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A REAPPRAISAL OF THE GENERIC STATUS OF *GASTROCOTYLE*, *HORMUZAKIA* AND *PHYLLOCARA* (*BORAGINACEAE*) IN THE LIGHT OF MICROMORPHOLOGICAL AND KARYOLOGICAL EVIDENCE

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The results of karyological analyses and SEM micromorphological observations on vegetative and reproductive structures of the critical genera *Gastrocotyle*, *Hormuzakia* and *Phyllocara* (*Boraginaceae*: *Boragineae*) are presented and discussed in relation to their taxonomic position within the tribe. Each of these monotypic genera is characterized by distinctive features in stigma morphology, papillar pattern, structure of faucal scales, development of the hairy annulus at the base of the corolla tube and ornamentation of the mericarp coat surface. *G. hispida* and *H. aggregata* showed $2n = 16$ and complements of medium-sized and very large chromosomes, respectively. *P. aucheri* was characterized by a tetraploid set of 32 chromosomes including a group similar to those of *Anchusa* and a group closer to those of *Nonea* in terms of size and centromeric position. This species could have an allopolyploid origin, resulting from the hybridization of members of these two genera. Along with distinctive traits in inflorescence structure, fruit shape and pollen morphology, karyological and micromorphological characters determine sharp phenetic discontinuities from *Anchusa*, from which these plants should be kept generically separated. At the same time, the presence of relevant autapomorphies in each of the three genera prevents them from being merged in a single genus.

Keywords. *Boragineae*, karyology, pollination mechanisms, style micromorphology, systematics.

INTRODUCTION

In a recent survey of pollen morphology in the *Boraginaceae* tribe *Boragineae* (Bigazzi & Selvi, 1998), the finding of 14 different palynological types only partially corresponding to the currently accepted taxonomic units shed new light on the systematic relationships within this critical tribe. One of the key-points was that *Anchusa* L. sensu lato is highly polymorphic from a palynological viewpoint, in terms of shape of grains, tectum sculpture, stereostructure and number of apertures. Most species of this large genus were included within the basic *Pulmonaria obscura*-type recognized by Clarke (1977), characterized by a psilate-punctate tectum with usually four apertures and a reticulated equatorial band. However, *Anchusa* s.l. included six distinct pollen morphologies, three of which were: the *A. hispida*-type, with spheroidal grains and 8–9 rhomboidal apertures covered by conic processes; the *Nonea vesicaria*-type,

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which included *A. aggregata*, with a scrobiculate tectum and 6 7 apertures; and the *A. aucheri*-type, with prolate grains rugulate at the mesocolpia and punctate at the apocolpia and only three rectangular apertures. *Anchusa hispida*, *A. aggregata* and *A. aucheri* are annual relatives of *Anchusa* occurring in arid habitats of the south-eastern Mediterranean basin and Irano-Turanian territory, whose taxonomic position has always been uncertain, especially after their separation into the monotypic genera *Gastrocotyle* (Bunge) Benth. & Hook. (Bentham, 1876), *Hormuzakia* Guşul. (Guşuleac, 1923, 1928, 1929) and *Phyllocara* Guşul. (Guşuleac, 1928, 1929), respectively. Palynological data (see also Dìez, 1983, 1994; Perveen *et al.*, 1995) therefore supported the view of the authors (Popov, 1953; Tackhölm, 1954; Riedl, 1963, 1967, 1968; Kazmi, 1971; Feinbrun-Dothan, 1978; Qaiser, 1979) who kept the above three entities separated at the genus rank. In the more recent taxonomic literature, however, *Anchusa* is treated in a broad sense despite its internal heterogeneity, these genera being treated as heterotypic synonyms of *Anchusa* (Chater, 1972; Greuter *et al.*, 1984; Heller & Heyn, 1986 except *Gastrocotyle*) or included in it as separate subgenera (Chamberlain, 1977, 1979). These contrasting taxonomic opinions arise from an objective difficulty connected to a reticular variation of those morphological traits which have a potential systematic value, but also from a limited knowledge currently available on these little-known plants of the eastern Mediterranean flora. As a complement to the palynological survey, we focus here on karyology and micromorphology of vegetative and reproductive structures in order to extend our comparative basis for more objective taxonomic evaluations. Previous studies on the *Boraginaceae* (Strey, 1931; Smith, 1932; Britton, 1951; Heslop-Harrison, 1981; Luque, 1983, 1989, 1995; Bigazzi *et al.*, 1997; Selvi *et al.*, 1997; Selvi & Bigazzi, 1998) showed that these aspects, together with palynology, have a great potential of information on the systematic relationships, possible origins and taxonomic status of several critical groups or single entities.

MATERIAL AND METHODS

Gastrocotyle, *Hormuzakia* and *Phyllocara* were described as monotypic genera, consisting respectively of the species: *G. hispida* (Forssk.) C.B. Clarke, in Hook. f., *Fl. Brit. Ind.* 4: 168, 1885 (Fig. 1); *H. aggregata* (Lehm.) Guşul., *Publ. Soc. Nat. Roman.* 6: 8, 1923 (Fig. 2); *P. aucheri* (DC.) Guşul., *Bull. Fac. Şti. Cernauţi* 1: 120, 1927 (Fig. 3).

The provenance of the material used in this study is as follows:

G. hispida: Israel, northern Negev, c.6km NW of Yeroham, humid desert sands along a uadi; 17 iv 1996, *Bigazzi & Selvi*.

H. aggregata: Israel, maritime dunes c.2km S of Ashqelon, 14 iv 1996, *Bigazzi & Selvi*; Israel, sandy dunes of Cesarea, 16 iv 1996, *Bigazzi & Selvi;* Italy, southern Sicily, maritime sands of Manfria, 6 v 1997, *Bigazzi & Selvi*; Turkey, maritime sands c.2km W of Alanya, 7 vi 1997, *Bigazzi & Selvi.*

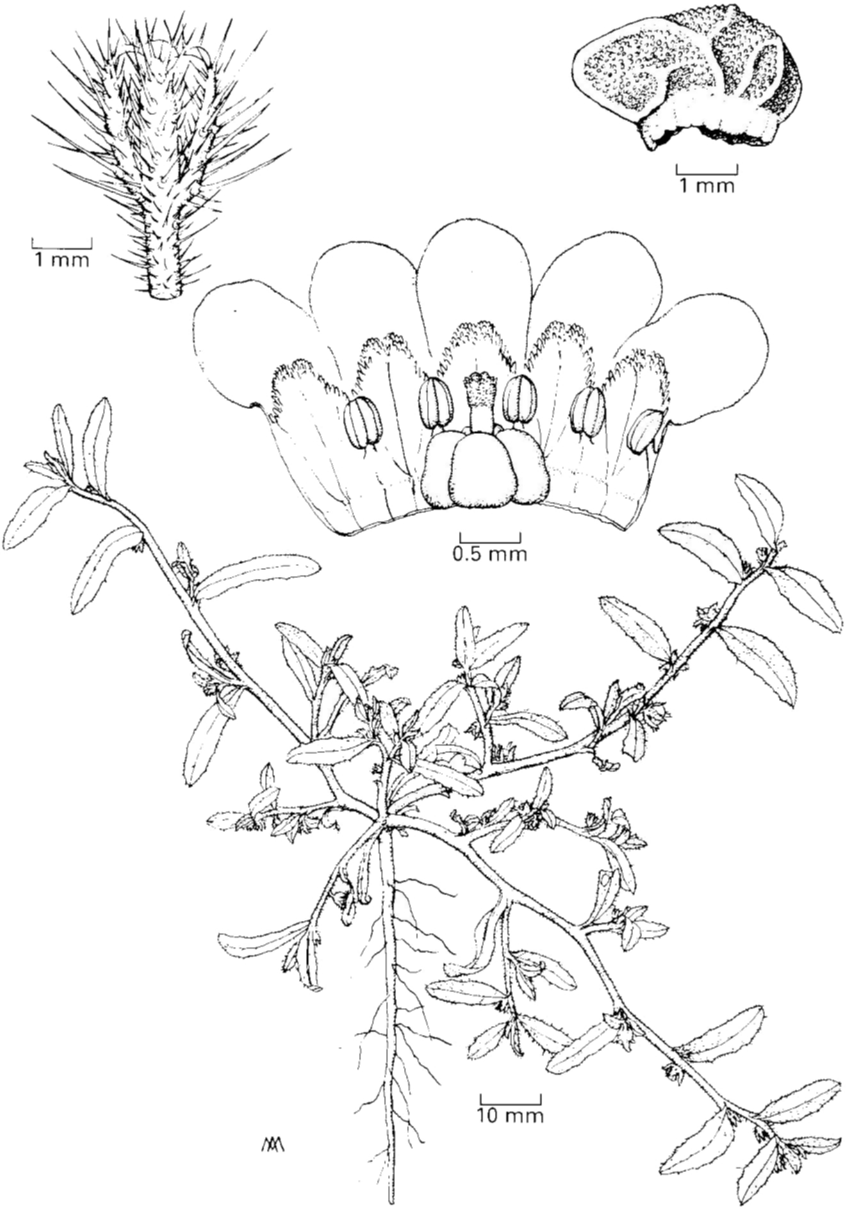


FIG. 1. *Gastrocotyle hispida*: habit, flower with calyx, open corolla showing faucal scales, stamens and pistil, and mericarp in lateral view.

