P (2) subclade (BS = 74, PP = 1), DAD plus *Limnas* are moderately supported as sister to *Alopecurus* (BS = 85; PP = 1). PA-P, *Poa* (BS = 98, PP = 1), *Phleum* (BS = 100, PP = 1), *Milium* (BS = 100, PP = 1), and Coleanthinae (BS = 72, PP = 1) form five distinct lineages in the PPAM clade (BS = 95, PP = 1). Relationships among the latter five lineages collapsed in the MP Bootstrap analysis (BS < 50), while the BI analysis resolved the same five clades differently, with PP = 0.93 for a clade of *Milium* as sister to *Phleum* plus *Poa* (PP = 0.67), and PP = 0.69 for Coleanthinae as sister to PA-P.

In a separate MP analysis performed on the ITS alignment including cloned ITS sequences of *Dupontia* from Brysting et al. (2004), the two divergent ITS clones (CAN2 c4, SVA5 c13) both resolved within the *Puccinellia* clade with strong support, as sister to *Pucc. arctica* (tree not shown). The other clones from the same two individuals of *Dupontia* resolved with our sequences of *Dupontia*, *Arctophila*, and *Poa hayachinensis* in a well resolved DAD clade. MP analysis of our larger ITS alignment confirmed the two divergent clones' position in a clade with *Pucc. arctica* and *Pucc. banksiensis* Consaul (tree not shown).

We justified combining the plastid and nuclear alignments (except for two taxa, discussed below) based on the high

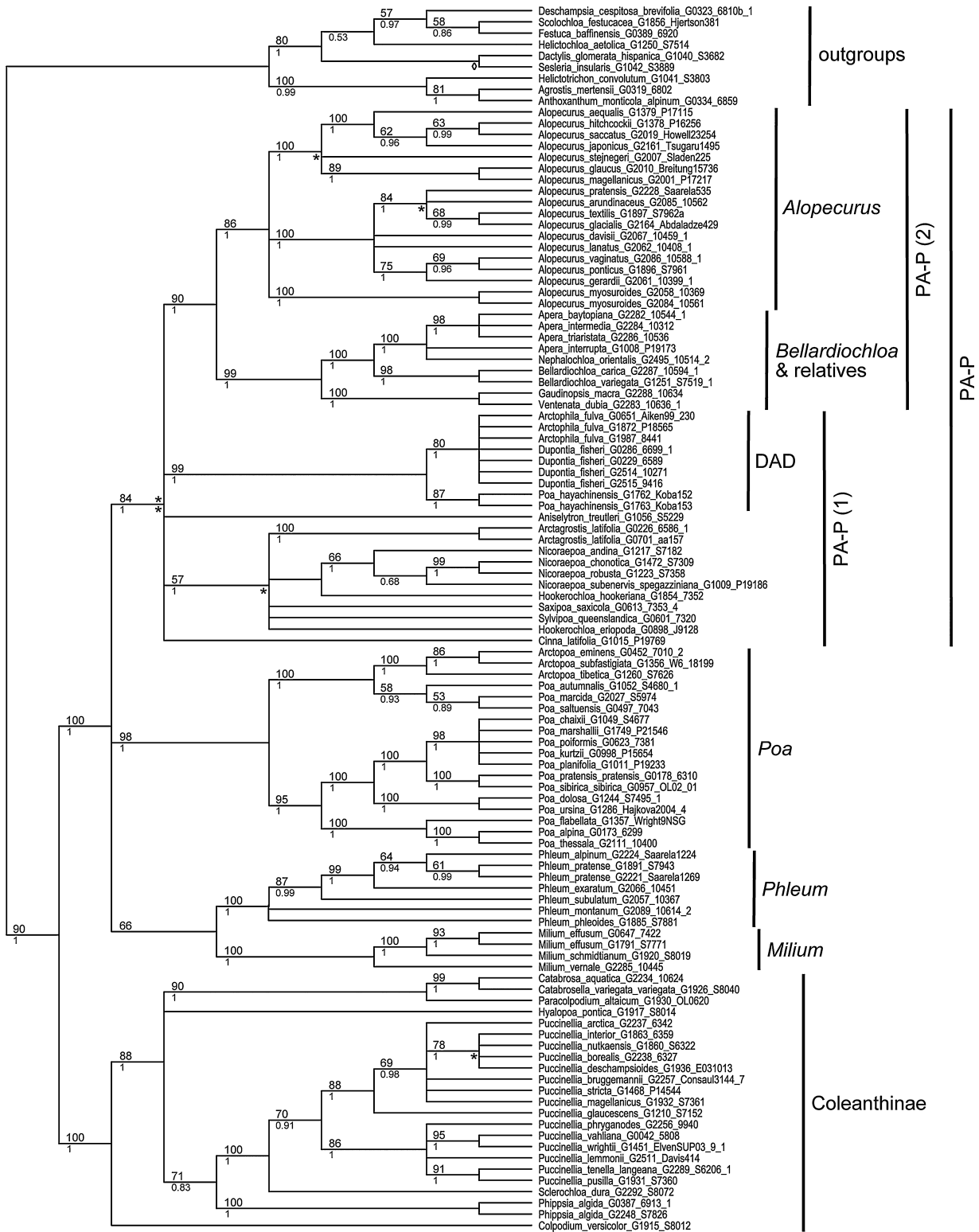


Fig. 1. Maximum parsimony strict consensus tree (SCT) based on *trnTLF*, *matK*, *rpoB-trnC* plastid sequences of tribe Poeae PPAM clade and out-groups. See Table 1 for parsimony tree statistics. Numbers above branches are bootstrap values, below branches are Bayesian Inference (BI) posterior probabilities. Asterisks above or below branches leading to the SCT polytomies indicate more resolution in the bootstrap or BI analyses, respectively. Diamonds “◇” above or below branches leading to SCT bifurcations indicate that incongruent topologies were resolved in the bootstrap or BI, respectively. The labels PA-P, PA-P (1), and PA-P (2) indicate the “Poinae+Alopecurinae minus *Poa*” clade, and its two subclades.

Table 1 Summary statistics for PAUP maximum parsimony analyses of nuclear ribosomal DNA (ITS + ETS), plastid DNA (*trnTLF* + *rpoB-trnC* + *MatK*), and combined datasets. Number of most parsimonious trees is given for both PAUP (search strategy saving a maximum of 100,000 trees), and Winclada analyses of nr and plastid data

	Nuclear	Plastid	Combined
#Total aligned characters	1566	4694	6260
#Sequences	110	109	122
#Parsimony informative characters (PI)	498	593	1091
%PI	32	13	17
Length of most parsimonious trees	2262	1730	4094
#Most parsimonious trees (PAUP)	100 000	100 000	100 000
#Most parsimonious trees (Winclada)	288	1728	—
Consistency Index (CI)	0.46	0.73	0.56
CI excluding uninformative characters	0.41	0.62	0.47
Retention Index (RI)	0.78	0.89	0.82

degree of congruence of plastid and nuclear trees. Both data sets strongly support a clade comprising all PPAM genera, and resolved the same major clades (labeled in Figs. 1, 2) within PPAM with mostly moderate to strong support. Relationships among major clades differed somewhat between plastid and nuclear trees, but support for the incongruent positions was weak or insignificant (e.g., positions of *Poa*, *Phleum*, *Milium*, and *Coleanthinae* in the nuclear tree). *Arctopoa* and the DAD clade were found to have highly inconsistent placements between plastid and nrDNA trees; plastid and nuclear partitions were analyzed separately for samples of the taxa involved. *Arctopoa* was strongly supported as a member of the *Poa* clade, sister to *Poa* subgen. *Sylvestres* in the plastid tree, but aligned with strong support within the PA-P clade, as sister to *Cinna*, in the nrDNA tree (as observed in previous studies; see Gillespie et al., 2008, 2010). The DAD clade was moderately supported as sister to *Alopecurus* within the PA-P (2) clade in the nuclear analysis, but did not resolve in the PA-P (2) clade in the plastid analysis. Although its position was poorly resolved on the MP plastid tree, DAD was sister to *Cinna* in the Bootstrap and BI analyses, suggesting a relationship with the PA-P (1) clade.

In analyses of the combined plastid-nrDNA data (SCT shown in Fig. 3) the PPAM clade was strongly supported (BS = 100, PP = 1), with *Coleanthinae* (BS = 100, PP = 1) strongly supported as sister to a clade of *Milium*, *Phleum*, *Poa* and PA-P (BS = 100, PP = 1). *Poa*, *Phleum*, and *Milium* are strongly supported lineages (BS = 100, PP = 1) that make up a moderately supported clade (BS = 80, PP = 1). The strongly supported PA-P clade (BS = 100, PP = 1) comprises two subclades, PA-P (1) (BS = 0.84, PP = 1) and PA-P (2) (BS = 100, PP = 1). Nuclear sequences of *Poa hayachinensis*, *Arctophila*, and *Dupontia* form a strongly supported clade (DADnr; BS = 90, PP = 1); relationships within this clade were poorly supported. DADnr resolved within the strongly supported PA-P (2) clade, in a strongly supported clade with *Alopecurus* and *Limnas* (BS = 99, PP = 1). Relationships among DADnr, *Alopecurus*, and *Limnas* (nuclear data only) were unresolved (SCT) or only weak supported; DADnr was sister to *Limnas* (BS = 51) in the Bootstrap analysis, and sister to *Limnas* and *A. myosuroides* (PP = 0.76) in the BI analysis. Plastid sequences of DAD (DADpl) resolved with moderate support within the PA-P (1) clade (BS = 84, PP = 1), in a subclade with *Aniselytron*, *Cinna*, and nuclear sequences of *Arctopoa* (*Arctopoa*-nr)

(BS = 58, PP = 0.92). Although relationships within this subclade were unresolved in the MP strict consensus tree, DADpl resolved as a moderately supported clade (BS = 85), sister to *Cinna* and *Arctopoa*-nr (BS = 51), in the Bootstrap analysis. In the BI analysis DADpl plus *Arctopoa*-nr (PP = 0.9; this grouping an artifact as the sets shared no characters) was sister to *Cinna* (PP = 1). *Poa hayachinensis*-pl resolved as sister to a clade of *Arctophila*-pl and *Dupontia*-pl (BS = 72) in the Bootstrap analysis; whereas it was sister to a clade of *Arctophila*-pl, *Dupontia*-pl, and *Arctopoa*-nr (PP = 0.57) in the BI analysis.

Morphological study

Our aim here is to highlight gross morphological and anatomical differences between *Poa hayachinensis* and the closely related, monotypic genera *Arctophila* and *Dupontia*, along with differences from *Poa*. In order to provide broader context for the documented character state distributions in PPAM, we sometimes note the states in additional genera. Electronic Supplement: Appendix S2 gives morphological states for many characters discussed for all accepted genera in the PPAM clade (Soreng et al., 2014), and includes many new observations along with compilations from the literature (Tzvelev, 1976; Watson & Dallwitz, 1992 onwards; Probatova, 2003; Cayouette & Darbyshire, 2007; Darbyshire & Cayouette, 2007; Soreng, 2007; Soreng et al., 2007, 2009).

