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Phylogeny and systematics of Indian *Polygonum sensu lato* in the subfamily Polygonoideae based on ITS sequences of nuclear ribosomal DNA

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ABSTRACT. The nuclear ribosomal DNA internal transcribed spacer (ITS) sequences from 44 Indian *Polygonum* taxa were examined to investigate relationships among various sections proposed by earlier researchers. The maximum parsimony trees obtained from analysis of the ITS sequences suggested eight major groups of the Indian *Polygonum* spp. The relationships among different sections were largely congruent with those inferred from morphological characters as described by Hooker. Also, the treatment of the *Persicaria* suggested by Haraldson on the basis of anatomical characters proved to be nearly in line with that based

on our molecular data. We provide a high resolution of phylogeny of the Himalayan *Polygonum sensu lato* and support merger of the section Amblygonon in the section Persicaria. Moreover, we made the first phylogenetic analysis of many of the less known Himalayan Polygonums, including *Polygonum microcephalum*, *P. assamicum*, *P. recumbens*, and *P. effusum*. Molecular differences were detected among *Persicaria barbata* collected from different geographical locations of India, although these were not differentiated at the morphological level.

Key words: *Polygonum sensu lato*; Phylogeny; Indian Himalaya; ITS

INTRODUCTION

The Polygonaceae family comprises approximately 46 genera and 1200 species (Mabberley, 2008) around the world, and is mostly distributed in northern temperate regions. They are either annual or perennial herbs, shrubs, or trees, often with unusual vascular structure and apparent swollen nodes. The leaves are simple, alternate, seldom opposite or whorled, usually entire and revolute, usually in spirals, petiolate to sessile, and with generally entire margins. The presence of ochrea is the most distinguishing feature, which is reduced in size or absent in the Eriogonoideae subfamily. Variation is seen in the axillary or terminal inflorescence, which is composed of simple or branched thyrsi that appear panicle-, raceme-, or spike-like but are, however, formed of dichasia or helicoid cymes. The flowers are small, trimerous, hermaphrodite, or unisexual with two or six tepals, forming two whorls of three elements or one whorl of five elements with characteristic quincuncial aestivation. The number of stamens ranges from two to nine, or rarely, more, whereas the pollen character varies from tricolporate to pantoporate. The ovary is superior, two to four carpellate (generally three-carpellate) and unilocular, whereas the fruits are a trigonous or lenticular achene.

To date, classification of the Polygonaceae has been mainly based on morphological characters such as presence or absence of ochrea, which is a nodal sheath, woodiness, tepal arrangements, etc., which have been widely debated. Ultimately, the two subfamilies Polygonoideae and Eriogonoideae have been universally accepted based on the presence or absence of ochrea (sometimes rudimentary in Eriogonoideae). All Indian genera belong to Polygonoideae, a subfamily of almost 790 species defined by the presence of ochrea, a monopodial branching pattern, and the lack of an involucre. They are distributed primarily in the Himalayan region, with a few species in tropical regions (Figure 1). The Eriogonoideae (330 species) are found only in the New World (Li et al., 2003). In addition to other commercial utilities, the members of the Polygonaceae family are highly valued for their medicinal uses. Kirtikar and Basu (1935) recorded medicinal uses for 31 species belonging to seven genera *viz. Calligonum*, *Pteropyrum*, *Polygonum*, *Fagopyrum*, *Rheum*, *Oxyria*, and *Rumex*. Thirty-four species of *Polygonum sensu lato* have been reported to be of medicinal use, e.g., *Polygonum aviculare*, which occurs in the west Himalayan region from Kashmir to Kumaon at elevations of approximately 1800-3600 m, is used variously in medicine (Choudhary et al., 2011b).

