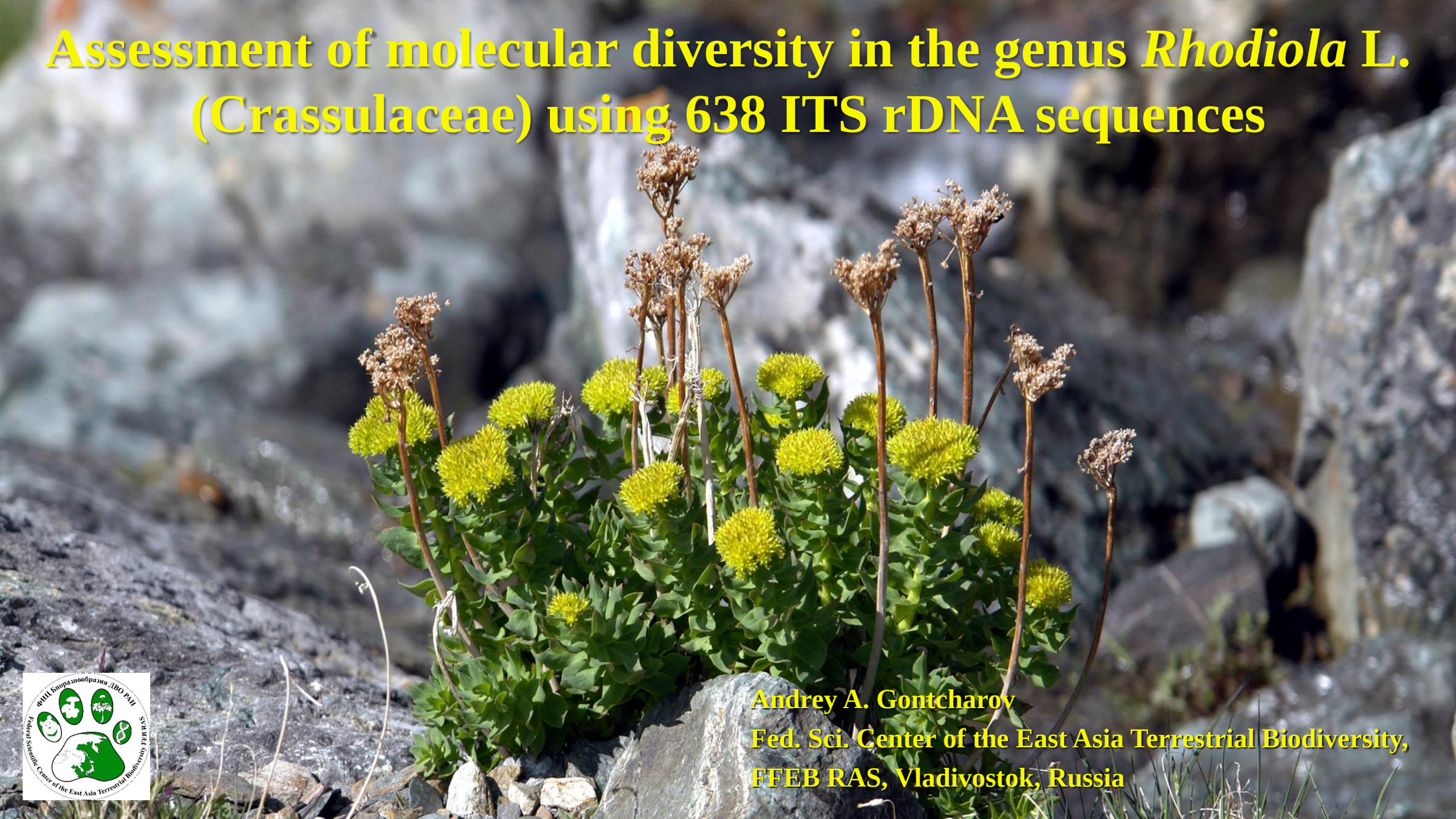
Assessment of molecular diversity in the genus *Rhodiola* L. (Crassulaceae) using 638 ITS rDNA sequences