Spikelets

The spikelets of *Poa hayachinensis* (Fig. 4) have several characteristics of note: (i) they have 2–4 (5) florets; (ii) the glume and lemma apices are narrowly acute to acuminate, entire, often attenuated to a slender, short awn, the glumes are slightly shorter than the adjacent lemmas; (iii) the lemmas are 3(5)-nerved (intermediate veins obscure to faint if present), 5–7 mm long, distinctly keeled, the base of the keel is gradually tapered or minimally arched to the callus, and the apex is scarious to hyaline for more than ¼ to ½ the length; (iv) the lemmas are pubescent on the keel and marginal veins, and sometimes exhibit sparse, shorter hairs on the surfaces between the veins; (v) the glume, lemma, and palea edges (i.e., on the rim of the flanges) have scattered antrorse prickles; (vi) the callus of the lemmas has a crown (sometimes slightly bearded) of soft, slightly wavy hairs to ca. 0.4–1 mm

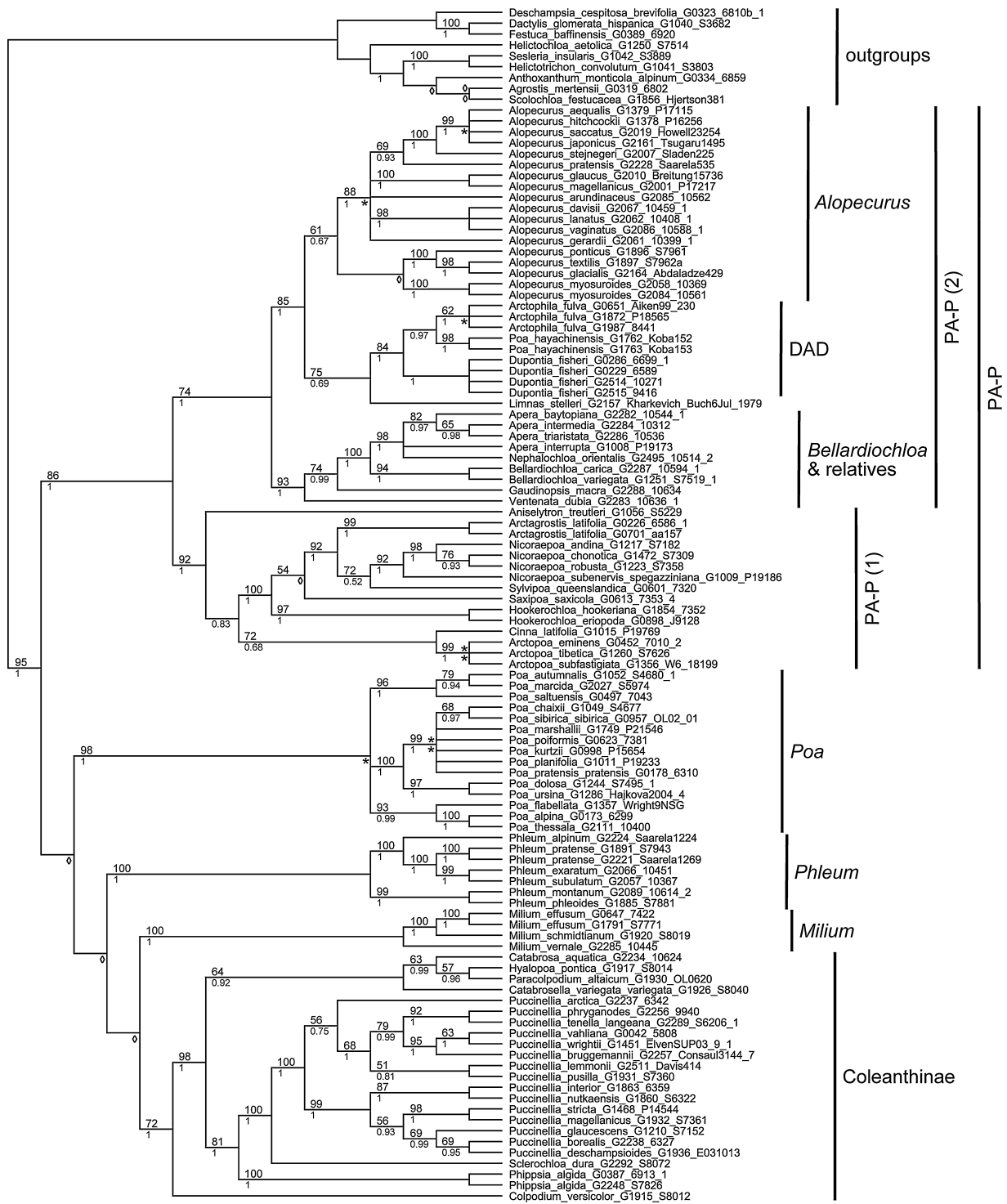


Fig. 2. Maximum parsimony strict consensus tree (SCT) based on the analysis of ITS and ETS nrDNA sequences of tribe Poeae PPAM clade and out-groups. See Table 1 for parsimony tree statistics. Numbers above branches are bootstrap values, below branches are Bayesian Inference (BI) posterior probabilities. Asterisks above or below branches leading to SCT polytomies indicate more resolution in the bootstrap or BI analyses, respectively. Diamonds “◇” above or below branches leading to SCT bifurcations indicate that incongruent topologies were resolved in the bootstrap or BI analyses, respectively. The labels PA-P, PA-P (1), and PA-P (2) indicate the “Poinae + Alopecurinae minus Poa” clade, and its two subclades.



Fig. 4. A spikelet of *Dupontiopsis hayachinensis* (H. Koba 153, US; Japan).

long; (vii) the rachilla internodes are up to 1.8 mm long, smooth or slightly scabrous, glabrous or pilulose with hairs to ca. 0.5 mm long; (viii) the palea keels are regularly scabrous, glabrous. In addition, the callus is slightly elongated and angled downward dorsally, and varies within spikelets, from slightly laterally compressed and oval (adaxially broadest) in proximal florets, to slightly, or even distinctly, dorsiventrally compressed and elliptical in distal florets.

Arctophila spikelets have 2–6 florets; glume and lemma apices weakly pointed to obtuse and blunt, lemmas entire or sometimes slightly erose, rarely with a central mucro (to 0.8 mm long), glumes are subequal to the adjacent lemmas; the lemmas are 3(5)-nerved, 2.5–4 mm long, weakly keeled, the base of the keel is slightly gibbous-arched to the callus; the lemmas are glabrous; the glume, lemma, and palea edges are smooth; the callus has a sparse crown of somewhat coarser hairs (sometimes reduced to 1 or a few hairs) 0.3–0.7 mm long, and the callus is short, blunt, and rounded on back; the rachilla internodes are up to ca. 1 mm, long smooth and glabrous; the palea keels are smooth and glabrous.

Dupontia spikelets have 1–2 (–4) florets; glume and lemma apices are narrowly acute to attenuate, the lemma apex frequently terminating with a cusp or infrequently a short slender awn 1 (–2.2) mm long; the glumes usually slightly exceed the adjacent lemmas, or are sometimes equal to them; the lemmas are 3(5)-nerved, 3–6.5 mm long, weakly keeled, the base of the keel is gradually tapered or minimally arched to the callus, the apex is scarious to hyaline for up to $\frac{1}{2}$ the length;

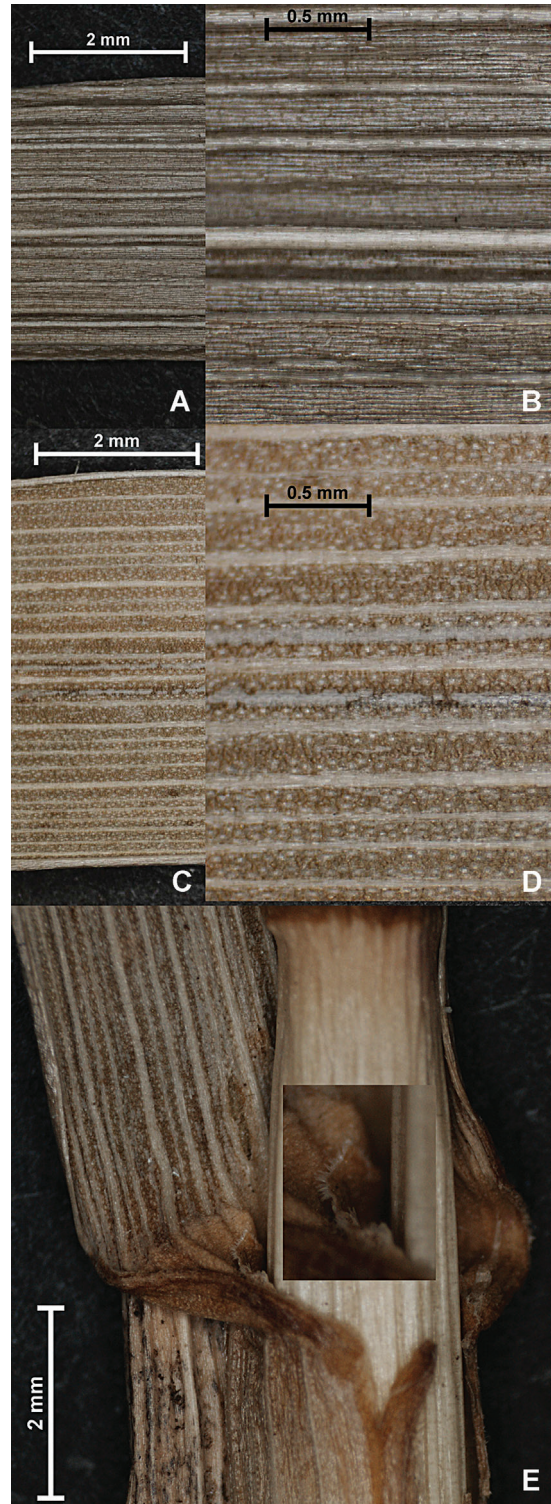


Fig. 5. Leaf-blade surfaces and the ligule region of *Dupontiopsis hayachinensis*. **A, B**, Abaxial view of leaf-blade; **C, D**, Adaxial view of leaf-blade; **E**, Ligule (and insert of ligule), bottom of one node, culm below node, and adaxial blade surface (H. Koba 153, US; Japan).

the lemmas are glabrous or loosely pubescent on the keel and sometimes the marginal veins and proximal surfaces; the glume, lemma, and palea edges are smooth; the callus has a crown of somewhat coarser hairs 0.5–1.2 mm long, and the callus is short, blunt, and rounded on back; the rachilla internodes are 1–1.5 mm long, smooth, glabrous, or sparsely pilulose; the palea keels are smooth or rarely sparsely scabrous, glabrous.

Poa spikelets have (1) 2–5 (7–10) florets; glume apices are usually broadly to narrowly acute, entire usually distinctly to slightly shorter than the adjacent lemmas (or one or both exceeding the lemmas as in sects. *Abbreviatae* Nannf. ex Tzvelev, *Dissanthelium* [Trin.] Refulio), the lemma apices are usually blunt or abruptly pointed, obtuse or acute, rarely acuminate, entire (exceptionally short stiff awned in *P. flabellata*); the lemmas are (3) 5 (7–11)-nerved (intermediate veins obscure to pronounced), 2.5–10 mm long, distinctly keeled (indistinctly keeled in sect. *Secundae* p.p.), the base of the keel is slightly to distinctly gibbous-arched to the callus, the apex is rarely scarious-hyaline for more than 1/5 the length; the lemmas glabrous or variously pubescent; the glume, lemma, and palea edges are smooth or finely scabrous with scattered antrorse prickles; callus (see following paragraph); rachilla internodes are variously smooth, scabrous, glabrous, or pilose; the palea keels are scabrous, glabrous, or pilose, rarely smooth but then pilose (*P.* sect. *Micrantherae* Stapf).

Description of the callus in *Poa* requires additional detail. The callus is glabrous (ca. 35% of species) or pubescent (ca. 65% of species), and then usually with a dorsal tuft of soft, slender, crinkly (or plicate in *P.* sect. *Dioicopoa* Hack.) hairs (ca. 58% of all species), which arise from a concentrated zone of hair initiating cells on the back of the callus (the tuft is often referred to as a “web,” or infrequently as a “comma”). The dorsal web occurs in all subgenera except *Pseudopoa* (K. Koch) Stapf (7 spp.). In ca. 30 other species (ca. 7% of all species) there is a diffuse or distinct crown of hairs, or a beard in one species (*P.* [subsect. *Austrofestuca* (Tzvelev) Soreng, L.J. Gillespie & S.W.L. Jacobs] *billardierei* St.-Yves). A crown of hairs in *Poa* is sometimes better characterized as a diffused dorsal tuft (e.g., in *P.* [sect. *Madropoa* Soreng] *piperi* Hitchc.), or an amplification of the number of hairs to the point, where any gap along the callus is unrecognizable and the crimp is lost (e.g., *P.* [sect. *Dioicopoa*] *obvallata* Steud.). Callus shape (whether hairy or not) in most species of *Poa* is short and blunt, and generally laterally slightly pinched-in on the dorsal side (indicating shared ancestry, and repeated secondary losses of hairs in different lineages). A few species of *Poa* appear to have a true crown of hairs on a dorsally rounded or slightly dorsiventrally compressed callus (e.g., in *P.* sect. *Secundae*, where the crown is best developed in *P. stenantha* Trin. and *P. hartzii* Gand.). The latter condition closely approximates that in *P. hayachinensis* and other PPAM genera with a well-developed crown of hairs (see Elect. Suppl: Appendix S2, and Discussion below).