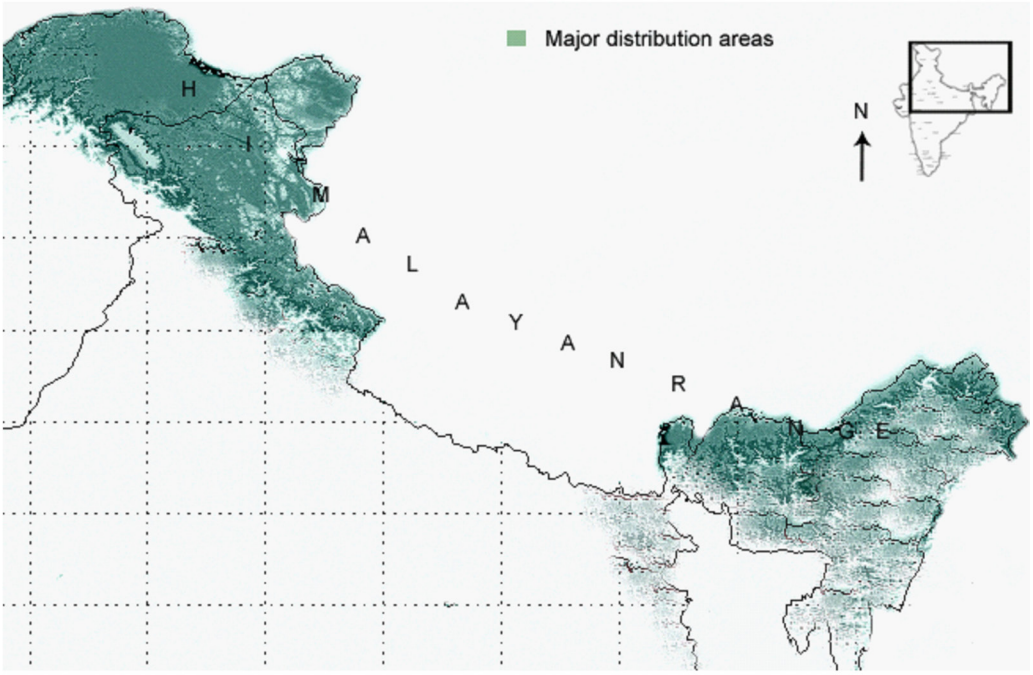

Figure 1. Major distribution areas of *Polygonum sensu lato* in India.

P. sensu lato (commonly known as knotweed) has long been a taxonomic puzzle and remains widely debated. It is represented by almost 230 species worldwide and is distributed mostly in the northern temperate regions (Li et al., 2003). It is the largest genus in Polygonaceae and is a member of the core eudicots in the flowering plants (Judd et al., 2002). Over the years, the generic concept for *Polygonum* has changed because the rationale for these varying approaches also changed and was never based on phylogenetic analyses. Rather, classifications were based on similarity (or dissimilarity) or subjectively "important" characteristics; the overall similarity might have been drawn from comparisons of shared ancestral traits rather than shared derived traits. Therefore, the traditional definitions of *Polygonum* and other polygonaceous taxa are useful for identification, but not necessarily for elucidation of evolutionary relationships. The traditional method of classification has led to disagreement among taxonomists over which species should be included in the *Polygonum* and which taxa should be elevated to their own genus because of the presence of at least one distinguishing characteristic (Meisner, 1826; Bentham and Hooker, 1880; Dammer, 1892; Gross, 1913; Hedberg, 1946; Graham and Wood, 1965; Holub, 1971; Haraldson, 1978; Ronse De Craene and Akeroyd, 1988; Hassan, 1997; Ronse De Craene et al., 2000; Hong et al., 2005). Morphological, cytological, palynological, and anatomical data, although useful in resolving some questions in the circumscription and relationship of genera, have not provided a consensus regarding relationship within the genera. Multiple base chromosome numbers have been documented in some genera, and polyploidy is common (Brandbyge, 1993). Recent biomolecular studies (Cuenound et al., 2002; Lamb Frye and Kron, 2003; Kim et al., 2005; Kim and Donoghue,

2008; Sanchez and Kron, 2008) have revealed that *P. sensu lato* is polyphyletic and should be divided into several genera. The treatment of the Polygonoideae subfamily by Haraldson (1978) and Ronse De Craene and Akeroyd (1988) have suggested that the *Polygonum* species, in the broad sense, be segregated into two separate tribes: Polygoneae and Persicarieae. The Persicarieae tribe is characterized by the presence of tepals with three nervatures departing from the base (with some exceptions in *Fagopyrum*), rectangular to elongate epidermic cells with straight or undulating anticlinal walls, and longitudinal and often continuous smooth or striate cuticles. The Polygoneae tribe is characterized by tepals with one principal nervature, more or less branched; irregular to elongate epidermic cells, rarely rectangular with mostly sinuate anticlinal walls, and cuticles rarely with longitudinal striations but with strong orthogonal to reticulate ridges or striae, often without correlation between cells. In Indian context, as classified in Flora of British India by Hooker (1886), the former is restricted to the *Avicularia* section (true *Polygonum*). The remainder of *Polygonum* in the broad sense is classified under 10 different sections *viz. Koenigia*, *Eleutherosperma*, *Amblygonon*, *Tovara*, *Bistorta*, *Persicaria*, *Cephalophilon*, *Echinocaulon*, *Aconogonon*, and *Tiniaria* (Figure 2). While dealing with the genus in a broad sense, Hooker (1886) was also slightly hesitant with the delimitation of the *Persicaria* and *Avicularia* sections, as evident from his special note: "... a very troublesome genus, the Indian species of which have been much confused, and I cannot hope that I have finally settled the limits of those especially of the *Persicaria* and *Avicularia* sections."