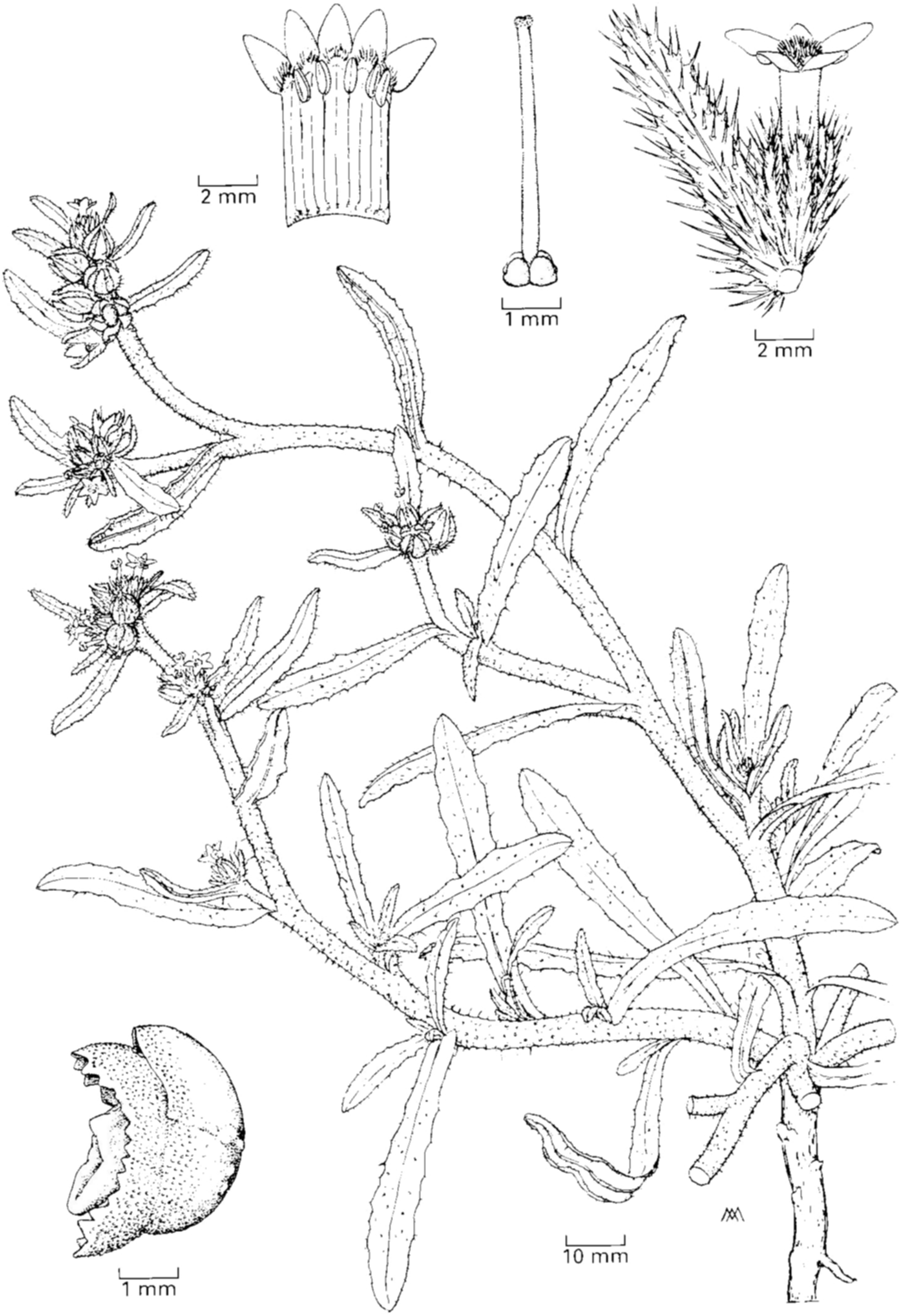


FIG. 2. *Hormuzakia aggregata*: habit; flower with bract and calyx; open corolla with scales and anthers, style and ovary, and mericarp in lateral view.

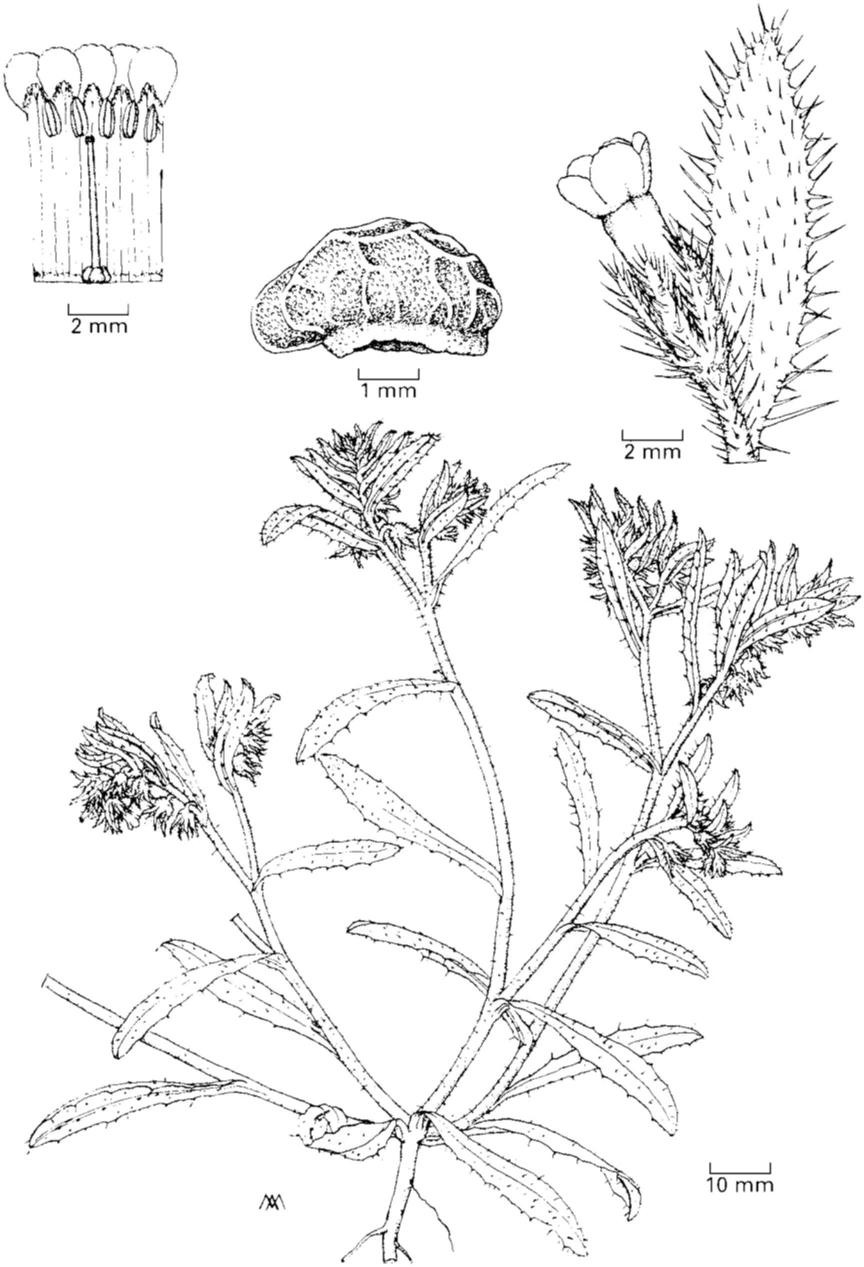


FIG. 3. *Phyllocara aucheri*: habit, flower with bract and calyx, open corolla with faucal scales, stamens and pistil, and mericarp in lateral view.

