

Polyphyly of the grass tribe Hainardieae (Poaceae: Pooideae): identification of its different lineages based on molecular phylogenetics, including morphological and cytogenetic characteristics

Julia Schneider · Grit Winterfeld · Martin Röser

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Abstract The small pooid grass tribe Hainardieae comprises six genera with approximately ten species; however, this tribe was not accepted by all previous taxonomic treatments. To study the relationships among these genera and to infer the phylogeny and evolutionary patterns, we used sequence variation of the internal transcribed spacers (ITS) of nuclear ribosomal and chloroplast (cp) *matK* DNA and morphology. Many genera of the Aveneae/Poeae tribe complex additionally were included. Both molecular datasets showed Hainardieae to be highly polyphyletic, and its genera to branch with different groups of the Aveneae/Poeae. *Parapholis* and *Hainardia* are corroborated as being closely related, and belonging to a firmly supported Eurasian clade together with *Catapodium* incl. *Scleropoa*, *Cutandia*, *Desmazeria*, *Sphenopus*, *Vulpiella* (subtribe Parapholiinae) and with *Cynosurus* as sister to this assemblage. The other genera of traditionally recognised Hainardieae are positioned phylogenetically distant: Mediterranean *Narduroides* is verified as more or less related to *Festuca* and relatives (subtribe Loliinae), whereas the west Eurasian *Pholiurus* is close to the lineage of *Poa* and relatives (subtribe Poinae). North American *Scribneria* is sister to *Deschampsia* and both genera should be unified under a common subtribe (Aristaveninae or Holcinae). The phylogenetic position of the Algerian genus *Agropyropsis* (close to *Narduroides* and within the Loliinae) is suggested on morphology only, because no molecular data was obtained

for it. Considering classification, we support the abandonment of tribe Hainardieae and argue to abandon Poeae subtribe Scribneriinae. Poeae subtribe Parapholiinae is redefined with a novel genus content, due to the exclusion of *Agropyropsis* and *Pholiurus* and the inclusion of *Vulpiella*.

Keywords Cytogenetics · Grasses · Hainardieae · ITS · *matK* · Poaceae

Introduction

The economic and ecological importance of the grass subfamily Pooideae (Poaceae) for humans and animals has induced many scientists to focus on its molecular phylogeny and evolution patterns. Many recent studies on classification and phylogeny have resulted in changes in circumscription and taxonomic treatment (Hsiao et al. 1995a, 1999; Catalán et al. 1997; Soreng and Davis 1998, 2000; Hilu et al. 1999; Mathews et al. 2000; GPWG 2001; Soreng et al. 2003; Davis and Soreng 2007, 2010; Döring et al. 2007; Duvall et al. 2007; Bouchenak-Khelladi et al. 2008; Schneider et al. 2009, 2011). The tribe complexes of Bromeae/Triticeae and Aveneae/Poeae were analysed especially extensively (Baum and Appels 1992; Hsiao et al. 1995b; Kellogg et al. 1996; Grebenstein et al. 1998; Soreng and Davis 2000; Catalán et al. 2004; 2007; Mason-Gamer and Kellogg 2000; Röser et al. 2001; Mason-Gamer 2005; Davis and Soreng 2007; Döring et al. 2007; Quintanar et al. 2007; Saarela et al. 2007; 2010; Seberg and Petersen 2007; Soreng et al. 2007; Gillespie et al. 2008, 2010; Döring 2009).

Hainardieae is a further, species-poor tribe of the subfamily Pooideae, characterised by common morphological characters like spicate to racemose inflorescences and

J. Schneider · G. Winterfeld · M. Röser (✉)
Institute of Biology, Department of Systematic Botany,
Martin Luther University Halle-Wittenberg,
Neuwerk 21,
06108 Halle (Saale), Germany
e-mail: martin.roeser@botanik.uni-halle.de

spikelets arranged in two rows. According to the circumscription of Clayton and Renvoize (1986), the tribe encompasses six genera (*Agropyropsis*, *Hainardia*, *Narduroides*, *Parapholis*, *Pholiurus*, *Scribneria*) and approximately ten species. The taxa are distributed from the Mediterranean to Central Asia with the exception of *Scribneria* from western North America. Most species are annuals and adapted to saline soils. Based on morphological and molecular data, other classifications were also proposed (Table 1). Some treatments did not acknowledge Hainardiaceae and summarised all genera under a broad tribe Poeae (e.g., Tzvelev 1989; Soreng et al. 2007 with several subtribes). Alternatively, *Scribneria* was excluded from Poeae and placed in Aveneae (Macfarlane 1987; Watson and Dallwitz 1992; 1992 onwards). Molecular phylogenetic studies have included *Hainardia*, *Narduroides* and *Parapholis* to date (cf. Table 1): *Parapholis* and *Hainardia* appeared to be closely related to the likewise annual genera *Desmazeria*, *Catapodium*, *Cutandia*, and *Sphenopus* (Soreng and Davis 2000; Catalán et al. 2004; 2007; Quintanar et al. 2007) and were treated with them as subtribe Parapholiinae (Soreng and Davis 2000; Catalán et al. 2004; 2007; Soreng et al. 2007). *Narduroides* was placed in broad molecular analyses of Poeae subtribe Loliinae within a clade of ‘fine-leaved *Festuca*’ (Torrecilla et al. 2004; Catalán et al. 2004; 2007). The general affiliation of *Narduroides* with Poeae was corroborated by several subsequent analyses (Soreng et al. 2007; Bouchenak-Khelladi et al. 2008).

Scribneria was classified under a separate subtribe Scribneriinae (Soreng et al. 2003; 2007), because cpDNA restriction site analysis provided a pattern different from that of *Hainardia*, *Parapholis* and relatives (Soreng and Davis 2000). For the genera *Agropyropsis* and *Pholiurus*, molecular data were not available until now.

In this study, we analysed nuclear ITS and cp *matK* sequence data to verify the phylogeny of Hainardiaceae among the Pooideae as a part of a more extended study with focus on the phylogeny of the Aveneae/Poeae tribe complex. Due to this we included many Aveneae/Poeae taxa for comparison. In addition to molecular phylogenetic information we examined morphological and cytogenetic characters to obtain a deeper insight into the evolution of the Hainardiaceae and their relation to the Aveneae/Poeae tribe complex.

Materials and methods

Plant selection

This analysis includes eight species of the tribe Hainardiaceae (*Agropyropsis lolium*, *Hainardia cylindrica*, *Parapholis filiformis*, *P. incurva*, *P. strigosa*, *Narduroides salzmannii*, *Pholiurus pannonicus*, *Scribneria bolanderi*) and encompasses all

genera (Table 2). *Parapholis gracilis*, *P. marginata* and *P. pycnantha* have not been sampled. Additionally, our study encompasses 36 species of the Aveneae/Poeae tribe complex, which represent close relatives of the Hainardiaceae taxa. *Neomolinia japonica* and *N. fauriei*, members of the tribe Diarrheneae were selected as outgroup, because previous molecular phylogenetic studies showed Diarrheneae consistently within subfamily Pooideae, but distant to the genera of Aveneae/Poeae and Hainardiaceae analysed (e.g. Catalán et al. 1997; Soreng and Davis 1998; Hsiao et al. 1999; GPWG 2001; Davis and Soreng 2007; Soreng et al. 2007; Döring 2009; Schneider et al. 2009, 2011). For this study, 46 new sequences were generated. Table 2 lists all taxa investigated with origin, voucher number and EMBL/GenBank sequence accession numbers.

Furthermore, at least one taxon of each lineage identified by molecular data was chosen to determine the chromosome numbers and ploidy levels. According to our previous study the plants used for cytogenetics were collected in their natural habitats or grown from fruits and planted in pots in the greenhouse (Schneider et al. 2011).

Molecular phylogenetics

DNA extraction from herbarium specimens or from silica gel-dried fresh leaves was carried out using the NucleoSpin Plant Kit (Macherey-Nagel, Düren, Germany). The *matK* gene–3′*trnK* exon was amplified with five oligonucleotide primer pairs: S5-1F/PO-*matK* 620R, PO-*matK* 470F/PO-*matK* 1070R, PO-*matK* 860F/PO-*matK* 1420R, PO-*matK* 1300F/*trnK*-2R and PO-*matK* 1900F/*psbA*R. The primer PO-*matK* 1900F (5′-ACA TAG GGA AAG TCG TGT GC-3′) was newly designed for this study, all other primer sequences are listed in Schneider et al. (2009). The ITS1–5.8S gene–ITS2 region was amplified using the primers ITS-SF and ITS-SR (Blattner et al. 2001). The PCR reaction was performed according the protocol described by Schneider et al. (2009). Due to difficulties in some samples, up to fourfold PCR reactions were pooled to obtain enough template DNA for sequencing. The PCR products were purified with the NucleoSpin Extract II Kit (Macherey-Nagel) and sequenced with the DYEnamic ET Terminator Cycle Sequencing Kit (GE Healthcare, Dallas, TX) using the PCR primers. After ethanol precipitation, the sequencing products were electrophoresed on a MegaBACE 1000 capillary sequencer (GE Healthcare). For *Pholiurus pannonicus*, only ITS2 could be sequenced directly. Therefore we cloned the column-purified ITS PCR product of *P. pannonicus* into the pGEM-T Easy Vector (Promega, Mannheim, Germany) according to the manufacturer’s protocol. Ten individual white colonies were picked and analysed. Plasmid DNA was isolated using the Wizard Plus SV Minipreps DNA Purification System (Promega) and sequenced in our

Table 1 Comparison of different classifications for genera of the traditionally circumscribed Hainardieae (supertribe, tribe, subtribe or informal rank). Only studies that included at least three Hainardieae genera are considered

