SHARED MOLECULAR SIGNATURES SUPPORT THE INCLUSION OF *CATAMIXIS* IN SUBFAMILY PERTYOIDEAE (ASTERACEAE).

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ABSTRACT

Two conserved indels and one consistent nucleotide substitution provide evidence to choose between competing hypotheses of relationship for a rare Himalayan endemic sunflower. Indel patterns in the intergenic spacer *ndh*I-*ndh*G of the chloroplast DNA as compared across all major lineages of Asteraceae show that *Catamixis* shares with the genera *Ainsliaea*, *Myripnois*, and *Pertya* a 145 base pair deletion. In addition, sequence data of the chloroplast gene *mat*K show that *Catamixis*, *Ainsliaea*, and *Pertya* share a mutation unique to the Pertyoideae lineage. These molecular signatures support the inclusion of *Catamixis* in subfamily Pertyoideae. *Phytologia 90(3): 418-424 (December, 2008).*

KEY WORDS: Rare genomic changes, conserved indels, indel pattern, molecular signature, *Catamixis*, Pertyoideae, Asteraceae, Himalayas

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Catamixis baccharoides is a rare shrub endemic to steep limestone canyons of the Himalayan region of north central India and westernmost Nepal and considered endangered due to habitat loss (Nayar and Ahmedullah, 1985). Because of its ligulate corollas, tailed anthers, and imbricate involucres with multiple series of phyllaries, *Catamixis* has traditionally been viewed as a member of tribe Mutisieae sensu lato (Hansen, 1991; Bremer, 1994), a grouping of some 82 genera (Hind, 2007) that has been shown in molecular phylogenetic analyses to consist of a paraphyletic grade of lineages (Panero and Funk, 2008). The name given to this monotypic genus by Thomson (1867) meaning "mixed affinity" refers to its combination of characteristics of several genera belonging to this large grade. Consequently, the position of

Catamixis among these lineages has not been easy to ascertain based on morphological comparisons. The genus has been variously allied to *Leucomeris* (Thomson, 1867), viewed as an isolated member of Mutisieae s.l. (Bremer, 1994; Hind, 2007), or as a member of tribe Pertyeae (Jeffrey, 2007). Perhaps owing to the rarity of tissue for analysis, no molecular phylogenetic study has yet included *Catamixis*.

To clarify the position of *Catamixis* in the tree of life of Asteraceae and distinguish between these alternative hypotheses DNA was extracted from a single leaf included in the fragment package of specimen Parker s. n. collected in northern India (HUH barcode 00263953). Although it would be desirable to amplify multiple genes and include *Catamixis* in a supermatrix analysis of phylogeny, we were unable to obtain enough high quality DNA to amplify many genes. Phylogenetic analysis of only two chloroplast DNA markers was insufficient to resolve the placement of *Catamixis* among the lineages of Asteraceae. Alternatively, we screened for rare genomic changes in short standardized DNA regions that might allow us to either eliminate or identify known clades of Asteraceae to which *Catamixis* could belong. *Catamixis* was compared with homologous sequences sampled from 108 species representing all subfamilies and tribes of Asteraceae and corresponding to the ingroup taxon sampling of Panero and Funk (2008).

MATERIALS AND METHODS

DNA was extracted from 0.25g dried leaf material of *Catamixis* using Qiagen's DNeasy Plant Mini Kit following the manufacturer's protocol for dried leaf material. Efficacy of the DNA extract was tested empirically using standard polymerase chain reaction (PCR) protocols and primers detailed in Panero and Crozier (2003) and Panero and Funk (2008) for the following chloroplast loci: *ndh*D, *ndh*F, *rbc*L, *rpo*B, *rpo*C1, *trn*T-*trn*L IGS, *ndh*I gene, *ndh*I-*ndh*G IGS, and *mat*K. PCR reactions were screened using agarose gel electrophoresis. Reactions with visible results were cleaned using QIAquick PCR purification columns and 4 microliters of each used as template DNA in cycle sequencing reactions following the protocols of the ABI Big Dye Terminator 3.1 Cycle Sequencing Kit. Cleaning of the sequencing reactions using the Millipore MultiScreen 96-Well Filtration Plate and

