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Molecular Evidence for the Compilospecies Model of Reticulate Evolution in *Armeria* (Plumbaginaceae)

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Abstract.—Cladistic analyses of the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences from 55 samples corresponding to 34 taxa in the genus *Armeria* reveal that ITS sequence diversity among and within species utterly conflicts with patterns of morphological similarity. Three facts are apparent from the results here reported: (1) different samples of a single subspecies, *A. villosa* subsp. *longiaristata,* appear in three of the five major clades; (2) samples of at least one of the six subspecies of *A. villosa* appear in four of the five major clades; and (3) the composition of major clades shows greater congruence with the geographic origin of plants than with the traditional systematic arrangement based primarily on morphology. Specifically, the clades here termed Ia, II, III, and IV each encompass terminals restricted to geographically delimited areas. There are alternative explanations for the ITS pattern, but the most likely one is that nucleotide positions supporting the major clades are due, in some of the samples, to concerted evolution following horizontal transfer (gene flow) rather than to recency of common ancestry. This interpretation is consistent with previous systematic and experimental evidence and implies that reticulation in *Armeria* may be extensive. Harlan and de Wet (1963, Evolution 17:497–501) proposed the *compilospecies* concept to account for situations in which a genetically "aggressive" species captures portions of the genome of other sympatric species by means of extensive introgression. Evidence of extensive reticulation, ecological diversification, and geographic pattern indicates that *A. villosa* may fit the compilospecies concept, which is here supported on molecular grounds for the first time. {*Armeria;* compilospecies; concerted evolution; hybridization; Plumbaginaceae; ribosomal DNA; reticulate evolution.}

The occurrence of past and recent events of hybridization is now well documented in different groups of animals and especially in vascular plants because of the large number of molecular markers available (Kaneshiro, 1990; Arnold, 1992, 1997; Grant and Grant, 1992; Dowling and DeMarais, 1993; Rieseberg, 1997). Further evidence, however, is needed to clarify the prevalence of hybridization (Ellstrand et al., 1997) and its evolutionary consequences (Rieseberg and Soltis, 1991; Rieseberg and Wendel, 1993; Rieseberg et al., 1995, 1996a; Wendel et al., 1995a; Arnold, 1997). Natural hybridization and introgression (the gradual infiltration of the genetic material of one species into another as a consequence of hybridization and repeated backcrossing; Anderson, 1949) may have different evolutionary consequences (Arnold, 1992). One effect is negative in terms of diversity, namely, the merging of the hybridizing forms and the genetic assimilation of geographically restricted species (Levin et al., 1996). Other outcomes of the process may include the reinforcing of reproductive barriers through selection for assortative mating (Dobzhansky, 1970) or the production of more or less fit introgressed genotypes that can colonize a novel habitat relative to that of the two parental species (Lewontin and Birch, 1966; Rieseberg, 1991; Cruzan and Arnold, 1993). The major possible evolutionary contribution of hybridization is the formation of new hybrid species. This process has been documented with various kinds of evidence mainly in cases where polyploidy is involved because of the advantages that polyploidy may provide to the hybrid genotypes (Grant, 1981; Arnold, 1992). Much rarer is the documentation of hybrid speciation at the diploid level (Rieseberg et al., 1990; Rieseberg, 1991; Wendel et al., 1991; Arnold, 1993; Wang and Szmidt, 1994; Sang et al., 1995; Wolfe et al., 1998).

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To account for a situation where a "genetically aggressive" species captures portions of the genome of other sympatric species by introgression, the concept of compilospecies (from the Latin *compilo,* to plunder or to rob) was proposed (Harlan and de Wet, 1963). Originally, the concept was applied to polyploid complexes in which a tetraploid cytotype captured genes on a regular basis from geographically restricted peripheral species (Harlan and de Wet, 1963; Heiser, 1965; Stebbins, 1971). In subsequent years, the concept was ignored, most likely because of a lack of conclusive supporting evidence (Jackson, 1976; Lewis, 1980; Grant, 1981; Arnold, 1997). Other authors mentioned the term either without explicitly endorsing it (Levin, 1971, 1978) or to question it (Heiser, 1973; Favarger, 1984). More recently, the compilospecies model was invoked to explain patterns in *Helianthus* at the homoploid level (Rieseberg and Soltis, 1991; Rieseberg et al., 1991; Rieseberg and Brunsfeld, 1992) as well as in *Draba* at the polyploid level (Brochmann, 1992). Previously, the concept was suggested to account for eco-morpho-geographical patterns in *Armeria villosa* (Nieto Feliner, 1987).

Armeria is primarily a holarctic genus, whose center of diversity is the western Mediterranean area. Sixty percent of ~120 taxa in this genus occur in the Iberian Peninsula (Nieto Feliner, 1990). Virtually all species are diploid (Moore, 1982; Castroviejo and Valdés-Bermejo, 1991) and, with a few exceptions not represented in our sampling, are obligate outcrossers, thanks to an efficient mechanism of heteromorphic self-incompatibility (Baker, 1966). The occurrence of extensive hybridization is consistent with our previous studies using various lines of evidence but lacking molecular data (Nieto Feliner, 1987, 1988, 1997; Nieto Feliner et al., 1996).

In recent years, nuclear ribosomal DNA (nrDNA) internal transcribed spacers (ITS) have been used as an efficient tool for phylogenetic studies and have also proven successful in detecting hybridization events (Rieseberg, 1991; Sang et al., 1995; Wendel et al., 1995a; O'Kane et al., 1996). However, an important gap is observed in the literature. Knowledge of within-species variability of ITS sequences from natural populations is limited (Baldwin et al., 1995). The vast majority of the published studies use only one sequence per taxon, and therefore the occurrence of polymorphisms within species and its implications for phylogenetic analysis may be neglected (Ritland and Eckenwalder, 1992). In our article, we have undertaken a broad sampling within *A. villosa* as compared to other species.

We think the ITS region may provide molecular evidence supporting an evolutionary scenario within the genus *Armeria* that fits the compilospecies concept. The proposed compilospecies is *A. villosa* Girard, a southern Spanish endemic (Nieto Feliner et al., 1996) in which six subspecies have been recognized (Nieto Feliner, 1990). Five of these subspecies are geographically very restricted or even localized, whereas subsp. *longiaristata* spans the whole distributional range of the species.

Materials and Methods

Sampling

The sampling was designed to include all the taxa potentially involved in hybridization events with *A. villosa.* To accomplish this, we included in the study 55 samples belonging in 34 species and subspecies of *Armeria,* including those whose distribution area contacts that of *A. villosa* (Appendix 1). Of those 55 samples, 20 correspond to *A. villosa.* The data set also contained all of the Iberian and one of the two North African representatives of section *Macrocentron* Boiss., a small but morphologically well-differentiated group with long-spurred calyces. What we here term *Armeria* "a" refers to populations that were identified as *A. alpina* (Devesa and Pinto da Silva, 1984). Such identification was rejected with a suggestion that the origin of such populations could be a cross between *A. villosa* subsp. *longiaristata* and another taxon (Nieto Feliner, 1987). Taxonomy follows Nieto Feliner (1990). In this treatment of *Armeria,* a genus with low internal reproductive barriers, the species rank is used for groups of populations that maintain a diagnostic combination of characters and some ecological preferences in at least part of their geo-

graphic area. Subspecific rank is used for populations that, morphologically, are clearly linked to another entity even if the link is due to introgression. Plants were collected in the field or grown in the greenhouse. Vouchers for morphometric studies are deposited in the herbarium at the Real Jardín Botánico in Madrid.

