Homenaje al Prof. Dr.

WOLFREDO WILDPRET DE LA TORRE

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INSTITUTO DE ESTUDIOS CANARIOS
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Smilax canariensis, *S. azorica* (Smilacaceae) and the genus *Smilax* in Europe

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Resumen: Un análisis morfológico y de sistemática molecular de las especies Europeas de *Smilax* dio como resultado el reconocimiento de una especie endémica de las Azores, *Smilax azorica* H. Schaef. & P. Schoenfelder, nom. nov. (= *S. divaricata* Sol. ex H. C. Wats., nom. illegit.). Su pariente mas cercano es *S. canariensis* Brouss. ex Willd. de Canarias y Madeira. Junto con la especie del este de Europa *S. excelsa* L., todas ellas forman un grupo dentro del clado de las especies norteamericanas, las cuales parecen pertenecer a un linaje asiático.
Palabras claves: Azores, biogeografía, Islas Canarias, refugio glacial, *Smilax azorica, Smilax canariensis*, *Smilax divaricata.*

Abstract: A morphological and molecular systematic analysis of the European species of *Smilax* results in the recognition of a species endemic to the Azores, *Smilax azorica* H. Schaef. & P. Schoenfelder, nom. nov. (= *S. divaricata* Sol. ex H. C. Wats., nom. illegit.). Its closest relative is *S. canariensis* Brouss. ex Willd. from the Canaries and Madeira. Together with the Eastern European *S. excelsa* L. they are nested in a clade of North American species, which seem to belong to an Asian lineage.
Key words: Azores, biogeography, Canary Islands, glacial refugia, *Smilax azorica, Smilax canariensis*, *Smilax divaricata.*

INTRODUCTION

The genus *Smilax* comprises about 200 species distributed mainly in the Northern hemisphere from the temperate regions to the Subtropics (CAMERON & FU, 2006). Diversity centres of the genus are located in Northern and Central America and East Asia, while the European region harbours only four species and Africa and Australia only two each (CAMERON & FU, 2006). The comparatively reduced diversity in European *Smilax* is presumably a result of the ice ages and is well known from other genera like *Quercus*, *Acer,* and *Cornus* (e.g. SVENNING *et al*., 2008; XIANG *et al*., 2006).

Of the four European species, one, *Smilax aspera* L., is widespread and often common throughout the Mediterranean region (Fig. 1). The three remaining species are restricted to glacial refugia: (i) *Smilax canariensis* Brouss. ex Willd. is endemic to the Canary Islands and Madeira (SCHOENFELDER & SCHOENFELDER, 2005), (ii) *Smilax azorica* H. Schaef. & Schoenfelder, nom. nov. (= *S. divaricata* Sol. ex H. C. Wats., nom. illegit., see DISCUSSION)

is known only from the Azores, and (iii) *Smilax excelsa* L. is found mainly in the Black Sea and Caspian Sea region from Greece and Bulgaria to Iran (BROWICZ, 1988).

Material and methods

Morphology

The authors studied herbarium material at AZU, BM, K, LISU, M, and REG. Both authors spent many months of fieldwork in the Mediterranean region and the middle-Atlantic Islands and studied morphology and ecology of *Smilax* in many different countries and habitats.

Sampling and DNA extraction

Total genomic DNA was isolated from herbarium specimens or, more rarely, silica-dried material following the standard CTAB method of DOYLE & DOYLE (1987). We amplified the *rbcL* and *matK* genes, the *trnL* intron and the *trnL-F* intergenic spacer. Polymerase chain reactions (PCR) were performed with the standard protocol and primers described in SCHAEFER *et al.* (2008), and products were purified with the Wizard SV PCR clean-up kit (PROMEGA GmbH, Mannheim, Germany). Cycle sequencing was performed with BigDye Terminator cycle sequencing kits on an ABI Prism 3100 Avant automated sequencer (Applied Biosystems, Foster City, California, USA).

In addition to these plastid regions, we sequenced the nuclear internal transcribed spacer region using the ITS primers of CAMERON & FU (2006). Direct PCR amplification of ITS yielded single bands and unambiguous base calls. Twenty-seven sequences were generated for this study. Table 1 lists the relevant taxonomic names with authors and plant sources. All new sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/). Additional sequences for Asian and American species (mostly generated by CAMERON & FU, 2006) were downloaded from GenBank.

