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Pollen grain morphology of *Coris* (*Primulaceae*)

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Abstract: Pollen grain morphology, sculpturing, and wall ultrastructure are investigated in two species of *Coris* (*Primulaceae*), *C. monspeliensis* L. and *C. hispanica* LANGE. The study includes both acetolysed and unacetolysed pollen. No evidence of any major palynological difference is recorded between these two species, apart from a somewhat larger pollen in *C. monspeliensis*. However, *Coris* can be distinguished from the remaining members of the *Primulaceae* by the conjunction of relatively large pollen grains, prominent margo, and particular tectal pattern causing a reticulate surface with minute luminal perforations decreasing towards the colpi. From both these distinctive features, and others typically primulaceous, some evolutionary considerations are inferred. Finally, the higher proportion of irregular grains in *C. hispanica* is interpreted in light of environmental stress.

Coris is a Mediterranean genus of *Primulaceae*, singularly possessing shrubby habit and zygomorphic flowers with spinose calyx. Although some taxonomical confusion arises when undue weight is attributed to the indument (MASCLANS 1968), since FERGUSON's (1972) treatment most floras recognize two species, namely *C. monspeliensis* L., and *C. hispanica* LANGE. Major morphological distinction deals with the number of calyx teeth as well as colouring and shape of the flower. Both species are also distinguishable with regard to biogeography and ecology. *C. hispanica* is endemic to south-eastern Spain, particularly Almería province, whereas *C. monspeliensis* is widely spread throughout the western and Central Mediterranean. Furthermore, notwithstanding their common affinity with dry, marly substrates, *C. hispanica* is distinctively gypsophilous.

The present paper aims at investigating the pollen grains of *Coris* using light (LM), scanning electron (SEM), and transmission electron microscopy (TEM). Earlier accounts were presented of *C. monspeliensis* by NOWICKE & SKVARLA (1977, 1979) and FERNANDEZ (1987), but no description is available of pollen grain morphology in *C. hispanica*, as far as we know. Moreover, no surveys have been found in the literature which exhaustively include pollen sculpturing and ultrastructure for the whole genus.

Material and methods

Material was either directly collected in the field or, less frequently, taken from herbarium sheets. All the samples were studied from locations in southeastern Spain, in Almería, Murcia, Albacete, and Alicante provinces. Voucher specimens are deposited at the Murcia University Herbarium (MUB). For LM grains were prepared by acetolysis and subsequently mounted in glycerine jelly. Measurements were made under oil immersion and are based upon at least 30 pollen grains per population.

For SEM, acetolysed grains were progressively dehydrated up to 100% acetone, then critical-point dried, sputter-coated with gold, and viewed with a Jeol JSM T300 scanning electron microscope operated at 15–20 kV. Likewise, from flowers of both species, stigmatic surfaces, together with the pollen naturally deposited on them, were observed after similar treatment.

For TEM, acetolysed and unacetolysed pollen grains were fixed in sodium cacodylate-buffered glutaraldehyde, incorporated into agar, dehydrated in an acetone series, and embedded in epon or Spurr resins. Ultrathin sections were mounted on copper grids and stained with uranyl acetate and lead citrate. Examination and photography were made with a Zeiss EM 10C and a Zeiss EM 109 Turbo, operated at 60–80 kV.

Specimens studied. *Coris hispanica* LANGE. 1: Almería, Posada de los Castaños, MUB 34757. 2: –, Venta de los Yesos, MUB 34761. 3: –, Río Aguas, MUB 34764. 4: –, Cortijada de Río Aguas, MUB 34751. *Coris monspeliensis* L. 1: Almería, Río Aguas, MUB 34768. 2: –, Molinos de Río Aguas, MUB 34766. 3: –, Agua Amarga, MUB 34763. 4: –, Cerro Molatas, MUB 34752. 5: Murcia, Bullas, MUB 34759. 6: Albacete, Las Rejas, Letur, MUB 34760. 7: –, Sierra de los Donceles, MUB 27191. 8: Murcia, Sierra de Espuña, MUB 15268. 9: –, Sierra de Altaona, MUB 21129. 10: Almería, Carboneras, MUB 18030. 11: Murcia, Los Asperos, Santomera, MUB 29943. 12: –, Sierra Larga, Jumilla, MUB 8676. 13: Alicante, Sierra del Maigmó, MUB 20079. 14: Albacete, Calar del Mundo, MUB 25914.

Results

All the collections studied have pollen grains which are essentially similar in shape, exine surface sculpturing, and ultrastructure. The only distinctive character is the pollen grain size, but it provides no taxonomic usefulness since the difference is recognizable only in statistical terms, showing a somewhat longer polar axis of *C. monspeliensis* (P 27.2–44.8 µm, mean 34.8 µm) than of *C. hispanica* (P 25.8–36.7 µm, mean 31.6 µm). Discrimination is worse what concerns the equatorial diameter (*C. monspeliensis*: E 17.6–28.6 µm, mean 22.2 µm; *C. hispanica*: E 16.3–27.2 µm, mean 21.4 µm). In the contrary, generic pollen characters offer interesting features for wider discussion, from phylogenetic, purely morphological, and ecological perspectives.

