

MOLECULAR STUDIES IN THE GENUS *IRIS* L.: A PRELIMINARY STUDY

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ABSTRACT - Preliminary analyses of molecular sequence data from *rps4* and *trnL-F* are presented. The results accord well with previous thinking about groupings within *Iris*. Several species generally accepted as distinct genera, *Belamcanda*, *Pardanthopsis* and *Hermodactylus*, fall within the genus *Iris*. *Scorpiris* also falls within *Iris* and should not be considered a separate genus. The series *Sibiricae* could be divided into two groups, *Sibiricae* and *Chrysographes*. *Iris kolpakowskiana* is closely related to subgenus *Xiphium*, and *I. anguifuga* belongs to series *Tenuifoliae*.

KEY WORDS - *Iridaceae*, *Iris*, systematics, DNA

INTRODUCTION

In the past, the large genus *Iris* L. (c. 225 species) has been subdivided many times. The bulbous groups of irises are often treated as separate genera (see Hall *et al.*, this volume), and even now the genera *Belamcanda* Adans., *Pardanthopsis* (Hance) Lenz and *Hermodactylus* Mill. are maintained by most authors, so it is clear that there are still different points of view about the classification of the genus *Iris* and its relatives. Although most authors have used morphological characters as the basis for their classifications, their interpretations and emphasis have varied. Some have viewed floral traits as most important, whereas others have focused more on the root system and leaf morphology, and this has brought about differences in delimitation of the genus and how it has been subdivided.

To evaluate these competing ideas on the treatment of the genus *Iris*, we have investigated the use of two regions in plastid (chloroplast) DNA, a protein-coding gene, *rps4*, and a largely noncoding region, *trnL-F*. We have produced DNA sequences for these two regions using standard automated techniques and used these data to construct an estimate of relationships for *Iris* and its closest relatives. These two datasets have been combined and used to produce a phylogenetic tree using parsimony methods to estimate their phylogenetic relationships.

To interpret the results of the DNA analyses, we use as our point of reference the classification of the genus by Taylor (1976) as published in the book by Mathew (1981). Following this classification, species from almost every *Iris* group were included in our sampling, but no species were used from the section *Hexapogon*.

