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## ***Tamarix minoa* (Tamaricaceae), a new species from the island of Crete (Greece) based on morphological and plastid molecular sequence data**

### **Abstract**

Villar J. L., Turland N. J., Juan A., Gaskin J. F., Alonso M. A. & Crespo M. B.: *Tamarix minoa* (Tamaricaceae), a new species from the island of Crete (Greece) based on morphological and plastid molecular sequence data. – Willdenowia 45: 161–172. 2015. – Version of record first published online on 17 June 2015 ahead of inclusion in August 2015 issue; ISSN 1868-6397; © 2015 BGBM Berlin.

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*Tamarix minoa* is described from material collected on the S Aegean island of Crete (Kriti), Greece. A morphological comparison with the species considered to be closest, *T. africana* and *T. hampeana*, is provided. An original illustration showing the main morphological characters of the new species is also given, as are photographs of the new species in its habitat. The isolated phylogenetic position of *T. minoa* is shown to be strongly supported by plastid molecular sequence data (*trnS-trnG*, *trnQ-rps16* and *ndhF-rpl32*), thus warranting its recognition at specific rank.

Additional key words: Mediterranean flora, Aegean flora, molecular phylogeny, taxonomy, *Tamarix africana*, *Tamarix hampeana*

### **Introduction**

The recent revision of the genus *Tamarix* in Crete (Villar & al. 2014) confirmed the presence of at least four species on that island: *T. hampeana* Boiss. & Heldr., *T. nilotica* (Ehrenb.) Bunge, *T. parviflora* DC. and *T. smyrnensis* Bunge. Among these, the record of *T. hampeana* was considered to be controversial and its identity was left in need of further clarification. It was difficult to obtain a precise identification of the specimens initially assigned to *T. hampeana* (Turland 2010; Villar & al. 2014). In fact, the different taxonomic works consulted (Boissier 1849, 1867; Bunge 1852; Baum 1968, 1978) did not lead to

satisfactory results in respect to the morphology of the studied specimens or their identity with *T. hampeana* or any other described taxa. The specimen MO6207620 (herbarium codes according to Thiers 2015+) was first identified as *T. africana* Poir. However, on B. Baum's advice, it was finally identified and reported as *T. hampeana* (Turland 2010; Villar & al. 2014). On the other hand, the specimens ABH54194, ABH54195 and ABH54196 (see Appendix) were first identified as *T. tetragyna* Ehrenb., due to the high proportion of tetramerous and pentamerous flowers present in the late-season racemes. Nevertheless, after examining several herbarium specimens of *T. tetragyna*, its morphological features showed clear dif-

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ferences from the Cretan specimens and, therefore, that identification was proven to be clearly erroneous. These specimens were finally assigned, with reservations, to *T. hampeana* (Villar & al. 2014).

On account of the doubts held about the taxonomic identity of these specimens from Crete, a deeper morphological study was conducted. In addition, a preliminary phylogeny, based on three plastid DNA regions (intergenic spacers *trnS-trnG*, *ndhF-rpl32* and *trnQ-rps16*), was conducted to identify which of the existing taxa could be genetically closest to the controversial Cretan samples.

The combination of morphological and molecular evidence allows the description of a new species, *Tamarix minoa*, apparently endemic to Crete. The morphological features of the new species, as well as its taxonomic relationships, are described below.

## Material and methods

The material on which this study is focused was collected at Georgioupoli, on the N coast of W Crete. The precise locations for all samples are not more than 1 km apart, so they can be considered to belong to a single population. The specimens collected by Turland and Bareka are kept at UPA, with duplicates at B and MO; the ones collected by Villar, Alonso and Vicente are conserved at ABH.

In order to clarify the morphological identity of the Cretan specimens, over 120 herbarium specimens of the morphologically closest taxa (*Tamarix africana* and *T. hampeana*) were studied, including type material (Villar & al. 2015) kept at G, K, MA and P. Moreover, different European and American herbaria (ABH, B, G, MO, MPU, P, PR, PRC, VAL and W) (Thiers 2015+) were visited in order to obtain a wider view of the morphological characterization of the whole genus. In addition, field collections were made around the W Mediterranean region, where *T. africana* is mainly distributed, as well as around the coasts of continental Greece, where *T. hampeana* is widespread. Those field collections are kept at ABH. Detailed observations and morphological measurements of vegetative and flower parameters were undertaken according to Villar & al. (2012). The main floras and taxonomic works dealing with *Tamarix* in the Mediterranean region were consulted (Candolle 1828; Boissier 1849, 1867; Bunge 1852; Baum 1966, 1968, 1978).

A total of 23 samples of *Tamarix*, belonging to 10 species from 11 countries, were used for the molecular analyses (Table 1). *Reaumuria alternifolia* (Labill.) Britten, *Myricaria bracteata* Royle and *Myrtama elegans* (Royle) Ovcz. & Kinzik., all belonging to *Tamaricaceae*, were also included as the outgroup. Regarding *Tamarix* samples, in addition to three of the Cretan samples, we included the vaginate-leaved *T. aphylla* (L.) H. Karst. and *T. usneoides* E. Mey. ex Bunge; the generic type species *T. gallica* L.; species occurring geographically nearby (*T. dalmatica* B. R. Baum and *T. parviflora*); and those spe-

cies once thought to match with the Cretan samples (Villar & al. 2014): *T. africana*, *T. hampeana* and *T. tetragyna* (along with its close relative *T. boveana* Bunge). All the studied samples are kept at the herbaria ABH or MO, with the exception of the outgroup samples corresponding to *R. alternifolia* and *M. bracteata*, which belong to John F. Gaskin, kept at the USDA ARS at Sidney (MT, U.S.A.), soon to be deposited at MO (Table 1).

Genomic DNA was extracted from silica-gel-dried leaf material (Chase & Hill 1991), following a modified 2xCTAB method (Doyle & Doyle 1987). Total DNA is kept at the ABH-DNA bank. The species-level phylogeny is based on three plastid intergenic spacers (*trnS-trnG*, *trnQ-rps16* and *ndhF-rpl32*), which were amplified using the primers described in Hamilton (1999) and Shaw & al. (2007). The amplifications were performed in a reaction volume of 25 µl containing 22.5 µl ABGene 1.1× Master Mix 2.5 mM MgCl<sub>2</sub> (Thermo Scientific Waltham, MA, U.S.A.), 0.5 µl of 0.4% bovine serum albumin (BSA), 0.5 µl of each primer (10 pmol/µl) and 1 µl of template DNA. The PCR programme used for all three regions was an initial denaturation of 94°C for 2 min, followed by 35 cycles at 94°C (1 min 15 sec), 55°C (1 min 30 sec) and 72°C (2 min); and a final elongation at 72°C for 10 min. PCR products were purified using Ultraclean® PCR Clean-Up Kit (MOBIO, Carlsbad, CA, U.S.A.) micro-columns, following the instructions provided by the manufacturer. Both strands were sequenced with the same primers for each region and for all samples at Macrogen Inc. Korea ([www.macrogen.com](http://www.macrogen.com)).

