Revised systematics of Mediterranean *Arundo* (Poaceae) based on AFLP fingerprints and morphology

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Abstract The systematics of the genus *Arundo* (Poaceae) remains poorly resolved because of the overlap of morphological characters classically used in the taxonomy of the genus and insufficient variation of DNA sequences. The main aim of this study is to delimit genetic clusters with AFLP fingerprints and to compare them with morphological data. On the basis of extensive sampling in the Mediterranean area, AFLP markers clearly showed five clusters. Despite overlapping values, morphometric analyses strongly confirm these clusters, and new qualitative features allow the recognition of five taxa: the Taiwanese *A. formosana*, the cosmopolitan *A. donax*, the circum-Mediterranean *A. micrantha*, the Italo-Balkan *A. plinii* and the Franco-Ligurian *A. donaciformis* (Loisel.) Hardion & al. comb. nov. Additionally, this study shows a correlation between genetic diversity, caryopsis production and ploidy level. However, the lack of genetic diversity for *A. micrantha* and *A. donax* in the Mediterranean area remains enigmatic.

Keywords *Arundo*; clonal reproduction; Mediterranean biogeography; polyploidy; taxonomy

INTRODUCTION

Arundo L. (Poaceae) includes three to five taxa distributed from the Mediterranean Basin to tropical Asia (Conert, 1961; Grass Phylogeny Working Group, 2001; Danin, 2004). It is the generitype of Arundinoideae, the most unresolved grass subfamily, historically known as a dustbin group (Prat, 1932; Renvoize, 1981; Barker & al., 1995, 1999; Linder & al., 1997). The genus is defined by high, rigid culms, clonal rhizomatous propagation and wetland affinities, rare features shared with Bambusoideae. However, the systematics of *Arundo* has remained poorly elucidated despite their use since ancient times, as described by Theophrastus and Pliny the Elder and shown by archeological work (Figueiral & al., 2010).

Formerly the only reed genus, *Arundo*, included up to 238 taxa (Govaerts, 1999), but almost all of these were moved to 31 other genera since (e.g., *Calamagrostis* Adans., *Phragmites* Adans., *Bambusa* Schreb.). A decade ago, *Arundo* was reduced to only three species: *Arundo donax* L., the subtropical Eurasian Giant Reed, invasive in many warm regions; *A. formosana* Hack., a decumbent grass from Taiwan and Ryukyu Islands; and the circum-Mediterranean *A. plinii* Turra s.l. In 2004, Danin divided the latter into three species: *A. plinii* s.str., a taxon from Bologna (N Italy; locus classicus), Sainte-Lucie Island and Fréjus (S France); *A. collina* Ten. (= *A. hellenica* Danin & al., 2002), an Italo-Balkan species described from Napoli; and *A. mediterranea* Danin (= *A. mauritanica* Desf. nom. illeg.) found in Algeria, Cyprus, Greece and Palestine. However, the latter is a later synonym of *A. micrantha* Lam. (1791) described from N Africa (Hardion & al., 2012).

This study was undertaken because of the uncertain taxonomic status of several Mediterranean reeds despite Danin's revision (2004). In fact, his "deliberately preliminary" work was based only on some vegetative (height, habit, leaf shape) and ecological characters rarely present or mentioned in herbarium specimens. More generally, the high variability of morphological characters among *Arundo* species has led to many erroneous identifications, as shown by our re-identification of the population from Sainte-Lucie as *A. micrantha* (Hardion & al., 2012). Moreover, our recent cytological investigations are incongruent with previous taxonomic treatments (Hardion & al., 2011). Finally, *A. plinii* s.l. is protected in France and in-depth knowledge (e.g., taxonomic delimitation, structure of genetic diversity and reproduction system) is urgently needed for conservation purposes (Olivier & al., 1995; Callmander & al., 2005).

Molecular studies in *Arundo* had already shown the lack of genetic variation using chloroplast spacers (*rps16-trnK*, *rpl32-trnL*, *psbA-trnH*) and ISSR markers (Grassi & al., 2008; Grassi, pers. comm.). Our preliminary investigations confirm these results through the absence of informative mutations in three additional cpDNA sequences (*matK* gene, *trnL-trnF* and *rps4-trnT2* spacers). Despite the high potential of AFLP fingerprinting for homoplasy and its drawbacks for phylogenetic use (dominant markers with only two states and uncertain homology between fragment sizes; Koopman, 2005), we chose this method in order to generate several polymorphic markers distributed across the whole genome, a feature particularly powerful for clonal species and in case of reticulate evolution. The AFLP method had already been used to reconstruct species relationships in evolutionarily complex groups (Desprès & al., 2003). Within Poales, these molecular fingerprints provided a basis for delimiting phylogenetic clusters in *Phragmites* (Lambertini & al., 2006), *Carex* L. (Jiménez-Mejías & al., 2011) and *Typha* L. (Lamote & al., 2005; Na & al., 2010). Moreover, a recent study has shown the capacity of the AFLP method to distinguish different genotypes within *Arundo donax* (Mariani & al., 2010).

The aim of this study is (1) to delimit genetic groups in *Arundo* using AFLP fingerprinting, (2) to test the congruence of genetic delimitations with multivariate analyses of quantitative morphological characters, and (3) to present a new identification key using the most reliable morphological features.

■ MATERIALS AND METHODS

Plant material. — On the basis of localities reported in the literature, by naturalists and on herbarium specimens (BM, E, G, K, MARS, MPU, P), a total of 96 sites were investigated (Appendix), including 79 sites for *A. plinii* s.l. and 16 for *A. donax*. In order to include all *Arundo* species, one individual of the morphologically differentiated *A. formosana* was included in the molecular analyses. The taxonomic assignment of Mediterranean reeds to *A. donax* or *A. plinii* s.l. is our basal hypothesis for the purpose of the molecular and morphological analyses. In addition, many herbarium samples were considered in order to estimate species distributions. At each site, material was collected for voucher specimens (MARS), and budding rhizomes for DNA extraction. These cuttings were greenhouse-cultivated in pots of loam soil to obtain healthy leaves without fungal contamination (Zhang & al., 1997). After one year, all dead rhizomes were counted to estimate survival rates reflecting specific vegetative vigor. Then, plants were cultivated in the Botanical Garden of Aix-Marseille University.

DNA extraction and AFLP fingerprints. — *Arundo* species form densely clonal patches by rhizome propagation. Given this predominately vegetative reproduction, we consider only one sample per patch for molecular analyses. About 50 mg of silica-dried leaves from greenhouse-cultivated samples were crushed with liquid nitrogen. Total DNA was extracted following Doyle & Doyle (1987) with the following modifications: 1.4 mM NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8 and 4% hexadecyltrimethyl-ammonium bromide. After precipitation with 95% ethanol, the centrifuged pellets were washed in 70% ethanol, dried and suspended in TE-buffer with RNase. DNA concentrations were measured using a photometer (Biophotometer, Eppendorf, Hamburg, Germany) and diluted to 50 ng/µL.

