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Anchusa limbata Boiss. & Heldr. (Boraginaceae): contribution to the knowledge of an enigmatic species from SW Turkey

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ABSTRACT

BIGAZZI, M., H. DUMAN & F. SELVI (2003). *Anchusa limbata* Boiss. & Heldr. (Boraginaceae): contribution to the knowledge of an enigmatic species from SW Turkey. *Candollea* 58: 339-349. In English, English and French abstracts.

Anchusa limbata Boiss. & Heldr., a very rare species endemic to SW Anatolia, has been rediscovered after more than 150 years since its original collection by T. von Heldreich. Based on the new collection, a detailed morphological description and an original iconography are given to allow a better knowledge of this plant. Micromorphological and karyological aspects are also illustrated for the first time. Geographical and ecological data are reported together with an evaluation of the conservation status of this species. The taxonomic position and possible systematic relationships within the genus *Anchusa* are briefly discussed in the light of the new data.

RÉSUMÉ

BIGAZZI, M., H. DUMAN & F. SELVI (2003). *Anchusa limbata* Boiss. & Heldr. (Boraginaceae): contribution à la connaissance d'une espèce énigmatique du sud-ouest de la Turquie. *Candollea* 58: 339-349. In English, English and French abstracts.

Anchusa limbata Boiss. & Heldr., une espèce très rare et endémique du sud-ouest de l'Anatolie, a été retrouvée plus de 150 ans après sa découverte par T. von Heldreich. Une nouvelle description et une illustration originale permettent de mieux connaître cette espèce. Ses caractéristiques chromosomiques et sa micromorphologie sont étudiées pour la première fois. Des données sur sa distribution et son écologie sont fournies, ainsi qu'une évaluation de l'état de conservation de la seule population existante. La position taxonomique et les relations systématiques de cette espèce au sein du genre *Anchusa* sont discutées à la lumière de ces nouvelles données.

KEY-WORDS: BORAGINACEAE – *Anchusa* – Systematics – Turkish flora.

Introduction

Anchusa limbata Boiss. & Heldr. was first collected in March 1845 by T. von Heldreich during one of his botanical trips through Southwest Anatolia. The species was described four years later by BOISSIER (1849), who wrote “hab. rarissima in colle calcareo consito Pamphylliae inter Adalia et Jenidjè Khan”. Boissier based this remark on Heldreich's handwritten label attached to the original collection (G-BOISS !) where one can read: “468 // *Anchusa streptoloba* // colline calcaire couverte de *Pinus halepensis* sur la route d'Adalia au Yenidsche Khan // paraît très rare, je ne l'ai jamais revue”.

The plants collected by Heldreich were in early flowering stage and did not have ripe fruits, so that Boissier could not describe the morphology of the nutlets. Nevertheless, he was able to

highlight the peculiar structure of the flowers of this plant: “species distincta corolla 3 lin. longa sensim attenuata in limbum,...rectiusculum,...vix manifeste crenulatum nec lobatum. Fornices ut et antherae corollam superantes”.

In more recent times, CHAMBERLAIN (1977) instituted the monotypic *Anchusa* subg. *Limbata* Chamb. & R. Mill to accomodate the distinctiveness “of this remarkable species with a much reduced corolla limb and exserted scales wich are unique in *Anchusa*”. However, since no later collections were available after the original one by Heldreich, *A. limbata* has been known only through brief and incomplete descriptions for more than 150 years, and this has hindered a better understanding of its systematic relationships and taxonomic position (cf. GUŞULEAC, 1928).

The recent rediscovery of this species by one of us (HD) gave the opportunity to collect additional material and to examine in detail its morphological characters, chromosome features, ecological aspects and conservation status. The results of these observations are reported and discussed in this paper to give a contribution to the systematics of the genus *Anchusa* and to the flora of Turkey.

Description

Anchusa limbata Boiss. & Heldr. in Boiss., Diagn. Pl. Orient. 11: 99. 1849.

Type: “*Anchusa streptoloba* // colline calcaire couverte de *Pinus halepensis* sur la route d’Adalia au Yenidsche Khan” March 2, 1845, *T. Heldreich* 468 (holo-: G-BOISS !; iso-: B).

Additional records. – **Turkey.** C3 Antalya: Kepez Gevresi, 250 m, *Pinus brutia* agikliklari, 24.VI.1993, *H. Duman* & *F. Karaveliogullari* 4898 (GAZI); C3 Antalya: Antalya, hill of Kepez, 245 m, in semi-shaded sites under natural *Pinus brutia* canopy, on crumbled calcareous soil, 5.VI.2002, *M. Bigazzi*, *H. Duman* & *F. Selvi* 02.01 (ANTALYA, BSB, E, FI, GAZI, G, W).