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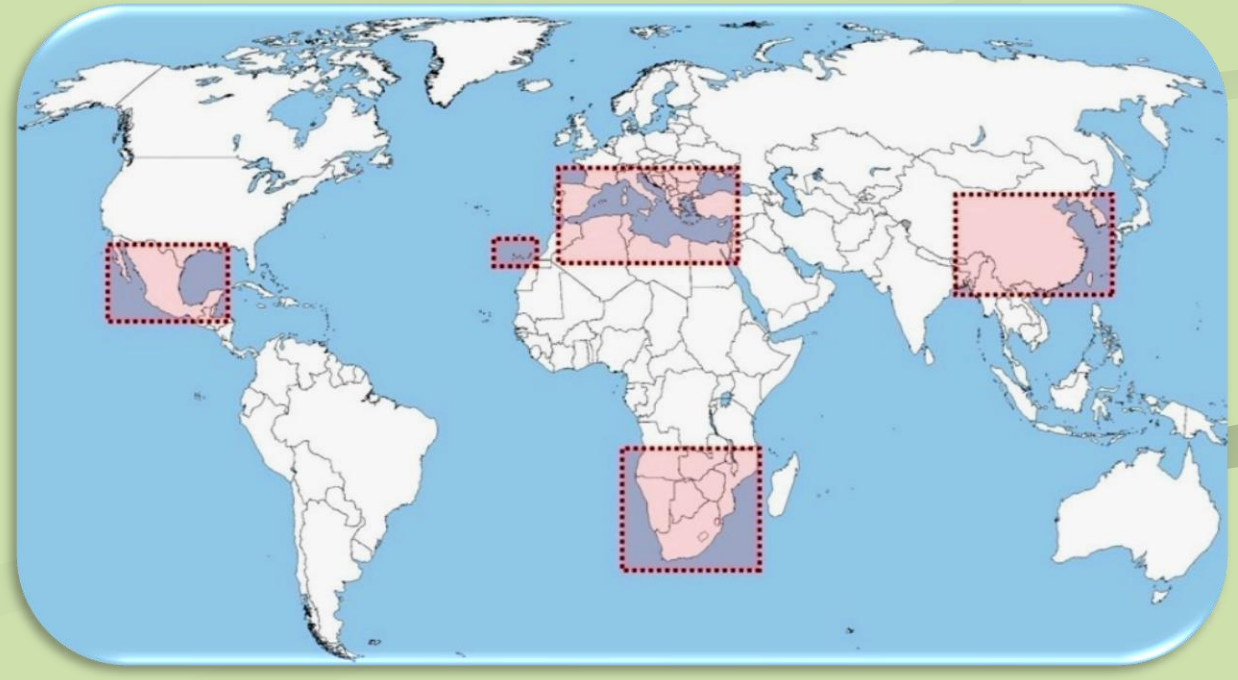
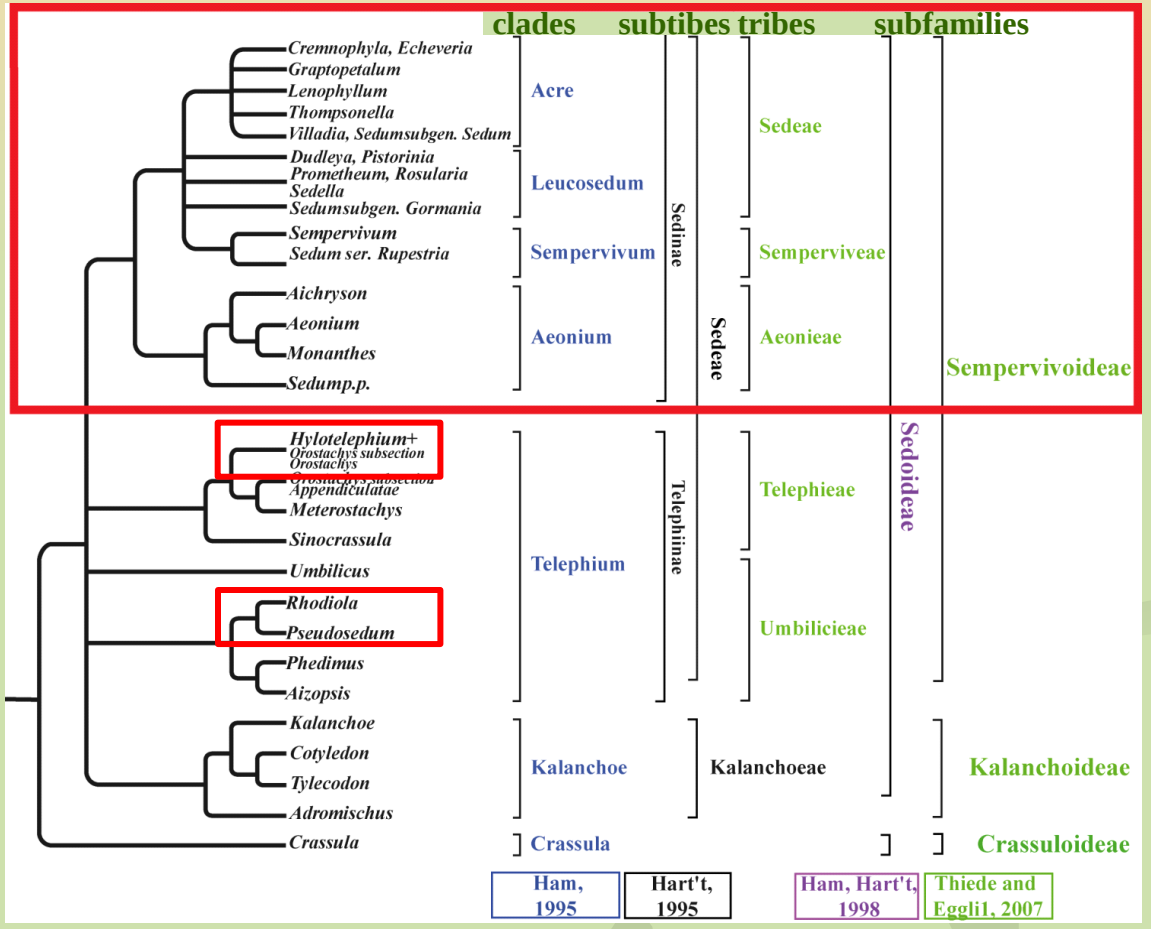
Family Crassulaceae DC - stonecropes

