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ANATOMICAL AND ECOLOGICAL ASPECTS IN ITALIAN TAXA OF THE GENUS *URTICA*

Abstract - The nettles native of Italy have been searched for microcharacters, such as cystoliths, mucilage cells, stomata, laminar hydathodes, non-glandular hairs, pearl glands, stinging hairs, etc, and for the anatomy of the vegetative organs. Information drawn from this work is useful not only for the current biosystematic revision of the genus Urtica, but also for a more accurate definition of the ecology of the different taxa. The isolated taxonomic position of *U. pilulifera* and *U. rupestris* is confirmed.

Keywords - *Urtica,* anatomy, ecology.

Riassunto - Aspetti anatomici ed ecologici dei taxa italiani del genere *Urtica.* Nelle ortiche italiane sono stati esaminati sia microcaratteri quali cistoliti, cellule a mucillagini, stomi, idatodi laminari, peli di rivestimento, ghiandole perlifere, peli urticanti, etc. sia l'anatomia degli organi vegetativi. Tale studio ha fornito informazioni utili non solo per la revisione biosistematica del genere *Urtica* che è in corso, ma anche per una più accurata definizione dell'ecologia dei differenti taxa. L'isolata posizione tassonomica di *U. pilulifera* e di *U. rupestris* viene confermata.

Parole chiave: *Urtica,* anatomia, ecologia.

INTRODUCTION

The genus *Urtica* comprises, in Italy, six specific units (Pignatti, 1982): *U. atrovirens* Req. ex Loisel., endemic to Sardinia, Corsica, and Balearic Islands, with some stations in coastal and insular Tuscany; *U. dioica* L., subcosmopolite, common throughout Italy, from 0 to 2000 m a.s.l.; *U. membranacea* Poir. ex Savigny in Lam., a stenomediterranean element, whose range is similar to that of *U. dioica*; *U. pilulifera* L. found in peninsular and insular Italy; *U. rupestris* Guss., endemic to eastern Sicily, and *U. urens* L., subcosmopolite, present throughout Italy, from coastal zones to highlands. Pignatti (1982) includes *U. sicula* Gasp. in Guss. in the intraspecific variations of *U. dioica*, while other authors (Parlatore, 1869; Arcangeli, 1894; Fiori e Paoletti, 1898; Fiori, 1923) consider it as a variety or subspecies.
The taxonomy of *Urtica* is traditionally based on vegetative and floral morphology, but in the recent years the use of other characters, such as the shape of cystoliths (Moreno, 1987; Paiva, 1993) and the ratio stinging structure/pedestal (Moreno, 1987), has been suggested. As a first contribution to a biosystematic revision of the genus *Urtica* in Italy, we consider these new microcharacters, along with others - such as stomata, mucilage cells, non-glandular hairs, pearl glands, laminar hydathodes - together with anatomy of vegetative organs - such as leaves, stem, root and, if present, rhizome - as convenient diagnostic characters.
The present work could also contribute, along with the current biosystematic revision, to a more accurate definition of the ecology of the various taxa. In fact, though nettles as a whole are generically designated as nitrophilous ruderal plants, *U. atrovirens*, as an example, is also found in woods and shady places (Arrigoni, 1983), *U. rupestris* is typical of rocky substrates and of volcanic (Gussone, 1821) and calcareous rocks, *U. pilulifera* prefers humus-rich soils, and *U. membranacea* thrives in any kind of substrate.

MATERIALS AND METHODS

The research is based on samples, currently grown in the Botanical Garden of the University of Pisa (*exsiccata* in PI), collected in various stations in the italian peninsula and islands, as follows.
U. atrovirens: Torre Vecchia, Gorgona Island (Livorno), Tuscany;
U. dioica: Petina (Salerno), Campania;
U. membranacea: Asciano (Pisa), Tuscany;
U. pilulifera: S. Antioco di Bisarcio (Sassari), Sardinia;
U. rupestris: Militello Val di Catania (Catania), Sicily (*locus classicus*);
U. sicula: Polizzi Generosa, on the Madonie range (Palermo), Sicily (*locus classicus*);
U. urens: Maremma Regional Park (Grosseto), Tuscany.

