

# CYTOTAXONOMIC NOTES ON SOME GALIUM SPECIES. A

BY

E. KLIPHUIS

(Communicated by Prof. F. A. STAFLEU at the meeting of March 30, 1974)

## INTRODUCTION

Within the frame work of general cytotaxonomic studies of various *Galium* species the following species were included: *Galium rotundifolium* L., *Galium arenarium* Lois., *Galium triflorum* Mich., *Galium uliginosum* L., *Galium pumilum* Murr., *Galium sternerii* Ehrend., *Galium oelandicum* (Stern. et Hyl.) Ehrend., *Galium spurium* L., *Galium tricornutum* Dandy, *Galium verrucosum* Huds. and *Galium purpureum* L.

Combined cytological and morphological studies were made on plants cultivated under uniform conditions during several years.

The results of these investigations are described and discussed for each species separately.

## MATERIAL AND METHODS

The chromosome numbers determined were made on roottips mitosis. The roottips were fixed in Karpechenko's fixative, embedded in paraffin, sectioned at 15 micron and stained according to Heidenhain's haematoxylin method.

In some cases a number of plants from one population was investigated. These plants are mentioned under the same collection number. Such collection numbers are indicated by an asterisk.

## RESULTS AND DISCUSSIONS

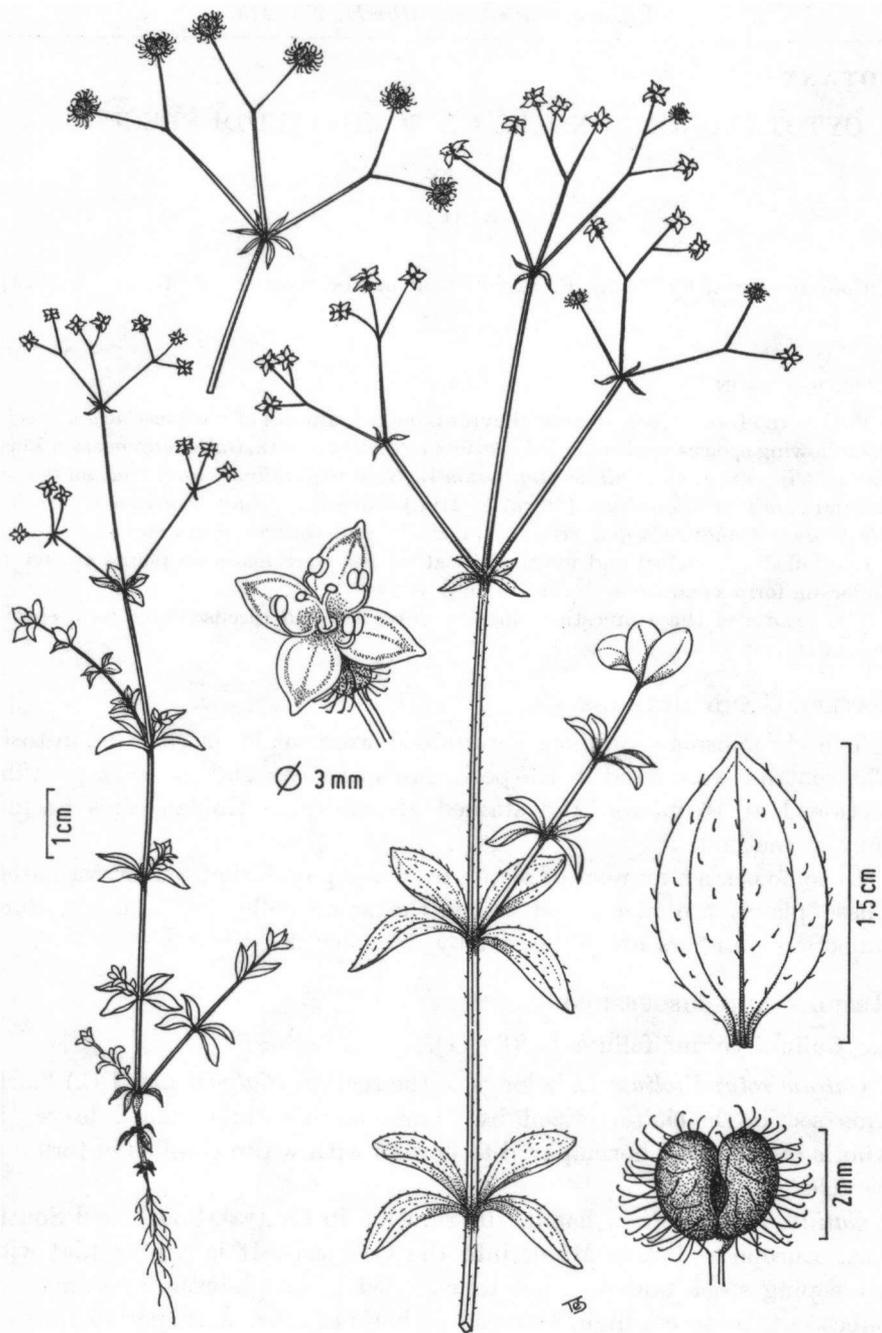
### 1. *Galium rotundifolium* L. (fig. 1).

