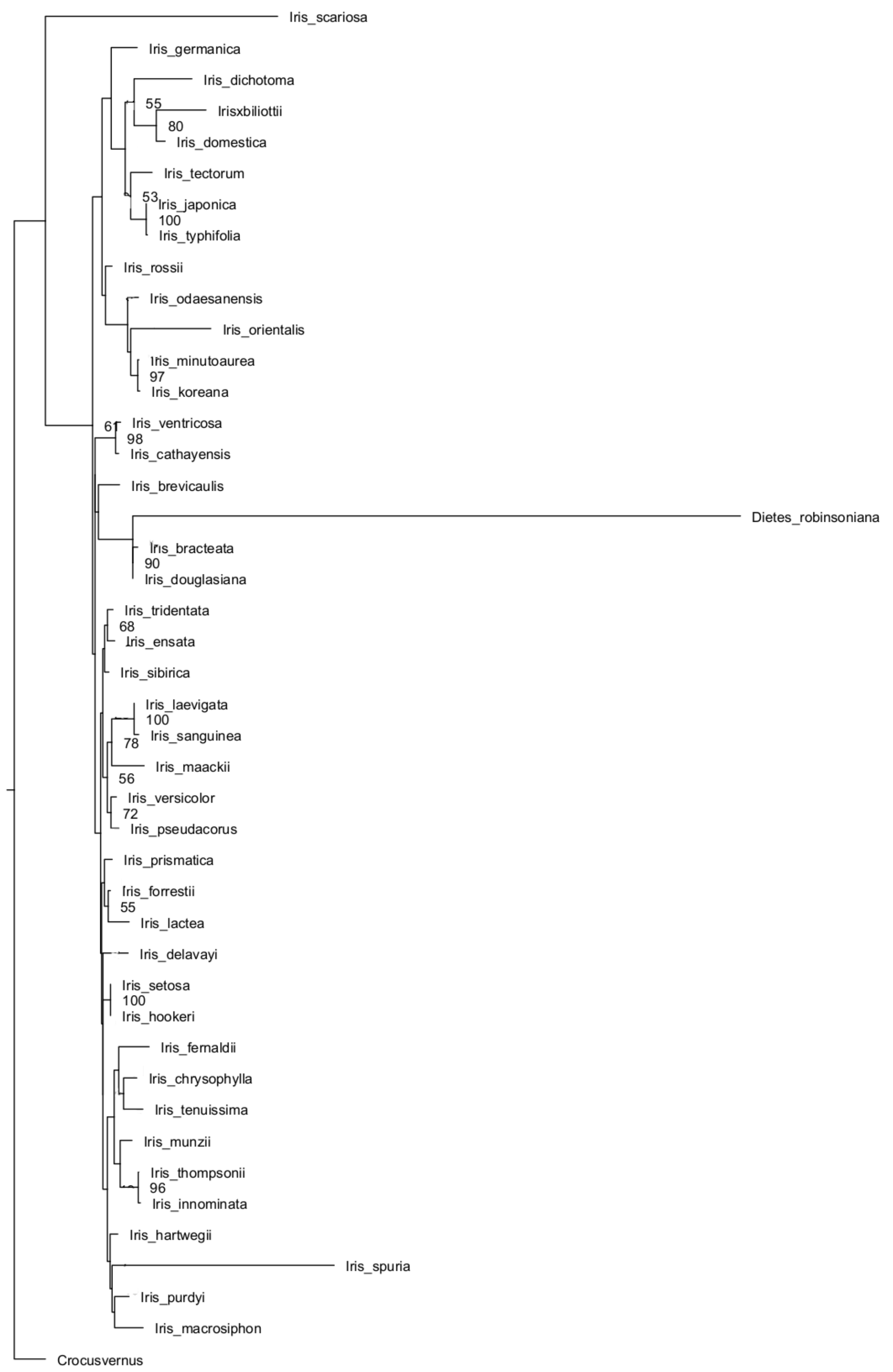
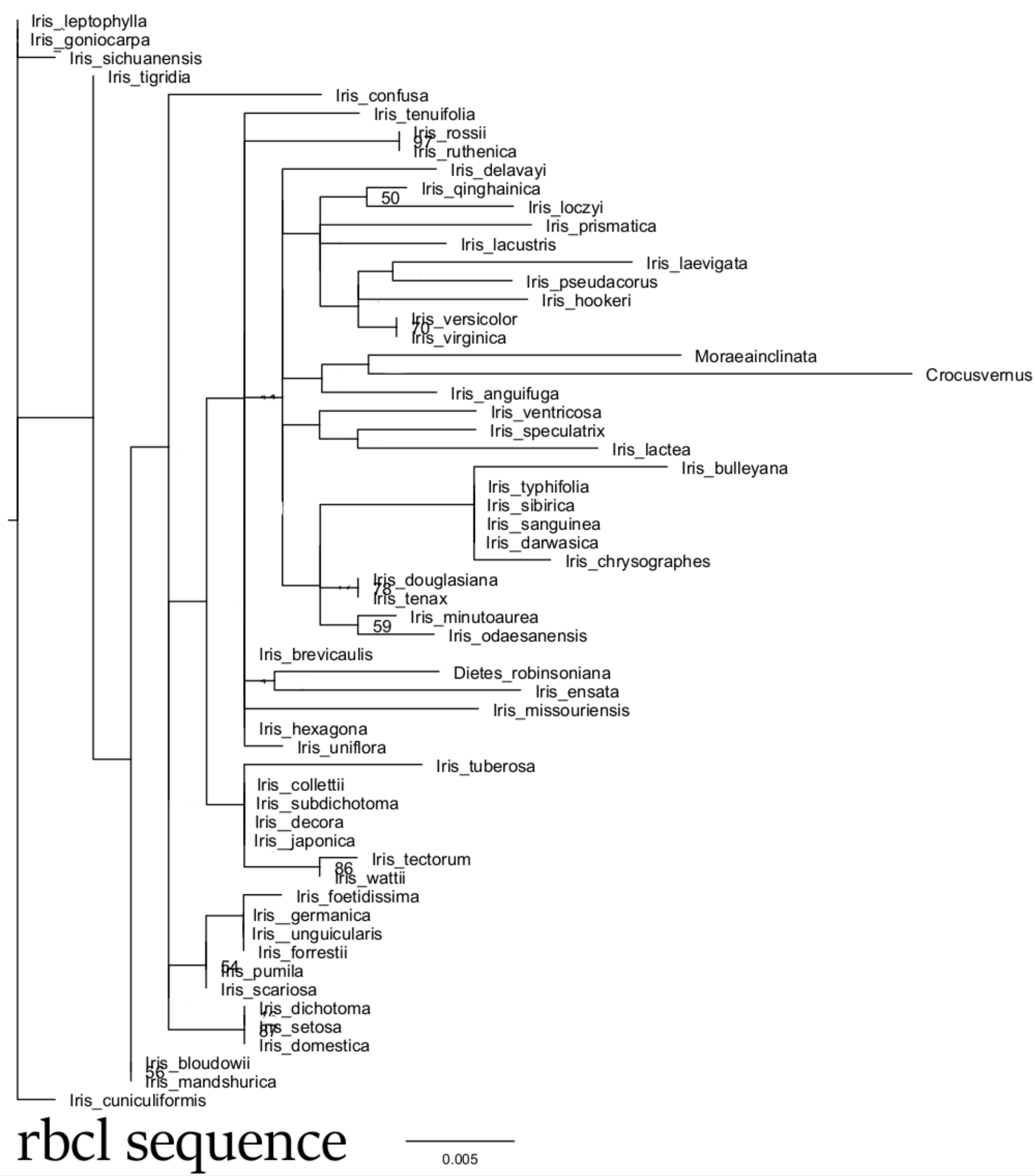