Figure 2. Different inflorescence patterns in *Polygonum sensu lato* **A.** *Fallopia dentatoalata*. **B.** *Bistorta amplexicaulis*. **C.** *Koenigia nepalensis*. **D.** *Polygonum aviculare*. **E.** *Persicaria capitata*. **F.** *Persicaria barbata*.

Hooker (1886) reported approximately 72 species in India with wide infraspecific diversity amongst some species. Later, Gage (1903) also carried out a census of Indian Polygonums and provided details on the occurrence of 79 species in India, but many of them were merged over time. The molecular studies carried out by Kim and Donoghue (2008) segregated *Persicaria* as a distinct genus in agreement with the morphological and anatomical studies carried out by Haraldson (1978).

In the present paper, the previous taxonomic hypotheses on the classification of *P. sensu lato* were tested using phylogeny reconstruction with the help of internal transcribed spacer (ITS) sequences of the nuclear ribosomal RNA (nrRNA) gene. The ITS region in the nrRNA gene is a suitable target to investigate the phylogenetic relationship among closely related plant genera and species because of rapid evolution of the ITS region (Sang et al., 1994; Baldwin et al., 1995) and homogenization among repeat units through concerted evolution (Arnheim, 1983). We also discuss specific systematic issues such as the validity of taxonomic groups in previous classification schemes, and circumscription of the true *Polygonum*. To date, there has been no descriptive phylogenetic analysis on the Himalayan Polygonums that addresses circumscription of *Polygonum* belonging exclusively to this region. The overarching purpose of the study was to trace the interspecific relationships of members of Indian Polygonums using phylogenetic methods in order to assess the generic definitions that have been applied to them. The specific objective of this study was to assess phylogenetic relationships within the Polygoneae and Persicarieae with emphasis on circumscription of *P. sensu lato* of the Indian Himalayas. We have also investigated Himalayan endemics such as *Bistorta amplexicaulis* ssp *sinensis*, *Persicaria assamica*, *Polygonum recumbens*, *P. effusum*, *Koenigia nepalensis*, and *K. delicatula*, whose phylogeny has never been attempted.

MATERIAL AND METHODS

Taxon sampling and outgroup selection

The leaf materials for the present study were collected from around India: Assam, Arunachal Pradesh, Karnataka, Bihar, Andaman and Nicobar, Himachal Pradesh, and Uttarakhand. The materials were identified using published taxonomic keys and compared to herbarium specimens housed at Botanical Survey of India, Arunachal Pradesh Regional Center, Itanagar (ARUN); Botanical Survey of India, Eastern Regional Center, Shillong, Meghalaya, Botanical Survey of India, Central National Herbarium, Kolkata, and Botanical Survey of India, Northern Circle, Dehra Dun. Voucher specimens were deposited at ARUN. Previously published ITS sequences of *P. sensu lato* (Kim and Donoghue, 2008) were retrieved from NCBI GenBank and included in the analysis (Table 1). Two *Rumex* species were selected as outgroups because of their close phylogenetic relationship with *Polygonum* and as a follow up to earlier references (Kim and Donoghue, 2008).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, The Netherlands). ITS sequences of nrDNA were amplified using the primers of White et al. (1990): ITS1 (forward, 5'-GTCCACTGAACCTTATCATTTAG-3' and ITS4 (reverse, 5'-TCCTCCGCTTATT

GATATGC-3') via polymerase chain reaction (PCR) using AccuPower HF PCR PreMix (Bioneer, South Korea). One round of amplification consisted of denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 49°C for 1 min, and extension at 72°C for 1 min, with a final extension step of 72°C for 5 min. Prior to sequencing, PCR products were purified using a SolGent PCR Purification Kit-Ultra (SolGent, South Korea).

The purified fragments were directly sequenced using dye terminator chemistry following the manufacturer protocol. Cycle sequencing was conducted using the same primers used for amplification and BigDye v.3.1 reagents and an ABI PRISM 3730XL DNA Analyzer (Applied Biosystems Division, Perkin-Elmer, USA) following manufacturer instructions. Cycling conditions included an initial denaturing set at 94°C for 5 min, followed by 30 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Each sample was sequenced in the sense and antisense direction. The sequences were analyzed with ABI Sequence Analysis and the ABI Sequence Navigator software (Applied Biosystems Division). Nucleotide sequences of both DNA strands were obtained and compared to ensure accuracy.

