

AN OVERVIEW OF THE PHYLOGENETIC RELATIONSHIPS OF *BROMUS PUMILIO* (POACEAE) AND ALLIES BASED ON nrDNA ITS AND trnL-F SEQUENCES

Z. Pourmoshir, A. Amirahmadi & R. Naderi

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According to various taxonomic treatments, *Bromus* has been divided into 8 to 9 sections, subgenera or even separate genera. The relationship of *Bromus pumilio* (syn.: *Boissiera squarrosa*) with other species of *Bromus* has long been a matter of dispute. To clarify this problem, the nuclear ribosomal DNA ITS and chloroplast DNA trnL-F sequences for 31 and 28 species respectively were used to reconstruct the phylogenetic relationship and reveal the infrageneric boundaries. Molecular analysis performed by Maximum Parsimony analyses and Bayesian method. The results based on nrDNA ITS show that sects. *Bromus*, *Ceratochloa*, *Genea*, *Mexibromus* and *Neobromus* are monophyletic, while the plastid data doesn't support the monophyly of any sections in the genus *Bromus*. Evidence based on the nrDNA ITS data doesn't show any close relationships between *B. pumilio* (sect. *Boissiera*) and *B. danthoniae* or *B. lanceolatus* (sect. *Bromus*) and instead robustly supports *B. pumilio* as the sister group of *B. gracillimus* (sect. *Nevskiella*), while in the plastid data *B. pumilio* is the sister group of a clade comprising *B. inermis* (sect. *Pnigma*), *B. gunckelii* (sect. *Neobromus*) and species of sect. *Ceratochloa*.

Zahra Pourmoshir, Atefe Amirahmadi (correspondence < a.amirahmadi@du.ac.ir >) & Reza Naderi, Faculty of Science and Institute of Biological Science, Damghan University, Damghan, Iran.

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بررسی اجمالی روابط فیلوژنتیکی *Bromus pumilio* (Poaceae) و خویشاوندان براساس توالی‌های nrDNA ITS و trnL-F

زهرا پورموشیر: فارغ التحصیل کارشناسی ارشد، گرایش سیستماتیک-بوم‌شناسی، دانشگاه دامغان

عاطفه امیراحمدی: استادیار دانشکده زیست‌شناسی و پژوهشکده علوم زیستی، دانشگاه دامغان

رضا نادری: استادیار دانشکده زیست‌شناسی و پژوهشکده علوم زیستی، دانشگاه دامغان

بر پایه مطالعات تاکسونومیک متعدد جنس *Bromus* به ۸ تا ۹ بخش، زیرجنس و یا جنس‌های مجزا تقسیم‌بندی می‌شود. مدت مدیدی است که رابطه بین گونه *Bromus pumilio* (syn: *Boissiera squarrosa*) با گونه‌های دیگر جنس *Bromus* موضوع بحث است. جهت بازسازی روابط فیلوژنی و آشکارسازی مرزهای درون جنسی از ۳۱ توالی ریبوزومی هسته‌ای ITS و ۲۸ توالی کلروپلاستی trnL-F استفاده شد. تحلیل‌های مولکولی به وسیله روش پارسیمونی و بایسین اجرا شد. نتایج بر پایه توالی ITS هسته‌ای ریبوزومی نشان می‌دهد که بخش‌های *Bromus*, *Genea*, *Ceratochloa*, *Mexibromus* و *Neobromus* تک‌نیا هستند در حالیکه داده‌های کلروپلاستی تک‌نیا بودن هیچ یک از بخش‌های جنس *Bromus* را نشان نمی‌دهد. شواهد بر پایه داده‌های ریبوزومی ITS هیچ رابطه نزدیکی بین گونه *B. pumilio* (sect. *Boissiera*) با گونه‌های *B. danthoniae* یا *B. lanceolatus* (sect. *Bromus*) نشان نمی‌دهد و به‌جای آن گونه *B. pumilio* را با حمایت بالا به‌عنوان گروه خواهری *B. gracillimus* (sect. *Nevskiella*) در نظر می‌گیرد، همچنین در داده‌های کلروپلاستی گونه *B. pumilio* گروه خواهری کلادی متشکل از *B. inermis* (sect. *Pnigma*), *B. gunckelii* (sect. *Neobromus*) و گونه‌های بخش *Ceratochloa* است.

