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TRIBE ANDROPOGONEAE FROM NEELUM VALLEY, AZAD JAMMU AND KASHMIR: PHYLOGENY BASED ON MORPHO-ANATOMY

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Abstract

The monophyletic tribe Andropogoneae (Poaceae) is a diverse group, which comprises economically important and environmentally essential clade of C_4 grasses. To find out the phylogenetic relationships, both morphological and molecular data sets were used. The morphological matrix including26 characters, were used to build a UPGMA trees implement in PAUP* 4.0b. For molecular studies, combined quality nuclear (ITS) and plastid loci (*ndhF*, *rbcL*, *trnL-trnF*, *atpB-rbcL* and *matK*) were used for phylogenetic analysis using Maximum Likelihood (ML) as implemented in RAxML-VI-HPC and Bayesian inference (BI) analyses in MrBayes v.3.2. Based on 19 key morphological characteristics, anatomical data was subjected to multivariate cluster analysis. We found overall topologies between the morphological tree and ML/BI tree were incongruent. However, Andropogoneae was found to be monophyletic. Astrong support crown clade, including *Bothriochloa*, *Capillipedium*, *Heteropogon*, and *Schizachyrium*, was recovered within Andropogoneae, and *Apludamutica* (1.00/100) was the first branch within this tribe. Significant variations for anatomical characteristics were exceedingly high and most of the characters seem to be related to environmental conditions. However, characteristics like shape of bulliform cells in leaf blade, presence of silica bodies in leaf epidermis, and more importantly the nature of pubescence can be related to systematics, at species level.

Key words: Anatomical Markers; Andropogoneae; Phylogenetics; Sclerification; Western Himalaya.

Introduction

During the last few decades, Poaceaehas been contemplated as a standard plant family for studying comparative genomics in plants. In particular, in grasses comparative genome analyses have been instrumental to perceive knowledge on different processes that lead to synteny maintenance or impairment. Many attempts have been done to determine the phylogeny of tribes of family Poaceae on the basis of form and structural characteristics. However, the Grass Phylogeny Working Group (2001) applied cladistic principles to the whole family using structural as well as molecular characteristics.

The Poaceae comprises on around 10 000 species (Soreng *et al*., 2003), falling in either of the two major clades, i.e., BEP (Bambusoideae, Ehrhartoideae, Pooideae) and PACCMAD (Panicoideae, Arundinoideae, Centothecoideae, Chloridoideae, Micrairoideae, Aristidoideae, Danthonioideae). Poaceaeis believed to have been evolved around 70 million years ago in a warm climate ecosystem (Anon., 2001; Edwards & Smith, 2010), but most of its members have adapted to diverse environmental regimes quite different from that of their origin. As a testament, most of grasses are seen adapted to multiple ecological regimes ranging from very low to very high temperatures (Stromberg & Mclnerney, 2011).

The monophyletic tribe Andropogoneae is a morphologically diverse comprising a variety of high value C_4 grasses (Kellogg, 2000). The group in fact comprises central food crops such as *Sorghum bicolor* and *Zea mays*, speciesdominating the North American prairie, *Sorghastrumnutans, Schizachyriumscoparium* and *Andropogongerardii,* and African grasslands *Cymbopogon* spp., and *Hyparrhenia* spp., and several poisonous weeds *Sorghastrumhalepense* and *Imperatacylindrica* (Orr *et al*., 2001). Based on morphological traits, this group of tropical grasses is distinct containing more than 900 morphologically diverse species (Clayton & Renvoize, 1986; Anon., 2001).

Morphology based phylogenetic reconstruction of Andropogoneae has shown that this tribe is not monophyletic (Kellogg & Watson, 1993). However, in contrast, a number of molecular studies have strongly supported its monophyly (Clayton & Renvoize, 1986), and also supported its close association with *Arundinella* (e.g., Spangler *et al*., 1999, Mathews *et al*., 2002, Sánchez-Ken *et al*., 2007; Christin *et al*., 2008; Vicentini *et al*., 2008). Although several molecular studies support the monophyly of the tribe, little information exists in the literature on the distinctiveness and relationships of its subtribes. Some recently conducted phylogenetic analyses have provided a strong clue that the small branches emerging from the spine of their trees and the changes in nucleotide concentrations on terminal branches in the Andropogoneae clade are believed to have been triggered by a rapid evolutionary emission near the base of the clade (Kellogg, 2000; Mathews *et al*., 2002). Many of these studies suggested that better sampling of lineages within the tribe, or the addition of more phylogenetic characters (more nucleotides), may help resolve the relationships.

In phylogeny, various assumptions and factors may influence on tree topologies (Luo *et al*., 2011), including

taxon sampling, alignment methodology, gene selection, gene sequence length, data treatment, optimality criterion, etc., (Dalevi *et al*., 2011; Ware *et al*., 2008). There are many complications in studying systematics and evolutionary studies in family as compared to other families which are listed elsewhere (Stebbins, 1956, 1982; Hilu & Wright, 1982). Due to these complications, the systematics and evolution of family Poaceaeis investigated considerably during the last few years by using a variety of approaches ranging from mere morphological to advanced molecular approaches. Among the molecular approaches, DNA sequencing is considered a prominent approach. The rationale of carrying out this study was to address the apparent conflicts between morphology, anatomy and molecules often seen in phylogenetic analyses, resolve major grouping within the tribe Andropogoneae, investigate their inter-relationships, and improve phylogenetic understanding of Andropogoneae.

