

Phylogenetics of *Swertia* L. (Gentianaceae-Swertiinae) and Molecular Differentiation of *Swertia* Species in Nepalese Medicinal Herbs

Kunjani Joshi* and † and Jianhua Li†

*Tribhuvan University, Botany Department, Kathmandu, Nepal. † Arnold Arboretum and Harvard University Herbaria, 22 Divinity Avenue, Cambridge, MA 02138



Swertia multicaulis
1 fimbriate gland



Swertia angustifolia
1 fimbriate gland



Swertia nervosa
1 fimbriate gland



Swertia chirayita
2 fimbriate gland



Swertia lurida
2 non-fimbriate gland



Swertia racemosa
1 fimbriate gland



Swertia dilatata
1 non-fimbriate gland



Swertia paniculata
1 non-fimbriate gland



Swertia pedicellata
1 non-fimbriate gland



Swertia macrosperma
2 fimbriate gland

Background

- Swertia* L. (Gentianaceae) is a morphologically diverse genus especially in floral merosity and petal gland shape and form (see photos).
- Diversity: 150 species worldwide. 30 species in Nepal with one endemic *S. acutis* (see Fig. 1).
- Distribution: Cosmopolitan with its center of species diversity in the Sino-Himalayan region. *Swertia* species predominantly occur in the mountainous regions of the 54 districts in Nepal.

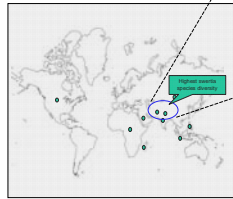
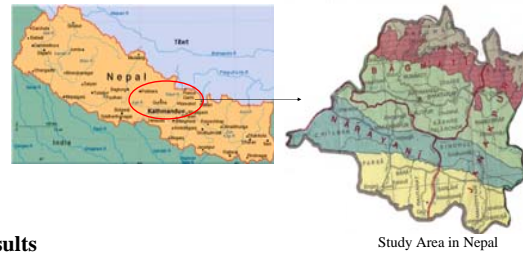


Fig. 1. Geographical distribution of *Swertia*

Materials and Methods

- 92 samples representing the major sections of *Swertia* and closely related genera were included in the study
- Nepalese species were collected from both wild habitats and local herbal markets
- Standard molecular techniques (PCR and DNA Sequencing) were used to obtain data from both nuclear ribosomal internal transcribed spacer (ITS) and chloroplast (*trnL-F*) regions
- Additional ITS and *trnL-F* sequence data was obtained from Genbank
- Distance, parsimony, and Bayesian analyses were used to reconstruct phylogenetic trees in PAUP* or MrBayes



Study Area in Nepal

Results

Molecular Differentiation

- nDNA ITS sequences are useful in differentiating Nepalese species commonly used in herbal medicine (Fig. 2).

- Many *Swertia* species in Nepal are used for medicinal purposes. Among them *S. chirayita* holds the greatest medicinal value.
- The evolutionary relationships among the species within the genus has been debated (Chassot, 2000; Gilg, 1895; Ho & Liu, 1990; Ho *et al.*, 1994; Shah, 1990, 1992; Struwe *et al.*, 2002; von Hagen and Kaderet, 2001; Yuan and Kupfer 1995).
- The major questions that are yet to be resolved include: species delimitation, section delimitation, *Swertia*'s relation with allied genera, and the domestication of economically important *Swertia* species

Objectives

- To study the morphological variation and distribution pattern of the species within *Swertia*
- To test the monophyly of *Swertia* and its sections
- To use DNA barcoding to identify the *Swertia* species used in Nepalese herbal medicine