INTRODUCTION

Bromus L. contains more than 160 annual and perennial species that are found in temperate regions worldwide (Saarela & al. 2014). There are considerable variations in chromosome size and ploidy levels ranging from $2n = 14-122$ which indicate the role of interspecific hybridization in the evolution of many species in the genus (Stebbins 1981; Armstrong 1991). In the most comprehensive phylogenetic-taxonomic studies of the genus, Saarela (2007, 2014) and Naderi & Rahiminejad (2015) recognized 9 sections in total within *Bromus*: sects. *Boissiera* (Hochst. ex Steud.) P.M.Sm., *Bromus*, *Ceratochloa* (P.Beauv.) Griseb., *Genea* Dumort., *Mexibromus* Saarela, P.M.Peterson & Valdés-Reyna, *Neobromus* (Shear) Hitchc., *Nevskiella* (V. I. Krecz. & Vved.) Tournay, *Pnigma* Dumort. (as sect. *Bromopsis* Dumort.) and *Triniusia* (Steud.) Nevski. Naderi & al. (2016) proposed a reduction to 8 sections by including sect. *Triniusia* in sect. *Bromus*, on the basis of the presence of a transitional series of appearing awns in the lemma apex. The monotypic section *Boissiera* including *Bromus pumilio* (Trin.) P.M.Sm. is naturally distributed in Central and SW Asia and eastern Mediterranean area (Bor 1970 as *Boissiera squarrosa* (Sol.) Nevski). The species is used as fodder plants for spring ephemeral pastures, but spoils the wool of sheep because of its multiple awns on the lemma (Tzvelev 1976).

Nomenclatural history of *Bromus* sect. *Boissiera*

The species under *Bromus* sect. *Boissiera* (Hochst. ex Steud.) P.M.Sm. now known as *Bromus pumilio* (Trin.) P.M.Sm. was first named *Pappaphorum squarrosus* Sol. by Solander (1794). Thereafter, *P. pumilio* Trin. was named by Trinius (1830). The genus *Boissiera* was legitimately described by Hochstetter ex Steudel (1854) with its basionym *P. pumilio*, while the epithet "*pumilio*" was not used in the illegitimate name *Boissiera bromoides* Hochst. & Steud. Hackel (1885) in the work of Stapf made the combination *Boissiera pumilio* (Trin.) Hackel. Nevski (1934) placed the specific epithet of Solander into the new combination *Boissiera squarrosa*. Smith (1969) used the next earliest epithet "*pumilio*" because of the presence of a similar specific epithet in *Bromus squarrosus* L., and transferred the species of Trinius to *Bromus pumilio*. Finally, Smith (1985) placed *B. pumilio* in *Bromus* sect. *Boissiera*, based on different dispersal mechanism.

From the morphological point of view, the placement of *B. pumilio* in its own section *Boissiera* and its affinity to sect. *Bromus* especially with *B. danthoniae* Trin. and *B. lanceolatus* Roth have long been a matter of dispute (Smith 1969, 1985; Naderi &

Rahiminejad 2015; Naderi & al. 2016; Pourmoshir & al. 2017). Hitherto, no phylogenetic studies have been depicted relationship of *B. pumilio* with its allies in a cladogram, though the position of *Boissiera* within *Bromus* has been implicitly mentioned by some studies (Saarela & al. 2007; Soreng & al. 2015; Kellogg 2015). A total of 39 species including 41 taxa (2 subspecies and 2 varieties) are currently accepted for the flora of Iran (Naderi & Rahiminejad 2015; Naderi & al. 2016). In order to prepare a revision of Poaceae (tribe Bromeae) in Flora of Iran, it is necessary to specify the position of sect. *Boissiera* (syn. gen. *Boissiera*) with molecular data. The recent morphological studies (e.g. Naderi & Rahiminejad 2015; Naderi & al. 2016; Pourmoshir & al. 2017) highlighted the taxonomic circumscription of sect. *Boissiera* within *Bromus*. Hence, the aim of this study is to clarify the phylogenetic relationships of *Bromus* sect. *Boissiera* among other sections of the genus.

MATERIALS AND METHODS

In this study we included *Bromus* species from 8 sections (out of 9 sections) based on treatment by Naderi & Rahiminejad (2015). Ten samples (representing 4 species and 2 varieties) were sequenced. Additional sequence data obtained from GenBank. *Triticum aestivum* L. and *Hordeum vulgare* L. were chosen as outgroups. Voucher specimens and GenBank accession numbers are listed in table 1.