The pollen grains of *Coris* are tricolporate, radially symmetrical, and isopolar. The shape in polar view is circular to nearly hexagonal. The lateral outline is elliptic, sometimes obtusely rhombic in equatorial mesocolpium view (Fig. 1).

The compound aperture (Figs. 2, 3) is sharply defined by colpi, colpus membrane, and ora. The colpi are long, mostly between 20 and 30 µm, at the equator approximately up to 3 µm wide, with acute ends. The apocolpia are small, usually 3–5 µm in side. The colpus membrane is beset with more or less densely spaced minute granules, especially in the proximity of the endoaperture, where they are usually larger. The ora are elliptic to rectangular, lalongate, occasionally overarched by a marginal bridge of ektexinous character, but well-defined in sufficiently expanded grains; costae present. In LM it can be seen that the meridional elongation of the endoaperture clearly surpasses the colpus boundaries, sometimes causing small

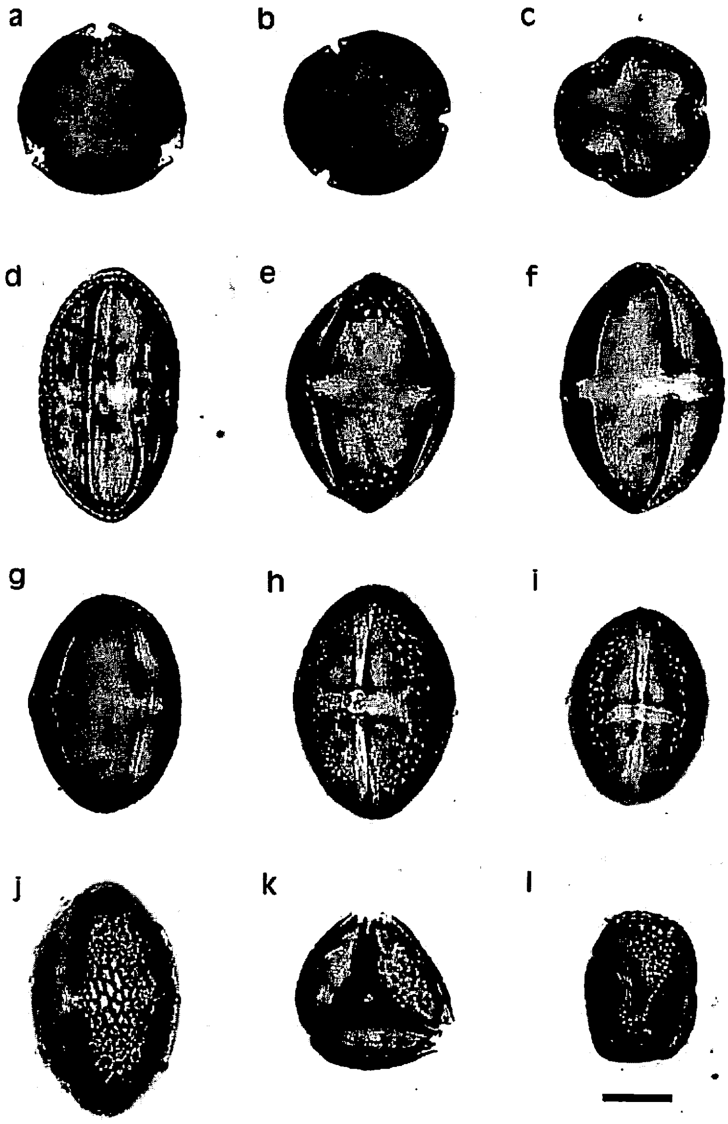

Fig. 1. LM micrographs of *Coris* pollen grains. *a, b, d–f, h, j C. monspeliensis. c, g, i, k, l C. hispanica. a–c* Outline in polar view. *d* Outline in equatorial view. *e–g* Equatorial views showing costae. *h, i* Endoaperture and margo. *j* Reticulum. *k* 3-syncolporate grain. *l* Irregularly shaped grain. — Bar: 10 µm

horns. The margo is distinct, 2–4.5 µm wide in the equator, psilate in surface, uneven in its border with the membrane.

The surface sculpturing is reticulate, with thin muri (below 0.5 µm), narrower than the width of the lumina, psilate. The lumina are more or less angular, with minute perforations mainly in their periphery, up to 2 µm in the mesocolpium, but clearly decreasing towards the margo, whose borders have a distinct perforate pattern.