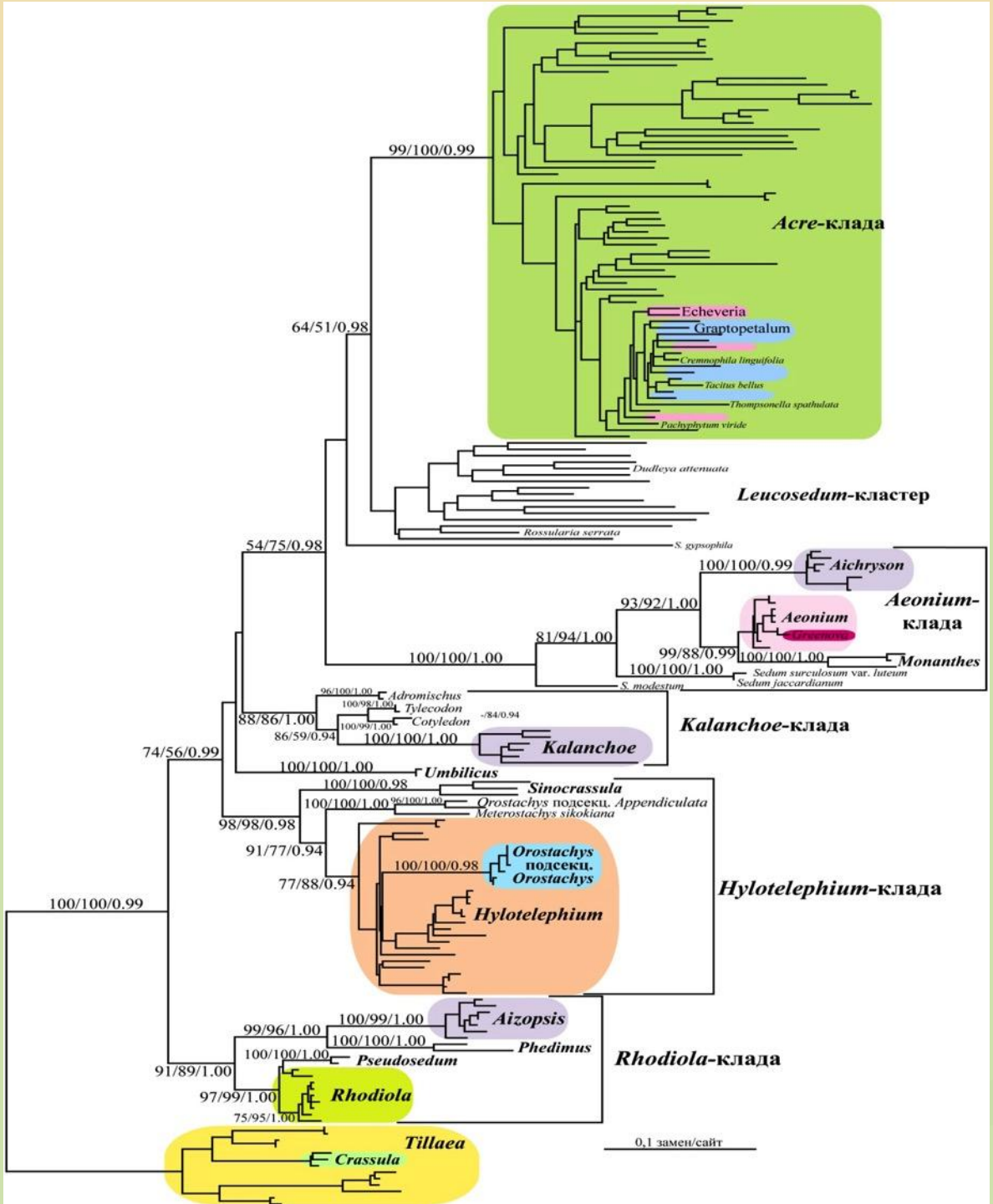
- ✓ Plants of succulent appearance, adapted to xeric habitats, have specific CAM metabolism.
- ✓ The family is monophyletic and accounts for ca. 1400 spp. / 33 genera.
- ✓ Its taxonomic and phylogenetic structure remains challenging due to homoplasy of phenotypic traits traditionally used in the family taxonomy.
- ✓ There are several geographically and phylogenetically defined lineages but only some of these are recognized as suprageneric taxa.



✓Tribe Umbiliceae Meisn. occupies unresolved (likely basal) position in the subfam. Sempervivoideae Arn. Comprises predominantly temperate Asian genera (except *Umbilicus* DC.) and *Rhodiola* L. is its core member.

✓Monophyly of the tribe was supported only in the early DNA data analyses (Mort et al., 1998) while in most sequence-based phylogenies robust *Umbilicus* and *Rhodiola* subclades showed little affinity to each other.

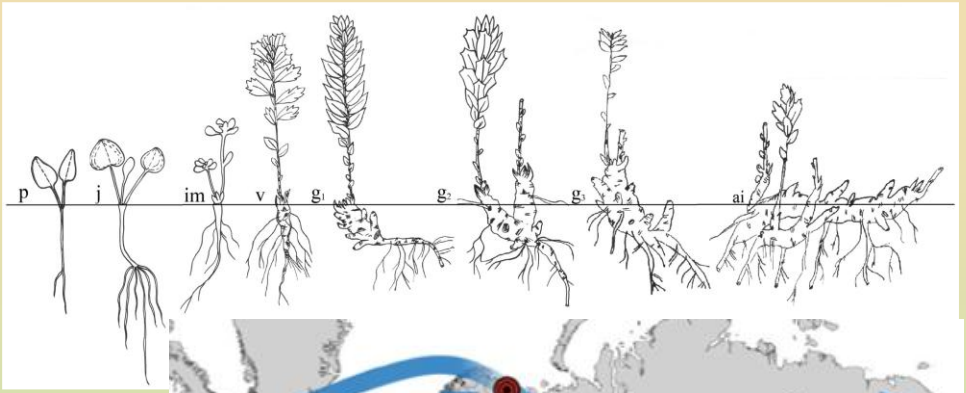




- ✓ **Close relationships between *Rhodiola*, *Pseudosedum*, and *Phedimus/Aizopsis* are undisputable now (Thiede, Eggli, 2007), however, there are few phenotypic characters in common between them.**

