NAT. CROAT. | VOL. 6 | No 4 | 367–380 | ZAGREB | December 31, 1997

ISSN 1330-0520
UDK 582.998:581.45/.522.5(497.5)

CONTRIBUTION TO KNOWLEDGE ABOUT THE LEAF ANATOMY OF THE CROATIAN ENDEMIC TAXON *Centaurea ragusina* L. SUBSP. *ragusina*

TOMISLAV BAČIĆ, DALIBOR VLADOVIĆ*, ŽELJKO POPOVIĆ

J. J. Strossmayer University, Faculty of Education, Department of Biology, L. Jägera 9, 31000 Osijek, Croatia
*»V. Nazor« High School, Zagrebačka 2, 21000 Split, Croatia

Bačić, T., Vladović, D. & Popović, Ž.: Contribution to knowledge about the leaf anatomy of the Croatian endemic taxon *Centaurea ragusina* L. subsp. *ragusina*, Nat. Croat., Vol. 6, No 4., 367–380, 1997, Zagreb

The main anatomical features of the leaf lamina and leaf petiole the Croatian endemic species *Centaurea ragusina* subsp. *ragusina* from the region of Split have been examined by light and scanning electron microscopes. Besides the many xeromorphic properties which have arisen as a result of the species' life in conditions of aridity and high light intensity, those which might characterize the species and therefore could have taxonomic significance hve to be identified. The last include: the microornamented cuticle of ordinary hairs, stomata with a stomatal and peristomatal rim and a long and narrow aperture, parenchymatous bundle sheaths in the leaf lamina, sclerenchymatous caps adjacent to the xylem and phloem in the petiole bundles, and the arrangement of bundles in the petiole.

Key words: *Asteraceae*, *Centaurea ragusina* L., leaf anatomy.

Bačić, T., Vladović, D. & Popović, Ž.: Prilog poznavanju anatomije lista hrvatske endemične vrste *Centaurea ragusina* L. subsp. *ragusina*, Nat. Croat., Vol. 6, No 4., 367–380, 1997, Zagreb

Istražene su glavne osobine anatomije plojke i peteljke lista hrvatske endemične vrste *Centaurea ragusina* subsp. *ragusina* s područja Splita svjetlosnim i rasterskim elektronskim mikroskopom. Osim kseromorfnih osobina, nastalih kao posljedica života u uvjetima vodnog deficita i jakog intenziteta svjetlosti, identificirane su i osobine koje bi mogle biti svojstvene vrsti te stoga imati taksonomsku važnost. Posljednje su: mikroornamentirana kutikula običnih dlaka u obliku brazda i ureza, puči sa stomatalnim i peristomatalnim obrubom te uskim i dugim stomatalnim otvorom, parenhimski žilni ovoji u plojci, sklerenhim u obliku kapa na ksilemu i floemu žila u peteljci te lučni raspored žila u peteljci.

Ključne riječi: *Asteraceae*, *Centaurea ragusina* L., anatomija lista.

Hrvatski prirodoslovni muzej, Demetrova 1, Zagreb, Croatia

INTRODUCTION

Centaurea ragusina L. (*Asteraceae*) is a Croatian endemic species, growing in the gapes of the perpendicular limestone clifts above the Adriatic Sea. Its *locus classicus* is the environs of Dubrovnik (in latin Ragusa) where it is a characteristic species of the *Phagnalo-Centaureetum ragusinae* (H-at) H-ić association (HORVATIĆ 1963). As well as in Dubrovnik, it can also be found in a few other localities (Split, the islands of Biševo, Šolta, Hvar and Vis).

Taxonomically based on it's morphological properties, it has been treated differently during time; firstly as two species (GINZBERGER 1912, 1920) and then as one (HAYEK 1927–33) comprising several subspecies or varieties. According to newer data (PEVALEK 1930, DOMAC 1956), supported by PAVLETIĆ (1965), *Centaurea ragusina* L. is one species consisting of three subspecies; *ragusina, padelini* and *baumgartneri. C. ragusina subsp. ragusina* has divided exclusively leaves, subs. *baumgartnery* has only entire leaves, whereas subsp. *padelini* has both (Fig. 1).

Contrary to the relatively numerous morphological data, there are no studies concerning the anatomy of the species that would contribute to a wider knowledge of it. The lack of such data was the primary point of the investigations, presented here.