Ligules

The ligules of vegetative shoot and lower culm leaves of *Poa hayachinensis* (Fig. 5E) are 0.2–0.5 mm long, relatively thick-scarious, brown, abaxially smooth, glabrous, with apices truncate, the rim densely, and coarsely asperous (ligules of upper culm leaves vary from 0.5–3.3 mm long, the longer

ones varying to off-white, with acute and smooth apices). The culms and nodes are smooth. Ligules in *Dupontia* are 1–4 (–5.5) mm long, scarious-hyaline, brownish, smooth, glabrous, truncate to acute; in *Arctophila* are 2–6 (–8) mm long, scarious-hyaline, brownish, smooth, glabrous, truncate, and erose-dentate to fimbriate-lacerate. Their culms and nodes are also smooth. *Poa* ligules are usually scarious and white or hyaline, with a diverse range of vestitures and apices, however, coarsely asperous margins as in *P. hayachinensis* (Fig. 5E) are unknown, and lacerate margins as in *Arctophila* are rare (*P.* sects. *Parodiochloa* and *Siphonocoleus* Hitchc.).

Leaf-blade cross-sections

The leaf-blade cross-section of *Poa hayachinensis* (Fig. 6F) shows moderately wide and tall sclerenchyma strands forming girders to the 1st order (1°) vascular bundles (VBs) abaxially and adaxially, to 2nd order (2°) VBs adaxially and sometimes abaxially, to 3rd order (3°) VBs not at all or occasional adaxially. The keel abaxial strand is sometimes low and narrow, and discontinuous to the VB (Fig. 6F), sometimes wide and continuous to the keel VB (Fig. 6G, and in which all girders were slightly stouter). Third order VBs are absent between the keel and adjacent two VBs, between the latter and the first 1° VBs. 1° and 2° VBs range between 11 and 23 in total (in herbarium samples). Fans (f) of bulliform cells occur between the keel and first and sometimes second VBs on the adaxial (ad) side (in a broadest blade—from keel to marginal sclerenchyma cap: kf^{ad} 2° (f^{ad}) 1° 3° 2° 3° 1° 3° 2° 3° 1° 2° 1° 2° cap). The surfaces are flat or have low, slightly-raised and rounded costal ridges. The margins are slightly inrolled. Kawano and Tatewaki (1961, fig. 2) illustrated cross sections of *P. hayachinensis* and their new *P. yezoalpina*. They also noted additional fans in the former species, but these were absent in the latter. Also the leaves of *P. yezoalpina* appear to have less sclerenchyma, with sclerenchyma strands abaxially and adaxially discontinuous to the keel VB, and abaxially discontinuous to the first lateral VBs, but are otherwise similar to Fig. 6F.

Arctophila fulva leaf-blades (Figs. 6A–6C) have tall, moderately-wide sclerenchyma strands forming girders to the keel and to lateral 1° VBs abaxially and adaxially, 2° and 3° VBs are not developed (at least near the mid-section of the broad leaves illustrated). Abaxially, fans of bulliform cells occur between each VB (adapted from Lohaus, 1905; taf. XII, as *Colpodium pendulinum* (Laest.) Griseb. [figs. 81, 82], and *C. fulvum* (Trin.) Griseb. [fig. 83]): (kf^{ad} 1° f^{ad} 1° f^{ad} 1° f^{ad} 1° ...). Both surfaces express prominently to moderately raised and rounded, costal ridges (ridges slightly taller abaxially). An SEM image of a blade cross-section of *A. fulva* (Darbyshire & Cayouette, 1992, fig. 2) matches well with our Figs. 6A–6C.

Dupontia fisheri leaf-blades (Figs. 6D, 6E) have moderately-wide abaxial and narrow adaxial sclerenchyma strands forming girders to the keel and to lateral 1° VBs abaxially and adaxially, 2° VBs abaxially and adaxially or only abaxially; 2° and 3° VBs are developed mainly in the outer margins. Adaxial fans of bulliform cells occur between the keel and adjacent VBs (Lohaus, 1905; XII, figs. 84, 85): (kf^{ad} 2° 1° 1° 1° 2° 3° cap). Metcalfe (1960: 167) described Lohaus' results as “Both surfaces flat. VBs: large and small present in equal numbers.” However, from examination of herbarium specimens (RJS pers. observ.) it is apparent that moderate abaxial raised and

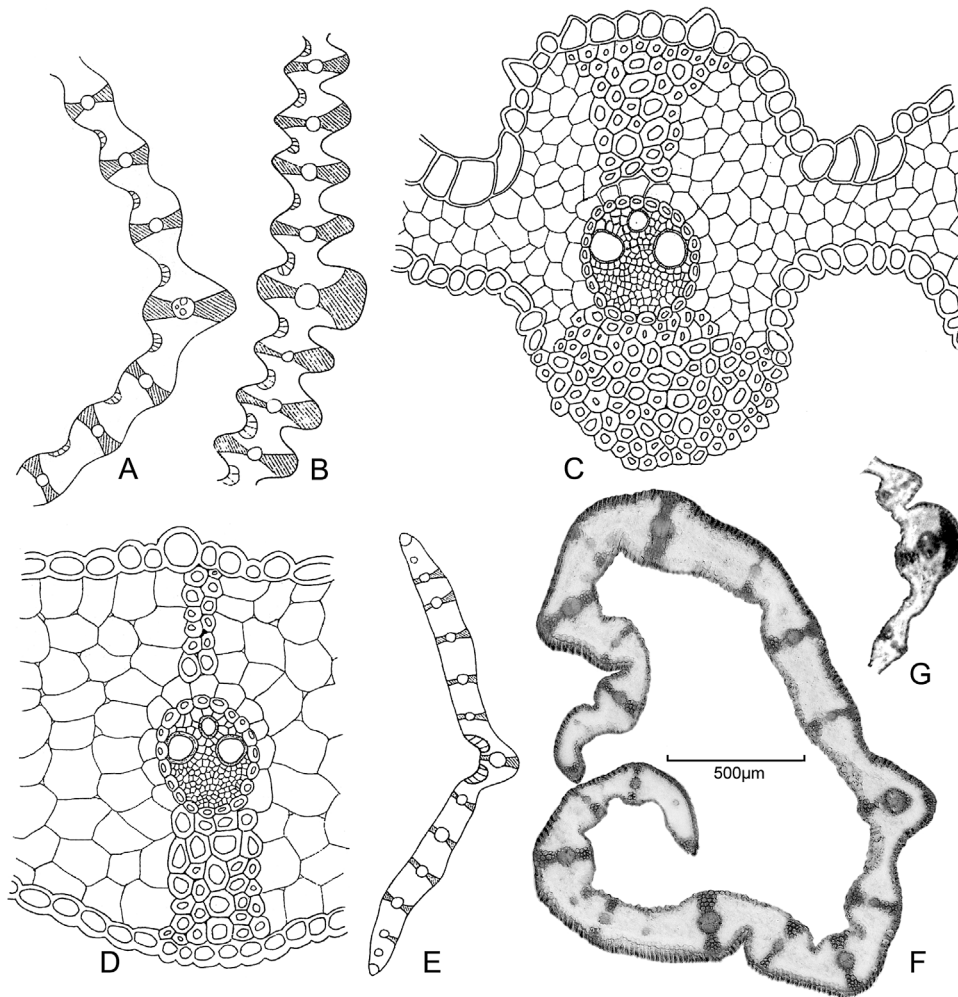


Fig. 6. Leaf blade cross-sections. **A–C**, *Arctophila fulva*; **D**, **E**, *Dupontia fisheri*; **F**, *Dupontiopsis hayachinensis* (H. Koba & H. Koba s.n., DAO; Japan); **G**, *Dupontiopsis hayachinensis*, keel (H. Koba 153, US; Japan). **A–E**, Adapted from Lohaus, 1905 Taf. XII, 81–85. **F**, Adapted from photo contributed by S. Darbyshire.

rounded costal ridges similar to those in *Arctophila* are frequently developed, and adaxial raised and rounded ridging is sometimes weakly developed.

Poa leaf-blades typically have one row of bulliform cells on either side of the mid-vein ($k^{\text{ad}} 2^{\circ} 1^{\circ} 3^{\circ} 2^{\circ} 3^{\circ} 1^{\circ} \dots \text{cap.}$), but a few species exhibit additional rows that are weakly developed (Lohaus, 1905; Metcalfe, 1960; Kawano & Tatewaki, 1961, fig. 2, *P. macrocalyx* Trautv. & C.A. Mey). Distinctly raised, rounded or blocky adaxial (but not abaxial) costal ridges are rare, but appear in *P.* sect. *Parodiochloa* (C.E. Hubb.) Soreng.

Leaf epidermal surfaces

The epidermis of *Poa hayachinensis* is shown in light (Figs. 5A–5D) and SEM (Figs. 7C, 7F) micrographs. Abaxial surfaces are smooth, with long (ca. 200 μm) and narrow (ca. 15 μm wide) rectangular intercostal long-cells with slightly sinuous walls, these alternate with mostly solitary short-cells; low-angled, stubby prickle-hairs are scattered and infrequent to rare, and stomata are infrequent or rare. The costal long-cells are frequently slightly narrower (ca. 10–15 μm), and smoother walled. Adaxial surfaces are moderately densely scabrous,

with short (ca. 100 μm , ca. 15–25 μm wide), smooth walled, irregularly hexagonal to elliptical to fusiform long-cells, these occasionally alternating with short-cells or without them, with regularly occurring, low-angled, stubby prickle-hairs, and frequent stomata; costal region long-cells tend to be longer (ca. 150 μm), narrower (ca. 15–20 μm), rectangular to fusiform, smooth-walled, frequently alternating with similar prickle hairs. Papillae are absent.

The epidermis of *Arctophila* has distinct costal and intercostal zones (Figs. 7A, 7D). The abaxial costal long-cells are shorter (ca. 100 μm), and are rectangular, or slightly irregularly fusiform in outline, and then often slightly inflated, and locally slightly bulbous in relief. The intercostal long-cells are mostly irregularly fusiform in outline, but of lower relief, or collapsed due to their thin walls (bulliform cells). Stomata and narrow, slender, nearly erect (high-angled) prickle-hairs are frequent in the junction between the costal and intercostal zones. The adaxial surface is similar to the abaxial surface with the exception of the absence of regular rectangular costal long-cells, and the intercostal long-cells average shorter (ca. 50–75 μm) and are more commonly somewhat hexagonal

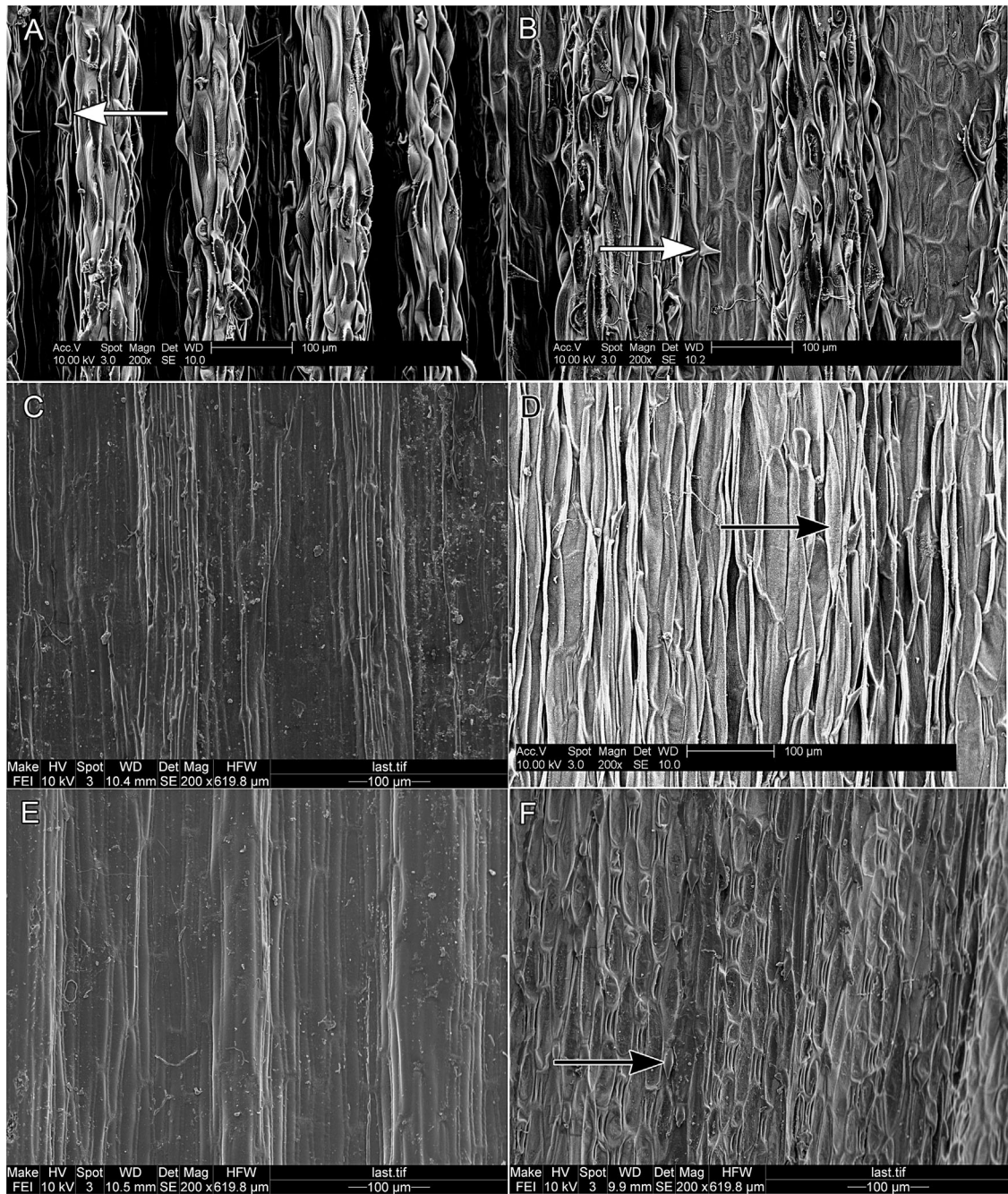


Fig. 7. SEM images of abaxial (left column) and adaxial (right column) leaf blade surfaces. Arrows point to prickles. **A, D,** *Arctophila fulva* (R. Soreng 6196, US; Alaska); **B, E,** *Dupontia fisheri* (R. Soreng 6198, US; Alaska); **C, F,** *Dupontiopsis hayachinensis* (H. Koba 153, US; Japan).

in outline and collapsed (bulliform cells). Stomata and prickles are somewhat more frequent adaxially. Papillae are absent or weakly developed.

