



Allium canariense (Amaryllidaceae), a species endemic to the Canary Islands

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Abstract

A revision of the *Allium* species of the section *Molium* in the Canary Islands is presented. As part of the phylogenetic revision of the Eurasian representatives of the subgenus *Amerallium* we found large disagreements in the nomenclature and taxonomy of *Allium roseum* in the floristic publications on the flora of the Canary Islands. At least four species of the section *Molium* are mentioned for the flora of the Canary Islands: *Allium roseum*, *A. subvillosum*, *A. subhirsutum* and *A. trifoliatum*. To learn more about the phylogenetic relationships within a group of closely related species of the section *Molium*, we used maximum parsimony and Bayesian analyses of combined nuclear (ITS—internal transcribed and ETS—external transcribed spacers of rRNA genes) and three chloroplast (*rpl32-trnL* and *trnL-trnF* intergenic spacers and *rps16* intron) datasets of 7 taxa. For comparison of the relationships of *A. canariense* populations between the islands we used the ISSR method. We found that only one species of the section *Molium* occurs in the Canary Islands—the endemic species *Allium canariense*, closely related to *Allium subvillosum*. Independence of this new species has been confirmed by morphological and molecular features. Discussion on phylogeny, origin and geographic distribution is provided.

Key words: *Molium*, phylogeny, ITS, ETS, *rpl32-trnL*, *trnL-trnF*, *rps16*, divergence time

Introduction

As part of the phylogenetic revision of the Eurasian representatives of *Allium* Linnaeus (1753: 294) subgenus *Amerallium* Traub (1968: 159) we found large disagreements in the nomenclature and taxonomy of *Allium roseum* Linnaeus (1753: 296) in the floristic publications on the flora of the Canary Islands. Ten *Allium* species were identified by Acebes Ginovés *et al.* (2009), at least four of them belong to the section *Molium* G. Don ex Koch (1837: 715): *Allium roseum*, *A. subvillosum* Salzm. ex Schult. & Schult.f. in Roemer & Schultes (1830: 1104), *A. trifoliatum* Cirillo (1788: 11) and *A. subhirsutum* Linnaeus (1753: 295). In the first classic flora by Webb & Berthelot (1848), two species were listed: *Allium roseum* and *A. trifoliatum*. (The latter was sometimes subsequently treated as a variety of *Allium subhirsutum*). The description of *Allium subvillosum* β *canariense* Regel (1875: 249) was overlooked by all the following authors, except Wilde-Duyfjes (1976) and listed as such in the recent “Flora de Gran Canaria” (<http://www.jardincanario.org/busqueda-de-la-flora-de-gran-canaria>) as a synonym for *Allium subhirsutum* L. subsp. *subvillosum* (Salzm. ex Schult. & Schult.f.) Duyfjes (1976: 137).

Pitard & Proust (1908) and Lindinger (1926) mentioned only *Allium roseum* and *Allium trifoliatum*, while *Allium trifoliatum* subsp. *obtusitepalum* Sventenius (1960: 3) was described from the small island Alegranza, later recombined under a different species: *Allium subhirsutum* subsp. *obtusitepalum* (Svent.) Kunkel (1971: 55). Eriksson *et al.* (1974) listed *Allium roseum*, *A. subhirsutum* and *A. trifoliatum* subsp. *obtusitepalum*, but Wilde-Duyfjes (1976) indicated for the first time that *Allium roseum* is not indigenous to the Canary Islands and included all accessions of *A. roseum* auct. non L. as well as *A. subvillosum* β *canariense* into *A. subhirsutum* subsp. *subvillosum*. In the 2. and 3. edition of “Flora of Macaronesia: checklist of vascular plants” by Hansen & Sunding (1979, 1985) *Allium roseum*, *A. subhirsutum* with subsp. *subvillosum* and subsp. *obtusitepalum* were accepted, while in the 4th edition (Hansen & Sunding 1993) *A. subvillosum* was accepted at species level. Kunkel *et al.* (1992) mentioned *Allium roseum* and *A. subhirsutum* subsp. *obtusitepalum*, and Hohenester & Welss (1993) *A. roseum*, *A. trifoliatum* and *A. subhirsutum*. In all three editions of the “Lista de especies silvestres de Canarias” by Acebes Ginovés *et al.* (2001; 2004; 2009) three species are listed:

A. roseum, *A. subhirsutum* with the two subspecies and *A. subvillosum*. As noticed by Anthos (<http://www.anthos.es/index.php?lang=en>), *Allium subhirsutum* subsp. *obtusitepalum* is recorded in two recent publications for Fuerteventura und Lanzarote: Scholz (2005) and Trujillo & Gilberto (2003), but in the latter work, only *Allium* sp. is mentioned. Stierstorfer & v. Gaisberg (2006) treated all records for El Hierro of *Allium roseum* and *A. subhirsutum* as belonging to a single widespread species: *A. subvillosum*.

In April 2013, one of us (NF) was on a botanical excursion with students on La Palma where he collected several accessions of *Allium* “*roseum*” and found disagreements within the nomenclature of the *Allium* taxa in Canary Islands. Schönfelder & Schönfelder (2012) pointed out that, on the Canary Islands, *Allium* “*roseum*” stamens are often longer than the tepals, which is not the case of the Mediterranean *A. roseum* (Schönfelder & Schönfelder 2008).

ITS sequences of accessions from La Palma showed that they are identical to *Allium subvillosum* from Tenerife, Mallorca (Spain) and Portugal. Morphologically they were also similar to accessions from Tenerife. Detailed phylogenetic analysis was needed to elucidate the taxonomical status of *Allium* “*roseum*” on the Canary Islands. In the present study we analysed all *Allium* taxa of section *Molium* mentioned for the Canary Islands and included two related taxa: *A. chamaemoly* Linnaeus (1753: 301) and *A. neapolitanum* Cirillo (1788: 13). We included accessions from all islands of the Canarian archipelago to check their relationships and the possible distribution pattern of the Canarian *Allium* taxa of sect. *Molium*. We sequenced two nuclear (ITS and ETS) and three plastid (*trnL-trnF*, *rpl32-trnL* spacers and *rps16* intron) regions and performed a phylogenetic analysis. For comparison of the relationships of *Allium* taxa between islands we used the ISSR (inter-simple sequence repeat) method. ISSR markers were chosen because the technique is very simple, fast, cost effective, highly discriminative, reliable and requires only a small quantity of sample DNA (Godwin *et al.* 1997).

The Canary Islands are one of the best studied volcanic archipelagos, concerning both their geological history (Ancochea *et al.* 1990, Acosta *et al.* 2003), phylogeography (Juan *et al.* 2000, Hochkirch & Görzig 2009, Husemann *et al.* 2014) and phytogeography (Böhle *et al.* 1996, Francisco-Ortega *et al.* 1996, Vargas *et al.* 1999, Francisco-Ortega *et al.* 2001, Kim *et al.* 2008, Mairal *et al.* 2015). Many taxa have undergone radiation within the archipelago subsequent to a single colonization event. Most of these radiations follow a simple stepping-stone model from east to west following the prevailing ocean currents and wind directions (Kim *et al.* 1996, Mort *et al.* 2002). However, multiple variants of this pattern have been found, including back-colonization, within-island speciation or reverse colonization from Canary Islands to the African mainland (Juan *et al.* 2000, Kim *et al.* 2008).

Material and Methods

Plant material:—The DNA was extracted from leaves and flowers of vouchers from herbarium specimens deposited in B, FR, FB, GAT, HUI, ORT, OSBU, TFC and W. Additional samples were extracted directly from the *Allium* collections in the Botanical Garden of the University of Osnabrueck. Origin and corresponding GenBank accession numbers of specimens used for DNA sequencing and fingerprint analyses are presented in Table 1.

Karyotype analysis:—Bulbs were planted in pots and growing root tips were used for the chromosome studies in mitosis. Excised roots were kept in distilled water on ice overnight. They were then transferred to room temperature for 20 min and pre-treated for 2 h at room temperature in an aqueous 0.1% solution of colchicine. The tissue was fixed in a freshly prepared mixture of 96% ethanol and glacial acetic acid (3:1). Haematoxylin staining according to Smirnov (1968) was used for imaging. For chromosome morphology, the classification of Levan *et al.* (1964) and Tzanoudakis (1983) was followed. Karyotype intrachromosomal asymmetry was determined calculating the M_{CA} index (Peruzzi & Eroğlu 2013) and interchromosomal asymmetry was determined calculating the CV_{CL} index (Paszko, 2006). The karyotype was studied on five metaphase plates from two bulbs.

DNA Sequencing:—Total genomic DNA was isolated from herbarium specimens using the “InnuPREP Plant DNA Kit” (Analytic Jena AG) according to the instructions of the manufacturer and used directly in PCR amplifications. The ITS region was amplified using primers ITS-A and ITS-B (Blattner 1999). The ETS region was amplified using the primers 18S-IGS (Baldwin & Markos 1998) and ETS-all-f (Nguyen *et al.* 2008). The PCR condition was identical to that described in Herden *et al.* (2012). Primers for the chloroplast regions were as follows: for the *rpl32-trnL* region described in Shaw *et al.* (2007), for *rps16* intron described in Oxelman *et al.* (1997), for *trnL-trnF* described in Taberlet *et al.* (1991). PCR products were sent to SeqLab (Göttingen, www.seqlab.de) for sequencing. Forward and reverse sequences from each individual were manually edited in CHROMAS Lite 2.1 (Technelysium Pty Ltd) and combined in single consensus sequences. The sequences of all samples were aligned with CLUSTAL X (Thompson *et al.* 1997), and the alignment subsequently corrected manually in MEGA 6 (Tamura *et al.* 2013).