MATERIAL AND METHODS

Fully developed leaves were gathered from examples growing on the Saint Jere clifts of the Marjan hill in Split (Dalmatia).

Herbarium materials were used for the investigations. The leaves were examined with light and scanning electron microscopes.

For light microscopy, leaf laminae and petioles were boiled in water, then transversally sectioned (TS) on a Reichert OME sliding microtome, cleared in bleach, stained with safranin, sudan III and alcien blue, dehydrated through an alcohol series and finally mounted in euparal.

For scanning electron microscopic (SEM) examinations small pieces of lamina were fixed to stubs with double-sided adhesive tape, coated with gold in a Sputter Biorad system coater and photographed in a Philips 515 SEM at x 71,5 x 341 x 1559 and x 6500 magnifications. The examinations were done in the SEM Laboratory of the INA-Naftaplin Company in Zagreb.

RESULTS

The results obtained have revealed the following:

***Leaf lamina structure* (in TS)**: isobilateral (isolateral) (Fig. 2).

***Lamina thickness* (in TS)**: 418 to 513 m.

Cuticle and cutinized layers of outer epidermal cell wall **(in TS and SEM)**: the surface is smooth; nearly equally thick on both adaxial and abaxial sides (about 13.3 μm); in some places even thicker than the lumen of epidermal cell (Figs 9, 10); microornamented only on the ordinary trichomes (Fig. 5).

Wax (in SEM): amorphous (Fig. 6).

Epidermis **(in TS and SEM)**: both are single layered with straight anticlinal and convex, smooth periclinal walls (Fig. 6); the size of cells is nearly the same on adaxial and abaxial surfaces (Fig. 2).

Stomata **(in TS and SEM)**: present on both surfaces (Figs 7, 8); guard cells are remarkedly raised in relation to other epidermal cells; substomatal chamber is usually very large; stomatal rim is broadly oval (Fig. 6); aperture is long and narrow; the stomatal rim surrounds a single circular peristomatal rim formed like a ridge; this is wider than the stomatal rim.

Nonglandular trichomes **(in light microscope and SEM)**: very long and very numerous, simple, filiform, unbranched, dead, (Fig. 3); they densely cover both surfaces; the trichome cuticle (in SEM, magnification 6500) is microornamented by folds and furrow-like sculpturing streching longitudinally (Fig. 5).

Glandular trichomes **(in TS):** short stalked with a head consisting of several layers of yellow spherical cells (Fig. 9); present in both epidermises; these trichomes were used by PAVLETIĆ (1965) for the distinction of subspecies.

Mesophyll **(in TS)**: palisade parenchyma consists of typicaly long cells forming three or four adaxial and two or three abaxial layers; spongy parenchyma is in the central portion, and made of irregularly shaped but somewhat longer cells with vacuoles for water storage; spongy parenchyma forms up to 1/3 or less of leaf thickness (Fig. 2); the distinction between palisade and spongy parenchyma is not always easy; on leaf margins palisade parenchyma prevails (Fig. 12)

Midrib **(in TS):** broadly ovate and extended dorsiventrally; both ridges are rounded; epidermal cells on the ridges are nearly twice as high as wide; bundle sheat is parenchymatous; adjacent to the xylem and phloem pole it is developed as multilayered caps; laterally it is usually one-layered; the sheat is strongly developed abaxially (Fig. 11).

Lateral veins **(in TS):** collateral, surrounded by one continuous or discontinuous parenchimatous sheat (Fig. 2) which is sometimes lacking.

Petiole **(in TS):** bifacial, basal part is horizontally extended and without »wings« (Fig. 15); middle part is dorsiventrally extended and has two »wings« (Fig. 13), one on each side between abaxial and adaxial ridge; in both basal and middle part bundles are free and arranged in an arc; on the xylem and phloem pole of three central vascular bundles, sclerenchymatous caps are present (Fig. 14); under the epidermis there is a continuous collenchyma tissue developed as one layer; this becomes multilayered in the »wings« and often in the ridge regions; the parenchyma is placed in the central part and among the bundles (Figs. 13, 14, 15)

DISCUSSION

The research presented here suggests that the leaves of *C. ragusina* subsp. *ragusina* have some features that could be attributed to those properties which have obviously arisen as the consequence of the species' life in conditions of aridity. They are known as xeromorphic properties and serve to protect the plant to an important extent from further water loss (CUTTER, 1978).