Complementary strands were assembled using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI, U.S.A.). The three plastid regions were aligned automatically by ClustalW, conducted in MEGA 5.05 (Tamura & al. 2011). Minor manual corrections were made to obtain the final alignments, and the three regions were joined in a single matrix. Maximum parsimony analyses (MP) and Bayesian analyses were performed on the plastid dataset.

MP analyses were conducted in PAUP v.4.0b10 (Swofford 2002), using heuristic search options. Searches included 1000 random addition replicates and tree-bisection-reconnection (TBR) branch swapping, with MULTREES in effect (keeping multiple most-parsimonious trees). All characters were treated as having equal weight. The shortest trees held in the heuristic search were used as initial trees for a final heuristic analysis, with the previously commented options. MP support was assessed by 1000 bootstrap replicates, TBR branch swapping, simple addition sequence and MULTREES on, keeping 10 trees per replicate (Salamin & al. 2003). For the MP analyses, the consistency index (CI) and retention index (RI) were calculated excluding uninformative characters. Clades showing bootstrap (BS) values between 50%–74% were considered as weakly supported, 75%–89% moderately supported and 90%–100% strongly supported.

A Bayesian Inference (BI) analysis was conducted using MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001;

Table 1. Materials used in the molecular analysis.

Label on Fig. 3	Taxon	Origin	Voucher	GenBank ref. <i>trnS-trnG</i>	GenBank ref. <i>trnQ-rps16</i>	GenBank ref. <i>ndhF-rpl32</i>
<i>R. alternifolia</i>	<i>Reaumuria alternifolia</i> (Labill.) Britten	Iran	Gaskin 919*	KP244371	KP244397	KP244423
<i>M. bracteata</i>	<i>Myricaria bracteata</i> Royle	Kazakhstan	Gaskin 1148*	KP244372	KP244398	KP244424
<i>M. elegans</i>	<i>Myrtana elegans</i> (Royle) Ovcz. & Kinzik.	China	MO5799414	KP244373	KP244399	KP244425
<i>T. aphylla</i> TA1Mo	<i>Tamarix aphylla</i> (L.) H. Karst.	Morocco	ABH54280	KP244374	KP244400	KP244426
<i>T. aphylla</i> TACD	<i>Tamarix aphylla</i> (L.) H. Karst.	Italy (Sardinia)	ABH54422	KP244375	KP244401	KP244427
<i>T. usneoides</i> T1NMB	<i>Tamarix usneoides</i> E. Mey.	Namibia	ABH58684	KP244376	KP244402	KP244428
<i>T. usneoides</i> TSA7	<i>Tamarix usneoides</i> E. Mey.	South Africa	ABH58683	KP244377	KP244403	KP244429
<i>T. africana</i> T13Mo	<i>Tamarix africana</i> Poir.	Morocco	ABH54205	KP244378	KP244404	KP244430
<i>T. africana</i> T2V	<i>Tamarix africana</i> Poir.	Spain	ABH55366	KP244379	KP244405	KP244431
<i>T. minoa</i> T2CR	<i>Tamarix minoa</i> J. L. Villar & al.	Greece (Crete)	ABH54194	KP244382	KP244408	KP244434
<i>T. minoa</i> T4CR	<i>Tamarix minoa</i> J. L. Villar & al.	Greece (Crete)	ABH54195	KP244383	KP244409	KP244435
<i>T. minoa</i> NT	<i>Tamarix minoa</i> J. L. Villar & al.	Greece (Crete)	MO6207620	KP244384	KP244410	KP244436
<i>T. tetragyna</i> W07-28	<i>Tamarix tetragyna</i> Ehrenb.	Egypt	W2007-25728	KP244385	KP244411	KP244437
<i>T. boveana</i> T7AG	<i>Tamarix boveana</i> Bunge	Algeria	ABH56326	KP244387	KP244413	KP244439
<i>T. boveana</i> T18Mo	<i>Tamarix boveana</i> Bunge	Morocco	ABH54183	KP244388	KP244414	KP244440
<i>T. boveana</i> Tb2Eb	<i>Tamarix boveana</i> Bunge	Spain	ABH54330	KP244389	KP244415	KP244441
<i>T. gallica</i> TC1Eb	<i>Tamarix gallica</i> L.	Spain	ABH54331	KP244396	KP244422	KP244448
<i>T. gallica</i> T2Fr	<i>Tamarix gallica</i> L.	France	ABH57865	KP244395	KP244421	KP244447
<i>T. tetragyna</i> W07-48	<i>Tamarix tetragyna</i> Ehrenb.	Egypt	W2007-14048	KP244386	KP244412	KP244438
<i>T. parviflora</i> T8CR	<i>Tamarix parviflora</i> DC.	Greece (Crete)	ABH54197	KP244393	KP244419	KP244445
<i>T. parviflora</i> TCR10	<i>Tamarix parviflora</i> DC.	Greece (Crete)	ABH54321	KP244394	KP244420	KP244446
<i>T. hampeana</i> T6GRE	<i>Tamarix hampeana</i> Boiss. & Heldr.	Greece (Epirus)	ABH59025	KP244390	KP244416	KP244442
<i>T. hampeana</i> T72GRE	<i>Tamarix hampeana</i> Boiss. & Heldr.	Greece (Central)	ABH59877	KP244391	KP244417	KP244443
<i>T. hampeana</i> T3MNE	<i>Tamarix hampeana</i> Boiss. & Heldr.	Montenegro	ABH57891	KP244392	KP244418	KP244444
<i>T. dalmatica</i> T1ALB	<i>Tamarix dalmatica</i> B. R. Baum	Albania	ABH57830	KP244380	KP244406	KP244432
<i>T. dalmatica</i> T6MNE	<i>Tamarix dalmatica</i> B. R. Baum	Montenegro	ABH57844	KP244381	KP244407	KP244433

\* Vouchers soon to be deposited at MO.