Materials and Methods

Study site: The Neelum Valley lies in the north-east of Muzaffarabad with an altitude ranges between 900-6,325 m above sea level and between 73-75° E longitude and 32-35° N latitude with temperate climate, covering an area of 3,737 km^2 (Mahmood *et al*., 2011). The mean annual rainfall is about165 cm. This mountain based area is excellent for adventure and tourism, and the inhabitants of the area have unique customs and way of living. The area is characterized by small plateaus and different types of landforms. Soil texture of the area varies from loamy to sandy loam because of which the soil can retain reasonable amount of moisture, which is beneficial for optimum growth of plants. Thus, resultantly, the area is fully covered with thick vegetation (Ahmad *et al*., 2012).

Taxon sampling and out group selection: Grasses were sampled by the quadrat method using 1 m x 1 m quadrats at 5 sampling points along a straight transect line, and each separated by 20 m from a subsequent quadrat. For morphological studies, nine genera of Andropogoneae were included for phylogenetic analysis. *Arundinella* and *Agrostis,* were selected as out groups. We followed Hart (1987) and Stefanovic *et al*. (1998) for the out grouping selection. The morphological matrix was made by following the data method described in (Zuloaga *et al*., 2011).

Morphological data analysis: Characters for morphological matrix were used mainly for a reasonable argument of similarity; character-state transformation based on out groups analysis and discrimination of genera. Twenty-six characters were considered and data matrix prepared for phylogenetic analysis (Table 1) following Watrous & Wheeler (1981). Cladistic analysis of the data was conducted using PAUP* 4.0b (Swofford, 2000). All characters were considered as equally weighted. Heuristic searches for most parsimonious trees used 100 random addition sequences.

Anatomical studies: For the study of anatomical characters, the plant samples were first fixed in a fixative i.e., formalin acetic alcohol (FAA) for 36 hours and for the long term preservation it was shifted to acetic alcohol solution. Anatomical sections were cut by free hand sectioning was used and safra nine and fast green stains were selected to stain different types of tissues. Micrographs of stained sections were taken with a digital camera equipped microscope (Nikon 104, Japan).Nineteen characters were measured and data matrix was arranged for multivariate cluster analysis based on the absence (0) and presence (1) of the characters (Table 1).

Molecular studies: For molecular studies, we used the existing GenBank sequences as a starting point for alignment, and downloaded all the available nucleotide sequences from GenBank. Each genus was covered by at least one available species. We collected 11 species which were available in deposited data of GenBank. We included only one sequence per species, keeping the longest sequence or the most recently added if sequences were of the same length. In total, our GenBank data alignment consisted of 6 quality nuclear (ITS)and plastid loci(*ndhF*, *rbcL*, *trnL-trnF*, *atpB-rbcL* and *matK*). The selected taxon and gene loci are shown in Table 2. Some loci were unavailable in GenBank, the gene loss was 44% of plastid and *Capillipedium* lacked ITS. We combined the plastid and nuclear loci for phylogenetic analysis. Specially, the gene regions were difficult to align in many places or indels appearance in the alignments were excluded: *trn* L-*trn* F (24-25, 121-208, 235-238, 262-271, 322-325, 530-533, 541-544, 582-593, 598-602, 695-699, 721-766, 801-803, 861-868,897-901), *mat*K (71-94), *atp*B-*rbc*L (176-187, 609-624, 665-676), and ITS (36-40, 48-55, 73-88, 100-105, 398-411, 510-513, 597-600).

Phylogenetic analysis: Phylogenetic analysis was performed on the combined dataset using Maximum Likelihood (ML) as implemented in RAxML-VI-HPC (Stamatakis, 2006). The ML analyses employed the GTRCAT nucleotide substitution model, with the default settings for the optimization of individual per-site substitution rates. Due to the difficulties of bootstrapping datasets with large amounts of non-randomly distributed missing data, we also used Bayesian Inference (BI) to assess nodal support values for our phylogenies. Bayesian analyses were performed in the program MrBayes v.3.2 (Huelsenbeck & Ronquist, 2001). We used the default settings (GTR + G + I model) analysis for the combined dataset. For the BI analysis, we ran two separate analyses for 2 million generations each with tempt 0.05, then removed burin of 25% generations.

Table 1. Character states used in the phylogenetic and cluster analysis of Andropogoneae.

