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Anchusa formosa (Boraginaceae), a new species from Southern Sardinia (Italy)

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Anchusa formosa (Boraginaceae), a new species from Southern Sardinia (Italy)

F. SELVI, M. BIGAZZI and G. BACCHETTA

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ABSTRACT - A new *Anchusa* species was discovered in the massif of Mt. Lattias in South-Western Sardinia. This species, here described as *A. formosa*, shows a sharp phenotypic differentiation from the other Sardinian endemic taxa of *Anchusa*, especially in habit, leaf shape and size, inflorescence and calyx morphology. Ecological traits are also peculiar, the plant being restricted to siliceous debris that accumulates in small depositional areas of dry stream beds. *A. formosa* is likely to represent a paleogenic schizoendemism originated through isolation and genetic drift from the same genic pool of the other Corso-Sardinian endemic taxa of *Anchusa*. SEM analyses provide additional information on the micromorphology of the stigma and its papillar pattern, pollen grains, mericarp coat surface and trichomes. The anatomical structure of stem, root and leaf were also analysed. Due to its extreme localization and ecological specialization, *A. formosa* needs total protection in its natural habitat and should be added to National and International Red Lists of plants at risk of extinction.

KEY WORDS - *Anchusa formosa*, Boraginaceae, karyology, micromorphology, Sardinian flora, taxonomy

Anchusa L. is one of the largest genera of the tribe Boragineae Bercht. & J. Presl., with about 45 entities centering in the Mediterranean basin and extending through Europe, Western Asia and tropical Africa, with a second minor distribution center in the Cape Region. The great diversity of forms exhibited by the genus (JOHNSTON, 1924; SMITH, 1932), led the monographer GUSULEAC (1927, 1928, 1929) to separate the three monotypic genera *Hormuzakia*, *Gastrocotyle* and *Phyllocara* and to recognize six subgenera within *Anchusa* sensu stricto. The complex and reticular variation of phenotypic traits with a potential taxonomic value, however, induced later authors to contrasting

interpretations which caused different generic delimitations and a variable number of accepted entities. Also the relevant floristic literature of this century concerning the Italian territory (FIORI, 1926; CHATER, 1972; PIGNATTI, 1982; GREUTER *et al.*, 1984) is affected by uncertainties relating to the nomenclature and biological aspects of some little known taxa. The only contributions on Italian representatives of *Anchusa* were by ILLARIO (1935) and VALSECCHI (1976, 1988), both limited to the Sardinian territory. The latter author came to recognize seven entities for Sardinia, of which three exclusive to the island, *A. undulata* L. subsp. *capellii* (Moris) Vals., *A. maritima* Vals. and *A. littorea*

Moris, and a fourth one endemic to Corsica and NW Sardinia, *A. crispa* Viv. Ongoing studies on the Boragineae on a broader geographical scale (BIGAZZI *et al.*, 1997; BIGAZZI & SELVI, in press) highlighted, however, that the systematic relationships of some Italian taxa with other Mediterranean entities are still unclear and in need of further investigation. During recent field researches in Sardinia, we found an *Anchusa* species in a locality of the Sulcis montane area falling well outside the known distribution range of all the other endemic taxa. After a close comparative examination, it was recognized as a species new to science in view of its peculiar phenotypic, ecological and chorological features.

Anchusa formosa Selvi, Bigazzi & Bacchetta, *sp. nova*

Herba biennis, valde hispida. Caulis basi ramoso, ramis longe adscendentibus. Folia oblanceolata, plana. Inflorescentia ramosa, conferta. Bractea ciliata, calyce paulo brevior. Calyx fructifer urceolatus, ventricosus,

costatus, lobis acutis brevibus. Corolla caerulea, rotata, lobis rotundatis, tubo calycem subaequante. Stamina epicorollina, partim squamis superposita. Stigma capitatum, ovoideum, parce bilobum. Mericarpia parva, nigra, rostrata, areola basali tenui.

Nomen ad plantae elegantiam relatum, propter caules adscendentes, folia laete viridia et copiosa, cinnos caeruleis floribus repletos.

Type - Holotypus: Sardegna, Sulcis Nord-Orientale (Cagliari), versante NE del Monte Lattias, fra le rocce granitiche nell'alveo secco del Rio Su Fundu, su suolo siliceo sabbioso-detritico, c. 850 m, 18 Mai 1997, Selvi & Bigazzi (FI; isotypes in FI, CAG, SASSA and K).

