Phylogenetic and developmental study of *Iris* subgenus *Limniris* section *Lophiris* and related species

2008-2010 Grant Proposal Presented to the American Iris Society

May 28, 2007

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Background

The genus *Iris* L., the largest genus of Iridaceae, is a group of perennial herbs with about 300 species and many infraspecific taxa. This genus occurs in varied habitats and is widely distributed throughout the northern temperate zone from Europe, the Middle East and northern Africa to Asia and across North America. Modern classification of this genus started with Dykes (1913) whose groupings of sections were essentially retained as subgenera by later authors. According to growing habit of creeping rhizomes (subgenera *Iris* and *Limniris*), swollen tubers (subgenus *Nepalensis*) and bulbs (subgenera *Xiphium*, *Scorpiris* and *Hermodactyloides*), they are classified into six subgenera, of which five are restricted to the Old World. Subgenus *Limniris* which has a holarctic distribution is further divided into sections and series (Mathew, 1981). The most recent classification comes from the Species Group of British Iris Society (1997) which mostly followed the classification of Mathew.

Section *Lophiris* Tausch is placed within *Iris* subgenus *Limniris*. Currently, 11 species are recognized: *I. confusa* Sealy, *I. cristata* Solander, *I. formosana* Ohwi, *I. gracilipes* Gray, *I. japonica* Thunberg, *I. lacustris* Nuttall, *I. latistyla* Y.T. Zhao, *I. milesii* Foster, *I. tectorum* Maximowicz, *I. tenuis* Watson and *I. wattii* Baker. Of these 11 species, seven are native to eastern Asia while three of them, *I. cristata*, *I. lacustris* and *I. tenuis*, are native to North America. The characteristics of members of section *Lophiris* are quite diverse: the growing habit of the eight eastern Asia species are evergreen while the North America species are cold tolerant with winter dormancy; plant size ranges from dwarf (*I. lacustris*) to tall (*I. wattii*); plants of *I. milesii*, *I. wattii* and *I. confusa* have leafy stems while other species have mostly based leaves; and their habitats include Himalaya regions (*I. milesii*), high mountains (*I. wattii* and *I. latisyla*), medium altitudes (*I. formosana*) and moist valleys (*I. confusa*). There is one common distinguishing feature within this section: cockscomb-like crests on the sepal midvein that may serve as pollinator guides. However, even this common character is quite diverse within section *Lophiris*: usually, the sepal crest is a single linear structure either with an entire margin (simple crest) or dissected margin (dissected crest); in *I. cristata* and *I. lacustris* there are three crests; and in *I. tenuis* the crests are two slightly elevated ridges. Based on morphology, habit and range, the relationships of these 11 species are not apparent which indicates that section *Lophiris* may not be a natural group. Based on morphological studies, several researchers have considered this section to be artificial (Lawrence, 1953; Wu & Culter, 1985).

Molecular phylogenetic research carried out by Wilson (2004) based on chloroplast *matK* gene and *trnK* intron sequence data showed that subgenus *Limniris* section *Lophiris* was not a monophyletic group because the North American species (*I. tenuis* and *I. cristata*) and the eastern Asian species (*I. wattii*) were nested in two different clades. Her study included three species within section *Lophiris* and she pointed out that additional sampling both within section *Lophiris* and other groups in the genus was required to

resolve the placement of section *Lophiris* species. Wilson's (2004) study also showed that *I. wattii* (section *Lophiris*) was basal in a clade that also included *I. collettii* Hook. f. (subgenus *Nepalensis*), and species in subgenus *Scorpiris* and subgenus *Iris* section *Hexapogon*. My preliminary studies based on chloroplast *trnL-trnF* sequence data showed that *I. decora* (subgenus *Nepalensis*) and *I. latisyla* (subgenus *Limniris* section *Lophiris*) were sister species indicating that subgenus *Nepalensis* species may be included within section *Lophiris*.