Molecular Biology

Extractions were made from leaf tissue (fresh, frozen, silica-gel preserved, or herbarium specimens) as described in Doyle and Doyle (1987). Double-stranded amplification of the ITS region was made with primers ITS7A (White et al., 1990) slightly modified by Panero and Plovanovich-Jones (pers. comm.) (5′-GGAAG GAGAAGTCGTAACAAGG-3′) and ITS 4 (White et al., 1990). After amplification, polymerase chain reaction (PCR) products were purified with a Gene Clean-Up kit (Boehringer Mannheim). Nucleotide sequences of both strands were determined directly from PCR fragments by using the dideoxy chain termination method (Sanger et al., 1977). Sequencing primers were the same as those used for amplification. Standard protocols of the manufacturer for *Taq* DNA polymerase-initiated cycle-sequencing reactions with fluorescently labeled dideoxynucleotide terminators (Applied Biosystems) were followed. The sequencing reactions were analyzed by using a Model 377 automated DNA sequencer (Applied Biosystems) and chromatograms were examined with EditView (Applied Biosystems). All the sequences were recorded in both strands with a 100% overlap. Alignment of the resulting sequences was straightforward and performed manually, taking *Psylliostachys suworowii* as reference taxon for site numbers. Although it makes no difference to the analysis whether an IUPAC ambiguity symbol indicates a poor signal or the presence of two bases in a single site (individual single-nucleotide polymorphisms), in our data matrix we reserved those symbols for the latter situation. We have reported the occurrence of single-nucleotide polymorphisms in either of the following cases: (1) when double peak signals occur in the same position (the higher being ~30% greater than the other) on both strands, and bases were complementary between both strands, e.g., K (G+T) in 5'→3' and M (A+C) in 3'→5'; (2) when unequivocal noncomplementary base pairs appear on the two complementary strands (e.g., A in 5′→3′and C in 3′→5′). After excluding cases that matched any of these two criteria across the data, only four remained as ambiguities that presumably resulted from bad readings; the four were coded as "?". All sequences have been submitted to the EMBL database; accession numbers are listed in Appendix 1.

Phylogenetic Analyses

Parsimony analyses were conducted by running PAUP 3.1.1 (Swofford, 1993). The data set is available in the *Systematic Biology* Website (http://www.utexas.edu/depts/systbiol/). Character-states (A, C, G, T) were treated as unordered, and polymorphisms—as well as gaps resulting from the alignment—were treated as missing data. Options used were MULPARS and TBR, for tree searching, and ACCTRAN, for character optimization. Heuristic searches (100 replicate searches with random taxon addition) were chosen to find the most-parsimonious trees in the different analyses. Additionally, two other types of analysis were made: a constrained analysis ("backbone" option in PAUP), to examine the costs of forcing all *A. villosa* accessions to be monophyletic, and a weighted parsimony analysis using a stepmatrix based in the transition/transversion ratio. Transition/transversion ratios were obtained by using MacClade (Maddison and Maddison, 1992) with the "arbitrary resolution of polytomies" option to overcome underestimates resulting from unresolved polytomies. MacClade was also used for mapping nucleotide substitutions and indels. Bootstrap analysis (100 replicates each with 10 random taxon addition replicate starting trees) was run on PAUP to assess relative branch support. A Wilcoxon signed range (WSR) test was run, using SPSS 7.5 for Windows (SPSS Inc.), to compare the trees resulting from the unconstrained analysis with those in the constrained analysis. Selection of the outgroup followed a phylogeny of Plumba-

ginaceae based on *rbc*L sequence data (Lledó et al., 1998). According to these results, the genus *Psylliostachys* is sister to *Armeria,* and the resulting clade has a strong bootstrap support (80%) within the subfamily Staticoideae. Genetic distance values were calculated with the Kimura two-parameter model and were used to construct a neighbor-joining tree, by using MEGA (Kumar et al., 1993), which was compared with the parsimony trees.

Morphometric Analyses

For comparing the topology of the ITS tree with the morphological evidence, a principal component analysis, based on the correlation matrix, was performed on 55 herbarium specimens corresponding to each of the DNA samples (Appendix 1). Each specimen comes from the same population as the ITS except for accessions 15 and 31 (*A. filicaulis* and *A. pubigera*), which were sampled for morphometry in adjacent populations. Eighteen characters (Appendix 2) were recorded. In addition, a discriminant analysis using the same 18 variables was performed, in which two groups were considered: *A. villosa* accessions versus the rest. Also, to check for the morphological cohesiveness of *A. villosa,* we performed an ANOVA of two variables in the two groups considered in the discriminant analysis. Analyses were conducted by using NTSYS-pc (Rohlf, 1992) and SPSS 7.5 for Windows.

RESULTS

ITS sequences within *Armeria* show great homogeneity. The total length of the aligned ITS + 5.8S sequences with *Psylliostachys suworowii* as reference is 625 bp. The region within *Armeria* ranges from 603 to 605 bp (ITS-1: 202 to 204 bp; 5.8S: 157 bp; ITS-2: 244 bp). Of the seven indels resulting from the alignment, only two (1 bp long) are present among the members of the ingroup. Both are in the ITS-1, and one of them is cladistically informative. Divergence within the ingroup, calculated with pairwise gap-deletion, varies between 0 and 1.49% (ITS-1: 0–1.01%; ITS-2: 0–3.47%). The greatest divergence is found between sample 18 (*A. bigerrensis* subsp. *losae*) and samples 48 (*A. villosa* subsp. *longiaristata*) and 22 (*A. splendens*). Mean G+C content in ITS-1 and ITS-2 for *Armeria* was 48.3% and 48.8%, respectively, increasing to 54.1% in the 5.8S segment. The range of unambiguous transitions as inferred from the 15 most-parsimonious trees was 11–14 and that of transversion was 7–10, the average estimated transition/transversion ratio being 1.38 (maximum 1.62, minimum 1.20). Overall, 47 variable sites were detected within the ingroup, of which 18 are potentially informative in the parsimony analysis.

Previous analyses of the data set, containing 55 samples plus the outgroup ("56 samples matrix"), resulted in 105 most-parsimonious trees of 103 steps. The consistency index (CI), excluding uninformative characters, was 0.84 and the retention index (RI) was 0.96. Topology of the strict consensus tree recognized three of the five major clades discussed below and placed everything else in a basal polytomy. Inspection of those 103 trees showed that part of the lack of resolution and the high number of equally parsimonious reconstructions was due to a single sample (sample 51 from *A. villosa* subsp. *longiaristata*), which presents polymorphic sites for five of the informative positions (367, 388, 389, 517, and 599), all of them supporting major clades (see below). This sample jumps between different branches in the most-parsimonious trees and shows behavior typical of a recent hybrid (McDade, 1990). Because this sample can be detected as a recent hybrid both by the five relevant polymorphic positions and by its effects on the most-parsimonious trees, we removed it from the data set (Humphries and Funk, 1984; Funk, 1985) to see what would be the effects on the analysis. A posteriori, it can be added to the trees as a reticulation.

When the reduced data set, containing 54 samples plus the outgroup ("55 samples matrix") was analyzed, the number of most-parsimonious trees dropped to 15, the length and levels of homoplasy remaining the same (103 steps, CI = 0.84, RI = 0.96). The number of synapomorphies in the most-parsimonious trees, excluding those of the whole ingroup, varied between 14 and 15, as reported in PAUP. The number of homoplastic characters varied between 3 and 4.