P. aucheri: Turkey, calcareous screes near Gözne (Mersin), c.1000m a.s.l., 5 vi 1997, *Bigazzi & Selvi*. Turkey, screes near Karamustafa, Gümüshane, c.1300m, 14 vi 1998, *Bigazzi & Selvi*.

All voucher specimens from the above localities are currently kept in FI. Plant drawings were made from living plants in the case of *A. aggregata* and *A. aucheri*, while *A. hispida* was illustrated from the Negev specimens.

For micromorphological analyses, we collected flowers in different developmental stages from live plants in the field, which were then fixed and preserved in 2% glutaraldehyde in phosphate buffer 0.1M (pH 7.2) at 4°C. The material was then dehydrated in acetone series, critical point-dried with liquid CO_2, mounted on aluminium stubs, coated with gold and observed by means of a Philips XL 20 Scanning Electron Microscope. Mericarps were directly mounted on stubs and sputter-coated with gold.

Karyological analyses were carried out on mitotic metaphase plates of meristematic cells taken from roots of seedlings. The seeds of *H. aggregata* and *G. hispida* germinated without any preparatory treatment, but in the case of *P. aucheri* the seeds germinated only after a chilling treatment of one month at 3–4°C. After a pretreatment of about 2h in a 8-hydroxyquinoline solution, the material was fixed in Carnoy, hydrolysed in HCl 1N at 60°C for 6min and stained with lactopropionic orcein (18h). Idiograms were prepared on enlarged prints of the original micrographs. Measurements and values were computer-processed in order to obtain chromosome ordering and homologue recognition, karyotype formula (Levan *et al.*, 1964) and the intra-(A_1) and interchromosomic (A_2) asymmetry indices proposed by Romero Zarco (1986).

RESULTS

Indumentum

A typical feature of *Anchusa* is the more or less dense indumentum covering vegetative and reproductive structures. SEM observations reveal that in all three species the indumentum consists of four types of trichomes varying in density and distribution. The first type is a stout, pluricellular trichome consisting of a pointed head cell c.1mm long or more, inserted on a prominent tubercle of 15–20 basal cells (Fig. 4a–c). The surface of the head cell is finely scabrid as a result of the presence of fine, granular thickenings on the wall (Fig. 4c). These trichomes are especially abundant along stems, margins and nerves of leaves, bracts and sepals of *Hormuzakia aggregata* and *Phyllocara aucheri*, while they are sparser in *Gastrocotyle hispida*. Mixed with this type, there are similar eglandular hairs, differing in their shorter head cells (0.2–0.5mm) and basal tubercles of usually only 5–6 cells (Fig. 4d), although intermediate forms are sometimes present. In all three species these hairs are distributed all over the plant, except the internal surface of sepals. The relative density of these two types of trichome contributes in determining the consistency of the indumentum, which can be from relatively short and soft (*G. hispida*, Fig. 4a) to

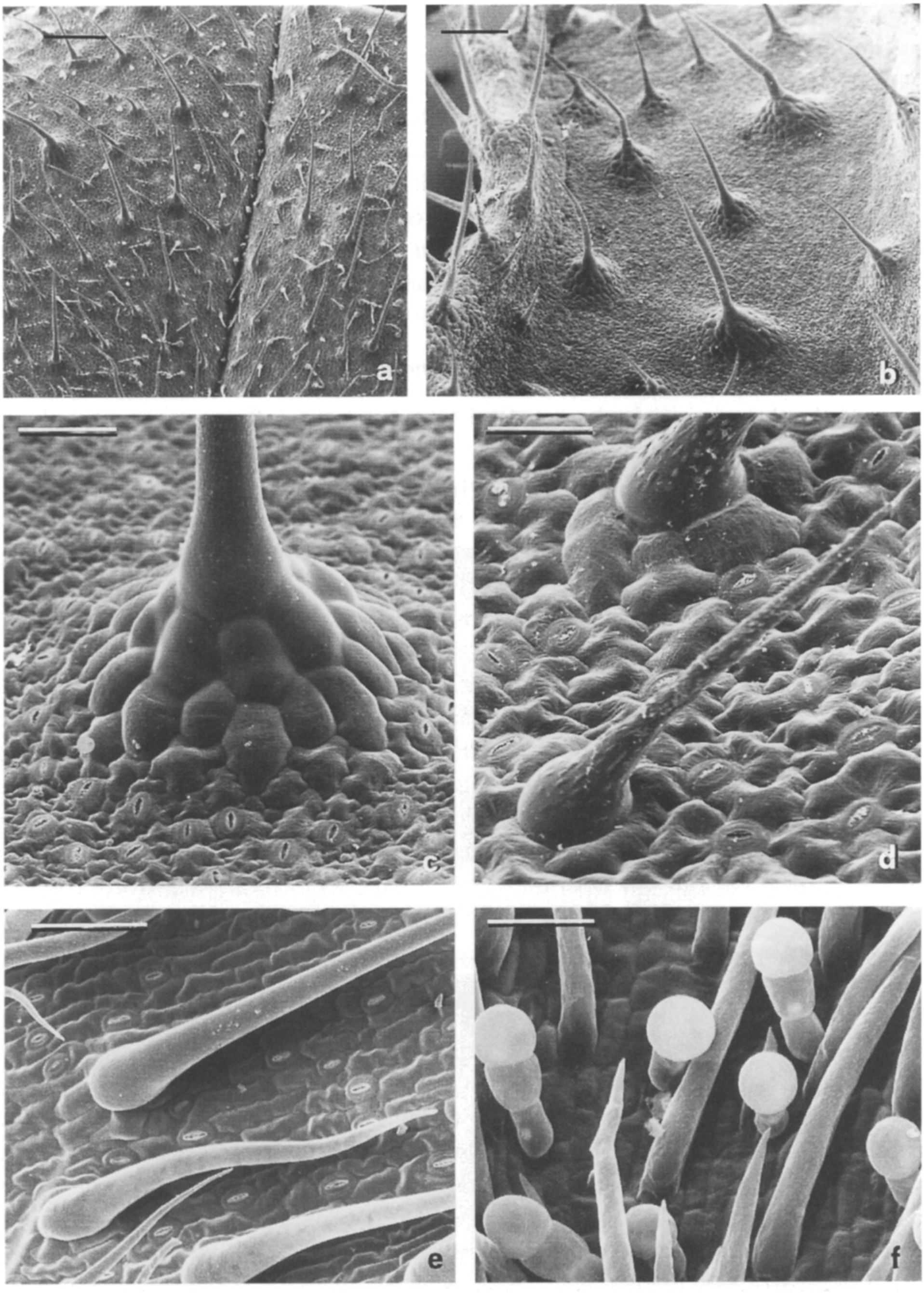


FIG. 4. SEM micrographs of trichomes on leaves and sepals. *G. hispida*: a, adaxial leaf surface with trichome types 1 and 2; d, internal surface of sepals with type 2 trichomes. *H. aggregata*: b, abaxial leaf surface with type 1 trichomes; f, internal sepal surface with glandular hairs. *P. aucheri*: c, type 1 trichome magnification of basal tubercle on abaxial leaf surface; e, internal sepal surface with type 3 trichomes. Bars: a,b 0.5mm; c,e 0.1mm; d,f 50μm.

long and almost pungent (*H. aggregata,* Fig. 4b). A third type of trichome consists of an eglandular, unicellular head cell directly inserted on the epidermal tissue, without basal cells (Fig. 4e). Unlike the previous types, the trichome surface is devoid of granular thickenings and perfectly smooth. In all three species, this hair type is found exclusively on the internal surface of sepals, where the hairs are appressed to the epidermal tissue. Finally, there are small, glandular hairs consisting of a basal cell, one or two stalk cells, and a spherical head cell (Fig. 4f). In *G. hispida,* this trichome type is restricted to the basal portion of the internal surface of sepals, while in *H. aggregata* and *P. aucheri* it is found also on their external surface.