The abaxial epidermis of *Dupontia* (Fig. 7B) is similar to that of *Poa hayachinensis*, low in topography, smooth, and with elongated, rectangular long-cell. The long-cells are similar in form, but somewhat narrower, and the lateral walls of the costal long-cells are smooth and intercostal long-cells are weakly sinuous to smooth. Prickles are absent, and stomata

are infrequent to rare along the costae and intercostae. The adaxial surface (Fig. 7E) differs from *P. hayachinensis* in having fusiform-elongate to somewhat hexagonal, smooth-walled, intercostal long-cells, and narrow fusiform smooth-walled costal long-cells. Prickles are frequent and confined to the costae, and are narrower, more slender, and more erect (high-angled) than those found in *P. hayachinensis*. Costal and intercostal stomata are common. Papillae are absent. Prickle-hairs in *Dupontia*, from our review of general collections, are

generally rare and at most infrequent on the abaxial surface, and are usually regularly occurring but sparse to moderately dense adaxially (Fig. 7E).

The epidermis of *Poa* is highly variable among species and covers most cases above. Prickles come in many shapes and sizes. Long-cells can be rectangular with sinuous walls, fusiform with smooth walls, or somewhat hexagonal. Papillae are known from only a few species. For SEM images of leaf surfaces of *Poa* and several other Poinae genera see; Gillespie (2007, figs. 1–3; *Bellardiochloa*, *Festucella*, *Hookerochloa*, *Nicoraepoa*, and *Poa* spp.), and Smithsonian, Department of Botany Images—Plant Image Archive (<http://collections.mnh.si.edu/search/botany/?ti=5>), select Photographer = Soreng, R.J. (for the above, and also *Arctagrostis*, *Arctopoa*, *Catabrosa*, *Hyalopoa*, *Puccinellia*).

Caryopses

In *Poa hayachinensis* the hilum is 20%–25% the length of the caryopsis (0.6–0.7 mm long), and narrowly elliptical. In *Arctophila* the hilum is 20%–25% the length of the caryopsis (0.3–0.4 mm long), and oval to narrowly elliptical, and in *Dupontia* it is 18%–27% the length (0.5–0.6 mm), and oval to elliptical. In *Poa* the hilum is mostly between 10 and 20% the length of the caryopsis (0.2–0.4 mm long), and round, oval or elliptical. *Poa billardierei* is an exception within *Poa* with a hilum 25%–30% the length of the caryopsis (0.8–1 mm long) and narrowly elliptical. The caryopsis is hard and glabrous in each of these genera. See Elect. Suppl: Appendix S2 for other PPAM genera.

Butt sheath hairs

Poa hayachinensis butt sheaths (near the base of the culms) express strigose hairs, both minute (ca. 0.05 mm) and longer (0.15 to ca. 0.3 mm). Similar hairs are present in *Dupontia*, but butt sheaths are glabrous in *Arctophila*. Butt sheath hairs are present in at least some species of other PPAM genera, including (* = minute only) *Alopecurus*, *Aniselytron*, *Arctagrostis*, *Arctopoa*, *Beckmannia* Host, *Bellardiochloa*, *Cinna*, *Coleanthus**, *Cyathopus**, *Hookerochloa*, *Limnas*, *Nicoraepoa*, *Phleum*, *Poa* *(rarely), *Simplicia* Kirk, and *Sylvipoa*. Butt sheaths are glabrous in all examined species of *Agrostopoa*, *Apera*, *Catabrosa*, *Catabrosella*, *Colpodium*, *Cornucopiae*, *Gaudinopsis*, *Hyalopoa* s.s., *Nepheleochloa*, *Paracolpodium*, *Parvotrisetum*, *Pholurus*, *Pseudopheleum*, *Rhizocephalus*, *Sclerochloa*, *Ventenata*, and *Zingeria*. In *Poa* they are known only in *P. glauca* Vahl, and there they are only of the shortest length.

Discussion

Reconstructing relationships among the genera in the Poinae, Alopecurinae, Phleinae, Miliinae, Coleanthinae clade (PPAM) has been challenging on both morphological and molecular grounds. Although we will expand on this topic in future papers, here we are concerned primarily with evaluating the relationships of *Poa hayachinensis*. However, even in doing this we must keep in mind that 11 of the 40 genera in this group are known only as polyploids, and may have reticulate origins (chromosome numbers are unknown for 11 other genera). Here we use DNA sequences, chromosome numbers, and comparative morphology and anatomy to support our interpretation of *P. hayachinensis* as a new genus related to

Arctophila and *Dupontia*. DNA plastid sequences tell us one side of the story of PPAM genera. DNA nuclear ribosomal sequences tell us another side of their story, but we are mindful that it is one which may be clouded by concerted evolution resulting in loss of one or the other parental contribution, along with possible recombination events (Stace 2005; Romaschenko et al., 2013). Reticulate histories in the PPAM clade have already been demonstrated for *Aniselytron*, *Arctopoa*, and *Nicoraepoa pugionifolia* (Speg.) Soreng and L.J. Gillespie, based on contradictory, but well supported, plastid and nrDNA phylogenies (Gillespie et al., 2008, 2010), and there are several nothogenera in the PPAM clade: \times *Arctodupontia* Tzvelev., \times *Duarctopoa* Soreng and L.J. Gillespie, \times *Pucciphippsia* Tzvelev. *Dupontia* has long been postulated to be of reticulate origin (Tzvelev, 1976; Kellogg & Watson, 1993; Soreng & Davis, 2000; Brysting et al., 2004), but only *Arctophila* has been confirmed as a probable parent, while the other parent, if there is one, has remained elusive (Brysting et al., 2004). The discovery of a new close relative of *Arctophila* and *Dupontia* sheds new light on this cold case.

Relationships according to DNA sequence data

DNA sequence data strongly support a close relationship of *Poa hayachinensis*, endemic to Japan, with *Arctophila* and *Dupontia*, two circumarctic monotypic genera placed in subtribe Poinae. These three taxa resolved together in a moderately to strongly supported clade (DAD clade) in plastid, nrDNA, and combined plastid-nrDNA analyses (Figs. 1, 2; Fig. 3 with separate nuclear and plastid DAD clades, the latter with moderate bootstrap support, although SCT collapsed here).

Within DAD *Poa hayachinensis* was moderately supported as sister to an *Arctophila*–*Dupontia* clade in the plastid analyses and the combined bootstrap analysis. Two unique plastid indels (not included in the analyses) provide further support for the *Arctophila*–*Dupontia* clade. Although the nuclear tree shows *P. hayachinensis* as sister to *Arctophila*, this relationship lacks BS support, and is likely erroneous (apparently based only on one shared but polymorphic ETS mutation). *Arctophila* and *Dupontia* resolve as monophyletic genera based on nrDNA data, but not based on plastid data; neither genus is distinguished by unique bp synapomorphies. All previous DNA studies that included *Arctophila* and *Dupontia* also showed their close relationship (Brysting et al., 2004; Döring et al., 2007; Gillespie et al., 2007, 2008, 2009, 2010; Döring, 2009; Kim et al., 2009; Schneider et al., 2009, 2012; Hoffmann et al., 2013). *Arctophila* and *Dupontia* are usually adjacent genera in taxonomies (Tzvelev, 1976; Clayton & Renvoize, 1986; Cayouette & Darbyshire, 2007; Darbyshire & Cayouette, 2007). In summary, we believe *Poa hayachinensis* is best understood as a new genus (*Dupontiopsis*, described in the Taxonomic treatment section) that is sister to *Arctophila* and *Dupontia*.

Our DNA results clearly show that the *Poa hayachinensis*–*Arctophila*–*Dupontia* clade (DAD) belongs to the major clade comprising all sampled subtribe Poinae and Alopecurinae genera but excluding *Poa* (PA-P clade). The genus *Poa* constitutes a distinct lineage apart from the PA-P clade, and thus *P. hayachinensis* is clearly excluded from *Poa*. Previous analyses also support inclusion of *Arctophila* and *Dupontia* in a Poinae–Alopecurinae clade, separate from the *Poa* clade (Döring et al., 2007; Gillespie et al., 2007, 2008, 2009, 2010; Döring, 2009; Kim et al., 2009; Schneider et al., 2009, 2012).

The position of DAD within the PA-P clade differs between plastid and nuclear analyses, supporting a reticulate origin for the DAD clade. The combined analysis (Fig. 3) clearly shows that DAD nuclear sequences resolve within PA-P (2), whereas DAD plastid sequences resolve within PA-P (1). DAD nuclear sequences resolve in a strongly supported clade with *Alopecurus* and *Limnas stelleri* (only nuclear data available). A sister relationship between DAD and *Limnas* was only weakly supported, and we found that *Limnas* shares no unique sequence synapomorphies with the DAD clade, suggesting that this relationship may not be stable. DAD plastid sequences resolve in a weakly supported clade with *Aniselytron*, *Cinna*, and nuclear sequences of *Arctopoa*, with weak Bootstrap support for a closer relationship with the latter two genera. Previous analyses that included sufficient density of taxa to narrow down the position of *Arctophila* and *Dupontia* among Poinae and Alopecurinae genera gave similar results to ours or were less resolved. Most studies suggested a relationship with *Alopecurus* (nrDNA analyses in Gillespie et al., 2008, 2009, 2010; and combined plastid-nrDNA analyses in Gillespie et al., 2010), or were insufficiently resolved (plastid analyses in Gillespie et al., 2008; Döring, 2009). In a large ITS analysis of PPAM genera, Hoffmann et al. (2013) found two *Limnas* species to be sister to a clade of *Dupontia* and *Arctophila*, these collectively nested within *Alopecurus*, but again with weak support values. A separate ETS analysis based on a different PPAM dataset, without *Poa hayachinensis*, showed strong support for *Limnas* as sister to an *Arctophila*-*Dupontia* clade, while relationships of these were unresolved in a separate ITS analysis (Boudko, 2014).

Chromosomes in DAD

We believe the common denominator of hexaploidy is key to understanding the origin of the DAD clade. All three members are hexaploid or higher, with a base number of $x = 7$. *Poa hayachinensis* has 71 counts of $2n = 42$ from 13 populations on Hokkaido and one on Honshu (Tateoka, 1985). *Arctophila* consists of one species, *A. fulva*, with $2n = 42$ (at least nine reports from across the Arctic: Flovik, 1938, 1040; Sokolovskaya & Strelkova, 1960; Löve & Ritchie, 1966; Hedberg, 1967; Zhukova 1967, 1980; Krogulevich, 1976; Cayouette & Blondeau, 1997; Brysting et al., 2004), and $2n = 63$ (one count from Wrangel Island; Zhukova & Petrovsky, 1972). *Dupontia* is more complex. Although sometimes separated into two or three subspecies or species with different ploidy levels and subtle morphological distinctions (Flovik, 1938; Bowden, 1960; Löve & Ritchie, 1966; Tzvelev, 1976; Probatova, 2003), Brysting et al. (2003, 2004) concluded that *Dupontia* consists of one morphologically and cytologically variable species. *Dupontia fisheri* s.l., has $2n = 42, 44, 63-66, 80, 84, 85, 88, 105-110$, and $126-132$ chromosomes (Flovik, 1938, 1940; Jorgensen et al., 1958; Bowden, 1960; Sokolovskaya & Strelkova, 1960; Löve & Ritchie, 1966; Zhukova, 1966, 1969; Petrovsky & Zhukova, 1978, 1081; Engelskjon, 1979; Darbyshire et al., 1992; Brysting et al., 2004), with hexaploid counts the most common. Ploidy level is sometimes interpreted as $x = 22$ (Flovik, 1938; Bowden, 1960; Tzvelev, 1976), although $x = 7$ is now viewed as the base number, with B chromosomes accounting for dysploid variations (Brysting et al., 2003, 2004).