We produced a dataset of nrDNA ITS with 35 (5 new sequences and 30 obtained from GenBank) accessions representing 31 species, and a dataset of cpDNA *trnL-F* with 30 (5 new sequences and 25 obtained from GenBank) accessions representing 28 species. DNA was extracted from dried leaf material using a Plant DNA extraction kit (GeneAll, China) according to the manufacturer's protocol. Amplification of the ITS region was performed using the primer pair ITS5m (Sang & al. 1995) and ITS4 (White & al. 1990). For the plastid region the universal primers c and f (Taberlet & al. 1991) were used. All PCR amplifications were performed by 1659 Eppendorf Master Cycler (Germany). PCR product then sequenced by commercial sequencing service provider (Pishgam Biotech Co.). We aligned sequences by muscle using the web-based version of MUSCLE (Edgar 2004; at <http://www.ebi.ac.uk/Tools/msa/muscle/>) under default parameters followed by manual adjustment. As the two data set were incompatible ($p = 0.01$, $p < 0.05$) therefore we analyzed the nrDNA ITS and *trnL-F* matrix separately. For each data set two kinds of analyses were run separately: maximum parsimony and Bayesian inference.

Table 1. Taxa included in the nrDNA ITS and *trnL-F* analyses.

	ITS Accession number	Origin-Voucher number or Source	<i>trnL-F</i> Accession number	Origin-Voucher number or Source
<i>Bromus alopecuroides</i> Poir.	KM077300	Morocco: Tiznt, Jbel Imzi, Llamas and Gomiz 11 2004	EU036177	Fortune & al. (2008)
<i>B. anomalus</i> E.Fourn.	AY367905	USA: Plant introduction Station, Pullman, Wash., U.S.A 232199 (WRPIS)/ Keane 49 (ALTA)	AY367955	USA: PI 232199 (WRPIS)/ Keane 49 (ALTA)
<i>B. arvensis</i> L.	KM077301	Germany: accession 06-116-74-74 Botanischer Garten Oldenburg, Alonso 344 2011	EU036175	Fortune & al. (2008)
<i>B. berteroi</i> Colla	KP987430	Alonso & al. (unpubl.)	AY367995	Chile: PI 224789 (WRPIS)/ Keane 37 (ALTA)
<i>B. brachystachys</i> Hornung	AH005736	Iran: Plant introduction Station, Pullman, Wash. U.S.A 229598	-	-
<i>B. briziformis</i> Fisch. & C.A.Mey.	KP987317	Alonso & al. (unpubl.)	EU036182	Fortune & al. 2008
<i>B. carinatus</i> Hook. & Arn.	AY367948	Mexico: Durango/ Peterson & al. 15421 (US)	KX372390	Lee & Kim (unpubl.)
<i>B. catharticus</i> Vahl	KP987384	Alonso & al. (unpubl.)	KX372391	Lee & Kim unpubl.
<i>B. commutatus</i> Schrad.	KP987323	Alonso & al. (unpubl.)	AB732923	JAPAN: Kouyou-cho-higashi, Kobe, Hyogo Pref., 3 m alt./ M. Mizuta
<i>B. danthoniae</i> Trin. var. <i>danthoniae</i> 2	LC467154	Iran: Razavi Khorassan, Chenaran, Akhlamad olia, Naderi 1315 (DU00008)	LC467159	Iran: Razavi Khorassan, Chenaran, Akhlamad olia, Naderi 1315 (DU00008)
<i>B. danthoniae</i> Trin. var. <i>danthoniae</i> 4	LC467153	Iran: South Khorassan, 20 km to Ghaen from Birjand, Naderi 1210 (DU000145)	LC467158	Iran: South Khorassan, 20 km to Ghaen from Birjand, Naderi 1210 (DU000145).
<i>B. danthoniae</i> Trin. var. <i>pauciaristatus</i> Naderi	LC467155	Iran: Khorassan Razavi, Mohsenabad Taheri, Douleh village, Naderi 1468 (DU000138)	LC467160	Iran: Khorassan Razavi, Mohsenabad Taheri, Douleh village, Naderi 1468 (DU000138)
<i>B. densus</i> Swallen	AY367910	Mexico: Tamaulipas/ Nuevo Leon/ Peterson 15926 & Valdes-Reyna (US)	AY367960	Mexico: Tamaulipas/ Nuevo Leon/ Peterson 15926 & Valdes-Reyna (US)
<i>B. dolichocarpus</i> Wagon	AY367911	Mexico: Michoacan/ Peterson 16128 (US)	AY367961	Mexico: Michoacan/ Peterson 16128 (US)
<i>B. gracillimus</i> Bunge	KM077289	Tajikistan: Pamir/ E. Tolmatcheva (1958) CAN	-	
<i>B. gunckelii</i> Matthei	AY367947	Chile: Region I/ Peterson 15697 & Soreng (US)	AY367996	Chile: Region I/ Peterson 15697 & Soreng (US)
<i>B. hordeaceus</i> L.	KP987342	Alonso & al. (unpubl.)	KJ529407	Spain. León
<i>B. inermis</i> Leyss.	KP987409	Alonso & al. (unpubl.)	AY829228	China: Kunming, Yunnan / S20004
<i>B. intermedius</i> Guss.	KP987346	Alonso & al. (unpubl.)	HQ130343	Jacquemin & al. (unpubl.)
<i>B. japonicus</i> Thunb.	924878821	Alonso & al. (unpubl.)	KX372392	Lee & Kim unpubl.