Inflorescences and flowers

Each of the three species is characterized by a distinctive inflorescence morphology, that can each be regarded as a different modification of the basic scorpioid type occurring in the rest of *Anchusa.* A common trait shared by *Hormuzakia aggregata* and *Phyllocara aucheri* is a considerable shortening of the inflorescence axis, while *Gastrocotyle hispida* is characterized by a production of single flowers in the axils of the cauline leaves (Figs 1–3). The two former species produce aggregated inflorescences, usually with short, compact branches not elongating in fruit, typically provided with foliaceous bracts. In *P. aucheri* the bracts are erecto-patent, much longer than the flowers and densely alternate on the two sides of the inflorescence, in such a way as to hide most of the small corollas (Fig. 3). It was mainly on the basis of these characters, along with habit and fruit morphology, that Guşuleac (1928, 1929) proposed the generic separation of *Phyllocara* from *Anchusa.* In a strict sense *G. hispida* does not produce true inflorescences, because the flowers are isolated and distanced at the axils of leaves along the diffuse stems. Within *Anchusa* s.l. a similar habit and arrangement of flowers is found only in *A. littorea* Moris, a psammophytic species endemic to western Sardinia (Valsecchi, 1976).

Compared with *Anchusa,* the flowers of the three species appear reduced in size, especially in limb width. In *G. hispida* the corolla is included in the calyx owing to the shortened tube, while *H. aggregata* and *P. aucheri* have exserted corollas because of the longer tubes. In *H. aggregata* the limb is substantially rotate, the lobes being patent, while in *P. aucheri* and *G. hispida* the limb is narrowly infundibuliform owing to the (sub)erect lobes. This contributes to the low attractiveness to insects of the two latter species, which is determined also by the small size and the pale colour of the flowers, as well as to their usually reclined position. By contrast, *H. aggregata* bears erect flowers of cobalt-blue colour, which are frequently visited by territorial Hymenoptera, mostly bees, and long-tongued Diptera (pers. obs.).

All three species have five epicorolline stamens, with short filaments and small, ovoid anthers placed in the upper part of the tube and partly overlapping the faucal scales.

The tissue at the base of the corolla tube is more or less thickened along the line of attachment to the receptacle. In *H. aggregata* this tissue portion is differentiated into a densely hairy annulus (Fig. 5a) formed by 10 thickenings whose cells develop into long, acute, unicellular trichomes with a smooth surface (Fig. 5b). The annulus is placed in front of the nectaries at the base of the ovary, and has the probable function of holding the nectar to slow down evaporation induced by the arid habitat. *G. hispida* and *P. aucheri* are devoid of a continuous annulus (Fig. 5c): in the former there are only five, widely separated thickenings made up of cells developing into trichomes much shorter than in *H. aggregata* (Fig. 5d), whereas in *P. aucheri* only a few cells tend to produce inconspicuous trichomes (Fig. 5c). This incomplete development of a strategy to save the nectar in the two latter species may represent further evidence of their probable shift to autogamic breeding systems shown in their inconspicuous, unattractive flowers.

Faucal scales

These typical structures of many *Boraginaceae* are internal folds of the corolla tissue with a vascular system derived from the corolla parenchyma (Lawrence, 1937). There

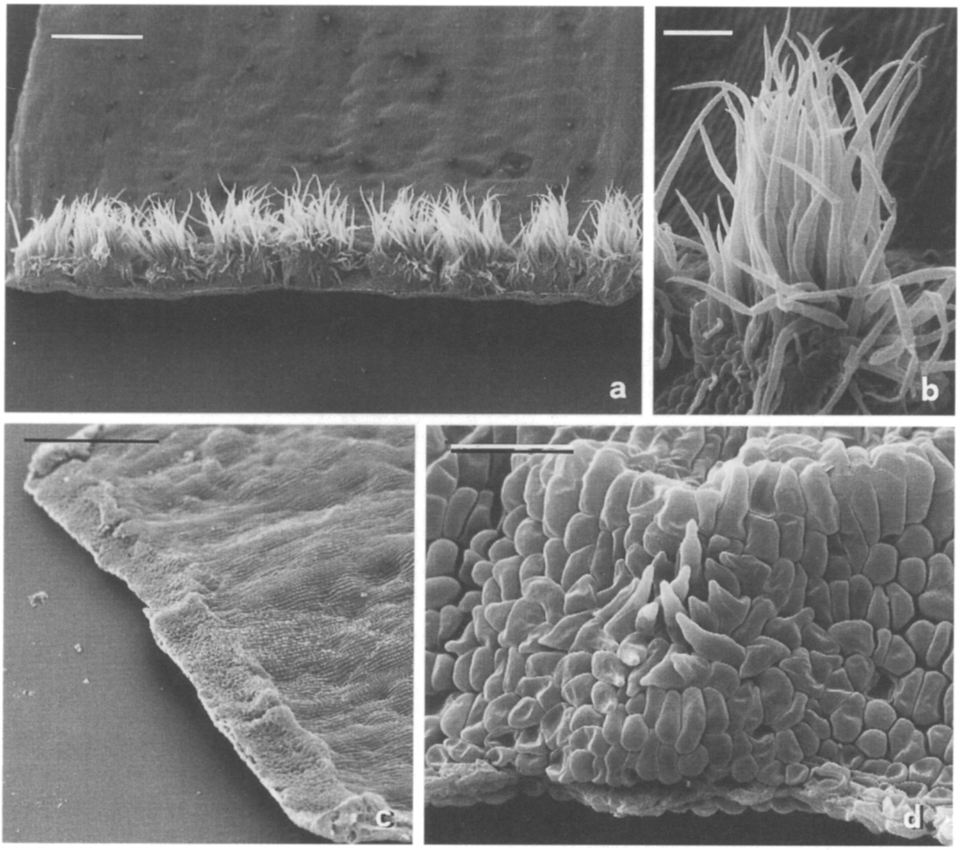


FIG. 5. SEM micrographs of the basal thickenings and hairy annulus of the corolla tube. a,b, *H. aggregata*; c, *P. aucheri*; d, *G. hispida*. Bars: a,c 0.5mm; b,d 0.1mm.

are several types of scales (Schaefer, 1942) but, when present, they are always placed at the junction between the tube and the limb, where they play a role in pollination mechanisms and probably in the avoidance of self-fertilization of many species.

Gastrocotyle hispida has small, erect and slightly bilobed scales of pale colour, formed by a basal part of corolline tissue and by a central portion of papillose cells differentiated into short trichomes along the margins (Fig. 6a). Both the papillose cells, and to a lesser extent, the marginal trichomes, show microgranules distributed all over the surface (Fig. 6b).

The scales of *Hormuzakia aggregata* are white, erect, and slightly exserted so that they close the throat completely. They are formed by a small, basal portion of corolla tissue bearing dense, unicellular trichomes 0.8 1.0mm long along the margins (Fig. 6c). These faucal trichomes have a tubulose and obtuse shape, and their surface is finely scabrid because of a sprinkling of dense, granular thickenings of cutin (Fig. 6d). This feature was observed also in the Sardinian endemic *Anchusa formosa* Selvi, Bigazzi & Bacchetta (Selvi *et al.*, 1997), where it was thought to play a role in the pollination mechanism. In this species, pollen grains released by the anthers become temporarily enmeshed in the scales owing to the rough surface of the faucal scale cells; the grains are then probably loaded onto the body of visiting insects that brush against the scales while struggling to reach the nectar secreted at the base of the ovary.

Phyllocara aucheri shows small, erect scales with a peculiar yellow-green colour. They are formed by three types of cells. Whereas the central part is made up of flattened, papillose cells, along the lower margins there are large, swollen cells with a subspherical shape that intergrade into short, conical trichomes forming two apical lobes oriented outwards (Fig. 6e). Also in this case, the surface of the scale cells show a dense, fine granulation of cutin (Fig. 6f).

Style, stigma and ovary

Stigmatic structure and papillar pattern show an extraordinary range of variation within the *Boraginaceae*, probably reflecting different adaptive strategies in pollen capture and in the early nurture of the male gametophyte (Heslop-Harrison, 1981). Owing also to their role in determining reproductive barriers and in controlling self-incompatibility systems, stigma characteristics are considered to have relevant significance in the systematics of the *Boraginoideae* (Heslop-Harrison, 1981).