Taxon	Tzvelev (1976): morphology	Clayton and Renvoize (1986): morphology	Tzvelev (1989): morphology	Macfarlane (1987); Watson and Dallwitz (1992, 1992 onwards): morphology	Soreng and Davis (2000): cpDNA restriction sites, morphology	Catalán et al. (2004): cp and ITS DNA sequences	Soreng et al. (2007): cpDNA sequences, morphology	Bouchenak-Khelladi et al. (2008): cpDNA sequences	This study: cp and ITS DNA sequences, morphology
<i>Agropyropsis</i> — ^a		Hainardieae	Poeae	Poodae: Poeae	—	—	Poodae: Poeae: Parapholiinae ^b	—	Poeae: <i>Festuca</i> lineage (Loliinae ^{b, c})
<i>Hainardia</i>	Monermeae (syn. Hainardieae)	Hainardieae	Poeae	Poodae: Poeae	Poeae: Ammochoiinae or Parapholiinae	Poeae: Parapholiinae	Poodae: Poeae: Parapholiinae	Poeae	Poeae: <i>Parapholis</i> lineage (Parapholiinae ^c)
<i>Narduroides</i> —		Hainardieae	Poeae	Poodae: Poeae	—	Poeae: Loliinae	Poodae: Poeae: Loliinae	Poeae	Poeae: <i>Festuca</i> lineage (Loliinae ^c)
<i>Parapholis</i>	Monermeae (syn. Hainardieae)	Hainardieae	Poeae	Poodae: Poeae	Poeae: Ammochoiinae or Parapholiinae	Poeae: Parapholiinae	Poodae: Poeae: Parapholiinae	Poeae	Poeae: <i>Parapholis</i> lineage (Parapholiinae ^c)
<i>Pholurus</i>	Monermeae (syn. Hainardieae)	Hainardieae	Poeae	Poodae: Poeae	—	—	Poodae: Poeae: Parapholiinae ^b	—	Poeae: <i>Poa</i> lineage (Poiinae ^c)
<i>Scribneria</i> —		Hainardieae	Poeae	Poodae: Aveneae	Poeae	—	Poodae: Poeae: Scribneriinae	—	Poeae: <i>Deschampsia</i> lineage (Aristaveninae ^c or Holcinae ^c)

^a Genera not analysed or unplaced^b Placement based on morphology^c Preliminary subtribe assignment

Table 2 Taxa and provenances analysed for DNA sequences, morphology (= morph) and cytogenetics (= cyto), herbarium vouchers, and GenBank DNA sequence accession numbers. *BG* Botanical Garden

Taxon	Origin/source	Collector and Index Herbariorum code for voucher specimens	<i>matK</i> gene– 3' <i>trnK</i> exon	ITS	Non-molecular data
<i>Agropyropsis lolium</i> (Balansa) A. Camus	Algeria, Dj. Milogh, Laghouat	Cosson <i>s.n.</i> (JE)			Morph
<i>Agropyropsis lolium</i> (Balansa) A. Camus	Algeria, Sidi Tiffour	Kralik <i>s.n.</i> (JE)			Morph
<i>Arctophila fulva</i> Nym.	Russia, Yakutiya	Doronkin 81 (NSK)	AM234606 ^a	FM179387 ^b	
<i>Arrhenatherum elatius</i> (L.) J. & C. Presl	Germany, Saxony	Winterfeld 77 (HAL)	AM234543 ^a	FM179388 ^b	
<i>Avena macrostachya</i> Coss. & Durieu	Algeria	accession CC 7068 of IGER Wales (HAL)	FM253118 ^b	FM179443 ^b	
<i>Bellardiocloa polychroa</i> (Trautv.) Roshev.	Armenia, Monte Agaraz lac	Voskonja <i>s.n.</i> (LE)	FM253119 ^b	FM179390 ^b	
<i>Bellardiocloa violacea</i> Bellardi (Chiov.) var. <i>aetnensis</i> (C. Presl) Chiov.	Italy, Sicily	Röser 6032 (HAL)	AM234605 ^a	FM179391 ^b	
<i>Briza media</i> L.	Germany, Thuringia	Röser 11072 (HAL)	AM234610 ^a	FM179393 ^b	
<i>Catabrosa aquatica</i> P. Beauv.	Germany, Baden-Wuerttemberg	Röser 2007 (HAL)	AM234589 ^a	FM179396 ^b	
<i>Catapodium marinum</i> (L.) C. E. Hubb.	Spain, Alicante	Röser 4299 (HAL)	HE646574 ^c	HE646600 ^c	
<i>Catapodium rigidum</i> (L.) C. E. Hubb. subsp. <i>rigidum</i>	Greece, Macedonia	Röser 2571 (HAL)	AM234586 ^{a, d}	FM179399 ^{b, d}	
<i>Colpodium versicolor</i> Woronow ex Grossh.	Georgia, South Ossetia	Bush <i>s.n.</i> (LE)	FM253122 ^b	FM179397 ^b	
<i>Colpodium versicolor</i> Woronow ex Grossh.	Armenia	Kotseruba <i>s.n.</i> , cultivated in BG Halle (R 620)			Cyto
<i>Cutandia maritima</i> (L.) K. Richt.	France, Frontignan	Dubuis (HAL 48831)	HE646572 ^c	HE646601 ^c	
<i>Cynosurus cristatus</i> L.	Germany, Baden-Wuerttemberg	Röser 9965 (HAL)	HE646575 ^c	HE646602 ^c	
<i>Deschampsia cespitosa</i> (L.) P. Beauv.				AF532929 ^e	
<i>Deschampsia cespitosa</i> (L.) P. Beauv.	Germany, Brandenburg	Röser 10737/1 (HAL)	AM234546 ^a		
<i>Deschampsia cespitosa</i> (L.) P. Beauv.	Germany, Saxonia	Röser 10734			Cyto
<i>Desmazeria philistaea</i> (Boiss.) H. Scholz	Israel, Ashkelon	Danin et al. (B 10 0240417)	HE646573 ^c	HE646603 ^c	
<i>Desmazeria sicula</i> Dumort.	Malta, Dwejra Point	Hansen 409 (C)	HE646576 ^c	HE646604 ^c	
<i>Dupontia fisheri</i> R.Br.				AY237848 ^f	
<i>Dupontia fisheri</i> R.Br. subsp. <i>pelligera</i> (Rupr.) Tzvelev	Russia, Central Taymyr	Pospelov 95-516 (NSK)	AM234602 ^a		
<i>Festuca altissima</i> All.	Germany, Lower Saxony	Röser 1227 (HAL)	AM234585 ^a	FM179404 ^b	
<i>Festuca gigantea</i> (L.) Vill.	Germany, Lower Saxony	Röser 5719 (HAL)	AM234720 ^a	HE646615 ^c	
<i>Festuca gigantea</i> (L.) Vill.	Plants grown from fruits obtained from BG Copenhagen	R 903 (no voucher)			Cyto
<i>Hainardia cylindrica</i> (Willd.) Greuter				AF532941 ^{e, g}	
<i>Hainardia cylindrica</i> (Willd.) Greuter	Plants grown from fruits obtained from BG Göttingen (no. 1850) in 2006	A 23 (HAL)	HE646577 ^c		Morph
<i>Hainardia cylindrica</i> (Willd.) Greuter	Plants grown from fruits obtained from BG Copenhagen	R 904 (HAL)			Cyto

Table 2 (continued)

Taxon	Origin/source	Collector and Index Herbariorum code for voucher specimens	<i>matK</i> gene– 3' <i>trnK</i> exon	ITS	Non-molecular data
<i>Hookerchloa eriopoda</i> (Vickery) S. W. L. Jacobs	Australia, Nimmitabel	Pullen 4003 (AD 96435171)	HE646578 ^c	HE646605 ^c	
<i>Hookerchloa hookeriana</i> (F. Muell. ex Hook. f.) E. B. Alexeev	Tasmania, Macquarie River	Buchanan 15711 (HO 507299)	HE646579 ^c	HE646606 ^c	
<i>Koeleria capensis</i> Nees	Uganda, Mt Elgon	Wesche (HAL 20026)	AM234558 ^a	FM179413 ^b	
<i>Koeleria cristata</i> Pers.	Greece, Epirus	Röser 10727 (HAL)			Cyto
<i>Koeleria loweana</i> Quintanar, Catalán & Castrov.	Portugal, Madeira	L. & V. Dalgaard 13276 (C)	HE646580 ^c	HE646607 ^c	
<i>Megalachne berteroniana</i> Steud.	Chile, Juan Fernández Islands	Wiens et al. 11203 (MO 5259377)	HE646581 ^c	FR692028 ^h	
<i>Megalachne masafuerana</i> (Skottsberg & Pilg.) Matthei	Chile, Juan Fernández Islands	I. & C. Skottsberg 183 (NY)	HE646582 ^c	FR692029 ^h	
<i>Narduroides salzmannii</i> (Boiss.) Rouy	Spain, Málaga	Rivas-Martínez 17742 (BASBG)	HE646583 ^c	HE646608 ^c	Morph
<i>Narduroides salzmannii</i> (Boiss.) Rouy	Spain, Sa. de Cazorla	Hernández <i>s.n.</i> (C)			Morph
<i>Narduroides salzmannii</i> (Boiss.) Rouy	Spain, Jaén	Lewejohann SEESP-88-064 (GOET)			Morph
<i>Narduroides salzmannii</i> (Boiss.) Rouy	Spain, Málaga	Lewejohann And-97-250 (GOET)			Morph
<i>Neomolinia fauriei</i> Honda	Russia, Primorskiy Kray	Tzvelev 191 (LE)	FR694881 ^h	FR692031 ^h	
<i>Neomolinia japonica</i> (Franch. & Savat.) Probat.	Russia, Primorskiy Kray	Tzvelev 446 (LE)	FR694882 ^h	FR692033 ^h	
<i>Nephelochloa orientalis</i> Boiss.	Turkey, Anatolia	Simon 76900 (BASBG)	HE646584 ^c	HE646609 ^c	
<i>Parapholis filiformis</i> (Roth) C. E. Hubb.	France, Montpellier	Streilberg & Stohr (HAL 81242)	HE646585 ^c	HE646610 ^c	Morph
<i>Parapholis incurva</i> (L.) C. E. Hubb.	Greece, Macedonia	Röser 2517 (HAL)	AM234583 ^a	FM179422 ^b	Morph
<i>Parapholis strigosa</i> Dumort.	Germany, Baltic Sea	Werner 3477 (HAL 71657)			Morph
<i>Phippsia concinna</i> (Th. Fries) Lindeb.	Russia, Taymyr	Vodopjanova 224 (NS)	AM234582 ^{a, h}	FM179425 ^{b, i}	
<i>Phleum phleoides</i> (L.) Karsten	Plants grown from fruits obtained from BG Oslo (no. 669) in 2003	Röser 11076 (HAL)	AM234552 ^a	FM179426 ^b	
<i>Phleum phleoides</i> (L.) Karsten	Plants grown from fruits obtained from BG (no. 376) in 2003	R 108 (HAL)			Cyto
<i>Pholiurus pannonicus</i> (Host) Trin.	Hungary, Hortobágy	Hilbig (HAL 67272)	HE646586 ^c	clones 1–10: HE646616 ^c –HE646625 ^c	Morph
<i>Pholiurus pannonicus</i> (Host) Trin.	Romania, Tibiscum	Sadler <i>s.n.</i> (HAL 112062)			Morph
<i>Psilurus incurvus</i> (Gouan) Schinz & Thell.	Spain, Salamanca	Amich & Sánchez 19923 (RO)	HE646587 ^c	HE646611 ^c	
<i>Puccinellia fasciculata</i> (Torr.) E. P. Bicknell	Hungary, Hajdú-Bihar	Röser 7633 (HAL)	AM234588 ^a	FM179431 ^b	
<i>Scribneria bolanderi</i> Hack.	USA, California	Wilken 16163 & Painter (RSA 695253)	HE646588 ^c	HE646612 ^c	Morph
<i>Scribneria bolanderi</i> Hack.	USA, California	Ahart 10180 (RSA 692211)			Morph
<i>Sphenopus divaricatus</i> (Gouan) Rehb.	Spain, Huesca	Montserrat 38080 (RO 12841)	HE646589 ^c	HE646613 ^c	
<i>Ventenata macra</i> (Bieb.) Boiss.	Greece, Peloponnisos	Röser 10688 (HAL)	AM234555 ^a	FM179440 ^b	
<i>Vulpia bromoides</i> (L.) Gray	plants grown from fruits obtained from BG Dijon	R 45 (HAL)			Cyto
<i>Vulpia unilateralis</i> (L.) Stace				AF478492 ^j	