Two rarely studied species within subgenus *Limniris* section *Limniris* series *Chinenses* may also be closely aligned with section *Lophiris* species. Although *I. proantha* Diels and *I. speculatrix* Hance are currently placed within series *Chinenses*, this placement is considered controversial (The Species Group of the British Iris Society, 1997). In the Flora of China, Zhao (1985) identified these two species as possessing crests and classified them with species from subgenus *Limniris* section *Lophiris*.

The presence of sepal crests, the defining feature, of section *Lophiris*, is not exclusive to this section. Three taxa in subgenus *Nepalensis* (*I. collettii*, *I. collettii* var *acaulis*, and *I. decora*) and 42 taxa in subgenus *Scorpiris* are also described as possessing a crest (The Species Group of the British Iris Society, 1997). By mapping this character on the phylogenetic tree based on *matK* sequence data, Wilson (2006) showed that the presence of a crest was homoplastic and had evolved multiple times. Several developmental and anatomical studies have been reported on petal elaboration (Vaes, 2006) and petal appendages (Brown, 1992; Ronse, 2002) in other plant families. Petal appendages have also been reported in Bromeliaceae (Sajo, 2004). Sepal appendages are uncommon and developmental studies of crests have not been reported for this genus. The identification of developmental patterns for crests may be helpful in identifying lineages and phylogenetic events.

The objective of this molecular phylogenetic and developmental study is: 1) to test if the eastern Asian species from section *Lophiris* form a monophyletic group, 2) to investigate the relationships among species of section *Lophiris*, and 3) to identify the tissue responsible for the sepal crests in section *Lophiris* and compare development of crests in species of section *Lophiris* with crested species from subgenera *Nepalensis* and *Scorpiris*, and subgenus *Limniris* section *Limniris* series *Chinenses*.

Material and Methods

1. Molecular studies

1.1 Taxa selected

I will include 31 taxa in my molecular phylogeny. The following 20 ingroup taxa will be sampled for the molecular study:

1. *Iris* subgenus *Limniris* section *Lophiris* (9): *I. confusa*, *I. japonica*, *I. latistyla*, *I. milesii*, *I. tectorum*, *I. tectorum* f. *alba*, *I. tenuis*, *I. wattii* and *I. cristata*. I have material for each of these species.
2. *Iris* subgenus *Limniris* section *Limniris* series *Chinenses* (3): *I. proantha*, *I. speculatrix* and one non-crested species. I have collected these species during my present (May 2008) trip to China.
3. *Iris* subgenus *Nepalensis* (3): *I. collettii*, *I. collettii* var *acaulis* and *I. decora*. I will collect these taxa during my May 2009 trip to China.
4. *Iris* subgenus *Scorpiris*: 5 species from Dr. Wilson's collection.

Another 11 *Iris* species will be included from Dr Carol Wilson's collections as outgroups.

1.2 Gene regions

My preliminary studies employed the chloroplast *trn*L-*trn*F region for seven taxa which resulted in a partially resolved phylogenetic tree. I will gather *trn*L-*trn*F sequence data for the remaining taxa and also sequence the chloroplast *matK* region for each ingroup taxon except the five subgenus *Scorpiris* species included in my study (Dr. Wilson has *matK* data for the outgroup and subgenus *Scorpiris* species.). I anticipate that additional regions will be required to fully resolve relationships among the ingroup taxa. I will trial eight chloroplast marker regions that are typically more variable than the *trn*L-*trn*F and *matK* regions using five representative ingroup species in order to maximize the informational quality of markers chosen. I will then choose two of these regions that are promising and gather sequence data for each taxon included in my study.

I will extract total DNA using a modified CTAB method, the gene regions of interest will be amplified by PCR, and the resulting products will be purified and sequenced using an ABI 3130 XL automated sequencer. All the equipment required for the above experiments are available at the molecular laboratory of the Rancho Santa Ana Botanic Garden.

1.3 Analysis

DNA sequence data will be analyzed by using a variety of quantitative phylogenetic method including maximum parsimony, maximum likelihood and Bayesian posterior probability algorithms.

2. Development of crests

2.1 Taxa selected

The following eight taxa will be sampled for my developmental study:
1. *Iris* subgenus *Limniris* section *Lophiris*: *I. japonica*, *I. latistyla*, *I. tectorum*, and *I. tenuis*.

