



## A taxonomic reassessment of *Viburnum* (Adoxaceae) in the Azores

MÓNICA MOURA<sup>1</sup>, MARK A. CARINE<sup>2</sup>, VALÉRY MALÉCOT<sup>3,4,5,6</sup>, PAULA LOURENÇO<sup>7</sup>, HANNO SCHAEFER<sup>8</sup> & LUÍS SILVA<sup>1</sup>

<sup>1</sup>CIBIO Research Center in Biodiversity and Genetic Resources, CIBIO-Azores, Department of Biology, University of the Azores, Portugal.  
E-mail: moura@uac.pt

<sup>2</sup>Department of Life Sciences, The Natural History Museum, Cromwell Road, London, SW7 5BD, UK.

<sup>3</sup>Agrocampus Ouest, UMR1345 IRHS, 2 rue Le Nôtre, F-49045 Angers, France

<sup>4</sup>INRA, UMR1345 IRHS, F-49071 Beaucouzé, France.

<sup>5</sup>Université d'Angers, UMR1345 IRHS, F-49100 Angers, France.

<sup>6</sup>LUNAM Université, France.

<sup>7</sup>SEEBMO, HSEIT, Angra do Heroísmo, Ilha Terceira, Portugal and Genetics & Arthritis Research Group (GARG), IBMC-Institute for Molecular and Cell Biology, Porto, Portugal.

<sup>8</sup>Plant Biodiversity Research, Technische Universität München, D-85354 Freising, Germany.

### Abstract

The taxonomic status of the Azorean endemic *Viburnum tinus* subsp. *subcordatum* is reassessed, using morphological characters and new molecular data from the ITS region and the *trnK* intron. A survey of morphological variation supports the recognition of *V. tinus* subsp. *subcordatum* as distinct from *V. tinus* subsp. *tinus* and the Canary endemic *V. rugosum* (formerly known as *V. tinus* subsp. *rigidum*) based on leaf shape, the shape of the leaf base and apex, the sub-entire and revolute leaf margins, blistered upper leaf surfaces, trichome density and type, and fruit size. Molecular data also confirm this distinctiveness within section *Tinus*. Taken together, our morphological and genetic data presented in this paper support the recognition of the Azorean taxon at the species level under the name of *Viburnum treleasei*. A description of the species is provided and nomenclatural issues relating to the two Macaronesian *Viburnum* taxa are discussed.

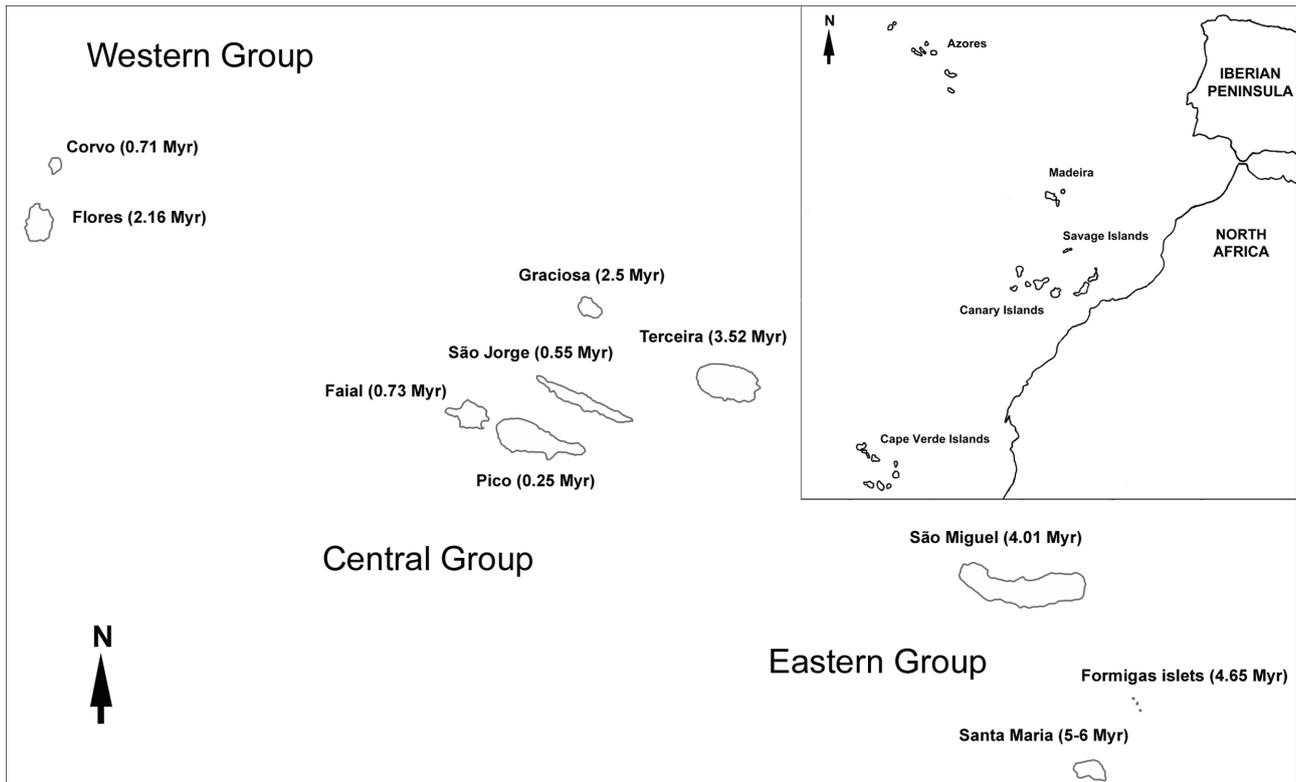
**Key words:** Endemic, taxonomy, Azores Islands, Canary Islands, Mediterranean

### Introduction

The genus *Viburnum* Linnaeus (1753: 267–268) (Adoxaceae) is estimated to contain between 175 to 230 species occurring primarily in the temperate regions of the Northern Hemisphere and secondarily in subtropical portions of Asia and Latin America (Malécot 2002, Donoghue *et al.* 2004). Traditionally, the genus has been subdivided into ten sections on the basis of morphological characters (Oersted 1861, Hara 1983). The monophyly of all sections was supported by morphological data and molecular studies, except sections *Megalotinus* and *Odontotinus* (Donoghue 1983, Baldwin *et al.* 1995, Donoghue *et al.* 2004, Winkworth & Donoghue 2005, Clement & Donoghue 2011, 2012, Schmerler *et al.* 2012). *Viburnum tinus* Linnaeus (1753: 267–268), placed in section *Tinus*, is a morphologically variable taxon in which two to three subspecies have been recognised. In addition to *V. tinus* subsp. *subcordatum* (Trelease 1897: 118) P. Silva in Palhinha (1966: 115–116) that is endemic to the Azores, *V. tinus* subsp. *tinus* is distributed throughout the Mediterranean basin including most of the Mediterranean islands. A third taxon, endemic to the Canaries, has been known as *Viburnum tinus* L. subsp. *rigidum* (Ventenat 1805: 98) P. Silva in Palhinha (1966: 116) but as we show below, the correct name is *Viburnum rugosum* Persoon (1805: 326). It was resolved as sister taxon to *V. tinus* subsp. *tinus* in studies by Clement & Donoghue (2011, 2012) and Schmerler *et al.* (2012).

The Azores archipelago comprises nine islands located in the North Atlantic Ocean, on a WNW-ESE axis between 37–40°N and 25–31°W (Fig. 1). The archipelago is volcanic and oceanic in origin and is relatively young, the oldest island being Santa Maria (5–6 Myr, Ávila *et al.* 2012) and the youngest Pico (0.25 Myr, França *et al.* 2003). The Azores span 615 km and the archipelago is isolated from other landmasses as it is situated 1,435 km west from the European coast (mainland Portugal), 3,380 km east from North America and 942 km northwest of Madeira.

The occurrence of a *Viburnum* in the Azores was first mentioned by the chronicler Gaspar Frutuoso (158?) as “folhado”, while the earliest botanical references were made by Seubert & Hochstetter (1843), and Seubert (1844), who called it *V. tinus*. Watson (1844, 1847, 1870) suggested that the differences shown by the Azorean specimens consisted only of more obtuse and coriaceous leaves, not significant enough for defining it a variety or a species, so he maintained the name *V. tinus*. However, Trelease (1897) recognised an Azorean endemic variety, *V. tinus* var. *subcordatum*, based on leaf morphology and Gandoger (1899) later considered it to be a distinct species, under the name *V. treleasei* Gandoger (1899: 255). Both Gandoger (1899) and Trelease (1897) suggested that the Azorean endemic *Viburnum* showed greater similarity with the Canarian endemic *V. rugosum* than with the Mediterranean *V. tinus* subsp. *tinus*. Pinto da Silva in Palhinha (1966) proposed that the variety described by Trelease should be recognised at the subspecific level, whilst Rivas-Martínez *et al.* (2002) raised the taxon to species rank proposing the name *V. subcordatum* Rivas-Martínez *et al.* (2002: 709), a *nomen superfluum*, given the validly published name at this rank (*V. treleasei*) proposed previously by Gandoger (1899).



**FIGURE 1.** Map of the Azores archipelago with geological ages. Inset: the location of the Azores relative to other land masses.

Recently, a population genetic study published by Moura *et al.* (2013) revealed that the Azorean populations of *V. tinus* subsp. *subcordatum* are genetically distinct from *V. tinus* subsp. *tinus* from Serra da Arrábida in mainland Portugal, although the *V. tinus* subsp. *subcordatum* population of the easternmost island Santa Maria showed a significant admixture pattern with the Arrábida population.

In this paper, we examine the status and relationships of the Azorean endemic *Viburnum tinus* subsp. *subcordatum*. We use DNA sequence data from the nuclear ribosomal internal transcribed spacer (ITS) region and the chloroplast *trnK* intron region, together with morphological data to address two questions: 1) Is the recognition of an endemic Azorean *Viburnum* taxon justified and, if so at which rank? 2) What are the relationships of the Azorean taxon within section *Tinus* and specifically with the Canary Islands endemic?

