Molecular phylogeny and detection of diploid hybrids in *Scorzoneroides* (Asteraceae)

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The genus *Scorzoneroides* of tribe Cichoriae, subfamily Cichorioidae, family Asteraceae comprises approximately 26 species distributed mainly in the Mediterranean and the mountain areas of Europe, from Sierra Nevada to the Carpathians. Previous, molecular studies seperated subgenus Oporinia from the genus *Leontodon* into a genus of its own, *Scorzoneroides*. The present investigation on *Scorzoneroides*, using Plastid DNA markers, such as *ndhF-rpl32*, *atpH-atpI* and the *rpl16* Intron, divides the genus *Scorzoneroides* into two groups, one of which is well supported (BS 95). The phylogeny of the nuclear ribosomal rDNA, ITS parallels that of the plastid. Work on two low copy markers namely *GAPDH* (Glycerine Aldehyde 3-Phosphate Dehydrogenase) and *A39* locus is in progress, to identify suspected homoploid hybridization within the genus *Scorzoneroides*.

Previous studies on *Scorzoneroides* (in chronological order)

Widder (1975) classified the genus *Leontodon* based on morphological characters as well as chromosome numbers. *Leontodon* is divided into two subgenera, *Leontodon* subg. *Leontodon* and *Leontodon* subg. *Oporinia* (Fig. 5).

A phylogenetic study based on cpDNA and ITS (Samuel *et al*., 2006) revealed that Leontodon, as described by Widder (1975), is diphyletic. Greuter *et al*. (2006) confirmed *Leontodon* subgenus *Oporinia* as a separate genus, *Scorzoneroides*.

A new investigation, based on molecular data, ecological distribution and morphological characters by Cruz-Mazo *et al*. (2009) confirmed the generic status of *Scorzoneroides* and also showed the genus could be divided into two subgenera (Fig. 10).

Present study

We focus on developing the most appropriate low-copy nuclear genes from the list of genes so far used in Asteraceae, to detect homoploid hybridization and to establish a more robust phylogeny of *Scorzoneroides* and related genera (*Leontodon* , *Hypochaeris*, *Picris*, *Helminthotheca*) with low-copy nuclear markers.

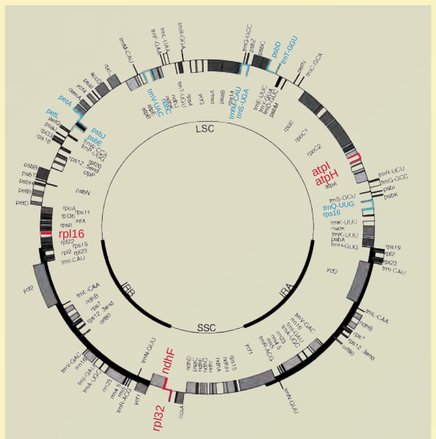

Fig. 1:Chloroplast genome. colored regions were tested, red indicates application in our study, blue stands for not used except for primer trials

Three chloroplast markers, namely *atpH-atpI*, *ndhF-rpl32* intergenetic spacer regions and the *rpl16* Intron were chosen from 9 tested regions to create a robust framework. The location of these regions in the chloroplast genome is shown in Fig. 1.

To detect possible hybridization events, we applied two low copy nuclear gene markers, *GAPDH* (Glycerine Aldehyde 3-Phosphate Dehydrogenase), published by Vaezi & Brouillet in 2009 and the *A39* locus published by Chapman *et al*. (2007). Details to these regions are given in Fig. 2 and 3.

Glyceraldehyde 3-Phosphate Dehydrogenase (*GAPDH*)

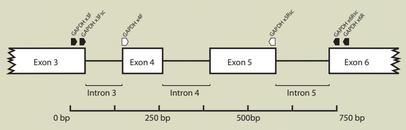

Fig. 2: Diagram of the *GAPDH* gene based on sequences of Scorzoneroides taxa. Primers used for amplification and sequencing are shown in black, while additional internal sequencing primers are shown in white.

A39 locus

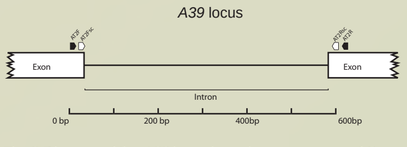

Fig. 3:Diagram of the *A39* locus based on sequences of *Scorzoneroides* taxa. Primers used for amplification are shown in black, while additional internal primers for sequencing are shown in white.

