

Phylogenetic analysis of *Jarava* (Poaceae, Pooideae, Stipeae) and related genera: testing the value of the awn indumentum in the circumscription of *Jarava*

Sergio E. Sclovich · Liliana M. Giussani ·
Ana M. Cialdella · Silvana M. Sede

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Abstract *Jarava*, a genus of the tribe Stipeae with 31 American species, is considered polyphyletic. In previous phylogenetic analyses, despite a few species of *Jarava* were used, they were included in the Major American Clade (MAC) together with species of *Achnatherum*, *Amelichloa* and *Nassella*. The main goal of this study is to test the monophyly of *Jarava* and to estimate relationships of *Jarava* species and allied genera. Eighty-nine species of Stipeae (17 species of *Jarava*) were included in the analyses of *trnH-psbA* and ITS regions from plastid and nuclear genomes, respectively. Morphological characters were optimized on one of the shortest trees derived from the combined analysis. *Jarava* splits into different lineages. *Jarava ichu*, the type species of the genus, and other eight species of *Jarava* were grouped into subclade B; another four species were associated with *Achnatherum* in subclade A. Both subclades A and B were included into the MAC. Three species: *Jarava neaei*, *J. psylantha*, and *J. subplumosa*, were grouped with *Pappostipa* in subclade C. The close relationship between *Jarava* and *Pappostipa* appears as a novelty and they should be considered within this genus or recognized as a new genus sister to *Pappostipa*. The awn indumentum (hair length and position of long hairs, either in part or the entire awn) and the subacute or acute callus are useful to recognize the group (subclade C). Geographical distribution of *Jarava* from subclade C overlaps

completely with that of *Pappostipa*. The shared geographical distribution is in accordance with major groupings in our phylogenetic topology.

Keywords ITS · *Jarava* · Molecular phylogeny · Morphological character optimization · Stipeae · *TrnH-psbA*

Introduction

Stipeae Dumort., a well-defined tribe of the subfamily Pooideae within the grass family Poaceae, has ca. 600 species with a worldwide distribution along temperate and warm temperate grasslands (Barkworth et al. 2008; Cialdella et al. 2010; Hamasha et al. 2011). Morphologically, the tribe is characterized by the single-flowered spikelets with terminally awned lemmas and the awn usually geniculate and twisted in the proximal segment. Many regional taxonomic revisions have been accomplished for Stipeae, and based on morphological characters and molecular phylogenies, some sections and subgenera have been clarified for particular areas (Rojas 1997; Peñailillo 2002; Cialdella et al. 2007, 2010, 2014; Romaschenko et al. 2008, 2010, 2012; Hamasha et al. 2011). However, generic and infrageneric boundaries within the whole tribe are still unresolved, and they are in need of a deeper review. Some species of *Achnatherum* P.Beauv., *Amelichloa* Arriaga & Barkworth, *Jarava* Ruiz & Pav., *Nassella* (Trin.) É.Desv., *Pappostipa* (Speg.) Romasch., P.M.Peterson & Soreng, and *Stipa* L. have been alternatively assigned into different genera or subgenera depending on the author criteria (Barkworth 1990; Peñailillo 2002; Arriaga and Barkworth 2006; Romaschenko et al. 2008; Cialdella et al. 2010).

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S. E. Sclovich · L. M. Giussani · A. M. Cialdella ·
S. M. Sede (✉)
Instituto de Botánica Darwinion, Labardén 200 Casilla de Correo
22, San Isidro, B1642HYD Buenos Aires, Argentina
e-mail: ssede@darwin.edu.ar

Jarava, originally described by Ruiz and Pavón (1794) and based on the single species *Jarava ichu* Ruiz & Pav., was characterized by having long hairs on the upper portion of the lemma forming an apical pappus (Cialdella et al. 2010). The genus was then treated as a subgenus of *Stipa* by Trinius and Ruprecht (1842), by Spegazzini (1901), and as a section of *Stipa* by Steudel (1855). Many of Spegazzini's observations were considered by different authors (Peñailillo 2002; Romaschenko et al. 2008; Cialdella et al. 2010). Moreover, Jacobs and Everett (1997), based on the morphological characterization by Caro and Sanchez (1973), considered that *Stipa* subg. *Jarava* should be reinstated at generic rank. Rojas (1997), when revising the Bolivian species of *Jarava*, added six new combinations and a new species. Afterwards, the genus was treated by Peñailillo (2002) who included approximately 40 species previously treated by Spegazzini (1901) in *Stipa*. He expanded the generic concept of *Jarava*, considering species with florets adapted to anemochory, not only those species having long hairs at the top of the lemma forming a pappus, but also, those species with plumose awns: completely covered by hairs, or only present in the proximal segment (column) or in the terminal segment (subula) of the awn. Peñailillo (2002) included in *Jarava* three subgenera treated by Spegazzini (1901): *Stipa* subg. *Jarava* (Ruiz & Pav.) Trin. & Rupr., *Stipa* subg. *Pappostipa* Speg., and *Stipa* subg. *Ptilostipa* Speg.

Currently, *Jarava* is recognized as polyphyletic; its species are intermingled with *Achnatherum*, *Amelichloa*, and *Nassella* (Cialdella et al. 2007, 2010, 2014; Romaschenko et al. 2008, 2012; Hamasha et al. 2011). Romaschenko et al. (2008), based on molecular studies and the micromorphology of the lemma, reduced the boundaries of *Jarava* and transferred 23 species with hairs on the column of the awn to *Pappostipa*. Finally, *Jarava*, as accepted by Soreng et al. (2003) for the American taxa, by Morrone et al. (2008) for the austral South American species, and by Cialdella (2012) for the Argentinean species, includes 31 species. The genus concept was restricted to species with the awn 1–2 geniculate or slightly curved, generally scabrous, or with long hairs at the top (subula) or in all its extension; the antherium has glabrous apex or a few short hairs, sometimes with long hairs forming a pappus at the lemma apex and the border of the crown (Cialdella 2012). As the morphological variability of *Jarava* is linked to the hairiness of the lemma and the awn, it is necessary to phylogenetically evaluate more species with pubescent awns. In this work, we increase the sampling of *Jarava* to test the monophyly of the genus, to reveal the evolution of diagnostic characters, the distribution of species, and to discuss the generic circumscription.

Materials and methods

Taxon sampling

A total of 17 out of 31 species of *Jarava* were included in this study. Samples were either collected in the field or obtained from herbarium material. Five species were included for the first time: *Jarava filifolia* (Nees) Ciald., *J. hypsophila* (Speg.) Peñail., *J. illimanica* (Hack.) F.Rojas, *J. psylantha* (Speg.) Peñail., and *J. subplumosa* (Hicken ex F.A.Roig) Peñail. The identity of the specimen of *J. neaei*, previously included by Hamasha et al. (2011) in a phylogenetic study, could not be corroborated; therefore several specimens from different locations were herein included. We used four individuals of *Jarava neaei* and three of *J. psylantha* from different geographical regions. We selected representative species of Stipeae available in GenBank to include all monophyletic groups according to previous phylogenetic studies (Cialdella et al. 2007, 2010, 2014; Romaschenko et al. 2008, 2012). The selection was guided by the aim of representing all genera within the tribe Stipeae including the diversity found in America: *Aciachne* Benth., *Amelichloa*, *Anatherostipa* (Hack. ex Kuntze) Peñail., *Hesperostipa* (M.K.Elias) Barkworth, *Jarava*, *Nassella*, *Ortachne* Nees ex Steud., *Pappostipa*, *Piptatheropsis* Romasch., P.M.Peterson & Soreng, *Piptochaetium* J.Presl, and in the Old World: *Aristella* (Trin.) Bertol., *Austrostipa* S.W.L.Jacobs & J.Everett, *Oloptum* Röser & Hamasha, *Ptilagrostis* Griseb., *Stipa*, and *Trikeriaia* Bor. Voucher information and GenBank accession numbers are listed in Table 1. Selected species of *Achnatherum* and *Piptatherum* P.Beauv. were sampled to represent the polyphyly of these genera.