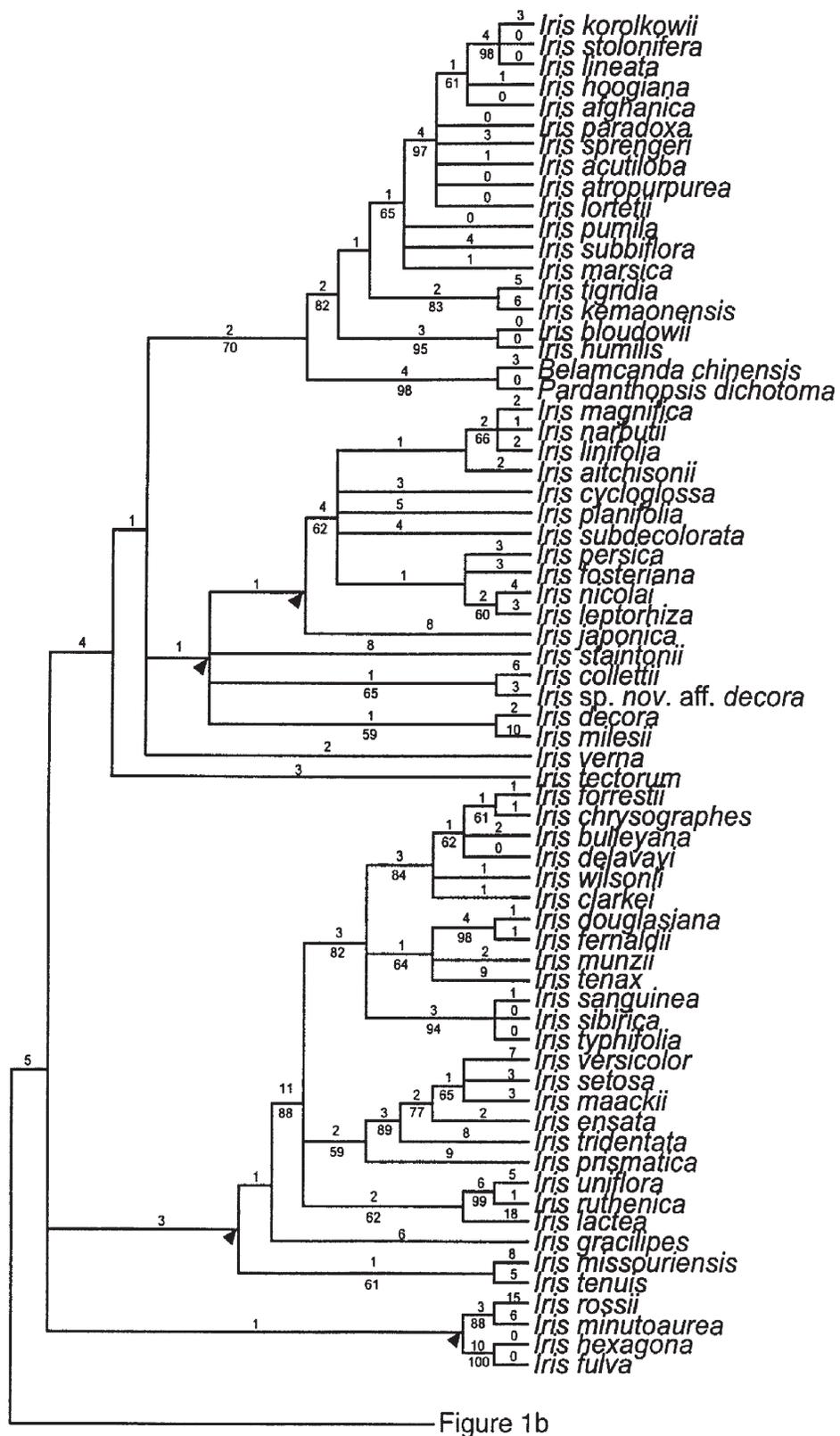


Figure 1 (continued)

reasonably reliable. With the low levels of sequence divergence detected, it is clear that more data must be collected before we can have great confidence in these results, but we can point to the results of Reeves *et al.* (this volume) in which the patterns found with one gene or sequence region were repeated quite consistently with the others, and when these were combined nearly all groups were strongly supported. We must also admit that some of the relationships in these trees appear somewhat unlikely, but can only report our results and look forward to the patterns that will be found when more data are brought to bear on these problems. In all figures we exhibit one of the shortest trees found with successive weighting (which downweights positions that change frequently); branch lengths (hypothesised changes in the sequences) are shown above the branches, whereas numbers below the branches are bootstrap percentages. Arrows are used to mark groups that are not present in the strict consensus tree of all shortest trees. Similar patterns were found with both regions, so we will not focus on the *trnL-F* results, but rather will emphasise the patterns obtained with the combined matrix.

Other molecular research has shown that the genus *Iris* is monophyletic (Reeves *et al.*, this volume), and with respect to the outgroups used here we confirm this finding, although bootstrap support is lacking. We will divide our results into three groups (Groups 1, 2 and 3 in Fig. 2).

GROUP 1. Group 1 (Fig. 2a) is monophyletic with very strong bootstrap support (100 %). In the strict consensus tree, *I. gracilipes* A. Gray is sister to the whole complex of group 1, although there is no bootstrap value for this. This species has always been considered extraordinary; Rodonienko (1961, 1987) placed it in its own section, *Monospatha*.

It is interesting that species from the series *Sibiricae* split into two monophyletic groups, since Mathew (1981) has noted that in some classifications the *Sibiricae* contain only *I. sanguinea* Hornem. ex Donn, *I. sibirica* L. and *I. typhifolia* Kitagawa, and the rest (including *I. chrysographes* Dykes and some other species) are placed in *Chrysographes*. This division had previously been proposed by Werckmeister (1967) and others, and cytological research shows differences between these groups. Species of series *Californicae* form one monophyletic group and come together with the two groups of *Sibiricae*. There are hybrids between the *Californicae* and *Sibiricae* species, the so called “Cal-Sibe hybrids”.