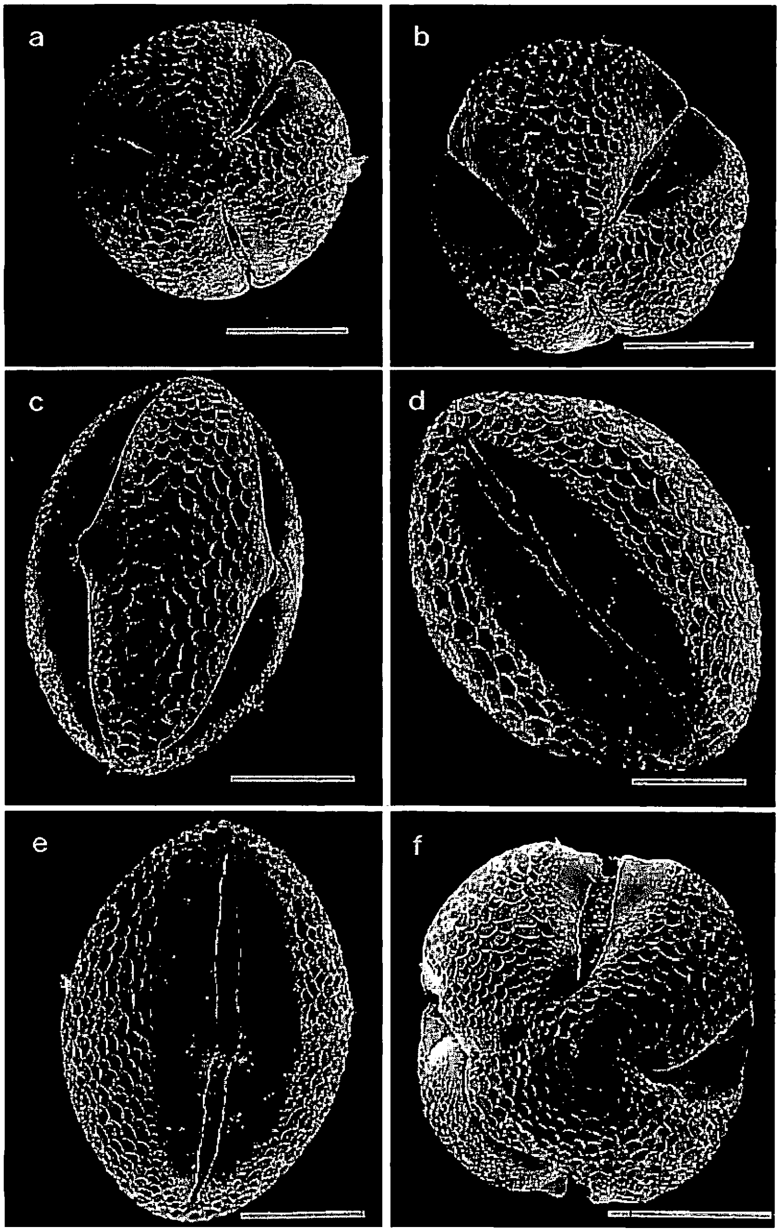

Fig. 2. SEM micrographs of *Coris* pollen grains. *b, d C. monspeliensis. a, c, e, f C. hispanica. a–b* Polar exine surface. *c* Mesocolpium centred view. *d, e* Surface view of apertural region. *f* Polar surface of abnormal grain. – Bars: 10 μm

The exine of non-apertural region is characteristically stratified and composed of ektexine and endexine (Figs. 4, 5). The ektexine is differentiated into tectum, columellae, and footlayer, a better development being observable at the poles and directly beneath the muri. The tectum is well-defined, with an undulating pattern, largely angular in outline, forming the reticulate surface. It is unevenly interrupted by minute perforations. The columellae are irregular, more consistent under the