TABLE 1. Accessions of *Allium* species used in the study.

Accession	Species	Origin	Voucher	nrITS	nrETS	Rpl32-trnL	trnL-trnF	rps16
Am-70	<i>A. canariense</i>	Spain, Canary Islands, Tenerife	OSN 06-09-0034-20	HF934337	HF934565	HF934672	HF934785	HF934226
Am-123	<i>A. canariense</i>	Spain, Canary Islands, Tenerife	OSN 08-02-0090-20	HF934297	HF934528	HF934632	HF934738	HF934179
Am-124	<i>A. canariense</i>	Spain, Canary Islands, Tenerife	OSN 08-02-0091-20	HF934298	HF934529	HF934633	HF934739	HF934180
Am-434	<i>A. canariense</i>	Spain, Canary Islands, Tenerife	FR 14.01.02	HF934299	HF934530	LN823671	LN823700	LN823642
Am-536	<i>A. canariense</i>	Spain, Canary Islands, Tenerife	Hb Schönfelder 10-28	LN823584	LN823613	LN823672	LN823701	LN823643
Am-596	<i>A. canariense</i>	Spain, Canary Islands, Tenerife	Schönfelder 2013-12-30	LN823585	LN823614	LN823673	LN823702	LN823644
Am-615	<i>A. canariense</i>	Spain, Canary Islands, Tenerife	OSBU23340	LN823586	LN823615	LN823674	LN823703	LN823645
Am-616	<i>A. canariense</i>	Spain, Canary Islands, Tenerife	OSBU 23341	LN823587	LN823616	LN823675	LN823704	LN823646
Am-617	<i>A. canariense</i>	Spain, Canary Islands, Tenerife	OSBU 23342	LN823588	LN823617	LN823676	LN823705	LN823647
Am-619	<i>A. canariense</i>	Spain, Canary Islands, Tenerife	OSBU 23339	LN823589	LN823618	LN823677	LN823706	LN823648
Am-474	<i>A. canariense</i>	Spain, Canary Islands, La Palma	OSBU 22349	LN823590	LN823619	LN823678	LN823707	LN823649
Am-475	<i>A. canariense</i>	Spain, Canary Islands, La Palma	OSBU 22423	LN823591	LN823620	LN823679	LN823708	LN823650
Am-531	<i>A. canariense</i>	Spain, Canary Islands, La Palma	Hb Schönfelder 03-35	LN823592	LN823621	LN823680	LN823709	LN823651
Am-544	<i>A. canariense</i>	Spain, Canary Islands, La Palma	OSBU 22388	LN823593	LN823622	LN823681	LN823710	LN823652
Am-614	<i>A. canariense</i>	Spain, Canary Islands, Gran Canaria	Hb Schönfelder 14-15	LN823594	LN823623	LN823682	LN823711	LN823653
Am-532	<i>A. canariense</i>	Spain, Canary Islands, La Gomera	Hb Schönfelder 10-3	LN823595	LN823624	LN823683	LN823712	LN823654
Am-533	<i>A. canariense</i>	Spain, Canary Islands, La Gomera	Hb Schönfelder 10-15	LN823596	LN823625	LN823684	LN823713	LN823655
Am-547	<i>A. canariense</i>	Spain, Canary Islands, El Hierro	B 100245045	LN823597	LN823626	LN823685	LN823714	LN823656
Am-548	<i>A. canariense</i>	Spain, Canary Islands, El Hierro	B 100245046	LN823598	LN823627	LN823686	LN823715	LN823657
Am-534	<i>A. canariense</i>	Spain, Canary Islands, Lanzarote	Hb Schönfelder 09-21	LN823599	LN823628	LN823687	LN823716	LN823658
Am-535	<i>A. canariense</i>	Spain, Canary Islands, Lanzarote	Hb Schönfelder 09-44	LN823600	LN823629	LN823688	LN823717	LN823659
Am-590	<i>A. canariense</i>	Spain, Canary Islands, Lanzarote	ORT 42417	LN823601	LN823630	LN823689	LN823718	LN823660
Am-592	<i>A. canariense</i>	Spain, Canary Islands, Montaña Clara	ORT 39750	LN823602	LN823631	LN823690	LN823719	LN823661
Am-594	<i>A. canariense</i>	Spain, Canary Islands, Fuerteventura	TFC 46482	LN823603	LN823632	LN823691	LN823720	LN823662
Am-595	<i>A. canariense</i>	Spain, Canary Islands, Fuerteventura	TFC 46506	LN823604	LN823633	LN823692	LN823721	LN823663
Am-94	<i>A. subvillosum</i>	Portugal, Algarve	OSBU 15067	LN823605	LN823634	LN823693	LN823722	LN823664
Am-430	<i>A. subvillosum</i>	Spain, Mallorca, Lluçmajor	FR-0034934	HF934338	HF934566	HF934673	HF934786	HF934227
Am-538	<i>A. subvillosum</i>	Spain, Mallorca, Cabo Blanco	Hb Schönfelder 99-3	LN823606	LN823635			
Am-670	<i>A. subvillosum</i>	Morocco, Prov. Tanger	FB 04746	LN823607	LN823636	LN823694	LN823723	LN823665
Am-671	<i>A. subvillosum</i>	Morocco, Prov. Tetouan	FB 04747	HF934339	HF934567	HF934674	HF934787	HF934228
Am-672	<i>A. subvillosum</i>	Morocco, Prov. Al Hoceima	FB 04748	LN823608	LN823637	LN823695	LN823724	LN823666
Am-673	<i>A. subvillosum</i>	Morocco, Prov. Khefira	FB 04749	LN823609	LN823638	LN823696	LN823725	LN823667
Am-81	<i>A. chamaemoly</i>	Greece, Corinthia pr. Kalamata	HUJ 2359	HF934271	HF934500	HF934609	HF934711	HF934152
Am-90	<i>A. chamaemoly</i>	Italy, Monte Testaccio, Roma	W 1974/02005		HF934501	HF934610	HF934712	HF934153
Am-126	<i>A. chamaemoly</i>	Spain, Malaga, Ardales	OSN 07-11-0032-20	HE962504	HE859943	HE859954	HF934713	HF934154
Am-26	<i>A. subhirsutum</i>	Turkey, Kusadasi	GAT 5322	HF934333	HF934561	HF934668	HF934781	HF934222
Am-228	<i>A. subhirsutum</i>	Greece, Lakonia,	B 100073951	HF934335	HF934563	HF934670	HF934783	HF934224
Am-283	<i>A. subhirsutum</i>	Italy, Monte Argentario	GAT 1461	HF934336	HF934564	HF934671	HF934784	HF934225
Am-222	<i>A. roseum</i>	Greece, Peranthe	B 100257074	HF934328	HF934557	HF934662	HF934775	HF934216
Am-243	<i>A. roseum</i>	Tunesia, Tabarka, Tell Atlas	GAT 3266	HF934329	HF934558	HF934663	HF934776	HF934217
Am-357	<i>A. roseum</i>	Spain, Alicante, Denia	HUJ, 28.01.96	HF934330	HF934559	HF934664	HF934777	HF934218
Am-36	<i>A. neapolitanum</i>	Turkey, Vilajet Antalya, Side	OSN 12-15-0043-20	HF934305	HF934533	HF934639	HF934748	HF934189
Am-341	<i>A. neapolitanum</i>	Israel, Mt. Gilboa	OSBU 20938	HF953353	HF953352	HF934642	HF934752	HF934193
Am-77	<i>A. moly</i>	Spain, Jaen, Sierra de Cazorla	FR, 4.06.99	HE962501	FN551226	HE859953	HF934740	HF934410

Phylogenetic analyses:—Phylogenetic analyses were carried out on both combined data sets (nuclear (ITS and ETS) and plastid DNA sequences) using parsimony and Bayesian methods. *Allium moly* Linnaeus (1753: 295) has been chosen as the outgroup based on the analyses of Friesen *et al.* (2006) and taxonomical revisions of Wilde-Duyfjes (1973) and Pastor & Valdes (1983). Parsimony analysis was performed with PAUP* 4.0b10 (Swofford 2002) using heuristic searches with TBR and 100 random addition sequence replicates. Bootstrap support (BS; Felsenstein 1985) was estimated with 100 bootstrap replicates, each with 100 random addition sequence searches. Bayesian analyses were conducted with MrBayes 3.1.23 (Ronquist & Huelsenbeck 2003). Sequence evolution models were evaluated using Akaike information criterion (AIC) with the aid of Modeltest 3.7 (Posada & Crandall 1998). Two independent runs each of eight chains, 10 million generations, sampling every 100 trees. 25% of initial trees were discarded as burn-in. The remaining 15,000 trees were combined into a single data set, and a majority-rule consensus tree was obtained. Bayesian posterior probabilities (BP) were calculated for that tree in MrBayes 3.1.23.