The origin of DAD from a hexaploid ancestor is consistent with its monophyletic origin based on DNA sequences.

However, this needs to be couched in terms of the distribution of polyploidy among surrounding genera (see Elect. Suppl: Appendix S2). Diploid taxa within PPAM are common in *Phleum*, *Milium*, Coleanthinae, and *Alopecurus*, and genera in the *Bellardiocloa* clade are all diploid, but only polyploids are known in the PA-P (1) clade (where several genera are without counts). Thirty diploids are recorded in *Poa*, along with many tetraploids, higher polyploids, and polyploid series (Soreng et al., 2010).

Evidence for the hybrid origin of DAD and against the independent hybrid origin of *Dupontia*

The discovery of a new lineage so closely related to *Arctophila* and *Dupontia* led us to revisit hypotheses of the probable hybrid origin of *Dupontia* (Tzvelev, 1976; Soreng & Davis, 2000; Brysting et al., 2004). Multiple nrDNA copies were detected in all samples of *Poa hayachinensis*, *Arctophila*, and *Dupontia* suggesting that all three taxa may have originated via hybridization. As discussed above, a common hexaploid base number is consistent with a single origin hypothesis of DAD. Strong support for the DAD clade and its different positions in the nuclear, plastid, and combined analyses also suggests a single hybrid origin. The lack of sequence differentiation in *Arctophila* and *Dupontia*, combined with shared indels support their placement as sister taxa with a complex evolutionary history, including possibly incomplete separation or introgression, a situation where reciprocal monophyly is called into question (Kiziriana & Donnelly, 2004). In contrast, greater sequence divergence, and absence of the above indels suggest an independent history for *P. hayachinensis*. We postulate here that the DAD clade originated at the hexaploid level from a single ancient hybridization event. Judging by the geography of the taxa, this event can reasonably be localized to western Beringia, with the branch leading to *P. hayachinensis* becoming isolated in Japan, and the branch leading to *Arctophila* and *Dupontia* diversifying in the Arctic. All three taxa favor wet habitats. Any alternative hypotheses we considered for the origin of these three taxa would require two or three reticulate events, and would not explain how they share a hexaploid base number, particularly one that is stable in *P. hayachinensis*, and fairly stable in *Arctophila*, and common in *Dupontia*; nor would they provide a simple geographic explanation for the origin of DAD.

But what of the long-standing hypotheses that *Dupontia* arose from wide hybridization? Tzvelev (1976, p. 488 [1983, p. 737]) suggested different origins for the three subspecies of *D. fisheri* that he recognized: subsp. *psilosantha* (Rupr.) Hultén (hexaploid) anciently derived from *Arctophila* × *Deschampsia cespitosa* s.l.; subsp. *pelligera* (Rupr.) Tzvelev ($2n=88$) from a cross between subsp. *psilosantha* and an Arctic species of *Puccinellia* sp.; and subsp. *fisheri* (the highest polyploids), possibly an autopolyploid derived from subspecies *pelligera* × *psilosantha*. An allopolyploid origin of *Dupontia* was considered likely by Kellogg & Watson (1993) and Soreng & Davis (2000) due to the unusual morphology of the genus and its unusually high and dysploid chromosome numbers. Brysting et al. (2004) evaluated molecular and cytogenetic evidence for a reticulate origin of *Dupontia* using plastid and cloned ITS sequences, in situ hybridization (FISH), and AFLP fingerprinting approaches. They (ibid., p. 379, 365) concluded

that *Dupontia* had “a possible origin within the Arctic area as a result of reticulate evolution (e.g., intergeneric hybrid speciation),” and “that *Dupontia* and *Arctophila* are closely related and that the latter might have been involved in a possible intergeneric hybrid origin of *Dupontia*.” They soundly rejected the hypothesis that a species of *Deschampsia* P. Beauv. was the second parent, and suggested that *Poa eminens* J. Presl (\equiv *Arctopoa eminens*) was a more likely candidate (based in part on overlapping geography, preference for wet arctic habitat, and near basal position in a plastid phylogeny of *Poa*; Soreng, 1990). This connection was bolstered by morphological similarities of *Dupontia* and *Arctopoa eminens* and the fact that they do occasionally hybridize (\times *Duarctopoa* Soreng & L.J. Gillespie; \times *Dupoa* J. Cay. & Darbysh.; Darbyshire et al., 1992; Cayouette & Darbyshire, 1993; Darbyshire & Cayouette, 2007). Using DNA sequence data Brysting et al. (2004) showed that *Dupontia* together with *Arctophila* was sister to *Arctagrostis*, but phylogenetically isolated from *Deschampsia* s.s. or s.l. (incl. *Avenella* [Bluff & Fingerh.] Drejer). Their FISH data revealed that *Arctophila* DNA had a strong affinity for *Dupontia* chromosomes, but *Poa* and *Deschampsia* nuclear DNA did not bind with *Dupontia* chromosomes. Although 29 of their 31 unique cloned *Dupontia* ITS sequences (cloned from individuals of ten widely geographically distributed populations) aligned around *Arctophila* (with few bp differences), Brysting et al. (2004) also cloned two very divergent ITS copies whose positions were unresolved in their strict consensus tree. The latter clones resolved in some of their MP trees as sister to *Poa*, in others at the base of the *Dupontia*–*Alopecurus*–*Arctagrostis* clade. When included in our dataset and analyzed via MP, these two divergent ITS sequences resolved within *Puccinellia*, specifically with the diploid *Pucc. arctica* complex. Given our hypothesis of a shared hexaploid origin for the DAD clade and the higher ploidy level of these two *Dupontia* samples ($2n = 84$ – 88 , duodecaploids; Brysting et al., 2004), the presence of these divergent ITS sequences suggests secondary hybridization with *Pucc. arctica*, or a related species, rather than a separate hybrid origin for *Dupontia* (as they interpreted it). The cloned sequence from Melville Island, Canada (CAN2 c4) closely matches *Pucc. arctica* (differing only in a single base) and *Pucc. banksiensis* (differing in two bases). Although *Pucc. arctica* and *Pucc. banksiensis* were not found at the site (LJG, pers. observ.), nor are they known from Melville Island, both species occur on nearby Banks and Victoria Islands. *Puccinellia arctica* sequences have also been detected in ITS copies of two polyploid species, *Pucc. angustata* (R. Br.) E.L. Rand & Redfield (hexaploid) and *Pucc. andersonii* Swallen (mostly octoploid), both of which occur on Melville Island (Consaul et al., 2010), suggesting the possibility that one of these species may have formed hybrids with *Dupontia*. The cloned sequence from Svalbard (SVA5 c13) is somewhat more divergent, but still aligned with the Nearctic *Pucc. arctica* complex; *Pucc. angustata* is known from Svalbard and may have contributed the *Pucc. arctica* sequence, but there has been insufficient sampling of *Puccinellia* from Svalbard to determine if another species might be involved. We agree with results of the Brysting et al. (2004) that hexaploid *Dupontia* is closely related to *Arctophila*, but consider the divergent sequences cloned from duodecaploid *Dupontia* are indicative of secondary hybridization with *Puccinellia* (as

predicted by Tzvelev, 1976), rather than pointing to the of origin of the genus.

Our separate and combined DNA data analyses, by expanding on the limited generic sampling employed by Brysting et al. (2004), fully corroborates and strengthens the hypothesis that *Arctophila* and *Dupontia* are inextricably linked, and suggests that *Dupontia* did not originate independently via reticulation involving a genus outside of the DAD clade and that *Poa hayachinensis* is only slightly removed from the previous pair.

What then might the parents of DAD have been? All data suggest *Arctophila* and *Dupontia* evolved within the Poinae PA-P alliance; based on phylogenetic studies (Soreng & Davis, 2000; Döring et al., 2007; Soreng et al., 2007; Gillespie et al., 2008, 2009, 2010; Döring, 2009; Kim et al., 2009; Schneider et al., 2009, 2012; Hoffmann et al., 2013) the PA-P alliance, has grown to ca. 26 genera (Soreng et al., 2014). Since no single genus has been strongly supported as sister to DAD in nrDNA or plastid analyses we also consider morphology, cytology, and geography as guides to parentage of the clade. Our analyses of DAD nuclear sequences support *Limnas* or an ancestor of *Limnas* and *Alopecurus* as one parent of DAD (Figs. 2, 3), whereas analyses of DAD plastid sequences point towards *Cinna* or *Arctopoa* as a possible second parent (Figs. 1, 3; relationship with *Cinna* and *Arctopoa* has weak Bootstrap support, not shown on the SCTs).

Limnas and *Alopecurus* have little in common morphologically with DAD; unlike DAD genera, they have 1-flowered spikelets that disarticulate below the glumes, first and second glumes fused on one side, lemmas without a crown of hairs at the base and with a dorsal geniculate awn, caryopses with soft endosperm (RJS, new observation) and styles fused at the base. *Limnas* (with three closely related species) has traditionally been closely allied to *Alopecurus* (Tzvelev, 1976; Clayton & Renvoize, 1986—where *Alopecurinae* included only these two genera). Ploidy level and geographic distribution are not inconsistent with a relationship with DAD. *Alopecurus* species range from diploid to 18 ploid, while for *Limnas* only one chromosome number ($2n = 28$) is known for only one species (*L. stelleri*—Probatova & Sokolovskaya, 1984) as far as we are aware. *Limnas* is confined to arctic and alpine northeast Asia where DAD probably arose; *Alopecurus* ranges circumboreally. Members of the *Bellardiachloa* clade are geographically isolated in southern Europe and adjacent Near East Asia, which also makes them unlikely candidates for DAD parents. Their morphology is coherent and reasonably transitional from PA-P genera with plesiomorphic character states to that of *Alopecurus* and *Limnas*. The genera are diploid, with soft or liquid endosperm, leaf-blades often involute, adaxially pronouncedly ribbed and coarsely scabrid-hispidulous, spikelets disarticulating above (rarely also below) the glumes, lemmas awned (awn dorsal and geniculate, or terminal), spikelets sometimes 1-flowered, callus usually with a crown of hairs, and annual except for *Bellardiachloa*. Nothing in the morphology of the above genera suggests a parental relationship to DAD, other than that all have awned lemmas and many have hairy calluses.

Cinna, or an ancestor close to it, might have contributed its plastid DNA to the immediate ancestor of DAD. The genus has three species, of which one ranges circumboreally in wetlands. Morphologically, however, we note that spikelets of

Cinna, like *Alopecurus* and *Limnas*, are 1-flowered, with disarticulation below the glumes, and their caryopses have soft endosperm, characteristics that make for an awkward scenario to arrive at the multi-flowered spikelet with disarticulation above the glumes, and hard endosperm, found in DAD. An early ancestor (or parent) of *Arctopoa* (i.e., before capturing a *Poa* plastid; Gillespie et al., 2008, 2010) remains a logical candidate for a plastid parent of DAD on both morphological and geographical grounds (Brysting et al., 2004), and is supported here by the sister relationship of *Arctopoa* to *Cinna* in our nrDNA tree (Fig. 2). *Arctopoa* species are mainly reported to be hexaploid, with a few tetraploid counts known in two species (it would be interesting to verify whether the latter have *Poa* or *Cinna*-like plastids). Currently, our best hypothesis for the origin of the DAD clade has the plastid type donated by an early lineage of the PA-P(1) clade, and the dominant nrDNA type contributed by a species that was a progenitor of the clade that contains *Alopecurus* and *Limnas*.