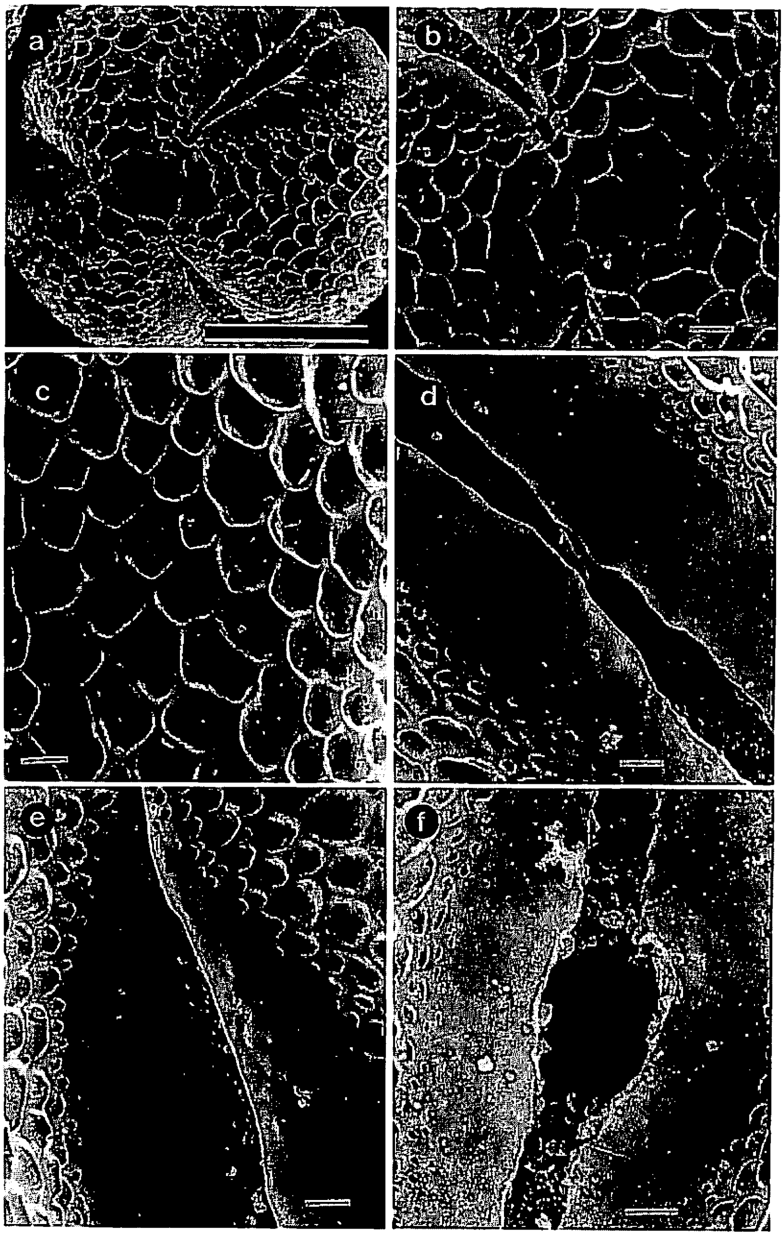

Fig. 3. SEM micrographs of *Coris* pollen grains. *a, d C. monspeliensis. b, c, e, f C. hispanica. a* Polar view, bar: 10 µm. *b–c* Reticulate sculpturing with minute perforations in the lumina of the apocolpium (*b*) and mesocolpium (*c*). *d–f* Different views of apertural area. Note lumina decreasing towards the broad margo, granular membrane, ektexinous bridge (*d*), and opened endoaperture (*f*). – Bars: 1 µm

tectal risings, and becoming branched where the ektexine is thickened, principally in the polar region. The footlayer is compact, continuous, like the columellae frequently thicker beneath the muri and at the poles, with the lower margin more or less smooth but somewhat irregular over its outer surface. In the aperture margin