References

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Phylogeny

- Results from nDNA ITS (Fig. 3a) are generally congruent with those of the chloroplast *trnL-F* sequences (Fig. 3b)
- Obolaria* and *Gentiana* are the closest genera to *Swertia*, which is paraphyletic including *Halenia*, *Comastoma*, *Gentianella*, and *Lomatogonium*. There are a few well-supported clades (Fig. 3b): Clade 1: *S. multicaulis*, *S. chirayita*, and *S. lurida*; Clade 2: *S. ciliata*, *S. dilatata*, *S. paniculata*, *S. pedicellata*, and *S. racemosa*; Clade 3: *Swertia cuneata* and *S. engelii*; Clade 4: *Gentianopsis* and *Pterygocalyx*; Clade 5: *Halenia* and *Swertia tetrapetra*; Clade 6: *S. kilimandscharica*, *S. angustifolia* and *S. nervosa*; Clade 7: *Swertia petiolata*, *S. perennis*, and *S. Calycina*
- Clades 1, 2, and 3 form a clade with *Comastoma*, *Gentianella*, *Lomatogonium*, *Swertia hispidicalyx*, and *S. macrosperma*. However, relationships among them are not resolved
- There is one well-supported discrepancy between the two genomes. In the ITS tree, *S. ciliata* is positioned in the clade containing *S. chirayita* and *S. lurida*, while in the *trnL-F* tree, it is in the clade of *S. dilatata*, *S. paniculata*, *S. pedicellata*, and *S. racemosa*. This suggests that *S. ciliata* may have evolved from a hybridization event with *S. dilatata*, *S. paniculata*, *S. pedicellata*, or *S. racemosa* serving as the possible maternal donor
- Both ITS and *trnL-F* data support the close relationship of *S. lurida* with *S. chirayita*
- We find support for the paraphyly of *Swertia* relative to other genera of Swertiinae, highlighting the need for a re-evaluation of the sections. *Ophelia* is highly polyphyletic, while section *Kingdon-Wardia* may be derived from within the polyphyletic section *Platyna* (Fig. 4)
- Both the floral merosity and petal glands are homoplasious in *Swertia*

Fig. 4. Majority Bayesian Consensus (ITS data)

Key: Flower 4 or 5 merous / Number nectaries or corollas / Types of nectaries (F = fimbriate, N = non-fimbriate / Infrageneric sections

Section abbreviations: Oph - Ophelia; Het - Heteranthus; Mon - Montana; Rug - Rugosa; Kin - Kingdon-wardia; Pla - Platyna; Swe - Swertia; Mac - Macranthus

Swertia species in the Nepalese herbal market



Fig. 2. Sites in color showing species-specific nucleotides.

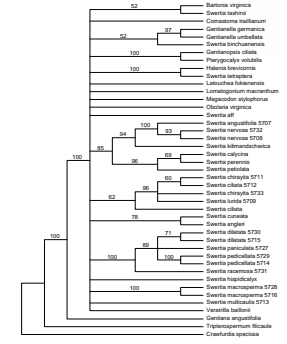


Fig. 3a. Strict consensus of 12 trees (Consistency index=0.57) from ITS data. Branches with less than 50% bootstrap support were collapsed.

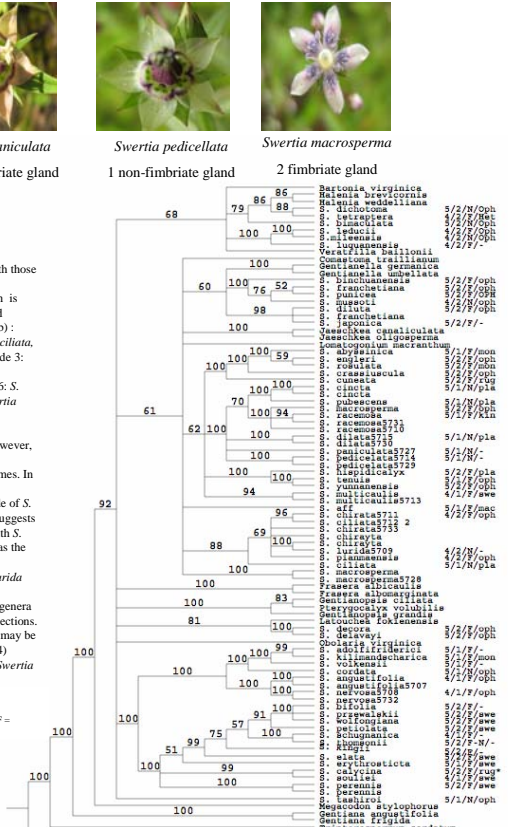


Fig. 3b. Strict consensus of 10,000 trees reconstructed from *trnL-F* sequence data. Numbers on branches represent bootstrap support of 100 replicates.

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