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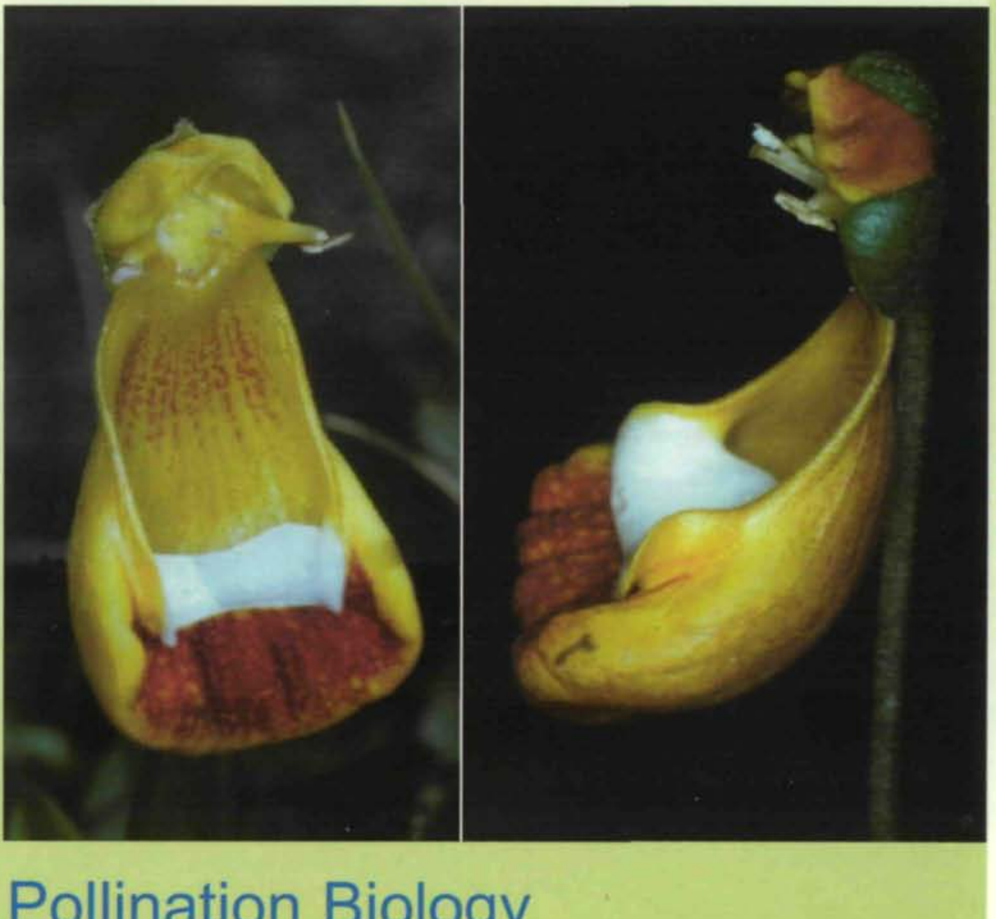

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Pollination biology in the genus *Calceolaria* L. (Calceolariaceae)

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Abstract: A comparative analysis of the floral biology in 48 species of the genus *Calceolaria* (*Calceolariaceae*) was done; 29 of them were studied at their native habitats. Cultivated plants, dried and formaldehyde-preserved specimens were also used. An analysis of the floral structures which have an anthoecological importance, are described. Features of corolla, elaiophor, androecium, gynoecium and anthesis are treated in detail; floral oils were studied by thin layer chromatography; the food body for *C. uniflora* is described. Oil collectors of the two bee pollinators (genera *Centris* and *Chalepogenus*) are described based on specimens collected in the field. An account on visitation of 19 species in natural populations and the behavior of the bee-visitors is given. Sternotribic pollination is documented and described for five species of section Rugosae and one of section Integerrimae. Bird pollination with a food body as reward is also described and documented for the genus. Based on the available data, the floral syndromes of *Calceolaria* appear to be restricted to:

1. Melittophily
    a. with oils as reward
    b. with pollen as reward
2. Bird pollination

The distribution of floral syndromes in different sections of the genus is analyzed and its relation to the geographical distribution of *Calceolaria* in South America is considered.

Keywords: bird pollination, *Calceolaria*, *Centris*, *Chalepogenus*, oil bees, oil flowers, pollination.

Introduction

Nectar is by far the most widespread flower reward or primary attractant since it is required by all main groups of anthophilous animals (birds, bats and insects). However, this concept is of limited value in the study of pollination by bees or melittophily. It has to be born in mind that the relationships between bees and plants are diverse since these insects depend on flowers at several points in their life history. The adults not only take nectar for their own energy requirements, but in addition they obtain pollen that is utilized as the only protein source to feed the brood. Since SPRENGEL (1793) pollen and nectar were recognized as the rewards of bee pollinated flowers and for a long period of time they were considered the only ones. Only recently flower biologists have proven that, though these flower products are the fundamental primary attractants, the array of rewards is much broader. The flower attractants of melittophilous flowers include both substances and services. The latter is exemplified by a few cases of considerable importance in general, but can be left aside in the context of the present investigation.

Resins and perfumes (volatile oils) are additional primary attractants of bee pollinated flowers. Resins as reward were studied in more or less detail by ARMBUSTER (1984), ARMBRUSTER & WEBSTER (1979), BITTRICH & AMARAL (1996a), BITTRICH & AMARAL (1996b), PORSCH (1905), VAN DER PIJL & DODSON (1966), who reported that resins are foraged by females of wild bees of the families Apidae (tribes Meliponini, Euglossini) and Megachilidae for the purpose of nest building. The biology of flowers offering perfumes as rewards was first correctly interpreted and extensively studied by DODSON (1967), DODSON & FRYMIRE (1961) DODSON (1962), GERLACH & SCHILL (1989), GERLACH & SCHILL (1991), GRACIE (1993), KUNDSEN & MORI (1996), SAZIMA & al. (1993), VOGEL (1963a,b), VOGEL (1966), among others. The flowers that offer this kind of reward are pollinated by males of the bee tribe Euglossini, which obtain the volatile substances and store them in highly specialized structures of the hindlegs. The collected substances, probably in a modified form, are ventilated during part of the still ill-understood mating process.

Fixed oils represent a third kind of flower substance acting as reward. It is the most recently discovered primary attractant and is now placed third in order of importance after nectar and pollen. The term "oil flower" was coined for this new flower class by VOGEL (1974). The occurrence of this type of reward is now well documented for eight angiosperm families and about 1400 species and 73 genera. *Calceolaria* represents the largest among these genera.

KIRCHENER (1925) and POHL (1935) first suggested that oils could act as flower reward after studying orchid species of the genera *Oncidium*, *Eria* and *Bulbophyllum*. On the basis of observation in *Angelonia*, VOGEL (1964) reported for the first time that flower oils were involved in pollination by oil-collecting bees. He also screened through representatives of many plant families searching for flower oils. He found them in *Malpighia* (*Malpighiaceae*), *Oncidium* (*Orchidaceae*), in *Bowkeria* and *Diascia* (*Scrophulariaceae*) and in *Calceolaria* (*Calceolariaceae*). After a trip to South America (1969, 1970) he could provide overwhelming evidence of the close mutualism between bees of the genera *Centris*, *Chalepogenus* (formerly *Tapinotaspis*) and *Paratetrapedia* and several oil flowers. He further added the genera *Stigmatophyllum* (*Malpighiaceae*), and several iridaceous genera (*Allophia, Cypella, Sisyrinchium* and *Sphenostigma*) to the already known cases. VOGEL (1974) was the first to interpret correctly the flower biology of *Calceolaria* for which previous reports were contradictory. While CORRENS (1891a) and KRÄNZLIN (1907) left open the question about the nature of the flower secretion, other authors (DESCOLE & BORSINI 1954, EDWIN 1971, HILDEBRAND 1867, KERNER V. M. 1886-1891, KNUTH 1899, RATNALIKAR & PAI 1969, URBAN 1934, and WITASEK 1906, 1907) wrongly regarded the flower glands as nectaries. VOGEL's (1974) conclusions were based on direct observations of eight *Calceolaria* species.

The present work aims to give a broader account on the diversity of the flower biology in *Calceolaria* based on observations of pollination mechanisms in other species, the study of functional morphology, considering also aspects of oil chemistry and breeding systems.

Geographical distribution

According to recent molecular studies (JUDD & al. 1999; OLMSTEAD & al. 2001) the genera *Calceolaria*, *Porodittia* and *Jovellana* (Fig. 2D) constitute a new family, Calceolariaceae. *Calceolaria* with 250-270 species ranges from Mexico to Tierra del Fuego, and is distributed mainly at high altitudes, between 2000 and 4000 m in tropical and subtropical regions; in temperate regions of southern Argentina and Chile certain species reach the sea level at both the Atlantic and Pacific coasts (Fig. 62). Taking in consideration the latitudinal variations of the genus, three more or less well defined phytogeographical zones can be distinguished within the genus. Each zone has its own species assemblage and only a few species occur in more than one zone (MOLAU 1988, PENNELL 1945). Two geographical discontinuities are interposed along the ecological continuum of the high Andes: in northern Peru the so called Piura divided is determined by the Huancabamba valley that cuts the cordillera abruptly building a barrier difficult for *Calceolaria* to cross (MOLAU 1978, 1981). A second barrier is built up by the Atacama desert between 18° and 20° S which represents a "dead zone" that can only be crossed along isolated oases (MOLAU 1979, VUILLEUMIER & SIMBERLOFF 1980). A similar latitudinal discontinuity is also known for other genera of *Scrophulariaceae*, such as *Alonsoa* R. & P., *Bartsia* L. and *Castilleja* L., for *Mutisia* L. among *Asteraceae*, and for *Bomarea* MIRB. among the *Alstroemeriaceae* (SIMPSON 1975).

Two centers of diversification are recognized in *Calceolaria*, one in central Chile, and the other one in northern Peru (MOLAU 1988, GRAU pers. comm.). After cytological studies (EHRHART 1997, 2000) two diversity centers are also distinguishable; one includes predominantly tetraploid tropical species and the other contains mostly diploid temperate species of Argentina and Chile. There is almost no overlap between both groups of species.

Calceolaria and *Jovellana* are members of subantarctic affinity (CLEEF 1979). By the end of the Cretaceous and beginning of the Tertiary, when South America, Australia and New Zealand were still connected (RAVEN & AXELROD 1974) *Jovellana* probably had a wider distribution in the southern hemisphere. After Mid Tertiary its original range was divided into two disjunct areas of New Zealand and South America. *Calceolaria* is regarded as apomorphic in relation to *Jovellana* and is considered to have evolved after the continent split. Its distribution along the Andes to tropical zones would have occurred more recently at the end of the Tertiary and beginning of the Quaternary, 1.5-2 million years before present.

Infrageneric classification

The most recent infrageneric classification of *Calceolaria* is that of MOLAU (1988) who recognizes 24 sections and three subgenera:

- Subgenus **Calceolaria** contains most of the sections previously placed in subgenus Cheiloncos by PENNELL (1951), and includes 19 sections and 184 species mainly from the tropical Andes.
- Subgenus **Cheiloncos** is restricted to two sections and 30-35 species and probably builds up a monophyletic group distributed almost exclusively in temperate zones of Chile and Argentina (MOLAU 1988).
- Subgenus **Rosula** contains three sections and 35-40 species of temperate and cold regions of Chile and Argentina.

MOLAU's monograph (1988) clarifies the taxonomy of all species growing north of the tropic of Capricorn. EHRHART (2000) made an exhaustive study of the Chilean species, clarifying the taxonomy of many southern species, whereas those from Argentina still need detailed revisionary studies to identify the actual number of species, their phylogenetic affinities and geographical distribution. Since it is not intended here to make any taxonomical analysis, the species studied are placed in the sections defined by MOLAU (1988) according to their distinctive characters:

Tribus Calceolarieae
- **Genus** *Jovellana*
  - *J. violacea* (CAV.) G. DON
- **Genus** *Calceolaria*
  - **Subgenus** Calceolaria
    - **Section** Calceolaria
      - *C. chelidonoides* H. B. K.
      - *C. rivularis* KRÄNZ.
      - *C. tripartita* R. & P.
    - **Section** Englerina
      - *C. irazuensis* DONNELL SM.
    - **Section** Integerrimae
      - *C. hypericina* BENTH.
      - *C. pinifola* CAV.
      - *C. stellariifolia* PHIL.
      - *C. thyrsiflora* GRAH.
      - *C. ascendens* LINDL. subsp. *ascendens*
    - **Section** Lobatae
      - *C. rhacodes* KRÄNZ.
    - **Section** Parvifoliae
      - *C. schickendantziana* KRÄNZ.
    - **Section** Perfoliatae
      - *C. perfoliata* L.
    - **Section** Polyclada
      - *C. polyclada* KRÄNZ.
    - **Section** Salicifoliae
      - *C. tetragona* BENTH.
    - **Section** Teucriifoliae
      - *C. lossenii* KRÄNZ.
      - *C. ruiz-pavonii* DESCL. & BORS.
      - *C. santolinoides* KRÄNZ.
      - *C. teucrioides* GRIS.
    - **Section** Urticopsis
      - *C. bicolor* R. & P.

Subgenus Cheiloncos
- **Section** Rugosae
  - *C. andina* BENTH.
  - *C. plectranthifolia* WALP.
  - *C. dentata* R. & P.
  - *C. integrifolia* L.
  - *C. lepida* PHIL.
  - *C. polifolia* HOOK
  - *C. petioalaris* CAV.
  - *C. robusta* DIETR.
  - *C. latifolia* BENTH.
  - *C. volckmanii* PHIL.
- **Section** Micranthera
  - *C. tucumana* DESCL.

Subgenus Rosula
- **Section** Bellidifoliae
  - *C. mendocina* PHIL.
  - *C. brunellifolia* PHIL.
  - *C. parviflora* BENTH.
  - *C. umbellata* WEDD.
- **Section** Kremastocheilos
  - *C. uniflora* LAM.
  - *C. prichardii* (RENDLE) KRÄNZ.
  - *C. lanceolata* CAV.
  - *C. lagunae-blancae* KRÄNZ.
  - *C. biflora* LAM.
  - *C. filicaulis* CLOS. subsp. *luxurians* (WIT.) C. EHRHART
  - *C. polyrhiza* CAV.
- **Section** Corymbosae
  - *C. valdiviana* PHIL.
  - *C. arachnoidea* GRAH.
  - *C. paralia* CAV.
  - *C. corymbosa* R. & P.
  - *C. tenella* POEPP. & ENDL.
  - *C. crenatiflora* R. & P.
  - *C. williamsii* PHIL.

According to the recent taxonomic revision of the Chilean species (EHRHART 2000) the following changes are suggested: *C. mendocina* PHIL., *C. prichardii* (RENDLE) KRÄNZ. and *C. lanceolata* CAV. = *C. polyrhiza*; and *C. robusta* DIETR. and *C. andina* BENTH. = *C. integrifolia* L.

Methods

Investigation of flower structures

Anatomical studies with light microscopy (LM): To study stamens and corolla appendages, hand sections were made from living material. Serial thin sections (10 µm thick) were made on formaldehyde-fixed (FAA) material, following standard paraffin-embedding and hemathoxylin-safranin-fast green-staining (JOHANSEN 1940). Embedding in synthetic resins (Technovit 7100, Kulzer) was used for a detailed analysis of glandular hairs.

Morphological studies with scanning electron microscopy (SEM): Occurrence, dimensions, shapes and glandular hair densities, as well as structural characters of the androecium were determined on SEM photographs obtained from critical point-dried and gold-sputtered excised flower parts. For critical point drying a modified version of the technique performed by GERSTERBERG & LEINS (1978) was used. FAA-fixed samples were dehydrated twice for 10 min in formaldehyde dimethyl acetal (FDA); FDA or acetone was used as the medium prior to drying with a Polaron E 3000 or a Balzers CPD 030 equipment. A Polaron E 5100 instrument was used to sputter the samples with gold. For observations a Cambridge Stereoscan 250 Mk2 was used.

Elaiophore area and number of trichomes: The total area covered by glandular trichomes was calculated from SEM photographs by cutting out the corresponding area of paper, weighting on an analytical balance, and comparing it with the weight of a piece of paper of the same kind of known surface. The absolute number of trichomes was counted, when possible, from SEM photographs. Otherwise, trichome density was measured under LM on whole mounts of the appendages by comparison with a square of known area.

Oil secretion: To determine the beginning of oil secretion, appendages were excised from living buds and flowers at different stages of development and stained with Sudan IV. This analysis was only performed with cultivated plants. To measure the weight of secreted oils, 20 flowers at the same developmental stage were sampled. The oil was collected with small triangular strips of Wathmann #1 chromatography paper previously dried at 60° C for a minimum of 24 h and then weighted with an analytical balance (precision 1 µg). After collecting the oil, the oil-soaked paper strips were dried again at 60° C and the difference in weight was recorded. With the aid of this technique the following measurements were performed:

- Oil secretion dynamics along the anthesis in *C. latifolia*: 35 buds of 0.4 cm in length were marked in cultivated plants excluded from pollinators. Each 24 hours, oil weight was measured in 5 flowers, thus covering the days of anthesis. For this procedure flowers had to be cut from plants.
- Dynamics of oil reposition after extraction in *C. parviflora*: 25 buds prior to anthesis were identified in cultivated plants excluded from pollinators. Oil was collected periodically without cutting the flowers. This procedure was performed gently without damaging the glandular area, so that some oil was left on the glands. Therefore, measurements were calculated on a relative but not on an absolute basis.

Oil chemistry: Thin layer chromatography (TLC) was performed according to the methods developed by VOGEL (1974) for this particular kind of oils. Raw extracts were obtained by eluting the excised corolla appendages with chloroform or hexane. For each species 2-351 appendages were submerged for 2-3 min in the solvents and gently shaked. Extracts obtained during field trips were filled in 25-30 cm long capillary tubes, heat-sealed and stored at about 5°C until analysis. Extracts were spotted on pre-coated TLC plates (Reichelt Chemietechnik, 20 x 20 cm) 5 cm apart from the lower margin and developed at 24°C in chloroform-ethanol-acetic acid-methanol (96:4:2:1) for 60 min. Iodine vapors or carbonization (1 h at 150°C) after spraying with sulfuric acid-methanol (1:1) was alternatively used for spot visualization.

Numerical analysis of TLC spots: To make comparisons of chromatographic spot patterns among the 22 species of *Calceolaria* and the 3 oil-collecting bees, a cluster analysis was performed. For the numerical treatment the RR association coefficient (1.1 agreements/total number of agreements) and the unweighted pair-group method of arithmetic averages (UPGMA) was used as a clustering strategy.

Investigation of mating systems

Pollen viability, in particular to reveal differences between thecae of single anthers, was assayed by cotton blue or acetocarmine staining of fixed or living samples (JOHANSEN 1940). Alternatively, the procedure of GREISSL (1989) using fluorescein diacetate was performed on living samples. During progress of anthesis, sequence and duration of the following events were determined: 1) stigma receptivity, 2) onset of oil secretion (see above), and 3) pollen shedding. Hydrogen peroxide (6%) was used to test stigma receptivity (KEARNS & INOUYE 1993). Anthesis was followed in the species *C. latifolia* and *C. tripartita*. Their flowers never open, so the stage of flower development was determined by measuring corolla length. The beginning of oil secretion was arbitrarily set as the beginning of anthesis. Percentages of fruit set were determined after cross and self-pollination of plants excluded from visitation. Percentages of seed viability for each cross were determined by germinating seeds (n=100) on wet filter paper in Petri dishes.

Analysis of insect structures

To study oil-collecting structures of bees under SEM, pinned specimens were cleaned by sonication in water and chloroform, allowing the specimens to dehydrate and rehydrate along an ethanol series. After drying at room temperature the legs were mounted and gold sputtered and viewed with the same instruments as above. For more details of this procedure especially developed for oil-collecting organs see NEFF & SIMPSON (1981).

Field observations

Pollinator activities on flowers were observed in natural populations of 21 *Calceolaria* species. Their behavior was documented with a 35 mm camera equipped with a 100 mm macro lens and ring light, or a 205 mm tele lens. These processes were also recorded in some cases with a super 8 film camera or a video camera.

Results

Analysis of flower structures

Each floral cycle is treated separately in the following anylsis stressing its modifications and adaptations related to floral biology.

Calyx: The calyx does not show much variation within the genus. It consists of four sepals (one dorsal, two lateral, and one ventral). The ventral sepal is the product of the fusion of the two ventro-lateral lobes. Its color is normally green or pale green, except for some species of sections Perfoliatae (Fig. 2C) and Rugosae (MOLAU 1988), where it is completely yellow. This coloration enhances the visual effect of the yellow color of the corolla.

Corolla: The corolla is two-lipped. The lips arise from a short and thick ring of tissue common to the corolla and androecium, the so called stapetum (RITTERBUSCH 1976), a tissue complex that appears between the calyx and the ovary. The lower lip is larger than the upper lip, it is always inflated and bears a median lobe, the appendage or lap. This lap is folded inwards in most cases and carries a cushion of glandular trichomes, the elaiophore (Fig. 1A), on its morphological underside. Two theories about the origin of the corolla lips have been considered:

- Traditionally, the lips have been interpreted as being formed by the congenitally united flaps of the corolla limb, also normally present in the related family Scrophulariaceae, whereas the upper lip is formed by the two dorsal ones (Fig. 3C), and the lower lip by the two lateral and the ventral limb flaps: "...tubus subnulus, limbus concavus bilobus..." (BENTHAM 1846, KRÄNZLIN 1907, REICHE 1911, WETTSTEIN 1891).

- RITTERBUSCH (1976) considers each corolla lip as being formed by the corolla tube and not by the limb and regards the individual petals not being identifiable. During the primordial stages, after the development of the stapetum, the ring-shaped meristem of the corolla tube divides into two portions, a dorsal and a ventral one, which correspond to the upper and the lower lip, respectively. Both meristems develop further independently and give the impression that the tube is absent. The only reminiscence of a true corolla limb would be represented by the appendage.

A bubble-shape or slipper-like structure is always formed by the lower lip, and sometimes (section Rugosae) also by the upper lip. RITTERBUSCH (1976) has thoroughly documented that this morphological character is achieved by means of a process called "fimatosis" in which the middle portions of the tube between stapetum and rim grow intensively, while growth of the rim remains inhibited. In the lower lip this differential growth causes an inward folding of the appendage (RITTERBUSCH 1976).

The appendage is single-lobed, but is considered to be formed by the congenital union of three (VOGEL 1974) or two (VARGHESE 1967) corolla flaps. Certain teratological flower forms with a three-lobed appendage carrying one elaiophore each (Fig. 3B) support the former interpretation, while the existence of only two vascular bundles supplying the appendage in normal flowers supports the latter.

Considerable variation and complexity is present in the shape of the corolla within the genus. Figure 1A shows a flower with a large dorso-ventrally flattened lower lip and a much smaller upper lip (UL) that only partially covers the fertile organs. In the lower lip two main parts are recognized: a throat (T) and a slipper-shaped or calceiform part. The latter is built up by the following parts: instep (I), apex (A), and sole (S). The heel (H), which is not always present, is an inflection point formed between the sole and the throat. The instep runs into the cavity of the lower lip and ends in the appendage (a) carrying the elaiophore (E). In flowers in which even during anthesis the lower and upper lip keep in touch, the instep may have a depression which houses the fertile parts. The space bound by the rims of the throat, which extends between the slipper-like part and the attachment of the lower lip to the stapetum is called opening (o). The space extending between the appendix and the sole is the entrance (e) to the slipper-like part (Fig. 1).

The appendage may exhibit two inflections, one at the base, and less frequently an apical one (Fig. 1F). The basal inflection is formed by growth of the abaxial side of the corolla and thus the appendage is folded inside. Exceptionally (*C. uniflora*), growth of the adaxial instead of the abaxial side of the corolla is fostered at this inflection point, causing the appendage to fold outwards (Fig. 1H). On the basis of this ground plan, several modifications are found (Fig. 1B-H) by a proportional increase in the development of either the upper lip (Fig. 1B), the appendage (A in Fig. 1C), the opening (o in Fig. 1E, H), the apex and the heel (A, H in Fig. 1G), or the basal inflection of the appendage (Fig. 1H).

Coloration of the corolla: Almost all species show yellow corollas (varying between yolk-yellow and lemon light-yellow) combined in many cases with red marks such as spots, thin strips, or bands of irregular shape. White corollas are present in ten species, whereas violet corollas are found in three species only (GRAU & BAYER 1984, MOLAU 1988, Fig. 2A-D).

Upper lip: Two general types of upper lip can be distinguished: the "Rugosae type" and the "hood type".

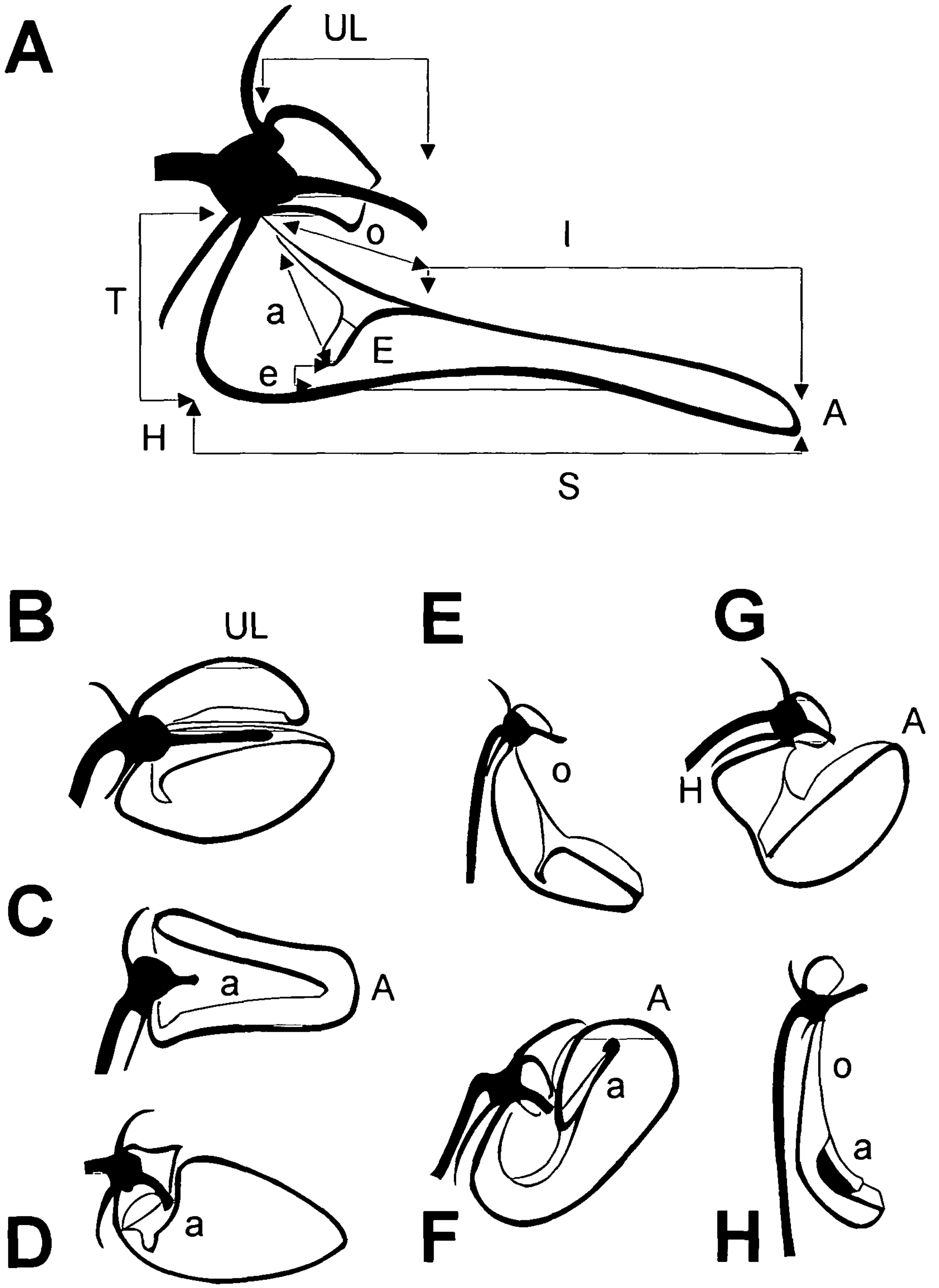


Fig. 1. Flower morphology in *Calceolaria*. **A**, schematic drawing of a flower showing the different parts of the corolla (based on RITTERBUSCH 1976). **B-H**, changes in the shape of the corolla. The most modified part of the flower is indicated. Abbreviations: UL, upper lip; T, throat; I, instep; A, apex; S, sole; H, heel; E, elaiophore; a, appendage; o, opening; e, entrance.

"Rugosae type": This kind of upper lip is characterized by a fimatous development, though not as strong as in the lower lip. The upper lip is well developed being 2/3 times as long as the lower lip and completely encloses the fertile parts. The edge of the lip is strong and involute, two characters important for pollination. Upper and lower lips are in contact and the opening remains closed during the whole life of the flowers ("cleistopetaly"; SÉRSIC 1991). This type is present in all species of section Rugosae (Figs. 6A-G, 7A-E) and in *C. thyrsiflora* of section Integerrimae (Fig. 7F).

"Hood type": In other sections of *Calceolaria* the upper lip is normally not well developed. In most cases, it does not appear as a bubble-like structure, but is seen as a relatively open, hood-like construction. It covers the fertile parts and protects them against rainfall and/or pollen robbery. Considering the way how the upper lip covers the fertile parts, three conditions are distinguishable:

- The upper lip broadly covers the stamens without touching them: *C. hypericina, C. lagunae-blancae, C. lanceolata, C. polyclada* (Figs. 55E, F), *C. parviflora, C. pinifolia, C. ascendens* subsp. *ascendens* (Figs. 4A, B). *C. ruiz-pavonii, C. thyrsiflora, C. uniflora, C. tenella* and *C. tucumana*.
- The upper lip tightly covers the sides of the stamens, completely protecting the upper thecae, while the lower ones remain totally or partially exposed. To render the upper thecae free a hinge mechanism is necessary to allow a balancing movement of the anther. This condition is present for example in *C. lossenii* (Fig. 4E), *C. teucrioides, C. williamsii, C. corymbosa, C. umbellata, C. schickentanziana, C. arachnoidea, C. pavonii, C. polyrhiza, C. prichardii* (Figs. 4C, D*)* and *C. brunellifolia*.
- The upper lip completely covers the stamens leaving just a thin longitudinal slit, which allows the exposition of the upper thecae, generally one at a time. A flexible hinge is also necessary for this kind of flowers. This condition is evident in *C. tripartita* (Fig. 4F), *C. chelidonoides, C. filicaulis* subsp. *luxurians* , *C. mendocina,* and others.

Lower lip: The lower lip is better developed than the upper one. Because of its strong fimatous growth it is always bubble-shaped. As already mentioned, the lower lip carries a lap, where normally an elaiophore is placed. This lap is more or less prominently folded inside (involute) of the saccate part of the lip (Fig. 1). The lap may have different angles of inflection (Fig. 1A). This angle is determined by the positions of the internal surfaces of the lap and the instep.

- Angles between 180° to 360°: The appendage is folded to the outside of the lower lip (e. g., *C. uniflora*, Fig. 1H, Fig. 60A-B; *C. fothergillii* AITON).
- Angles of 180° (Fig. 1G): The lap is practically not folded but appears as a continuation of the instep and does not extend into the saccate portion of the lower lip (e. g., *C. prichardii*, Fig. 9F; *C. tripartita*).
- Angles of 90° (Figs. 1B, E): The lap is short and partially or totally occludes the entrance to the bubble. The bubble-like portion is generally flat, not very inflated (e. g., *C. dentata, C. hypericina, C. integrifolia, C. latifolia, C. lossenii, C. parviflora, C. petioalaris, C. pinifolia, C. plectranthifolia, C. polifolia, C. polyclada, C. ruiz-pavonii, C. stellariifolia, C. teucrioides, C. thyrsiflora, C. umbellata,* Figs. 5A-F, 7A-F).
- Angles between 90° and 45°: This kind of appendage is long and partially or totally occludes the entrance to the bubble, which is always inflated (e. g., *C. biflora, C. brunellifolia, C. lagunae-blancae, C. lanceolata,* Fig. 8A-D).

- Angles between 45° and 0°: The lap is completely folded to the inside of the bubble and appears almost parallel to the instep. The lower lip is always inflated (e. g., *C. corymbosae, C. arachnoidea, C. filicaulis* subsp. *luxurians* , *C. pavonii, C. schickendantziana, C. ascendens* subsp. *ascendens* , *C. paralia,* Figs. 1C, F, 9A-E).

Furthermore, the appendage can show a second fold or an apical curvature as in *C. schickendantziana* (Figs. 10A, B) where the tip of the lap rolls outwards to the upper side (involute). In *C. lossenii* (Fig. 20A) or *C. ruiz-pavonii* (Fig. 14A) on the other hand, the margins of the lap appear revolute, surrounding the elaiophore which thus covers a shallow depression. The lap in *C. santolinoides* (Fig. 12G), *C. dentata* (Fig. 12H), *C. brunellifolia* and *C. polifolia* is saddle-like with the central portion of the lap being elevated while the sides remain at a lower level.

Elaiophore: The elaiophore is always located on the abaxial side of the lap of the lower lip. Among the 270 species of the genus, 49 (c. 18%) lack oil glands. Location, size and shape of the elaiophore, as well as type and density of glandular hairs are variable and characteristic for each species.

Location of the elaiophore: The elaiophore can be coincident in size and shape with the lap or can be restricted to its apical border. The glandular area can extend to the distal border of the lap, as seen in *C. bicolor, C. biflora* (Fig. 12C), *C. hypericina* (Fig. 12A), *C. lagunae-blancae* (Fig. 14F), *C. lanceolata* (Fig. 14G), *C. paralia, C. pinifolia, C. plectranthifolia, C. polyclada, C. prichardii, C. schickendantziana* and *C. dentata* (Fig. 10D), *C. petioalaris* (Fig. 10H), *C. polifolia* (Fig. 10G), *C. polyrhiza, C. rhacodes* (Fig. 14H), *C. santolinoides, C. tetragona, C. teucrioides* (Fig. 14B), *C. thyrsiflora, C. tripartita* (Fig. 13F), *C. umbellata* (Fig. 14D) and *C. arachnoidea* (Figs. 13C & D).

Size of the elaiophore: The average surface area of the elaiophore of all species studied is 6.63 mm^2. The smallest glandular area was detected for *C. tripartita* with 1.24 mm^2, whereas the largest elaiophore was found in *C. biflora* with 22.64 mm^2 (Tab. 1).

Shape of the elaiophore: Three groups of elaiophore types can be recognized based on their outlines (these groups are in agreement with those proposed by VOGEL 1974):

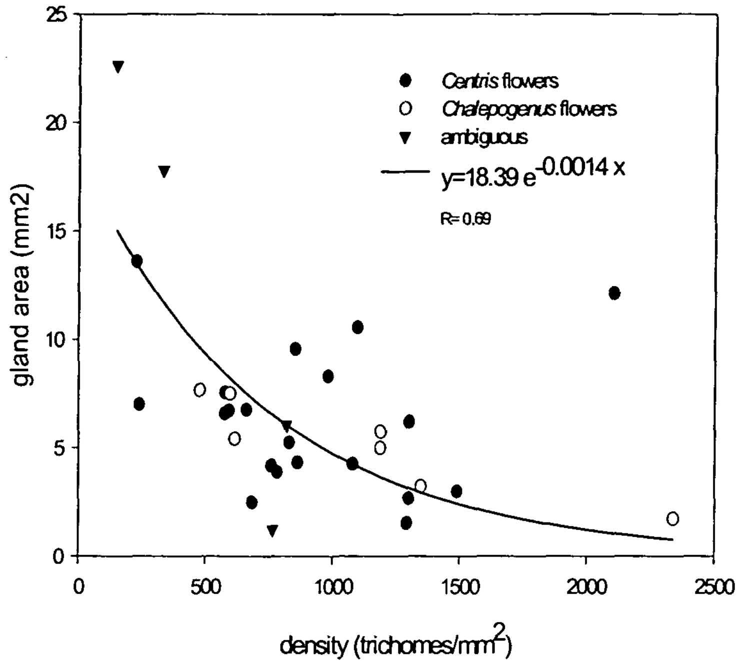
- Banded elaiophore: This type of elaiophore is wide and short. With the exception of *C. schickendantziana,* the glandular area covers the whole lap including the inflection line. It is found in *C. bicolor, C. petioalaris, C. pinifolia, C. ascendens* subsp. *ascendens* , *C. schickendantziana, C. stellariifolia, C. volckmannii* (Figs. 10A-J, 11A, B).
- Trapezoidal elaiophore: The margins of the elaiophore are not curved and it does not necessarily cover the whole area of the appendage. Examples: *C. biflora, C. chelidonoides, C, corymbosa, C. hypericina, C. polifolia, C. prichardii and C. santolinoides* (Figs. 12A-F).
- Oval elaiophore: This type is characterized by curved outlines and does not cover the whole lap. Examples: *C. crenatiflora, C. lagunae-blancae, C. lanceolata, C. lossenii, C. parviflora, C. polyclada, C. rhacodes, C. ruiz-pavonii, C. teucrioides, C. tripartita, C. umbellata* (Figs. 13B-F, 14A-H).

Density of glandular trichomes: The density of trichomes is uniform on the whole elaiophore. Only in a few species (*C. biflora*, *C. corymbosa, C. crenatiflora,* and *C. ruiz-pavonii,* Figs. 12C, D, 14E, and 14A, respectively) the density decreases towards the tip of the appendage. In very dense elaiophores, the trichomes are tightly arranged and build up two layers, one with short hairs, where their heads are interlocked between the feet of a

Table 1. Density of glandular hairs, total number of glandular hairs, and surface area of elaiophores of 31 *Calceolaria* species.

Species	Density of hairs (hairs/mm^2)	Total no. of hairs (n = 10)	Elaiophore surface area (mm^2)
C. arachnoidea	852.27	8139.18	9.55
C. bicolor	2104.59	25507.63	12.12
C. biflora	149.28	3379.70	22.64
C. brunellifolia	1347.53	4352.50	3.23
C. corymbosa	827.27	3515.90	4.25
C. crenatiflora	227.58	3095.09	13.60
C. chelidonoides	682.30	1705.75	2.50
C. dentata	1077.75	4591.22	4.26
C. lagunae-blancae	476.95	3658.21	7.67
C. lanceolata	613.23	3323.71	5.42
C. lossenii	glandular: 660.00 simple: 1388.30	4461.61 384.71	6.76
C. filicaulis subsp. *luxurians*	331.06	5896.23	17.81
C. paralia	1491.48	4474.44	3.00
C. parviflora	1301.41	8068.74	6.20
C. petioalaris	575.76	3788.50	6.58
C. plectranthifolia	1290.52	1987.40	1.54
C. polifolia	757.99	3183.60	4.20
C. polyclada	1186.90	5934.50	5.00
C. prichardii	818.18	4949.99	6.05
C. rhacodes	1298.82	3480.84	2.68
C. ascendens subs. *ascendens*	781.29	3031.41	3.88
C. ruiz-pavonii	595.70	4468.00	7.50
C. santolinoides	239.50	1676.50	7.00
C. schickendantziana	575.52	4345.18	7.55
C. stellariifolia	1188.11	6819.75	5.74
C. tetragona	1095.58	11558.37	10.55
C. teucrioides	979.90	8142.90	8.30
C. thyrsiflora	860.80	3727.30	4.33
C. tripartita	764.27	925.04	1.24
C. umbellata	2338.32	4045.30	1.73
C. volckmanii	592.11	3978.98	6.72

second layer of longer trichomes. This stratification is seen in *C. umbellata, C. brunellifolia,* and *C. stellariifolia* (Fig. 16A, B, Tab. 1). The size of the elaiophore is not correlated to the density of trichomes, because *C. biflora*, for example, with an expanded glandular surface area of 22.64 mm^2 shows one of the lowest densities (149.28 trichomes/mm^2), the hairs are loosely spaced and do not cover the lap surface. In *C. bicolor*, on the other hand, a large elaiophore surface area (12.12 mm^2) is combined with a high trichome density (2104.59 hairs/mm^2). *C. umbellata* shows a very small elaiophore surface area (1.73 mm^2) but has the highest trichome density of all species studied (2338.32 hairs/mm^2). The correlation between density and size of elaiophore for different pollinators is shown in Graphic 1. The highest species concentration is in the range of small elaiophores (4 to 7 mm^2) with a medium trichome density (between 500 and 1400 hairs/mm^2).



Graphic 1. Correlation between trichome density and area of elaiophore of 31 *Calceolaria* species.

Anatomy and kind of trichomes: The glandular trichomes are multicellular structures and contain a foot and a glandular head[1]. Table 3 summarizes their dimensions.

The foot: is uniseriate, multicellular with a variable number of cells depending on the species and on its position within the elaiophore. In general, the marginal trichomes are shorter. The number of cells varies from 1 (or 2) as in *C. biflora* (Fig. 19C, D) to 8 as in *C. polifolia* (Fig. 15C). The foot cells are strongly vacuolated. A large vacuole, surrounded by many smaller ones, is located in the middle, and the cytoplasmic stroma is relegated to a peripheral position. Cchloroplasts are frequent in these cells and provide a greenish coloration to the elaiophores in fresh material. CORRENS (1891a) has proven that these chloroplasts do not provide assimilation material for the metabolism of the glandular head *in C. pinnata*. He supposed that the presence of these plastids functions as a visual guide for pollinators. VOGEL (1974) without rejecting the assimilatory function, associated the presence of these organelles with the abundant leucoplasts of the glandular head, which derive ontogenetically from chloroplasts. Nutrition of the elaiophore is predominantly accomplished by the dense vascular network in the appendage. The apical cell of the foot is a constant feature in all glandular hairs. This so-called neck cell shows strongly cutinized outer cell walls, which, after SCHNEPF (1969), could serve to avoid the flow of excessive oil to the bottom of the glandular head.

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[1] The ontogenic development of the glandular hairs was studied by RAMAN (1989), and their fine structure by SCHNEPF (1969)

The glandular head develops above - and partly covers - the neck cell. The number of head cells is variable (Tab. 2), reaching from 4 in *C. tripartita* to 35 in *C. biflora*. The head cells originate from anticlinal divisions of an initial cell, thus the whole head resembles a peeled orange. The number of cells is also variable among the trichomes of one elaiophore.

Table 2. Average number of cells of the glandular head of some species of *Calceolaria* (n = 10 trichomes).

Species	Average number of cells/head
C. parviflora	20.6
C. ruiz-pavonii	11.2
C. hypericina	8.9
C. polifolia	10.8
C. lagunae-blancae	17.4
C. tripartita	6.4
C. biflora	25.4
C. stellariifolia	15.3

Following the code proposed by RAMAN (1987) for classification of trichomes in family Scrophulariaceae, the glandular hairs of the elaiophores of *Calceolaria* correspond to types 34 2101 or 34 2601. That means, they are glandular trichomes with a secretory head formed by cells divided many times vertically (anticlinally), with a neck cell, long and cylindrical, or short and doliiform intermediary cells (of the foot), with smooth cellular walls and without ornamentation.

In hand-cuts of fresh material the head cells show an abundant and foamy cytoplasm without chloroplasts and poorly visible nuclei (Fig. 21A, B). In microtome sections of paraffin- and resin-embedded material, it is possible to distinguish small nuclei (c. 5 µm in diameter in *C. parviflora*, Fig. 21A, B) not larger than those found in parenchyma cells. The nuclei are positioned at the same level in the lower half of all cells of the head. The cytoplasm shows abundant spherosomes and small vacuoles with an oily content. The cell walls are thin and the cuticle is evident (Fig. 20H).

In SEM-analyzed samples it is possible to recognize a subcuticular space, delimited by the cuticula and the outer periclinal walls of the cells of the glandular head. Seen in sections of fresh material, is in this cavity were the secreted oils are accumulated. Normally there's only one such cavity for the whole head (Figs. 15D-F, 16E, G). *C. lagunae-blancae* is an exception in having one subcuticular cavity for each cell of the head. Longitudinal paraffin sections of the elaiophores of this species show short trichomes with large glandular heads. The cells of the head are distally divergent and their tips are well separated from each other (Fig. 20G, H). No pore is distinguishable for secretion of the fluids. According to SCHNEPF (l. c.) the cuticle presents fine fibrils, which permit the oil to flow out of the subcuticular cavity.

The glandular heads are of different shapes and proportions. They can be longer than wide as in *C. bicolor* and *C. tripartita* (Fig. 15A, B), spherical as in *C. polifolia, C. parviflora, C. ascendens* subsp. *ascendens* , *C. corymbosa, C. ruiz-pavonii*, and *C. umbellata* (Figs. 15C, D, 16E-H). Or they can be wider than long as in *C. lanceolata, C. lagunae-blancae, C. filicaulis* subsp. *luxurians*, and *C. biflora* (Figs. 15E-H, 19B-D, Tab. 3).

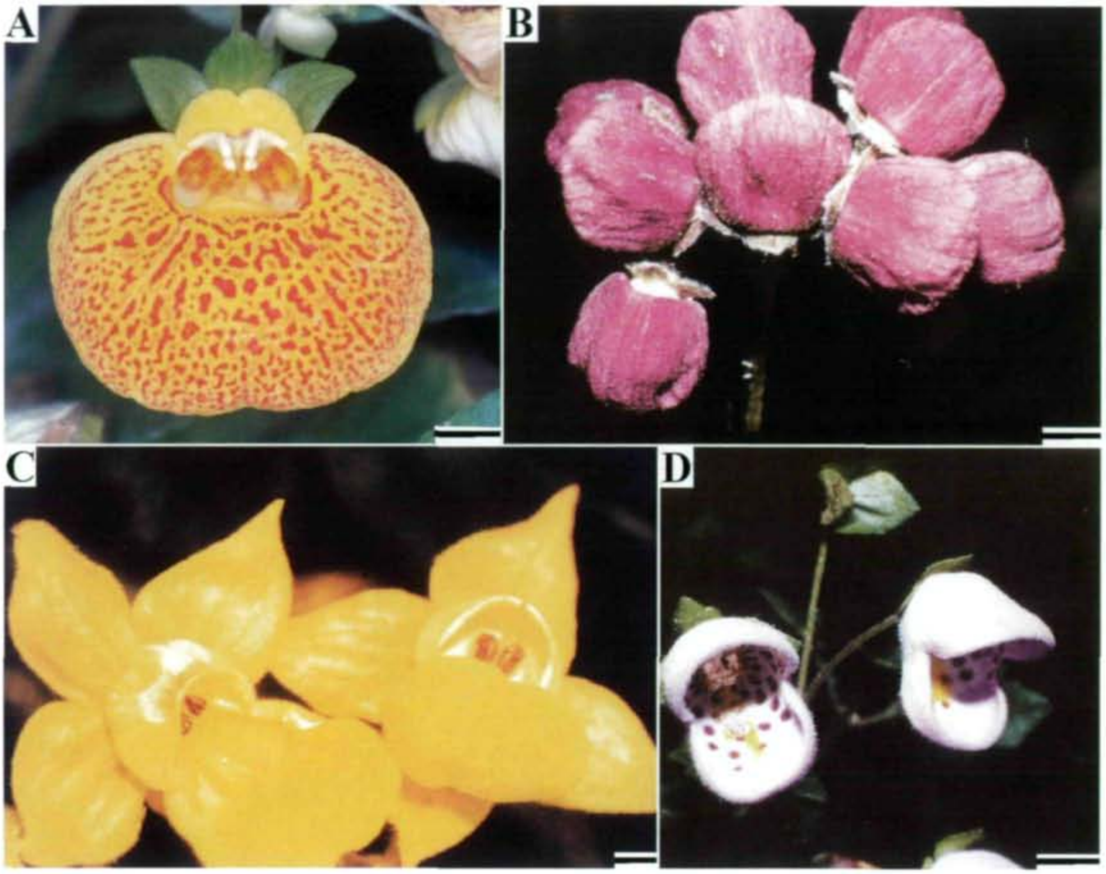


Fig. 2. Coloration in Calceolariaceae. **A,** *Calceolaria* x *herbeohybrida* VOSS. **B,** violet flowers of C. arachnoidea. **C,** yellow calyx and corolla in *C. calycina* BENTH. **D,** light violet flowers in *Jovellana violacea*. Scale bars: 0.5 cm.