Sequence alignment and phylogenetic analyses

Sequences were edited with Sequencher (4.6; Gene Codes, Ann Arbor, Michigan, USA) and aligned by eye, using MacClade 4.06 (MADDISON & MADDISON, 2003). The aligned plastid matrix comprised 3517 nucleotides. The aligned ITS matrix comprised 867 nucleotides. Maximum likelihood (ML) tree searches and ML bootstrap searches were performed using RAxML 7.0.3 (STAMATAKIS *et al.* 2008, available at http://phylobench.vital-it.ch/raxml-bb/). RAxML searches relied on the GTR + G + I model (six general time-reversible substitution rates, assuming gamma rate heterogeneity and a proportion of invariable sites), with model parameters estimated over the duration of specified runs. Analyses in RAxML were run both with the combined un-partitioned data and with a model that partitioned the plastid regions from the ITS region. Trees were rooted on *Philesia magellanica* (sequences from GenBank). The data matrix and trees have been deposited in TreeBASE (http://www.treebase.org/).

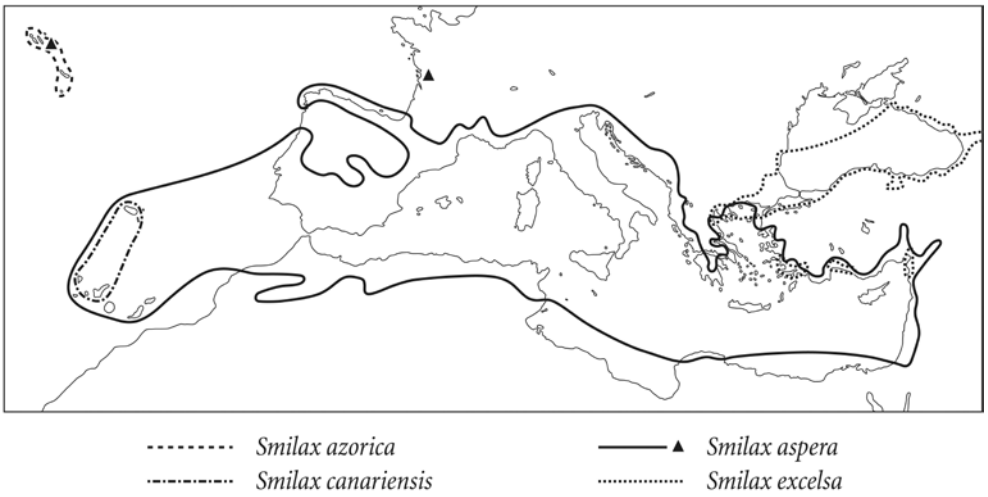

Figure 1. European distribution of *Smilax* species (modified after BOLÓS & VIGO, 2001; BROWICZ, 1988).

Table 1. Species sampled and their origin.

SPECIES	ORIGIN
Smilax aspera L. subsp. *aspera*	France, Camargue
Smilax aspera L. subsp. *balearica* (Willk.) Romo	Spain, Balearic Islands, Mallorca
Smilax aspera L. subsp. *mauritanica* (Desf.) Malag.	Spain, Canary Islands, Tenerife
Smilax canariensis Brouss. ex Willd.	Portugal, Madeira
Smilax canariensis Brouss. ex Willd.	Spain, Canary Islands, Tenerife
Smilax azorica H. Schaef. & P. Schoenfelder	Portugal, Azores, São Miguel
Smilax excelsa L.	Georgian Republic
Smilax hispida Muhl. ex Torr.	United States, seeds bought from "BT World Seeds"
Smilax lasioneura Hook.	United States, seeds bought from "BT World Seeds"

Results

Morphology

The mediterranean *Smilax aspera* differs from the remaining European species above all in its inflorescences, which are composed of several umbel-like sub-inflorescences and often more than 20 cm long (fig. 2a). All other European taxa have their flowers in simple umbels (SCHOENFELDER & SCHOENFELDER, 2005; our Fig. 2 c-d, Fig. 3).