Morphological characters	Character states
Root type	Rhizome (0)/fibrous (1)/stolniferous (2)
Root length	(1) <2cm, (2) 2-3 cm, (3) 3-4 cm, (4) >4cm
Culm origin	Caespitose (0)/solitary (1)
Culm shape	decumbent (0)/erect (1)/geniculate (2)
Culm surface	Glabrous (0)/node beared (1)
Culm length	(1) <2cm, (2) 2-3 cm, (3) 3-4 cm, (4) >4cm
Leaf Length	(1) <2cm, (2) 2-3 cm, (3) 3-4 cm, (4) >4cm
Leaf apex	Attanuate (0)/acute (1)/acuminate (2)
Leaf origin	Cauline (0)/basal (1)
Leaf hairiness-adaxial	(1) none, (2) sparse, (3) dense
Leaf roughness-abaxial	(1) smooth (2) rough (3) very rough
Leaf sheath hairiness	(1) none, (2) sparse, (3) dense
Blade type	Attanuate (0)/lanceolate (1)/linear
Blade surface	Glabrous (0)/scabrous (1)
Blade shape	Flat (0)/lanceolate (1)/folded (2)/ convolute (3)
Sheath type	Smooth (0)/smooth & keeled (1)/hairy(2)/ papery (3)/slightly scabrid (4)
Auricle	Absent (0)/present (1)
Ligule type	memberanous fringed (0)/ring of hairs (1)
Ligule Length	(1) <2mm, (2) 2-3 mm, (3) 3-4 mm, (4) >4mm
Inflorescence length	(1) <2mm, (2) 2-3 mm, (3) 3-4 mm, (4) >4mm
Spikelet length	(1) <2cm, (2) 2-3 cm, (3) 3-4 cm, (4) >4cm
Spikelet number	(1) <15, (2) 15-20, (3) >20
Inflorescence type	Panicle (0)/raceme (1)/spike (2)
Tiller number	(1) <15, (2) 15-20, (3) >20
Awn length	(1) <2mm, (2) 2-3 mm, (3) 3-4 mm, (4) >4mm
Habitat	Annual (0)/perennial (1)
Anatomical characters	**Character states**
Thick prominent midrib	Absence (0)/presence (1)
Enlarge lower epidermis in midrib	Absence (0)/presence (1)
Sclerification inside adaxial midrib epidermis	Absence (0)/presence (1)
Enlarge endodermis in leaf midrib	Absence (0)/presence (1)
Enlarge epidermis at adaxial leaf surface	Absence (0)/presence (1)
Deeply inserted bulliform cells	Absence (0)/presence (1)
Extended bulliform tissues	Absence (0)/presence (1)
Hairs at abaxial surface	Absence (0)/presence (1)
Hairs at adaxial surface	Absence (0)/presence (1)
Arenchyma in leaf sheath	Absence (0)/presence (1)
Extensive sclerification in leaf sheath	Absence (0)/presence (1)
Intensive sclerification in stem hypodermis	Absence (0)/presence (1)
Sclerification in root pith	Absence (0)/presence (1)
Central portion hollow stem	Absence (0)/presence (1)
Lacunar cavity in stem vascular bundle	Absence (0)/presence (1)
Numerous metxylem vessels	Absence (0)/presence (1)
Sclerified inner tangential wall	Absence (0)/presence (1)
Central metaxylem vessel in root pith	Absence (0)/presence (1)
Prickly trichomes on leaf surface near secondary veins	Absence (0)/presence (1)
Silica bodies	Absence (0)/presence (1)

Table 2. Accession number of the gene markers from GeneBank.

Taxon	*atp*B-*rbc*L	*atp*F-*atp*H	*mat*K	ITS	ndhF	*psb*A-*trn*H	*rbc*L	*trn*L-*trn*F
Apludamutica	GQ870094	-	-	DQ005016	-	-	JQ933221	GQ869980
Arthraxonhispidus	GQ870072	HQ594554	KF163811	DQ006027	-	DQ006213	KC164295	DQ004956
Bothriochloapertusa	GQ870107	-	-	DQ005028	-	-	-	GQ869993
Bothriochloabladhii	-	-	-	-	AF117396	-	-	DQ004960
Capillipediumparviflorum	-	-	HE574008	-	AF117396	-	AM849398	-
Heteropogoncontortus	-	-	FR821324	AF190759	AF117411	-	HE575844	DQ004984
Saccharumsinense	AB731987	-	-	AB2811454	-	HQ876977	-	AB732009
Saccharumspontaneum	AB731984	-	-	AB281142	-	-	-	JN642308
Sorghum scoparium	-	-	HE586094	DQ005072	AF117420	-	HE577863	DQ004994
Schizachyriumscoparium	-	-	-	DQ005081	AF117429	-	-	DQ005003
Sorghum nitidum	GQ870112	-	-	GQ856354	AF117428	-	-	GQ869998
Arundinellanepalensis	GQ870039	-	-	AF019816	AF117394	-	-	DQ004958
Agrostisgigantea	-	FJ395261	DQ146802	EF565133	-	HQ596584	HQ589940	EU639571

Results

We employed 26 morphological characters to build a UPGMA tree implement in PAUP* 4.0b. The *Schizachyrium* was the first branch within Andropogoneae, clustered with [*Apluda* + *Arthraxon*]; then was sister to [*Bothriochloa* + *Heteropogon*], collectively sister to the remaining crown clade ([*Saccharum* + *Sorghum*] + *Capillipedium*). *Heteropogon* spp., showed a close relationship with two *Bothriochloa* spp., whereas, *Capillepidium* was found much closer to the species of *Sorghum* and *Saccharum* (Fig. 1). The members of tribe Andropogoneae showed variation in root system that was either rhizomatous or stolniferous (e.g. *Apludamutica*, *Arthraxonhispidus*), fiberous (e.g. *Bothriochloapertusa*, *Bothriochloabladhii*) or typically stolniferous (*Sorghum arundinaceum*).

Based on molecular studies, a total of 7446 characters and 14 taxa (11 in groups and 3 out groups) were included in phylogeny reconstruction. Because of loss of many data and close relationship appearing within the tribe, the support values were not good both in the ML and BI trees. But, we got same monophyletic topology of ML and BI trees of tribe Andropogoneae. Here, we only showed the BI tree with PP and BS values. The *Apluda* (*Apludamutica*) was at the first branch within tribe Andropogoneae, sister to the remaining genera with robust support (PP = 1.00, BS = 100; or 1.00/100). *Arthraxon* (*Arthraxonhispidus*) was sister to the left 6 genera (*Saccharum*, *Sorghum*, *Capillipedium Schizachyrium*, *Heteropogon*, and *Bothriochloa*) with high PP value (0.96), but no bootstrap values. The *Saccharum* + *Sorghum* clade was sister to the crown clade (1.00/64) without PP and BS. Within the crown clade, *Schizachyrium* clustered with the left genera ([*Heteropogon* + *Capillipedium*] + *Bothriochloa*) as sister (0.97/53); *Heteropogon* was sister to the genus *Bothriochloa* with strong support values (1.00/91; Fig. 1).