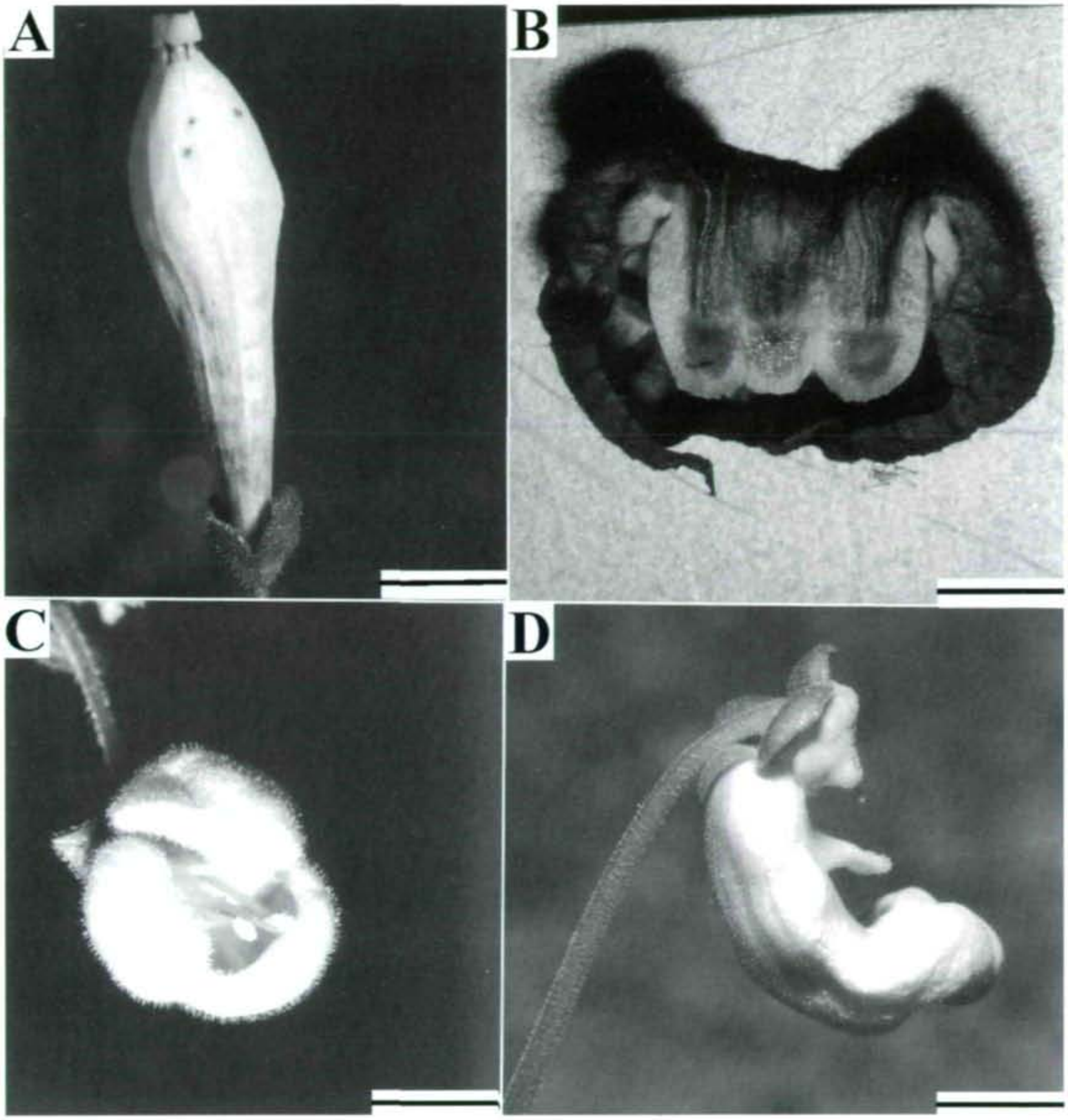


Fig. 3. Teratological forms of flowers. **A**, peloria in *Calceolaria lagunae-blancae*. **B**, three lobed appendage, each with an elaiophore in *C. arachnoidea*. **C**, divided upper lip in *C. latifolia*. **D**, appendage folded outwards in *C.* spp. Scale bars: 0.5 cm.

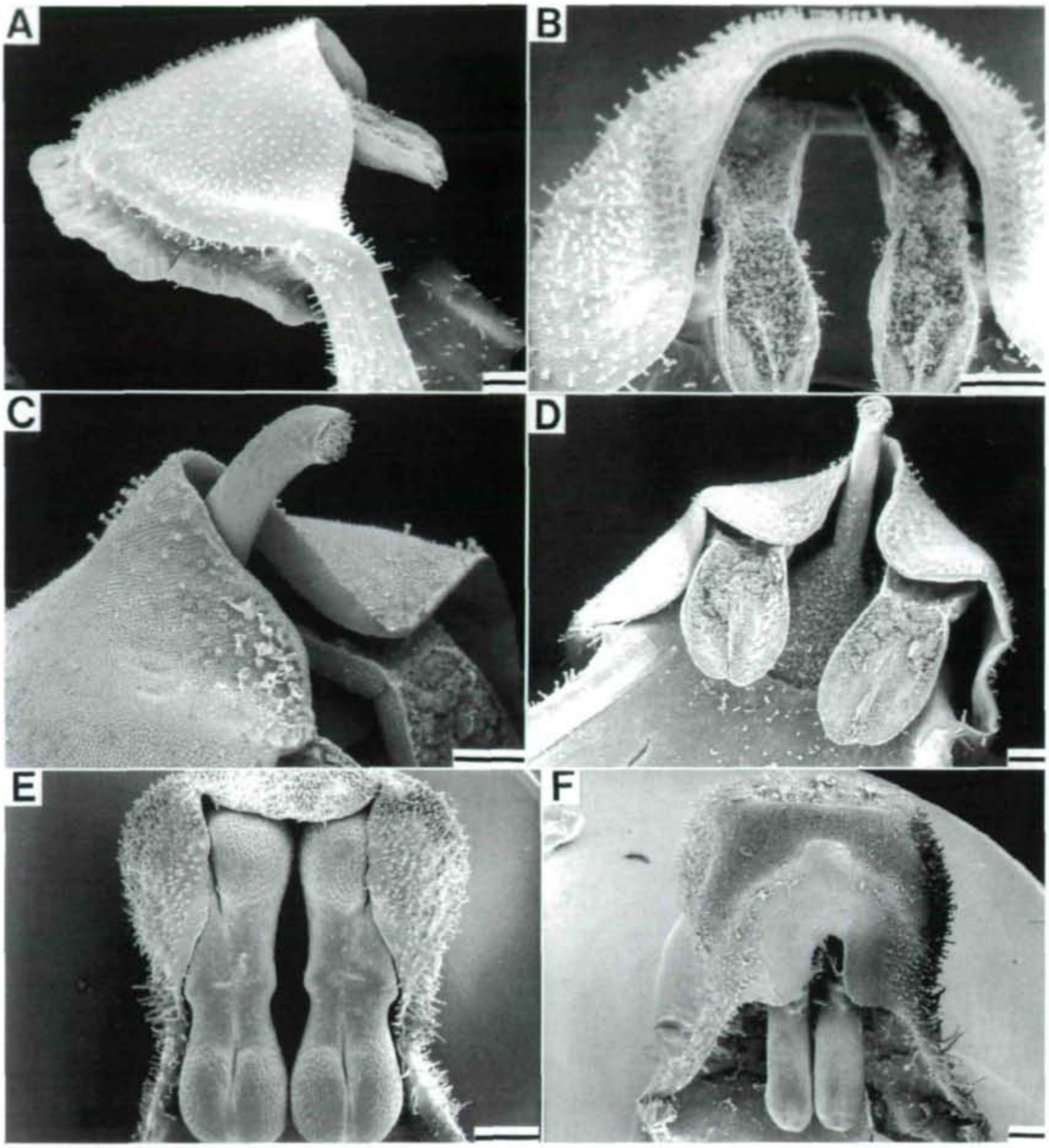


Fig. 4. Hooded upper lips. **A** & **B**, *Calceolaria. ascendens* subsp. *ascendens*, type **1**, lateral and frontal views, respectively. **C** & **D**, *C. prichardii*, type **2**, lateral and frontal views, respectively; note partially covered adaxial thecae. **E**, *C. lossenii*, type **2**, with lever stamens. **F**, *C. tripartita*, type **3**, lever stamens with adaxial thecae totally covered; only one theca can alternatively pass through the longitudinal slit. Scale bars: 500 µm.

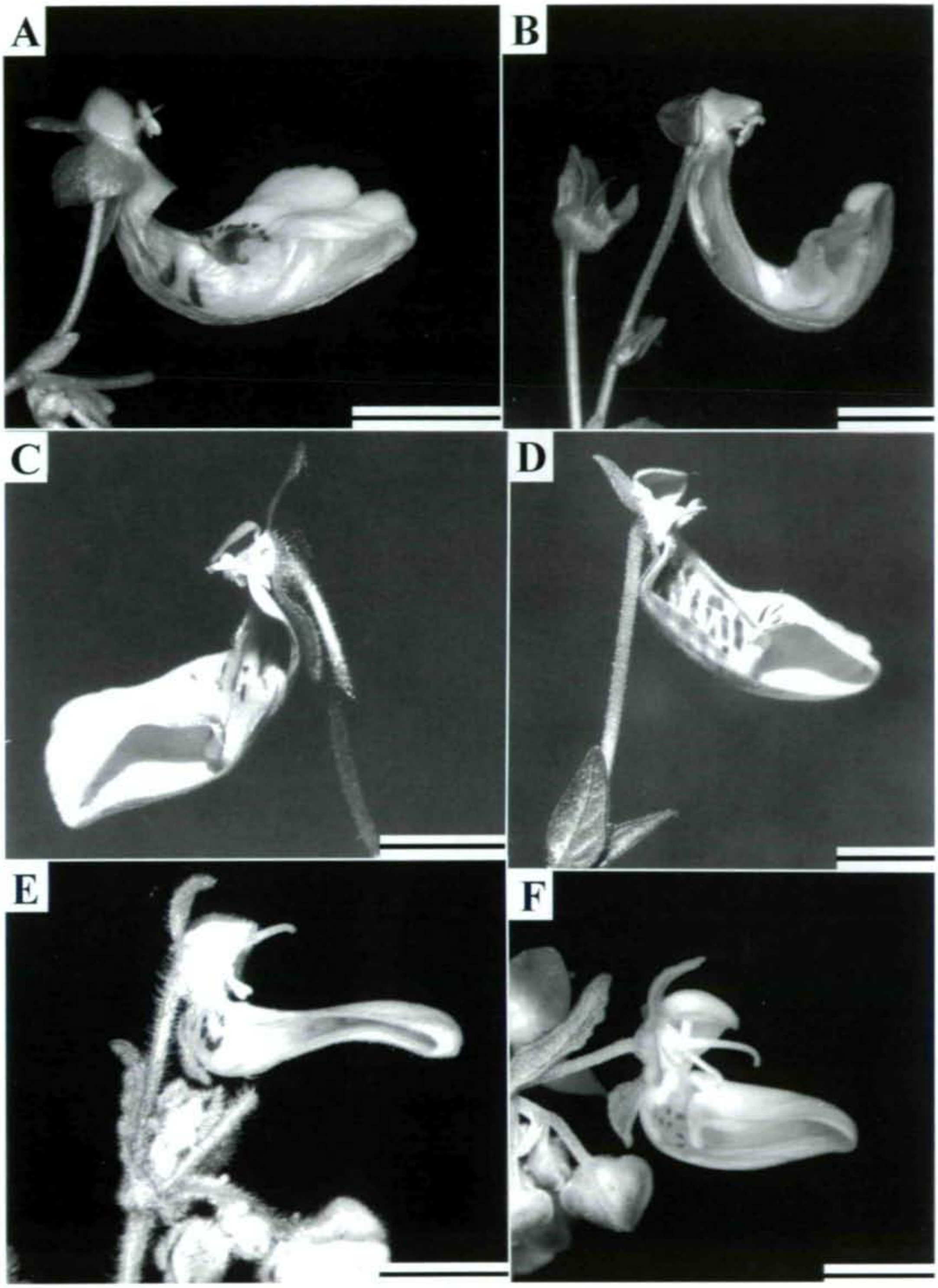


Fig. 5. Tangential sections through flowers showing appendages with an inflection angle of c. 90°. **A**, *Calceolaria pinifolia*. **B**, *C. hypericina*. **C**, *C. parviflora*. **D**, *C. stellariifolia*. **E**, *C. ruiz-pavonii*. **F**, *C. polyclada*; note the lever mechanism in the stamens of **E** & **F**. Scale bars: 0.5 cm.

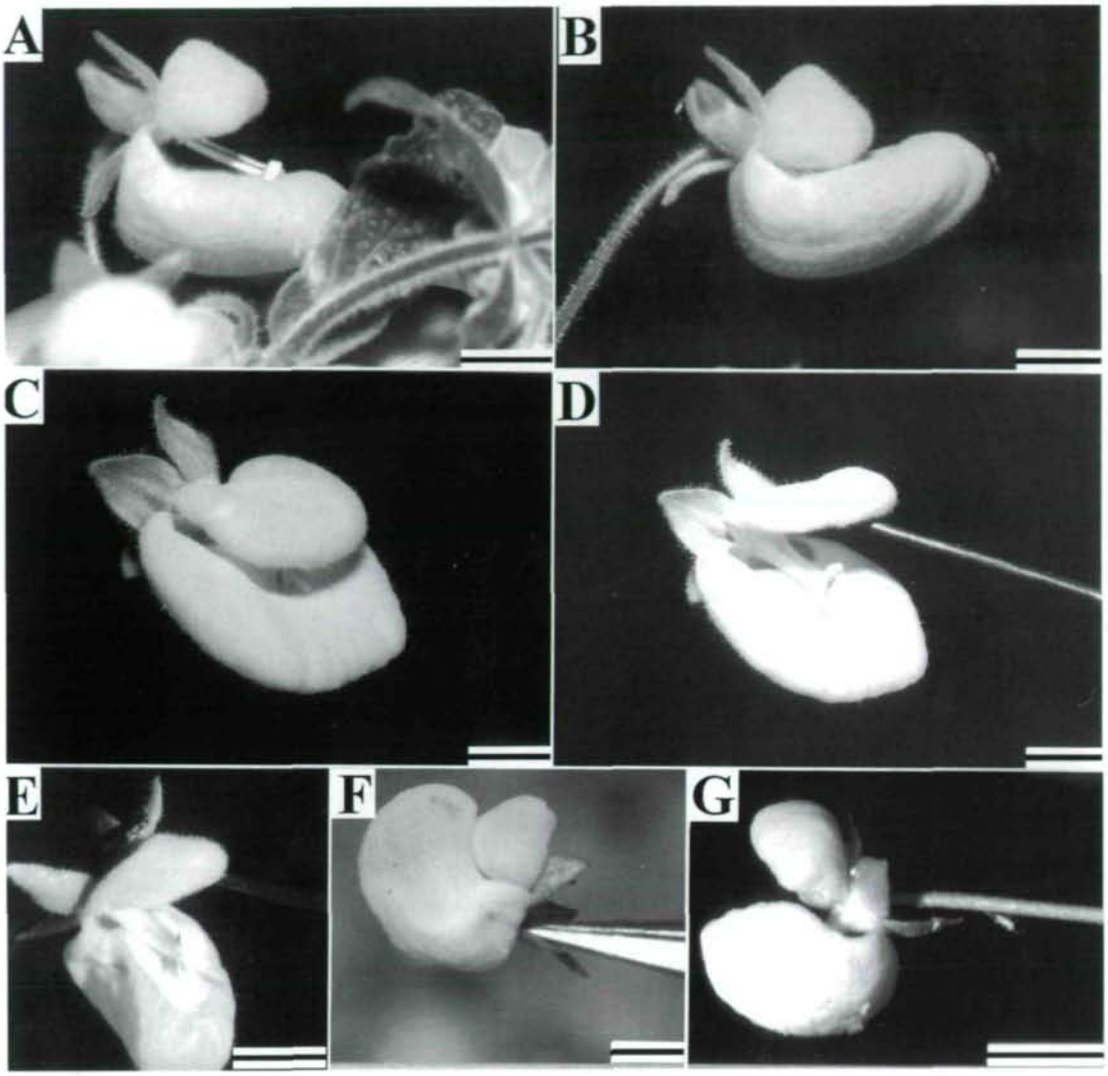


Fig. 6. Sternotribic Centridini flowers. **A** & **B**, *Calceolaria andina*. **C** & **D**, *C. dentata*. **E**, *C. petioalaris*. **F**, *C. thyrsiflora* and **G**, *C. integrifolia*. Scale bars: 0.5 cm.

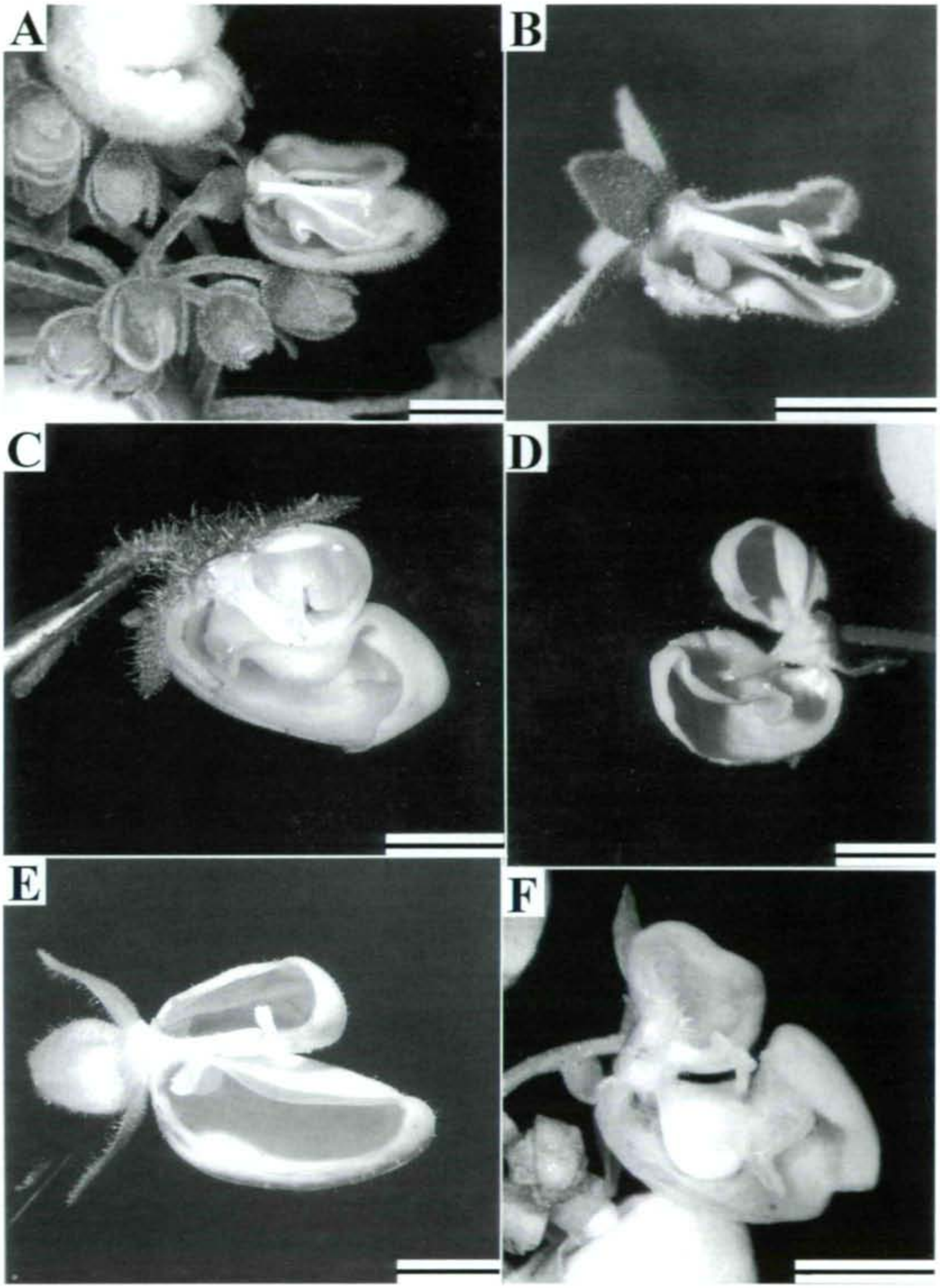


Fig. 7. Tangential sections of flowers showing appendages with an inflection angle of ca. 90°. **A-E**, section Rugosae, note the long filaments of the stamens, the development of the upper lip and its involute margin. **A**, *Calceolaria latifolia*. **B**, *C. petioalaris*. **C**, *C. plectranthifolia*. **D**, *C. integrifolia*. **E**. *C. dentata*. and **F**. Sect. Integerrimae, *C. thyrsiflora*. Scale bars: 0.5 cm.

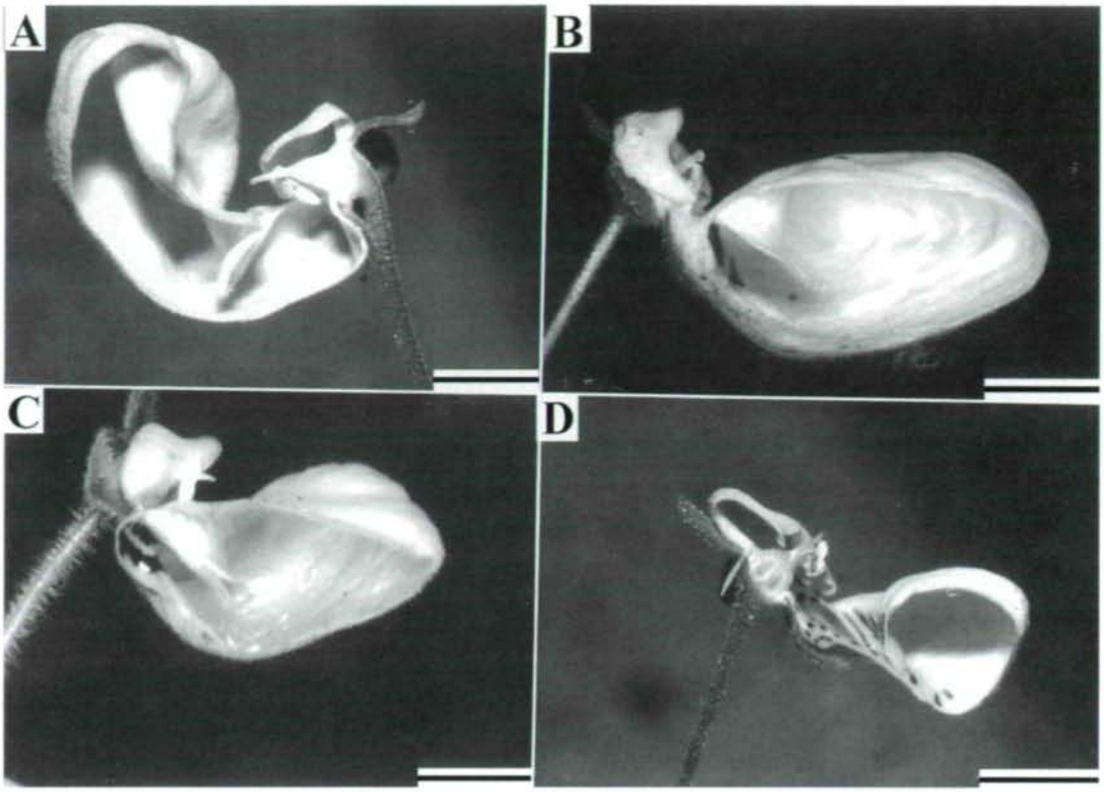


Fig. 8. Tangential sections of flowers showing appendages with an inflection angle of ca. 45º. **A**, *Calceolaria lanceolata*. **B**, *C. biflora*. **C**, *C. brunellifolia*. **D**, *Calceolaria* sp. Scale bars: 0.5 cm.

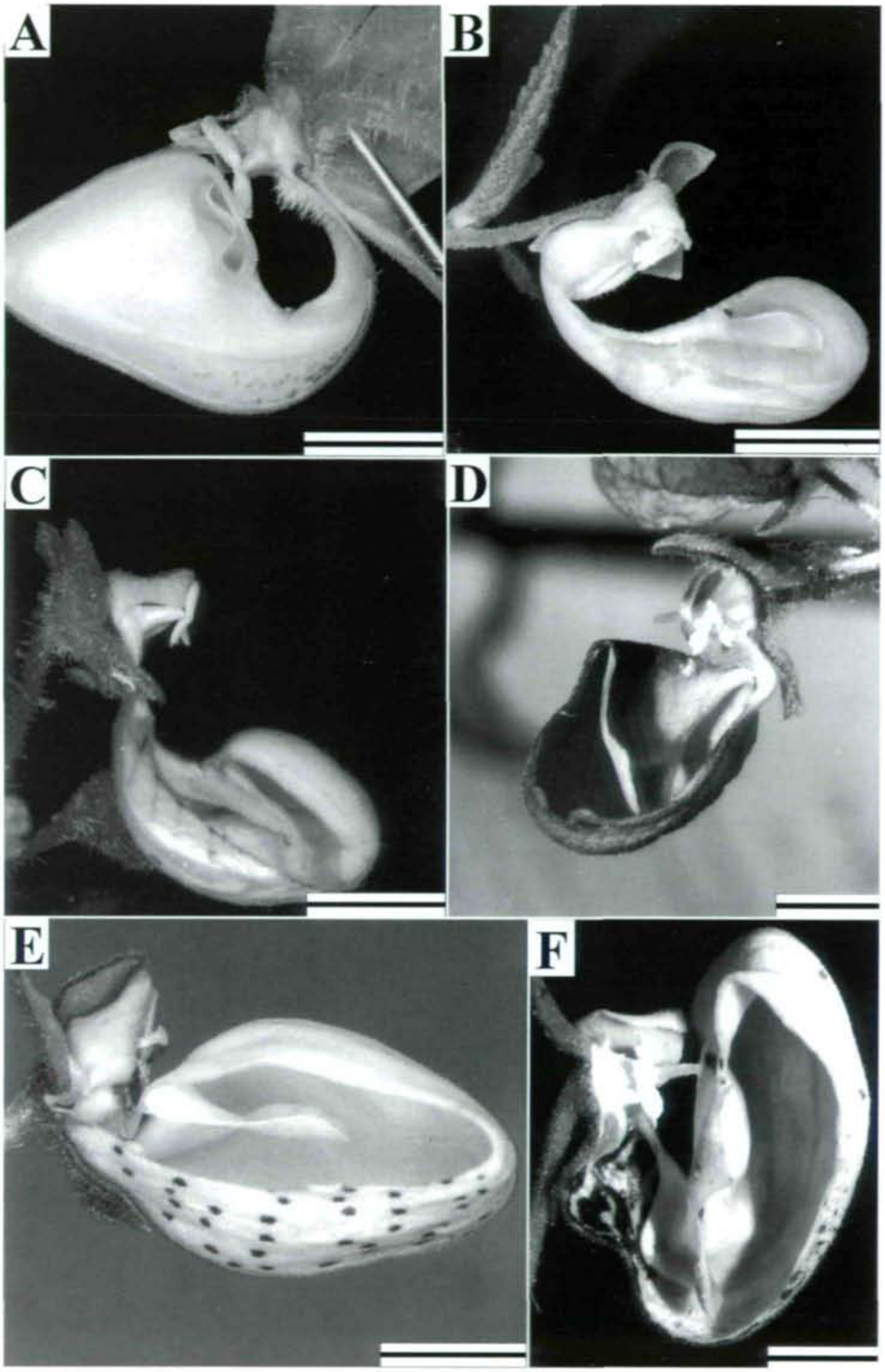


Fig. 9. Tangential sections of flowers showing the position of the appendage. **A-E**, appendages with an inflection angle between 45° and 0°; note also the inflated lower lip. **A**, *Calceolaria pavonii*. **B**, *C. schickendantziana*. **C**, *C. corymbosa*. **D**, *C. arachnoidea*. **E**, *C. filicaulis* subsp. *luxurians* . **F**, *C. prichardii*, appendage with an inflection angle of ca.180°. Scale bars: 0.5 cm.

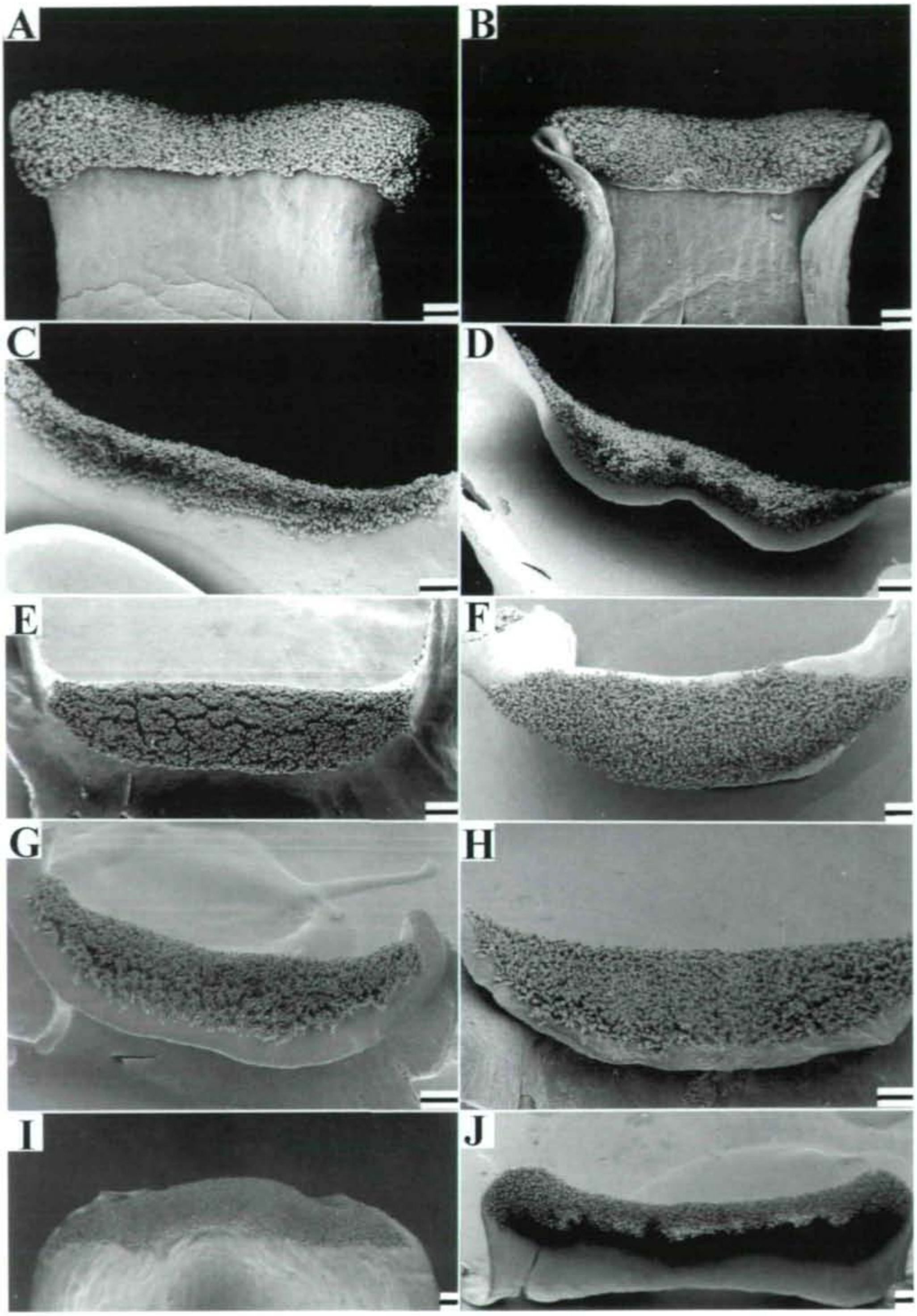


Fig. 10. Banded elaiophores. **A** & **B**, *Calceolaria schickendantziana*, ventral and dorsal views, respectively; in **B** note a second fold of the appendage. **C** & **D**, *C. dentata*, dorsal and ventral views, respectively. *C. stellariifolia*. **F**, *C. volckmanii*. **G**, *C. polifolia*. **H**, *C. petioalaris*. **I**, *C. paralia*. **J**, *C. bicolor*. In **B**, **D**, **G** & **H** the elaiophore is submarginal. Scale bars: 400 µm.

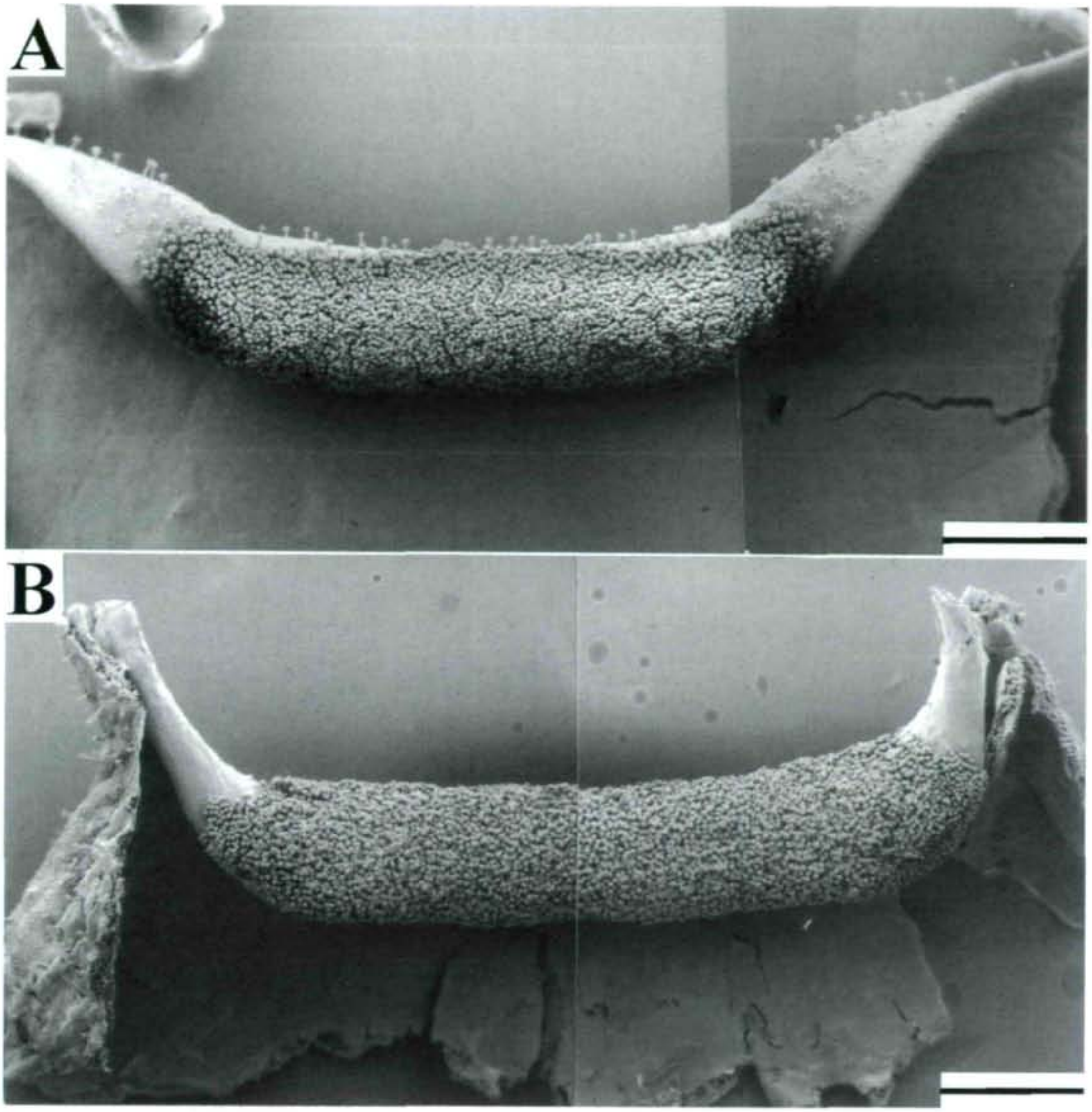


Fig. 11. Banded elaiophores. **A**, *Calceolaria ascendens* subsp. *ascendens*. **B**, *C. pinifolia*. Scale bars: 1 mm.

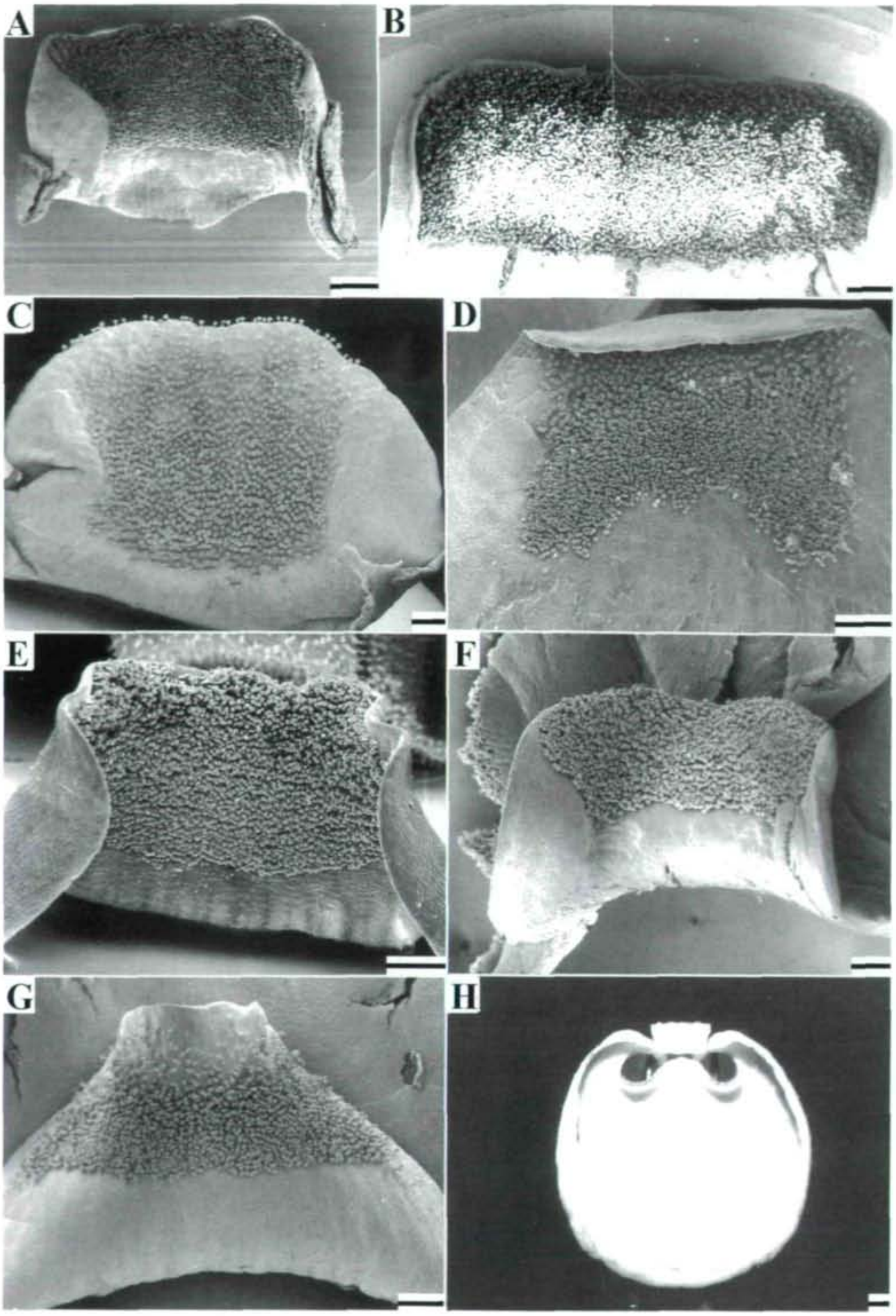


Fig. 12. Shapes of elaiophores. **A-F**, trapezoidal-elaiophores. **A**, *Calceolaria hypericina*. **B**, *C. polyrrhiza*. **C**, *C. biflora*. **D**, *C. corymbosa*. **E**, *C. prichardii*. **F**, C. *chelidonoides*. **G** & **H**, saddle-like appendages. **G**, *C. santolinoides*. **H**, *C. dentata*, longitudinal section of the lower lip in ventral view. Scale bars: A-G: 600 µm; H: 2 mm.

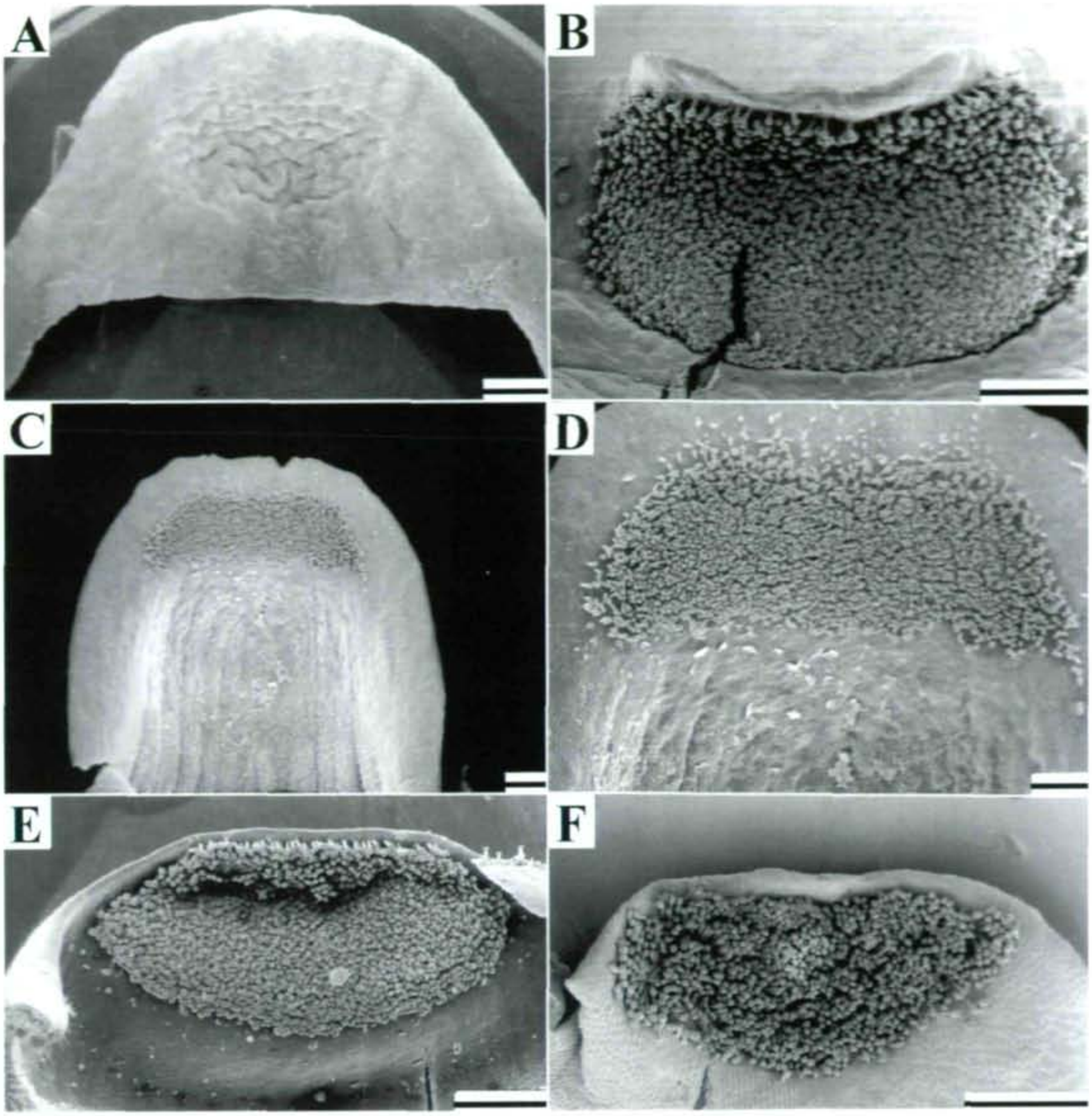


Fig. 13. Elaiophores in *Calceolaria*. **A**, *Calceolaria perfoliata*, note the absence of elaiophore. **B-F**, submarginal oval-elaiophores **B**, *C. brunellifolia*. **C** & **D**, *C. arachnoidea*. **E**, *C. parviflora*. **F**, *C. tripartita*. Scale bars: A & C: 1 mm; B, D-F: 600 µm.

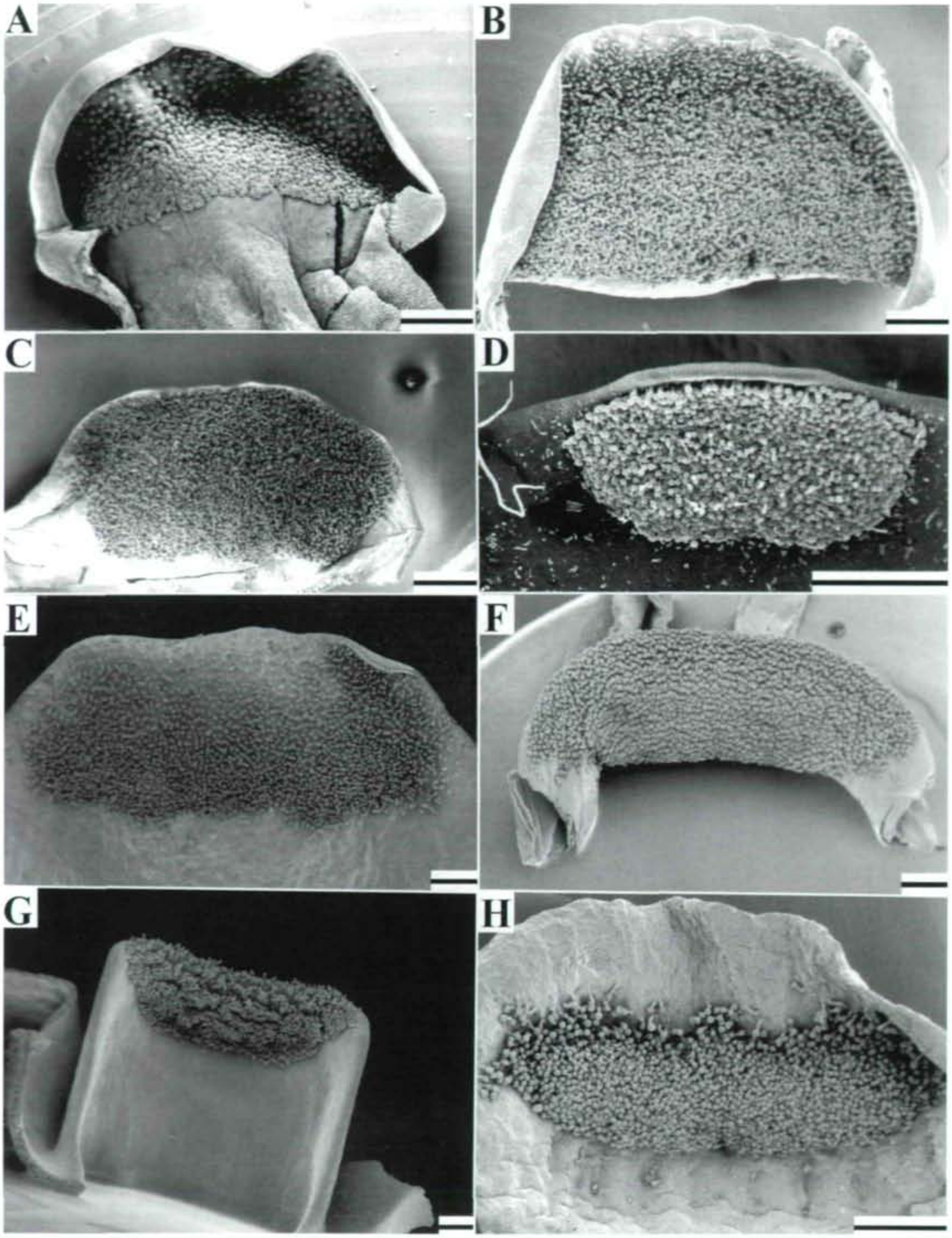


Fig. 14. Oval elaiophores. **A**, *Calceolaria ruiz-pavonii*. **B**, *C. teucrioides*. **C**, *C. polyclada*. **D**, *C. umbellata*. **E**, *C. crenatiflora*. **F**, *C. lagunae-blancae*. **G**, *C. lanceolata*. **H**, *C. rhacodes*. In **B**, **D** & **H** the elaiophore is submarginal. In **A** & **E** the distribution of trichomes is not uniform. Scale bars: 600 µm.

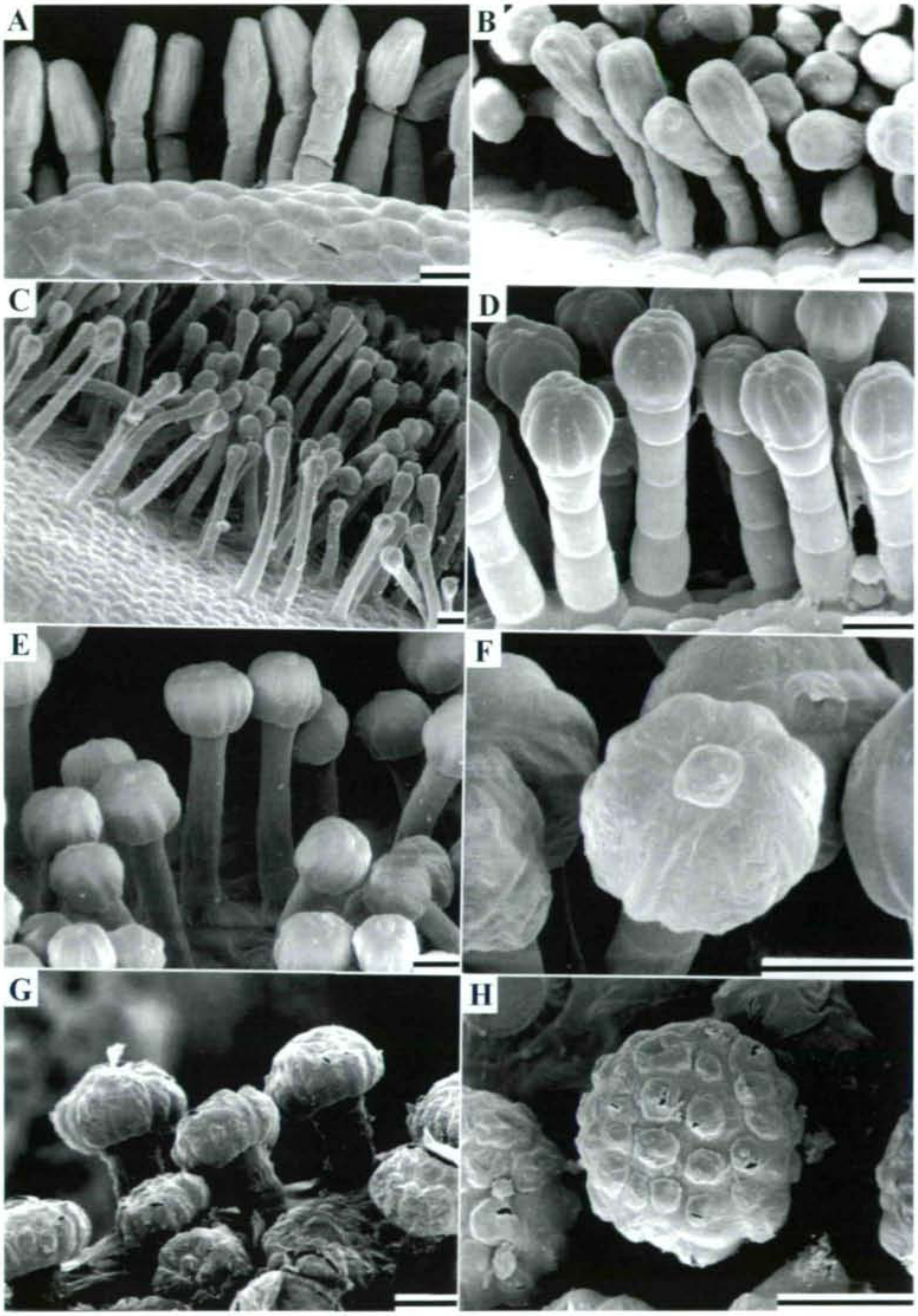


Fig. 15. Diversity of glandular hairs of the elaiophores. **A** & **B**, long and thin glandular heads. **A**, *Calceolaria bicolor*. **B**, *C. tripartita*. **C** & **D**, spherical glandular heads, **C**, *C. polifolia*. **D,** *C. parvifolia*. **E** & **F**, *C. lanceolata*. **E**, general view. **F**, detail of a glandular head showing one subcuticular cavity for each head. **G** & **H**, *C. lagunae-blancae*. **G**, general view. **H**, glandular head in a superior view, showing one subcuticular cavity for each cell of the head.