Divergence time estimation:—Beast 1.8 (Drummond & Rambaut 2007) was used to estimate the divergence times. The Beauti 1.8 interface was used to create input files for Beast and the XML files were adjusted manually if necessary. For the estimation, a reduced subset of 18 species was selected for the combined cpDNA markers (see Table 1). We used the uncorrelated lognormal relaxed clock (ucl) with the substitution models selected by AIC. The Yule process was chosen as speciation process. As there are no fossils in the genus *Allium*, we used the calculated nucleotide substitution rates in chloroplast DNA for grasses. The ucl.mean was set to a normal distribution with a mean of 1.52×10^{-9} substitutions per site per year (sub/site/yr) and a standard deviation (SD) of 0.06×10^{-9} according to the works of Yamane *et al.* (2006). The user-specified starting tree was calculated in the same manner as the phylogenetic tree for the cpDNA and inserted in the XML files in the Newick format. Other parameters were set to default. Several short Beast runs were first conducted to examine the performance of the *Markov Chain Monte Carlo (MCMC)* methods. Finally, three Beast runs were performed with the MCMC chain length of 10^8 generations and a sample frequency of every 100 generations. We also ran an analysis with an empty alignment to ensure that the priors alone were not determining the results. The effective sample size (ESS) was >200 with a 25% burn-in for all parameters as confirmed by analysing the output file with Tracer 1.5. The tree output files from Beast were summarized with LogCombiner 1.8, annotated with the program TreeAnnotator 1.8 and burn-in set to 25% with the aid of Tracer. The mean node heights option was selected and the posterior probability set to 0.5. The trees were visualized using FigTree 1.4.0 with means and 95% highest posterior density (HPDs) age estimates.

ISSR amplification and analysis:—Fifteen ISSR primers were screened for amplifiability and amount of polymorphism; finally, six primers (Table 2) which amplified polymorphic DNA fragments were selected for analysis. Amplification was performed in a volume of 25 μ l containing 2.5 mM of each dNTP, 1 U Taq DNA polymerase, 6 μ g of primer, 1.5 mM of MgCl₂, and ca. 100 ng of genomic DNA. Amplification began with initial denaturation of 4 min at 95 °C, followed by 35 cycles of 30 sec denaturation at 94 °C, 1 min annealing at 45–55 °C (Table 2), 2 min extension at 71 °C, and a final extension step of 10 min at 72 °C. PCR products were separated by gel electrophoresis in 1.5% agarose gel at a fixed voltage (80 V) in 0.5 X TBE buffer for approximately 120–130 min. Gels were stained with ethidium bromide and the size of the produced fragment defined by comparing it to a size marker (100 bp extended ladder, T835.1, Carl ROTH). The polymorphic bands were scored as absent (0) or present (1) for all samples. A similarity matrix was generated based on the Dice/Nei+Li model. ISSR data were analyzed using NTSYSpc version 2.02 (Rohlf 2000). The similarity matrix was used for cluster analysis and construction of the UPGMA and NJ trees.

TABLE 2. List of ISSR primers, their sequence motifs, annealing temperatures, number of the amplified fragments generated. R= AG.

No.	Primers	5'-3' motif	Annealing temperature	Number of polymorphic bands	Total number of bands amplified
1	MAO	(CTC)4-RC	45°C	21	25
2	HB 10	(GA)6-CC	45°C	25	28
3	HB12	(CAC)3-GC	44°C	14	18
4	HB14	(CTC)3-GC	44°C	13	15
5	17898A	(CA)6-AC	45°C	10	13
6	UBC807	(AG)8-T	50°C	26	29

Results

Karyotype Analysis:—The chromosome studies on two accessions from La Palma (Am474 and Am544) and three accessions from Tenerife (Am615, Am616 and Am619) revealed the chromosome number $2n = 28$ (Fig. 1a). Karyotype was studied on accession Am474 from La Palma. Seven chromosomes pairs are submetacentric, six pairs subtelocentric and only one metacentric (Fig. 1b; Table 3).

Phylogenetic Analysis:—The alignment of ITS sequences of the 48 taxa (Table 1) generated a matrix of 665 characters, of which 111 were parsimony informative. For the Bayesian analyses, the substitution model TrN+G was chosen by AIC in Modeltest 3.7. Parsimony analysis resulted in 2 most parsimonious trees of 189 steps (consistency index (CI) = 0.8660; retention index (RI) = 0.933). All accessions from the Canary Islands built a monophyletic clade together with *A. subvillosum* without resolution. Only two weakly supported clades are formed inside the Canarian plants: one clade with plants from La Gomera and El Hierro (BS = 62%, BP = 0.81); the second clade with plants from Fuerteventura and Montana Clara (BS = 59%, BP = 0.79). One accession of *A. subvillosum* from Morocco (Am671) is sister group to all other *A. subvillosum* (tree not shown). The alignment of ETS sequences generated a matrix of

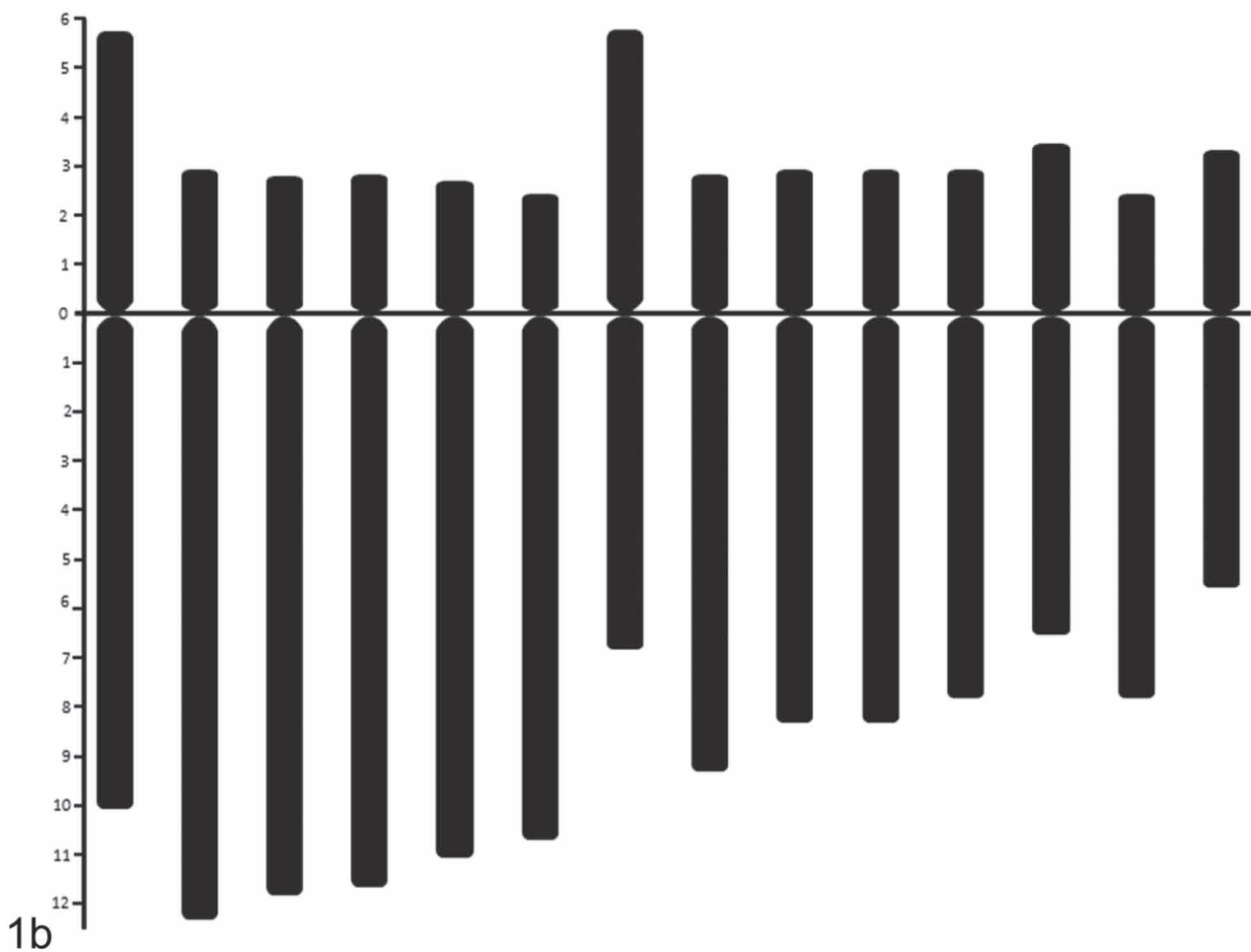
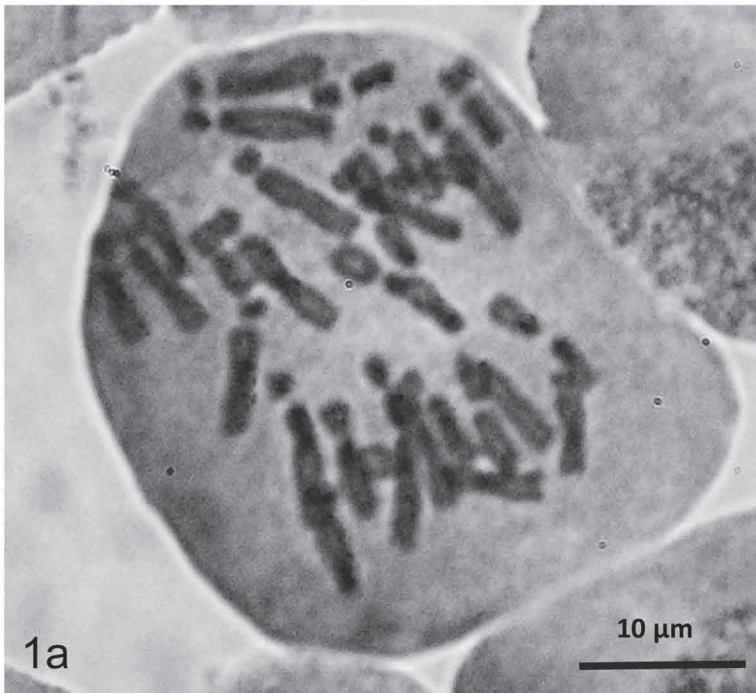


FIGURE 1. Metaphase chromosomes of *Allium canariense* from La Palma, $2n = 28$ (a); Idiogram of *Allium canariense*, $n = 14$ (b).