The leaves of *S. aspera* are coriaceous and very variable, usually with 7-9 main nerves and a ± cordate base. The leaves of the remaining species are laurophyllous (*S. canariensis*, *S. azorica*) or deciduous (*S. excelsa*) with usually 3-5 main nerves and a cuneate to rounded or shallowly cordate base (BROWICZ, 1988; S. Arndt, Jena Botanical Gardens, pers. comm.). Leaf shape is variable, especially on young shoots. Leaves on second-year or older shoots are more uniform in shape and in general broadly ovate in *S. azorica* and more narrowly ovate in *S. canariensis*. The older stems of *S. excelsa* carry considerable thorns (Fig. 2d), while thorns on stems of *S. canariensis* and *S. azorica* are small or absent.

A taxon with extremly narrow leaves that has been described as *S. aspera* subsp. *balearica* (Willk.) Romo is apparently restricted to the Balearic Islands and accepted as an endemic variety in BOLÒS & VIGO (2001), but not accepted as a separate taxon by AEDO (2005). Forms with broadly cordate leaves that lack thorns almost completely are known as *S. aspera* subsp. *mauritanica* (Desf.) Malag. (= *S. altissima* Roxb.). They are found in the Western Mediterranean region and in the Canary Island's laurel forest but also in dry lowland areas and cliffs on Madeira (PRESS & SHORT, 1994), and the Azores (Terceira Island). The Madeiran plants have been described as endemic species *S. pendulina* Lowe but they do not differ considerably from *S. aspera* subsp. *mauritanica*.

Phylogenetic analyses

The topologies of the best likelihood tree for the plastid and ITS datasets (not shown) were not contradicting in any well-supported node. We therefore combined the data and in the following focus on the result of the combined data (Fig. 4). Resolution and bootstrap support was in general low, a problem already reported in previous studies (CAMERON & FU, 2006). However, the placement of *S. aspera* as sister to all other analysed ingroup taxa is moderately supported. Furthermore, we found support for a clade consisting of the North American *S. herbacea* and other American species, a clade of Asian species, and a clade consisting of *S. china*, two North American species, *S. excelsa*, and the middle-Atlantic island species. The Azorean plants are cleary different from *S. canariensis*, while the sample from Madeira seems to be genetically very close to the Canary Island plants.