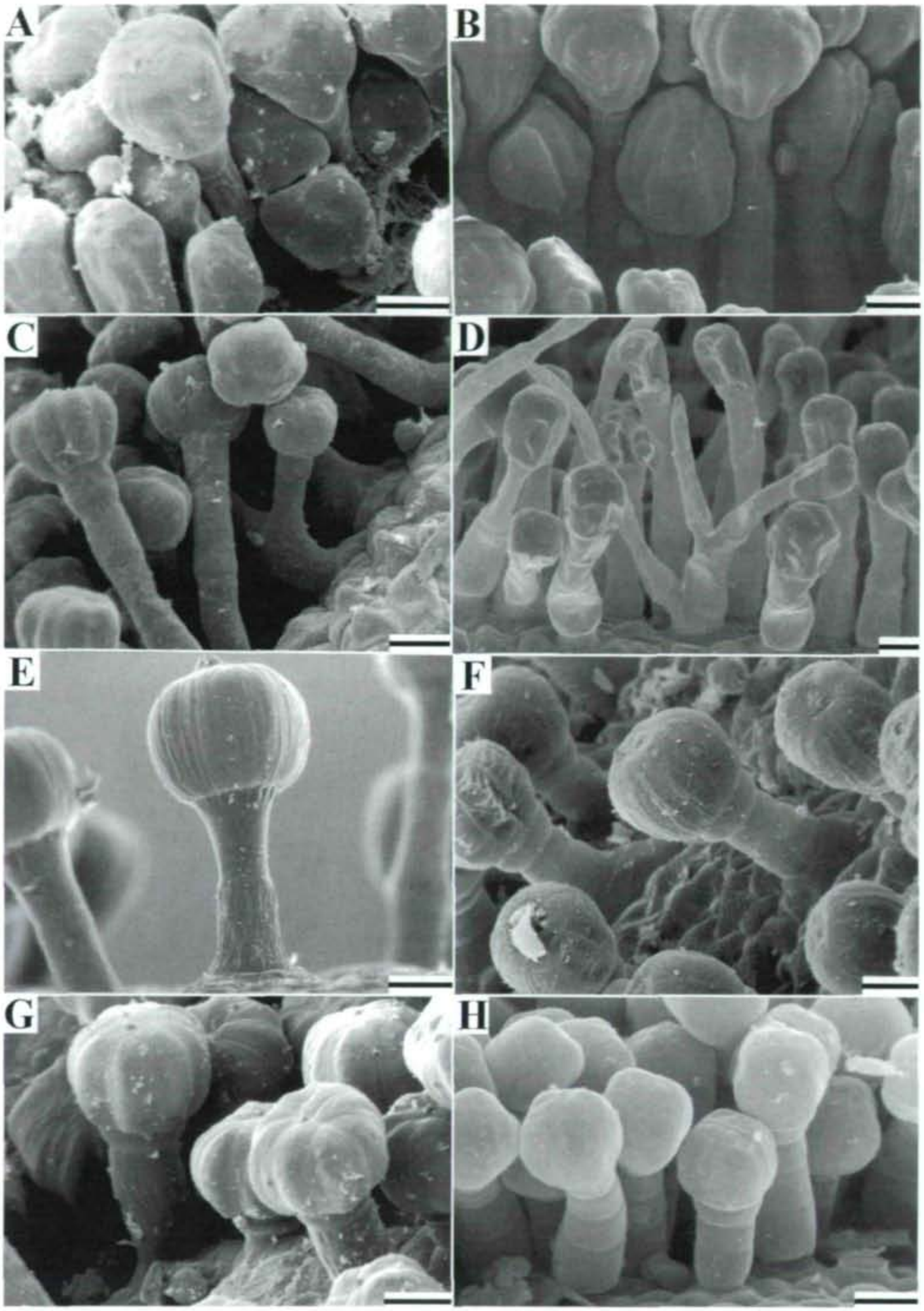


Fig. 16. Diversity of glandular hairs of the elaiophores. **A** & **B**, glandular hairs densely arranged building two layers. **A**, *Calceolaria brunellifolia*. **B**, *C. stellariifolia*. **C** & **D**, branched hairs. **C**, *C. hypericina*, branches end in glandular heads. **D**, *C. lossenii*, two simple branches, and one with a small glandular head. **E**, *C. ascendens* subsp. *ascendens*. **F**, *C. corymbosa*. **G**, *C. ruiz-pavonii*. **H**, *C. umbellata*. Scale bars: 20 µm.

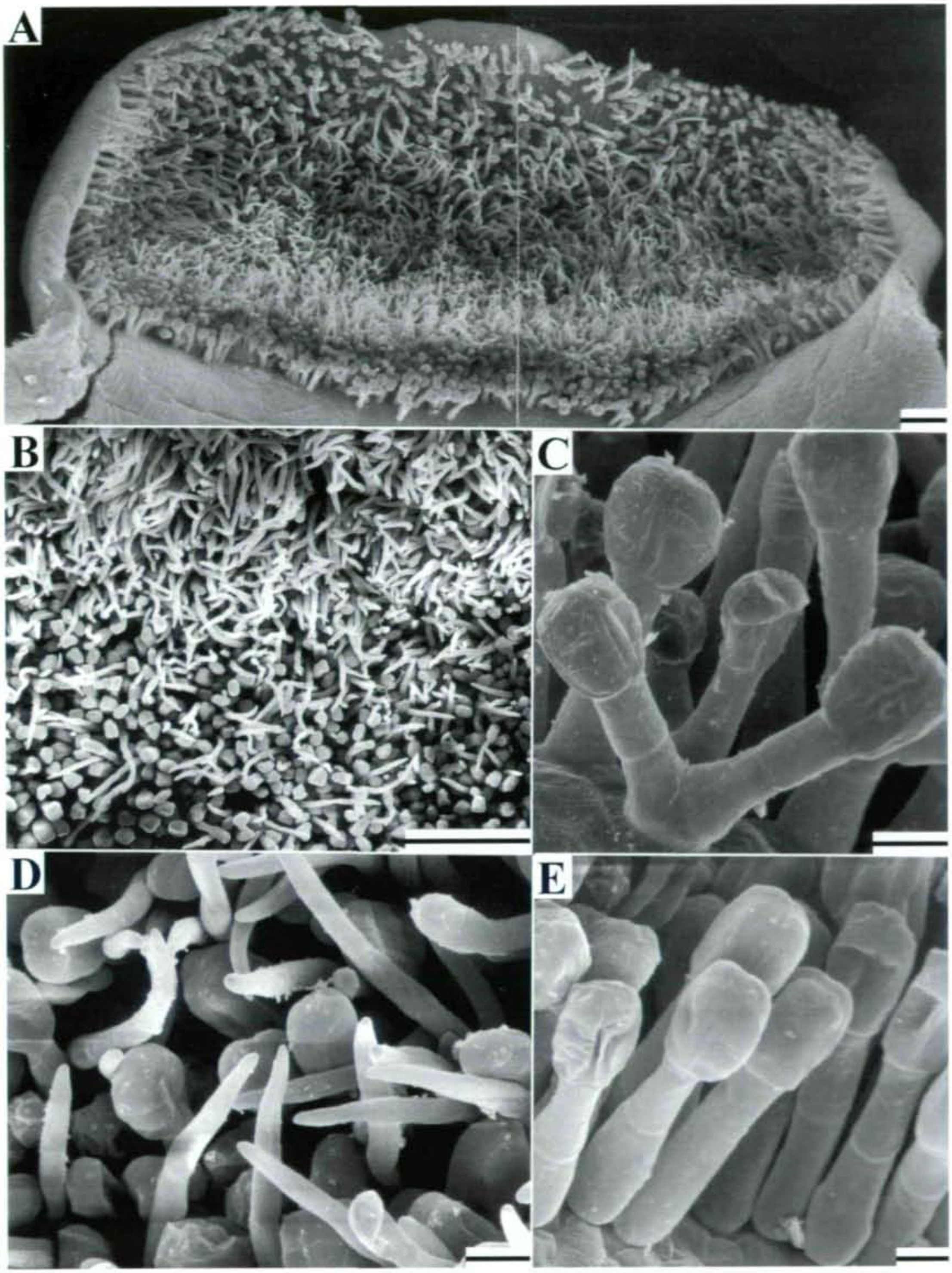


Fig. 17. Elaiophore of *Calceolaria lossenii*. **A**, general view. **B**, detail of A showing simple trichomes mixed with glandular ones. **C**, detail of one branched glandular hair. **D**, detail of B. **E**, normal glandular hairs. Scale bars: A & B: 200 µm, C-E: 20 µm.

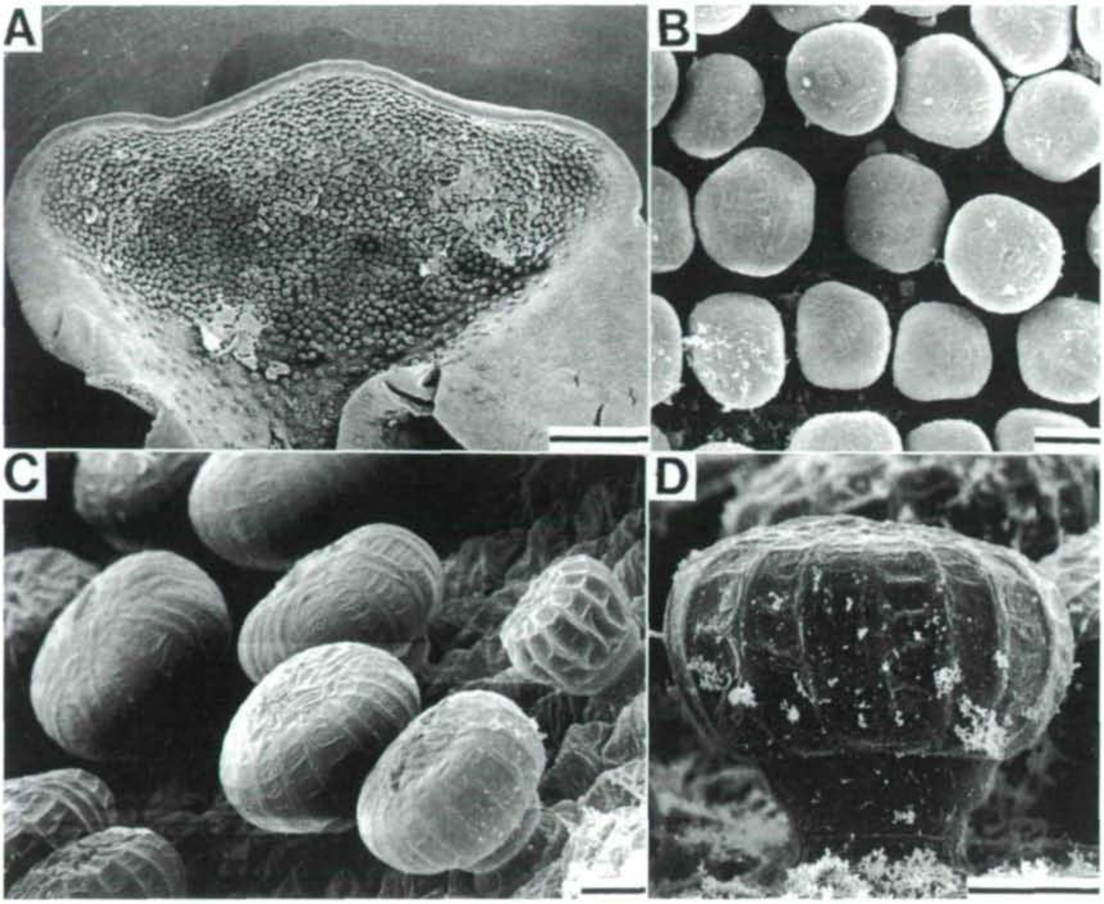


Fig. 18. Elaiophore of *Calceolaria filicaulis* subsp. *luxurians*. **A**, general view of the appendage. **B**, detail of A. **C**, glandular hairs showing a short foot and a short but wide glandular head. **D**, detail of one trichome. Scale bars: A: 1 mm, B: 40 µm, C & D: 20 µm.

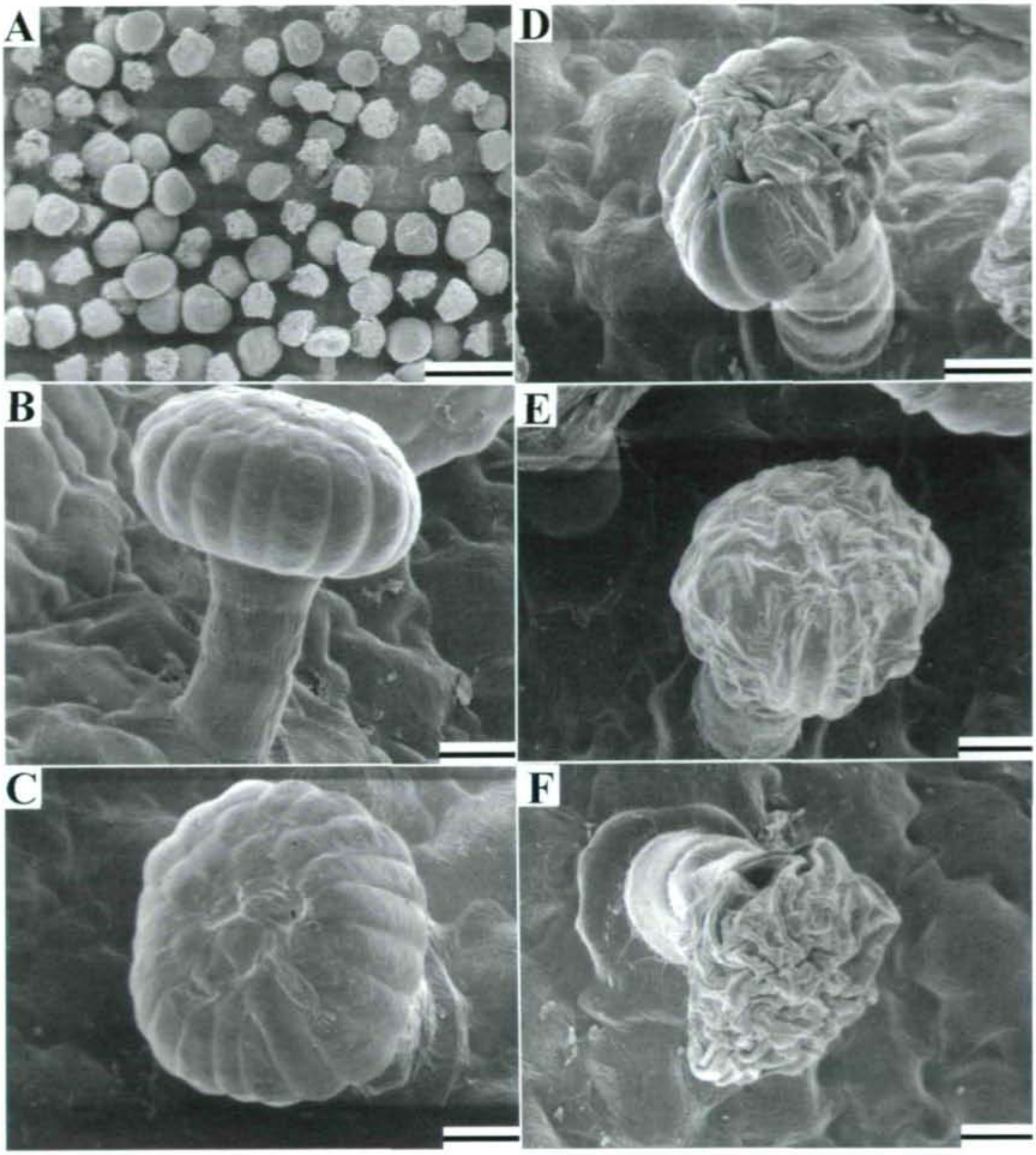


Fig. 19. Elaiophore of *Calceolaria biflora*. **A**, Sector of the elaiophore showing a low density of trichomes. **B**, lateral view of a glandular hair. **C**, frontal view of a glandular trichome showing about 30 secretory cells. **D-F**, progressive senescence of the glandular head. Scale bars: 20 µm.

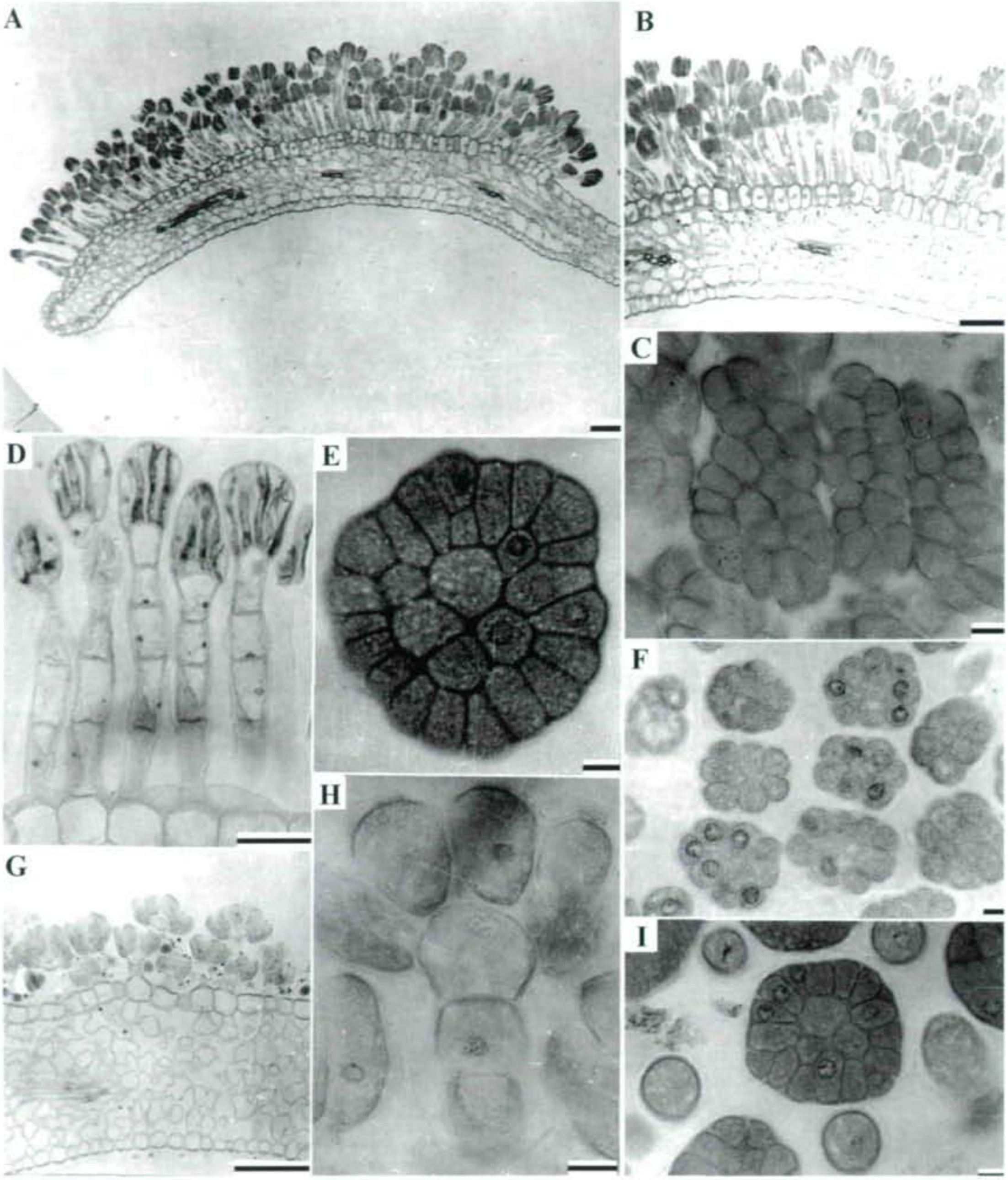


Fig. 20. Light microscopy photographs of microtomic sections of elaiophores. **A-C**, *Calceolaria polifolia*. **A,** tangential section of the trichome cushion. **B**, detail of A**,** note the uniseriate, multicellular foot and the multicellular secretory head. **C**, transection through three glandular heads. **D**, *C. stellariifolia*, longitudinal section of glandular trichomes. **E**, *C. biflora* transection of a 27-celled head. **F**, *C. tripartita*, transection of glandular heads with fewer cells. **G** & **H**, *C. lagunae-blancae*, **G**, tangential section of the elaiophore showing the flabellate glandular heads. **H**, detail of one trichome, note the disposition of the cells of the head and the cuticle covering them; at the right cell it is possible to see the perforated cuticle. **I**, *C. parviflora*, transection of a glandular head. Scale bars: A & B: 100 µm, D & G: 50 µm, C, E, F & I: 10 µm, H: 5 µm.

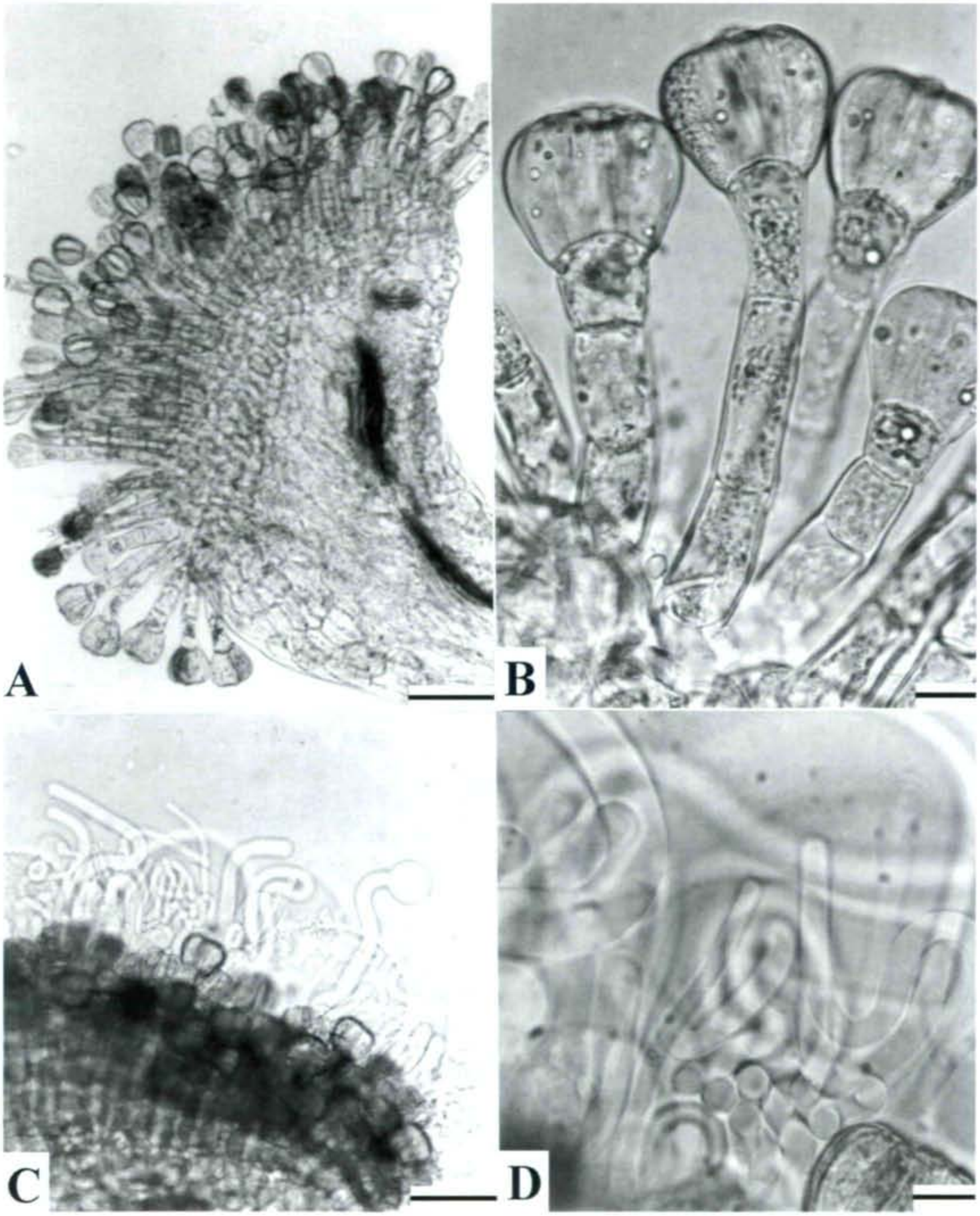


Fig. 21. Light microscopy photographs of hand sections of the elaiophore of fresh material of *C. latifolia*. **A**, tangential section of the appendage showing the cushion of glandular trichomes. **B**, detail of the glandular trichomes showing the uniseriate foot and the multicellular head. **C-D**, oils producing myelin patterns in presence of tap water. Scale bars: A & C: 100 µm, B & D: 20 µm.

Table 3. Length of glandular trichomes and dimensions of the secretory heads in some species of *Calceolaria* (n = 10 trichomes/species).

Species	Trichome length (µm)	Head width (µm)	Head length (µm)
C. umbellata	50.38	29.89	24.81
C. crenatiflora	-	43.09	-
C. biflora	69.74	10.41	44.93
C. ascendens	77.55	47.27	41.28
C. stellariifolia	107.70	35.00	49.06
C. tripartita	93.80	23.28	43.20
C. hypericina	111.59	33.12	24.13
C. ruiz-pavonii	52.20	41.68	34.67
C. lagunae-blancae	75.58	54.37	32.76
C. parviflora	129.87	34.48	38.62
C. corymbosa	66.82	41.94	36.96
C. bicolor	100.23	29.34	56.88
C. polifolia	189.58	34.27	44.49
C. brunellifolia	58.66	39.95	32.48
C. lanceolata	137.44	41.88	39.06
C. lossenii	115.41	31.06	44.61
C. prichardii	132.39	42.52	40.00
C. paralia	-	27.77	-
C. pinifolia	-	34.09	-
C. thyrsiflora	-	38.97	-
C. schickendantziana	148.00	48.00	57.50
C. polyclada	-	31.74	-
C. teucrioides	-	37.45	-
C. dentata	-	35.34	-
C. petioalaris	-	47.28	-
C. volckmanii	134.77	57.96	35.31
C. polyrhiza	-	29.19	-
C. plectranthifolia	164.00	41.00	49.40

The presence of branched multicellular trichomes mixed with normal glandular hairs is noteworthy in the elaiophores of *C. hypericina, C. lossenii,* and *C. parviflora* (Figs. 16C, D, 17C). In *C. parviflora,* each branch ends in a reduced glandular head. In *C. lossenii,* a broad variation of trichomes can be distinguished, from branched multicellular trichomes, without glandular heads to branched and completely glandular ones, with intermediary stages with branches and with or without glandular heads (Fig. 17A-E). There is some variation existing between populations of *C. lossenii.* Plants growing in El Durazno (Córdoba, Argentina) show all kinds of trichomes in the same elaiophore, whereas plants growing in Copina (Córdoba, Argentina) do not have branched trichomes.

Oils: Flowers in anthesis show the elaiophore covered by a shiny yellowish oil film. Due to the density of the oils they draw menisci between the neighboring glandular heads. Sudan

IV stains the oils accumulated outside of the glandular hairs but does not stain the interior of glandular cells. In the field it is possible to detect the presence of oils with filter paper or cigarette paper by touching the elaiophore with the paper. If oils are present, a little diffuse fleck is formed which does not evaporate even when exposed to heat.

Table 4. Weight of oils (mg/flower) of 16 species of *Calceolaria*.

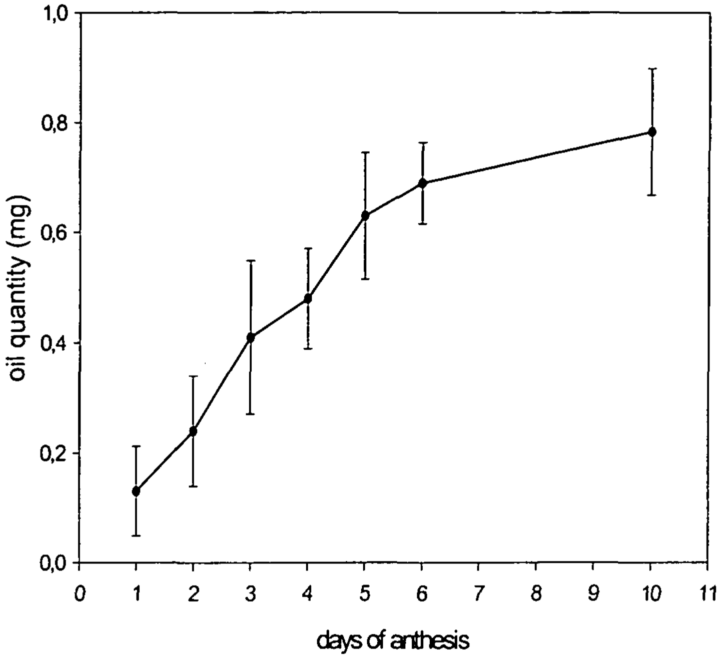
Species	mg/flower n = 20
Section Bellidifoliae	
C. brunellifolia	0.230
C. parviflora	0.450
C. umbellata	0.075
Section Integerrimae	
C. stellariifolia	0.100
Section Kremastocheilos	
C. biflora	0.230
C. lanceolata	0.350
C. polyrhiza	1.550
C. prichardii	0.300
Section Rugosae	
C. dentata	1.350
C. integrifolia	0.685
C. latifolia	0.494
C. paralia	1.400
C. petioalaris	0.900
C. polifolia	1.275
C. volckmanii	0.880
Section Corymbosae	
C. arachnoidea	0.270

The floral oils of *Calceolaria*, like those of other oil-flower species, build myelin figures in tap water (Fig. 21C, D). The oils form thick filaments of circular section which grow and extend with wavy movements, building small heads and rings, which curve many times and change their shapes. This behavior of the oils reveals their hydrophilous nature. In *Calceolaria*, after 45 min the myelin figures contract and form small foam-like drops. Myelin figures are not formed in media at pH 5 or lower.

<u>*Weight:*</u> The weight of oils per flower (n = 20) of 16 species was measured (Tab. 4). The flowers had not been visited and their anthesis had already started, since the anthers were just dehiscent.

Oil production during anthesis was measured in 35 flowers of *C. latifolia* during the period of anthesis (9 days) at intervals of 24 hours. During the first 5 days the secretion increases about 0.170 mg/day; during the last 4 days the increase was at a rate of 0.093 mg/day (flattening the resulting curve, Graphic 2). The total amount of oils accumulated during anthesis was 0.783 mg.

Effect of oil removals: The relative production of oils per flower was measured in 24 flowers of *C. parviflora* performing extractions of oils in 24 hours intervals. The results of the removal of oils every 24 hours during four days (Graphic 3) indicates that although a reposition of oils occurred after each removal, the differences between oil quantities obtained in each extraction is not significant.



Graphic 2. Oil production during anthesis of *Calceolaria latifolia*.

The effect of oil removal in three different schedules every 24 hours during oil production in *C. parviflora* is shown in Graphic 4. The results show that the removals performed in schedule 1 and 2 are significantly different from the other two schedules, where only two removals (b) in an interval of 24 hours or only one removal (c) were done. The oil produced by the one removal schedule is significantly lower than the other schedules. From these data it is evident that the frequency of removals stimulates the production of oils per flower.

Chromatographic analysis: VOGEL (1974) studied in detail the chemistry of the principal components of the floral oils of *Calceolaria pavonii*. The most abundant fraction corresponds to a diglycerid of ß-acetoxypalmitic acid and acetic acid. A saponification process reveals that there are also free fatty acids of the same kind present in *C. pavonii*.

Thin layer chromatography (TLC) was practiced with samples of 22 *Calceolaria* species and 3 samples obtained from eluted pollinators. The results show that the oil is a complex mixture of different lipid fractions along a broad polarity range (Fig. 22). A total of 19 different flecks were detected in the species studied (Tab. 5). The more hydrophobic triglycerides were detectable at the upper part (Fig. 22, spots 15–19), while the more hydrophilic lipids characteristic of the floral oils develop at the lower part of the plate (spots

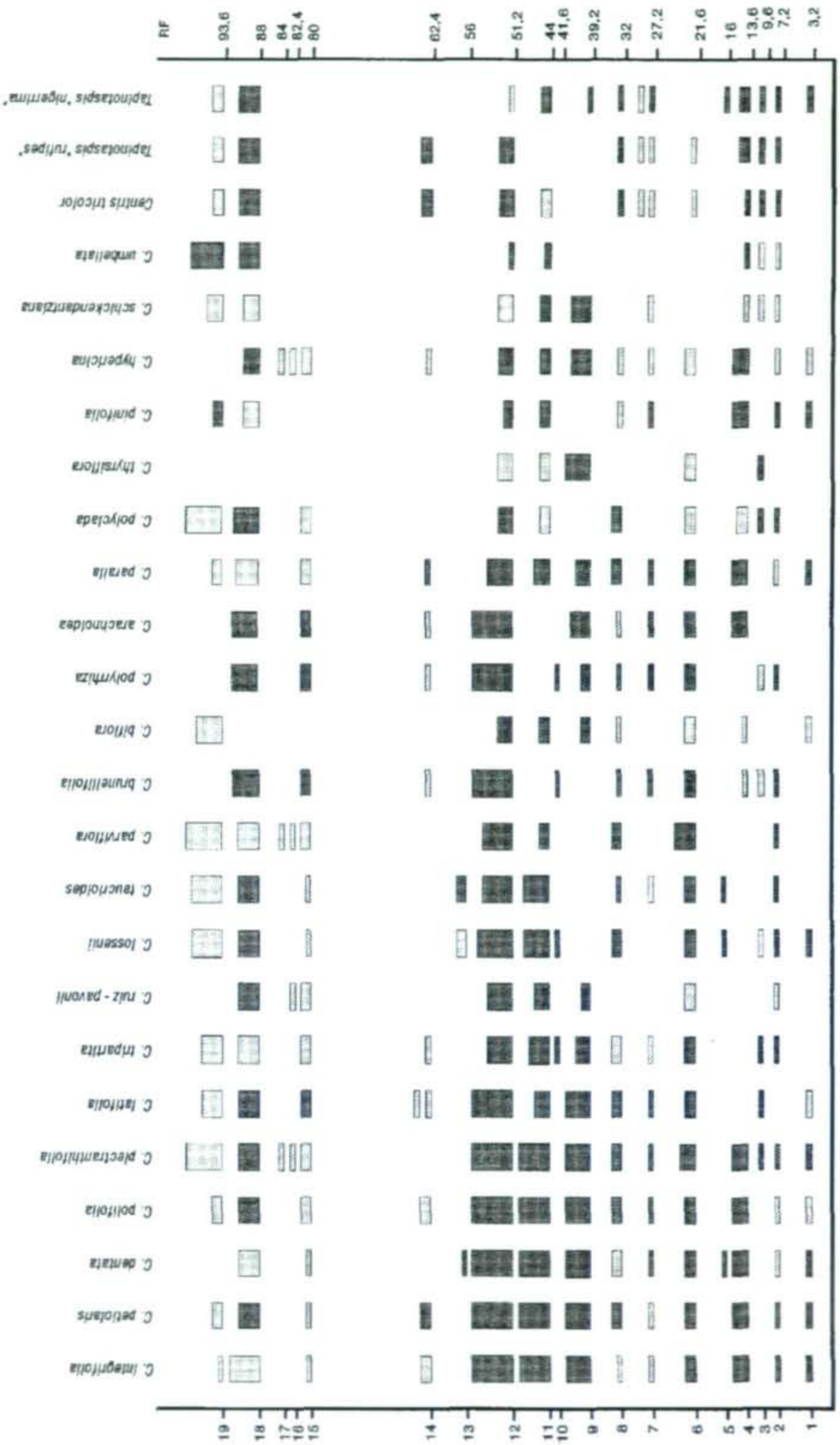
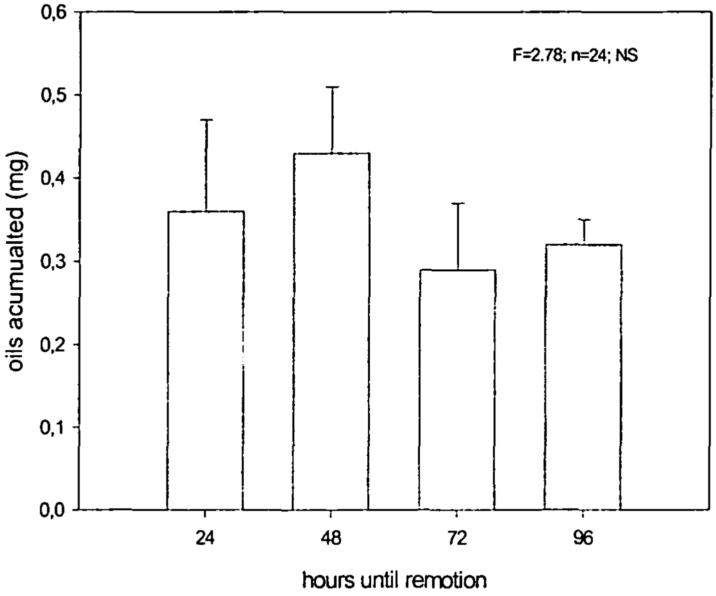


Fig. 22. TLC analysis of floral oils of 22 species of *Calceolaria* and three oil samples of bees. Left scale, number of the fleck. Right scale, RF value corresponding to each fleck.

1–14). Spots 9, 11 and 12 correspond to the more abundant and widespread types of floral oils. Spot number 12 is present in all samples studied. Spot 11 is absent in *C. brunellifolia, C. polyrhiza,* and *C. arachnoidea.* Spot 9 is absent in *C. lossenii, C. teucrioides, C. parviflora, C. brunellifolia, C. polyclada, C. pinifolia,* and *C. umbellata.* These three oils are the same oil fractions that VOGEL (1974) identified as the most important ones. Fractions number 2, 6 and 8 appear very frequent, but are present only in small quantities.



Graphic 3. Effect of removal of oil every 24 hours during oil production in *Calceolaria parviflora.*

Extracts of a few flowers (2-3) gave satisfactory results although their oil concentration was low. Five years old extracts were also successfully used in the chromatographic study. *C. schickendantziana* and *C. lossenii* showed a peculiar uniform and dark track along the whole run, irrespectively if the extracts were old or fresh. Fresh extracts of the latter species were resin-like and more dense, than those of other species.

Samples obtained from eluted insects showed a similar pattern as those of the floral oils directly. The most important spots were developed: spot 12 was present in all insects, while spot 11 appeared in *Centris tricolor* and *Chalepogenus vogeli* and spot 9 was present only in *Ch. vogeli.* Spot 7 appeared as a double band in all three cases.

Phenetic study: The spot patterns were variable and constant for each species (Fig. 22). The chromatographic pattern was used to make a numerical analysis of the species studied. Cluster analysis could demonstrate if the floral oil patterns allowed the association of species by means of phylogenetic affinities or pollination syndromes (visited by *Centris* or *Chalepogenus*). The extracts obtained from the bees could show an eventually relation

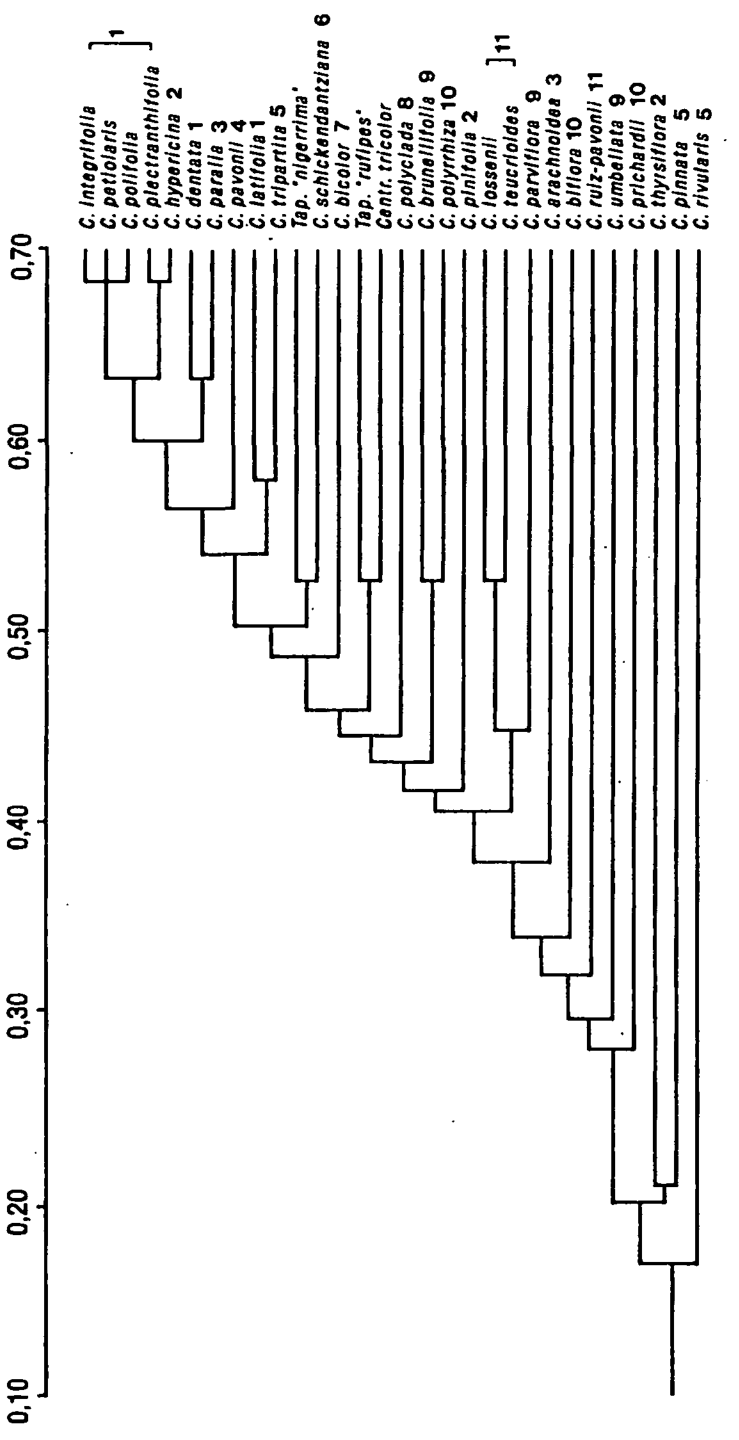


Fig. 23. Phenetic diagram obtained from the TLC data shown in Fig. 22, and from five additional oil patterns obtained from VOGEL (1974). References: 1, section Rugosae, 2, section Integerrimae, 3, section Corymbosae, 4, section. Perfoliatae, 5, section Calceolaria, 6, section Parvifoliae, 7, section Urticopsis, 8, section Polyclada, 9, section Bellidifoliae, 10, section Kremastocheilos, 11, section Teucriifoliae.

between the bees and the floral oils of the flowers they visited. To the 22 species of *Calceolaria,* three extracts of the insects were added, making a total of 25 spot patterns. The coefficient of association (RR) was used to establish similarities between all oil patterns; this coefficient indicates the relation between number of coincidences by presence, over total number of coincidences. The unweighted pair-group method of arithmetic averages (UPGMA) was used as a clustering strategy.

Table 5. Comparison of RF-values obtained in this work with those of VOGEL (1974). Values are expressed in percentage of the total length of the run.

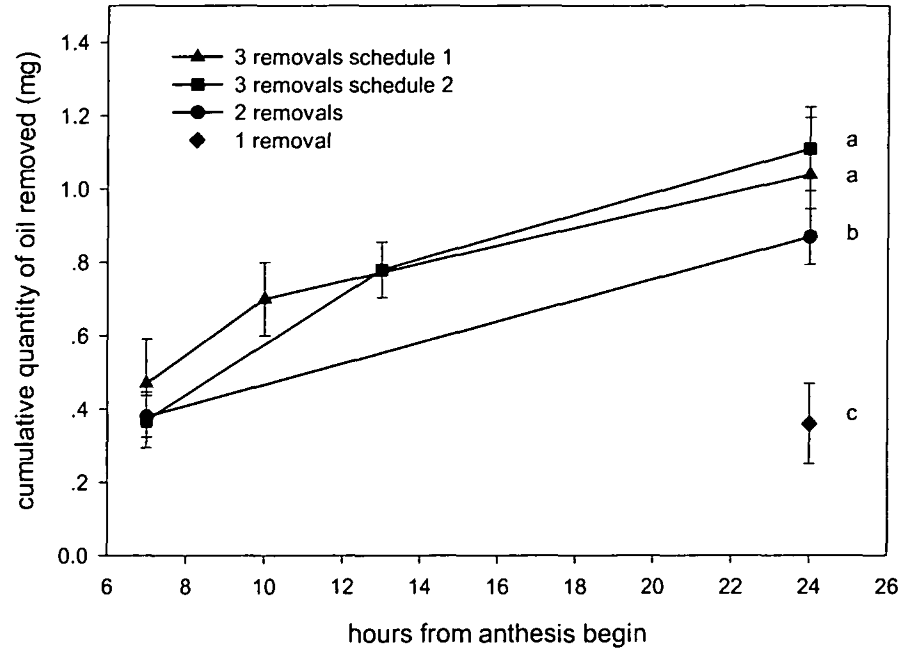
Spot	RF-values (precoated plates, Mainz)	RF-values (hand-coated plates, Córdoba)	RF-values VOGEL (1974)
1	3.2	-	-
2	7.2	7.9	8
3	9.6	9.6	-
4	13.6	20	21
5	16	26	-
6	21.6	29.4	32
7	27.2	32	37
8	32	42	41
9	39.2	49	48
10	41.6	56	-
11	44	58	61
12	51.2	65.9	72
13	56	-	-
14	62.4	66.14	-
15	80	76.1	-
16	82.4	77.2	-
17	84.0	82.56	-
18	88	86.45	94
19	93.6	92.68	100

The clusters obtained did not show any relationship with the pollination syndromes (Fig. 23). In only a few cases the chromatographic patterns correlate with phylogenetic alliances, as for example with most species of section Rugosae, although *C. dentata* and *C. latifolia* of the same section appear somewhat distant. *C. teucrioides* and *C. lossenii*, both of section Teucriifoliae, build a cluster but appear separated from *C. ruiz-pavonii* of the same section.

Centris tricolor was collected on *Calceolaria lossenii, Chalepogenus rufipes* on *C. umbellata* and *Ch. vogeli* in *C. polyclada* but the oils obtained from these insects did not show similar patterns as those of the flowers they were visiting.

The food body of Calceolaria uniflora

The saccate lower lip of *C. uniflora* shows a notorious lap folded to the outside of the flower. This lap is thick, juicy and almond-tasted, is sharply bounded and white in color, which contrasts optically against the yellow and red or brownish-red spotted lower lip. It is rectangular in shape (0.7 cm in length, 1.4 cm wide and 0.3 cm thick) and is located at a distance of 1.8 cm from the anthers. Non-functional glandular hairs are hidden between the abaxial side of the lap and the instep of the corolla (Fig. 24A-B). The absence of oils was tested directly in the field. No translucent spot was formed while slightly pressing the hairs on a cigarette paper, hand cuts didn't produce myelin figures in a diluted solution of KOH in tap water, and hand cuts stained with Sudan IV gave negative results. Hand cuts of the appendage were immersed in I_2/IK to detect presence of starch in the cells of the appendage but the results were also negative.



Graphic 4. Effect of oil removal during oil production in *Calceolaria parviflora* (four different schedules).

Only sugars were detected in the appendage cells (Tab. 6). Sugar measurements were compared with measurements on the fruits of *Empetrum rubrum* VAHL. (Empetraceae), a species growing in the same plant community with *C. uniflora*.

Sections of the appendage were studied with LM and SEM. A well irrigated, uniform parenchyma of large, vacuolized, loosely arranged cells with many intercellular spaces was detected (Fig. 24B). There are no glandular cells of any kind in the body of the tissue.

The glandular hairs have smaller glandular heads (c. 4.6 µm in diameter) with only four cells (Tab. 3, Fig. 24B).