Ronquist & Huelsenbeck 2003). The most accurate evolutionary model required for Bayesian estimation was selected using the AIC (Akaike Information Criterion) (Posada & Crandall 1998; Posada & Buckley 2004), conducted in JMODELTEST 2.1.5 (Darriba & al. 2012). Two simultaneous and independent analyses were performed. For each analysis, the Markov and Monte Carlo chains were run for  $2 \times 10^6$  generations and sampled every 100 generations. The chains became stationary and the average standard deviation of the split frequencies stabilized in 250 000 generations. Thus the first 250 trees were excluded (“burn-in”) and the remaining trees were used to compile a posterior probability (PP) distribution using a 50% majority-rule consensus.

## Results and Discussion

*Tamarix minoa* J. L. Villar, Turland, Juan, Gaskin, M. A. Alonso & M. B. Crespo, **sp. nov.** – Fig. 1 & 2.

Holotype: Greece, Crete, Nomos Chanion, Eparchia Apokoronou, Georgioupoli beach, by river mouth at E edge of village, 35°21'34.3"N, 24°15'59.6"E, 0 m, sandy river bank on beach with springs emerging beneath the *Tamarix* trees, 28 Mar 2009, *N. J. Turland 1778 & P. Bareka* (UPA-Herb. Phitos & Kamari; isotypes: B, MO6207620).

– *Tamarix hampeana* sensu Turland in Willdenowia 40: 198. 2010, non Boiss. & Heldr. 1849.

*Diagnosis* — Species distincta primo aspectu ad *Tamaricem africanam* Poir. aemulans et cum *T. hampeana* Boiss. & Heldr. plerumque confusa, sed eis singulari characterum combinatio valde diversa et multo differt; racemis vernalis e ramorum vetustiorum productis aliquando junioribus hornotinis ramulis alternantibus; bracteis lineari-oblongis, concavis, subpatulis, calyces paulo superantibus; floribus plerumque pentameris, sed ad postremum tempus florendi cum aliis tetrameris floribus simul commixtis; sepalis acutis, marginibus denticulatis; petalis oblongo-obovatis vel obovatis, concavis vel leviter carinatis; antheris mucronatis.

*Description* — *Shrubs* or low shrubby *trees*, up to 5 m tall. *Bark* reddish brown to dark burgundy. *Leaves* green to glaucous green, lanceolate, (1–)1.5–2.5(–3.5) mm long, surface slightly papillose, bearing salt glands, more evident and abundant in older leaves, base narrow, slightly decurrent, broader and auriculate in leaves remaining from previous year, apex acuminate, slightly incurved. *Racemes* loosely arranged on previous year’s branchlets, sometimes alternating with new-leaved twigs (Fig. 2B), 2.5–4.5(–6) cm long, 6–7.5 mm wide. *Peduncle* 3–5 mm long, with 4–8 scarious bracts grouped at base, sometimes showing 2–3 mm of naked rachis before first floral bract; *rachis* lacking papillae on all studied specimens. *Bracts* patent, yellowish brown, linear-oblong, narrow-

ing at apex, concave, (2–)2.5–3.5(–4.5) mm long, usually slightly surpassing calyx, sometimes not reaching it, base narrow, slightly decurrent, sometimes showing 2 small auricles, margin sparsely papillose-ciliate, apex obtuse to acute, slightly incurved. *Pedicels* 0.5–0.75 mm long, always shorter than sepals. *Calyx* of 5 sepals (sometimes 4, very rarely 6)\*. *Sepals* green, ovate-triangular, 1.5–2 mm long, 1–1.2 mm wide, 2 outer ones slightly larger than 3 inner ones, margin broadly membranous, markedly denticulate, apex acute; pentamerous calyx sepals almost alike; tetramerous\* calyx outer sepals sometimes with a marked central nerve and apex apiculate, inner sepals evidently smaller and apex obtuse. *Corolla* of 5 petals (sometimes 4, very rarely 6)\*. *Petals* deciduous at fruit maturity, white, oblong-obovate to obovate, concave to keeled, recurved at late anthesis, 2.8–3.2 mm long, 1–1.5 mm wide, margin sometimes irregular, base sometimes cuneate, not unguiculate. *Staminal disk* dark brown, 5-lobed (rarely 4-lobed, exceptionally 6-lobed)\*; *lobes* antesepalous, broadly ovate, apex somewhat progressively narrowed into filaments; *filaments* white, 1.5–2 mm long; *anthers* pale yellow to light pink, 0.8–1 mm long, 0.5–0.6 mm wide, apex mucronate. *Gynoecium* of 3 styles and 3 carpels, (rarely 4)\*; *styles* nearly 0.6 mm long.

\* More common in late-season racemes.

*Phenology* — Flowering mainly in March and April, though some late-season racemes may extend the flowering period until the end of May.

*Distribution and biogeography* — At present, *Tamarix minoa* is known only from the type locality at Georgioupoli on the N coast of W Crete in the S Aegean region, Greece. Further exploration in the field and examination of herbarium material are needed to clarify if its distribution is confined to Crete or if it extends to other territories. According to Rivas-Martínez & al. (2004), the type locality of *T. minoa* is located in the Cretan Subprovince, which belongs to the Graeco-Aegean Province, Eastern Mediterranean Subregion and Mediterranean Region. The biogeographical unit Cretan Subprovince matches the “Cretan area” (“Cr”) of *Flora europaea* (Tutin & al. 1964–1980) and the Euro+Med PlantBase (Euro+Med 2006+) as well as the floristic region “Kriti+Karthos” (“KK”) of *Flora hellenica* (Strid & Tan 1997, 2002) and the recent checklist of Greek vascular plants (Dimopoulos & al. 2013).

*Ecology* — Growing on sandy river banks close to the sea, with springs of percolating (fresh or brackish?) groundwater emerging beneath the *Tamarix* trees, exposed to inundation by seawater during storms (Fig. 2A).

*Etymology* — The epithet *minoa* makes reference to the Minoan civilization, which existed in Crete in the Bronze Age between 3500–2500 BCE and c. 1100 BCE. The term “Minoan” itself is derived from Minos (Μίνωϛ), a mythical king of Crete.



