

Glandular hairs of the ovary: a helpful character for Asteroideae (Asteraceae) taxonomy?

Daniela Ciccarelli*, Fabio Garbari & Anna M. Pagni

Department of Biology, University of Pisa, via Luca Ghini 5, I-56126, Italy (*corresponding author's e-mail: dciccarelli@biologia.unipi.it)

Received 19 Dec. 2005, revised version received 9 June 2006, accepted 14 June 2006

Ciccarelli, D., Garbari, F. & Pagni, A. M. 2007: Glandular hairs of the ovary: a helpful character for Asteroideae (Asteraceae) taxonomy? — *Ann. Bot. Fennici* 44: 1–7.

Surface microcharacters of the ovary of the Asteraceae were studied, especially biseriate glandular hairs. Of 34 species belonging to the Asteroideae, only *Dittrichia viscosa*, *Pulicaria dysenterica*, *Bellis perennis*, *Tanacetum parthenium*, *Achillea maritima*, *Matricaria chamomilla*, and *Eupatorium cannabinum* possess glandular hairs on the ovary. Additional characters considered are hair morphology and distribution, cell number, and presence of subcuticular chambers and chloroplasts. The usefulness of glandular hairs of the ovary for taxonomic purposes is discussed.

Key words: Asteraceae, Asteroideae, glandular hairs, light microscopy, ovary, SEM, taxonomy

Introduction

In the Asteraceae both non-glandular and glandular hairs are present (Metcalf & Chalk 1950). The non-glandular hairs are represented by numerous morphological types, whereas the glandular hairs are more homogeneous. The latter are usually formed of a peduncle and a head of one or many cells. The most common type, found in numerous species, is a biseriate hair formed of five pairs of cells. Detailed studies of this type of hair, its morphology and ultrastructure have been carried out on plants of economic interest, such as *Dittrichia viscosa* (Werker & Fahn 1981), *Artemisia annua* (Duke & Paul 1993), *Artemisia nitida* (Corsi & Nencioni 1995), *Artemisia campestris* (Ascensão & Pais 1987), *Achillea millefolium* (Figueiredo & Pais 1994), *Helichrysum aureonitens* (Afolayan & Meyer 1995), and *Helichrysum stoechas*

(Ascensão *et al.* 2001). Investigations referring to a supraspecific taxon are scarce. Carlquist (1958) analysed the structure and ontogenesis of glandular hairs of *Madinae*, demonstrating their taxonomic and evolutionary significance. Napp-Zinn and Eble (1980) took into consideration the glandular and non-glandular hairs of twenty genera of the Anthemideae. In the genus *Artemisia*, features of the glandular hairs are considered useful for the separation of *A. nova* from *A. arbuscula* and *A. tridentata* (Kelsey 1984).

Glandular hairs, in the Asteraceae, may be present both on the vegetative and floral parts. In the capitula they are found on the receptacle, bracts, corolla and also on the ovary. On the ovary surface, other types of hairs may also be present: *twin hairs*, of a characteristic double structure, and *slime hairs*, usually multicellular, involved in the production of mucilaginous

material (Roth 1977). The characters of the surface of the ovary and fruit have repeatedly been used for the delimitation of taxa at various levels in several families (Karcz 1996, Lane 1985). However, in the Asteraceae they are seldom used as key characters in floras, and when they are, the different types of hairs are only occasionally considered. The importance of cypsela features

for the phylogeny and classification of the family is underlined in several recent works (Anderberg 1991, Karis 1993, Zhang & Bremer 1993, Bremer 1994). The present paper is a contribution to the study of the surface microcharacters of the ovary, focusing on biseriate glandular hairs. Its particular aim is to verify, through examples, the potential usefulness of glandular hairs for taxonomical and phylogenetic studies.

Table 1. Species of subfamily Asteroideae collected from Migliarino-San Rossore-Massacciuccoli Natural Park. Species marked with an asterisk (*) possess glandular hairs on the ovary.