*Galium rotundifolium* L. belongs to the section *Platygalium* (DC.) Koch. This section is characterized by plants having three-veined leaves in whorls of four, and hermaphrodite flowers with white corollas in terminal panicles.

*Galium rotundifolium* has its distribution in Central, South and South-East Europe and Asia Minor into the Caucasus. It is a perennial with a creeping stock and an erect four-angled stem, glabrous or sometimes pubescent, to 40 cm high. Leaves in whorls of four, short-petioled, ovate to sub-orbicular, 1-1.5 cm long and 0.5-0.8 cm wide, widest just above the middle, often turning reddish-brown, and black when dried.

Flowers up to 3 mm in diameter in spreading axillary cymes forming a loose panicle, greenish white or white, ovate with apiculate lobes.

Fruits 2 mm long, densely covered with uncinata hairs.



*Galium rotundifolium* L.

Fig. 1.

Flowers from the middle of May until July.

The species is uniform in its morphology. This was confirmed on plants collected from different parts of the distributional area and cultivated during several years in the experimental garden.

Cytological investigation on this material showed without exception diploids with  $2n = 22$  chromosomes. This number  $2n = 22$  was also found by PIOTROWICZ (1958) and SKALINSKA et al. (1964) in plants from different parts of Poland. KHOSHOO and BHATIA (1963) and HSU (1968) reported diploids from the Himalayas and Taiwan respectively. However, these regions do not belong to the area of *Galium rotundifolium*. According to EHRENDORFER (1973) the plants investigated by these authors belong to closely related species. The chromosome portrait is regular without satellites or additional chromosomes. (Fig. 10.3)

**ORIGIN OF THE MATERIAL INVESTIGATED:**

ANDORRA: K-1019, Ponta d'Envalina.

AUSTRIA: K-289, Manhartsberg, Nieder-Oesterreich; K-1083, Herschaftswald, Oberwart, Burgenland.

GERMANY: K-545, near Steinach, Ober-Franken, Bavaria; K-717, Dübner Heide, Bad Dübren.

PORTUGAL: K-1267, near Gerês, Serra do Gerês; K-1268, K-1269, Rio d'Homen, Serra do Gerês; K-1047, K-1270, near Leonte, Serra do Gerês.

POLAND: K-696, Tatri, Dolina Lejowa.

SWITZERLAND: K-566, Wallis; K-1238, near Mettmen, ca. 1360 m, Glarus.

TSJECHOSLOVAKIA: K-714, Northern part of Moravia; K-569, Posazavi, Jihlavské Vrehy, Bohemia; K-713, Eastern part of Bohemia; K-728, Predjumavi, Bohemia.

YUGOSLAVIA: K-525, near Zagreb; K-281, near Serajewo.

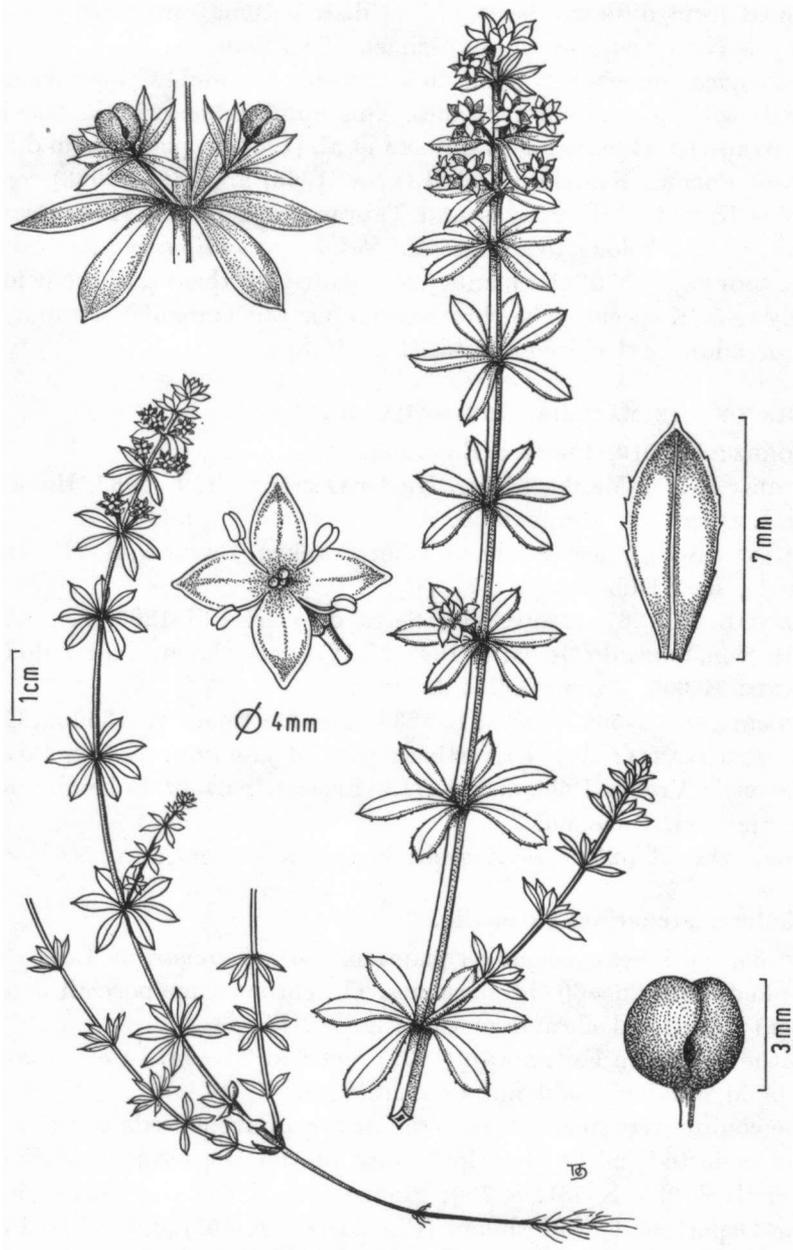
**2. *Galium arenarium* Lois. (fig. 2).**