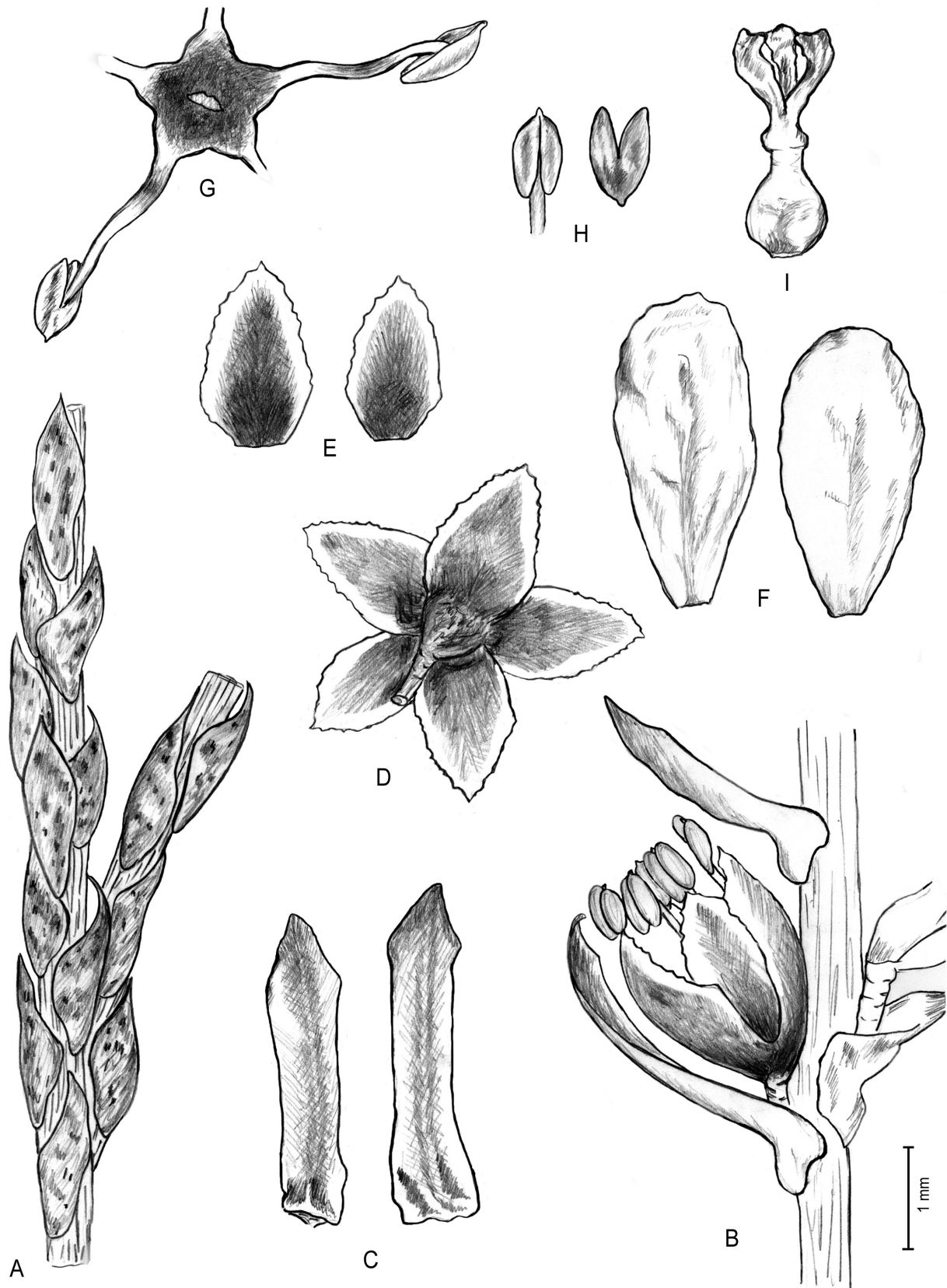


Fig. 1. *Tamarix minoa* – A: young branchlet with leaves; B: portion of branchlet with bracts and flower (petals removed); C: bracts, adaxial (left) and abaxial (right) surfaces; D: flower, basal view, showing pedicel and calyx; E: sepals, abaxial surface, outer sepal (left), inner sepal (right); F: petals, adaxial surface; G: staminal disk, apical view with filaments and anthers; H: anthers, lateral views, with distal part of filament (left), with apex at bottom (right); I: gynoecium, lateral view. – Drawn by María García Rivas from the isotype MO6207620.





Fig. 2. *Tamarix minoa* – A: tree growing on sandy ground beside river (left), springs emerging beneath tree, sea in distance; B: flowering branchlets. – Type locality, 28 Mar 2009, photographs by N. J. Turland.