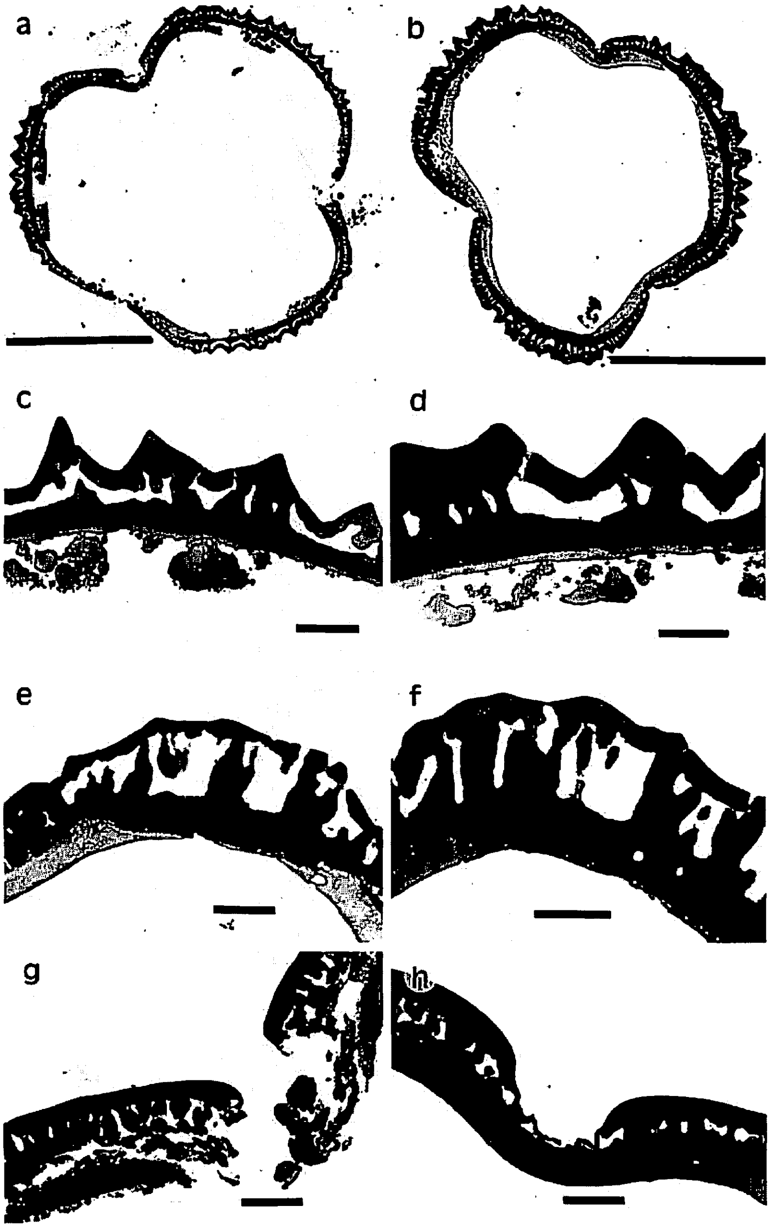

Fig. 4. TEM micrographs of acetolysed *Coris* pollen grains. *b, d, f C. monspeliensis. a, c, e, g, h C. hispanica. a–b* Radial sections of whole pollen grains affecting the endoaperture (*a*) and ectoaperture (*b*). *c–d* Mesocolpium in radial section. The ektexine appears differentiated into footlayer, columellae, and conspicuous tectum interrupted by minute perforations. The endexine is mainly fragmented although a thin layer remains attached to the footlayer. *e–f* Apocolpium in polar section. The endexine is consistent in subpolar areas but thinned in the centre of the apocolpium wherein the footlayer is thick and the columellae higher and branched. *g* Radial section through endoaperture, note interruption and the break in both ektexine and endexine. *h* Radial section through ectoaperture, note increase in endexine, the tectum parallel to footlayer, shorter columellae and the ektexinous granules over the endexinous colpal membrane. – Bars: *a, b*: 10 µm, *c–h*: 1 µm