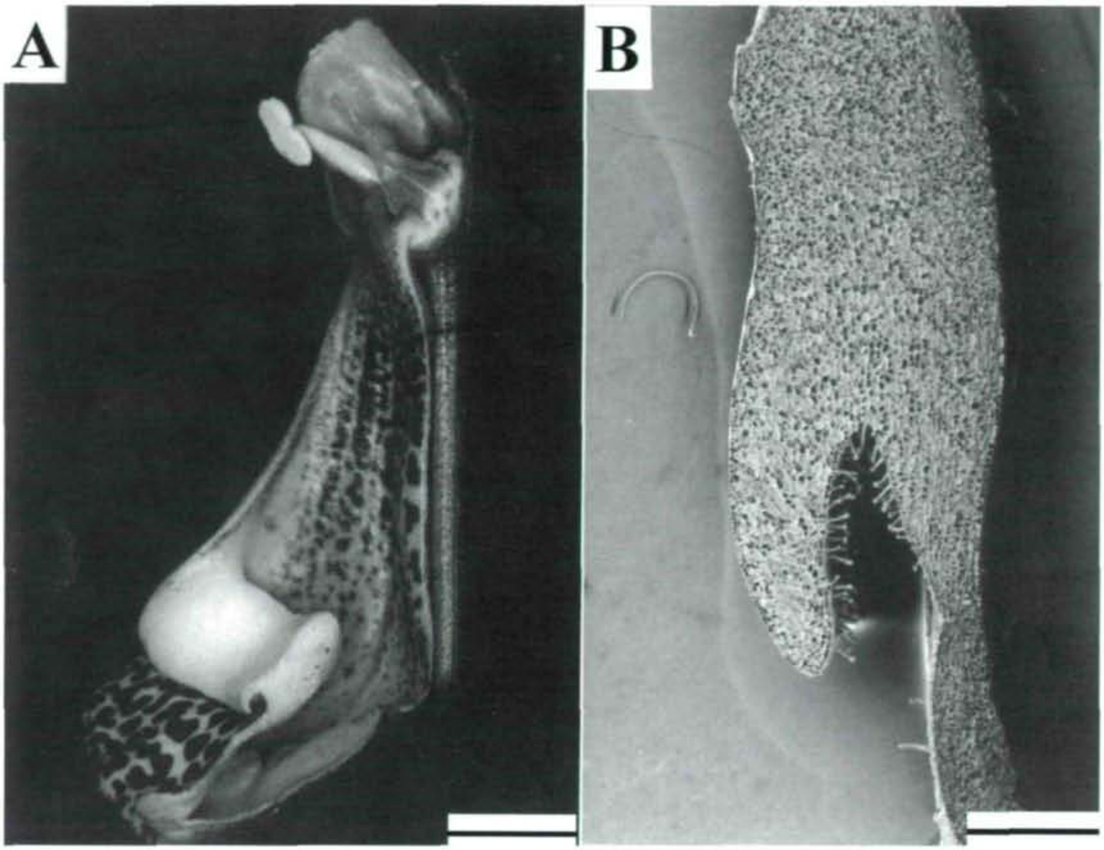


Fig. 24. Food body of *Calceolaria uniflora*. **A**, longitudinal section of the flower showing the white and thick food body. **B**, detail showing parenchymatic tissue and the hidden, non functional trichomes. Scale bars: A: 0.5 cm, B: 1 mm.

***Androecium*:** The androecium of *Calceolaria* consists of only two dorso-lateral stamens (Fig. 35B). They are shortly fused with the corolla along the stapetum and normally do not overpass the corolla length.

Filaments: With the exception of section Rugosae most species have filaments shorter than the anthers (0.5 mm)-1.39 mm-(3 mm) and are relatively wide. Stamens with short filaments are related to a hood-like upper corolla lip, which protects them partially or totally. The filaments of section Rugosae, which are 3–5 mm in length, are not exserted and are always related to a "Rugosae-type" upper lip, which completely covers the fertile parts.

Table 6. pH, refraction index, presence of proteins and glucose in the food body of *C. uniflora* and fruits of *E. rubrum*.

	Food body of *C. uniflora*	Fruits of *E. rubrum*
n	10	10
pH	5	5
Proteins	- (or traces)	-
Glucose (gr/gr)	+ (2%)	+ (2%)
Refraction index (gr/gr)	2.14%	2.58%

Anther insertion and movability: The insertion zone of the anther into the filament may show no modified tissues; in this case the anther is fixed to the end of the filament and the possibilities of movements are scarce. In most species the tissues at the insertion point are modified, rendering it flexible but resistant and functions as a true hinge, which allows anther movements. In most species this hinge is short, wide, but thin and binds the anther to the filament along a transversal band. At this point parenchyma cells of the filament become necrotic, while their cell walls become strong, building a flexible and resistant band, which allows only dorso-ventral movements.

Versatile anthers are more rare in the genus. At least in *C. uniflora* and probably *C. fothergillii* the hinge is like a cord of circular section and is built by necrotic cells. This tissue joins the connective in a small spot, which allows circular movements of the anther.

Anthers: With a few exceptions, the anthers are always bithecic and tetrasporangiate. The connective can be formed in two different ways:

- Commonly, it develops like a thin wall that separates the two thecae at their apical part. When the anther dehisces, the connective shrinks and the dehiscence lines of both thecae become contiguous, originating in the appearance of one stomium (Figs. 26G, H, 27B, D, F, 28D, F).
- Less frequently, the connective does not shrink, and each theca remains separated with its own dehiscence line (Fig. 30A, B, D). This separation between both thecae is variable, depending on the species. Sometimes, both thecae are very distant from each other as it occurs in the lever mechanism (Figs. 34A-G, 35A, 36A, 37A).

In *Caceolaria,* a morphological variability of the anthers related to the phenomena of divergence, reduction, lateral and facial secondary fusion is evident:

Divergence: All species have divergent thecae. The divergence angle is determined by imaginary lines running longitudinally along each theca and joining at the apex of the anther. During early development, the thecae separate from each other at their basal part and stabilize at an angle of 90° (deflexed thecae, Fig. 25C, E), or at 180° (divaricate thecae, Figs. 26A-H, 27A-F, H, 28C, E). In a few cases, the divergence angle exceeds 180° (ascendant thecae) as in *C. parviflora* (Fig. 27G) and *C. dentata* (Fig. 28A, B)[2].

Lateral fusion of the pollen sacs of the same thecae: In all species of *Calceolaria* studied, as in most other Angiosperms, the pollen sacs of the same thecae are completely separated by a septum in early stages of development. During anther development, the septum contracts and looses connection with the anther walls, so just before dehiscence both pollen sacs appear secondarily united. Old empty anthers still show rests of the contracted septum.

In some species the septum persists and the cells do not collapse into the anther locule, but become thin and necrotic instead, maintaining only their cellulose walls. The transversal anticlinal walls become perforated, while only the periclinal and the radial ones persist, thus building a net-like structure[3]. During anther dehiscence these walls are still attached to the stomium cells; the anther walls contract, stretching the net and pressing it onto the pollen masses so that they disattach from the septal portion, but stay attached along the stomium walls. At the stomium level this tissue is almost not perforated and appears like a stronger membrane (Fig. 35B-G). Three species of section Calceolaria (*C. tripartita, C. rivularis, C. chelidonoides*) (Figs. 35A-G, 36B-D, 37D) showed this cellulose net fixed along the margins of the dehiscence line. The possible ecological significance of this net could lay in the regulation of pollen quantities available for pollinators in each visit. Another interpretation can also be considered: the upper lip of these species tightly encloses the stamens (Fig. 4F), leaving a thin slit as the only exit of the anther when it is tilted outwards. The stamens of these species have a lever mechanism (as will be discussed later), so that when an insect visits a flower the upper theca of the anther descends and contacts the dorsal part of the insect. The slit of the upper lip is so narrow that only one anther at a time passes through. When the anther is forced through the slit the thin net is retained by the margins of the slit, the theca widens and exposes the pollen. When the anther tilts back inside the upper lip its margins press the anther walls so that the nets of both margins are directed to the median line, and fold back again on the theca.

Facial fusion: VAN TIEGHEM (1903) studied the stamens of some species within family Scrophulariaceae and described two types: the "type tetrathèque" and the "type dithèque", which correspond actually to the "tetrasporangiate" type (bithecic) and the "bisporangiate" type (monothecic), respectively. He described monothecic stamens for *Verbascum, Celsia, Chaenostoma, Manulea, Nemesia, Limosella,* and *Scrophularia* and considered the monothecic type being derived from the bithecic one through a complete reduction of one thecae and displacement ("dèplacement") of the other to reach a terminal position, perpendicular to the filament. Later, TRAPP (l. c.) studied the anthers of *Scrophularia* in detail and confirmed the supposition of GOEBEL (1933) that the anthers in this genus are

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[2] The presence of divergent thecae is frequent in many Scrophulariaceae (TRAPP 1956). Angles of 90° characterize the anthers of *Digitalis*, *Linaria*, *Nemesia*, *Maurandya* and *Penstemon*; *Russellia* and *Mazus* show divergence angles of 180° and ascendant thecae (more than 180°) were mentioned for *Ceranthera linearifolia* (Bignoniaceae), a feature, which is known as "Überspreizung der Anthere".

[3] A similar phenomenon was registered in *Impatiens* (VOGEL & COCUCCI 1988b)

bithecic and not monothecic as thought by VAN TIEGHEM. At the first stages of the ontogenic development, he distinguishes four masses of sporogenic tissue separated by the septa and the connective. During differentiation of the mother cells of the pollen grains, the connective reduces and the pollen sacs of each theca fuse, giving the impression of a monothecic anther. These "pseudo-monothecic" anthers were named by TRAPP (l. c.) as "synthecic anthers", and he distinguishes two types. One corresponds to the case already mentioned of *Scrophularia* with "primary synthecic anthers" (primary confluent thecae or primary facial fusion of the thecae). The other comprises the "secondary synthecic anthers" (secondary confluent or secondary facial fusion of the thecae), which TRAPP describes for species of *Digitalis, Antirrhinum, Misopates, Mimulus, Tetranema, Russelia,* and *Penstemon.*

In *Calceolaria* the fusion of both thecae of an anther occurs late in the ontogenic development. Only when the pollen grains are mature and just before dehiscence of the anther, the connective contracts and produces the connection between both thecae. This secondary facial fusion is present in most species studied here; the dehiscent anthers show a wide surface where neither septa nor connective are distinguishable, and the thecae appear as primarily confluent. However, in freshly dehiscent anthers of *C. latifolia* and *C. petioalaris* four pollen masses corresponding to the four pollen sacs are easily distinguishable. Serial microtome sections of young stamens with already differentiated pollen grains show that at this stage both thecae are well separated by a thin connective in *C. hypericina* and *C. schickendantziana.*

Species like *C. revoluta* PENNELL, *C. helianthemoides* H.B.K., *C. lavandulifolia* H.B.K., *C. jujuyensis* BOTTA, *C. paralia*, (Fig. 30C-D), *C. lepida,* and *C. tetragona* (Fig. 30A, B) do not exhibit facial fusion of their anthers and one independent dehiscence line corresponds to each thecae. The thin connective separates both dehiscence lines. Obviously, no fusion is seen in the anthers with lever mechanism where both thecae are widely separated by an elongated sterile tissue.

Lateral reduction[4]: Lateral reduction occurs when there is no development of a whole theca. This type of modification is present in some of the *Calceolaria* species with a lever mechanism.

Stamen types: Considering the different modifications found in the stamens of *Calceolaria*, it is possible to characterize the types of stamens which are important in their adaptations to the pollination mechanisms in the genus. Angle of divergence, dehiscence and position of the thecae, length of the filament, lateral reduction, presence and kind of hinge, are the most important features to be considered in the different stamen types. It is possible to distinguish 5 types of stamens:

Basic type: Most widespread and apparently most primitive in the genus. It is characterized by a short filament (Fig. 40C), if the hinge is absent the stamens are incapable of movements. When the hinge is developed, it is a short and wide band of necrotic cells, which allows the dorsal-ventral movements (Figs. 26A-H, 27A-F, H). The thecae are equal in size and have a divergence angle of 180°; they are separated by a thin connective, but

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[4] GOEBEL (1933) considered the facial reduction to be also included as another kind of reduction process. Facial reduction is present when one pollen sac of each theca does not develop, as it occurs in Asclepiadaceae and Lauraceae p. p. (*Cryptocarya, Bellota, Ajouea,* and *Hueflandia*). Cases of reductions of this kind are not present in *Calceolaria.*

dehiscence is complete due to the secondary facial fusion; the walls of the anther shrink notably in a way that the pollen is exposed in a wide surface. About 55% of all *Calceolaria* species show this type of stamens.

Jovellana type: The filaments are short. No hinge is developed, the filament is wide and has a circular section at the point where it joins the connective, maintaining the anther fixed in the same position (Fig. 40A). The thin connective holds the thecae with a divergence angle of < 90° and a secondary facial fusion. When dehiscence occurs the anther walls do not shrink and the pollen masses are not completely exposed (Fig. 25E-F). Stamens with these features are present in about 15% of the species, 68% of them correspond to species without elaiophore, they are autogamous or function as pollen flowers. *Jovellana violaceae* (Fig. 25A-D) shows this type of stamens.

Long filament stamens: the main feature of this type is the presence of extremely long filaments (Figs. 28A-F, 40D). The average length of the filaments is (2)-2.49-(5) mm. The hinge can be present or absent, when present the movements of the anthers are dorso-ventral as in *C. dentata, C. integrifolia*, and *C. plectranthifolia*. *C. latifolia* (Fig. 28C, D) has fixed anthers. The thecae have a divergence angle from 90° to over 180° (*C. dentata*, Fig. 28A, B). This kind of stamens with long filaments are restricted to section Rugosae in relation to sternotribic pollination (see later).

Versatile stamens: The filament is comparatively long (3 mm) but its distal tip becomes very thin and necrotic, and it contacts the connective at a tiny spot allowing circular movements of the anther. The divergence angle of c. 180° of the thecae, their facial fusion and the strong shrinkage of the anther walls allow the exposition of the pollen masses on a wide open surface. This kind of stamen is present in bird pollinated *C. uniflora* (Figs. 29A-D, 40E). The wide range of movements of the anthers probably favored the deposition of pollen on the body of the bird.

Stamens with lever mechanisms ("Hebelmechanismus", Fig. 40B): Filaments of this type of stamens are very short and joined transversally to the connective by a thin membrane-like band. This feature limits lateral movements, making them possible only in dorso-ventral direction. Each stamen moves independently. Young stamens with indehiscent anthers have no differentiated hinge; microtome sections at this level (Fig. 38A, B) show under the epidermis a well developed parenchyma formed by cells containing nuclei and vacuolized cytoplasm. In dehiscent stamens the anatomy of the hinge is completely different (Fig. 38C, D), the parenchymatic cells become necrotic, they loose their cytoplasmatic contents, and their cell walls appear thicker, more flexible and resistant.

Both thecae are separated by a bar of sterile tissue. The apical face of this sterile tissue does not grow as much as the basal face, thus twisting the thecae upwards in a way that their dehiscence lines end parallel to the apical bar face and along the same line (Fig. $32B_{2-5}$). The posterior (adaxial) end of this sterile bar has a well developed and always fertile theca, while the anterior (abaxial) theca may be reduced, sometimes sterile or completely absent.

There are many cases in the development of the anterior theca and the sterile bar, which reflect a tendency to more apomorphic stages:

- Both thecae are equally developed and fertile, but separated by a moderately grown bar of sterile tissue (*C. tetragona*, Figs. 30A, B, 40B left).
- The abaxial theca is reduced, but still fertile; the sterile tissue separates both thecae. For example: *C. ruiz-pavonii, C. lossenii, C. teucrioides, C. santolinoides,* and *C. polyclada* (Figs. 34A-G, 40 B middle).

- True lever stamens: the abaxial theca is almost or completely absent, without remnants of fertile tissue. The sterile tissue is well extended to the sides (Figs. 35A, 40B right). In the most advanced species the abaxial theca is completely sterile as in *C. tripartita, C. mandoniana* KRANZ. (section Calceolarieae) and *C. chaetostemon* PENNELL (section Urticopsis).

Origin of the sterile tissue: The sterile tissue separating both thecae was always considered in *Calceolaria* as being derived from the connective (DELPINO 1868, CORRENS 1891b, TRAPP 1956), due to an extrapolation of the lever stamens in *Salvia*. Two different origins of the sterile tissue are distinguished here through studies of anther anatomy of *C. polyclada* (section Polyclada), *C. lossenii, C. teucrioides,* and *C. ruiz-pavonii* (section Teucriifolieae), *C. pavonii* (section Perfoliatae), and *C. tripartita* (section Calceolaria):

- Connective origin of the lever: Microtome sections show that the epidermis of the sterile bar is different from the epidermis of the thecae. There is no dehiscence line differentiated, and there is a normal parenchyma tissue surrounding the vascular bundle (Fig. 33A). These features were found only in *C. polyclada*, and *C. tripartita*.
- Thecae origin of the lever: The anthers are divaricate with divergence angles of 180° or more; the apical portion of the thecae is sterile in a variable degree and forms the sterile bar that functions as the arm of the lever. The epidermis of this sterile part maintains the same aspect as on the fertile part of the theca. The dehiscence line is also developed along the sterile bar, although it is not functional. Layers of endothecium are well developed not only in the fertile part of the thecae, where it is functional, but also at the sterile part. There is no vascular bundle along the whole structure (Fig. 33B). This kind of anthers was found in all other species studied.

The double nature of the lever anthers reveals the independent origin of the lever anthers in different sections.

Distribution within the genus: VOGEL (1974) already mentioned the presence of lever anthers for sections Calceolaria, Parvifoliae, and Perfoliatae. After MOLAU (1988) and own observations, it was possible to map the distribution in the genus (Fig. 39) and put in evidence its evolutionary appearance. The lever mechanism is restricted to subgenus Calceolaria; it is present in 45 species (18–16 % of the total number of species). The frequency increases in more advanced sections. With the exception of two species, the species of section Teucriifoliae all have lever anthers in different degrees of development. The lever mechanism shows its highest developmental stage in section Calceolaria where all species have the most advanced type of lever anthers. Although there are phylogenetic relations between sections Polyclada, Urticopsis, Lobatae, Perfoliatae, Teucriifoliae, and Calceolaria, the origin of lever anthers appears to be independent in each section. The lever mechanism is not present in section Parvifoliae as already mentioned by VOGEL (1974).

Organographic and functional comparison with the lever anthers of other families: The ecological significance of lever stamens were first described by SPRENGEL (1793) for some species of *Salvia*. This genus has only two fertile stamens corresponding to the anterior pair (Fig. 32A_1). The filament is short and united through a small flexible hinge to the stretched and curved connective. The upper (anterior or abaxial) tip of the connective, which is hidden in the flower by the upper corolla lip, ends in a completely developed fertile theca. This theca is subjected to two movements during development, the inferior end of the theca rotates to reach an apical position and the whole theca turns around 90° to place the dehiscence line downwards (Fig. 32$A_{2\text{-}5}$). The inferior (posterior or adaxial) end of the connective is situated at the entrance of the floral tube; the theca that it carries is reduced in

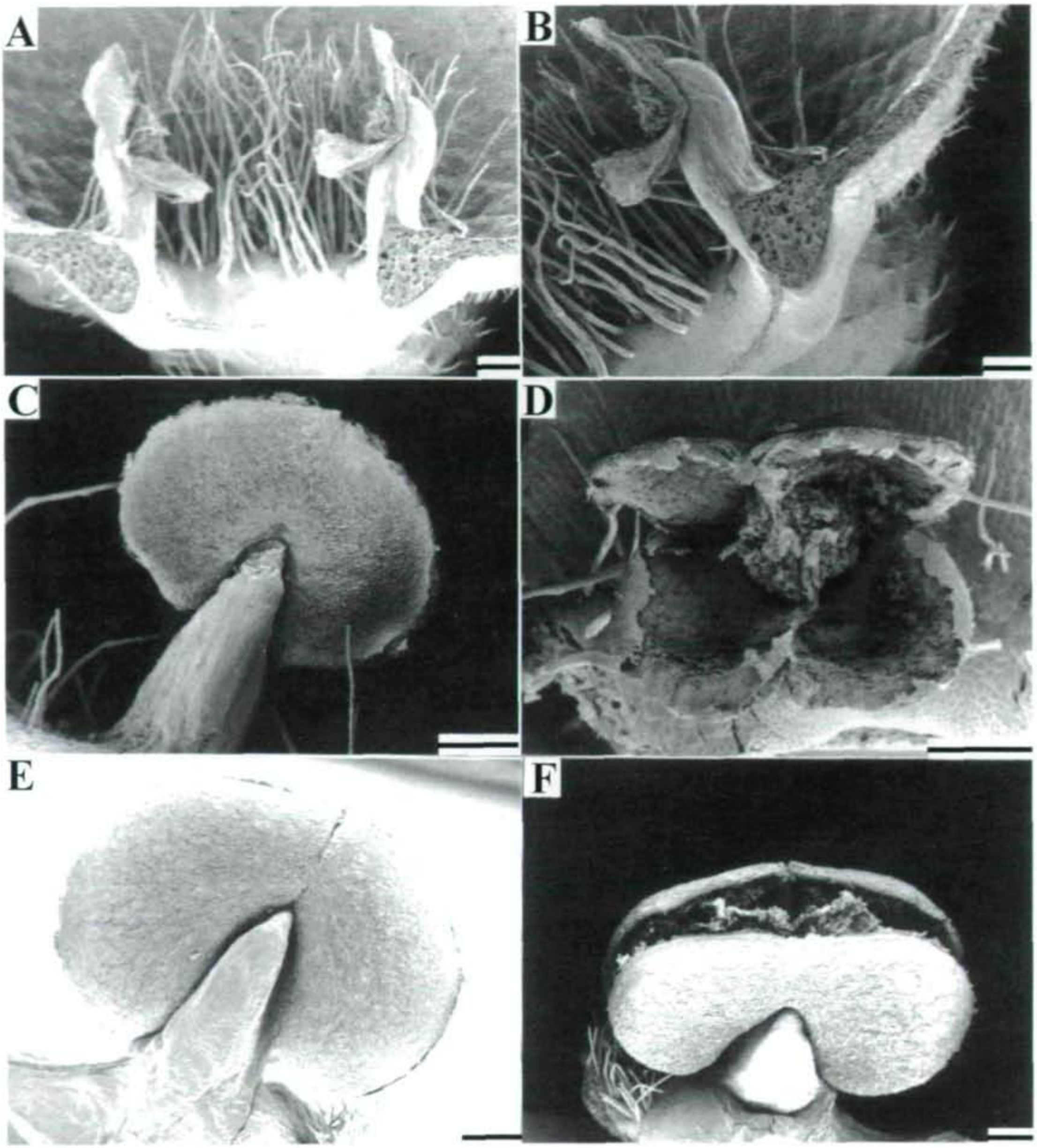


Fig. 25. Different types of stamens. **A-D**, *Jovellana violaceae*, **A**, adaxial view of the androecium. **B**, stamen showing the thick filament and the dehiscent anther. **C**, ventral view of the stamen showing the anther walls not retracted. **D**, stomium, rests of the connective are distinguishable. **E** & **F**, *Calceolaria irazuensis*, **E**, dorsal view. **F**, frontal view showing the stomium. The anther walls do not retract after dehiscence. Scale bars: 400 µm.

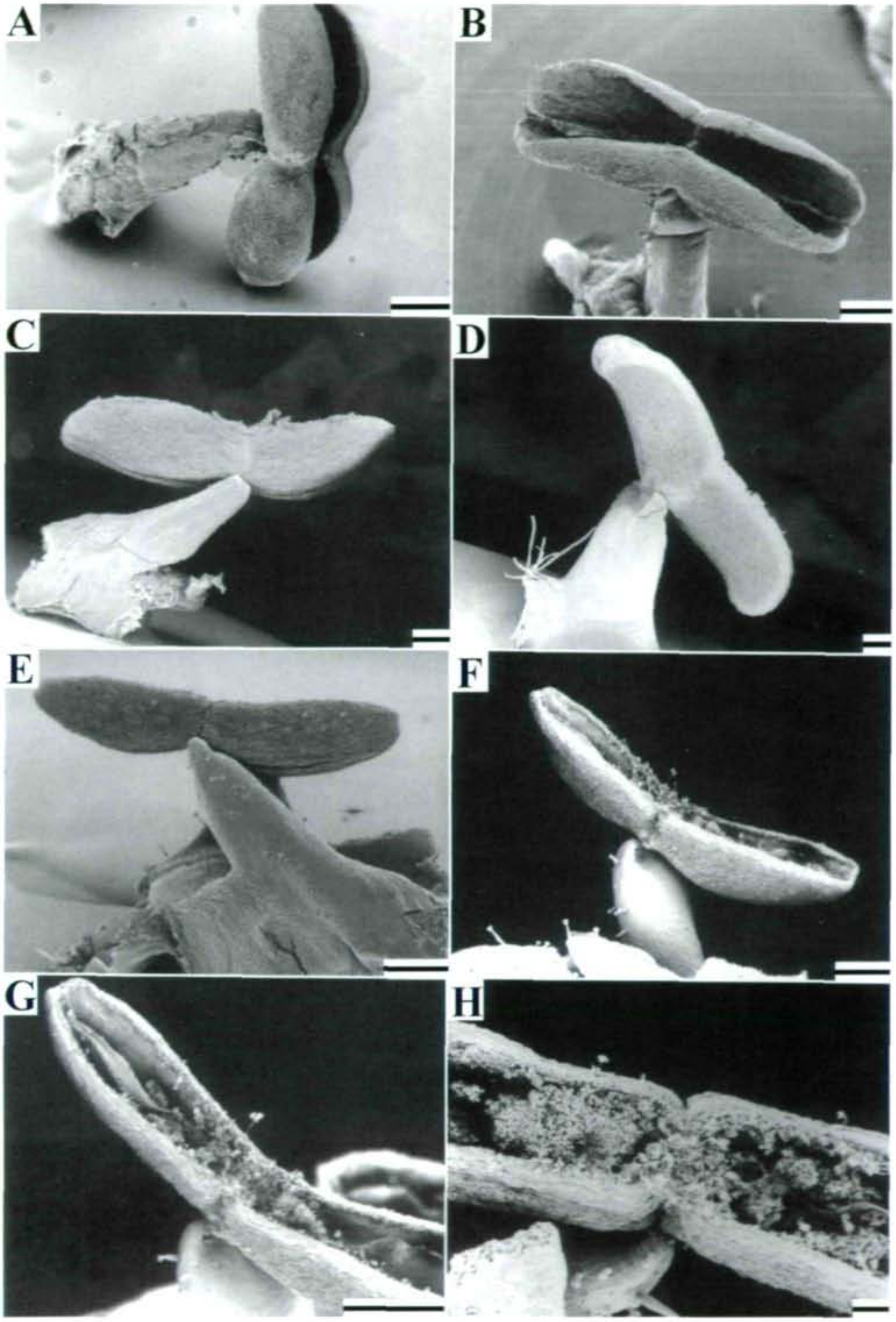


Fig. 26. Anther divergence angle of c. 180°. **A**, *Calceolaria pinifolia*. **B**, *C. hypericina*, frontal view, note the thin connective and the retracted septa between the pollen sacs. **C**, *C. rhacodes*. **D**, *C. ascendens* subsp. *ascendens* . **E** & **F**, lateral views of the anther showing its articulation with the filament. **G**, detail in frontal view, note the septa between the pollen sacs. **H**, connective area covered with germinated pollen. Scale bars: 400 µm.

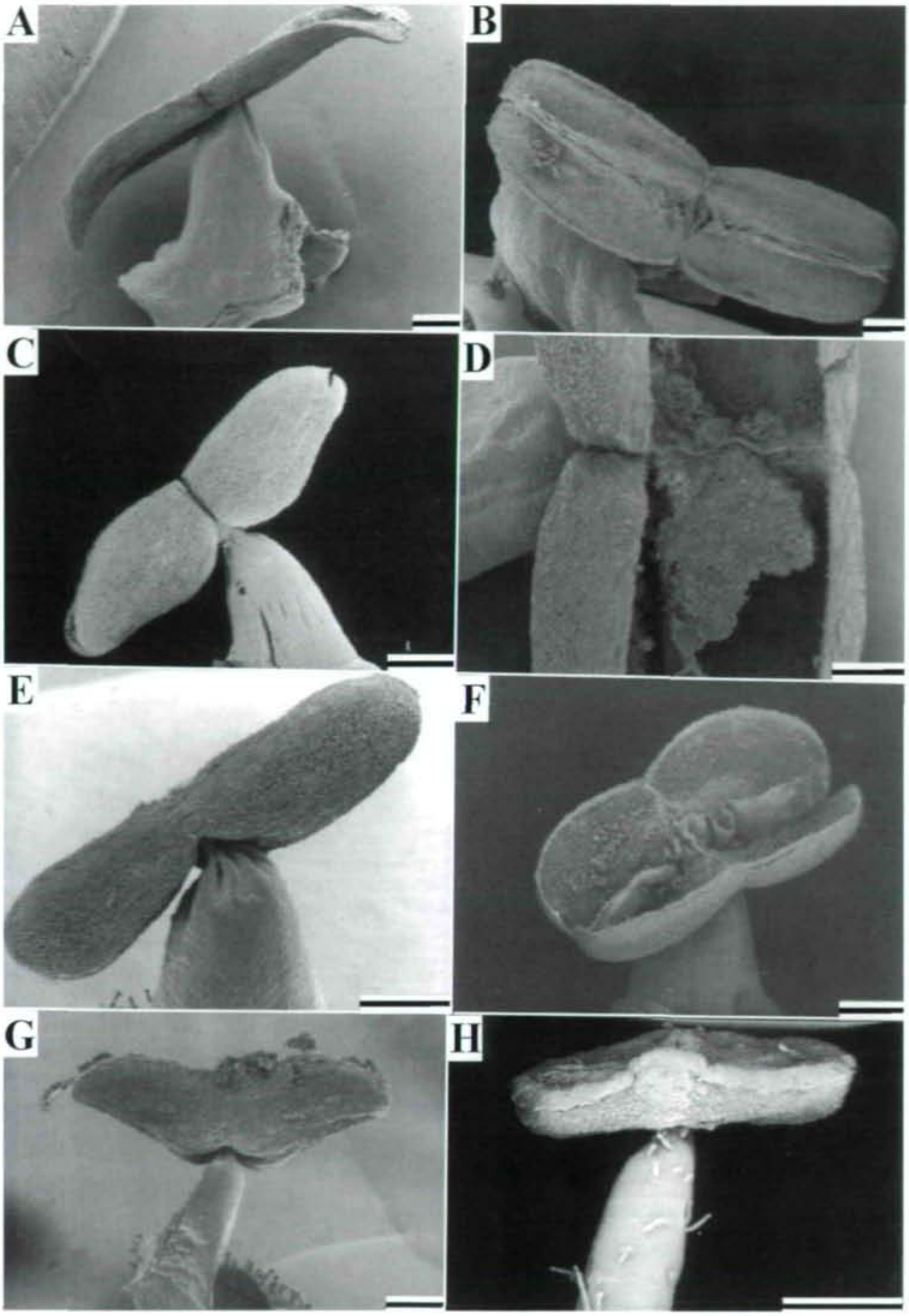


Fig. 27. A-F & H, divergence angle of 180°. **A & B**, *Calceolaria prichardii*, **A**, dorsal view. **B**, frontal view; note the septa and the connective totally reduced. **C & D**, *C. lagunae-blancae*. **C**, dorsal view. **D**, detail of the connective area, note the connective like a thin layer separating both thecae. **E & F**, *C. stellariifolia*. **E**, dorsal view showing the hinge. **F**, dehiscent anther showing rests of septa and connective. **H**, *C. thyrsiflora* stamen in dorsal view, note the thick edge of the stomium. **G**, *C. parviflora*, divergence angle > 180°. Scale bars: A-C & E-H: 400 µm, D: 200 µm.

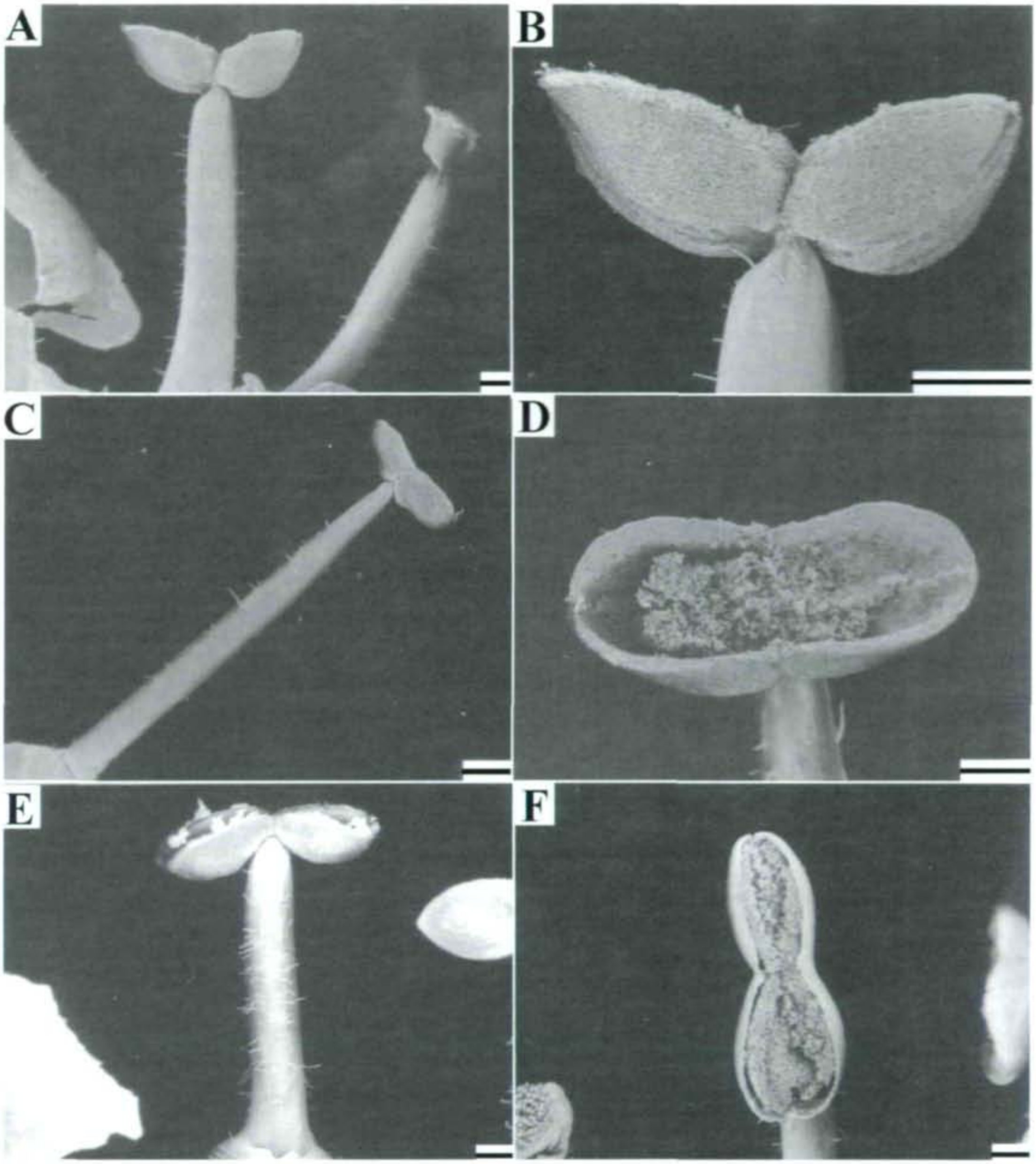


Fig. 28. Long stamens of section Rugosae. **A & B**, *Calceolaria dentata*. **A**, general view. **B**, detail of anther dehiscence, showing the lateral walls partially retracted, divergence angle > 180°. **C & D**, *C. latifolia*. **C**, general view. **D**, detail of the dehiscent anther. **E**, *C. polifolia*. **F**, *C. petioalaris*, frontal view of the anther, note the adaxial thecae more developed and located parallel to the filament. Scale bars: A-C, E & F: 500 μm, D: 200 μm.

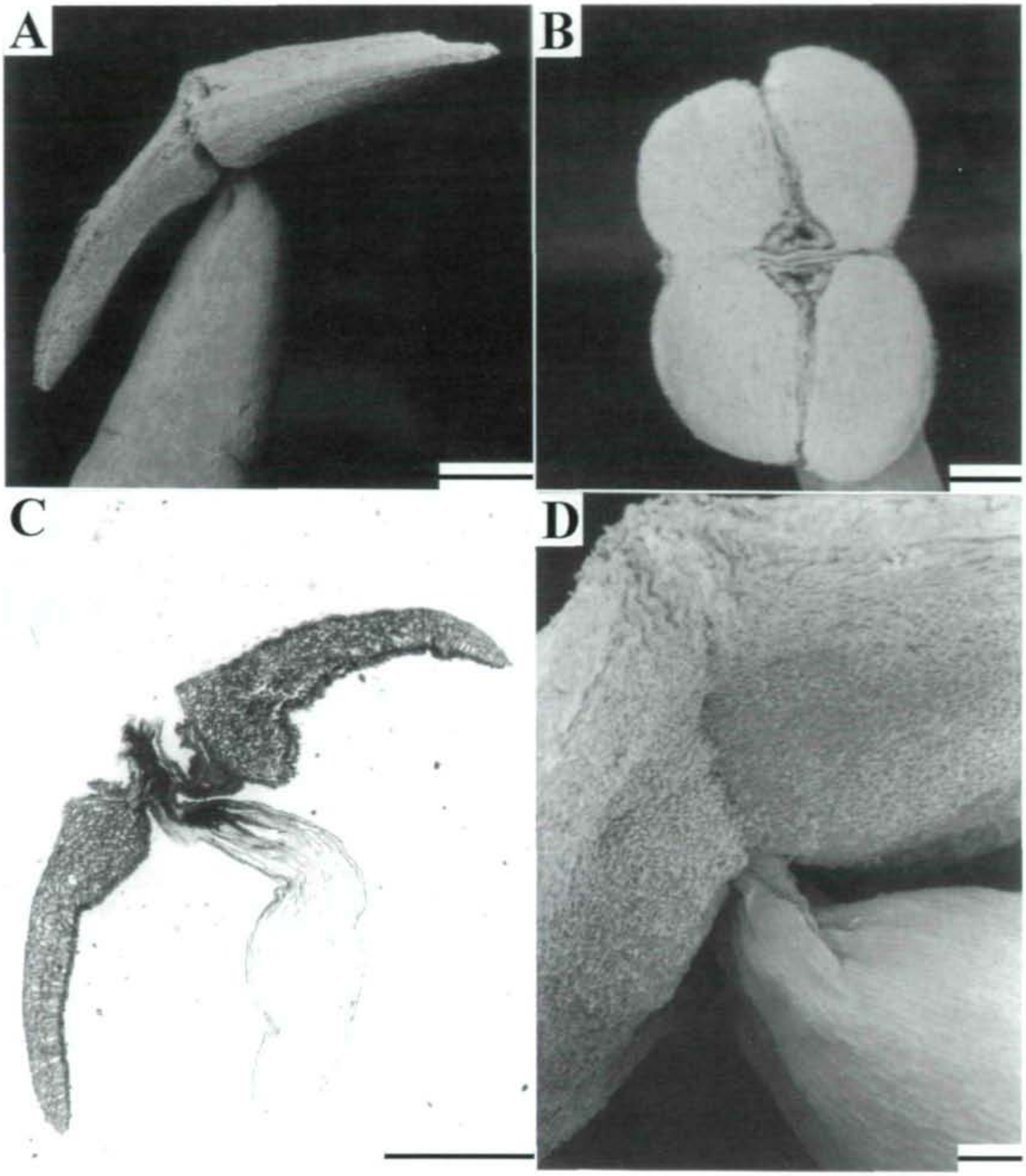


Fig. 29. Stamen of *Calceolaria uniflora*. **A**, dorsal view. **B**, dehiscent anther showing the collapsed connective tissue in the middle and rests of the septa retracted in each thecae. **C**, LM photograph of a longitudinal section, note the necrotic tissue at the apical end of the filament building the hinge. Scale bars: A & B: 500 µm, D: 100 µm, C: 1 mm.

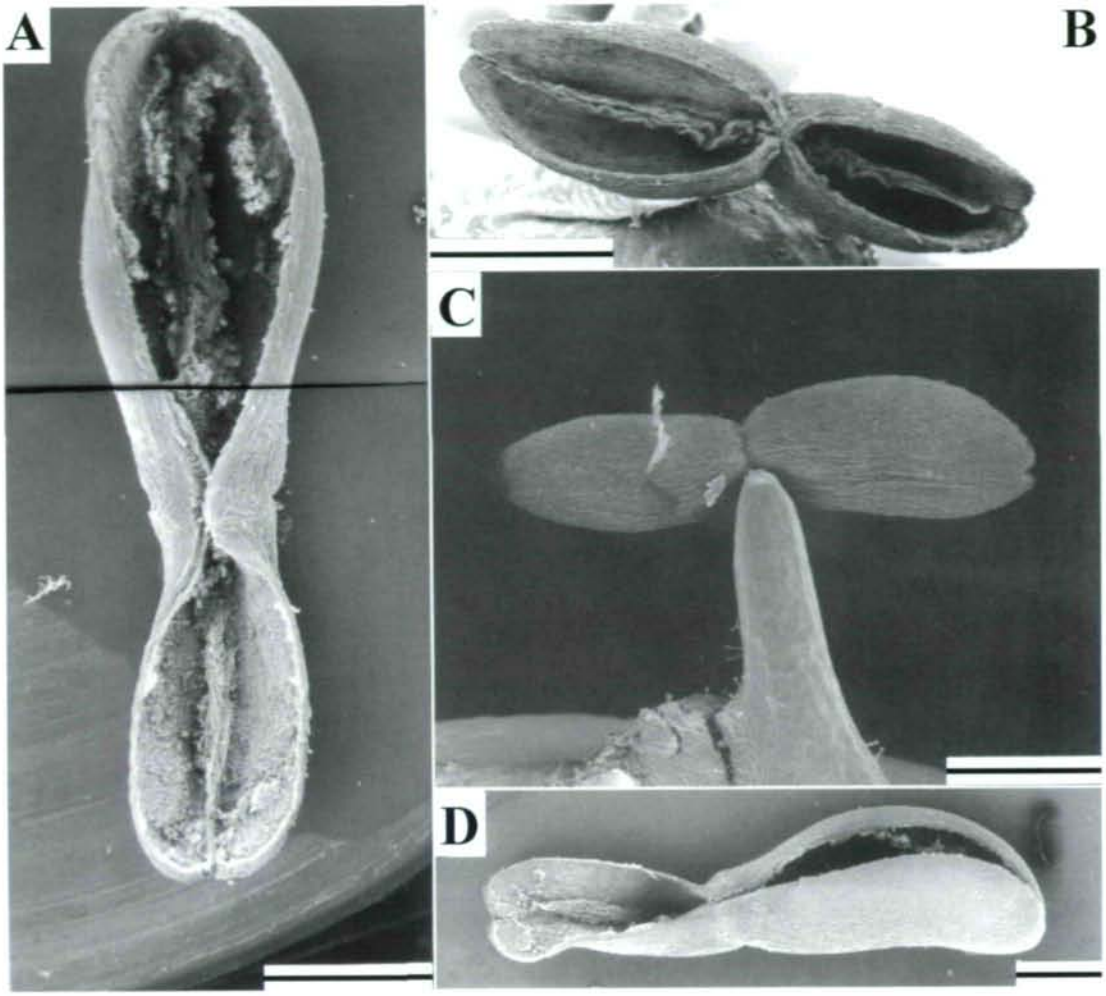


Fig. 30. Stamens with two dehiscence lines, connective not collapsed. **A** & **B**, *Calceolaria tetragona*. **C** & **D**, *C. paralia*. **C**, dorsal view showing the hinge. **D**, frontal view. Scale bars: 1 mm.

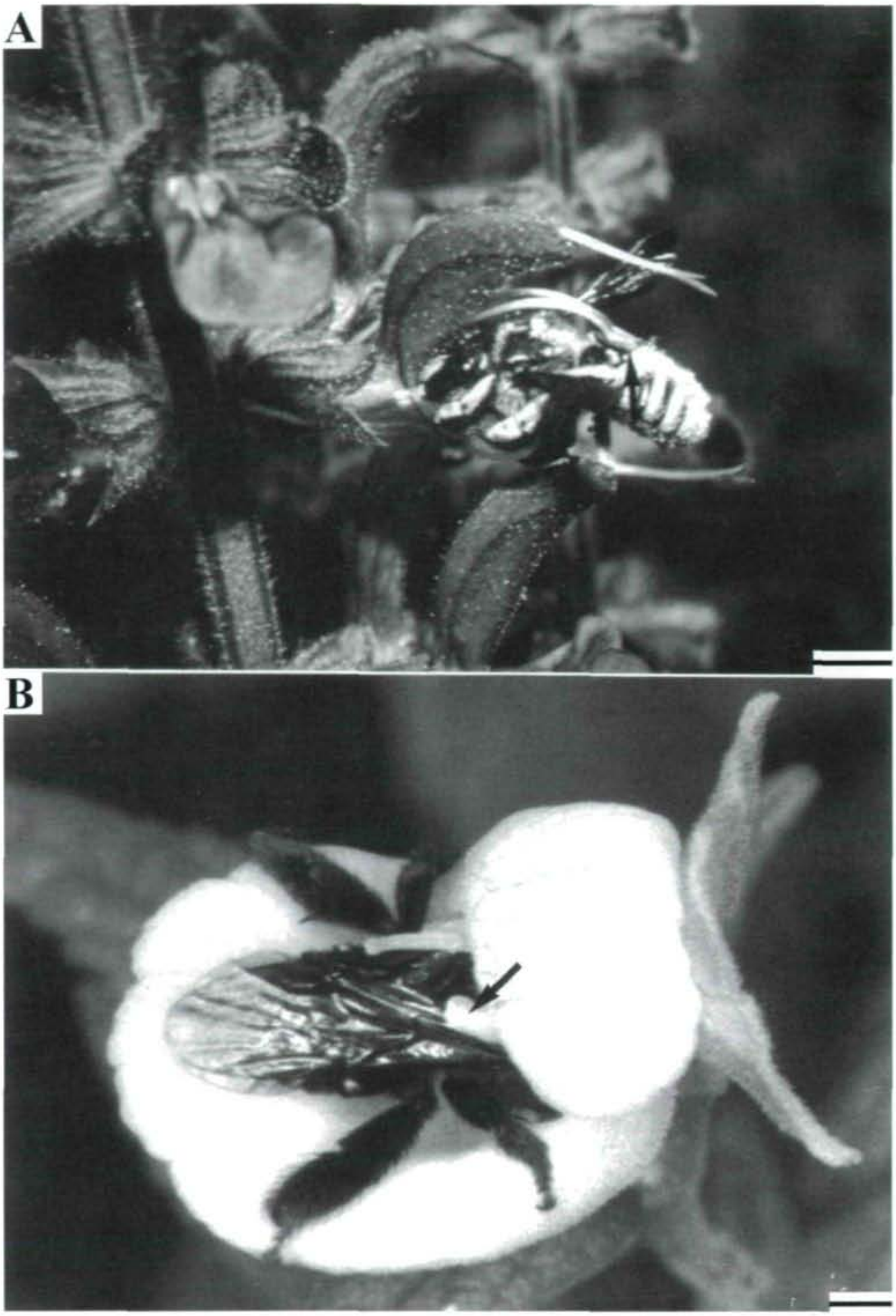


Fig. 31. Lever mechanism in *Salvia* and *Calceolaria*, note how the fertile thecae descend, being pushed by the insect and touching the dorsal part. **A**, *Salvia pratensis* L. visited by *Anthidium manicatum* (Linnaeus 1758). **B,** *Calceolaria polyclada* visited by *Chalepogenus vogeli*. Scale bars: A: 0.5 cm, B: 0.1 cm.

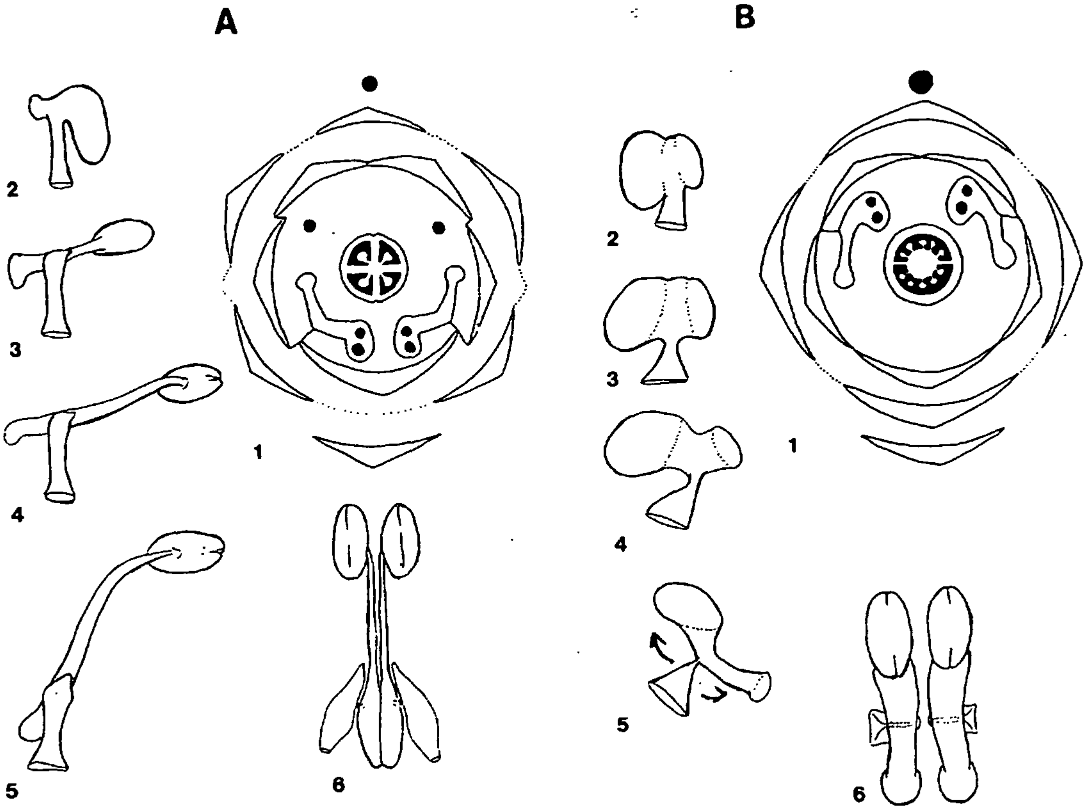


Fig. 32. **A_1** & **B_1**, flower diagrams of *Salvia* and *Calceolaria* respectively. Analogies of the lever mechanism between both genera. **$A_{2\text{-}5}$** & **$B_{2\text{-}5}$**, comparative development of the lever stamens. **A_6** & **B_6** frontal view of the pair of stamens to indicate the differences of the hinges in both genera.