Anatomical studies of root, stem and leaf of all studied grasses are presented in Figs. 2, 3, 4. *Agrostisgigentea* shows different arrangement of vascular bundles in stem, which are deeply seated in cortical region, far from epidermis. The all other grasses have vascular bundles near stem periphery. Large proportion of adaxial epidermis in *Apludamutica* and *Arthraxonhispidus* comprises of large bulliform cells, in later case adaxial and abaxial epidermis has of elongated epidermal cells. Bulliform cells in *Arundinellanepalensis* are arranged in large groups, which cover slarge portion in leaf lamina between adjacent vascular tissues. *Capillipediumparviflorum* has very specific arrangement of vascular tissue on adaxialside is completely covered with large bulliform cells, whereas abaxialside has long tichomes. *Heterpogoncontortus* has similar bulliform cells arrangement as was recorded in *Capillipedium,* but trichomes are present only at the adaxial surface. *Polypogonmonspeliensis* and *Saccharumsinense* has prickle-shaped trichomes, which are restricted on the leaf veins. *Schizachyriumscoparium* shows prominent aerenchyma in leaf lamina between vascular tissues on abaxial side and thick layer of storage parenchyma on adaxial surface. Central metaxylem vessel in roots is recorded in *Polypogonmonspeliensis* and *Arundinellnepalensis*.

Two of the out groups *Agrostisgigantea* and *Polypogonmonspeliensis*, are C_3 grasses, whereas *Arundinellanepalensis* is C_4. All grasses studied in tribe Andrpogoneae are C_4. *Arundinella* is a tall grass and showed extra-ordinarily large bundle sheath cells, which are quite different from that recorded in the grasses of Andropogoneae. Among tribe Andropogoneae, the largest cells were recorded in *Saccharumsinense* and *Bothriochloapertusa*. The transverse sections of leaves of grasses showed mesophyll (MC) and bundle sheath cells (BSC) that were stained darker, thicker walled forming rings surrounding the veins. Enlarged BSC, relative to MC and closed vein spacing are the characteristic feature of C_4 leaves (Fig. 4).

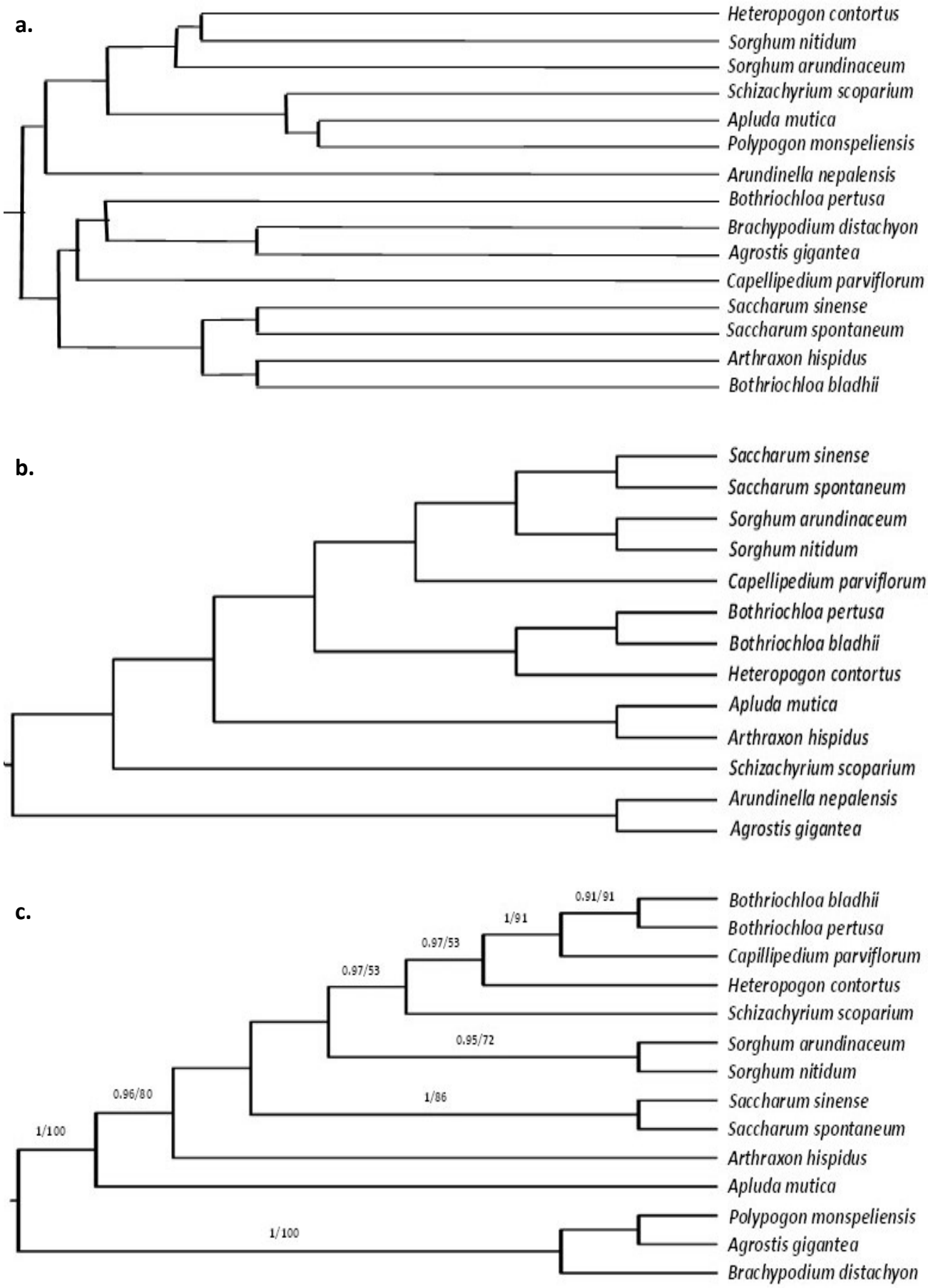

Fig. 1. Trees based on (a) morphological characteristics based on UPGMA tree, (b) anatomical characteristics and (c) molecular data combined 6 loci dataset.