In comparison with the list of typical xeromorphic properties given by MAXIMOV (1929, 1930) and BARANOV (1925) the xeromorphic features observed in the *C. ragusina* subsp. *ragusina* leaves can be listed as follows: very dense hair cover, markedly thick cuticle including cuticularized layers and cutinized outer epidermal cell wall and entire leaf, palisade parenchyma more developed than spongy parenchyma, which has developed as water storing tissue. Among this group of features an additional one should be mentioned: the stomata on both adaxial and abaxial surfaces. It is believed that this position of the stomata may be the result of the species' life in conditions of high light intensity. Some experiments, e. g. those done on tomato plants, support this view. Plants grown in conditions of high light intensity have amphistomatous leaves with a slighter stomata density on the abaxial epidermis than plants grown in low light intensity (CUTTER 1978, GAY and HURD 1975). The position of stomata in regard to the level of the epidermis in the leaves of the taxon investigated is also in accordance with that which can be find in some xerophytes. Although the stomata are not sunken or in grooves, as in most xerophytes, but strongly raised above the leaf surface, as in some mesophytes or even in hygrophytes, this feature nevertheless characterizes xerophytes, especially those growing in habitats exposed to strong winds. At first this feature seems to be a counter-productive one. However, when the stomatal aperture is open in such raised stomata, the chimney-like structure below is subjected to reduced air pressure, when the wind blows over the leaf surface, as in the Venturi effect. It enhances the removal of water vapour and hence increases the transpiration stream (personal explanation of Dr. Cutler, the head of the Anatomy Section of Jodrell Laboratory at the Royal Botanic Gardens, Kew).

The other group of leaf properties of *C. ragusina* L. subsp. *ragusina* includes some which could be of taxonomic significance. These certainly comprise microornamentation of the ordinary hair cuticle surface, the shape and structure of stomata, as well as characteristics of the bundle sheaths and of the vein arrangement. As has previously been pointed out (BAČIĆ 1996) cuticular sculpturing is little affected by environmental conditions; according to CUTLER and BRANDHAM (1977), CUTLER (1979) and BEHNKE and BARTHLOTT (1983), it is under strong genetic control and constant at the species level and as such may serve as an excellent diagnostic character (STACE 1965, PAGANELLI CAPELLETI 1975, BARTHLOTT 1981, 1990 etc), like the form of the stomatal and peristomatal rims (BARANOVA 1972, WILKINSON 1979) as well as the form and arrangement of veins and bundle sheats (SOLEREDER 1908).

ACKNOWLEDGEMENT

We are deeply grateful to Mr. sc. R. Holub, director of the INA- Naftaplin Company from Zagreb, as well as to Dipl. ing. R. Slavković from the same institution, for screening and photographic facilities in their Laboratory.

We also thank to Professor Ana-Zlata Štefanac of the Faculty of Science of the University in Zagreb for her comments and critical reading of the manuscript and to dr. David Cutler, the head of Anatomy section of the Jodrell Laboratory, Royal Botanic Gardens, Kew, England, for his expert advices.

Received October 1, 1997

REFERENCES

BAČIĆ, T., 1996: Note on use of some micro-morphological features in distinction of three pubescent oaks in Croatia. – Acta Biol. Cracov. Series: Bot., **38**, 67–72.

BARANOV, P., 1925: K poznaniju rastitelnosti gornih kamenistih osipey. – Juletin SAGU. Tashkent.

BARANOVA, M. A., 1972: Systematic anatomy of the leaf epidermis in the *Magnoliaceae* and some related families. – Taxon, Vol. 21, **4**, 447–469.

BARTHLOTT, W., 1981: Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. – Nordic. J. Bot., **1**, 345–355.

BARTHLOTT, W., 1990: Scanning Electron Microscopy in Taxonomy and Functional Morphology (ed. D. Claugher), Systematic: Association Special Volume 41, 69–83. – Clarendon Press. Oxford.

BEHNKE, H. D. & BARTHLOTT, W., 1983: New evidence from the ultrastructural and micromorphological fields in Angiosperm classification. – Nordic. J. Bot., **3**, 43–66.

CUTTER, E. G., 1978: Plant Anatomy. Part 1. Cells and Tissues. – Contemporary Biology. William Clowes & Sons, Limited, London.

CUTLER, D. F., 1979: Leaf surface studies in *Aloe* and *Haworthia* species (*Liliaceae*): taxonomic implications. – Trop. Subtrop. Pflanzenwelt (Akad. Wiss. Lit. Mainz), **28**, 449–471.

