

**HOROLOGICAL AND ANATOMICAL INVESTIGATION  
ON *LINDERNIA PROCUMBENS* AND *LINDERNIA DUBIA*  
(SCROPHULARIACEAE)**

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**ABSTRACT** . The horology of *Lindernia procumbens* (Krocker) Philcox and *L. dubia* (L.) Pennell in Bulgarian flora was established. A comparative anatomical study of leaves, stems and flower stalks, as well as scanning electron microscopic analysis of the spermoderm were carried out. Variability of the studied characteristics was presented.

**KEY WORDS:** horology, spermoderm, anatomy, leaf, stoma, stem.

**INTRODUCTION**

Two *Lindernia* species are growing in Bulgaria: *L. procumbens* (Krocker) Philcox and *L. dubia* (L.) Pennell, spreading in wet sandy places, in puddles and rice-fields. The first species was established for the country in 1889 by Georgiev and the second one – in 1984 by Delipavlov and Cheshmedziev. During that period a significant amount of horological information was collected without carrying out studies of another character. *L. procumbens* is a protected species (Order No. RD 402 of 1955 and Governmental Decree No. 442 of 1997) and it was included in the Red Book of Bulgaria (Bozhilova, 1984), having the status of a “rare” plant. The aim of the present paper was to summarize the data about the distribution of both species in the country, to study the spermoderm and the anatomic structure of the vegetative organs.

**MATERIAL AND METHODS**

The horological information of the species has been updated using herbarium materials /SOM, SOA, SO/, literature sources and own collections. The spermoderm was studied under scanning electronic microscope Jeol (gold coating) by samples from Plovdiv (the Maritsa river bed). The plants for anatomy originated from: *L.*

*procumbens* – Plovdiv, wet sands and paddles along the Maritsa river bed (02.08.2004 – IČ, GS); *L. dubia* – from the same places (01.08.2004 - IČ); the village of Tsalapitsa, Plovdiv region, in rice-fields (21.09.2004 – IČ, GS) and Ivaylo quarter, Pazardjik region in rice-fields (17.09.2004 – IČ, GS). Leaves (*ad* and *ab* epidermis, mesophyll), stems and flower stalk fixed in 75 % ethyl alcohol have been studied. 30 reports per index from 10 plants of a population have been carried out on temporary preparations. Variability of leaf characteristics was expressed by the *max* : *min* index (up to 1,4 - poor, 1,5-2,4 - average, 2,5-3,4 - increased, above 3,5 - high) and the summarized degrees of the variation coefficient – S % (up to 5 - poor, 5,1-15,0 - average, 15, 1-25,0 - increased, above 25,1 - high).

## RESULTS AND DISCUSSION

**H o r o l o g y** . Checking the herbaria showed the availability of the deposits cited here.

***L. procumbens*** (Map 1). *The Danube plain*: LJ-24 (Cherkovitsa village, region of Nikopol, Tsonev) SO 100673. *Balkan foothill region*: LH-17 (region of Lovech, Baev) SOM 67004. *West Stara planina mountain*: GN-25 (Botevgrad, Urumov) SO 67007. *West Frontier Mountains*: FL-69 (Ograzhden mountain, Gega village, Bozhilova & Stoyanov) SO 96662. *Struma valley (South)*: FL-89 (Strumeshnitsa river, DD\*) SOA 45375. *Thracian plain*: KG-77 (Pazardjik, Georgiev) SO 65414; KG-67 (Kovachevo village, region of Pazardjik, DD, IČ) SOA 40402, 40403; KG-78 (Golemenovo village, Pazardjik region, DD, IČ) SOA 40401; KG-86 (Ognyanovo village, Pazardjik region, DD, IČ) SOA 38207, 38208, 41954, 41955, 41960; KG-04 (Harmanli, DD, IČ) SOA 37132; LG-16 (Plovdiv, Vihodtsevski) SOM 56633; LG-16 (there, DD) SOA 18104, 18106; LG-16 (there, Gramatikov) SOA 18107; LG-17 (there, IČ, GS) SOA 56633; LG-26 (town of Sadovo, Stribrny) SOA 28554; LG-09 (Tseretelevo village, region of Plovdiv, DD, IČ) SOA 45573, 45577, 45579, 45582; LG-27 (Manole village, region of Plovdiv, DD) SOA 38346.