Voucher specimens - Sardegna, Sulcis Nord-Orientale (Cagliari), versante NE del Monte Lattias, fra le rocce granitiche nell'alveo secco del Rio Su Fundu, su suolo siliceo sabbioso-detritico, c. 850 m, 18 Mai 1997, Selvi

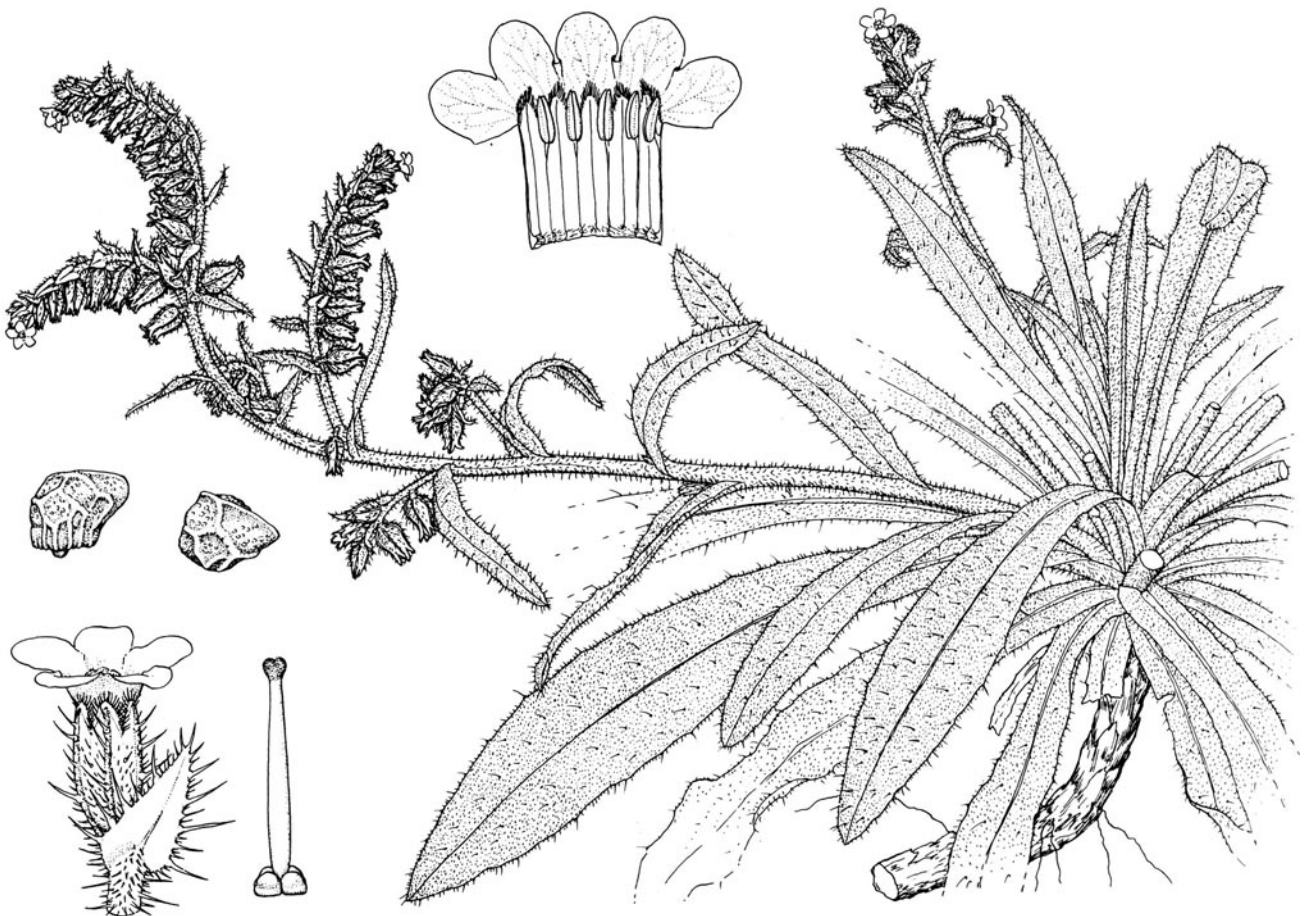


FIGURE 1 - *Anchusa formosa*: whole plant (x 0.6), flower with peduncle and bract (x 3), ovary and style (x 6), mericarps in lateral and dorsal views (x 6), open corolla showing stamen position and faucal scales (x 3); drawing by A. Maury.

& Bigazzi (FI, holotype; FI, CAG, SASSA and K, isotypes); Mt Lattias nell'alveo del Rio Su Fundu, detrito granitico, c. 780 m, 18 Mai 1997, Selvi & Bigazzi (FI); Mt Lattias (Agro di Uta, CA), nel Canale de Longufresu su suolo siliceo scheletrico, esp. E-SE, c. 720 m, 13 Jul 1997, Bacchetta & Mossa (CAG); Mt Lattias, alla base della cresta nel canale di Sennamanna, detrito granitico, c. 830 m, 13 Jul 1997, Bacchetta & Mossa (CAG).