Biennial with parenchymatous tap root, producing a basal rosette the first year and flowering in spring of the second year. Indumentum very dense and long throughout the plant and especially on bracts and calyces; whitish-monomorphic with harsh, hyaline bristles up to 4 mm long, patent. Basal leaves present at anthesis, narrowly oblanceolate and tapering into an indistinct petiole, up to 5-8 cm long and 4-6 (-8) mm wide, with entire and flat or faintly undulate margins, without distinct nerves, subobtusate at the apex; the cauline almost linear and semiamplexicaul, slightly shorter. Flowering stems decumbent, branched in the upper half. Cymes subdistich, dense at early stages but elongated and loose in fruit. Bracts ovate-lanceolate, about as long as flower peduncles, c. 4 × 2 mm. Calyx divided to c. 1/3 into obtuse teeth, c. 8 mm long and tubulose in flower, but much accrescent in fruit and reaching up to 13 mm in width and 15 mm in length, with peduncle up to 12 mm, deflexed. Corolla 12 mm long, with tube 9 mm (slightly longer than calyx), limb 2 mm and 1 mm of exserted faucal scales; tube slightly irregular, narrow, whitish in the lower half and pale purple in the upper half; limb much reduced, without distinct lobes, forming a narrow fringe with crenulate margins, bright red; throat closed by five connivent scales of white color, exserted from the limb. Anthers inserted at the throat and interposed to the scales, c. 1.3 mm; style 12 mm long, reaching the base of scales. Stigma capitate, slightly bilobed. Mericarpids brown, stocky, transversely ovoid with blunt lateral beak, c. 3 × 2 mm; basal ring thick, minutely tuberculate and denticulate-rugose; coat surface papillose, with a reticulation of few, low wrinkles (Fig. 1 & 2).

Micromorphology

Micromorphological characters of reproductive parts were examined by means of Scanning Electron Microscopy. The material was fixed in the field and preserved in 2% glutaraldehyde in phosphate buffer 0.1 M (pH 7.2) at 4°C. It was then dehydrated in an acetone series, critical point-dried with liquid CO₂, mounted on aluminium stubs, sputter-coated with gold and observed with a Philips XL 20 Scanning Electron Microscope.