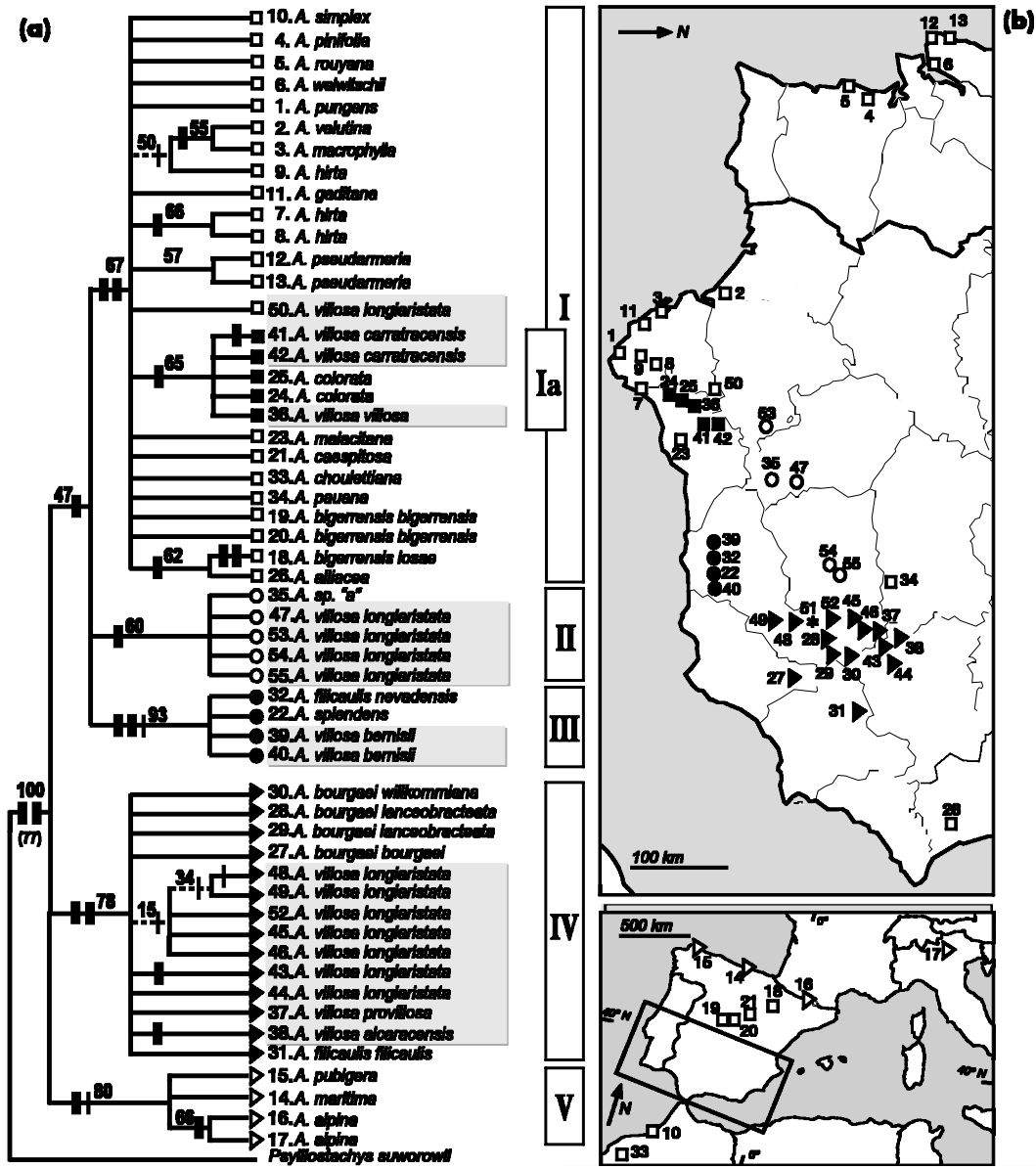

FIGURE 1. (a) One of the 30 most-parsimonious trees based on nrDNA internal transcribed spacers (ITS1 + 5.8S + ITS2) sequences of 54 samples of *Armeria* with *Psylliostachys suworowii* as outgroup (length = 103, CI (excluding uninformative characters) = 0.84, RI = 0.96). Numbers above branches indicate bootstrap values. Dashed lines indicate branches collapsing in the strict consensus tree. Roman numerals identify the five major clades, each of which is associated with a different symbol. Samples belonging in the compilospecies *A. villosa* are shaded. Solid rectangles represent nonhomoplastic changes, thin bars indicate homoplastic changes (see Table 1). The number in parenthesis in the basal node indicates the number of changes separating the outgroup from the ingroup. (b) Geographic origin of the samples (both those sequenced and those studied morphometrically). To facilitate comparison with the ITS results, each sample is identified by its accession number (Appendix 1) and a symbol corresponding to the major clade in the tree.

TABLE 1. Nucleotides of the ITS1 + 5.8S + ITS2 region supporting the major clades (in bold) resulting from a parsimony analysis of 54 samples in *Armeria.*

	Nucleotide number												
Clades	93	129	169	367	378[a]	387	388	389	471[a]	499	517	550	599
Outgr.	-	A	T	A	C	C	C	C	G	G	G	G	G
I	-	A	T	A	C (T)	C	**T**	**T**	G	G	**A**	G	G
Ia	-	A	T	A	C	C	**T**	**T**	G	G	**A**	**T**	G
II	-	A	**A**	A	C	C	C	C	G	G	**A**	G	G
III	**A**	**T**	T	A	C	C	C	C	**A**	**T**	**A**	G	G
IV	-	A	T	**T**	C	C	C	C	G (A)	G	G	G	**A**
V	-	A	T	A	**T**	**T**	C	C	G	G	G	G	G

[a]Homoplastic character in the most-parsimonious trees. Character states in parentheses mark the clade where the homoplastic change also occurs.

The topology in all the trees is consistent in recognizing five major clades (bootstraps between 60% and 93%), the 15 most-parsimonious trees differing only in terminal or subterminal clades. Additionally, a subclade of clade I (clade Ia), which is relevant to our discussion, is highlighted (Fig. 1). Character-state changes supporting the major clades in all the most-parsimonious trees are: site 388 (C→T) and 389 (C→T) for clade I; site 550 (G→T) for clade Ia; site 517 (G→A) for clade I + II + III; site 169 (T→A) for clade II; sites 129 (C→T), 471 (G→A), and 499 (G→T) for clade III; sites 367 (A→T) and 599 (G→A) for clade IV; and sites 378 (C→T) and 387 (C→T) for clade V (Table 1). All of these 12 characters except 2 are nonhomoplastic. For site 378, *A. velutina* and *A. macrophylla* (from clade I) share a T with clade V. Also, *A. hirta* (also from clade I) shows an additive polymorphism (C/T) for this site. The homoplasy in the second character (471) is due to two accessions of *A. villosa* subsp. *longiaristata* (samples 48 and 49), which share character-state A with members of clade III. These two accessions are the closest geographically to the terminals in clade III. Besides these substitutions supporting major clades, an informative insertion at site 93 (A for samples 22, 32, 39, and 40) provides additional support for clade III.

Of the five major clades, clade I is the largest in number of samples and number of species contained. Geographically, it includes plants from the southwestern, eastern, and central part of the Iberian Peninsula, plus the two accessions from Morocco. Morphologically, it also contains a wide sample of the diversity in the genus, including one sample of *A. villosa* subsp. *longiaristata.* Nested within clade I is clade Ia, which contains accessions from two taxa that have been the subject of previous studies (Nieto Feliner et al., 1996) and are restricted to a reduced area in the western end of the Andalusian ranges. Clade II involves only accessions from the central part of the Andalusian ranges. All of them belong to *A. villosa* subsp. *longiaristata* except for what we term *Armeria "a"* (sample 35). Clade III, which is restricted to the Sierra Nevada range in the eastern end of the Andalusian massifs, includes accessions from three different species. It has the highest bootstrap value, receiving support from three base substitutions plus the indel mentioned above. Clade IV includes samples from a geographically delimited area comprising the massifs collectively known as the Subbaetic ranges, northeast of Andalusia. It contains accessions from three different subspecies of *A. villosa* plus all samples of *A. bourgaei* (three subspecies) and one of *A. filicaulis.* Clade V contains four accessions from three species, one of which, *A. maritima,* is the most widespread in the genus, occurring in Eurasia and the New World (Moore and Yates, 1974; Lefèbvre and Vekemans, 1995).