The AFLP procedure followed Vos & al. (1995) with slight modifications: 300–1000 ng of DNA was digested for 3 h at 37°C with 6 units of EcoRI (MBI Fermentas, Mundolsheim, France) and for 3 h at 65°C with 4 units of Tru9I (MBI Fermentas) in a total volume of 25 µL. Digestion products were immediately ligated for 8 h at room temperature by adding 2.5 pmol and 25 pmol of EcoRI and MseI adaptors, respectively, 0.5 units of T4 DNA ligase and 10 mM of ATP (MBI Fermentas) in a final volume of 25 µL. Preamplification was performed in 50 µL volumes containing 5 µL of 8-fold diluted ligation product, 10 pmol of EcoRI (+A) and MseI (+C) primers, 0.16 mM of dNTPs, 0.65 mM of $MgCl_2$ and 1.5 units of *Taq* DNA polymerase (Q-Biogen, Illkirch, France). The preamplification thermocycle profile was 94°C for 2 min, followed by 20 cycles at 94°C for 45 s, 56°C for 45 s, 72°C for 1 min and 72°C for 10 min.

Six primer combinations were chosen for the selective PCR: EcoRI-AAC/MseI-CAA, EcoRI-AGC/MseI-CTG, EcoRI-ACG/MseI-CAC, EcoRI-ATG/MseI-CTA, EcoRI-ATC/MseI-CTC and EcoRI-AGG/MseI-CGG, dyed with 6-FAM fluorescence at 5′ end (Eurofins MWG Operon, Ebersberg, Germany). Selective amplification was performed in 20 µL volumes with 5 pmol of each primer, 0.65 mM of $MgCl_2$, 0.5 mM of dNTPs, 1 unit of *Taq* DNA polymerase (Q-Biogen) and 5 µL of 100× diluted preamplification. The selective amplification thermocycle profile was 94°C for 2 min, 10 cycles of 94°C for 30 s, 65°C for 30 s (step −0.7°C per cycle), 72°C for 1 min, followed by 20 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 1 min and 72°C for 5 min. PCR products were separated and quantified on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, California, U.S.A.). The reliability of AFLP markers was checked by repeating the complete analysis from DNA amplification to AFLP screening on 10 samples for each pair of primers.

Analyses of genetic structure. — AFLP fingerprints were generated from electrophoretogram alignments using GeneMapper v.4.1 (Applied Biosystems). Putative loci were defined as any fragment with a minimum amplitude of 200 Relative Fluorescent Units (RFU) occurring in at least one electrophoretogram. All samples were scored for peak absence or presence for each marker from 50 to 500 bp, and all overlapping peaks were discarded. Unfortunately, electrophoretograms from old herbarium specimens (>20 years) had to be removed from the analyses because they show clear DNA damage and a lack of fragments larger than 200 bp. To delimit *Arundo* taxa, a two-step approach was adopted. First, AFLPs were explored without any a priori clustering hypothesis, using a principal coordinate (PCo) analysis on Jaccard distances with ade4 R-package (Thioulouse & al., 1997) in R software v2.14.1 (R Development Core Team, 2010). Second, a discriminant Analysis of principal components (DAPC) tested the previously inferred clustering, using adegenet R-package (Jombart & al., 2010). This method uses principal components to perform a discriminant analysis on genetic variation. Whenever group prior information is unknown, the DAPC tries different numbers of clusters based on a K-means clustering of principal components and uses a Bayesian information criterion (BIC) to assess the best number of clusters. Because DAPC is a model-free approach which does not require prior information on the clustering, its use is particularly appropriate for systematic analysis. For each cluster, descriptive statistics such as number of fragments (F), number of specific fragments (SF), percentage of polymorphic fragments (%P) and Nei's genetic diversity index (*D*) were obtained using the AFLPdat package (Ehrich, 2006). Moreover, the Clones-function from this package offered a basis to detect multi-locus lineages (MLLs) and discredits genotypes generated by somatic mutations or genotyping errors (De Witte & al., 2011). According to our taxonomic objectives, the largest pairwise genetic distance among the 10 reliability tests was defined as a consistent MLL threshold.

Morphology. — At each site, 15 samples from the same clonal patch were measured, i.e., a total of 930 samples of *A. plinii* s.l. (in 62 sites) and 45 samples of *A. donax* (in

3 sites). The 14 quantitative characters were height of culm with panicle (C), node number/culm (N), internode length (measured halfway along the culm; eC), culm basal diameter (∅C), panicle length (P), rhizome internode length (eR) and diameter (∅R), length (Lf) and width (lf) of the leaf blade from the middle part of the main axis, lengths of lower (G1) and upper (G2) glumes, lemma (L), palea (pa) and lemma hairs (pL) of spikelets collected in the middle part of the panicle. All observed caryopses were counted to estimate a rate of seed production.

To delimit *Arundo* taxa, a two-step approach was adopted for the morphometric data. First, a principal component analysis (PCA) of quantitative data shows potential clusters among all measured samples. The aim of the second step is to test and explain these taxonomic hypotheses by a discriminant analysis (DA) in order to study intergroup information. DA generates jackknife validations (JV) based on half of the samples and leave-one-out cross-validations (CV) for each sample to test cluster robustness. Then, DA identifies the most explicative variables for an a priori clustering on the basis of correlation coefficients (Pearson) between variables and coordinates. According to the hypothesis of site clonality, DA was first generated on individual samples, and then on the site means in order to reinforce specific features and to improve the clarity of DA representation. Box-and-whisker plots were generated for each variable to reveal size overlaps between *Arundo* species. All these analyses were performed with ade4 and MASS R-packages (Venables & Ripley, 2002).

All qualitative morphological features mentioned in Floras (Rouy, 1913; Coste, 1937; Fiori, 1969; Tutin & al., 1980; Davis & al., 1985; Feinbrun-Dothan, 1986; Bolos & Vigo, 2001) and other literature (Danin & al., 2002, 2005; Danin, 2004; Danin & Naenny, 2008) were observed, and we retained the most discriminant features delimiting taxonomic clusters. In addition, we searched for new features useful for identification in the field and of herbarium specimens. Only the most reliable of them were included in the new determination key and illustrated with Scanning Electron Microscopy (PRATIM, Aix-Marseille University).

■ RESULTS

Genetic differentiation. — The six AFLP primer pairs generated 1046 fragments (from 141 to 208 each one), of which 83% were polymorphic. The threshold delimiting MLLs (i.e., the maximum distance between pairwise controls) was assessed as 11 mismatches which corresponds to an error rate of 2%.

The three first axes of PCo represent 67% of the overall variation (respectively, 35.8%, 26.9% and 5.2%) and clearly discriminate five clusters (Fig. 1A–B) corresponding to *A. formosana*, *A. donax*, and to three clusters within *A. pliniii* s.l.: a circum-Mediterranean cluster (*A. micrantha*), and a second group divided by the third principal coordinate into an Italian-Balkan (*A. plinii* s.str.) and a Franco-Ligurian (*A. donaciformis*) cluster (see Taxonomic treatment). Using 35 principal components, model selection and the K-means method, the DAPC supports the consistency of five clusters (Fig. 1C). The exact (100%) reassignment of all samples strongly confirms the cohesion and the differentiation of each.

In the Mediterranean Basin, *A. plinii* s.str. stands apart by having a higher number of fragments (F = 787; Table 1), linked to a much higher polymorphism rate (%P = 50.1%) and higher genetic diversity ($D = 0.132$, MLL = 37). In contrast, *A. donax*, *A. micrantha* and *A. donaciformis* have unexpectedly and dramatically low diversity indices ($D < 0.01$), with only one MLL per taxon. These three taxa do not form caryopses but show higher rhizome survival rates than the fertile *A. plinii* s.str. (86%–90% vs. 51%; Table 1). Even with only one sample, *A. formosana* has the highest number of specific markers (SF = 101).