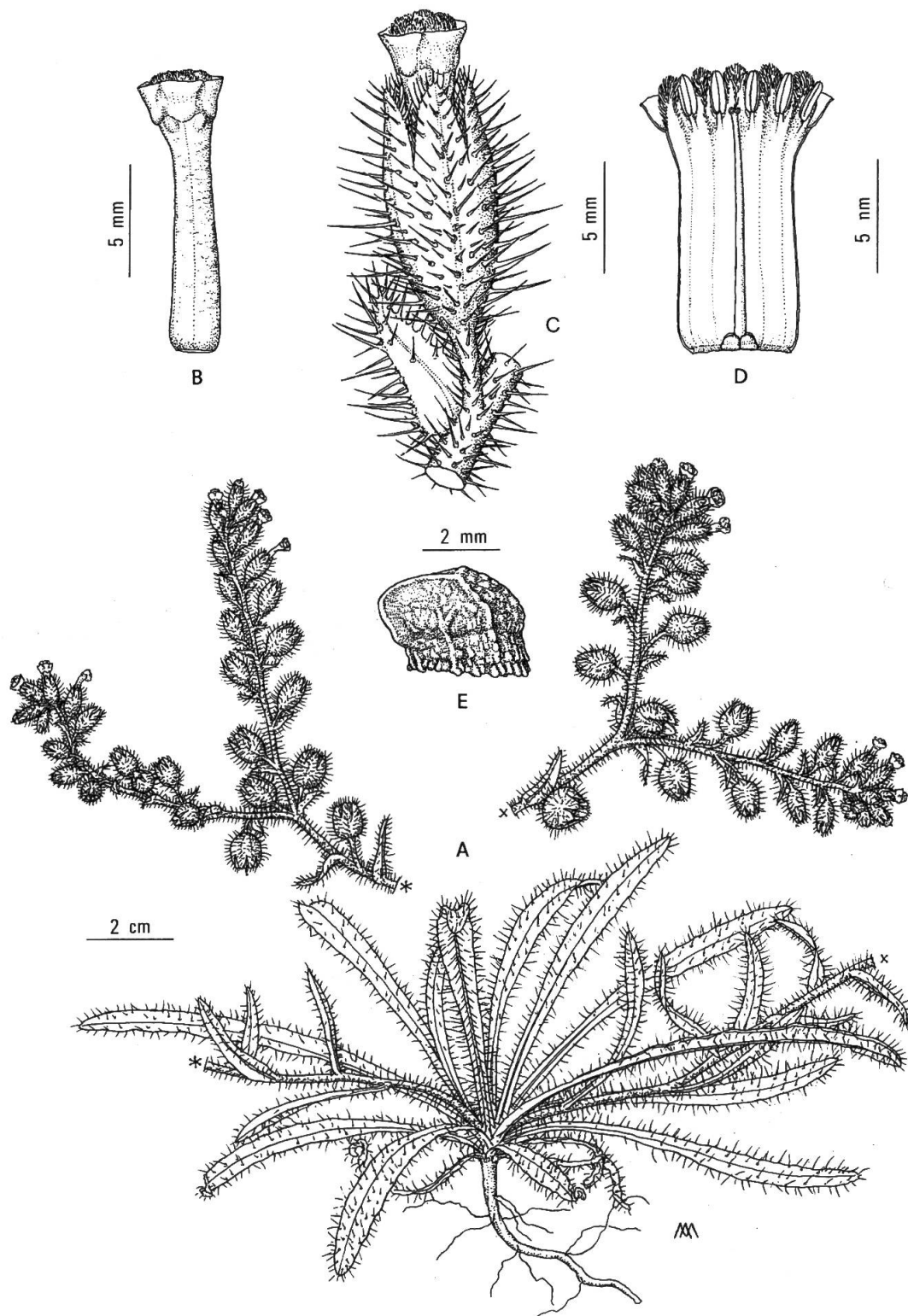


Fig. 1. – *Anchusa limbata* Boiss. & Heldr. **A**, habit; **B**, corolla; **C**, flower with peduncle and bract; **D**, open corolla with stamens and pistil; **E**, mericarpid. [Drawing by A. Maury]

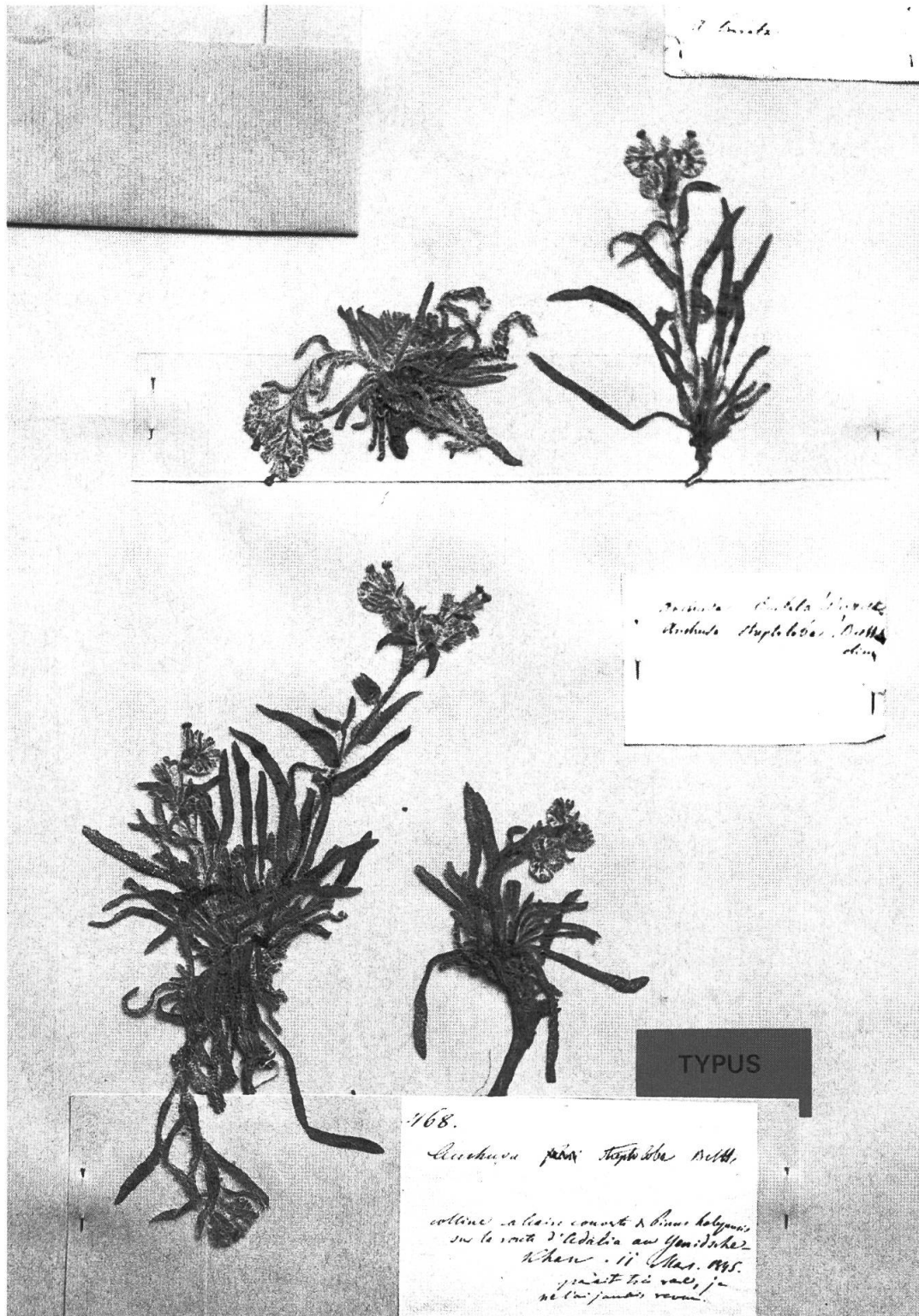


Fig. 2. – The type sheet of *Anchusa limbata* Boiss. & Heldr. in G-BOISS.