CUTLER, D. F. & BRANDHAM, P. E., 1977: Experimental evidence for the genetic control of leaf surface characters in hybrid *Aloineae* (*Liliaceae*). Kew Bul., **32**, 23–42.

DOMAC, R., 1956: Noviji pogledi na svojtu *Centaurea ragusina* L. – Glas Biol. sekc. Hrv. prir. dr., **7**, 128–131.

GAY, A. P. & HURD, R. G., 1975: The influence of light on stomatal density in the tomato. – New. Phytol., **75**, 37–46.

GINZBERGER, A., 1916: *Centaurea lungensis*, nov. spec. – Verh. d. zool.-bot. Ges., **66**, 463.

GINZBERGER, A., 1920: Über einige Centaurea-Arten der adriatischen Küsten und Inseln. – Öster. bot. Zeit., **4**, 89–110.

HAYEK, A., 1927–1933: Prodromus Florae Peninsulae Balcanicae, 2:756, Berlin.

HORVATIĆ, S., 1963: Vegetacijska karta otoka Paga s općim pregledom vegetacijskih jedinica Hrvatskog primorja. – Prirodosl. istraž. JAZU, **33**, Acta Biol., **4**.

MAXIMOV, M. A., 1929: The plant in relation to water. A study of the physiological basis of drought resistance (English translation by R. H. Yapp). – George Allen and Unwin. London.

MAXIMOV, M. A., 1931: The physiological significance of the xeromorphic structure of plants. – J. Ecol., **19**, 272–282.

PAGANELLI CAPELLETI, E. M., 1975: Studio morphologico al microscopio elettronico a scansione di foglie di *Atropa belladonna* L. – Bot. Ital., **7**, 24–25.

PAVLETIĆ, Z., 1965: Morfološka istraživanja roške vrste *Centaurea ragusina* L. – Acta Bot. Croat., **24**, 103–167.

PEVALEK, I., 1930: Vaskularna flora otoka Dugog. Prirodosl. istraž. Kralj. Jugosl., **16**, 119–158.

SOLEREDER, H., 1908: Systematic anatomy of the Dicotyledonen. – Ferdinand Enke. Stuttgart.

STACE, C. A., 1965: Cuticular studies as an aid to plant taxonomy. – Bull. Brit. Mus. (nat. Hist.), Bot., **4**, 1–78.

WILKINSON, H. P., 1979: The plant surface (Mainly leaf). Part I: Stomata. In: Anatomy of the Dicotyledones. Vol I. (C. R. Metcalfe and L. Chalk) 2nd edition. – Clarendon Press. Oxford.