DNA isolation, amplification and sequencing

Genomic DNA was isolated from silica-dried leaf tissue following a modified CTAB protocol (Doyle and Doyle 1987) and from herbarium material with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The plastid DNA region *trnH-psbA* [primers *trnH*^(GUG) and *psbA* (Shaw et al. 2005)] and nuclear ribosomal DNA regions ITS1 and ITS2 [primers ITS4, ITS5, ITS2, and ITS3 (White et al. 1990)] were amplified. The profile consisted of 94 °C for 3 min followed by 30 cycles of 94 °C for 1 min, 48 °C (*trnH-psbA*) or 61 °C (ITS) for 1 min, and 72 °C for 1 min. PCR reactions were performed in 25 µl final volume with approx. 50–100 ng of DNA template, 0.6 µM of each primer, 50 µM of dNTPs, 5 mM MgCl₂, 1X Taq buffer and 1.25 units of Taq polymerase (Invitrogen, Sao Paulo, Brazil). The selection of the plastid region was based on the percentage of informative characters as used in

Table 1 Voucher specimens, geographical location, and GenBank accession numbers of the species included in this study

Species	Collector (herbarium)	Geographical location	<i>trnH-psbA</i>	ITS
<i>Achnatherum caragana</i> (Trin.) Nevski	Goloskokov s.n. (US)	Kazakhstan, Dzhungar Alatau	GU254852	GU254631
<i>Achnatherum chinense</i> (Hitchc.) Tzvelev	Petrov s.n. (LE)	China, Gansu	GU254860	GU254630
<i>Achnatherum eminens</i> (Cav.) Barkworth	Peterson & Annable 10952 (US)	Mexico, Zacateca	JF698523	JF697693
<i>Achnatherum nelsonii</i> (Scribn.) Barkworth	Saarela 593 (UBC)	Canada, British Columbia	GU254854	GU254633
<i>Achnatherum multinode</i> (Scribn. ex Beal) Valdés-Reyna & Barkworth	Hoge 264 (US)	Mexico, Coahuila	JF698530	JF697699
<i>Achnatherum occidentale</i> (Thurb. ex S. Watson) Barkworth	Saarela et al. 594 (UBC)	Canada	EU489238	EU489090
<i>Achnatherum pekinense</i> (Hance) Ohwi	Nefedova & Paschenko s.n. (LE)	Russia, Far East	JF698531	JF697700
<i>Aciachne acicularis</i> Laegaard	Peterson & Rodríguez13931 (US)	Peru, Ancash	GU254865	GU254625
<i>Aciachne flagellifera</i> Laegaard	Laegaard 19436 (AAU)	Ecuador, Tungurahua	GU254877	GU254654
<i>Amelichloa caudata</i> (Trin.) Arriaga & Barkworth	Peterson & Annable 11398 (US)	Argentina, Mendoza	EU489241	EU489095
<i>Amelichloa clandestina</i> (Hack.) Arriaga & Barkworth	Barkworth 5103 (US)	USA, Texas	GU254853	GU254636
<i>Anatherostipa hans-meyeri</i> (Pilg.) Peñail.	Peterson et al. 20645 (US)	Bolivia, La Paz	EU489244	EU489098
<i>Anatherostipa rosea</i> (Hitchc.) Peñail.	Laegaard 10864 (AAU)	Ecuador, Azuzay	GU254867	GU254652
<i>Aristella bromoides</i> (L.) Bertol.	Romaschenko & Didukh 439 (KW)	Ukraine, Crimea	GU254845	GU254624
<i>Austrostipa flavescens</i> (Labill.) S.W.L.Jacobs & J.Everett	Spjut 7182 (US)	Australia, Western Australia	JF698542	JF697710
<i>Austrostipa hemipogon</i> (Benth.) S.W.L.Jacobs & J.Everett	Symon 13449 (US)	Australia, south Australia	JF698543	JF697711
<i>Austrostipa pycnostachya</i> (Benth.) S.W.L.Jacobs & J.Everett	Peterson et al. 14318 (US)	Australia, western Australia	EU489247	EU489101
<i>Austrostipa semibarbata</i> (R. Br.) S.W.L.Jacobs & J.Everett	Symon 13439 (US)	Australia, south Australia	GU254849	GU254628
<i>Austrostipa tenuifolia</i> (Steud.) S.W.L.Jacobs & J.Everett	Peterson et al. 14248 (US)	Australia, western Australia	EU489250	EU489104
<i>Hesperostipa comata</i> (Trin. & Rupr.) Barkworth	Saarela et al. 595 (UBC)	Canada, British Columbia	EU489252	EU489106
<i>Hesperostipa neomexicana</i> (Thurb.) Barkworth	Peterson & Valdes-Reyna 18934 (US)	Mexico, Nuevo León	GU254840	EU489107
<i>Hesperostipa sparteae</i> (Trin.) Barkworth	Holmes 214 (US)	USA, Missouri	EU489253	EU489108
<i>Jarava annua</i> (Mez) Peñail.	Peterson 15614 (US)	Chile, Region I	JF698552	JF697720
<i>Jarava castellanosis</i> (F.A.Roig) Peñail.	Peterson & Annable 10336 (US)	Argentina, Jujuy	EU489256	EU489112
<i>Jarava filifolia</i> (Nees) Ciald.	Zuloaga et al. 11423 (SI)	Argentina, Buenos Aires	KP082990	KP083003
<i>Jarava hysophila</i> (Speg.) Peñail.	Morrone et al. 5969 (SI)	Argentina, Neuquén	KP082991	KP083004
<i>Jarava hystericina</i> (Speg.) Peñail.	Peterson et al. 19550 (US)	Argentina, Jujuy	KP082992	EU489122
<i>Jarava ichu</i> Ruiz & Pav.	Peterson et al. 20745 (US)	Peru, Puno	EU489267	EU489125
<i>Jarava illimanica</i> (Hack.) F.Rojas	Beck 27048 (SI)	Bolivia, Tarija	KP082993	KP083005
<i>Jarava leptostachya</i> (Griseb.) F.Rojas	Peterson & Annable 11714 (US)	Argentina, Salta	EU489270	–
<i>Jarava leptostachya</i> (Griseb.) F.Rojas	Villavicencio & Sukopp 52 (US)	Bolivia	–	EU489128
<i>Jarava media</i> (Speg.) Peñail.	Peterson et al. 19337 (US)	Argentina, La Rioja	EU489272	EU489129
<i>Jarava neaei</i> (Nees ex Steud.) Peñail. (A)	Sede et al. 379 (SI)	Argentina, Mendoza	KP082994	KP083006
<i>Jarava neaei</i> (Nees ex Steud.) Peñail. (B)	Sede et al. 322 (SI)	Argentina, Neuquén	KP082995	KP083007

Table 1 continued

Species	Collector (herbarium)	Geographical location	<i>trnH-psbA</i>	ITS
<i>Jarava neaei</i> (Nees ex Steud.) Peñail. (C)	Sede et al. 635 (SI)	Argentina, Santa Cruz	KP082996	KP083008
<i>Jarava neaei</i> (Nees ex Steud.) Peñail. (D)	Sede et al. 484 (SI)	Argentina, San Juan	KP082997	KP083009
<i>Jarava plumosa</i> (Spreng.) S.W.L.Jacobs & J.Everett	BA 80072	–	EU204709	–
<i>Jarava plumosa</i> (Spreng.) S.W.L.Jacobs & J.Everett	Morrone et al. 5151 (SI)	Argentina, Córdoba	–	FJ461265
<i>Jarava plumosula</i> (Nees ex Steud.) F.Rojas	Cialdella et al. 258 (SI)	Argentina, Salta	KP082998	KP083010
<i>Jarava polyclada</i> (Hack.) Peñail.	Peterson & Annable 11569 (US)	Argentina	EU489276	EU489134
<i>Jarava pseudoichu</i> (Caro) F.Rojas	Peterson et al. 20736 (US)	Peru, Puno	EU489277	EU489135
<i>Jarava psylantha</i> (Speg.) Peñail. (A)	Sede et al. 567 (SI)	Argentina, Chubut	KP082999	KP083011
<i>Jarava psylantha</i> (Speg.) Peñail. (B)	Sede et al. 580 (SI)	Argentina, Santa Cruz	KP083000	KP083014
<i>Jarava psylantha</i> (Speg.) Peñail. (C)	Sede et al. 704 (SI)	Argentina, Santa Cruz	KP083001	KP083012
<i>Jarava scabrifolia</i> (Torres) Peñail.	Peterson & Annable 11712 (US)	Argentina, Salta	EU489278	EU489136
<i>Jarava subplumosa</i> (Hicken ex F.A.Roig) Peñail.	Peterson 17280 (SI)	Argentina, Santa Cruz	KP083002	KP083013
<i>Nassella caespitosa</i> Griseb.	Peterson et al. 19540 (US)	Argentina, Salta	EU489288	EU489146
<i>Nassella filiculmis</i> (Delile) Barkworth	Soreng 7009 (US)	Chile, Region VIII	EU489292	EU489150
<i>Nassella meyeri</i> Torres	BA 91099	–	EU204718	–
<i>Nassella meyeri</i> Torres	Cialdella et al. 488 (SI)	Argentina, Jujuy	–	FJ461278
<i>Nassella neesiana</i> (Trin. & Rupr.) Barkworth	BA 80071	–	EU204719	–
<i>Nassella neesiana</i> (Trin. & Rupr.) Barkworth	Peterson & Annable 10258 (US)	Argentina	–	EU489155
<i>Nassella pfisteri</i> (Matthei) Barkworth	Soreng 7017a (US)	Chile, Region VIII	EU489299	EU489157
<i>Nassella pubiflora</i> (Trin. & Rupr.) Barkworth	Peterson & Annable 11618 (US)	Argentina, Tucumán	EU489300	EU489158
<i>Nassella punensis</i> Torres	UTC 210644	–	EU204722	–
<i>Nassella punensis</i> Torres	Cialdella 428 (SI)	Argentina, Salta	–	FJ461283
<i>Nassella sanluisensis</i> (Speg.) Barkworth	Roig 3293 (US)	Argentina	EU489303	EU489161
<i>Nassella tenuissima</i> (Trin.) Barkworth	BA 91106	–	EU204725	–
<i>Nassella tenuissima</i> (Trin.) Barkworth	Pyke 992 (BC)	Argentina	–	EU489163
<i>Nassella trichotoma</i> (Nees) Hack. ex Arechav.	BA 81421	–	EU204726	–
<i>Nassella trichotoma</i> (Nees) Hack. ex Arechav.	Peterson & Annable 11506 (US)	Argentina	–	EU489164
<i>Oloptum miliaceum</i> Röser & Hamasha	Gillett 16094 (US)	Jordan, Azraq	GU254838	GU254643
<i>Ortachne breviseta</i> Hitchc.	Laegaard 12802 (AAU)	Argentina, Neuquén	GU254868	GU254666
<i>Ortachne rariflora</i> (Hook.f.) Hughes	Mariz 924 (CONC)	Chile, Region XI	GU254864	GU254665
<i>Pappostipa atacamensis</i> (Parodi) Romasch.	Peterson et al. 15447 (US)	Chile, Region III	EU489254	EU489109
<i>Pappostipa barrancaensis</i> (F.A.Roig) Romasch.	Peterson & Annable 11371 (US)	Argentina, Mendoza	EU489255	EU489111
<i>Pappostipa chrysophylla</i> (É.Desv.) Romasch.	Peterson et al. 19220 (US)	Argentina, Mendoza	EU489258	EU489114
<i>Pappostipa frigida</i> (Phil.) Romasch.	Peterson et al. 19360 (US)	Argentina	EU489260	–
<i>Pappostipa frigida</i> (Phil.) Romasch.	Peterson & Annable 10397 (US)	Argentina	–	EU489117
<i>Pappostipa hieronymusii</i> (Pilg.) Romasch.	Peterson & Annable 11488 (US)	Argentina, San Juan	EU489262	EU489118
<i>Pappostipa humilis</i> (Cav.) Romasch.	Peterson & Annable 11317 (US)	Argentina, Mendoza	EU489264	EU489120
<i>Pappostipa ibarii</i> (Phil.) Romasch.	Soreng 7331 (US)	Chile	EU489266	EU489123
<i>Pappostipa nicorae</i> (F.A.Roig) Romasch.	Peterson & Annable 11408 (US)	Argentina, Mendoza	EU489273	EU489130
<i>Pappostipa speciosa</i> (Trin. & Rupr.) Romasch.	Peterson & Annable 11549 (US)	Argentina, San Juan	EU489279	EU489137