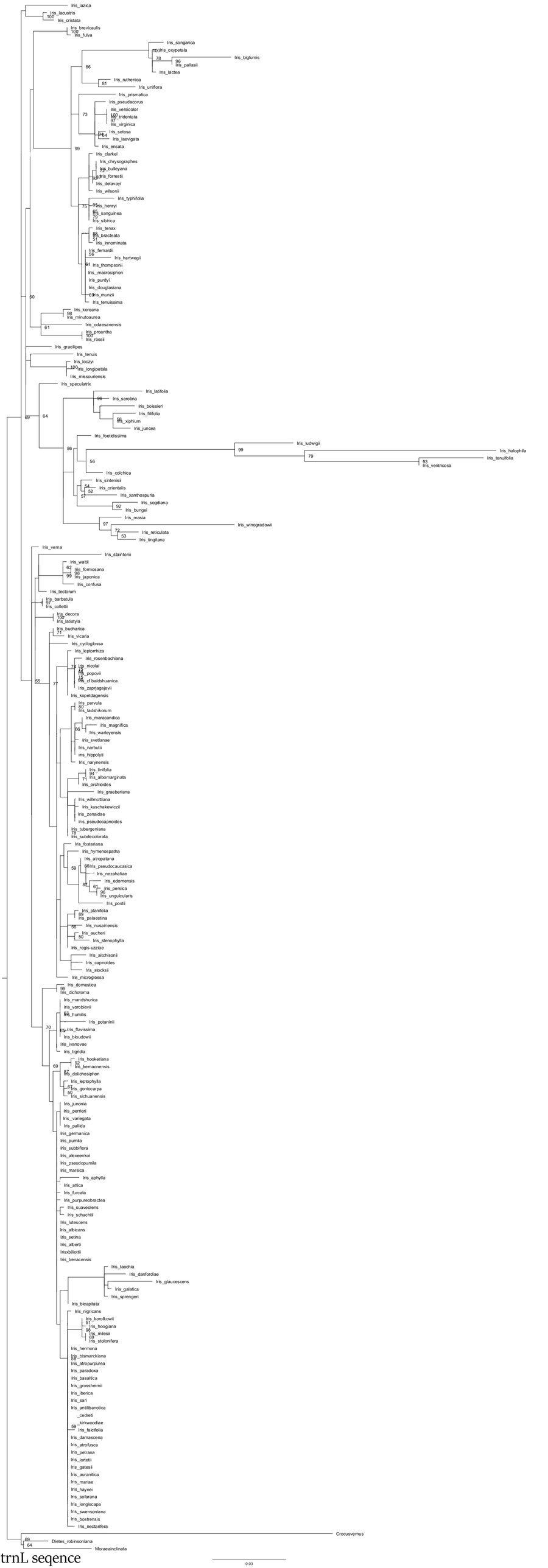