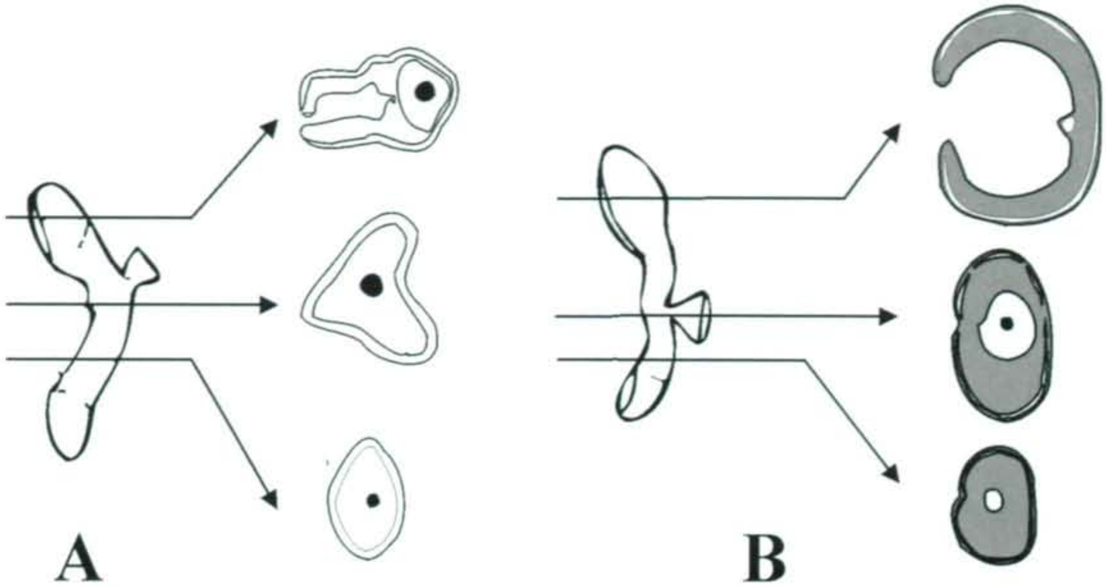


Fig. 33. Origin of the sterile tissue in lever stamens. **A**, connective origin (white area), **B**, anther origin, the endothecium is recognisable at every section level (hatched). The white outer layer represents the epidermis, while the dots are the conductive bundles.

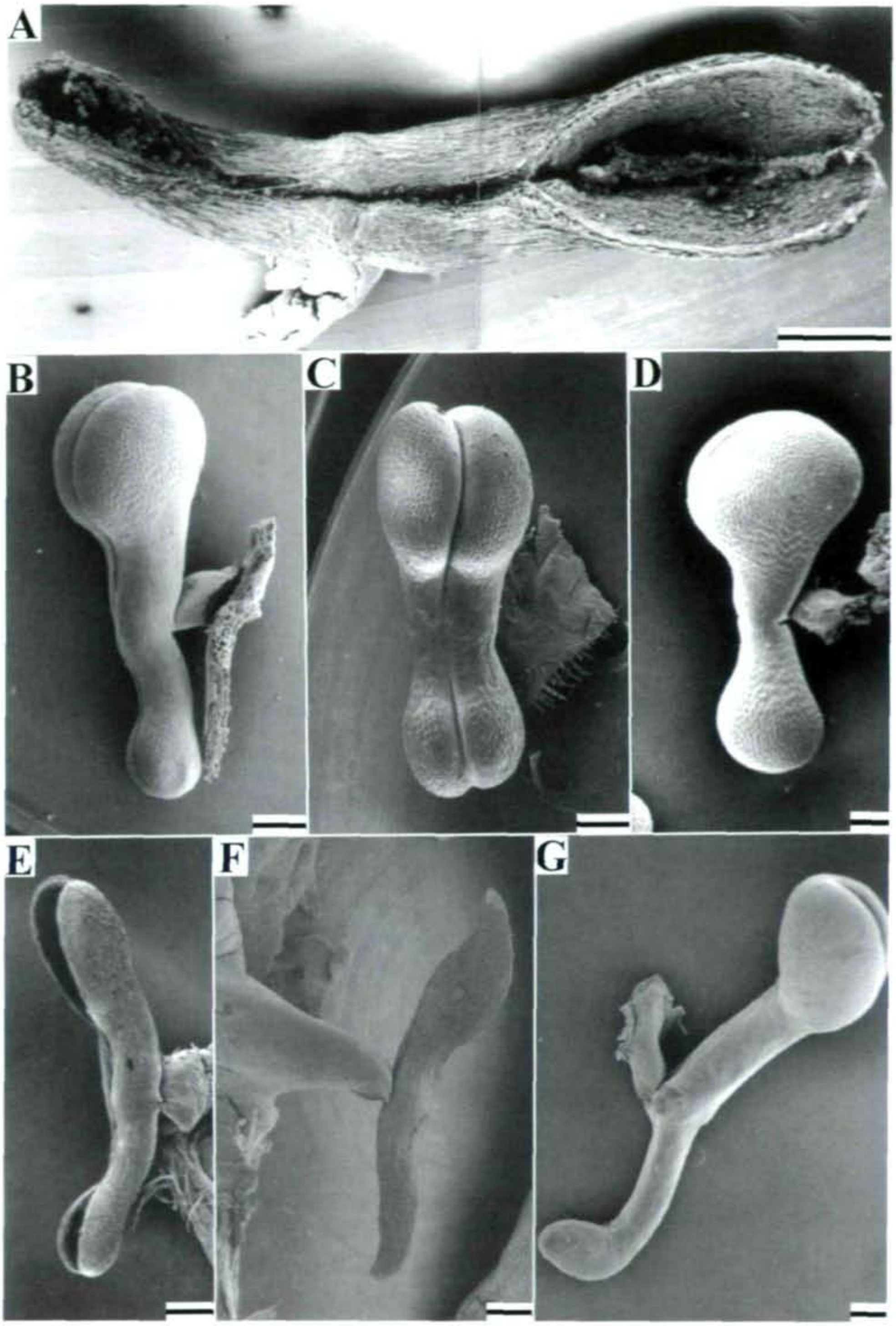


Fig. 34. Lever stamens. **A & B**, *Calceolaria ruiz-pavonii*, **A**, front view. Note the prolonged dehiscence line of each theca over the sterile tissue. **B**, dorsal view of a young indehiscent stamen. **C & D**, *C. lossenii* frontal and dorsal views, respectively. **E**, *C. teucrioides* in dorsal view. **F**, *C. santolinoides*. **G**, *C. polyclada*, sterile tissue derived from the connective. Scale bars: 400 µm.

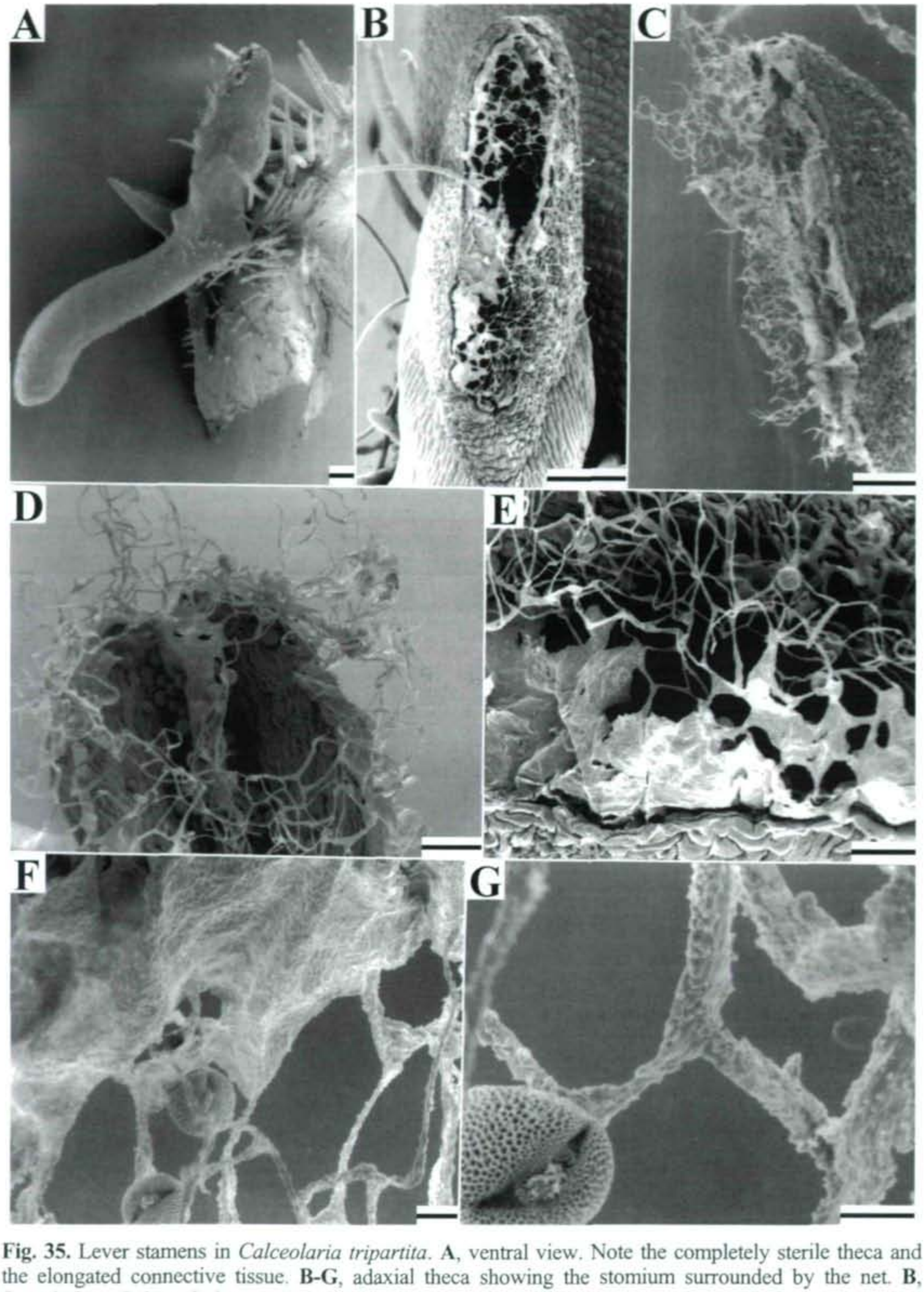


Fig. 35. Lever stamens in *Calceolaria tripartita*. **A**, ventral view. Note the completely sterile theca and the elongated connective tissue. **B-G**, adaxial theca showing the stomium surrounded by the net. **B**, frontal view. **C**, lateral view, note the contracted anther wall after dehiscence. **D**, detail of the net attached to the septum and edge of the stomium. **E**, detail of the net at the edge of the stomium. **F** & **G**, pollen grains attached to the net. Scale bars: A-C: 200 µm, D & E: 40 µm, E & G: 10 µm.

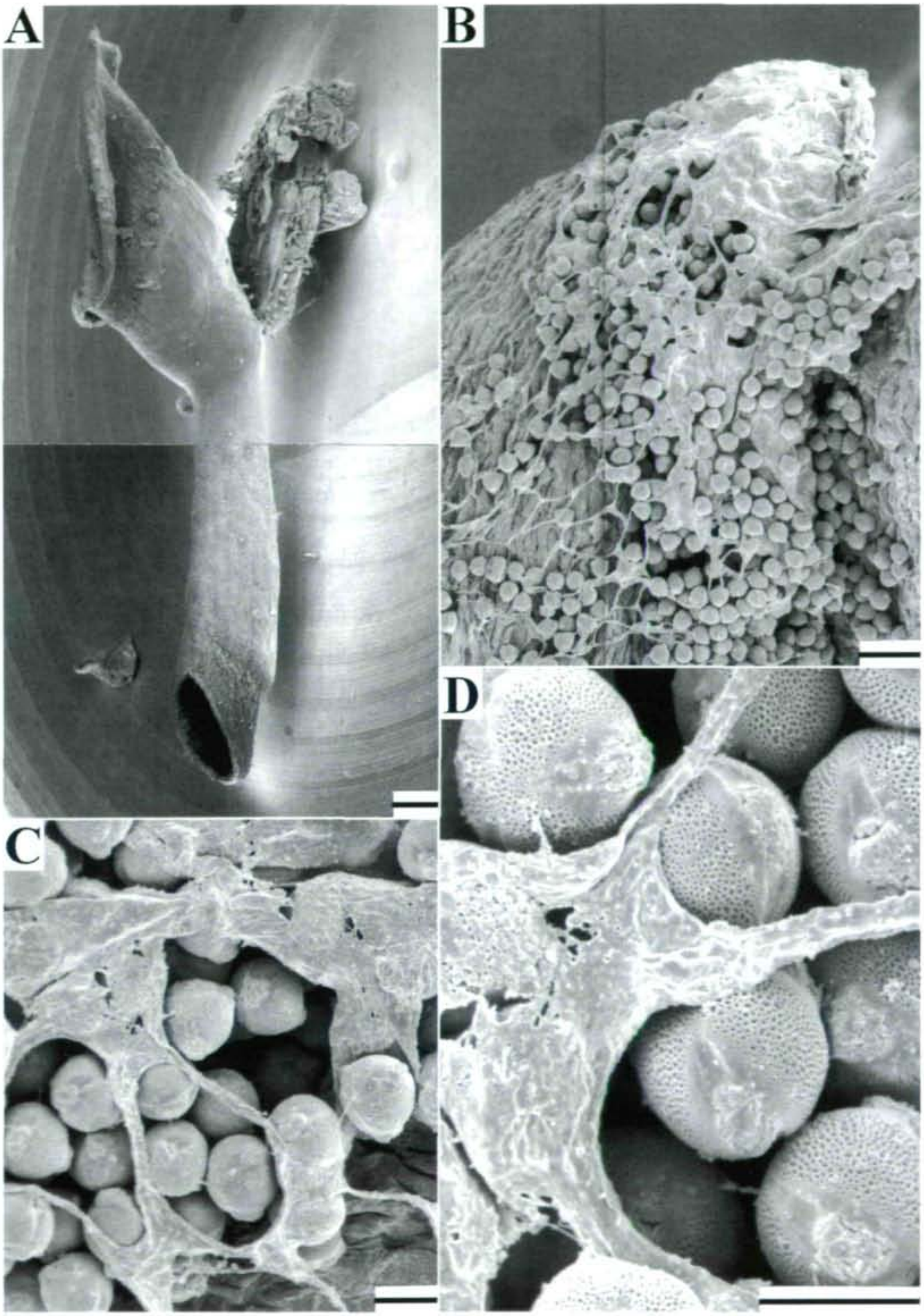


Fig. 36. Lever stamens in *Calceolaria rivularis*. **A**, general view, note the reduced but not sterile abaxial theca and the elongated connective. **B-D**, details of the adaxial theca showing the pollen grains attached to the net. Scale bars: A: 400 µm, B: 40 µm, C & D: 10 µm.

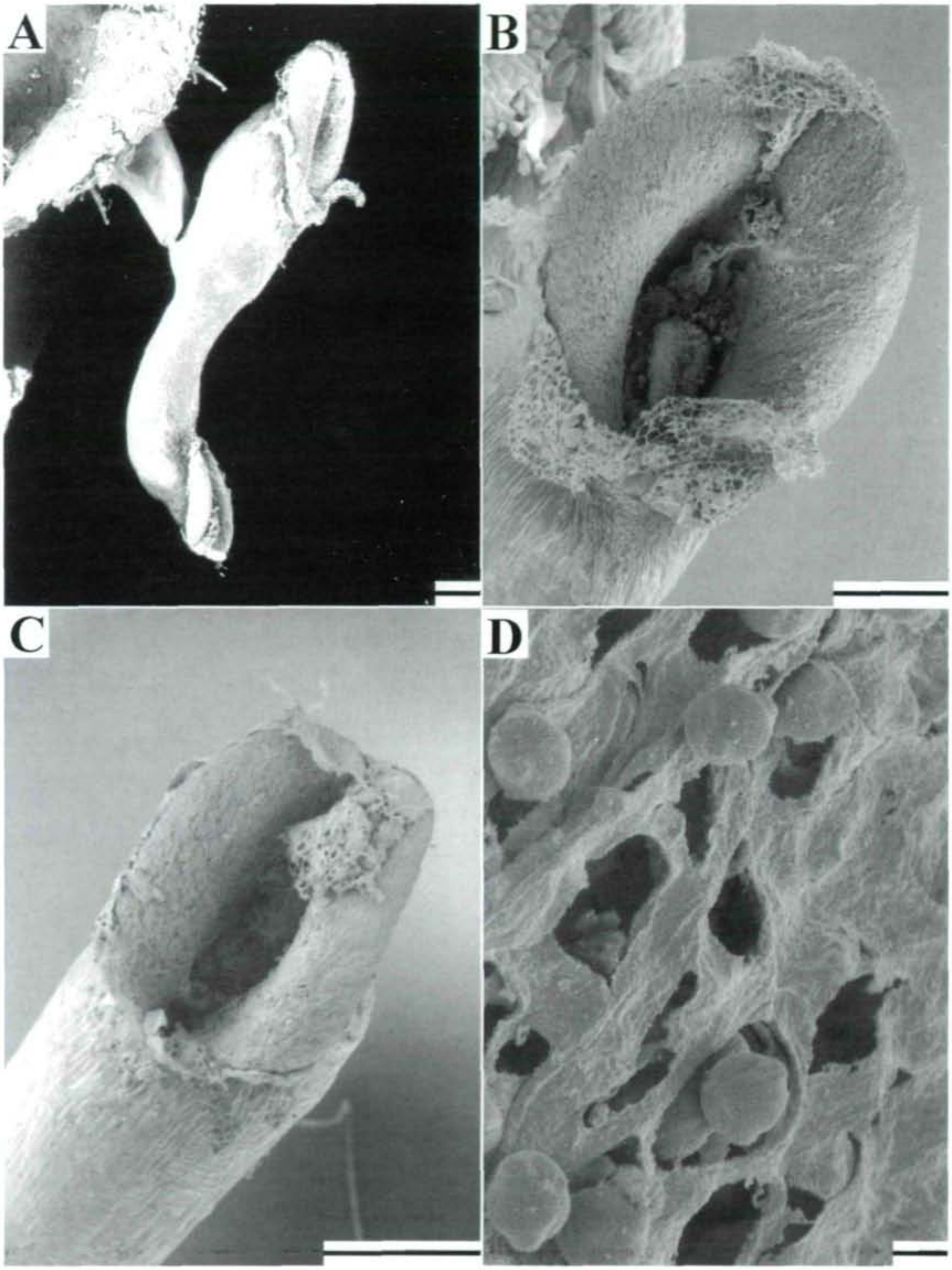


Fig. 37. Lever stamens in *Calceolaria chelidonoides*. **A**, general view. **B** & **C**, adaxial and abaxial thecae, respectively, showing the net attached to the stomium and the septum. **D**, detail of the net with attached pollen grains. Scale bars: A-C: 400 µm, D: 10 µm.

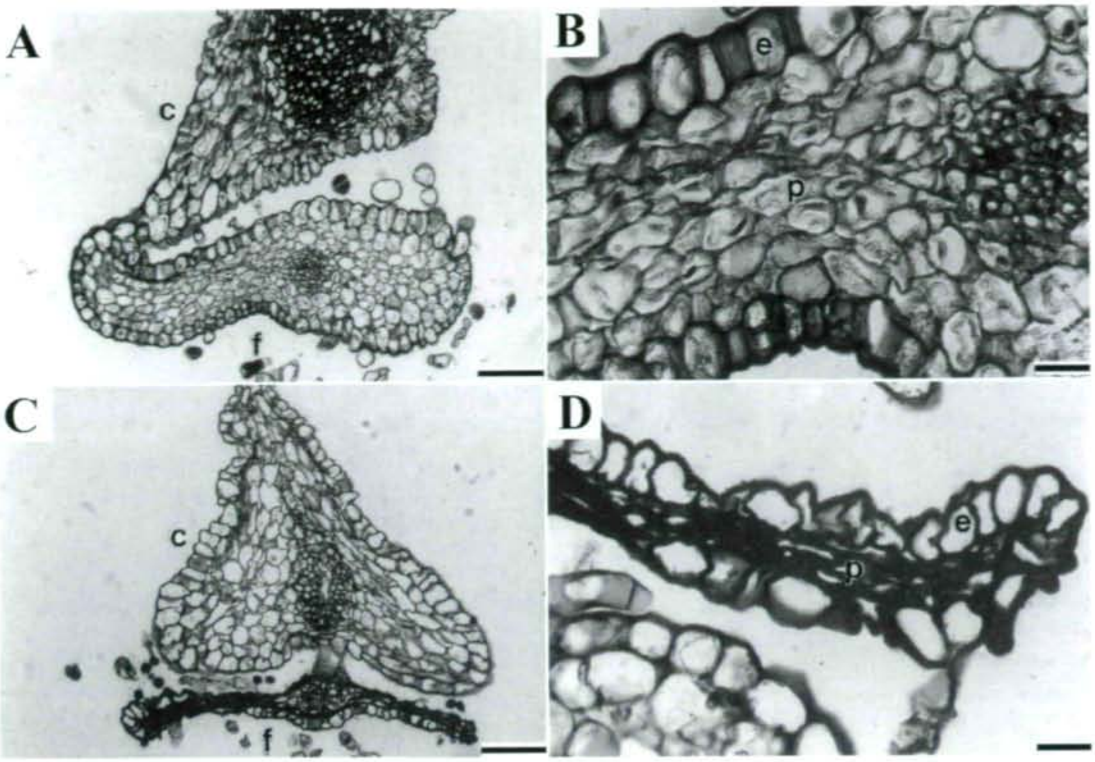


Fig. 38. Transection of the stamens of *Calceolaria tripartita* at the hinge level. **A**, young indehiscent stamen, hinge not differentiated. **B**, detail of A showing normal parenchymatic cells (p) under the epidermis (e). **C**, dehiscent stamen showing a well developed hinge. **D**, detail of C showing the necrotic parenchymatic cells. Scale bars: A & C: 100 μm, B & D: 20 μm.

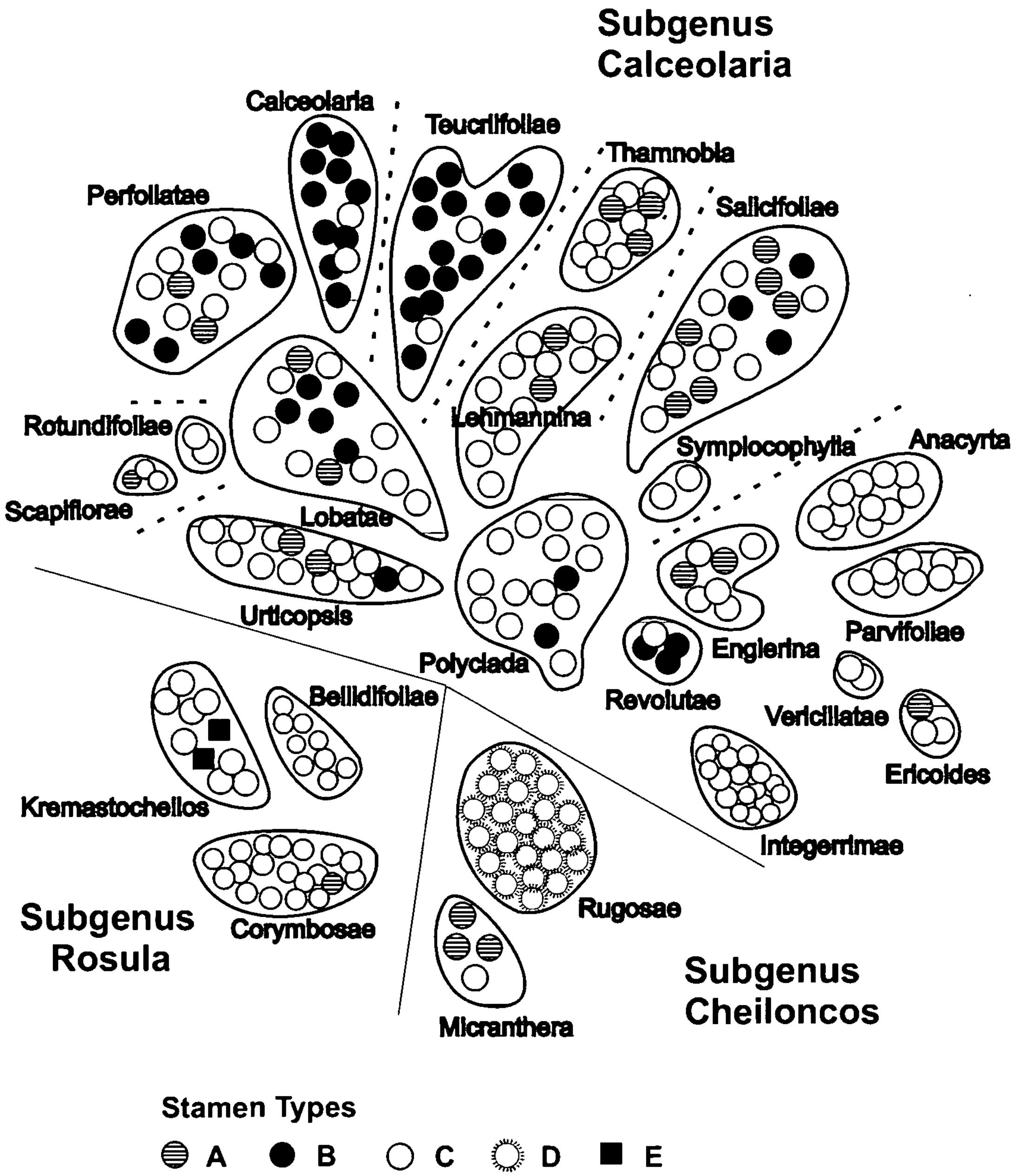


Fig. 39. Position of *Calceolaria* species with lever stamens in the phylogenetic tree. Each dot corresponds to one species.

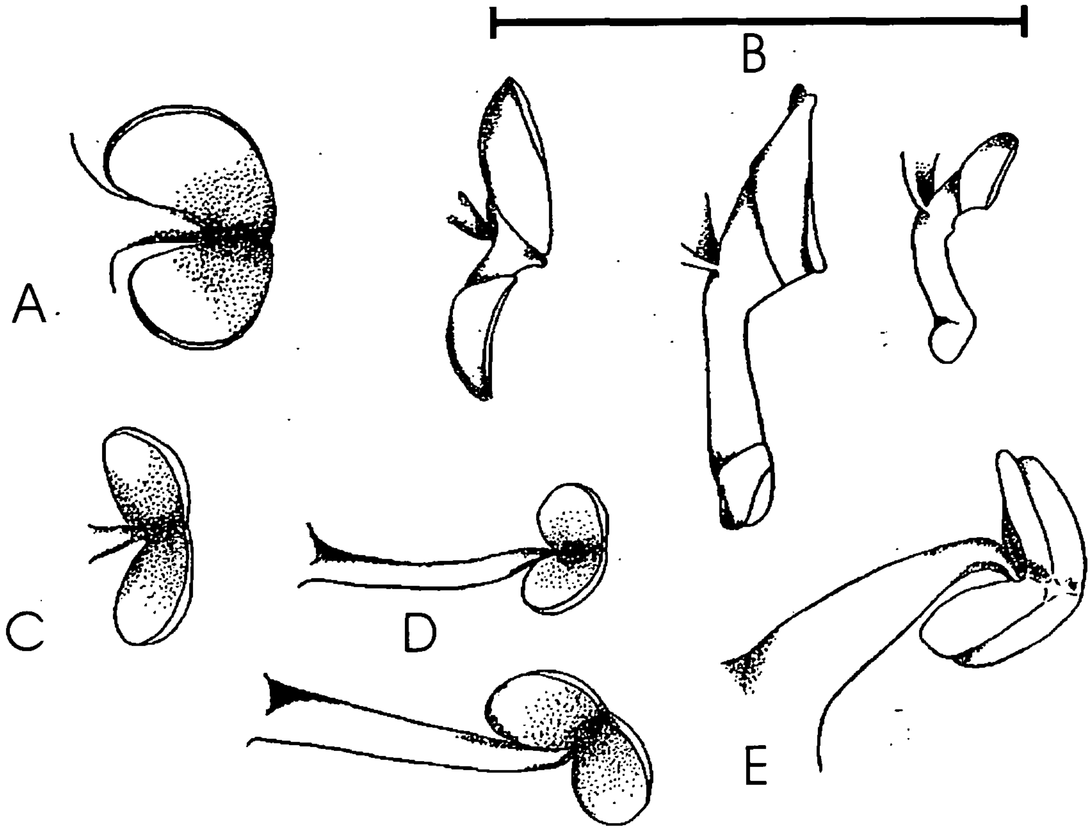


Fig. 40. Different types of stamens in the genus. **A,** *Jovellana*-type. **B**, different levels of lever stamens. **C**, basic type. **D**, elongated stamens. **E**, versatile stamen.

different degrees, fertile or sterile, depending on the species. The thecae of both stamens adhere to each other in a way that they build a structural unit so that both stamens move simultaneously dorso-ventrally due to the hinge which is a flexible and membranous cord which works by torsion.

Table 7. Average fruit production in four species of *Calceolaria* (three different treatments).

Treatment	*C. tripartita* % (n)	*C. latifolia* % (n)	*C. parviflora* % (n)	*C. polyclada* % (n)
Natural autogamy	36 (150)	39.18 (171)	0.00 (20)	0.00 (50)
Artificial autogamy	100 (50)	100 (50)	22.22 (20)	50.00 (50)
Artificial allogamy	100 (50)	100 (50)	55.55 (20)	93.99 (50)

The inferior thecae form a pushing surface, which is pressed by the visitors while introducing itself in the flower to take nectar. While pressing the lever, the upper thecae become exposed, which touch and deposit pollen on the dorsal part of the pollinator (notoribic pollination, Fig. 31A). *Salvia* is characterized by having proteranderous flowers. The long and curved style that runs between the stamens exceeds their length and receives the pollen when the flower is in the receptive stage.

Similar lever mechanisms are rare in Angiosperms. Besides *Salvia,* they were described by DELPINO (1873), LYNCH (1882) and TROLL (1929) for *Roscoea purpurea* SM. (Zingiberaceae), by TRAPP (1956) for *Pseudosopubia obtusifolia* ENGL. (Scrophulariaceae) and HILDEBRAND (1867) mentioned it briefly for *Calceolaria pinnata* L., while CORRENS (1891b) described the mechanism in more detail not only for *C. pinnata* but also for *C. tripartita*.

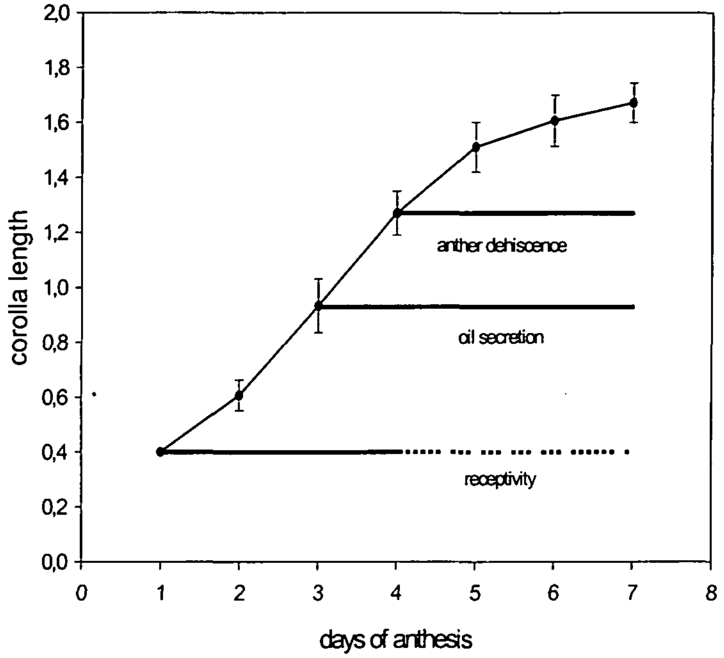
Gynoecium: The gynoecium has two united carpels, the ovary can be inferior or semi-inferior, the style runs between the stamens slightly surpassing them. The stigma is terminal, papillose and wet (group III of stigma sensu HESLOP-HARRISON & SHIVANNA 1977). In the material studied the receptivity of the stigma starts 1 to 4 days before the stamens dehisce, and its receptivity (it remains wet and tests positive with H_2O_2) continues until the end of anthesis.

Table 8. Percentage of seed germination in *C. tripartita* and *C. latifolia*.

Treatment	*C. tripartita* % (n = 100)	*C. latifolia* % (n = 100)
Spontaneous autogamy	96.7	85
Artificial autogamy	96.5	61
Artificial allogamy	94.4	88

Autogamy and allogamy (Tab. 7): These tests were performed with greenhouses plants cultivated at the Botanical Garden of Mainz where they did not receive visits either from native oil-collecting bees nor other insects. Four species, *C. parviflora, C. polyclada, C. tripartita* and *C. latifolia* were studied.

Natural autogamy: Whole inflorescences without manipulation were checked during anthesis. Table 7 shows that *C. parviflora* and *C. polyclada* formed no fruits, while *C. tripartita* and *C. latifolia* had low levels of fruit set.



Graphic 5. Anthesis in *Calceolaria tripartita*. Receptivity of the stigma, strong reaction to H_2O_2 (solid line), slight reaction to H_2O_2 (dotted line); oil production period, and anther dehiscence period.

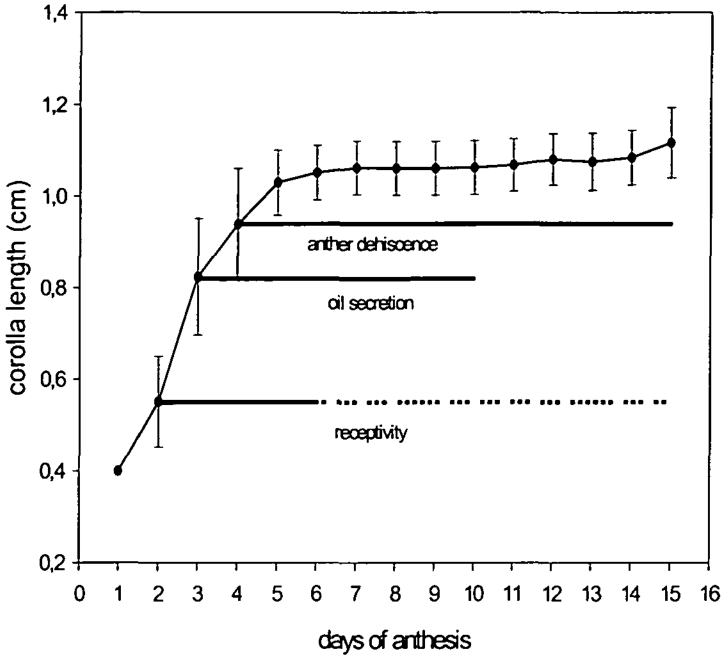
Artificial autogamy: All manual self-pollinated flowers of *C. tripartita* and *C. latifolia* set fruits, while the fruit set of the other two species was considerably lower (Tab. 7).

The results of these tests showed that *C. tripartita* and *C. latifolia* are selfcompatible but have a low level of autonomous self-pollination since under certain circumstances, the contact of pollen and stigma can take place. *C. parviflora* and *C. polyclada* have a low level of selfcompatibility but autonomous self-pollination is precluded by hercogamic mechanisms.

Artificial allogamy: Pollen of the same species but from different plants was used to pollinate artificially the flowers of the four species. Average fruit set was high for *C.*

tripartita, *C. latifolia*, and *C. polyclada*, while only 55% of the flowers of *C. parviflora* formed fruits (Tab. 7).

Seed viability: Germination of seeds obtained from different treatments was performed for *C. tripartita* and *C. latifolia* (Tab. 8). In all cases average germination was high.



Graphic 6. Anthesis in *Calceolaria latifolia*. Receptivity of the stigma, strong reaction to H_2O_2 (solid line), slight reaction to H_2O_2 (dotted line); oil production period, and anther dehiscence period.

Anthesis in Calceolaria tripartita (Graphic 5): Flowers cultivated in the Botanical Garden of Mainz were followed during whole anthesis. As in this species both lips are in contact during the life of the flower, changes in the corolla were followed before anthesis begins, arbitrarily starting from a corolla length of 0.4 cm. The development of the flower follows a logarithmic pattern, it begins to grow very fast and stabilizes for about 2 days after the corolla length reaches 1.7 cm. During the control period stigma receptivity, oil production and stamen dehiscence were followed. The beginning of the stigma receptivity was considered as the beginning of anthesis (day 1). The average duration of development until anthesis ends is 8 days. The stigma matures prior to maturation of anthers, and maintains its receptivity (strong reaction to hydrogen peroxide) for the first 4 days of anthesis. Oil production starts on day 3, while the anthers start dehiscence one day later, that means that the period of stigma receptivity overlaps partially with oil production (1 day).

Anthesis in Calceolaria latifolia (Graphic 6): As above, the control period of the flowers started with a corolla length of 0.4 cm. At this point the elaiophore does not produce oils, the appendage is not folded to the inside of the saccate lower lip and the stigma is not receptive. The mean length of the corolla reaches 1.06 cm; at this point the upper lip covers about 76% of the lower lip. The receptivity of the stigma starts very early, when the flower is about 0.6 cm long and the anthers are still indehiscent. The hydrogen peroxide test reveals a strong receptivity until day 6, and a weaker receptivity during the last days of anthesis. A small quantity of oil is detectable on day 3, and the maximum of secretion occurs between days 6 and 9 reaching a production of 0.783 mg per flower. The dehiscence of the stamens starts around day 4. The anthesis period lasts about 14 days. The lower lip of *Calceolaria latifolia* has a depression on the instep in which the fertile parts are housed. If pollen is not removed during the last days of anthesis it falls down onto this depression. The style, which normally runs parallel to the stamens, bends to the bottom, comes into contact with the pollen masses, and favors spontaneous self-pollination.

Visitors of *Calceolaria*

Genus Centris: The genus *Centris*, together with *Epicharis,* build the tribe Centridini (Hymenoptera: Apidae, ROIG-ALSINA & MICHENER 1993). *Centris* is mainly neotropical with two important diversity centers, one in Central America and the other in Brazil. Texas, in the United States (SNELLING 1984), represents the northern distribution range of the genus, while the most southern locality is given by HOLMBERG (1903) when describing the visits of *Centris cineraria* on the flowers of *Lathyrus* L. in the Province of Santa Cruz (Argentina).

The northernmost range of *Centris* that visits *Calceolaria* was published by MOLAU (1988) between Tarma and Huaricolca in northern Peru, where *Calceolaria cuneiformis* R.et P. was pollinated by *Centris autrani.* The southern range was recorded for this work in El Bolsón (Argentina) at 42° S where *Centris* cfr. *autrani* visited *Calceolaria prichardii.*

The c. 200 species of *Centris* are distributed into 12 subgenera (MICHENER 1951); only in two of them there are species known, which visit *Calceolaria.*

Subgenus Wagenknechtia:	*Centris cineraria* SMITH *Centris orellanai* RUIZ
Subgenus Paracentris:	*Centris autrani* VACHAL. *Centris nigerrima* SPINOLA *Centris tricolor* FRIESE *Centris* aff. *tricolor* nov. sp.

Only three of these species where morphologically studied in this work: *Centris autrani, Centris nigerrima,* and *Centris tricolor.* VOGEL (1974) and NEFF & SIMPSON (1981) already studied different aspects of morphology and function of the oil-collecting structures of *C. autrani*, *C. cineraria* and *C. tricolor.*

Oil collectors[5]*:* Most of the females of the genus are equipped with special structures to collect floral oils. Depending on the position of these structures it is possible to distinguish two different kinds of bees:

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[5] The terminology used for the topographic localizations follows NEFF & SIMPSON (1981)

- Four-legged bees: these have oil-collectors on the fore- and the midlegs (Fig. 41B). This kind of bees was first described as "Vierbeinsammler" by VOGEL (1974).
- Two-legged bees (Zweibeinsammler): These bees have oil collectors only on the forelegs, while the collectors of the midlegs are vestigial or completely absent.

Centris tricolor is a four-legged bee (Fig. 42A-F), while *C. autrani* (Fig. 44A-F) is a two legged bee. *C. nigerrima* is a two-legged bee, although the midlegs show a poorly developed and probably non-functional comb (Fig. 43A-D).

Table 9. Position of oil-collectors of bee species studied.

Species	Foreleg		Midleg
	Anterior and posterior combs	"Tarsal pad"	Comb
C. tricolor	+	+	+
C. nigerrima	+	-	reduced
C. autrani	+	-	almost absent

Collector of the foreleg: The oil-collector is located on the ventral surface of the basitarsus; it consists of 2 combs (Tab. 9 and 10), one on the posterior leg side and the other on the anterior leg side. The posterior comb is short and formed by 3-5 strong, spathulate and imbricate giant setae. The primary anterior comb is longer than the posterior one, built by a variable number of thinner, spathulate and imbricate setae. The setae of both combs are curved to the middle line of the basitarsus in a way that they delimit a spoon-like concavity. This structure was first named by VOGEL (1974) as "Kapuze" and by NEFF & SIMPSON (1981) as "hood organ" or "elaiospathe".

As it is interpreted from its form, this hood organ is used by the bee to take the oils from the elaiophore with sweeping movements. The posterior comb sweeps the oils to the inside of the concavity, while the anterior one functions as a barrier to prevent the loss of the oils.

This structure shows some variations among the studied species (Figs. 42A, B; 46A, B; 47A, B).

Modifications of the oil-collector

- On the postero-ventral surface of the forebasitarsus of *C. tricolor* there is a cushion of dense and branched hairs (Fig. 42C, D) called "tarsal pad". This pad was already recognized in *C. brethesii* SCHROTTKY as an additional oil absorbing structure (SIMPSON & NEFF 1990). Branched hairs like those ones are combined with simple hairs in the forebasitarsus of *C. autrani*, but they do not build a cushion. *Centris nigerrima* completely lacks a tarsal pad.
- In *Centris nigerrima* the proximal and ventral fringe of each foremeditarsus shows smaller combs formed by spathulate and inwards curved setae (Fig. 43A).

Collector of the midleg: The presence of an oil-collector on the midleg is variable (Tab. 9, Tab. 10). In the four-legged bees the oil-collector is well developed and located on the ventral surface of the basitarsus. It crosses the segment from the basal posterior edge to the apical anterior edge. The setae of this comb are also spathulate, imbricate and apically curved to the middle line of the tarsus. *Centris tricolor* shows a comb built by 22, well developed setae. On the dorsal surface of the midbasitarsus there are some branched hairs with simple, stout setae (Fig. 42E, F). The midbasitarsus comb of *C. nigerrima* is reduced to simple, straight, non-spathulate setae (Fig. 43C, D). In *C. autrani* this comb is quite rudimentary formed by a row of 11 thin, distant setae (Fig. 44C-E). In the last two species the associated branched hairs are very rare.

Table 10. Number of setae and length of comb of fore- and midlegs of three species.

	Foreleg				Midleg		
	Anterior comb		Posterior comb		Antero-posterior comb		
	No. of setae	Length (mm)	No. of setae	Length. (mm)	No. of setae	Length (mm)	Spathulate
C. tricolor	22	0.29	3	0.45	22	0.24	+
C. nigerrima	20	0.28	3	0.44	23	0.27	-
C. autrani	10	0.22	3	0.59	10	0.13	-

Behavior of the bees on the flowers: The bees fly very rapidly, following fixed routes and covering large areas. They usually perform short visits to almost all flowers of the inflorescence following certain sequence, in a trap-lining fashion. It could be seen that they alternatively visit many *Calceolaria* species that grow together in the area, but they do not visit other oil flowers such as species of *Nierembergia* or *Sisyrinchium,* which may coexist with them. Their flights follow always the same directions, their routes are long and they need 20 to 30 minutes to complete the journey. *Centris* species use only the forelegs to collect oils on flowers of *Calceolaria.* The oils are passed during the flight from the front legs to the hindlegs with the mediation of the midlegs. Once landed on the flowers, the midlegs may be used to clutch the flower for a firm hold.

Genus Chalepogenus: The genus *Chalepogenus* belongs to the tribe Tapinotaspidini of family Apidae. It is distributed from southern Brazil to the temperate regions of Argentina and Chile (MICHENER & MOURE 1957; ROIG-ALSINA 1999). The most northern observations of visits of *Chalepogenus* on *Calceolaria* were made by MOLAU (pers. comm.) on *Calceolaria rosmarinifolia* LAM, southern Ecuador (Province of Azuay). He also collected *Chalepogenus rasmusseni* on *Calceolaria tripartita* in northern Peru. The most southern observations of this relationship (this work) were made in Argentina at 43° S, where *Chalepogenus caeruleus* visited *Calceolaria filicaulis*. Possibly, this range can be extended to 49° southern latitude where I could see the corollas of *C. prichardii* having evident signs of visitation by this particular bee.

There are 21 species in the genus, seven of which are involved in the pollination of some species of *Calceolaria* (COCUCCI & al. 2000): *Chalepogenus caeruleus* (FRIESE 1906), *Ch. perimelaena* (COCKERELL 1916), *Ch. rozeni* (ROIG-ALSINA 1999), *Ch. vogeli* (ROIG-ALSINA 1999), *Ch. rufipes* (ROIG-ALSINA 1999), *Ch. calceolariae* (ROIG-ALSINA 1999), *Ch. rasmusseni* (ROIG-ALSINA 1999). Four of these bees were studied in this work: *Chalepogenus caeruleus, Ch. vogeli, Ch. rufipes* and *Ch. rasmusseni.* These bees are relatively small compared with *Centris* bees. On average, they are c. 8 mm long.

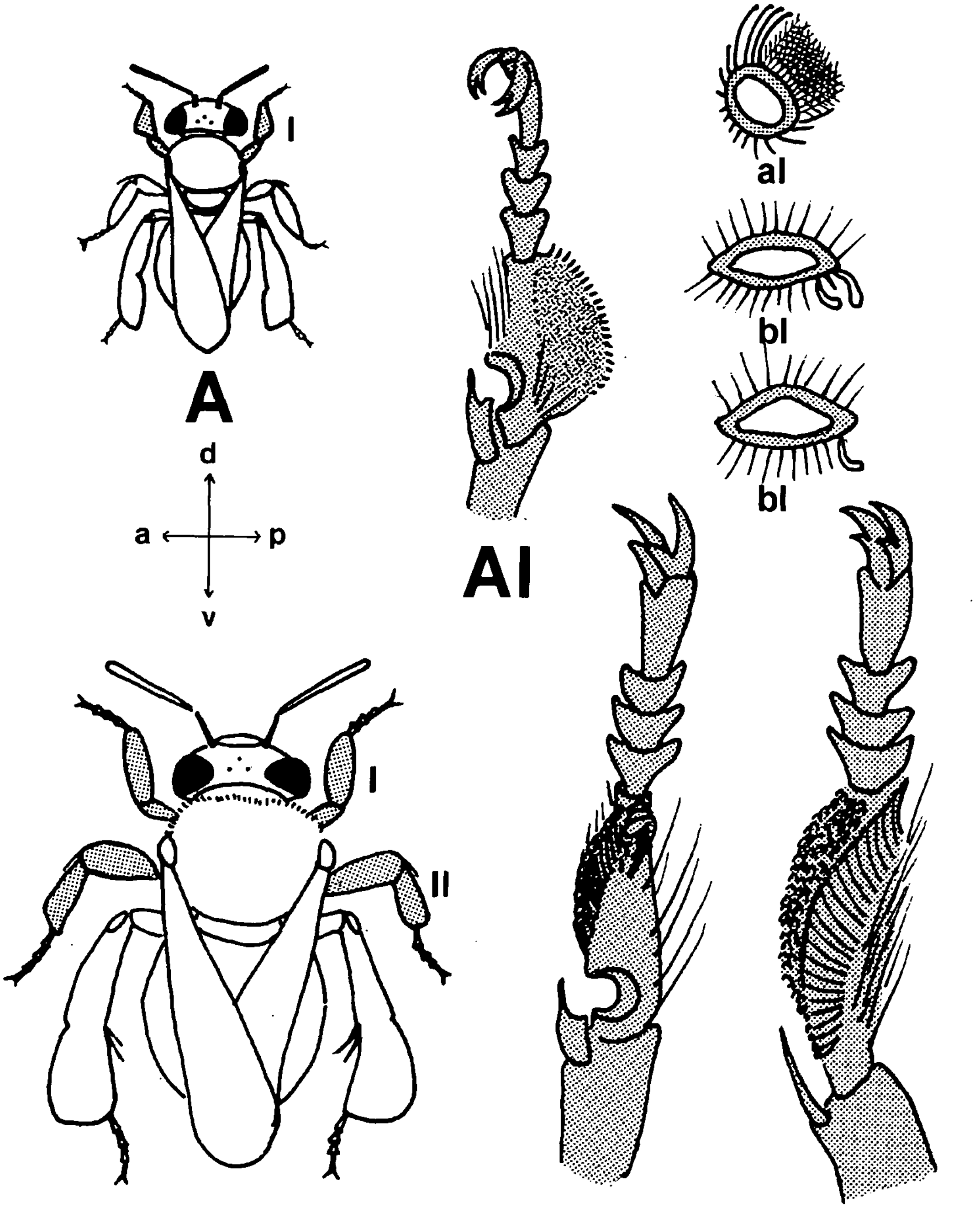

Fig. 41. Position of the oil collectors in *Chalepogenus* and *Centris*. A, *Chalepogenus*, **I** indicates the leg pair with collectors. **AI**, ventral view of a foreleg showing the main position of the collector on the basitarsus; **aI**, transection of the basitarsus. **B**, *Centris*, **I** & **II** indicate the leg pairs with collectors. **BI** & **BII**, Ventral view of the oil-collecting combs of the fore- and midlegs respectively; **bI** & **bII**, transections of the of the fore- and midbasitarsus respectively, showing the location of the combs. Abbreviations: a, anterior, p, posterior, d, dorsal, v, ventral.