469 characters, of which 127 were parsimony informative. For the Bayesian analyses, the substitution model TrN+G was chosen by AIC in Modeltest 3.7. Parsimony analysis resulted in 26 most parsimonious trees of 234 steps (CI =

0.8751; RI 0.9619). All Canary Islands accessions were grouped into a weakly supported monophyletic clade (BS = 67% and BP = 0.89) sister to accessions of *A. subvillosum* from the Iberian Peninsula and Mallorca. Four accessions of *A. subvillosum* from Morocco formed also a monophyletic clade with 75% bootstrap support and 0.96 BP (tree not shown). As the topology of both trees (ITS and ETS) did not produce contradictory results, we combined the nuclear sequences of both regions generating a combined matrix of 1134 characters, of which 238 were parsimony informative. For the Bayesian analyses, the substitution model TrN+G was chosen by AIC in Modeltest 3.7. Parsimony analysis of the 48 sequences resulted in 6 most parsimonious trees of 427 steps (CI = 0.8475; RI 0.9574). The consensus phylogenetic tree of combined ITS and ETS regions is shown in Figure 2. All accessions from the Canary Islands were grouped into a weakly supported Canarian clade (BS = 68% and BP = 0.92). This clade, in turn, is divided into four clades with unresolved relationships: one greater clade including all accessions from Tenerife, La Palma and Gran Canaria (BS = 62%; BP = 0.70); a clade with accessions from La Gomera and El Hierro (BS = 78%; BP = 0.92); a clade with accessions from Montana Clara and Fuerteventura (BS = 61%; BP = 0.63) and, not resolved, accessions from Lanzarote. The Canary clade is a sister group to the clade of *A. subvillosum* accessions from the Iberian Peninsula. Four accessions of *A. subvillosum* from Morocco form the sister group to *A. subvillosum* from the Iberian peninsula and Canarian clade (BS = 80%, BP = 0.98). Sister group to *A. subvillosum* and plants from the Canary Islands is the clade with accessions of *A. chamaemoly*. Monophyletic clades of *A. neapolitanum* and *A. subhirsutum* formed a strongly supported clade which is sister to the *A. subvillosum* / *A. chamaemoly* clade and the clade of *A. roseum*.

TABLE 3. Karyo-morphometric parameters for *A. canariense* (Am474). Mean values come from 5 good metaphase plates. Abbreviations: TAL = total absolute length; LA = long arm; SA = short arm; RL = relative length; CI = centromeric index; Type = chromosome nomenclature according to Levan *et al.* (1964) and Tzanoudakis (1983); THL = total karyotype haploid length; M_{CA} = mean centromeric asymmetry; KCI = karyotype centromeric index; CV_{CL} = coefficient of variation of chromosome length.

Pair n.	TAL μm	RL	LA μm	SA μm	CI %	Type
I	18.0 \pm 0.4	8.8 \pm 0.4	12.1 \pm 0.4	5.9 \pm 0.5	19.3	st
II	15.8 \pm 0.6	9.3 \pm 0.2	9.9 \pm 0.3	5.9 \pm 0.4	37.3	sm
III	14.2 \pm 0.6	8.3 \pm 0.3	11.8 \pm 0.5	2.4 \pm 0.4	16.9	st
IV	14.0 \pm 0.5	8.2 \pm 0.4	11.6 \pm 0.4	2.4 \pm 0.5	17.1	st
V	13.5 \pm 0.4	7.9 \pm 0.2	11.0 \pm 0.3	2.5 \pm 0.4	18.5	st
VI	12.9 \pm 0.6	7.6 \pm 0.4	10.6 \pm 0.5	2.3 \pm 0.5	17.8	st
VII	12.4 \pm 0.3	7.3 \pm 0.2	6.8 \pm 0.3	5.6 \pm 0.3	45.1	m
VIII	11.8 \pm 0.4	6.9 \pm 0.4	9.2 \pm 0.3	2.6 \pm 0.3	22.0	st
IX	11.2 \pm 0.6	6.6 \pm 0.3	8.4 \pm 0.6	2.8 \pm 0.6	25.0	sm
X	11.0 \pm 0.5	6.4 \pm 0.4	8.2 \pm 0.4	2.8 \pm 0.4	25.4	sm
XI	10.3 \pm 0.4	6.0 \pm 0.2	7.5 \pm 0.5	2.8 \pm 0.3	27.2	sm
XII	10.0 \pm 0.6	5.9 \pm 0.4	6.5 \pm 0.4	3.3 \pm 0.5	33.6	sm
XIII	9.8 \pm 1.0	5.7 \pm 0.5	7.7 \pm 0.7	2.3 \pm 0.7	23.0	sm
XIV	8.7 \pm 0.4	5.1 \pm 0.5	5.5 \pm 0.5	3.2 \pm 0.4	36.8	sm
THL = 173.6 \pm 1.4; M_{CA} = 46.4; KCI = 26.95; CV_{CL} = 3.31						

The sequence lengths of the three chloroplast regions analyzed for this study are: 287–313 bp for *trnL-trnF*; 770–877 bp for *rpl32-trnL* and 805–840 bp for *rps16* within section *Molium*. Alignment length of 2134 bp consisted of position 1–321 for *trnL-trnF*; 322–1273 for *rpl32-trnL* and 1274–2134 for *rps16*, 1947 characters were constant, 57 variable characters are parsimony-uninformative and 130 were parsimony informative. Parsimony analysis resulted in up to 20,000 trees of 222 steps (CI = 0.8784, RI = 0.9551). For the Bayesian analyses, the substitution model K81uf+I was chosen by AIC in Modeltest 3.7. The consensus tree of combined plastid regions is shown in Figure 3. All plants from the Canary Islands formed a weakly supported clade (BS = 54%, BP = 0.88), and were sister to continental accessions of *A. subvillosum*. Within the unresolved clade with plants from the Canary Islands, a few small groups appeared: three accessions from La Palma and both accessions from El Hierro formed a clade (BS = 71% and BP = 0.98); all accessions from Lanzarote and accession from the neighboring Island Montana Clara formed also a not strongly supported clade (BS = 65% and BP = 0.99) and one accession from Fuerteventura is sister to this clade (BS = 65% and BP = 0.95). The sister clade of the strongly supported common clade (BS 100%, BP 1.0) of *A. subvillosum* with Canarian clade is *A. neapolitanum*.

