

**Progress Report to Israel Taxonomy Initiative Project:
Taxonomy, Systematics and Phylogeny of Radishes – *Raphanus* L. (Cruciferae)**

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1. Introduction

Though great advance has been made in modern classification of Brassicaceae, the status of many genera is still unresolved. A large number of species in the tribe Brassicaceae, including *Brassica* and *Erucastrum* was studied with molecular methods (Warwick and Sauder 2005). However the genus *Raphanus* – of which two out of three wild species do not widely occur and are poorly known were not included in the analysis.

The genus *Raphanus* comprises according to classical taxonomy (Zohary 1966), three wild species: *R. raphanistrum*, *R. pugioniformis*, *R. confusus* – and one cultivated species, the garden radish, *R. sativus* (Schulz 1919). The common attribute that places these three taxa under the genus *Raphanus* is the fruit's structure and function. In *Raphanus* mature fruits have a very short, persisting rudimentary lower silique segment which contains no seeds. The upper segment is indehiscent and contains the seeds. When ripe it abscises and falls from the plant, leaving the peduncle and lower segment on the mother plant.

While the fruit attributes unite all *Raphanus* species, there are other floral and ecological attributes in which the species differ from each other, but resemble to other members of other genera. For instance, flower venation appears on all *Raphanus* apart from *R. confusus*, but also in *Eruca sativa* and *Enarthrocarpus* spp.; the yellow, veinless flowers *R. confusus* resemble those of *Brassica* and *Hirschfeldia*.

The classification of species according to subjectively selected morphological characteristics, mainly of fruits, has been the main method in taxonomy (Bailey et al 2006). In recent years molecular genetic methods have provided insights into the

phylogeny in plants and indicate much more objectively the kinship of taxa and thus rejected some paradigms in crucifer classification.

In this study we aimed to resolve the generic and infra-generic phylogeny in the apparent genus *Raphanus*, as it has been recently accepted in the world Brassicaceae checklist (Warwick et al. 2006). We aim to resolve which morphological and biogeographic attributes best associate with phylogenetic divergence. We also aim to resolve the relationship between wild *Raphanus* species and the garden radish and to assign its potential wild ancestor.

Target species: The survey encompasses the whole genus *Raphanus*, namely *R. raphanistrum*, *R. sativus*, *R. pugioniformis* and *R. confusus*. All of them occur in Israel.

2. Methods

In this project we conducted a floristic survey and mapped populations of the target species. Ripe seeds were collected from select populations and will be used for phenotypic characterization in common garden experiments in future project. These will be used to identify subspecific variation. Whole plants were collected and were deposited at the Hebrew University of Jerusalem herbarium. Molecular phylogeny was used to examine the molecular classification of the three species by comparing the 5.8S rDNA and its Internal Transcribed Spacers (ITS). Several other species included in the analysis were *Hirschfeldia*, *Sinapis* sp., *Brassica* sp. and *Arabidopsis*.

In a common garden experiment we assessed morphometric variations within *R. raphanistrum*. We grew plants originated from three wild populations of *R. raphanistrum* in experimental field site in Bet Dagan, where each accession is represented by 14-15 plants. After fruit ripened we harvested five siliquae from each plant and measured: 1) length; 2) width; 3) the ratio between the pod and beak; 4) number of seed nodes; and 5) presence of trichomes. We then analyzed the polymorphism among accessions using ANOVA or contingency analysis.

3. Results

3.1. Analysis of GIS data: Survey population accessions mark very distinct distribution ranges to the wild *Raphanus* species (Fig. 1). *R. raphanistrum* is allopatric to its sibling species as its populations occur only on Hamra and Kurkar hills along the coastal plain

of Israel. *R. pugioniformis* and *R. confusus* (= *R. aucheri*) are sympatric, as they occur on mountain slopes mostly east of the watershed, from north Samaria to the Golan and Upper Galilee, with the exception of Menashe Hills. The allopatry of *R. raphanistrum* rules out *in natura* crossings with its sibling species, so in order to verify that these species are not interfertile, crossing trials are needed. However, its geographical isolation suggests earlier adaptive radiation. The sympatry of *R. confusus* and *R. pugioniformis* indicates strong reproductive separation between the two species and asserts their taxonomic separation.