Sequence alignment and phylogenetic analysis

Initially, sequence alignments were performed using ClustalX version 1.81 (Thompson et al., 1997). Subsequently, they were adjusted manually using BioEdit (Hall, 1999). Insertion-deletions (indels) were scored as single characters when we had confidence in positional homology. The boundaries between ITS1, 5.8S, and ITS2 were determined by comparisons with earlier published related sequences available at NCBI GenBank. All sequences generated in the present study were deposited in GenBank and the accession Nos. are included in Table 1.

Tree searches under parsimony were conducted using PAUP* 4.0b10 (Swofford, 2002) and a combined data set of ITS1 and ITS4 sequences. Searches were performed with both the inclusion and exclusion of indels. We excluded 451 characters from all analyses due to alignment ambiguity. Initial searches excluding indels used a heuristic search strategy with tree-bisection-reconnection branch swapping and a random addition of taxa for 1000 replicates. Clade support was assessed by bootstrap values (Felsenstein, 1985).

Each putative event was treated as independent of overlapping gaps. Once indels were coded, all gap regions were excluded using the "exclude gapped" command in PAUP. Long indels sometimes spanned areas containing potentially phylogenetically informative nucleotide or indel sites in other sequences. In such cases, "N" coding was used in the gap-containing sequence to ensure the inclusion of that site in the analysis. Consequently, it was possible for the number of parsimony-informative sites to exceed the total number of sites minus gap-containing sites (Simmons and Ochoterena, 2000; Simmons et al., 2001).

RESULTS

Characteristics of ITS sequences

The combined length of the entire ITS region (ITS1, 5.8S and ITS2) from taxa included in the present study ranged from 533 to 666 bp, with ITS1 spanning 168-246 bp and ITS2 spanning 138-259 bp. Indels were necessary to align the sequences. The indels ranged in length from 1 to 47 bp. The complete sequences of the ITS regions were deposited in GenBank (Table 1).

Table 1. Sources of *Polygonum* and out-group accessions examined for nuclear ribosomal DNA ITS.

	Taxon	Voucher/source	GenBank accession No.
Ingroups			
1	*Aconogonon campanulatum*	Sun W and Zhou Z, 2006	DQ406630
2	*Aconogonum molle*	Kim ST and Donoghue MJ, 2008	EF653687
3	*Bistorta vivipara*	Yurtseva OV et al., 2010	GQ339919
4	*Bistorta macrophylla*	Sun W and Zhou Z, 2008	EU718496
5	*Bistorta amplexicaulis* var. *speciosa*	Lee J and Choudhary RK, 025 (BSD)	JX144673
6	*Bistorta amplexicaulis* ssp *sinensis*	Lee J and Choudhary RK, 011 (BSD)	JX144673
7	*Bistorta griffithii*	Sun W and Zhou Z, 2006	DQ406632
8	*Fallopia convolvulus*	Won H and Park CW, 1997	AF040064
9	*Fallopia dentatoalata*	Won H and Park CW, 1997	AF040066
10	*Fallopia dumetorum*	Won H and Park CW, 1997	AF040068
11	*Knorringia sibirica*	Sanchez A et al., 2009	GQ206253
12	*Koenigia islandica*	Kim ST and Donoghue MJ, 2008	EF653686
13	*Koenigia delicatula*	Lee J and Choudhary RK, A1 (BSD)	JX144675
14	*Koenigia nepalensis*	Lee J and Choudhary RK, A2 (BSD)	JX144676
15	*Persicaria amphibia*	Kim ST and Donoghue MJ, 2008	EF653700
16	*Persicaria assamica*	Choudhary RK, 127b (ARUN)	JX144669
17	*Persicaria barbata*	Choudhary RK, 101 (ARUN)	HQ709159
18	*Persicaria capitata*	Kim ST and Donoghue MJ, 2008	EF653690
19	*Persicaria chinensis*	Choudhary RK, 115 (ARUN)	JX144672
20	*Persicaria glabra*	Choudhary RK, 102a (ARUN)	JX144667
21	*Persicaria hydropiper*	Choudhary RK, 112 (ARUN)	JX144666
22	*Persicaria kawagoeana*	Kim ST and Donoghue MJ, 2008	EU196886
23	*Persicaria lapathifolia*	Kim ST and Donoghue MJ, 2008	EU196888
24	*Persicaria limbata*	Kim ST and Donoghue MJ, 2008	EU196889
25	*Persicaria longiseta*	Kim ST and Donoghue MJ, 2008	EU196890
26	*Persicaria maculosa*	Kim ST and Donoghue MJ, 2008	EU196892
27	*Persicaria microcephala*	Choudhary RK, 108 (ARUN)	JX144671
28	*Persicaria minor*	Kim ST and Donoghue MJ, 2008	EU196895
29	*Persicaria nepalense*	Kim ST and Donoghue MJ, 2008	EF653691
30	*Persicaria orientalis*	Choudhary RK, n11 (ARUN)	JX144668
31	*Persicaria perfoliata*	Choudhary RK, 114 (ARUN)	JX144670
32	*Persicaria posumbu*	Kim ST and Donoghue MJ, 2008	EU196900
33	*Persicaria pubescens*	Choudhary RK, 106 (ARUN)	JX144665
34	*Persicaria runcinata*	Kim ST and Donoghue MJ, 2008	EF653692
35	*Persicaria sagittata*	Sun W and Zhou Z, 2006	DQ372904
36	*Persicaria virginiana*	Kim ST and Donoghue MJ, 2008	EF653698
37	*Polygonum aviculare*	Kim ST and Donoghue MJ, 2008	EF653684
38	*Polygonum cognatum*	Yurtseva OV et al., 2010	GQ339994
39	*Polygonum effusum*	Lee J and Choudhary RK, LR019 (BSD)	JX144678
40	*Polygonum paronychioides*	Yurtseva OV et al., 2009	GQ340029
41	*Polygonum plebeium*	Yurtseva OV et al., 2009	GQ339946
42	*Polygonum recumbens*	Lee J and Choudhary RK, LR010 (BSD)	JX144677
43	*Polygonum rottboellioides*	Yurtseva OV et al., 2010	GQ340045
44	*Polygonum molliiforme*	Yurtseva OV et al., 2009	GQ340018
Outgroups			
1	*Rumex hastatus*	Zheng S et al., 2001	AF338218
2	*Rumex acetosa*	Chen S-L et al., 2008	FJ503011