There are literature data about the distribution of the species in other regions of the country, too, without herbarium materials being preserved, i.e.: Kuklen, region of Asenovgrad, LG-15 (Velenovsky, 1902), later also mentioned by other authors; region of Sevlievo – the villages of Suhindol LH-58, Bara LH-46, Malkochevo LH-75, Gabene LH-64 (Urumov, 1928), not mentioned by other authors; Etropole, KN-54 (Stoyanov and Stefanov, 1925, 1933) and Zagore village, region of Stara Zagora, LG-98 (Vihodtsevski, 1962). The localities mentioned need to be confirmed. The horology summarized by floristic regions, was presented in most details by Dimitrov, (2002) and Cheshmedziev (2003). The supplemented and corrected distribution of *L. procumbens* in Bulgarian flora is the following: Danube plain, Balkan foothill region, Stara planina mountain (West), West frontier mountains, Struma valley (South) and Thracian plain. It was mentioned for the central Rhodopes mountains.

***L. dubia*** (Map 2). *Danube plain*: LJ- 24 (Cherkovitsa village, region of Nikopol, Tsonev) SOM 15874. *Thracian plain*: KG-77 (Ivaylo quarter, Pazardjik, IČ,

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\* D. Delipavlov

GS) SOA 56631; KG-97 (Tsalapitsa village, region of Plovdiv, IČ, GS) SOA 56632; LG-16 (Plovdiv, DD) SOA 36342-36345, 36355, 36356; LG-18 (Graf Ignatievo village, region of Plovdiv, DD, IČ) SOA 44743; LG-27 (Manole village, region of Plovdiv, DD) SOA 38364, 38346, 38586, 36588; LG-36 (Popovitsa village, region of Plovdiv, DD) SOA 39833; LG-38 (town of Rakovski, DD) SOA 43456 and there (IČ) SOA 44742; LG-38 (Papukchi village, region of Stara Zagora, DD, IČ) SOA 43048; LG-98 (Grudovo village, region of Stara Zagora, DD, IČ) SOA 43847; MG-04 (Harmanli, DD, IČ) SOA 37131, 37133, 37134; MG-05 (town of Maritsa, DD) SOA 38586; MG-13 (Biser village, region of Haskovo, DD, IČ) SOA 37135.

The distribution of the species by floristic regions is as follows: Danube plain, Thracian plain.

A n a t o m y. (Figs. 1, 2, Table 1)