Table 1 continued

Species	Collector (herbarium)	Geographical location	<i>trnH-psbA</i>	ITS
<i>Pappostipa vaginata</i> (Phil.) Romasch.	Peterson et al. 19222 (US)	Argentina	EU489283	–
<i>Pappostipa vaginata</i> (Phil.) Romasch.	Peterson & Annable 11744 (US)	Argentina	–	EU489142
<i>Piptatheropsis micrantha</i> (Trin. & Rupr.) Romasch., P.M.Peterson & Soreng	Peterson et al. 18437 (US)	Canada, Alberta	GU254866	GU254664
<i>Piptatheropsis pungens</i> (Torr. ex Spreng.) Romasch., P.M.Peterson & Soreng	Hermann 13407 (US)	Canada, Alberta	JF698562	JF697730
<i>Piptatherum aequiglume</i> (Duthie ex Hook.f.) Roshev.	Koelz 1389 (US)	India, Himachal Pradesh	JF698555	JF697723
<i>Piptatherum angustifolium</i> Munro ex Boiss.	Nepli 144 (LE)	Tajikistan, Khorog	GU254825	GU254659
<i>Piptatherum fedtschenkoi</i> Roshev.	Botschantzev 1559 (LE)	Tajikistan, Pamir	JF698557	JF697725
<i>Piptatherum ferganense</i> (Litv.) Roshev. ex Nikitina	Kamelin 100 (LE)	Tajikistan, Guissar	JF698558	JF697726
<i>Piptatherum hilariae</i> Pazij	Potaliev 343 (LE)	Tajikistan, Pamir	GU254828	GU254660
<i>Piptatherum munroi</i> (Stapf) Mez	Soreng 5686 (US)	China, Sichuan	JF698561	JF697729
<i>Piptatherum paradoxum</i> (L.) P.Beauv.	Pyke 831 (BC)	Spain, Catalonia	GU254862	GU254622
<i>Piptatherum sogdianum</i> (Grigorj.) Roshev.	Konkov 1637 (LE)	Tajikistan, Sughd	JF698563	JF697731
<i>Piptatherum songaricum</i> (Trin. & Rupr.) Roshev. ex Nikitin	Goloskokov s.n. (LE)	Kazakhstan, Dzhambulskaya	JF698564	JF697732
<i>Piptatherum virescens</i> (Trin.) Boiss.	Romaschenko & Didukh 445 (KW)	Ukraine, Yalta	GU254837	GU254644
<i>Piptochaetium brachyspermum</i> (Speg.) Parodi	Peterson & Annable 11252 (US)	Argentina, La Pampa	EU489306	EU489165
<i>Piptochaetium montevidense</i> (Spreng.) Parodi	Peterson et al. 20486 (US)	Peru, Ayacucho	JF698565	JF697733
<i>Piptochaetium panicoides</i> (Lam.) É.Desv.	Soreng 7011 (US)	Chile, Region VIII	EU489307	EU489166
<i>Ptilagrostis dichotoma</i> Keng ex Tzvelev	Soreng et al. 5647 (US)	China, Xizang	GU254874	GU254618
<i>Ptilagrostis junatovii</i> Grubov	Kotukhov s.n. (LE)	Russia, West Altai	GU254842	GU254619
<i>Ptilagrostis luquensis</i> P.M.Peterson, Soreng & Z.L.Wu	Soreng 5383 (US)	China, Gansu	GU254875	GU254620
<i>Ptilagrostis mongholica</i> Griseb.	Koloskov s.n. (LE)	Kyrgyzstan	GU254876	GU254621
<i>Sinochasea trigyna</i> Keng	Soreng 5644 (US)	China, Xizang	GU254824	GU254645
<i>Stipa barbata</i> Desf.	Pyke 704 (BC)	Spain, Granada	JF698570	JF697738
<i>Stipa brauneri</i> (Pacz.) Klokov	Romaschenko 418 (BC)	Ukraine, Crimea	JF698571	JF697739
<i>Stipa bungeana</i> Trin. ex Bunge	Soreng 5397 (US)	China, Gansu	JF698573	JF697741
<i>Stipa caucasica</i> Schmalh.	Romaschenko et al. 635 (BC)	Tajikistan, Zeravshan	JF698576	JF697744
<i>Stipa pennata</i> L.	Romaschenko 466 (BC)	Ukraine, Lugansk	GU254857	GU254637
<i>Trikeria hookeri</i> (Stapf) Bor	Koelz 2328 (US)	India, Jammu and Kashmir	GU254830	GU254650
<i>Trikeria pappiformis</i> (Keng) P.C.Kuo & S.L.Lu	Soreng et al. 5653 (US)	China, Xizang	GU254831	GU254649

Romaschenko et al. (2008), (2012). Alternatively, nuclear DNA regions from herbarium material were amplified using a touchdown PCR strategy (Korbie et al. 2008) with a profile consisting of denaturation at 94 °C for 1 min followed by 10 cycles decreasing annealing temperature from 61 °C to 56 °C at 0.5 °C per cycle and 30 cycles at 54 °C, and 72 °C for 1 min. Automated sequencing was performed by Macrogen, Inc. (South Korea). Electropherograms were edited and assembled in BioEdit 5.0.9 (Hall 1999).

Sequence alignment and phylogenetic analyses

Sequences were aligned using the program MAFFT ver. 6 (Kato and Toh 2008) available on line (<http://mafft.cbrc.jp/alignment/server/>), using the default settings. Indels were coded as presence/absence characters, except in regions of ambiguous alignment, using the simple method of Simmons and Ochoterena (2000) implemented in Seq-State 1.4.1 (Müller 2005). Data matrices were submitted to TreeBASE (Study number 16524).

TNT v1.1 (Goloboff et al. 2008) was used for phylogenetic analyses of the individual and combined datasets under the parsimony criterion. All characters were considered unordered and parsimony-uninformative characters were excluded from the analyses. For both matrices, we used a driven search finding the minimum length 20 times with default settings for sectorial searches and tree fusing (Goloboff 1999). The resulting trees were additionally TBR swapped, retaining a maximum of 10,000 total trees. A strict consensus tree was generated from the most parsimonious trees. Branch support was calculated by Jackknifing (Farris et al. 1996), with a character removal probability of 36 %, performing 10,000 replicates and a heuristic search strategy of five addition sequences swapped with TBR with 10 trees saved per replication. The sequence of *Sinochasea trigyna* Keng was used to root the trees, guided by previous molecular phylogenetic analyses (Romaschenko et al. 2012).

Additionally, because of the low level of sequence variation of the plastid region, we constructed a network in a parsimony framework using TCS (Clement et al. 2000). To identify shared haplotypes among species of *Jarava*, a reduced matrix of 61 terminals was used including species of *Achnatherum*, *Amelichloa*, *Austrostipa*, *Jarava*, *Nassella*, *Oloptum*, *Pappostipa*, and *Piptatherum*.