Table 1. Continued.

	ITS Accession number	Origin-Voucher number or Source	<i>trnL-F</i> Accession number	Origin-Voucher number or Source
<i>B. lanceolatus</i> Roth	LC467156	Iran: Khuzestan, 15 km to Shush from Dezful, Naderi 1467 (DU000137).	LC467161	Iran: Khuzestan, 15 km to Shush from Dezful, Naderi 1467 (DU000137).
<i>B. pectinatus</i> Thunb.	KP987364	Alonso & al. (unpubl.)	AY367988	Belgium: PI 442453 (WRPIS)
<i>B. pumilio</i> (Trin.) P.M.Sm.	KP987312	Alonso & al. (unpubl.)	-	
<i>B. pumilio</i> (Trin.) P.M.Sm.	KP987313	Alonso & al. (unpubl.)	-	
<i>B. pumilio</i> (Trin.) P.M.Sm.	LC467157	Iran: North Khorassan: 15 km Shirvan to Quchan, Naderi 1336 (DU000139)	LC467162	Iran: North Khorassan: 15 km Shirvan to Quchan, Naderi 1336 (DU000139)
<i>B. racemosus</i> L.	KM077299	Spain: Leon, Cofinal, Alonso 29 2011	-	-
<i>B. ramosus</i> Huds.	KP987418	Alonso & al. (unpubl.)	KJ529405	Spain. León, Macizo Central. LEB WGS84
<i>B. scoparius</i> L.	KP987374	Alonso & al. (unpubl.)	EU036176	Fortune & al. (2008)
<i>B. secalinus</i> L.	KM077304	Germany: accession 07-104-07-74 Botanischer Garten Oldenburg, Alonso 339 2011	AB732927	JAPAN: Okayama Harbor, Okayama Pref., 2 m alt./ H. Kobatake
<i>B. squarrosus</i> L.	KP987381	Alonso & al. (unpubl.)	EU036173	Fortune & al. 2008
<i>B. sterilis</i> L.	KM077296	Israel: Sou, Philistean Plain/ A. Danin, T. Raus, W. Sauer, S. Brullo, B. Valdes, F. Amich, S.G. Gardner, R.C.H.J. van Ham, A. Gambino, F. Axelrod, Battia Pazy and Rivka Nokrian (1981) SALA	EU036167	Fortune & al. (2008)
<i>B. striatus</i> Hitchc.	AY367945	France: PI 477988 974 (WRPIS)/ Keane 6 (ALTA)	AY367994	Chile: PI 224789 (WRPIS)/ Keane 37 (ALTA)
<i>B. tectorum</i> L.	KP987445	Alonso & al. (unpubl.)	KX372395	Lee & Kim (unpubl.)
<i>Hordeum vulgare</i> L.	KU513496	Uzbekistan: R. Fritsch	EU036163	Fortune & al. (2008)
<i>Triticum aestivum</i> L.	AF521903	Corach & al. (unpubl.)	AF148757	Marchant s.n. Briggs & al. (2000)

Maximum Parsimony (MP) analyses were carried out using PAUP* v.4.0b10 (Swofford 2002). For the analyses all characters were unordered and equally weighted, heuristic search was with random sequence addition and tree-bisection-reconnection (TBR) branch swapping with 100 random-addition-sequence replicates, and MAXTREES option was set to 50,000. The obtained trees were summarized in a strict consensus tree. Branch support values were calculated using a full heuristic search with 1000 bootstrap replicates (Felsenstein 1985) each with simple addition sequence.

In Bayesian inference (BI), the optimal substitution models of sequence evolution were estimated using the program MrModeltest version 2.3 (Nylander 2004) based on the Akaike information criterion (AIC) (Posada & Buckley 2004). This program indicated

General time reversible model with gamma shaped rate variation and a proportion of invariable sites (GTR + G + I) as the best-fit model for both nuclear and chloroplast markers. For the Bayesian phylogenetic analyses, the program MrBayes version 3.2.4 (Ronquist & al. 2012) was used with the number of Markov chain Monte Carlo (MCMC) generations for ITS and *trnL-F* datasets set to 8 million. MrBayes performed two simultaneous analyses starting from different random trees (Nruns = 2) each with four Markov chains and trees sampled at every 100 generations. The first 25% trees were discarded as burn-in. The remaining trees were summarized in a 50% majority rule consensus tree accompanied with posterior probability (PP) values with each dataset. Tree visualization was carried out using TreeView version 1.6.6 (Page 2001).