Tribe	Species
Inuleae	* <i>Diitrichia viscosa</i> (L.) Greuter <i>Inula conyzae</i> Griesselich <i>Limbarda crithmoides</i> (L.) Dumort. * <i>Pulicaria dysenterica</i> (L.) Bernh. <i>Pulicaria odora</i> (L.) Rchb. <i>Pulicaria sicula</i> (L.) Moris
Gnaphalieae	<i>Filago pyramidata</i> L. <i>Filago germanica</i> L. <i>Gnaphalium uliginosum</i> L. <i>Helichrysum italicum</i> (Roth.) Don. <i>Helichrysum stoechas</i> (L.) Moench
Astereae	<i>Tripolium pannonicum</i> (Jacq.) Dobroc. * <i>Bellis perennis</i> L. <i>Erigeron canadensis</i> L. <i>Erigeron acris</i> L. <i>Erigeron karwinskianus</i> DC.
Anthemideae	* <i>Tanacetum parthenium</i> (L.) Sch.-Bip. <i>Artemisia coerulescens</i> L. <i>Achillea millefolium</i> L. * <i>Achillea maritima</i> (L.) Ehrend. <i>Anthemis arvensis</i> L. <i>Anthemis cotula</i> L. <i>Coleostephus myconis</i> (L.) Cass. <i>Leucanthemum pallens</i> (Gay) DC. <i>Leucanthemum vulgare</i> Lam. * <i>Matricaria chamomilla</i> L.
Senecioneae	<i>Jacobaea erratica</i> (Bertol.) Fourr. <i>Senecio lividus</i> L. <i>Senecio vulgaris</i> L.
Heliantheae	<i>Bidens frondosa</i> L. <i>Bidens tripartita</i> L. <i>Xanthium italicum</i> Moretti <i>Xanthium strumarium</i> L.
Eupatorieae	* <i>Eupatorium cannabinum</i> L.

Material and methods

The plant material examined originated from a protected area (Migliarino-San Rossore-Massacciuccoli Natural Regional Park, Pisa, Italy) characterised by a high biological diversity. Restricted by the Park regulations, we randomly collected capitula from at least five individual plants of each species during the flowering and fruiting phases (years 2001–2003). The species studied are listed in Table 1. The 34 species examined belong to seven tribes: Inuleae, Gnaphalieae, Astereae, Anthemideae, Senecioneae, Heliantheae, Eupatorieae (classification and nomenclature in accordance with Bremer 1994 as modified by Greuter 2003a, 2003b, 2003c, Greuter *et al.* 2003).

Light microscopy (LM)

Sections (25 μm) of fresh capitula were cut using a Leitz 1720 cryostat at -14° – -16°C . Other sections (3 μm) were cut using a Leica 2055 microtome, after fixing the material in FAA (Sass 1958) and embedding it in LR White acrylic resin (SIGMA). All the material was stained with Toluidine Blue (TBO) (O'Brien & McCully 1981), a generic dye for DNA, cytoplasm and some cell wall components.

Fresh material was also observed under a light microscope without any staining procedure.

Scanning electron microscopy (SEM)

All the material was fixed in glutaraldehyde (2% with buffer solution at pH 7.4), dehydrated in a gradient of an alcohol and acetone mixture, criti-

cal point dried and sputter-coated with gold. The samples were examined at 15 KV using a Cambridge Stereoscan 90 SEM.

Results

Biseriate glandular hairs were found on the ovary of *Dittrichia viscosa*, *Pulicaria dysenterica*, *Bellis perennis*, *Tanacetum parthenium*, *Achillea maritima*, *Matricaria chamomilla*, and *Eupatorium cannabinum*. The typical base model (made up of ten cells: two basal cells, two of the peduncle and six of the secretory head) is present on the ovary of *B. perennis*, *T. parthenium*, *A. maritima*, *M. chamomilla* and *E. cannabinum*. In *B. perennis* and in *E. cannabinum* the number of head cells varies from six to eight, in *D. viscosa* and in *P. dysenterica* each series of the head hair is formed of six to nine cells; the apical cells are very large (Fig. 1). The hair morphology does not show any within-species variation.