Table 2 (continued)

Taxon	Origin/source	Collector and Index Herbariorum code for voucher specimens	<i>matK</i> gene– <i>3'trnK</i> exon	ITS	Non-molecular data
<i>Vulpia unilateralis</i> (L.) Stace	France, Montpellier	Schubert (HAL 81028)	HE646590 ^c		
<i>Vulpiella stipoides</i> (L.) Maire	Libya, Tripolitania	Eckerlein (HAL 16576)	HE646591 ^c	HE646614 ^c	
<i>Zingieria trichopoda</i> (Boiss.) P. Smirn.	plants grown from fruits obtained from Institute of Plant Genetics and Crop Plant Research, Gatersleben in 2002	Röser 11074 (HAL)	AM234551 ^a	FM179441 ^b	

^a Döring et al. (2007)^b Schneider et al. (2009)^c This study^d Sequence entry under *Desmazeria rigida* (L.) Tutin subsp. *rigida*^e Catalán et al. (2004)^f Brysting et al. (2004)^g Sequence entry under *Monerma cylindrica* (Willd.) Coss. & Dur.^h Schneider et al. (2011)ⁱ Sequence entry under *P. algida* R. Br. subsp. *concinna* (Th. Fries) Á. Löve & D. Löve^j Torrecilla and Catalán (unpublished)

laboratory as described above, or by StarSEQ GmbH (Mainz, Germany).

Sequences were edited and aligned manually with the program Sequencher 4.5 (Gene Codes, Ann Arbor, MI). Ambiguous positions in the alignment of the ITS and the *matK* gene–*3'trnK* exon (intron region) were excluded from phylogenetic analyses. Potentially parsimony-informative indels were coded as 0/1 matrix for each of the two datasets.

Phylogenetic trees were obtained by Bayesian and maximum parsimony (MP) analyses. Nuclear and cpDNA sequence datasets were analysed separately.

MrModeltest 2.3 (Nylander 2004) was chosen to test the nucleotide substitution models. For both datasets, the GTR + *I* + *I* substitution model was selected as best-fit by Akaike information criterion (AIC) and hierarchical likelihood ratio tests (hLRTs) and was used in Bayesian phylogenetic analyses with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Four parallel chains were run for 1 million (*matK* dataset) or 2 million generations (ITS dataset), respectively, and every 100th tree was sampled and saved. Standard deviation of split frequencies and potential scale reduction factor accorded to the recommendations of the MrBayes program manual (Ronquist et al. 2011). Bayesian majority rule consensus trees with posterior probabilities (PP) were generated for both datasets after the first 25% of the trees were discarded as “burn-in”.

The MP analyses were conducted with PAUP* 4.0b10 (Swofford 2002) using the following settings: heuristic search with TBR branch swapping, MULTREES option in

effect, stepwise taxon addition, maxtrees=10,000, RANDOM addition, and 300 replications. Strict consensus trees were generated from the most parsimonious trees. To test the robustness of branch topology, bootstrap analysis (Felsenstein 1985) was carried out with 500 replications using settings maxtrees=1,000, TBR and closest addition.

Morphology

Structural characters of the inflorescences and spikelets were studied in herbarium material. Spikelets and spikelet parts were rehydrated in boiling water. Morphological preparations were examined and photographed on a Zeiss Stemi 2000-C microscope with a computer-assisted camera and Zeiss Axiovision software.

Cytogenetics

Root tips were cut and processed in iced water for 20 h to accumulate metaphases, followed by fixing in absolute ethanol:glacial acetic acid (3:1) for 3 h. Samples were stored in absolute ethanol at –20°C until preparation. Root tips were enzyme-treated according to Schwarzacher et al. (1980), squashed on slides in a drop of 45% propionic acid with 2% carmine and covered with a coverslip. Metaphase chromosomes were photographed using a Zeiss Axioskop microscope.

Results

Molecular phylogeny

Chloroplast dataset (*matK* gene–3'*trnK* exon)

The alignment comprised 2,122 positions, of which 40 characters in the intron region were excluded from the phylogenetic analyses because of ambiguities in the alignment. The topology of the Bayesian tree (Fig. 1) corresponded with the MP strict consensus tree (not shown), except for one difference marked by a bold line. Fifteen indels were coded and included in the MP analysis with 449 (21.4%) variable and 270 of them (60.1%) parsimony-informative characters. The heuristic search resulted in 30 equally parsimonious trees with a length of 669 steps (consistency index=0.79, retention index=0.88).

The phylogenetic tree of Fig. 1 showed a highly supported lineage of tribes Aveneae/Poeae [(Bayesian posterior probability (PP) 1.00, percentage bootstrap support (BS) 100] branching after the outgroup taxa. Clade 1, representing the Aveneae clade (PP 1.00, BS 100), comprised genera with a shared 4 bp insertion in the intron region between the *matK* gene and 3'*trnK* exon.

Members of the traditionally defined Hainardieae (*Hainardia*, *Narduroides*, *Parapholis*, *Pholiurus*, *Scribneria*) were nested in Clade 2, the Poeae clade (PP 1.00, BS 100). It was divided in five lineages (I–V in Fig. 1). Lineage I comprised *Parapholis filiformis*, *P. incurva* and *Hainardia cylindrica* together with the genera *Catapodium*, *Cutandia*, *Desmazeria*, *Sphenopus*, *Vulpiella*, and *Cynosurus*, which shared a 6 bp deletion in the *matK* gene region (Fig. 1). *Narduroides salzmanii* was member of lineage II (PP 1.00, BS 99), which encompassed also *Festuca*, *Megalachne*, *Psilurus*, and *Vulpia*. *Vulpia unilateralis* and *Psilurus incurvus* had a common 7 bp insertion in the intron region. Lineage III consisted of *Scribneria bolanderi* in a strongly supported clade with *Deschampsia cespitosa* (PP 1.00, BS 100) and both genera were characterised by a 3 bp deletion in the intron region. Within lineage IV (PP 1.00, BS 100), also characterised by a deletion of 1 bp, *Phleum phleoides* was sister to an assemblage of *Pholiurus*, *Arctophila*, *Bellardiachloa*, *Dupontia*, *Hookerachloa* (incl. *Festucella*), *Nephelochloa* and *Ventenata*, additionally characterised by a 6 bp insertion in the intron region (PP 1.00, BS 92). *Bellardiachloa* and *Nephelochloa* shared a further 4 bp insertion in the intron. In lineage V *Catabrosa*, *Phippsia* and *Puccinellia* appeared together with *Colpodium* and *Zingeria* (PP 1.00, BS 100). This lineage was characterised by a 7 bp insertion in the intron region. *Colpodium* and *Zingeria*

were sister and shared a 9 bp deletion in the *matK* gene (PP 1.00, BS 100).

Nuclear dataset (ITS region)

The alignment of the nuclear sequence data ITS1–5.8S rDNA gene–ITS2 encompassed 536 positions after exclusion of 76 characters. The Bayesian phylogenetic tree is shown in Fig. 2 with differences to the MP strict consensus tree marked in bold. MP analysis with inclusion of 8 indels resulted in 276 equally parsimonious trees with a length of 623 steps (consistency index=0.53, retention index=0.75). A total of 228 (41.9%) of the 544 characters included were variable and 164 (71%) of them parsimony-informative.

Ten ITS clones sequenced for *Pholiurus pannonicus* were nearly identical and appeared in a single branch as a polytomy (maximum support, tree not shown). Two of them were selected randomly and included for the final analyses (Fig. 2).