Light microscopy

For the purposes of light microscopy, the following parts have been used:
epidermal leaf strips, taken from both the adaxial and the abaxial surface;
20 µm sections of leaves, stem, root, and, if applicable, rhizome, obtained with a Leitz 1720 Digital Cryostat at -8° C;
2÷3 µm sections of leaves, stem, root, and, if applicable, rhizome, obtained with an Autocut Automatic

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Microtome Leica 2055, after fixation in FAA for 12 hrs, dehydration and infiltration in epoxydic resins (LR White resin - London Resin Company).
The following stains have been used: Toluidine Blue (O' Brien and McCully, 1981), for general purposes; Phloroglucine + HCl (Jensen, 1962), for the lignified parts; Ruthenium Red (Jensen, 1962) and Delafields hematoxylin (Faure, 1914), for the mucilage cells.
In order to locate laminar hydathodes, leaves were placed on a wet paper towel on a stereomicroscope stage, with light transmitted from below, as suggested by Lersten and Curtis (1991).

Scanning electron microscopy

For examination with scanning electron microscope (SEM), leaf blades of each entity were fixed in glutaraldehyde (2% with pH 7.4 phosphate buffer), dehydrated in increasing concentrations of alcohol and aceton mixtures, then dried in a critical point drying apparatus before being sputter-coated with gold and examined at 15 KV in a Cambridge Stereoscan 90.

RESULTS

Mucilage cells

Usually located on the abaxial surface of leaves, they are especially frequent in *U. rupestris* (fig. 1a) and *U. sicula*. They have never been found in *U. pilulifera* and in *U. urens*.

Cystoliths

Present in every entity reviewed in the present work, they have a roundish shape in *U. atrovirens* (fig. 1b), *U. pilulifera* and *U. urens*, while they are elongated in *U. membranacea* (fig. 1c) and in *U. rupestris*, and finally can be of either type (round and elongated) in *U. sicula* and *U. dioica*.
They are especially large in *U. dioica*, *U. sicula* and *U. rupestris*, and particularly numerous in *U. dioica*, *U. rupestris* and *U. urens*.

Laminar hydathodes

They are present in all entities, always on the upper side of leaves, usually at vein branchings, near the apex or the edge. They are quite numerous and large-sized in *U. atrovirens*, and, especially, in *U.pilulifera* (fig. 1d), in which they are not necessarily located at vein branchings nor near the apex or the edge of leaves. Associated with laminar hydathodes, in all the entities reviewed, a glandular hair can be observed, made up of an unicellular stalk and a head, usually made of 4 to 6 cells (fig. 1e). This kind of glandular hair, besides the association with hydathodes, is also found almost everywhere on the leaf epidermis, in all the entities reviewed, among which it doesn't seem to show morphological differences, with the exception of *U. pilulifera*, whose hairs are always larger than other species'.

Non-glandular hairs

Unicellular in all entities (fig. 2a) - seldom bicellular in *U. urens* - these hairs are, nearly always, bi- or tricellular in *U. pilulifera* (fig. 2b). In this species they are also very numerous, especially on both sides of the leaf, while they're quite scarce in the other entities.

Pearl glands

These structures, already studied by Mathwieser et al. (1987) in *U. dioica* and by Corsi and Maffei (1992) in *U. membranacea*, are present in the wild only in the latter (in which they are particularly frequent), in *U. sicula*, in *U. rupestris*, in *U. urens* and in *U. atrovirens*. In *U. dioica* pearl glands, in practice, are present only if the plants are kept for some time in a warm-moist greenhouse, while they are nearly absent in *U. pilulifera*. In all the entities reviewed, pearl glands appear to be always associated with a glandular hair.

Stomata

Anisocytic in all the entities reviewed, stomata are always slightly raised above the leaf epidermis (fig. 2c), and are present only on the abaxial leaf surface. In *U. atrovirens* (fig. 2d) and in *U. pilulifera* they seem somewhat smaller and particularly frequent (300÷320/mm^2), compared with the ones in other species (fig. 2e).

Stinging structures

These are not much frequent in *U. atrovirens* and in *U. rupestris*, and numerous in *U. dioica*, *U. membranacea*, *U. pilulifera* and *U. sicula*. They show glandular hairs (identical to those associated with laminar hydathodes, pearl glands and scattered on the leaf epidermis) on the pedestal in *U. membranacea* (cf. Corsi and Garbari, 1990). The ratio between the overall length of the structure and that of the pedestal changes among the different species: it is 1:3 in *U. atrovirens*, *U. membranacea*, *U. pilulifera* and *U. urens*; 1:4 in *U. dioica* and *U. rupestris*; 1:5 in *U. sicula*.