Description (Figure 1)

Biennial hemicryptophyte 15-35 cm high, prickly, forming a rich rosette of basal leaves from which originate several prostrate-ascending branches; indumentum dimorphic, consisting of short hairs and long, stiff, tubercle-based trichomes; tap root fusiform, deep and stout; basal leaves 8-18 x 1-3 cm, bright green, lanceolate to oblanceolate, tapering into a short petiole, hispid especially along veins and margins, which are entire and flat; cauline leaves similar but progressively smaller, sessile; flowering stems branching in their upper part into 2-4 short and dense, curved cymes remaining compact in fruit; flowers tendentially oriented all in the same direction; bracts triangular-ovate, cordate at the base, acute, slightly shorter than the flowering calyx (4-5 mm) and accrescent in fruit, margins densely ciliate; flowers subsessile, with calyx 5-6 mm long, tubulose, prickly along veins and teeth margins, teeth 1-1.5 mm long, narrowly triangular, acute, purplish along the margins; fruiting calyx ventricose-urceolate, 8-10 mm long, strongly ribbed, with teeth reflexed outwards; corolla tube 4.5-5.5 mm long, straight, whitish, purplish-variegated at the throat, with internal tufts of short hairs ringing the base; limb rotate, 7-9 mm in diameter, light blue-violet (colour type: Methuen 19 C7-8; KORNERUP & WANSCHER, 1978), lobes rounded; faucal scales white-pilose, closing the throat; stamens inserted onto the upper part of the corolla tube through tapering filaments, anthers 1.7 mm long, inserted on the filament slightly below the middle, light brown, overlapping scales for about 0.6 mm; style included within the tube, ca. 5 mm long, tapering, bearing a capitate-ovoid, slightly bilobed stigma; mericarps (nutlets) obliquely ovoid, small, ca. 1.5 x 2 mm, with a lateral pointed apex and a weak basal rim; surface blackish, minutely papillose-tuberculate, with a reticulation of blunt ridges; Fl. April-June; Fr. May-July.

Chorology and ecology

A. formosa is a stenochorous endemism currently known for a single narrow area in the North-Eastern part of the Sulcis subregion, in South-Western Sardinia (Figure 2). It was not found in the nearby Mt Arcosu, whose

flora has recently been studied by MOSSA *et al.* (1996). The species was discovered in the upper part of the Rio Su Fundu dry bed, a seasonal stream originating at the base of the NE-facing ridge system forming the highest peaks of Mt Lattias (1086 m a.s.l.), in the Mt Arcosu Nature Reserve and in the so called "Agro di Uta" area (Cagliari Province). A second, smaller population was found in the bed of Su Canali de Longufresu, a secondary branch of the upper Rio Su Fundu. Both localities are included in the old I.G.M. maps 1:25.000 Santa Barbara No. 233 II NE, while in the 1992 1°edn. series xxv°, they are reported in the Capoterra map No. 565 I. Both populations have an extremely limited distribution, stretching over about 500 m of stream length, from about 600 to 860 m a.s.l.

Mt Lattias is a major peak in the NE sector of the Sulcis montane area, and is characterized by a very rough morphology with sharp ridges and cliffs. From a bioclimatic viewpoint the area belongs to the subhumid mesomediterranean type; mean annual temperatures range from 13-15°C, and rainfall reaches about 1000 mm (MOSSA *et al.*, 1996). The NE slopes of Mt Lattias are characterized by frequent mists and by humid currents throughout the year. From a geological viewpoint Mt Lattias consists of a Late-Hercinian formation of leucogranites belonging to the ancient Corso-Sardinian batholite. The average mineralogical composition of these rocks is quartz (35%), biotite (2-5%) and small percentages of potassic feldspar and orthoclase (BARCA *et al.*, 1986). Water erosion leads to the formation of a siliceous debris (Dystric-Xerorthens, cf. ARU *et al.* 1991) that accumulates in small depositional areas among stones along the narrow margins of the stream bed. In these niches, evapotranspiration creates a humid and warm microclimate. *A. formosa* is exclusive of this habitat type, in which it is also partially protected from stream floodings. Basal leaves are often shaded but flowering stems elongate enough to reach full sunlight. In some cases, plants are almost completely overwhelmed by dense bushes of *Rubus ulmifolius* that tend to colonize the stream bed and offer a physical protection against running water.

At Rio Su Fundu *A. formosa* grows in association with: *Cymbalaria aequitriloba* (Viv.) Cheval., *Scrophularia trifoliata* L., *Vincetoxicum hirundinaria* Medicus, *Hypericum hircinum* L., *Teucrium marum* L., *Stachys corsica* Pers., *Digitalis purpurea* L., *Euphorbia characias* L., *Ferula communis* L., *Phagnalon saxatile* (L.) Cass., *Rumex thyrsoides* Desf., *Galium scabrum* L., *Rubus ulmifolius* Schott, *Cistus incanus* L., *Erica arborea* L., *Nerium oleander* L. and other species of rocky, siliceous soils. In the uppermost part of the stream, right at the