RESULTS

Since the assessment of topologies and support for the nuclear ITS and plastid *trnL-F* regions show major conflicts also the ILD test indicated that nrDNA ITS and *trnL-F* data yield incongruent results ($p < 0.01$), therefore these datasets were not combined. Descriptive statistics for individual data sets (nrDNA

ITS and *trnL-F* data) are given in table 2. Visual inspection of all clades and sister group relationships revealed no major differences among tree topologies obtained from MP and BI. Therefore, we will focus on the better resolved BI trees. In nrDNA ITS sequences a total of 35 taxa were used in the phylogenetic analyses.

Table 2. Alignment characteristics and statistics for ITS and *trnL-F* dataset.

	nrDNA ITS	cpDNA <i>trnL-F</i>
Sequences (n)	35	30
Nucleotide sites	608	1156
Informative characters	135	51
Uninformative characters	473	1105
CI of MPTs	0.732	0.683
RI of MPTs	0.916	0.855
RI-CI MPTs	0.184	0.172
Number of MPTs	81	10000
Length of MPTs	250	82

In the nrDNA ITS tree topology (fig. 1) the genus *Bromus* formed a well-supported monophyletic group (PP = 1.00, MPS = 97) that comprising two distinct clades. The first clade included two subclades, in the first subclade *B. densus* Swallen and *B. dolichocarpus* Wagnon form the sect. *Mexibromus* formed a well-supported monophyletic group (PP = 0.93, MPS = 57). In the second subclade, three representatives of *B. pumilio* formed a clade (PP = 1.00, MPS = 100) that is sister group with *B. gracillimus* Bunge of monotypic sect. *Nevskiella* (PP = 1.00, MPS = 100). In the second clade several subclades could be identified: (i) the 17 members of the sect. *Bromus* from *B. secalinus* L. to *B. hordeaceus* L. (PP = 1.00, MPS = 100), (ii) *B. catharticus*, *B. striatus* Hitchc. and *B. carinatus* Hook. & Arn. from the sect. *Ceratochloa* (PP = 1.00, MPS = 93), (iii) *B. berterioanus* Colla and *B. gunckelii* Matthei from the sect. *Neobromus* (PP = 1.00, MPS = 93), (iv) *B. sterilis* L. and *B. tectorum* L. from the sect. *Genea* (PP = 1.00, MPS = 100), (v) *Bromus inermis* Leyss. from the sect. *Pnigma* (PP = 1.00, MPS = 99) and (vi) *B. ramosus* Huds. and *B. anomalus* E. Fourn. from the sect. *Pnigma* (PP = 0.51).

In the *trnL-F* tree (fig. 2) the genus *Bromus* was inferred as monophyletic (PP = 0.97, MPS = 88) and formed a polytomy of four independent lineages. First and second is corresponding to *B. dolichocarpus* and *B. densus* of the sect. *Mexibromus*. The third clade (PP = 0.92, MPS = 54) is a polytomy comprising the members of the sect. *Ceratochloa* (*B. striatus*, *B. carinatus*, *B. catharticus*) along with *B. inermis* (sect. *Pnigma*), *B. gunckelii* (sect. *Neobromus*, PP = 0.89, MPS = 83) and *B. pumilio* (Sect. *Boissiera*, PP = 0.92, MPS = 54) that

formed the successive sister lineages to the sect. *Ceratochloa*. In the fourth and biggest clade the members of the sect. *Bromus* (from *B. commutatus* Schrad to *B. intermedius*) along with *B. berterioanus* (Sect. *Neobromus*) formed a polytomy (PP = 0.98, MPS = 67). The two subclades including the members of the sect. *Genea* (*B. sterilis*, *B. tectorum*, PP = 0.57), sect. *Bromus* (*B. pectinatus* Thunb., PP = 1.00, MPS = 96) and sect. *Pnigma* (*B. ramosus* and *B. anomalus*, PP = 1.00, MPS = 78) are successive subclades to the above mentioned polytomy (PP = 0.61 and PP = 0.93), respectively.

DISCUSSION

The results of our analyses based on nrDNA ITS and cpDNA *trnL-F* indicate the monophyly of the genus *Bromus* which are congruent with previous studies (e. g. Ainouche & Bayer 1997; Saarela & al. 2007; Pourmoshir & al. 2017).