Anatomy of vegetative organs

a) leaf
In all the entities reviewed, the leaf stalk is roughly horseshoe-like. Lacunar collenchyma is arranged in 1÷2 layers, just underneath the epidermis. On the contrary, *U. pilulifera* has 3 collenchymatic layers. *U. urens* (fig. 3a) and *U. rupestris* have 3 fibrovascular bundles, with small amount of woody vessels; the other entities have, instead, 5÷7 bundles. In *U. pilulifera* the 5 bundles are very thick and so close

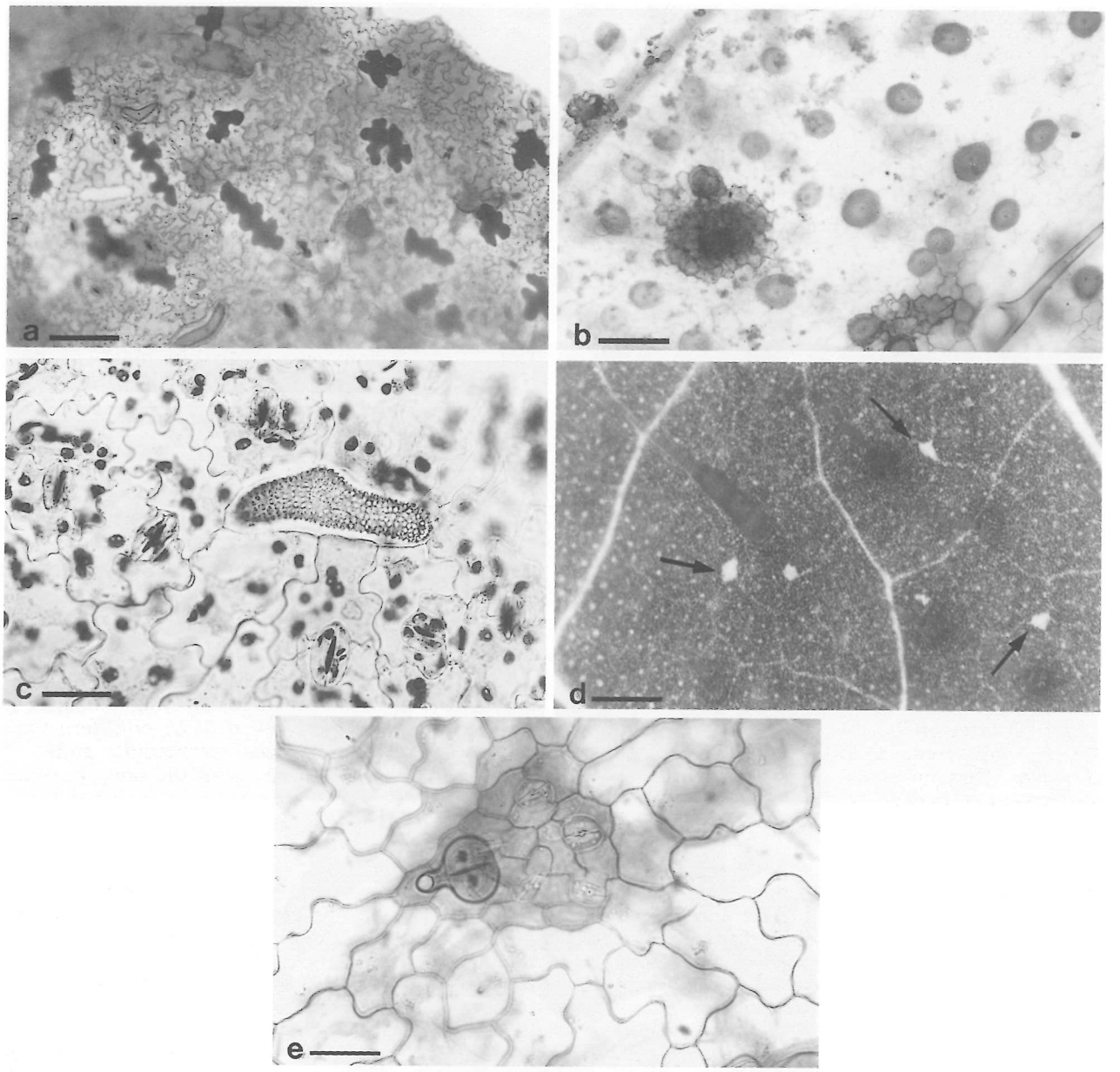

Figure 1a. *U. rupestris*: mucilage cells (ruthenium red) (bar=100 μm)
Figure 1b. *U. atrovirens*: roundish cystoliths (ruthenium red) (bar=40 μm)
Figure 1c. *U. membranacea*: elongated cystolith (hematoxylin) (bar=45 μm)
Figure 1d. *U. pilulifera*: laminar hydathodes (arrows) (locating in vivo) (bar=330 μm)
Figure 1e. *U. urens*: glandular hair associated with a laminar hydathode (hematoxylin) (bar=25 μm)

to each other that they look like a single, large bundle (fig. 3b). The veins in the leaf blade are highly prominent in *U. atrovirens*, *U. pilulifera*, *U. sicula* and, mostly, in *U. dioica*, in which they form a very thick net (fig. 3c).