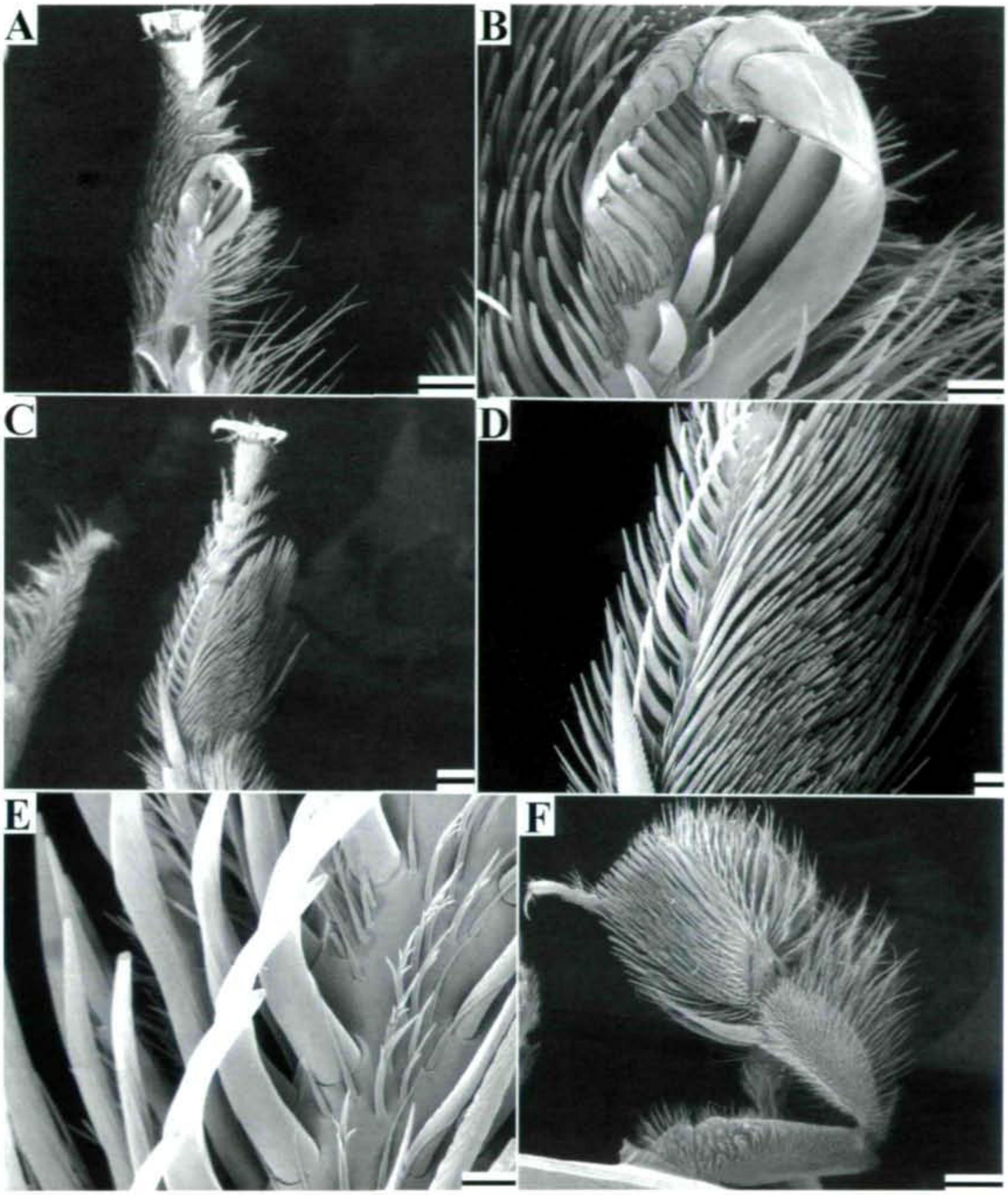

Fig. 42. Oil collecting organs of *Centris tricolor*. **A** & **B**, ventral view of the left foreleg. **A**, general view of the oil collector. **B**, detail of the anterior and posterior combs (elaiospathe). **C** & **D**, left foreleg in lateral (posterior) view, note the tarsal pad behind the elaiospathe. **E** & **F**, left midleg in ventral view, note the well developed comb. Scale bars: B, D & F: 100 µm, A, C & E: 400 µm.

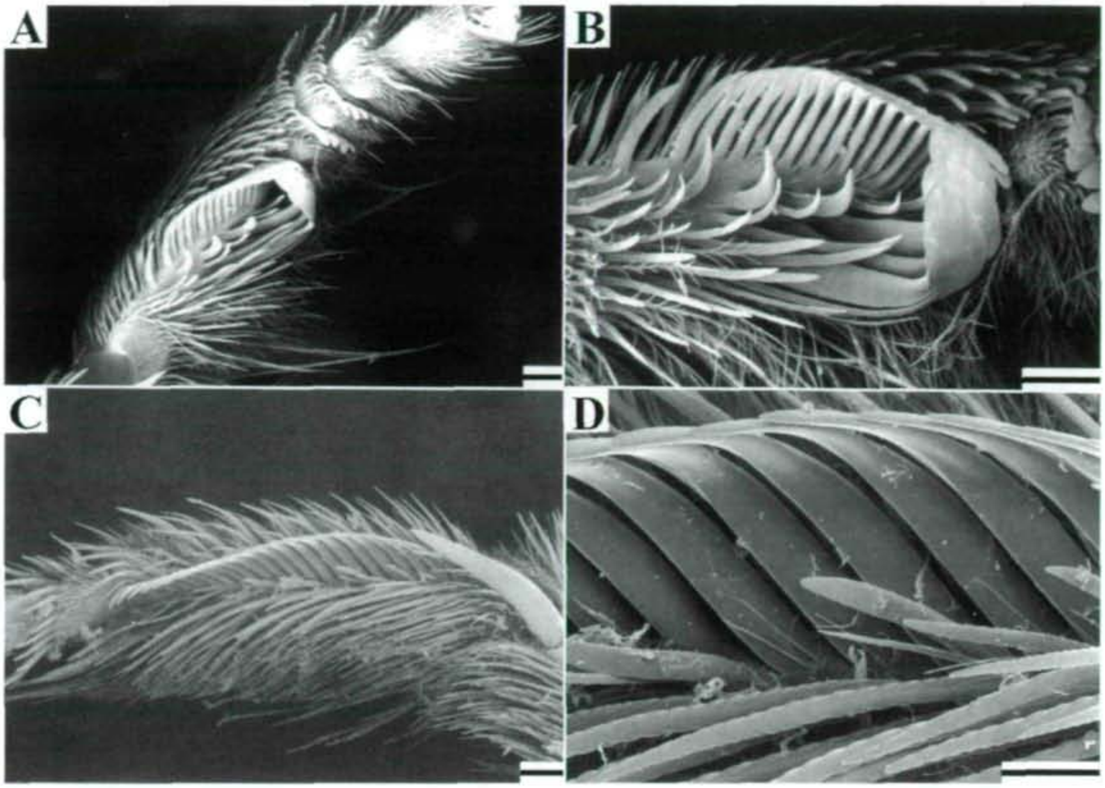

Fig. 43. Oil collectors of *Centris nigerrima*. **A** & **B**, ventral views of the left foreleg. **A**, general view of the basitarsus, the elaiospathe, and rudimentary combs on the meditarsus. **B**, detail of the anterior and posterior combs of the elaiospathe. **C** & **D**, ventral view of the midleg basitarsus showing a slightly reduced comb along the anterior edge. Scale bars: A-C: 200 µm, D: 100 µm.

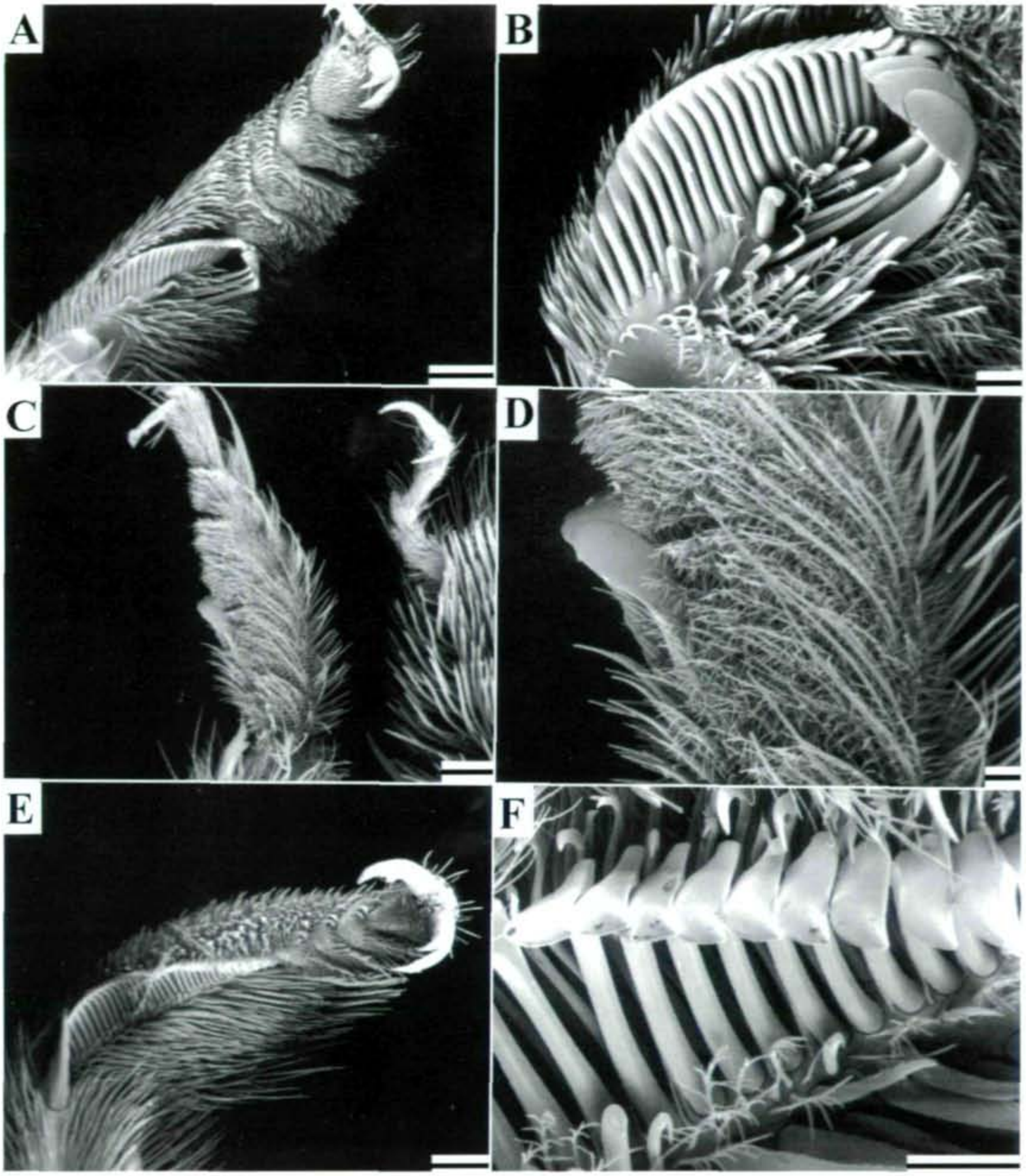

Fig. 44. Oil collectors of *Centris autrani*. **A** & **B**, ventral view of the left foreleg. **A**, general view. **B** detail of the elaiospathe. **C**-**E**, ventral view of the left midleg. **C**, general view showing a rudimentary comb on the anterior edge. **D** & **E**, detail of the setae of the comb. **F**, general view of the left hindleg, showing the scopae extended on the basitarsus and the tibia. Scale bars: A & C: 400 µm, B & D: 100 µm, E: 40 µm, F: 1 mm.

Oil collectors: *Chalepogenus* bees show oil-collectors only on the forelegs (Fig. 41A). The collectors are well described in COCUCCI & al. (2000). It consists basically of a well defined pad of finely branched hairs, located mainly at the posterior surface of the forebasitarsus. This general pattern may vary depending on the bee species (COCUCCI & al. 2000). In all cases studied here, the pad is dense and well developed, it is restricted to the basitarsus in *Ch. rufipes* and *C. vogeli* (Fig. 45A, B, D), but it is extended to the tibia and meditarsus in *Ch. caeruleus* and only to the meditarsus in *Ch. rasmusseni* (Fig. 45C).

In *Chalepogenus caeruleus* the absorbing surface is not bulging and is fringed at the dorsal margin by one or few poorly differentiated rows of stout unbranched setae (compound comb). This comb is completely absent in other species studied here.

While the hood organ of *Centris* was adapted to perform sweeping movements, this pads of *Chalepogenus* are better adapted to collect by tap movements ("Abtüpfen", VOGEL 1974) on the elaiophore, absorbing the oils by capillary action.

Behavior of the bees on the flowers: The behavior of *Chalepogenus* bees is quite different from *Centris* bees. They do not usually cover extended areas during their flights, and their routes are not fixed. They limit the visits to small plant populations and they usually visit all flowers they can find returning many times to the flowers already visited in the same spot. The approaching flight to individual flowers is vague and not precise. This behaviorial pattern is especially notable in *Ch. rufipes* and *Ch. vogeli*, while the bees of *Ch. caeruleus* perform their flights more rapidly and more precise. These bees are apparently not able to clutch the corolla with the legs. Intervals of oil collection on the flower are interrupted by movements of oil transference to the hindlegs without leaving the flower.

Nidification: Two nests of *Ch. caeruleus* were found near a dense population of *Calceolaria prichardii* in El Bolsón (Río Negro, Argentina). Only one of them could be successfully excavated; the soil was formed by a powdery, mostly inorganic soil, mixed with clasts of about 10 cm in diameter; the uppermost layers were dry and consequently the soil structure was very loose, but the deeper layers were humid and more compact. Following the entrance, which was protected under a rock, there was a small chamber, 1.5 cm long, which continued with a gallery 0.46 cm in diameter for about 11.5 cm. The gallery built an angle of 45° with the soil surface, while the vertical depth was 7.5 cm (Fig. 46). At the end of the gallery there were two egg-shaped cells c. 1.2 cm long and 0.68 cm wide. They were located one over the other[6]. The deepest one was already closed and provisioned with a small mass c. 0.48 cm in diameter made up of pollen and oil mixture; at the top of this provision there was a white and elongated egg of about 4 mm long. The 1 mm thick cell walls were smooth, shiny inside and almost black, presumably built by a mixture of mud and oils. The upper cell was not finished and was occupied by the female during the night. During the excavation, which was performed before sunrise, the bee did not abandon the nest, trying to hide under the earth and permanently emitting a characteristic buzz. The bees normally make this buzz while searching for the entrance, which was useful to localize the nests. VOGEL (1974) observed, as I also did, that the bees made this buzz ("Tüten"), not only during the nest building, but also during flower visitations.

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[6] JANVIER (1926) describes the nest of the same bee species being formed by a helicoidal gallery towards which 2-3 series of 3-6 cells are arranged linearly. This type of nest is called "parodal" (STEPHEN & al. 1969) The nest I found probably corresponds to early stages of construction of this kind of nests, where some series of cells were not yet built. VOGEL (1974) instead describes the nest of *Ch. caeruleus* as "heterodal" which is formed by a main gallery towards which 4-6 individual cells open.

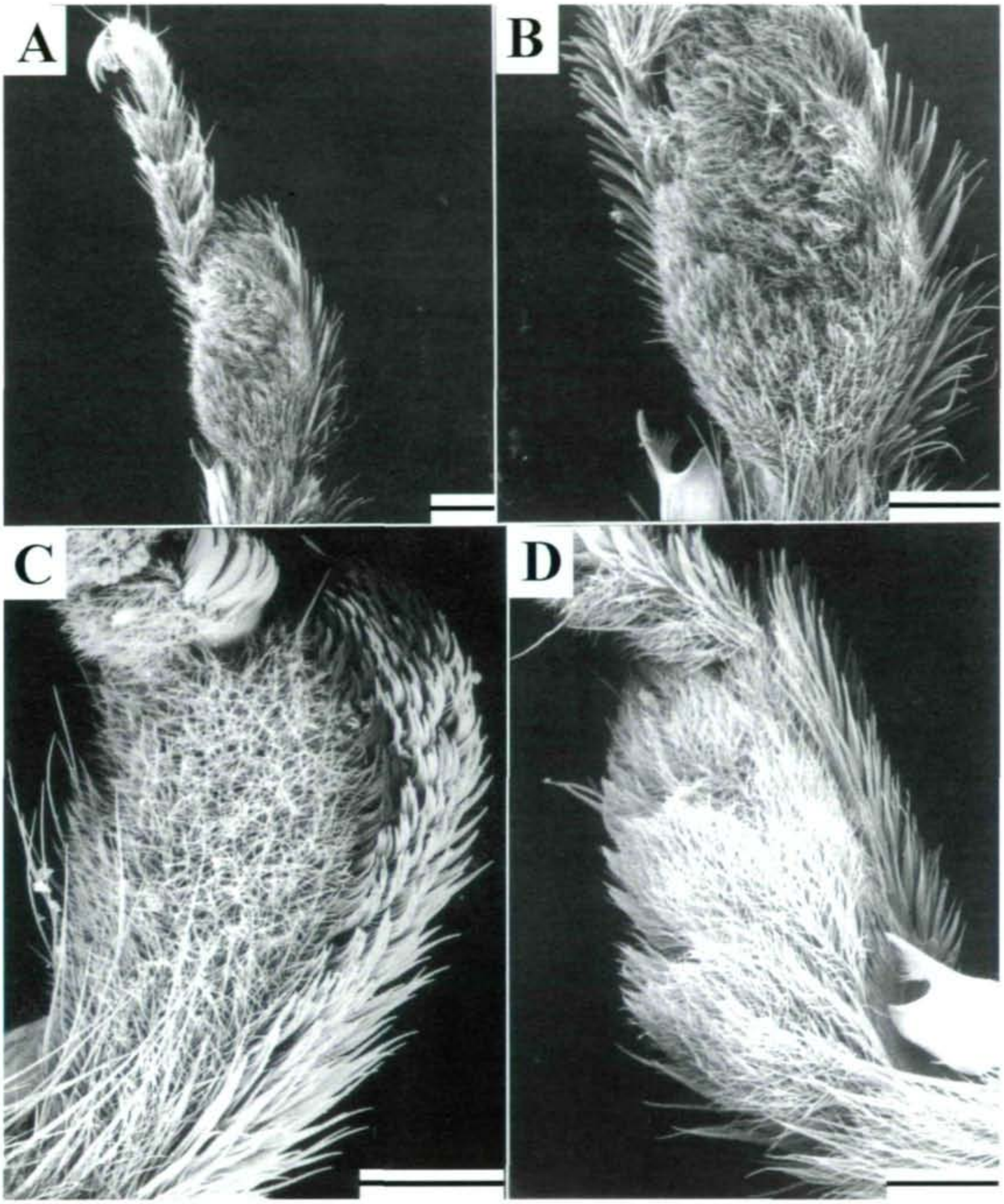

Fig. 45. Oil collectors of *Chalepogenus*. **A** & **B**, *Ch. rufipes*, **A**, posterior view of the forebasitarsus showing the oil collector. **B**, detail of A, showing the branched hairs of the collector. **C**, *Ch. rasmusseni*, posterior view of the oil collector on the forebasitarsus, extended to the meditarsi. **D**, *Ch. vogeli*, posterior view of the oil collector on the forebasitarsus and meditarsi. Scale bars: 200 µm.

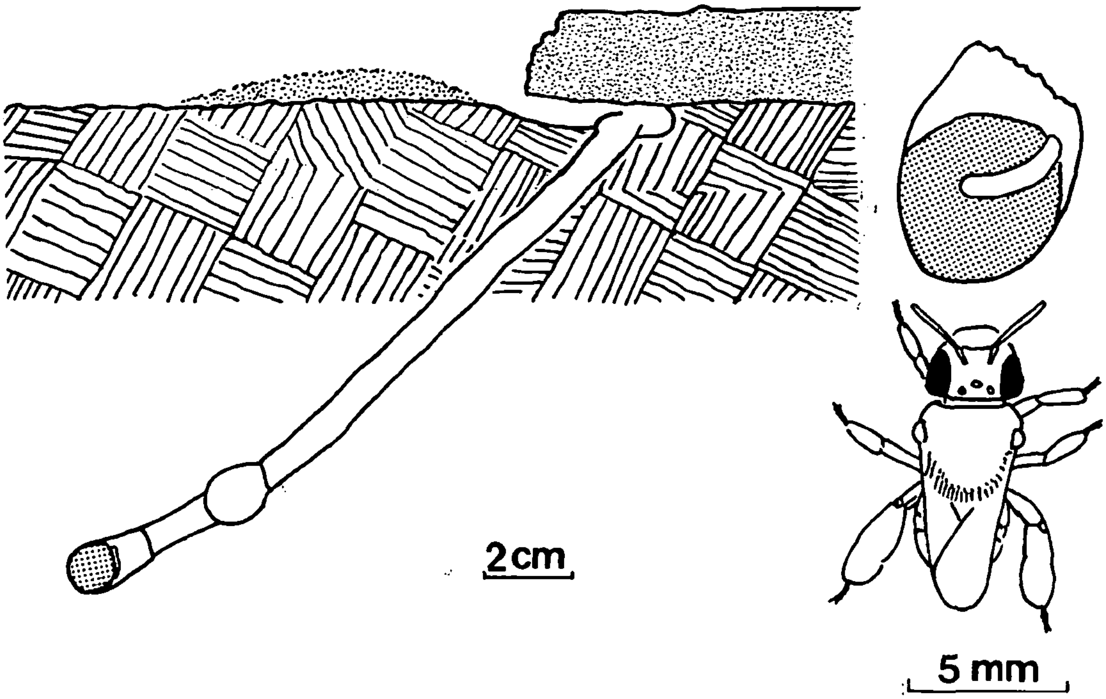

Fig. 46. Nest of *Chalepogenus caeruleus*. Left: longitudinal dig through the gallery and cells. Right: detail of a cell with an egg on the provision mass. Right below: scetch of the bee.

***Thinocorus rumicivorus* ESCHOLTZ:** *Thinocorus rumicivorus* is a bird of family Thinocoridae commonly named "agachona" or "seedsnipe"; this family is related to those families of the lapwings and plovers, snipes and sandpipers among others in the order Charadriiformes. This family has probably originated in South America (GRIZMEK 1969) and inhabits the low vegetated plains of Patagonia, Falkland Islands, the pampas and the arid regions of the High Andes from Chile to Ecuador. During winter they migrate in big flocks to the north searching for warmer regions (AUSTIN 1961).

Thinocorus rumicivorus is a bird of the size of a pigeon (c. 12 cm from the apex of the beak to the end of the tail). The male has a gray neck and breast; a characteristic black line runs along the middle of the breast like a "tie" (Fig. 60). This line ends in a black collar, which separates the breast with the white under parts. The female has no black tie or collar. The wings are pointed, long and narrow, with a white band and black coverts (NAROSKY & YZURIETA 1987); the upper part of the body is cryptically brownish in color. The legs are short and the beak is short and thick. A distinctive feature of these birds is the presence of a small membrane that covers each nasal hole, which could function to avoid the entrance of sand and dust blown by strong winds common in the habitats where they live (AUSTIN 1961; GRIZMEK 1969).

Thinocoridae is the only family of the order that has specialized their diet to grains, seeds, fruits and other parts of plant origin (OLROG 1984; AUSTIN 1961); all other members of the order are carnivorous. Apparently, this vegetarian birds obtain all their water requirements from the plants they eat and do not drink water directly, since they have never been observed drinking. In fact, SCOTT (1977) mentions that they cover their water requirements eating leaves of fleshy plants and young shoots.

The nest building habit of these birds is not well known. The nests of *T. rumicivorus* were discovered by BELCHER in 1936 (GRIZMEK 1969; VIGIL 1977); they are shallow depressions built on the ground, surrounded by a ring of small sticks; the eggs are covered with sand, debris and grasses. Oviposition occurs during August and September; they lay 3-4 pyriform eggs, which are shiny and show creamy, brown or olive green flecks. It is not yet known, which sex incubates them or how long the incubation time is. The chicks are nidifuge, but stay with their parents at least for some time (VIGIL 1973, SCOTT 1977).

<u>Behavior of the birds in relation to flower visits:</u> As far as could be seen in the study site, these birds had terrestrial habits and during nesting periods they moved in couples (or together with the chicks). They fed principally on grains of grasses and seeds of *Armeria maritima* WILLD. During this study, the birds were never seen drinking water, but instead they ate the fruits of *Empetrum rubrum* and the food bodies of *Calceolaria uniflora* (Fig. 60A-C, SÉRSIC & COCUCCI 1996a). They run describing zigzag routes following the narrow pebbled paths formed between the plant patches, halting here and there picking to their food. The sighting of the birds in the field, when they were not moving around was rather difficult because of their cryptic plumage (Fig. 60). Commonly, the female walks stooped over the ground, eating, while the male walks upright or stands over cushions of plants or on low promontories surveying the area. When it moves from one place to another it stops briefly to feed on seeds or flowers it finds on its way. With short whistles it announces any possible danger to advice the female. If the danger is too close, the male flies away from the female, whistling, and lands far from the female, but they communicate with each other by characteristic whistles. In the presence of danger the female rarely flies away, but rather stays very quiet, hidden under a bush, or she moves very rapidly without halting to eat. Normally, the male moves back to join the female.

Visits to species of *Calceolaria*

The visits of the different pollinators (*Centris, Chalepogenus,* and *Thinocorus*) to the flowers of *Calceolaria* will be described separately. There are some exceptions of flowers that are pollinated indistinctively by both bee genera. In those cases both will be described together.

Species visited by members of the genus *Centris*

There are different types of flowers adjusted to the bees of *Centris*: open flowers and closed flowers. The open flowers deposit the pollen on the dorsal part of the bee (notoribic pollination, Fig. 47A, B), while the closed flowers deposit the pollen either on the dorsal part Fig. 47C-E) or on the ventral part (sternotribic) of the bee (Fig. 47F).

Species with open nototribic flowers

Calceolaria parviflora.

Collecting data: Argentina. Córdoba Province, Punilla department. Cuesta Blanca. AAC & ANS 206. 21-10-1987.

This species includes rosulate to subrosulate plants, with petiolate elliptic, obtuse leaves. Bracteose inflorescence, HF and CoF[7] pauperized, with only one flowering node, and 2-6 flowers per cyme.

The upper lip of the flower is hooded, and encloses the upper theca, while the lower one remains exposed; the thecae have a divergence angle of more than 180° (Fig. 27G). The lower lip shows a wide opening, the appendage is almost vertical (with an inflection angle of 90° or less, Fig. 5C). The margins of the instep are constricted at the level where the lap folds (Fig. 48A). Flowers are yellow and bear red markings (points and irregular lines) at the inside of the throat in addition to small radial arranged red lines on the inflection line of the lap.

Flowering season: from October to February. Observations were done from 22-10-1987 until the last week of January 1988. The maximum flowering period was between November 23 and 29.

Visitors: *Centris tricolor* females were the only legitimate pollinators seen. An undetermined species of Halictidae as an illegitimate visitor was also seen.

The first observations started at the end of October. Visits were very rapid and seldom, scarcely halting in the plant population. The first bees to be seen around the flowers were males of *C. tricolor* which were patrolling the flowers probably in search of females. They were frequently seen taking nectar from the flowers of *Lepechinia floribunda* (BENTH.) EPLING. Just at the beginning of November some females started visiting the flowers, and at the end of the month the number of bees and visits increased markedly. Records of 3 to 4 visits simultaneously to neighboring plants were very common, while the males were constantly patrolling the plants. Most of the flowers showed damaged lower lips, a pair of injuries matched with the constrictions already mentioned, and another pair was located on

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[7] HF="Hauptflorescenz" and CoF="Coflorescenz" following the terminology used by TROLL (1964) and WEBERLING (1965), for more details about the inflorescence in *Calceolaria* see SÉRSIC & COCUCCI (1996b), EHRHART (2000).

the margin of the instep at the distal part near the tip. These injuries are due to the worn-out by the mid- and the hindlegs of the bees, while they grasp the flowers during the visits. Sometimes the injuries are so strong that the corolla ends were perforated.

The activity normally starts at 9:30 a. m. The females make their flying route in the same direction, from East to West. When the bee lands on the lower lip it introduces the forelegs into the opening to extract the oils from the elaiophore (Fig. 48B-D), while the other legs hold firmly the lower lip. The elaiophore is swept alternatively by the oil-collector of the basitarsi. During each visit the oil gland is swept approximately six times (3 times each leg). The visits are very rapid, remaining an average of 1.7 sec (n = 13) in each flower. This could be calculated through a film made at a speed of 24 frames/second.

The film obtained shows clearly when the bee gathers the oils with the forelegs, and occasionally moves the midlegs in a similar manner rubbing the lower lip. This phenomenon occurs because *C. tricolor* has oil-collectors in the first and the second pair of legs. As the bee uses only the first pair, the second is moved reflexly. The performance of a behavior not adjusted to the floral structure is known as "carry-over" and was first observed by BUCHMANN (1983) on bees that, after vibrating flowers with anthers dehiscing by pores, continued vibrating other flowers where the buzz was not necessary.

The collected oils are transferred to the scopae of the hindlegs during the flight from one flower to another. These movements are very rapid, but evidently the midlegs intervene in the oil transference. The flowers of the inflorescences are visited systematically, and occasionally they land on old flowers that fell down under the weight of the bee. Freshly fallen flowers may also be visited on the ground by the bees, which adopted the same posture mentioned before. Pollen is deposited on the front of the head and the anterior parts of the thorax (Fig. 48B-D). The last visits were recorded at 17:30.

Calceolaria lossenii

Collecting data: Argentina. Córdoba Province, San Alberto department. Copina, second bridge on the old road. 1600 m. AAC & ANS 164. 12-11-1988. Pampa de Achala, between Giulio Cesare and the telephone antenna. 2000 m. AAC & ANS 236. 16-02-1988.

This species includes low shrubby plants, 0.40 m tall, with horizontal rhizomes and ascending, leafy stems; bearing petiolate or subsessile leaves with a blade oval-triangular, cordate at the base, the margins being crenate and revolute. Bracteose inflorescence; the HF and CoF have 3 flowering nodes and many-flowered cymes.

The flowers are open and relatively small (0.9-1.0 cm in length); the upper lip is hooded, covering almost completely the adaxial thecae of the stamens (Fig. 4E). The lower lip is long, tongue-shaped. The opening is wide, but the lip constricts distally and the calceiform part continues laterally compressed (Fig. 49A); the wide mouth allows the bees to enter and reach the elaiophore, while the slipper-shaped part functions as a handle. The flowers are yellow, with irregular red markings on the throat. The stamens have a lever mechanism (Figs. 4E; 34C, D); the thecae are separated, and the adaxial one is more developed than the abaxial one (Fig. 34C, D). All pollen of both thecae was fertile. The differentiation of the hinge occurs late in the development of the flowers and the anthers are almost fixed in flower buds or during the carpelate stage. The appendage is placed vertically (inflection angle of 90°) or slightly folded at the apex.

Flowering season: the flowering season is from November to March. The maximum peak of flowering season is December. Observations of the pollinators were made between 11-11-1987 and 01-01-1988.

Visitors: Females of *Centris tricolor* were the legitimate pollinators seen. One species of *Augochloropsis* and another unidentified Halictidae collected pollen and pollinated illegitimately, without taking oils. Some individuals of *Bombus opifex* F. SM. were also seen.

Early in the morning between 7:00 and 9:00 a. m. the visits were scarce. Females of *C. tricolor* flew over the flowers without staying long on them. From 9:30 to 17:00 the number of visits increased, the intervals of visits were between 30 to 60 minutes. During the maximum of the flowering season, in December, visits were very frequent; up to 4 individuals could be seen simultaneously pollinating flowers of the same inflorescence. Collection of oils from flower buds was also common, to get into these buds the bee had to force their entrance separating both lips by pushing them with the head.

When the bee lands on the flower it clutches the lower lip with the midlegs at the constricted lateral edges, while the hindlegs hang without support (Fig. 49B, D). The collection of oil is identical to that described for *C. parviflora*. Each foreleg makes 6-8 sweeping movements in c. 2.94 sec (n = 14) on one elaiophore. Carry-over movements of the midlegs were also seen. The collected oils are transferred to the hindlegs during the short flights between one visit and another. Pollen is deposited on the frontal part of the bee between the antennae (Fig. 49B, D).

Halictidae were seen collecting pollen actively from the anthers (Figs. 49A, 54D). They land on the lower lip and hang from the upper lip with the legs; in this position they collect pollen with the forelegs and transfer it to the scopae of the hindlegs with the help of the midlegs.

Bombus opifex was seen in three opportunities landing briefly on the lower lip, maybe erroneously searching for nectar.

Calceolaria teucrioides

Collecting data: Argentina. Salta Province. Chicoana department. Cuesta del Obispo. AAC & ANS 378. 07.03.1989. Rosario de Lerma department. Quebrada del Toro, Province road 51, from km 31.7 to 6.7, NW of Campo Quijano. 1600-1680 m. LN 7552. 16-01-1988. Tucumán Province, Monteros department, province road 307, from km 49 to 50. 1800 m. 19-02-1988.

This species includes low shrubby plants c. 0.4-0.6 m tall, bearing horizontal rhizomes with erect leafy and dense villous stems. Leaves are petiolate to subpetiolate with crenate to subcrenate, sometimes revolute, margins. Inflorescences are dense, HF and CoF with 3-4 flowering nodes with many flowered cymes. Flowers are open; the hooded upper lip covers tightly the adaxial thecae of the anthers. Stamens have lever mechanism, where the abaxial thecae, though with pollen, is reduced and functions as a pushing surface (Fig. 34E). The lower lip is tongue-shaped with a broad and stretched opening. The appendage has an inflection angle of 90°.

Flowers are yellow with red spots on the throat. The population studied was found adjacent to a small population of *Calceolaria umbellata*.

Flowering season: November to April. Observation period: 17 to19-02-1988, from 7:00 to 19:00 h.

Visitors: *Centris tricolor* as legitimate pollinator and *Chalepogenus vogeli* as illegitimate visitor.

Visits were seen briefly and seldom. During the whole observation time *C. tricolor* was seen only once between 14:00-15:00 h. It came very rapidly and could not be photographed, but its behavior on the flowers was similar to that observed for *C. lossenii.* Carry-over movements were not seen. Many other approaches to the small plant population were also seen, but there were no visits to the flowers.

Chalepogenus vogeli was seen collecting oil and pollen from the flowers of *Calceolaria* species. While collecting oil, these bees did not touch the fertile parts (Fig. 54C) because of the reduced dimensions of the bee in relation to the flowers. It collects also pollen while hanging upside down from the upper lip.

Calceolaria hypericina

Collecting data: Chile. V Region. Juncal. AAC & ANS 340. 15-01-1989.

The species includes dense and resinous shrubs, 0.9-1.20 m tall. The leaves are scattered, but especially developed at the lower part of the stems. The inflorescences are pauperized with only one flowering node in the HF and CoF, with 2 to many flowers per cyme. The cymes are elevated in a way that the flowers are exposed above the foliage (Fig. 50A). The flowers are relatively small, open, with a hooded upper lip. Stamens correspond to the basic type (Fig. 26B), the anthers are completely exposed (Figs. 5B; 47B; 50B). The lower lip is yellow with small red spots inside the throat. The calceiform part is curved upwards, and the apex is 4-crenated (Fig. 50B). The lower lip constricts laterally where the instep starts. The oil gland is trapezoidal and the appendage is slightly folded at the apex (Figs. 12A; 5B).

Flowering period: January and February. Observation period: 7 hours, from 7:00 a. m. to 14:00 p. m.

Visitors: *Centris* cfr. *orellanai* as the legitimate pollinator and an unidentified Syrphid fly as illegitimate visitor.

At 9:30 h the plant population started to receive the sunlight, and simultaneously a large quantity of syrphids appeared, which systematically visited the flowers, searching for pollen with the proboscis. Probably they transferred pollen illegitimately to other flowers (Fig. 54E, F).

A male started patrolling the plants at 11:00 a. m., following the same route. At 12:00 the first female of *Centris* cfr. *orellanai* came. During the collection of oils they emitted a particular buzz, which favored probably the deposit of pollen on the front of the head, between the eyes and at the base of the antennae (Fig. 50C, D). The midlegs fasten the constricted part of the lower lip, which frequently appears damaged. Transference of the collected oils to the hindlegs occurs during the flight.

Calceolaria pinifolia

Collecting data: Argentina. Mendoza Province, Luján de Cuyo department. Vallecitos, near Río Blanco. AAC & ANS 329. 11-01-1989.

This species includes glandular-resinous, strongly glutinous chasmophytes, forming dense cushions growing on rocky walls. The woody stems are branched from the base, with exfoliable bark. Leaves are up to 5cm in length, lineal, crenate and revolute, the abaxial side densely glandular. The inflorescence has two or more flowering nodes in the HF and the CoF, the cymes have many flowers. The corolla is externally glandular, yellow with transversal red lines inside the throat and red spots on the inflection margin of the appendage. The upper lip is small, hooded and does not cover the anthers. The lower lip is

1.0-1.5 mm long, narrowly oblong, with a wide opening (Fig. 5A). The anthers, with a divergence angle of 45°-90°, can move slightly dorso-ventrally. Dehiscence is complete, but the anther walls do not contract, in a way that the pollen is not openly exposed (Fig. 26A). The appendage is wide and short, with an inflection angle of 90°, the elaiophore is very dense and green in color (Figs. 5A; 11B).

Flowering season: January.

Visitors: *Centris* cfr. *tricolor* is the probable pollinator of this species. Although the plants were controlled for eight hours, effective visits couldn't be seen. Only rapid flights of *Centris* cfr. *tricolor* were observed, and due to the floral features, bees of this genus are probably the legitimate pollinators of *C. pinifolia*.

Calceolaria filicaulis subsp. *luxurians*

Collecting data: Argentina. Mendoza Province. Luján de Cuyo department. Vallecitos. A.A.C. & A.S. 330. 12-01-1989. Las Heras department. Laguna de los Horcones. A.A.C. & A.S. 335 13-01-1989.

Chile. V Region. Argentine-Chile border, Caracoles. A.A.C. & A.S. s/n. 14-01-89.

This species includes rosulate plants with flavelliform leaves, prolonged in wide petioles; the blade is pilose, has prominent nerves on the abaxial side and toothed-lacerated margins. Inflorescence is pauperized; the HF and CoF with only one flowering node and few-flowered cymes. Flowers are inflated, externally glandular; upper lip hooded with folded margins enclosing the anthers inside the upper lip. The lower lip has red spots on the sole and the opening is small. The appendage is folded with an inflection angle of 0°-45°, the elaiophore is widely extended, but with a low density of glandular trichomes (Figs. 9E; 18A-D).

Visitors: This is one of the few species in the genus in which it could be seen that both oil-gathering bee genera are involved in legitimate pollination. *Chalepogenus perimelaena* and *Centris nigerrima* were observed in different plant populations. *Centris* was seen in Caracoles (Chile) near the boundary with Argentina and *Chalepogenus* in Laguna de los Horcones (Mendoza, Argentina).

Centris nigerrima visited systematically all the flowers of this species. With the forelegs it swept the elaiophore 5 to 8 times. Almost all flowers were injured; the damages were seen on the lower lip and are produced especially by the midlegs, which are principally used to grasp the flowers. A second species of *Centris* was also seen on the flowers, but it could not be captured for identification; it could have been *Centris muralis*, because of its size and the grayish pilosity of the notothorax. The elongated shoots of the inflorescence bend, and the lower lip descends under the bee's weight. The small opening allows the bee to introduce only its forelegs. Pollen is deposited on the frontal and dorsal parts of the head (Fig. 51A-D). A male patrolled the plant population.

The visits of females of *Chalepogenus perimelaena* started at 14:00 h. They operate the flowers in two distinct steps: 1) The bee lands on the lower lip and introduces itself between both lips, maintaining a horizontal position, thus the anthers make contact with the head, then it starts to vibrate the anthers making short high pitched and repetitive buzzes. In this way pollen is actively deposited on the bee. 2) After collecting pollen, the bee curves itself entering even more in the flower to reach the elaiophore, which is located inside the calceiform part of the lower lip. During oil collection the fertile parts make contact with the notothorax of the bee.

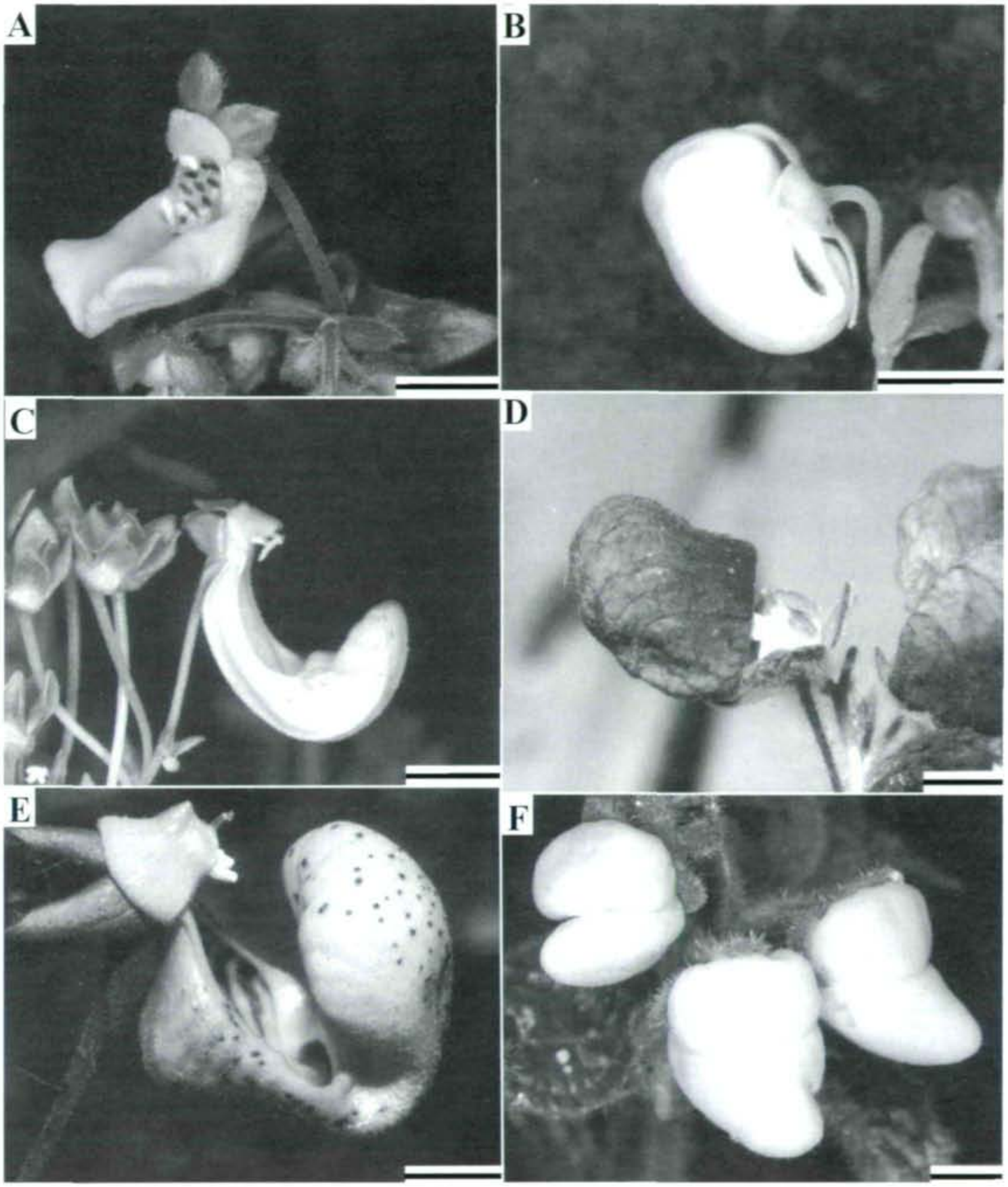

Fig. 47. General view of Centridini flowers. **A** & **B**, open flowers. **A**, *Calceolaria parviflora*. **B**, *C. hypericina*. **C-F**, closed flowers. **C**, *C. prichardii*. **D**, *C. schickendantziana*. **E**, *C. arachnoidea*. **F**, *C. plectranthifolia*. Scale bars: 0.5 cm.

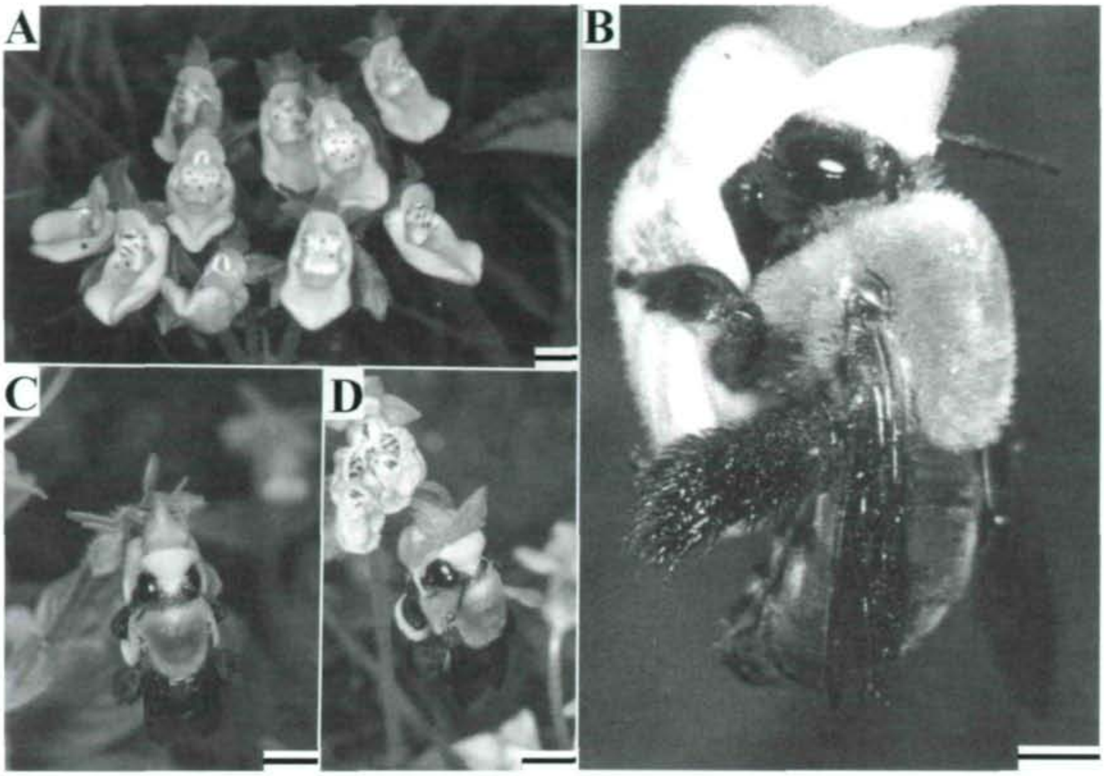

Fig. 48. Pollination of *Calceolaria parviflora* by *Centris tricolor*. **A**, general view of the open flowers with hooded upper lips. **B-D**, position of the females collecting oils, note the upper lip contacting the dorsal part of the head and the pollen deposited on the bee (nototribic pollination). The midlegs clutch at the constriction of the lower lip, while the forelegs are inside the flower collecting oils. Scale bars: A, C & D: 0.5 cm, B: 0.2 cm.