Cytological investigations on plants of *Galium arenarium* Lois. showed hexaploids with  $2n = 66$  chromosomes. The chromosome portrait is regular without additional chromosomes. (Fig. 10.1)

In the literature FAGERLIND (1937) reported  $2n = 44$ . In our study this tetraploid number could not be confirmed.

The counts were made on material grown up from seeds as well as from plants collected in the wild in France of the Department of Finistère (Lesconil: K-295, K-561, K-700; Plougernan: K-554 and Cléder: K-697), of the Department of Morbihan (Plouharnel: K-556) and of the Department of Loire Atlantique (near Nantes: K-312, K-350, K-474 and K-522) and of the Department of Les Landes (Labenne Océan: K-1230, K-1259 and K-1260; Vieux-Boucau les Bains: K-1261, K-1262 and K-1263; Messagnes Plage: K-1231; St. Giron Plage: K-1264; Mimizan Plage: K-1265 and K-1266).

The plants were cultivated during several years in the Botanical Garden



*Galium arenarium* Lois

**Fig. 2.**

of the State University of Utrecht, (with exception of the nrs. K-1259-K-1266, which were collected in 1973). The plants showed a remarkable uniformity in their morphology. All characters mentioned below remained constant over the years of cultivation.

*Galium arenarium* is a typical representative of the coastal regions of France from the Channel to San Sebastian in the North of Spain. It is a perennial, 10–20 cm high, stem prostrate to ascending, reddish at the base, glabrous, smooth, quadrangular with ribbed angles, much branched, with numerous leaves apically, usually defoliated at the base. Internodes to 1.5 cm. Leaves in whorls of 5–8 rarely 10, to 0.8 cm long and 0.3 cm wide, widest just above the middle, mucronate, linear-lanceolate to rarely linear, bearing small stout teeth, fleshy, darkgreen, shiny. Flowers in short cymes at the end of the branches, deep yellow, up to 4 mm in diameter. Petals acute to obtuse, not mucronate. Pedicels short, fruit to 3 mm, black, shining.

Flowering period: juin–september.

### 3. *Galium triflorum* Michaux (fig. 3).

Plants obtained from two seed samples received from the botanical gardens from Montreal (Canada) and Lund (Sweden) and collected in nature both on the North-American continent turned out to be hexaploid with  $2n = 66$  chromosomes. (K-722, K-353, respectively).

The chromosome portrait is given in fig. 10.6. It shows a normal regular pattern and no accessory chromosomes could be detected.

In morphological respect these hexaploid plants are similar in their characters. Over the years of cultivation these characters remained constant.