In all three species studied the style is simple, gynobasic, erect and straight. In *Gastrocotyle hispida* it is rather short (2 3mm), consistent with the reduced size of the flowers (Fig. 7a); it reaches the height of the anthers but remains well below the level of the faucal scales (Fig. 1). The stigma is rather peculiar in terms of both shape and papillar pattern and differs from all 22 genera of *Boraginaceae* investigated by Heslop-Harrison & Shivanna (1977) and Heslop-Harrison (1981). In particular, it is strongly differentiated from genuine species of *Anchusa*, including *A. officinalis*,

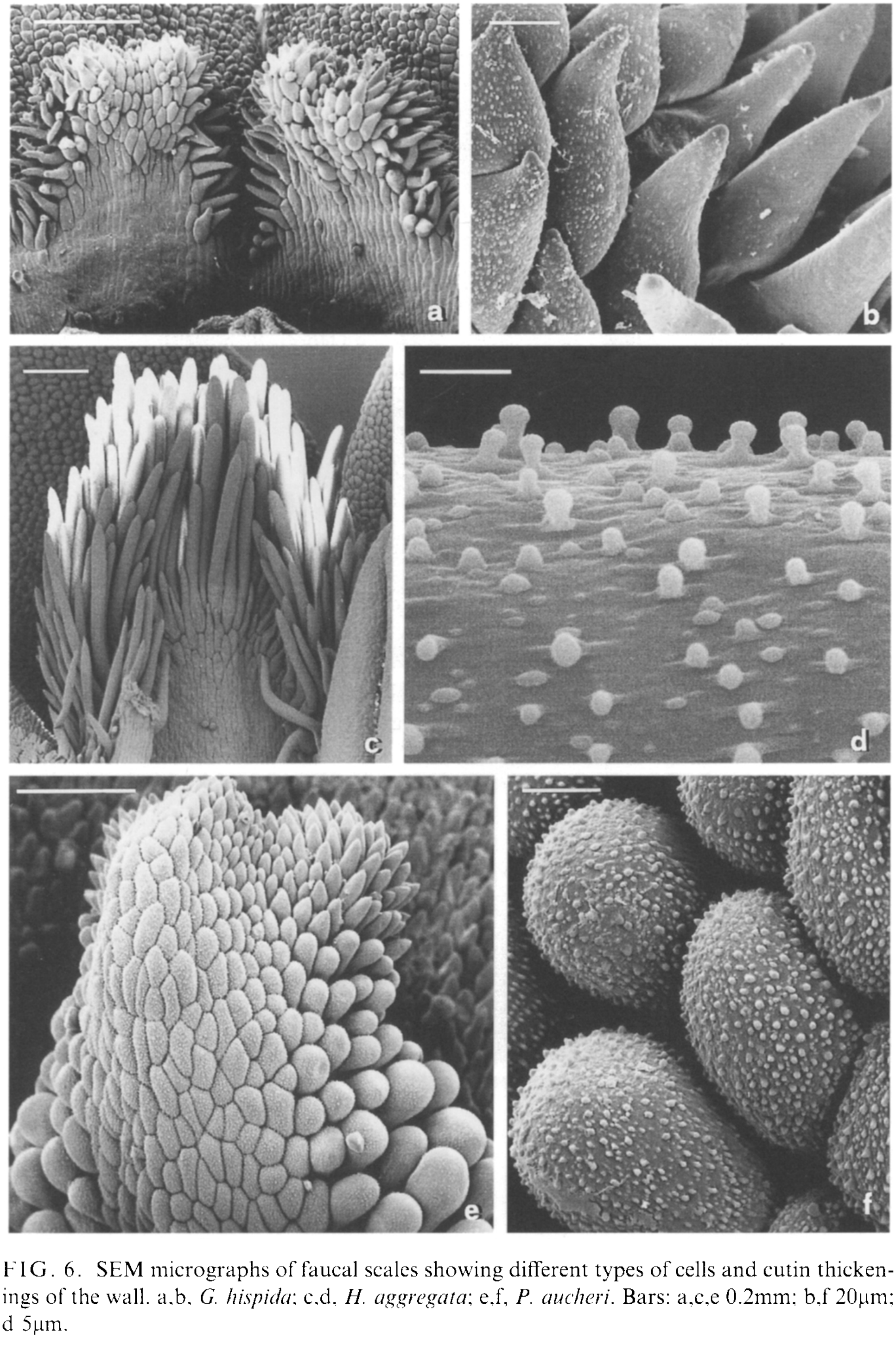
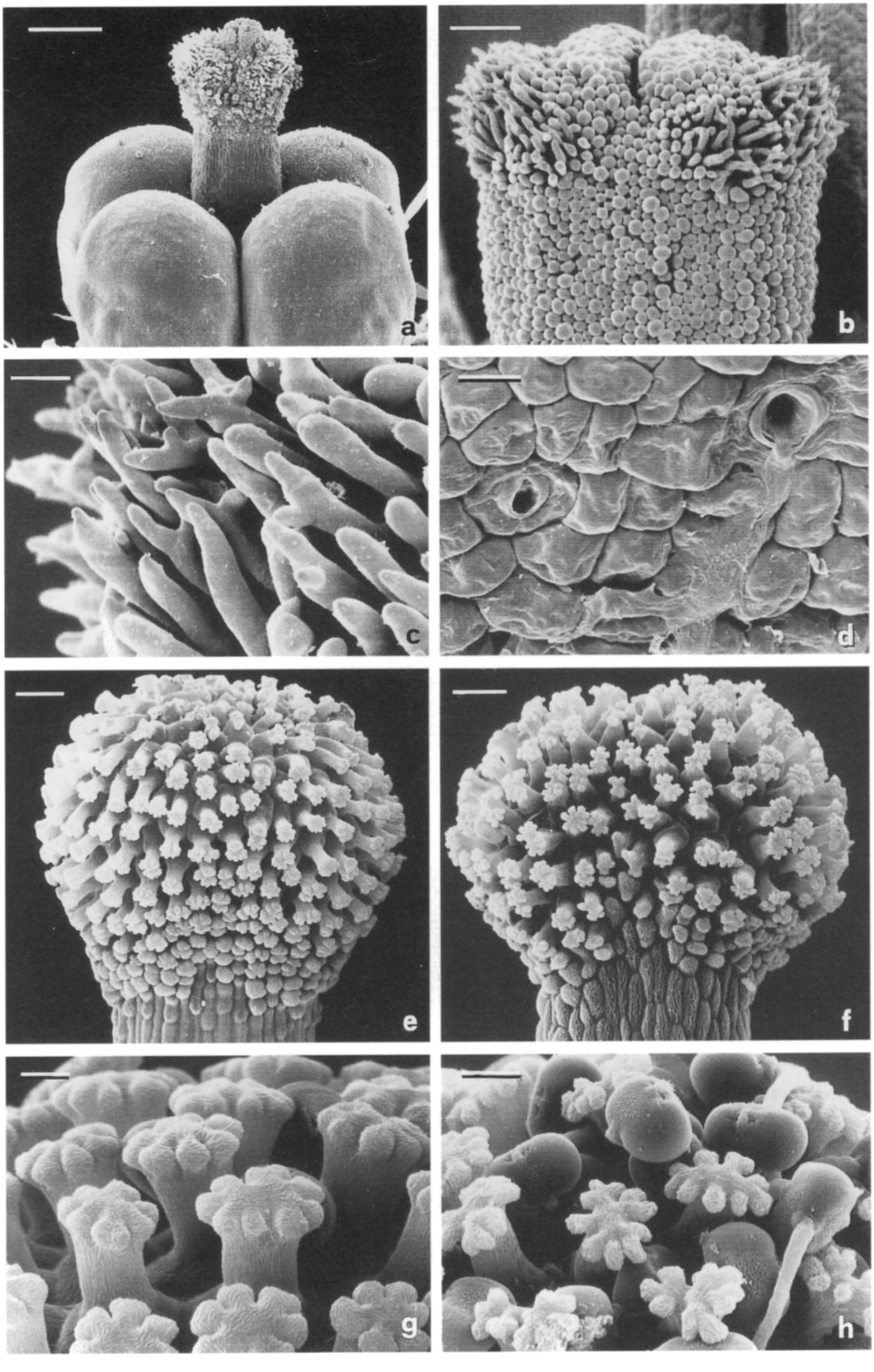


FIG. 6. SEM micrographs of faucal scales showing different types of cells and cutin thickenings of the wall. a,b, *G. hispida*; c,d, *H. aggregata*; e,f, *P. aucheri*. Bars: a,c,e 0.2mm; b,f 20µm; d 5µm.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the type species of the genus (Selvi *et al.*, 1996). The receptive surface is essentially cylindrical, and bears at the tip a protrusion showing an apical notch (Fig. 7a,b). Curiously, this apical stigmatic portion consists of spherical cells not differentiated into functional papillae. Well-developed papillae are found only below the apical portion, where they form a sparse, discontinuous ring around the stigma. Each papilla has a smooth surface and shows an elongated shape oriented horizontally (Fig. 7c); in some cases the papillae are distally branched. Unlike most of *Anchusa* s.l., the stigmatic papillae of *G. hispida* are devoid of the apical cap, that represents a lock and key mechanism for preventing receipt of foreign pollen with different shape or size (Heslop-Harrison 1981). Below the functional papillar ring, the stigma shows a large portion of tissue of unknown function, consisting of spherical cells with a smooth surface (Fig. 7b). However, the scarcity of well-developed papillae, their discontinuous arrangement, and their simplified structure, give to the papillar pattern of *G. hispida* a regressive character, which might be associated with the shift to an autogamic breeding system. This hypothesis is not supported by the fact that each locule of the ovary shows at the base a few nectariferous ducts, although it remains to be ascertained whether this is a residual or a still functional feature.