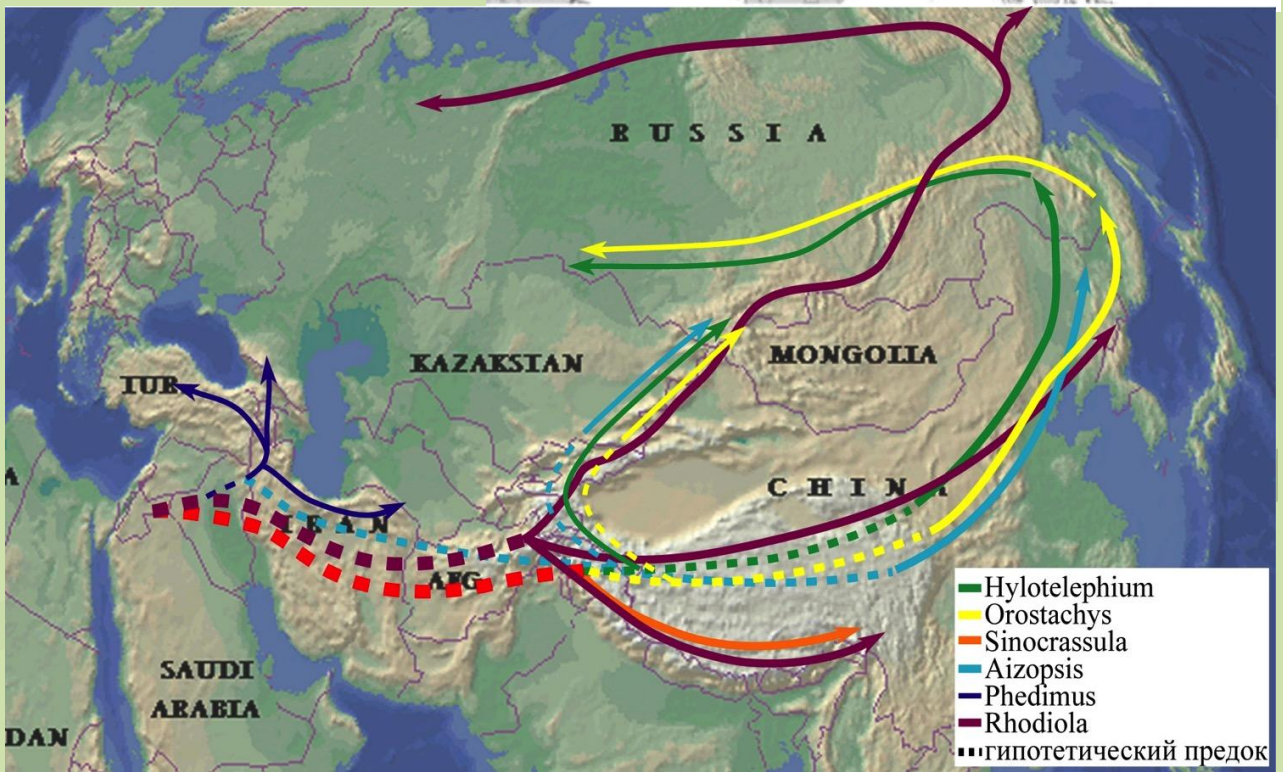


- ✓ ***Rhodiola*** **spp. herbaceous perennials distinct in presence of thick rhizomes and annual flowering stems arising from the axils of the scaly radical leaves, mostly thyrsoid inflorescences.**
- ✓ **According to the morphology-based taxonomy, the genus accounts for 55 to 100 spp. that are mostly confined to alpine and high altitude regions of the Northern Hemisphere.**
- ✓ **Most *Rhodiola* spp. are restricted to the Qinghai-Tibetan Plateau (QTP), its adjacent areas and Central Asian mountains (Tianshan and Altai).**









Taxonomy

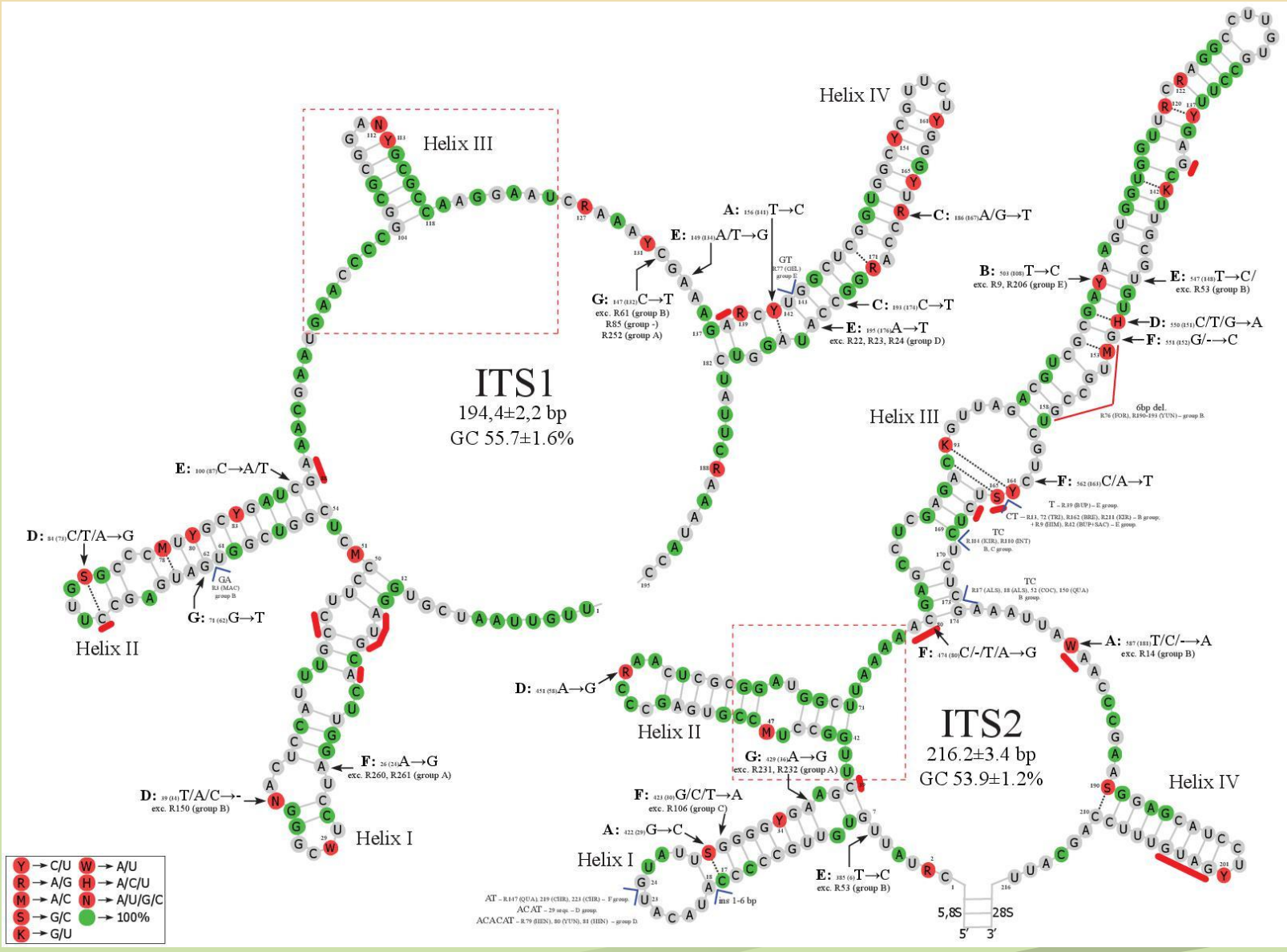
Rh. rosea accounts for as many as 50 synonyms:

Rh. officinarum **Crantz**
Rh. elongata **Fisch. & C.A.Mey.**
Rh. hideoi **Nakai**
Rh. imbricata **Edgew.**
Rh. iremelica **Boriss.**
Rh. maxima **Nakai**
Rh. minor **Mill.**
Rh. odora **Salisb.**
Rh. odorata **Lam.**
Rh. roanensis **Britton**
Rh. rosea **subsp.** ***arctica*** **(Boriss.) Love =** ***Rh. arctica*** **Boriss.**
Rh. rosea **subsp.** ***borealis*** **(Boriss.) A.P.Khokhr. & Kuvaev =** ***Rh. borealis*** **Boriss.**
Rh. rosea **subsp.** ***elongata*** **(Led.) H.Jacobsen**
Rh. rosea **subsp.** ***krivochizhinii*** **(Sipliv.) S.B.Gontch. =** ***Rh. krivochizhinii*** **Sipliv.**
Rh. rosea **subsp.** ***roanensis*** **(Br.) H.Jacobsen**
Rh. rosea **subsp.** ***sachalinensis*** **(Boriss.) S.B.Gontch. =** ***Rh. sachalinensis*** **Boriss.**
Rh. rosea **subsp.** ***tachiroi*** **(Franch. & Sav.) H.Jacobsen =** ***Rh. tachiroei*** **(Franch. & Savat.) Nakai**
Rh. rosea **var.** ***scopolii*** **(Kern. ex Simonk.) Soó =** ***Rh. scopolii*** **A.Kern. ex Simonk.**
Rh. sibirica **Sweet**
Rh. telephioides **(Maxim.) S.H.Fu**



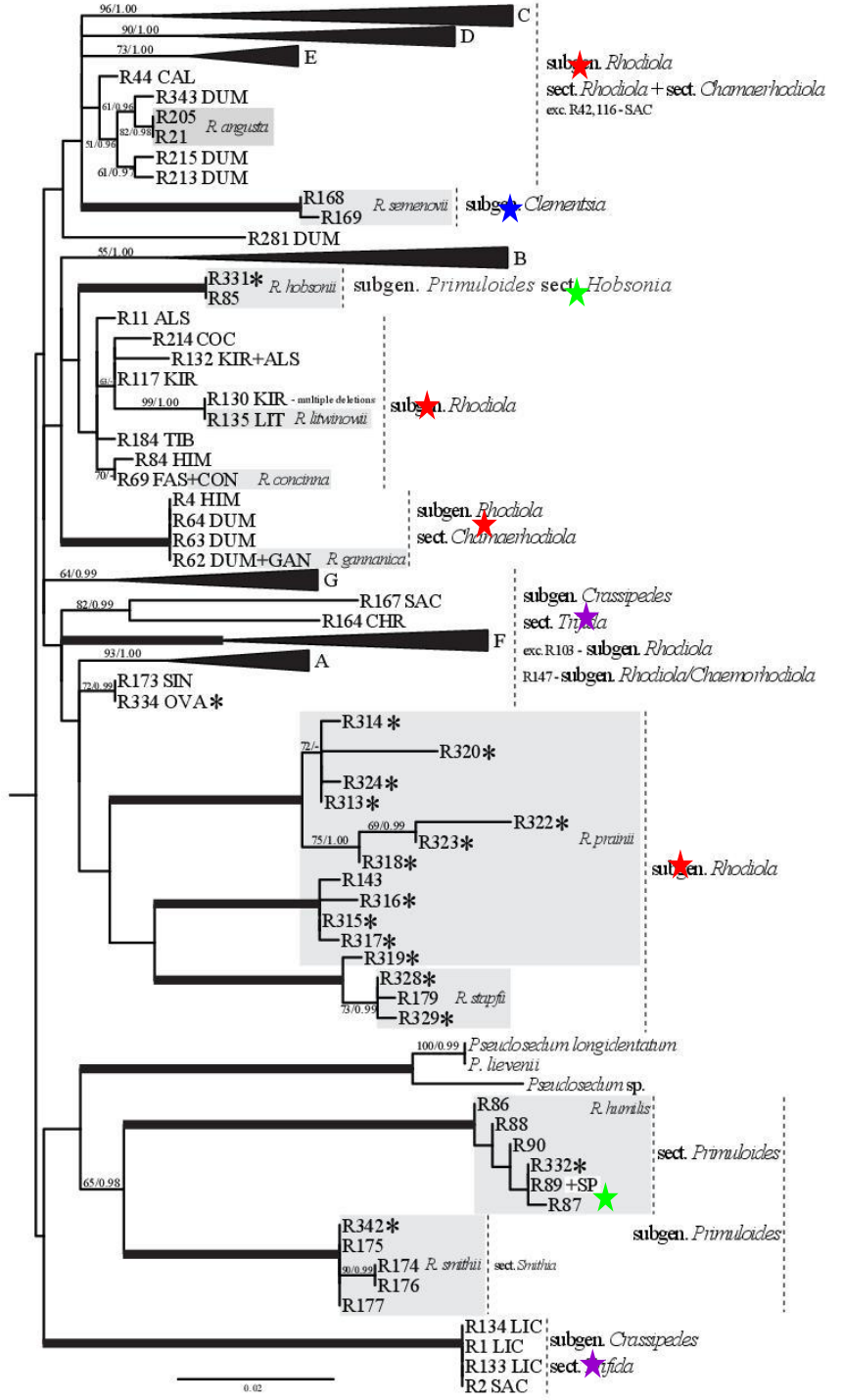
- ✓ ***Rhodiola*** **infrageneric classification is controversial (morphological diversity and convergent evolution of characters).**
- ✓ **There are four subgenera (*Primuloides* H. Ohba, *Crassipedes* H. Ohba, *Rhodiola*, and *Clementsia* H. Ohba), seven sections and two groups. Many of these taxa are artificial (genetic data).**
- ✓ **Intra- and interpopulational phenotypic plasticity sometimes is comparable in range to, or even greater than, differences between species (species delimitation is difficult).**
- ✓ **Complex taxonomy, specific distribution pattern and medicinal properties of the genus *Rhodiola* attracted much attention in recent years. However, most studies were focused toward understanding the pattern of genetic diversity in a particular species at a local or regional scale with only a few broad-scale analyses. Phylogenetic structure remains largely unexplored.**
- ✓ **To feel this gap we constructed the first densely sampled phylogeny of *Rhodiola* based on 638 ITS rDNA sequences (58 spp.). The objective of this study were: 1) to assess genetic diversity in the genus; 2) reveal its phylogenetic structure; 3) test the species concept in most genus members; 4) test ITS rDNA informativeness.**