## Materials and Methods

**Morphological data:**—Morphological variation in *V. tinus* subsp. *subcordatum*, *V. rugosum* and *V. tinus* subsp. *tinus* was initially assessed based on 91 characters described by Seubert (1844), Trelease (1897), Gandoger (1899), Tutin *et al.* (1976), Franco (1984) and Donoghue (1983). Eighteen characters exhibited some degree of variation among the

samples and these were subsequently scored for the following sample: (i) a total of 73 individuals of *V. tinus* subsp. *subcordatum* sampled fresh from five populations growing on the island of São Miguel in addition to herbarium specimens of this taxon from Santa Maria, São Miguel, Terceira, Pico and Flores at AZB, AZ, MO and NY (55 specimens); (ii) fresh specimens of *V. tinus* subsp. *tinus* from two Portuguese mainland populations located in Serra da Estrela Natural Park and in Serra da Arrábida, together with herbarium material of this taxon from Spain, France, Italy, Slovenia, Croatia, Greece, Russia (Altai Republic), Turkey, Israel, Tunisia, Libya, Algeria and Morocco held at BM, B and M (50 specimens); (iii) fifteen fresh specimens of *V. rugosum* from a population on Gran Canaria together with BM and NY specimens of this taxon from the islands of Tenerife, Gran Canaria and La Palma (17 specimens). Qualitative leaf characters were evaluated using mature leaves with leaf shape classification following Hikey & King (2000). Leaf colour was assessed with the RHS Colour Chart (5<sup>th</sup> Edition) for both fresh and dried herbarium specimens. When a perfect match with the available chart colours was not possible the closest values were selected.

To investigate geographical patterns of morphological variation in the complex, we first grouped accessions by region as follows: (i) Azores (*V. tinus* subsp. *subcordatum*), (ii) Canaries (*V. rugosum*), (iii) Europe (mainland Portugal, Spain, France, Italy, Slovenia, Croatia, Greece), (iv) Mediterranean Islands (Corsica, Sardegna, Elba, Corfu, Lussino, Rab, Mljet, Lokrum), (v) Middle East (Turkey and Israel), (vi) Asia (Russian Altai Republic), and (vii) North Africa (Tunisia, Morocco, Libya, Algeria) (regions (iii) to (vii) all *V. tinus* subsp. *tinus*).

ANOVA was used to analyse variation between these regions for the following character sets: (i) leaf characters (length, width and the ratio width/length (W:L) of leaves), (ii) inflorescence characters (length of peduncles), and (iii) fruit characters (length, maximum and corresponding minimum diameter at cross-section, the ratio between diameters ( $D_{\max}:D_{\min}$ ), and the ratio length/mean diameter (L:D) of fruits). When ANOVA indicated significant differences, Tukey HSD test or Dunnett T3 test were used for multiple comparisons between regions.

The analysis of leaf traits did not include Asia, while the fruit trait analysis did not include Asia or the Middle East, due to the low number of replicates available. A Principal Component Analysis for Categorical Data (CATPCA) was applied to the leaf trait data, including also general leaf shape and the shape of leaf apex and leaf base. Specimens were represented in a scatter plot based on scores for the first two factors. A Principal Component Analysis (PCA) was also applied to the inflorescence and fruit data and specimens were represented in a scatter plot based on scores for the first two factors.

**Molecular data sampling:**—Sampling of *V. tinus* subsp. *subcordatum* was conducted in all three Azorean island groups (Western, Central and Eastern). In the Eastern group, populations in São Miguel and Santa Maria were sampled. In the Central group, samples were taken from Pico and in the Western group, material was sampled from Flores. In total, leaf samples from 13 individuals pertaining to nine Azorean *V. tinus* subsp. *subcordatum* populations were included in the analyses. For *V. tinus* subsp. *tinus*, leaves of two individuals were sampled from populations in mainland Portugal (Setúbal, Arrábida), France (Hérault, Montpellier) and Morocco (Meknés-Tafilalet, Aïn-Leuh). Leaf samples were also obtained from herbarium specimens collected in mainland Spain (Málaga, S. Pedro), Italy (Toscana, Livorno), Croatia (Rab), Israel (Har Ha’Karmel), Greece (Preveza, Kato Myrsini), Turkey (Boğaziçi, Tarabya), Algeria (Annaba, Laverdure), Tunisia (Zaghuan, Djebel Zaghuan) and Morocco (Beni-Mellal, El-Ksiba). Finally, one or two samples per population of *V. rugosum* from the Canary Islands were collected in Gran Canaria (Moya, Barranco de Los Tilos), Tenerife (Güímar, Barranco de Badajoz), El Hierro (Frontera, Tábano), La Palma (Barlovento, Las Mimbreras) and La Gomera (Hermigua, El Rejo). GenBank sequences for other taxa pertaining to section *Tinus* were also included in the analysis, namely *V. propinquum* Hemsl. in Forbes & Hemsley (1888: 355), *V. cinnamomifolium* Rehder (1913: 31), *V. atrocyanum* Clarke (1882: 7), *V. calvum* Rehder (1911: 310) and *V. davidii* Franchet (1885: 251), all native from China. Based on the results of Donoghue *et al.* (2004) and Schmerler *et al.* (2012) we selected the following outgroup taxa: *V. acerifolium* Linnaeus (1753: 268), *V. flavescens* Smith (1916: 139), *V. edule* (Michaux 1803: 180) Rafinesque (1808: 354), *V. ellipticum* Hooker (1833: 280), *V. dentatum* Linnaeus (1753: 268), *V. farreri* Stearn (1966: 22), *V. lutescens* Blume (1825: 655) and *V. taiwanianum* Hayata (1911: 137). The GenBank sequences for the outgroup taxa and additional taxa within section *Tinus* were from the studies of Donoghue *et al.* (2004) and Clement & Donoghue (2011, 2012). A list of the accessions used in the molecular study is available in the Appendix.

**DNA extraction:**—DNA from Azorean material was extracted from fresh leaves using DNeasy Plant Mini kit (Qiagen, Portugal), while DNA from herbarium and silica gel dried material was extracted using the modified CTAB protocol outlined by Carine *et al.* (2004).

**PCR and sequencing:**—Primer selection followed Donoghue *et al.* (2004). All amplification reactions were performed in 25 µL reactions containing 10 µmol/L (1 µL) of the respective primers, and approximately 35 ng of purified DNA (1 µL). The ITS region was amplified using either (i) the protocol of Carine *et al.* (2004) or (ii) 0.2 ml puReTaq illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare, Portugal) and the following thermocycling PCR

profile: i) initial denaturation at 98°C for 3 min; ii) 35 cycles consisting of denaturation, 1 min at 95°C; annealing, 1 min at 56°C and extension, 1 min at 72°C; iii) final extension of 5 min at 72°C. Amplification of the *trnK* intron followed either the second ITS protocol outlined above, with the thermocycling PCR profile modified to include an annealing temperature of 54°C and a 2 min extension time at 72°C or the PCR protocol described by Carine *et al.* (2007) for the *trnH-psbA* region. All amplification products were purified using the illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Portugal). Sequencing of PCR fragments was done by STABVida (Portugal) and at the Natural History Museum Sequencing Facility (London).

**Alignments and phylogenetic analyses:**—Sequence data were assembled, edited and aligned using Geneious ver. 5.6.6 (Biomatters) and the Geneious alignment algorithm. The alignments were then inspected and manually optimized in Geneious. Phylogenetic analysis of the combined data set followed the same methodology as used for each individual data set. Maximum parsimony (MP) analysis were conducted in PAUP\* ver. 4.0b10 (Swofford 2003). The analysis used 100 heuristic searches, random stepwise addition, and TBR branch swapping. A 50% majority rule consensus tree was calculated. Branch support values were assessed by bootstrap analysis, using 1,000 bootstrap replicates each comprising 100 heuristic searches, random stepwise addition and NNI branch swapping. jModeltest ver. 2.1.3 (Darriba *et al.* 2012) was used to determine the best-fitting model of sequence evolution based on the Akaike Information Criterion (AIC). A Maximum Likelihood analysis (ML) was conducted using RAxML-HPC2 ver. 7.4.4 (Stamatakis *et al.* 2008) on the CIPRES Science Gateway (Miller *et al.* 2010) with 1000 bootstraps and a partitioned dataset for the combined matrix. All ribotypes and haplotypes have been submitted to GenBank (see supplementary material for accession numbers).

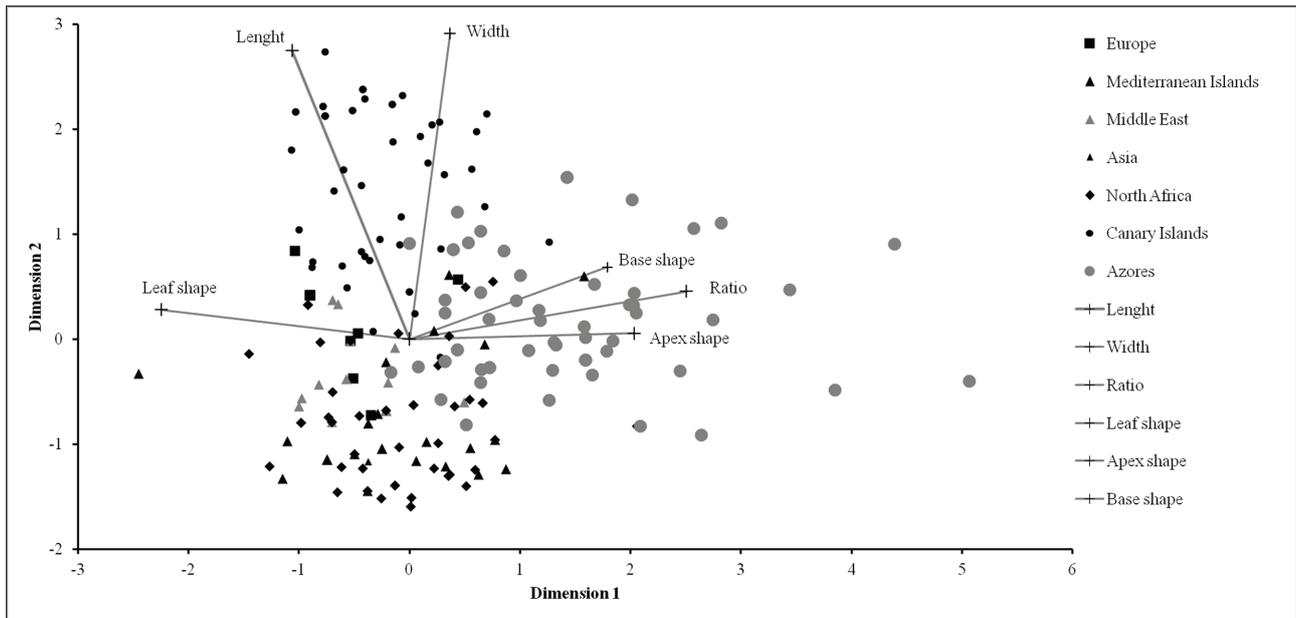
**TABLE 1.** Variability found between regions in the leaf characters. W:L = width/length ratio. An asterisk indicates insufficient data for ANOVA analysis. Means followed by the same letter are not significantly different (Tukey,  $p > 0.05$ ).