In *Hormuzakia aggregata* the style is included in the corolla tube, where it reaches the height of the faucal scales. The ovary locules at the base of the stigma are provided with many secretory ducts producing abundant nectar, which is the reward for pollen vectors (Fig. 7d). According to the classification of Heslop-Harrison (1981), the stigma of *H. aggregata* is of the DPU-type, namely dry and papillate with unicellular papillae. The style bears a capitate-globose stigma head (Fig. 7e), a shape substantially different from the bilobed forms occurring in *Anchusa* s.l. The papillae are erect and widely separated one from the other and each of them has a lageniform (flask-like) shape, with a swollen base and neck surmounted by a plate-like cap bearing 8 11 radial crenulations (Fig. 7g). In comparison with other *Anchusa* species (cf. Heslop-Harrison & Shivanna, 1977; Heslop-Harrison, 1981; Bigazzi *et al.*, 1997; Selvi *et al.*, 1997), the neck of the papillae appears substantially longer and the cap lobes are less developed.

In *Phyllocara aucheri* the style is as long as the corolla tube (c.5.5mm) reaching the bases of the scales and anthers (Fig. 3). As in the other species, it arises from the middle of the ovary, which is formed by four subspherical locules provided at the base with numerous nectaries. In view of its bilobed structure with globose lobes, the stigma shows a closer resemblance to *Anchusa* (Fig. 7f) than the two

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FIG. 7. SEM micrographs of style, stigma and ovary. *G. hispida*: a, gynoecium; b, stigma; c, lateral stigmatic papillae. *H. aggregata*: d, nectariferous ducts; e, stigma; g, papillae. *P. aucheri*: f, stigma; h, papillae with germinating pollen grains. Bars: c,d,g 10µm; a 0.2mm; b,e,f 50µm; h 20µm.

former species. In comparison with *H. aggregata*, the stigmatic papillae show a shorter neck and a wider cap with longer lobes (Fig. 7h) as found in most typical Anchusas.

Mericarps

Owing to the wealth of shapes and surface ornamentations, the fruits (schizocarp of four mericarps) of the *Boraginaceae* have been widely used for taxonomic purposes (Lawrence, 1937; Shmida, 1978; Hilger, 1985; Hilger *et al.*, 1985; Al-Shebhaz, 1991), and studied with regard of evolutionary trends (Johnston, 1924; Fabre & Nicoli, 1974), ecological implications and dispersal strategies (Fabre, 1966).

The three species here considered share a basic ovoid, curved-reniform shape and a ventral attachment on the gynobase, both peculiar characters within the *Boragineae*. As already noted by De Candolle (1846) and Boissier (1879), an additional common feature of these plants is that they usually bear only one or two mericarps per flower at maturity, the others being precociously abortive (Fig. 8a). This fact may be related to the relatively large size of the fruits in respect to the size of the plants, to their brief life-cycles and to the generally arid habitats in which they grow.

The mericarps of *Gastrocotyle hispida* have a lateral, obtuse beak, are provided at the base with a thin annulus and bear in the upper part a reticulum of blunt ridges (Fig. 8b). The coat surface is densely covered by lobed papillae in which are dispersed spherical protuberances with a rugose surface (Fig. 8c).

The peculiar structure of the mericarps of *Hormuzakia aggregata* was the main reason for its separation at the generic rank by Guşuleac (1923, 1928, 1929). In the ventral part each nutlet bears a plicate-dentate, not incrassate, skirt-like annulus (Fig. 8d,e). A deep horizontal cleft at one side of the nutlet divides the basal annulus from the upper portion, which has a concave, pointed apex. This makes the nutlet resemble the visor of a helmet. The coat surface is devoid of papillae but is densely covered by conical protuberances (Fig. 8f). The protuberances are especially dense over the basal annulus and become progressively sparser toward the upper part of the nutlet, which shows an almost smooth surface and obscure reticulum of low, blunt ridges. Field observations suggested that the presence of the numerous teeth and the rough surface of the basal annulus have probably two functions: to maximize anchorage of fruits in the unstable maritime sands and to offer ants a hold for seed dispersal. As temperature rises during the day, the fruits fall to the ground because of opening of calyces and detachment of the strophioles caused by dehydration of the tissue. In the warmer hours of the day the plants of *H. aggregata* become a centre of intense activity by ants, that pick up the mericarps by the basal annulus and carry them to their underground nests (pers. obs., Fig. 9).

FIG. 8. SEM micrographs of mericarps with particulars of the coat surface. a c, *G. hispida*; d f, *H. aggregata*; g,h, *P. aucheri*. Bars: a,b,d,e,g 0.5mm; c,f,h 20μm.

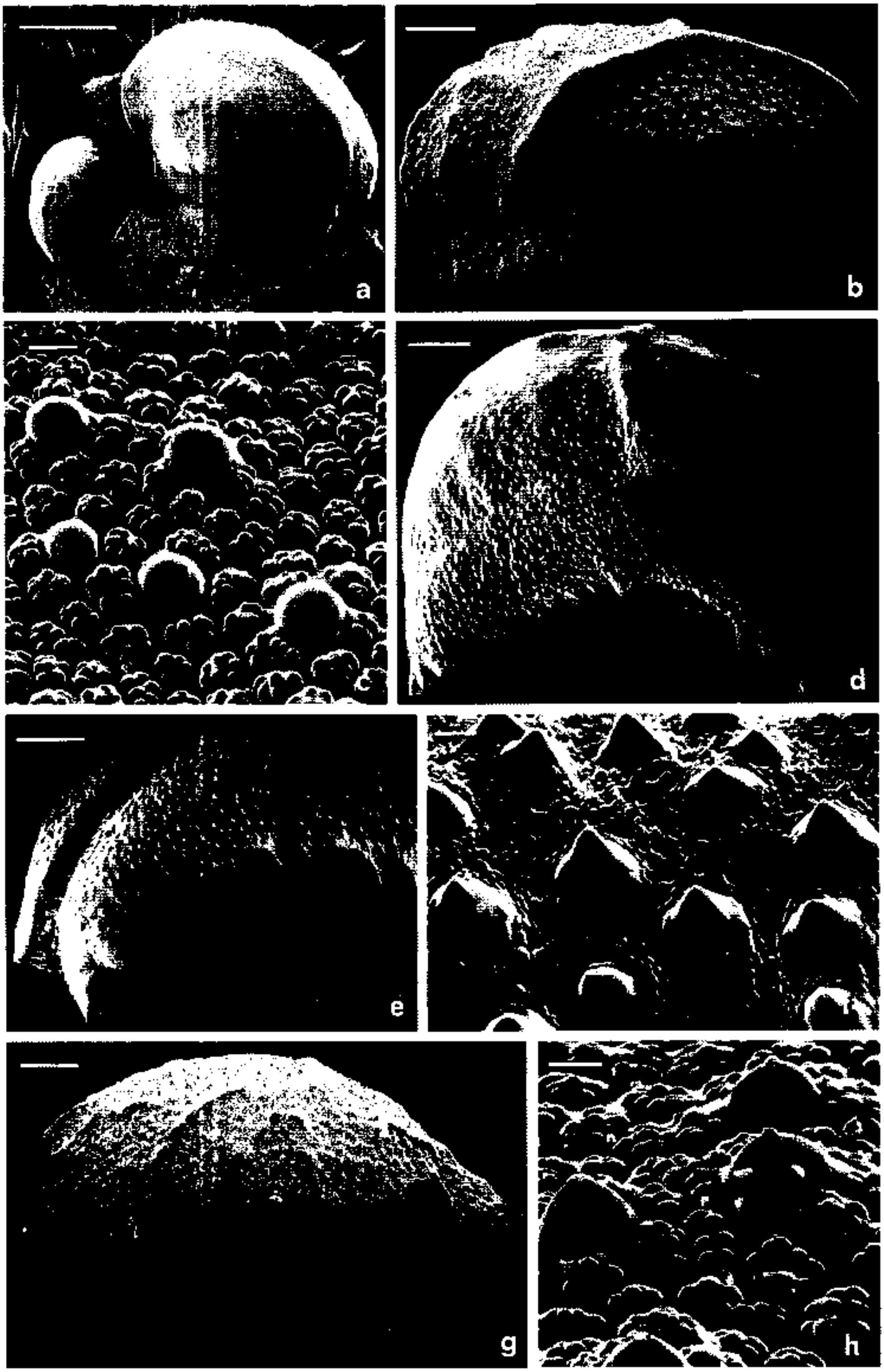
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FIG. 9. Dispersal by ants of a mericarp of *H. aggregata* on beaches in southern Sicily.