Iris_scariosa
Iris_germanica
Iris_dichotoma
55
Irisxbiliottii
80
Iris_domestica
Iris_tectorum
53
Iris_japonica
100
Iris_typhifolia
Iris_rossii
Iris_odaesanensis
Iris_orientalis
Iris_minutoaurea
97
Iris_koreana
61
Iris_ventricosa
98
Iris_cathayensis
Iris_brevicaulis
Dietes_robinsoniana
Iris_bracteata
90
Iris_douglasiana
Iris_tridentata
68
Iris_ensata
Iris_sibirica
Iris_laevigata
100
Iris_sanguinea
78
Iris_maackii
56
Iris_versicolor
72
Iris_pseudacorus
Iris_prismatica
Iris_forrestii
55
Iris_lactea
Iris_delavayi
Iris_setosa
100
Iris_hookeri
Iris_fernaldii
Iris_chrysophylla
Iris_tenuissima
Iris_munzii
Iris_thompsonii
96
Iris_innominata
Iris_hartwegii
Iris_spuria
Iris_purdyi
Iris_macrosiphon
Crocusvernus
ITS sequence
0.3

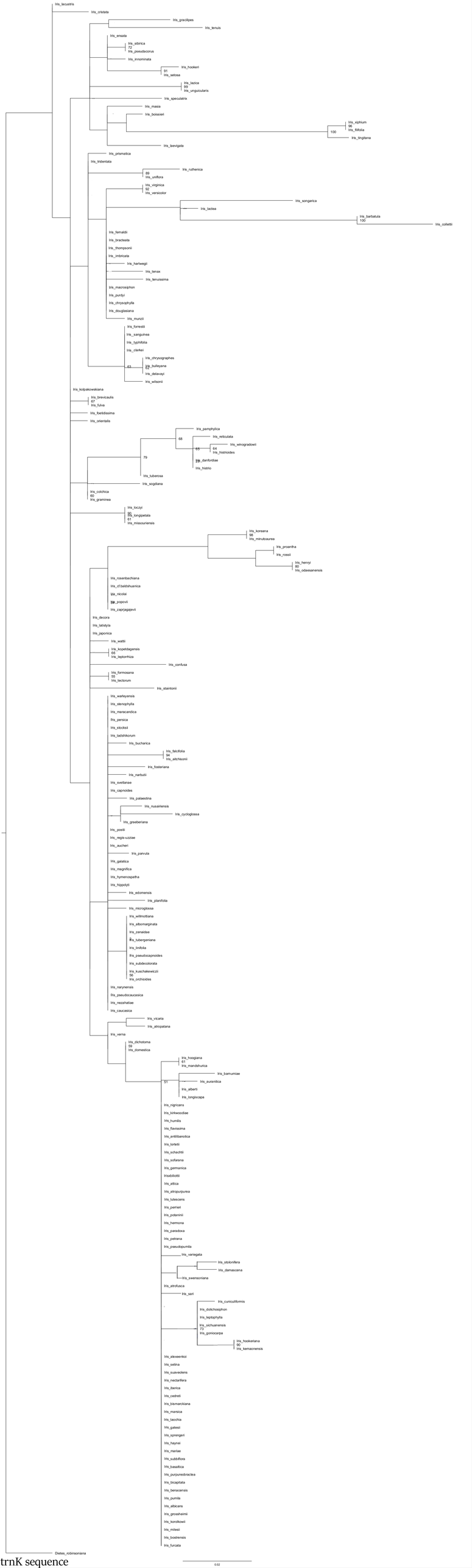
Iris_crisata
Iris_boissieri
Iris_lazica
Iris_unguicularis
Iris_vema
Iris_dolichosiphon
Iris_cuniculiformis
Iris_hookeriana
Iris_kemaonensis
Iris_sichuanensis
Iris_goniocarpa
Iris_leptophylla
Iris_imbricata
Iris_potaninii
Iris_tigridia
Iris_flavissima
Iris_humilis
Iris_bloudowii
Iris_longiscapa
Iris_iberica
Iris_grossheimii
Iris_paradoxa
Iris_petrana
Iris_auranitica
Iris_sofarana
Iris_mariae
Iris_antiblanoitica
Iris_nigricans
Iris_hermona
Iris_cedretii
Iris_atrofusca
Iris_atropurpurea
Iris_bismarckiana
Iris_haynei
Iris_damascena
Iris_swensoniana
Iris_basaltica
Iris_bostrensis
Iris_kirkwoodiae
Iris_gatesii
Iris_nectarifera
Iris_lortetii
Iris_sprengeri
Iris_barnumiae
Iris_sari
Iris_korolkowii
Iris_stolonifera
Iris_milesii
Iris_mandshurica
Iris_hoogiana
Iris_subbiflora
Iris_pumila
Iris_setina
Iris_lutescens
Iris_attica
Iris_alexeenkoi
Iris_bicapitata
Iris_taochia
Iris_furcata
Iris_albicans
Iris_benacensis
Iris_alberti
Iris_marsica
Iris_pseudopumila
Iris_scariosa
Iris_suaveolens
Iris_perrieri
Iris_pallida
Iris_variegata
Iris_junonia
Iris_germanica
Iris_ventricosa
Iris_purpureobractea
Iris_schachtii
Iris_aphylla
Iris×biliottii
Iris_dichotoma
Iris_domestica
Iris_tectorum
Iris_leptorrhiza
Iris_kopetdagensis
Iris_cf_baldshuanica
Iris_nicolai
Iris_zaprjagajevii
Iris_rosenbachiana
Iris_popovii
Iris_fosteriana
Iris_hymenospatha
Iris_caucasica
Iris_pseudocaucasica
Iris_nezahatiae
Iris_postii
Iris_atropatana
Iris_edomensis
Iris_persica
Iris_narbutii
Iris_parvula
Iris_tadshikorum
Iris_maracandica
Iris_warleyensis
Iris_svetlanae
Iris_hippolyti
Iris_magnifica
Iris_narynensis
Iris_knifolia
Iris_orchioides
Iris_albomarginata
Iris_oncocyclus
Iris_subdecolorata
Iris_kuschakewiczii
Iris_tuberogeniana
Iris_willmottiana
Iris_zenaidae
Iris_pseudocapnoides
Iris_graeberiana
Iris_vicaria
Iris_bucharica
Iris_galatica
Iris_regis-uzziae
Iris_aucheri
Iris_nusairiensis
Iris_palaestina
Iris_planifolia
Iris_stenophylla
Iris_stocksii
Iris_cycloglossa
Iris_atchisonii
Iris_falcifolia
Iris_microglossa
Iris_confusa
Iris_formosana
Iris_japonica
Iris_wattii
Iris_staintonii
Iris_decora
Iris_latistyla
Iris_barbatula
Iris_colettii
Iris_subdichotoma
Iris_tenuis
Iris_gracilipes
Iris_bozyi
Iris_missouriensis
Iris_longipetala
Iris_odaesanensis
Iris_hennyi
Iris_minutoaurea
Iris_koreana
Iris_rossii
Iris_proantha
Iris_speculatrix
Iris_tenuifolia
Iris_foetidissima
Iris_colchica
Iris_graminea
Iris_orientalis
Iris_anguifuga
Iris_spuria
Iris_qinghainica
Iris_sogdiana
Iris_kolpakowskiana
Iris_bosseri
Iris_tingitana
Iris_xiphium
Iris_filifolia
Iris_masia
Iris_tuberosa
Iris_pamphylica
Iris_histrio
Iris_danfordiae
Iris_reticulata
Iris_winogradowii
Iris_histrioides
Iris_brevicaulis
Iris_fulva
Iris_hexagona
Iris_ruthenica
Iris_uniflora
Iris_songarica
Dietes_robinsoniana
Iris_lactea
Iris_chrysophylla
Iris_macrosiphon
Iris_purdyi
Iris_thompsonii
Iris_tenuissima
Iris_innominata
Iris_tenax
Iris_brackiata
Iris_douglasiana
Iris_forrestii
Iris_hartwegii
Iris_fernaldii
Iris_munzii
Iris_wilsonii
Iris_clarkei
Iris_delavayi
Iris_chrysographes
Iris_bulleyana
Iris_typhifolia
Iris_sanguinea
Iris_clarkeana
Iris_prismatica
Iris_tridentata
Iris_pseudacorus
Iris_sibirica
Iris_virginica
Iris_versicolor
Iris_laevigata
Iris_pardanthopsis
Iris_ensata
Iris_setosa
Iris_hookeri
Crocusvernus
matK sequence
0.3