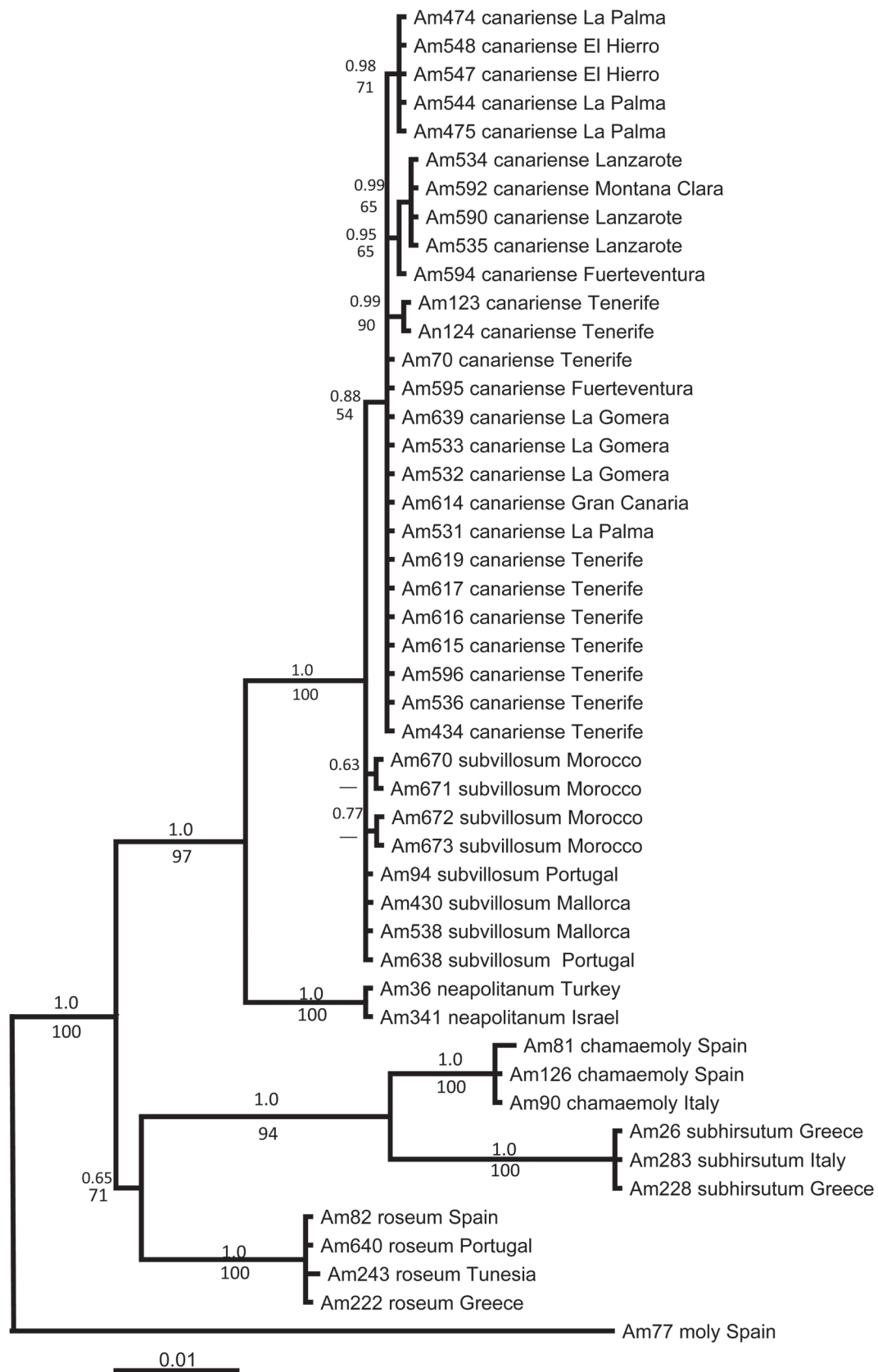


FIGURE 3. Phylogenetic tree based on a combined plastid DNA (*trnL-trnF*, *rpl32-trnL* and *rps16*) sequence data set. Bayesian posterior probabilities above branches, bootstrap support over 50% below branches.

ISSR analysis:—The six selected ISSR primers resulted in 109 polymorphic DNA fragments (Table 2) from genomic DNA of 30 individuals with fragment sizes ranging from 300 to 2300 bp. Nineteen fragments were monomorphic in all samples and were not included in the analysis. The number of fragments varied from 13 (17898A) to 29 (UBC807). The ISSR data clearly divided all accessions studied into two groups: continental *Allium subvillosum* (Iberian Peninsula and Morocco) in one and all Canarian *Allium* plants in the other (Figure 4). All Canarian accessions are also clearly divided into two groups: plants from the eastern islands (Lanzarote, Fuerteventura and Montana Clara) and plants from the western islands (Tenerife, La Palma, Gran Canaria, El Hierro and La Gomera). All accessions derived from single islands are not identical but clearly monophyletic. The most variable pattern have the eight accessions from Tenerife.

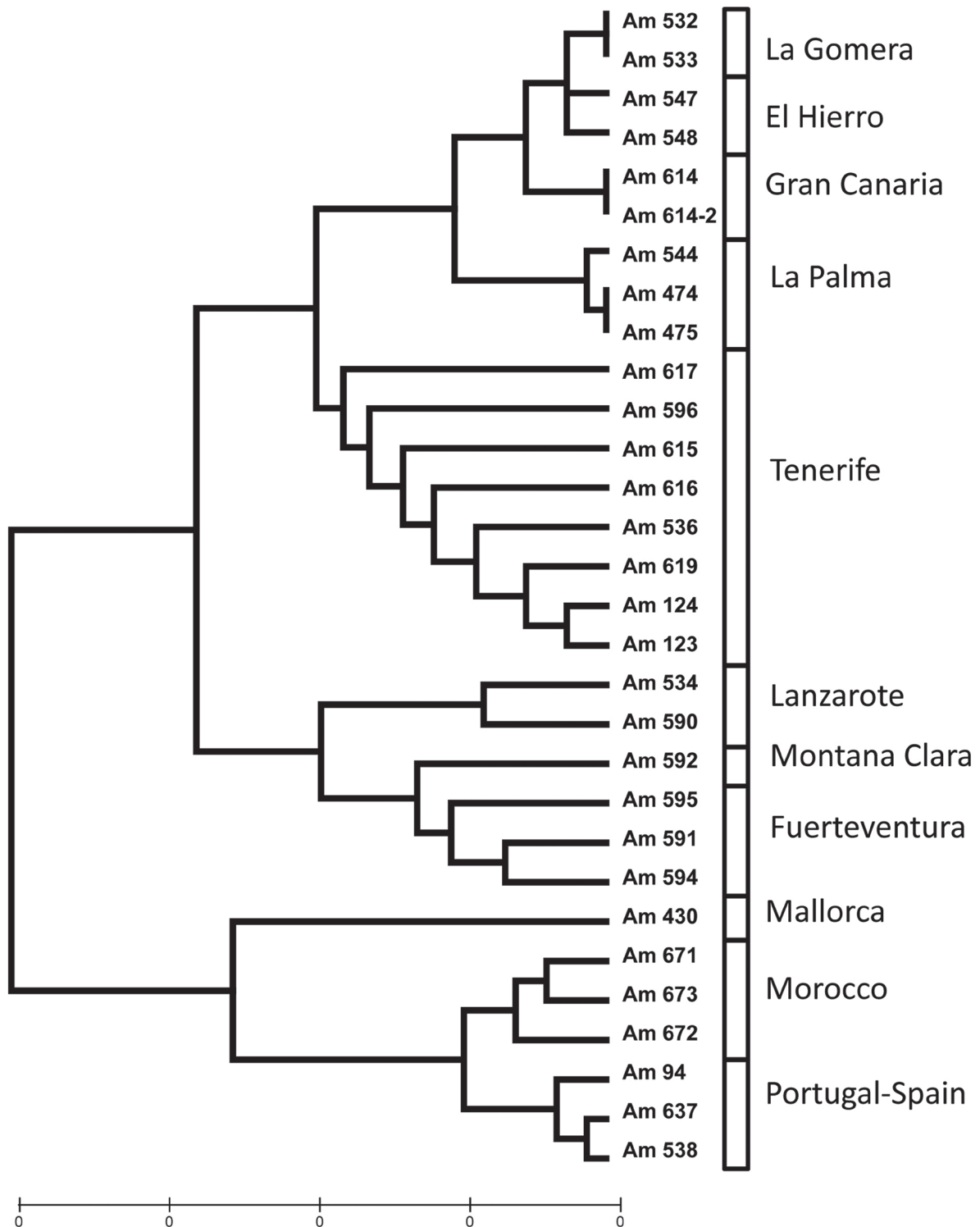


FIGURE 4. UPGMA tree based on 109 polymorphic ISSR marker.