Morphological considerations for the placement of *Poa hayachinensis* and DAD

Our molecular results supporting *Poa hayachinensis* as a distinct lineage closely allied to *Dupontia* and *Arctophila* led us to reexamine its morphological characteristics in the context of this relationship (see Results). *Arctophila* and *Dupontia* are adjacent in modern classifications, and here we provide evidence that *P. hayachinensis* is reasonably placed with these two genera on morphological grounds, and is most similar to *Dupontia*. Morphological character evolution in Poeae and subtribe Poinae remains poorly understood and many characters are known to show extensive homoplasy (Soreng et al., 2007). Poinae genera are often distinguished by subtle morphological differences (see Elect. Suppl: Appendix S2 for a tabulation of some characteristics of genera of the PPAM clade). Some of the following characteristics must be framed in terms of their distribution within PPAM in order to understand their significance to the taxonomy of DAD.

In aspect and morphology *Poa hayachinensis* approaches *Dupontia fisheri* more closely than it does any other species in PPAM, but it is generally more robust and has larger spikelets than *Dupontia*. *Poa hayachinensis* most obviously differs from *Arctophila* and *Dupontia* in having glumes shorter than the lowest floret, keeled lemmas, and scabrous (versus smooth) palea keels. In these details it agrees with *Poa*. It differs from *Poa* by having lemmas principally 3-nerved, distally golden-brown scarious to hyaline for $\frac{1}{3}$ to $\frac{1}{4}$ the length, the keel scarcely curved at base and the apex narrowly and sharply pointed, occasionally to frequently with a slender apical micro or short awn, callus with a crown of hairs, and a longer hilum, all features shared with *Dupontia*, and some with *Arctophila*. The lemmas of *Arctophila*, *Dupontia*, and *Poa hayachinensis* are 3 (5)-nerved, but the intermediate veins, when present, are obscure to faint in these taxa (see also Elect. Suppl: Appendix S2). *Poa hayachinensis* is described as having 5-nerved lemmas (Ohwi, 1965; Osada, 1993), however, in our review of specimens the normal state is 3-nerved. In the vast majority of *Poa* species the lemmas are 5-nerved, sometimes with faint or rarely obscure intermediate veins (rarely up to 7–11-nerved). Only a few *Poa* species are primarily 3-nerved.

Details of the callus in DAD and *Poa* are provided in Results, and are discussed here in a broader context. Callus hairs are important for dispersal by wind or animals, and lodging of the diaspores into suitable habitat (Davidse, 1987). Presence of a hairy callus at the base of the lemma is presumably plesiomorphic in PPAM (present in 21 of 40 genera, Elect. Suppl: Appendix S2, and widespread among out-groups). Callus hair presence and diversity is substantial but often subtle in the PPAM clade, and is critical to the identification of genera. A hairy callus is present in 13 PPAM genera with perennial species and multiple-flowered spikelets that disarticulate above the glumes (see key to these genera below), and many of the genera with only annuals. Callus hairs are distributed as a crown around the base of the lemma in most of these genera (including all DAD taxa and some *Poa*), as a beard of hairs on an elongated angled callus in a few genera (sometimes slightly bearded in *Poa hayachinensis*), or only in *Poa* as a dorsal tuft (see Results, Spikelets for more detail on *Poa*). The callus hairs in *Arctophila* and *Dupontia* are straighter, stouter and sparser than in *P. hayachinensis*, and are never diffused down the callus sides (sub-bearded). Callus shape is also important. In PPAM genera other than *Poa*, the callus is generally short and blunt, and smoothly rounded on the back to slightly dorsiventrally compressed (never dorsally pinched as in many *Poa*). Calluses in *P. hayachinensis* are slightly elongated and angled downward dorsally, a rare condition in PPAM.

How *Poa* sect. *Secundae* (to which we initially guessed *P. hayachinensis* might be related) came to have a callus that is abaxially dorsiventrally compressed and with a crown of hairs AND rounded lemma keels is a mystery. However, the hypothesis that reticulation was involved (Stebbins, 1950; p. 404 with *Puccinellia* suggested) has some merit (but probably only between groups of *Poa* in this case, as *Secundae* species share N plastid genotypes and ss nrDNA genotypes; see Soreng et al., 2010). Possibly the mixing of divergent genomes in this allopolyploid lineage of *Poa* disrupted the normal pattern of development giving rise to the reappearance of these plesiomorphic, states. Such re-patterning of developmental morphology may apply widely to hybrids (Soreng & Davis, 2000; Rogers Ackermann et al., 2006), including DAD, and obscure their parentages (Kellogg & Watson, 1993).

Lipid in the grass caryopsis endosperm is confined in subfamily Pooideae to the tribe Poeae, but is widespread there (Rosengurt et al., 1972; Clayton & Renvoise, 1986; Watson & Dallwitz, 1992 onward). Owing to the absence or presence and amount of lipid in the endosperm, caryopses in PPAM vary from hard to soft (Elect. Suppl: Appendix S2), but in genera with hard endosperm a trace of lipid can be tested for by staining (Rosengurt et al., 1972). Within PAM, 16 genera including all in PA-P (2) (except *Cornucopiae* and DAD taxa), have soft or semisoft endosperm in some or all species, while *Poa*, DAD taxa, and 11 other genera have hard endosperm in all species. Of the latter only three were tested for lipid by Rosengurt et al. (1972) (*Cornucopiae*, *Milium*, and *Poa*), and lipid was present. Further testing would be useful.

Caryopses of DAD taxa have longer hila, and hila that are proportionally longer (*Arctophila* the shorter of the three), than almost all *Poa*. The plesiomorphic states in Pooideae are of a linear hilum about as long as the grain, but these vary within tribe Poeae. In PPAM the hilum is rarely linear, and is

66% or less the length of the grain, longest in the Coleanthinae clade and *Millium*, with *Poa* among the genera with the shortest (see Elect. Suppl: Appendix S2).

Ciliate tipped lodicules are rarely found in Poinae, but are known from *Poa hayachinensis* (Kawano & Tatewaki, 1961 fig. 3, and RJS pers. observ.), *Bellardiochloa*, *Hookerchloa*, and *Nicoraepoa* (Soreng & Gillespie, 2007), *Arctopoa eminens* (RJS pers. observ.), *Saxipoa* (Soreng et al., 2009), but lodicules are glabrous in *Arctophila*, *Dupontia* and *Poa* (Elect. Suppl: Appendix S2).

Leaf anatomy is sometimes diagnostic for taxa in PPAM (see also Soreng & Gillespie, 2007; for SEM epidermal images of several other genera and species of *Poa*), and shows some differences between *Poa hayachinensis*, *Arctophila*, and *Dupontia*. *Poa hayachinensis* has leaf abaxial epidermis anatomy (Fig. 5) similar to *Dupontia fisheri* (Fig. 7), but differs in the intercostal long-cell shape in the adaxial epidermis (Fig. 7), and in the distribution and shape of prickly hairs. The description of the epidermis of *Dupontia* by Metcalfe (1960, p. 167), derived from Lohaus's description (1905, p. 75, without figure), includes "Prickles alternating with rectangular long-cells over the veins on both surfaces." This description suggests that both epidermal surfaces have lots of prickles, but our observations indicate prickles are consistently rare on the abaxial surface of both *P. hayachinensis* and *Dupontia*. These genera and *Poa* differ markedly from *Arctophila*, by the lack or limited development of prominently raised and rounded ab- and adaxial costal ridges, which is best seen in blade cross-sections (Fig. 6), and presence of 2nd and 3rd order vascular bundles (absent at least centrally in *Arctophila*). In cross-sectional leaf anatomy Darbyshire et al. (1992, fig. 3: C) noted there are parenchymal air spaces in *Dupontia* blades, which are typical of many aquatic grasses, but these were not observed by us or reported by others in *Arctophila* or *P. hayachinensis*. Darbyshire (pers. comm.) suggested the collapse of such air spaces in *Dupontia* may account for the somewhat raised and rounded ridging in blades noted by us in herbarium material of the genus (see Results). More extensive survey of leaf anatomy of PPAM taxa, including *Poa*, is needed, and at this point we cannot say the anatomy of *P. hayachinensis* differs from the majority of *Poa* by any particular characteristic other than in the tendency to have one or two extra central rows of bulliform cells.

Ligules in DAD taxa are similar in form, generally truncate, smooth and glabrous on the back, with brownish coloration, but these vary to acute and off-white in *P. hayachinensis* uppermost culm leaves. *Poa* ligules differ in being almost uniformly clear hyaline or scarious white (rarely tinted), and vary from truncate to acuminate (rarely lacerate). The upper ligule rim is asperous in at least the ligules of lower culm and lateral shoot leaves of *P. hayachinensis*. The latter state is rare in *Poa* and absent in *Arctophila* and *Dupontia*. The distribution of butt sheath hairs is incompletely known in PPAM. These hairs are long in *P. hayachinensis* and *Dupontia*, and minute in the only species of *Poa* where they were seen. These hairs are also present in at least some species of 16 other PPAM genera, but were absent in examined species of 18 other genera including *Arctophila*.

Overall, morphological features of *Poa hayachinensis* point towards a relationship with *Dupontia* and *Arctophila*. Although many morphological states found in *P. hayachinensis* are also

found in *Poa*, most are rare and occur only in one or several species of *Poa*. It is the combination of these rare states in *P. hayachinensis* that makes it stand out from *Poa*. Cumulatively, along with DNA phylogenetic evidence, these character differences show that *P. hayachinensis* is something special, related to *Arctophila* and *Dupontia*, and deserving of generic status. In the following section a new genus is described for *P. hayachinensis* and a key is provided to separate the perennial genera of PPAM that have multiple flowered spikelets and hairy calluses.

Taxonomic treatment

Dupontiopsis Soreng, L.J. Gillespie and Koba, **gen. nov.**

Type: *Dupontiopsis hayachinensis*. Monotypic.

Genus Diagnosis: Differing from *Poa* by ligules brownish, asperous-margined, calluses slightly elongated, slightly pointed, with a dense crown of hairs 0.4–0.8 mm long, lemmas principally 3-nerved, keel not at all gibbous-arched near the base, apices narrowly acute to acuminate and frequently tapered to a slender awn up to 0.5 mm long, lodicules frequently with apical hairs, hilum $\frac{1}{4}$ to $\frac{1}{5}$ the grain in length. Differing from *Arctophila* and *Dupontia* by lemmas distinctly keeled, palea keels regularly scabrous for most of the length, lodicules with apical hairs, glumes distinctly shorter than the lowest floret, and from *Arctopoa* by lemmas principally 3-nerved, distally scarious to hyaline for $\frac{1}{5}$ to $\frac{1}{4}$ the length, apices narrowly acute to acuminate and frequently tapered to a slender awn up to 0.5 mm, glume and lemma margins eciliate.

Genus Description: Perennial. Hermaphroditic. Rhizomatous. Vegetative shoots extravaginal. **Culms** decumbent to sub-erect, terete or slightly elliptical, hollow. **Leaves: sheath** margins of upper culm leaves fused up to $\frac{1}{5}$ their length; **collars** smooth, glabrous, auricles absent; **ligules** of proximal and sometimes upper culm leaves bronzy-brownish, abaxially smooth, glabrous, apical margins entire or erose, asperous or smooth; **blades** flat (to folded), with slightly incurved margins, keeled, adaxially with 1(–2) grooves on either side of the midrib, blade apex gradually tapered. **Inflorescence** simple paniculate, loosely contracted to open, broadly lanceolate to laxly pyramidal, sparsely flowered; **branches** 2–4 per node, ascending to spreading, sometimes reflexed below, somewhat flexuous, smooth, angled. **Spikelets** 2–4 (–5)-flowered, laterally compressed, disarticulating above the glumes and between florets; **glumes** lanceolate, sub-equal, or slightly unequal, slightly shorter than the adjacent lemmas, weakly keeled, apex narrowly acute to acuminate, sharply pointed, sometimes briefly awned, 1–3-nerved; **rachilla** internodes terete or slightly dorsally compressed; **calluses** slightly elongated, with a dense crown (to sub-bearded) of slightly sinuous short hairs; **lemmas** lanceolate, the base tapered or only slightly arched (not at all gibbous at base), 3 (infrequently obscurely to faintly 5)-nerved, laterally compressed, keeled, pubescent, apex narrowly acute to acuminate to a smooth point or frequently a slender awn; **paleas** distinctly 2-keeled, keels regularly and closely scabrous, glabrous; **flowers** perfect; **lodicules** 2, broadly oblanceolate to sub-flabellate, margins with 1 or 2 lateral lobes, and 0 or 1–4 minute hairs;

stamens 3; **ovary** glabrous, **styles** 2, apical, free, adjacent, erect, stigmas white, lanceoloid, plumose; **caryopses** laterally compressed, firm, free from the palea, hilum short. Chromosome number: $x = 7$, $2n = 42$.