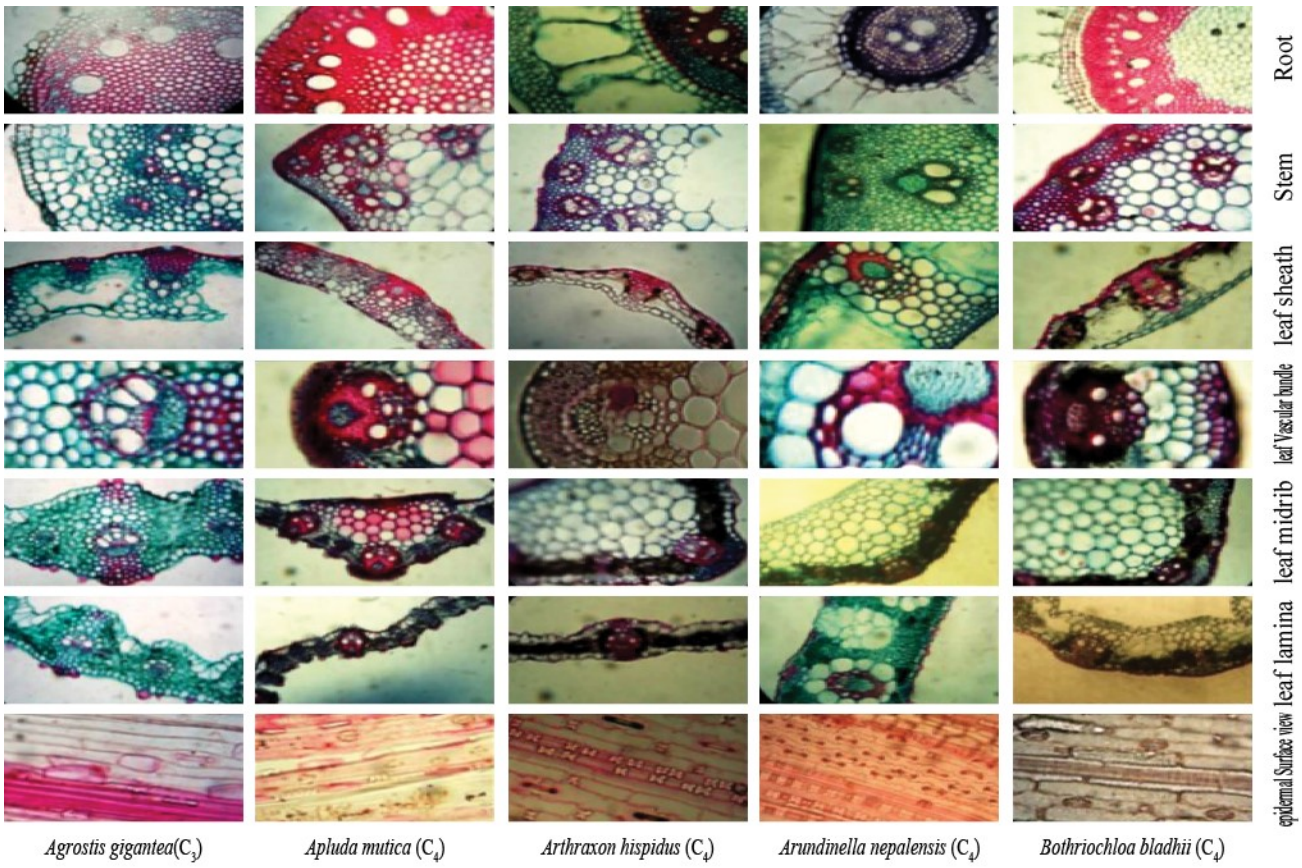

Fig. 2. Anatomical studies of *Agrostis, Apluda, Arthraxon, Arundinella* and *Bothriochloa* species from Neelum Valley, Azad Jammu and Kashmir.