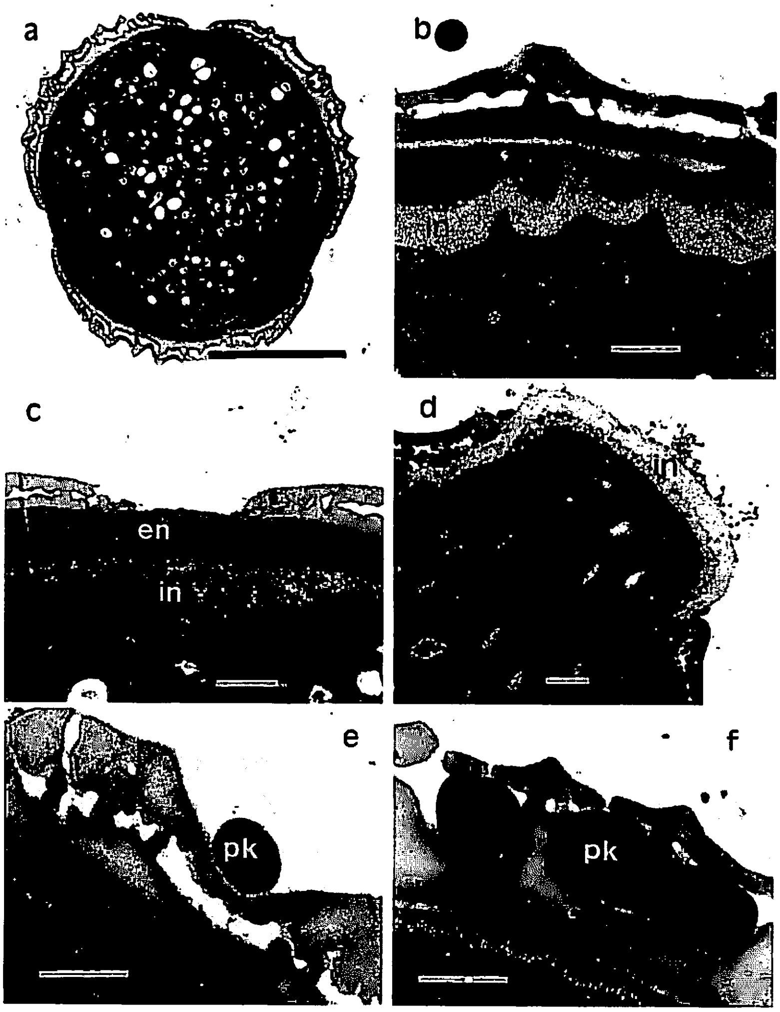

Fig. 5. TEM micrographs of unacetolysed *Coris* pollen grains. *a, b, c C. monspeliensis. d, e, f C. hispanica. a* Low magnification view of a whole pollen grain. The intine is consistently thickened beneath aperture whereas the endexine is continuous under the entire apertural region including the margo. *b* Mesocolpal view. The intine (in) is wavy and adjusted to the fragmented endexine. *c* Radial section through ectoaperture, note intinous (in) and endexinous (en) thickenings. *d* Radial section through germinal aperture. The intine (in) is protruding, note its lower zone somewhat more electron-dense. *e* Very electron-dense pollenkitt (pk) deposited on a luminal area. *f* Pollenkitt (pk) filling intercolumellar cavities. – Bars: *a*: 10 µm, *b–f*: 1 µm

the whole ektexine decreases in thickness, and is virtually absent in the colpus, where ektexinous material is deposited causing the afore-mentioned granular pattern over the membrane. Towards the aperture, the undulating and minutely perforate tectum decreases, runs nearly parallel to the endexine, lacks perforations, the columellae become shorter, and the footlayer thinner.

The more electron dense endexine is prominent and continuous in the aperture, but uneven in the mesocolpium, where it can be represented merely by random fragments together with a thin layer attached to the inner footlayer. Moreover, it undergoes some thinning in the apocolpium, where a less undulating tectal pattern is sometimes observed. Structurally, the colpal exine is composed of underlying, well-developed endexine, often surmounted by scattered, ektexinous elements. Conversely, at the endoaperture, entire interruption of the exine is seen, with dismemberment of the endexine in the adjacent areas.

Most of the features described hitherto can be observed in both acetolysed and unacetolysed pollen grains. However, there are some characteristics, namely the intine and the pollenkitt, which are revealed exclusively without acetolysis. The intine is a continuous layer, variable in thickness, but consistently thickened in the aperture where it becomes thicker than the endexine. It has a more lamellate than massive appearance, made up of channel-like structures. Its outline is sinuous and adjusted to the exine configuration. Thus, it can penetrate between the fragmentary endexine in the mesocolpial areas, or protrude through the endoaperture towards the exterior. In the latter case, some inner stratification can be seen, with the lower zone more dense.

In ultrathin sections stained with uranyl acetate, the pollenkitt appears primarily as a highly electron-dense material filling up the cavities between the columellae as well as deposited as droplets over the tectum surface, generally on the luminal areas (Fig. 5 e, f). Since SEM micrographs reveal extensive surface coatings of pollenkitt (Fig. 6 d), we assume that it may have been partially removed during the TEM pretreatment. On the other hand, the individual pollen grains show a strong tendency to link together and cohere with the stigmatic papillae, although the copious stigmatic exudates must play their own role. As far as the papillae are concerned, they show no appreciable variation in morphology between *C. monspeliensis* and *C. hispanica* (Fig. 6 a, b).

Although the pollen grain morphology of *Coris* can be considered as relatively uniform in the material studied, some irregularities occur. Abnormal types include regularly shaped 3-syncolporate and 4-colporate grains, but most often grains with hazardously arranged apertures that may cause asymmetry. The irregular grains are often smaller than the normal ones. On the other hand, SEM and TEM observations of irregular pollen show no substantial difference in surface sculpturing and wall stratification. Attention should be paid to the proportion of irregular grains, a higher mean percentage being noticeable in *C. hispanica* (15.3%) than in *C. monspeliensis* (4.1%).

Discussion

Palynological surveys of *Primulaceae* have been primarily aimed at evaluating the controversial relationship of the family to the order *Centrospermae* s.l., a treatment based mainly on the common free central placentation (HUTCHINSON 1959). ROLAND (1971) reviewed the ultrastructure of the pollen apertures in *Primulaceae* and suggested that this family might be derived from the *Centrospermae*. However, no critical argument was given to justify such an affinity. More recently, SKVARLA & NOWICKE (1976) and NOWICKE & SKVARLA (1977, 1979) substantiate, palynologically, the current opinion that similarities among *Primulaceae* and the centrospermous members are, rather, the result of parallel evolution. The long-suspected, alleged affinity between *Primulaceae* and *Theales* seems more likely, from em-

Fig. 6. SEM micrographs of *Coris* stigmas. *a* Part of the style and stigma of *C. hispanica* before pollen deposition showing papillae and stigmatic exudates. *b* Detail of papillae, identical in both *C. hispanica* and *C. monspeliensis*. *c* Stigma surface with pollen attached. *d* Individual pollen grain in mesocolpal view showing the exine coated with highly viscous pollenkitt (arrows), which also forms droplets on the lumina. – Bars: *a*: 100 μm, *b–d*: 10 μm

bryological, chemical, and serological studies (Philipson 1974; Dahlgren 1977, 1983; Cronquist 1988).