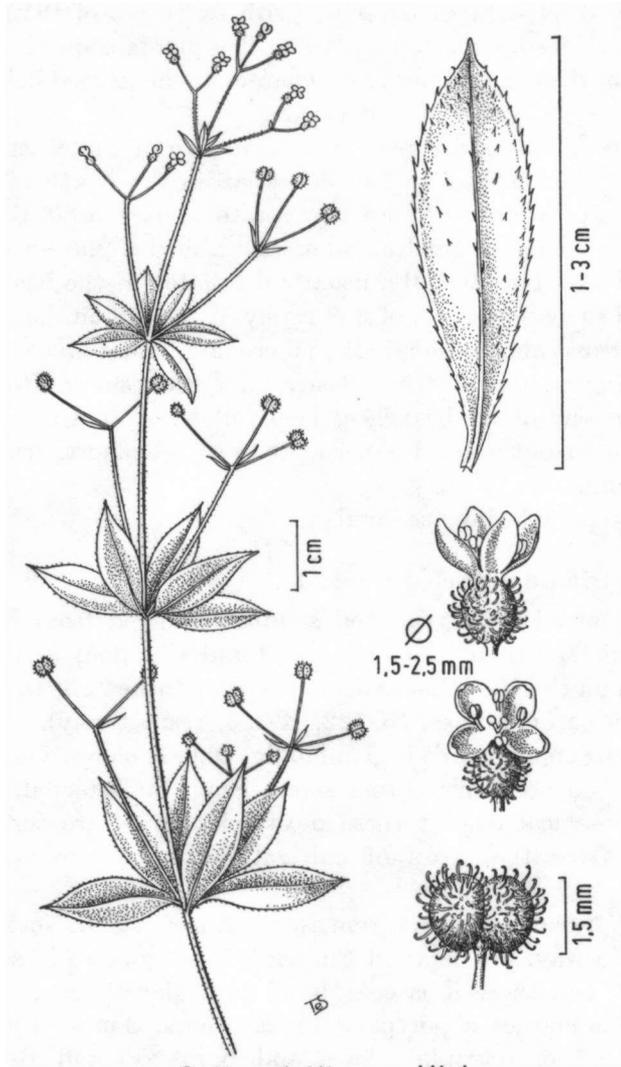
*Galium triflorum* is a plant from North-America and Northern-Europe (Northern-Norway, Sweden and Finland), it occurs also in some parts of Switzerland, and there it is considered as a glacial relic.

Within this species a polyploid series occurs. SORSA (1963) found in Finland plants of tetraploid level and KHOSHOO and BHATIA (1963) reported diploids from the Himalayas.

The finding of these cytotypes in separated parts of the area of the species indicates a certain correlation between chromosome number and chorology. However, should this be the case, then still too little is known about the distribution of plants of different ploidy level to give a clear picture.

*Galium triflorum* is a perennial, 30–60 cm high. Stem decumbent, ascending or erect, quadrangular, the angles sometimes rough by retrorse prickles. Leaves in whorls of 6–8, large, 1–3 cm long and to 1.2 cm wide, ovate-lanceolate, sessile or shortly petiolate, mucronate, usually thin with clear midvein, otherwise reticulate-veined, glabrous, mostly dark green, margins rough by antrorse prickles or smooth.

Flowers mostly in cymes of three in the leaf axils. Corolla small, greenish.



*Galium triflorum* Michaux

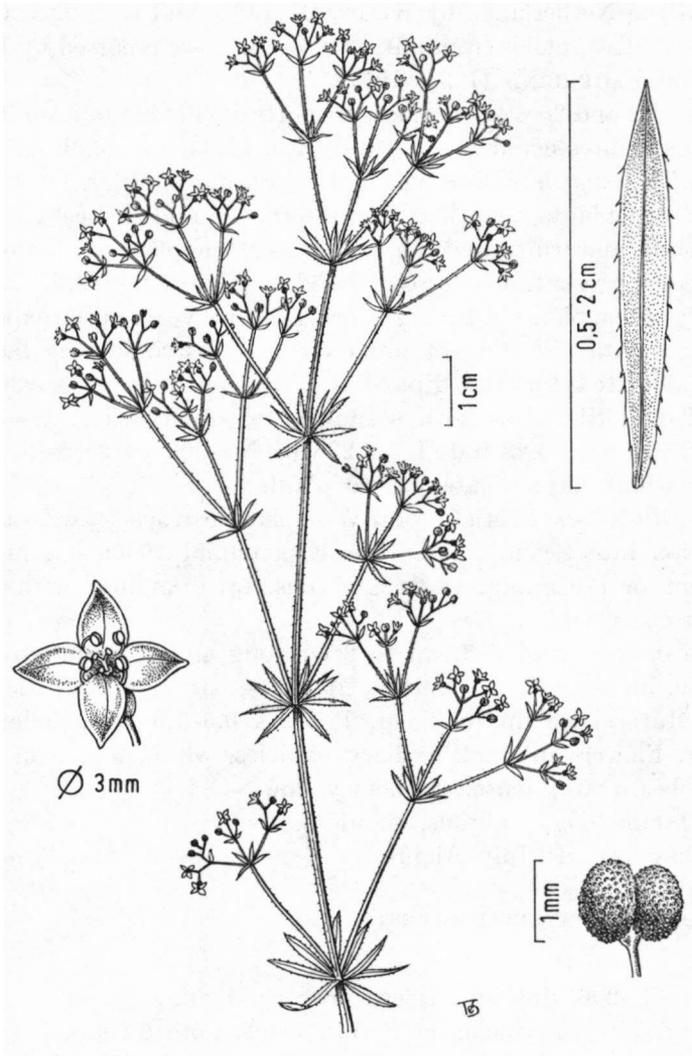
Fig. 3.