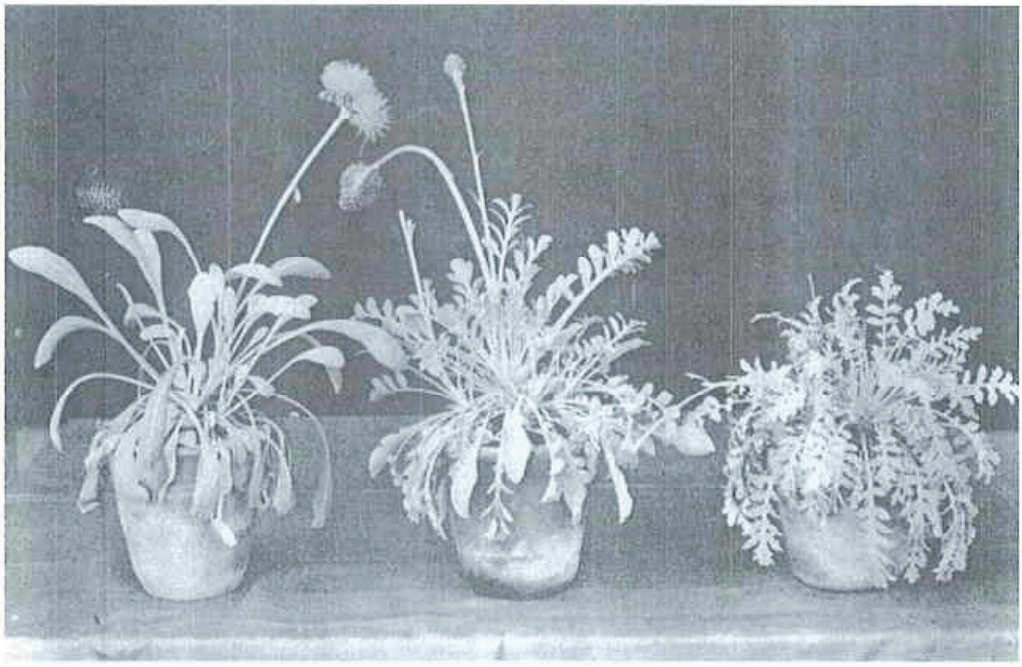
SAŽETAK

Prilog poznavanju anatomije lista hrvatskog endema dubrovačke zečine (*Centaurea ragusina* subsp. *ragusina*)

T. Bačić, D. Vladović i Ž. Popović

Među endemima Hrvatske važno mjesto pripada dubrovačkoj zečini (*Centaurea ragusina* subsp. *ragusina*). Taksonomsko tretiranje te svojte bilo je tijekom vremena različito. Svojta se prvo smatrala dvijema vrstama s više podvrsta i varijeteta, a potom, sve do danas, jednom vrstom s tri podvrste: *baumgartneri*, *padelini* i *ragusina*. Nasuprot razmjerno brojnim podacima o njezinoj morfologiji, podataka o njezinoj anatomskoj građi nema mnogo.

Istraživanje anatomije listova spomenute vrste obavljeno je svjetlosnim i rasterskim elektronskim mikroskopom. Rezultati istraživanja pokazuju da su njezini listovi odlikuju, prije svega, kseromorfnim svojstvima: njihova površina prekrivena je debelim slojem običnih dlaka, na obje su epiderme brojne žljezdane dlake, kutikula zajedno s kutikularnim slojevima i kutiniziranom vanjskom stijenkom epidermalnih stanica izrazito je debela, kao i list u cjelini, više je palisadnog nego spužvastog parenhima, spužvasti je parenhim razvijen kao tkivo za pohranjivanje pričuvne vode. Međutim, osim kseromorfnih svojstava, listovi imaju i osobine koje bi mogle biti svojstvene toj svojti i stoga imati taksonomsku važnost. Tim osobinama pripadaju, primjerice: mikroornamentirana kutikula običnih dlaka u obliku uzdužnih brazda i ureza, puči sa stomatalnim i peristomatalnim obrubom te uskim i dugim stomatalnim otvorom, parenhimski ovoji oko žila u plojci, sklerenhim u obliku kapa na ksilemu i floemu žila u peteljci te lučni raspored žila u peteljci.



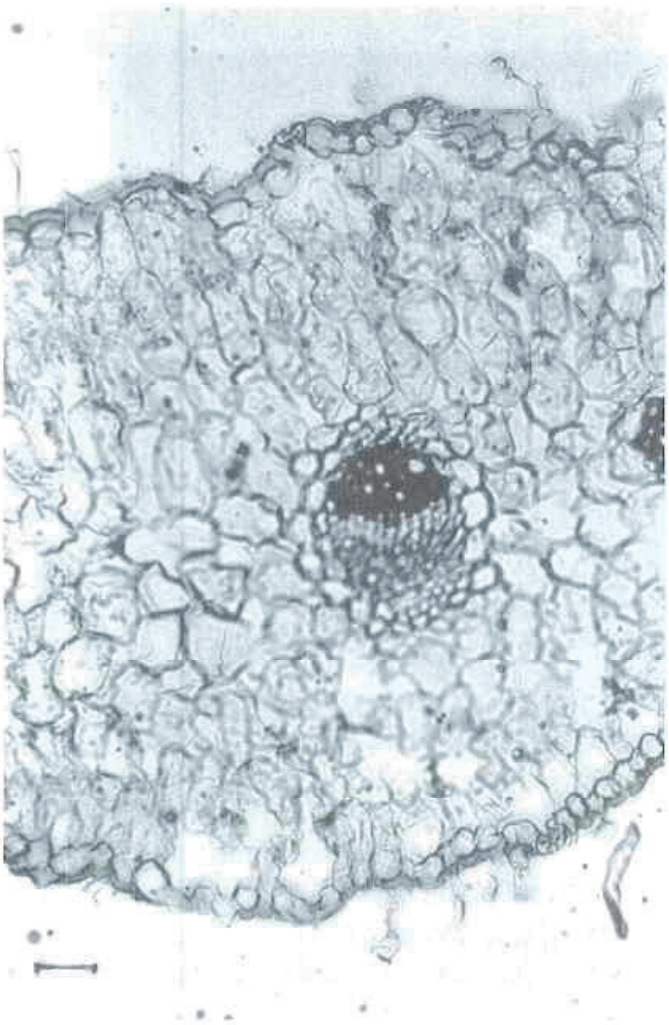


Plate 1

Fig. 1. Pavletić's original photograph of *Centaurea ragusina* L. From left to right are: subsp. *baumgartneri,* subsp. *padelini* and subsp. *ragusina.*