Morphological traits. — In the morphometric analysis, the first axis of PCA (48.6%) clearly shows the morphological differentiation of *A. donax* in the Mediterranean (Fig. 2). Within the *A. plinii* s.l. complex, three groups appear which are relatively congruent with the clusters revealed by AFLPs: (1) *A. plinii* s.str., (2) *A. donaciformis* and (3) *A. micrantha*. Although these three taxa overlap, CV and holdout validations on morphometric data both reassign 97.5% of samples according to the molecular clustering assignment. The DA generated from

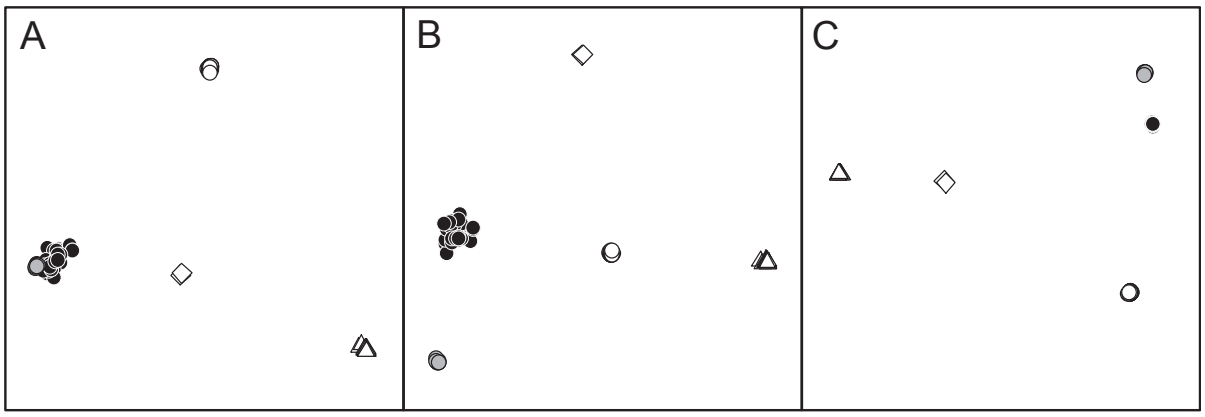

Fig. 1. Analysis of AFLP data of one sample of *A. formosana* (diamond), 16 samples of *A. donax* (triangles) and 79 samples of *A. plinii* s.l. (circles). White circles, *A. micrantha* (n = 30); grey circles, *A. donaciformis* (n = 8); black circles, *A. plinii* s.str. (n = 41). **A,** principal coordinates (PCo) analysis of Jaccard distances between samples, axis 1 (35.8%) and axis 2 (26.9%); **B,** PCo axis 1 (35.8%) and axis 3 (5.2%); **C,** discriminant analysis of principal components (axis 1: 59.5%; axis 2: 22.3%).

Table 1. Descriptive statistics based on AFLP data, reproductive strategy index and chromosome numbers.

	n	F	SF	%P	*D*	MLL	%R	%S	2*n* [a]
Arundo plinii s.l.									
A. plinii s.str.	41	787	78	50.1	0.132	37	51 (343)	12 (495)	72–76 (17)
A. donaciformis	8	542	0	2.0	0.007	1	86 (113)	0 (210)	108 (21)
A. micrantha	30	599	20	5.3	0.006	1	88 (189)	0 (255)	72 (11)
Arundo donax	16	570	57	4.4	0.008	1	90 (122)	0 (165)	ca. 108 [b] (9)
Arundo formosana	1	563	101	–	–	–	–	–	72 (2)

Column headers are as follows: n, number of samples; F, number of fragments; SF, number of specific fragments; %P, percentage of polymorphic fragments; *D*, Nei's genetic diversity index; MLL, number of multi-locus lineages; %R, survival percentage of cultivated rhizomes; %S, percentage of seed production; 2*n*, chromosome numbers from Pizzolongo (1962), Gorenflot & al. (1972), Hardion & al. (2011) and our unpublished data.
[a] Number of sites studied in parentheses.
[b] In the Mediterranean.

site means clearly distinguishes the three clusters of *A. plinii* s.l. (Fig. 3). The first axis (74.4%) is positively correlated with vegetative characters and discriminates the *A. micrantha* cluster from the two others. The second axis (25.6%) is explained by spikelet variables and discriminates *A. donaciformis*.

Box-and-whiskers plots clearly illustrate the amplitude of all morphometric characters (Fig. 4), and the greater size of *A. donax* in almost all variables, except for rhizome internode length (eR). Within the *A. plinii* s.l. complex, *A. micrantha* has the most robust vegetative parts, *A. donaciformis* has the longest floral organs, and *A. plinii* s.str. has the smallest values for all morphometric variables, except eR.

Since morphometric characters are strongly overlapping, eight (including five new ones) discriminant qualitative features, relating to rhizome, flower number by spikelet, and indumentum of culms, leaves and spikelets, have been selected for the determination key (Fig. 5; Taxonomic treatment).

■ DISCUSSION

Systematics and distribution of *Arundo* taxa. — On the basis of Mediterranean-wide samples and a molecular and morphometric approach, our study revealed the existence of five taxa which are better discriminated by AFLP fingerprinting than by morphometry due to character overlap. As the Taiwanese *A. formosana* and the cosmopolitan *A. donax* are well differentiated, the taxonomic challenge in *Arundo* is to distinguish taxa within *A. plinii* s.l. Despite its coherence supported by the lemma plinii-type (i.e., lemma with hairs horizontally inserted on a ring upon its first quarter; Fig. 5C) and 1(2)-flowered spikelets, our data imply the division of this group into three distinct taxa (Fig. 6): (1) the circum-Mediterranean *A. micrantha*, (2) the Italo-Balkan *A. plinii* s.str. (3)

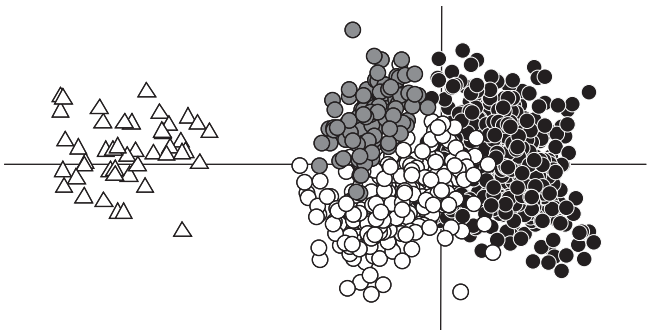

Fig. 2. Principal component analysis based on 14 morphological characters and 930 individuals of *A. plinii* s.l. (circles) and 45 of *A. donax* (triangles). Circle colours indicate the clustering by AFLP fingerprints (Fig. 2). The first two axes explain 48.6% and 10.9% of total variation, respectively. White circles, *A. micrantha* (n = 270); grey circles, *A. donaciformis* (n = 105); black circles, *A. plinii* s.str. (n = 555).