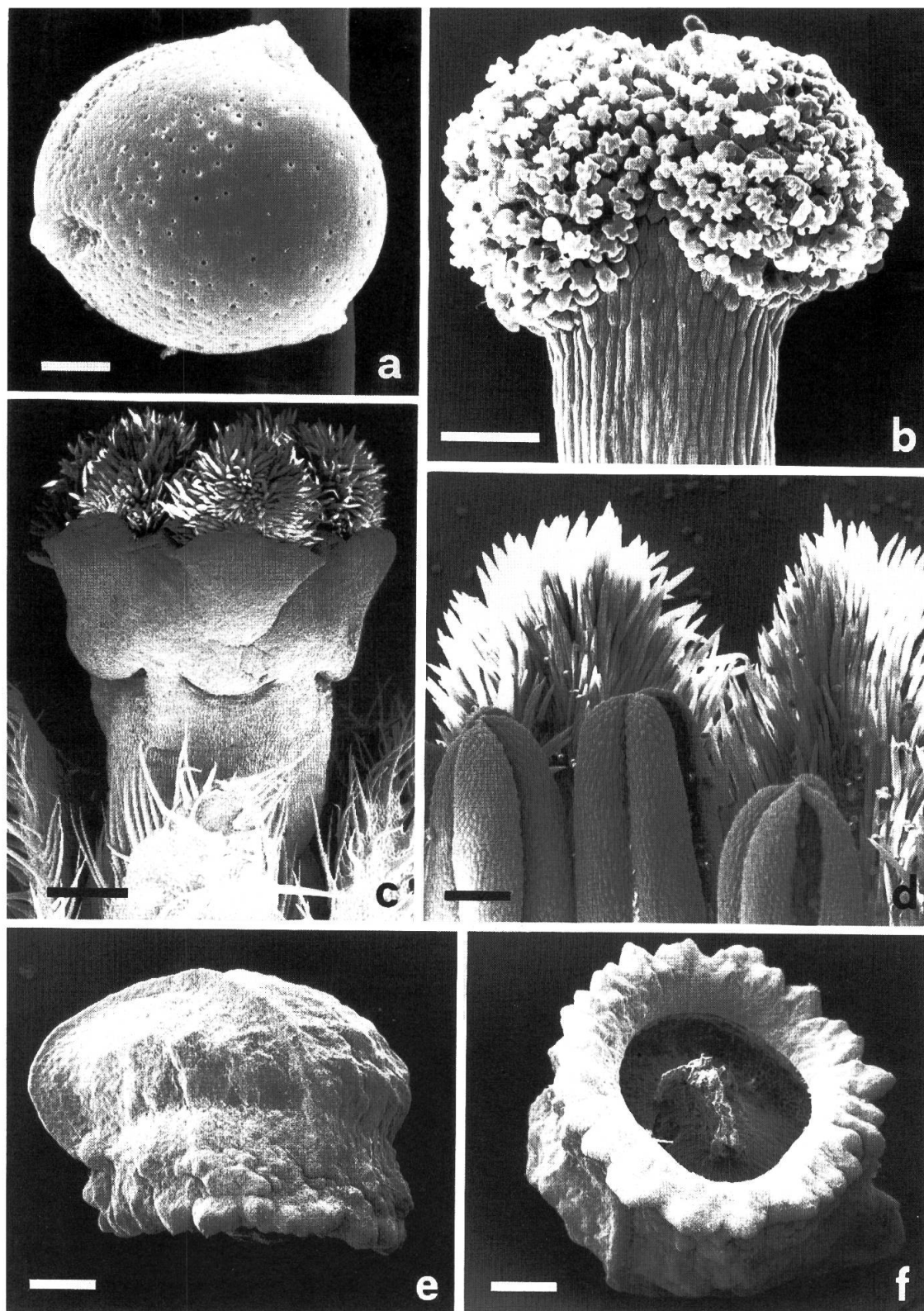


Fig. 3. – *Anchusa limbata* Boiss. & Heldr. SEM micrographs of: **a**, pollen grain; **b**, stigma; **c**, corolla; **d**, detail of faucal scales and anthers; **e**, mericarp in lateral view; **f** mericarp in ventral view. Scale bars: **a** = 5 μ m; **b** = 100 μ m; **d**, 200 μ m; **c**, **e**, **f** = 0.5 μ m.