The hairs can be distributed on the whole surface of the ovary as in *D. viscosa*, in *B. perennis* and in *E. cannabinum* (Fig. 2A–C); between the ribs as in *T. parthenium*, *A. maritima* (Fig. 2D, E) and *M. chamomilla* (A. Andreucci pers. comm.); and in the apical part of the ovary as in *P. dysenterica* (Fig. 2F). The hair distribution pattern seems to remain constant within a species.

The shape of the hair tip can vary from globular in *T. parthenium* (Fig. 3A) to fungiform in *E. cannabinum* (Fig. 3B), more or less clavate in *B. perennis* (Fig. 1) or cylindrical in *D. viscosa* (Fig. 3C) and in *P. dysenterica* (Fig. 3D).

All glandular hairs have, at full growth, a subcuticular chamber, a space coming out when the two layers of the cuticle become detached from the pecto-cellulosic wall (Fahn 2000). Its size can be very different among species while being usually uniform within a species. Among the plants analysed, *B. perennis* has the smallest subcuticular chamber (Fig. 1C) with regard to only the apical cells; *E. cannabinum* has a bigger chamber that gives the hair a fungiform appearance (Fig. 3B); a large spherical chamber is also present in *D. viscosa*, *P. dysenterica* and *A. maritima*. In the last species the chamber regards not only the apical but also the lower cells (Fig. 1F).

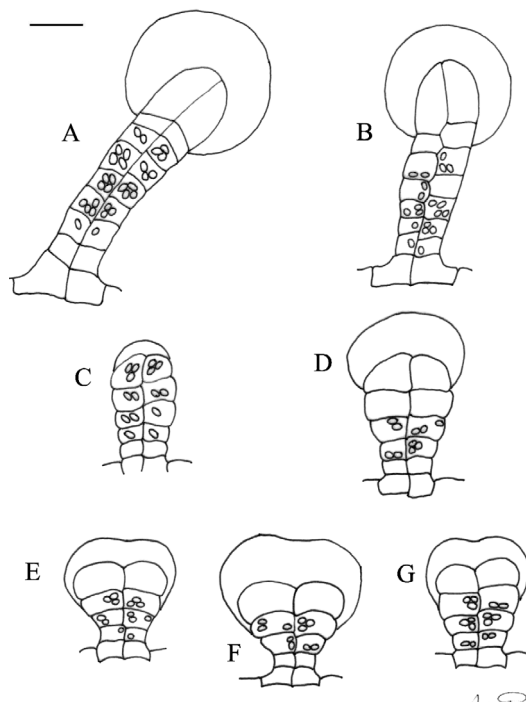


Fig. 1. Biseriate glandular hairs. — A: *Dittrichia viscosa* (scale bar = 18.3 μm). — B: *Pulicaria dysenterica* (scale bar = 8.3 μm). — C: *Bellis perennis* (scale bar = 3.4 μm). — D: *Eupatorium cannabinum* (scale bar = 4.6 μm). — E: *Tanacetum parthenium* (scale bar = 4.1 μm). — F: *Achillea maritima* (scale bar = 2.6 μm). — G: *Matricaria chamomilla* (scale bar = 3.0 μm). Chloroplasts are present in all the cells of the head with the exception of the apical cells, in *B. perennis* also the apical cells have chloroplasts.

In the species studied, chloroplasts are present in all cells that form the head of the hair, except in the apical cells; in *B. perennis* also the apical cells are green.

When the fruit matures the glandular hairs remain, but their structure often degenerates.