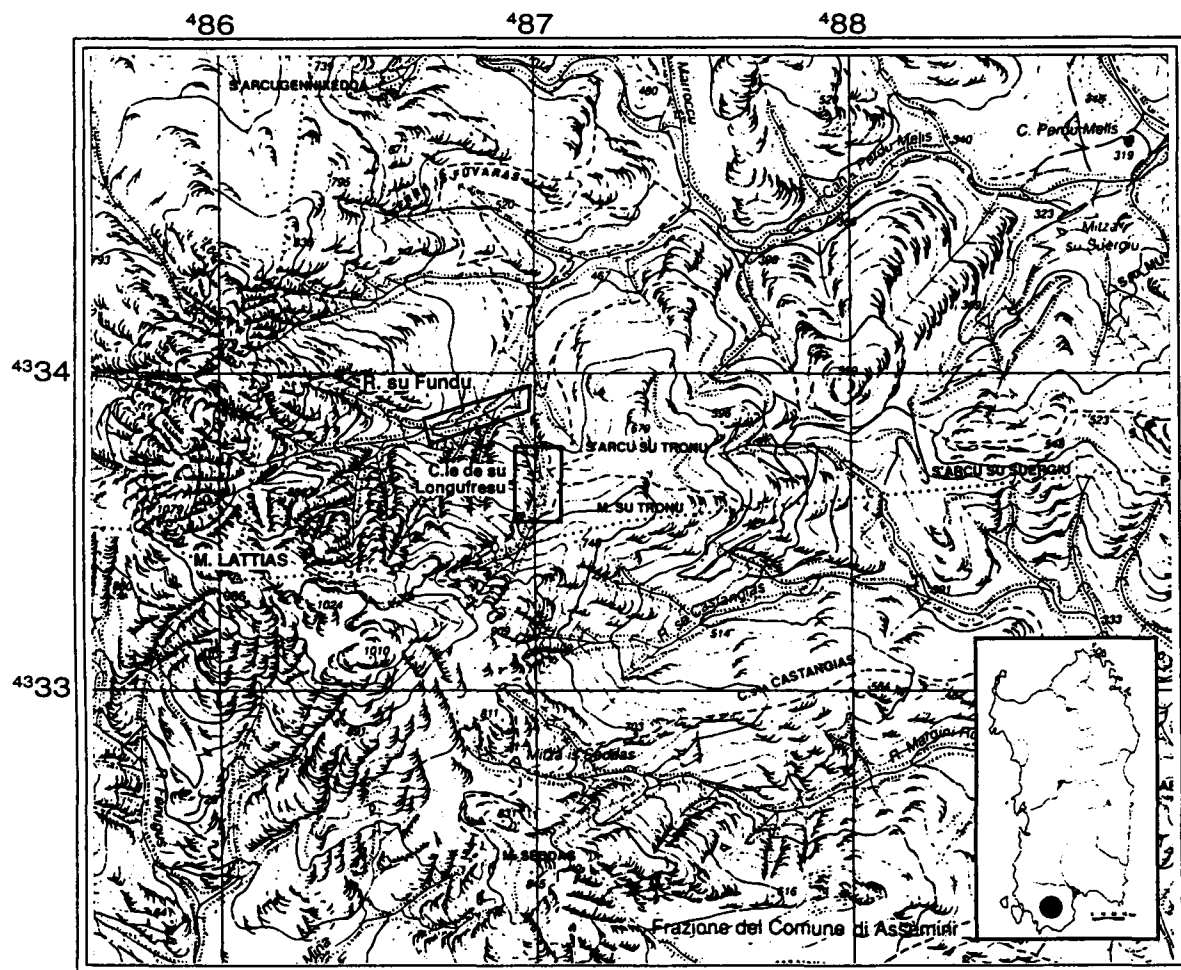


FIGURE 2 - Distribution of *Anchusa formosa* in South-Western Sardinia.

base of the Mt Lattias cliffs (Canale di Sennamanna), other associated plants of particular phytogeographic interest are *Armeria sulcitana* Arrig. and *Mentha requienii* Benth. From a phytosociological viewpoint the riverbed vegetation, of which *A. formosa* is a part, can be referred to the Rubo-Nerion oleandri Bolòs 1985 alliance, while the surrounding communities are acidophilic maquis of the Quercion ilicis Br.-Bl. 1936 em. Riv.-Mart. 1975 or degraded aspects of the Ericion arboreae (Riv.-Mart., Costa et Izco 1984) Riv.-Mart 1987 (MOSSA *et al.*, 1991; MOSSA *et al.*, 1996).

Karyology

Karyological analyses were carried out on mitotic metaphase plates of meristematic cells taken from root apices of germinating seeds. After a pretreatment of about 2 h in a 8-hydroxychinolin aqueous solution, the material was fixed in Carnoy, hydrolyzed in HCl

1N at 60°C for 6 min. and stained with lactopropionorcein (18 h). Idiograms were prepared on enlarged prints of the original micrographs. Measures and values were computer-processed in order to obtain chromosome ordering and homologue recognition, the karyotype formula following LEVAN *et al.* (1964), and the general asymmetry index by ARANO & SAITO (1980).