Parsimony analysis under a transition: transversion stepmatrix (1:1.38) reduced the number of most-parsimonious trees (now nine) but the topology of the strict consensus remained identical.

The topology of the neighbor-joining tree recognized the five major clades (and clade

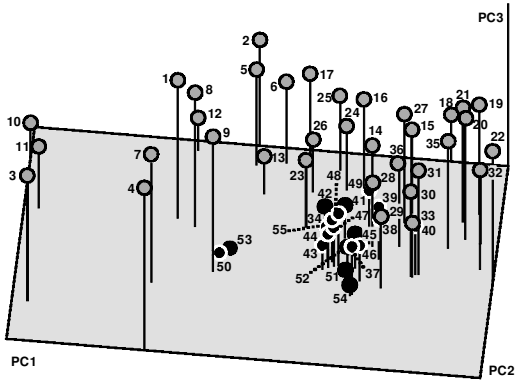

FIGURE 2. Morphometric multivariate analysis of *Armeria.* Plot of 55 specimens in the morphospace defined by the first three principal component (PC) axes. Numbers for each item correspond to those of specimens sampled for DNA (Appendix 1, Fig. 1). Black circles represent specimens belonging in any of the various subspecies of *A. villosa.*

Ia) but differed slightly from the parsimony trees in the basal arrangement. The basal trichotomy for the ingroup in the parsimony analysis (Fig. 1) was resolved by placing clade V as sister to the rest and letting the four remaining major clades form a polytomy.

Results of the morphometric analysis largely reflected the traditional systematic arrangement of the species sampled (Fig. 2). In the plot of the 55 specimens against the first three principal components axes (explaining 41.2%, 13.8%, and 11.1% of the variance, respectively), most dots corresponding to any of the subspecies of *A. villosa* (in black) tended to cluster.

DISCUSSION

Phylogenetic Approach and Reticulate Evolution in Armeria

Two facts become apparent from the cladistic analysis based on ITS sequences. First, different subspecies of *Armeria villosa,* and even different accessions of the same subspecies, do not cluster in the same lineage (Fig. 1a). Specifically, samples of *A. villosa* subsp. *longiaristata* appear in three of the five major clades, and samples of at least one of the subspecies of *A. villosa* appear in four of the five major clades. Second, composition of the five major clades shows more congruence with the geographic origin of the studied plants than with the systematic arrangement based on morphology; i.e., clades Ia, II, III, and IV each encompasses terminals restricted to geographically delimited areas (Fig. 1b). In other words, there is a pattern of morphological similarity that overlies, and conflicts with, ITS sequence diversity and an underlying geographical structure that fits the ITS diversity. Several alternative, although not equally likely, explanations for this pattern are possible.

First, we might argue that the cladistic analysis based on ITS reflects true relationships and that the subspecies subordinated under *A. villosa* are unrelated, their similarity resulting from convergence. To consider this explanation, we need first to examine whether *A. villosa* s.l. is a morphologically cohesive group. Before the systematic revision for the Iberian Peninsula (Nieto Feliner, 1990), in which an infraspecific taxonomy for *A. villosa* was proposed, most authors did not recognize any infraspecific subdivision and so all the populations were considered as a single entity (Lawrence, 1940; Pinto da Silva, 1972; Devesa, 1987), implying that the group has morphologic cohesiveness (but see Bernis, 1955, who did recognize infraspecific taxa). This morphological cohesiveness can also be examined with the multivariate analysis (sampling adapted to that of ITS sequences). In the plot of the specimens against the first three principal component axes, most dots belonging to *A. villosa* cluster together (Fig. 2). That some specimens, belonging in different subspecies of *A. villosa* (subspp. *villosa, alcaracensis,* and *bernisii*), lie relatively apart reflects the fact that, despite being morphologically close to the rest of the group, they exhibit diagnostic characters. The different accessions from the same subspecies are close to each other (e.g., samples 41 and 42 of subsp. *carratracensis,* 39 and 40 of subsp. *bernisii*), except for two (samples 50 and 53) of subsp. *longiaristata,* which are apart with respect to the first principal component axis. However, principal component analysis is not designed to produce discrete groups (Marcus, 1990) when a relatively heterogeneous sample, such as a whole genus, is analyzed, and it should be complemented with other analyses. Discrimi-

nant analysis, using the same morphometric characters and considering two groups (*villosa* vs. the rest of the taxa), correctly classifies every specimen into its original group (results not shown). At the univariate level, the same two groups differ significantly when ANOVAs of two variables (length of calyx lobe including awn, and lobe length to calyx length ratio) are performed, thereby confirming that *A. villosa* is morphologically cohesive.

The next questions is, Could this cohesiveness be due to convergence? The greater the similarity, the more compelling the convergent explanation would be. Therefore, we will concentrate on the widespread subsp. *longiaristata,* from which morphologically indistinguishable populations appear in different clades. Diagnostic features for this subspecies include the following combination: short lanceolate ciliate leaves, straw-colored awned involucral bracts, almost fully scarious spikelet bracts, long awns in calyx lobes (lobe length to calyx length ratio >0.3), and white corollas. This set of features is related mostly to reproductive organs but also to vegetative. Is it possible that they have been acquired independently in three different areas through convergence? It might be, but it is not very likely that such a set of seemingly independent characters have converged so precisely as to result in morphologically indistinguishable plants.

A possible second explanation is to assume, as in the first alternative, that the ITS tree reflects true relationships but in addition that the subspecies subordinated under *A. villosa* are disconnected genetic lineages that have retained a plesiomorphic phenotype. To address properly this interpretation, we would need a cladistic analysis based on another independent data set on which to map or trace back those possible plesiomorphies. Unfortunately, the morphological data in the genus are not suitable for a cladistic analysis. A great portion of the characters that distinguish species are continuous and their ranges overlap largely; thus, even if some type of gap-coding were applied, the matrix would be very inappropriate. Besides, high levels of intraspecific variability require that a large number of characters are coded as polymorphic or missing. In fact, an unpublished analysis we tried in a group (sect. *Macrocentron*) that possesses more qualitative characters than average reveals much incongruence. This complex morphological pattern might have to do with the fifth possible explanation for the ITS tree (see below).

Even in the absence of a cladistic analysis based on a different data set, some considerations on the possibility that characters distinguishing *A. villosa* are plesiomorphic can be made. The long awned calyx lobes, departing from the parachute-like fruit-enclosing calyx, are exceptional within *Armeria* and absent in other genera. Likewise, white corollas are much rarer or even absent in related genera such as *Psylliostachys, Acantholimon, Limoniastrum,* or *Limonium* (Rechinger and Schiman-Czeika, 1974; Davis, 1982). Furthermore, if the characters diagnosing *A. villosa* are plesiomorphic and populations from different areas share more-recent common ancestors with other species, we should be able to find other morphological characters shared by the different *A. villosa* populations and the other species. For instance, some morphological feature should be shared by all the terminals of clade I. The discovery of any such features, so far unknown, could give some support to this possibility.

A third alternative is that the subspecies of *A. villosa* are currently a genetically connected group but have maintained large amounts of genetic polymorphism relative to other species, perhaps because of having larger populations. Lineage sorting could theoretically account for patterns like the ITS in *Armeria* (Pamilo and Nei, 1988; Doyle, 1992; Maddison, 1997). In fact, it can explain many different situations.

For lineage sorting to explain discrepancies between molecular evidence and, in this case, morphological evidence, we need two conditions. First, ancient polymorphisms must have co-occurred within *A. villosa.* It seems unlikely with the current levels of ITS diversity that one species spanned almost the whole range of variation. However, let us assume that at the base of our ITS tree, several ITS copies existed. Still, for lineage sorting to account for our observations, we also need a second

condition, namely, random sorting of such ancestral polymorphisms. In our case, this implies four events of selective loss (or failure to sample), each one involving three ITS copies: (1) loss of three ITS copies (those presently occurring in clades II, III, and IV) in terminals appearing in clade I; (2) loss of the three ITS copies occurring in clades I, III, and IV in terminals appearing in clade II; (3) loss of the three ITS copies occurring in clades I, II, and IV in terminals appearing in clade III; and (4) loss of the three ITS copies occurring in clades I, II, and III in terminals appearing in clade IV. This is certainly possible but is it likely? If added to this are the requirements that those clades sharing an ITS contain different species and that three of them are geographically very restricted, lineage sorting remains as a possible explanation but we think not the most likely one.