Discussion

The classification of Asteraceae remains in a state of flux. In a revision of the family, Bremer (1994) pointed out that, to provide a complete classification, new research and taxonomic revisions were necessary especially at the lower taxonomical levels. Even if molecular research has provided an important contribution to under-

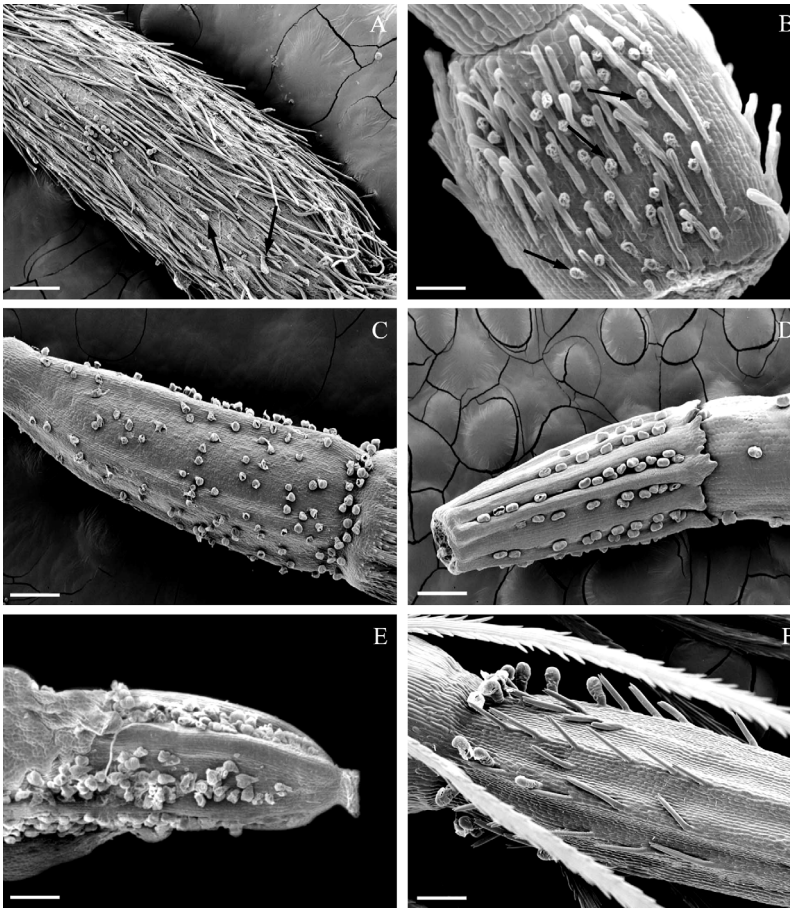


Fig. 2. — **A:** *Dittrichia viscosa*: few glandular hairs (arrows) are distributed on the ovary among non-glandular hairs (scale bar = 100 μ m). — **B:** *Bellis perennis*: biseriate glandular hairs (arrows) are distributed on the whole surface of the ovary (scale bar = 80 μ m). — **C:** *Eupatorium cannabinum*: glandular hairs are distributed on the whole surface of the ovary (scale bar = 130 μ m). — **D:** *Tanacetum parthenium*: biseriate glandular hairs are distributed between the ribs of the ovary (scale bar = 130 μ m). — **E:** *Achillea maritima*: glandular hairs are distributed between the ribs of the ovary (scale bar = 160 μ m). — **F:** *Pulicaria dysenterica*: biseriate glandular hairs are present in the apical part of the ovary (scale bar = 50 μ m). All SEM.