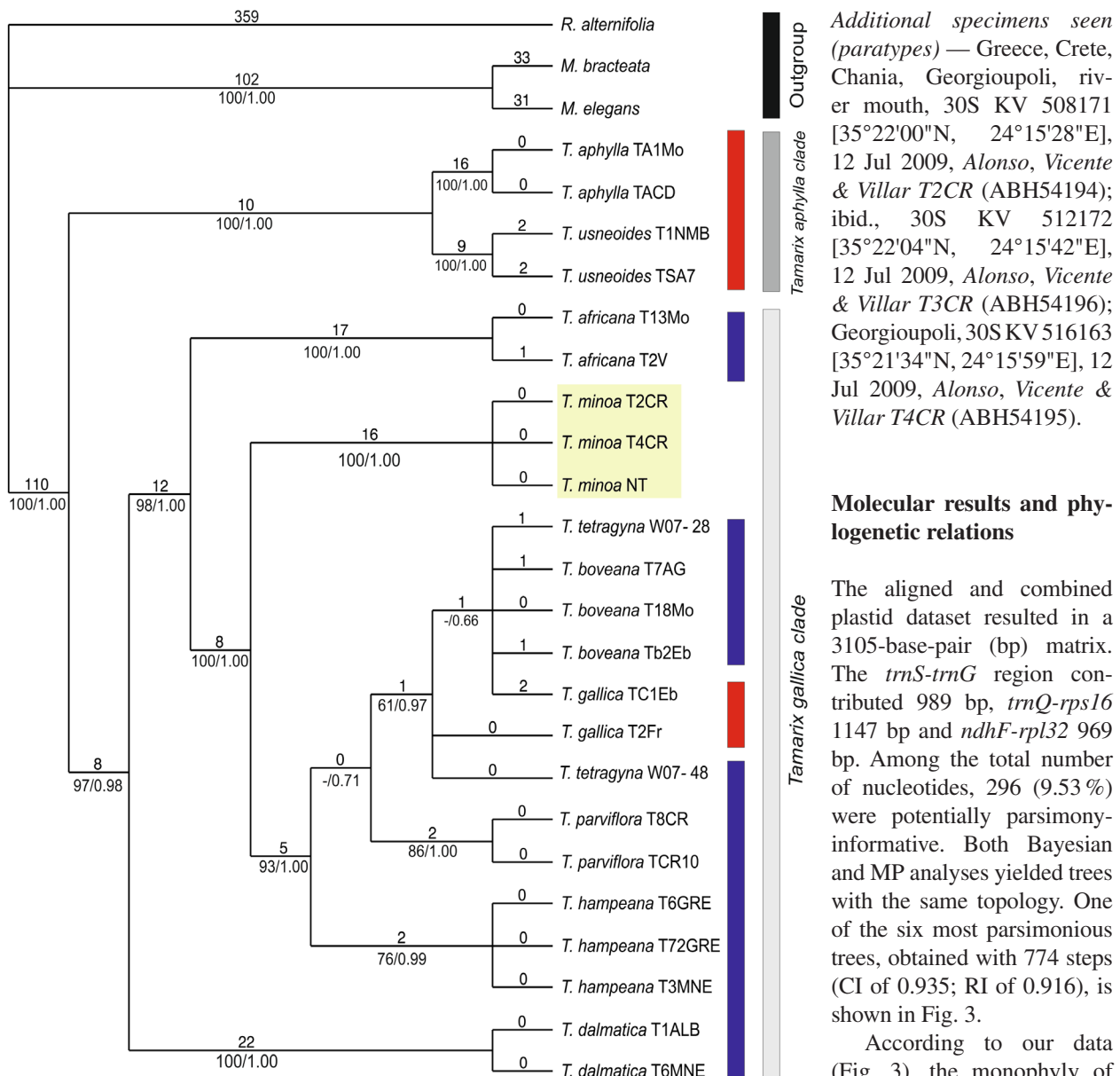


Fig. 3. One of the six most parsimonious trees obtained (774 steps, CI 0.935, RI 0.916). Branch lengths for parsimony analysis are shown above branches. Bootstrap values (BS) > 50% and Bayesian posterior probability (PP) > 0.90 are shown below branches (BS/PP). The outer vertical bars represent the outgroup and the two first split clades in *Tamarix*. The inner vertical bars represent the sections accepted by Baum (1978).

**Remarks** — According to our observations, *Tamarix minoia* is mostly a pentamerous-flowered species, with 5 sepals, 5 petals, 5 stamens and 3 styles. A few rare flowers, though, can be found to be tetramerous or hexamerous in some of the floral whorls, more frequently on late flowering racemes. Often tetramery or hexamery does not affect all whorls at the same time. Most of the examined flowers with a tetramerous calyx had a pentamerous staminal disk. Most of the hexamerous staminal disks were found in pentamerous flowers in relation to sepals and petals. In a few cases, tetramery of sepals was related to hexamery of petals (or vice-versa). No special pattern was found in the occurrence of a 4-styled/-valved gynoecium.

1.00) and the *T. gallica* clade (BS 97 %, PP 0.98). Within the *T. gallica* clade, *T. minoia* formed an independent and strongly supported clade (BS 100 %, PP 1.00). Its phylogenetic position is well resolved, being a sister group of a large clade (BS 93 %, PP 1.00) that includes *T. hampeana* (BS 76 %, PP 0.99), *T. parviflora* (BS 86 %, PP 1.00) and a third clade formed by *T. boveana*, *T. gallica* and *T. tetragyna* (BS 61 %, PP 0.97). The phylogenetic relationships within this third clade are not adequately resolved. Consequently the phylogenetic position of these three species appeared collapsed. Finally, *T. africana* and *T. dalmatica* are placed in the outermost positions of this



*T. gallica* clade, as independent branches (BS 100%, PP 1.00, for both taxa).

The current work is not intended to offer a complete phylogeny of the genus *Tamarix*, since many species were not sequenced for this study and, specifically, no specimens characterized by amplexicaul leaves (e.g. *T. passerinoides* Delile ex Desv., *T. pycnocarpa* DC.) were included. Further phylogenetic studies are needed in order to obtain a better overview of the whole genus, including a larger number of samples and species. Nonetheless, and as Gaskin & Schaal (2003) already pointed out, the sections of *Tamarix* accepted by Baum (1978) do not correspond to monophyletic groups in this preliminary molecular study. In Fig. 3, *T. gallica* (*T. sect. Tamarix*, *T. ser. Gallicae* B. R. Baum) is included in a large group of species belonging to *T. sect. Oligadenia* (Ehrenb.) B. R. Baum, and separated from *T. aphylla* and *T. usneoides* (*T. sect. Tamarix*, *T. ser. Vaginantes* (Bunge) Bunge). Moreover, *T. parviflora* (*T. sect. Oligadenia*, *T. ser. Arbusculae* B. R. Baum) shares the same clade with *T. tetragyna* and *T. boveana* (*T. sect. Oligadenia*, *T. ser. Anisandrae* Bunge), as well as with *T. gallica*. *Tamarix africana*, the closest species morphologically to *T. minoa*, is included in *T. sect. Oligadenia*, *T. ser. Anisandrae*. These results suggest that a reanalysis of sections and series should be undertaken. Consequently, we prefer not to classify the new species *T. minoa* in any of the existing sections.

### Morphological comparisons

Despite the taxonomic complexity of the genus, and the diffuse borders between similar species, we can highlight some morphological features to separate *Tamarix minoa* from the morphologically close *T. africana*, as well as from its previous identification of *T. hampeana* (Table 2).

**Leaves** — Leaves are similar between *Tamarix minoa* and *T. africana*, though those of *T. africana* tend to show a broader auriculate base. In both species the longer leaves rarely reach 3.5 mm. The leaves of *T. hampeana* are narrower, and it is common to find some longer ones (4–7 mm) at the base of the first young twigs of the year.

**Racemes** — *Tamarix hampeana* racemes are among the larger ones in the genus (usually 4–10 cm long × 7–12 mm wide), some reaching up to 16 cm long × 14 mm wide. The racemes of *T. minoa* and *T. africana*, are smaller (usually less than 5 cm long × 8 mm wide), although the smallest *T. hampeana* racemes can overlap in size with *T. africana* and *T. minoa*. However, those of *T. minoa* show the peculiarity of frequently alternating with new leafy twigs.

**Bracts** — The bract size of the three species falls into a similar range. However, the bracts of *Tamarix minoa* are patent, linear-oblong, only narrowing at the apex, with a

narrow slightly decurrent base sometimes with two small auricles. On the other hand, the bracts of *T. africana* are triangular-oblong, always narrowing progressively toward the apex, with a narrow decurrent base sometimes calcarate with two small auricles that can even be slightly decurrent. The bracts of *T. hampeana* can be ovate-oblong, oblong or narrowly triangular; they are so variable that they are not very reliable for comparison with the other two species.