Iris_unguicularis
100
Iris_lazica
Iris_cristata
100
Iris_lacustris
Iris_japonica
100
Iris_wattii
Iris_orchioides
Iris_cycloglossa
90
100
Iris_falcifolia
83
Iris_kuschakewiczii
93
Iris_magnifica
55
88
Iris_persica
88
Iris_planifolia
100
Iris_aucheri
Iris_decora
82
Iris_barbatula
100
Iris_collettii
97
Iris_domestica
97
Iris_dichotoma
Iris_hookeriana
100
Iris_kemaonensis
76
Iris_purpureobractea
64
Iris_aphylla
Iris_germanica
68
Iris_pallida
91
Iris_alberti
Iris_taochia
83
Iris_pumila
90
Iris_alexeenkoi
64
Iris_bicapitata
Iris_subbiflora
Iris_humilis
87
Iris_flavissima
99
Iris_tigridia
86
Iris_potaninii
Iris_hoogiana
99
Iris_mandshurica
Iris_bismarckiana
63
Iris_haynei
74
Iris_hermona
Iris_swensoniana
100
Iris_bostrensis
Iris_nigricans
Iris_barnumiae
Iris_sari
Iris_kirkwoodiae
Iris_gatesii
Iris_sprengeri
Iris_nectarifera
Iris_longipetala
100
Iris_missouriensis
53
Iris_gracilipes
67
Iris_tenuis
Iris_rossii
100
Iris_proantha
96
Iris_odaesanensis
100
Iris_henryi
66
Iris_koreana
100
Iris_minutoaurea
Iris_foetidissima
52
82
96
Iris_graminea
100
Iris_colchica
86
Iris_spuria
100
Iris_orientalis
Iris_tingitana
100
Iris_xiphium
99
Iris_filifolia
100
100
Iris_boissieri
100
Iris_masia
100
Iris_tuberosa
100
Iris_pamphylica
74
100
Iris_reticulata
50
Iris_winogradowii
98
Iris_danfordiae
57
Iris_histrio
Iris_kolpakowskiana
Iris_brevicaulis
100
Iris_fulva
Iris_delavayi
91
Iris_wilsonii
99
Iris_forrestii
Iris_sanguinea
Iris_typhifolia
Iris_purdyi
75
Iris_chrysophylla
58
89
Iris_munzii
Iris_innominata
94
Iris_tenax
80
Iris_prismatica
Iris_songarica
100
Iris_lactea
95
Iris_uniflora
Iris_ensata
81
Iris_setosa
Iris_pseudacorus
100
Iris_sibirica
56
Iris_virginica
100
Iris_tridentata
Crocusvernus
55
Dietes_robinsoniana
ndhF sequence
0.02