Fig. 2. Transverse section (TS) of *Centaurea ragusina* subsp. *ragusina* lamina showing palisade parenchyma on both sides, spongy parenchyma in central portion and a collateral vein surrounded by a parenchymatous sheath. Scale bar = 10 μm.

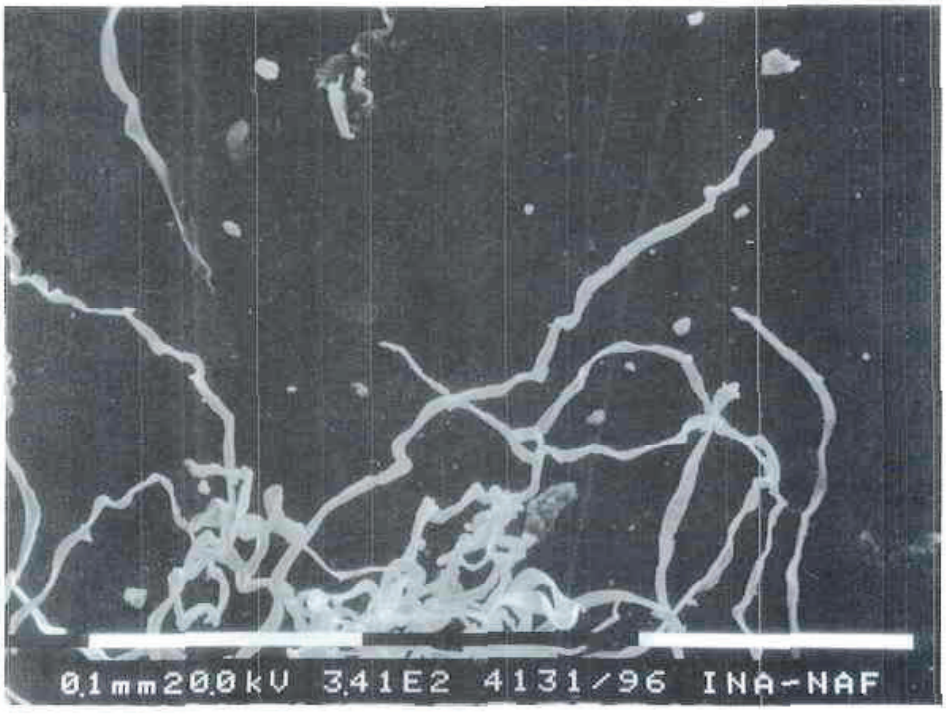
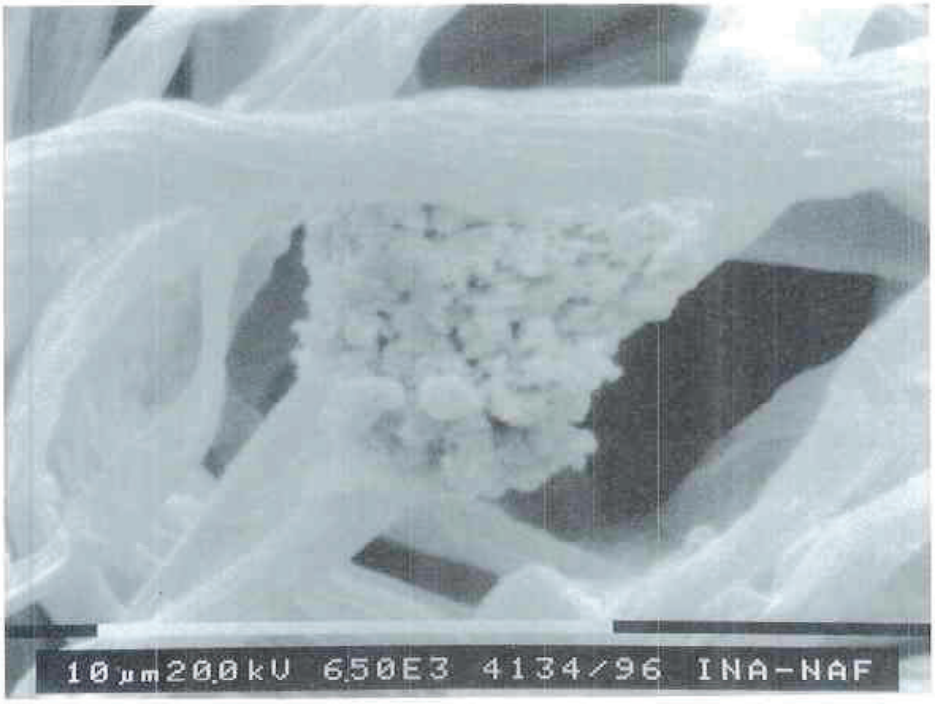