Taxonomic position of *B. pumilio* (the monotypic section *Boissiera*) and its relationship with other species of *Bromus* such as *B. danthoniae* and *B. lanceolatus* have been previously discussed based on serological and morphological characteristics as well as a morphological cladistic analysis (Smith 1969, 1972, 1985; Oja & Jaaska 1998; Naderi & Rahiminejad 2015; Naderi & al. 2016; Pourmoshir & al. 2017), but no study has hitherto been present a detailed phylogenetic analysis of *B. pumilio*. In our nuclear ribosomal tree *B. pumilio* (sect. *Boissiera*: lemmas with 7–9 awns) is the sister group of *B. gracillimus* (the monotypic sect. *Nevskiella*: lemmas 1 awn, short, 3.5–5 mm long and awns 3–5 times the length of lemmas), whereas in the

plastid tree *B. pumilio* is the sister group of a clade comprising *B. inermis* (sect. *Pnigma*) and species of sect. *Ceratochloa* and *B. gunckelii* (sect. *Neobromus*). As a result, evidence based on DNA sequences does not show any close relationships between *B. pumilio* and *B. danthoniae* or *B. lanceolatus* and instead, robustly supports sect. *Boissiera* as a section within the genus *Bromus*.

The present molecular study shows that sect. *Bromus* s.l. forms a monophyletic group with high support which is consistent with chromosomal and molecular data (Armstrong 1991; Ainouche & Bayer 1997). *Bromus* sects. *Bromus*, *Genea*, *Ceratochloa*, *Neobromus* and *Mexibromus* are appeared to be monophyletic based on nrDNA ITS data (fig. 1), whereas these sections along with sect. *Pnigma* are not monophyletic by the *trnL-F* sequences (fig. 2). ITS data shows better resolution and more informative characters than *trnL-F* data. The most important reasons for the success are biparental inheritance of nuclear DNA in which nuclear chromosomes are inherited from each parents, the presence of high number of copy in almost all organisms (excluding vertebrate) genomes and rapid rate of evolution in nrDNA ITS marker (Calonje & al. 2009).

Scholz (1998) recognized sect. *Triniusia* as a distinct group with three awns on each of the upper most lemmas of the spikelets, including four taxa namely *B. danthoniae* subsp. *danthoniae*, *B. danthoniae* subsp. *pseudodanthoniae* (Drobov) H. Scholz, *B. danthoniae* subsp. *rogersii* C.E.Hubb. ex H. Scholz, and *B. turcomanicus* H. Scholz. Serological (Smith 1972) and molecular studies (Ainouche & Bayer 1997) show a close relationship between *B. danthoniae* and *B. lanceolatus*. Our results revealed that all representative of *B. danthoniae* (sect. *Triniusia*) are nested within sect. *Bromus*. Likewise, it was found that if *B. danthoniae* be considered as a separate section, sect. *Bromus* will not be monophyletic. Naderi & Rahiminejad (2015) and Naderi & al. (2016) considering the transitional series of appearing and producing awn in the lemma apex of spikelet from *B. lanceolatus* to *B. danthoniae*, proposed sect. *Triniusia* is a synonym of sect. *Bromus*. Our molecular

phylogenetic studies corroborate the taxonomic position of *B. danthoniae* within sect. *Bromus* and certainly confirm the synonymy of sect. *Triniusia* to sect. *Bromus*. In our plastid tree, *B. berterioanus* (sect. *Neobromus*) is nested within sect. *Bromus*. Due to mismatch of accession numbers of some species e.g. *B. berterioanus*, *B. densus*, *B. anomalus* and *B. attenuatus* Swallen in the GenBank with the accession numbers of the study of Saarela & al. (2007), the position of *B. berterioanus* within sect. *Bromus* needs further investigation.

In this study, sect. *Genea* (with two representatives) based only on nrDNA ITS analysis is monophyletic. A molecular phylogenetic study by Fortune & al. (2008) showed that the nrDNA ITS and plastid *trnT-L* and *trnL-F* sequences support the monophyly of sect. *Genea*. Whereas based on the nuclear *Waxy* gene sequences, this section retrieved as a paraphyletic group. Saarela & al. (2007) sequenced the ITS region and the plastid *trnL* intron and 3'-end of *ndhF* genes to test the monophyly of sect. *Genea*. The two sources in their study were in conflict with each other; the nuclear ribosomal data supported the monophyly of sect. *Genea*, while the *trnL* intron and 3'-end of *ndhF* data did not show its monophyly. Our analyses corroborate the study of Saarela & al. (2007) and indicate that *trnL-F* data doesn't support the monophyly of sect. *Genea*, due to the position of *B. pectinatus* (sect. *Bromus*, fig. 2). This species with florets that taper toward the apex is morphologically similar to species of sect. *Genea* and is an intersectional hybrid which has been produced between species of sects. *Bromus* and *Genea* (Smith 1972; Scholz 1981; Stebbins 1981).