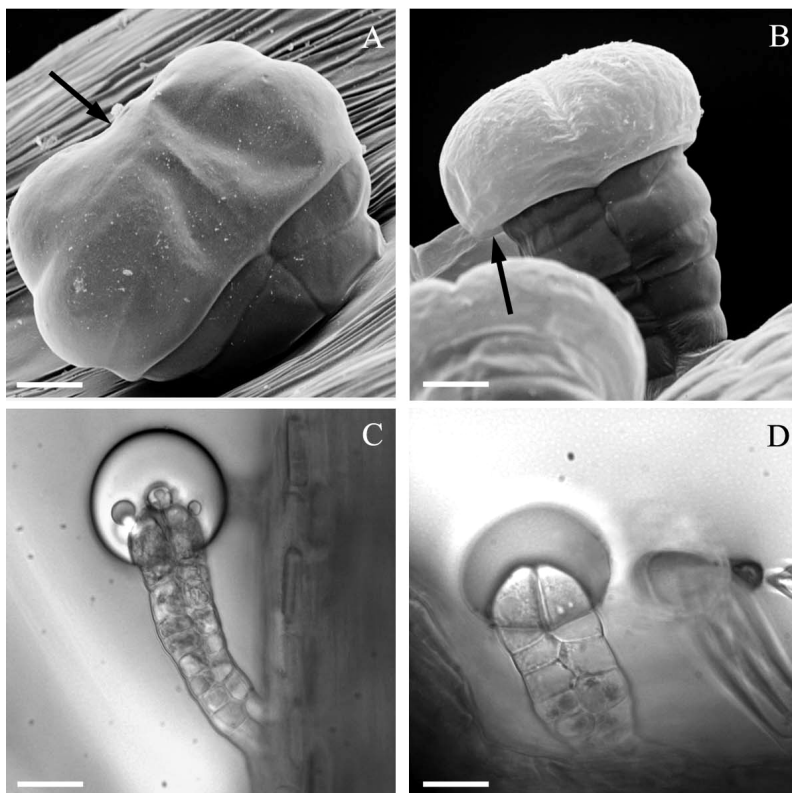
standing the phylogeny of Asteraceae and the position of controversial taxa, morphological characters continue to be an irreplaceable source of information for phylogenetic studies. Currently, there is a sort of renaissance of morphology in plant systematics (Weber 2003).

The importance of cypsela characters for the systematic study of taxa, for clarifying their delimitation and establishing their phylogeny, has repeatedly been emphasised (Grau 1977, Heywood & Humphries 1977, Robinson & King 1977, Anderberg 1989, 1991, Karis 1993, Bremer 1994). The characters that are given most importance are general morphology, the presence of ribs, crystals in the epidermal cells, the structure of the carpodium, the shape of the pappus, and the presence of cells with phytomelanin in the fruit wall. Lane (1985) and Crawford *et al.* (2001) reported that in the Asteraceae the morphological characters of the fruit were stable and

genetically controlled, as had been demonstrated experimentally for other families. The non-glandular and glandular hairs, which are part of the surface microcharacters, are occasionally used in the classification, especially at generic and specific level (Källersjö 1986, Sanders & Clark 1987, Mukherjee & Sarkar 2001).

We studied 34 species of Asteroideae to verify if “glandular hairs of the ovary” can be used as a diagnostic character for taxonomical purposes. Biseriate hairs were found in species belonging to the tribes Inuleae (*D. viscosa* and *P. dysenterica*), Astereae (*B. perennis*), Anthemideae (*T. parthenium*, *A. maritima* and *M. chamomilla*), and Eupatorieae (*E. cannabinum*). We considered hair morphology and distribution, cell number, subcuticular chamber and the presence of chloroplasts. The morphology and the number of cells that form the tip of the hair, while stable within species, show considerable

Fig. 3. — **A:** *Tanacetum parthenium*: glandular hair has a globular morphology, the subcuticle chamber (arrow) is evident (scale bar = 7.4 μm). — **B:** *Eupatorium cannabinum*: biseriata glandular hair with a fungiform appearance, the subcuticle chamber (arrow) is visible (scale bar = 6.5 μm). — **C:** *Ditrichia viscosa*: cylindrical biseriata hair with a large spherical subcuticle chamber (scale bar = 13.3 μm). — **D:** *Pulicaria dysenterica*: cylindrical glandular hair with a spherical subcuticle chamber (scale bar = 8.3 μm). **A** and **B** SEM, **C** and **D** LM.