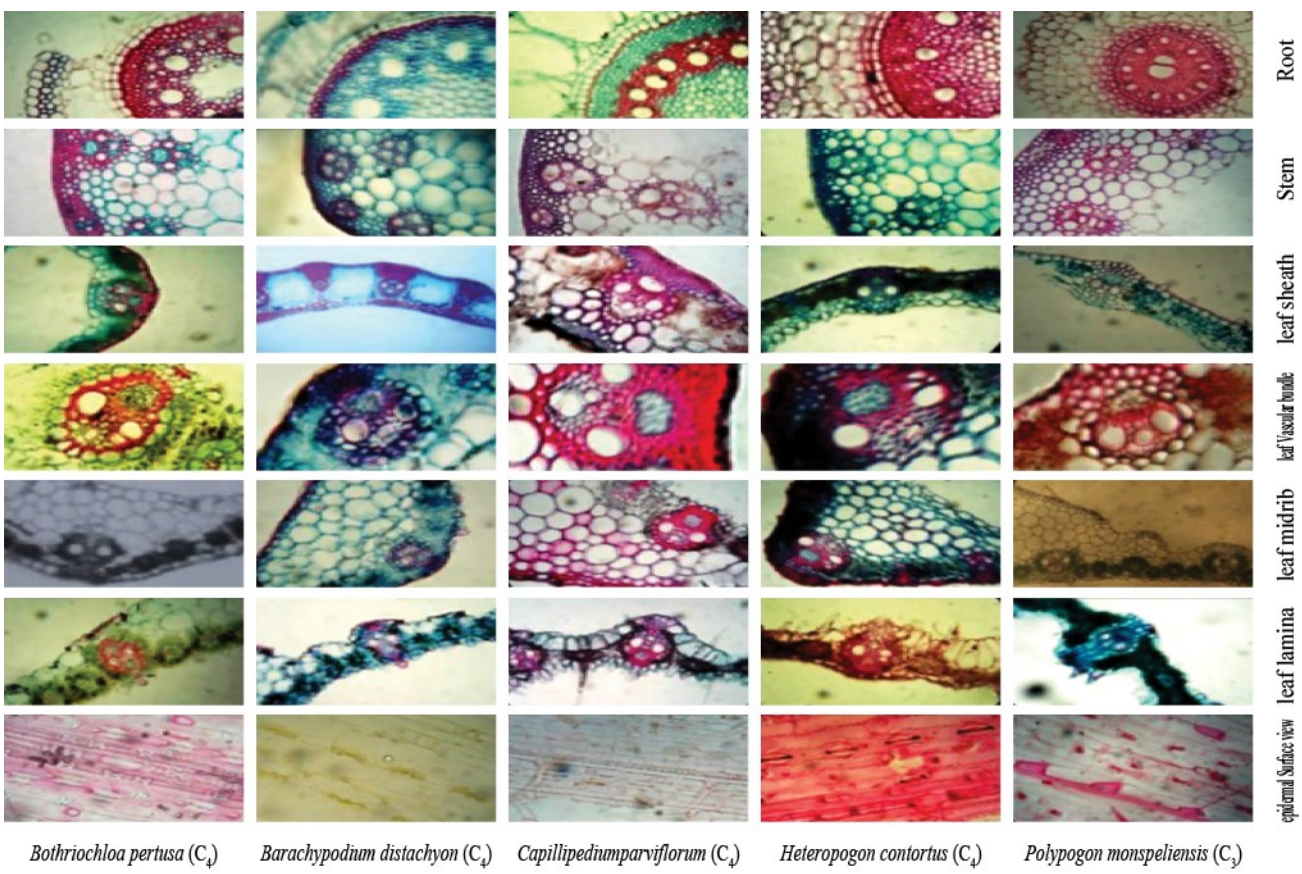

Fig. 3. Anatomical studies of *Bothriochloa, Brachypodium, Capillipedium, Heteropogon*and*Polypogon* species from Neelum Valley, Azad Jammu and Kashmir.