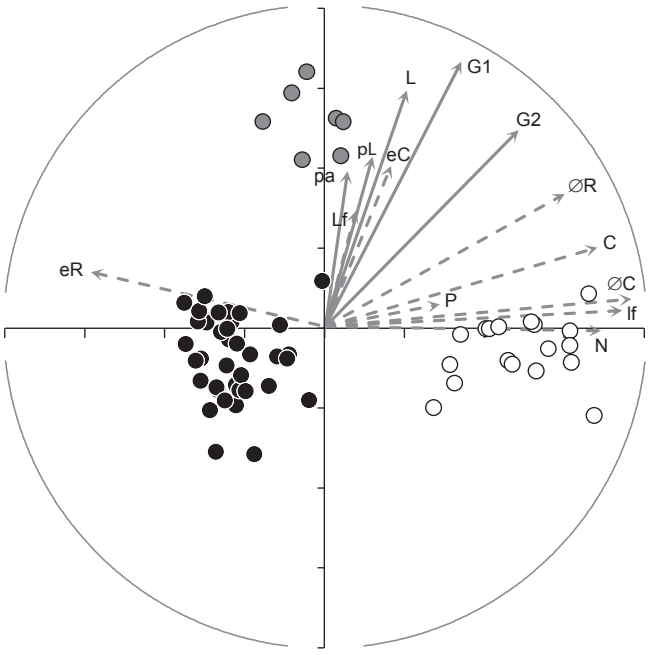

Fig. 3. Discriminant analysis (74.4% and 25.6%) based on the mean values of the 62 sites of *A. plinii* s.l. for 14 morphological characters. White circles, *A. micrantha* (n = 18); grey circles, *A. donaciformis* (n = 7); black circles, *A. plinii* s.str. (n = 37). Vegetative characters (dashed arrows): height of culm with panicle (C), node number/culm (N), internode length (eC), culm basal diameter (∅C), panicle length (P), rhizome internode length (eR) and diameter (∅R), length (Lf) and width (lf) of the leaf blade. Spikelet characters (solid arrows): lengths of lower (G1) and upper (G2) glumes, lemma (L), palea (pa) and lemma hairs (pL).

and the Franco-Ligurian *A. donaciformis*. Their morphological, geographical and genetic characteristics justify the chosen specific rank, reinforced by the absence of interspecific hybrids (Sites & Marshall, 2003; Crawford & al. 2005). Moreover, our results are in accordance with chromosome data from the literature (Hardion & al., 2011) showing a higher ploidy level for *A. donaciformis* ($2n = 108$; Table 1).

While *A. micrantha* is a synonym of *A. mediterranea* Danin, *A. plinii* s.str. and *A. donaciformis* clearly differ from the taxa proposed by Danin (2004). In contrast to previous studies (Danin 2004; Mariani & al., 2010), no genetic or morphological difference between samples from Bologna and the remaining Italo-Balkan samples supports the distinction of *A. collina* Ten. (locus classicus: Napoli) from *A. plinii* s.str. (locus classicus: Bologna): both taxa belong to the same species. We tested and rejected the only character supporting *A. collina* in Danin's key, "lemma with entire apex", because this feature is too variable and barely visible.

Relationships between genetic diversity, fertility and polyploidy. — This study reveals a high correlation between genetic diversity, fertility and polyploidy in *Arundo* (Table 1). In the literature, fruit formation has been reported only for $2n = 72$ cytotypes: *A. formosana*, Asian *A. donax* ($2n$ = ca. 72 in Thailand: Larsen, 1963; India: Christopher & Abraham, 1971; Mehra & Chaudhary, 1975; Uzbekistan: Bochantseva, 1972; fertile in Iran: Bor, 1970; China: Wu & Raven, 1994; Pakistan: Brach & Song, 2006), and *A. plinii* s.str. (Hardion & al., 2011). In contrast, with $2n$ = ca. 108 (e.g., Gorenflot & al., 1972; Hardion & al., 2011), Mediterranean *A. donax* and *A. donaciformis* do not show fructification or genetic variation. The most parsimonious hypothesis to explain the formation of $2n = 108$ is the fusion of reduced ($n = 36$) and unreduced ($n = 72$) gametes from diploidized progenitors ($2n = 72$). This event produces pseudotriploids which are often sterile due to strong meiotic irregularities (Thompson & Lumaret, 1992; Bretagnolle & Thompson, 1995). Polyploid formation frequently occurs on

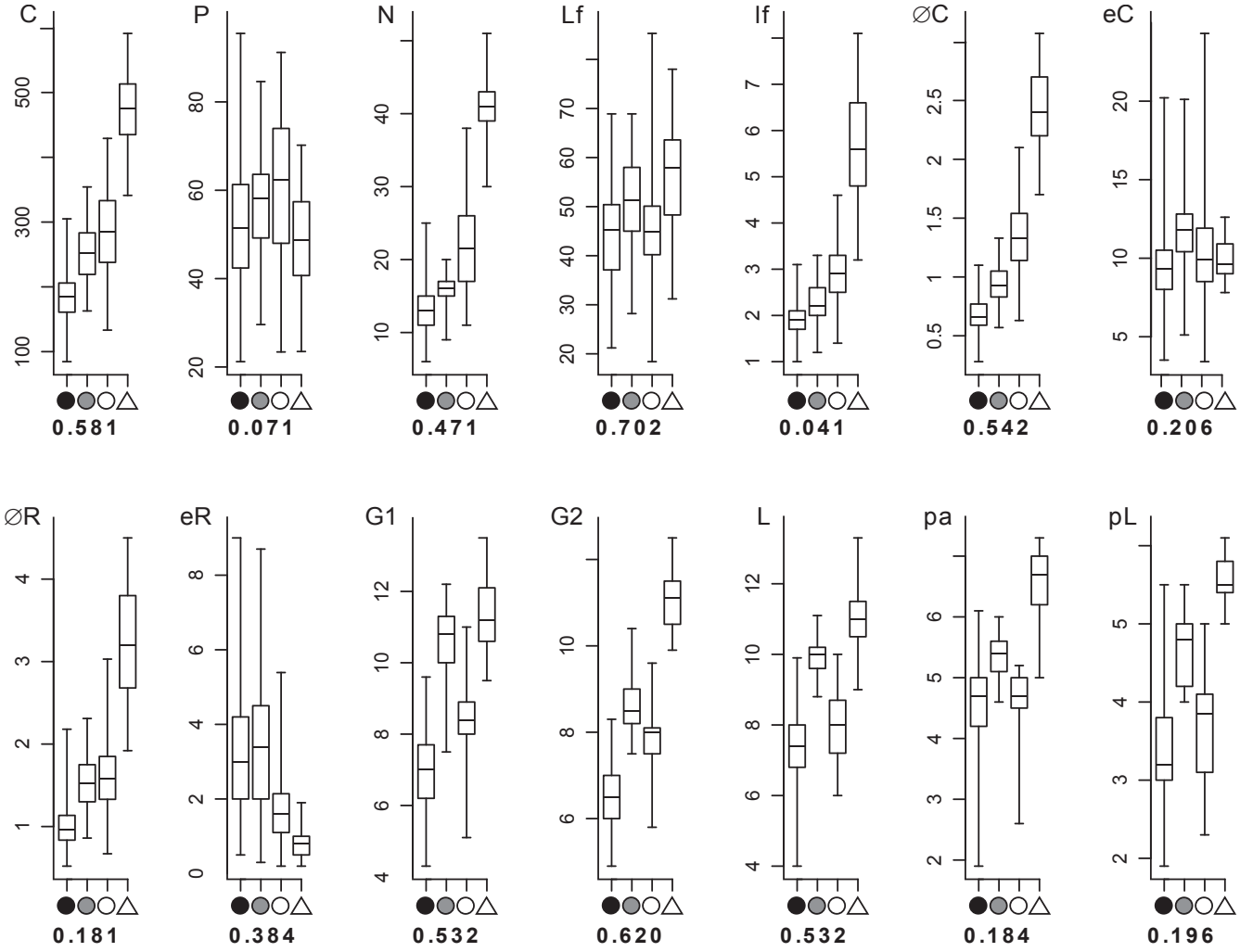

Fig. 4. Box-and-whiskers plots of morphological variables measured on 930 samples of *A. plinii* s.l. (circles) and 45 samples of *A. donax* (triangles). Bold numbers indicate R^2 values between *A. plinii* s.l. clusters. White circles, *A. micrantha* (n = 270); grey circles, *A. donaciformis* (n = 105); black circles, *A. plinii* s.str. (n = 555). Variables from left to right, top row: Height of culm with panicle (C), panicle length (P), node number/culm (N), length (Lf) and width (lf) of the leaf blade, culm basal diameter (∅C), internode length (eC); bottom row: rhizome diameter (∅R) and internode length (eR), lengths of lower (G1) and upper (G2) glumes, lemma (L), palea (pa) and lemma hairs (pL).