Phyllocara aucheri has also transversely reniform mericarps attached ventrally on the gynobase (Fig. 8g). They are morphologically close to those of *G. hispida*, from which they differ in their larger size, their more pronounced reniform shape and their very reduced basal rim without denticulation. As in *G. hispida*, the mericarps show a prominent reticulum of blunt ridges and a coat surface consisting of dense, lobed papillae. However, the tubercles dispersed on the coat surface show the same conical shape of those found in *H. aggregata* (Fig. 8h).

Karyology

Only a few metaphasic plates could be obtained in *Gastrocotyle hispida*. They showed a set of $2n=16$ chromosomes arranged according to the formula (Fig. 10a,a′): $2n=2x=16=4\ M+4m+6\ sm+2$ st, with mean chromosome size of c.5.2 × 2μm. The A_2 asymmetry, measured as the ratio standard deviation of chromosome lengths/mean chromosome length, was relatively low, 0.12, as a consequence of the small difference between the longer (c.5.7μm) and the shorter (c.3.5μm) chromosomes. The A_1 asymmetry was even lower, because of the presence of only two subtelocentric chromosomes. Unlike in most *Anchusa* s.l. species, no satellites were observed.

Our observation does not confirm the only chromosome count published for *G. hispida*, $2n=20$, obtained on plants from the Maidan province, Afghanistan (Podlech & Bader, 1974). The existence of distinct cytotypes can not be excluded, also in consideration of the vast distribution range of *G. hispida*, but additional studies are necessary to ascertain the real cause of such different numbers. Within the *Boragineae* $2n=16$ is one of the most common numbers, for example in *Anchusa* s.l. (Britton,

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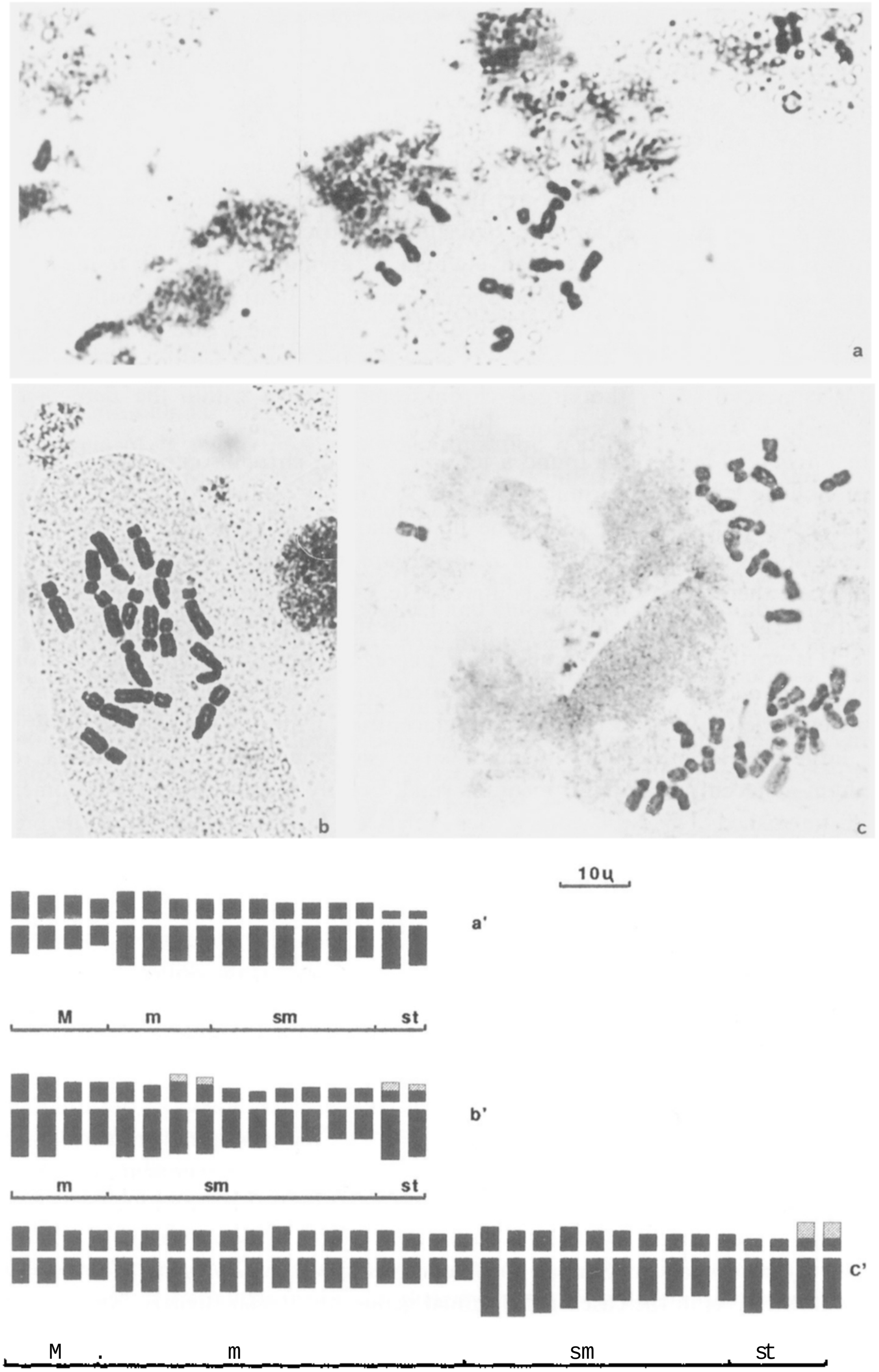
FIG. 10. Metaphasic chromosome plates and idiograms. a,a′, *G. hispida* ($2n=16$); b,b′ *H. aggregata* ($2n=16$); c,c′, *P. aucheri* ($2n=32$). Satellites are indicated by arrows. Bar: 10μm.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1951; Valsecchi, 1976; Luque, 1983; Bigazzi *et al.*, 1997; Selvi & Bigazzi, 1998), while $x=10$ is known only for a few species of *Nonea* and *Symphytum* (see Luque, 1995).

Hormuzakia aggregata was characterized by a diploid set of chromosomes (Fig. 10b,b′) made up of: $2n=2x=16$: 4m + 8 sm + 2 sm sat + 2 st sat. Such a karyotype formula was obtained on material from either Ashqelon, Israel and Manfria, Sicily. To our knowledge, these are the first karyological data about this species. The presence of 10 submetacentric, two subtelocentric and of only four metacentric chromosomes accounted for a relatively high A_1 asymmetry, 0.52. In terms of size, there was a difference of c.3.6μm between the larger (9.3μm) and the smaller (5.7μm) chromosomes, with an A_2 asymmetry of 0.15. With a mean size of c.7.5 × 3.3μm, the chromosomes of *H. aggregata* are amongst the largest within *Anchusa* s.l., a genus characterized by the largest chromosomes known within the *Boraginaceae* (D'Amato & Trojani, 1985; Luque, 1995).

In *Phyllocara aucheri* we found a tetraploid set of chromosomes with $x=8$, and the following karyotype formula (Fig. 10c,c′): $2n=4x=32=4$ M + 14m + 10 sm + 2 st + 2 st sat. These appear to be the first data on the karyology of this species. Chromosomes were smaller than in *H. aggregata* (mean size c.5.6 × 1.8μm), but also in this case there was a substantial difference (c.3.4μm) between the smaller (c.3.9μm) and the larger (c.7.3μm) ones. This accounted for a relatively high A_2 asymmetry, 0.18. It is worthy of note that, unlike in *H. aggregata*, asymmetry and size of chromosomes in *P. aucheri* were positively correlated, with a decreasing mean size from the subtelocentric (7.3μm) to the exactly metacentric (3.9μm). This allowed groups of (i) large-subtelocentric, (ii) medium–large submetacentric chromosomes, (iii) medium-metacentric, and (iv) medium–small exactly metacentric chromosomes to be distinguished. The A_1 asymmetry was relatively low, 0.38, because of the prevalence of metacentric chromosomes.