Variables	Region	Mean	SD	SE	Min	Max	Tukey	
Length (cm)	Asia	4.90	0.96	0.56	4.20	6.00	*	
	Azores	6.95	1.35	0.17	3.30	10.40	ab	
	Canary Islands	12.48	3.60	0.49	7.00	20.30	c	
	Europe	7.91	2.22	0.22	4.40	13.40	b	
	Med. Islands	6.12	1.19	0.23	4.00	8.90	a	
	Middle East	7.85	1.06	0.31	6.20	9.90	b	
	North Africa	6.40	1.69	0.24	3.30	10.10	ab	
	Width (cm)	Asia	2.27	0.40	0.23	1.90	2.70	*
Width (cm)	Azores	4.43	0.80	0.10	2.70	6.00	b	
	Canary Islands	6.44	1.72	0.24	3.60	10.50	c	
	Europe	3.60	0.89	0.09	2.10	6.60	a	
	Med. Islands	3.03	0.77	0.15	2.30	5.30	a	
	Middle East	3.52	0.47	0.14	2.90	4.40	a	
	North Africa	3.16	0.78	0.11	1.70	4.70	a	
	W:L ratio	Asia	0.46	0.02	0.01	0.45	0.49	*
		Azores	0.65	0.09	0.01	0.47	0.86	c
Canary Islands		0.52	0.07	0.01	0.44	0.79	b	
Europe		0.46	0.07	0.01	0.31	0.66	a	
Med. Islands		0.50	0.07	0.01	0.35	0.63	ab	
Middle East		0.45	0.05	0.01	0.36	0.53	a	
North Africa		0.50	0.07	0.01	0.35	0.69	ab	

## Results

**Morphological data:**—ANOVA showed significant differences for all analyzed leaf traits ( $p < 0.001$ ). Post-hoc tests showed that the Canarian material had significantly longer and wider leaves than material from the other regions, that specimens from all *V. tinus* subsp. *tinus* regions displayed significantly narrower leaves, while specimens from the Azores had a significantly different intermediate leaf width and higher width/length ratio (Table 1).

In CATPCA (Fig. 2) two factors were extracted explaining a total of 68% of the variance observed in leaves. A scatter plot showed a separation of the Azorean specimens, although with some overlap with specimens from other regions. Azorean material was associated with larger values for the leaf width/length ratio, and to peculiar attributes related to leaf base and leaf apex shape.



**FIGURE 2.** Analysis of leaf traits by region. Biplot resulting from a Categorical Principal Component Analysis, representing both individuals and variables based on two principal components. Scores of all variables were multiplied by three to increase plot legibility.

A frequency analysis showed that leaf apex in the Azores material was often acute, but that obtuse-rounded apex types were more frequent in the Azores than in other regions (Fig. 3). Regarding leaf bases, the Azores again showed some overlapping of base types with other regions but a larger percentage of obtuse, cordate, and exclusive subcordate types were observed (Fig. 3). Leaf shape analysis corroborated the tendency for rounder types in the Azores with ca. 60% of leaves displaying orbicular, ovate, obovate or intermediary shapes between the latter two types (Fig 3). Differences between the *V. tinus* subsp. *tinus* accessions from different regions were limited to leaf shape traits that occurred with low frequencies. Leaf shapes in Canarian material mostly overlapped with those observed in *V. tinus* subsp. *tinus*, with the exception of the occurrence of an intermediate, elliptic-ovate shape, with a frequency of ca. 30%.

With regards to other leaf traits not included in the ANOVA (Table 2), leaves of *V. tinus* subsp. *subcordatum* were found to be coriaceous and the leaf margin was markedly revolute, undulate, and sub-entire, while *V. rugosum* and *V. tinus* subsp. *tinus* mainly had sub-undulate to undulate, and entire leaf margins. The upper leaf surface of *V. tinus* subsp. *subcordatum* showed larger protrusions when compared to the Mediterranean and Canarian material which led us to consider it blistered and not, respectively, rugose or sub-blistered.

**TABLE 2.** Discriminating qualitative leaf characters found between *V. tinus* subsp. *subcordatum* (= *V. treleasei*), *V. rugosum* and *V. tinus* subsp. *tinus*. Leaf colour codes are from the RHS Colour chart (5<sup>th</sup> edition).

Taxon	Leaf colour (fresh)	Leaf margin	Upper leaf surface	Trichomes on lower leaf surface
<i>V. tinus</i> subsp. <i>subcordatum</i>	Shiny green (146A)	Subentire; Undulate; Revolute	Blistered	Simple hairs, occasionally sessile stellate; hirsutulous indumentum, often near glabrous over the veins
<i>V. rugosum</i>	Dull green (146A, 146B or 147A)	Entire; Subundulate	Mainly sub-blistered	Long, simple, bi-, tri- or more rarely, tetra-stellate hairs, and short, sessile stellate hairs; veins and petiole with villous-tomentose indumentum
<i>V. tinus</i> subsp. <i>tinus</i>	Dark green (N137A)	Entire; mainly subundulate	Rugose	Long, simple, bi-, tri- or more rarely, tetra-stellate hairs, and short, sessile stellate hairs; veins with hirsutulous-hirtellous indumentum

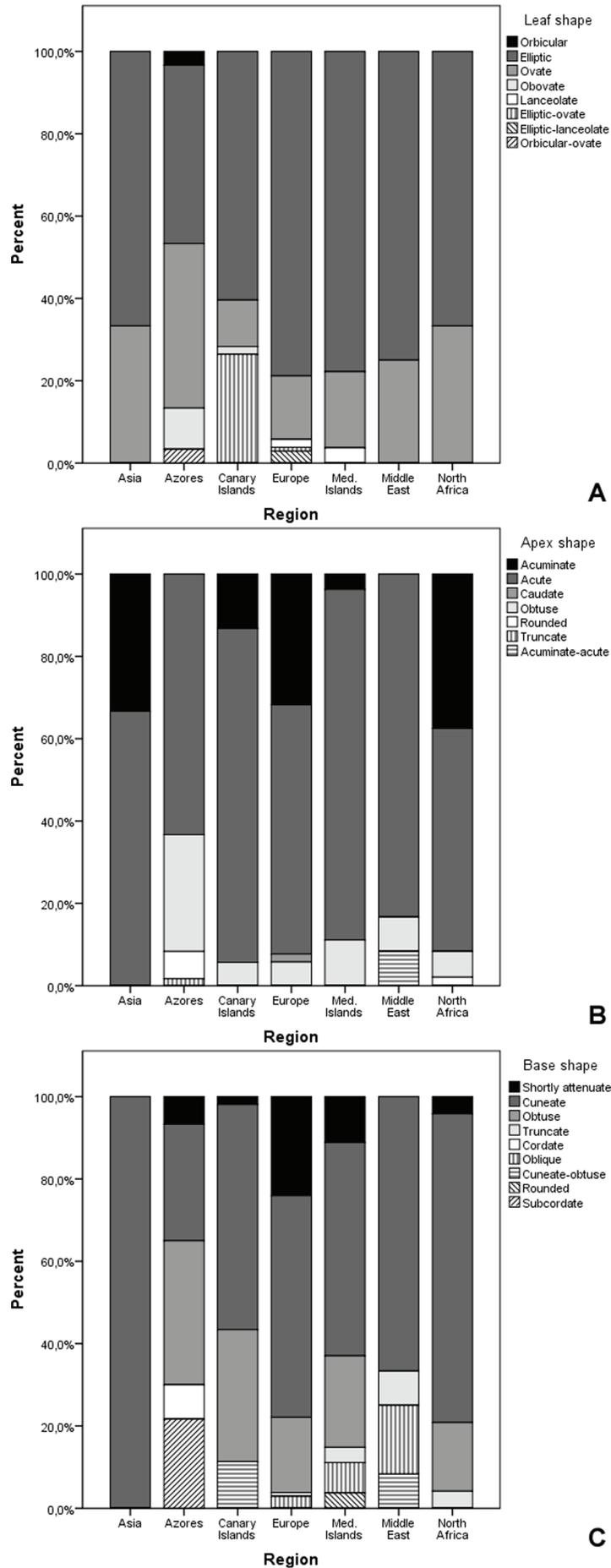


FIGURE 3. Frequency analysis of leaf shape, leaf apex shape and leaf base shape by region.

The colour of fresh leaves of the three taxa showed variability, mainly between Macaronesian taxa and continental *V. tinus* subsp. *tinus* (Table 2). The leaves of *V. tinus* subsp. *subcordatum* displayed a shiny bright green colour (146A), and *V. rugosum* displayed a similar, albeit duller, colour (146A, 146B or 147A), while leaves of *V. tinus* subsp. *tinus* specimens from the Portuguese mainland had a darker green colour (N137A). However, when comparing herbarium specimens, it was not possible to conclude if the observed differences for *V. tinus* subsp. *tinus* were constant within the Mediterranean distribution area of the taxon due to the impact of herborization on specimens.