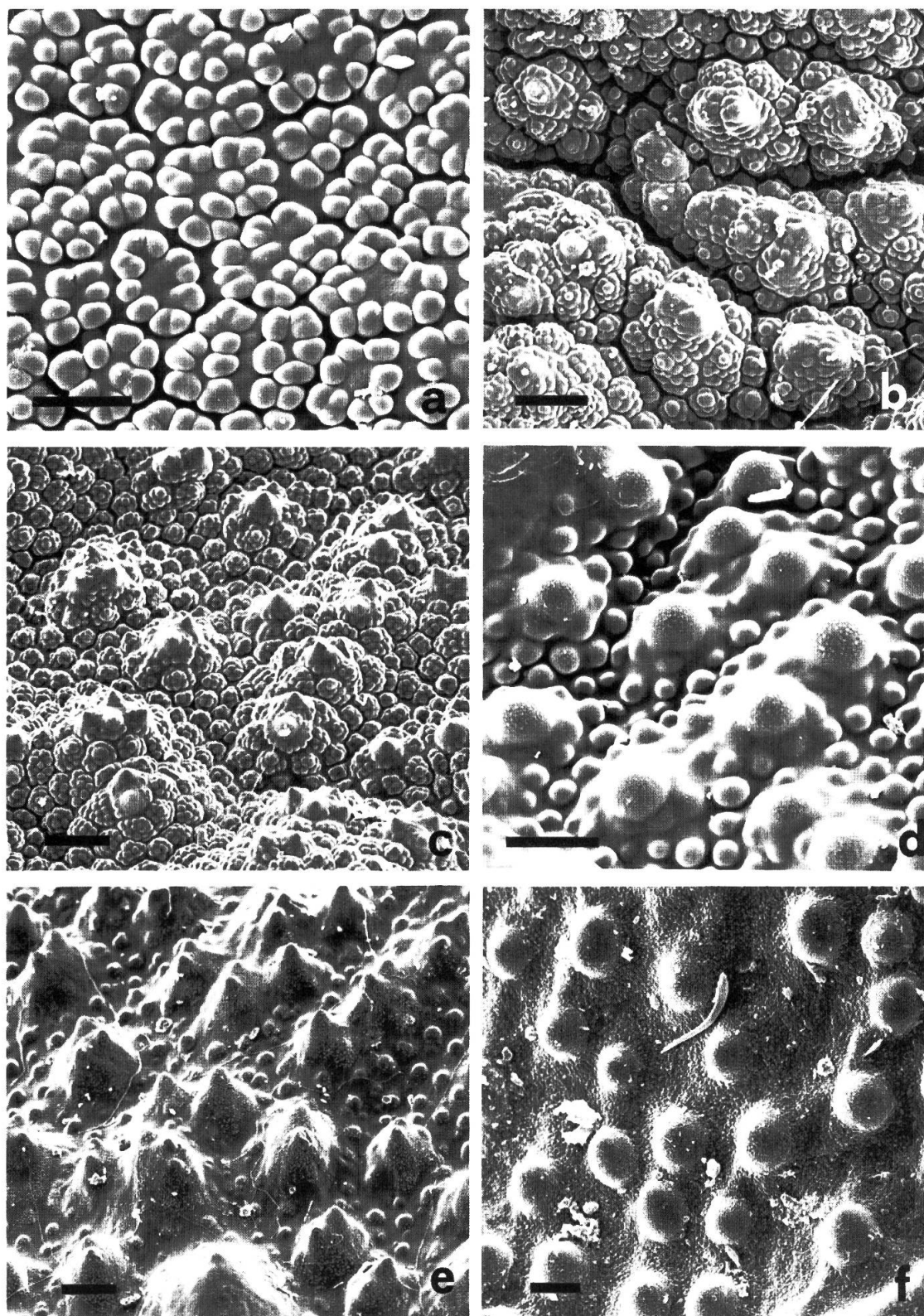


Fig. 4. – SEM micrographs of different types of coat surface of nutlets in *Anchusa*: **a**, *A. limbata* Boiss. & Heldr.; **b**, *A. leptophylla* Roem. & Schult.; **c**, *A. procera* Besser; **d**, *A. puechii* Valdés; **e**, *A. strigosa* Banks & Sol.; **f**, *A. azurea* Mill. Scale bars: **a** = 20 μ m; **b**, **c**, **d**, **e**, **f** = 50 μ m.

Non-acetolyzed pollen grains of *A. limbata* are medium-sized (polar diameter, $P = 36.5 \mu\text{m}$; equatorial diameter, $E = 23.5 \mu\text{m}$), prolate in shape ($P/E = 1.55$) and with three narrowly fusiform ectoapertures c. $11.4 \mu\text{m}$ long. The tectum is psilate-punctate at the polar and equatorial regions (Fig. 3a), but distinctly reticulated along the equatorial line of the grain. This pollen morphology corresponds to the “*Pulmonaria obscura*-type” (CLARKE, 1977), which is typical of *Pulmonaria*, *Anchusa* subg. *Anchusa*, and *Nonea* sect. *Nonea* (BIGAZZI & SELVI, 1998). It is worth of note, however, that 3-aperturate pollen is uncommon within the *Pulmonaria obscura*-type, as it is found only in Iberian populations of the *Anchusa undulata* group (DÍEZ, 1994). Most of the other taxa within this pollen type have 4-aperturated grains. A second minor difference is that the equatorial reticulum in *A. limbata* is smooth, while it is finely sculptured-granulose in all the other taxa of *Anchusa* sensu lato.