**Pedicels** — The pedicels of *Tamarix hampeana* (usually 1–4 mm long) are frequently longer than the sepals and significantly larger than those of *T. africana* and *T. minoa* (in both species sessile to 0.75 mm long).

**Calyx** — *Tamarix africana* and *T. minoa* show essentially a pentamerous calyx, although both species can show exceptions. Tetramerous calyces are more common in *T. minoa* than in *T. africana*. *Tamarix hampeana* shows flowers with normally 4 or 5 sepals, but sometimes 6 or 7 or even 8 or 9. This variability makes it difficult to identify *T. hampeana* as tetramerous or pentamerous. The sepals of *T. minoa* look similar, the two outer ones are slightly larger than the three inner ones, all are clearly denticulate at the margin and most are acute at the apex. The two outer sepals of *T. africana* tend to be narrower and usually show a marked central nerve, and all sepals usually have a subentire margin and obtuse apex. The two outer sepals of *T. hampeana* tend to be narrower and sometimes show a marked central nerve, and all sepals have a subentire margin and acute to obtuse apex.

**Corolla** — *Tamarix africana* and *T. minoa* are essentially pentamerous, with rare exceptions. In *T. hampeana*, the number of petals is as variable as the number of sepals (4–6(–9)). The petals of *T. hampeana* tend to be elliptic-oblong, whereas those of *T. minoa* are oblong-obovate to obovate and concave to keeled, and those of *T. africana* tend to be broadly ovate and flat (in the typical variety) to oblong-unguiculate. In a single flower, for all three species, all petals are the same size.

**Staminal disk** — The configuration of the disk is very similar in *Tamarix minoa* and *T. africana*, both showing disks with the filaments rising progressively from the apex of disk lobes. They are also quite stable in showing 5 stamens, rarely 6, exceptionally 7 or 8 (Villar & al. 2012). On the other hand, the filaments of *T. hampeana* rise abruptly from the apex of the disk lobes. Moreover, *T. hampeana* shows an extremely high variability in the staminal disk features: disks with 4 or 5 staminal filaments are not always easy to find, whereas disks with 6–8(–10) well-developed stamens are common.

**Anthers** — Only the anthers of *Tamarix minoa* are clearly mucronate. Those of *T. africana* and *T. hampeana* are not, although submucronate anthers can sometimes be found.

Table 2. Some key morphological features for distinguishing among *Tamarix minoa* and the related *T. africana* and *T. hampeana*.

	<i>Tamarix minoa</i>	<i>Tamarix africana</i>	<i>Tamarix hampeana</i>
Raceme disposition	loosely arranged on previous year's branchlets, sometimes alternating with new leafy twigs	loosely arranged on previous year's branchlets	loosely arranged on previous year's branchlets and branches
Raceme size [mm]	25–45(–60) × 6–7.5	20–50(–80) × 5–8(–9)	(30–)40–100(–160) × (6–)7–12(–14)
Pedicel length [mm]	0.5–0.75	<0.75(–1)	1–4
Sepal shape	ovate, margin denticulate, apex acute; 2 outer sepals slightly larger than inner ones	ovate, margin subentire, apex obtuse; 2 outer sepals slightly narrower than inner ones	ovate, margin subentire, apex acute to obtuse; 2 outer sepals slightly narrower than inner ones
Number of sepals	(4 or)5(or 6)	5(or 6)	4 or 5(–9)
Number of stamens	(4 or)5(or 6)	5(or 6(–8))	4–7(–10)
Anthers mucronate	yes (markedly)	no (rarely minutely)	no (rarely minutely)

According to the molecular and morphological results obtained, we can conclude that *Tamarix minoa* is indeed an independent species well separated from any other taxa of *Tamarix*. The closest species with respect to morphology is *T. africana*. However, *T. minoa* can be separated from *T. africana* by the linear-oblong concave bracts, acute and denticulate sepals, concave-keeled petals and apiculate anthers. Our phylogenetic results have pointed out the well-supported, isolated position of the studied samples, clearly separated from the morphological close taxa *T. africana* and *T. hampeana*, as well as from geographically close taxa such as *T. dalmatica* and *T. parviflora*. Regarding morphology, the correct identification of *Tamarix* collections is known as a troublesome matter, and *T. africana* is one of the most widely distributed and morphologically variable species in the Mediterranean region. Taking these points into consideration, we would recommend being careful with future records of apparent *T. minoa* from outside the Aegean region, in order to avoid mistaken identifications, as happened with *T. dalmatica* in the Iberian Peninsula (Villar & al. 2012).

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## Appendix: *Tamarix africana* and *T. hampeana*, specimens examined

*Tamarix africana* (for more specimens see Villar & al. 2012)

ALGERIA: “Cote de Barbarie”, *Poiret* (lectotype of *Tamarix africana*, P-LA, P00287249); “*T. africana* (n.), 5. andr. 3.g. ex Numidia”, *Poiret* (syntype of *T. africana*, Herb. *Poiret* in Herb. Moquin-Tandon, P00166702). — MOROCCO: Taza-Al Hoceima, Tizi Ouasli, road R505 from Tizi Ouasli to Aknoul, 30S VD 2545 [34°44'39"N, 03°49'09"W], 1252 m, 30 Apr 2013, *Crespo, Alonso, Vicente & Villar T53MO & T54MO* (ABH69534, ABH69535); Meknes, Khenifra, From Azrou to Khenifra, Oum Rabii sources, 30S TB 748608 [33°03'44"N, 05°24'43"W], 1223 m, 1 May 2013, *Crespo, Alonso, Vicente & Villar T55MO & T56MO* (ABH69594, ABH69593); Casablanca, Mohammedia, Oued Mefifik, below the bridge of N1 road, 29S PT 548298

[33°41'48"N, 07°19'47"W], 5 m, 3 May 2013, *Crespo, Alonso, Vicente & Villar T57MO & T59MO* (ABH69591, ABH69589). — SPAIN: Valencia, Cullera, Cap de Cullera, 30S YJ 397414 [39°11'19"N, 00°13'28"W], 4 m, 10 May 2012, *Vicente & Villar* (ABH67412, ABH67410); Almería, Vera, between Coto de Vera and Puerto Rey, 30S XG 0418 [37°12'09"N, 01°49'41"W], 4 m, 14 May 2012, *Alonso, Vicente & Villar T26AL* (ABH67465); Almería, Pulpí, beside the sea, 30S XG 1639 [37°23'25"N, 01°41'22"W], 11 m, 15 May 2012, *Alonso, Vicente & Villar T35AL* (ABH69653); Almería, Pulpí, Pilar de Jaravía, 30S XG 1639 [37°19'33"N, 00°57'25"W], 84 m, 15 May 2012, *Alonso, Vicente & Villar T39AL & T42AL* (ABH69649, ABH69646); Alicante, Albaterra, on the way to Rambla Salada, 30S XH 8133 [38°13'36"N, 00°55'55"W], 230 m, 18 Mar 2013, *Villar T127A, Agulló, Vicente, Quinto & Marchant* (ABH648456); Alicante, Mutxamel, Río Monnegre, Next to Riopark, 30S XH 2157 [38°27'11"N, 01°36'47"W], 105 m, 17 Apr 2013,



Moreno, Terrones & Villar (ABH69576, ABH69568, ABH69574, ABH69564).