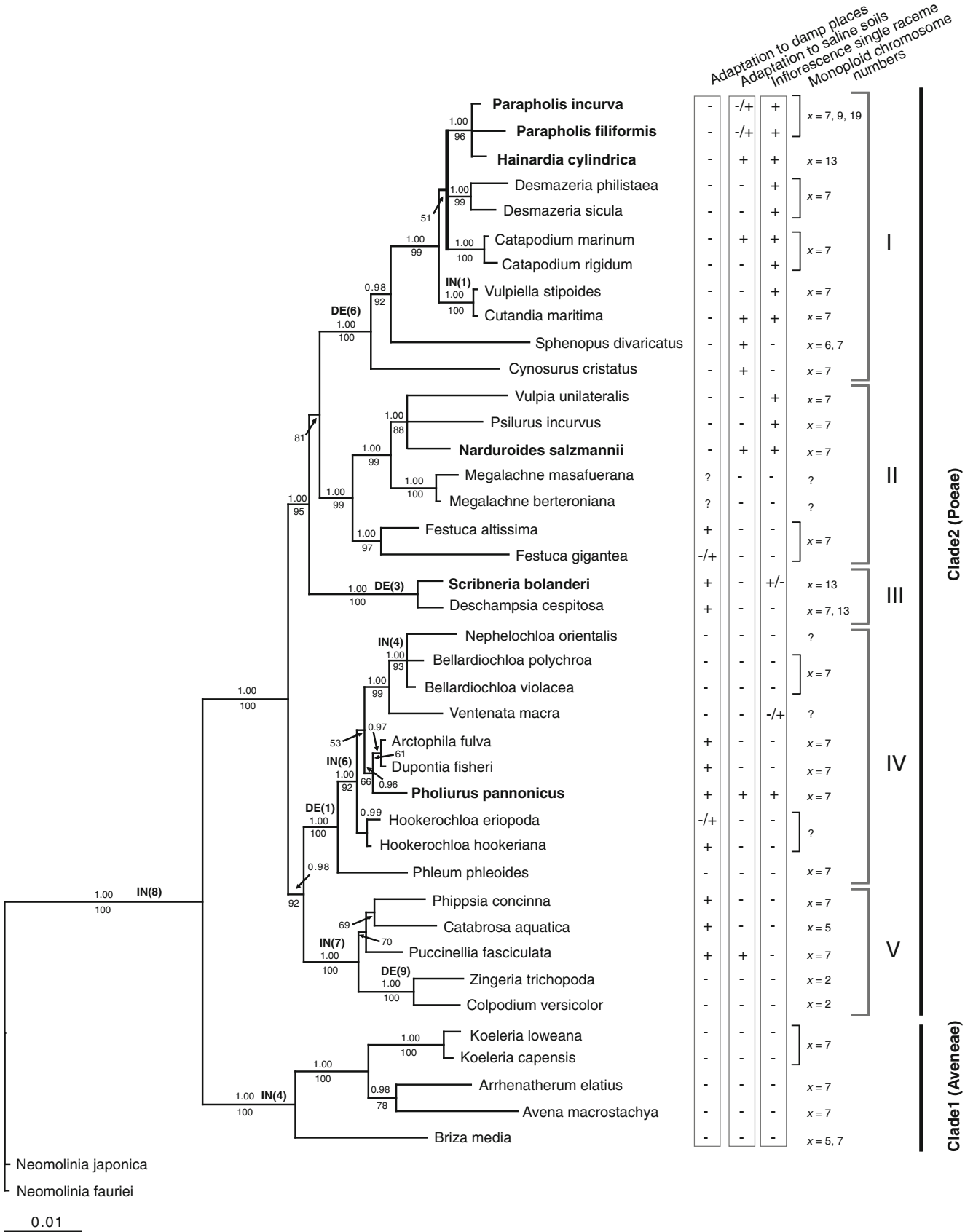
The ITS tree had less resolution of the backbone than the *matK* tree and the split between Clade 1 (Aveneae) and Clade 2 (Poeae) was not obvious. Additionally, it showed minor differences in some taxon positions, but without strong support. However, the traditionally Hainardieae representatives were nested in four different lineages with rather high support (Fig. 2), but with merging of lineages I and II and IV and V, respectively, relative to the cpDNA data (Fig. 1).

Morphology

The genera were surveyed especially for structural characters of the inflorescences, spikelets and parts of the spikelets (glumes, lemma, palea, ovary, karyopsis). They displayed considerable variation, which is described in detail in Table 3, including comments on previous observations in the footnotes. Important characters are exemplary illustrated in Fig. 3 (inflorescence details, disarticulation of inflorescence axes, disarticulation of spikelets, entirely falling spikelets), Fig. 4 (spikelets, lemma, palea) and Fig. 5 (ovary, karyopsis). Taxonomically relevant characters are detailed below in context with the different lineages resolved in this study by molecular data (see Discussion).

Cytogenetics

Chromosome numbers studied in selected taxa from the different lineages among the Aveneae/Poeae tribe complex and the estimated polyploidy levels are summarised in Table 4 and included in Fig. 1. We obtained various monoploid chromosome numbers ranging from $x=2$ (*Colpodium*) to $x=13$ (*Hainardia*, *Deschampsia*) with large metaphase chromosomes in all examples (Fig. 6).



0.01

◀ **Fig. 1** Bayesian phylogenetic tree of representative members of the Aveneae/Poeae tribe complex and genera of traditionally recognised Hainardieae (*bold*) based on *matK* sequence data. The branch indicated by a *bold line* is not obtained in maximum parsimony analysis. Posterior probabilities ≥ 0.95 are shown above branches and bootstrap values are indicated as percentage node supports ≥ 50 below branches. Ecological preferences (adaptation to damp places or to saline soils) and the occurrence of inflorescences as spikes or racemes are indicated as + for presence and – for absence. Monoploid chromosome numbers for the genera according to Watson and Dallwitz (1992) and this study. Insertions/deletions with the numbers of base pairs are indicated on the corresponding branches

Discussion

In the past, *Agropyropsis*, *Hainardia*, *Narduroides*, *Parapholis*, *Pholiurus* and *Scribneria* were sometimes unified under a separate tribe Hainardieae based on conspicuous morphological traits, such as spicate inflorescences and spikelets arranged in two rows (e.g. Clayton and Renvoize 1986). Alternatively, all genera that had been included in Poeae or *Scribneria* was excluded from Poeae and placed in Aveneae (cf. Table 1). Based on molecular sequence variation and morphological characters, the present study supports that Hainardieae represent a highly polyphyletic grouping. The six genera concerned belong to no less than four different lineages within the Aveneae/Poeae tribe complex.

Molecular phylogenetics and morphology

The cp sequence data of this study show a bifurcation of the Aveneae/Poeae tribe complex into Clades 1 (Aveneae) and 2 (Poeae) with strong support (Fig. 1) in agreement with all previous molecular studies conducted on plastid DNA in this tribe complex, irrespective of the methods and molecular markers used (RFLP, sequencing of *atpB*, *matK*–*trnK* region, *ndhF*, *ndhH*, *rbcL*, *trnT*–*F* region; Soreng and Davis 2000; Döring et al. 2007; Quintanar et al. 2007; Soreng et al. 2007; Bouchenak-Khelladi et al. 2008; Gillespie et al. 2008; Döring 2009; Schneider et al. 2009, 2011; Saarela et al. 2010). As discussed in some of these studies, both clades are not congruent with the traditional morphological delineation of the tribes Aveneae and Poeae (cf. Clayton and Renvoize 1986; Watson and Dallwitz 1992; 1992 onwards).

The Hainardieae genera sampled in this study (no molecular data obtained for *Agropyropsis*) are nested in Clade 2 (Poeae) of the Aveneae/Poeae tribe complex and none of them in Clade 1 (Aveneae). This result is similarly seen in the nuclear ITS analysis, which, however, does not show monophyly of Clade 1 (Fig. 2). The five strongly supported lineages (I–V) resolved within Clade 2 by the cpDNA tree (Fig. 1) are also retrieved largely by the nuclear ITS sequence data (Fig. 2) except for lineages II and IV, which are

becoming paraphyletic due to the changing positions of *Festuca* and *Phleum*, respectively (Fig. 2). These placements in ITS, however, are unsupported and most likely due to homoplasy (low consistency index for ITS; see Results) or paralogy-associated problems (Schneider et al. 2009).

Parapholis and *Hainardia* (lineage I)

Parapholis (*P. incurva* and *P. filiformis* sampled) and monotypic genus *Hainardia* (only *H. cylindrica*) are related closely with each other and with *Catapodium* (incl. *Scleropoa*), *Cutandia*, *Desmazeria*, *Sphenopus*, and *Vulpiella*. *Cynosurus* is a sister of this group within lineage I (Figs. 1 and 2). Furthermore, all these lineage I taxa are characterised by a 6 bp deletion in the *matK* gene region (Fig. 1). Previous molecular phylogenetic studies generally found a similar structure of this group, but had included fewer genera (Soreng and Davis 2000; Catalán et al. 2004; 2007; Davis and Soreng 2007; Döring et al. 2007; Quintanar et al. 2007; Bouchenak-Khelladi et al. 2008; Döring 2009; Schneider et al. 2009). Most current classifications of subtribes treat *Cynosurus* under monotypic subtribe Cynosurinae, whereas the other genera are unified under subtribe Parapholiinae (Table 1; cf. Tzvelev 1976; Soreng and Davis 2000; Soreng et al. 2003; 2007; Catalán et al. 2004; 2007; Quintanar et al. 2007). *Agropyropsis* and *Pholiurus*, affiliated with Parapholiinae in Soreng et al. (2007), are phylogenetically distant to this assemblage. These genera belong to the lineages of *Festuca* and relatives (subtribe Loliinae) and *Poa* and relatives (subtribe Poinae), respectively (see below; cf. Table 1). *Vulpiella*, conversely, is distant to subtribe Loliinae, in which it was placed by Soreng et al. (2007). It belongs to the *Parapholis* lineage as stated implicitly by Clayton and Renvoize (1986), who pointed to the similarity of *Vulpiella* and *Cutandia*.

Hainardia and *Parapholis* are formed only in the *matK* tree a clearly supported clade (Fig. 1), whereas the ITS data are ambiguous and even show *Parapholis* as paraphyletic (Fig. 2). Both genera share morphologically well-defined characters, such as the spicate inflorescences with the rachis disarticulating transversely at fruit maturity into segments, each of which bears an entire spikelet (Table 3; Fig. 3a, b). Additionally, the palea has glabrous and only weakly developed keels (Table 3; Fig. 4a, b). This contrasts to the other genera of lineage I (subtribe Parapholiinae) with more or less paniculate inflorescences, spikelets with two or more florets, and disarticulating below each floret, and with paleas having hairy keels (*Catapodium* incl. *Scleropoa*, *Cutandia*, *Desmazeria*, *Sphenopus*, *Vulpiella*; personal observations; Clayton and Renvoize 1986; Watson and Dallwitz 1992; 1992 onwards). The spikelet-bearing axes are disarticulating in *Vulpiella* and