BSD = Botanical Survey of India, Dehra Dun; ARUN = Botanical Survey of India, Arunachal Pradesh Regional Center, Itanagar.

The bootstrap strict consensus tree inferred from 220 maximally parsimonious trees is shown in Figure 3. Branches corresponding to partitions reproduced in less than 50% trees are collapsed. The consistency index was 0.553 and the retention index was 0.813 for all sites and parsimony-informative sites. The percentage of parsimonious trees in which the associated taxa clustered together is shown beside the branches. All trees resulting from the analysis of ITS sequences were resolved in two major clades, clades I and II (>99% bootstrap support,

BS); clade I (100% BS) was represented by sub-clades *Persicaria* (66% BS), *Bistorta* (99% BS), and *Aconogonon-Koenigia* (84% BS), whereas clade II (66% BS) was represented by *Fallopia* (99% BS) and *Polygonum* (93% BS). *Knorringia*, which was represented by only one species, exhibited a lesser affinity with *Polygonum* (50% BS) (Figure 3).

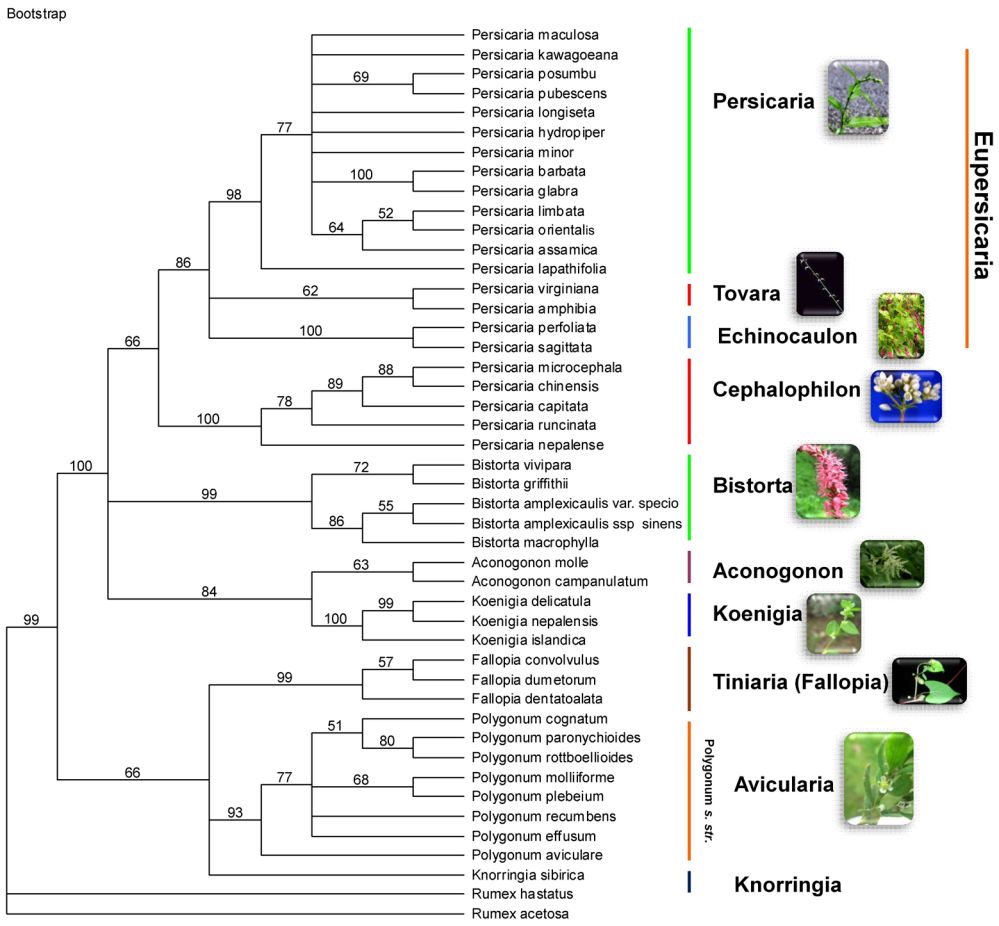

Figure 3. Bootstrap strict consensus tree of 220 maximally parsimonious trees with a total length of 1075 steps, a consistency index of 0.553, and a retention index of 0.813. Bootstrap values greater than 50% in 100 bootstrap replicates are shown above lines. *Rumex hastatus* and *R. acetosa* were considered as outgroups.

DISCUSSION

Hooker (1886) recognized 10 sections of *P. sensu lato* in Flora of British India *viz*. *Koenigia*, *Eleutherosperma*, *Avicularia*, *Tovara*, *Bistorta*, *Persicaria*, *Cephalophilon*, *Echinocaulon*, *Aconogonon*, and *Tiniaria*, whereas, in our study all trees resulting from ITS sequence analysis resolved into eight distinct clades, representing *Polygonum*, *Persicaria*, *Bistorta*, *Tovara*, *Aconogonon*, *Koenigia*, *Knorringia*, and *Fallopia*.

The bootstrap tree indicated two major taxonomic categories in the *Persicaria*. In one group, the racemes are spike-like cylindrical to filiform and pollen grains are tricolporate to pantoporate; in the other group, the inflorescence is ovoid or subglobose, capitate and pollen grains are three to four colpate. The true *Polygonum* (treated as sect. *Avicularia* by Hooker, 1886) appeared as a distinct clade. Of 44 taxa analyzed, 22 species were grouped under the *Persicaria* clade, three species under the *Koenigia* clade, two under *Aconogonon*, five under *Bistorta*, three under *Fallopia*, eight under *Polygonum* and one under the *Knorringia* clade. The Himalayan species *Persicaria