The epidermis of the adaxial surface shows larger cells than the one of the abaxial side, in *U. atrovirens*, *U. rupestris (fig. 3d)*, *U. dioica* and *U. sicula*; cell sizes are similar in both leaf sides of the other species. Mesophyll is thick, with multiple layers of palisade tissue (seldom with a single layer, in which case palisade cells are quite large) in *U. atrovirens (fig. 3e)*; it is somewhat less thick in *U. dioica* and in *U. sicula*, where the palisade tissue can also be single-layered, in which case cells are very closely packed, and chloroplasts are particularly abundant; it is rather thin in *U. urens*, *U. membranacea*, *U. rupestris* and *U. pilulifera* (fig. 3f), where palisade

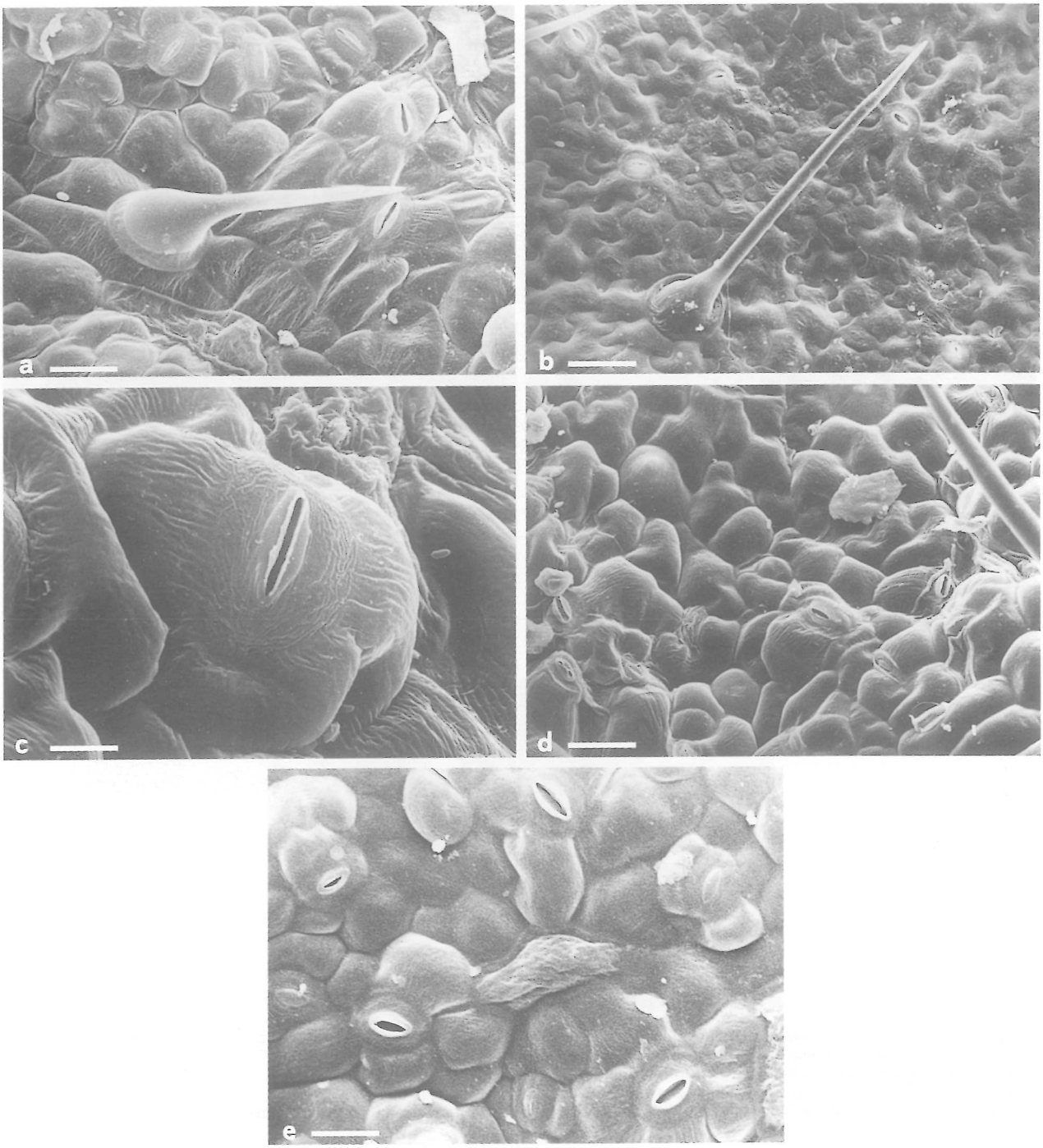

Figure 2a. *U. dioica*: unicellular non-glandular hair (SEM, bar=19 μm)
Figure 2b. *U. pilulifera*: pluricellular non-glandular hair (SEM, bar=30 μm)
Figure 2c. *U. dioica*: stoma slightly protruding above other epidermal cells (SEM, bar=5 μm)
Figure 2d. *U. dioica*: abaxial side epidermis, with stomata (SEM, bar=20 μm)
Figure 2e. *U. atrovirens*: abaxial side epidermis, with stomata (SEM, bar=17 μm)

tissue shows rather undifferentiated, large cells, and large intercellular spaces. In *U. atrovirens*, just outside the fibrovascular bundle of the main nervation, there is a big secretory duct (fig. 3g) whose content reacts positively to histochemical stains for lipids. On the same nervation, on the opposite side of the