DISCUSSION

As realized by Johnston (1924), an objective taxonomy of the critical genera of the Old-World *Boraginoideae* needs a wide comparative basis, including as many sources of information as possible. Typical cases are *Anchusa* s.l. and *Nonea*, which show a great diversity of forms radiating from basal, homogeneous groups. *Gastrocotyle hispida*, *Hormuzakia aggregata* and *Phyllocara aucheri* represent cases of this mosaic-like evolutionary divergence, probably induced by the ecological pressure of arid, sandy habitats in the first two species and by hybridization phenomena in the third one. As in the majority of *Anchusa* species, *H. aggregata* is a diploid with $n=8$ and does not show any substantial differentiation in karyotype morphology, except a larger chromosome size. Similar considerations apply to *G. hispida*, if $2n=16$ will be confirmed. As in the case of the annual genus *Anchusella* Bigazzi, Nardi & Selvi (Bigazzi *et al.*, 1997), the derivation of these evolutionary lines from basal groups of *Anchusa* has not involved substantial changes at the karyological level. This suggests that the differentiation of annual, diploid forms with apomorphies originating through adaptive reduction in response to arid conditions is one of the main evolutionary tendencies of *Anchusa* s.l. By contrast, the perennial relatives of *Anchusa*, i.e. *Brunnera*, *Cynoglottis* and *Pentaglottis* are characterized by different diploid numbers and consist of few species with symplesiomorphic traits indicating a more ancient origin (Guşuleac, 1928; Popov, 1953; Chamberlain, 1979; Luque, 1995). In *P. aucheri*, the occurrence in the complement of a group of large, asymmetrical chromosomes of the *Anchusa* type, besides a group of medium and medium–small metacentrics as found in *Nonea* (Grau, 1971; Luque, 1995; Constantinidis, 1996), agrees with the morphological intermediacy between these two genera. As observed by Boissier (1875), this plant is provided with faucal scales as in *Anchusa*, to which it is closer also in stigma morphology and papillar pattern, but is clearly related to *Nonea* in the habit, the foliaceous, shortened inflorescence, and in the tubular corolla with a reduced, infundibular limb. This provides circumstantial evidence for an allotetraploid origin of *P. aucheri*, following hybridization between *Anchusa* and a *Nonea* species with $2n = 16$. *N. echioides* (L.) Roemer & Schultes (= *Nonea ventricosa* (Sm.) Griseb.) for example, could represent one of the possible parent species, as it is partially sympatric with *P. aucheri*, is the only species of *Nonea* producing curved-reniform mericarps with a ventral attachment to the gynobase, and has $2n = 16$ (Grau, 1971; Luque, 1995). Ecological and chorological considerations also support the allotetraploid hypothesis. As with many *Nonea* species, *P. aucheri* grows in montane screes (Guşuleac, 1928), and this leads to exclude the hypothesis that it originated under the selective pressure of aridity as in the case of *G. hispida* and *H. aggregata*. Furthermore, its distribution range stretches from the highlands of central Anatolia eastwards to northern Iran, an area largely corresponding to the centre of highest species diversity of *Nonea* and, in part, of *Anchusa*.

From a morphological and biological viewpoint, the three taxa share some synapomorphic traits that probably originated through adaptive reduction, such as the brief annual life-cycle, the reduced size of the flowers, especially of limb, and the usual abortion of 2–3 mericarps. Other common characters are the ventral attachment of the mericarps and the leafy inflorescence, which however may represent simple parallelisms. This body of common features was more or less considered by past and recent authors (De Candolle, 1846; Boissier, 1879; Guşuleac, 1928; Popov, 1953; Riedl, 1967) to reflect a close relationship between the three plants and led Johnston (1924) to consider them as 'obvious relatives and clearly congeneric'. Nevertheless, *G. hispida*, *H. aggregata* and *P. aucheri* differ in the habit and in the structures of inflorescences, flowers and fruits. On this basis, several authors maintained the three plants in separate genera (Popov, 1953; Riedl, 1963, 1968; Feinbrun-Dothan, 1978; Qaiser, 1979; Valdés, 1992), while others (Gürke, 1897; Melchior, 1964; Chater, 1972; Chamberlain, 1979; Pignatti, 1982; Greuter *et al.*, 1984; Meikle, 1985; in part Heller & Heyn, 1986) did not consider their morphological differentiation sufficiently marked to accept their separation from *Anchusa*. The results reported in this work support the interpretation of the former group of authors, as each of the three entities shows unique features also in the micromorphology of reproductive structures. Of particular relevance is that in all three species distinct pollen morphologies are associated with stigmatic forms and papillar patterns clearly differentiated from most of *Anchusa* (Dulberger, 1970; Heslop-Harrison, 1981; Phillip & Schou, 1981; Bigazzi *et al.*, 1997; Selvi *et al.*, 1997). This correlation indicates the existence in the three species of functional differences in pollen-stigma interactions and in fecundation processes. In *G. hispida* there is also a correlation between the small pollen size (polar diam. c.20μm) and the reduced length of the functional papillae (c.38μm), which was supposed (Heslop-Harrison, 1981) to have the mechanical function of ensuring the most favourable contact between the grains and the papillae. By contrast, in *P. aucheri* and *H. aggregata*, large- and medium-sized grains (polar diams c.50μm and c.34μm, respectively) are associated with similar papillar lengths (50–60μm). In the three investigated species, especially in *P. aucheri*, another distinctive feature is represented by the structure of the faucal scales, with cells sharply differentiated from those found in other members of the tribe (Schaefer, 1942). Also in this case, functional implications in pollination mechanisms and breeding systems are probable. *H. aggregata* is especially distinct in inflorescence and fruit morphology, as well as in the strong development of a hairy annulus at the base of the corolla tube. A similar fruit morphology is found also in *Anchusa negevensis* Danin, recently described from the Israeli Negev desert and referred to subg. *Hormuzakia* (Guşul.) Chamb. (Danin, 1995). Nevertheless, *A. negevensis* differs from *H. aggregata* mainly in having succulent, perennial roots, an elongated, lax inflorescence, and other distinctive characters in floral morphology. Preliminary observations on pollen morphology (pers. obs.) revealed that the grains of *A. negevensis* belong to the *Pulmonaria obscura*-type occurring in the great majority of *Anchusa* (Bigazzi & Selvi, 1998). The correct generic placement of *A. negevensis* should therefore be ascertained through additional comparative studies.

In the light of these data, we consider the three genera as distinct evolutionary lines, with common traits besides autapomorphic characters that probably reflect specific adaptive features in pollination ecology, seed dispersal and breeding systems. *Hormuzakia*, *Gastrocotyle* and *Phyllocara* are separated from the other members of the tribe by sharp gaps in morphological, palynological and karyological traits of outstanding taxonomic value. These discontinuities reflect a substantial evolutionary distance from the putative ancestors and appear of the same level as those separating, for example, *Anchusa* from *Cynoglottis* Vural & Kit Tan, a small 'satellite' genus delimited on the basis of the shortened corolla tube and the erect mericarps (Vural & Kit Tan, 1983). Another example is the monotypic genus *Pentaglottis* Tausch, whose autonomy in respect of *Anchusa* has always been unanimously accepted in view of the stipitate mericarps, even before knowing its peculiar chromosome number ($2n = 22$) and pollen morphology. Another case within the *Boragineae* is finally represented by the genus *Elizaldia* Willk. (two species), which was separated from *Nonea* by the exserted stamens. On the basis of these considerations, to include *Gastrocotyle*, *Hormuzakia* and *Phyllocara* within *Anchusa* produces heterogeneity in this genus and fails to recognize taxonomic and phylogenetic diversity. At the same time, the presence of autapomorphies in each of the three taxa prevents merging them into a single genus as proposed by Johnston (1924). This is especially true if 2n = 20 will be observed again in *G. hispida*, because in this case the three species would have each different chromosome numbers. In the light of these considerations, the only balanced and objective solution in respect of the general taxonomic arrangement of the tribe is therefore to recognize their generic status.

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