Table 2 Morphological characters and character states

Morphological characters	States
1. Pungent basal leaves	Absent (0); present (1)
2. Shape of the callus	Acute, subacute (0); blunt (1)
3. Callus indumentum	Glabrous (0); sparsely pubescent (1); densely pubescent (2)
4. Crown	Absent (0); inconspicuous (1); conspicuous (2)
5. Palea texture	Membranous (0); indurated (1)
6. Lemma margins	Strongly overlapping (0); not or only slightly overlapping (1)
7. Palea length	Half to equal the length of the lemma (0); shorter than half the length of the lemma (1)
8. Lemma texture	Membranous (0); indurated (1)
9. Lemma apex	Minutely 2-toothed or without any tooth (0); with 2 conspicuous teeth (1)
10. Disarticulation of the awn	Persistent (0); deciduous (1)
11. Awn shape	Straight or curved but not geniculate (0); 1-geniculate (1); 2-geniculate (2)
12. Awn indumentum	Long hairs (0); short hairs (1)
13. Long hairs location	All along the awn (0); only at the upper portion (subula) (1); only at the lower portion (column) (2)

Morphological characters

A total of 13 morphological characters were optimized on one of the shortest trees from the combined nuclear and plastid data analysis of the same reduced data set used for the network construction. The characters were chosen to represent diagnostic features for *Jarava* and allied genera and were extracted from previously published contributions (Cialdella et al. 2007, 2010, 2014; Cialdella 2012) (Table 2). Data were either obtained from the literature (Matthei 1965; Bentham 1878; Jessop 1981; Watson and Dallwitz 1992; Zanín et al. 1992; Barkworth 1993; Jacobs and Everett 1996; Torres 1997; Clayton et al. 2006; Zhenlan and Phillips 2006; Barkworth 2007; USDA and NRCS 2013), or from our own observations.

Results

Plastid analysis

The *trnH-psbA* data matrix consisted of 94 terminals and 649 characters. The matrix contained 0.90 % missing data and 5.85 % of the characters were parsimony informative. Five gaps were binary coded and added to the matrix. The analysis yielded 1,800 most parsimonious trees (MPT) ($L = 60$; $CI = 0.68$; $RI = 0.95$; Table 3). The strict consensus tree is shown in Fig. 1a. The topology is mostly unresolved at the base; *Jarava* was not recovered as monophyletic and all specimens of *J. neaei*, *J. psylantha* and *J. subplumosa* were included in a basal polytomy. The Major American clade (MAC) obtained in previous studies (Romaschenko et al. 2008, 2010, 2012) was here recovered with low support ($JK = 64$), which includes the rest of the *Jarava* species: *J. annua*, *J. castellanosii*, *J. filifolia*, *J. ichu*, *J. illimanica*, *J. hypsophila*, *J. hystricina*, *J.*

Table 3 Summary information of the parsimony analyses for individual and combined molecular data sets

	<i>trnH-psbA</i>	ITS	Combined
Taxa	94	94	94
Aligned length (bp)	649	626	1275
Informative characters (%)	5.85	33.86	19.62
Number of coded indels	5	18	23
Missing data (%)	0.90	2.26	1.57
Number of MPT	1800	288	8192
Length (steps)	60	600	688
CI	0.68	0.48	0.55
RI	0.95	0.86	0.82

bp base pairs, *CI* consistency index, *RI* retention index, *MPT* most parsimonious trees

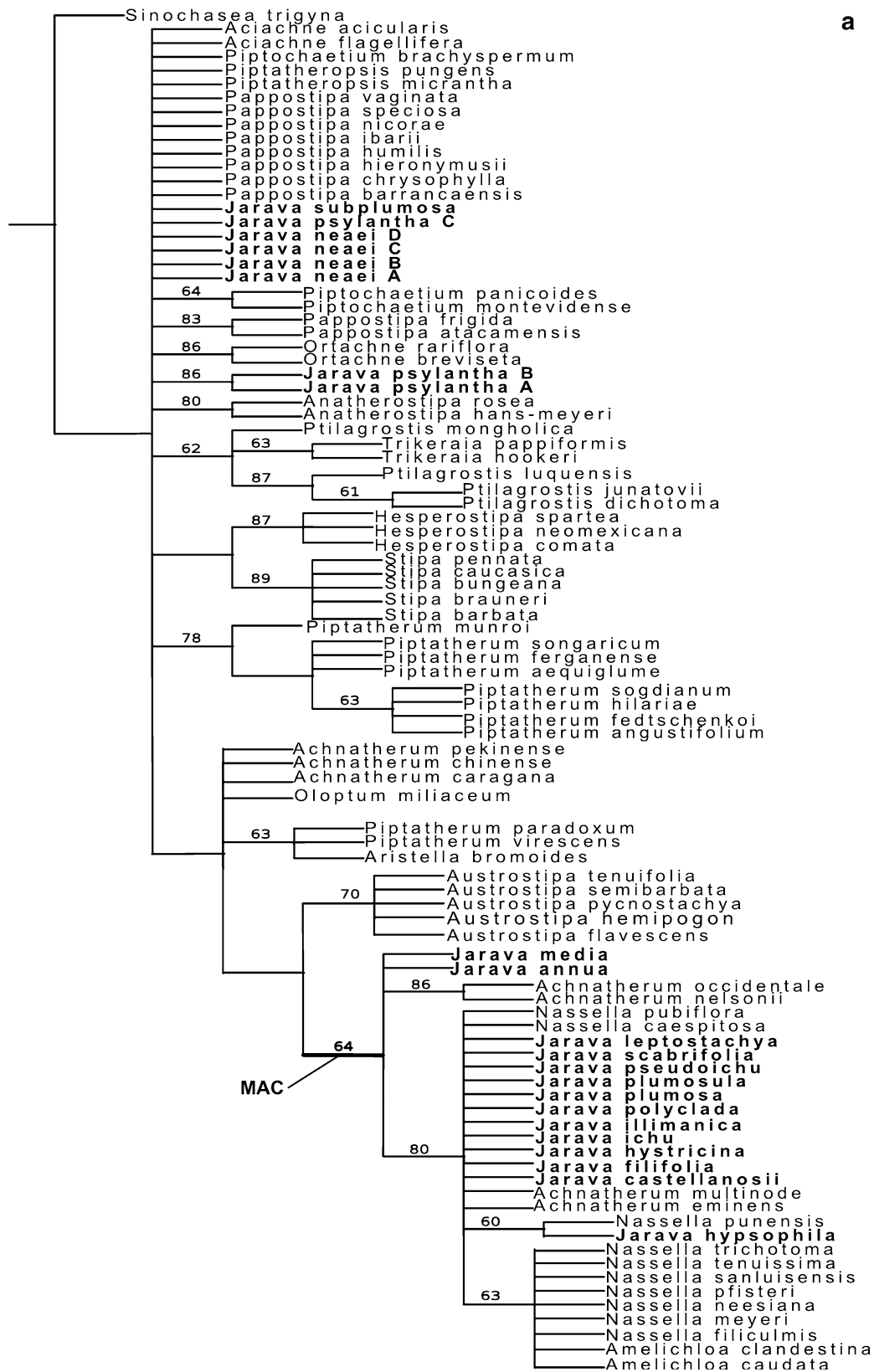


Fig. 1 Strict consensus trees from the analyses of *tmH-psbA* (a) and ITS (b) data sets. Numbers above branches are jackknife values. Gray boxes show *Jarava* groups according to the text