the edge of a species' distribution area (Soltis & al. 2007), such as in *A. donaciformis* at the northwest edge of the range of the diploidized *A. plinii* s.str., or in Mediterranean *A. donax* at the western edge of its Asian range.

With a single MLL in the Mediterranean Basin, origin and dispersal of non-fructiferous *A. donax* and *A. micrantha* raise challenging questions. Such genetic uniformity has rarely been observed in widespread native species, e.g., *Spartina maritima* (Curtis) Fernald (Raybould & al., 1991; Yannic & al., 2004), but more frequently in invasive taxa, such as *Pennisetum setaceum* (Forssk.) Chiov. (Poulin & al., 2005; Le Roux & al., 2007), *Fallopia japonica* (Houtt.) Ronse Decr. (Hollingsworth & Bailey, 2000), *Spartina anglica* C.E. Hubb. (Baumel & al., 2001) or *A. donax* in the U.S.A. (Ahmad & al., 2008). In case of *A. donax*, an ancient introduction (as an archaeophyte) to the Mediterranean Basin, linked to drastic founder effects (Dlugosch & Parker, 2008), could explain its genetic uniformity in this area. In contrast to *A. donax* in the Mediterranean area, the species shows AFLP genotype variation in Asia (Mariani & al. 2010). On the other hand, the sterility of *A. micrantha* and its lack of genetic diversity remain more enigmatic given its ploidy level ($2n = 72$) and its restriction to the Mediterranean Basin. In conclusion, the systematics and evolution of *Arundo* needs further investigations in western Asia.

■ TAXONOMIC TREATMENT

Key to species of the genus *Arundo*:

1. Lemma with hairs obliquely spread on its lower quarter, spikelets with 3–5 florets …… 2
1. Lemma with hairs perpendicularly inserted on a ring on its first quarter; spikelets with 1(2) florets …… 3
2. Stems erect, rhizome tuberous with internodes <1cm, glumes, nodes, limbs and sheaths glabrous …. ***A. donax***
2. Stems decumbent, rhizome stem-like, glumes and nodes glabrous, sheaths and limbs hairy …… ***A. formosana***
3. Culms erect, rhizomes stem-like, nodes, sheaths, limbs and glumes glabrous…… ***A. micrantha***
3. Culms erect, rhizomes stem-like, nodes pubescent, sheaths and limbs glabrous …… 4
4. Upper glumes glabrous ……***A. plinii*** s.str.
4. Upper glumes hairy …… ***A. donaciformis***

Arundo donaciformis (Loisel.) Hardion, Verlaque & B. Vila, **comb. nov.** ≡ *Calamagrostis donaciformis* Loisel., Fl. Gall., ed. 2, 1: 53. 1828 – Holotype: France. Fréjus, *Perreymond s.n.* (AV!; isotype: MARS [no. MARS00004]!).

This rare taxon from southern France and Liguria was

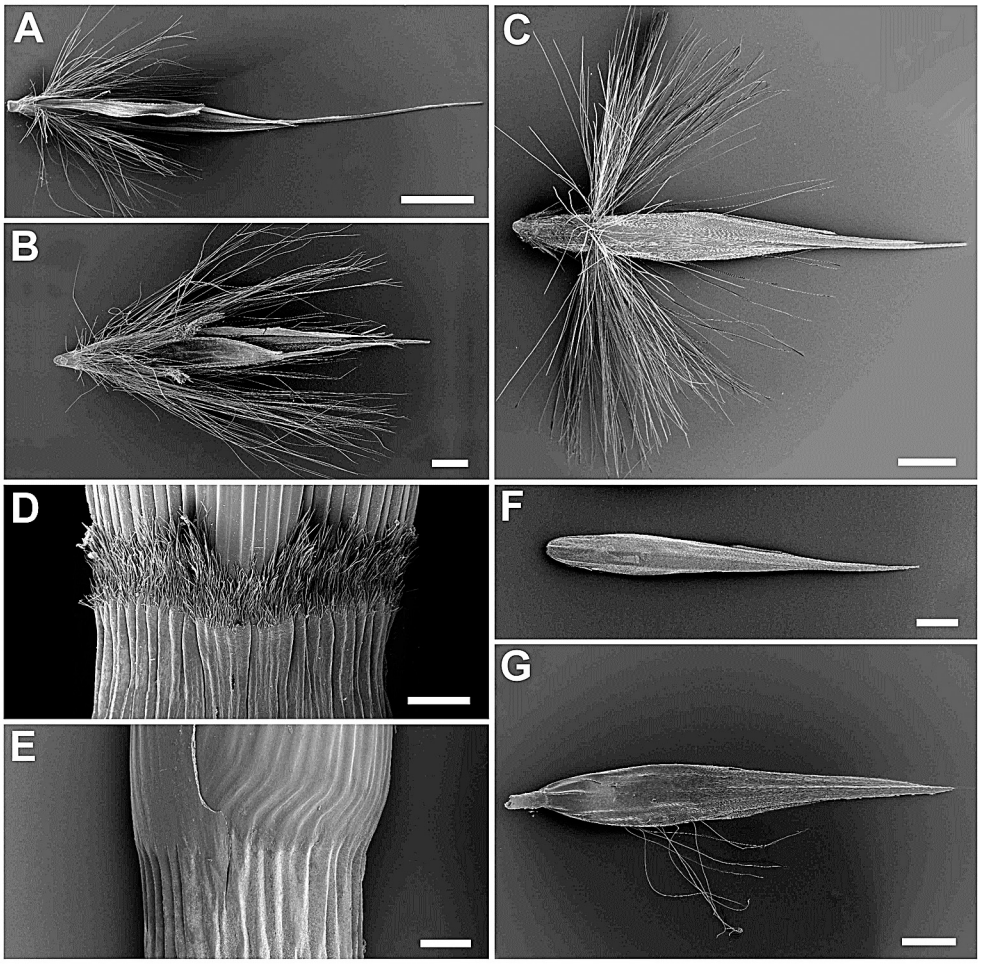

Fig. 5. Discriminant morphological characters in *Arundo* (SEM). **A,** lemma of *A. formosana*; **B,** lemma of *A. donax*; **C,** lemma of *A. donaciformis*; **D,** pubescent node of *A. donaciformis*; **E,** glabrous node of *A. micrantha*; **F,** glabrous upper glume of *A. plinii* s.str.; **G,** hairy upper glume of *A. donaciformis*. — All scale bars = 1 mm.

already described from Fréjus by Jean-Louis-Auguste Loiseleur-Deslongchamps as a *Calamagrostis* species because of its 1-flowered spikelet.