### *Tamarix hampeana*

ALBANIA: Lushnje, beside the road to Durres, 34T CL 704007 [40°38'49"N, 19°28'05"E], 7 m, 26 Apr 2011, *Martínez & Villar T4ALB* (ABH58162); Lushnje, Babujne, beside the road, 34T CL 810268 [40°53'02"N, 19°35'15"E], 9 m, 26 Apr 2011, *Martínez & Villar T5ALB* (ABH58161). — GREECE: Phalère, au bord des fosses, 14 Apr 1844, *Heldreich 64* (lectotype of *Tamarix hampeana* G-BOIS G00330259); Phalareus, 1841, *Spruner*, (syntype of *T. hampeana* G-BOIS G00330260); Attica, 1842, *Spruner* (syntype of *T. hampeana* G00380106); Attica, Phalareus, 1844, *Spruner* (syntype of *T. hampeana* G00380107); Phalareus, *Spruner* (syntype of *T. hampeana* G00380108); Astros ad mare, Apr 1842, *Boissier* (syntype of *T. hampeana* G-BOIS G00330258); ad Phalerum, 1844, *Heldreich* (syntype of *T. hampeana* P06618760); Grèce Phalere, 1846, *Heldreich* (syntype of *T. hampeana* P05144597); in maritimis ad Phalerum, Apr 1844, de *Heldreich* (syntypes of *T. hampeana* G00380104, G00380104\_a, G00380105, K000641864, P06618759); Attica, Apr 1844, de *Heldreich* (syntype of *T. hampeana* P06618441); Attica: in subpaludosis et ad ripas in halipedo Phaleri, 8 Apr 1847, “De Heldreich Herbarium Graecum Normale”, *Heldreich* (syntype of *T. hampeana* MA78790); Attica, Phaleri, 3 Apr 1890, *Heldreich* (G); Attica ad Phalerum, 3 May 1854, *Heldreich* (PR); ad Cephissi ostia, Phalaerum, May 1856, *Heldreich* (G); Attica, in subpaludosis et ad ripas in halipedo Phaleri, 5 Apr 1893, *Heldreich 927* (G, 2 vouchers); Phaleres, Jun 1852, *W. Barbey* (G); ad Phalaerum, Jun 1852, *Heldreich* (G); in maritimis ad Phaleri, 18/30 Apr 1852, *Orphanides 318* (G); in maritimis Phaleri Atticae, 30 Mar 1852, *Orphanides* (PR); Regio aegaea orientalis, Insula Hios, ad litus orientale inter coenobium Panajia Mirtidhiotissa Mersinidhiou et pagum Virsi Passa, ad margines viae, forsan culta, 16 Apr 1976, 50 m, *Greuter* (G); Thessaloniki, in arenosis maritimis, Apr 1909, *Dimonie* (G, 2 vouchers); PR, a complex voucher with 3 *T. parviflora* and 1 *T. hampeana* branches); Zakynthos, oppidi et ad Kryonero, 28 May 1926, *Bornmüller* (G, 2 vouchers), Samos, in limosis ad ruinam templi Herae, prope Colonna, 12 Apr 1934, *K. H. Rechinger & F. Rechinger 4002* (G); Phanarion prope Porto Lagos, in arenosis maritimis, *Rechinger 22419* (G, as *T. tetrandra*); Kastades, Corfú, an der Strasse nach Gasturi in der Nähe des Hyllaeischen Hafens, 22 Apr 1896, *Baenitz* (PR); Epiro, Igoumenitsa, coastal salt marshes north-west of Igoumenitsa, 34S DJ 331738 [39°30'40"N, 20°13'19"E], 1 m, 17 Apr 2011, *Martínez & Villar T3GRE* (ABH59028); *ibid*, 34S DJ 330737 [39°30'39"N, 20°13'15"E], 5 m, 17 Apr 2011, *Martínez & Villar T4GRE* (ABH59027); *ibid*, 34S DJ 315746 [39°31'06"N, 20°12'13"E], 0 m, 17 Apr 2011, *Martínez & Villar T5GRE* (ABH59026); *ibid*, 34S DJ 311754 [39°31'32"N, 20°11'57"E], 1 m, 17 Apr 2011, *Martínez*