In the next monophyletic group (91% bootstrap) two series, *Tripetalae* and *Laevigatae*, come together. One of the main characteristics of *Tripetalae* is a much reduced inner (standard) tepal. As this is also the case with some other *Iris* species, and indeed is characteristic of the majority of junos, this cannot be called a defining character. *Iris prismatica* is sister to this group. Species from the series *Ruthenicae* form a monophyletic group with *I. lactea* Pallas (series *Ensatae*).

GROUP 2. *I. bungei* Maxim., which is the only species examined of series *Tenuifoliae*, forms a monophyletic group with *I. anguifuga* Y.T. Zhou, which was placed in its own section, *Ophioiris* by Zhao (see Mathew, 1981). These results (Fig. 2a) confirm that *I. anguifuga* Y.T. Zhao belongs to the *Tenuifoliae* as suggested by Mathew (1981). The species of series *Spuriae* examined here form a monophyletic group, apart from *I. graminea* L., which previously has been placed

in other groups. *Iris graminea* and *I. foetidissima* L. form a sister pair. Rodonienko (1961, 1987) placed these two species in his subgenus *Xyridion* and also mentioned that *I. graminea* and *I. foetidissima* were put in the *Spathula* group by Tausch in 1823. These species show some similarities; for example, both are evergreen.

Iris speculatrix Hance is strongly supported as sister to the species of group 2, but this species, for which relationships have always been problematical, is highly sequence-divergent from all the others in this group.

GROUP 3. Almost all *Iris* species with a beard or crest (and intermediates) can be found in this group (Fig. 2b), although there is no bootstrap support for this assemblage. Subgenus *Iris* is monophyletic but not with strong bootstrap support. Sections *Iris*, *Pseudoregelia* and *Psammiris* are also monophyletic groups, but only the last two have strong support. Sections *Oncocyclus* and *Regelia* together form a strongly supported monophyletic group, and two subgroups can be seen within this complex. The *Regelia* species *I. hoogiana* Dykes and *I. afghanica* Wendelbo are closer to the *Oncocyclus* species than other *Regelia* species. *Iris afghanica* has, just like the *Oncocyclus* species, only one flower per stem. All other *Regelia* species have two flowers per stem.

Belamcanda Adams. and *Pardanthopsis* Lenz. form a single monophyletic group. Although there is no bootstrap value for this pair or for their position within the genus *Iris*, they are present in the strict consensus tree in this position. From this one can conclude that these two genera are species of *Iris* that have switched to a different pollination system.

Iris verna L. occurs in this group, and it too has beard, although this is minute and velvety in texture. The species from subgenus *Nepalensis* are also present in this group; *I. milesii* Foster forms a sister pair with *I. decora* Wallich. The *Scorpiris* species also form a monophyletic group, but this is not strongly supported (see Hall *et al.*, this volume).

OTHER TAXA. In addition to the groups discussed above, there are some others that we have not identified as major groups (Fig. 2a). The two species examined of series *Chinensis* come together with the species of the *Hexagonae* examined: *I. hexagona* Walter and *I. fulva* Ker-Gawl. It is interesting that *I. tenuis* Watson forms a sister pair with *I. missouriensis* Nuttall; both are North American species, and *I. tenuis* would appear to be an isolated relic. The *Unguiculares* species, *I. lazica* Alboff and *I. unguicularis* Poir., also form a monophyletic group, which is sister to groups 1 and 2. The *Unguiculares* are also isolated and highly divergent from all other species of *Iris*. The species of this set of taxa are all often morphologically diverse and have in the past been problematical.