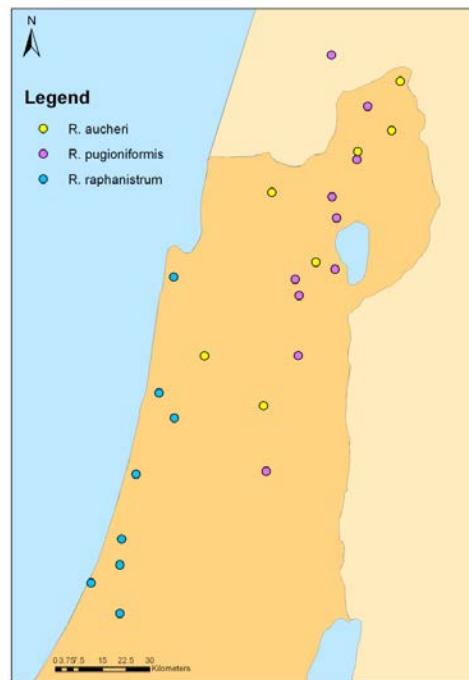


Figure 1. Distribution map of *Raphanus* species in Israel.

3.2. Multimorphometric analysis of *Raphanus raphanistrum* siliquae: A large number of subspecies, varieties and forms were assigned to wild radish (*R. rapahnistrum*). Our impression is that many taxonomic descriptions were based on fruit morphology which is highly variable within species. Here we aimed to determine the extent of fruit polymorphism within and among populations.

Siliqua dimensions were well correlated to geographical location but trichome presence seems to be unrelated to the accessions (Table 1). The most accession-related feature is the length of the siliqua. Number of seed nodes seems to be related to

accession, however the results should be interpreted with caution, as the variance is barely by one seed.

Table 1. Morphological evaluation of siliquae of *Raphanus raphanistrum*. Values present the mean and standard error of 215 measurements.

| Accession | length (mm) | Width (mm) | Pod/Beak | # Nodes | Trichomes* |
|------------------------|-------------|-------------|------------|------------|------------|
| Carmel, Makura | 51; 1.33 | 3.36; 0.094 | 2.54; 0.13 | 7.15; 0.25 | P=49 A=21 |
| Sharon, Givat Hayim | 47; 1.28 | 3.81; 0.091 | 3.03; 0.13 | 7.79; 0.24 | P=50 A=20 |
| Sharon, Hadassim | 41; 1.33 | 3.24; 0.094 | 2.27; 0.13 | 6.49; 0.25 | P=38 A=32 |
| R ² | 0.11 | 0.091 | 0.078 | 0.06 | 0.01 |
| P | <0.0001 | <0.0001 | 0.0002 | 0.0009 | 0.12 |

* P- presence, A-absence

3.3. Molecular phylogeny analysis: Since the submission of the last progress report we elaborated the molecular study and included several accessions as well as other genetically related species in order to elucidate the genetic relations among the *Raphanus* species. The ITS-based phylogenetic analysis (Fig. 2) clearly indicate that *Raphanus* species in a distinctively different order from that of classical systematics. *R. raphanistrum*, *R. sativus* and *R. pugioniformis* are placed one lineage, where *R. sativus* shows close relationship to *R. raphanistrum*. All these three seem to be congeneric. The *sativus-raphanistrum-pugioniformis* cluster is closer to *Hirschfeldia* than to *R. confusus*. This suggests that *R. confusus* should belong to a different genus and that the siliqua structure which was used as a diagnostic character of *Raphanus* is homoplastic. The closely related *R. sativus* and *R. raphanistrum* support the theory that the *R. raphanistrum* is the wild ancestor of the garden radish.