Fruiting pedicel straight, to 5 mm. Fruit densely covered with uncinuate hairs.

Flowering time: July–August.

4. *Galium uliginosum* L. (fig. 4).

From each of eighteen populations of *Galium uliginosum* in the Netherlands and from one in the Belgian Ardennes 7–8 plants were dug out and transported to the experimental garden. The plants from two populations in the Netherlands and those from the population from Belgium turned out to be tetraploids with  $2n = 44$  chromosomes. The plants from the



*Galium uliginosum* L.

Fig. 4.

other sixteen populations were diploids with  $2n=22$  chromosomes.

Plants from one location in Denmark and from one in France all obtained from seeds collected in the wild, were diploids. Two other plants collected in Norway were also diploids. Both cytotypes are also reported in the literature. Diploids by HOMEYER (1932) in material from unknown origin; by HANCOCK (1942) for Britain; for Central-Europe by GARAJOVA (1959) and EHRENDORFER, (in LÖVE and LÖVE, 1961) and for the Netherlands by KLIPHUIS (1962) and GADELLA and KLIPHUIS (1963), tetraploids by LÖVE and LÖVE (1956) for Iceland, by ROHWEDER (1937) for Germany

and from the Netherlands by KLIPHUIS (1962) and GADELLA and KLIPHUIS (1963). Tetraploids from unknown origin were reported by HOMEYER (1932) and FAGERLIND (1934, 1937).

The  $2n = 22$  and  $2n = 44$  chromosome portraits are regular, no B-chromosomes or satellites could be observed (figs. 10.8a and 10.8b respectively) *Galium uliginosum* is rather constant in its morphology. Characters as: height of the plants, length of the internodes and pedicels, size of the leaves, petals and fruits, and the indument of the plant are little variable. Differences if present, are not correlated with ploidy level. This could be demonstrated on plants cultivated during several years in the experimental garden. Stomata size did not prove to be a discriminating factor. The values calculated for the diploid and tetraploid are:  $\bar{X} = 30.15 \mu \pm SE = 0.22$  and  $SD = 3.10$  with a number of observation:  $N = 200$ ; and  $\bar{X} = 30.42 \mu \pm SE = 0.23$  and  $SD = 3.23$  with  $N = 200$  respectively. (Number of observations: 20 stomata per 10 plants).

*Galium uliginosum* L. is a Central-West European species with a northern distribution into Scandinavia. It is a perennial 10–60 cm high. Stem decumbent or ascending, weak, glabrous, quadrangular with four ribs with retrorse prickles.

Leaves in whorls of 5–8, up to 2 cm long and 2.5 mm wide, linear-lanceolate, mucronate, one veined, glabrous, the margins with recurved prickles. Internodes up to 7 cm. Pedicels 0.5–1.5 mm, deflexed after flowering. Flowers in small axillary panicles, white, 3 mm in diameter corolla lobes ovate, acute. Anthers yellow.

Fruit 1 mm long, glabrous, rugulose.

Flowering period: July–August.

#### ORIGIN OF THE MATERIAL INVESTIGATED:

##### Diploids:

DENMARK: K-206, Jutland, Iglesø near Rold Skov.

FRANCE: K-786, La Roche en Brenil. Dept. Côte d'Or.

THE NETHERLANDS: K-247\*, near Duurswoude, prov. of Friesland; K-189\*, K-190\*, Putten, Arboretum: "De Schovenhorst", prov. of Gelderland; K-630\*, K-631\*, K-633\*, K-634\*, Speuld, near Putten, prov. of Gelderland; K-660\*, K-666\*, "de Muy", Isle of Tessel, prov. of Noord-Holland; K-152\*, between Maarn and Maasbergen, prov. of Utrecht; K-960\*, Eempolder, near Eemnes, prov. of Utrecht; K-371\*, K-372\*, Oost Voorne, prov. of Zuid-Holland; K-373\*, K-1007\*, former airstrip, near Oost Voorne, prov. of Zuid-Holland; K-374\*, "de Beer", Goeree, prov. of Zuid-Holland.

NORWAY: K-1148, roadside near Høvringen, near Dombas; K-1156, 2 km South of Dovre.

##### Tetraploids:

BELGIUM: K-407\*, Robbertsville, Ardennes.

THE NETHERLANDS: K-32\*, near Weerdinge, prov. of Drenthe; K-188\*, between Ermelo and Nieuw-Milligen, prov. of Gelderland.

5a. *Galium pumilum* Murr.; 5b. *Galium sternerii* Ehrend. and 5c. *Galium oelandicum* (Stern. et Hyl.) Ehrend. (figs. 5a, b, c).