sequencing was performed by the University of Texas ICMB Core DNA Facility on an ABI 3730 DNA analyzer. Raw sequence data was proofread using Sequencher 4.8 (Gene Codes Corporation). *Catamixis* sequences were aligned by eye with the 108-taxon *mat*K and *ndh*I-*ndh*G intergenic spacer alignments used in previous phylogenetic studies (Panero and Funk, 2008).

Phylogenetic trees were constructed for the 108-taxon *ndh*I and *mat*K data sets individually and in combination using the maximum parsimony criterion and TBR branch swapping was implemented in PAUP*4b10 and limiting the heuristic search to 10,000 most parsimonious trees saved. The strict consensus of each set of trees was then constructed and checked for the resolution of *Catamixis* relationships.

Indel characters observed during alignment of *Catamixis* with homologous *ndh*I-*ndh*G sequences of other Asteraceae were checked for character consistency (sensu Farris, 1969) when character states were optimized on the tree topology of Panero and Funk (2008) including *Catamixis* in the Pertyoideae. Observing this, the *mat*K alignment was then inspected visually for nucleotide characters that might also be 100% consistent on a tree including *Catamixis* in the Pertyoideae. To confirm the visual observation, a Neighbor Joining tree based on *mat*K data using the Maximum Parsimony criterion was constructed and described including a list of apomorphies for each branch using PAUP*. The resulting list of characters supporting Pertyoideae, including *Catamixis,* was checked for phylogenetically consistent characters. Apomorphies with 100% consistency due to gaps (scored as missing data in our analysis), or those not completely consistent, were ignored.

RESULTS AND DISCUSSION

Parsimony analysis was unable to resolve the placement of *Catamixis* to any subfamily of Asteraceae. However, rare genomic changes mapped to a statistically well-supported best estimate of Asteraceae phylogeny previously published in Panero and Funk (2008) and compared with new data for *Catamixis* revealed two phylogenetically consistent indel characters in the *ndh*I-*ndh*G intergenic

spacer region. Presence of a rare mutation shared consistently by members of a single clade and *Catamixis* is interpreted as evidence for the inclusion of *Catamixis* in that clade (shared ancestry). Shared insertions/deletions (indels) have provided diagnostic signatures for such identifications in the Asteraceae (Panero and Funk, 2008) and in widely divergent organisms from microbes (Gupta and Johari, 1998) to placental mammals (De Jong et al., 2003). The Asteraceae topology of Panero and Funk (2008) conveniently distinguishes between the lineages to which *Catamixis* has been historically allied, including *Leucomeris* (Wunderlichioideae: Hyalideae) and Pertyeae (Pertyoideae) as well as six other lineages of Mutisieae s. l.

Using the *ndh*I-*ndh*G intergenic spacer as a reference molecular marker, we were able to exclude *Catamixis* from the Gymnarrhenoideae-Asteroideae clade of Asteraceae (Fig. 1) and provide evidence in support of Jeffrey's (2007) assignment of *Catamixis* to the Pertyoideae (GenBank, Accession # FJ154843). We found that *Catamixis* lacks the 17 bp deletion located 4-20 nucleotide sites downstream of the *ndh*I stop codon that is shared by all members of the clade Gymnarrhenoideae-Asteroideae sampled in the chloroplast phylogenetic study of Panero and Funk (2008; Fig. 1). This result confirms historical hypothesis of relationships based on morphology that allied the genus to members of Mutisieae s. l. grade. Further, *Catamixis* shares with *Pertya*, two species of *Ainsliaea*, and *Myripnois* a 145 bp deletion not found in any lineage except Pertyoideae.