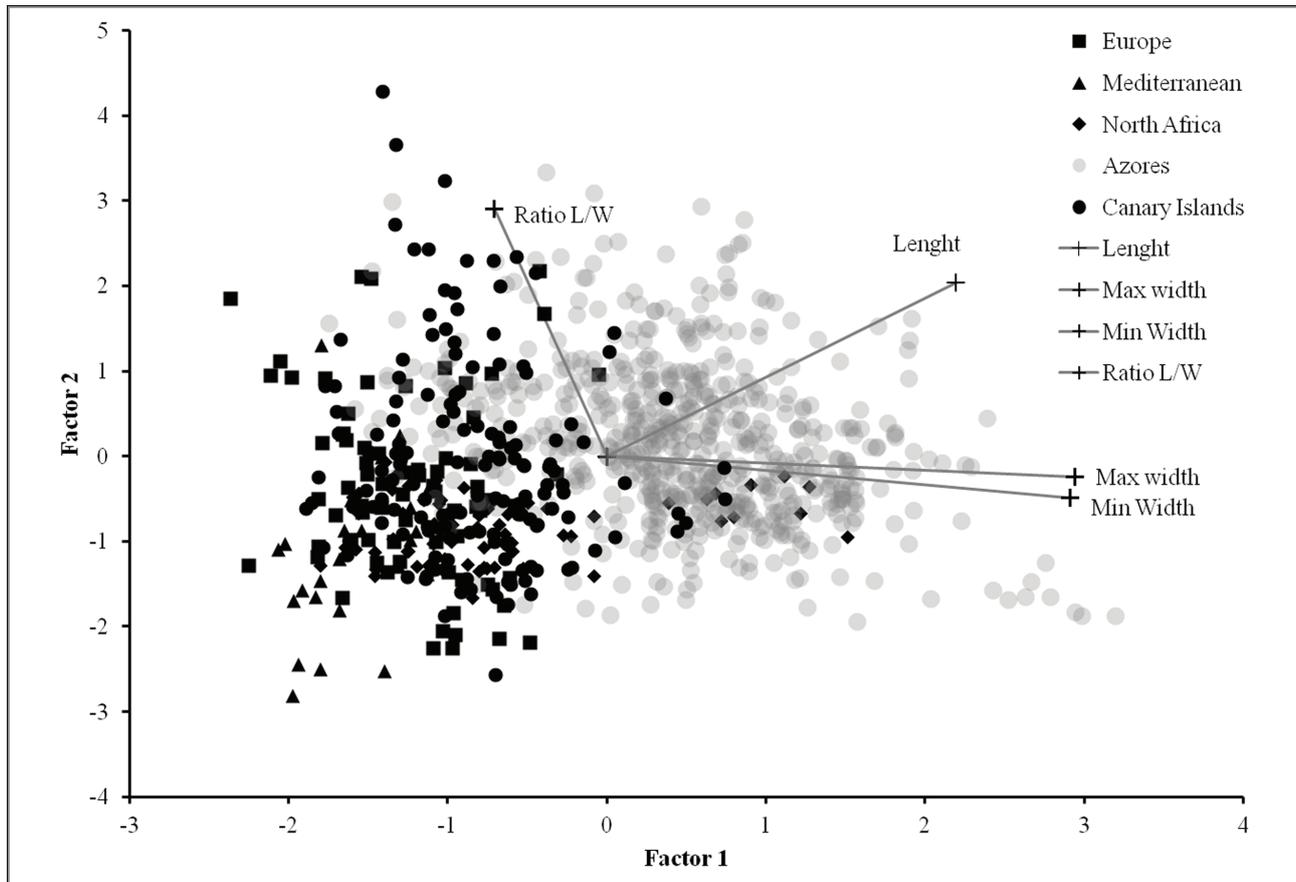
The lower leaf surface of *Viburnum tinus* subsp. *tinus* and *V. rugosum* possessed long, simple, bi-, tri- or more rarely, tetra-stellate hairs, and also short, sessile stellate hairs, although the *V. tinus* subsp. *tinus* population from Arrábida showed a particular trichome pattern with only short sessile stellate trichomes on the lower leaf surface (Table 2). In all three taxa the axils between midrib and secondary veins typically displayed tomentose hairs, with increasing density near the leaf base. *Viburnum tinus* subsp. *subcordatum*, possessed tomentose trichomes on the axils near the base, and simple hirsutulous hairs and occasionally sessile stellate trichomes, on the lower leaf surface. Density of indumentum was highly variable, from almost glabrous to villous-tomentose on the midrib and secondary veins of the lower leaf surface. *Viburnum tinus* subsp. *tinus* veins generally displayed denser, hirsutulous-hirtellous indumentum when compared with *V. tinus* subsp. *subcordatum*, which were frequently near glabrous. *Viburnum rugosum* displayed the highest levels of indumentum density, with hirsute trichomes on the upper leaf surface and villous-tomentose on the lower leaf surface veins and petiole (Table 2).

**TABLE 3.** Variability found between regions in fruit characters.  $D_{max}:D_{min}$  = ratio of maximum and minimum fruit diameter; L:D = ratio of length and mean diameter of fruits. An asterisk indicates insufficient data for ANOVA analysis. Means followed by the same letter are not significantly different (Tukey,  $p > 0.05$ ).

Variables	Region	Mean	SD	SE	Min	Max	Tukey
Length (cm)	Azores	9.03	0.99	0.04	6.22	12.06	d
	Canary Islands	7.49	1.08	0.09	5.32	10.37	c
	Europe	6.80	0.98	0.11	5.02	9.94	b
	Med. Islands	5.80	0.93	0.19	4.07	7.54	a
	North Africa	7.14	0.94	0.11	5.14	10.04	bc
Max. diameter (mm)	Azores	6.47	0.80	0.03	4.21	9.25	c
	Canary Islands	5.30	0.53	0.04	4.20	7.43	b
	Europe	4.90	0.46	0.05	3.70	5.73	a
	Med. Islands	4.59	0.35	0.07	4.08	5.54	a
	North Africa	5.39	0.73	0.09	4.28	7.48	b
Min. diameter (mm)	Azores	5.88	0.75	0.03	4.00	8.91	d
	Canary Islands	4.75	0.53	0.04	3.50	6.33	bc
	Europe	4.52	0.48	0.06	3.25	5.50	ab
	Med. Islands	4.23	0.25	0.05	3.82	4.67	a
	North Africa	5.00	0.71	0.09	3.71	6.88	c
$D_{max}:D_{min}$ ratio	Azores	0.91	0.05	0.00	0.73	1.00	ab
	Canary Islands	0.90	0.07	0.01	0.53	0.99	a
	Europe	0.92	0.06	0.01	0.73	0.99	ab
	Med. Islands	0.92	0.06	0.01	0.73	0.99	b
	North Africa	0.93	0.06	0.01	0.75	1.00	b
L:D ratio	Azores	1.48	0.19	0.01	1.02	2.18	bc
	Canary Islands	1.50	0.25	0.02	0.98	2.46	c
	Europe	1.45	0.23	0.03	1.06	2.01	bc
	Med. Islands	1.32	0.20	0.04	0.97	1.85	a
	North Africa	1.38	0.14	0.02	1.10	1.78	ab
Peduncle length (mm)	Asia	9.00	1.73	1.00	7.00	10.00	*
	Azores	5.83	3.51	1.01	5.00	12.00	a
	Canary Islands	18.00	10.45	2.28	9.00	48.00	b
	Europe	11.64	5.25	0.74	5.00	30.00	ab
	Med. Islands	11.50	6.60	1.90	5.00	26.00	ab
	Middle East	10.20	4.76	2.13	5.00	18.00	*
	North Africa	9.19	2.58	0.51	5.00	14.00	a

Significant differences ( $p < 0.001$ ) were found for all of the fruit traits included in the ANOVA (Table 3). Post-hoc tests showed that the Azorean material had significantly longer and wider fruits than the other regions. Within

*V. tinus* subsp. *tinus*, the Mediterranean Island samples displayed significantly shorter fruits, while fruits from the Mediterranean region and from Europe had significantly narrower maximum diameters. In PCA, two factors were extracted explaining a total of 98% of the variance. A scatter plot showed a separation of the Azorean specimens, although with some overlap with specimens from other regions (Fig. 4).



**FIGURE 4.** Analysis of fruit traits by region. Biplot resulting from a Principal Component Analysis, representing both individuals and variables based on two principal components. Scores of all variables were multiplied by three to increase plot legibility.

Finally, habit may also be used to discriminate the three taxa. *Viburnum tinus* subsp. *subcordatum* is a nanophanerophyte, typically not reaching taller than 2 m under natural conditions, while *V. rugosum* and *V. tinus* subsp. *tinus* are microphanerophytes with a maximum height range in natural habitat of, respectively, 4–5 m and 2–3 m.

**Phylogenetic analyses:**—The ITS alignment comprised 40 ingroup accessions and eight outgroup accessions, and had a length of 620 bp with 548 ingroup identical sites. The *trnK* intron alignment was composed by 42 ingroup accessions and eight outgroup accessions, and had a length of 1146 bp, of which 1069 sites were constant in the ingroup. Matrix cells scored as gaps or missing data corresponded to 1.7 % of the ITS matrix and to 0.3 % of the *trnK* data matrix.

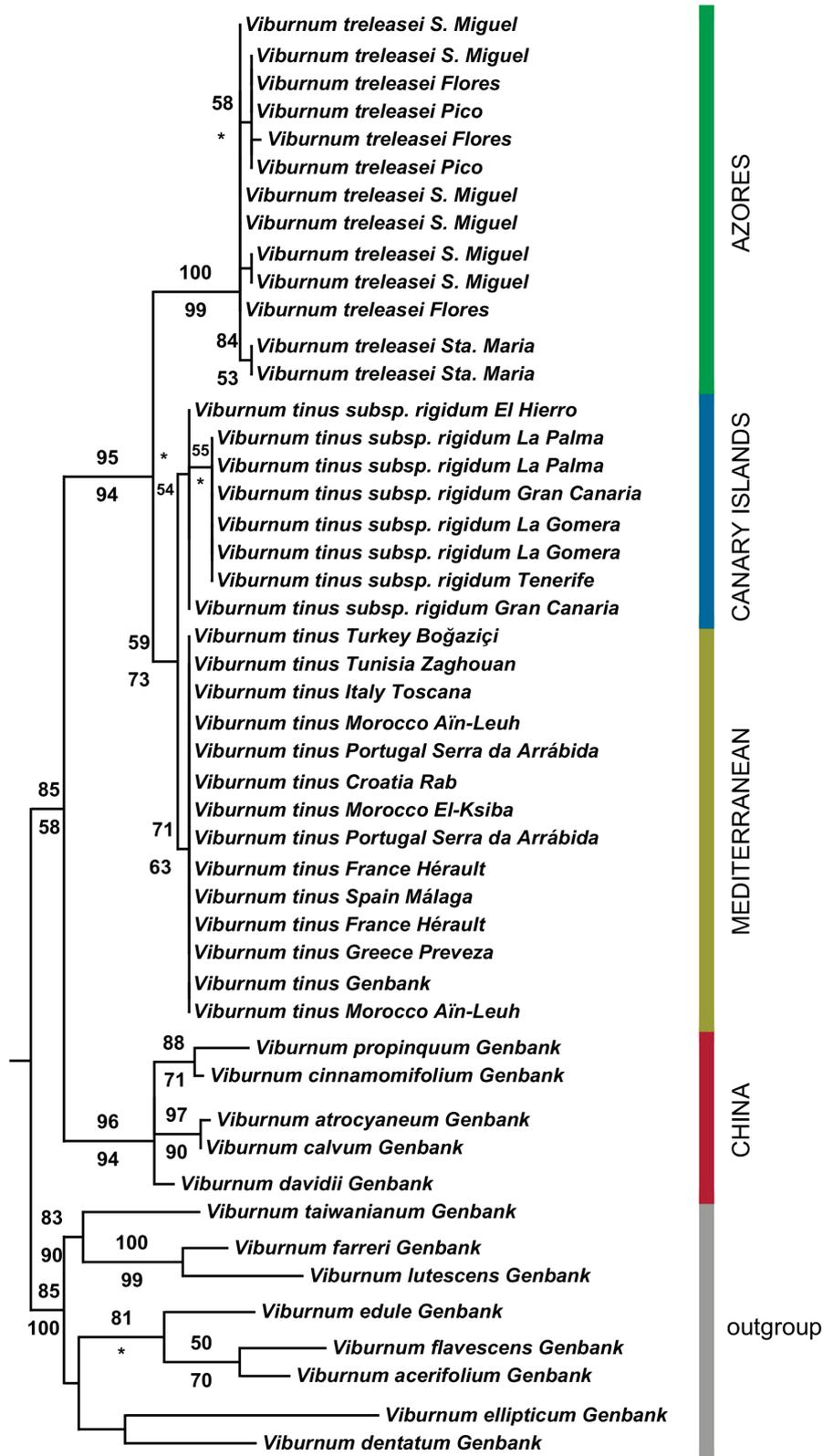
For the ITS region, MP analyses resulted in 186 best trees, 154 steps long, and a consistency index (CI) of 0.609 and a retention (RI) index of 0.814 (Fig. 5). MP analyses of the *trnK* intron data resulted in 2 best trees, 57 steps long, with a CI of 0.947 and a RI of 0.977 (Fig. 6).