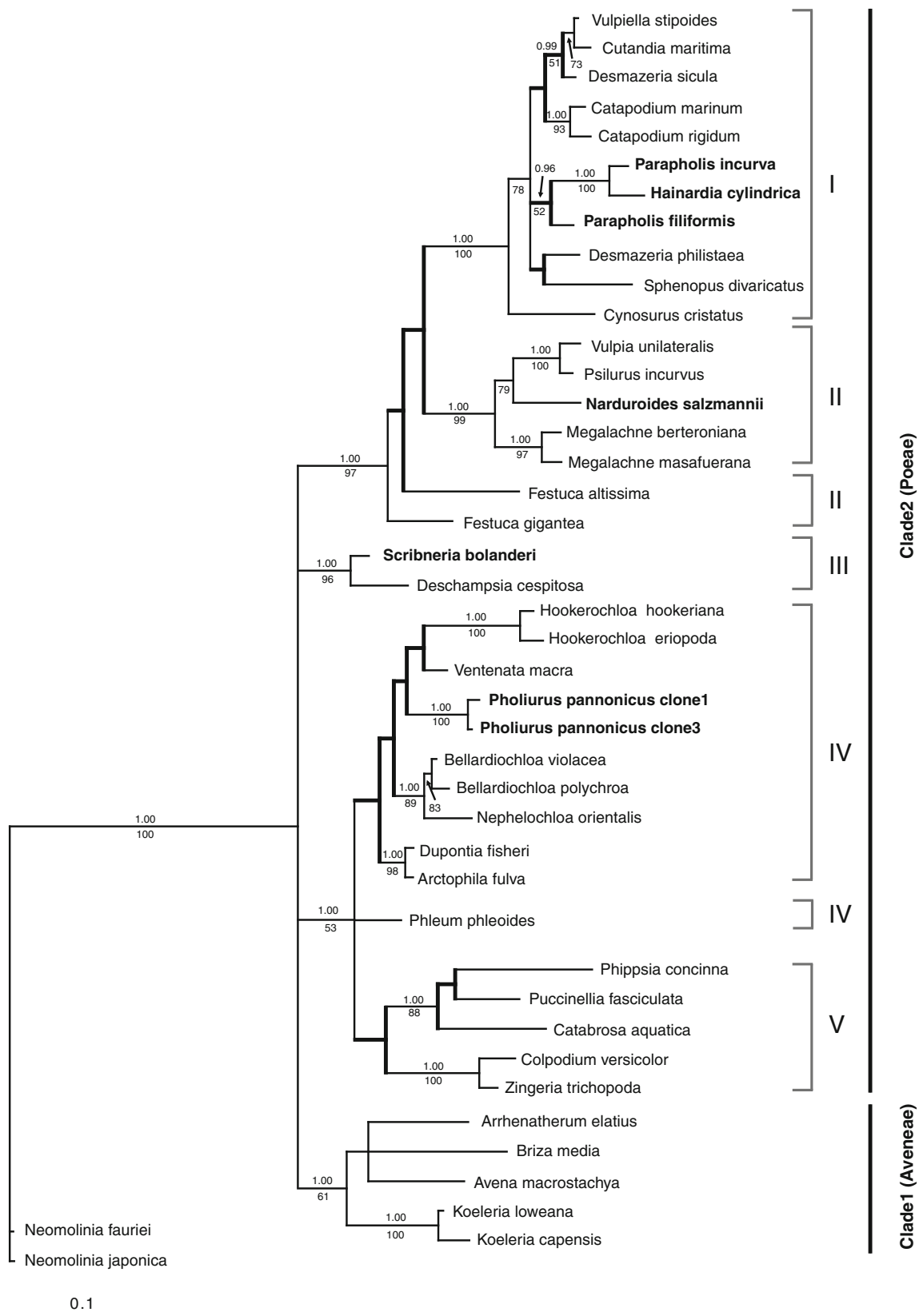


Fig. 2 Bayesian phylogenetic tree obtained from nuclear ITS DNA sequences. Branches collapsed in the strict consensus tree based on maximum parsimony analysis are in *bold*. Posterior probabilities ≥ 0.95

are shown above branches and numbers below are bootstrap supports ≥ 50 . Genera of the traditionally defined Hainardiaceae indicated in *bold*

Table 3 Morphological variation, chromosome numbers, biogeographical and ecological features of the traditional Hainardieae genera according to own results, and those of Clayton and Renvoize (1986), Watson and Dallwitz (1992; 1992 onwards)

	<i>Hainardia</i>	<i>Parapholis</i>	<i>Narduroides</i>	<i>Agropyropsis</i>	<i>Scribneria</i>	<i>Pholiurus</i>
Life form	Annual	Annual	Annual	Perennial	Annual	Annual
Inflorescence shape	Spicate; spikelets without pedicel ^a	Spicate; spikelets without pedicel ^b	Racemose; spikelets briefly pedicelled ^c	Spicate; spikelets without pedicel ^d	Spicate to racemose; sometimes several spikelets per rachis node (reduced panicle) ^e	Spicate; spikelets without pedicel ^f
Arrangement of spikelets	Distichous (bilateral) ^a	Distichous (bilateral) ^b	Distichous (bilateral), but frequently oriented \pm secund (unilateral) ^c	Distichous (bilateral) ^d	Distichous (bilateral) ^e	Distichous (bilateral) ^f
Spike or raceme rachis	Fragile ^a	Fragile ^b	Tough ^c	Tough ^{d, g}	Tough ^e	Tough ^f
Spikelet	1-Flowered	1-Flowered	2- to 4-Flowered ^{c, h}	3- to 6-Flowered ^d	1-Flowered ^e	2-Flowered ^f
Disarticulation of spikelet	Absent; spikelets falling entire together with the rachis fragments ^a	Absent; spikelets falling entire together with the rachis fragments ^b	Beneath the florets ⁱ	Beneath the florets ^j	Beneath the floret ^k	Below the glumes; spikelets falling entire with the glumes staying together ^f
Number of glumes	1 (Lower glume suppressed); 2 glumes present only in the terminal spikelet of the inflorescence ^a	2 ^b	2 ^c	2 ^d	2 ^e	2 ^f
Lemma	Awnless ^a	Awnless ^b	Awnless ^{c, i}	Awnless ^{d, j}	Awned; awn straight, arising from the sinus of the bidentate lemma tip ^{e, k}	Awnless ^f
Callus of the lemma	Absent	Absent	Present, glabrous ⁱ	Present, glabrous ^j	Present, hairy ^k	Absent
Keels of the palea	Glabrous, smooth ^l	Glabrous, smooth ^m	Ciliolate	Ciliolate ⁿ	Ciliolate	Ciliolate ^f
Ovary (karyopsis) with conspicuous apical appendage	Yes ^o	Yes ^p	Yes ^q	Yes ^r	No ^s	Yes ^t
Ovary (karyopsis) tip with two decurrent wings of the appendage	No ^o	No ^p	Yes ^q	Yes ^r	No ^s	No ^t
Karyopsis with deep longitudinal furrow and adherent to the palea	No	No	Yes ^q	No	No	No
Hilum shape	Shortly linear	Shortly linear ^p	Shortly linear	Shortly linear ^r	Punctiform	Oval ^t
Chromosome base number	$x=13$	$x=7, 9$ and 19	$x=7$?	$x=13$	$x=7$
Habitat	Saline or alkaline soils, coastal sands	Sandy soils, salt marshes	Dry places	Damp saline soils	Damp sandy soils in mountains	Damp saline or alkaline soils

Table 3 (continued)

	<i>Hainardia</i>	<i>Parapholis</i>	<i>Narduroides</i>	<i>Agropyropsis</i>	<i>Scribneria</i>	<i>Pholiurus</i>
Distribution	Mediterranean	Middle East and Mediterranean	Mediterranean	Mediterranean (Algeria)	Western USA, Washington to California	Eastern Europe to Central Asia

^a Fig. 3a^b Fig. 3b^c Fig. 3c^d Fig. 3d^e Fig. 3e^f Fig. 3f

^g Unclearly reported as "spikelets falling entire, deciduous with accessory branch structures; readily shedding fertile florets..." by Clayton et al. (2006 onwards). The fragility of the rachis (Clayton and Renvoize 1986) could be an artefact judging from the herbarium material examined in our study (see Fig. 3d). Our observation of a tough rachis agrees with the detailed description by Maire (1955), in which no disarticulation of the rachis was mentioned

^h Spikelets were reported as 3–6- or 4–6-flowered (Guinochet and de Vilmorin 1978; Clayton and Renvoize 1986; Watson and Dallwitz 1992; Clayton et al. 2006 onwards; Stace 1980; Devesa 1987; Romero 2009). In the herbarium material examined for this study we observed spikelets with consistently only 2–4 florets

ⁱ Fig. 4c, d^j Fig. 4e^k Fig. 4g, h^l Fig. 4a^m Fig. 4bⁿ Fig. 4f^o Fig. 5a^p Fig. 5b^q Fig. 5d, e^r Fig. 5f, g^s Fig. 5h^t Fig. 5c

some species of *Catapodium* in addition to the spikelets disarticulating between the florets. This might represent a transitional character to the disarticulation of the inflorescence axis in *Hainardia* and *Parapholis*, as argued by Soreng and Davis (2000), Soreng et al. (2007). *Hainardia* and *Parapholis* belong to the few genera within the Aveneae/Poeae tribe complex that deviate from the typical monoploid chromosome number of $x=7$ (Table 4, Figs. 1 and 6c).

Narduroides (lineage II) and *Agropyropsis*

Our analyses resolved the monotypic genus *Narduroides* (only *N. salzmannii*) as a member of lineage II, which is clearly monophyletic from the cp *matK* data but paraphyletic with respect to the placement of *Festuca* in the nuclear ITS tree (Figs. 1 and 2). A close relation of *Narduroides* to *Festuca*, *Psilurus* and *Vulpia* was also found by previous molecular studies (Catalán et al. 2004; 2007; Torrecilla et al. 2004; Soreng et al. 2007; Bouchenak-Khelladi et al. 2008), and these genera were accommodated under subtribe Loliinae

(Soreng et al. 2003; 2007; Catalán et al. 2004; 2007). *Megalachne* is a further element of the *Festuca* lineage (Figs. 1 and 2; Schneider et al. 2011). Although it was not considered an element of the Loliinae in recent classifications (Soreng et al. 2003; 2007; Catalán et al. 2007), the molecular data corroborate placement in the *Festuca* lineage as suggested by Clayton and Renvoize (1986).