CONCLUSIONS

This preliminary study of DNA sequences has produced a series of relationships that accord well with a great deal of previous thinking about groupings within *Iris*. Taxa that have often been problematical, such as *Unguiculares*, *I. speculatrix* Hance and *I. gracilipes*, are isolated and highly divergent here as well. Section *Lophiris* appears to have been a dumping ground for many distantly related species, all of which here have clear relationships to

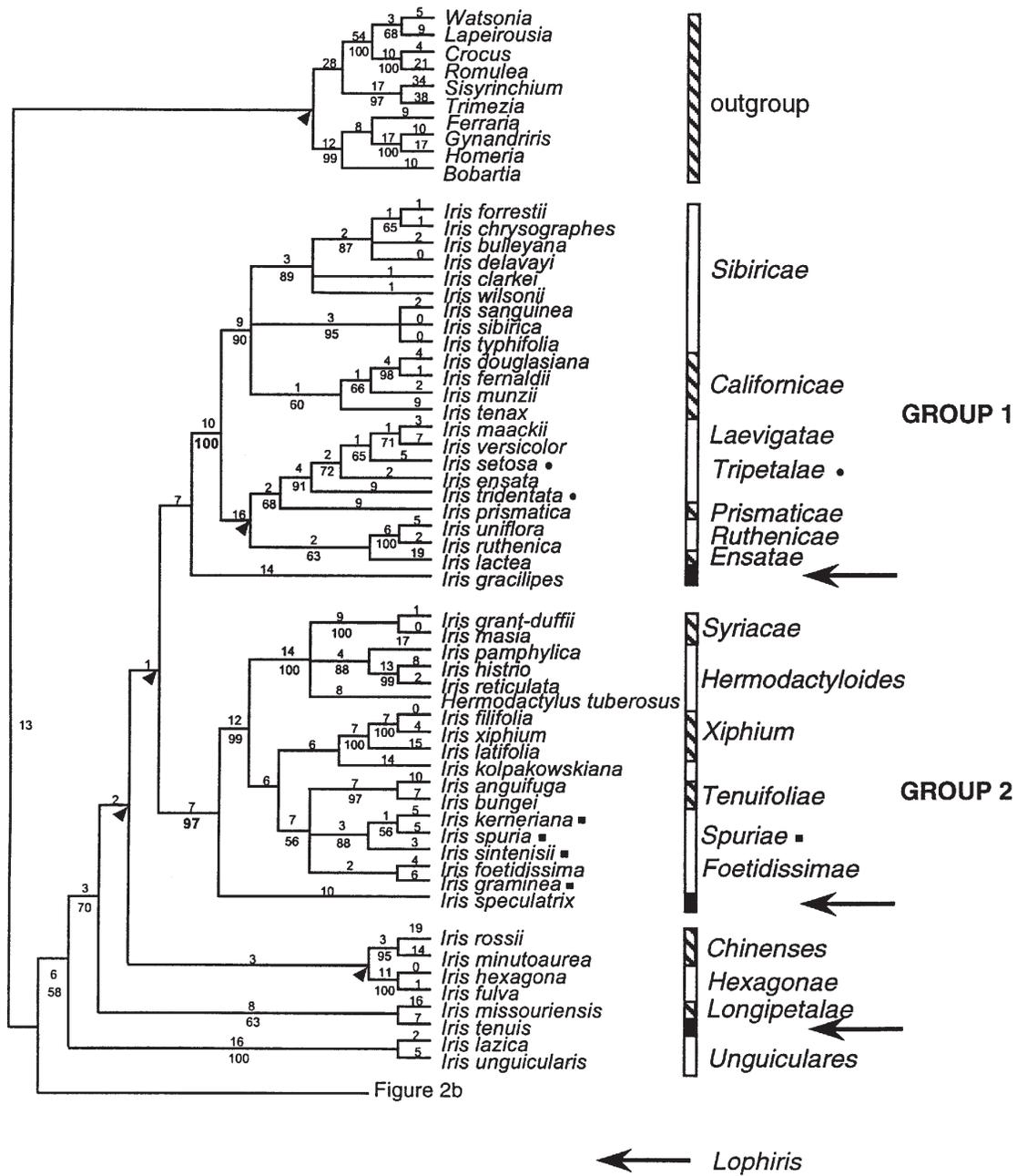


Figure 2 (a and b). Figure 2a - One of the most parsimonious successively weighted trees for the combined *trnL-trnF* and *rps4* data. Arrows indicate groups that are not present in the strict consensus tree. Numbers below branches are bootstrap values. Numbers above branches are the number of substitutions.