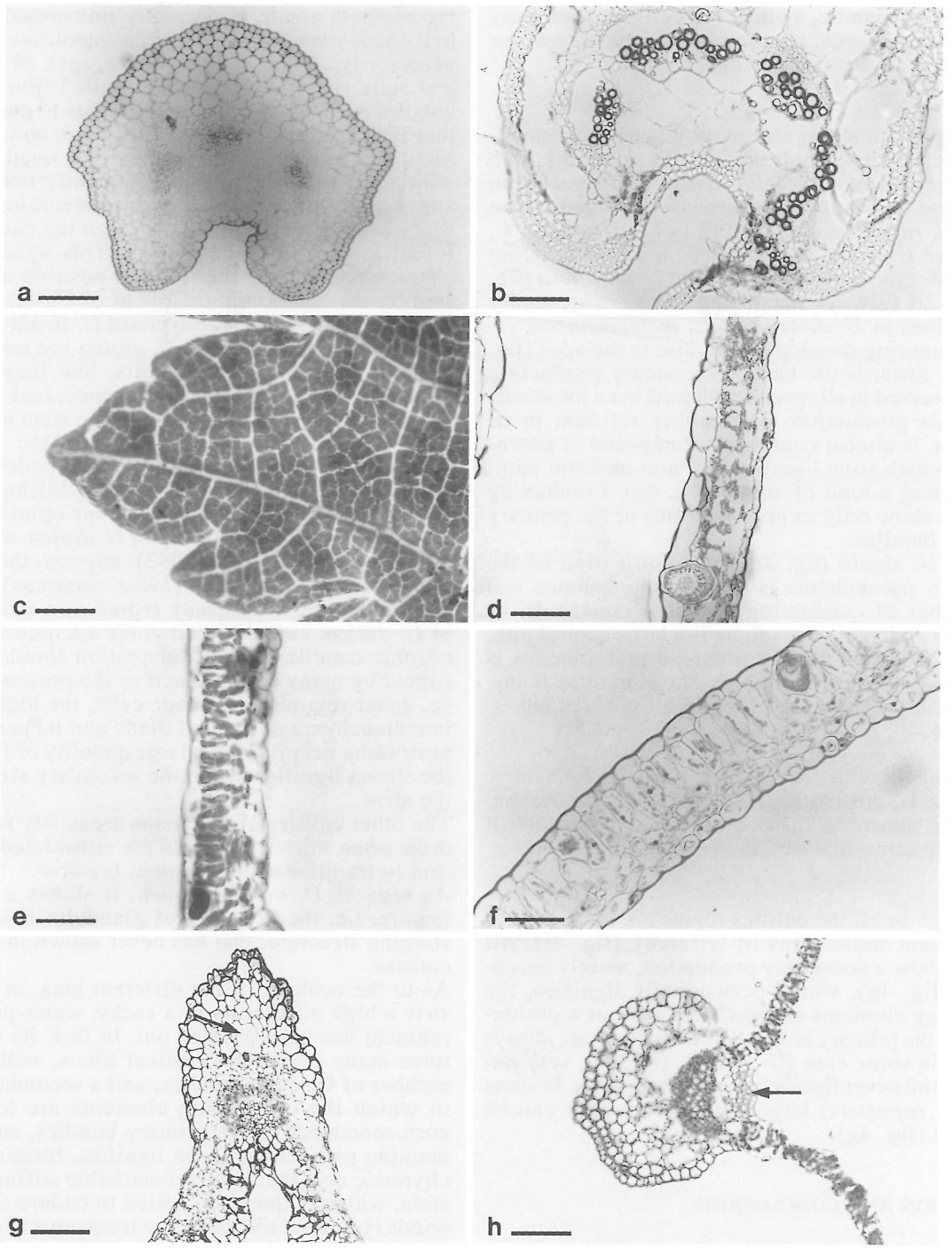

Figure 3a. *U. urens*: leaf stalk with three fibrovascular bundles (toluidine blue) (bar=50 μm)
Figure 3b. *U. pilulifera*: leaf stalk with five large fibrovascular bundles, close to each other so that they resemble a single, very large fibrovascular bundle (phloroglucine + HCl) (bar=50 μm)
Figure 3c. *U. dioica*: leaf blade with a very thick net made up by the nervations (locating in vivo) (bar=500 μm)
Figure 3d. *U. rupestris*: cross section of the leaf blade; the epidermal cells of the adaxial side larger than the ones of the abaxial side (toluidine blue) (bar=50 μm)
Figure 3e. *U. atrovirens*: cross section of the leaf blade, showing the two-layered palisade tissue (hematoxylin) (bar=50 μm)
Figure 3f. *U. pilulifera*: cross section of the leaf blade, with large palisade cells and wide intercellular spaces (toluidine blue) (bar=31 μm)
Figure 3g. *U. atrovirens*: secretory duct (arrow) at main nervature level, underneath the fibrovascular bundle (toluidine blue) (bar=125 μm)
Figure 3h. *U. atrovirens*: group of cells with gelified walls (arrow), at main nervature level, above the fibrovascular bundle (hematoxylin) (bar=125 μm)

fibrovascular bundle, a cluster of cells with strongly thickened cell walls, reacting positively to tests for pectine-like matter, can be observed (fig. 3h).

b) stem
It is roughly quadrangular, with lacunar collenchyma at each corner, in all the entities reviewed, with the exceptions of *U. pilulifera* and *U. rupestris*, in which case it is roundish. Fibrovascular bundles can be 6 in *U. rupestris* (fig. 4a), 12 in *U. atrovirens*, *U. sicula* and *U. urens*, 12÷16 (depending on whether we consider the apex or the base) in *U. pilulifera* (fig. 4b), 12÷20 (always according to the zone under observation) in *U. dioica* and *U. membranacea*.