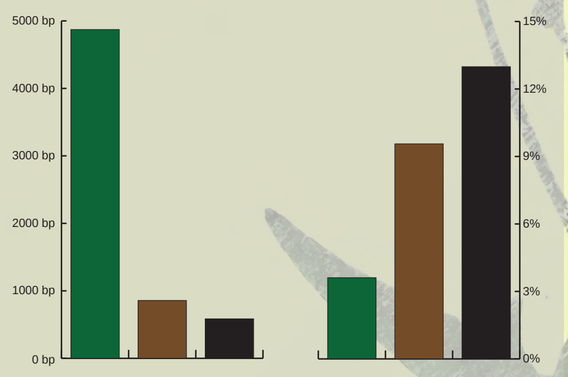

Fig. 4: Table showing length (left) and pecentage of potentially informative characters (PIC, right), comparing the three combined chloroplast markers (*ndhF-rpl32*, *atpH-atpI* intergenetic spacer region and *rpl16* Intron) labelled green, the low-copy nuclear marker *GAPDH* (brown) and the *A39* locus(black)

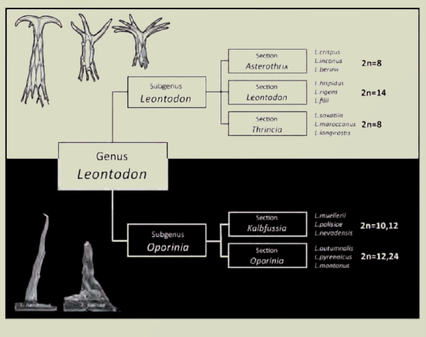

Fig. 5: Classification of *Leontodon* by Widder

Fig. 6: Distribution map of the genus *Scorzononeroides*. Dark brown colored areas indicate a high number of taxa, light brown colored areasindicating occurrence of several taxa. Estimated species number in a country given in white digits

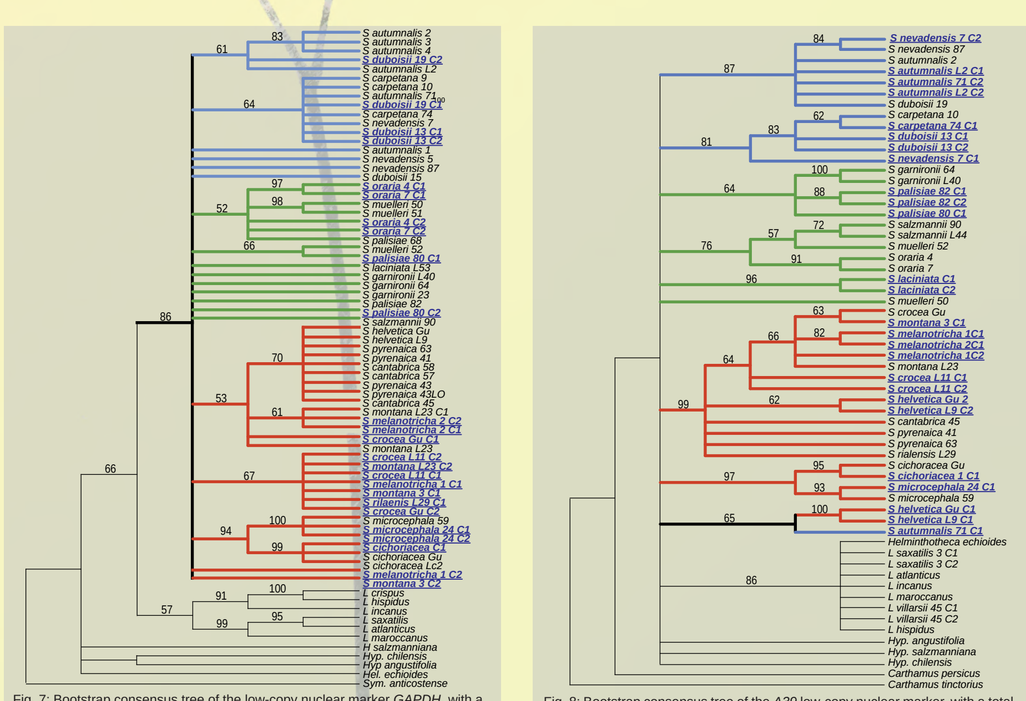

Fig. 7: Bootstrap consensus tree of the low-copy nuclear marker *GAPDH*, with a total length of 857 base pairs. Bootstrap values >50% are given above branches. Broad lines indicate membership to *Scorzoneroides*. Taxa which inhabit mediterranean or subdesertic areas are labelled in green, the perennial european mountain group (*S. autumnalis*, *S. duboisii*, *S. nevadensis*, and *S. carpetana*) is colored blue, whereas the group formed by exclusively perennial european mountain taxa with unbranched flowering stems is marked red. Bold, underlined names indicate that these sequences were obtained by cloning.

Fig. 8: Bootstrap consensus tree of the *A39* low-copy nuclear marker, with a total length of 585 base pairs. Bootstrap values >50% are given above branches. Broad lines indicate membership to *Scorzoneroides*. Taxa which inhabit Mediterranean or subdesertic areas are labelled in green, the perennial european mountain group (*S. autumnalis*, *S. duboisii*, *S. nevadensis*, and *S. carpetana*) is colored blue, whereas the group formed by exclusively perennial european mountain taxa with unbranched flowering stems is marked red. Bold, underlined names indicate that these sequences were obtained by cloning.