The stigma of *A. limbata* corresponds to the *Pulmonaria-Anchusa* type, which is the most common one in the *Boragineae* (BIGAZZI & SELVI, 2000). It is capitate, slightly bilobed and well distinct from the stylar region (Fig. 3b). The receptive surface consists of lageniform (“flask-like”) papillae bearing an apical plate-like cap with 8-37 crenellated lobes. In early stages of flower development, the stigma is slightly exserted owing to the earlier growth of the style with respect to the corolla tube. At this stages, the papillae of the stigma are densely packed and the surface is not receptive. The corolla tube undergoes a later but rapid growth that results in the inclusion of the stigma between the faucal scales. During full anthesis the papillae are typically spaced out and the receptive surface is ready for the capture of pollen grains.

Anchusa limbata possesses strongly developed faucal scales, which are typical structures of the *Boragineae* originating as internal folds of the corolla tissue (SCHAEFER, 1942). In this species, the five scales close the throat and form a structure that protrude from the flower also due to the reduction of the corolla limb (Figs. 3c-d). The showiness of this structures is increased by its white color that contrasts with the bright red of the corolla. This chromatic effect probably plays a role in the biology of pollination by means of long-tongued *Diptera* or, secondarily, *Hymenoptera*. The cells of the scales are transformed into spreading, tubulose trichomes 0.8-1.0 mm long. Like in most other *Anchusa* species the external surface of the trichomes is finely scabrid because sprinkled with granular thickenings of cutin. This may play an important role in the transfer of pollen grains from the anthers to the body of visiting insects that brush against the scales while struggling to reach the nectar at the base of the ovary (SELVI & BIGAZZI, 1998).

The mericarpids of *A. limbata* are medium-sized, stocky, with a blunt lateral beak and a thick basal annulus bearing small, irregular teeth (Figs. 3e-f). The coat surface is covered by complex papillae that form tightly packed “insulae” with subcircular shape (Fig. 4a). Each complex papilla has a diameter of 25-30 μm and is formed by 5-12 spheroidal gemmae c. 5 μm in diam. lying side by side. When compared with other species of *Anchusa*, this pattern seems unique to *A. limbata* (see Figs. 4b-f). Most commonly the coat surface consists of warts (Fig. 4f) or a mixture of warts and papillae combined in different ways (Figs. 4b-e). The warts, with a diameter between 60 μm and 120 μm , can be conic-cuspidate (Figs. 4b-c, 4e) or rounded (Figs. 4d, 4f), close to each other (Fig. 4b) or fairly distanced (Figs. 4c, 4e-f). The papillae are smaller than the warts (10-15 μm diam.) and can be spheroidal (Figs. 4d-e) or characteristically lobed and “rosette” shaped (Figs. 4b-c).

Karyology

Determination of the somatic chromosome number was done using root tips in active growing of a transplanted plant currently cultivated in the Botanical Garden of the University of Firenze. Tips were treated with 0.05% colchicine, 2.5 h at room temperature and then fixed overnight in ethanol:glacial acetic acid 3:1. They were then rinsed in distilled water, hydrolyzed in 1N HCl at 60°C for 6-7 min., stained in lactopropionic orcein overnight, dissected and squashed on glass slides in a drop of 45% acetic acid.

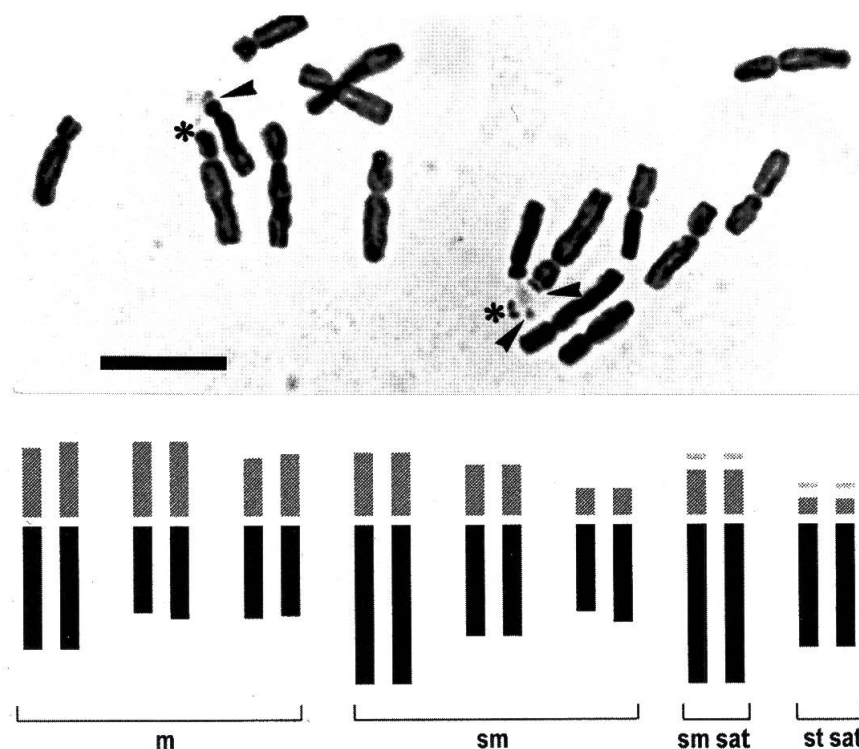


Fig. 5. – Metaphase chromosomes and karyotype idiogram of *Anchusa limbata* Boiss. & Heldr. ($2n = 16$). Arrows indicate satellites; asterisks indicates the submetacentric satellited chromosome and its detached satellite. Scale bar: 10 μ m.