Two representatives from sect. *Mexibromus* including *B. dolichocarpus* and *B. densus* were analyzed. These two species along with *B. attenuatus* are endemic to México and differ from other sections of *Bromus* by their 3(-5)-nerved lemmas (Saarela & al. 2014). The nuclear ribosomal and plastid data are in conflict regarding the monophyly of sect. *Mexibromus*. In the nuclear ribosomal tree, the two species of sect. *Mexibromus* form a well-supported monophyletic group, while in the plastid tree the two above mentioned species do not form a monophyletic group.

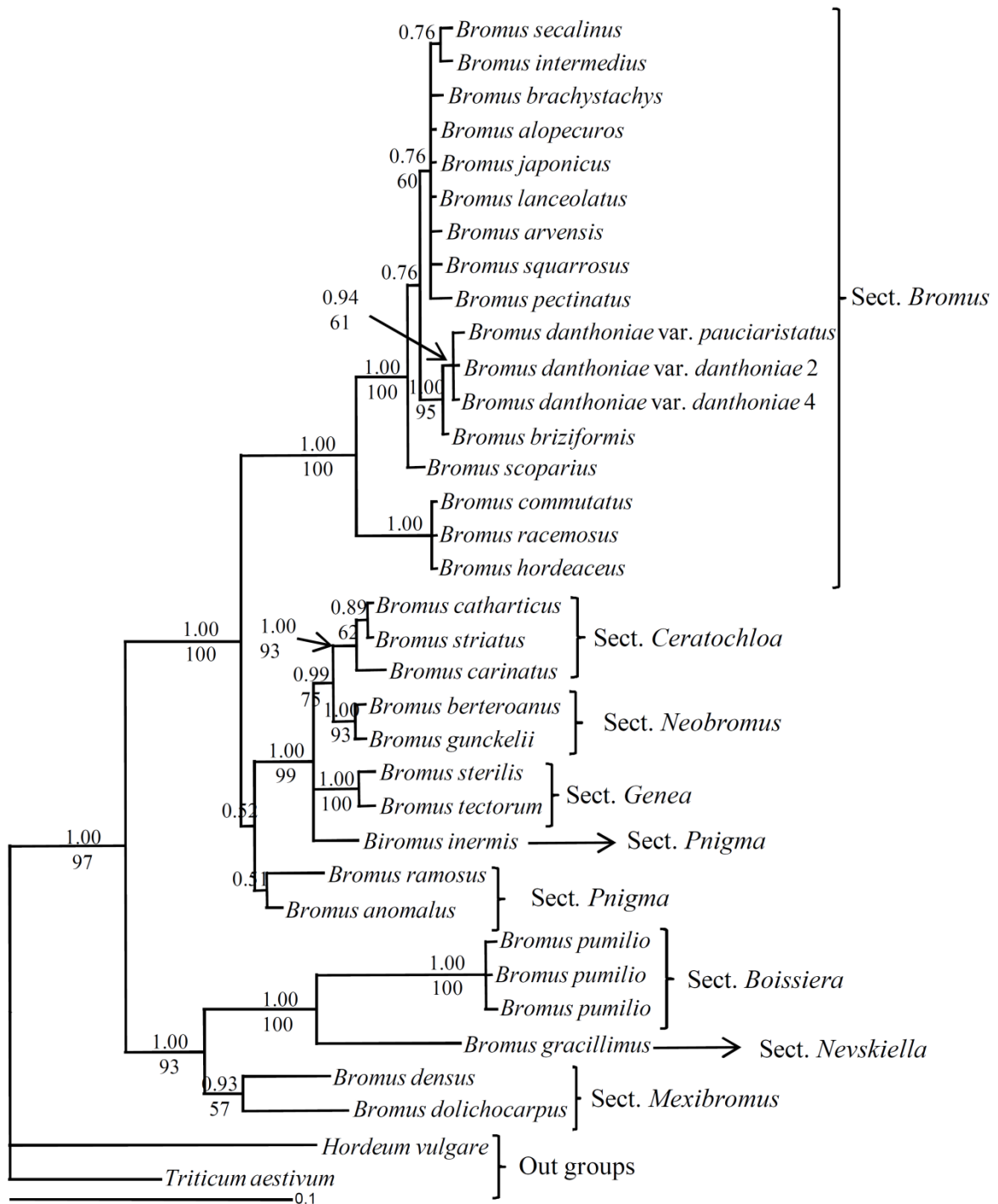


Fig. 1. Bayesian 50% majority-rule consensus tree inferred from nrDNA ITS data. Numbers above branches are Bayesian posterior probability and below branches are bootstrap support values.