2. *Iris* subgenus *Limniris* section *Limniris* series *Chinenses*: *I. speculatrix*.
3. *Iris* subgenus *Scorpiris*: one species chosen from Dr. Wilson's living collection at the Rancho Santa Ana Botanic Garden.
4. Outgroups: *Belamcanda chinensis* and *I. lactea* var. *chinensis*.

2.2 Anatomy-morphology

Mature flower buds for light microscopic study will be fixed in FPA, dehydrated in an ethanol series, embedded in paraffin wax, cut by rotary microtome, mounted on slides, stained and observed. Crest development will also be studied using a Scanning Electron Microscopy (SEM). Developing flower buds will be collected in FPA, dehydrated in an ethanol series, critical-point dried, sputter-coated with gold, observed and photographed using International Scientific Instruments WB06 Scanning Electron Microscope. All the developmental studies will be carried out in the anatomy/morphology laboratory of Rancho Santo Ana Botanic Garden which has all the equipment required for this portion of my study.

Proposed Products of this Research

1. A resolved phylogentic tree including taxa from *Iris* subgenus *Limniris* section *Lophiris*, targeted species from subgenus *Limniris* section *Limniris* series *Chinenses*, subgenera *Nepalensis* and *Scorpiris*, and several outgroup species.
2. A preliminary survey of crest developmental patterns for representative species from subgenus *Iris* section *Lophiris*, subgenus *Scorpiris,* subgenus *Iris* section *Limniris* series *Chinensis* and two outgroup taxa.

Literature Cited:

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Lawrence, G. H. M. 1953. A reclassification of the genus *Iris*. Gentes Herbarum 8: 346-371.

Mathew, B. 1981. The *Iris*. Universe Books, New York, N.Y.

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The Species Group of the British Iris Society. 1997. A Guide to Species Irises: their identification and cultivation. Cambridge University Press, Cambridge, UK.

Vaes E., A. Vrijdaghs, E. F. Smets and S. Dessein. 2006. Elaborate petals in Australian *Spermacoce* (Rubiaceae) species: morphology, ontogeny and function. Annals of Botany 98: 1167-1178.

Wilson, C. A. 2004. Phylogeny of *Iris* based on chloroplast *mat*K gene and *trn*K intron sequence data. Molecular Phylogenetics and Evolution 33: 402-412.

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Wu, Q. J. and D. F. Cutler. 1985. Taxonomic, evolutionary and ecological implications of the leaf anatomy of rhizomatous *Iris* species. Botanical Journal of the Linnean Society 90: 253-303.

Zhao, Y. T. 1985. Flora Reipublicae Popularis Sinicae, vol. 16, Part 1, pp. 48-60

Timeline for Study Period

Fall/2008:

- DNA extraction of *I. tenuis, I. proantha*, *I. speculatrix* and *I. cristata.*
- Sequencing *matK* region for *I. confusa*, *I. japonica*, *I. latistyla*, *I. milesii*, *I. tectorum*, *I. tectorum* f. *alba*, *I. tenuis*, *I. wattii*, *I. proantha*, *I. speculatrix* and *I. cristata.*
- Sequencing *trn*L-*trn*F region for *I. tenuis*, *I. proantha*, *I. speculatrix* and *I. cristata.*

Winter-Spring/2009:

- Crest developmental study of *I. japonica*, *I. latistyla*, *I. tectorum* and *I. tenuis.*
- Trials of eight relatively fast-evolving chloroplast regions.
- Begin sequencing of two additional chloroplast regions chosen from the trialed regions.

Spring-Early Summer/2009:

- Fieldwork in China to collect subgenus *Nepalensis* species (*I. collettii*, *I. collettii* var *acaulis* and *I. decora*).