Iris_leptophylla
Iris_goniocarpa
Iris_sichuanensis
Iris_tigridia
Iris_confusa
Iris_tenuifolia
Iris_rossii
97
Iris_ruthenica
Iris_delavayi
Iris_qinghainica
50
Iris_loczyi
Iris_prismatica
Iris_lacustris
Iris_laevigata
Iris_pseudacorus
Iris_hookeri
Iris_versicolor
70
Iris_virginica
Moraeainclinata
Crocusvernus
Iris_anguifuga
Iris_ventricosa
Iris_speculatrix
Iris_lactea
Iris_bulleyana
Iris_typhifolia
Iris_sibirica
Iris_sanguinea
Iris_darwasica
Iris_chrysographes
Iris_douglasiana
78
Iris_tenax
Iris_minutoaurea
59
Iris_odaesanensis
Iris_brevicaulis
Dietes_robinsoniana
Iris_ensata
Iris_missouriensis
Iris_hexagona
Iris_uniflora
Iris_tuberosa
Iris_collettii
Iris_subdichotoma
Iris_decora
Iris_japonica
Iris_tectorum
86
Iris_wattii
Iris_foetidissima
Iris_germanica
Iris_unguicularis
Iris_forrestii
54
Iris_pumila
Iris_scariosa
Iris_dichotoma
Iris_setosa
87
Iris_domestica
Iris_bloudowii
56
Iris_mandshurica
Iris_cuniculiformis
rbcl sequence
0.005