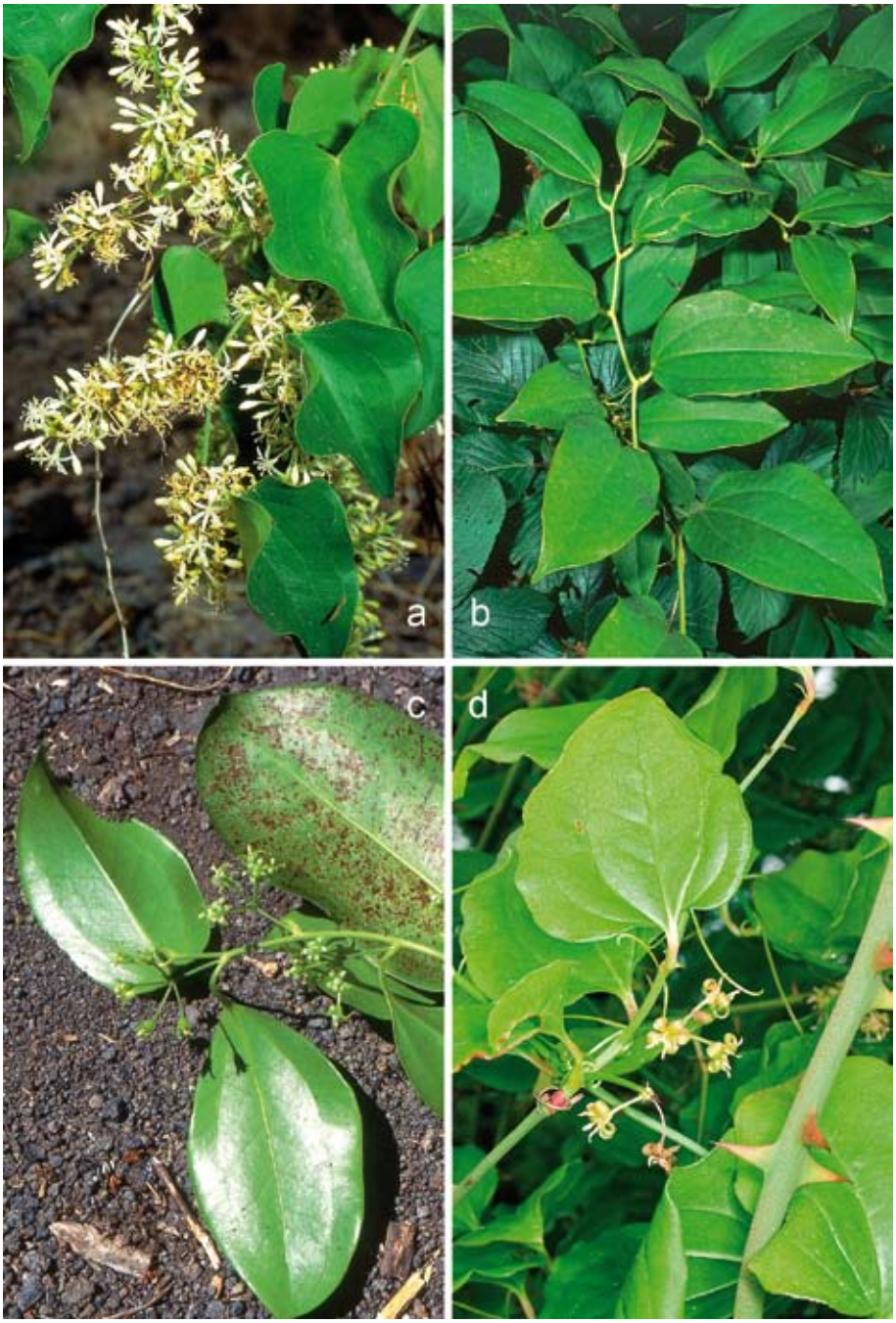

Figure 2. Inflorescences and leaves of European *Smilax*: a) *S. aspera* subsp. *mauritanica* (La Palma, Canary Islands, 10-10-1993); b) *S. canariensis* (La Palma, Canary Islands, 7-10-1993); c) female inflorescence of *S. azorica* (Faial Island, Azores, 17-7-1999); d) male inflorescence of *S. excelsa* (Botanical Garden, Jena, 31-5-2003).