Fall/2009:

- DNA extraction of *I. milesii*, *I. wattii, I. collettii*, *I. collettii* var *acaulis* and *I. decora.*
- Sequencing *matK* and *trn*L-*trn*F region for *I. milesii*, *I. wattii, I. collettii*, *I. collettii* var *acaulis* and *I. decora.*

Winer-Spring/2010:

- Crest developmental study of *I. speculatrix,* one species from *Iris* subgenus *Scorpiris*, *I. lactea* var. *chinensis* and *Belamcanda chinensis*.
- Complete sequencing for the two additional chloroplast regions for all taxa included in my phylogenetic study

Two-Year Budget

Year 1 (Fall 2008-Summer 2009)

Supplies	
Extraction: 4 samples	35
PCR & cleaning: ~ 70 amplifications	105
Cycle sequencing & cleaning: ~160 amplifications	480
Anatomy & SEM supplies	200
Partial travel expenses within China	500
Subtotal Year 1	1320

Year 2 (Fall 2009-Summer 2010)

Supplies	
Extraction: 5 samples	40
PCR & cleaning: ~ 100 amplifications	150
Cycle sequencing & cleaning: ~190 amplifications	570
Anatomy & SEM supplies	200
Subtotal Year 2	960
Total	$2280

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Education

- **09/2007- Present：** Graduate Student，Botany; Rancho Santa Ana Botanic Garden, Claremont Graduate University
- **09/2004-06/2007:** Master of Science, Cell Biology; Botanical Garden, Institute of Botany, Chinese Academy of Sciences (CAS)
- **09/2000-07/2004:** Bachelor of Agronomy, Horticulture; College of Agriculture and Biotechnology, China Agricultural University

Research Experience

- **01/2008-present:** Research Assistant, Rancho Santa Ana Botanic Garden. Sequencing of chloroplast and nuclear regions in the genus *Iris*.
- **09/2007-12/2008:** Research Assistant, Rancho Santa Ana Botanic Garden. Relationship between parasite and epiparasite species pairs in *Phoradendron*.
- **05/2006-11/2006:** Research Assistant, Botanical Garden, Institute of Botany, CAS. Effect of synthetic plant growth regulator on endogenous hormone levels in *Iris germanica*.
- **03/2006-05/2006:** Research Assistant, Botanical Garden, Institute of Botany, CAS. Rooting ability of *Iris germanica* offset as stem cuttings.
- **03/2005-02/2006:** Research Assistant, Botanical Garden, Institute of Botany, CAS. Stimulatory effect of 6-BA on axillary and rhizomic budbreak and offset formation of *Iris germanica*.

- **09/2004-01/2005:** Research Assistant, Botanical Garden, Institute of Botany, CAS. Descriptors and data standard for herbaceous medicinal plants (part of national project of databases of natural resources of medicinal plants).

- **04/2004-06/2004:** Research Assistant, Botanical Garden, Institute of Botany, CAS. Hybridization of *Iris germanica* varieties with domestic wild *Iris* species.

- **01/2003-01/2004:** Research Assistant, Beijing Institute of Forestry and Fruit Trees. Peach genetic resources and their heredity characters of fruit quality.

Field Trip

- **06/2004-07/2004:** collection of endemic *Iris* species in the northeastern China of Jilin and Liaoning Provinces.

- **05/2008:** collection of endemic *Iris* species in the southeastern China of Zhejiang Province.

Publications

- **Guo Jinyan**. 2004. Genetic diversity analysis of soluble solid content of peach fruit. China Agricultural University. (Undergraduate dissertation)

- **Guo Jinyan**, Sun Guofeng, Zhang Jinzheng. 2005. Advances in horticultural study of bulbous iris. Advances in Ornamental Horticulture of China, 2005. China forestry Press: Beijing. p74-80. (Review)

- **Guo Jinyan**, Zhang Jinzheng, Sun Guofeng, Shi Lei. 2006. Advances of horticultural study of rhizomatous irises. Acta Horticulturae Sinica. 33(5): 1149-1156. (Review)

- **Guo Jinyan**, Zhang Jinzheng, Sun Guofeng, Shi Lei. 2007. Effects of 6-BA application on promotion of offset formation in *Iris germanica*. Acta Horticulturae Sinica. 34(2): 461-464.

- **Guo Jinyan**. 2007. Effect of 6-BA on the efficient vegetative propagation of *Iris germanica* L. The Institute of Botany, Chinese Academy of Sciences. (Master dissertation)