trnL seqence




trnK sequence

## Results

There were differences in the topology were observed between trees based on all six-locus trees and the trees based on each of used locus separately. In *mat*K and ITS trees, rooted on *Crocus vernus*, *Dietes robinsoniana* resolved as nested within subgenus *Limniris* (low bootstrap, hereafter Bp). Most of the described large-scaled relationships (Wilson, 2004; 2009; Wilson et al., 2016; Jiang et al., 2018) were recovered in present study.

The topology of all studied species evidenced two major subgenera (*Limniris* and *Iris*) and five minor (*Hermodactyloides, Nepalensis, Pardanthopsis, Scorpiris* and *Xiphium*). All but one genera were resolved as monophyletic, with *Hermodactyloides, Pardanthopsis* and *Scorpiris* being highly supported (Bp 86, 99 and 91 respectively). Only subgenus *Limniris* proved to be polyphyletic.

The *Scorpiris* subgenus (91 Bp), third by size and native to Middle East and Central Asia (Mathew, 1990), was represented by two highly supported sections *Juno* (80 Bp) and *Physocaulon* (100 Bp). This genus resolved as sister to two separated parts of subgenus *Limniris*.

Within subgenus *Iris,* distributed from eastern Asia through Southern or eastern Europe to North America (Mathew, 1990) six sections were resolved *Pardanthopsis* (99 Bp), *Psammiris* (56 Bp), *Pseudoregelia* (99 Bp), *Oncocyclus* (86 Bp), *Regelia* (87 Bp), *Hexapogon* (86 Bp) and *Pogon*. *Iris falcifolia*, described as belonging to the section *Hexapogon* was resolved in the section *Limniris*. Also *I. tigridia* and *I. ivanovae* classified as representing section *Pseudoregelia* resolved in section *Psammiris*. *Iris milesii* described as part of the subgenus *Limniris* resolved in the section *Pseudoregelia*. *Iris mandsuhgarica* described in *Psammiris* resolved in *Pogon*.

Within *Limniris,* genera widely distributed around Northern Hemisphere (Mathew, 1990), two sections were resolved *Limniris* (71 Bp) and *LophIris*, with the first section containing the majority of the species belonging to genera *Limniris*. Several species classified as belonging to section *LophIris* resolved as part of the section *Limniris*.