*Galium pumilum* Murr., *Galium sternerii* Ehrend. and *Galium oelandicum* (Stern. et Hyl.) Ehrend. are closely related species which forwardly all were included in *Galium pumilum* Murr.. Confusion around *Galium pumilum* has always been great because taxa of various rank within this complex have been based on unclear and often overlapping morphological characters. (JORDAN, 1846; BRAUN, 1885; SCHUSTER, 1909; BRIQUET, 1900; BRIQUET et CAVILLIER, 1915). STERNER (1944) in a well founded morphological study has tried to bring more clarity in the taxonomy of this complex species, especially with regard to its representatives in North-Western Europe.

He distinguished a central European group morphologically corresponding with *Galium pumilum* Murray s.str., and a Northern-European

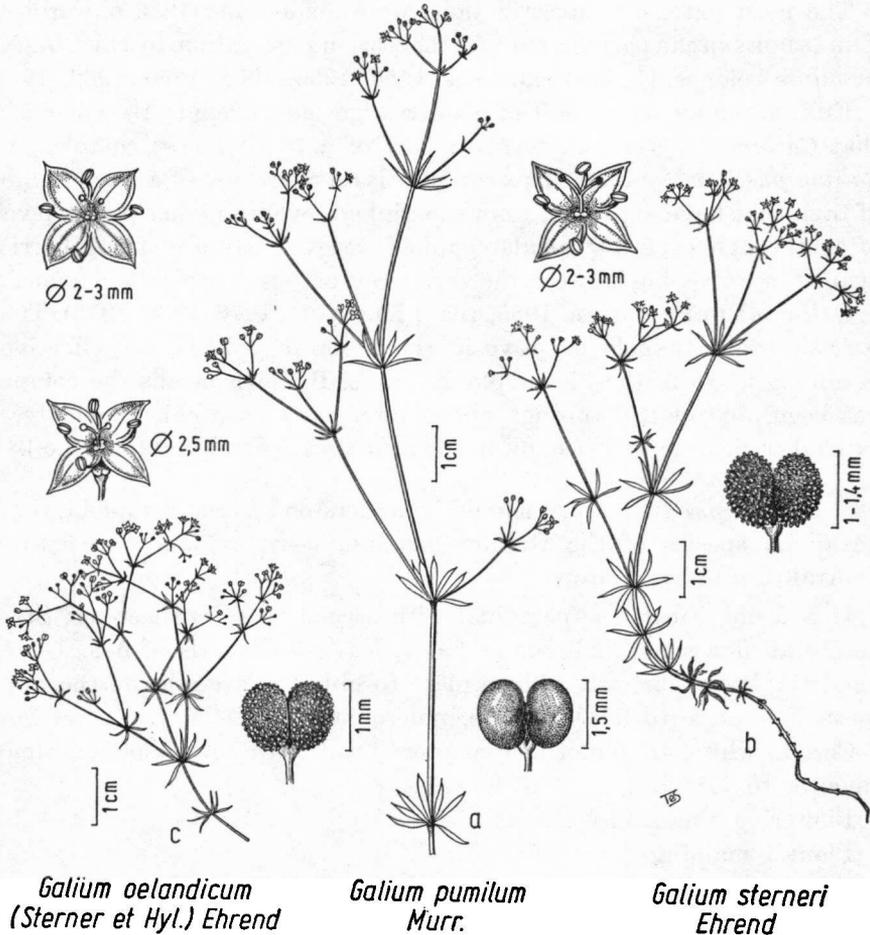


Fig. 5.

group comprising six geographically isolated and morphologically different taxa on the subspecific level, one of them with one variety (ssp. *normanii* (O. Dahl) Nordh.; ssp. *islandicum* Stern. et Hyl.; ssp. *septentrionale* Stern.; ssp. *slesvicence* Stern.; ssp. *oelandicum* Stern. et Hyl. and ssp. *suecicum* Stern. with the variety *vestrogoticum* Stern.)

Of these the subspecies *septentrionale* has the largest area comprising South-Norway, Denmark and the Northern part of the British Isles. This subspecies was elevated to specific rank (as *Galium sternerii* Ehrend.) by EHRENDORFER (1960). *Galium sternerii* Ehrend. is mainly represented by tetraploids, diploids occur in Britain in disjunct areas. The diploids are smaller and of a more slender habit than tetraploids. On the other hand the tetraploids are very variable and the degree of overlapping is such that a distinction is not always possible. (GOODWAY, 1955, 1957).

In the Flora of the British Isles the two cytotypes for that reason are united under one subspecies: ssp. *sternerii* (CLAPHAM, TUTIN and WARBURG, 1962).

The most extensive investigations were made from 1946 onwards by EHRENDORFER as a part of a study of the section *Leptogalium* to which *Galium pumilum* belongs. (1949, 1953, 1954, 1955, 1956, 1958, 1960, 1962, 1963).