A cambium ring develops very close to the apex (fig. 4c), and, towards the base, a secondary production can be observed in all species reviewed, even the annual ones. This production, particularly relevant in *U. pilulifera*, is almost completely composed of parenchyma, which soon lignifies in a non-uniform manner, forming a kind of annuli (fig. 4d). Conducting elements show only as prolongations of the primary vascular bundles.
Only in *U. sicula (fig. 4e)* the lignification of the secondary parenchyma is more homogeneous, and the number of conducting elements greater. In *U. pilulifera* and in *U. urens* there is a large central pith, that is reabsorbed in the former, so that a lacuna is formed (fig. 4c). Cortical fibers, showing little, if any, lignification, can be observed in all entities but *U. pilulifera*.

c) rhizome
Present in *U. atrovirens*, *U. dioica*, *U. rupestris* and *U. sicula*; absent in the other entities. If present, it shows the same features as the stem.

d) root
It is biarch in all the entities reviewed, though there are frequent indications of tetrarchy (fig. 4f). All entities show a secondary production, mainly parenchyma (fig. 4g), which occasionally lignifies; the conducting elements are produced only as a prolongation of the primary arches. Cortical fibers are always present, in some case (*U. sicula*), (fig. 4f), very numerous, and never lignify but in *U. atrovirens*. In some case (*U. rupestris*) large amounts of cork can be observed (fig. 4g).