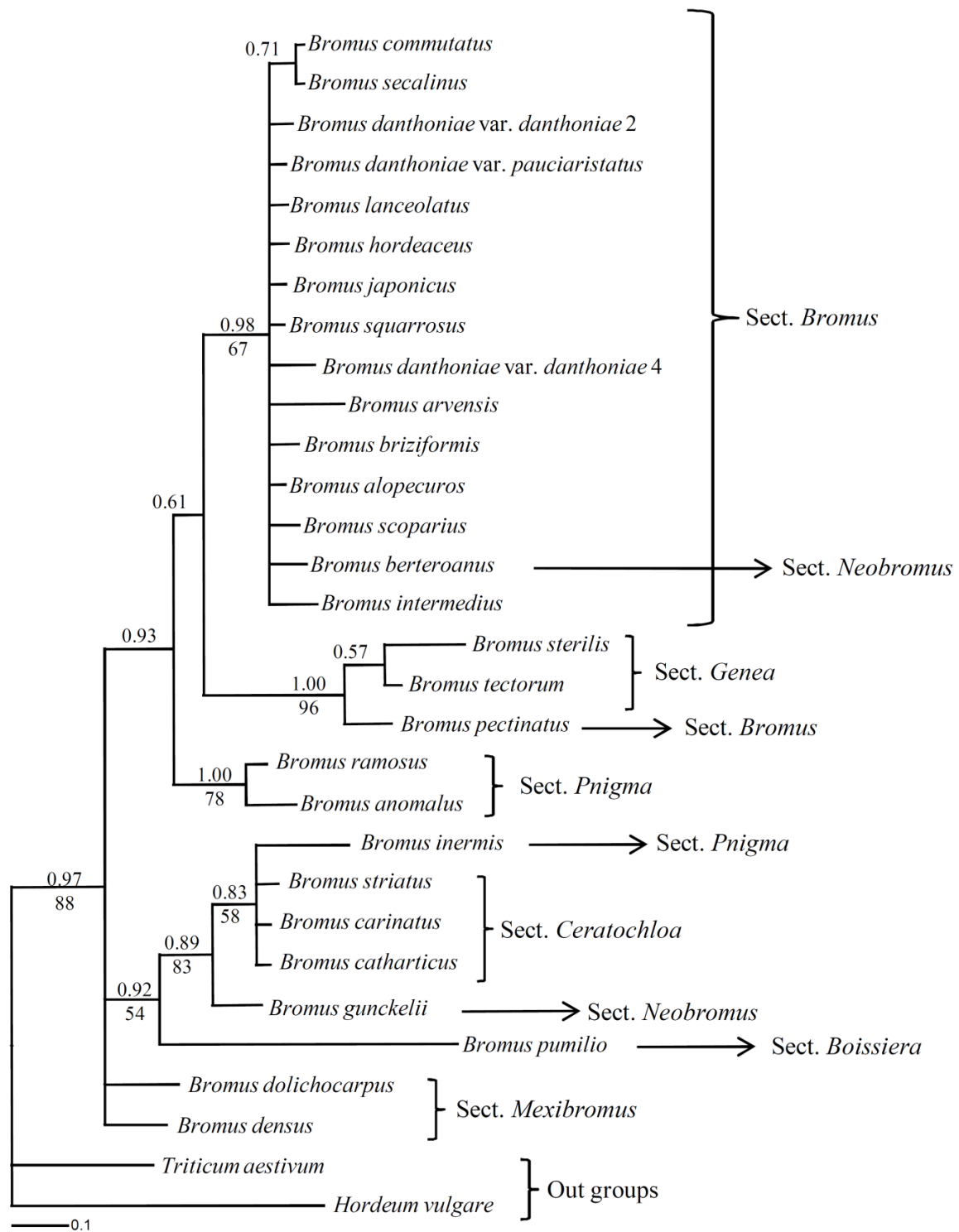


Fig. 2. Bayesian 50% majority-rule consensus tree inferred from *trnL-F* data. Numbers above branches are Bayesian posterior probability and below branches are bootstrap support values.

Section *Pnigma* (*Bromopsis*) with three representatives of the Old World and the New World taxa were analyzed. Based on the nuclear ribosomal data, our results show that *B. inermis* from the Old World is closely related to sect. *Ceratochloa* and *Neobromus*. *B. anomalus* from the New World with *B. ramosus* from the Old World form a strongly supported clade. Overall, sect. *Pnigma* constitutes two different and independent evolutionary lineages due to difficulties in crossing the Old World and New World taxa; these two groups differ from each other, with some exceptions, in life cycle, size of anther and size and number of chromosomes (Wagnon 1952; Armstrong 1991; Oja 2006; Saarela & al. 2007). Our analyses indicate that sect. *Pnigma* is not monophyletic which are consistent with the studies of Pillay & Hilu (1995) and Saarela & al. (2007) based on restriction site analysis and nuclear ribosomal and plastid data, respectively.

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