Dupontiaopsis hayachinensis (Koidz.) Soreng, L.J. Gillespie and Koba, **comb. nov.**—*Poa hayachinensis* Koidz., Bot. Mag. (Tokyo) 31: 254–255. 1917—**Type:** G. Koidzumi, Japan: Honshu: Prov. Rikutsiu [Itwate Pref.], Mt. Hayachinesan, alpine belt, Jul 1914 (holotype, TI-M04-03-3, photo, US!; isotype, TI-M04-03-4, photo, US!).

Poa yezoalpina Tatew. and Kawano, J. Jap. Bot. 36: 337, f. 1, f. 2b, f. 3c. 1961—**Type Protologue:** S. Watanabe, Japan: Hokkaido: Prov. Tokachi [Tokachi Sub-Pref.]: Mt. Tottabetsu, 15 August 1959 (holotype: SAPS).

Images: Holotype (Fig. 8), spikelet (Fig. 4), ligule (Fig. 5), leaf-blade anatomy (Figs. 5–7). **Iconography:** Kawano and Tatewaki (1961, fig. 1: A–E; as *P. yezoalpina*); Osada (1993, p. 199).

Species Description: Plants densely to loosely tufted, rhizomes short. Roots up to 5 per node, not curly. Vegetative



Fig. 8. *Poa hayachinensis* Koidz. holotype: TI-M04-03-3. (US photo), ≡ *Dupontiaopsis hayachinensis* (Koidz.) Soreng, L.J. Gillespie & Koba, type species of *Dupontiaopsis* Soreng, L.J. Gillespie and Koba.

shoots with up to seven scarious, brown cataphylls. **Culms** 50–60 cm tall, 1.5–2.25 mm diam. below, culms and nodes smooth, (1–) 2–3 nodes exposed. **Leaves: sheaths** with moderately pronounced veins, proximal sheaths cataphyllous, bronze colored, glabrous, or the lowest (butt sheaths) with some stiffly strigose, appressed, or hooks (these sometimes obscure and present only adjacent to the subtending node), hairs ca. 0.05–0.3 mm long, proximal few bladed sheaths with a few diagonal cross-veins, sparsely to moderately scabrous mainly on longitudinal-veins, middle and upper culm sheaths, bladed, smooth or nearly so, sheaths in age becoming papery to a little fibrous, closed to near the collar or for most of their length, except the uppermost 1–2 culm ones closed $\frac{1}{2}$ to $\frac{3}{4}$ their length, the uppermost one 6–8 cm long, shorter than its blade by $\frac{1}{2}$ to $\frac{3}{4}$; **ligules** of lower culm leaves and lateral shoots 0.2–0.5 mm long, apex truncate, margins asperous, of upper culm leaves to 0.5–3.3 mm long and bronzy-brownish to off-white, apex truncate to acute, margins asperous in shorter ligules, smooth to sparsely asperous in longer ligules; **blades** of vegetative shoots up to 30 cm long, but otherwise like those of the culms, of culms 5–8 above the short proximal initial two to three leaves, gradually reduced upward from the longest one (the top one equaling or longer than its sheath), longest 10–25 cm long, 2.5–5.5 mm wide, distally narrow and scabrous, margins proximally smooth, distally scabrous, *abaxially* keeled, keel raised, proximally smooth, distally scabrous, lateral veins narrow (ca. 10–22 total), moderately pronounced (to low-rounded) costal ridges, smooth throughout or distally moderately scabrous, *adaxially* the surface nearly flat or slightly undulating in cross-section, smooth or moderately scabrous with short low-angled stubby hooks scattered over the surface, intercostal gaps mostly 2–3 times broader than the costae, blade apex not or only very slightly naviculate. **Inflorescence** 7–14 cm long, with 2–4 cm long proximal internodes, spikelets 20–50; **branches** slender, longest branches 3–8 cm, naked below, with 2–8 spikelets in the distal half, pedicles 1–10 mm, smooth or occasionally with one or a few hooks in the distal 0.5 mm, with occasional subulate to glume-like bracts (to 5 mm long) at panicle branch secondary nodes and below glumes. **Spikelets** 5.5–9.5 mm; **glumes** proximally herbaceous membranous, distal $\frac{1}{2}$ to $\frac{3}{4}$ scarious to hyaline in the margins, keel smooth or infrequently sparsely scabrous, surfaces smooth, sparsely muriculate, margins smooth or sparsely to moderately densely scaberulous, *first* glume 2.9–4.5 mm, 1–1.5 mm wide in side view, *second* glume 4–6.5 mm, 1.3–1.8 mm wide in side view; **rachilla** internodes smooth or sparsely scabrous, glabrous (or with a sparse hairs up to 0.5 mm long, like those of the callus), but not concentrated into an apical crown), proximal internode short, 0.2–0.4 mm, second internode 1.3–1.8 mm, upper internodes ca. 1.4–1.7 mm, with or without a reduced terminal floret; **calluses** to 0.3 mm long, of distal florets angled to about 45°, of proximal floret slightly laterally compressed, oval (widest *adaxially*), of distal 2–3 florets progressively slightly to strongly dorsiventrally compressed, elliptical, densely pubescent around the sides and back with a crown of hairs that is sometimes slightly diffused along the elongated callus surface, hairs soft, white, slightly sinuous, ca. 0.4–1 mm long; **lemmas** 5–7 mm long, thinly herbaceous, regularly pubescent on the lower $\frac{1}{2}$ to $\frac{3}{4}$ of keel and $\frac{1}{4}$ to $\frac{3}{5}$ of

marginal veins with soft, white, sinuous hairs 0.2–0.8 mm long, keel sparingly scabrous above, distally thinly herbaceous for $\frac{1}{4}$ to $\frac{3}{5}$ the length, and golden-brown, scarious to hyaline above, surfaces smooth or sparingly to moderately scabrous between veins, glabrous, or with sparse short hairs near the lower back, margins inrolled below, regularly to irregularly scaberulous on the outer edge above, apex of the proximal ones narrowly acute to acuminate, tapered to a smooth point or a awn to 0.5 mm long; **paleas** apically distinctly notched, keels projecting as points or slender awns to 0.5 mm long, shorter (by up to 1 mm in proximal florets) to sub-equaling their lemmas, keels chlorophyllous, regularly and closely scabrous for up to $\frac{1}{2}$ the length in proximal florets (hooks diffuse, not in a single file), but distally smooth for up to 1 mm (hooks moderately large in comparison to most *Poa*, progressively reduced in number, size and density in distal florets), inter-keel ca. 0.8 mm wide, outer flanges 0.3–0.4 mm wide; outer flanges and inter-keel surfaces thin-herbaceous to whitish-scarious, to hyaline at the edges, smooth or with few hooks; **lodicules** 0.6–0.8 mm long; **anthers** (1.8–) 2.5–3.3 mm long; **stigmas** with secondary branching, moderately dense; **caryopses** [Furuse 1992b] 2.8 mm long, fusiform in lateral view (0.9 mm deep), hard, light brown, translucent, free from the palea, ventrally shallowly sulcate, hilum 0.6–0.7 mm long ($\frac{1}{4}$ to $\frac{1}{2}$ the grain length), narrowly elliptical.

Leaf-blade Anatomy & Epidermis and cross-section: (see RESULTS; Figs. 3–5).

Distribution: *Dupontioipsis hayachinensis* is endemic to Japan, occurring on one mountain on northern Honshu (Iwate Pref., Mt. Hayachine), and several mountains on Hokkaido (Hidaka, Kamikawa, Sorachi, Tokachi sub-prefs.).

Habitat: Alpine, in wet gravelly soils, among boulders, and along streams. Koyama (1987, p. 101) wrote that the species is “considered a *relict* in limestone areas,” with an elevation range of around 1,900 m on Honshu, and 1,400–2,200 m on Hokkaido. However, Kitamura (1952) listed *Poa hayachinensis* among serpentine endemics on Mt. Hayachine, and other sources suggest it co-occurs with other serpentine endemics on both Honshu and Hokkaido (Kawase et al., 2009). Our coauthor, Hidehisa Koba, agrees that serpentine, not limestone, is correct.

Conservation Status: Osada (1993) comments that the species is uncommon. The species (as *Poa hayachinensis*) is listed as Endangered on the Red List of Threatened plants of Japan (available online http://www.biodic.go.jp/english/rdb/rdb_e.html). The Japanese Ministry of the Environment used IUCN 1994 criteria for this listing. The current status using 2014 IUCN criteria is Rare (IUCN Global Species Programme Red List Unit, 2014). The species is not listed under CITES.

Additional specimens examined

Japan. Hokkaido: Kamikawa Sub-Pref.—Daisetsu Mts., Mt. Chubetsu-dake Takanegahara, 28 July 1955, S. Kawano (SAPS, paratype of *P. yezoalpina*); Sorachi-gun, Yamabe-mura [Furano-shi], Mt. Ashibetsu, 1,720 m, 28 July 1956, M. Furuse 31036 (K); vicinity and summit Mt. Ashibetsu, 1,700 m, 27 July 1957, M. Furuse 32648 (K); Minami-furano-mura [Minami-furano-cho], about summit of Mt. Yubari-dake, 1,400–1,668 m, 31 July 1956, M. Furuse 31198 (K); Yubari-dake, 9 August 1936, Kakuo Uno 16851 (US); Mt. Yubari, July 1934, J. Ohwi (US);

Tokachi Sub-Pref.—National Park Daisetsu, Mt. Hakuun-dake, 1,900–2,200 m, 30 July 1960, *M. Furuse* 37282 (K); National Park Daisetsu, summit from half-way up Mt. Nisei-kaushuppe, 1,500–1,850 m, 3 August 1960, *M. Furuse* 37380 (K); National Park Daisetsu, summit Mt. Nisei-kaushuppe, ca. 1,850 m, 3 August 1960, *M. Furuse* 37411 (K); Daisetsu-sanke, Mt. Tomuranshi, Kamuishuzan, in alpine herbages, 1,700 m, 2 August 1965, *T. Yamazaki* 5038 (BM ex Univ. Tokyo). **Honshu:** Iwate Pref., Shimo-heiji-gun Kawai-mura, Mt. Hayachine, 1,913 m, 16 August 1948, *M. Furuse* 19926 & 19926 (K); Mt. Hayachine, alpine belt, 1,900 m, 14 July 1954, *T. Tateoka* (C, US [presumably a cytological voucher, $2n = 42$; Tateoka 1955]); Hienuki-gun, Ohasama-machi, Summit of Mt. Hayachine, 39° 33' 29" N 141° 29' 21" E, 1,913 m, 1 August 2005, *Hidehisa Koba and Hiroko Koba* (KPM, DAO, US); DNA vouchers—39° 33' 30" N 141° 29' 20" E, 1,913 m, 25 July 2009, *Hidehisa Koba* 151 (TI, US), 152 (US), 153 (US).

Other material (not seen by us): Kawano and Tatewaki (1961) also list for the distribution of *P. yezoalpina*—**Hokkaido:** Tokachi Sub-Pref. [Hidaka Range]—Mt. Esaomantottabetus 28 July 1927, *M. Tatewaki*; Upper branch, Tottabetsu River, Pirikapetan, 26 Jul 1927, *M. Tatewaki*; Mt. Porojiri, 10 Jul 1929, *M. Sakamoto and Y. Tokunaga*; and Mt. Tottabetsu, 15 August 1959, *S. Watanabe* (**Type** of *P. yezoalpina*). Also see cytological vouchers in Notes (below).

Notes: Chromosome numbers of *Poa hayachinensis* were studied by Tateoka (1955, 1985); 71 counts from Hokkaido and one from Honshu were all $2n = 42$. His vouchers (TNS) are from **Hokkaido:** Tokachi Sub-Pref.—Daisetsu Range [boundary between Kamikawa & Tokachi sub-prefs.]—Mt. Aka-dake (9 vouchers), Mt. Hokkaidake-Mt. Hakuun-dake (3 vouchers), Mt. Kuro-dake (5 vouchers); Hidaka Sub-Pref. [Hidaka Range]—Mt. Poroshiri-dake (12 vouchers) and Mt. Tottabetsu-dake (10 vouchers); Sorachi, Sub-Pref.—Mt. Yubari-dake (4 vouchers). **Honshu:** Mt. Hayachine (cited above). Tateoka (1985) concluded *P. yezoalpina* was synonymous with *P. hayachinensis*, and this synonymy was accepted by Koyama (1987) and Osada (1993) and by us. Pollen fertility was found to be less than full in Honshu and Hokkaido material tested (52%–90%) (Kawano & Tatewaki, 1961).

Generally material from Hokkaido differs from Honshu plants by longer ligules in upper culm leaves (2–3.3 vs. 0.5–2 mm) that are acute and smooth or sparingly asperous (versus truncate and distinctly asperous). However, these states vary in both sets of populations.