EHRENDORFER, as a result of his investigations, came to the conclusion that *Galium pumilum* s.l. forms a part of a much larger complex, the *Galium pusillum* complex. This complex is an aggregate of a large number of taxa that have expanded from the diploid level to higher ploidy levels, often through perfectly regular euploid series. There are strong barriers against intercrossing between the various cytotypes, a normal phenomenon in *Galium* (EHRENDORFER, 1955, 1958; KLIPHUIS, 1970, 1972, 1973). These barriers are extremely effective in the lower polyploids, the effectively becoming lesser in the higher ploidy levels. By such means the complex has been able, partly through autopolyploidy, partly through allopolyploidy, to expand with time and occupy its present area. (EHRENDORFER, l.c.).

5a. *Galium pumilum* Murr. should be understood as the octoploid, rarely hexaploid species of the *Galium pusillum* complex, and it occurs in Central- and West Europe.

It is a non caespitose perennial with ascending to erect stems. Stems nearly all flowering, glabrous or hairy, leaves in whorls of 6-9, 1-2 cm long, the lower narrowly oblanceolate to oblanceolate-falcate, the upper linear-lanceolate to linear-falcate, mucronate.

Corolla white, to 4 mm in diameter. Fruit with tiny papillae, almost smooth, to 1.5 mm.

Flowering time: May-August.

Plant remaining green when dried.

*Galium pumilum* Murr. occurs, on dry to moderately dry well drained basic to slightly acid soil, in grassland vegetations which are rich in species and which belongs to the *Mesobromium* (EHRENDORFER, 1953).

Probably because of less favourable climatological circumstances the species is restricted in the Netherlands to neutral or distinctly basic soils.

*Galium pumilum* is regarded by WESTHOFF and DEN HELD (1969) as faithful species of a kind of limestone-grasslandvegetation ("het krijthellinggrasland") belonging to the *Koelerio-Gentianetum*.

Plants from Belgium, France and the Netherlands were investigated cytologically. Only octoploids with  $2n = 88$  chromosome were found. The chromosome portrait is regular, no B-chromosomes or satellites were observed. (fig. 10.7c).

From each population 7–10 plants were dug out and transported to the experimental garden. The plants retained their morphological characters during the years of cultivation. Existing differences among the plants were of minor importance and consisted largely in the indument and the plants size.

#### MATERIAL INVESTIGATED:

BELGIUM: K-169, K-170, K-171\*, near Baelartshoven.

FRANCE: K-785, Thoisy la Berchère (Dyon).

THE NETHERLANDS: K-990\*, South of the province of Limburg, Schweiberg; K-991\*, K-992\*, idem Kunraderberg; K-993\*, K-994\*, K-995\*, idem, slope near Eyserbos.

#### 5b. *Galium sternerii* Ehrend. (fig. 5b).

Ten plants taken from three populations of *Galium sternerii* and five obtained from seeds all from Jutland, Denmark were investigated cytologically. These plants turned out to be tetraploids with  $2n = 44$  chromosomes. The chromosome portrait is regular, no accessory chromosomes were observed. (fig. 10.7b). The tetraploid is a perennial, caespitose, non flowering shoots prostrate, the flowering shoots ascending or erect, up to 25 cm, glabrous or hairy. Internodes up to four cm long.

Leaves in whorls of 7–8, to 1.5 cm long, and 1.5 mm wide, oblanceolate to linear, mucronate, margins with curved, retrorse prickles.

Flowers to 4 mm, creamy white, pedicels 1–2 mm. Inflorescence forming a compact pyramidal panicle.

Fruits to 1.25 mm long, glabrous with acute tubercles.

Flowers in June and July.

#### ORIGIN OF THE MATERIAL INVESTIGATED:

DENMARK, Jutland: K-233\*, Vorgod (obtained from seeds) K-1195\*, Blåvand, Dunes K-1196\*, Klim, near the coast; K-1199\*, near Løgstør.

#### 5c. *Galium oelandicum* (Sterner et Hyl.) Ehrend. (fig. 5c).

This species is endemic on the isle of Öland (Sweden). It is a diploid, postglacial relic (EHRENDORFER, 1960).

The plant is a perennial, densely caespitose, up to 15 cm high, flowering shoots ascending with short internodes. Leaves in whorls of 6–9, linear-