Arundo plinii Turra, Farsetia: 11. 1765 ≡ *Calamagrostis plinii* (Turra) J.F. Gmel., Syst. Nat. 1: 172. 1796 ≡ *Donax plinii* (Turra) K. Koch, Dendrologie 2(2): 352. 1873 ≡ *Arundo donax* subsp. *plinii* (Turra) Mateo & R. Figuerola in Fl. Analit. Prov. Valencia (I.A.M. Investig. 14): 368. 1987– **Neotype (designated here):** Italy. Bologna, On the banks of the Reno, *Hardion s.n.* (MARS [no. MARS00005]!; isoneotypes: BM!, E!, K!, P!).

= *Arundo collina* Ten., Fl. Napol. 3: 101. 1822 – **Lectotype (designated here):** Italy. In collibus neapolis, *Tenore s.n.* (MARS [no. MARS00006]!).

= *Arundo hellenica* Danin, Raus & H. Scholz in Willdenowia 32: 191. 2002 – Holotype: Greece. Sterea Ellas, Nomos of Viotia, 2 km West of Livadia, 10 m off the road, clayey rock outcrop, 3 Nov 2000, *Danin G2000/09/16N* (HUJ; isotypes: B!, E!).

Arundo micrantha Lam., Tabl. Encycl. 1: 196. 1791 – Holotype: Africa, *Desfontaines s.n.* (P-LA!; isotypes: B!, G!, K!, MPU!, P!).

= *Arundo mauritanica* Desf., Fl. Atlant. 1: 106. 1798, nom. illeg., non Poir. 1789 ≡ *Calamagrostis mauritanica* Spreng., Syst. Veg. 1: 252. 1825 – Holotype: Algeria, *Desfontaines s.n.* (P!; isotypes: B!, G!, K!, MPU!, P-LA!).

= *Arundo mediterranea* Danin in Willdenowia 34: 362. 2004 – Holotype: Israel, Nahal Sorek, 18 Sep 2004, *Danin s.n.* (HUJ; isotypes: B!, E!, MARS [no. MARS00007]!).

Arundo formosana Hack. in Bull. Herb. Boissier 7(9): 724. 1899 – Holotype: Taiwan. Shinchiku, 24 Nov 1896, *Makino 322* (W; isotype: US).

= *Arundo parviflora* Ohwi in Repert. Spec. Nov. Regni Veg 36: 40. 1934 – Holotype: Japan. Shikoku, Mai 1933, *Ohwi 1597* (US).

Arundo donax L., Sp. Pl. 1: 81. 1753 1753 ≡ *Cynodon donax* (L.) Raspail in Ann. Sci. Nat., Bot. 5: 302. 1825 ≡ *Scolochloa donax* (L.) Gaudin in Fl. Helv. 1: 202. 1828 – Lectotype (designated by Renvoize in Regnum Veg. 127: 21. 1993): Herb. A. van Royen, 912.356-93.

= *Arundo bifaria* Retz., Observ. Bot. 4: 21. 1786 ≡ *Amphidonax bifaria* (Retz.) Nees ex Steud. Syn. Pl. Glumac. 1: 197. 1854 ≡ *Donax bifarius* (Retz.) Trin. ex Spreng., Neue Entdeck. Pflanzenk. 2: 73. 1821 – Holotype: India Orientali, *König s.n.* (K!; isotype: BM!).

= *Arundo bengalensis* Retz., Observ. Bot. 5: 20. 1789 ≡ *Aira bengalensis* (Retz.) Gmel., Syst. Nat.: 174. 1791 ≡ *Donax bengalensis* (Retz.) P. Beauv., Ess. Agrostogr.: 78, 152, 161. 1812 ≡ *Amphidonax bengalensis* Roxb. ex Nees in Nat. Syst. Bot.: 449. 1836 ≡ *Amphidonax bengalensis* (Retz.) Nees ex Steud., Syn. Pl. Glumac. 1: 197. 1854, hom. illeg. – Holotype: India, Bengala, *König s.n.* (LD).

= *Arundo donax* var. *coleotricha* Hack. in Bull. Herb. Boissier 7: 724. 1899 ≡ *Arundo coleotricha* (Hack.) Honda in Bot. Mag. (Tokyo) 41: 14. 1927 – Holotype: Taiwan, Tamsui, *Makino 343* (TI).

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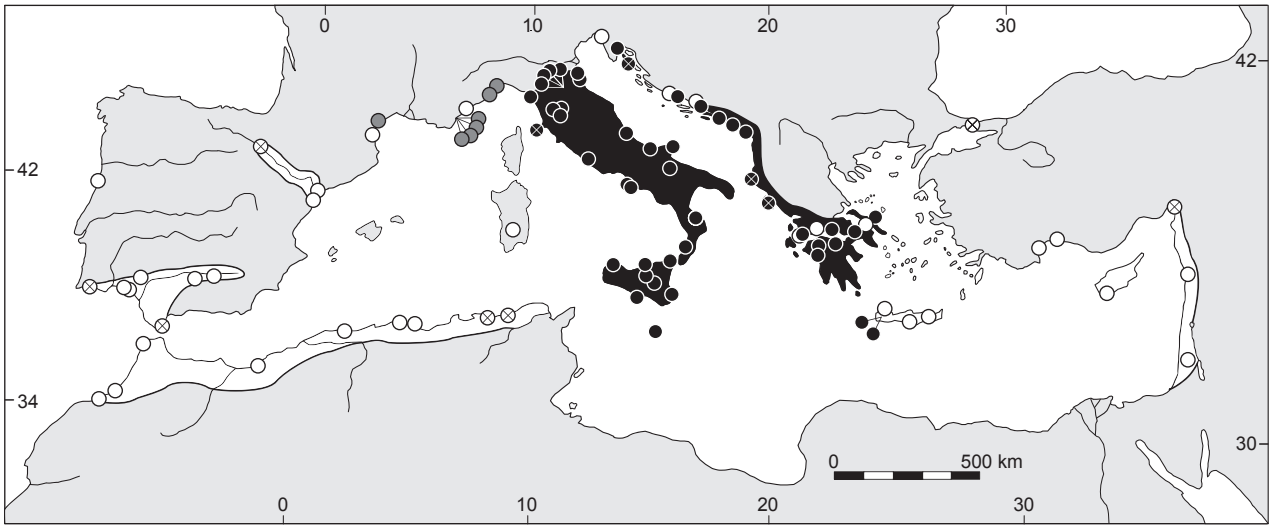

Fig. 6. Origin of samples (circles) and estimated distribution areas of *A. micrantha* (white), *A. donaciformis* (grey) and *A. plinii* s.str. (black) in the Mediterranean Basin according to AFLP fingerprints and morphology. Crossed circles indicate herbarium samples not included in the AFLP analysis (Appendix).

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Appendix. List of samples studied and included in morphometry and AFLP analyses.