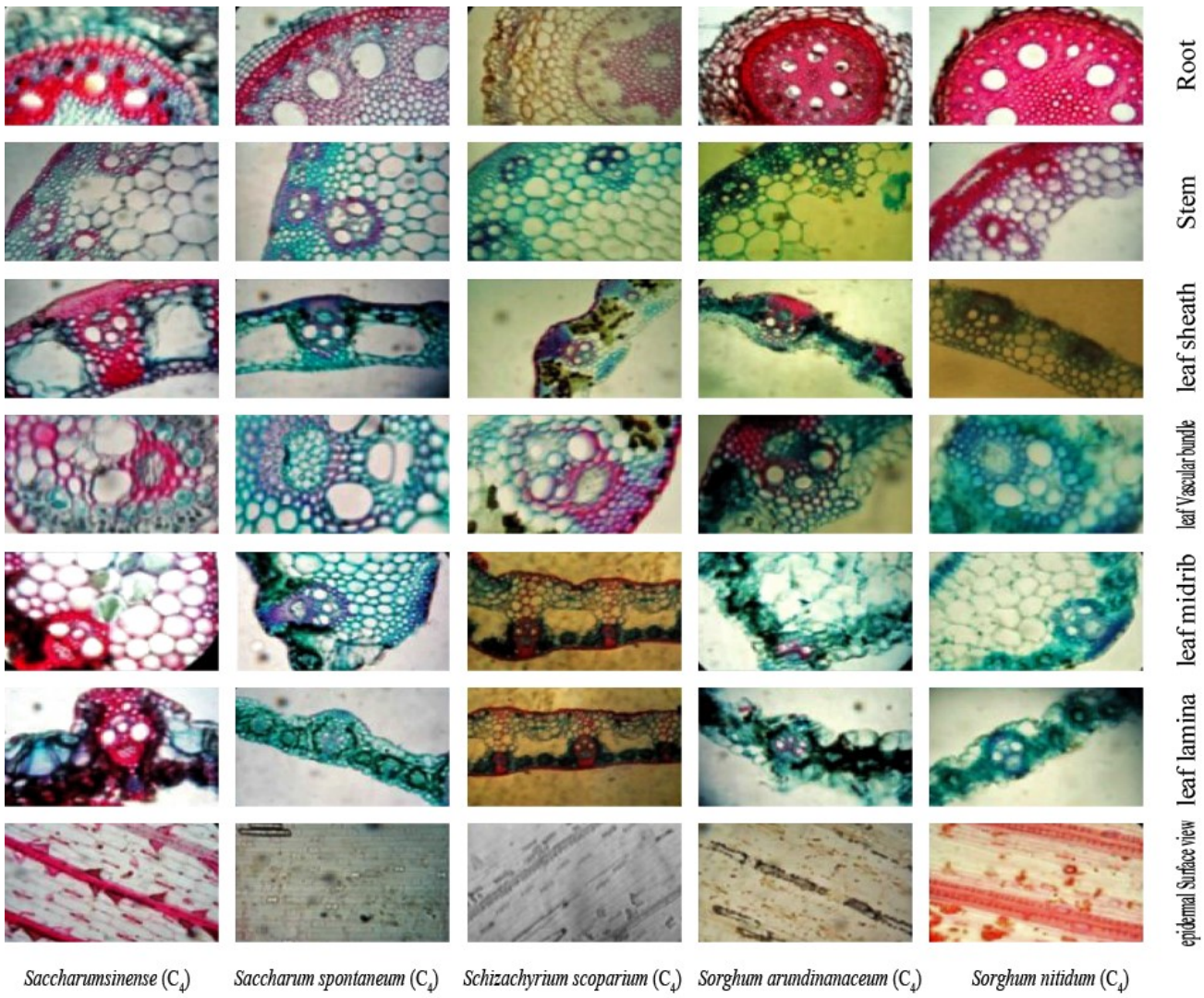

Fig. 4. Anatomical studies of *Saccharum, Schizachyrium,* and *Sorghum* species from Neelum Valley, Azad Jammu and Kashmir.

Discussion

Grasses have been more rigorously subjected tophylogenetic analyses, using both morphological as well as molecular data, than many other groups. Classical early morphological studies for the whole Poaceae family was conducted by Kellogg & Campbellin, 1987 and its subfamilies by Kellogg and Watson in 1993using data collected by Watson & Dallwitz (1992), Linder &Verboom (1996) and Snow (1997). With the refinement of techniques in genomic analyses, many phylogenetic analyses based on molecular data were followedin the 1990s. Some of the best known and most frequently cited papers are Hamby & Zimmer (1988) using ribosomal RNA sequences, Doebley *et al*. (1990) using plastid *rbc*L sequence data, Davis & Soreng (1993) using plastid DNA restriction site variation, Nadot *et al*. (1994) usingp lastid rps4 sequences, Clark *et al*. (1995) using plastid ndhF sequence data, Duvall & Morton (1996) using plastid *rbc*L sequences, Soreng & Davis (1998) using plastid DNA restriction site data, Barker *et al*. (1999) using plastid *rpo*C2 sequences, Hilu *et al*. (1999) using *mat*K sequences, Hsiao *et al*. (1999) using nuclear ribosomal ITS, Zhang (2000) using plastid *rpl*16 intron sequences, and Mathews & Sharrock (1996) using the nuclear phytochrome gene family.

For most species, the spikelets of a pair are dissimilar, rarely; the entire inflorescence is reduced to a triad (e.g., *Apluda*). The phylogenetic tree, however, did not support the subtribal classification of Clayton & Renvoize (1986). In our analysis, we selected 6 quality nuclear (ITS) and plastid loci (*ndh*F, *rbc*L, *trn*L-*trn*F, *atp*B-*rbc*L and *mat*K) from the GeneBank for alignment. Among these, some loci were unavailable in GenBank, with 44% gene loss of plastid and *Capillipedium* spp., lacked of ITS. The lack of morphological support for most subfamilies of Poaceae does not appear to be of concern to some grass phylogeneticists. It is true that many of the subfamilies as defined by the GPWG do not have any morphological synapomorphies. It is probably an inevitable consequence of a cladistic classification, since there is not any particular reason why evolution should always produce a handy macroscopic character at every speciation event.

We selected a total of 26 morphological characters that were grouped as: root (2), culm (4), leaf (10), including leaf blade and leaf sheath, spikelet (5), auricle (1), ligule (1) and habitat (1). That is an agreement with

the Anon. (2001). They assessed 46 structural characters that could be interpreted to be of use as measures of phylogenetic signal in the grasses. These characters can be grouped as follows: culm (2 characters), leaf (5), spikelet (10), floret (14), fruit and embryo (9), seedling (6). A major argument against a phylogenetic classification is that it is not always practical. In most cases, they are two-state characters indicating absence or presence. These characters and their states were optimized on the overall phylogeny, but the results suggest that some of them may be useful for delimiting groups within tribes or subfamilies, but are too variable to be useful in delimiting subfamilies (Anon., 2001). Clayton & Renvoize (1986) used morphological characters of the Andropogoneae inflorescence, rachis inter node, and spikelet pair to suggest intratribal relationships along axes of increasing complexity. Spicate racemes of Andropogoneae showing typical spikelet arrangement and disarticulation usually arranged in pairs.

There are some intricate genera that cannot be easily differentiated only on the basis of morphological characters. The genera *Bothriochloa* and *Dicanthium* are closely associated but confusion prevails for identification and discrimination between these genera. Likewise, *Saccharum bengalense* is confused with *Arundo donax,* whereas *Sorghum halepense, Chrysopogon serrulatus* and *Cymbopogon jwarancusa* cannot be precisely identified solely on the basis of morphological characters (Ahmad *et al*., 2010). In such circumstances, anatomical characteristics may provide assistance in proper identification of these taxa.