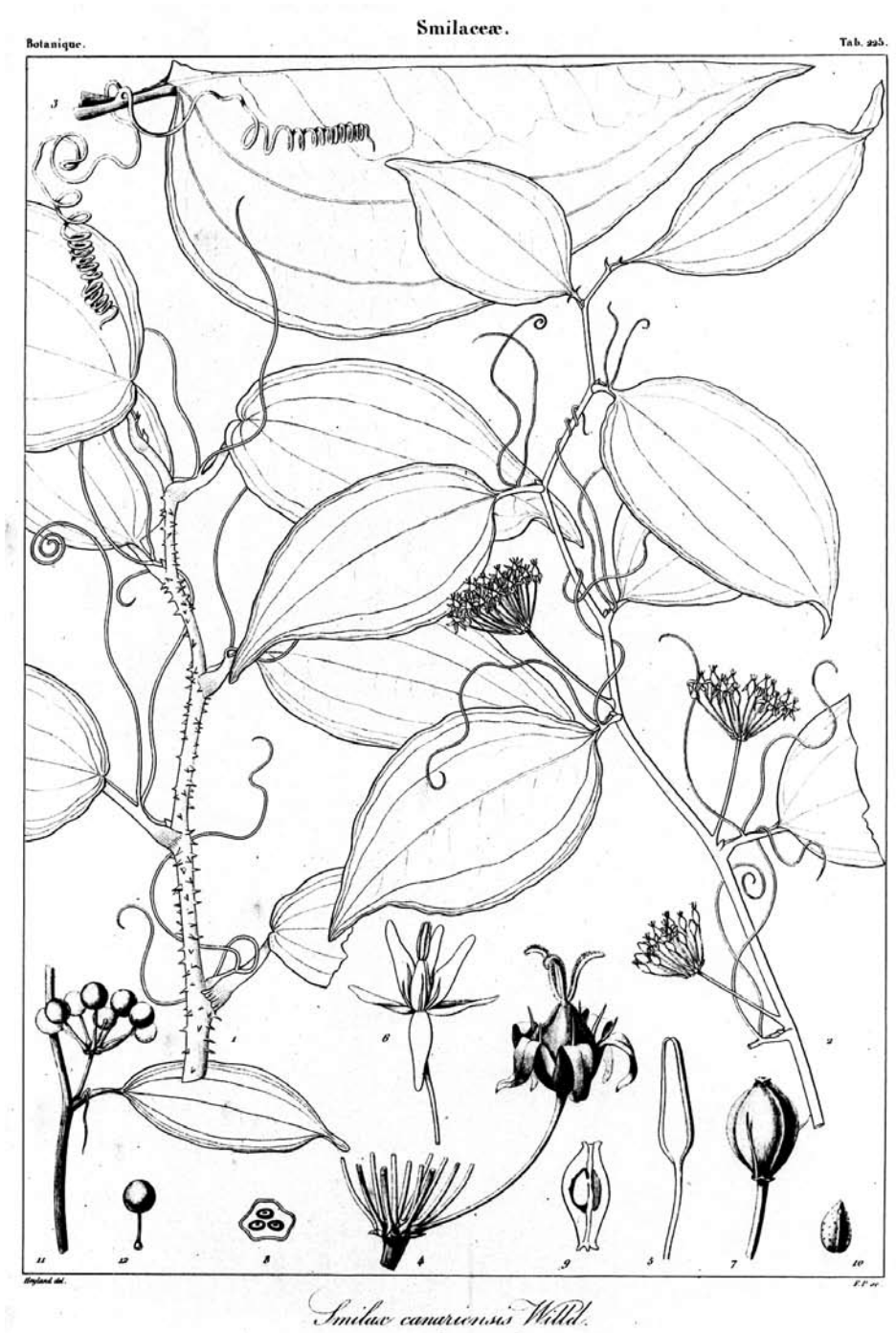

Figure 3. Illustration of *S. canariensis* reproduced from WEBB & BERTHELOT (1847).

KEY TO THE EUROPEAN SPECIES OF *SMILAX*

1 Leaves usually with 7-9 main nerves. Male and female inflorescences compound of several umbel-like sub-inflorescences. Flowering time VIII-XI. Fruit ripening blackish-red..... *S. aspera*

1* Leaves usually with 3-5 main nerves. Male and female inflorescence a simple umbel..... 2

2 Plant deciduous, older stems with strong thorns. Flowering time V-VI. Black sea and Eastern Mediterranean region .....*S. excelsa*

2* Leaves wintergreen, thorns on older stems weak or absent. Flowering time V-VIII. Middle-Atlantic Islands 3

3 Leaf blades on older branches broadly cordate-ovate, almost as broad as long (relation length:width c. 1:0.9). Fruit ripening red. Endemic to the Azores..... *S. azorica*

3* Leaf blades on older branches narrower (relation c. 1: 0.6). Fruit ripening black (fide WEBB & BERTHELOT, 1847). Endemic to the Canary Islands and Madeira .....*S. canariensis*

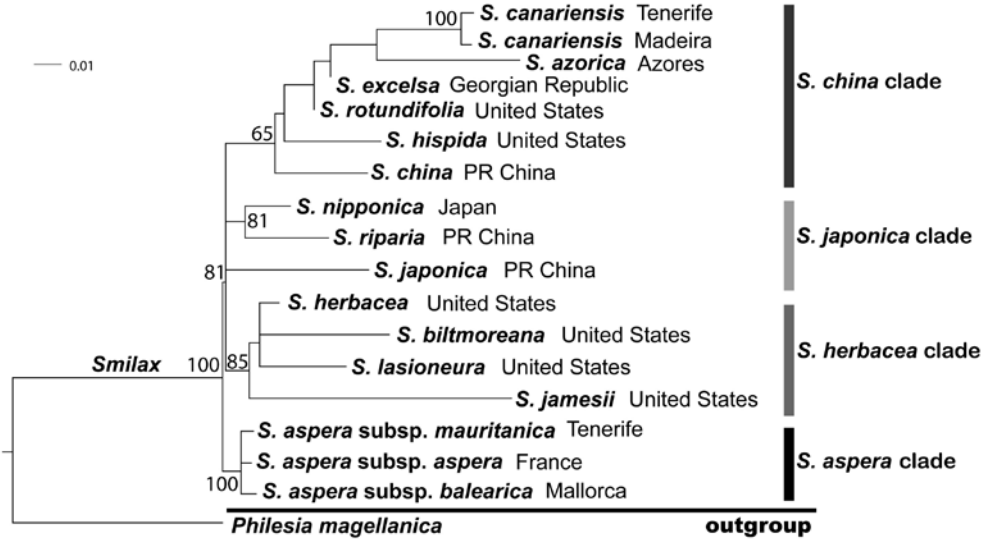

Figure 4. Maximum likelihood phylogram of *Smilax* plastid and ITS sequences produced with RAxML 7.0.3 (STAMATAKIS *et al.* 2008). Likelihood bootstrap support values > 60% are given at the nodes.