- ✓ 638 ITS rRNA sequences
- ✓ 58 *Rhodiola* spp. + *Pseudosedum* – 3 seqs
- ✓ ITS region - 546 to 579 nt (571.5±5.6), GC from 51.9 to 55.7% (54.1±0.9%).
- ✓ 5.8S rRNA 161 bp and GC-content from 50.9% to 53.5%.
- ✓ *R. integrifolia* (112 seqs), *R. chrysanthemifolia* (58 seqs), and *R. rosea* (55 seqs) - nearly 1/3 of the total number of seqs.
- ✓ 30 spp. - 1 to 4 seqs only.



Genetic diversity

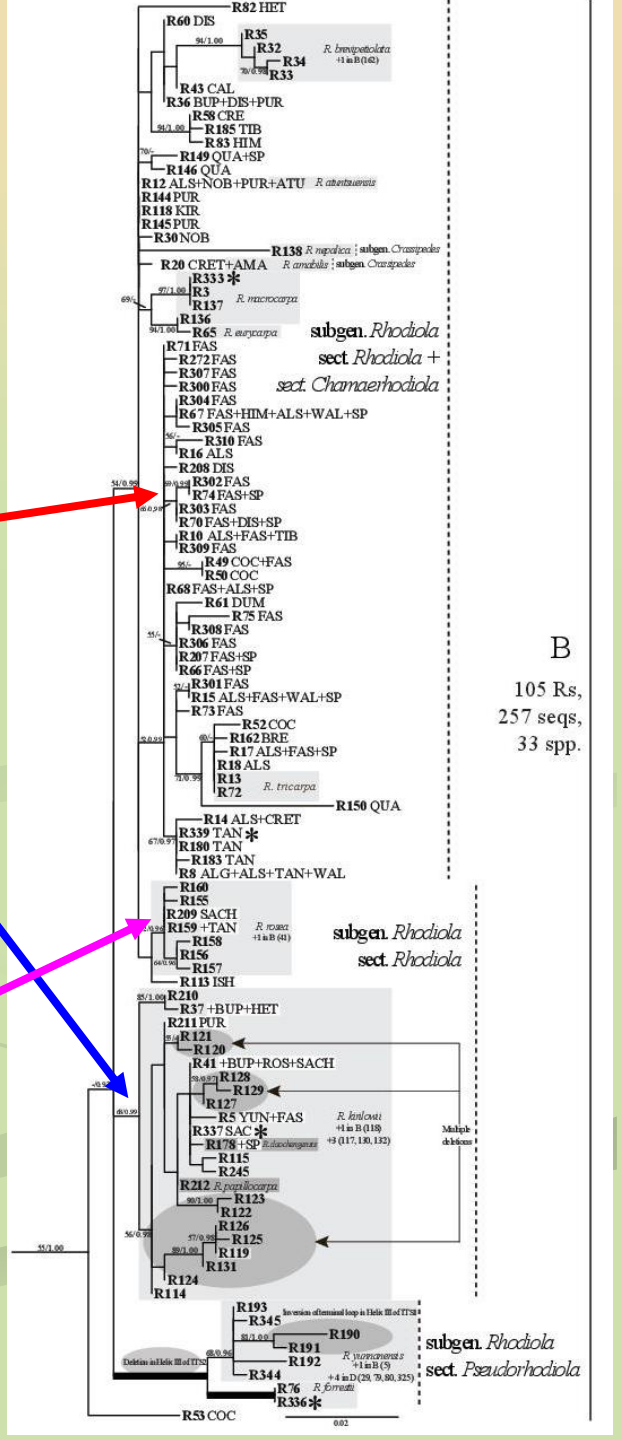
- ✓ Sequence divergence was relatively low 0,0368±0,0038
- ✓ 299 Ribotypes (Rts) were revealed in *Rhodiola* and 3 Rts in *Pseudosedum*.
- ✓ 208 (70%) Rts were private (a single seq) and the number of seqs per Rt decreased almost exponentially.
- ✓ Only 8 Rts had more than 10 seqs with 39 seqs in R112 of *R. integrifolia*.
- ✓ Most species carry more than one Rt.
- ✓ 8 spp. were represented by a single seq/Rt; 7 spp. with 2 to 5 seqs - monomorphic (one Rt).
- ✓ In remaining 43 spp. (15 spp. - 2 to 7 seqs) from 2 to 44 Rts (*R. chrysanthemifolia* with 58 seqs) were found.
- ✓ 7 larger clades (A–G; from 10 to 105 Rts), ca. 86% of Rts.