microcephala* grouped with *P. chinensis*, *P. capitata*, *P. runcinata*, and *P. nepalense* with 100% BS. *P. pubescens* also nested within the *Persicaria* clade with an affinity to *P. hydropiper*, *P. kawagoeana*, *P. posumbu*, *P. minor*, *P. lapathifolia*, *P. orientalis*, and *P. assamica*. This study marks the first time ITS sequencing of *K. delicatula* and *K. nepalensis*. They formed a distinct clade along with *Aconogonon* showing close affinity (84% BS) of the genera. Two infraspecific taxa of *B. amplexicaulis viz.* ssp *sinensis* and var. *speciosa*, which are often confused with one another, were also collected from Himachal Pradesh, India, and analyzed. Their ITS sequences exhibited a difference of only 1 bp. Hence, we recommend further studies on these taxa using chloroplast markers to obtain a clear picture of the molecular differentiation. Moreover, we also collected *P. effusum* and *P. recumbens*, two narrowly endemic taxa of the Himalayan region and sequenced them for study. It is hoped that this study confirms the taxonomic identity of this taxon and proves its phylogenetic distinctness. It was earlier considered a variety of *P. plebeium* (Hooker, 1886). Later, Steward (1930) reduced it to the synonymy of *P. plebeium*, but based on morphological studies, Qaiser (2005) argued that it should be a separate species. Our data also showed the distinctness of this species as evidenced from the bootstrap value, which is considerably low (68%) in comparison with the *P. plebeium* clade. The plant also shows affinity with *P. rottboellioides*, *P. cognatum*, and *P. paronychioides*. *Knorringia sibirica* formed a separate clade from all other Himalayan species.

Kim and Donoghue (2008) conducted an elaborate cladistic analysis of *Persicaria* and allied genera that supported the polyphyly of *P. sensu lato*. Sanchez and Kron (2008) also confirmed that *Polygonum* is a polyphyletic group according to the systematic tradition and previous molecular analysis. We included representatives from all sections in the analysis, excluding *Fagopyrum* as per Hooker's circumscription and subsequent morphological and molecular studies that placed them under a separate genus. The present study illustrates the relationship between Himalayan *Polygonum* that formed 8 blocks, not completely corresponding to the older system of classification that consisted of 10 taxonomic sections (Hooker, 1886). They represent *Koenigia*, *Persicaria*, *Tovara*, *Bistorta*, *Fallopia*, *Aconogonon*, *Polygonum*, and *Knorringia*.