Taxon	Post treatment	Locality, *collector*, voucher	Metric	AFLP
A. donax	*A. donax*	Chypre, Limassol, *Constantinou*, MARS00046		+
A. donax	*A. donax*	Croatia, Pag, 44°30′42″N, 14°56′03″E, *Fridlender*, MARS00047		+
A. donax	*A. donax*	France, Bonifacio, 41°24′04″N, 9°10′13″E, *Verlaque*, MARS00048		+
A. donax	*A. donax*	France, Marseille, 43°20′04″N, 5°08′26″E, *Verlaque*, MARS00049	+	+
A. donax	*A. donax*	Greece, Kolimpari, 35°32′11″N, 23°47′30″E, *Vila*, MARS00050		+
A. donax	*A. donax*	Greece, Kavassilas, 37°52′34″N, 21°16′02″E, *Hardion* & Vila, MARS00051	+	+
A. donax	*A. donax*	Italy, Volterra, 43°24′05″N, 10°59′53″E, *Hardion & Vila*, MARS00052		+
A. donax	*A. donax*	Italy, Napoli, 40°53′50″N, 14°02′38″E, *Hardion & Vila*, MARS00053		+
A. donax	*A. donax*	Italy, Cannitello, 38°13′48″N, 15°38′58″E, *Hardion*, MARS00054		+
A. donax	*A. donax*	Lebanon, Jadra, 33°38′03″N, 35°24′01″E, *Abdel Samad*, MARS00055		+
A. donax	*A. donax*	Malta, Marsaskala, 35°50′48″N, 14°33′51″E, *Médail & Gambin*, MARS00056		+
A. donax	*A. donax*	Morocco, Rabat, 34°01′46″N, 6°44′20″W, *Hardion*, MARS00057	+	+
A. donax	*A. donax*	Portugal, Figueira da foz, 40°12′19″N, 8°53′56″W, *Engels*, MARS00058		+
A. donax	*A. donax*	Spain, Bonares, 37°20′39″N, 6°40′37″E, *Hardion*, MARS00059		+
A. donax	*A. donax*	Spain, Amposta, 40°42′20″N, 0°35′55″E, *Hardion*, MARS00060		+
A. donax	*A. donax*	Tunisia, Sidi bou said, 36°52′00″N, 10°20′36″E, *Hardion*, MARS00061		+
A. formosana	*A. formosana*	Taiwan, *Lyonnet*, MARS00045		+
A. plinii s.l.	*A. donaciformis*	France, Fréjus, 43°25′55″N, 6°45′52″E, *Hardion*, MARS00062	+	+
A. plinii s.l.	*A. donaciformis*	France, Saint Raphaël, 43°27′47″N, 6°47′52″E, *Hardion*, MARS00063	+	+
A. plinii s.l.	*A. donaciformis*	France, Puget/Argens, 43°26′38″N, 6°42′28″E, *Hardion*, MARS00064	+	+
A. plinii s.l.	*A. donaciformis*	France, Les Arcs, 43°27′09″N, 6°31′13″E, *Hardion*, MARS00065	+	+
A. plinii s.l.	*A. donaciformis*	France, Lespignan, 43°16′36″N, 3°09′03″E, *Hardion*, MARS00066	+	+
A. plinii s.l.	*A. donaciformis*	Italy, Cervo, 43°55′38″N, 8°07′37″E, *Hardion*, MARS00067	+	+
A. plinii s.l.	*A. donaciformis*	Italy, Andorra, 43°57′49″N, 8°10′00″E, *Hardion*, MARS00068	+	+
A. plinii s.l.	*A. donaciformis*	Italy, Finale Ligure, 44°10′39″N, 8°22′12″E, *Hardion*, MARS00069		+
A. plinii s.l.	*A. micrantha*	Algeria, Tipasa, 36°35′34′′N, 2°27′24′′E, *Baumel*, MARS00070		+
A. plinii s.l.	*A. micrantha*	Algeria, Tizi Ouzou, 36°52′13″N, 4°02′48″E, *Ait-Saïd*, MARS00071		+
A. plinii s.l.	*A. micrantha*	Algeria, Tlemcen, 35°17′10″N, 1°27′51″W, *Youssef*, MARS00072		+
A. plinii s.l.	*A. micrantha*	Algeria, Sidi Aich, 36°35′00″N, 4°39′00″E, *Vela*, MARS00073		+
A. plinii s.l.	*A. micrantha*	Algeria, Bône, *Tribout*, P02620644		
A. plinii s.l.	*A. micrantha*	Algeria, La Calle, *Lefranc*, P02620659		
A. plinii s.l.	*A. micrantha*	Chypre, Limassol, *Constantinou*, MARS00074		+
A. plinii s.l.	*A. micrantha*	Croatia, Ploce, 43°02′56″N, 17°28′43″E, *Hardion*, MARS00138		+
A. plinii s.l.	*A. micrantha*	Croatia, Split, 43°31′42″N, 16°30′07″E, *Hardion*, MARS00136	+	+
A. plinii s.l.	*A. micrantha*	France, Golfe Juan, 43°33′48″N, 7°04′08″E, *Hardion*, MARS00075	+	+
A. plinii s.l.	*A. micrantha*	France, Ste Lucie, 43°3′25″, 3°1′51″, *Argagnon, Michaud & Molina*, MARS00076		+
A. plinii s.l.	*A. micrantha*	Greece, Kissamos, 35°29′46″N, 23°38′57″E, *Vila*, MARS00077	+	+

Appendix. Continued.