- ✓ ***R. chrysanthemifolia*** **and some other sequence-abundant species (*R. discolor* (31 seqs/28 Rts), *R. sacra* (30/29), *R. kirilowii* (24/21), *R. sexifolia* (19/17), and *R. fastigiata* (33/17)) - high ITS seq diversity and many private Rts.**
- ✓ **In the most densely sampled *R. integrifolia* (112 seqs) 20 Rts were revealed.**
- ✓ **Among Rt-rich spp. only *R. integrifolia* has a wide distribution (from Siberia to N. America) while distribution of the remaining taxa is narrower and mostly confined to the Himalaya and/or Tibet.**
- ✓ **In contrast to that, in the third abundant in the data set *R. rosea* (55 seqs), only 7 Rts were found.**
- ✓ **Most Rts were species-specific; 91 Rts found in 2 and more spp.**
- ✓ **29 Rts (10%) were shared between two (20 Rs) to four different spp. (3 Rs).**
- ✓ **Mostly, these Rts included 2 to 4 seqs except for the R67, R68, and R159, comprised by 14, 13, and 24 seqs respectively.**
- ✓ ***R. fastigiata*** **and *R. rosea* seqs prevailed in R67 and R159, respectively, while in R68 *R. fastigiata* and *R. alsia* were represented equally.**
- ✓ **Surprisingly, species with relatively narrow disjunct distribution areas (except *R. alsia*), (e.g. *R. fastigiata* (3 parts), *R. alsia*, *R. discolor*, and *R. bupleoroides* (4 parts)) were the most frequent in the shared Rts (10, 9, 8, and 5, respectively).**

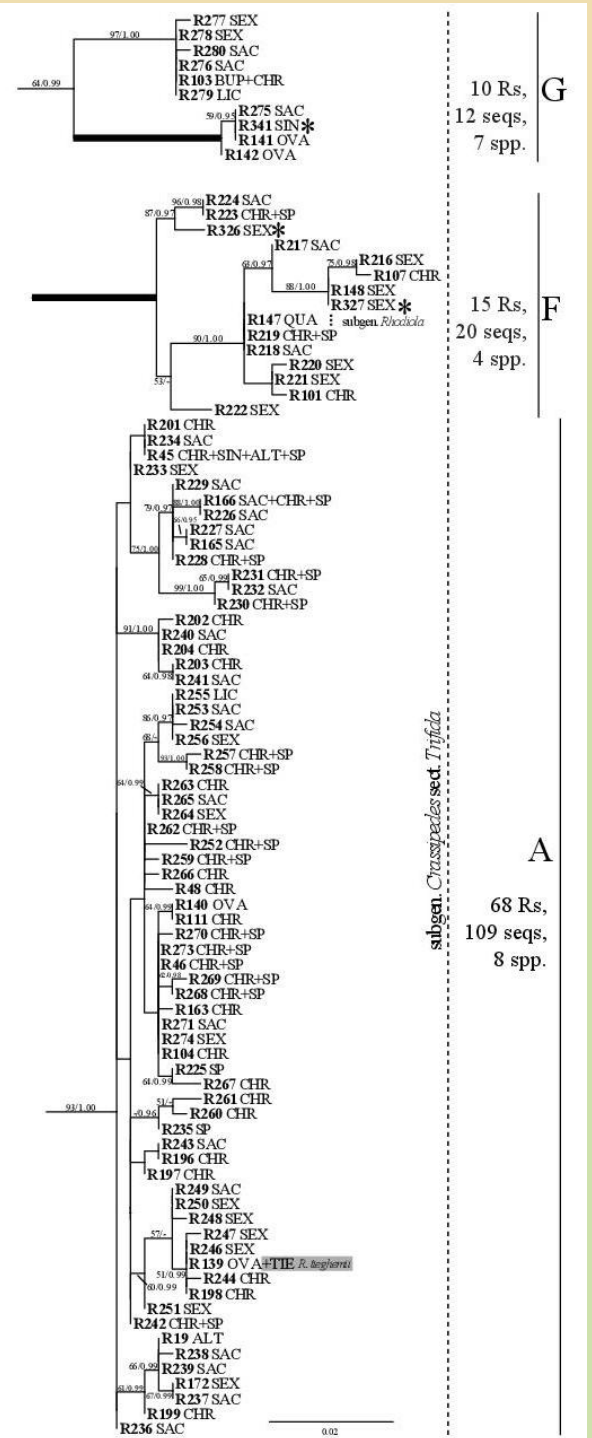
Clade B

- ✓ 105 Rts (33 spp.), 2 subgen., *Rhodiola* (sect. *Rhodiola* (subsect. *Rhodiola* and *Pseudorhodiola*) and *Chamaerhodiola*) and *Crassipedes*.
- ✓ Seq. divergence was relatively high (0,0185±0,0028)
- ✓ Several significant subclades and numerous individual branches.
- ✓ The largest subclade comprised 35 mostly pure *R. fastigiata* Rts.
- ✓ *R. kirilowii* subclade - 15 "pure" Rts and those shared with (R37, R41) or representing other species (*R. papillocarpa*, *R. purpureoviridis*, and *R. daochengensis*). Three *R. kirilowii* accessions were positioned in clades A–G as individual branches.
- ✓ *R. rosea* subclade - 5 "pure" Rts, R159 shared with *R. tangutica* (22 seqs + 1 seq), and a single *R. sachalinensis* accession (R209). Besides that, one accession attributed to *R. rosea* was found in R41 shared by four species (*R. kirilowii*, *R. bupleuroides*, *R. rosea*, and *R. sachalinensis*) in *R. kirilowii* subclade.



Clades A, F, and G

- ✓ Members of the subgen. Crassipedes sect. Trifida, mostly *R. chrysanthemifolia* (41 Rts, 4 shared), *R. sacra* (24 Rts, 1 shared), and *R. sexifolia* (18 Rts) comprised a bulk of the clades A (68 Rts, 8 spp.), F (15 Rts, 4 spp.), and G (10 Rts, 6 spp.).
- ✓ Clade A was well supported (94/1.00) but relationships remained mostly unresolved. Low sequence divergence (0,0097±0,0019).
- ✓ The clades F and G were better structured; (p-distances 0.0121±0.0027 and 0.016±0.0027, respectively). Yet, no species-specific lineages and only a few shared Rts were revealed in the three clades.