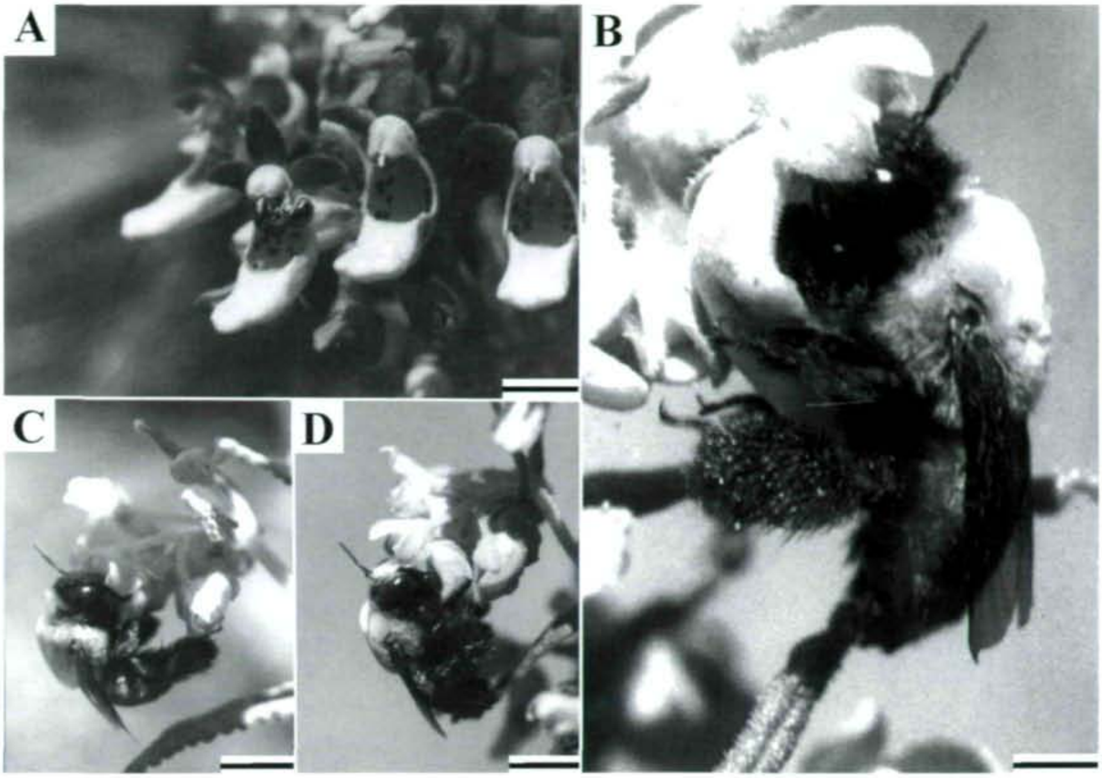

Fig. 49. Visits on *Calceolaria lossenii*. **A**, general view of the flowers; one of them shows an Halictidae bee taking pollen from the stamens. **B-D**, visits of *Centris tricolor*. **B**, midlegs are clutched at the constriction of the lower lip, while the hindlegs are hanging. **C**, visits on a flower bud; note the right hindleg grasping a neighbor flower. **D**, note the pollen deposited on the head of the bee (nototribic pollination). Scale bars: A-D: 0.5 cm, B: 0.2 cm.

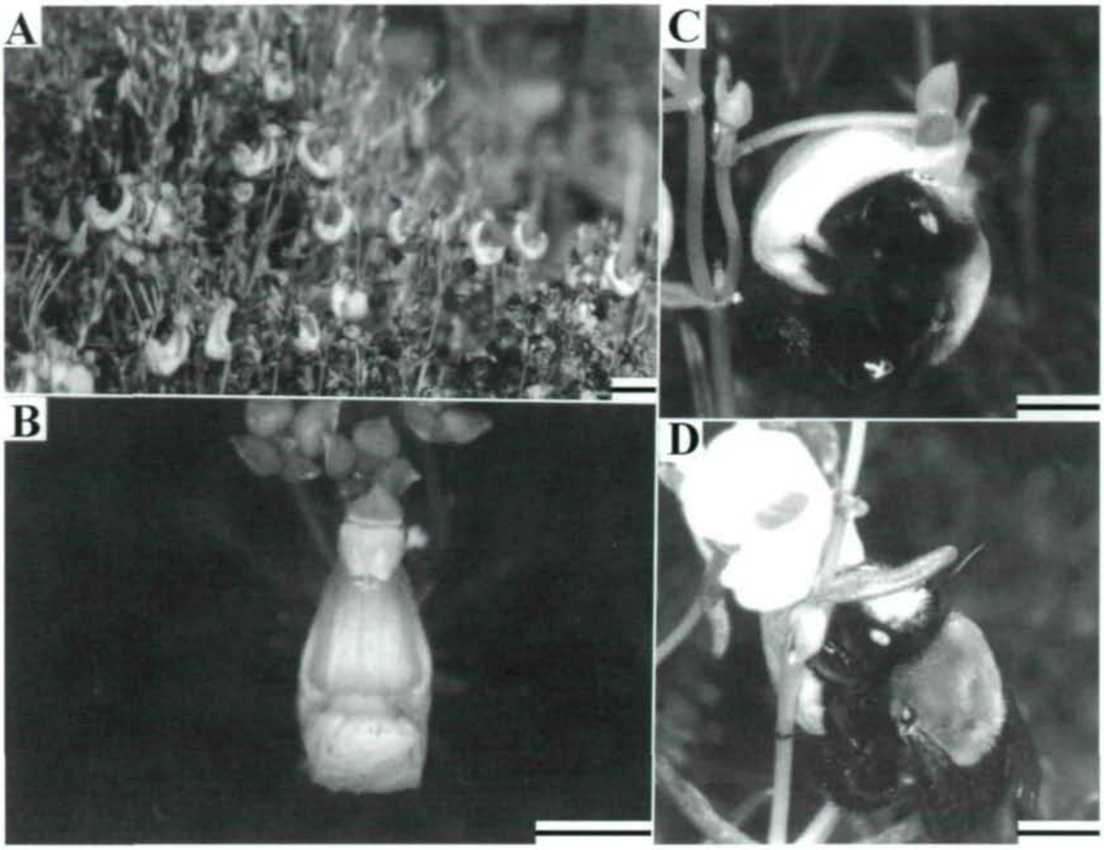

Fig. 50. Pollination of *Calceolaria hypericina*. **A**, general view of the flowering plant. **B**, frontal view of one flower. **C** & **D**, visits by *Centris* cf. *chilensis*, note the white pollen on the head (nototribic pollination). Scale bars: A: 1 cm, B-D: 0.5 cm.

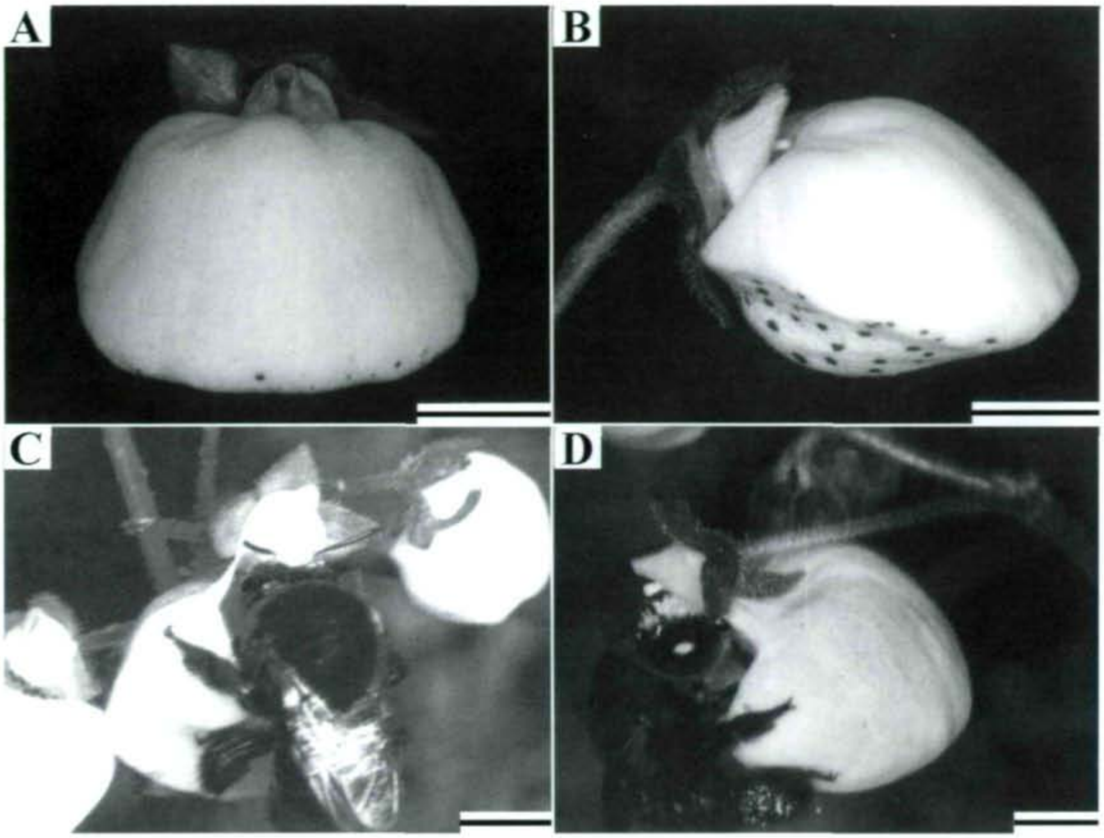

Fig. 51. Pollination of *Calceolaria filicaulis* subsp. *luxurians* by *Centris sp.* **A**, front view of a flower. **B**, lateral view of the flower showing the different size of both lips. **C & D**, entrance of the bee by its weight. Note the pollen on the head and the forelegs inside the flower. Scale bars: 0.5 cm.

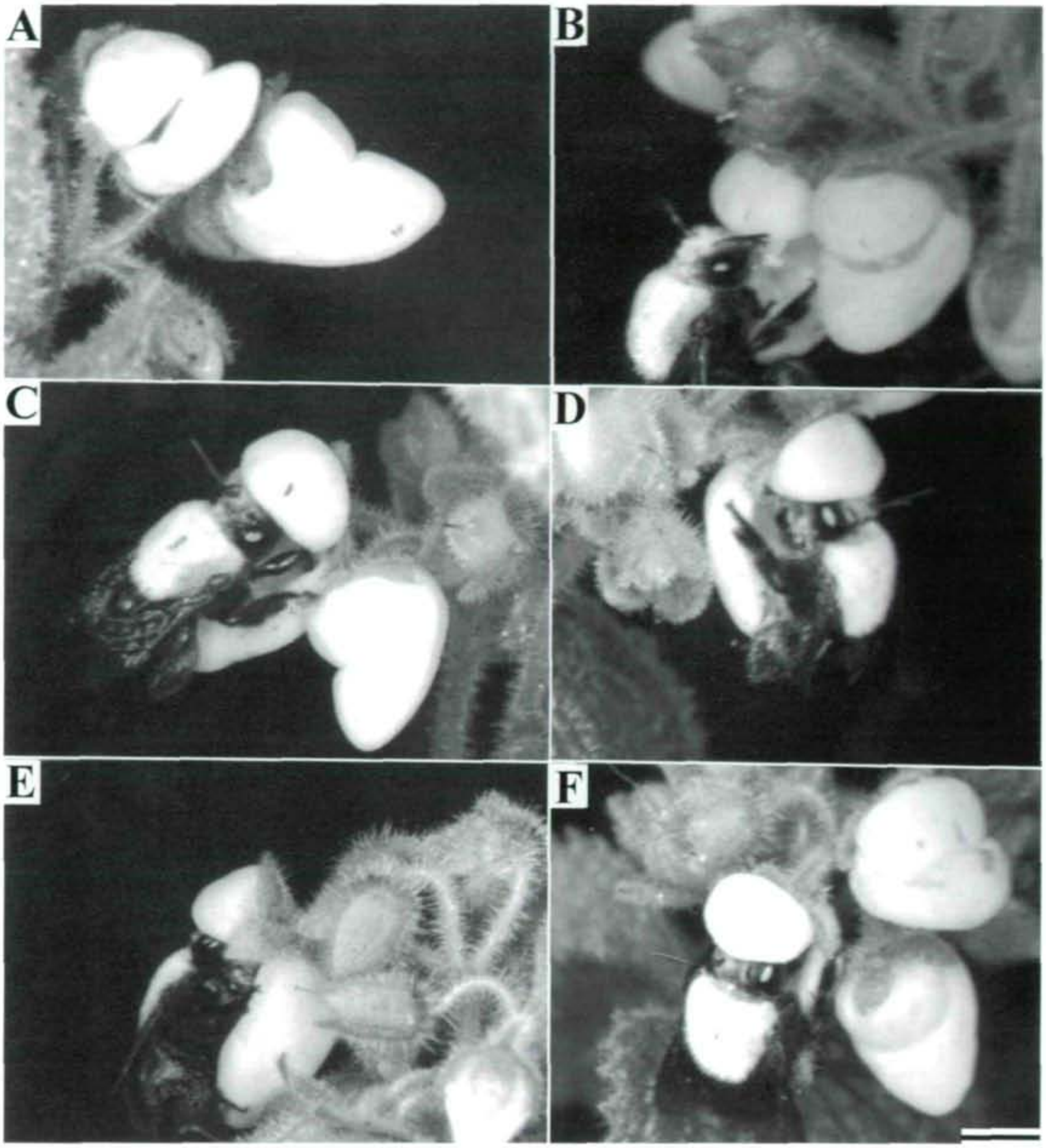

Fig. 52. *Calceolaria plectranthifolia* visited by *Centris autrani*. **A**, general view of the flower; note the large and inflated upper lip. **B**, oil collection; note the mandibles elevating the upper lip and exposing the fertile parts. **C**, right foreleg inside the flower, and midleg clutching and damaging the lower lip. Upper lip laying on the head of the bee. **D**, deposition of pollen on the intercoxal region (sternotribic pollination). **F**, injuries on the corolla, on the upper lip performed by the mandibles, and on the lower lip by the legs. Scale bars: 0.5 cm.

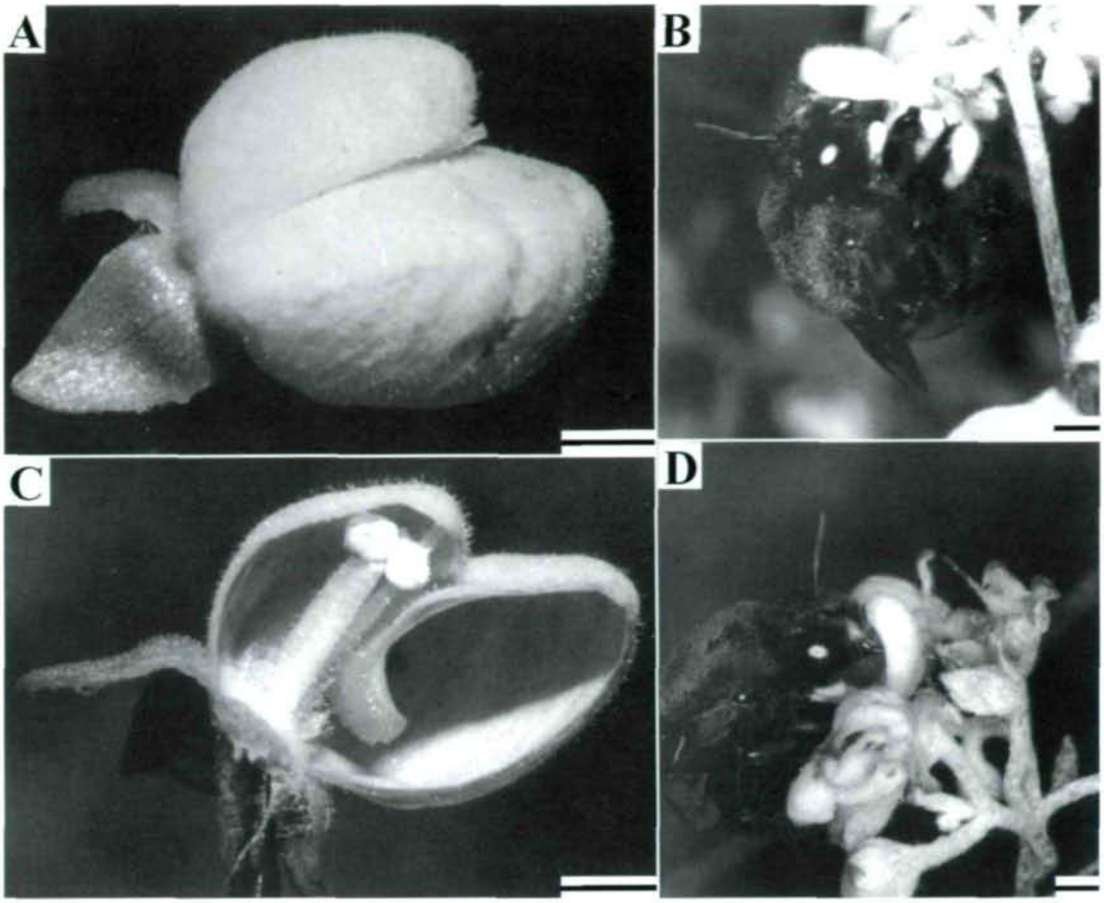

Fig. 53. Pollination of *Calceolaria polifolia* by *Centris nigerrima*. **A**, general view of the flower, note the well developed upper lip. **B**, tangential section of a flower to show the hidden elongated fertile parts. **C** & **D**, visits by *Centris nigerrima*. Note the extended mandibles maintaining the upper lip opened, the fertile parts touch the ventral side of the bee (sternotribic pollination). Scale bars: 0.2 cm.

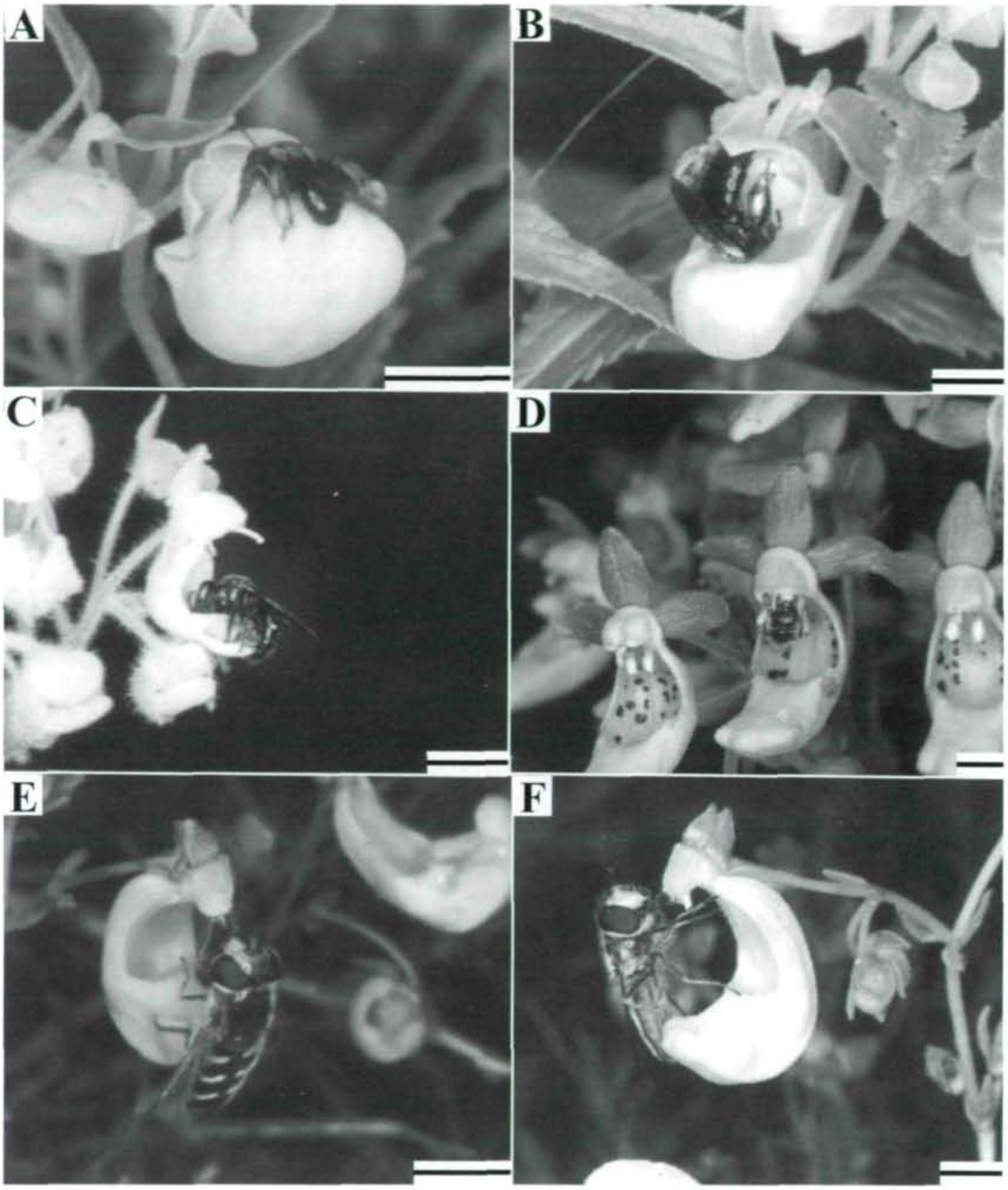

Fig. 54. Illegitimate visits to different species of *Calceolaria,* which are normally pollinated by *Centris*. **A** & **B**, *C. schickendantziana* visited by *Chalepogenus rufipes*. **A**, bee forcing its entrance between the flower lips. **B**, entrance is only possible in an upside down position and permits only the collection of pollen. **C**, *C. teucrioides* and *Chalepogenus vogeli*, the small bees do not touch the stamens during oil collection. **D**, *C. lossenii* visited by an unidentified species of Halictidae which takes pollen. **E** & **F**, *C. hypericina*, visited by an unidentified species of Syrphidae, which consumes pollen. Scale bars: 0.5 cm.

A male was permanently patrolling the plant population from the early morning on; it made the same route every time, halting in the same place from time to time. In presence of a female it followed it permanently in search of copulation opportunities.

Calceolaria corymbosa

Collecting data: Chile. V Region. La Campana National Park. A.A.C. & A.S. 16-01-1989.

This species includes herbaceous, rosulate plants with big, obtuse, cordiform and petiolate leaves. The pauperized HF and CoF have only 1 flowering node and many flowered cymes. The flowers are open with a hooded upper lip, anthers being partially exposed. The lower lip is yellow with transversal red lines and red dots inside the throat. The appendage is strongly inflexed to the interior of the calceiform part with an inflection angle of 0°-45° (Fig. 9C).

During the observation period there were no visits to the flowers. Only a male *Centris nigerrima* was seen patrolling the plants. The size and the morphological features of the flowers may allow to infer that the possible pollinator is a bee of the genus *Centris*.

Species with closed sternotribic flowers (Fig. 47F)

Calceolaria plectranthifolia

Collecting data: Tucumán Province. Tafí del Valle department. RP 307. Between Carapunco and La Bolsa. c. 2300 m. A.A.C. & A.S. 301. 19-02-1988.

This species includes herbaceous annual plants, 0.3 to 0.5 m tall, covered with glandular hairs. The pubescent leaves are sessile or with a winged petiole. The leaf blade is well developed (up to 20 cm long), ovate to spathulate, with serrate margins. The HF and CoF have 3 to many bracteous flowering nodes, and many-flowered cymes. The upper lip is inflated (Rugosae type) and as large as the lower lip. Both lips keep closed during whole anthesis, hiding the fertile parts inside; filaments are 3-5.2 mm long. The elaiophore is banded and occupies the whole surface of the appendage. The instep of the lower lip shows a depressed area before giving place to the appendage; the style and the long stamens are housed in this depression (Figs. 7C; 52A).

Flowering period: December to March. Observation period: 19 to 21-02-1988

Visitors: Females of *Centris autrani* were the only legitimate visitors.

A small population of 5 plants was controlled. The plants grew along a stream and showed big, many-flowered inflorescences. At 9:30 in the morning, when the sunlight still had not reached the valley, oil collecting bees first arrived. The bee lands on the flowers, clutches the basal part of the lower lip with the midlegs (Fig. 52B-E), while the hindlegs remain without hold. Moving the head forwards and upwards, and with the help of the forelegs, it forces its way into the flower. The bee elevates the upper lip and let it lay on the head, in this position it lays mounted on the fertile parts (Fig. 52B, E, F). The bee takes the oils by sweeping the forelegs 6 to 8 times on the elaiophore. It stays about 4 sec in each flower. Pollen is deposited on the ventral part of the bee, between the first pair of coxas, a place difficult to reach while the bee cleans its body. The bee searches for all flowers, coming back two or three times to the same flowers during the same foraging route.

Calceolaria polifolia

Collecting data: Chile. V Region. National Park Cerro Campana. A.A.C. & A.S. 344. 16-01-1989.

This species includes low, branched bushes, 0.3-0.4 m tall; with loosely arranged ascending stems, fasciculate and small, oval-lanceolate and short petiolate leaves. Stems, leaves and inflorescence are covered with a dense and woolly pubescence. Flowers are small, yolk-yellow. The upper lip is inflated (Rugosae type) almost as big as the lower lip. As already seen for *C. plectranthifolia*, both lips enclose the fertile parts (Fig. 53A, B). Stamens have also long filaments. The appendage is wide, short and folded. The elaiophore covers the whole appendage except the margin, which is trichome free (Fig. 10G).

Visitors: Females of *Centris nigerrima* were the only legitimate pollinators seen during the observation period.

The activity started already in the morning (at c. 9:30 a. m.), although the plants were not in sunlight. Up to three females could be seen visiting simultaneously flowers of the same plant. The insects were easy to localize because of their bright black color, the low pitch buzzing they performed while flying from one flower to the other and the peculiar high pitch buzz they also performed while visiting the flowers. The flowers are relatively small compared with the size of the bee. When it lands on the flower it grasps it with the midlegs from the articulation point between both lips. The hindlegs surround the flower without holding it. The upper lip which is hooked at its margin by the open mandibles is held in an elevated position by the extended head all the time the visit lasts (Fig. 53C, D). The long stamens deposit the pollen on the ventral side of the bee, on the intercoxal region of the forelegs. This bee extracts the oils in the same way as described for *C. plectranthifolia*. Oils are also transferred to the scopae during the flight. The injuries produced by weaving down the corolla are located on the lower lip, corresponding to the midlegs and on the upper lip corresponding to the mandibles.

Many males were constantly patrolling the plants. Frequently, they felt rapidly onto the females, while they were on the flowers; they left the flowers flying together for a while, probably as part of the courtship. The activity notably decreased with direct sunshine, but continued until 16:00 h.

One individual of *Bombus chilensis* SCROTTKY was captured collecting pollen on the flowers. *Centris* bees collected also pollen illegitimately from the flowers of *Lobelia salicifolia* SWEET that grew in the same place. These big red and tubular flowers are in fact hummingbird-pollinated.

C. integrifolia, Calceolaria latifolia, C. petioalaris and *C. thyrsiflora.*

Collecting data: ***C. integrifolia***: Chile. V Region. Cerro Campana National Park. A.A.C. & A.S. 345. 16-01-1989. Puertas Negras View Point, south of Valparaíso. A.A.C. & A.S. 355. 20-01-1989. ***Calceolaria latifolia***: Chile. IV Región. Between Hurtado and Vicuña, Cordillera of Hurtado, c. 2000 m. A.A.C. & A.S. 358. 24-01-1989. ***C. petioalaris***: Chile. V Region. Lo Valdés. A.A.C. & A.S. 352. 18-02-1989. ***C. thyrsiflora***: Chile. V Region. Cerro Campana National Park. A.A.C. & A.S. 347. 16-01-1989. Puertas NegrasView Point, south of Valparaíso. 50 m A.A.C. & A.S. 355. 20-01-1989.

Several flower features of these species allow to infer that they also exhibit the same pollination mechanism described as for *C. polifolia*: The injuries left on the lower and upper lip corresponding to legs and mandibles, respectively; Inflated and well developed upper lip; Closed anthesic flowers; Fertile parts enclosed between both lips, and long stamens (Figs. 6A, D, E; 7A. B, E, F),

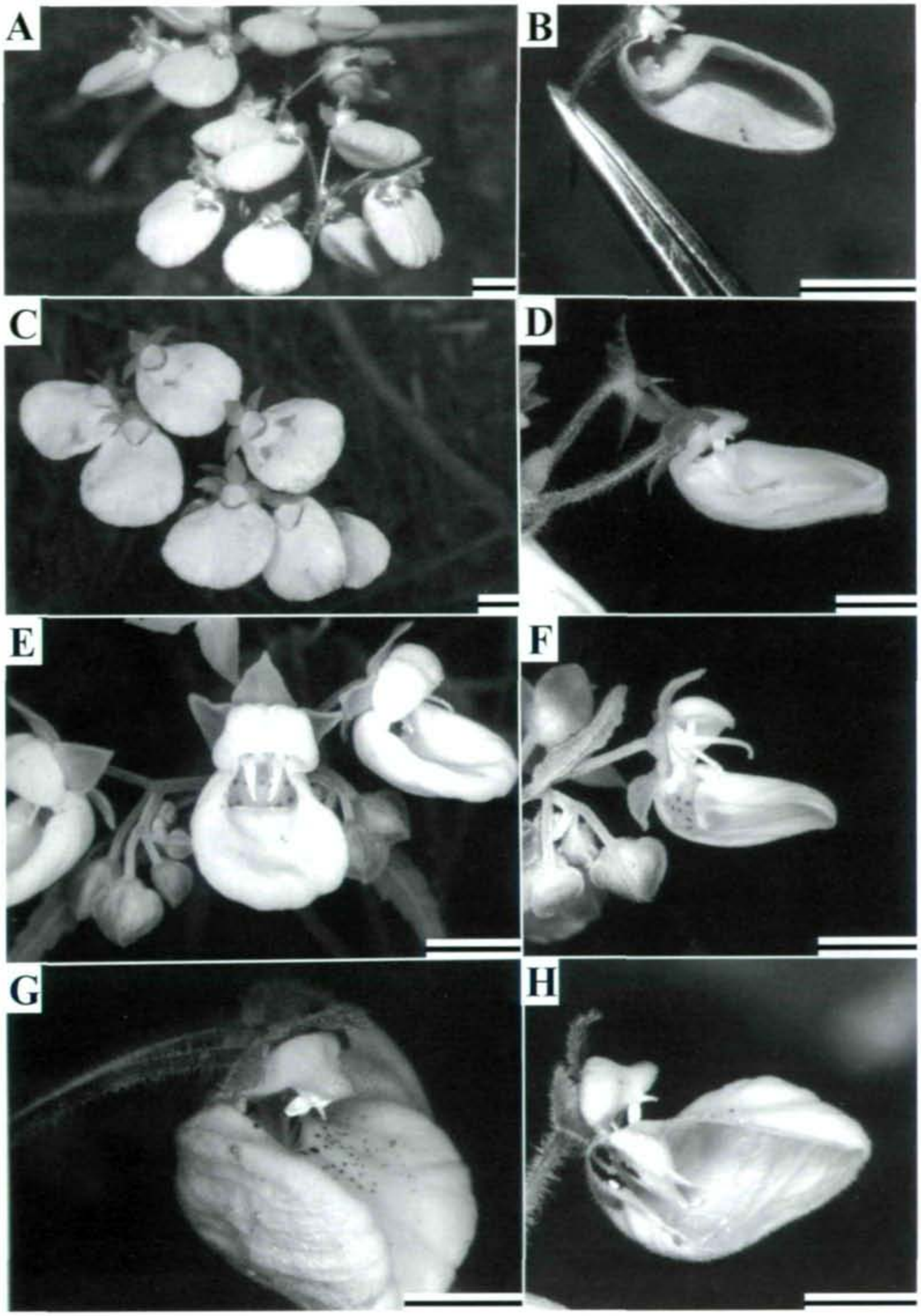

Fig. 55. General view of Tapinotaspidini flowers. Left: general view. Right: tangential section of the left one. **A & B**, *Calceolaria williamsii*. **C & D**, *C. umbellata*, see the injuries on the lower lip left by the bees. **E & F**, *C. polyclada*, in E see the reduced adaxial thecae acting as a pushing surface. **G & H**, *C. brunellifolia*. Scale bars: 0.5 cm.

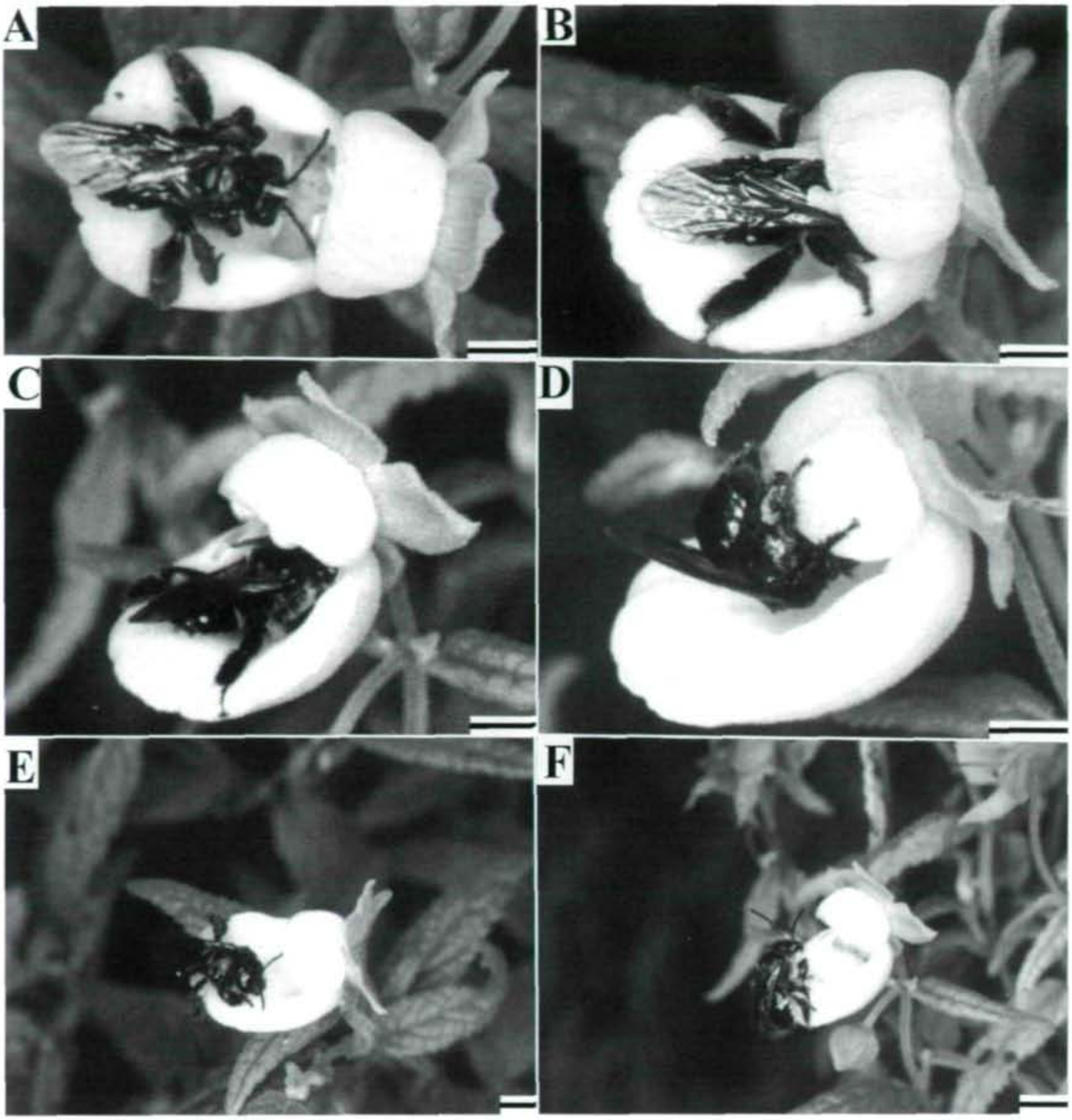

Fig. 56. *Calceolaria polyclada* visited by *Chalepogenus vogeli*. **A**, while entering the flower the bee touches the stigma. **B**, oil collection with the forelegs, the mid- and hindlegs grasp the margin of the lower lip. The adaxial thecae move downwards and deposit pollen on the back of the bee (nototribic pollination). **C**, visit of a flower with indehiscent stamens, note that the lever mechanism is not functional yet. **D**, characteristic posture of *Chalepogenus* while collecting pollen. **E** & **F**, transference of oils from the forelegs to the hindlegs with alternate movements. Scale bars: 2 mm.

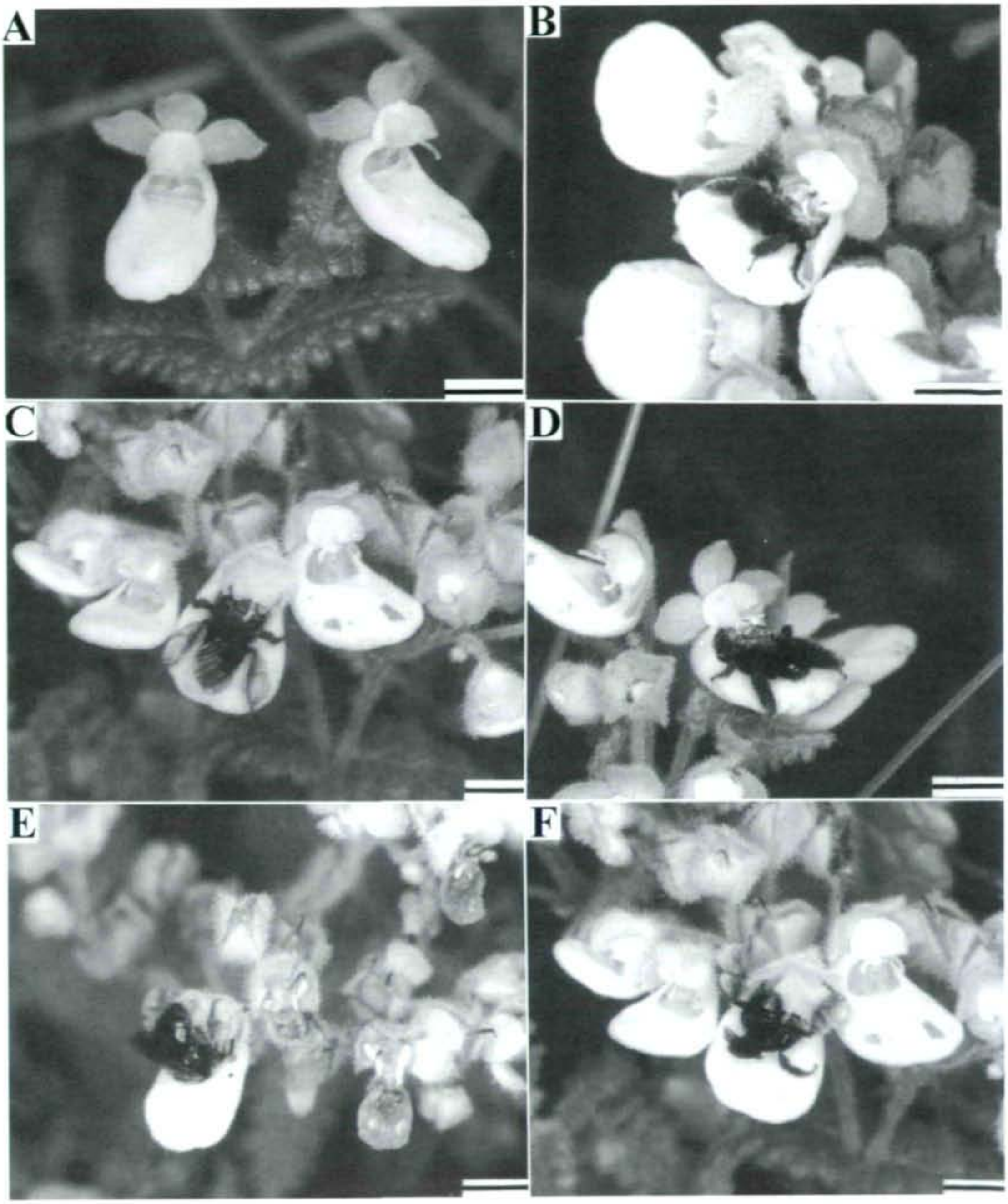

Fig. 57. *Calceolaria ruiz-pavonii* visited by *Chalepogenus vogeli*. **A**, general view of the flower. **B-D**, oil collection. The lever anthers descend to touch the back of the bee. **E** & **F**, inverted position of the bee for pollen collection. In **B**, **C**, **D** & **F** note the injuries on the lower lip produced by the mid- and hindlegs of the bee. Scale bars: 0.5 cm.

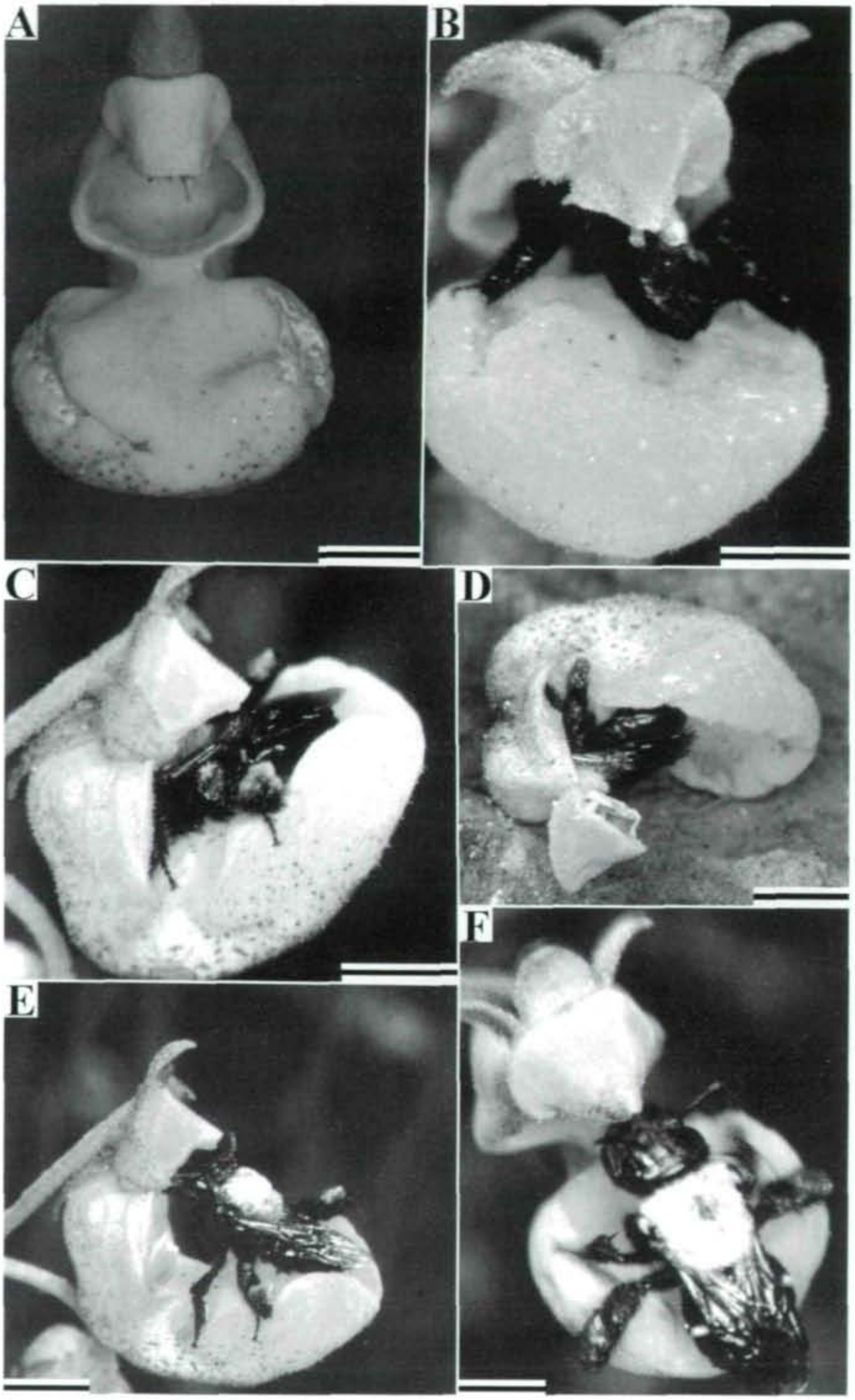

Fig. 58. Pollination of *Calceolaria prichardii* by *Chalepogenus caeruleus*. **A**, general view of the flower, note the injuries left on the lower lip by the hindlegs of the bee. **B-D**, oil collection, note in B one theca touching the back of the bee. **D**, visit of a fallen flower. **E** & **F**, collection of pollen. **E**, characteristic position of the bee, it stands with the mid- and hind legs, while collecting pollen with the forelegs. **F**, transference of pollen to the scopae, note the movement of the right midleg. Scale bars: 0.5 cm.

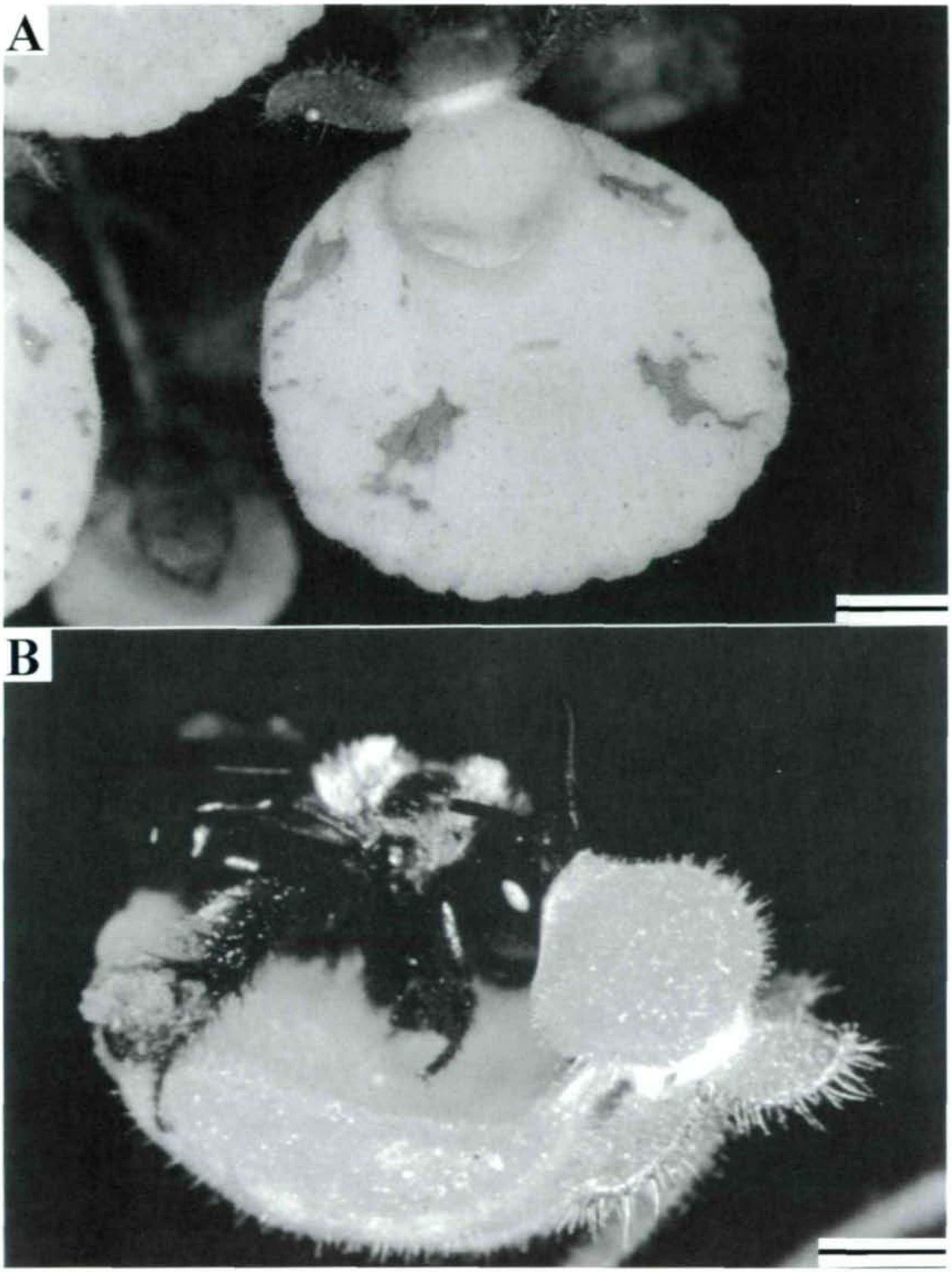

Fig. 59. Pollination of *Calceolaria valdiviana* by *Chalepogenus caeruleus*. **A**, general view of the flower showing four injuries on the lower lip left by the mid- and hindlegs of the bee. **B**, visit of *Ch. caeruleus*, note that the right midleg receives oils from the right foreleg. Scale bars: 0.3 cm.