interspecific variation. For example, *D. viscosa* and *P. dysenterica* (both in Inuleae) have cylindrical biseriata hairs made up of 12–18 cells, slightly different from the base model, i.e. five pairs of cells. The similar morphology could reflect the taxonomic closeness of the two taxa (Anderberg 1991, Eldenäs *et al.* 1996). Very complex hairs that represent an elaboration of the typical base model were observed by Carlquist (1958) in species of Madiinae. Glandular hairs formed by a big multicellular peduncle and a secretory head were found on the involucre bracts of *Siegesbeckia jorullensis* (Heinrich *et al.* 2002) of Heliantheae, which are dispersed as a whole, including the cypselas. The marked variability of hair morphology, also here evidenced, implies a need to improve our knowledge of glandular hairs of the ovary and the whole plant for a larger number of taxa. Nevertheless, also when we consider hairs that represent the base model, distribution, overall size, presence or absence of chloroplasts, and the subcuticular chamber show fine variations. Anyway, we did not observe any within-species variation of these

characters among the species studied.

Regarding hair distribution, for example, all Anthemideae species with biseriata hairs here studied (*T. parthenium*, *A. maritima* and *M. chamomilla*) show the same inter-costal pattern.

The cells of the glandular hairs are characterised by a highly specialised cytoplasm (Lüttge 1971, Cheniclet & Carde 1985). The presence of plastids and in particular chloroplasts in the head cells is easily observed under light microscope and indicates metabolic activity in the cells. Cheniclet and Carde (1985) associated the presence of plastids with the synthesis of terpenoids and/or their precursors.

The subcuticular chamber is quite variable among the species studied (Fig. 1). However, similar subcuticular chambers have been found in *Tanacetum parthenium*, *Achillea maritima* and *Matricaria chamomilla* (all Anthemideae), where several head cells are involved, and in *Ditrichia viscosa* and *Pulicaria dysenterica* (Inuleae), where only the apical cells are involved (Fig. 1). Also, the hairs of *E. cannabinum* are very similar in morphology, size and, especially, shape of the

subcuticular chamber to those of *Stevia rebaudiana* (Cornara et al. 2001). This is not surprising because both species belong to the Eupatorieae.

Glandular hairs, together with other characters, can thus indeed be of help in taxonomic and phylogenetic studies in the Asteraceae. Moreover, the presence or absence of glandular hairs may be of diagnostic value. For example, the separation of *Dittrichia* from *Inula* (Bremer 1994) is supported by our results, which show the presence of glandular hairs on the ovary of *D. viscosa* and their absence on the ovary of *I. conyzae*.

In the Asteraceae, glandular hairs are frequent not only on the capitula, but also on the vegetative parts of the plant. The secretion of terpenoids by these structures, shown by several studies (Ascensão et al. 2001, Heinrich et al. 2002, Pagni et al. 2003), may have many different functions. Some volatile terpenoids may attract pollinating insects to flowers, while others may protect the plant and its reproductive structures from destruction by herbivores and pathogens (Kelsey et al. 1984, Harborne 1993).

We plan to extend our studies on glandular hairs of the ovary to other Italian species of Asteroideae so as to assess the taxonomic usefulness of this character in a wider taxonomical context.

Acknowledgements

We are deeply grateful to Prof. Dr. Werner Greuter (Berlin) for his expert advice and helpful suggestions on the taxonomy of Asteroideae. Thanks are due also to Mr. Antonio Masini for his technical assistance.