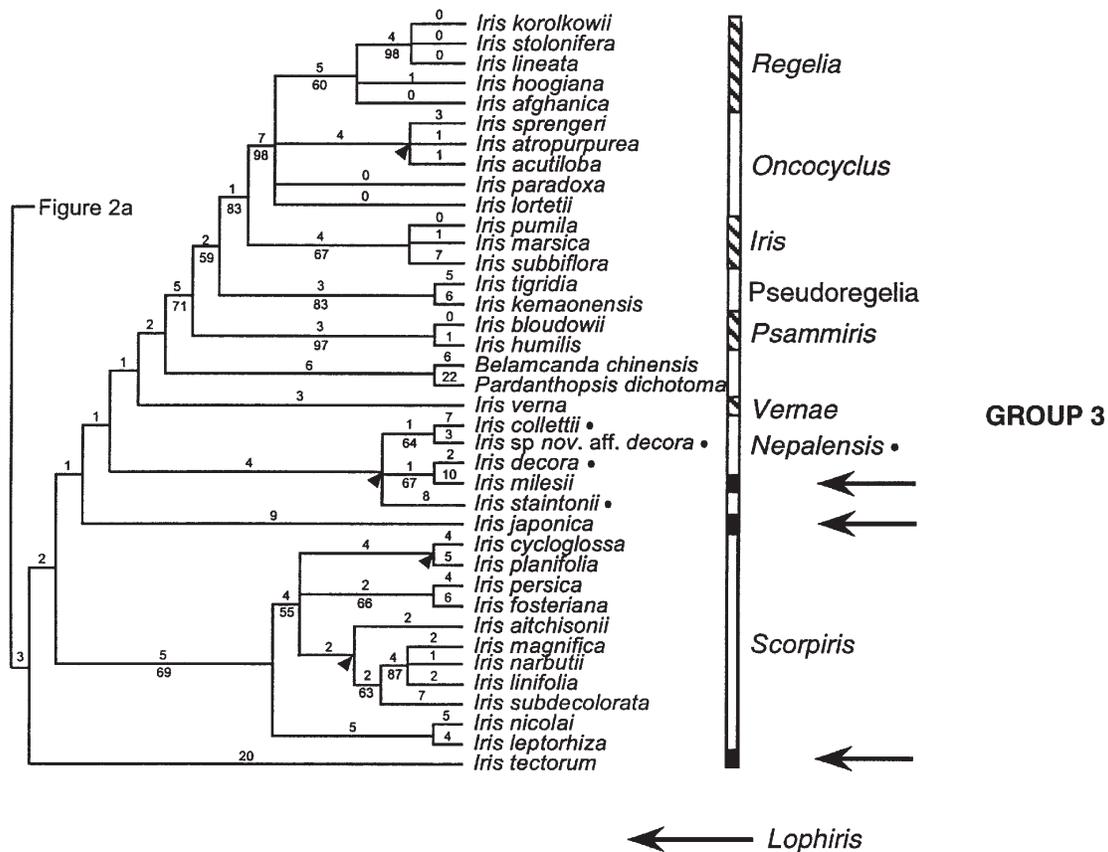


Figure 2b - One of the most parsimonious successively weighted trees for the combined *trnL-trnF* and *rps4* data.

other major groups within the genus. This indicates that their characteristics are likely to be ancestral for the genus *Iris*.

Several species generally accepted as distinct genera, *Belamcanda*, *Pardanthopsis* and *Hermodactylus*, fall within the genus *Iris* and should be treated as legitimate *Iris* species. *Scorpiris* also falls within *Iris* and should not be considered a separate genus. The series *Sibiricae* could be divided into two groups, *Sibiricae* and *Chrysographes*. *Iris kolpakowskiana* is closely related to subgenus *Xiphium*, and *I. anguifuga* belongs to series *Tenuifoliae*.

Although much of this pattern appears sensible and indicates that DNA sequences are useful in clarifying taxonomic problems, more data are required to confirm our findings and produce higher levels of bootstrap support.

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