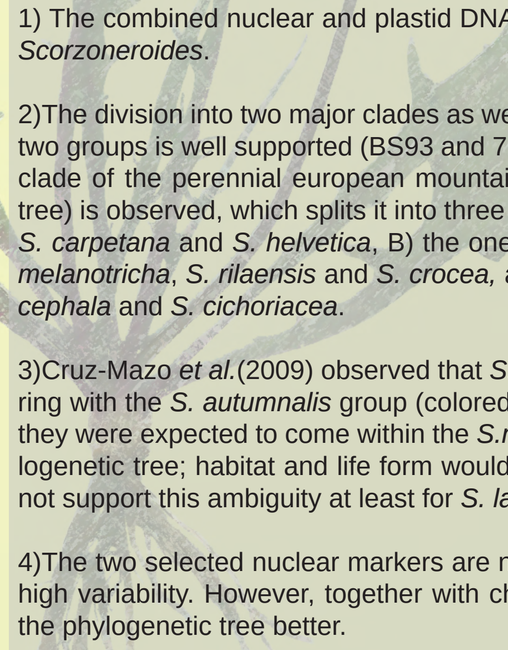

Fig. 9: Phylogenetic tree of the combined dataset of cpDNA (*ndhF-rpl32*, *atpH-atpI*, *rpl16* Intron) and low-copy nuclear markers (*GAPDH*, *A39* locus), comprising roughly 5000 base pairs in length. Number of changes and bootstrap values >50% are given above and below branches, respectively. Broad lines indicate membership to *Scorzoneroides*. Taxa which inhabit mediterranean or subdesertic areas are labelled in green, the perennial and european mountain group (*S. autumnalis, S. duboisii, S. nevadensis, and S. carpetana*) is colored blue, whereas the group formed by exclusively perennial european mountain taxa with unbranched flowering stems is marked red.

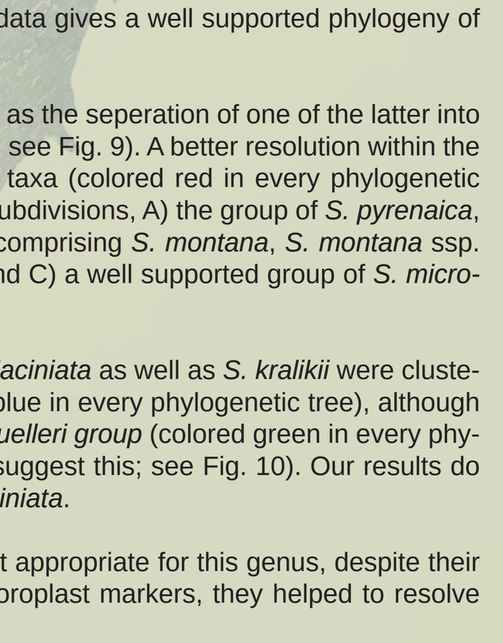

Fig. 10: Phylogenetic tree of the combined dataset of cpDNA (*ndhF-rpl32*, *rpl32-trnL*) and internal transcribed spacer (ITS) region sequenced by Cruz-Mazo *et al*. (2009). Number of changes and bootstrap values >50% are given above and below branches, respectively. Broad lines indicate membership to *Scorzoneroides* . Taxa which inhabit mediterranean or subdesertic areas are labelled in green, the perennial european mountain group (*S. autumnalis*, *S. duboisii*, *S. nevadensis*, and *S. carpetana*) is colored blue, whereas the group formed by exclusively perennial european mountain taxa with unbranched flowering stems is marked red.

Conclusions

1) The combined nuclear and plastid DNA data gives a well supported phylogeny of *Scorzoneroides*.

2)The division into two major clades as well as the seperation of one of the latter into two groups is well supported (BS93 and 71, see Fig. 9). A better resolution within the clade of the perennial european mountain taxa (colored red in every phylogenetic tree) is observed, which splits it into three subdivisions, A) the group of *S. pyrenaica*, *S. carpetana* and *S. helvetica*, B) the one comprising *S. montana*, *S. montana* ssp. *melanotricha*, *S. rilaensis* and *S. crocea,* and C) a well supported group of *S. microcephala* and *S. cichoriacea*.

3)Cruz-Mazo *et al.*(2009) observed that *S. laciniata* as well as *S. kralikii* were clustering with the *S. autumnalis* group (colored blue in every phylogenetic tree), although they were expected to come within the *S.muelleri group* (colored green in every phylogenetic tree; habitat and life form would suggest this; see Fig. 10). Our results do not support this ambiguity at least for *S. laciniata*.

4)The two selected nuclear markers are not appropriate for this genus, despite their high variability. However, together with chloroplast markers, they helped to resolve the phylogenetic tree better.

Acknowledgements:

This project was funded by KIÖS, Austrian Academy of Science, project number 2008-5

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