Furthermore, the probability that gene copies fail to coalesce depends on population size (Pamilo and Nei, 1988); the larger the population size, the greater the chances that intraspecific polymorphisms exist. In a genus like *Armeria* with low internal reproductive barriers (see below), such a favorable condition for lineage sorting to occur would also facilitate the occurrence of other mechanisms resulting in the observed pattern. In the Iberian peninsula, where the most diversity is concentrated and sympatric situations are frequent, the larger population size would also imply greater chances for gene flow to occur. Therefore, if lineage sorting can theoretically account for our ITS trees, the chance that it is the only cause is low.

Still a fourth possible explanation is that the ITS tree is estimated incorrectly, i.e., that the *A. villosa* accessions are monophyletic for this data set. However, the cost in terms of parsimony of forcing all *villosa* accessions to be monophyletic in a constrained analysis make this possibility untenable. Ten more steps for the most-parsimonious trees (only 18 characters are potentially informative for the ingroup), CI excluding uninformative characters decreasing to 0.6 (as compared to 0.84), the number of trees itself (519 vs. 15), and a polytomy involving everything else except *A. villosa* accessions are the comparative features of this constrained analysis. Furthermore, a WSR test comparing the number of changes required in the strict consensus of the most-parsimonious trees from the unconstrained analysis with those required in the strict consensus of the constrained analysis (Mason-Gamer and Kellog, 1996) gives highly significant differences between the trees ($P < 0.005$). This implies that monophyly of the different accessions of *A. villosa* for ITS is unrealistic.

We have also tried other outgroups that result in very little change in topology. When two species of *Limonium* were used (results not shown), the trichotomy in the base was resolved by placing clade VI as sister to the rest of *Armeria* as in the neighbor-joining tree. However, with the support that major clades receive in all the different analyses we have performed, a different rooting would not make a difference to the central theme of this article. It is true that the number of cladistically informative characters in our data set is relatively low, but this feature is coupled with the highly consistent nature of the data.

The fifth and last alternative explanation we can envisage is that all the subspecies subordinated under *A. villosa* are a genetically connected group (warranting the label "species") but that they have differentiated via introgression. This would imply that nucleotide positions supporting the major clades are due, at least in some of the samples, to horizontal transfer (gene flow) rather than to recency of common ancestry.

This explanation could account for the placement of the different geographically restricted subspecies of *A. villosa* in different clades by suggesting their origin from hybridization between the widespread subsp. *longiaristata* and some other sympatric taxa. Likewise, the placement of different specimens of subsp. *longiaristata* in three clades could be explained by the same process with other taxa, without resulting in the formation of new subspecies. For instance, the occurrence of one accession (sample 50) of *A. villosa* subsp. *longiaristata* in clade I could result from introgression from *A. hirta* because they are close both geographically (Fig. 1) and in the morphometric space (Fig. 2). Within clade Ia, the hy-

pothesis of the origin of *A. villosa* subsp. *carratracensis* from a cross between *A. colorata* and *A. villosa* subsp. *longiaristata* (Nieto Feliner et al., 1996) is compatible with the ITS sequence data, but further independent evidence is needed and the subject will be discussed elsewhere. We have no morphological clues about the possible origin of the other subspecies of the *A. villosa* group: *A. villosa* subsp. *villosa,* a dwarf plant restricted to a single mountain peak. The fact that the only sample from this rare plant appears together with subsp. *carratracensis* and *A. colorata* within clade Ia (Fig. 1) might suggest that subsp. *villosa* instead of subsp. *longiaristata* is, in fact, the progenitor of subsp. *carratracensis.* But this possibility does not fit the previous morphological evidence (Nieto Feliner et al., 1996) nor the preliminary RAPD data (unpubl.). Besides, populations from subsp. *longiaristata* are closer geographically to subsp. *carratracensis* than is subsp. *villosa.* The occurrence of three species sharing the same ITS sequence within clade IV also can be explained in terms of hybridization. On morphological and ecological grounds, the subspecies of *villosa* in this clade (subsp. *bernisii*) was hypothesized to have arisen from a cross between *A. villosa* subsp. *longiaristata* and *A. filicaulis* (Nieto Feliner, 1990), a possibility consistent with the ITS data presented here. Another putative hybrid taxon appears to be constituted by the populations of *A. filicaulis* from Sierra Nevada. These populations differ not only in ITS from the single accession from another part of its distribution area (sample 31) but also in morphological features (Nieto Feliner, 1990; Nieto Feliner et al., 1998). Both the molecular and morphological character distributions are compatible with the hypothesis that the Sierra Nevada populations of *A. filicaulis* originated from hybridization between *A. splendens* and other forms of *A. filicaulis.* Clade IV contains the greatest number of accessions of the *A. villosa* group. The taxon or taxa that, according to this fifth possible interpretation, could ultimately be responsible for the ITS sequence in this clade remains uncertain.

This interpretation of ITS cladograms, in terms of hybridization/introgression, has previous support from independent evidence. This can be summarized in two points. First, internal reproductive barriers are very weak and thus allow for interspecific gene flow. The formation and persistence of complex hybrids whenever populations come in contact are also facilitated by the effective restoration of pollen viability through backcrossing, although this is not always necessary: Some artificial F_1 hybrids display an average pollen stainability >90%. Experimental crosses resulting in these features included 9 of the 34 taxa sampled in the present study from clades I, Ia, IV and V, including *A. villosa* subsp. *longiaristata* and subsp. *carratracensis* (Nieto Feliner et al., 1996; Nieto Feliner, 1997). Second, in the same reports, it was also noted that some quantitative characters under multigenic control (e.g., length of calyx lobe, but several others as well) are reliable expressions of intermediate genotypes. To the extent that this finding minimizes the effect of phenotypic plasticity in morphological variability, it gives some support to reports of hybridization within *Armeria* in the literature (Bernis, 1954; Lefèbvre, 1969; Arrigoni, 1970; Philipp, 1974) as well as to reports of intrapopulation heterogeneity for morphological characters (Lefèbvre, 1969; Arrigoni, 1970; Philipp, 1974; Nieto Feliner, 1993) also explained in terms of hybridization.

Nevertheless, a question may arise concerning the point of the morphological cohesiveness of *A. villosa* (Fig. 2). If the different ITS types within *A. villosa* subsp. *longiaristata* are due to hybridization with other taxa, how can the species be so morphologically cohesive? A simple answer would be to suppose that backcrossing has always occurred towards subsp. *longiaristata,* but, if so, why would the ITS types, supposedly acquired through hybridization with other taxa, have been retained? Our suggestion would be that biased concerted evolution could account for this apparent discrepancy.