The best fitting model selected by AIC was GTR+ $\Gamma$ +I, however, addition of the invariable site parameter “I” is not advised for RAxML since it is already incorporated in the standard algorithm (Stamatakis *et al.* 2008), and the analysis was conducted without this parameter. The RAxML analysis resulted in an ITS tree with a likelihood of  $-\ln L = -1707.529$ , whilst the *trnK* intron data resulted in a tree with a likelihood of  $-\ln L = -1952.538$ .

Since separate analysis for both matrices produced similar results, combined analyses were also performed. The combined MP analyses recovered 194 best trees, 211 steps long, with a CI of 0.720 and a RI of 0.871, while the combined ML analysis resulted in a final optimization likelihood of  $-\ln L = -3833.586$  (Fig. 7).

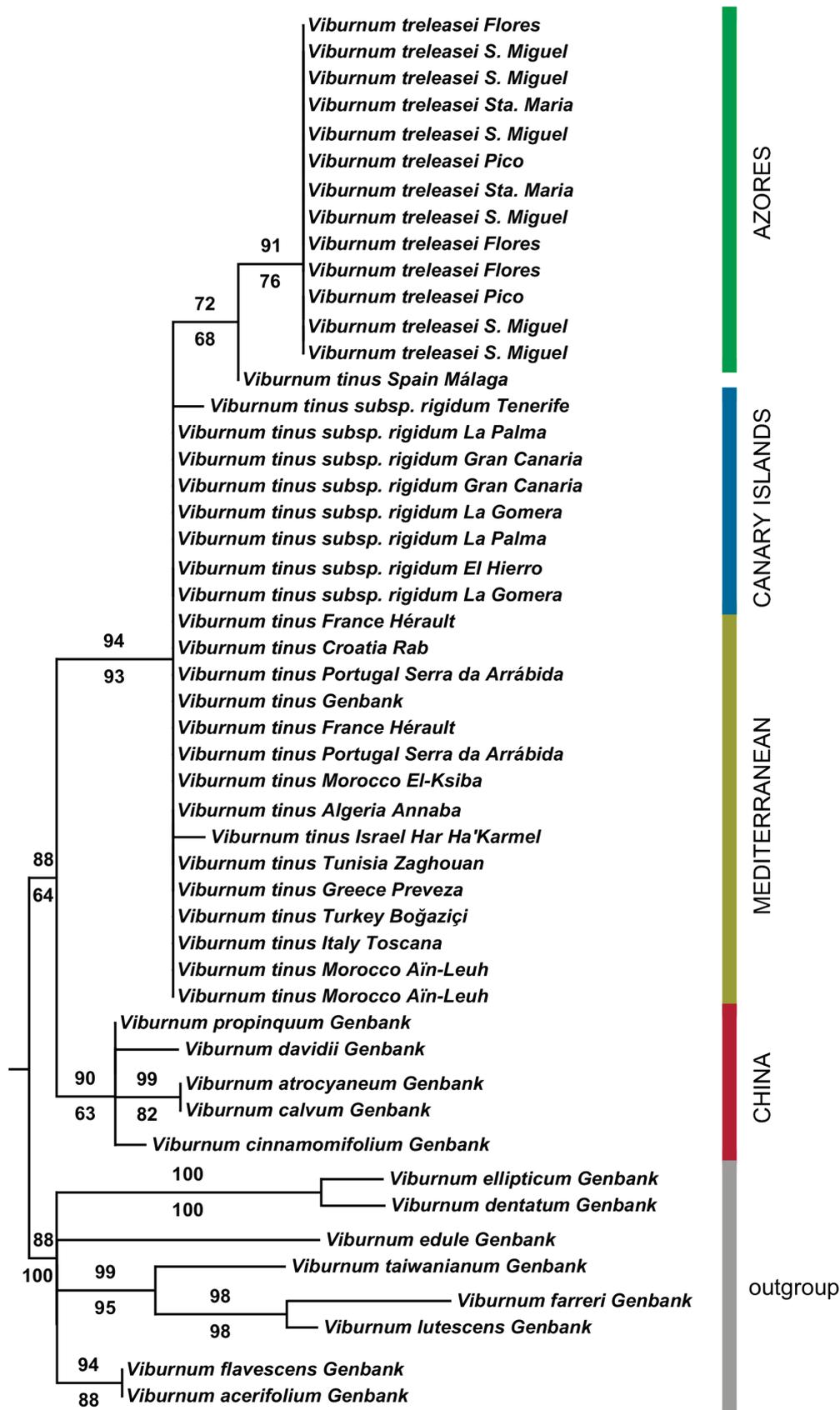
Bootstrap analysis of both the ITS and combined phylogenies strongly supported *V. tinus* subsp. *subcordatum* as monophyletic, with support values of 99 or 100% (Fig. 5 and 7). The monophyly of the taxon was also supported by the *trnK* intron although with weaker support (Fig. 6; ML = 91%; MP = 76%). Relationships between *V. rugosum* and *V. tinus* subsp. *tinus* were poorly resolved, particularly in the analysis of the less variable *trnK* intron (Fig. 6), wherein

the two taxa formed a polytomy. In the combined analysis (Fig. 7), accessions of *V. rugosum* and *V. tinus* subsp. *tinus* formed a monophyletic group with some support in the MP analysis (64%), but no support in the ML analysis. Within this clade, *V. rugosum* and *V. tinus* subsp. *tinus* were resolved as reciprocally monophyletic sister taxa although both with generally weak bootstrap support (51–77% support values).

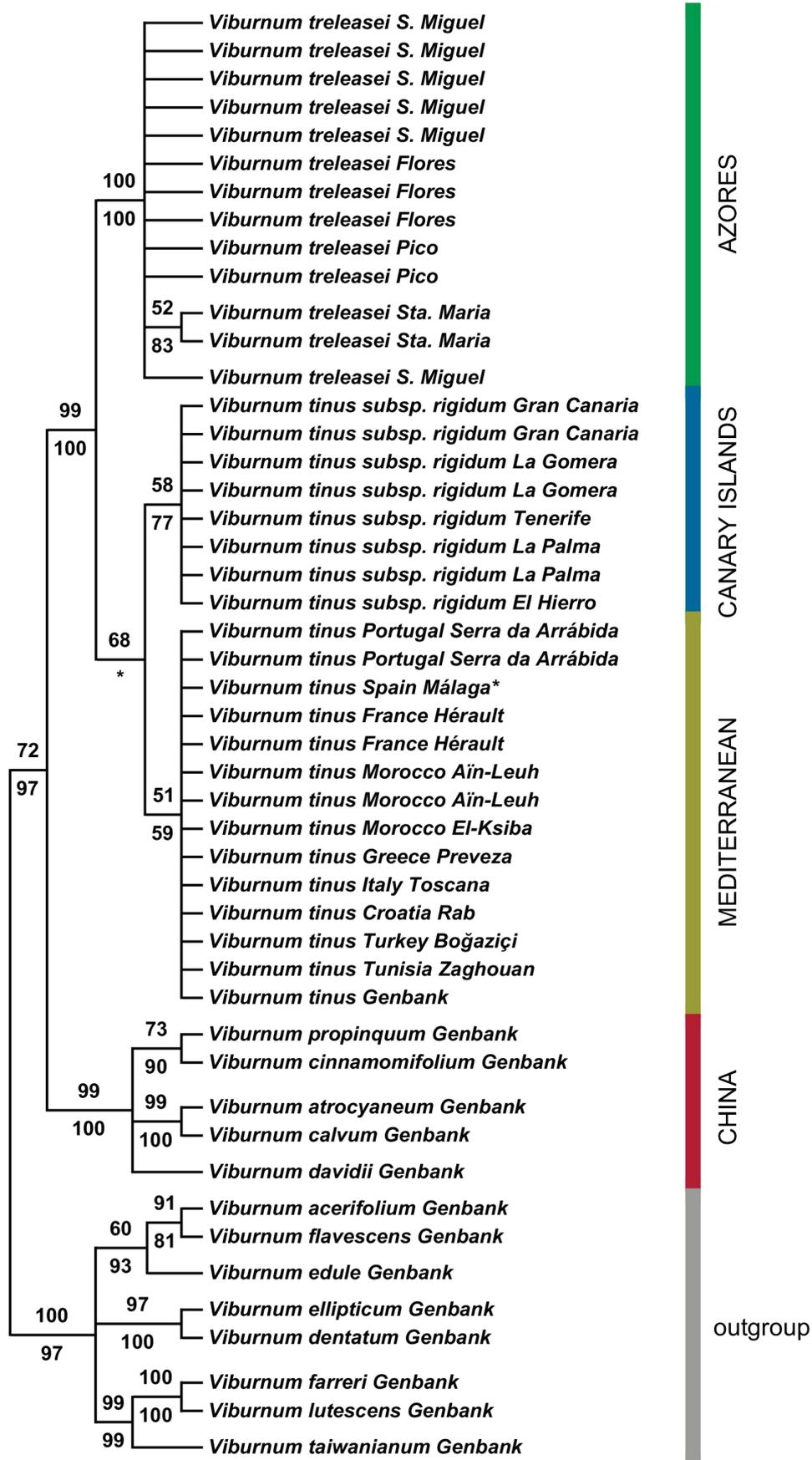


**FIGURE 5.** Best maximum likelihood tree obtained from nuclear ITS sequence data. Values above branches show ML bootstrap support; values below are the corresponding MP bootstrap support. \* indicates <50% bootstrap. Only values above 50% in at least one of the analysis criteria are shown. *Viburnum treleasei* = *V. tinus* subsp. *subcordatum*.