The monotypic genus *Agropyropsis* (only *A. lolium*) differs morphologically from the genera of lineage I (Figs. 1 and 2), which represents subtribe Parapholiinae as circumscribed in this study: Spikelets of *Agropyropsis* have several flowers instead of a single flower and ciliolate instead of glabrous keels of the palea (Table 3; Figs. 3d and 4f) as in *Hainardia* and *Parapholis*, which are the only genera of Parapholiinae with spicate inflorescences (after removal of *Agropyropsis* and *Pholiurus* from this subtribe). The spikelet rachilla in *Agropyropsis* disarticulates below each floret (cf. footnote to Table 3), whereas the spikelet axis is tough in *Hainardia* and *Parapholis* and the rachis of the inflorescence disarticulates (Table 3 with footnotes; Fig. 3a, b, d). Additionally, the rachilla internode beneath the uppermost floret is comparatively long in

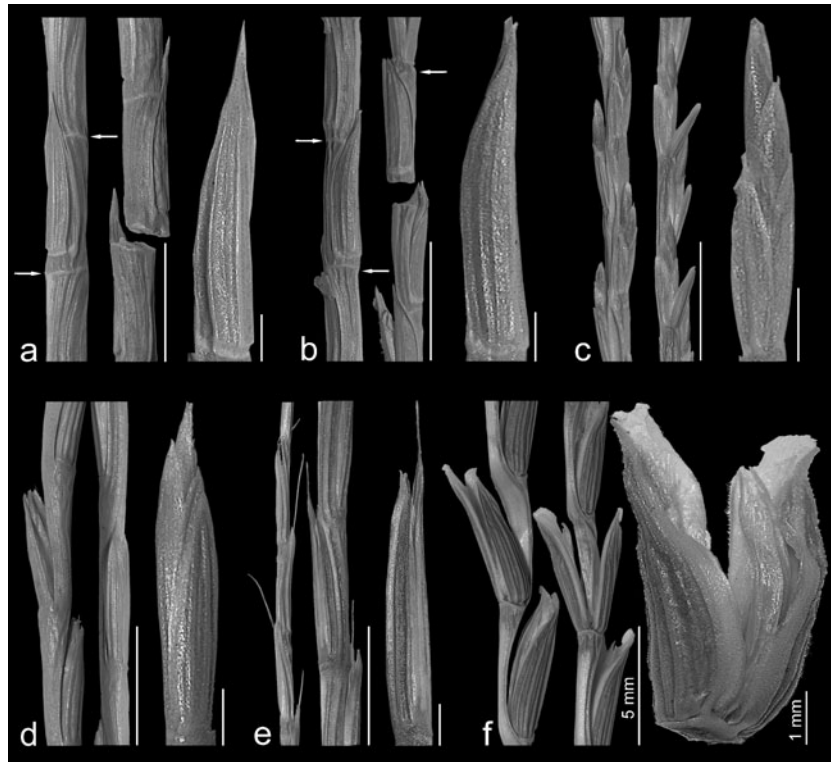


Fig. 3 a–f Spicate or racemose inflorescences in genera of traditionally recognised Hainardieae. **a** *Hainardia cylindrica* and **b** *Parapholis strigosa* with rachis of the inflorescence disarticulating at maturity. Disarticulation points are marked by *short arrows*. The spikelets are sunken in the rachis and have only a single (upper) glume in *Hainardia* except for the uppermost spikelet where both glumes are present. Both glumes are present in the spikelets of *Parapholis* and stand side by side. **c** *Narduroides salzmannii*, **d** *Agropyropsis lolium*, and **e**

Scribneria bolanderi have a tough rachis and spikelets disarticulating below each floret. **f** *Pholiurus pannonicus* with tough rachis and spikelets falling entire. Material used: **a** fruits from Göttingen Botanical Garden in 2006, no. 1850 (HAL); **b** Werner 3477 (HAL 71657); **c** Rivas-Martínez *s.n.* (BASBG); **d** left Kralik *s.n.* (JE), right Cosson *s.n.* (JE); **e** left Wilken 16163 and Painter (RSA 695253), right Ahart 10180 (RSA 692211); **f** Hilbig *s.n.* (HAL 67272)

Agropyropsis (Fig. 4e) and the appendage of the ovary tip has two conspicuous decurrent wings (Table 3; Fig. 5f, g). Altogether, in these morphological characters, perennial *Agropyropsis* thus rather resembles *Narduroides* (Table 3; Figs. 3c, 4c, d and 5d, e) or other annual or perennial taxa with spike-like inflorescences and prolonged upper rachilla internodes of the *Festuca* lineage (subtribe Loliinae), in which it seems to be positioned judging from the available data. Molecular information on *Agropyropsis*, however, is still wanting.

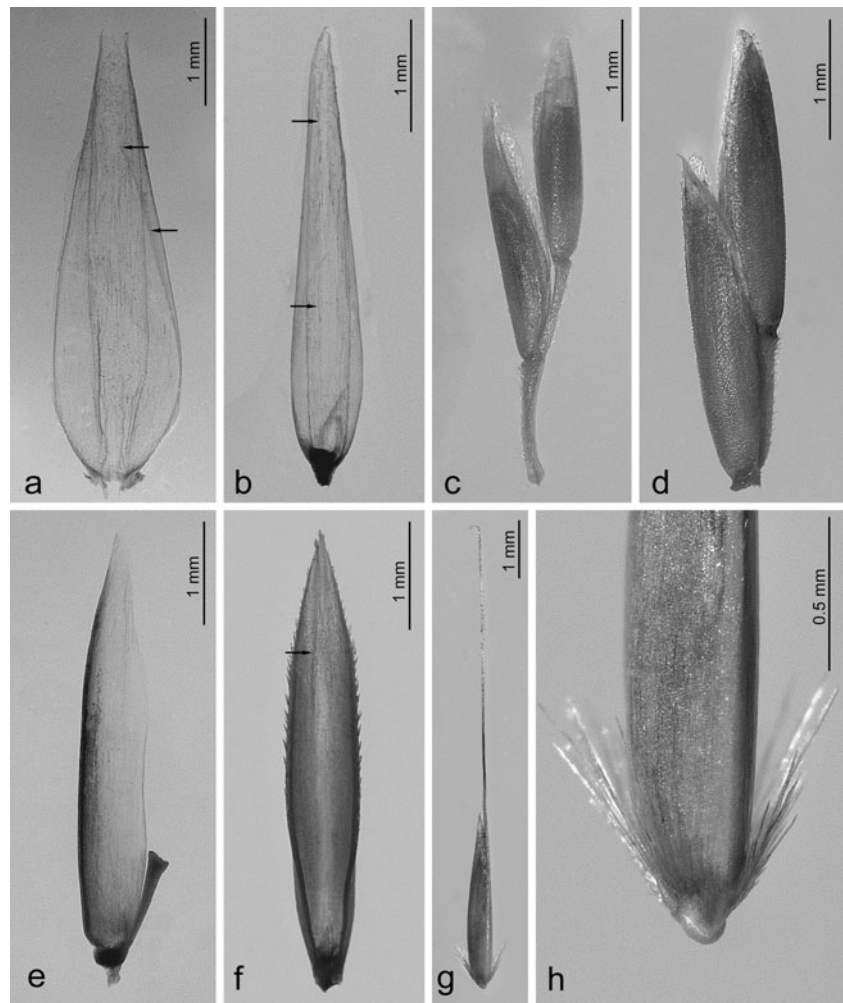
Scribneria (lineage III)

The placement of the monotypic genus *Scribneria* (only *S. bolanderi*) in a lineage together with *Deschampsia cespitosa* is obtained by cp *matK* as well as nuclear ITS (Figs. 1 and 2). Additionally, a 3 bp deletion in the intron region between the *matK* gene and the 3'*trnK* exon supports the close relatedness of both taxa. CpDNA restriction site data have shown *Scribneria* to be distant from the traditional Hainardieae genera *Hainardia*, *Parapholis* and their relatives, and placed in a clade with *Aira*, *Avenula*, *Holcus*, and *Molinierella*

(Soreng and Davis 2000). Unfortunately, the close relatedness of *Scribneria* and *Deschampsia* remained unnoticed in that study, because what the authors considered *Deschampsia cespitosa* had been misidentified material of *Agrostis* (Davis and Soreng; 2007: p. 347). Placement of *Deschampsia* in Clade 2 (Poeae; Figs. 1 and 2) is now firmly established by cp and nuclear DNA data (Davis and Soreng 2007; Döring et al. 2007; Quintanar et al. 2007; Bouchenak-Khelladi et al. 2008; Döring 2009; Schneider et al. 2009; Saarela et al. 2010).

Maintenance of a separate monotypic Scribneriinae for *Scribneria* (Soreng et al. 2003; 2007) is not required, due to the sister relation to *Deschampsia* revealed by the molecular data (Figs. 1 and 2). We suggest that both genera be accommodated in a common subtribe, because *Scribneria* having an awned lemma with a hairy callus (Table 3; Figs. 3e and 4g, h) resembles *Deschampsia* also morphologically as noted by Stebbins and Crampton (1961). Additionally, *Scribneria* does not always have spicate to racemose inflorescences. Inflorescences of vigorous specimens are reduced panicles with several branches at the lower nodes and comparatively long-pedicelled spikelets on partly branched

Fig. 4 a–h Spikelet details. Palea of **a** *Hainardia cylindrica* and **b** *Parapholis incurva* with glabrous keels (nerves). **c, d** florets of *Nardurooides salzmannii* with glabrous callus of the lemmas and long rachilla internodes. **e, f** *Agropyropsis lolium* with glabrous callus of the lemma (**e**) and ciliate keels of the palea (**f**). **g, h** *Scribneria bolanderi* with lemma awned from a sinus of the lemma tip (**g**) and hairy callus (**h**). The margin of the lemma is marked at one side in **a, b,** and **f** with *arrows*. Material used: **a** fruits from Göttingen Botanical Garden in 2006, no. 1850 (HAL); **b** Röser 2517 (HAL); **c** Hernández *s.n.* (C); **d** Rivas-Martínez *s.n.* (BASBG); **e, f** Cosson *s.n.* (JE); **g, h** Wilken 16163 and Painter (RSA 695253)



peduncles (personal observations; Stebbins and Crampton 1961; Smith 2007). A close relation of *Scribneria* and *Deschampsia* is reflected also in the occurrence of $2n=26$ chromosomes (Table 4, Figs. 1 and 6a) based on a monoploid chromosome number of $x=13$, which is rather uncommon within the whole subfamily Pooideae.