A. formosa has the following karyotype formula: $2n = 2x = 16: 2M + 4m + 8sm + 2sm\ sat$; asymmetry index 0.63; average chromosome length 5.8 μm , width 2.3 μm (Figure 3). $2n = 16$ is the most common diploid number in *Anchusa sensu stricto* (STREY, 1931; SMITH, 1932; BRITTON, 1951; LUQUE, 1983; D'AMATO & TROJANI, 1985). Karyotype morphology of *A. formosa* shows affinities with several other members of this genus, including the other Sardinian endemics (Valsecchi, 1976).

Micromorphology

Floral and vegetative structures were collected from living plants in the field, fixed and preserved in 2% glutaraldehyde in phosphate buffer 0.1 M (pH 7.2) at 4°C. Stem, leaf and root portions were embedded in Histo-resin, cut with a Microtome LKB and stained with Toluidine Blue in phosphate buffer. Observations and micrographs were made by means of a Zeiss Axiophot light microscope. For SEM procedures, samples were dehydrated in an acetone series, critical point-dried with liquid CO₂, mounted on aluminium stubs, coated with gold and observed by means of a Philips XL 20 Scanning Electron Microscope. Fruits were directly mounted on stubs and sputter-coated with gold. Pollen samples were preserved in glacial acetic acid and then prepared according to the acetolysis method (ERDTMAN, 1960).

According to the classification by HESLOP-HARRISON, (1981), the stigma head of *A. formosa* belongs to the DPU-type, i.e. dry, papillate with unicellular papillae. It

shows the basic shape of most *Anchusa* species, namely capitate-ovoid and slightly bilobed (Figure 4a). The two lobes are divided by a weak, apical depression extending to the frontal sides of the stigma head. The surface bears densely crowded lageniform papillae, consisting of a swollen base and a very short neck surmounted by a platelike cap deeply lobed into 6-9 digitate-crenulate extroflexions (Figure 4b). This basic papillar pattern is common to many Boragineae, but there is a wide variation in terms of density, spatial arrangement and shape (DULBERGER, 1970; HESLOP-HARRISON, 1981; PHILLIP & SCHOU, 1981; BIGAZZI *et al.*, 1997).

Pollen grains of *A. formosa* are medium-sized, with P \approx 32 μ m and E \approx 23 μ m; the shape is prolate (P/E \approx 1.4) and rectangular-elliptic in equatorial outline (Figure 4c). The four ectoapertures are fusiform, about 11 μ m long, while endoapertures are subelliptical. The tectum is homogeneously psilate-punctate, except along a narrow equatorial furrow which has a reticulate sculpturation. From a morphological viewpoint, this pollen belongs to the common *Pulmonaria obscura*-type (CLARKE, 1977), that includes the largest part of *Anchusa*, *Nonea* and *Pulmonaria* (BIGAZZI & SELVI, in press).

Faucal scales of *A. formosa* have the same structure and origin as the other *Anchusa* species (SCHAEFER, 1942). SEM observation reveals that unicellular hairs covering the scales have a rough surface due to the presence of granular thickenings of the cell wall (Figure 4d). Little is currently known about the presence of this character in other Boraginaceae, which is likely to be connected in some way to pollination biology and to the avoidance of self-fertilization. Owing to the rough surface of the crowded hairs (Figure 4d), pollen grains released by the anthers become temporarily enmeshed in the scales. They are then probably loaded onto the body of visiting insects, mainly long-tongued Diptera or Hymenoptera, that brush against the scales while trying to reach the nectar secreted at the base of the ovary (Figure 4e).

The base of the corolla tube is provided with an internal ring of ten tufts of hairs, two for each corolla lobe (Figure 4f). Unlike the trichomes of faucal scales, hairs of the basal tufts have a smooth surface, consistent with their probable function in slowing down the evaporation of the nectar secreted by the facing ducts. The mericarp coat surface is densely covered with small papillae in which are scattered more conspicuous, spinescent tubercles (Figure 4g,h).

Anatomy

Cross-sectioned stems in secondary structure (Figure 5a) show a single-layered epidermis enclosing a ring