The subgenera *Hermodactyloides* (86 Bp), distributed from East Europe to Central Asia (Mathew, 1990), is subdivided into two sections *Monolepsis* (represented by one species, 73 BP*),* and *Reticulatae* (86 Bp). A sister subgenus *Xiphium* (97 Bp), distributed in western Europe and North Africa, contains only *Xiphium* section.

The subgenus *Nepalensis,* (58 Bt), distributed only in south-western China (Mathew, 1990) and represented by *Nepalensis* section, resolved as closely related to *Pardanthopsis* (99 Bp),

the subgenus native to Asia (Mathew, 1990) and represented by two species. *Iris subdichotoma* described as species in section Loph*Iris* resolved in the section *Nepalensis*. *Iris lazica* and *I. unguicularis* resolved as separated subgenus, with high support (100 Bp) similarly as *I. imbricata* (78 Bp).

## Discussion

The phylogeny presented in this paper is the most comprehensive study of genus *Iris*. The topology of obtained trees is in most cases consistent with previously published data (Tillie et al., 2000; Makarevitch et al., 2003; Wilson, 2009; Wilson, 2011; Jiang et al., 2018). In some cases, using *trn*L-F may have resulted in lack of bootstrap support of some branches, which was described by Tillie et al. (2000). Previously described generic groupings are recovered in present study as subgenera and subsections (Tillie et al., 2000; Makarevitch et al., 2003; Wilson, 2009; Wilson, 2011; Jiang et al., 2018), with monophyletic subgenera *Hermodactyloides, Iris, Nepalensis, Pardanthopsis, Scorpiris* and *Xiphium*, and paraphyletic *Limniris*. This study, similar like Wilson (2011), resolved *I. dichotoma* and *I. domestica* as sister taxa in subgenus *Pardanthopsis* that is sister to subgenus *Iris*. Similarly like in Wilson (2011) study *I. falcifolia* resolved as species belonging to subgenus *Scorpiris*. Contrary to previous works (Dykes, 1913; Mathew, 1989; Wilson, 2011) *I. tuberosa* was recognized as species belonging to subgenus *Limniris*.

## References


Dykes, W.R. (1913). *The Genus Iris.* New York: Dover Publications.

Jiang, Y.L., Huang, Z., Liao, J.Q., Song, H.X., Luo, X.M., Gao, S.P., et al. (2018). Phylogenetic analysis of IRIS L. from China on chloroplast TRNL-F sequences. *Biologia (Poland)* 73(5), 459-466. doi: 10.2478/s11756-018-0063-0.

Makarevitch, I., Golovnina, K., Scherbik, S., and Blinov, A. (2003). Phylogenetic relationships of the siberian *Iris* species inferred from noncoding chloroplast DNA sequences. *International Journal of Plant Sciences* 164(2), 229-237.

Mathew, B. (1990). *The iris.* Portland, Ore.: Timber Press.

Tillie, N., Chase, M.W., and Hall, T. (2000). Molecular studies in the genus Iris L.: A preliminary study. *Annali di Botanica* 58(March), 105-112. doi: 10.4462/annbotrm-9068.

Wilson, C. (2006). Patterns in Evolution in Characters That Define Iris Subgenera and Sections. *Aliso* 22(1), 425-433. doi: 10.5642/aliso.20062201.34.

Wilson, C.A. (2004). Phylogeny of *Iris* based on chloroplast matK gene and trnK intron sequence data. *Molecular Phylogenetics and Evolution* 33(2), 402-412. doi: http://dx.doi.org/10.1016/j.ympev.2004.06.013.

Wilson, C.A. (2009). Phylogenetic relationships among the recognized series in *Iris* section Limniris. *Systematic Botany* 34(2), 277-284.

Wilson, C.A. (2011). Subgeneric classification in Iris re-examined using chloroplast sequence data. *Taxon* 60(1), 27-35.

Wilson, C.A., Padiernos, J., and Sapir, Y. (2016). The royal irises (Iris subg. Iris sect. Oncocyclus): Plastid and low-copy nuclear data contribute to an understanding of their phylogenetic relationships. *Taxon* 65(1), 35-46. doi: 10.12705/651.3.

Winkel-Shirley, B. (2002). Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology* 5(3), 218-223. doi: http://dx.doi.org/10.1016/S1369-5266(02)00256-X.