The precise placement of the genus pair *Deschampsia/Scribneria* (lineage III in Figs. 1 and 2) within Clade 2 (Poeae) should be investigated further with a more extended sampling of additional Clade 2 taxa. From previous investigations, albeit using a different sample of genera than ours, it is obvious that *Deschampsia cespitosa* and other species of *Deschampsia* s.str. are close to *Holcus* and *Vahlodea* and rather distant from *Aira* and *Avenella* (cpDNA: Döring et al. 2007; Quintanar et al. 2007; Soreng et al. 2007; Döring 2009; Schneider et al. 2009; Saarela et al. 2010; nuclear DNA: Schneider et al. 2009). Alternatively, *Deschampsia* was slightly distant also from *Holcus* and *Vahlodea*, but even more distant from *Aira* and *Avenella* (cpDNA: Davis

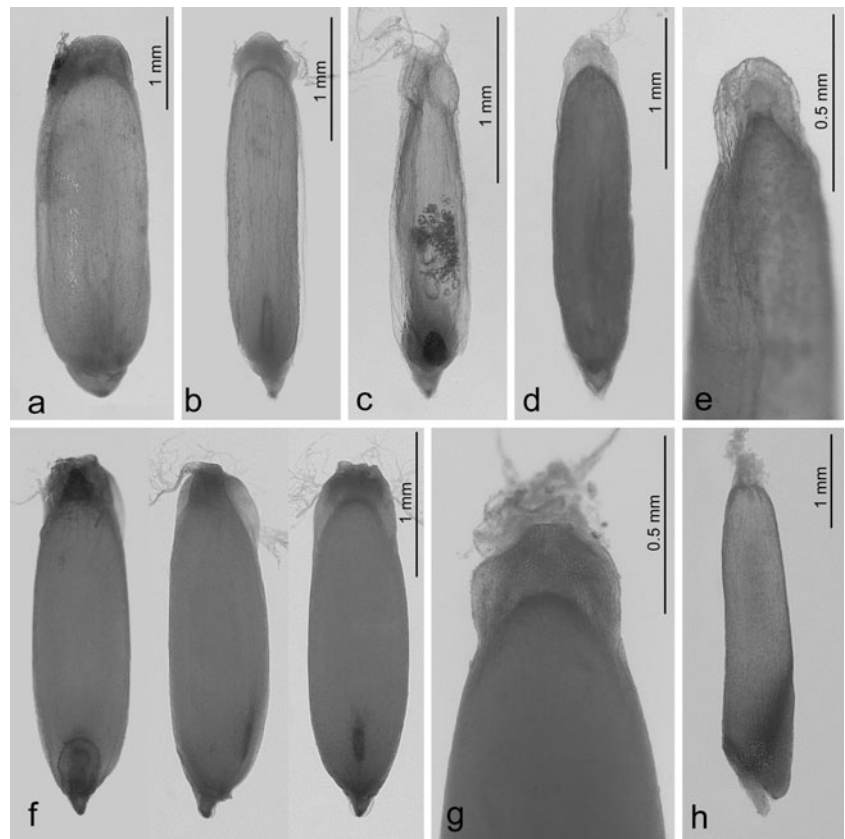
and Soreng 2007; nuclear DNA: Quintanar et al. 2007) or *Deschampsia*, *Avenella* and *Holcus* were part of more or less a polytomy (Saarela et al. 2010).

In view of the available molecular phylogenetic information (cf. also Garcia-Suárez et al. 1997) merging of *Deschampsia* with *Aira*, *Avenella* and other genera under subtribe Airinae thus should be discontinued. Further study is necessary to ascertain if the two genera *Deschampsia* and *Scribneria* represent together a separate subtribe; in this instance, its correct name would be Aristaveninae (cf. Albers and Butzin 1977) due to nomenclatural priority over Scribneriinae. Conversely, if *Deschampsia* and *Scribneria* are placed together with *Holcus* and *Vahlodea* in a common lineage, the older subtribe name Holcinae would be applicable.

Pholiurus and allies (lineage IV)

Based concordantly on both sequence datasets, the monotypic genus *Pholiurus* (only *P. pannonicus*) is firmly nested

Fig. 5 a–h Karyopses. **a** *Hainardia cylindrica* and **b** *Parapholis strigosa* with short linear hilum. **c** *Pholiurus pannonicus* with oval hilum. **d**, **e** *Narduroides salzmannii* with short linear hilum and longitudinal furrow of the karyopsis adherent to the palea. **f**, **g** *Agropyropsis lolium* with embryo, short linear hilum, and large apical ovary appendage. **h** *Scribneria bolanderi* with comparatively large embryo. Material used: **a** fruits from Göttingen Botanical Garden in 2006, no. 1850 (HAL); **b** Werner 3477 (HAL 71657); **c** Sadler *s.n.* (HAL 112064); **d**, **e** Hernández *s.n.* (C); **f**, **g** Cosson *s.n.* (JE); **h** Wilken 16163 and Painter (RSA 695253)



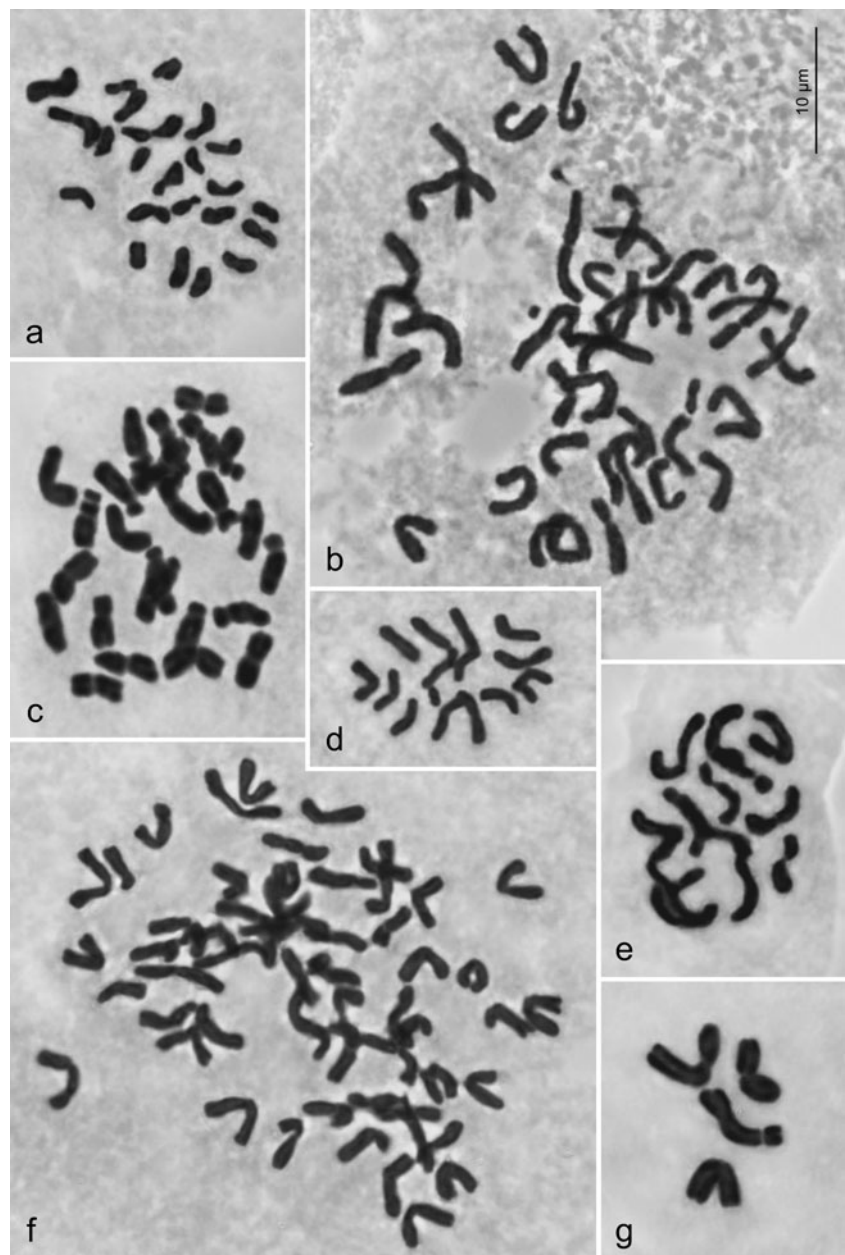
in lineage IV (*Poa* lineage), and is thus rather distant to *Hainardia* and *Parapholis*. *Pholiurus* is close to *Arctophila*, *Bellardiachloa*, *Dupontia*, *Hookerochloa* incl. *Festucella*, *Nepheleochloa*, and *Ventenata* (Figs. 1 and 2). Monophyly of these taxa is supported by a common insertion of 6 bp in the intron region between *matK* gene and the 3'*trnK* exon. Although *Nepheleochloa* and *Pholiurus* have not been investigated in any previous molecular phylogenetic study, this assemblage of genera and its comparatively close relationship to the genus *Phleum* was found principally also in a number of previous studies (Soreng and Davis 2000; Hunter et al. 2004; Gillespie et al. 2007, 2008, 2009, 2010; Döring

et al. 2007; Davis and Soreng 2007; Soreng et al. 2007; Döring 2009; Schneider et al. 2009). *Phleum* resolves with support in lineage IV based on the cpDNA sequence data, whereas ITS shows it next to this lineage in a polytomy with lineage V (Figs. 1 and 2). Such an unstable position of *Phleum* is encountered frequently: CpDNA usually suggests a placement compatible to that seen in Fig. 1, irrespective of the particular markers used (Döring et al. 2007; Gillespie et al. 2008, 2009; Döring 2009; Schneider et al. 2009). Nuclear ITS or combined ITS/ETS placed *Phleum* in more-or-less the same polytomy with taxa of lineage V as seen in Fig. 2 (Quintanar et al. 2007; Gillespie et al. 2008, 2009, 2010;

Table 4 Chromosome numbers and ploidy levels in exemplary species of the Aveneae/Poeae tribe complex

Taxa	Chromosome number found in this study and likely ploidy levels	Chromosome numbers reported by Fedorov (1969) and IPCN (2010)
<i>Colpodium versicolor</i>	$2n=2x=4$	$2n=4$
<i>Deschampsia cespitosa</i>	$2n=2x=26$	$2n=24, 26, 26+1-2B, 52$
<i>Festuca gigantea</i>	$2n=6x=42$	$2n=14, 42, 56$
<i>Hainardia cylindrica</i>	$2n=2x=26$	$2n=26$
<i>Koeleria cristata</i>	$2n=8x=56$	$2n=14, 14+1-2B, 28, 56, 70$
<i>Phleum phleoides</i>	$2n=2x=14+2B$	$2n=14, 14+1-4B, 28$
<i>Vulpia bromoides</i>	$2n=2x=14$	$2n=14$

Fig. 6 a–g Mitotic metaphase chromosomes. of **a** *Deschampsia cespitosa* ($2n=26$). **b** *Festuca gigantea* $2n=42$. **c** *Hainardia cylindrica* $2n=26$. **d** *Phleum phleoides* ($2n=14+2B$). **e** *Vulpia bromoides* ($2n=14$). **f** *Koeleria cristata* ($2n=56$). **g** *Colpodium versicolor* ($2n=4$)



Schneider et al. 2009). A combined analyses of respective cp and nuclear ITS or cp and nuclear ITS/ETS datasets placed *Phleum* (and *Milium*; cf. below) with moderate support even amongst other genera of the *Poa* lineage, where both genera were not placed by the separate analyses of the individual cp and nuclear datasets. This somewhat surprising result was obtained independently in different studies using a different set of taxa and different DNA regions (cp *matK* and nuclear ITS: Schneider et al. 2009; cp *trnT-trnF* and nuclear ITS/ETS: Gillespie et al. 2009, 2010). The precise position of *Phleum*, however, differed in both studies.