Plate 2

Fig. 3. Scanning electron microscopy (SEM) of ordinary tubular trichomes on the adaxial epidermis of *Centaurea ragusina* subsp. *ragusina* covering the whole leaf surface.

Fig. 4. Scanning electron microscopy (SEM) of several ordinary trichomes of *Centaurea ragusina* subsp. *ragusina*. Trichomes seem to be flattened as a result of pressure during the preparation of dry material.



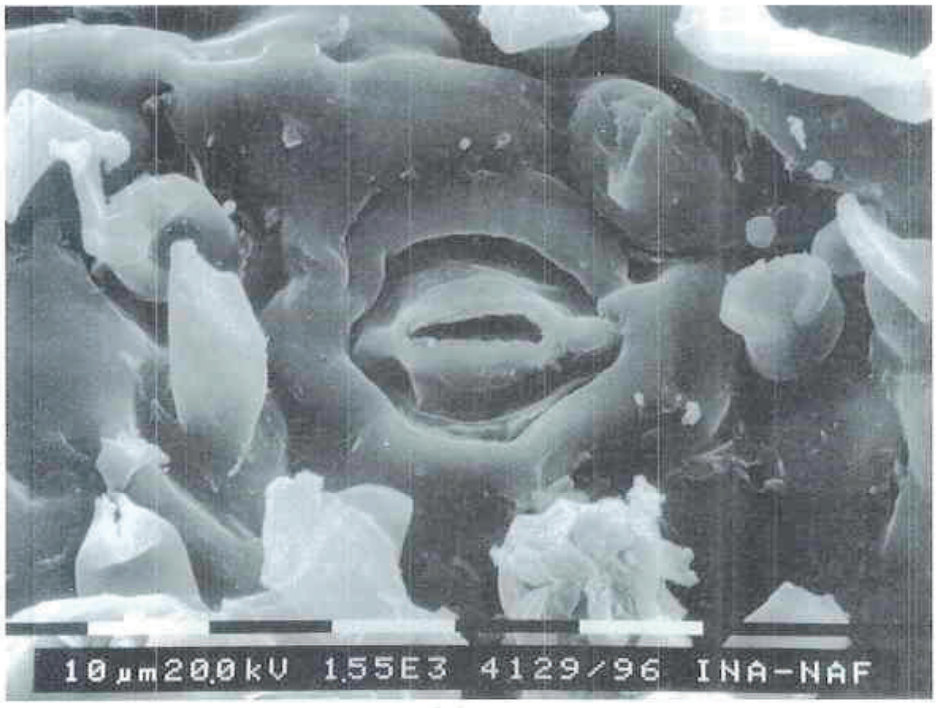


Plate 3

Fig. 5. Scanning electron microscopy (SEM) of an ordinary trichome segment of *Centaurea ragusina* subsp. *ragusina* with longitudinally oriented folds and furrow-like cuticular sculpturing.

Fig. 6. Scanning electron microscope (SEM) image of a stoma and of outline of epidermal cells of *Centaurea ragusina* subsp. *ragusina*. A broadly oval stomatal rim with a long and relatively narrow aperture and a broadly circular peristomatal rim are visible. From stomatal to peristomatal rim there are two extensions. Epidermal cells have straight and channel-like anticlinal walls and smooth periclinal ones. No crystaline wax. On the right is part of a sectioned tubular hair.