References

- Afolayan, A. J. & Meyer, J. J. M. 1995: Morphology and ultrastructure of secreting and nonsecreting foliar trichomes of *Helichrysum aureonitens* (Asteraceae). — *Int. J. Plant Sci.* 156: 481–487.
- Anderberg, A. A. 1989: Phylogeny and reclassification of the tribe Inuleae (Asteraceae). — *Can. J. Bot.* 67: 2277–2296.
- Anderberg, A. A. 1991: Taxonomy and phylogeny of the tribe Inuleae (Asteraceae). — *Plant Syst. Evol.* 176: 75–123.
- Ascensão, L. & Pais, M. S. S. 1987: Glandular trichomes of *Artemisia campestris* (ssp. *maritima*): Ontogeny and histochemistry of the secretory product. — *Bot. Gaz.* 148: 221–227.
- Ascensão, L., Da-Silva, J. A. T., Barroso, J. G., Figueiredo, A. C. & Pedro, L. G. 2001: Glandular trichomes and essential oils of *Helichrysum stoechas*. — *Israel J. Plant Sci.* 49: 115–122.
- Bremer, K. 1994: *Asteraceae: cladistics and classification*. — Timber Press, Portland, Oregon.
- Carlquist, S. 1958: Structure and ontogeny of glandular trichomes of *Madinae* (Compositae). — *Am. J. Bot.* 45: 675–682.
- Cheniclet, C. & Carde, J. P. 1985: Presence of leucoplasts in secretory cells and of monoterpene in the essential oil: a correlative study. — *Israel J. Bot.* 34: 219–238.
- Cornara, L., Bononi, M., Tateo, F., Serrato-Valenti, G. & Mariotti, M. G. 2001: Trichomes on vegetative and reproductive organs of *Stevia rebaudiana* (Asteraceae). Structure and secretory products. — *Plant Biosystems* 135(1): 25–37.
- Corsi, G. & Nencioni, S. 1995: Secretory structures in *Artemisia nitida* Bertol. (Asteraceae). — *Israel J. Plant Sci.* 43: 359–365.
- Crawford, D. J., Kimball, R. T. & Tadesse, M. 2001: The generic placement of a morphologically enigmatic species in Asteraceae: evidence from ITS sequences. — *Plant Syst. Evol.* 228: 63–69.
- Duke, S. O. & Paul, R. N. 1993: Development and fine structure of the glandular trichomes of *Artemisia annua* L. — *Int. J. Plant Sci.* 154(1): 107–118.
- Eldenäs, P., Anderberg, A. A. & Källersjö, M. 1996: Molecular phylogenetics of the tribe Inuleae s. str. (Asteraceae), based on ITS sequences of nuclear ribosomal DNA. — *Plant Syst. Evol.* 210: 159–173.
- Fahn, A. 2000: Structure and function of secretory cells. — *Adv. Bot. Res.* 31: 37–75.
- Figueiredo, A. C. & Pais, M. S. S. 1994: Ultrastructural aspects of the glandular cells from the secretory trichomes and from the cell suspension cultures of *Achillea millefolium* L. ssp. *millefolium*. — *Ann. Bot.* 74: 179–190.
- Grau, J. 1977: Astereae, systematic review. — In: Heywood, V. H., Harborne, J. B. & Turner, B. L. (eds.), *The biology and chemistry of the Compositae*, vol. 1: 539–565. Acad. Press, London.
- Greuter, W. 2003a: The Euro+Med treatment of Astereae (Compositae) — generic concepts and required new names. — *Willdenowia* 33: 45–47.
- Greuter, W. 2003b: The Euro+Med treatment of Gnaphalieae and Inuleae (Compositae) — generic concepts and required new names. — *Willdenowia* 33: 239–244.
- Greuter, W. 2003c: The Euro+Med treatment of Senecioneae and the minor Compositae tribes — generic concepts and required new names, with an addendum to Cardueae. — *Willdenowia* 33: 245–250.
- Greuter, W., Oberprieler, C. & Vogt, R. 2003: The Euro+Med treatment of Anthemideae (Compositae) — generic concepts and required new names. — *Willdenowia* 33: 37–43.
- Harborne, J. B. 1993: *Introduction to ecological biochemistry*, 4th ed. — Acad. Press, London.
- Heinrich, G., Pfeifhofer, H. W., Stabentheiner, E. & Sawidis,