DISCUSSION

Taxonomy

Our results support the separation of a species endemic to the Azores from *S. canariensis*, endemic to the Canaries and probably Madeira. The species from the Azores was first collected by Francis Masson in 1777 on São Miguel (specimens in BM) and later described as *Smilax divaricata* Sol. ex H. C. Watson (WATSON, 1844), a name that had already been

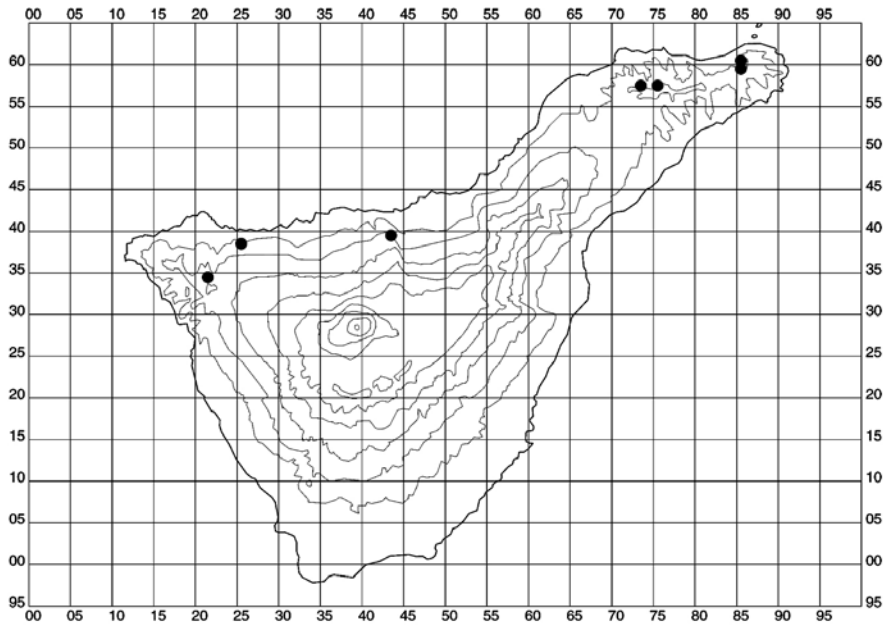

Figure 5. Distribution of *S. canariensis* on Tenerife, Canary Islands.

given to a species from the Philippines seven years earlier (BLANCO, 1837). Therefore the Solander name is a later homonym and illegitimate. A new epithet is required, which we propose as follows:

Smilax azorica H. Schaef. & P. Schoenfelder, nom. nov.
replaced synonym: *Smilax divaricata* Sol. ex H. C. Wats. in London J. Bot. 3: 608. 1844. – Type: F. Masson s.n. (holo BM!), Portugal, Azores, Sao Miguel 1777. – (non *Smilax divaricata* Blanco, Fl. Filip. 795. 1837).

HANSEN & SUNDING (1993) listed both species *Smilax canariensis* and *S. divaricata* (= *S. azorica* H. Schaef. & P. Schoenfelder, nom. nov.) in their Azores checklist and also added the East European *S. excelsa*, certainly a mistake. SCHAEFER (2003, 2005) based on morphology only, treated *S. divaricata* as a synonym of *S. canariensis* but with our new genetic data, this view is no longer supported. SEUBERT (1844) lists *S. tetragona* L.f. in his "Flora Azorica", a synonym for *S. aspera* subsp. *mauritanica*, but his description of plants from Pico Island (Azores) matches *S. azorica*. The specimen C. Hochstetter 121, cited by Seubert, was studied at BM and identified as *S. azorica*.