Taxon	Post treatment	Locality, *collector*, voucher	Metric	AFLP
A. plinii s.l.	*A. micrantha*	Greece, Mires, 35°04′05″N, 24°47′06″E, *Vila*, MARS00078	+	+
A. plinii s.l.	*A. micrantha*	Greece, Mirtos, 35°00′21″N, 25°35′16″E, *Vila*, MARS00079	+	+
A. plinii s.l.	*A. micrantha*	Greece, Vassiliko, 38°25′29″N, 23°39′31″E, *Hardion & Vila*, MARS00080	+	+
A. plinii s.l.	*A. micrantha*	Greece, Messolonghi, 38°22′42″N, 21°28′37″E, *Hardion & Vila*, MARS00081	+	+
A. plinii s.l.	*A. micrantha*	Greece, Itea, 38°26′14″N, 22°25′25″E, *Hardion & Vila*, MARS00082	+	+
A. plinii s.l.	*A. micrantha*	Italy, Trieste, 45°45′02″N, 13°39′16″E, *Hardion*, MARS00083	+	+
A. plinii s.l.	*A. micrantha*	Italy, Sanluri, 39°32′08″N, 8°55′07″E, *Maquet, Gauthier & Bouchet*, MARS00084		+
A. plinii s.l.	*A. micrantha*	Lebanon, Nahr el Kalb, 33°56′39″N, 35°36′44″E, *Médail*, MARS00085		+
A. plinii s.l.	*A. micrantha*	Morocco, Tanger, 35°44′44″N, 5°53′07″W, *Hardion*, MARS00086	+	+
A. plinii s.l.	*A. micrantha*	Morocco, Rabat, 34°01′43″N, 6°47′42″W, *Hardion*, MARS00087	+	+
A. plinii s.l.	*A. micrantha*	Israel, Nahal Sorek, *Danin*, MARS0007		+
A. plinii s.l.	*A. micrantha*	Portugal, Figueira da Foz, 40°12′19″N, 8°53′56″W, *Engels*, MARS00089		+
A. plinii s.l.	*A. micrantha*	Portugal, Silves, 37º06′N, 08º22′W, *Pistoor*, B		
A. plinii s.l.	*A. micrantha*	Spain, Amposta, 40°41′21″N, 0°34′31″E, *Hardion*, MARS00090	+	+
A. plinii s.l.	*A. micrantha*	Spain, Deltebre, 40°43′44″N, 0°41′04″E, *Hardion*, MARS00091	+	+
A. plinii s.l.	*A. micrantha*	Spain, Jaen, 38°01′32″N, 3°54′54″W, *Hardion*, MARS00092	+	+
A. plinii s.l.	*A. micrantha*	Spain, Cordoba, 37°56′42″N, 4°29′50″W, *Hardion*, MARS00093	+	+
A. plinii s.l.	*A. micrantha*	Spain, Huelva, 37°17′06″N, 6°56′44″W, *Hardion & Sánchez-Gullón*, MARS00094	+	+
A. plinii s.l.	*A. micrantha*	Spain, Huelva, 37°15′02″N, 6°56′05″W, *Hardion & Sánchez-Gullón*, MARS00095	+	+
A. plinii s.l.	*A. micrantha*	Spain, Bonares, 37°20′39″N, 6°40′37″W, *Hardion*, MARS00096	+	+
A. plinii s.l.	*A. micrantha*	Spain, Zaragoza, JACA		
A. plinii s.l.	*A. micrantha*	Turkey, Kumluca, 36°16′30″N, 30°24′13″E, *Médail*, MARS00097		+
A. plinii s.l.	*A. micrantha*	Turkey, Tasagil, 36°56′38″N, 31°13′16″E, *Médail*, MARS00098		+
A. plinii s.l.	*A. micrantha*	Turkey, Beilan, *Haussknecht*, G		
A. plinii s.l.	*A. micrantha*	Turkey, Constantinople, *Castagne*, G00163762		
A. plinii s.l.	*A. plinii* s.str.	Albany, Sazan Island, 40°30′17″N, 19°16′39″E, *Médail*, MARS00088		
A. plinii s.l.	*A. plinii* s.str.	Croatia, Brsec, 45°11′07″N, 14°14′31″E, *Hardion*, MARS00099	+	+
A. plinii s.l.	*A. plinii* s.str.	Croatia, Trsteno, 42°42′17″N, 17°59′23″E, *Hardion*, MARS00137	+	+
A. plinii s.l.	*A. plinii* s.str.	Croatia, Split, 43°27′53″N, 16°35′05″E, *Hardion*, MARS00139	+	+
A. plinii s.l.	*A. plinii* s.str.	Croatia, Cherso, *Noë*, P02656189		
A. plinii s.l.	*A. plinii* s.str.	Greece, Sirili, 35°29′56″N, 23°48′54″E, *Vila*, MARS00100	+	+
A. plinii s.l.	*A. plinii* s.str.	Greece, Lauzakies, 35°27′54″N, 23°37′53″E, *Vila*, MARS00101		+
A. plinii s.l.	*A. plinii* s.str.	Greece, Paralia Kimis, 38°34′16″N, 24°07′31″E, *Hardion & Vila*, MARS00102	+	+
A. plinii s.l.	*A. plinii* s.str.	Greece, Sicyone, 37°58′18″N, 22°44′32″E, *Hardion & Vila*, MARS00103	+	+
A. plinii s.l.	*A. plinii* s.str.	Greece, Kavassilas, 37°52′34″N, 21°16′02″E, *Hardion & Vila*, MARS00104	+	+
A. plinii s.l.	*A. plinii* s.str.	Greece, Patras, 38°14′26″N, 21°46′02″E, *Hardion & Vila*, MARS00105	+	+
A. plinii s.l.	*A. plinii* s.str.	Greece, Messolonghi, 38°22′42″N, 21°28′37″E, *Hardion & Vila*, MARS00106	+	+
A. plinii s.l.	*A. plinii* s.str.	Greece, Levadia, 38°24′00″N, 22°58′00″E, *Hardion & Vila*, MARS00107	+	+
A. plinii s.l.	*A. plinii* s.str.	Greece, Agios Stephanos, 38°07′30″N, 23°50′06″E, *Hardion & Vila*, MARS00108	+	+
A. plinii s.l.	*A. plinii* s.str.	Greece, Courfou, *Bally*, G		
A. plinii s.l.	*A. plinii* s.str.	Italy, Napoli, 40°50′09″N, 14°03′52″E, *Hardion & Vila*, MARS00109	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Pozzuoli, 40°53′50″N, 14°02′38″E, *Hardion & Vila*, MARS00110	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Candela, 41°08′41″N, 15°29′35″E, *Hardion & Vila*, MARS00111	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Rodi Garganico, 41°55′58″N, 15°55′16″E, *Hardion & Vila*, MARS00112	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Termoli, 41°57′55″N, 14°59′46″E, *Hardion & Vila*, MARS00113	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Lanciano, 42°16′09″N, 14°27′04″E, *Hardion & Vila*, MARS00114	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Ostia, 41°44′53″N, 12°15′26″E, *Hardion & Vila*, MARS00115	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Pisa, 43°42′34″N, 10°22′32″E, *Hardion & Vila*, MARS00116	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Montegabbro, 43°24′23″N, 11°03′01″E, *Hardion & Vila*, MARS00117	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, San Giminiano, 43°24′05″N, 10°59′53″E, *Hardion & Vila*, MARS00118	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Volterra, 43°23′43″N, 10°52′54″E, *Hardion & Vila,* MARS00119	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Bologne, 44°35′07″N, 11°19′20″E, *Hardion & Vila*, MARS00120	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Castel Maggiore, 44°37′49″N, 11°18′30″E, *Hardion & Vila*, MARS00121	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Sasso Marconi, 44°22′20″N, 11°15′10″E, *Hardion & Vila*, MARS00122	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Pianoro, 44°23′35″N, 11°18′59″E, *Hardion & Vila*, MARS00123	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Forli, 44°18′28″N, 12°06′51″E, *Hardion & Vila*, MARS00124	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Ravenne, 44°22′58″N, 12°11′34″E, *Hardion & Vila*, MARS00125	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Santo Stefano, 38°00′35″N, 14°21′38″E, *Hardion*, MARS00126	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Orto Liuzzo, 38°15′50″N, 15°28′21″E, *Hardion*, MARS00127	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Dinami, 38°33′38″N, 16°04′54″E, *Hardion*, MARS00128	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Siracusa, 37°18′31″N, 15°06′26″E, *Hardion*, MARS00129		+
A. plinii s.l.	*A. plinii* s.str.	Italy, Enna, 37°34′44″N, 14°17′12″E, *Hardion*, MARS00130	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Agrigente, 37°36′19″N, 13°48′52″E, *Hardion*, MARS00131		+
A. plinii s.l.	*A. plinii* s.str.	Italy, Scilliato, 37°36′19″N, 13°48′52″E, *Hardion*, MARS00132	+	+
A. plinii s.l.	*A. plinii* s.str.	Italy, Castellamare, 38°01′11″N, 12°53′55″E, *Hardion*, MARS00133	+	+
A. plinii s.l.	*A. plinii* s.str.	Montenegro, Herceg Novi, 42°26′52″N, 18°38′35″E, MARS00140	+	+
A. plinii s.l.	*A. plinii* s.str.	Montenegro, Bar, 42°06′56″N, 19°04′46″E, *Hardion*, MARS00142	+	+
A. plinii s.l.	*A. plinii* s.str.	Malta, Girgenti, 35°51′22″N, 14°24′59″E, *Gambin*, MARS00134		+