- T. 2002: Glandular hairs of *Siegesbeckia jorullensis* Kunth (Asteraceae): morphology, histochemistry and composition of essential oil. — *Ann. Bot.* 89: 459–469.
- Heywood, V. H., Harborne, J. B. & Turner, B. L. (eds.) 1977: *The biology and chemistry of the Compositae*, 2 vols. — Acad. Press, London, New York, San Francisco.
- Källersjö, M. 1986: Fruit structure and generic delimitation of *Athanasia* (Asteraceae-Anthemideae) and related South African genera. — *Nordic J. Bot.* 5: 527–542.
- Karcz, J. 1996: Fruit micromorphology and anatomy of *Valeriana officinalis* s. str. (Valerianaceae). — *Nordic J. Bot.* 16: 409–419.
- Kelsey, R. G. 1984: Glandular trichomes: a helpful taxonomic character for *Artemisia nova* (black sagebrush). — *J. Range Manage.* 37: 370–372.
- Kelsey, R. G., Reynolds, G. W. & Rodriguez, E. 1984: The chemistry of biologically active constituents secreted and stored in plant glandular trichomes. — In: Rodriguez, E., Healey, P. L. & Metha, J. (eds.), *Biology and chemistry of plant trichomes*: 187–241. Plenum Press, New York.
- Lane, A. L. 1985: Features observed by electron microscopy as generic criteria. — *Taxon* 34: 38–43.
- Lüttge, U. 1971: Structure and function of plant glands. — *Ann. Rev. Plant Physiol.* 22: 23–44.
- Metcalf, C. R. & Chalk, L. 1950: *Anatomy of the dicotyledons*, 2nd ed., vol. 2. — Clarendon Press, Oxford.
- Mukherjee, S. K. & Sarkar, A. K. 2001: Morphology and structure of cypselas in thirteen species of the tribe Astereae (Asteraceae). — *Phytomorphology* 51(1): 17–26.
- Napp-Zinn, K. & Eble, M. 1980: Beiträge zur systematischen Anatomie der Asteraceae — Anthemideae: die Trichome. — *Plant Syst. Evol.* 136: 169–207.
- O'Brien, T. P. & McCully, M. E. 1981: *The study of plant structure. Principles and selected methods*. — Termacarp Pty LTT, Melbourne.
- Pagni, A. M., Orlando, R., Masini, A. & Ciccarelli, D. 2003: Secretory structures of *Santolina ligustica* Arrigoni (Asteraceae), an Italian endemic species. — *Israel J. Plant Sci.* 51: 185–192.
- Robinson, H. & King, R. M. 1977: Eupatorieae, systematic review. — In: Heywood, V. H., Harborne, J. B. & Turner, B. L. (eds.), *The biology and chemistry of the Compositae*, vol. 1: 437–485. Acad. Press, London, New York, San Francisco.
- Roth, I. 1977: *Fruits of angiosperms. Encyclopedia of plant anatomy*. — Gebrüder Borntraeger, Berlin, Stuttgart.
- Sanders, D. L. & Clark, C. 1987: Comparative morphology of the capitulum of *Enceliopsis* (Asteraceae: Heliantheae). — *Am. J. Bot.* 74: 1072–1086.
- Sass, J. E. 1958: *Botanical microtechnique*. — Iowa State Univ. Press, Ames.
- Weber, A. 2003: What is morphology and why is it time for its renaissance in plant systematics? — In: Stuessy, T. F., Mayer, V. & Hörandl, E. (eds.), *Deep morphology: Towards a renaissance of morphology in plant systematics*: 3–32. A.R.G. Gantner Verlag, Ruggell.
- Werker, E. & Fahn, A. 1981: Secretory hairs of *Inula viscosa* (L.) Ait. — development, ultrastructure and secretion. — *Bot. Gaz.* 142: 461–476.
- Zhang, X. & Bremer, K. 1993: A cladistic analysis of the tribe Astereae (Asteraceae) with notes on their evolution and subtribal classification. — *Plant Syst. Evol.* 184: 259–283.