The somatic chromosome number of *A. limbata* is $2n = 16$ (Fig. 5). Karyotype morphology is expressed by the following formula (LEVAN & al., 1964): $2n = 2x = 16$: 6 metacentrics + 6 submetacentrics + 2 satellited submetacentrics + 2 satellited subtelocentrics. The intrachromosomal asymmetry according to ROMERO ZARCO (1986) is 0.51, while the interchromosomal asymmetry, measured as the ratio standard deviation of chromosome length/mean chromosome length, is 0.17. Chromosomes are euchromatic and large-sized, with length ranging between c. 6 and 9.5 μ m.

The base number $n = 8$ is the most common one in *Anchusa* (BRITTON, 1951; LUQUE, 1983), and *A. limbata* is diploid like most congeneric taxa. Thus, this endemic is karyologically close to the rest of the group in terms of base number, ploidy level, chromosome size and karyotype morphology. With respect to most members of *Anchusa* subg. *Anchusa*, however, *A. limbata* is characterized by the presence of two heteromorphic pairs of satellited chromosomes. While one pair of satellited subtelocentrics is found in most species of *Anchusa*, the couple of satellited submetacentrics is a rare feature in the genus.

Distribution, ecology and conservation status

In his account of *Anchusa* L. for *Flora of Turkey*, CHAMBERLAIN (1979) cited the type collection plus the following record: “Antalya to Elmali, Gümüş bucağı”, *Cedrus libani* forest, 1100-1300 m, Chetik 1990 (E !). Examination of Chetik’s record, however, revealed that the somewhat poor specimen is not *A. limbata* but instead an incomplete sample of the *A. undulata* group with reduced and withered flowers. This finding was supported by our field researches in June 1997 around Elmali, the collection area cited in the label, that confirmed the occurrence of the only *A. undulata* subsp. *hybrida* (Ten.) Bég.

On the basis of our field and herbarium researches, *A. limbata* is currently known for only a very restricted area (36°57'228" N, 30°38'154" E) at the foot of the south-western Anatolian plateau, close to the coastal town of Antalya (Fig. 6). This area lies on the first slopes of the hill of Kepez, along the road from Antalya to Isparta, and is likely to be the same spot of the original collection by Heldreich. The population is found at an altitude of 230-250 m, in the understory of a native, thermomediterranean forest of *Pinus brutia* developed on calcareous soil. Here, *A. limbata* colonizes the understory sites with finely crumbled substratum, avoiding either the outcrops of hard limestone and the areas with thick layers of pine needles.

At the time of our visit to the site (June 2002), we estimated that the population consisted of less than 500 plants distributed with variable density over ca. 2 hectares. The abundant fruiting of the adult plants and the presence of numerous juvenile individuals suggested that the population was not in demographic decline. Indeed, *A. limbata* seems a "naturally" rare plant in a steady demographic state since long time.

However, the extreme localization in close proximity of a densely populated area which is rapidly expanding its urbanized perimeter, makes the future of this species quite uncertain. Thus, its inclusion in the Critically Endangered category of the IUCN Red List (IUCN, 2001) seems fully justified. A more practical action that can be adopted is the collection of seed for cultivation in Botanical Gardens and conservation in Germoplasm banks.

Taxonomic position and systematic relationships

For more than 150 years *A. limbata* has been known only through incomplete descriptions based on the type collection, and this explains the reason why botanists have written only a few things about it. The monographer M. Guşuleac gave contrasted taxonomic interpretations of this species. In his two main revisions of *Anchusa*, GUŞULEAC (1927, 1929) referred *A. limbata* to the genus *Alkanna* Tausch, without giving any explanation for this interpretation. Perhaps he was influenced by a remark by BOISSIER (1879: 156) who wrote: "facies Alkannae, sed nuculae