Arguments presented by Nowicke & Skvarla (1977, 1979) in order to stress the strong dissimilarity between *Primulaceae* and the centrospermous families, refer to pollen grain sculpture and ultrastructure. In fact, they presented some pollen features of *C. monspeliensis* as illustrative of the dissimilarity, namely the compound aperture and consistent endexine. The aperture type and exine architecture in *Coris* fit excellently in the general patterns of the *Primulaceae*. Furthermore, its pollen does not suffer the high degree of variation seen in several other genera, such as *Primula* or *Hottonia* (Punt & al. 1974), a phenomenon often closely related to heterostyly.

Coris can be distinguished from the remaining genera of European *Primulaceae* by the conjunction of relatively large pollen grains, prominent margo, and the peculiarly perforate tectum. Apart from *Coris*, pollen grains commonly larger than 30 μm are only found in *Trientalis*, albeit associated with polyploidy and increased aperture number (Punt & al. 1974). The presence of a margo has been shown in *Anagallis, Asterolinon, Lysimachia* (Punt & al. 1974, Fernandez & Garcia-Martin 1985, Fernandez 1987), and *Naumbergia* (Nowicke & Skvarla 1977, 1979), but it is less sharply defines as in *Coris*. Moreover, as far as we know, there is no other primulaceous pollen where the reticulate pattern is added to luminal minute tectum perforations.

Although bearing in mind the above particularities in the pollen morphology of the genus *Coris*, little can be said at present about its phylogenetic affinities. In contrast to *Coris*, most *Primulaceae* are herbaceous and display actinomorphic flowers, although their evolutionary relationships with *Ebenales* and the woody *Theophrastaceae* and *Myrsinaceae* remain unclear (DOUGLAS 1936, SPANOWKSY 1962, CRONQUIST 1988). At first sight, the tendency towards larger pollen grains might parallel increased specialisation in other features, such as flower symmetry and growth habit, thereby placing *Coris* in an advanced status within the *Primulaceae*. Similarly, the conspicuous margo and large lumina, decreasing towards the colpi, as well as the distinct costae, could be viewed as derived characters according the apertural dicotyledoneous trends suggested by PUNT (1976). The great difficulty of interpreting the evolutionary significance of pollen characters without a complete set of additional data must be borne in mind. Secondary pollen-morphological trends may have intervened. In fact, the colpate aperture seen in some species of *Primula* (PUNT & al. 1976) may be a secondary character derived from the colporate type generalized in the thealean-primulalean line. Whether or not the seemingly specialised pollen of *Coris* reaches sufficient height to justify its advanced position within the family, and whether the shrubby habit can be associated to the irregular flower in order to support such a specialisation, is largely speculative, given that a comprehensive study of the *Primulaceae* systematics remains to be undertaken.

The matter becomes even more complicated with respect to the possible adaptation of the pollen features involved. As mentioned earlier, the margo is distinctly developed in *Coris, Anagallis, Asterolinon*, and some *Lysimachia* spp., all well represented in the Mediterranean area. Since the *Primulaceae* are centred in temperate and cold latitudes, it may be wondered whether the development of a margo in that family might have taken place as an adaptive response to increased summer dryness or greater insolation. This is further stressed by the possibility that a margo should provide some advantage in protecting the pollen grain from desiccation, perhaps by improving the harmomegathic mechanisms. Although a relationship of the margo to the aperture physiology seems plausible, we are still very far from understanding the forces involved in its development.

The study of ultrathin sections of unacetolysed grains is worthwhile because it substantiates the main characters perceived from acetolysed material (HESSE & WAHA 1989). In *Coris*, there are observable differences of density of endexine and footlayer as well as of the fragmentary character of the non-apertural endexine between acetolysed and unacetolysed pollen grains. Study of unacetolysed grains to describe the intine, characteristically thickened under the germinal aperture and thoroughly adjusted to the course of the endexine. The pollenkitt exhibits those features established by HESSE (1981 a, b) as diagnostic of strict entomophily. It is sticky electron-dense, homogeneous, distributed both between the columellae and over the tectum surface.

The higher proportion of irregular grains in *C. hispanica* is difficult to interprete. Previous studies (THOMAS 1970, CLARKE 1975) on significance of abnormal pollen grains in some *Hypericum* spp. have associated their abundance with low fertility, presumably as a consequence of meiotic disturbances. A relationship to ploidy level (BRONCKERS 1963, VAN DEN BERG 1985, CARRION & ALCARAZ 1988) or environmental stress (BLANCA 1981) has often been suggested. In the case of *H. hispanica*, the resolution of the dilemma might lie in the intrinsically stressful gypsic soils where the species grows. Without strict evidence this hypothesis is, of course, inconclusive. It has to be further evaluated.