Based on pollen morphological studies, Hedberg (1997) suggested *Koenigia* be given a separate taxonomic status because of the occurrence of the characteristic spinulose pollen type. In India, *Polygonum* sect. *Koenigia* is represented by four species. We included *Koenigia islandica*, *K. delicatula* and *K. nepalensis* in the present study as per availability of the materials. In congruence with the pollen morphological data, they are strongly supported to be monophyletic, and apparently, closely related. A close similarity of the *Koenigia* clade with *Aconogonon* (63%) supports the views forwarded by Hedberg (1997) stating that *Koenigia* is derived from the montane ancestors of a fast-dwindling genus displaying adaptive radiation to fit diverse alpine niches.

Hooker (1886) included eight species in *Polygonum* sect. *Avicularia*, which is distributed throughout the Himalayas. We included all species in the study except *P. salicornioi-*

des, which is reported from present-day Iran (erstwhile British India). Further, Hooker (1886) treated the taxon *P. plebeium* var. *effusum* merely on a variety level, which later was argued to be a separate species (Qaiser, 2005). We also collected fresh samples of the plant and studied its morphological variations from the typical variety. A number of variations were observed from *P. plebeium viz*. pedicellate flowers exerted from ochreae, equal and obtuse tepals, number of stamens, shape of leaf, etc. (Figure 4). As discussed above, we found considerable variation between the ITS regions of these two taxa and hence support their treatment as distinct species. *P. molliiforme*, *P. plebeium*, *P. effusum*, *P. recumbens*, *P. cognatum*, *P. rottboellioides*, and *P. paronychioides* nested within one clade, whereas the last two taxa appeared closer to each other.

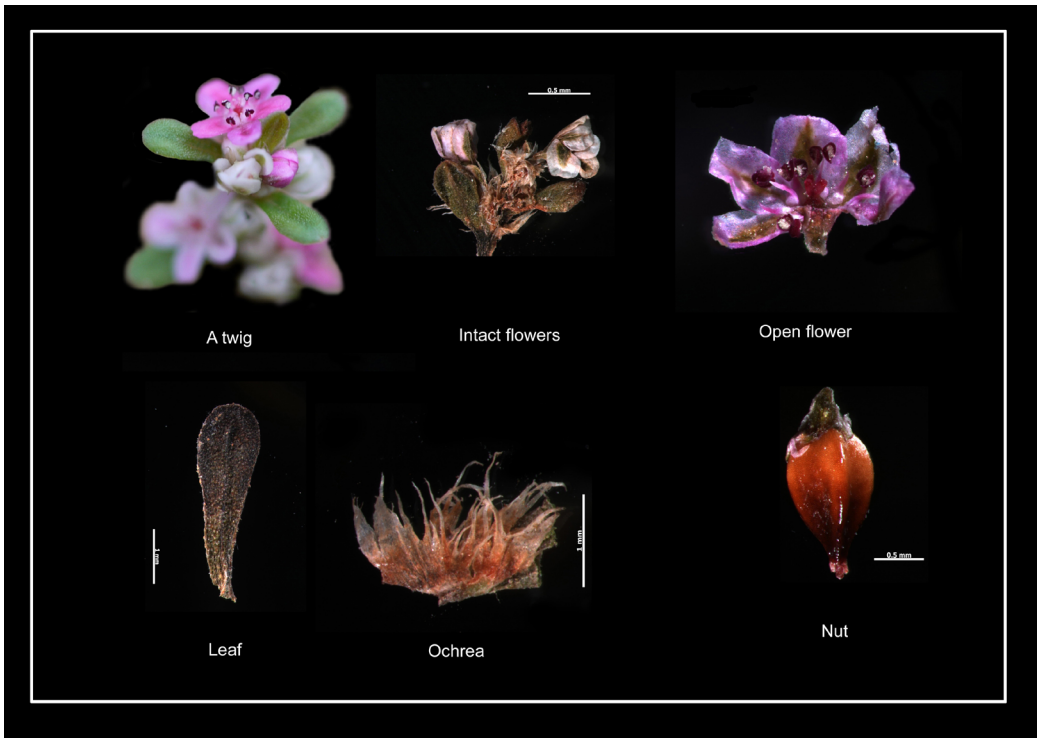

Figure 4. Habit and dissected parts of *Polygonum effusum* Meisn.