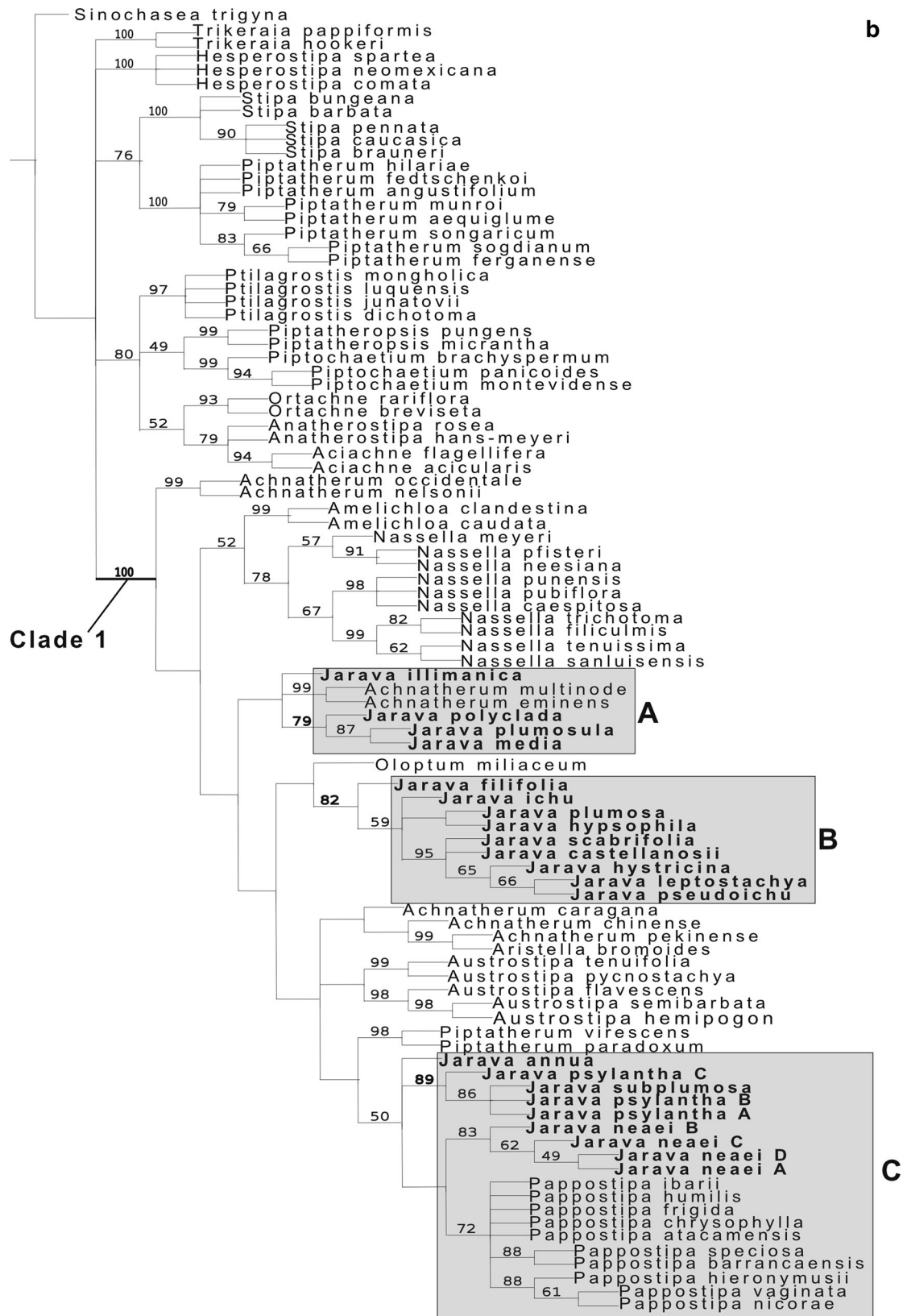


Fig. 1 continued

leptostachya, *J. media*, *J. plumosa*, *J. plumosula*, *J. polyclada*, *J. pseudoichu*, and *J. scabrifolia*. These *Jarava* species were gathered together with *Achnatherum*, *Amelichloa*, and *Nassella* species. All species of *Austrostipa*, grouped in a clade, were sister to the MAC in the strict consensus tree.

Nuclear analysis

The ITS data matrix consisted of 94 terminals and 626 characters. The matrix contained 2.26 % missing data and 33.86 % of parsimony informative characters. Eighteen gaps were binary coded and added to the matrix. The analysis yielded 288 MPT ($L = 600$; $CI = 0.48$; $RI = 0.86$; Table 3). Neither *Jarava* nor the MAC was recovered as monophyletic. Instead, the species were split in three subclades nested in a major clade, Clade 1 (JK = 100; Fig. 1b), which also includes species of *Achnatherum*, *Amelichloa*, *Aristella*, *Austrostipa*, *Nassella*, *Pappostipa*, and *Piptatherum*. The three subclades are: subclade A which includes *J. illimanica*, and *J. polyclada*-*J. plumosula*-*J. media* subclade (JK = 79), and *Achnatherum multinode*-*A. eminens* subclade (JK = 99). Subclade B (JK = 82) includes only species of *Jarava*: *J. castellanosii*, *J. filifolia*, *J. hypsophila*, *J. hystricina*, *J. ichu*, *J. leptostachya*, *J. plumosa*, *J. pseudoichu*, and *J. scabrifolia*; this clade is sister to *Oloptum miliaceum*. Subclade C (JK = 50) consists of a trichotomy that gathers *J. annua*, a subclade with *J. psylantha*-*J. subplumosa* (JK = 89) and a third subclade with *J. neaei* as sister to species of *Pappostipa*.

Combined analysis

The plastid and nuclear combined data matrix consisted of 94 terminals and 1275 characters. The matrix contained 1.57 % missing data and 19.62 % parsimony informative characters. Twenty-three gaps were binary coded and added to the matrix. The analysis yielded 8,192 MPT ($L = 688$; $CI = 0.55$; $RI = 0.82$; Table 3). The strict consensus tree showed a resolved topology (Fig. 2) although the monophyly of *Jarava* was not corroborated. Species of *Jarava* and allied genera were gathered into the major Clade 1 (JK = 100) and subclades A, B, and C were recovered. Forcing *Jarava* to be monophyletic added 18 steps to the most parsimonious trees.

The MAC clade was recovered with moderate support (JK = 73): an *Achnatherum occidentale*-*A. nelsonii* subclade was sister to a group (JK = 71) with subclades A (JK = 67; supported by two base pair substitutions) and B (JK = 84; with two base substitutions), both included in a polytomy together with *Nassella*-*Amelichloa* subclade.

Subclade C (JK = 77; with six base pair substitutions) included *J. neaei*, *J. psylantha* and *J. subplumosa* in a polytomy together with species of *Pappostipa*. *Jarava annua* was neither included in the MAC nor in subclade C. Instead, it was in an intermediate position as sister to *Achnatherum chinense*.

Haplotype network

A total of 22 different haplotypes were identified (H1-H22; Fig. 3) of which 12 were exclusive for single species, whereas the rest were shared among different species. Haplotypes H1, H2, and H18 occurred at higher frequencies. Twenty haplotypes were considered as missing intermediates. One position in the network revealed a closed loop that could not be unambiguously resolved.

Jarava species were gathered in two main groups: the MAC core, together with *Amelichloa*, *Achnatherum*, and *Nassella* species, and subclade C as described in previous analyses (Figs. 1, 2). Both principal groups were in a terminal position and included all *Jarava* species, except for *J. annua* and *J. media*, which were found in an intermediate position. Within the MAC core, there were three haplotypes shared among different genera: H1 was common to species of *Achnatherum*, *Jarava* and *Nassella*, H2 was shared by *Amelichloa* and *Nassella* species, while H3 was present in *J. hypsophila* and *Nassella punensis*. Within subclade C, H18 was shared by all specimens of *J. neaei*, one accession of *J. psylantha* and most species of *Pappostipa*.

Morphological character optimization

To analyze the evolution of morphological characters, we optimized 13 diagnostic characters (Table 2) on one of the MPT derived from the combined dataset. We selected those characters that represent the variation among species of *Jarava* and allied genera such as the shape of the callus (Fig. 4a), and two characters related to the awn (Fig. 4b, c): the awn indumentum and the location of long hairs.

The blunt shape of the callus is an ancestral state in the phylogeny while the acute or subacute shape has evolved several times (Fig. 4a). Short hairs appeared as ancestral in the reconstruction of the awn indumentum, while long hairs appeared at least three times in the evolution of the group: in *Pappostipa*, *J. neaei*, *J. psylantha*, and *J. subplumosa* group, in *J. plumosula*-*J. media* subclade, and in *Austrostipa hemipogon* (Fig. 4b). The distribution of long hairs all along the awn appeared twice independently in the phylogeny, while the presence of long hairs only in the upper part of the awn (subula) is a derived condition for *J. neaei*; the presence of long hairs only at the base of the awn (column) is a synapomorphy for *Pappostipa* (Fig. 4c).