Key to genera of PPAM that are perennial, has multiple-flowered spikelets, and a hairy callus

There are 13 genera in the PPAM clade with perennial species with more than one floret per spikelet and with a callus that is sometimes hairy (*Arctophila*, *Arctopoa*, *Bellardiochloa*, *Catabrosella*, *Dupontia*, *Dupontiopsis*, *Hookerochloa*, *Hyalopoa*, *Nicoraepoa*, *Poa*, *Puccinellia*, *Saxipoa*, *Sylvipoa*). In all of these there is a crown of hairs, but only in *Poa* (tufts, diffuse tufts, rarely a beard), *Hookerochloa* (beard), and *Bellardiochloa* (crown at the rachilla internode apices) are other arrangements found. Nearly all have glabrous ovaries (*Hookerochloa* and *Saxipoa* can have 1 or 2 minute hairs), and the hilum is always less than 66% as long as the grain. All have paleas that are well developed and thinly herbaceous (with chlorophyll) in part (firmer in a few) (i.e., not hyaline throughout except for

the keel veins as occurs in many subtribe Aveninae s.l. or Agrostidinae genera), with the inter-keel gap broader than the lateral flanges. The genera can be divided on numbers of nerves in the lemmas, the degree of lemma compression, vestiture of the palea keels, lemma texture, apex shape, edge vestiture, and percent of fusion of the upper culm sheath margins.

General circumscription of the group of 13 genera in the key: Genera with multiple flowered spikelets: **Perennial**; culms hollow, thin-walled; uppermost culm leaf sheath margins fused at the base least 1 mm (except *Sylvipoa*) or more, sometimes to the top; ligules membranous (rarely chartaceous in part), the apex glabrous and smooth or asperous to ciliolate to short ciliate (not long ciliate); inflorescences simple paniculate; **spikelets** laterally compressed (to narrowly sub-cylindrical in *Puccinellia*), **mostly <1 cm long, with 2 or more florets** (sometimes 1 in *Dupontia*, or in depauperate plants of some species *Poa*); florets disarticulating above the glumes and between the florets; rachilla internodes well developed above the proximal floret (usually >0.4 mm long), more or less terete or slightly dorsiventrally compressed; **callus hairy**, short, blunt, or slightly angled (not annulated as in *Festuca*); lemmas lanceolate to slightly oblanceolate, 3–5 (7–11)-nerved, **keeled or rounded on back**, sometimes with a solitary, straight, terminal awn (up to ca. 5 mm long); **palea well-developed, scarious to thin or thick herbaceous, or subcoreaceous** (surfaces, excluding keels, not hyaline throughout), with well-separated, prominent keels; lodicules 2, free, distally hyaline, lanceolate to oblanceolate (to flabellate), often lobed or dentate, glabrous (a few with 1–4 hairs at the apex), obscurely veined; styles plumose, lanceolate in outline (versus globose in tribe Meliceae, or linear), white, free to the base (except in *Poa flabellata*); **ovary glabrous** (with or without a few obscure short hairs at the apex in *Saxipoa* and *Hookerochloa*), without an apical appendage; **hilum sub-linear to round, 10%–30% the grain in length** (25%–66% in some Coleanthinae); embryo short (ca. 20%–25% the grain in length).

- 1a. Callus of lemma with a sparse to dense dorsal tuft of wooly or plicate hairs (infrequently with an additional tuft from beneath each marginal vein) (tip of rachilla internodes, if hairy, not with hairs concentrated at the tip or differentiated from those below it); lemma with (3) 5 (7–11) veins *Poa* p.p.
- 1b. Callus of lemmas (or tip of rachilla internodes) with a crown or beard of hairs (hairs infrequently reduced to only one or a few over 0.5 mm long, but then palea keels of proximal florets smooth); lemma with 3, 5, 7 (9–11) veins 2
- 2a. Palea keels smooth or with rare hooks (sometimes softly pilulose); lemmas often 3-nerved, sometimes faintly 5-nerved. 3
- 2b. Palea keels regularly scabrous in part, sometimes densely ciliate, or softly pilulose in part; lemmas 3–11-nerved 6
- 3a. First glume distinctly shorter than the proximal lemma; lemmas keeled, awnless; plants Asian, alpine 4
- 3b. First glume about as long or longer than the first lemma; lemmas rounded on back, sometimes mucronate

- or awned; plants circumboreal, boreal and arctic wetlands. 5
- 4a. Uppermost culm sheath closed more than $\frac{1}{5}$; basal sheaths papery. ***Hyalopoa***
- 4b. Uppermost culm sheath closed less than $\frac{1}{5}$; basal sheaths often reticulate fibrous. ***Catabrosella*** p.p.
- 5a. Lemma apex of lower florets narrowly acute to acuminate, tapered to a smooth, slender, scarios point, or briefly slender-awned; spikelets 1–3(4)-flowered; glumes equaling or longer than the proximal floret. ***Dupontia***
- 5b. Lemma apex of lower florets obtuse to acute, sometimes briefly cuspidate; spikelets 2–6-flowered; glumes equaling the proximal floret. ***Arctophila***
- 6a. Upper culm sheath open to the base; blades flat (4–) 7–17 mm wide, thin, costae narrow and not raised, except for a prominent, usually excentric, abaxial keel, surfaces with evident cross-venation (visible with back-lighting); lemmas 2.3–3.2 mm long, thin, with lateral veins prominently raised, unawned; callus hairs to 0.2 mm long; plants of eastern Australia, temperate rainforest margins. ***Sylvipoa***
- 6b. Upper culm sheath closed at least 1 mm at base; blades various, if flat and wide then differing from the above, costae narrow or wide, raised or not, abaxial keel centric, surfaces without evident cross-venation; lemmas various, but not short and thin, with prominently raised veins, awned or not; callus hairs various; plants widespread, sometimes of Australia, of various habitats. 7
- 7a. Rachilla internodes with a distinct crown of short, crisp hairs concentrated at the apex, and the callus of the lemma similarly hairy; caryopsis semi-soft to soft; lemmas rounded or weakly keeled on back, 2.7–5.2 mm long, 5-nerved, usually with a brief apical awn; leaf-blades narrow, involute, adaxially with rounded costae that are densely scabrous or coarsely short hairy; plants of non-saline, mountain grassland habitats in southern Europe to southwest Asia. ***Bellardiachloa***
- 7b. Rachilla internodes without a distinct crown of hairs concentrated at the apex, callus of the lemma variously hairy; caryopsis hard (semi-soft in *Nicoraepoa*); lemmas rounded to distinctly keeled on back, sometimes longer than 5.2 mm, 3–11-nerved, awned or not; leaf-blades various; plants widespread, if of Asia then of sub-saline to saline habitats. 8
- 8a. Lemmas rounded on back, usually thin, apex retuse, obtuse to acute, often blunt, infrequently sharply pointed (never awned), 1.5–5 mm long, 3–5-nerved, intermediate veins, when present, usually faint; callus with a short crown of hairs 0.05–0.2 mm long; lower glume rarely more than $\frac{1}{2}$ the length of the adjacent lemma, usually 1-nerved; plants of temperate to Arctic saline and alkaline wetland habitats world-wide (*Poa* sect. *Secundae* species that have weakly keeled lemmas will key here, but differ by their longer glumes, lower glume 3-nerved). ***Puccinellia*** p.p.
- 8b. Lemmas more or less distinctly keeled on back, thin or thick, apex obtuse to acuminate, sometimes awed, 3–9.4 mm long; callus hairs often longer than 0.2 mm; lower glume usually more than $\frac{1}{2}$ the length of the adjacent lemma, 1–3-nerved; plants sometimes of saline habitats. 9
- 9a. Lemmas, and often glumes, finely ciliolate to ciliate along the edges (at least in the lower half); plants of Australia or sub-Arctic coasts. 10
- 9b. Lemmas and glumes, smooth or scabrous (rarely densely so) along the edges; plants widely distributed. 11
- 10a. Lemmas indurate, sub-coriaceous; uppermost culm sheath margins fused 0.5%–3% the length; plants of southeastern Australia, alpine. ***Saxipoa***
- 10b. Lemmas thinly herbaceous; uppermost culm sheath margins fused 15%–33% the length (the higher percentages often by a hyaline membrane); plants of coastal beaches and estuaries of north Pacific and western Atlantic oceans, and Hudson Bay. ***Arctopoa*** sect. ***Arctopoa***
- 11a. Leaf-blades (at least the basal ones) thin soft, flat, folded or involute, 1–3(–5) mm wide, soon withering, adaxially at most modestly scaberulous, costae indistinct; uppermost culm sheath closed 5%–20% (25%); lemmas unawned, glabrous, or villous on the keel and marginal veins, and sometimes the intermediate veins, glabrous or pilulose between the veins; spikelets sometimes viviparous; plants mainly of Western North America (but *Poa stenantha* reaching the Commander Islands in the north Pacific, and the Patagonian Andes). ***Poa*** sect. ***Secundae*** subsect. ***Halophytae*** Soreng p.p.
- 11b. Leaf blades mostly firmer, flat to involute, not soft and soon withering, adaxially variously smooth or scabrous to coarsely hairy, costae often prominent; uppermost culm sheath closed 1%–70%; lemmas awned or not, variously glabrous or pubescent; spikelets sometimes viviparous; plants widespread. 12
- 12a. Uppermost culm sheath margins fused 20%–70% the length; leaf-blades adaxially flat (exclusive of central grooves), OR with low, rounded or slightly raised, narrow costal ridges that are much narrower than or equal to the intercostae in width. 13
- 12b. Uppermost culm sheath margins fused for 1%–18% the length; leaf-blades adaxially level (exclusive of central grooves) OR adaxially with prominent, rounded or blocky, costal ridges that are 1 to 2 or more times broader than the intercostae in width. 15
- 13a. Lemmas 3(5)-nerved, narrowly acute to acuminate sometimes slender awned, scarios to hyaline in the upper $\frac{1}{3}$ to $\frac{1}{4}$; lemma keel gradually and subtly arched; flowers perfect; plants of Japan, alpine. ***Dupontia***
- 13b. Lemmas 5–11-nerved, acute to acuminate, not awned, scarios to hyaline only in the upper margins; lemma keel slightly gibbous at base; plants of Australasia and the Americas; flowers perfect or unisexual (*Poa* p.p. minor). 14
- 14a. Flowers perfect, spikelets notviviparous; plants of Australia & New Zealand, coastal sands and meadows. ***P.*** (subsect. ***Austrofestuca***) ***billardierei***
- 14b. Flowers mostly unisexual, spikelets sometimes viviparous; plants of the Americas. ***P.*** sect. ***Dioicopoa*** p.p. (South America, callus hairs 1–5 mm long, spikelets often viviparous), and ***P.***

- sect. *Madropoa* p.p. (western North America, callus hairs mostly less than 1mm long, spikelets not viviparous).
- 15a. Uppermost culm sheath margins fused 3%–18% their length; leaf-blades flat folded, or involute, adaxially with distinct blocky or rounded costae that are two or more times broader than the intercostae; lemmas 3–5 veined; callus with a crown of hairs; plants of South America, Patagonian coastal to alpine (sometimes saline) wetlands. *Nicoraepoa* p.p.
- 15b. Uppermost culm sheath margins fused less than 2 mm, ca. 1% their length; leaf-blades flat or folded, thick, 2–8mm wide, adaxially level or of low indistinct topography (except for central grooves), OR narrow (to 0.7mm diam.), tightly involute, adaxially with rounded raised scabrous costae; lemmas (3) 5–7(11) veins; callus with a crown or beard of hairs; plants of southeastern Australia, of non-saline wetlands or drier habitats *Hookerchloa* (including *Festucella*)

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Supplementary Material

The following supplementary material is available online for this article at <http://onlinelibrary.wiley.com/doi/10.1111/jse.12146/supinfo>:

Appendix S1. Details of sequences used in this study with voucher information and GenBank accession numbers using the following format: taxon name, ploidy (2n), tribe-subtribe, country of origin, DNA extraction ID, voucher code, GenBank accession numbers [ITS, ETS, *trnT-trnL-trnF*, *matK*, *rpoB-trnC*].—missing data; *, new sequences.

Appendix S2. Characteristics including diagnostic morphological characters of the 40 accepted genera of Poaeae tribes Coleanthinae, Miliinae, and Poinae (including Alopecurinae, Beckmanniinae, Cinninae, Phleinae). Discrepancies noted: Fl TK, Davis, 1985; Tz, Tzvelev, 1976; W&D, Watson & Dallwitz, 1992.