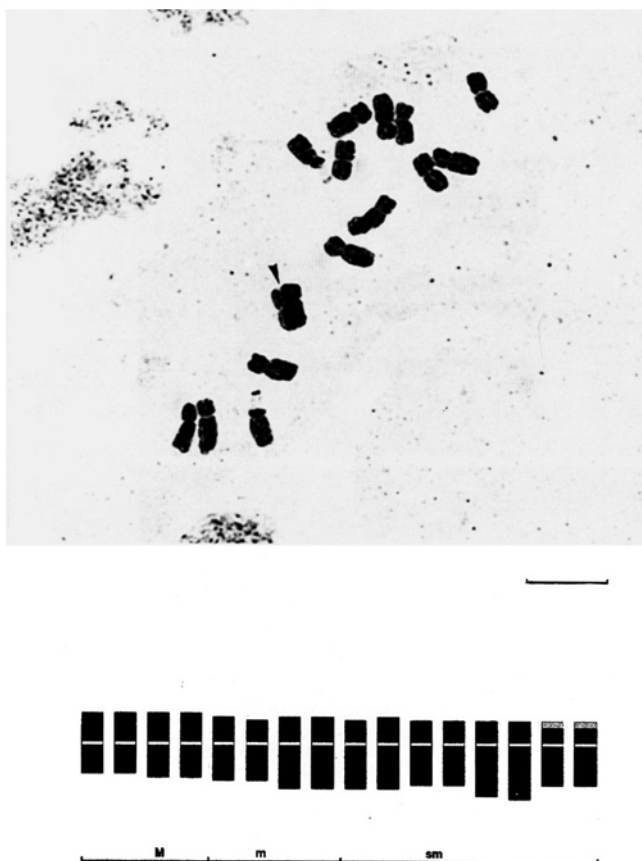


FIGURE 3 - Metaphasic chromosome plate and karyotype idiogram; the arrow indicates two overlapping chromosomes. Bar = 10 μ m.

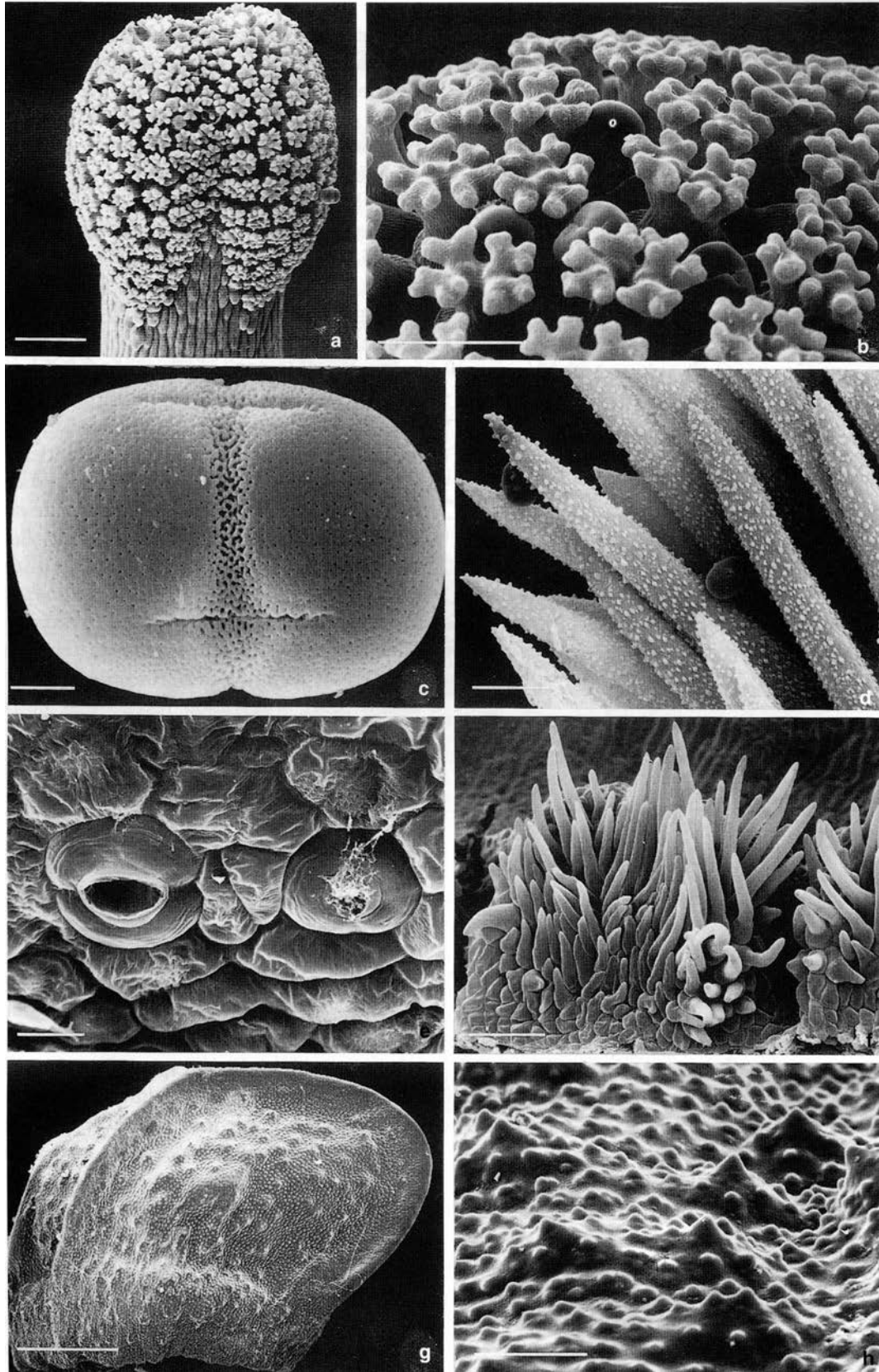


FIGURE 4 - SEM micrographs of a) stigma head, b) stigmatic papillae, c) pollen grain in equatorial view, d) trichomes of faucal scales, e) nectaries at the base of the ovary, f) tufts of trichomes ringing the base of the corolla tube, g) mericarp in lateral view, h) mericarp coat surface. Scale bars: a, f) 100 μ m; b, d, h) 50 μ m; e) 10 μ m; c) 5 μ m; g) 0.5 mm.