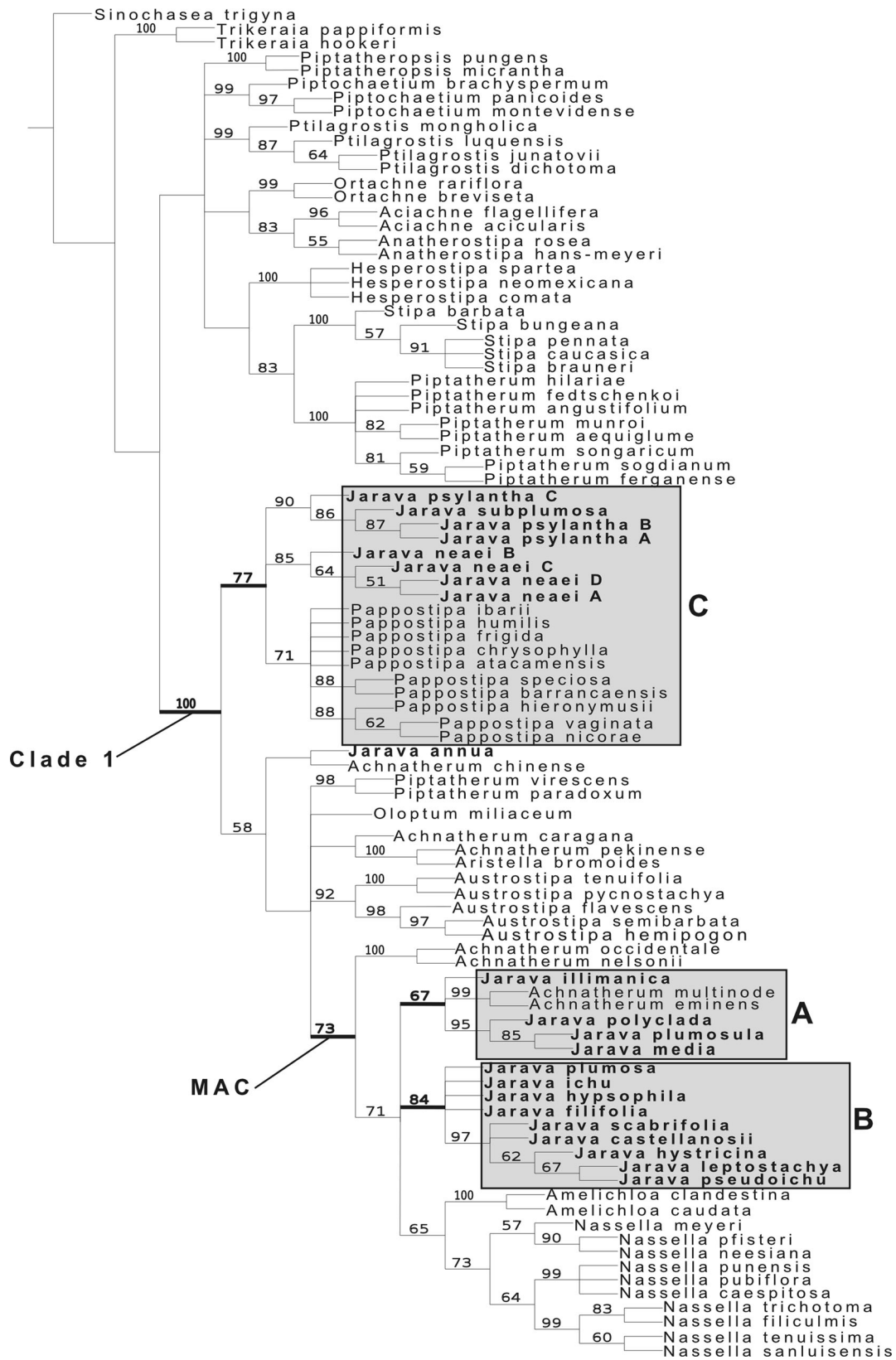


Fig. 2 Strict consensus tree from the analysis of the combined data set. Numbers above branches are jackknife values. *Gray boxes* show *Jarava* groups according to the text

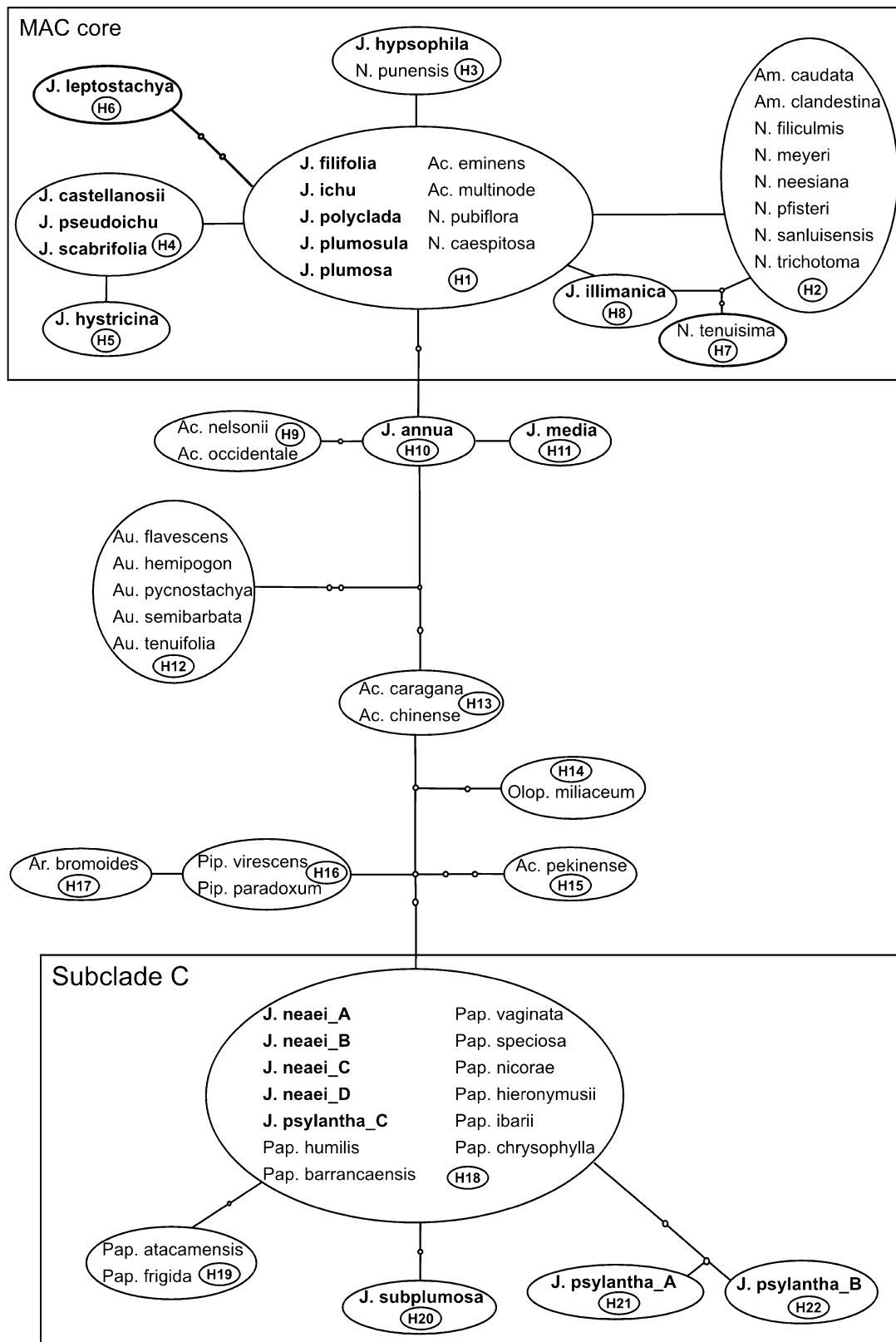


Fig. 3 Statistical parsimony network based on a reduced *trnH-psbA* data set (Clade 1 as in previous analyses). “H” and numbers within circles indicate sampled haplotypes. Small empty circles represent

hypothetical haplotypes. Genera abbreviations: *Ac* *Achnatherum*, *Am* *Amelichloa*, *Ar* *Aristella*, *Au* *Austrostipa*, *J* *Jarava*, *N* *Nassella*, *Olop* *Oloptium*, *Pap* *Pappostipa*, *Pip* *Piptatherum*

Discussion

The monophyly of *Jarava* was rejected in agreement with previous studies (Cialdella et al. 2007, 2010, 2014; Romaschenko et al. 2008, 2010, 2012). When analyzing the

phylogenetic relationships within Stipeae, species of *Jarava* were grouped in different major clades. Part of the South American species were included within the MAC core of Romaschenko et al. (2008, 2010, 2012), in which, we distinguished two subclades: subclade A with four

Fig. 4 Morphological character optimization on one of the most parsimonious trees obtained from the combined data set analysis. **a** Shape of the callus: blunt (*white*)/acute or subacute (*black*)/ambiguous (*dashed line*). **b** Awn indumentum: long hairs (*black*)/short hairs (*white*)/ambiguous (*dashed line*). **c** Long hairs location: all along the awn (*dashed line*)/only at the upper portion (subula) (*grey*)/only at the lower portion (column) (*black*)/ambiguous (*white*)