Polygonum sect. *Amblygonon* was created by Meisner (1826) and was mainly distinguished from the *Persicaria* section based on the incumbent, as opposed to accumbent, cotyledons. Several authors have rejected *Amblygonon*, as these distinctions have been shown to be variable (Steward, 1930). Similarly, based on the study of floral characters, Ronse De Craene and Akeroyd (1988) also did not find any difference between this section and *Persicaria*, except that the stamen number is constantly seven. In our study, all the species belonging to the *Amblygonon* section falls within the core of *Persicaria* s. str. in the cladogram, thus confirming the systematic invalidity of this section. This conclusion is also in line with the views proposed by Ronse De Craene and Akeroyd (1988). We also carried out studies on population structure

and genetic variation of *P. barbata*. The ITS sequences of nrDNA regions from 16 individuals were sampled from five geographical locations in India that exhibited polymorphism among the populations and evolution under reproductive isolation, probably due to long distance distribution and population fragmentation. The UPGMA tree revealed two major groups. In the first group, accessions collected from Bihar, Himachal Pradesh, Arunachal Pradesh, and Karnataka were grouped together, while accessions collected from Andaman Island were grouped in the second group, indicating that *P. barbata* evolved under reproductive isolation, probably due to long distance distribution and population fragmentation (Choudhary et al., 2011a).

Polygonum sect. *Tovara* is represented in India by a single species i.e., *Persicaria virginiana*. In our study, this taxon appeared as a distinct clade with an affinity towards the polyploid *Persicaria amphibia*. However, the long, hooked persistent style free to the base, serving to attach the fruit to foreign bodies, is unique in this genus. Our result supports its treatment under a separate genus.

Polygonum sect. *Bistorta* is represented by eight species in India (Hooker, 1886) and formed a distinct clade in our study. We included five taxa from this group, which were grouped under one section. Fresh samples of *Bistorta amplexicaulis* var. *speciosa* and *B. amplexicaulis* ssp *sinensis* were also collected from the Himachal Pradesh region and analyzed. We found a difference of only one base between the ITS sequences of these two taxa. We advocate further studies on mitochondrial and chloroplast genes to gain a clear picture on the molecular differentiation between these two taxa.

Polygonum sect. *Persicaria* was considered the most taxonomically debatable section. Our study concluded that it is paraphyletic, as evidenced from the parsimonious tree obtained from the sequence data. Hooker (1886) reported 15 species under this section. However, many species belonging to the *Cephalophilon* and *Echinocaulon* sections were grouped under the *Persicaria* section in the present study. Nevertheless, we advocate two major taxonomic categories in the *Persicaria* genus. In one group, the racemes are spike-like cylindrical to filiform, and pollen grains are tricolpate to pantoporate, whereas in the other group, the inflorescence is ovoid or subglobose, capitate and pollen grains are three to four colpate. The second group was placed under the *Cephalophilon* section (Hooker, 1886); however, we insist on following *Persicaria* in a broad sense to reduce the taxonomic puzzlement created with the genus.

Polygonum sect. *Tiniaria* was created by Meisner, which was later on circumscribed under the genus *Fallopia* by Adanson (1783). In our phylogenetic tree, *Fallopia* appears as a distinct clade represented by three species and is closest to the *Polygonum* s. str. clade.

Knorringia (Czukav) Tzvelev, a monotypic genus distributed in Central Asia, the former USSR, China, Afghanistan, Pakistan, and Nepal (Qaiser, 2005) has also been reported from Sikkim, India. Earlier this was treated under *Polygonum* sect. *Knorringia* (Czukav, Novosti Sist. Vyssh. Rast., 1966), but later conferred a separate generic status by Tzvelev in 1987. In our study, this also appeared as a distinct clade from the rest of the *Polygonum*.

Conclusively, the results of this investigation clarify the phylogeny of Indian Polygonums and indicate that several changes in the infra-generic systematics of *Polygonum* are warranted. The ITS sequence data presented here appear to be very useful for recognizing phylogenetic relationships and the level of differentiation among the species of different *Polygonum* sections. The phylogenetic analysis of ITS sequence variation in different *Polygonum* sections appears to have been resolved among the Himalayan species, as well as being an additional criterion for estimating their levels of differentiation. The relationships among the species depicted in

the ITS tree and their nucleotide divergence values support the results from earlier morphology studies except in some cases (Hooker, 1886; Haraldson, 1978). Our study supports the separate treatment of *Persicaria*, *Koenigia*, *Tovara*, *Bistorta*, *Fallopia*, *Aconogonon*, and *Knorringia* from *Polygonum*. We also advocate two major taxonomic categories in the *Persicaria* genus in support of the views forwarded by earlier researchers (Qaiser, 2005; Kim and Donoghue, 2008).

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