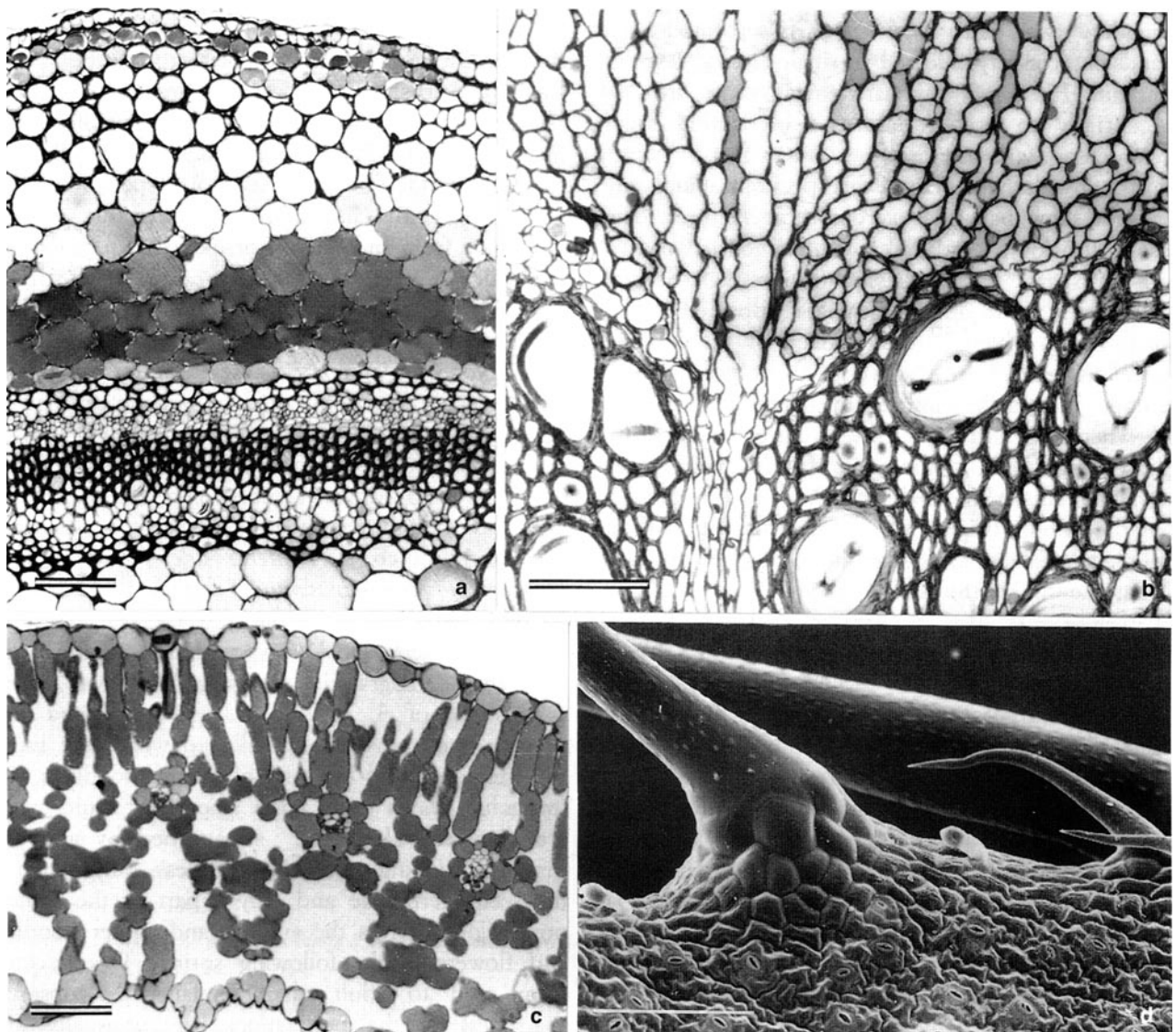


FIGURE 5 - Light microscope and SEM micrographs of a) stem in cross section, b) two-year old root in cross section, c) leaf mesophyll, d) leaf epidermis and trichomes of three types. Bars: a, d) 200 μ m; b, c) 100 μ m.