In this respect, the occurrence of polymorphic sites may be a problem. Sample 51 of *A. villosa* subsp. *longiaristata,* showing polymorphisms for five of the informative sites, represents a clear additive pattern with respect to extant taxa. In fact, because those sites are precisely the ones in which clade IV (where the sample geographically belongs) differs from clade I, it is reason-

able to suppose that it is a hybrid between *A. villosa* subsp. *longiaristata* from clade IV and some other species from clade I. Therefore, the sample could be added as a reticulation to the trees. Other polymorphisms following a clear additive pattern, as interpreted in the sequence chromatograms, are sites 378 (C/T) and 442 (C/G) for sample 9 of *A. hirta,* site 517 (A/G) for samples 49 and 52 of *A. villosa longiaristata,* and site 578 (A/C) for sample 45 of *A. villosa longiaristata.* The two polymorphic sites for sample 9 of *A. hirta* suggest introgression either from *A. macrophylla* or *A. velutina,* both of them sympatric *to A. hirta* and exhibiting the "rare" nucleotide (378T, 442C) involved in those two polymorphisms. Sample 49 of *A. villosa longiaristata* may have introgression from clade III, because this is not only consistent with polymorphism in site 517 but also with that in site 471 (Table 1). Sample 52 exhibits several other polymorphic sites, although only the one in site 517 shows an additive pattern with respect to our data set. This sample is likely to be a hybrid or introgressed individual. The polymorphic site 578 (A/C) for sample 45 of *A. villosa longiaristata* is unexpected and probably irrelevant, because the only C in our data set occurs in *Psylliostachys.*

However, this number of additive polymorphisms is few compared with those in other cases where hybridization is involved (Sang et al., 1995; Campbell et al., 1997). Therefore, for the hybridization/introgression scenario to be plausible, homogenization of hybrid sequences should proceed actively. Putative hybrids in *Gossypium* (Wendel et al., 1995b), *Saxifraga* (Brochmann et al., 1996), and the silversword alliance (Baldwin and Robichaux, 1995) are reported to undergo rapid concerted evolution, whereas retardation of this mechanism and thus a preservation of polymorphisms along the tandem copies of ITS are reported in groups where apomixis and particularly polyploidy are involved (Kim and Jansen, 1994; Sang et al., 1995; Campbell et al., 1997). In *Armeria,* where almost all species are diploid, we could expect that concerted evolution after hybridization proceeds rapidly. In this regard, we have unpublished data that support such expectations. We have examined sequences from artificial hybrids between *A. villosa* subsp. *longiaristata* (from clade IV) and *A. colorata* and from two generations of backcrosses. If we score only those sites that differ between the two parental sequences and are supporting any of the major clades in the cladistic analysis based on ITS, there turns out to be homogenization in the six nucleotide sites satisfying both conditions. This effect was apparent in F_2 hybrids and in the backcrosses.

Low homoplasy levels obtained in our parsimony analyses (Fig. 1a) might also be related to a high degree of concerted evolution (Sanderson and Doyle, 1992). Alternatively, low rates of molecular evolution, recent divergences, or both can also result in low homoplasy. But none of those two features by themselves explain the geographic structure of the data. If combined with lineage sorting, we would still have to assume the selective losses of ITS types outlined above. It thus appears that low rates may be one of the intervening forces, but not the only one. On the other hand, if low rates of molecular evolution or recent divergences (or both) were combined with introgression, we would expect that additive polymorphic patterns would tend to persist unless sequence homogenization is active. Therefore, concerted evolution is a significant piece in the fifth explanation for the ITS pattern.

From the above argumentation, we think the explanation for the ITS pattern involving extensive gene flow is more likely than the others. Certainly, it is also more challenging and, because the evidence is not absolutely conclusive, needs to be confirmed by future studies; in particular, sequences from a chloroplast gene (currently in progress) would be helpful. We point out that, even if extensive hybridization/introgression were not the main cause behind the ITS data, the geographical structure of the ITS sequence diversity and the overlying discordant morphological pattern is interesting in itself, regardless of the explanation.

Armeria villosa *as a Compilospecies*

Assuming the fifth explanation is the most likely of those entertained has several

implications. One of the possible outcomes of extensive gene flow is a compilospecies. Despite the fact that the term was originally proposed for tetraploids and that Stebbins even used the concept in a diagram of what he considered a "typical polyploid complex" (Stebbins, 1971), the concept was explicitly considered applicable for crosses at the diploid level (Harlan and de Wet, 1963).

On the basis of the ITS analysis, and morphological, ecological, and distributional evidence, we suggest that *A. villosa* can be considered a compilospecies. Our proposition rests on the following arguments. First, *A. villosa* subsp. *longiaristata* is the most widespread of the six subspecies, its area spanning the whole distributional range of the species, which covers a large proportion of the hilly territories in Andalusia (Nieto Feliner et al., 1996). Second, the remaining five subspecies are geographically marginal and restricted (even local except for subsp. *bernisii*). Third, these five subspecies are morphologically close to subsp. *longiaristata* but differ from it in sets of characters that, in three of them, are found in sympatric taxa not belonging in the *A. villosa* group. Those three sympatric taxa are *A. colorata* for subsp. *carratracensis*, *A. filicaulis* subsp. *nevadensis* for subsp. *bernisii,* and *A. alboi* (not sampled in this study) for subsp. *alcaracensis* (Nieto Feliner, 1987; Nieto Feliner et al., 1996). Fourth, ecological requirements of the three putative germplasm donors as compared with these three subspecies are consistent with the hypothesis of hybridization and introgression (see below). Fifth, ITS profiles are heavily dependent on the geographic area, thus suggesting the possibility that the similarities among sequences are partly due to gene flow. Sixth, in two cases, hypothetical gene flow from the donors into the subspecies of *A. villosa* (subsp. *carratracensis* and subsp. *bernisii*) is consistent with the ITS sequences of both the donors (*A. colorata* and *A. filicaulis* subsp. *nevadensis,* respectively) and the two subspecies. For the third one proposed (*A. villosa* subsp. *alcaracensis*), we lack data for comparison.

The possible occurrence of such a model is striking and its implications on the species concept noteworthy (see below). Nonetheless, even if the compilospecies model does fit to some extent the evolutionary scenario in *Armeria,* we do not claim it to be a frequent model in angiosperms.

Although this model might follow evenly from the assumption of the fifth explanation, it should also make sense ecologically. In this respect, it can be argued that a compilospecies in a genus of obligate outcrossers, with extremely weak internal reproductive barriers, might represent an alternative avenue for differentiation. It has been suggested, particularly for island scenarios, that failure to develop reproductive barriers facilitates the influx of genes through hybridization (Gillet, 1972; Francisco-Ortega et al., 1996). Species can colonize novel habitats through introgression with other sympatric congeners that have different ecological requirements. In this way, depending on the degree of assimilation of foreign genes, new genotypes can arise that differ from the core species both in ecological requirements and in morphological features.

In *Armeria,* we can infer a possible adaptive advantage in the putative hybrid origin of *A. villosa* subsp. *carratracensis* from a cross between subsp. *longiaristata* and *A. colorata.* Because *A. colorata* occurs exclusively on serpentine soils and subsp. *longiaristata* on limestone soils, the cross between these two taxa may have allowed the widespread subspecies (i.e., the compilospecies) to acquire tolerance to serpentine and thus occupy a new habitat through the formation of the new subspecies, subsp. *carratracensis.* Likewise, ecological differentiation of subsp. *bernisii,* as compared with the widespread subsp. *longiaristata,* has apparently occurred in the direction of colonizing higher altitudes and siliceous substrates. This differentiation may have proceeded through introgression from the high-altitude silicicolous endemic *A. splendens.*

Our data do not imply that all the introgression events detected result in adaptive advantages. Some of those events may be chance consequences of the evolutionary history of the two taxa involved in a given instance of gene flow. However, the whole scenario is better explained in adaptive terms. In particular, the exclusive occurrence of the subspecies *carratracensis* and

bernisii in ecologically differentiated areas with respect to the widespread subsp. *longiaristata,* coupled with the ITS data, is an argument for the adaptive advantage of their presumed origin and thus for the compilospecies model.

The original proposition of the concept attributed a "genetically aggressive" behavior to the compilospecies (Harlan and de Wet, 1963). In groups like *Armeria,* with weak internal reproductive barriers, we think such aggressiveness could be rather a consequence of a larger distribution that provides more sympatric situations. In fact, we think that other species with relatively large distribution areas, such as *A. transmontana* and *A. filicaulis,* may resemble the model as well.