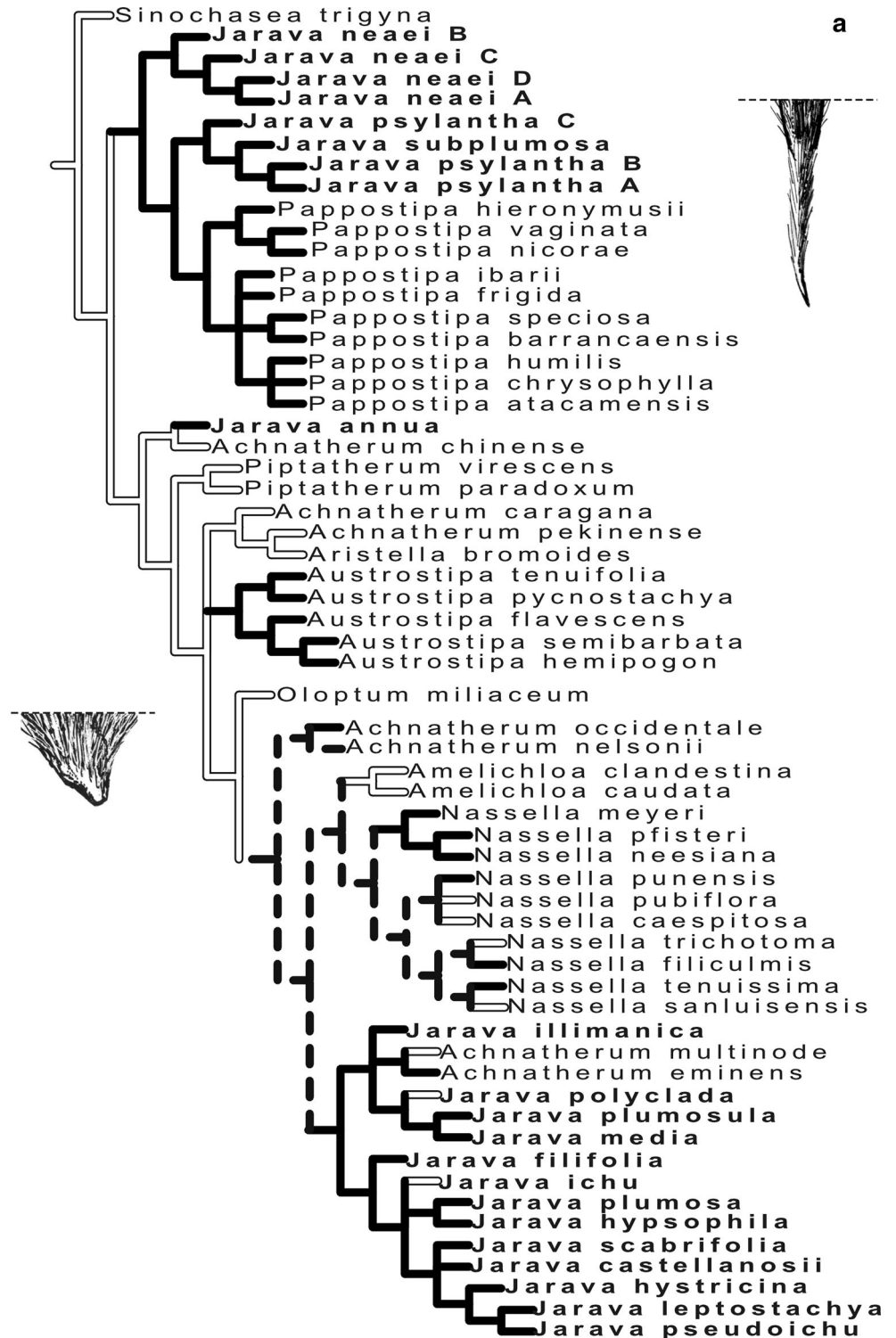
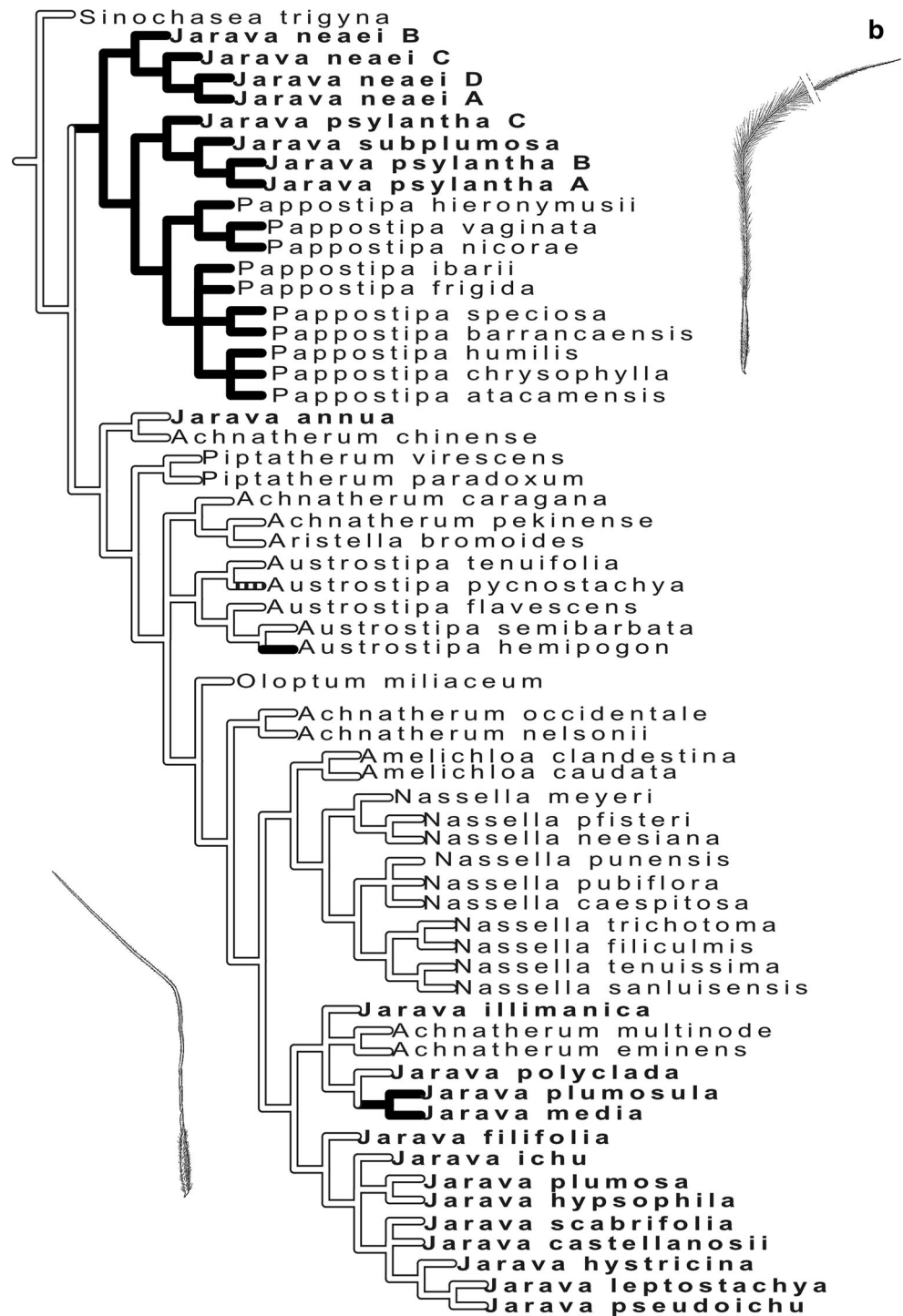


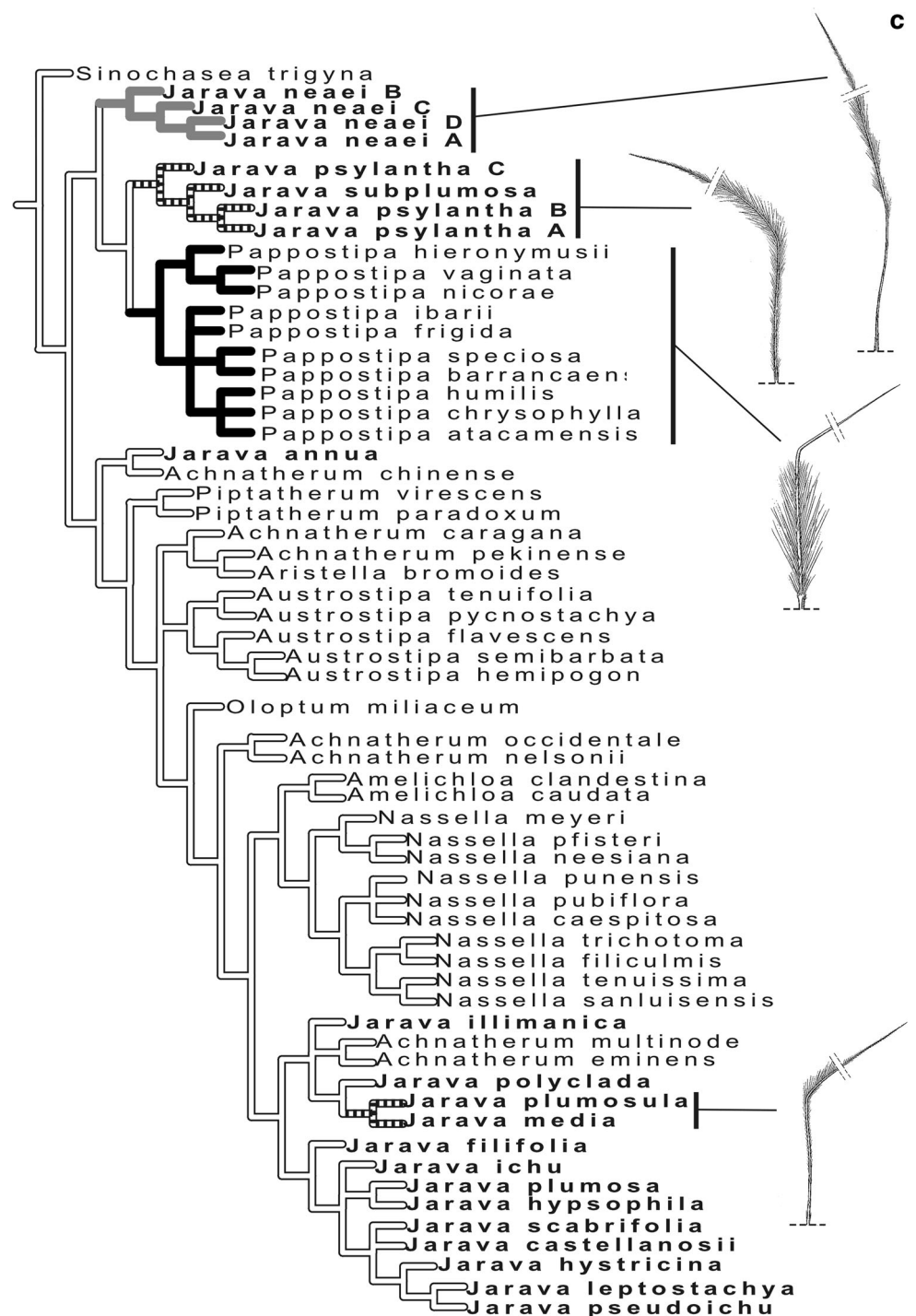
Fig. 4 continued



species of *Jarava* together with two species of New World *Achnatherum*, and subclade B, grouping the type species of the genus, *J. ichu*, together with other eight species of *Jarava*; the latter forming the *Jarava* s.s. subclade. The remaining species of *Jarava* were sister to *Pappostipa*, a clade that was not closely related to the MAC. Only the position of *J. annua* was incongruent between plastid and

nuclear topologies, while basal to the MAC in the plastid tree, it was included in subclade C in the nuclear hypothesis. In addition to the phylogenetic trees, both major *Jarava* groups were recovered in the haplotype network (Fig. 3), confirming that at least two lineages appeared independently in South America during the evolution of the Stipeae.