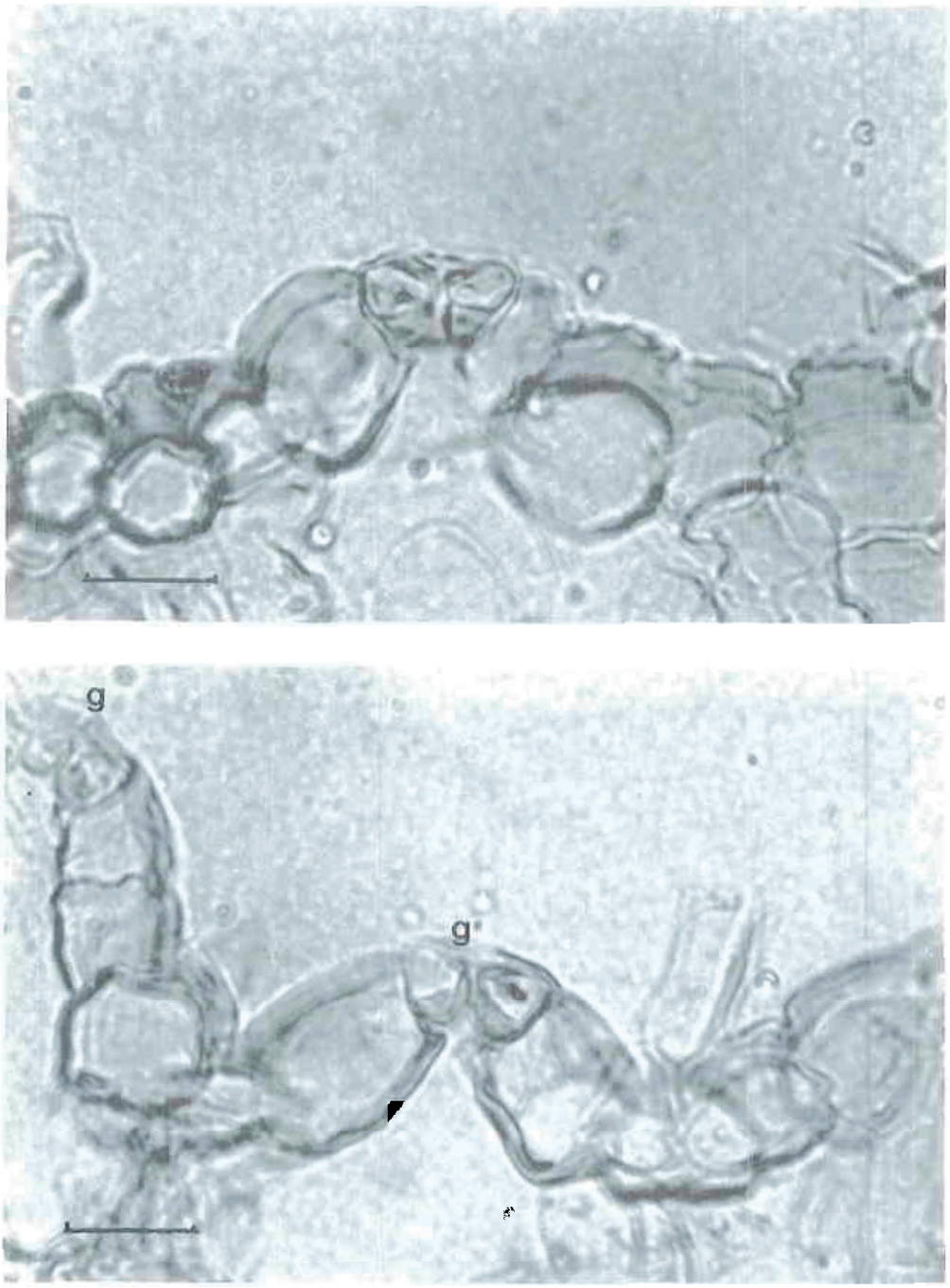


Plate 4

Fig. 7. Transverse section (TS) of a stoma of *Centaurea ragusina* subsp. *ragusina* above the level of the epidermis on the adaxial side. Under the guard cells there is a large substomatal chamber. Scale bar = 10 μm.

Fig. 8. Transverse section (TS) of stomata of *Centaurea ragusina* subsp. *ragusina* above the level of the epidermis on the abaxial side. Under the guard cells (g) there is also a large substomatal chamber. Scale bar = 10 μm.