Morphologically, *Pholiurus*—until now considered to be part of tribe Hainardieae or subtribe Parapholiinae—

resembles *Hainardia* and *Parapholis*, due to its spicate inflorescences with hollowed rachis, sunk-in spikelets and the strongly thickened glumes with prominent nerves (Fig. 3a, b, f). The inflorescence rachis, however, is tough in *Pholiurus* and the spikelets disarticulate below the glumes, which thus fall in their entirety (Table 3; Fig. 3f). The latter character is not found in lineage I with *Hainardia* and *Parapholis* (Figs. 1 and 2), but occurs in some genera of the *Poa* lineage (lineage IV), in which *Pholiurus* is placed by the molecular phylogenetic data. These genera with spikelets falling entire were previously ascribed to either a broadly defined tribe Phleaeae (Tzvelev 1989) or to broadly or narrowly defined subtribes Phleinae and/or Alopecurinae, respectively, and a further subtribe acknowledged only in

some classifications, namely Cinninae (cf. Tzvelev 1976; Clayton and Renvoize 1986; Soreng and Davis 2000; Quintanar et al. 2007; Soreng et al. 2003; 2007; Gillespie et al. 2008, 2010).

The genera *Alopecurus*, *Beckmannia*, *Cornucopiae*, and *Limnas* (subtribe Alopecurinae *sensu* Gillespie et al. 2010), *Cinna* and *Limnodea* (subtribe Cinninae *sensu* Soreng et al. 2007, or included with subtribe Poinae *sensu* Gillespie et al. 2010) and additionally *Rhizocephalus* (subtribe Phleinae *sensu* Tzvelev 1976 and Gillespie et al. 2010) all have spikelets falling entire like *Pholiurus* and not disarticulating above the glumes or below the floret(s). The spikelets, however, are always one- (or rarely two-) flowered in *Beckmannia* instead of two-flowered as in *Pholiurus*. The hilum of the caryopses is rounded and the endosperm seems to be soft in most if not all of these genera (Clayton and Renvoize 1986; Watson and Dallwitz 1992; 1992 onwards; Clayton et al. 2006 onwards) as newly reported here also for *Pholiurus* (Table 3; Fig. 5c), in contrast to *Agropyropsis*, *Hainardia*, *Narduroides*, *Parapholis* and *Scribneria* with punctiform or shortly linear hilum (Table 3; Fig. 5a, b, d–f). Fruit data are seemingly missing for *Limnas*.

Judging from morphology, we expect the closest extant relatives of *Pholiurus* among the assemblage around *Alopecurus* with allied genera showing the same type of spikelets falling entire and with a spikelet abscission zone below the glumes. To address this question using molecular phylogenetic data, we have started a denser sampling of potential target taxa.

Phleum, with its segregate genera *Maillea* and *Pseudophleum* (subtribe Phleinae *sensu* Tzvelev 1976; Gillespie et al. 2010), has spikelets disarticulating above the glumes (Conert 1979–1998; Watson and Dallwitz 1992; 1992 onwards) in contrast to *Rhizocephalus* (Bor 1970; Tzvelev 1976, 1982), although this has not always been reported correctly or was overlooked (e.g. Clayton and Renvoize 1986; Gillespie et al. 2010: p 591). Altogether a maintenance of the subtribes Alopecurinae and Phleinae as recently reinstated and delineated (Gillespie et al. 2010) needs to be examined further.

Puccinellia lineage (lineage V)

This lineage comprises taxa that were regarded as members of the separate subtribes Puccinelliinae (*Catabrosa*, *Puccinellia*, *Phippsia*) and Miliinae (*Colpodium*, *Zingieria*; cf. Tzvelev 1976; Soreng et al. 2003; 2007; Gillespie et al. 2010). *Zingieria* and *Colpodium* are supported strongly as sister taxa by cpDNA data, sharing a deletion of 9 bp in the *matK* gene region (Fig. 1; Schneider et al. 2009), and nuclear ITS sequences (Fig. 2; Quintanar et al. 2007; Gillespie et al. 2008; Schneider et al. 2009). They are related also in chromosomal aspects, as the *Colpodium versicolor* or a

closely related genome ($x=2$; Fig. 6g) was involved in the polyploid evolution of *Zingieria* (Kotseruba et al. 2010; cf. Kotseruba et al. 2003, 2005; Kim et al. 2009). Both genera are nested within the lineage V together with *Catabrosa*, *Phippsia* and *Puccinellia*, but in the ITS tree without support (Figs. 1 and 2). This result concurs with previous studies, in which *Catabrosa*, *Phippsia* and *Puccinellia* were placed in a common lineage with either *Zingieria* or with *Zingieria* and *Colpodium* (Döring et al. 2007; Döring 2009; Schneider et al. 2009) according to cpDNA data. Nuclear ITS data were less decisive and showed larger polytomies or branches without BS, which encompassed members of the *Poa* lineage (lineage IV in this study) and included *Milium*, not sampled in this study (Quintanar et al. 2007; Gillespie et al. 2008; Schneider et al. 2009). A similar structure was obtained from a combined ITS and ETS data matrix (Gillespie et al. 2010). According to cpDNA data, however, *Milium* is nested firmly in the *Poa* lineage (Döring et al. 2007; Quintanar et al. 2007; Gillespie et al. 2008; Schneider et al. 2009). Considering classification, this suggests that subtribe Miliinae should be abandoned, and *Colpodium* and *Zingieria* acknowledged as members of the *Puccinellia* lineage.

Cytogenetic analysis

The monoploid chromosome number of $x=7$ and ‘large’ chromosomes prevail within the Aveneae/Poeae tribe complex and the Triticeae incl. Bromeae, but there are exceptions. A previous study corroborated the tendency for a reduction in monoploid chromosome numbers from the early (tribes Brachyelytreae, Stipeae, Nardeae, Meliceae, Phaenospemateae, Duthieae, Diarrheneae, Brachypodieae) to the late diverging lineages of the subfamily Pooideae (tribe Triticeae, Aveneae/Poeae tribe complex; cf. Schneider et al. 2011). With a broader sampling of genera from the Aveneae/Poeae, the present study shows that chromosome numbers vary in different lineages (I–V; Fig. 1) of this tribe complex between $x=2$ and $x=13$, with even $x=19$ reported in the literature (Table 4; Fig. 1). The most frequent number is $x=7$, long recognised as characteristic of this group (Stebbins 1956, 1985; Clayton and Renvoize 1986; Hunziker and Stebbins 1987; Watson and Dallwitz 1992; 1992 onwards; Hilu 2004; Rodionov et al. 2007). Meta-phase chromosomes are typically large and more than 4 μm long (Fig. 6).

Chromosome numbers may be variable even within smaller phylogenetic groupings, as seen in lineage I, which usually has chromosome numbers of $x=7$ (Fig. 1), $x=13$ in *Hainardia* (Table 4, Figs. 1 and 6c), and different numbers of $x=7$, 9, 19 reported within the genus *Parapholis*. *Scribneria* in lineage III also has the uncommon chromosome number of $x=13$. Interestingly, this uncommon chromosome number is well-documented also for its evidenced neighbour

genus *Deschampsia* and is known to occur in *D. cespitosa* (Figs. 1 and 6a). In *Deschampsia*, this monoploid chromosome number was probably derived from a dysploid chromosome number reduction in a tetraploid from $2n=28$ to $2n=26$ and not from hybridisation between parental taxa with monoploid chromosome sets of $x=7$ and $x=6$ and subsequent genome doubling (García-Suárez et al. 1997; Winterfeld 2006; Winterfeld and Röser 2007). The origin of the monoploid chromosome number of $x=13$ in *Hainardia*, which has much larger chromosomes (Fig. 6c) than in *Deschampsia*, and of $x=19$ reported in *Parapholis*, has not yet been investigated. However, it seems likely that these high numbers are actually resting already on polyploidy as in *Deschampsia*.

Conclusions

Based on molecular and morphological evidence, the traditionally defined tribe Hainardiaceae is revealed as a polyphyletic grouping. Its six genera belong to no less than four different lineages within Clade 2 (Poeae) of the Aveneae/Poeae tribe complex. *Hainardia* and *Parapholis* represent strongly supported sister taxa within the *Parapholis* lineage (subtribe Parapholiinae) according to DNA sequence data. Both show a disarticulation of the inflorescence axis at maturity of the fruits into segments and have paleas with glabrous keels as synapomorphic characters, which are absent in *Agropyropsis*, *Narduroides*, *Pholiurus*, and *Scribneria*. *Narduroides* was confirmed as a member of the *Festuca* lineage (Loliinae). The same placement is suggested by morphological characters such as spike-like inflorescence and prolonged upper rachilla internodes for *Agropyropsis*. The genus *Scribneria* resembles *Deschampsia* widely in molecular and morphological features. Both are characterised by an awned lemma and a conspicuous hairy callus. A monoploid chromosome number of $x=13$, otherwise rare within the subfamily Pooideae, supports their close relatedness. Further studies are required to clarify if *Scribneria* and *Deschampsia* alone represent a separate subtribe, i.e. Aristaveninae, or have to be accommodated together with *Holcus* and *Vahlodea* in a reinstated subtribe Holcinae. Molecular phylogenetic data placed *Pholiurus* firmly within the lineage of *Poa* and relatives (Poinae). Disarticulation below the glumes, thus entirely falling spikelets, is found also in *Alopecurus*, *Beckmannia*, *Cornucopiae*, *Limnas*, *Cinna*, *Cyathopus*, *Limnodea*, and *Rhizocephalus*. A more detailed future investigation of the latter genera is needed to validate their current classification under different small-sized subtribes.

In conclusion, some, at first glance striking, characteristics of the former ‘Hainardiaceae’ genera such as unbranched inflorescences with spikelets in two rows, sunk-in spikelets

partly with strongly thickened glumes and adaptation to saline soils, have originated several times in parallel within the Aveneae/Poeae tribe complex and are not indicative of phylogenetic relatedness.

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