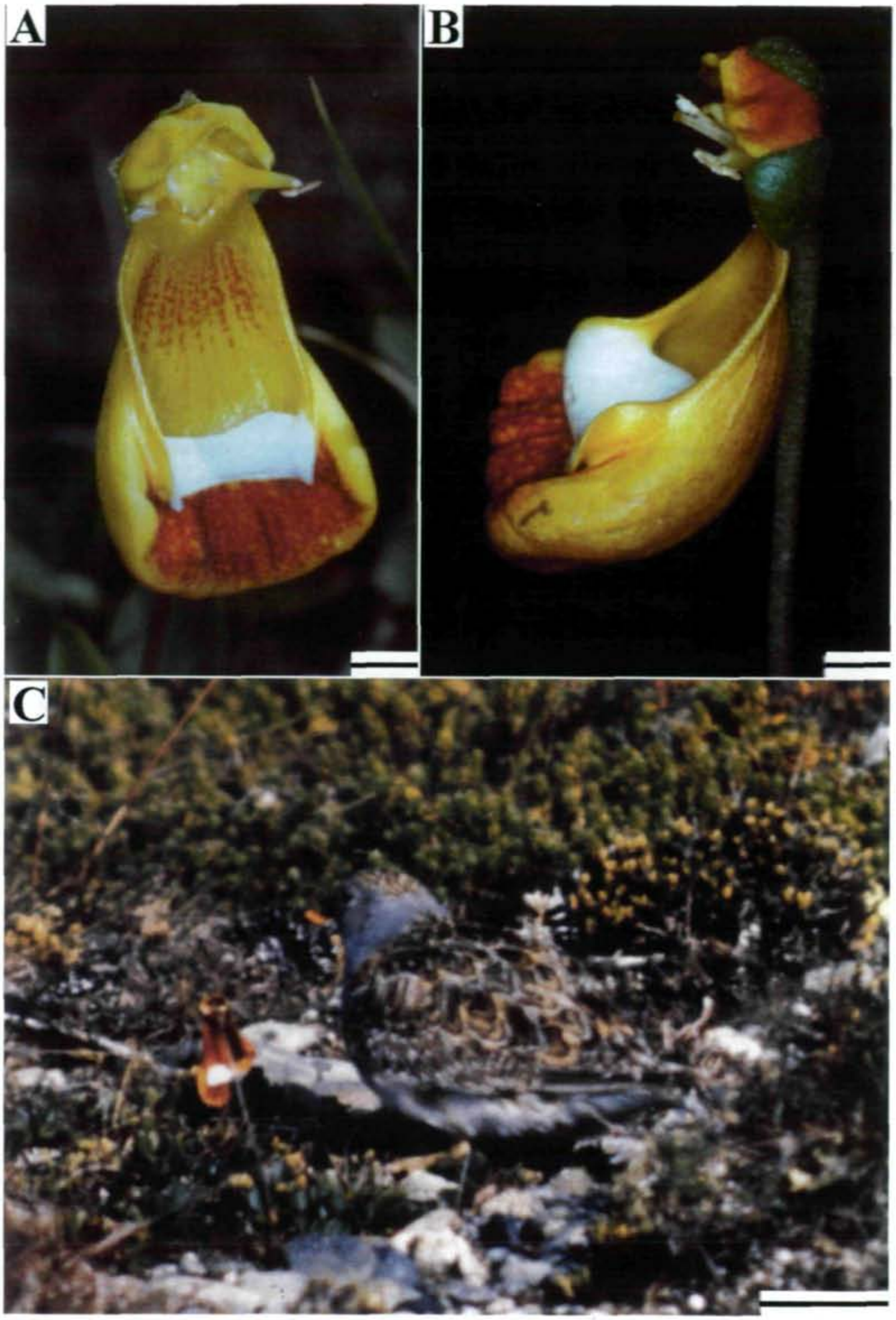

Fig. 60. *Calceolaria uniflora*. **A & B**, frontal and lateral views of the flower, note the contrasting colours and the white food body. **C**, *Thinocorus rumicivorus* eating the food body of one flower. Scale bars: A & B: 0.5 cm, C: 4 cm.

Visits of *Centris nigerrima* to the flowers of *Calceolaria latifolia* and *C. thyrsiflora* were registered. In the first case, visits were too fast and it was not possible to observe in detail the bee's behavior on the flowers. The pollination of *C. thyrsiflora* could be seen in more detail. The only difference with the other species is that the filaments are shorter (2 mm) and the pollen deposition is on the proboscidial fossa. The pollination of *C. petioalaris* and *C. integrifolia* by *Centris nigerrima* is very probable due to the constant patrolling by the males of this bee species over the plants.

Species with closed nototribic flowers

C. schickendantziana

Collecting data: Argentina. Tucumán Province. Tafí del Valle department. R.P. 307, Carapunco. A.A.C. & A.S. 295. 19-02-1988. Between Carapunco and La Bolsa. c. 2300 m. A.A.C. & A.S. s/n. 19-02-1988.

This species includes loosely branched 1 m tall shrubs. Small, oblong, short petiolate leaves, with serrate margins. Flowers are lemon-like in color. The upper lip is small, hooded and covers the adaxial thecae. The throat is stretched and drastically curved upwards, in a way that the calceiform part is at the same level and almost in contact with the upper lip (Figs. 1F; 47D). On this inflection line there is a flexible hinge that allows the flower to be opened. The appendage is very broad and folded to the inside of the lower lip with an inflection angle between 0°-45°, not closing the entrance. The elaiophore is banded and restricted to the tip of the appendage which exhibits a second inflection (Fig. 10A, B).

Flowering period: December to March.

Visitors: Legitimate visits of *Centris autrani* were well documented by VOGEL (1974). In this work these bees could also be observed visiting this species alternatively to *C. plectranthifolia*. Illegitimate visits of *Chalepogenus vogeli* and *Ch. rufipes* were also recorded (Fig. 54A, B).

Centris bees land on the lower lip and actively open the flower, partially assisted by their own weight. They introduce their head and thorax into the lower lip. At first the abaxial thecae, which are not covered by the upper lip, touch the head of the bee. To reach the elaiophore the bees have to progress to the inside. They have to place their heads at the inflection line of the lower lip to reach the elaiophore. In this position, the thorax pushes the upper lip exposing the adaxial thecae. Pollen is deposited also on the notothorax. The midlegs and hindlegs clutch the lower lip while the forelegs extract the oils with sweeping movements.

Females of *Ch. vogeli* and *Ch. rufipes* unsuccessfully tried to open the flowers. These small bees were not strong enough to separate the lips. With much effort they could reach the anthers upside down introducing the head into the flower, and pushing the thorax between the lower lip and the upper lip (Fig. 54B); in this position they collected pollen aided with a buzzing of the body. Many males of *Chalepogenus* patrolled the plants to copulate the females.

Calceolaria prichardii

Collecting data: Argentina. Río Negro Province. Bariloche department. El Bolsón, on the path to Cerro Piltriquitrón. A.A.C. & A.S. 529. 8 to 9-01-1994.

This species includes rosulate, glandular plants with leaves of variable sizes, bearing lanceolate and acute blades, attenuated in a petiole. Pauperized inflorescence, HF and CoF with one flowering node and 1 or 2 flowers per cyme. Flowers are about 1.5 cm in length. The upper lip is hooded and tightly covers the stamens (Fig. 4C, D). The lower lip is large and fiddle-shaped. The throat is stretched and curved upwards, yellow in color and sometimes inside with broad red stripes irregular in contour; the inside of the throat is concave in a way that it delimits a short chamber just behind the mouth. The calceiform part of the lower lip is yellow with red markings on the sole and articulates with the throat in a flexible hinge (Figs. 47C; 58A-F). At the level of this articulation there is a constriction on both sides of the lower lip, at this point the straight appendage with an inflection angle of 180° differentiates, carrying a dense trapezoidal elaiophore (Fig. 12E).

Visitors: The flowers of this species are pollinated indistinctively by *Centris* or *Chalepogenus*.

During 18 hours of observation, it was possible to see only two visits of *Centris autrani*; they were so rapid that no details of the behavior could be recorded.

VOGEL (1974) made very good observations on the visits of *C. nigerrima* and *C. cineraria* to these flowers in a population in Neuquén Province (Argentina).

Visits of *Ch. caeruleus* were more abundant and well documented here. The plant population was relatively widespread. At 9:30 a. m. many males were flying around the plants, and occasionally landing on the lower lip. At 10:00 h the first females appeared, which were recognizable from the distance by the buzzing they performed during the visits. The first activity was the collection of oils. At 11:30 h the activity decreased, remaining around only the males. Thirty minutes later visitations by females started again, this time actively collecting pollen alternatively to oils.

For oil collection, the females land on the widened tip of the lower lip, and have to force their way between the lips, pushing with the head and thorax. During this handling pollen is deposited on the bee. The flower appears relatively large compared to the dimensions of the bee, for this reason it has to enter quite deep into it to reach the elaiophore (Fig. 58B-D). Head and thorax fit in the concavity of the throat. While the forelegs reach the elaiophore to collect the oil, the midlegs fasten to the already mentioned constriction, and the hindlegs tightly clutch the widened part of the lower lip (Fig. 58A). While the bee is inside, only the abdomen and the hindlegs are visible from outside (Fig. 58B). The bee takes the oils making tapping movements on the glandular trichomes sweeping the oil surface from side to side. Without changing this position, the bee transfers the oils to the midlegs, when the forelegs approach each side during this tapping. To come out from the flower the bees take first the midlegs out, which fasten to the margin of the lower lip pulling the whole insect to the outside. Head and thorax touch the anthers. Before taking off, the bee passes the collected oils to the hindlegs (Fig. 58F).

During pollen collection the bees stand with the hindlegs on the tip of the lower lip partially elevating the upper lip with the head (Fig. 58E); with the forelegs they collect pollen, while the flower is kept open between the head and the hindlegs. They pass the pollen to the mid- and hindlegs ipsilaterally and simultaneously on both sides. During pollen collection the bee produces a vibration and a particular audible buzz. Before leaving the flower it cleans its body.

Many times visits of old fallen down flowers were also seen, taking oils and sometime also pollen from them, performing the same postures as already described even when the flowers were not horizontal.

Species visited by members of *Chalepogenus*

Calceolaria polyclada

Collecting data: Argentina. Salta Province. Chicoana department. Cuesta del Obispo. A.A.C. & A.S. 381, 287. 7-03-1989. Tucumán Province. Monteros department. RP 307. Km 48-49, 1800 m. A.A.C. & A.S. 290. 10-12-1988.

This species includes shrubs with loose and overhanging, slender branches, 0.5-1.2 m height with short petiolate leaves bearing oval to lanceolate, serrate blades. Inflorescence slightly pauperized, with only one flowering node in the HF and the CoF, but many flowered cymes. The flowers are about 1 cm long, yellow with small red flecks on the throat. The upper lip is hooded, widely covering the stamens. The stamens build a lever mechanism, the abaxial theca being reduced (Figs. 55E, F; 34G). The lower lip is large, but with a small opening, the appendage has an inflection angle of about 90° (Fig. 5F) and is completely covered with the elaiophore.

Flowering period: September to February

Visitors: Two bees were seen visiting legitimately this species: *Chalepogenus vogeli* and *Ch. rufipes*.

The first visits of *Ch. vogeli* started at 9:45 and took place until dusk without interruptions. Sometimes there were many individuals simultaneously foraging on the same plant. *Ch. rufipes* in contrast was less frequent. Only two visits could be recorded during 12 hours of observation. At noon many males were flying over the plants, almost without halting. Some of them fell on the females, which were collecting oils; both flew together to the same direction probably for the courtship.

When the bee arrived to the flower it landed on the wide lower lip and clutched its margins with the completely extended mid- and hindlegs. The bee alternates movements of elaiophore manipulations with the moments of oil transference to the hindlegs; this alternation occurs 2-3 times during the visit to one flower. Manipulation of the elaiophore takes each time about 8 sec after which the bee moves backwards on the flower and with almost undetectable movements the forelegs simultaneously glide on the midlegs which also simultaneously touch the hindlegs to transfer the oils to the scopae (Fig. 56A, E, F). This last movement is almost synchronic with the beginning of the next step of elaiophore manipulation.

During the first visits in the morning the bees were exclusively extracting oils; their scopae where shiny, and without pollen. Gradually they started to collect pollen from the same *Calceolaria* flowers. This is the first activity they perform when they land on them. For pollen collection, the bee adopts an inverted position hanging from the upper lip, with the back against the lower lip and the head inside the flower (Fig. 56D). In this posture pollen is collected with the forelegs and transferred to the hindlegs.

This species has lever stamens, the adaxial thecae being hidden inside the upper lip, while the abaxial thecae are exposed and occlude the entrance. The bee pushes them with the head making the anthers to turn and the adaxial thecae to emerge and descending onto the thorax, between the tegulae (Fig. 56B). After the visits the anthers turn to the starting position. Oil production begins before the anthers mature, when the stamens' hinge is not differentiated, therefore, they do not descend during this visits (Fig. 56C).

Calceolaria umbellata

Collecting data: Argentina. Tucumán Province. Tafí del Valle department. R.P. 307, Km 49-50, 1800 msm. A.A.C. & A.S. 285. 17-02-1988. Salta Province. Chicoana department. Valle Encantado, at the top of Cuesta del Obispo. A.S. s/n. 15-12-1989.

This species includes rosulate herbs with short to sessile, elliptic to lanceolate leaves. The inflorescence is pauperized, with one flowering node in the HF and the CoF and 2-6 flowering cymes. The yellow flowers have a small, hooded upper lip completely covering the reduced opening. The lower lip is wide, inflated, but with a horizontal instep (Fig. 55C, D), which functions as a landing surface. The appendage has an inflection angle of 90° and carries a reduced, circular and dense elaiophore (Fig. 14D).

Flowering period: November to March.

Visitors: The only legitimate pollinator observed was *Chalepogenus rufipes*.

The small bees of *Chalepogenus* are almost completely covered by the upper lip during the visits to the flowers. Only the abdomen and the reddish hindlegs are visible from outside. Injured flowers are commonly found, they show two pairs of damaged spots: one at both sides of the opening made by the midlegs and the other pair just at the beginning of the instep probably made by the hindlegs (Fig. 55C). The bees are very shy and difficult to observe, however, most visits were recorded between 13:30 and 15:00 h.

Calceolaria ruiz-pavonii

Collecting data: Argentina. Tucumán Province. Tafí del Valle department. R.P. 307 a few km north of La Bolsa. A.A.C. & A.S. 299. 18-02-1988.

This species includes overhanging branched bushes, 0.5 m tall. Leaves are polymorphic, sessile or shortly petiolate bearing ovate, crenate and pilose blades. The HF and CoF have 3-4 flowering nodes and many-flowered cymes. Flowers are yellow with red irregular flecks inside the throat. The upper lip is hooded (Figs. 5E; 57A), the stamens are provided with a lever mechanism, the abaxial theca is reduced (Fig. 34A, B), the wide lower lip has an horizontal instep. The opening is small but not hidden, and the throat is not stretched (Fig. 57A). The appendage has an inflection angle about 90° (Fig. 5E); the elaiophore covers almost the whole appendage, but the trichome density decreases to the apical border (Fig. 14A).

This species is very close to *C. teucrioides*, in fact, for MOLAU (1988) *C. ruiz-pavonii* is just a synonym, and he considers it as a clinal variation. But I could see that there are differences in the shape of the flowers, which determine the type of bee that pollinates this plant. For this reason I describe the species separately, however, other kind of studies are required to decide if these populations should be considered as belonging to the same or a different species.

Visitors: *Chalepogenus rufipes* and *Chalepogenus vogeli* were the only bees seen visiting this species.

This bee collects both oils and pollen from the flower (Fig. 57B-F) and the sequence of movements in both activities is the same as described for *C. polyclada*.

Calceolaria valdiviana

Collecting data: Argentina. Chubut Province. Futaleufú department. Futalaufquen National Park. A.A.C. & A.S. 522. 05-01-1994.

This species includes rosulate plants with ovate leaves, attenuated in a short petiole. The pauperized inflorescence shows the HF and CoF with only one node and 2-6 flowers per cyme. The flowers are yellow, 1-1.3 cm long. The upper lip is small and hooded; the lower lip is inflated but shallow and rounded in contour (Fig. 59A). The appendage is not folded (inflection angle of 180°), the elaiophore is oval-shaped.

Visitors: *Chalepogenus caeruleus* was the only pollinator seen for these flowers.

All the flowers controlled had injured corollas (Fig. 59A) indicating frequent visits, but during the whole observation period (from 8:30 to 19:00 h) only three visits were recorded. The behavior of this bee species is similar to that of *Centris*. It passes the plant population following the same route, the flight is rapid and precise. The bees are very shy, disappearing immediately in case of danger. In agreement with the description made by VOGEL (1974) for this species, the visit to the flowers comprises two activities: oil collection and pollen collection. The elaiophore is relatively near the opening and the bee does not need to enter very deep inside the flower (Fig. 59B), while entering, the head of the bee makes contact only with the stigma, because the anthers are hidden in the upper lip. Oil collection is identical to that already described for *C. filicaulis* subsp. *luxurians*. Pollen collection, which is not always performed, is decisive for the plant, because only during this activity the bee makes contact with the pollen; it has to elevate the body to press the anthers with the head; pollen discharging is actively assisted by a distinctive buzzing. Transference of oil and pollen to the hindlegs could not be seen.

Species visited by birds

Calceolaria uniflora

Collecting data: Argentina. Santa Cruz Province. Lago Argentino department. Los Glaciares National Park, Fitz Roy, trekking path to Río Blanco. A.A.C. & A.S. 456. 25-01-1990. Tierra del Fuego, Antártida e Islas del Atlántico Sur Province. Río Grande department. Estancia Las Violetas c. 10 km north of Río Grande on road 3. A.A.C. & A.S. 514. 23 to 28-12-1993. San Sebastián. Estancia Sara, on road 3. A.A.C. & A.S. 508. 23-12-1993.

The pollination mode of this species was already described (SÉRSIC & COCUCCI 1996a). Only a brief account of the particular pollination strategy is given here.

This species includes rosulate plants, forming loose and low, cushion-like patches of 0.1-0.5 m across. The leaves show a variety of sizes depending on the environmental conditions, they are lanceolate to ovate, attenuate in a short petiole. The inflorescence is extremely pauperized with only one flowering node and only one flower. The flowers are large, 2.5-3.5 cm long pending from the leafless flowering shoot. The upper lip is hooded and wide, slightly shift backwards, thus leaving the stamens completely exposed. The stamens diverge from the middle axis, so they are distant from the pistil (Fig. 60A). The filaments are relatively long and the anthers have divaricate thecae, which open completely when mature. The tip of the filament is very thin and the necrotic tissue allows versatile movements (Fig. 29A-D). The lower lip is large, vertical in position, with a stretched throat and a large opening (Fig. 60A-B). The calceiform section is wide and the appendage is folded to the outside (inflection angle of about 360°; Fig. 24A-B; Fig. 60A-B). This feature is unique in the genus and is only shared with the related species *C. fothergillii*. The large appendage (0.7 cm long x 1.4 cm wide x 0.3 cm thick) is rectangular, fleshy and white in color, which contrasts with the red and yellow color of the instep. The operative distance between the fertile parts and the appendage is 1.8 cm. There are still some glandular trichomes between the appendage and the instep, which are not functional. This appendage functions as a food body, eaten by a bird, as will be described later.

Prior evidence: The possible existence of this peculiar pollination mechanism had only been inferred. VOGEL (1974) predicted that this fleshy structure of these two species could function as a food body. He never saw the plant in nature, but he based his inference on appendage morphology and on anecdotes gathered by colleges from inhabitants of the Magellan region. They referred, that the juicy nut-tasting appendages were eaten by children; they also observed that there were no bees around the flowers, but birds similar to the Muscicapidae. Additionally, I could also see that photographs taken by MOORE (1983, plate 7) edited in the Flora of Tierra del Fuego, and by GROSSMANN (pers. comm.) from the surroundings of Calafate (Santa Cruz, Argentina) showed that part of the appendage was missing, as if it had been plucked off.

Table 11. Percentage of plucked flowers in four localities of *Calceolaria uniflora*.

Population studied	Damaged flowers		Intact flowers		Total
	n	%	n	%	n
Fitz Roy	31	54.38	26	45.61	57
San Sebastián	25	40.32	37	59.67	62
Ea. Las Violetas	84	68.85	38	31.14	122
Río Grande	66	81.48	15	18.51	81
Total	206	63.97	116	36.02	322

Visitor: Males and females of *Thinocorus rumicivorus* (Thinocoridae, Charadriiformes). Common names: "Seed snipe", "agachona chica", "chorlo aperdizado", in Chile: "perdicilla", "achatadera". This bird species was the only legitimate pollinator that could be recorded.

The plant population of the region of Cerro Fitz Roy (Santa Cruz, Argentina) grew under a forest of *Nothofagus pumilio* on a windy, S-SW exposed slope. Most of the flowers were already old, in the fruit period, only some flowers were in anthesis and had plucked off lower lips (Tab. 11). There were no flower buds or open flowers with dehiscent anthers. During 9 hours of observation in this site it was not possible to see any animal visiting the flowers; probably the harsh climatic conditions (low temperatures, rain) did not favor the visits. In the surroundings it was possible to see other birds different to *Thinocorus*, like *Melanodera xanthogramma* G.R. GRAY, *Melanodera melanodera* QUOY & GAIMARD, *Elaenia albiceps chilensis* HELLMAYR and some flocks of *Carduelis barbatus* MOLINA that did not approach the flowers. The presence of many red or white fleshy diaspores near the floor, as those from *Pernettya mucronata* (L.F.) GAUD., *Berberis empetrifolia* LAM., *Empertum rubrum* LAM., and *Ephedra frustillata* (G.DON.) TORT., indicated the occurrence of fruit-eating birds with terrestrial habits.

In the Tierra del Fuego populations, the plants grow in an open steep with low vegetation dominated by *Empetrum rubrum*, *Lycopodium magellanicum* (P. BEAUV.) SWARTZ, *Armeria maritima* and some Poaceae. The spaces without vegetation were completely covered by medium-size pebbles. Observation period was from 7:30 to 20:30, during 4 days. There was permanent wind with a velocity up to 180 km/h. *Thinocorus rumicivorus* was a frequent bird in these regions. During their walks between the vegetation they did not miss the opportunity to take the food body from the flowers they encounter on their way. When the birds found one or more flowers they normally stopped until they ate all the available appendages (Fig. 60C). The encounter of the birds with the flowers is not always casual, because frequently they intentionally look for them. The distance between the tip of the beak to the uppermost part of the head is about 1.8 cm, which is approximately the operating distance of the flowers. Most visited flowers had the calceiform part completely removed, remaining only a small fragment of the throat (SÉRSIC & COCUCCI 1996a).

Characterization of the pollination syndromes in *Calceolaria*

Based on the analysis of flower structure and on field observations, several flower syndromes can be recognized. Distinct assemblages of morphological, anatomical, and physiological characters can be tentatively assigned to certain pollinator types. *Calceolaria* has specialized flowers adapted to a very limited guild of pollinator species (eutropic flowers). None of the syndromes found in the genus requires nectar as reward and therefore, the flower syndroms can be classified as:

- Melittophily
  - With oils as reward
  - With pollen as reward
- Bird pollination with a food body as reward

Melittophily with oils as reward

All species of the genus that develop a functional elaiophore are probably legitimately visited by oil collecting bees. The estimated number of species with elaiophore is 210.

Only a few species of *Centris* and *Chalepogenus* appear to be associated to *Calceolaria.* Some oil collecting bees from these genera and oil collecting bees of other genera like *Lanthanomelissa* or *Tapinotaspis*, that overlap in some degree in their geographical range with species of *Calceolaria*, have not been associated with these plants. The observations available for 38 oil secreting *Calceolaria* species indicate that only nine bee species collect oils on these flowers. As already pointed out, the morphological traits and behaviors of both bee genera are different enough to allow the distinction of two flower subtypes.

Tapinotaspidini flowers. Visited by *Chalepogenus* species. These flowers normally have a small hooded upper lip, short stamens, with or without a lever mechanism. The lower lip is larger than the upper lip and functions as a landing surface; frequently it is not very much inflated, but quite flattened because the bees normally do not enter into the calceiform part and do not need to embrace it. The elaiophore is always directly accessible. The appendage has an inflection angle between 180° and 45°. The opening is small, in correspondence with the size of the bee. Pollination is always nototribic. The elaiophores are mainly oval shaped or trapezoidal. The mean density of secretory trichomes is 1054.57 trichomes/mm^2 (n = 10), and the mean quantity of oil secreted is 0.240 mg/flower (range: 0.075-0.350, n = 20 of 5 species).

Centridini flowers. There is more diversity in flower morphology because this bee genus has a higher behavioral and ecological plasticity. The larger size of these bees is not always correlated with larger flowers. Actually they commonly visit flowers much more reduced than those visited by Tapinotaspidini bees. The upper lip can be hooded or of Rugosae-type. The stamens may or may not be provided with a lever mechanism, the filaments are short or extraordinarily long. The lower lip is normally inflated, not flattened and not used as a landing surface, but rather as a handle. The appendage shows a variety of inflection angles (from 0° to 180°). The elaiophores have also a diversity of shapes and the mean density of secretory trichomes in the species studied is 760.85 trichomes/mm^2 ($n = 10$), while the mean quantity of oil secreted is 0.760 mg/flower (range: 0.270-1.550, $n = 20$ of 13 species).

The Centridini flowers can be open or closed.

Open flowers: These have a small upper lip, which encloses the stamens; the anthers may or may not be provided with a lever mechanism; the lower lip is narrow and elongated, with a wide opening (Fig. 47A-B), the elaiophore is placed at a distance from the stamens. Pollination is nototribic. The flowers may be visited by smaller bees but they do not make contact with the anthers while collecting oils.

Table 12. Comparison of the lengths of stamens between nototribic and sternotribic species.

	Nototribic flowers	Sternotribic flowers
n	141	25
x	1.39	2.49
s	0.49	1.26
Degrees of freedom = 164		
t = 7.56		
p = 0.001		

Closed flowers

Sternotribic closed flowers: They have a reduced lower lip, about as large as the upper lip. It serves just as a handle (*C. plectranthifolia*) or it is even so small that it cannot be embraced by the bee (*C. polifolia*). The upper lip is larger than in other flower types and also inflated, its margin is involute. This lip lays firmly on the lower one and encloses the fertile parts (cleistopetalia, SÉRSIC 1991). This particularity conditions the pollinators to force their entrance into the flowers. The stamens, which are with the longest filaments to be found in the genus, lay over the lower lip, favoring the deposit of pollen on the ventral surface of the pollinators. The comparison of the length of the stamens between nototribic and sternotribic flowers in the whole genus is summarized in Tab. 12. The data were obtained from MOLAU (1988), DESCOLE & BORSINI (1954) and own observations. The c. 50 elaiophore-less species were not considered.

Nototribic closed flowers: The fertile parts of these flowers are not easily accessible. The upper lip is small and the fertile parts are short, similar to the open flowers; but the upper lip and fertile parts are hidden by the lower lip in different manners. Sometimes the throat articulates the calceiform portion, elevating it to the same level of the upper lip, covering it partially (*C. shickendantziana*, Fig. 47D). *C. arachnoidea* together with other species of section Corymbosae, show also notoribic closed flowers, with a very reduced throat and an enlarged and tubular calceiform portion; the opening is big enough to surround and cover almost completely the upper lip.

Melittophily with pollen as reward

There are 49 species in the genus that lack elaiophores (18-20% in total). The absence of the oil-producing gland is apparently secondary and occurred independently in 20 of the 24 sections. Although, they are not necessarily related, they often share some morphological features. The upper lip is hooded but very wide, sometimes slightly bent backwards, thus exposing the anthers. The lower lip has a large opening, and a reduced calceiform part. The appendage is always developed and folded, but devoid of trichomes. The stamens have short filaments and deflexed anthers, which lack movability because the hinge is not developed. About 10% of the elaiophore-less species have lever mechanisms, what is in agreement with the secondary origin of this kind of flowers. The color of the flower is commonly light yellow or white, frequently without red flecks.

C. tucumana and *C. tenella* were the only elaiophore-less flowers studied in the field. During the observations of the first of them no pollinator was seen, and the corolla did not show signs of visitation. Visits of *Bombus chilensis* were registered for *C. tenella*. Not very frequently, but systematically these bees landed on the flowers. It was not possible to see in detail their activity, but evidently they were working on the flowers, probably collecting pollen. There were signs of visitation on the corolla of many flowers.

MOLAU (1988) recorded visits of *Bombus* sp. in *C. sonchensis* EDWIN, *C. adenanthera* MOLAU, *C. rhododendroides* KRÄNZ and *C. ballotifolia* KRÄNZ. and visits of *Xylocopa* sp. in *C. cypripediifolia* KRÄNZ. however this species has a well developed elaiophore. He also points out that in the label of an herbarium specimen of another oil-bearing species, *C. pisacomensis* MEYEN, collected by Straw visits of *Xylocopa* were recorded. VOGEL (pers. comm.) observed legitimate visits of *Bombus ephippiatus* SAY. on *C. irazuensis* DONNELL SMITH and *C. tripartita*. In some cases the elaiophore is rudimentary, although with some trichomes, it is not functional in terms of pollination, as in the case of *C. adenanthera*, *C. boliviana* (RUSBY) PENNELL, *C. odonthophylla* MOLAU, *C. williamsii*, *C. rariflora* MOLAU, and others (MOLAU 1988).

Bird pollination with a food body as reward

As already explained, there are only two *Calceolaria* species adapted to this peculiar bird pollination syndrome, growing in the southernmost regions of South America (*Calceolaria uniflora* and *C. fothergillii*). The main morphological adaptations are the large flowers born at about 5-10 cm above the ground level; they have exserted and versatile anthers, a wide and elongated opening, a fleshy white appendage folded to the outside, which optically contrasts with the red and yellow colored lower lip (VALLENTIN & COTTON 1921, SÉRSIC & COCUCCI 1996a, ROITMAN & al. 2002). This fleshy and sweet appendage acts as a food body to attract at least one bird species, *Thinocorus rumicivorus*.

Food bodies offered to birds are not common within the angiosperms. This case can be compared with the pollination strategy of *Acca* (*Feijoa*) *sellowiana* BERG and *Myrrhinium atropurpureum* (Myrtaceae) where the sweet fleshy petals are ingested by frugivorous birds (MÜLLER 1886, STEWART & CRAIG 1989, ROITMAN & al. 1997, 2002, and pers. obs.).

The following table summarizes all the observations of visits on *Calceolaria* made by different authors and by myself.

Subgenus Calceolaria			
Section Anacyrta			
C. virgata R. & P.	*Centris autrani*	Perú	MOLAU 1988
C. cfr. *glauca* R. & P.	*Centris* cfr. *autrani*	Perú	WEIHRAUCH VOGEL 1974
Section Calceolaria			
C. tripartita R. & P.	*Chalepogenus rasmusseni*	Perú	MOLAU l. c.
	Centris autrani	Perú	RASMUSSEN 1999
C. tenuis Benth.	*Chalepogenus* sp.		
Section Englerina			
C. irazuensis DONNELL SMITH	*Bombus ephippiatus*	Costa Rica	VOGEL (comm.)
Section Lehmannina			
C. cypripediiflora KRÄNZ.	*Xylocopa* sp.	Perú	MOLAU l. c.
Section Lobatae			
C. cumbemayensis MOLAU	*Centris autrani*	Perú	MOLAU l. c.
C. cfr. *incarum* KRÄNZ	*Centris* cfr. *Autrani*	Perú	WEIHRAUCH VOGEL 1974
Section Integerrimae			
C. hypericina BENTH.	*Centris* cfr. *orellanai*	Chile	SÉRSIC this work
C. pinifolia CAV.	*Centris tricolor*	Argentina	SÉRSIC l. c.
C. thyrsiflora GRAH.	*Centris nigerrima*	Chile	SÉRSIC l. c.
Section Parvifoliae			
C. cuneiformis R. & P. subsp. *cuneiformis*	*Centris autrani*	Perú	MOLAU l. c.
C. inamoena KRÄNZ. subsp. *inamoena*	*Centris autrani*	Perú	MOLAU l. c.
C. myriophylla KRÄNZ.	*Centris autrani*	Perú	MOLAU l. c.
C. schickendantziana KRÄNZ.	*Centris neffi* *	Argentina	VOGEL 1974
	Centris autrani	Argentina	SÉRSIC l. c.
		Bolivia	MOLAU l. c.
	Chalepogenus vogeli	Argentina	SÉRSIC l. c.
	Chalepogenus rufipes	Argentina	SÉRSIC l. c.
C. sparsiflora KUNTZE	*Centris autrani*	Perú	MOLAU l. c.
Section Perfoliatae			
C. pavonii BENTH.	*Chalepogenus rasmusseni*	Perú, Ecuador	MOLAU l. c.
Section Polyclada			
C. polyclada KRÄNZ.	*Chalepogenus vogeli*	Argentina	VOGEL l. c.
		Argentina	SÉRSIC l. c.
Section Salicifoliae			
C. deflexa R. & P.	*Centris* sp.	Perú	MOLAU l. c.
C. rhododendroides KRÄNZ.	*Bombus* sp.	Perú	MOLAU l. c.
C. salicifolia R. & P.	*Chalepogenus rasmusseni*	Perú	MOLAU l. c.
Section Teucriifoliae			
C. argentea H.B.K.	*Chalepogenus rasmusseni*	Perú	MOLAU l. c.
C. cajabambae KRÄNZ.	*Chalepogenus rasmusseni*	Perú	MOLAU l. c.
C. pisacomensis MEYEN ex WALPERS	*Xilocopa* sp.	Perú	STRAWEN MOLAU l. c.
C. ruiz-pavonii DESCL. & BORS.	*Chalepogenus vogeli*	Argentina	SÉRSIC l. c.
	Chalepogenus rufipes	Argentina	SÉRSIC l. c.

C. santolinoides KRÄNZ.	*Centris cordillerana* *	Argentina	VOGEL l. c.
C. lossenii KRÄNZ.	*Centris tricolor*	Argentina	SÉRSIC l. c.
C. teucrioides GRIS.	*Centris tricolor*	Argentina	SÉRSIC l. c.
	Chalepogenus vogeli	Argentina	VOGEL l. c.
	Chalepogenus rufipes	Argentina	VOGEL l. c.
	Chalepogenus vogeli	Argentina	VOGEL l. c.
Section Thamnobia			
C. adenanthera MOLAU	*Bombus* sp.	Ecuador	MOLAU l. c.
C. rosmarinifolia LAM.	*Chalepogenus rasmusseni*	Ecuador	MOLAU l. c.
Section Urticopsis			
C. ballotifolia KRÄNZ.	*Bombus* sp.	Perú	MOLAU l. c.
Subgenus Cheiloncos			
Section Rugosae			
C. cavanillesi PHIL.	*Centris cineraria*	Chile	SÉRSIC unpubl.
C. dentata R. & P.	*Centris nigerrima*	Chile	SÉRSIC 1991
C. integrifolia MURR.	*Chalepogenus caeruleus*	Chile	JANVIER 1926
C. latifolia BENTH.	*Centris nigerrima*	Chile	SÉRSIC l. c.
C. lepida PHIL.	*Centris nigerrima*	Chile	SÉRSIC this work
C. petioalaris CAV.	*Centris nigerrima*	Chile	SÉRSIC l. c.
C. plectranthifolia WALP.	*Centris autrani*	Argentina	SÉRSIC l. c.
C. polifolia HOOK.	*Centris nigerrima*	Chile	SÉRSIC l. c.
C. ascendens LINDL. subsp. *ascendens* .			
	Centris sp.	Chile	SÉRSIC l. c.
Subgenus Rosula			
Section Bellidifoliae			
C. parviflora WEDDELL	*Centris tricolor*	Argentina	SÉRSIC this work
C. umbellata WEDDELL	*Chalepogenus rufipes*	Argentina	VOGEL l. c.
	Chalepogenus rufipes	Argentina	SÉRSIC l. c.
Section Corymbosae			
C. arachnoidea GRAH.	*Centris nigerrima*	Chile	SÉRSIC unpubl.
C. corymbosa R. & P.	*Centris nigerrima*	Chile	SÉRSIC l. c.
	Centris cineraria	Chile	JANVIER 1926
C. tenella POEPP. & ENDL.	*Bombus chilensis*	Chile	SÉRSIC l. c.
Section Kremastocheilos			
C. crenatiflora CAV.	*Chalepogenus caeruleus*	Argentina	VOGEL l. c.
C. valdiviana PHIL.	*Chalepogenus caeruleus*	Argentina	VOGEL l. c.
			SÉRSIC l. c.
C. filicaulis CLOS subsp. *luxurians*			
	Chalepogenus perimelaena	Argentina	VOGEL l. c.
	Centris nigerrima	Chile	SÉRSIC l. c.
C. prichardii (RENDLE) KRÄNZ.			
	Centris nigerrima	Argentina	VOGEL l. c.
	Centris cineraria	Argentina	VOGEL l. c.
	Chalepogenus caeruleus	Argentina	VOGEL l. c.
			SÉRSIC l. c.
	Centris sp.	Argentina	SÉRSIC l. c.
C. uniflora LAM.	*Thinocorus rumicivorus*	Argentina	SÉRSIC 1996
Determinandae			
Calceolaria sp.	*Centris nigerrima*	Chile	JOHOW

* After descriptions made by ROIG-ALSINA (2000).

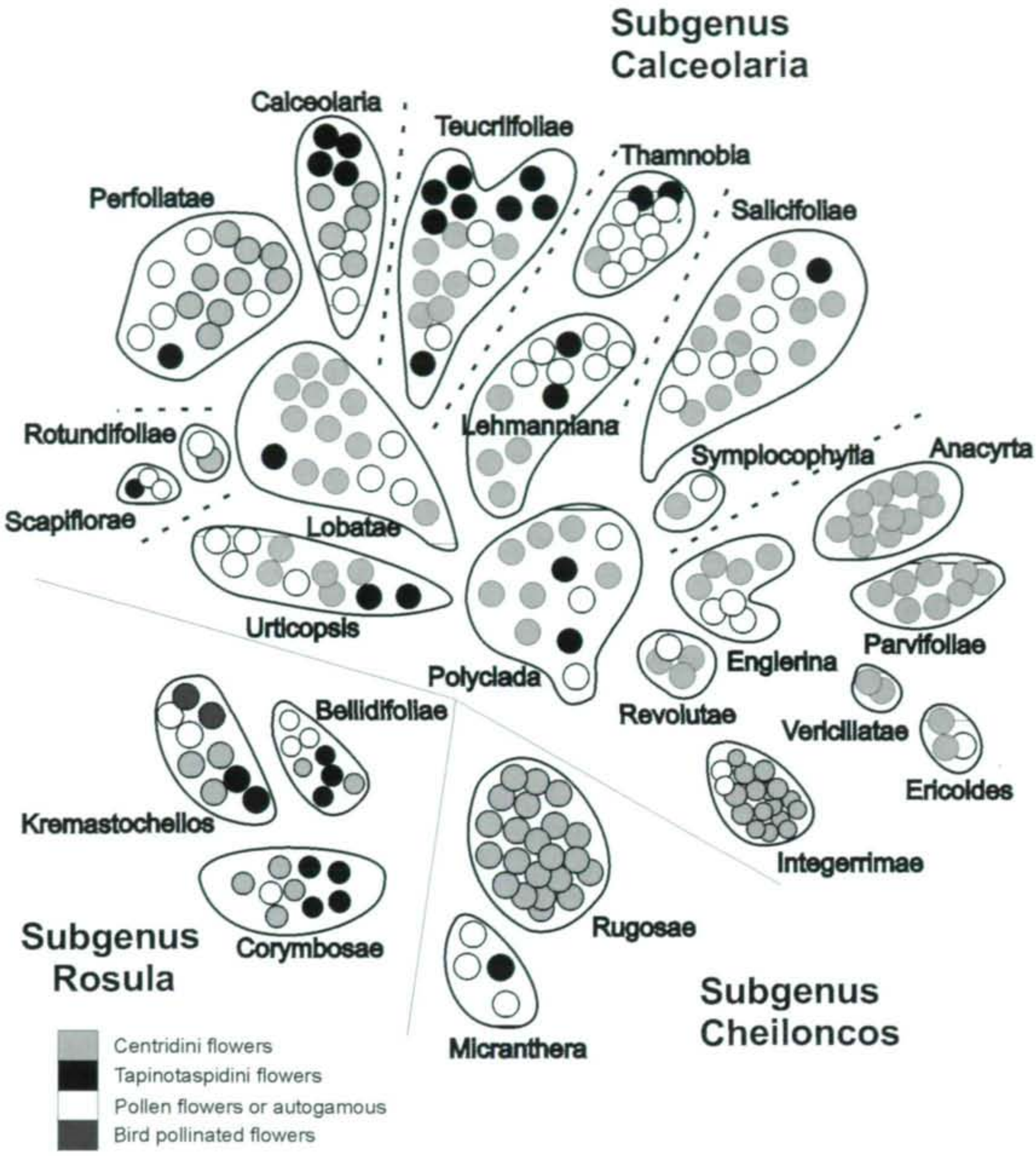

Fig. 61. Distribution of the flower syndromes in the phylogenetic tree of the genus *Calceolaria*.

Fig. 62. Geographical distribution of flower syndromes of *Calceolaria* The circles represent the percentage of species in each syndrome between the latitudes indicated by the horizontal lines.

Using the descriptions of the species contained in MOLAU (1988), DESCOLE & BORSINI (1954) and own observations is possible to tentatively map the distribution of these syndromes on the phylogenetic diagram of *Calceolaria* (Fig. 61).

Following aspects become evident therefrom:

- The section Kremastocheilos is the only one containing all four flower syndromes, and the only one with bird pollinated species. The recent monograph of EHRHART (2000) circumscribes this section only to *C uniflora* and *C. fothergillii*, the bird-pollinated species.
- Syndromes different from bird pollinatation are widely distributed in other sections.
- Section Rugosae has a preponderance of Centridini closed sternotribic flowers.
- The closely related sections Anacyrta, Parvifoliae, Verticillata and Ericoides, which are mainly distributed in the middle latitudes, are dominated by Centridini flowers.
- The sections Lehmannina and Thamnobia, of the northern biogeographical region have mainly elaiophore-less, probably pollen- or autogamous flowers.

Biogeographical distribution of the syndromes

The distribution of the flower syndromes in the genus is summarized in the Fig. 62. It becomes evident that there is a preponderance of *Centris* flowers in the middle zones, between 10° to 40° southern latitude. The Tapinotaspidini species are in general less represented in the genus, and their main abundance is located between 20° to 30° S where the geographical ranges of the bees and the plants overlap. Interestingly, at the most northern and southern latitudes pollen- or bird-pollinated flowers are dominant, in coincidence with zones were oil collecting bees are scarce or completely absent.

How could the relationships between *Calceolaria* and the oil-collecting bees have originated?

The co-evolution of *Centris* bees with the c. 950 south American species of Malpighiaceae is out of discussion (VOGEL 1987, VOGEL 1988a). The c. 200 *Centris* species are mainly distributed in the tropical rain forests of Brazil and Central America, overlapping with the New World Malpighiaceae. Most of these bees developed strong oil collectors in the fore- and the midlegs, especially adapted to extract oils from the epithelial elaiophores commonly found on the abaxial face of the sepals in the flowers of that family. The relation of *Centris* with epithelial elaiophores seems to be ancestral. It is NEFF and SIMPSON'S (1981) belief that the relationship between these bees and the flowers with trichome elaiophores started secondarily with lineages of small *Centris* (from the subgenera Paracentris and Wagenknechtia). These insects may have visited indistinctly plants with epithelial or trichomatic elaiophores; consequently, the geographical distribution of the bees may have extended to colder regions of the high mountains, where plants with trichomatic elaiophores were dominant. In this way, they became secondarily associated with some Iridaceae, Scrophulariaceae and Calceolariaceae, with the consequent loss of the oil collectors of the midlegs. This is the case at least in *Centris autrani* and *C. nigerrima*, bees that are closely associated not only to *Calceolaria*, but the last one, also to *Monttea*. These are secondarily two-legged oil collecting bees that inhabit the high Andes from northern Peru to Rio Negro in southern Argentina. Those *Calceolaria* species that grow away from the Andes are pollinated by four-legged oil collecting bees, which alternate between epithelial- and trichomatic elaiophore-bearing flowers. MICHENER (1979) suggested that the subgenera

Paracentris and Wagenknechtia included the most primitive forms in the genus, and that they probably had originated in dry environments, reaching the tropical regions secondarily. This hypothesis seems less probable, since both subgenera are closely related to tropical subgenera, and not between each other. Dessert environments appeared more recently, and the presence of rudimentary oil collectors in the midlegs do not make sense in regions without Malpighiaceae (NEFF & SIMPSON 1981).

The genus *Chalepogenus* seems to have originated in the temperate regions of Argentina, Paraguay and southern Brazil (Paranaense region). These basal species are particularly associated with Iridaceae; more advanced *Chalepogenus* species are mainly related to *Nierembergia* (Solanaceae) and distributed in the Chaco and Pampas regions. But the most recently evolved *Chalepogenus* species are distributed mainly on the Andean region and are associated with *Calceolaria* and Iridaceae, especially *Sisyrinchium* (COCUCCI & al. 2000; COCUCCI & VOGEL 2001). It seems, that bees migrated from East to West, following also a migration of an oil flower flora in the same direction, that allowed a secondary contact of these oil collecting bees with members of the subantarctic floras (*Calceolaria* and *Sisyrinchium*).

From both oil-collecting bee genera involved in the pollination of *Calceolaria*, the small bees of *Chalepogenus* seem to be the first ones to have established the connection (VOGEL 1988). It is possible to imagine a scenario during the Tertiary when the genus was mainly distributed in the southern regions of South America and started its relation with the *Chalepogenus* bees that were coming from the East. Later, during the migration of *Calceolaria* northwards, these plants started an association with the bee genus *Centris*. The highly evolved adaptation plasticity of these bees favored them to establish a more diverse and more successful relation than with *Chalepogenus*.

It is also possible to speculate that both relationships evolved nearly simultaneously, and the plants adapted themselves opportunistically to one or to the other bee type. This opportunistic evolution of the flowers and the bees influence the development of both syndromes in different sections. This plasticity of the plant to adapt alternatively to one or the other bee may have been an important speciation factor (VOGEL 1974, MOLAU 1981a, b, 1988).

Conclusion

Studies on the floral biology of the genus Calceolaria based on morphologic and functional aspects of both plants and pollinators were performed. The main chapters of the results will be treated separately to discuss previous results and concepts.

Flower structure

Corolla

- The features of the corolla become important to characterize the different pollination syndromes. Size, shape, types of upper lip, relation of the upper lip with the fertile parts or with the lower lip, proportions and features of the lower lip were all important characters, which combined in different ways, allow to establish the pollination syndromes. With the large variation of corolla types it is possible to establish tendencies in their adaptations to the pollinators.

Elaiophore

- The morphologic study of the elaiophore of 31 species made it possible to characterize three main types in relation to shape and location in the appendage. It is possible to conclude that the elaiophores of the Tapinotaspidini flowers are more accessible and keep a proportional size to the pollinator.
- The area of the oil gland varies between 22.64 mm^2 to 1.24 mm^2 and there is no evident correlation to the kind of pollinator.
- The density and kind of secretory trichomes are characteristic for each species. The Tapinotaspidini flowers commonly show a higher density per mm^2.

Oils

- The principal substances of the floral oils obtained from the studied species are in general identical to those already found by VOGEL (1974) in this genus and in other genera of other families with oils as reward. These are highly polar diglycerides, which form myelin figures in contact with water.
- The removal of oils in short and regular intervals stimulates the reposition of oil in the flower.
- The quantity of oil/flower varies from 0.073 mg to 1.55 mg and seems to be related to the kind of bee that pollinates the flowers. The Centridini flowers produce more oil than the Tapinotaspidini flowers.
- The chromatographic analysis of the oils of 22 *Calceolaria* species and 3 elutions of oil collecting bees shows that each species has a characteristic pattern of spots, and that there are three main components (spots 9, 11 and 12) in almost all species.
- The phenetic study of the chromatographic patterns are only partially in agreement with the phylogenetic relationships of the species studied, and have no direct relation with their pollinators.

Foodbody

- The foodbody or food tissue builds a unique structure in the genus, present only in *C. uniflora* and *C. fothergillii*. It derives from the appendage, which is fleshy and folded to the outside of the flower and resembles a fruit in color, consistence and taste. These flowers do not produce oils or starch grains.

Androecium

Variations in the androecium of *Calceolaria* species are based on different aspects:

- Length of the filaments. There are two main groups of flowers, those with short filaments with a mean length of 1.39 mm, and those with long filaments with a mean length of 2.49 mm.
- Presence or absence of a hinge, coupled with the capability of movements.
- Divergent anthers, lateral fusion of the pollen sacs, secondary facial fusion and cases of lateral reduction.

- Presence of a net derived from the septal cells and the stomium in *C. tripartita, C. rivularis* and *C. chelidonoides*. This structure, not mentioned before for the family, may be involved in the regulation of pollen deposition on the insects.
- Lever mechanism. It is distributed in 25% of the species and restricted to the subgenus *Calceolaria*. Its origin in the different sections may have been independent. This lever mechanism is not restricted to determine group of pollinators and the presence of this kind of stamens in *Calceolaria* species without oil glands agrees with the theory of the secondary loss of the elaiophore.

Anthesis

- Dichogamy. Although protogyny is evident in the species studied, there is an overlapping of the flower phases which allow autogamic pollination in the self-compatible species.
- Anthesis. It lasts until 7 to 10 days. During this period of time variables as stigma receptivity, oil secretion, and dehiscence of stamens were registered.

Pollinators

- The oil collecting bees that pollinate the flowers of *Calceolaria* are restricted to the genus *Centris* (*C. autrani, C. triclor, C. nigerrima, C. cineraria* and *Centris* nov. spec.), and *Chalepogenus* (*Ch. caeruleus, Ch. rufipes, Ch. rasmusseni, Ch. perimelenae* and *Ch. vogeli*).
- The elaiophore-less species, which offer pollen as reward, are visited by species of *Bombus* and *Xylocopa*.
- Visits by *Centris* on *C. parviflora, C. lossenii, C. teucrioides, C. hypericina, C. pinifolia, C. corymbosa,* and by *Chalepogenus* on *Calceolaria ruiz-pavonii*, are described for the first time. Visits on *C. filicaulis* subsp. *luxurians, C. schickendantziana, C. prichardii, C. umbellata, C. polyclada , C. filicaulis, C. petioalaris, C. polifolia, C. latifolia, C. thyrsiflora, C. integrifolia, C. plectranthifolia* are also described although they were already recorded by VOGEL (1974) and SÉRSIC (1981).
- Sternotribic pollination is described for 5 species of section Rugosae and 1 species of section Integerrimae.
- The pollination of *Calceolaria uniflora* by the bird *Thinocorus rumicivorus* with a food body as reward is described.

Syndromes

- Flower types are determined and described for the genus. Their geographical and taxonomical distribution is also considered. Flower biotypes appeared alternatively in different sections. Melittophily with pollen as reward predominates in the northern and southern regions of distribution, while bird pollination is exclusively found in the southernmost regions. Although the relationship between *Centris* and *Calceolaria* predominates, the relation between this plant genus and the oil collecting bees appears to have started with the small bees of genus *Chalepogenus*.

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