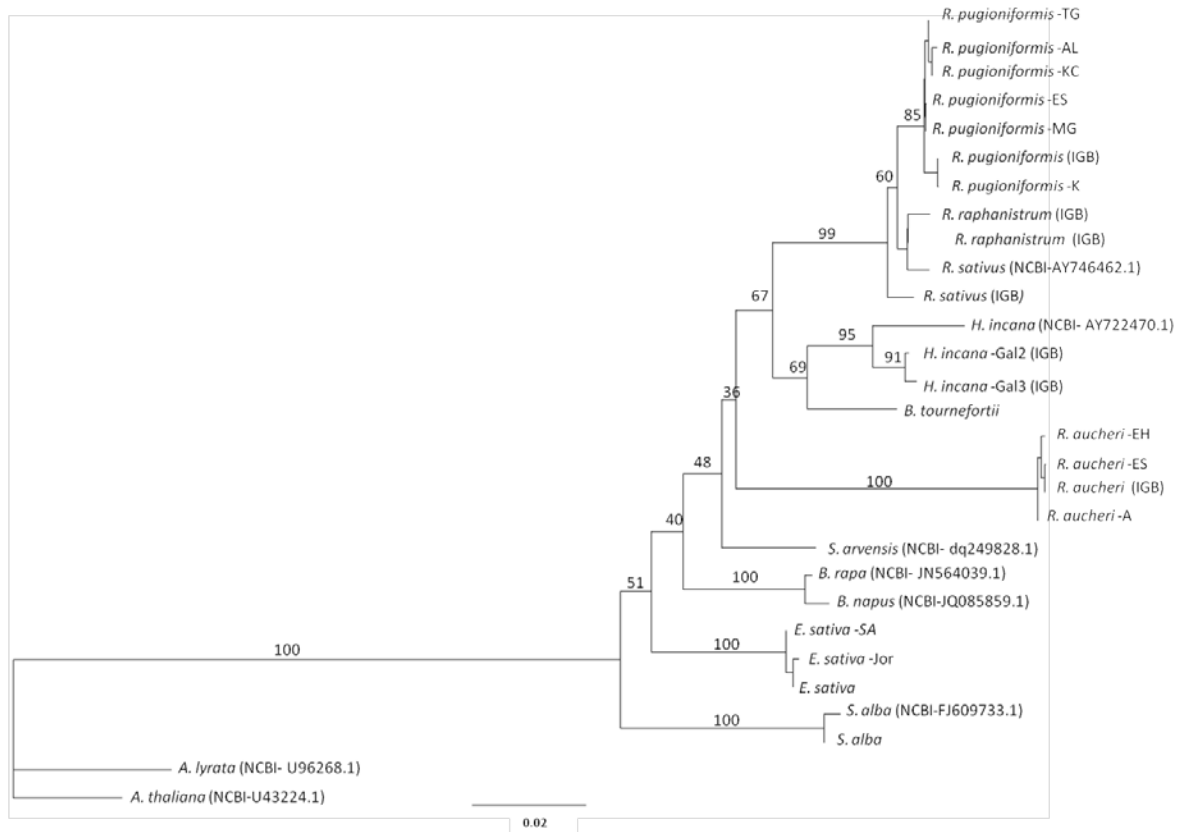


Figure 2. PHYML maximum likelihood tree of *Raphanus* spp.

The tree was constructed on the basis of the sequence of the ITS region of *R. raphanistrum*, *R. confusus* and *R. pugioniformis* used in this study, and sequences of other members of the Brassicaceae, several of them were sequenced and others derived from genebank (<http://www.ncbi.nlm.nih.gov>). Distance estimation was calculated using 100 bootstraps.

Abbreviations next to the species name indicate the collection site: ES- Ein ha'Shofet, MG- Ma'ale Gilboa, KC- Kefar Kana, TG, Tsomet Golani, A- Arbel, K- Kadori, EH, Emek ha'Shalom, AL- Alma; IGB- seeds were received from the Israel Plant Gene Bank.

4. Bibliography

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