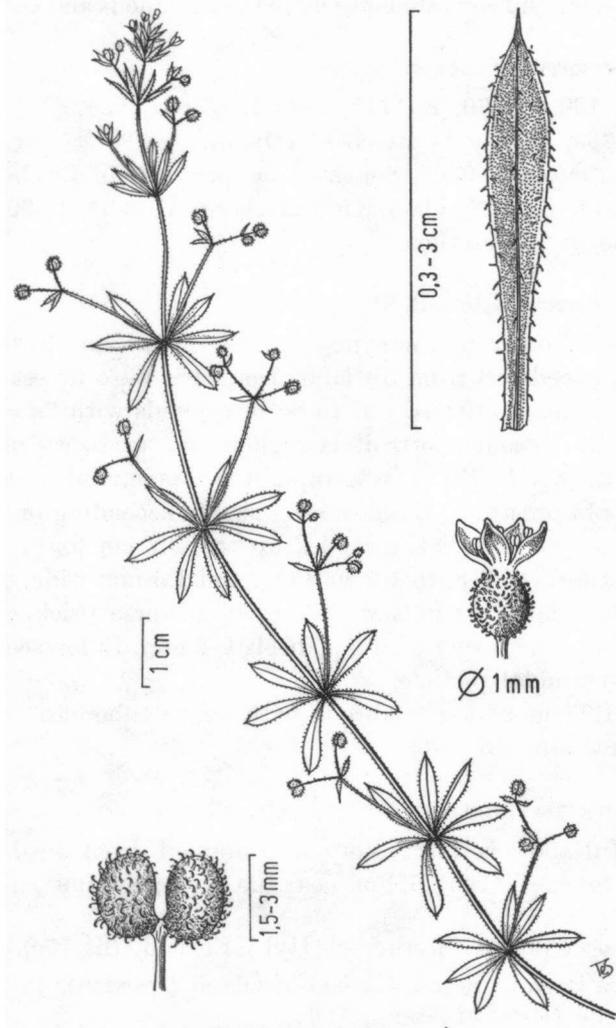
oblanceolate, to 1 cm long and 1.5 mm wide. Flowers white, in lax pyramidal panicles. Pedicels short, up to 0.5 mm. Fruits up to 1 mm long.

Two plants from Öland (K-488 and K-779) were investigated cytologically. Both were diploids with  $2n=22$  chromosomes. (fig. 10.7a).

6. *Galium spurium* L. (fig. 6).

Seeds from *Galium spurium* collected in nature and received from the Botanical Gardens of Udine (Italy), Stockholm, Erevan and Leningrad were sown.

Counts on metaphaseplates of roottip mitosis from plants obtained from these seeds, showed the chromosome number  $2n=20$ . This number is also reported by FAGERLIND (1934, 1937) in material from unknown



*Galium spurium* L.

Fig. 6.

origin and by PODLECH and DIETERLE (1969) in *Galium spurium* from the Andarab valley in Baghlan, Afghanistan. The number  $2n=20$  is remarkable in the genus *Galium*, because the common basic chromosome number in this genus is  $X=11$ . Only from two other species the basic number  $X=10$  is known, e.g. *Galium ibicinum* Boiss. et Hausskn. and *Galium molluginoides* E. H. L. Krause (PODLECH and DIETERLE, 1969).

The chromosome portrait is very regular (fig. 10.4). No striking differences in the length of the chromosomes are present and, therefore, fusion of chromosomes as suggested for *Galium aparine* by BÖCHER et al. (1955) seems unlikely. In this species the chromosome number  $2n=64$  is the most common (BÖCHER, LARSEN and RAHN, 1955; KLIPHUIS, 1962, 1967; GADELLA and KLIPHUIS, 1963) but also the number  $2n=66$  occurs (POUQUES, 1949; LÖVE and LÖVE, 1956; KLIPHUIS, 1962, 1967; GADELLA and KLIPHUIS, 1963; PODLECH and DIETERLE, 1969; STRID, 1971), even in the same plant (KLIPHUIS, 1967). In the metaphaseplates of *Galium aparine* with  $2n=64$  chromosomes, two large chromosomes are present which are absent in the  $2n=66$  plates (BÖCHER, LARSEN and RAHN, 1955; GADELLA and KLIPHUIS, 1963).

*Galium spurium* occurs through the whole of Europe (except the arctic parts), West-Asia, North and Central America.

The plant is an annual, up to 100 cm high, with decumbent or prostrate quadrangular stems, rough on the edges by retrorse prickles. Nodes glabrous or glabrate, internodes up to 6 cm.

Leaves in whorls of 5–10, up to 3 cm long, linear-lanceolate, mucronate, midrib and margins rough by retrorse prickles. Flowers in axillary few flowered (3–9) cymes, 1 mm in diameter, greenish-white or white with four acute lobes.

Pedicels straight.

Fruits 1.5–3 mm long, black, rugulose, glabrous or with white uncinata hairs with non tuberculate bases.

Flowering period: May–September.

According to GABRIELS (1965) there are two subspecies characterized by differences in fruit indument, e.g. the subspecies *spurium* with glabrous fruits and the subspecies *infestum* (Waldst. et Kit.) K. Janchen with uncinata fruit-indument. According to this subdivision the investigated plants used in this study belong to the subspecies *infestum*.

*Galium spurium* is closely related to *Galium aparine*. However, clear morphological differences are present, *Galium spurium* having smaller flowers and fruits, and axillary flowers with 2–3 leafy bracts.

#### MATERIAL INVESTIGATED:

ITALY: K-301, Udine, Val Tellina.

SWEDEN: K-455, Carlshäs.

U.S.S.R.: K-268, Esthonia; K-318, Armenia.