In addition to the indel patterns, a phylogenetically consistent single nucleotide substitution was observed in the *mat*K gene that is unique to members of Pertyoideae. This synapomorphy is found in aligned position 274 and characterized by a change from A to C in the sequences of *Ainsliaea*, *Pertya* and *Catamixis*. Alignment of the *mat*K sequence of *Catamixis* (GenBank, Accession # FJ179462). in this data matrix shows that the genus shares with *Ainsliaea* and *Pertya* this diagnostic substitution.

The indel patterns and nucleotide substitution described above appear to be diagnostic of members of Pertyoideae. These allow us to predict that molecular phylogenetic studies that include broad taxonomic sampling with all members of the subfamily represented and

concomitant character sampling would also include *Catamixis* in Pertyoideae.

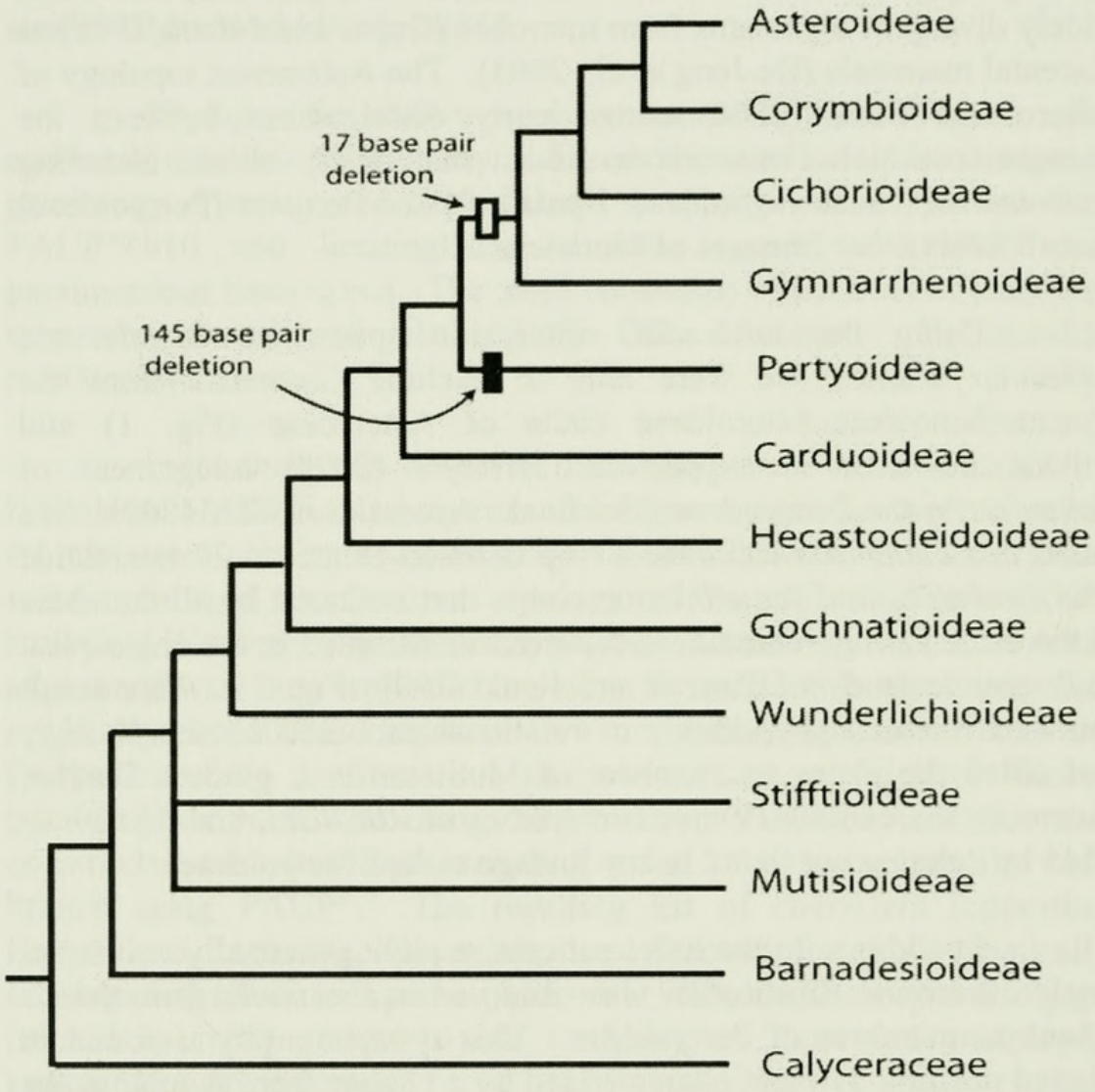

Fig. 1. *Ndh*I-*ndh*G IGS synapomorphies mapped to the Asteraceae tree. Indel shared with *Catamixis* (solid bar) and lacking in *Catamixis* (open bar) shown on topology of Panero and Funk (2008).

With the addition of *Catamixis*, tribe Pertyeae of subfamily Pertyoideae contains six genera of perennial herbs and shrubs of eastern and central Asia including *Ainsliaea*, *Diaspananthus*, *Macroclinidium*, *Myripnois* and *Pertya*. The molecular signatures observed in the *ndh*I-

*ndh*G IGS and the *mat*K gene unfortunately do not shed any light on the affinities of *Catamixis* within Pertyoideae.

ACKNOWLEDGEMENTS

I thank James Macklin of Harvard University Herbaria for permission to remove tissue samples for DNA analysis and T. Wendt, TEX-LL herbarium curator for locating specimens of *Catamixis*. I thank Bonnie S. Crozier, Guy Nesom, B. L. Turner, and A. Schwarzbach for reviewing the manuscript. Sequencing of the *mat*K and *ndh*I genes was funded by grant NSF DEB 0344116.