Discussion and conclusions

The study of the microcharacters and of the anatomy has allowed to get useful biosystematic informations. According to Chrtek (1979), the genus *Urtica* can be split in three subgenera: *Urtica*, *Sarcourtica* and *Dendrourtica*.
From the present study, *U. pilulifera* shows such features through which it can be clearly discriminated from the other entities of the same genus, notably: absence of mucilage cells and pearl glands; non-glandular hairs very frequent, long, bi- or tricellular; stomata small, particularly numerous; laminar hydathodes very large, in large quantities and, not necessarily, at the edge or at the apex of the leaf; leaf stalk rich in collenchyma, with 5 fibrovascular bundles particularly thick and so close to one another that they seem to form one very large bundle; stem section roundish, while it normally is quadrangular, with a large central pith (subsequently reabsorbed) and a quite relevant secondary production, and absence of cortical fibers, that can, on the contrary, be found in all the other species. This syndrome of characters confirms the isolated position as suggested by the taxonomic treatment of Chrtek (1979).
Similarities between specimens of *U. sicula* collected in the *locus classicus* and *U. dioica* are relevant, as to stomata, non-glandular hairs, low frequency of laminar hydathodes, cystoliths' shape, leaf anatomy, frequency of extraxylar fibers in the stem and in the root. The differences in the shape of the mucilage cells and in the ratio stinging structure/pedestal don't seem sufficient to motivate the establishment of a subspecific or varietal taxon. In our opinion, *U. sicula* is part of the variability of *U. dioica*, as already pointed out by Pignatti (1982); anyway, the population of Polizzi Generosa (*locus classicus*) is made up of individuals that may represent a morphotype of *U. dioica*, suited to particular microclimatic and edaphic conditions. This adaptation should be confirmed by many details, such as the presence of large, water-retaining mucilage cells, the high content in collenchyma of the leaf blade and its particularly protruding nervation, the large quantity of fibers and the strong lignification in the secondary structure of the stem.
The other entities of the genus are neatly separated, even when microcharacters are considered in addition to traditional diagnostical features.
As regards *U. membranacea*, it shows a peculiar feature, i.e. the presence of glandular hairs on the stinging structure, that has never shown in the other entities.
As to the ecology of the different taxa, in *U. rupestris* a high adaptation to a rocky, water-poor environment has been pointed out. In fact, its stem features many extraxylar cortical fibers, with a small number of vascular bundles, and a secondary wood, in which the conducting elements are located in correspondence to the primary bundles, and the remaining parenchyma soon lignifies, forming parenchymatic rays that lend a remarkable stiffness of the stem, which is thus well suited to endure the strong winds typical of the rocky environments (Francini & Messeri, 1956). A small amount of stomata per surface unit, and, above all, a relevant number of mucilage cells, that are able to effectively retain water, are other aspects of its successful adaptive process. Accordingly, the position in the subgen. *Dendrourtica* Chrtek seems acceptable.
In *U. atrovirens*, the leaf anatomy, distinguished by different layers of palisade tissue, with a very high number of chloroplasts per cell, gives a reason for the plant's ability to live in woods and in shady pla-