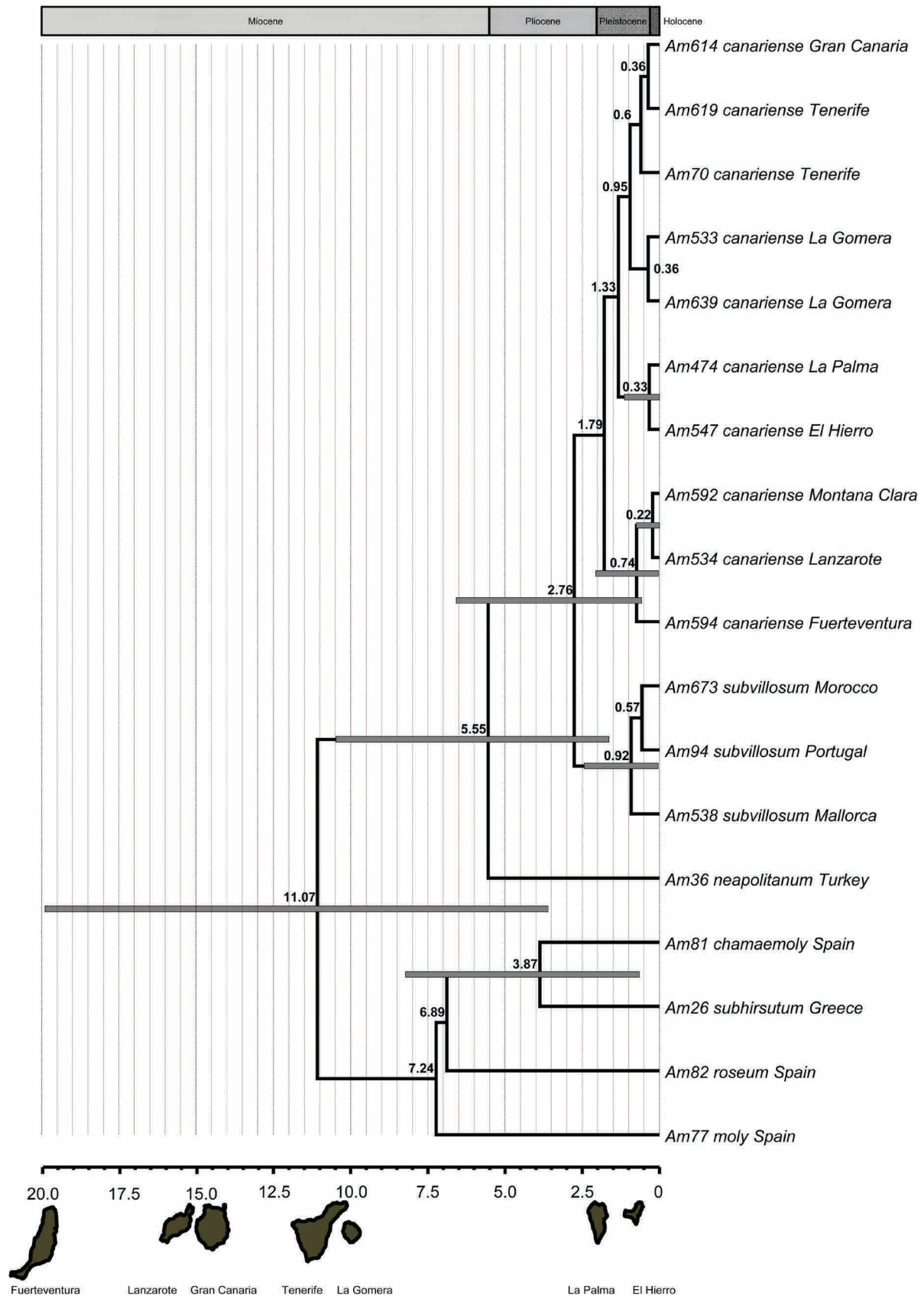


FIGURE 5. Chronogram with divergence time estimates based on cpDNA sequences of 18 taxa. The maximum clade credibility tree from the divergence times estimated with BEAST. Mean divergence time estimates are shown at the nodes. The 95% highest posterior density (HPD) estimates for each node are represented by bars. Island ages are displayed on the lower axis, whereas geological epochs are displayed on the upper axis. Geological epochs are according to the international commission on stratigraphy (ICS).

Divergence time estimation:—The root of the chronogram was computed to an age of 11.07 mya (95% HPD: 3.61–19.9) setting *A. moly*, *A. roseum*, *A. subhirsutum* and *A. chamaemoly* as outgroup taxa (Fig. 5). Around 5.5 mya (95% HPD: 1.64–10.48) the tree split into two branches from which *A. neapolitanum* emerged. About 3 mya later (2.76 mya, 95% HPD: 0.58–6.68) another divergence event took place from which one branch originated *A. subvillosum* (0.92 mya, 95% HPD: 0.04–2.44) and the other *A. canariense*. Around 1.79 mya (posterior probabilities < 0.5) the ancestor population of *A. canariense* branched out and spread to the Canary Islands. At this time it diverged into two lineages: firstly the Islands of Fuerteventura (0.74 mya, 95% HPD: 0.03–2.06), Lanzarote and Montana Clara (0.22 mya, 95% HPD: 0–0.75) and then El Hierro and La Palma (0.33 mya, 95% HPD: 0–1.14) and the rest of the Canary Islands.

Discussion

Colonization pattern and taxon ages:—Our molecular data show the monophyletic origin of Canarian *Allium* plants which are closely related to the clade of the continental *A. subvillosum* (Figs. 2–4). It is interesting that in the nuclear tree (Fig. 2), the sister clade to the Canarian plants is not the geographically closer clade with accessions of *A. subvillosum* from Morocco, but the clade with accessions from the Iberian Peninsula. Inside the Canary Islands we have weak differentiation. No clade is identical in either nuclear nor plastid trees. The poor resolution of the clade with *A. canariense* in the phylogenetic trees can only be interpreted to have had a single colonization event on the Canarian islands, followed by rapid radiation, which support similar findings in other plant groups (Kim *et al.* 2008). Molecular clock analysis suggests an age of *A. canariense* of 1.7 my and the oldest measurable radiation on Fuerteventura starting 0.74 mya. While we have to acknowledge that these dating estimates, in the absence of good fossil calibration points, are very rudimentary, the estimates are much younger than the ages of the island's origin (Fig. 5). The ISSR analyses (Fig. 4) clearly show differentiation of *A. canariense* into two groups: East (Lanzarote, Fuerteventura, Montana Clara) and West (Tenerife, Gran Canaria, La Palma, La Gomera and El Hierro).

Origin of *Allium subvillosum* and *A. canariense*:—All *A. canariense* plants which have been studied are tetraploid, with $2n = 28$ chromosomes, which is in agreement with the number found for plants from Gran Canaria (Dalgaard, 1991, as *A. subvillosum*). For *A. subvillosum*, two different chromosome numbers can be found: tetraploid ($2n = 28$) and triploid ($2n = 21$). Plants from the Iberian Peninsula are all tetraploid (Granada: Ruiz Rejon & Sanudo 1976, Cadiz: Pastor 1982). Triploid plants were found in Algeria (Arends & Laan 1979) and in Mallorca (Ohri *et al.* 1998). The karyotypes of *A. canariense* and *A. subvillosum* are very similar and extremely asymmetrical, which is anomalous in the section *Molium*. Most of the species of the section *Molium* display a high level of intrachromosomal symmetry with large-sized metacentric chromosomes (Kollmann 1973, Pastor 1982, Tzanoudakis 1985). The polyploid nature of the *A. subvillosum*—*A. canariense* species group and the phenomena causing conflicting topologies of plastid and nuclear DNA-based trees, in our case different sister group species for *A. subvillosum*—*A. canariense* clade in nuclear (*A. chamaemoly*) and plastid (*A. neapolitanum*) trees, could be explained by hypothesizing hybridization events between *A. neapolitanum* as the mother species and *A. chamaemoly*. *Allium neapolitanum* is a polyploid complex with $2n = 14, 21, 28, 35$ and 42 , wherein most frequent is the pentaploid number $2n = 35$ (Kollmann 1973, Badr & Elkington 1977, Pastor 1982, Özhatay 1990, Karavokyrou & Tzaunodakis 1991, Puizina *et al.* 1995, De Sarker *et al.* 1997, Tzanoudakis 1999). Several aneuploid chromosome numbers have also been reported ($2n = 27, 30–32, 34–36, 39, 40$; Pejčinović 1995). Several chromosomes counts in metaphase stages show that *A. chamaemoly* has only $2n = 22$ chromosomes (Mossa & Scrugli, 1970; Marchi *et al.* 1974; Tzaunodakis & Vosa, 1988; Speta 1989; Phitos *et al.* 1989; Brullo *et al.* 1997). Our own chromosome study on root tips of *A. chamaemoly* from Spain, Malaga, Ardales (Am-126) showed also only $2n = 22$ (Fig. 6.). Pastor (1982), however, found in pollen meiosis of 8 specimens from three Spanish populations a haploid chromosome number of $n = 14$. So it is possible that *A. chamaemoly* and *A. neapolitanum* can produce gametes with $n = 14$, possibly producing a homotetraploid hybrid.

Morphological variability of *Allium canariense*:—It is not surprising that the *Allium* plants from the Canary Islands were placed into several different species, since they are, despite genetic uniformity, morphologically very variable (Fig. 7). Plants of this species are 10–40 cm high including the inflorescence, the leaf blades are glabrous or finely ciliate at the base (only a few cm in length), with a very finely serrate edge, 2–10 mm wide and 4–40 cm long, sometimes longer than the associated inflorescence. The stem base is enclosed in a 3–12 cm long leaf sheath. Most plants have 2–3 (rarely up to 8) leaves. The oblong-oval bulb is 5–15 mm in diameter, the outer brownish-gray shell is very finely pitted, the inner skins are silvery-white, at the base often with small bulblets. The upright, umbel-like

inflorescences bear in small plants only 5–7 flowers, but often 20–25 flowers are found, rarely up to 50. The perigone segments vary in color from white to pink; sometimes there is every color graduation in one population. The stamens are equal or more-or-less longer than the perigone.