Our reappraisal of the compilospecies model is congruent with the conceptually relevant findings of Rieseberg et al. (1996a) in *Helianthus,* in which interactions between the genes of hybridizing parents constrain the genomic composition of the hybrids (both natural and artificial). That the genome of the hybrid lineages is, to a greater or lesser extent, being selected clearly increases the chances that the introgressants produced within the frame of a compilospecies persist in that ecogeographic milieu.

One of the main implications of the compilospecies model, as proposed for *Armeria,* is the species concept. In a genus where extensive gene flow may take place, the biological species concept is inadequate. The fact that a group of morphologically and ecologically differentiated entities are capable of interbreeding occurs in various groups within vascular plants (*Quercus, Pinus, Iris, Geum, Aquilegia;* Grant, 1981). In this regard, *Armeria* may be a syngameon in biosystematic terms, but we do not think adopting a reproductive criterion for species recognition would be appropriate or practical in this genus. Our use of taxonomic ranks is admittedly arbitrary (Nieto Feliner, 1987), but reducing the genus to a single taxonomic species is no better. A considerable amount of morphological variation is geographically structured and sorted in such a way that roughly half of the ~120 species are recognized by common botanists, although some of them are recognized only in parts of their geographical area. In any case, we agree with Arnold's (1997:21) statement that "it is of extreme importance that we do not let definitions . . . limit investigations into the evolutionary importance of the process of natural hybridization."

Recent studies (Sang et al., 1995; Buckler and Holtsford, 1996) have warned about the use of ITS in phylogenetic reconstruction when reticulate evolution and concerted evolution are present. In this paper we present what we consider insightful results based on an accurate sampling of natural populations combined with detailed morphological knowledge of the group involved. Detection of reticulate evolution in phylogenetically oriented studies is seriously hampered by the fact that there are no fully reliable predictable patterns (Rieseberg and Ellstrand, 1993; McDade, 1995). As a consequence, reticulation is inferred from discrepancies between analyses that were based on different data sets, in particular different genes (Rieseberg et al., 1996b). Reticulation is thus invoked only when other sources of such discrepancies are dismissed. One of the reasons we think the results presented may be relevant is that a single gene, with an underlying geographic structure and previous experimental and morphological evidence, provides credible, although perhaps not conclusive, arguments for reticulation. This is consistent with the claim of Baldwin et al. (1995) that ITS can provide direct evidence of reticulate evolution. Therefore, the compilospecies model, here supported on molecular data for the first time, illustrates the importance of a "phylogenetic approach" in studies of natural hybridization (Nieto Feliner and Fuertes Aguilar, 1998).

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APPENDIX 1. ORIGIN OF THE *ARMERIA* SPECIMENS SAMPLED FOR MOLECULAR AND MORPHOMETRIC DATA

EMBL/GenBank/ DDBJ accession no.	Sample no.	Taxon	Locality	Voucher no.
AJ225563	1	*A. pungens* (Link) Hoffmanns. & Link	Spain, Cádiz: Trafalgar cape, dunes	GN 3867
AJ225564	2	*A. velutina* Boiss. & Reut.	Spain, Huelva: Matalasca as, Do ana National Park, sandy shrubland	GN 3883
AJ225565	3	*A. macrophylla* Boiss. & Reut.	Spain, Cádiz: Chiclana, Laguna de la Paja, pinewoods on sand	GN 3877
AJ225566	4	*A. pinifolia* (Brot.) Hoffmanns. & Link	Portugal, Estremadura: Setubal, Brejos de Azeitao	Ind. Sem. Lisbon 1997, 552
AJ225567	5	*A. rouyana* Daveau	Portugal, Baixo Alentejo: Santiago do Cacem, lagoa de Santo Andre	Ind. Sem. Lisbon 1997, 555
AJ225569	6	*A. welwitschii* Boiss.	Portugal, Estremadura: Cascais, Guincho, Botanical Garden of Lisbon	Ind. Sem. Lisbon 1995, 515
AJ225568	7	*A. hirta* Willd.	Spain, Málaga: Manilva, punta de la Chullera, beach	GN 3856
AJ225571	8	*A. hirta* Willd.	Spain, Cádiz: between Arcos and Medina Sidonia, *Quercus suber* forest clearings	GN 3865
AJ225572	9	*A. hirta* Willd.	Spain, Cádiz: Conil de la Frontera, Pinar de Roche, pinewoods, on wet sandy soils	GN 3874
AJ225570	10	*A. simplex* Pomel	Morocco: Kenitra, between Kenitra and Mendiya beach, sands	Vogt 6085
AJ225589	11	*A. gaditana* Boiss.	Spain, Cádiz: Chiclana, Laguna de la Paja, pinewoods, on wet sandy soils	GN 3879
AJ225573	12	*A. pseudarmeria* (Murray) Mansfeld	Portugal, Estremadura: Sintra, Cabo da Roca, granite	Ind. Sem. Lisbon 1995, 511
AJ225596	13	*A. pseudarmeria* (Murray) Mansfeld	Portugal, Estremadura: Sintra, Cabo da Roca, granite	Jury s/n
AJ225574	14	*A. maritima* Willd.	Spain, Santander: Liencres, limestone rocks by the sea	GN 2866
AJ225590	15	*A. pubigera* (Desf.) Boiss.	Spain, Lugo: Ribadeo, Meirengos, 29PJ5124, 5 m, sea cliffs, on schists	Aedo 3410
AJ225576	16	*A. alpina* Willd.	Spain, Huesca: Bielsa, Macizo de la Maladeta, Aigualluts, wet pastures, granites	38Vargas97
AJ225575	17	*A. alpina* Willd.	Italy: Trento, Campitello di Val di Fassa, Coll Rodella, 2450 m, 46°30′N, 11°35′E stony pastures, limestone	Alvarez 1348
AJ225599	18	*A. bigerrensis* (C. Vicioso & Beltrán) Rivas Mart. subsp. *losae* (Bernis) Rivas Mart.	Spain, Soria: Covaleda, Picos de Urbión, rock crevices, conglomerates	GN 3843
AJ225577	19	*A. bigerrensis* (C. Vicioso & Beltrán) Rivas Mart. subsp. *bigerrensis*	Spain, Avila: Macizo Central de Gredos, prado de las Pozas, 30TUK1061, 1900 m, granite rock crevices	GN 3790

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APPENDIX 1. CONTINUED.