The major problem with the paired spikelets in both Andropogoneae and Pharoideaeis that if the development of the paired condition in these lineages is not equivalent, the paired spikelet character has to be split into two characters, with appropriate wording to define the nature of the pairing. Andropogoneae can be described as a 'natural group' based mainly on the presence of paired spikelets (sessile and pedicellate) and fragile racemes (Clayton & Renvoize, 1986). Knowledge of the assessment of homology in structural characters in grasses is very restricted and until much more is known about this subject, the use of morphological characters in grass phylogeny will probably have a limited application. Nonetheless, phenetic studies of the Poaceae, based on large morphological data sets, have provided the basis for grass taxonomy (Hilu & Wright, 1982; Watson & Dallwitz, 1992). Kellogg & Watson (1993) performed a cladistic parsimony analysis on a large data set comprising 72 Andropogoneae/ Maydeae genera and 220 characters (mostly morphological and anatomical). They found three groups roughly corresponding to Watson & Dallwitz's (1992) classification.

We selected *Arundinellanepalensis* an out group for morphological studies. Recent molecular studies using chloroplast and nuclear genes reported that the monophyly of the tribe and its sister group relationship with *Arundinella* are strongly supported (Kellogg, 2000; Mathews *et al*., 2002; Rondeau *et al*., 2005), but did not find support for the subtribal designations of Clayton & Renvoize (1986).

The phylogenetic analysis for molecular data showed 7446 characters and 14 taxa (11 in groups and 3 out groups) were included in phylogeny reconstruction. *Apludamutica* and *Arthraxonhispidus* formed were first and second branch within the tribe, respectively; that are sister to the remaining genera. *Schizachyrium* clustered with the genera ([*Heteropogon* + *Capillipedium*] + *Bothriochloa*) as sister (0.97/53). Moreover, *Heteropogon* was sister to the tip genus *Bothriochloa* with strong support values (1.00/91).Previous molecular studies showed two genera, *Bothriochloa*, and *Capillipedium*in amonophyletic group in the combined analysis (Skendzic *et al*., 2007). However, this relationship not supported our morphological data. Basedon molecular data, this clade was previously found to be within the core Andropogoneae (Mathews *et al*., 2002). The results from all analyses confirmed the monophyly of *Arthraxon*.

Morphologically, *Arthraxon* is distinguished from all other genera within Andropogoneae by its lemmas with a sub-basal awn. Although several well supported groups have been identified, the present matrix with three non-coding markers (*trn*L–*trn*F, *atp*β–*rbc*L and ITS) was insufficient to provide phylogenetic characters to resolve many evolutionary comploications at intergeneric level in Andropogoneae. Adding more data and more taxa are the only ways of resolving these difficult groups (Pirie *et al*., 2010). Choice of a gene is also critical and it would be worth while sequencing a large number of more slowly evolving genes to reconstruct the phylogenetic patterns inside these clades of Panicoideae (Moore *et al*., 2007).

Modern phylogenetic studies of the grasses have led to the formulation of a family tree that function as the basis of the taxonomic system and provide a reliable resolution of genealogical associations which precisely reflects patterns of evolution within the family. Most of the conclusions about grass phylogeny are, however, based on data obtained from the chloroplast genome (*rbc*L sequence data: Duvall & Morton, 1996; Barker, 1997; *ndh*F sequence data (Olmstead & Reeves, 1995); *rps*4 sequence data: Nadot *et al*., 1994; chloroplast restriction site variation: Soreng & Davis, 1998). A comprehensive nuclear dataset for comparison is lacking in grasses. Anatomical marker such as shape and size of bundle sheath cells showed huge variation among members of tribe Andropogoneae, with little unusual that all grasses from cooler climates are C_4 therefore, demand more precise studies. Christin *et al*. (2012) related size of bundle sheath cells with sub-types of C_4 pathway.

High altitudinal grasses are exposed to in the heterogeneous environmental conditions, therefore, they are an excellent model system to understand the potential response of the grasses to climate change (Mark *et al*., 2006), however, the structural responses are particularly important in plants occupying areas that are restricted geographically, like, high elevated alpine areas (Beniston, 2003), also some evidences suggested the alterations in the distribution of plant species due to global warming (Korner, 1999).

Variation for anatomical characteristics was exceedingly high and most of the anatomical characteristics seem to be related to environmental conditions. However, characteristics like shape of

bulliform cells in leaf blade, presence of silica bodies in leaf epidermis, and more importantly the nature of pubescence can be related to systematics, at least at species level. Presence of central large vascular bundle is recorded in only two species, *Polypogonmonspeliensis* and *Arundinellanepalensis*. Both species are restricted to moist habitats. This may be advantageous gaseous supply particularly under anaerobic conditions (Hameed *et al*., 2012). Plants at drier regions are generally equipped with dense hairiness that is known to be a protection against water loss (Hameed *et al*., 2013) or in controlling wind speed and leaf temperature (JianJing *et al*., 2012), as a result, a significant decline in transpiration rate.

Another prominent feature in grasses of drier habitats is the extensive sclerification, whether in outer cortex of roots, in stem hypodermis, around vascular tissues or pith region. This is a typical response of species under stress conditions (Hameed *et al*., 2012). Presence of bulliform cell play an important role in leaf rolling has been reported in many species of family Poaceae (Alvarez *et al*., 2008). Stomata are generally located on only one side of leaf surface, and these are protected in leaf cavity by leaf rolling, as a result can significantly enhance water use efficiency (Balsamo *et al*., 2006).

Conclusion

We can improve phylogenetic understanding within Andropogoneae and among subfamilies in the PACMAD clade by increasing the sampling of taxa and by using morphological to study inter-relationships of taxa within this group of plants. Formal nomenclatural and taxonomic variations should only be encouraged, especially at the species level, when the phylogenetic lineages correlate with marker morphological characterstics, i.e., when genotypic differences are reflected through the phenotype. Much more thought and research though need to be directed toward establishing the exact nature of morphological and anatomical characters and their interpretation in reflecting phylogeny. Furthermore, the homologous and developmental nature of these characters needs much more attention.

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