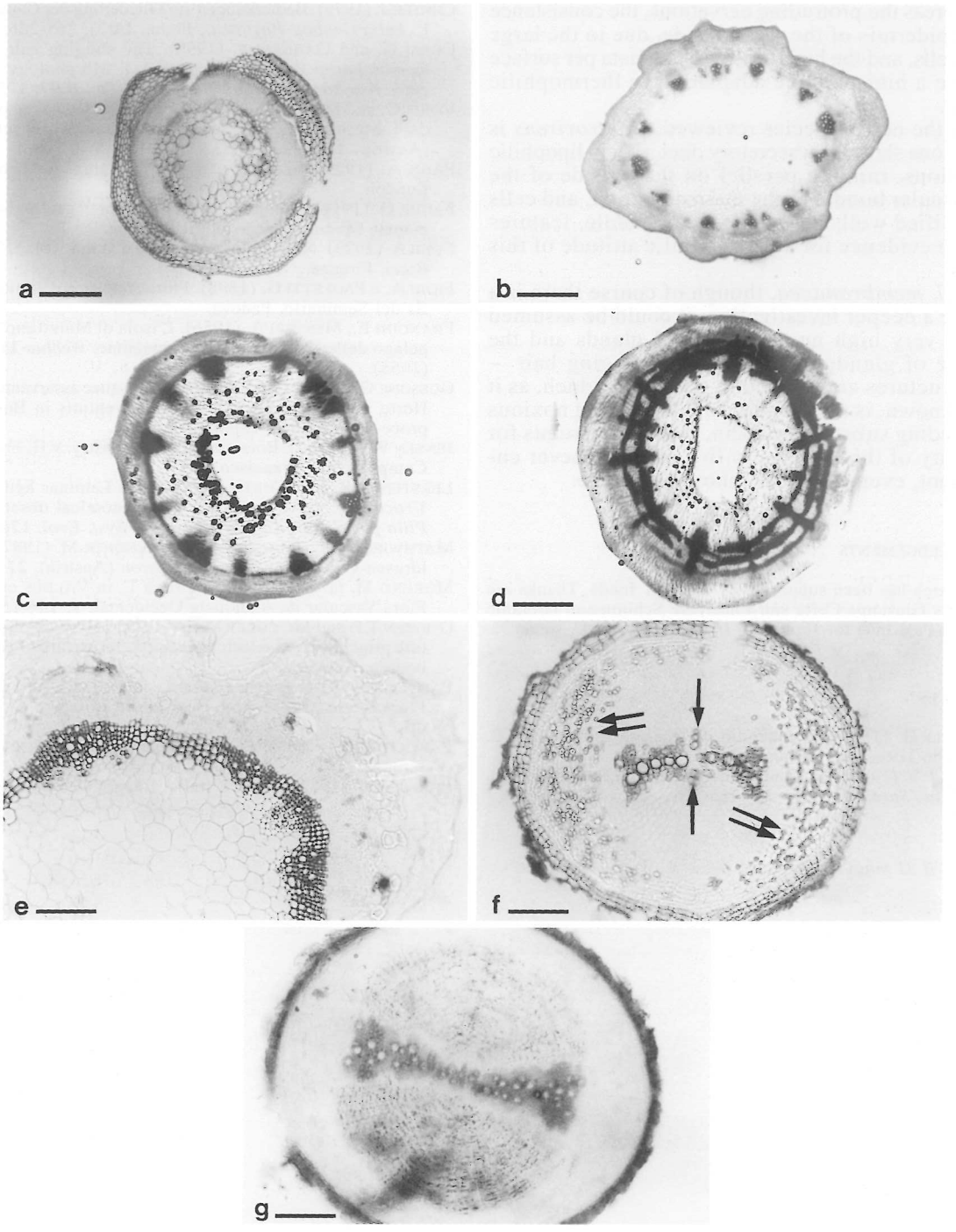

Figure 4a. *U. rupestris*: cross section of the stem, showing 6 fibrovascular bundles (hematoxylin) (bar=330 μm)
Figure 4b. *U. pilulifera*: cross section of the stem, showing >12 fibrovascular bundles (phloroglucine + HCl) (bar=250 μm)
Figure 4c. *U. pilulifera*: cross section of the stem, near the apex, showing the cambium ring and the big central lacuna (phloroglucine + HCl) (bar=250 μm)
Figure 4d. *U. pilulifera*: cross section of the stem, showing an unusual lignifying pattern of the parenchyma, discontinued by rays formed by the elongation of the fibrovascular bundles (phloroglucine + HCl) (bar=250 μm)
Figure 4e. *U. sicula*: uniform lignification of secondary parenchyma in the stem (phloroglucine + HCl) (bar=125 μm)
Figure 4f. *U. sicula*: biarch root, with two additional arches, just outlined (arrows), and a very high amount of fibers in the cortical zone (double arrows) (phloroglucine + HCl) (bar=125 μm)
Figure 4g. *U. rupestris*: root showing production of secondary parenchyma on both sides of the two arches (phloroglucine + HCL); a remarkable amount of cork marked by its morphological features is also shown (bar=125 μm)

ces, whereas the protruding nervations, the consistence of the epidermis of the adaxial side, due to the large size of cells, and the high density of stomata per surface unit give a hint of some adaptation to thermophilic habitats.

Among the nettle species reviewed, *U. atrovirens* is the only one showing a secretory duct, rich in lipophilic productions, running parallel on the outside of the fibrovascular bundle of the main nervation, and cells with gelified walls below the same bundle, features that give evidence for a thermophilic attitude of this species.

As for *U. membranacea*, though of course there is a need for a deeper investigation, it could be assumed that the very high number of pearl glands and the presence of glandular hairs on the stinging hair – both structures are implied in guttation, which, as it is well known, is also a mean for eliminating noxious or exceeding substances (Fahn, 1979) – accounts for the ability of this species to live on whatsoever environment, even the most inhospitable one.

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