& Villar T6GRE (ABH59025); Epiro, Ammoudia, Acherondas river delta, *Tamarix* grove between Mesopotamo and Ammoudia, 34S DJ 565436 [39°14'26"N, 20°29'46"E], 0 m, 17 Apr 2011, *Martínez & Villar T7GRE* (ABH59040); *ibid*, 34S DJ 5743 [39°14'15"N, 20°30'19"E], 0 m, 17 Apr 2011, *Martínez & Villar T8GRE & T9GRE* (ABH59039, ABH59038); *ibid*, 34S DJ 559428 [39°14'01"N, 20°29'21"E], 0 m, 17 Apr 2011, *Martínez & Villar T10GRE* (ABH59037); Epiro, Koronisia, beside the sea, 34S DJ 884231 [39°03'27"N, 20°51'57"E], 2 m, 17 Apr 2011, *Martínez & Villar T11GRE* (ABH59036); Epiro, Strongyli, *Tamarix* grove on coastal marsh, 34S DJ 840312 [39°07'48"N, 20°48'57"E], 17 Apr 2011, *Martínez & Villar T12GRE* (ABH59031); Epiro, road Strongyli-Petra, *Tamarix* grove on coastal marsh, 34S DJ 838334 [39°08'59"N, 20°48'45"E], 17 Apr 2011, *Martínez & Villar T13GRE* (ABH59032); Western Greece, Paleros, 34S DH 897919 [38°46'35"N, 20°52'56"E], 25 m, 18 Apr 2011, *Martínez & Villar T16GRE* (ABH59857); Western Greece, road from Astakos to Messolonghi, 34S EH 137610 [38°29'52"N, 21°09'29"E], 0 m, 18 Apr 2011, *Martínez & Villar T17GRE* (ABH59858); Western Greece, Aitoniko, degraded coastal *Tamarix* grove, 34S EH 303537 [38°25'54"N, 21°20'53"E], 0 m, 18 Apr 2011, *Martínez & Villar T18GRE* (ABH59859); Western Greece, Messolonghi, 34S EH 367484 [38°23'00"N, 21°25'14"E], 0 m, 18 Apr 2011, *Martínez & Villar T19GRE & T20GRE* (ABH59861, ABH59860); *ibid*, 34S EH 366485 [38°23'02"N, 21°25'11"E], 0 m, 18 Apr 2011, *Martínez & Villar T21GRE* (ABH59862); Western Greece, Kounoupelli, salt marsh beside the sea, 34S EH 307171 [38°06'06"N, 21°21'02"E], 0 m, 18 Apr 2011, *Martínez & Villar T25GRE, T26GRE & T27GRE* (ABH59863, ABH59864, ABH59865); Peloponnisos, Marathopoli, south of the town, 34S EG 5160012 [37°03'21"N, 21°34'52"E], 20 m, 19 Apr 2011, *Martínez & Villar T32GRE* (ABH58545); Peloponnisos, salt marsh close to Pylos, 34S EF 588897 [36°57'08"N, 21°39'40"E], 0 m, 19 Apr 2011, *Martínez & Villar T33GRE* (ABH58544); Peloponnisos, Itilo beach, 34S FF 241617 [36°41'34"N, 22°23'21"E], 0 m, 20 Apr 2011, *Martínez & Villar T35GRE* (ABH58622); Peloponnisos, Valtaki, *Tamarix* grove between Agios Konstantinos and Valtaki, 34S FF 4172 [36°47'21"N, 22°34'54"E], 0 m, 20 Apr 2011, *Martínez & Villar T36GRE & T37GRE* (ABH58623, ABH58624); Peloponnisos, Agios Andreas, Karakouvni, 34S FG 564356 [37°21'14"N, 22°46'00"E], 8 m, 20 Apr 2011, *Martínez & Villar T39GRE* (ABH58625); Peloponnisos, Agios Andreas beach, salt marsh, 34S FG 571387 [37°22'56"N, 22°46'28"E], 0 m, 20 Apr 2011, *Martínez & Villar T41GRE* (ABH58621); Peloponnisos, Agia Anastasia, big salt marsh near Astros, 34S FG 555391 [37°23'08"N, 22°45'26"E], 0 m, 20 Apr 2011, *Martínez & Villar T42GRE* (ABH58620); Peloponnisos, Drepano, coastal *Tamarix* grove close to Drepano, 34S FG 741541 [37°31'04"N, 22°58'13"E], 0 m, 21 Apr 2011,

*Martínez & Villar T44GRE* (ABH59002); Peloponnisos, Almyri, small grove in the mouth of a ravine, 34S FG 771903 [37°50'37"N, 23°00'48"E], 0 m, 21 Apr 2011, *Martínez & Villar T49GRE* (ABH58999); Attica, Glyfada, between Glyfada and Vouliagmeni, 34S GG 430914 [37°50'17"N, 23°45'44"E], 5 m, 22 Apr 2011, *Martínez & Villar T52GRE* (ABH59049); Attica, Saronida, 34S GG 522877 [37°48'07"N, 23°51'56"E], 3 m, 22 Apr 2011, *Martínez & Villar T55GRE* (ABH59016); Attica, Lavriou, degraded coastal *Tamarix* grove, 35S KB 403788 [37°43'11"N, 24°03'15"E], 9 m, 22 Apr 2011, *Martínez & Villar T61GRE* (ABH59051); Central Greece, Dilesi, beside the sea, 34S GH 378467 [38°20'14"N, 23°43'16"E], 6 m, 22 Apr 2011, *Martínez & Villar T65GRE* (ABH59021); Central Greece, Neo Thronio, *Tamarix* grove 200 m south of Camping Venezuela, 34S FH 494986 [38°49'27"N, 22°43'18"E], 9 m, 23 Apr 2011, *Martínez & Villar T66GRE*, *T67GRE*, *T68GRE*, *T69GRE* & *T70GRE* (ABH59875, ABH59871, ABH59872, ABH59873, ABH59874); Central Greece, Neo Thronio, 1 km north of Camping Venezuela, 34S FH 478997 [38°50'03"N, 22°42'12"E], 6 m, 23 Apr 2011, *Martínez & Villar T72GRE* (ABH59877); Central Greece, Anthili, in a river floodplain, 34S FJ 302025 [38°51'44"N, 22°30'05"E], 12 m, 23 Apr 2011, *Martínez & Villar T75GRE* (ABH59866); Thessalia, Nees Pagases, small grove close to Volos, 34S FJ 664551 [39°19'46"N, 22°55'52"E], 0 m, 23 Apr 2011, *Martínez & Villar T77GRE* (ABH59870); Central Macedonia, Limenas, degraded *Tamarix* grove, 34T FK 334498 [40°11'18"N, 22°34'2"E], 23 Apr 2011, *Martínez & Villar T78GRE* & *T79GRE* (ABH59868, ABH59869); Central Macedonia, Methoni, Aliakmona river delta, 34T FK 364833 [40°29'22"N, 22°36'34"E], 8 m, 24 Apr 2011, *Martínez & Villar T81GRE* (ABH59867). — MONTENEGRO: Ulcinj, Sveti Nikola, *Tamarix* grove in the Bojana river mouth, in the northern branch of the river, close to the Albanian border, 34T CM 632367 [41°52'16"N, 19°21'09"E], 27 Apr 2011, *Martínez & Villar T2MNE*, *T3MNE*, *T4MNE* & *T5MNE* (ABH57893, ABH57891, ABH57890, ABH57892). — TURKEY: Marais sales situés sur les bords de la route conduisant de Smyrne à Vorula, à 2 lieues environ à l'est de cette première ville, 29 May 1854, *Balansa 130* (type of *T. hampeana* var. *smyrneana* G-BOIS; G, 2 vouchers); "Constantinopla", 1845, *Noë* (G-BOIS, ut *T. parviflora*); Golfe de Smyrne, Echelle de Papa, 24 Apr 1854, *Balansa 133* (type of *T. hampeana* var. *composita*, G); Aydın, Söke, B. Menderes Agli, Avlon merlii, denis kendri, 10 Apr 1976, *Uslu 4173* (G); Trockenés Bachbett, Aydın, Samsun Dađı bei Güzelçlami, 8 Apr 1990, *Nydegger* (G440247).