- ✓ **Clades C, D, and E - 24 Rts (4 spp.), 34 (7 spp.), and 32 (10 spp.).**
- ✓ **Majority of the Rts in the clade C (19) - *R. integrifolia* (79/1.00); relatively high sequence divergence (0,0129±0,0023).**
- ✓ **American endemic *R. rhodantha* (83/0.98) was a sister to *R. integrifolia* (70/0.97) not Central Asian *R. algida*.**

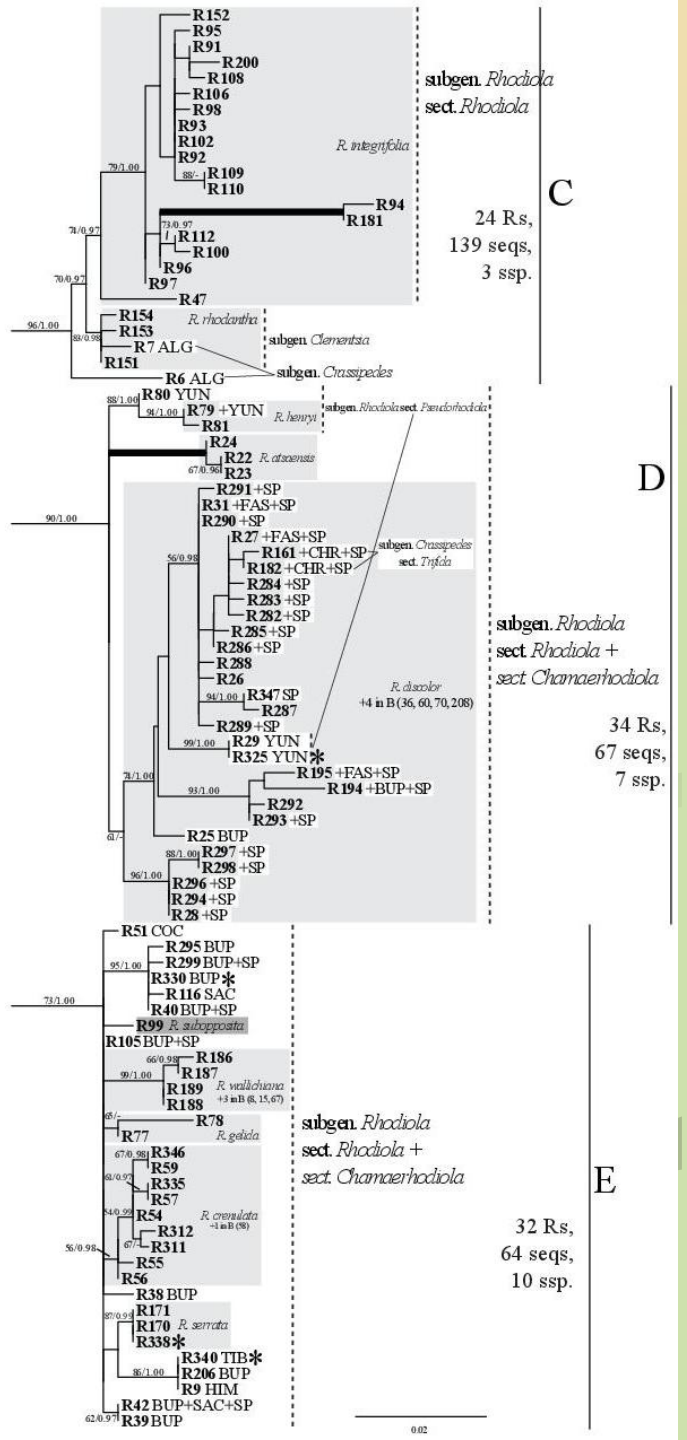












Conclusion

- ✓ **Although application of a single nuclear marker having biparental inheritance should be treated with certain caution, our analyses uncovered unusually high ribotypic diversity (299 Rts in 638 seqs) in the relatively small genus reflecting a complex history of its diversification and speciation.**
- ✓ **Most *Rhodiola* speies are not uniform genetically and comprised of several (multiple) ITS rDNA Rts.**
- ✓ **Two contrasting patterns of ribotypic structure were observed in the densely sampled species: 1) a species comprises one/few frequent and multiple private/unique ribotypes (e.g. *R. rosea, R. integrifolia,* and *R. crenulata*); 2) nearly each sequence carry a unique ribiotype (e.g. *R. sacra, R. sexifolia, R. kirilowii* and others with sequences/ribotypes ration <2).**
- ✓ **In *Rhodiola*, a wide distribution range and morphological polymorphism of a species not necessarily implies its high ribotypic diversity. Different patter was observed with cpDNA data (György et al,. 2018).**

- ✓ **Proportion of the shared Rts was not exceptionally high in *Rhodiola* (10%), half of the species (29 out of 58) participated in the ribotype sharing.**
- ✓ **There could be several explanations for the shared genetic diversity: 1) specimens/DNA sample confusion, 2) hybridization and/or introgression between species, 3) incomplete lineage sorting.**
- ✓ **Apparently, the data set includes sequences obtained from misidentified specimens but we can't judge to what extent.**
- ✓ **Confusion is more likely for specimens falling outside otherwise robust/long-branched species clades or showing affinity to distantly related species. Discrimination of shared genetic diversity that is due to ancestral polymorphism retention or to a continuous or secondary gene flow between species/populations is difficult and was not an aim of our study. In case of four species most frequently contributing to shared ribotypes and having overlapping distribution (e.g. *R. fastigiata, R. alsia, R. discolor,* and *R. bupleoroides*) these reasons should be considered.**


Thank you very much
for your attention!

Rh. rosea subsp. *rosea* vs. *Rh. rosea* subsp. *sachalinensis* vs. *Rh. sachalinensis*

61 (60) Plants 20-30 cm tall; stem leaves elliptic-lanceolate, oblong, or broadly oblong-ovate; sepals ca. 1/3 as long as petals ***R. rosea***

+ Plants 10-15 cm tall; stem leaves oblong-spatulate, oblong-lanceolate, or oblong-rhombic; sepals ca. 1/2 as long as petals ***R. sachalinensis***