Within the Azorean clade, analysis of the nuclear and combined data indicated a clear separation of the Santa Maria accessions with support values of ca. 83% for the ML analysis and ca. 52% for the MP.



**FIGURE 6.** Best maximum likelihood tree obtained from chloroplast *trnK* sequence data. Values above branches show ML bootstrap support; values below are the corresponding MP bootstrap support. Only values above 50% in at least one of the analysis criteria are shown. *Viburnum treleasei* = *V. tinus* subsp. *subcordatum*.



**FIGURE 7.** Maximum parsimony 50% majority-rule consensus tree obtained from combined data. Values above branches show MP bootstrap support; values below are the corresponding ML bootstrap support. \* indicates <50% bootstrap. Only values above 50% in at least one of the analysis criteria are shown. *Viburnum treleasei* = *V. tinus* subsp. *subcordatum*.

## Discussion

**Distinctiveness of Azorean *Viburnum*:**—Morphologically, some of the characters traditionally considered useful for distinguishing *V. tinus* subsp. *subcordatum* from *V. rugosum* and *V. tinus* subsp. *tinus* were found to be polymorphic (e.g. overall leaf shape) and exhibited overlap with the variation found in accessions from the Mediterranean basin and the Canary Islands.

The most discriminating characteristics for *V. tinus* subsp. *subcordatum* were however, leaf characters, namely an intermediate width between *V. tinus* subsp. *tinus* and *V. rugosum*, and overall rounder leaves, with sub-entire and revolute leaf margins, and blistered upper leaf surfaces. Leaf indumentum was also less dense, with the frequent occurrence of near glabrous specimens. The trichomes over the midrib and secondary veins were hirsutulous and simple. These character states in conjunction with the frequent presence of an obtuse, cordate, or subcordate leaf base, obtuse-rounded apices, larger fruits and smaller growth form, morphologically separate *V. tinus* subsp. *subcordatum* from *V. rugosum* and *V. tinus* subsp. *tinus*.

The results of the molecular analysis resolved the Azorean accessions in a strongly-supported clade, supporting the distinctiveness of the Azorean *Viburnum*. This is in accordance with a population structure analysis of *V. tinus* subsp. *subcordatum* (= *V. treleasei*) by Moura *et al.* (2013) which revealed distinct genetic patterns between the Azorean populations and a population of *V. tinus* subsp. *tinus* from Serra da Arrábida. The distinctiveness of Santa Maria accessions obtained in the present study is also in accordance with the results of Moura *et al.* (2013), which indicated that the only extant population on Santa Maria formed the sister clade to all other Azorean populations and partially shared the genetic pattern of the *V. tinus* subsp. *tinus* population sampled. This led Moura *et al.* (2013) to suggest that initial colonization of the Azores archipelago by mainland *Viburnum* might have occurred at Santa Maria. The remaining within-archipelago structure obtained by Moura *et al.* (2013) was not confirmed here, namely the reciprocal monophyly of São Miguel and Flores populations and of both these islands' populations with the populations from Pico, which might be related to the lower variability of the phylogenetic markers.

Although the analysis of our set of molecular markers did not always result in well supported reciprocal monophyly of *V. tinus* subsp. *tinus* and *V. rugosum*, the greater width and length of the leaves of *V. rugosum*, as well as extremely dense leaf indumentum, and larger habit allow for its differentiation from *V. tinus* subsp. *subcordatum* and *V. tinus* subsp. *tinus*. The lack of marked molecular differentiation between *V. tinus* and *V. rugosum* by comparison to a higher morphological separation might be connected to the recent speciation of *V. rugosum* or to the occurrence of gene flow between North Africa populations of *V. tinus* and Canary Islands populations of *V. rugosum*. Variation within the distribution regions of *V. tinus* subsp. *tinus* can be explained by the occurrence of low frequency leaf shape traits in European and Middle East specimens, and the smaller fruit size of European and Mediterranean Island specimens. All Chinese taxa belonging to section *Tinus* formed a strongly-supported sister clade to the Mediterranean-Macaronesian group in the molecular analysis, which is in accordance with Clement & Donoghue (2012).

In addition to molecular and morphological characters there are also ecological differences between the taxa, mainly between *V. tinus* subsp. *subcordatum* and *V. tinus* subsp. *tinus*. *Viburnum tinus* subsp. *tinus* occurs generally in Mediterranean scrub on dry calcareous sites (Berger & Walther 2006), most probably on decarbonated soils (see Torres *et al.* 2002), while *V. tinus* subsp. *subcordatum* occurs on the perimeter and clearings of humid to ultrahumid native forests, native scrubland and meadows, on andossols or lithosols. *Viburnum rugosum* occurs in similar, albeit drier, laurel forest and *Erica* scrubland on andossols (Rastad 1990, Fernández-Palacios & Nicolás 1995).

The three taxa also differ somewhat in flowering times although there is some overlap. Thus *V. tinus* subsp. *tinus* (Fig. 8A) flowers mainly from December to the beginning of April (Nebot & Mateu 1991, Castro-Díez & Monserrat-Martí 1998), *V. rugosum* (Fig. 8B) from December to March (Don 1834), while *V. tinus* subsp. *subcordatum* (Fig. 9) flowers from November to June, with a peak in April (Moura 2005). Additionally, whilst both *V. tinus* subsp. *tinus* (Nebot & Mateu 1991) and *V. tinus* subsp. *subcordatum* (Moura 2005) have been shown to be allogamous and self-incompatible, we found no putative hybrids when they were cultivated together and there is no historical report of such hybrids in horticultural literature (V. Malécot, personal observation).

**Taxonomic implications:**—Based on morphological, ecological, and molecular evidence, the Azorean *Viburnum* is distinguishable from Canarian, Mediterranean and Chinese taxa in the *V. tinus* section. A point of disagreement among earlier authors (see introduction) has concerned the most appropriate rank to treat the Azorean taxon. Whilst Palhinha (1966) considered that *V. tinus* subsp. *tinus*, *V. tinus* subsp. *rigidum* (= *V. rugosum*) and *V. tinus* subsp. *subcordatum* should be recognised at subspecific rank under *V. tinus*, this arrangement would fail to reflect the distinctiveness of the Azorean taxon from both a morphological and molecular perspective given that ITS and combined data MP analysis

indicate that the Azorean *Viburnum* is sister to a clade comprising both the Canarian *V. rugosum* and the continental *V. tinus* subsp. *tinus*. Given this pattern of relationships, it is therefore appropriate to recognise the Azorean endemic taxon at specific rank, under the oldest valid name of *Viburnum treleasei*. An account of the Azorean species is included below together with a nomenclatural discussion for the Canary island endemic *V. rugosum*.



**FIGURE 8.** Field photographs. A. *Viburnum tinus* subsp. *tinus*, Serra da Arrábida (Francisco Clamote). B. *Viburnum rugosum*, Breña Alta, La Palma, Canary Islands (Frank Vincentz).



**FIGURE 9.** Field photographs of *Viburnum tinus* subsp. *subcordatum* (= *V. treleasei*), Lombadas, São Miguel, Azores (M. Moura). A. Mature plant. B. Inflorescences with flower buds. C. Mature inflorescence. D. Infructescence with nearly ripe fruits.

## Taxonomic Treatment

*Viburnum treleasei* Gandoger (1899: 255)

Type (lectotype designated here):—AZORES. Flores: 4 August 1894, *Trelease 389* (MO1679100!, isolectotype AZ1336!).

≡ *Viburnum tinus* L. var. *subcordatum* Trelease (1897: 118) ≡ *Viburnum tinus* L. subsp. *subcordatum* (Trel.) P. Silva in Palhinha (1966: 115–116) ≡ *Viburnum subcordatum* (Trel.) Rivas-Martínez *et al.* (2002: 709), *nom. superfl.*

– “*Viburnum tinus*” *auct. non* Linnaeus (1753: 267–268): Seubert & Hochstetter (1843: 13).

– “*Viburnum tinus* L. var. *lucidum*” *auct. non* (Miller 1768: VIB-VIB) Aiton (1789: 372): Seubert (1844: 35).

**Note:**—We consider that *V. treleasei* Gandoger is a name at a new rank (*stat. nov.*) for *V. tinus* var. *subcordatum*, thus following Art. 7.3 of ICN, its type should be selected among the material cited by Trelease (1897). Trelease (1897) cites four specimens collected by himself in the Azores (São Miguel: 25 August 1894, *Trelease 388*, MO!; Flores: 4 August 1894, *Trelease 389*, MO! AZ!; Flores: 10 August 1894, *Trelease 390*, MO!; Santa Maria: 29 June 1896, *Trelease 390a*, MO!, *idem*, *Trelease 390b*, MO!), together with a Carreiro specimen “San Miguel (388; Carreiro)”. The only Carreiro collections older than the protologue that match are deposited at LY and AZ: Carreiro s.n. (São Miguel: Furnas, July 1891, at LY! [in Gandoger’s herbarium]), Carreiro s.n. (São Miguel: Furnas, July 1891, at AZ! [1331, *V. lucidum* sensu H. Christ]). From among these syntypes, *Trelease 389* is selected as lectotype. It is a mature fruiting collection, distributed at both MO, where Trelease worked, and AZ.