EMBL/GenBank/ DDBJ accession no.	Sample no.	Taxon	Locality	Voucher no.
AJ225613	20	*A. bigerrensis* (C. Vicioso & Beltrán) Rivas Mart. subsp. *bigerrensis*	Spain, Avila: Macizo Central de Gredos, prado de las Pozas,	GN 3791
AJ225594	21	*A. caespitosa* (Ortega) Boiss.	Spain, Segovia: Cerezo de Arriba, Pico del Lobo, 30TVL6160, 2100 m, schistose pastures	Martínez s/n.
AJ225591	22	*A. splendens* (Lag. & Rodr.) Webb	Spain, Granada: Sierra Nevada, lagunilla Juntillas, 30SVG7607, 2950 m, schistose pastures	Alvarez 1388
AJ225595	23	*A. malacitana* Nieto Fel.	Spain, Málaga: Sierra de Mijas, Jarapalo, 30SVF55, 650 m, dolomitic sandy soils	GN 1733
AJ225592	24	*A. colorata* Pau	Spain, Málaga: Estepona, Sierra Bermeja, Los Reales, 30SUF0240, 1400 m, serpentine rock crevices	GN 3683, 2
AJ225593	25	*A. colorata* Pau	Spain, Málaga: Estepona, Sierra Bermeja, Los Reales, 30SUF0240, 1400 m, serpentine rock crevices	GN 3683, 9
AJ225578	26	*A. alliacea* (Cav.) Hoffmanns. & Link	Spain, Alicante: Sierra de Aitana, 1100–1300 m, limestone	Vogt s/n
AJ225581	27	*A. bourgaei* Boiss. ex Merino subsp. *bourgaei*	Spain, Almería: Sierra de María, 1800 m, limestone	MA 306105
AJ225615	28	*A. bourgaei* Boiss. ex Merino subsp. *lanceobracteata* (Bernis) Nieto Fel.	Spain, Granada: Sierra de Guillimona, WH30, 1750 m, pastures on limestone	GN 1365, 1
AJ225614	29	*A. bourgaei* Boiss. ex Merino subsp. *lanceobracteata* (Bernis) Nieto Fel.	Spain, Granada: Sierra de Guillimona, WH30, 1750 m, pastures on limestone	GN 1365, 2
AJ225604	30	*A. bourgaei* Boiss. ex Merino subsp. *willkommiana* (Bernis) Nieto Fel.	Spain, Murcia: Benizar, Rincón de las Cuevas, 30SWH8835, 1000 m, pastures on limestone	Alvarez 1138
AJ225579	31	*A. filicaulis* (Boiss.) Boiss. subsp. *filicaulis*	Spain, Murcia: Muela de Moratalla, Sierra de la Muela 30SWH9134, 1400 m, pastures on limestone	Castroviejo 14566
AJ225610	32	*A. filicaulis* (Bernis) Nieto Fel. subsp. *nevadensis* Nieto Fel. et al.	Spain, Granada: Sierran Nevada, pe ón de Dílar, 30SVG6103, 2460 m, shrubland on schist	Alvarez 1365
AJ225580	33	*A. choulettiana* Pomel	Morocco: High Atlas, Oukaimedene, 31°12′11N, 7°51′28W, 2660 m, dry siliceous pastures	Güemes 1595
AJ225588	34	*A. pauana* (Bernis) Nieto Fel.	Spain, Jaén: Santa Elena, N-VI, schistose slopes	GN 3885
AJ225582	35	*A.* sp. "a"	Spain, Córdoba: Priego,Ti osa, 1550 m, UG 9038, limestone on summit area	SB 17550
AJ225597	36	*A. villosa* Girard subsp. *villosa*	Spain, Málaga: Sierra de las Nieves, UF2062, 1800 m, limestone	GN 1423
AJ225600	37	*A. villosa* Girard subsp. *provillosa* (Bernis) Nieto Fel.	Spain, Jaén: Villarrodrigo. La Mangada, 30SWH2756, 980 m, limestone	MA 403427
AJ225583	38	*A. villosa* Girard subsp. *alcaracensis* Nieto Fel.	Spain, Albacete: Vianos. 30SWH4177, 960 m, on loam	MA 586743

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APPENDIX 1. CONTINUED.

EMBL/GenBank/DDBJ accession no.	Sample no.	Taxon	Locality	Voucher no.
AJ225584	39	*A. villosa* Girard subsp. *bernisi* Nieto Fel.	Spain, Granada: Sierra Nevada, on the road to the Veleta, VG5910, 1450 m, stony pastures, limestone	GN 1370
AJ225598	40	*A. villosa* Girard subsp. *bernisi* Nieto Fel.	Spain, Almeria: Sierra Nevada, Laujar, 2000 m	MA 503922
AJ225585	41	*A. villosa* Girard subsp. *carratracensis* (Bernis) Nieto Fel.	Spain, Málaga: Carratraca, Sierra de Aguas, 30SUF4379, 800 m, serpentine outcrops	GN 3803, 1
AJ225587	42	*A. villosa* Girard subsp. *carratracensis* (Bernis) Nieto Fel.	Spain, Málaga: Carratraca, Sierra de Aguas, 30SUF4379, 800 m, serpentine outcrops	GN 3803, 2
AJ225586	43	*A. villosa* Girard subsp. *longiaristata*. (Boiss. & Reut.) Nieto Fel	Spain, Albacete: Calar del Mundo, 30SWH4753, 1300 m, pinewoods on limestone	GN 3346, 2
AJ225611	44	*A. villosa* Girard subsp. *longiaristata* (Boiss. & Reut.) Nieto Fel.	Spain, Albacete: Calar del Mundo, 30SWH4753, 1300 m, pinewoods on limestone	GN 3346, 13
AJ225607	45	*A. villosa* Girard subsp. *longiaristata*. (Boiss. & Reut.) Nieto Fel	Spain, Jaén: Segura de la Sierra, 30SWH3031, 1300 m, pinewoods on limestone	GN 3675, 5
AJ225609	46	*A. villosa* Girard subsp. *longiaristata* (Boiss. & Reut.) Nieto Fel.	Spain, Jaén: Segura de la Sierra, 30SWH3031, 1300 m, pinewoods on limestone	GN 3675, 7
AJ225603	47	*A. villosa* Girard subsp. *longiaristata* (Boiss. & Reut.) Nieto Fel.	Spain, Córdoba: Zuheros, Cueva de los Murciélagos, limestone rock crevices	GN 3850
AJ225606	48	*A. villosa* Girard subsp. *longiaristata* (Boiss. & Reut.) Nieto Fel.	Spain, Granada: Zújar, Cerro Javalcón, 30SWG1657, 1400 m, limestone rock crevices	GN 3678, 3
AJ225608	49	*A. villosa* Girard subsp. *longiaristata* (Boiss. & Reut.) Nieto Fel.	Spain, Granada: Zújar, Cerro Javalcón, 30SWG1657, 1400 m, limestone rock crevices	GN 3678, 2
AJ225601	50	*A. villosa* Girard subsp. *longiaristata* (Boiss. & Reut.) Nieto Fel.	Spain, Cádiz: Villaluenga del Rosario, roadsides on limestone	GN 3862
AJ225605	51	*A. villosa* Girard subsp. *longiaristata* (Boiss. & Reut.) Nieto Fel.	Spain, Jaén: Huéscar, Santiago de la Espada, 30SWH3914, 1200 m, roadsides on limestone	GN 3676, 2
AJ225612	52	*A. villosa* Girard subsp. *longiaristata* (Boiss. & Reut.) Nieto Fel.	Spain, Jaén: Huéscar, Santiago de la Espada, 30SWH3914, 1200 m, roadsides on limestone	GN 3676, 8
AJ225602	53	*A. villosa* Girard subsp. *longiaristata* (Boiss. & Reut.) Nieto Fel.	Spain, Sevilla: Estepa: Pico Becerrero, limestone rock crevices	GN 3853
AJ225616	54	*A. villosa* Girard subsp. *longiaristata* (Boiss. & Reut.) Nieto Fel.	Spain, Jaén: Valdepe as de Jaén, 30SVG3363, 1400 m, limestone	GN 3679, 7
AJ225617	55	*A. villosa* Girard subsp. *longiaristata* (Boiss. & Reut.) Nieto Fel.	Spain, Jaén: Valdepe as de Jaén, 30SVG3363, 1400 m, limestone	GN 3679, 5
AJ132446	56	*Psylliostachys suworowii* (Regel) Roshk.	Botanischer Garten und Alpengarten. Universität Innsbruck	Ind. Sem. 1997, 623

APPENDIX 2. MORPHOMETRIC VARIABLES

Character no.	Description
1	Leaf length
2	Ratio leaf length to leaf width
3	Scape length
4	Diameter of the scape at base
5	Diameter of the involucre
6	Ratio of the involucral diameter to the length of the involucral sheath
7	Number of involucral bracts
8	Length of inner involucral bracts
9	Ratio of the shortest to the longest involucral bracts
10	Width of inner involucral bracts
11	Length of spikelet bracts
12	Ratio of the length of spikelet bracts to the length of inner involucral bracts
13	Calyx length
14	Ratio of calyx lobe length (including awn) to total calyx length
15	Calyx tube length
16	Ratio of calyx tube length to calyx limb length
17	Length of calyx pedicel scar
18	Petal color