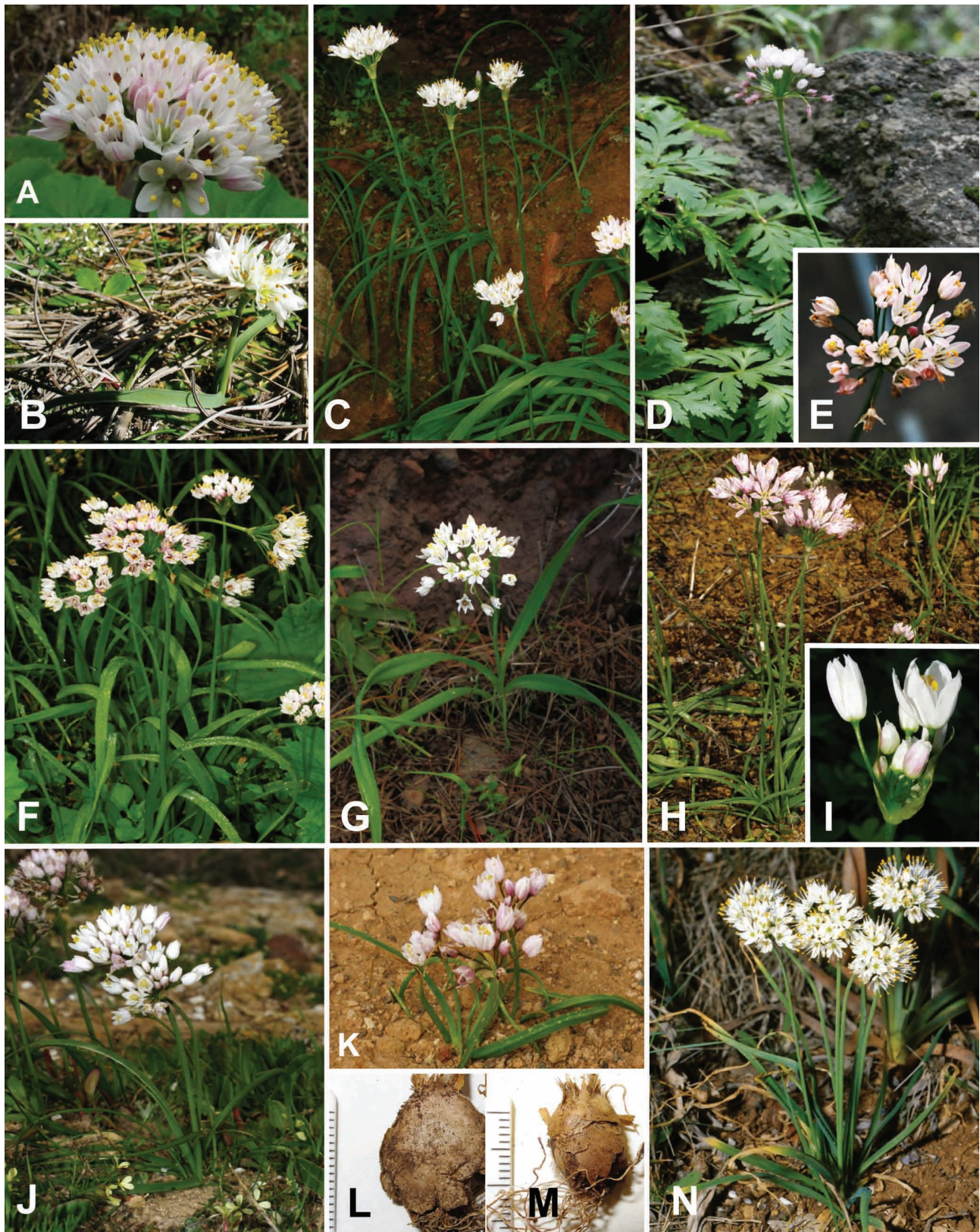


FIGURE 6. Photos of the *Allium canariense* from different islands and some related species. A–L *Allium canariense*: A–B. El Hierro (photos C. Stierstorfer); C–E. La Palma (photos N. Friesen); F–G. La Gomera, H–I. Tenerife (E–H photos P. Schoenfelder, I photo N. Friesen) J–K. Lanzarote; L. bulb of *A. canariense*; M. bulb of *A. roseum*, N. *A. subvillosum*—Mallorca (J–N photos P. Schoenfelder).

Comparing the herbarium specimens of Lanzarote and Fuerteventura with the type-specimen of the subspecies *obtusitepalum* of Alegranza (Fig. 8) reveals that indeed some plants are similar to this subspecies (Am-534, Hb Schoenfelder 09-21), but other plants from Lanzarote (Am-535, Hb Schoenfelder 09-44) and Fuerteventura (TFC 46506) correspond morphologically to those of the western islands. A taxonomic separation of *A. canariense* in two subspecies based on morphology is very difficult. The ecological amplitude of *A. canariense* is high reaching from the succulent scrub (particularly on the eastern islands) to open sites in the laurel forest zone and up to the Canarian pine forests. In the laurel forest zone, plants are usually well developed, while plants in the dry habitats of the lower zones and in pine forests are often smaller. The different forms can hardly be unambiguously distinguished and, therefore, we do not advocate to accept subspecies. On the other hand, based on DNA fingerprinting data, two geographical groups are obvious (Fig. 6), but they are not congruent with morphological characters. Divergence time estimates would appear to support a scenario of a young allopolyploid origin of *A. canariense*. The observed phenotypic variability may be the outcome of such a young hybridogenous radiation which maybe is still in progress. The Canary Islands would provide many ecological niches for morphological and ecological differentiation. To further substantiate this hypothesis, more comprehensive studies are necessary.

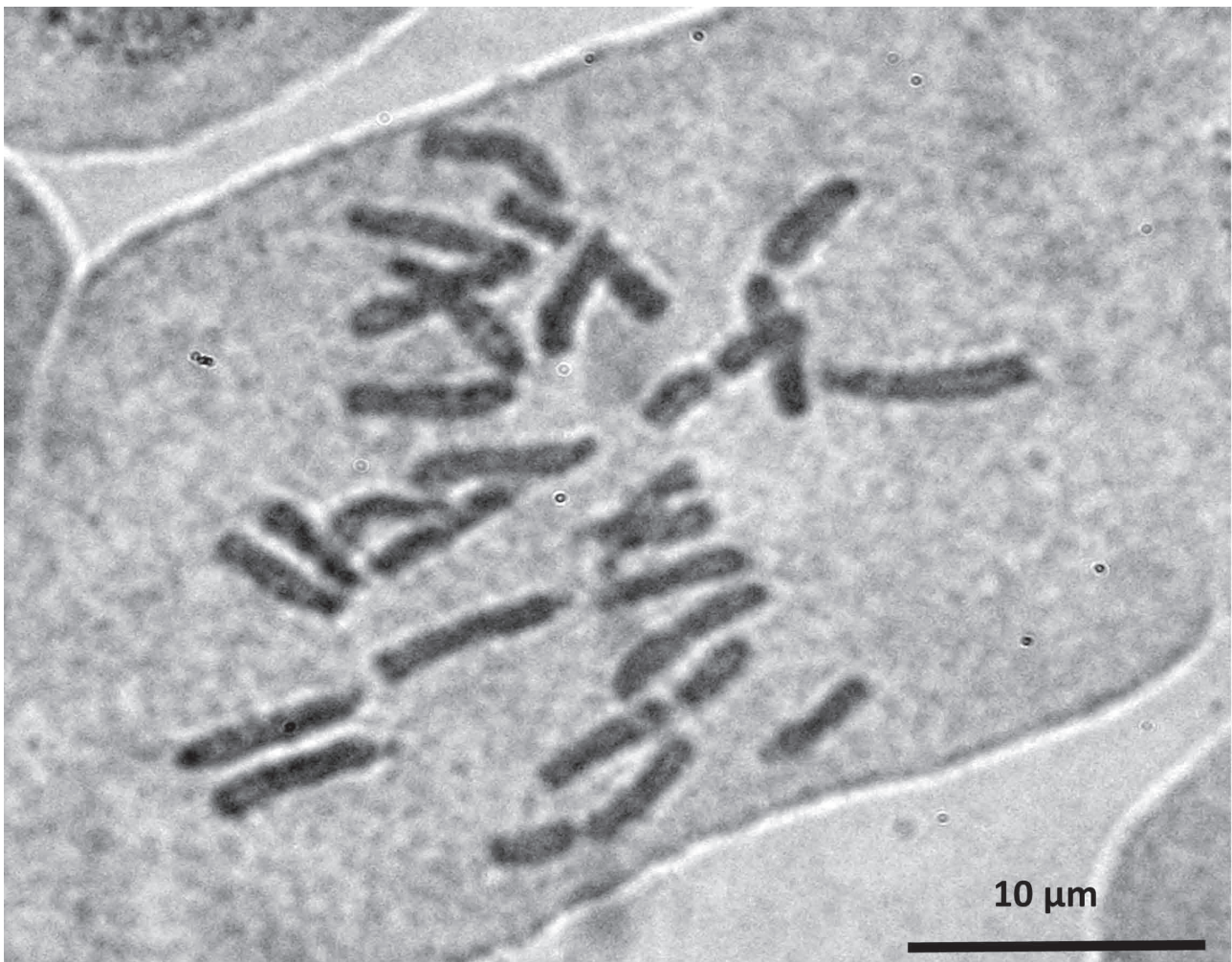


FIGURE 7. Metaphase chromosomes of *Allium chamaemoly*, (Acc.-Am126, Spain, Ardales) $2n = 22$.

Nomenclatural notes

Allium canariense (Regel) N.Friesen & P.Schoenfelder **comb. nov.**

Bas.: *Allium subvillosum* var. *canariense* Regel (1875: 249)

Type (lectotype, here designated):—SPAIN. **Canary Islands.** In Insulis canariensibus prope St. Sebastian, 18 November 1853, C. Maximowicz s.n. (LE 01010228!; Fig. 8).