References

BLANCA, G., 1981: Estudios taxonómicos en el género *Centaurea* L. Sección *Willkommia* G. BLANCA: Palinología. – Bot. Macaronesica **8–9**: 103–118.

BRONCKERS, F., 1963: Variations polliniques dans une série d'autopolyploides artificiels d'*Arabidopsis thaliana* (L.) HEYNH. – Pollen & Spores **5**: 233–238.

CARRION, J., ALCARAZ, F., 1988: Algunas anomalías polínicas en *Sideritis* del sudeste ibérico. – Acta Bot. Malacitana **13**: 179–188.

CLARKE, G. C. S., 1975: Irregular pollen grains in some *Hypericum* species. – Grana **15**: 117–125.

CRONQUIST, A., 1988: The evolution and classification of flowering plants. 2nd edn. – New York: New York Botanical Garden.

DAHLGREN, R., 1977: A commentary on a diagrammatic presentation of the angiosperms in relation to the distribution of character states. – Pl. Syst. Evol., Suppl. **1**: 253–283.

– 1983: The importance of modern serological research for angiosperm classification. – In JENSEN, V., FAIRBROTHERS, D. E., (Eds): Proteins and nucleic acids in plant systematics, pp. 371–394. – Berlin: Springer.

DOUGLAS, G. E., 1936: Studies in the vascular anatomy of the *Primulaceae*. – Amer. J. Bot. **23**: 199–212.

FERGUSON, L. F., 1972: *Coris*. – In TUTIN, T. G., HEYWOOD, V. H., BURGES, N. A., MOORE, D. M., VALENTINE, D. H., WALTERS, S. M., WEBB, D. A., (Eds): Flora Europaea **3**, pp. 29. – Cambridge: Cambridge University Press.

FERNANDEZ, I., 1987: *Primulaceae*. – In VALDES, B., DIEZ, M. J., FERNANDEZ, I., (Eds): Atlas polínico de Andalucía Occidental, pp. 168–174. – Sevilla: Excma. Diputación de Cádiz.

– GARCIA-MARTIN, F., 1985: Morfología polínica de la familia *Primulaceae* en Andalucía Occidental. – An. Asoc. Palinol. Leng. Esp. **2**: 107–115.

HESSE, M., 1981 a: The fine structure of the exine in relation to the stickiness of angiosperm pollen. – Rev. Palaeobot. Palynol. **35**: 81–92.

– 1981 b: Pollenkitt and viscin threads: their role in cementing pollen grains. – Grana **20**: 145–152.

– WAHA, M., 1989: A new look at the acetolysis method. – Pl. Syst. Evol. **163**: 147–152.

HUTCHINSON, J., 1959: Families of flowering plants. 1. Dicotyledons. – London: Oxford University Press.

MASCLANS, F., 1968: El género *Coris* en la Región Mediterránea Occidental. – Collect. Bot. **7**: 749–758.

NOWICKE, J. W., SKVARLA, J. J., 1977: Pollen morphology and the relationship of the *Plumbaginaceae, Polygonaceae*, and *Primulaceae* to the order *Centrospermae*. – Smithsonian Contrib. Bot. **37**: 1–64.

– – 1979: Pollen morphology: the potential influence in higher order systematics. – Ann. Missouri Bot. Gard. **66**: 633–700.

PHILIPSON, W. R., 1974: Evolutionary lines within the dicotyledons. – New Zealand J. Bot. **13**: 73–91.

PUNT, W., 1976: Evolutionary trends in the pollen grains of *Dichapetalaceae*. – In FERGUSON, I. K., MULLER, J., (Eds): The evolutionary significance of the exine, pp. 139–146. – London, New York: Academic Press.

– De Leeuw Van Weenen, J. S., Van Oostrum, W. A. P., 1974: The Northwest European pollen flora, 3. *Primulaceae*. – Rev. Palaeobot. Palynol. **17**: 31–70.

Roland, F., 1971: Données évolutives résultant de l'étude ultrastructurale des apertures de pollens appartenant au groupe *Ranales Centrospermales*. – Rév. Gén. Bot. **78**: 329–338.

Skvarla, J. J., Nowicke, J. W., 1976: Ultrastructure of pollen exine in Centrospermous families. – Pl. Syst. Evol. **126**: 55–78.

Spanowsky, W., 1962: Die Bedeutung der Pollenmorphologie für die Taxonomie der *Primulaceae-Primuloideae*. – Feddes Repert. **65**: 149–214.

Thomas, J. L., 1970: Haploid and diploid pollen in *Hypericum patulum*. – J. Arnold Arbor. **51**: 247–250.

Van Den Berg, R., 1985: Pollen morphology of the genus *Begonia* in Africa. Studies in *Begoniaceae* II. – Agric. Univ. Wageningen Papers **3**: 5–94.

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