Both Gandoger (1899) and Trelease (1897) cite the names *Viburnum tinus* and *V. tinus* var. *lucidum* in synonymy. For *Viburnum tinus*, Gandoger (1899) clearly stated “non L.,” thus he explicitly excluded Linnaeus’ type. This was not the case for *V. tinus* var. *lucidum*. However, we consider that their inclusion in synonymy is to record names previously used in the Azores to refer to the taxon described by Trelease (and renamed by Gandoger). Gandoger (1899) considers

*Viburnum treleasei* as endemic to the Azores, thus excluding the type *V. lucidum* Mill. (basionym) and *V. tinus* var. *lucidum* (Mill.) Aiton by implication [Art. 52.2(e)]. *Viburnum lucidum* was first reported by Clusius (1576) from Portugal, and cultivated in mainland Europe [as indicated by Oersted (1861) in his monograph of *Viburnum*]. We also note that Gandoger considered *V. lucidum* as a distinct taxon from southern Europe, in his *Flora Europae* [Gandoger, 1886: 40, as “*Tinus lucidus* (Mill.)], a work that he mentions in the introduction of his 1899 article containing the *V. treleasei* protologue, and in his *Novus Conspectus Florae Europae* (Gandoger 1910: 225, as *Viburnum tinus* subsp. *lucidum*), leaving no place for including its type under *V. treleasei*.

Evergreen shrub up to 2 m tall, with dense, ovate canopy and pubescent twigs with stellate trichomes, the most apical internodes slightly compressed. Leaf 3–10 × 2.5–6 cm, bright green, subcoriaceous, entire, ovate, obovate, orbicular-ovate or elliptic; base mostly subcordate or auriculate-cordate; apex acute or obtuse; margins revolute; venation pinnate, with secondary veins curving, not ending in margins, and anastomosing; blade surface blistered; upper leaf surface with simple pilose trichomes located on the mid rib and secondary veins; lower leaf surface with dense long tomentose trichomes on the mid rib and secondary veins axils, simple hirsutulous trichomes and occasional, small sessile stellate hairs, on the mid rib and secondary veins, trichome density variable with near glabrous forms occurring frequently. Petiole 0.5–4 cm in length, hemicylindrical, with simple trichomes on the upper surface and stellate trichomes on the lower. Inflorescence 4.5–7 cm wide, umbelliform. Peduncles with one axillary bract; primary peduncle very short, generally with less than 1 cm long, branching into 5–7 primary rays; secondary and tertiary peduncles 0.9–2.1 and 0.5–1.3 cm, respectively, branching into 4–6 secondary rays and pedicels. Pedicel 0.2–0.6 cm, with 1 axillary bract and 2 terminal bracteoles. Calyx 5-merous, herbaceous, funnellform, synsepalous, fused with the ovary, persistent. Corolla diameter 5–9 mm, actinomorphic, rotate, 5-merous, synpetalous, marcescent, white inside with pink or red streaks outside. Stamens 5, exerted, filament glabrous. Pollen exine with regular reticulum and psilate muri. Ovary inferior, with long, stellate trichomes at the base, 1-locular, excentric, with one fertile locule, 1-ovulate, matted with long fascicled hairs. Style less than 1 mm long, glabrous, or sessile. Stigma 3-lobed, generally pink. Fruit subglobose pseudodrupe with thin flesh, 7–11 cm long, metallic-blue. Stone generally moderately compressed in cross-section, slightly grooved. Endosperm ruminant. Glands upright with rounded apex on the inflorescences, mid rib and secondary veins of the upper leaf surface, less frequently on the lower leaf surface.

**Phenology:**—Flowering occurs from November to June, with inflorescences showing diminute dark red flower buds, which grow and open into white or white and pink streaked flowers. Fruiting occurs from May to February, with fruits initially bearing a green colouration and later turning a characteristic metallic-blue shade when ripe. Seedlings emerge from March to August. Annual growth develops from March to July.

**Distribution and Habitat:**—Native in all Azorean islands, except Graciosa, where it was recently translocated from other islands. Generally found in non-shaded locations, either on the margin or in clearings of natural forests (*Morella faya* woodland, laurel forest, *Ilex perado* subsp. *azorica* forest, *Juniperus brevifolia* forest, *Erica azorica* forest), on *Erica azorica* scrubland, *Holcus rigidus* natural meadows, pioneer scrubland, roadsides and on ravines and crater slopes, generally between 300 to 800 m, although altitudinal limits can reach below 100 m (Flores) and above 900 m (Pico).

### *Viburnum rugosum* Persoon (1805: 326)

≡ *Tynus rugosus* (Pers.) Presl in Berchtold & Presl (1825: 93) ≡ *Tinus rugosus* (Pers.) Spach (1839: 317). Type:—Persoon (L? n.v.).  
= *Viburnum rigidum* Ventenat (1805: 98) ≡ *Viburnum tinus* subsp. *rigidum* (Vent.) P. Silva in Palhinha (1966: 116). Type (lectotype designated here):—Ventenat (1805: plate 98!).

**Note:**—Both *Viburnum rugosum* Pers. and *V. rigidum* Vent. were published in 1805. According to Stafleu & Cowan (1983: 183) the first part of Persoon’s *Synopsis Plantarum* was published between 1<sup>st</sup> of April and 15<sup>th</sup> of June 1805. Stafleu and Cowan (1986: 702) stated that the publication date for Ventenat’s plate 98 of *Jardin de la Malmaison* was July 1805 (reproducing the approximate date provided by Stearn, 1939). One of the indexing journals of the period, the *Gazette nationale ou le Moniteur universel*, published by Panckoucke (1805), provides a description of Persoon’s *Synopsis* in its edition dated 15<sup>th</sup> June 1805, while in the edition dated 13<sup>th</sup> July 1805, Jussieu (1805) announces plates 91 to 96 Ventenat’s *Jardin de la Malmaison*. This is part 16 of *Jardin de la Malmaison*, the part preceding that which included pl. 98 (*Viburnum rugosum*). Additionally, Ventenat’s plate is cited on page 195 of volume 13(7) of the *Journal général de la littérature de France* (Anonymous 1805), consisting of the late summer fascicule of this journal. Here, it is cited together with delivery 4 and 5 of Boissieu’s *Flore d’Europe* and delivery 21<sup>st</sup> of Redouté’s *Liliacées*. This last

delivery was announced in the *Gazette Nationale* on 24<sup>th</sup> September 1805 while delivery 3 of Boissieu is cited in the 10<sup>th</sup> of July 1805 edition this journal. All these facts indicate that Persoon's name was published before 15<sup>th</sup> of June 1805 while Ventenat's name appears between 10<sup>th</sup> of July 1805 and 24<sup>th</sup> of September 1805 and thus, *V. rugosum* has priority over *V. rigidum*.

**Representative specimens examined:**—*Viburnum treleasei* Gand.: AZORES. Santa Maria: *Carreiro 333B* (AZ!); “abundant in serras”, *Treleave 390a* (MO!); *Treleave 390b* (MO!); Pico Alto, *Moura 101 to 110* (AZB!). São Miguel: s loc., *Hunt s.n.* (NY!); Nordeste, Tronqueira, roadside, *Moura 1 to 10* (AZB!); Sete Cidades, *Carreiro 333* (AZ!, LY!); Sete Cidades, *Carreiro 333A* (AZ!); Sete Cidades, Cumeeiras, *Moura 11 to 20* (AZB!); Lombadas, *Carreiro s.n.* (AZ!); Lombadas, roadside, *Moura 21 to 30* (AZB!); Furnas, *Carreiro s.n.* (AZ!, LY!); “above Furnas”, *Treleave 388* (MO). Terceira: Terra Brava, Bagacinas (Fajã), *P. Dansereau, A.R. Pinto da Silva & B.V. Rainha 124* (NY!). Pico: Mistério da Prainha, in *Erica azorica* and *Juniperus brevifolia* scrub, *Moura 81 to 90* (AZB!); Lajes, descent to Lajes, roadside, *Moura 71 to 80* (AZB!). Flores: *Treleave 389* (AZ!, MO!); *Treleave 390* (MO!); Mosteiros, descent to Mosteiros, roadside, *Moura 51 to 60* (AZB!); Ponta Delgada, descent to Ponta Delgada, roadside, *Moura 61 to 70* (AZB!). *Viburnum rugosum* Pers.: CANARY ISLANDS. Gran Canaria: Los Tillos de Moya, *Ruth Jaén-Molina, Moisés Soto & Oscar Saturno 1–16* (AZB!). Tenerife: Orotava valley, *Rev. Richard Thomas Lowe 25* (BM!); Aguamansa, above la villa d’Orotava *Rev. Richard Thomas Lowe 25bis* (NY!); s.loc., *C. E. Jarvis 449* (BM!); s.loc., *E. Bourgeau 61* (BM!); Tazanana, *Rev. H. E. Fox s.n.* (BM!); Anaga mountains, *J. F. M., M. J. & P. F. Cannon 4531* (BM!); Peninsula de Anaga, pr. *Locum dictum* Pico del Inglés, *J. Fernandez-Casas FC 27–78* (NY!); s.loc., *Herb A. H. Maude s.n.* (BM!); s.loc., *Herb A. H. Maude s.n.* (BM!); s.loc., *Herb A. H. Maude s.n.* (BM!); Barranco San Antonio, *Erik Asplund 177* (NY!); Barranco San Antonio, supre Orotava, *Einar & Ragnhild Wahlström s.n.* (NY!); Las Mercedes, *Alice Carter Cook 1003* (NY!); Las Mercedes, *Adelaide Stork s.n.* (NY!); Puerto de la Cruz, Las Rosas, *Hervé M. Burdet CAN 003* (NY!); Aquagarcía, *Otto Kuntze s.n.* (NY!). La Palma: dans les montagnes, *P. S. in herb. Torrey s.n.* (NY!). *Viburnum tinus* L. **subsp. tinus**: MAINLAND PORTUGAL. Serra da Arrábida: Parque Natural da Serra da Arrábida, s. col. *PNA 91A–D, 95A–C, 96A–C, 98A–C, 100A–B* (AZB!). Serra da Estrela: Ponte do Espinho, bridge, *J. Diamantino PNE 1* (AZB!); Alvoco, bridge over river Alvoco in Barriosa, *J. Diamantino PNE 2* (AZB!); Alvoco, farthest shore of near Vide, *J. Diamantino PNE 3* (AZB!); Levadas (EN n.º230), next to crossroad of Baloquinhas, *J. Diamantino PNE 4* (AZB!); Muro-Casal do Rei, roadside, *J. Diamantino PNE 5* (AZB!); Ribeiro de Forcados, Cabeça, *J. Diamantino PNE 6* (AZB!); Ponte de Jugais, bridge, *J. Diamantino PNE 7* (AZB!). SPAIN. Barcelona: *F. Sennen 3948* (BM!); *F. Sennen s.n.1* (BM!); *F. Sennen s.n.2* (BM!); *F. Sennen s.n.3* (BM!); *F. Sennen 4754* (BM!). Valencia: *C. Pau 4622* (BM!). Málaga: *G. Michel 7452* (BM!); *G. Michel 7288* (BM!). FRANCE. Hérault: Montpellier, *A. Neyra s.n.* (BM!). Cruzol: *R. Dechamps & H. Doutréle 2711* (BM!). Corsica: Folelli, *G. Bocquet 16930* (BM!). ITALY. Toscana: Bólgheri, *J. Grau & E. Bayer 21A* (M!); Livorno, Bibona, Maccia della Magona, *Dürbye, Weber & Meyer Dür 1061* (B!). Sardegna: Sarcidano, Laconi, *H. Merxmüller & F. Oberwinkler 9* (M!). Elba: Procchio, *H. Roessler 4591* (M!). SLOVENIA. South of Koper, *O. Angerer s.n.* (M!). CROATIA. Fisella: *K. Untchj s.n.* (B!); s.loc., s.col. s.n. (B!); Machis near Fisella, *K. Untchj s.n.* (M!). Split: *Fr. Petter 403* (B!). Lussino: Lussinpiccolo, Bocca Falsa, *C. Baenitz s.n.* (B!). Rab: *N. Kilian & M. Romebach 555* (B!); Rab, *A. Bamberger & P. Schultze Motel s.n.* (B!). Mljet: Sv. Marija, *Fr. Petter 403* (B!). Lokrum: *B. E. E. Duyffes, R. Hengeveld & C. W. van der Voet 83* (B!). GREECE. Preveza: South of Kato Myrsini, *R. & E. Willing 103.867* (B!). Pelopónnisos: *H. Wittmann 10* (M!). Corfu: Pelekas, *C. Baenitz s.n.* (B!). TURKEY. Boğaziçi: Tarabya, *H. & E. Walter 3322* (B!). ISRAEL. Har Ha’Karmel: *A. Danin, T. Raus, W. Sauer, S. Brullo, B. Valdés, F. Amich, S. G. Gardner, R. C. H. J. van Ham, A. Gambino, F. Axelrod, Battia Pazy, Rivka Nokrian 57.001* (B!); Wadi Shumariye, *M. Zohary 762* (M!). RUSSIA, ALTAI REPUBLIC. Altay: Osero, *Dr. Noë 1213* (B!). TUNISIA. Zaghuan: Djebel Zaghuan, *H. Hertel 8412* (M!); Djebel Zaghuan, *R. Vogt & Ch. Oberprieler RV 13862, CO 8167* (B!). LIBYA. Cyrene: *N. Douglas Simpson 39228* (BM!). No province: s.loc., *Dr. T. F. W. Gregory s.n.* (BM!). Wadi Kuf: *K. Guichard Cyr/57/37* (BM!). ALGERIA. El-Biar: *E. G. Paris 65* (BM!). Annaba: Laverdure, *Leippert 7104* (B!). MOROCCO. Al Hoceïma: *S. L. Jury, M. Rejdali, A. Taleb & T. M. Upson 12488* (BM!). Tisouka: *Davis 54832* (BM!). Metalza: *F. Sennen & H. Mauricio 8815* (BM!). Beni Mellal: *S. L. Jury, A. Abaouz, M. Ait Lafkih & A. J. K. Griffiths 17446* (BM!); *R. M. Harley & A. M. Harley 845* (BM!). Azrou: *James Bynon M/80–46* (BM!). Gurugú: *H. Mauricio 7590* (BM!); *H. Mauricio 7865* (BM!). Beni Medein: *H. Mauricio s.n.* (BM!).