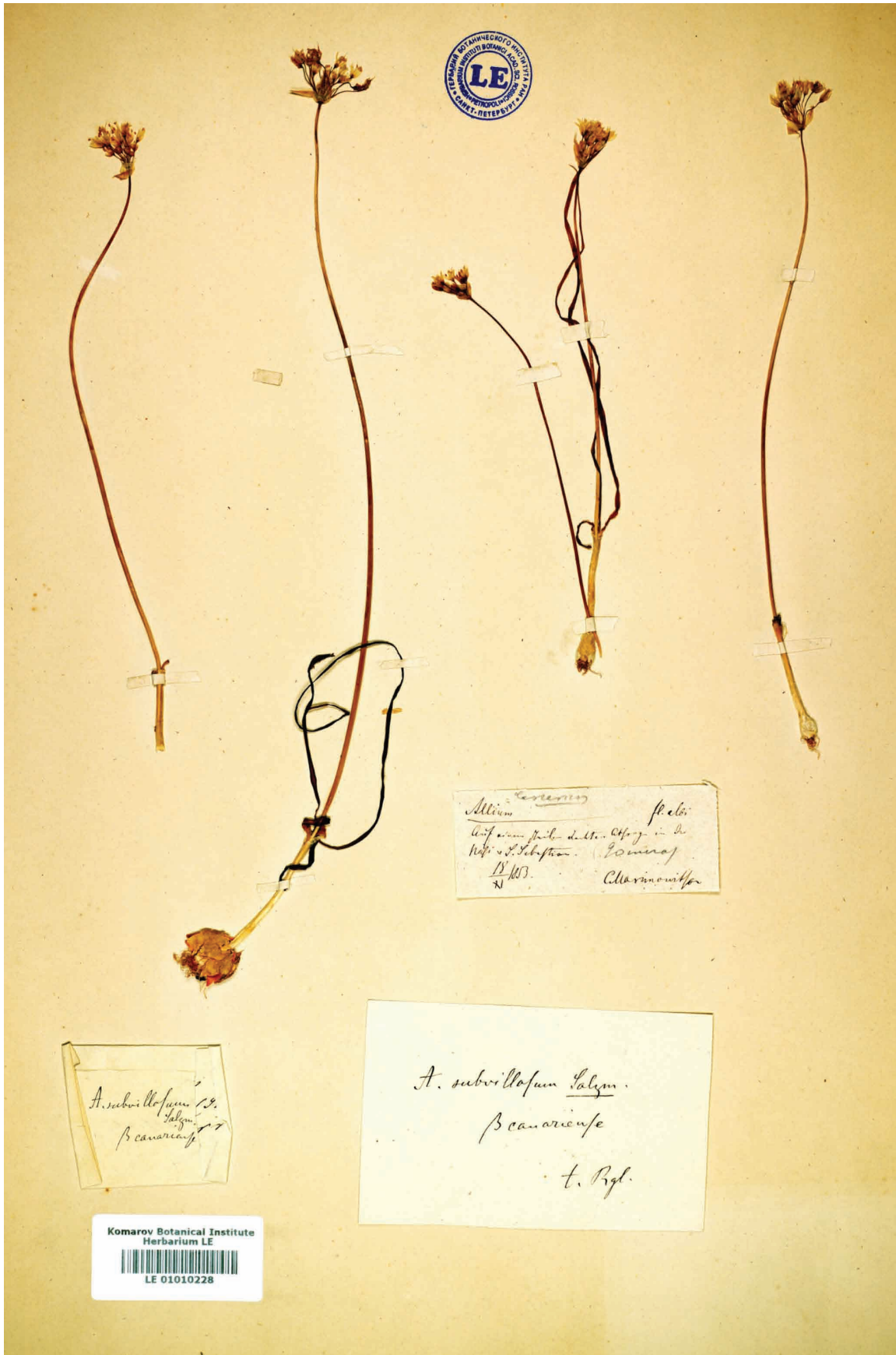


FIGURE 8. Lectotype of *Allium canariense* (LE 01010228).

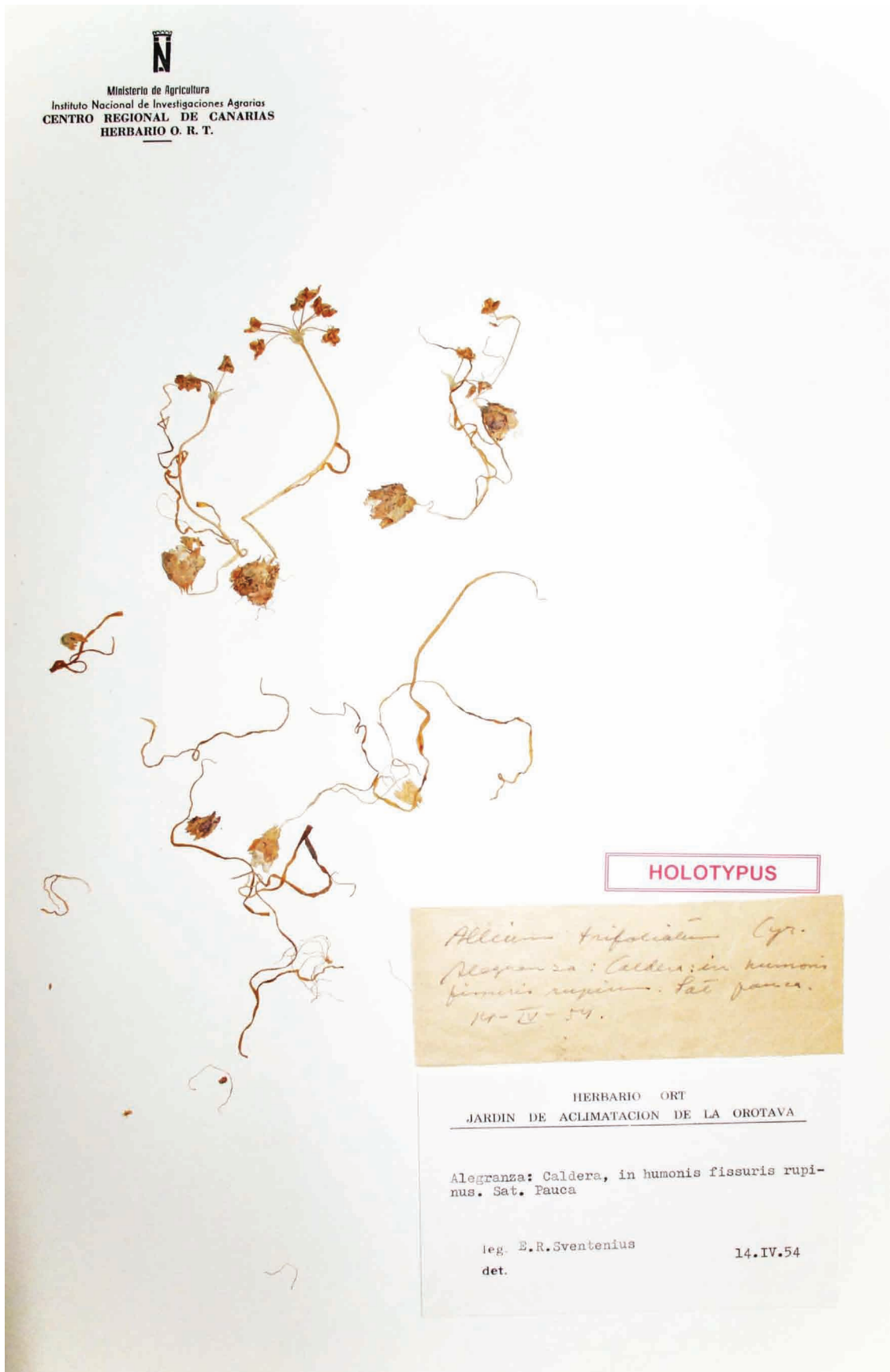


FIGURE 9. Lectotype of *Allium canariense* subsp. *obtusitepalum* (ORT).

If the subspecies *obtusitepalum*, despite the difficulties in morphological delimitation, will be accepted, the next nomenclature combination should be used:

Allium canariense (Regel) N.Friesen & P.Schoenfelder subsp. *obtusitepalum* (Svent.) N.Friesen & P.Schoenfelder *comb. nov.*

Bas.: *Allium trifoliatum* Cirillo subsp. *obtusitepalum* Sventenius (1960: 3)

Type (lectotype, here designated):—SPAIN. **Canary Islands**. Alegranza: Caldera, in humonis fissuris rupinus. Sat. Pauca, 14 April 1954, E.R. Sventenius s.n. (ORT!; Fig. 9).

Identification key to *Allium canariense* and its close relatives

1. Scape very short; umbel subsessile in a basal rosette of hairy leaves (Western and Central Mediterranean) ... *Allium chamaemoly*
- Umbel borne on conspicuous scape 2
2. Leaves glabrous, often scabridulous at margin 3
- Leaves (at least sheath) hairy or long-ciliate at margin 6
3. Scape triquetrous, rarely terete; umbel nodding in bud; perigone segments white; anthers pale green (Northern Mediterranean region to Egypt) *Allium neapolitanum*
- Scape terete; umbel not nodding in bud 4
4. Perigone segments unicolored pink, 9–13 mm long (Mediterranean) *Allium roseum*
- Perigone segments white or white to flushed pink, up to 7.5 mm long 5
5. Perigone segments white, inflorescence sub-globose, leaves full-length ciliate (Iberian Peninsula, Balearic Islands, Morocco) *Allium subvillosum*
- Perigone segments white to flushed pink, inflorescence fascicular to sub-fascicular, leaves only slightly ciliate at the base (Canary Islands) *Allium canariense*
6. Pedicels 3–5 times as long as the white perigone segments; anthers usually brown. (Northern Mediterranean region to Egypt) *Allium subhirsutum*
- Pedicels 1.5–3 times as long as the white, often pink flushed perigone segments; anthers yellow. (France to Eastern Mediterranean) *Allium trifoliatum*

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