of chlorenchyma cells. These are separated from the parenchymatous cortex by strands of an atypical lacunar and relatively thin-walled collenchyma. The cortex is sharply delimited by a ring of mechanical tissue with cells showing unevenly thickened walls of pectin and hemicellulose. Such an internal position of the collenchyma is somewhat atypical as this tissue is more often limited to the peripheral cortex and is discontinuous. The mechanical tissue encloses a continuous ring of phloem consisting of sieve tubes, companion and parenchyma cells. The phloem is separated from a thick secondary band of xylem by the vascular cambium. A ring of primary xylem with

vessels showing less lignified cell walls is also visible internally to the secondary xylem. The primary xylem, which is formed in the first months of growth, consists of an annular-spiralate protoxylem (tracheids) and a metaxylem with vessels showing scalariform thickenings. The vascular tissue is separated from the parenchymatous pith by an additional multi-layered ring of thick-walled collenchyma cells. The strong development of this tissue and its peculiar position gives to stems and branches a marked mechanical resistance that is likely to be connected with the particular habitat of the species. Cross-sectioned second-year roots show the typical

secondary structure of herbaceous dicots (Figure 5b). Secondary xylem with large vessels is separated from strands of secondary phloem by a single-layered vascular cambium. Flares of ray parenchyma cells, dividing tangentially to accommodate the increasing stem circumference, are also visible.

Consistent with the habitat of this species, leaf anatomy does not show particular xeromorphic features. The mesophyll below the adaxial epidermis consists of two layers of sparse palisade tissue (Figure 5c). Several veinlets with xylem and phloem are dispersed in the spongy tissue, which is characterized by rather large intercellular spaces. In main veins, xylem is located in the upper part of the bundle and phloem in the lower part, where it is surrounded by a ring of collenchyma cells. Tubercle-based trichomes and simple hairs occur on both surfaces, while small, glandular hairs are present along the margins (Figure 5d). Stomata are scattered on both the adaxial and abaxial surfaces, although they are more numerous on the latter.

OBSERVATIONS

A. formosa is a geographically and genetically isolated species differing from the other members of *Anchusa* subg. *Euanchusa* (Griseb.) Gusul. from a phenotypic and ecological viewpoint. Characterizing features of this species are: 1) the long prostrate-ascending branches originating laterally to a rich rosette of basal leaves, 2) the indumentum with dense, subpungent trichomes, 3) the flowering stems branching in their distal part into 2-4 short and dense, curved cymes which remain compact in the fruit, 4) the fruiting calyx ventricose-urceolate with five prominent longitudinal costae and short, acute teeth reflexed outwards. *A. capellii* Moris, which is the other montane species endemic to a single Sardinian relief, differs in having a much sparser indumentum all over the plant, smaller leaves with obscurely undulate-crispate margins, inflorescence branched near the middle into long branches becoming rather lax in fruit, bracts as long as or longer than the calyx, calyx divided almost to the base, not costate, distinctly campanulate in the fruit. *A. capellii* is isolated on the summit of Mt Santa Vittoria Esterzili in Central Sardinia, where it grows in stony pastures and rock crevices. *Anchusa formosa* occurs on a massif of different geological constitution about 60 km SW of M.S. Vittoria, where it grows on temporarily humid granitic incoherent debris accumulating in small depositional areas of stream beds between 750 and 900 m a.s.l. No other *Anchusa* species is known to have similar habitat requirements. On the other hand, *A. formosa* shares with *A. capellii*, *A. crispa*, *A. littorea* and *A. maritima* the following

traits: prostrate-ascending habit, the corolla with a rotate limb, rounded lobes and relatively short tube, the small anthers inserted in the upper half of the tube and the small mericarps. Karyological affinities among them are relevant, and from an ecological viewpoint they are all linked to mineral siliceous substrates. This combination of traits is not found in any other species complex within the genus, indicating their probable common derivation from an ancient Corso-Sardinian genic pool. In particular, *A. formosa* and *A. capellii* have strongly reduced allopatric distributions on paleozoic reliefs, show very little phenotypic variation and also a relatively low karyotype asymmetry. These elements may indicate a schizogenic origin "in situ" by genetic drift, which could have occurred after the fragmentation of a former continuous range following the Oligo-Miocenic rotation of the Corso-Sardinian complex. The Sulcis montane area has a high conservative power for paleogenic endemisms, such as *Armeria sulcitana* Arrig. and *Helichrysum montelinasanum* Schmidt (MOSSA *et al.*, 1996).

From a conservational viewpoint, it is worth noting that the demographic status of the two known populations of *A. formosa* is likely to depend year-by-year upon seasonal rainfalls, to which are linked the frequency and intensity of stream floodings. The few adult plants set a good amount of fruits which are dispersed along the stream bed by ants and probably running water. Nevertheless, only a part of the seeds germinate and only a part of the juvenile population survives the autumn and winter floodings and flowers in the following spring. This selection from seed to adult plant explains the observed sparseness of the populations, the unusually high number of sterile individuals amongst mature plants, and the fact that in the spring of 1996 no flowering individuals were seen after intense winter rainfalls. *A. formosa* is an extremely rare plant of great phytogeographic interest. Although it lives in the area of the WWF Natural Reserve of Mt Arcosu, it should be considered at risk of extinction in view of its extreme localization, ecological specialization and demographic fluctuations. The authors believe therefore that this species deserves total protection in its natural habitat and that it should be added to one of the new IUCN Red List categories (IUCN, 1994).

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