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## Appendix

List of taxa from section *Tinus* and outgroup used in the molecular analysis, voucher information, and GenBank accession numbers. All *Viburnum treleasei* Gand. samples are stored at the DNA Bank of Azorean Flora, which is part of AZB.

***Viburnum tinus* subsp. *subcordatum* (Trel.) P. Silva (= *V. treleasei* Gand.):**—1: São Miguel (Nordeste, Tronqueira), Moura 3 (ITS EF445116, *trnK* EF445122); 2: São Miguel (Nordeste, Tronqueira), Moura 7 (ITS EF445117, *trnK*=VTR1); 3: São Miguel (Sete Cidades, Cumeieiras), Moura 13 (ITS EF445118, *trnK*=VTR1); 4: São Miguel (Lombadas), Moura 22 (ITS EF445119, *trnK*=VTR1); 5: São Miguel (Lombadas), Moura 27 (ITS EF445120, *trnK*=VTR1); 6: Flores (descent to Mosteiros), Moura 58 (ITS EF445121, *trnK*=VTR1); 7: Flores (descent to Ponta Delgada), Moura 66 (ITS

HM563784, *trnK*=VTR1); 8: Pico (descent to Lajes), Moura 73 (ITS HM563785, *trnK*=VTR1); 9: Pico (descent to Prainha), Moura 84 (ITS HM563786, *trnK*=VTR1); 10: Santa Maria (Pico Alto), Silva 3 (ITS HM563787, *trnK*=VTR1); 11: Santa Maria (Pico Alto), Silva 7 (ITS HM563788, *trnK*=VTR1); 12: São Miguel (Sete Cidades, Cumeeiras), Moura 18 (ITS HM563789, *trnK*=VTR1); 13: Flores (descent to Ponta Delgada), Moura 62 (ITS KF443803, *trnK*=VTR1).

***Viburnum rugosum* Pers.**:—1: Gran Canaria (Barranco de Los Tilos, Moya), Caujapé-Castells & Navarro 721 (LPA) (ITS HM563790, *trnK* HM563800); 2: Gran Canaria (Barranco de Los Tilos, Moya), Caujapé-Castells & Navarro 725 (LPA) (ITS HM563791, *trnK*=VR1); 3: La Gomera (Hermigua, El Rejo), A. Santos 5A (ORT) (ITS HM563792, *trnK*=VR1); 4: La Gomera (Hermigua, El Rejo), A. Santos 5B (ORT) (ITS HM563793, *trnK*=VR1); 5: Tenerife (Güímar, Barranco de Badajoz), A. Santos 6A (ORT) (ITS HM563794, *trnK* HM563801); 6: La Palma (Barlovento, Las Mimbreras), A. Santos 3A (ORT) (ITS HM563795, *trnK*=VR1); 7: La Palma (Barlovento, Las Mimbreras), A. Santos 3B (ORT) (ITS HM563796, *trnK*=VR1); 8: El Hierro (Frontera, Támano), A. Santos 1A (ORT) (ITS HM563797, *trnK*=VR1).

***Viburnum tinus* subsp. *tinus***:—1: Mainland Portugal (Serra da Arrábida, Parque Natural), PNA 95A (AZB) (ITS HM563798, *trnK*=VR1); 2: Mainland Portugal (Serra da Arrábida, Parque Natural), PNA 96A (AZB) (ITS=VT1, *trnK*=VR1); 3: Mainland Spain (S. Pedro-Rondo, Málaga), C. Evrard 7908 (BM) (ITS=VT1, *trnK* HM563802); 4: France (Hérault, Montpellier), J. Mathez 1 (MPU) (ITS=VT1, *trnK*=VR1); 5: France (Hérault, Montpellier), J. Mathez 2 (MPU) (ITS=VT1, *trnK*=VR1); 6: Morocco (Aïn-Leuh, Meknés-Tafilalet), J.-F. Léger 2 (MPU) (ITS=VT1, *trnK*=VR1); 7: Morocco (Aïn-Leuh, Meknés-Tafilalet), J.-F. Léger 3 (MPU) (ITS HM563799, *trnK*=VR1); 8: Morocco (El-Ksiba, Beni-Mellal), Jury *et al.* 17446 (BM) (ITS=VT1, *trnK*=VR1); 9: Italy (Toscana, Livorno, Bibona, Maccia della Magona), Dürbye, Weber & Meyer Dür 1061 (B) (ITS=VT1, *trnK*=VR1); 10: Croatia (Rab) *N. Kilian & M. Romebach* 555 (B) (ITS=VT1, *trnK*=VR1); 11: Israel (Har Ha’Karmel) (ITS=VT1, *trnK*= KF430081); 12: Greece (Preveza, south of Kato Myrsini), R. & E. Willing 103.867 (B) (ITS=VT1, *trnK*=VR1); 13: Turkey (Boğaziçi, Tarabya), H. & E. Walter 3322 (B) (ITS=VT1, *trnK*=VR1); 14: Algeria (Annaba, Laverdure), Leippert 7104 (B) (ITS=VT1, *trnK*=VR1); 15: Tunisia (Zaghouan, Djebel Zaghouan), R. Vogt & Ch. Oberprieler RV 13862, CO 8167 (B) (ITS=VT1, *trnK*=VR1).

**GenBank accessions**:—*Viburnum cinnamomifolium* Rehder—R. Olmstead 2002-120 (WTU) (ITS AY265116, *trnK* AY265162). *Viburnum davidii* Franchet—M. J. Donoghue, voucher lacking (ITS AY265120, *trnK* AY265166). *Viburnum propinquum* Hemsl.—M. J. Donoghue 100 (ITS EF462987, *trnK* EF490250). *Viburnum atrocyaneum* Clarke—Boufford *et al.* 34956 (ITS HQ591950, *trnK* HQ591782). *Viburnum calvum* Rehder—Li & Soukup 934 (ITS HQ591955, *trnK* HQ591788). *Viburnum tinus* L. subsp. *tinus*—cultivated, M. J. Donoghue & R. C. Winkworth 39 (YU, A) (ITS AY265152, *trnK* AY265198). *V. acerifolium* L.—M. J. Donoghue & R. C. Winkworth 27 (ITS AY265114, *trnK* AY265160), *V. flavescens* W. W. Sm.—Boufford *et al.* 32758 (ITS HQ591962, *trnK* HQ591794), *V. edule* (Michx.) Raf.—NVI s.n. (ITS AY265123, *trnK* AY265169), *V. ellipticum* Hook.—NVI s.n. (ITS AY265125, *trnK* AY265171), *V. dentatum* L.—M. J. Donoghue & R. C. Winkworth 33 (ITS AY265121, *trnK* AY265167), *V. farreri* W.T. Stearn—M. J. Donoghue & R. C. Winkworth 18 (ITS AY265128, *trnK* AY265174), *V. lutescens* Blume—Wu *et al.* WP531 (ITS HQ591969, *trnK* HQ591802) and *V. taiwanianum* Hayata—W.-H. Hu *et al.* 2186 (ITS EF462989, *trnK* EF490253).