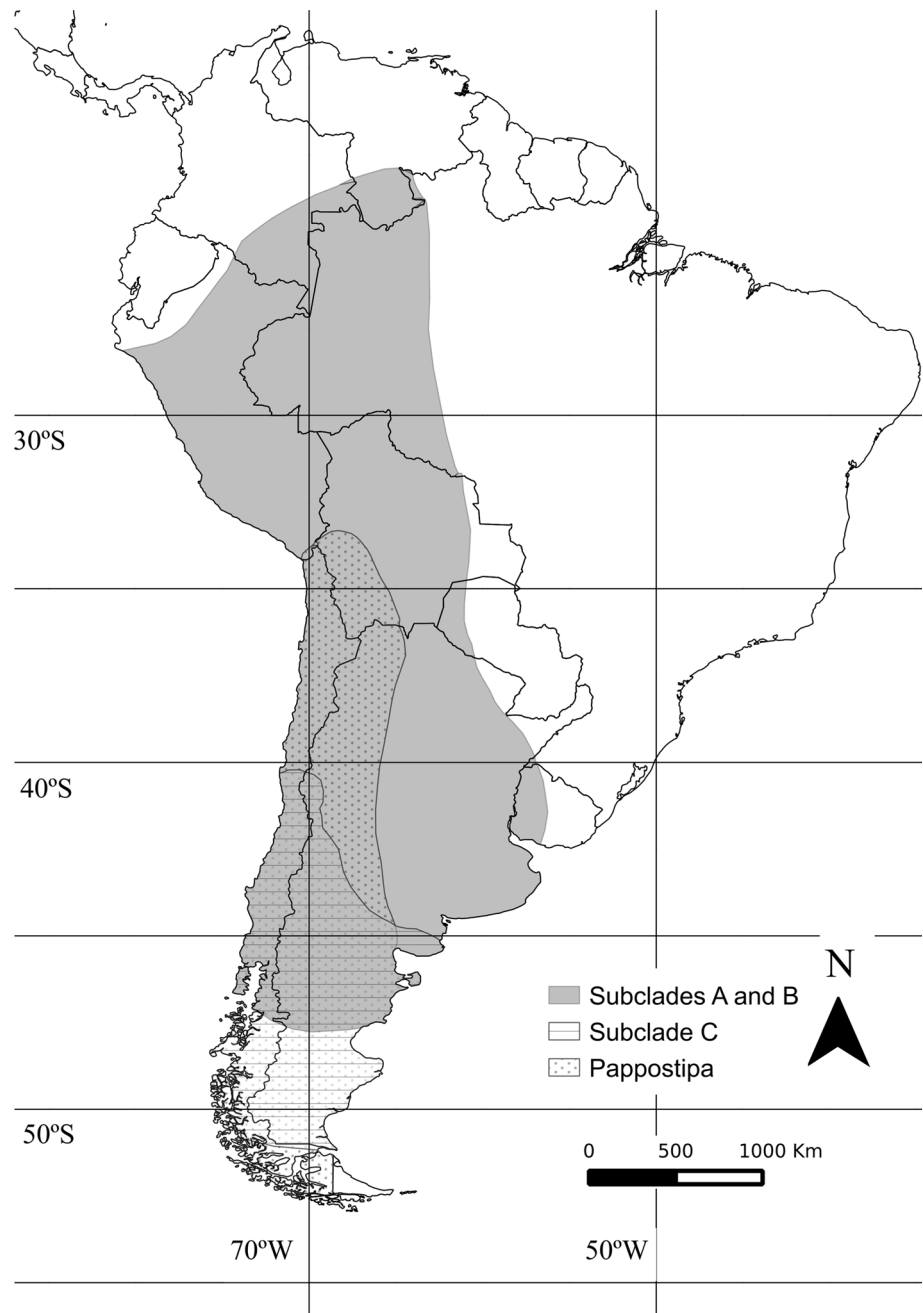
Fig. 4 continued



Diagnostic morphological characters for *Jarava* were considered by Jacobs and Everett (1997), authors who highlighted the importance of an apical pappus in the lemma and awns with short hairs all along to distinguish the genus; both characters are present in the *Jarava* s.s. subclade (subclade B) and in *Jarava* species of subclade A (*J. illimanica* and *J. polyclada*). However, Jacobs and Everett (1997) did not analyze species

of *Stipa* s.l. with plumose awns (long hairs all along or only in part). In our phylogeny, plumose awns appeared at least three times in the evolution of these Stipeae: in *Jarava media* and *J. plumosula* within subclade A, in species of subclade C, which includes species of *Pappostipa* and three species of *Jarava* (*J. neaei*, *J. psylantha*, and *J. subplumosa*), and in *Austrostipa hemipogon* (Fig. 4b).

Fig. 5 Distribution of major clades derived from the phylogenetic analyses in South America. *Jarava* subclades A and B (grey); *Jarava* subclade C (lines); *Pappostipa* subclade (dots)



In this work, *J. psylantha* and *J. subplumosa* were included in a molecular phylogeny for the first time. Moreover, the position of *J. neaei*, although included in the MAC clade by Hamasha et al. (2011), is here recovered within subclade C; the identity of the voucher specimen used by Hamasha et al. (2011) is doubtful and we could not verify it. Moreover, despite the unresolved topology within subclade C, the close relationship of *J. neaei*, *J. psylantha*, and *J. subplumosa* with *Pappostipa* appears also as a novelty. Morphologically, the species in subclade C, other than having plumose awns, share an acute or subacute callus (Fig. 4a, b).

Apart from these three species of *Jarava* included in subclade C, *Jarava pogonathera* (É.Desv.) Peñail. is another species which shares a plumose awn and an acute callus. However, we could not obtain any sequence of this species. In addition, *J. pogonathera* has larger spikelets and florets than those species of subclade A, *J. media* and *J. plumosula* (Cialdella 2012); therefore, it could be probably related to species of *Jarava* in subclade C. The phylogenetic position of *J. pogonathera* still needs further investigation.

Spegazzini (1901) first circumscribed *Stipa* subg. *Ptilostipa* to include species with awns covered with long

hairs all along, or only at its upper portion (subula, Table 2). The species *J. media*, *J. neaei*, *J. plumosula* and *J. psylantha*, placed by Spegazzini in subg. *Ptilostipa* (1901, 1925), are divided between subclade C (*J. neaei* and *J. psylantha*) and subclade A (*J. media* and *J. plumosula*). *Jarava pogonathera*, although we could not confirm its phylogenetic position, was also included in subg. *Ptilostipa* by Spegazzini (1901). *Jarava subplumosa* was first described by Roig (1972) under *Stipa*, however there is no mention of its subgeneric rank.

In the same treatments, Spegazzini (1901), (1925) defined *Stipa* subg. *Pappostipa* to include species with a particular indumentum of the awn: long hairs all restricted to the lower portion, and to differentiate these species from those included in subg. *Ptilostipa*. While Romaschenko et al. (2008) raised *Pappostipa* to the generic rank, we here consider that the species of *Jarava* from subclade C (*J. neaei*, *J. psylantha*, *J. subplumosa*, and probably *J. pogonathera*) should be treated under *Pappostipa* or included in a new genus based on two characters: the position of the long hairs in the awn, restricted to the upper portion or all along, and the acute or subacute callus of the floret. A decision should be made when the monophyly of this group is better supported, probably, by the inclusion of sequences from additional DNA regions.

Geographical distribution

Jarava s.l. is mainly distributed in South America, except for *J. ichu* (the type species of the genus) which extends to Central America and southern North America (Mexico, Veracruz; Valdés-Reyna & Barkworth, 2002; Arriaga, 2007). Both lineages in which the *Jarava* species split, subclades A and B and subclade C, differ in their geographical distribution. Species of subclade C are restricted to 31°–52° South latitude (S), in Argentina and Chile; species of *Jarava* from subclades A and B are distributed from North America to South America (approx. 40°S), except by one species (*J. ichu*) that extends from Mexico to 45°S (Arriaga, 2007). *Pappostipa* is widely distributed in southern South America from (0°–55°S) except for *P. speciosa* which is also distributed in Central and North America.

In South America, geographical distribution of *Pappostipa* completely overlaps with that of *Jarava* species (subclade C), while *Pappostipa* overlaps with *Jarava* species (subclades A and B) in the Andean region from 17° to 46°S (Soreng et al. 2003, Cialdella 2012) (Fig. 5). Species of *Jarava* (subclades A and B), mostly spread in temperate and subtropical areas, and have a wider distribution than *Jarava* species of subclade C, which are mostly restricted to the Patagonian region (Fig. 5). The shared geographical distribution between *Pappostipa* and *Jarava*-subclade C is

in accordance with the close relationship shown in our phylogenetic topology.

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