Biogeography

The European *Smilax* species clearly belong to two long separated lineages: the widespread *S. aspera* is sister to all other *Smilax* species (see also CAMERON & FU, 2006),

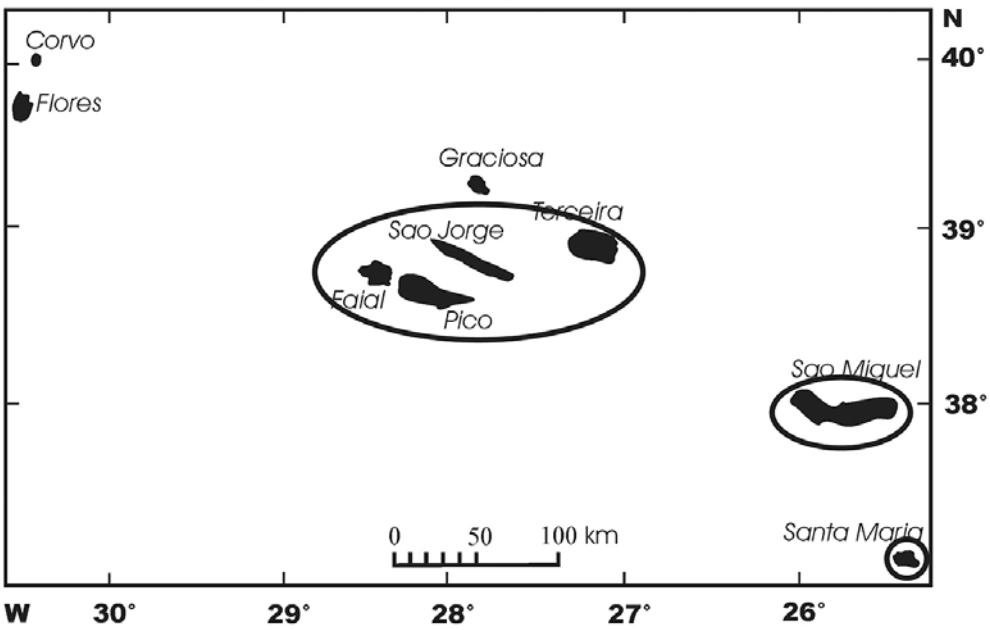

Figure 6. Distribution of *S. azorica* in the Azores archipelago.

while the remaining species *S. excelsa*, *S. canariensis*, and *S. azorica* form a monophyletic group. Their closest relatives seem to be North American species but these belong to an Asian lineage. All possible biogeographic scenarios locate the ancestors of the *S. canariensis* group in Asia. From there, in the most parsimonious scenario, the lineage spread via Beringia into North America and from there across the then narrow North Atlantic back into the European continent. During the glacial periods, this ancestral lineage was split into an eastern population in the Black sea region and a western population in the middle-Atlantic Islands. The lack of genetic exchange between the Azores and the Madeira/Canary islands population finally resulted in the evolution of two endemic species. An alternative, less parsimonious scenario would require at least two independent dispersal events from Asia into North America and one dispersal/range expansion of the *S. canariensis* lineage from Asia directly into the Mediterranean and the middle-Atlantic islands. A broader genetic analysis of *Smilax* samples from the islands, from North America, Asia, and especially from Africa (one or two endemic species) combined with molecular clock dating techniques will be required to confirm one of these scenarios.

Conservation

Today, *S. canariensis* is very rare in the Canaries and restricted to the central laurel forest regions on Tenerife (Fig. 5), La Palma, and La Gomera. On Madeira, it is a poorly known species that was collected only a few times (PRESS & SHORT, 1994). At least in the Canary Islands, it seems to be highly threatened. Despite years of search, the senior author could find

only one fruiting individual on La Palma and was unable to find a single flowering specimen in the archipelago. Notably, there is not a single picture of *S. canariensis* inflorescences in the contemporary literature on the Canary island flora and the flora of Madeira. An excellent description and a plate with flowers in all details, however, can be found in WEBB & BERTHELOT (1847) (our Fig. 3). Apparently, the species was reproducing more frequently in those days. Maybe the more intense use of the laurel forest in the 19th century produced more open spaces and clearings and favoured less competitive species like *S. canariensis*.

Smilax azorica, is known from six islands of the Azores archipelago (Fig. 6). While absent in the western group and rare in the central group, it is locally common on São Miguel and Santa Maria in the eastern group. Flowers and fruits are regularly found and the species seems to be not threatened. It is protected by the Berne convention on the conservation of European wildlife and natural habitats.

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