LITERATURE CITED

Bremer, K. 1994. Asteraceae: Cladistics and Classification. Timber Press, Portland, Oregon.

De Jong, W. W., M. A. M. van Dijk, C. Pux, G. Kappe, T. Van Rheede, and O. Madsen. 2003. Indels in protein-coding sequences of Euarchontoglires constrain the rooting of the eutherian tree. Mol. Phylogenet. Evol. 28: 328-340.

Farris, J.S. 1969. A successive approximations approach to character weighting. Systematic Zoology 18: 374-385.

Gupta, R. S. and V. Johari. 1998. Signature sequences in diverse proteins provide evidence of a close evolutionary relationship between the *Deinococcus-Thermus* group and Cyanobacteria. J. Mol. Evol. 46: 716-720.

Hansen, H. V. 1991. Phylogenetic studies in Compositae tribe Mutisieae. Opera Bot. 109: 1-50.

Hind, D. J .N. 2007. Compositae: Tribe Mutisieae. *in* Families and Genera of Vascular Plants, vol. VIII, Flowering Plants, Eudicots, Asterales. J. W. Kadereit and C. Jeffrey, eds., Springer-Verlag, Berlin, pp. 90-112.

Jeffrey, C. 2007. Compositae: Introduction with key to tribes. *in* Families and Genera of Vascular Plants, vol. VIII, Flowering Plants, Eudicots, Asterales. J. W. Kadereit. and C. Jeffrey, eds., Springer-Verlag, Berlin, pp. 61-87.

Nayar, M. P. and M. Ahmedullah. 1985. *Catamixis baccharoides* Thoms. an endemic chasmophyte of the W. Himalaya under threat. Bull. Bot. Sur. India 27: 248-250.

Panero, J. L. and B. S. Crozier. 2003. Primers for PCR amplification of Asteraceae chloroplast DNA. Lundellia 6: 1-9.

Panero, J. L. and V. A. Funk. 2008. The value of sampling anomalous taxa in phylogenetic studies: major clades of the Asteraceae revealed. Mol. Phylogenet. Evol. 47: 757-782.

Thomson, T. 1867. On two new genera of Compositae Mutisiaceae, from India. J. Linn. Soc. Bot. 9: 342-344.