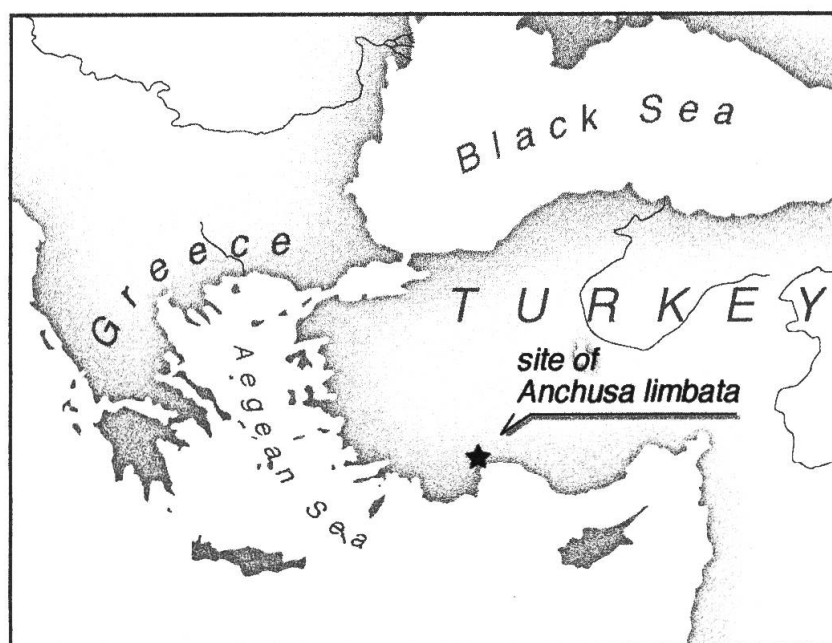


Fig. 6. – Punctiform distribution of *Anchusa limbata* Boiss. & Heldr. in SW Turkey.

juniores perforatae omnino videntur". However, *A. limbata* can not be placed in *Alkanna* because all species of this genus are typically devoid of faucal scales (like most members of the tribe *Lithospermeae*) and have unique mericarpids with curved shape. The lack of mature nutlets in Heldreich's collection probably facilitated such an erroneous interpretation. Indeed, in his work on the monotypic genera of the *Boragineae*, GUŞULEAC (1928) stressed the need for examining mature fruits for a better understanding of the systematics of *A. limbata*. In this work he referred it to the genus *Hormuzakia* Guşul. and made the new combination *H. limbata* (Boiss. & Heldr.) Guşul., though doubtfully ("species imperfecta"). The genus *Hormuzakia* was instituted for *H. aggregata* (Lehm.) Guşul., a species close to *Anchusa* but characterized by the congested-aggregate inflorescence with long bracts, the helmet shaped mericarpids with strongly toothed basal annulus, and the multiaperturate-scribulate pollen (BIGAZZI & al., 1999). Since none of these characters is found in *A. limbata*, neither this second interpretation can be accepted in the light of our data.

More recently, we showed the results of a phenetic analysis of *Anchusa*, where *A. limbata* was recognized as a monotypic group at a dissimilarity level of 1.4 (SELVI & BIGAZZI, 2000). Its position in the dendrogram was between the members of *Anchusa* subg. *Buglossellum* Guşul. (i.e. *A. stylosa* M. Bieb., *A. pusilla* Guşul.), and the more strongly diverging taxa of subg. *Buglossoides* Guşul. (*A. milleri* Spreng., *A. aegyptiaca* (L.) DC.), hence within the limits of *Anchusa* as currently accepted. In the light of palynological, chromosomal and fruit characters, this study confirms that the position of *A. limbata* is within *Anchusa*. Maintaining it in a separate subgenus as proposed by CHAMBERLAIN (1977) well accomodates its overall morphological distinctiveness with respect to the taxa of subg. *Anchusa* (i.e. *A. officinalis*, *A. undulata*), which is comparable with that shown by the species of the other subgenera *Buglossum*, *Buglossellum* and *Buglossoides*.

Apart from purely taxonomic aspects, it is difficult to make hypotheses on the possible phylogenetic relationships of this punctiform endemic. In terms of habit, pollen, and chromosomes characters, *A. limbata* is closer to the species of *A.* subg. *Anchusa* than to those of the other subgenera mentioned above. Also GUŞULEAC (1928) found characters of similarity with the genuine species of *Anchusa* in the morphology of the calyx and bracts.

In any case, the origin of the unique structure and colour of the corolla and the causes of such an extreme geographical localization appear obscure. We may assume that the reduction of the limb, the remarkable development of the faucal scales, the loss of warts from the coat surface of the fruits, and the presence of two heteromorphic couples of satellited chromosomes represent a set of "derivated" characters within *Anchusa* s.l. This does not sustain the hypothesis of a relict, paleoendemic species formerly distributed over a wider range and surviving today in a refugial area as a consequence of paleogeographic or paleoclimatic events. The above mentioned characters would suggest more an "in situ" origin through genetic drift involving small populations, possibly in relatively recent times. Considering the occurrence in the same spot of other notable endemics, among which the *Boraginaceae* *Alkanna macrophylla* Boiss. & Heldr., *Alkanna pinardii* Boiss. and *Onosma strigosissima* Boiss. we may assume that such area may have functioned as a centre of active, local speciation maybe due to geographical or ecological isolation.

Molecular studies on *Anchusa* s.l. currently on the way will hopefully provide new insights on this interesting aspect.

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