L E A F. In both species, the leaf is amphistomatic, dorsoventral, with a single-layered palisade tissue. B a s a l e p i d e r m a l c e l l s. They have an irregular shape (with a tendency to slight elongation), unevenly distributed. The anticlinal walls are wavy curved. The average number of cells per  $1 \text{ mm}^2$  for *L. procumbens* is: *ad*-epidermis 700, *ab*-epidermis 620, and for *L. dubia* – *ad*-627, *ab*-514, respectively. The mean data and the minimal and maximal values show that the epidermis of the first species is more small-celled. Variability of the basal cell density expressed by *max:min* index and the summarized degrees of the variation coefficient – S % (with some exceptions) is average. For the inter-population comparison of *L. dubia* it is high to increased for *ad*-axial epidermis. The mean values about the heights of the basal cells in *L. procumbens* are: *ad*-epidermis 15,84  $\mu\text{m}$ , *ab*-epidermis 16,5  $\mu\text{m}$ , the thickness of the external walls being: *ad*-3,8  $\mu\text{m}$ , *ab*-3,5  $\mu\text{m}$ . For *L. dubia* those values are: *ad*-19  $\mu\text{m}$ , *ab*-16,6  $\mu\text{m}$  and *ad*-2,7  $\mu\text{m}$ , *ab*-2,4  $\mu\text{m}$ , respectively. The latter is characterized by a bigger height of the cells in the upper epidermis and less thick external walls (the three characteristics studied by populations). Variability of those traits is average by the *max:min* index and average-increased by the summarized degrees of S %. S t o m a t a. For both species, they are of an anomocite type, i.e. oppositely placed. They border on 3-4 non-specialized adjacent cells. They are situated at the level of the basal epidermal cells or they are slightly protruding. It corresponds to the ecological conditions of the locations. The stoma shape is oval, one and the same for both species. The mean number of cells per  $1 \text{ mm}^2$  for *L. procumbens* is: for *ad*-surface 200, for *ab*-surface 182, and, for *L. dubia* - *ad*-145 и *ab*-155, respectively. Again the bigger density of stomata in *L. procumbens* correlates to the bigger density of the basal epidermal cells. Variability of both indices for *L. procumbens* is average. By *max:min* index it is average for *L. dubia* and, by S % - average-increased for the three populations, while for the inter-population comparison it is increased-high. The stoma sizes of the *ad*-epidermis of both species are very close: for *L. procumbens* = 25,89 X 15,4, for *L. dubia* = 25,3 X 15,7  $\mu\text{m}$ . Certain differences are found in the size of *ab*-epidermis. Size variability (with few exceptions) expressed by the *max:min* index is poor and by S % - average, becoming high for the inter-population comparison in *L. dubia*. Microscopic 4-cell sunk down glandular trichomes, rarely spread, are noticed on the *ad* and *ab* surfaces

of both species, at the level of the epidermal cells or slightly protruding. They are surrounded by several epidermal cells, a little bit elongated in a radial direction. Similar trichomes were mentioned for *Lindernia* genus by Metcalfe & Chalk (1957). When characterizing the genus, it is usually said the plants are naked.

**M e s o p h y l l.** The palisade layer is under the upper epidermis (sometimes lacking over the central vein). The average sizes for *L. procumbens* are 45,7 X 24,03  $\mu\text{m}$ , for *L. dubia* 43,2 X 25,3  $\mu\text{m}$ . The palisade coefficient is 30-50 %. The spongy parenchyma consists of 3-4 rows  $\pm$  round thin-walled cells, the intercellular spaces being not large. Variability of the palisade cell height is average by both indices and variability of their width – average to increased one. The leaf veins are covered by a single-layered parenchymous sheath, the biggest cells being at the central vein. Mechanical tissues are not observed.

**S T E M.** The cross section in both species is 4-sided. Two of the opposite sides are often  $\pm$  concave, sometimes quite obviously, so that clearly outlined ribs are noticed. The epidermis is single-layered with stomata and rarely spread trichomes as found in the leaves. There is an uninterrupted layer of 1-3-row thin-walled parenchymous cells under it, followed by well developed air-conducting parenchyma (forming large radially located cavities) and inside – a single-layered endodermis. The mechanical tissue is represented by 4 sclerenchyma bundles at the edges. Outside them, under the epidermis, several collenchymous cells are observed. The central cylinder is outlined by vascular bundles and the pith – by thin-walled parenchymous cells (the biggest one in the centre), forming the medullary rays outwards. The number of cavities and of the medullary rays is bigger in *L. dubia*. The presented anatomic characteristics is similar to that of Metcalfe & Chalk (1957) for *Lindernia* genus.

**F L O W E R   S T A L K.** It is 4-sided with rounded edges. Stomata and sunk down glandular trichomes are rarely to be found in the epidermis. There is a single sclerenchyma bundle in each corner under the epidermis. Air-conducting parenchyma is well developed. The conducting bundles in the cylinder are 4 in number, grouped by 2 in the upper side of the stalk. The core is thin-walled parenchyma.

**S E E D.** (Fig. 3). The seed is oblong in shape, yellowish, with longitudinal ribs, better expressed in *L. procumbens*. For both species the sizes are almost equal: *L. procumbens* – 0,33-0,38 X 0,16-0,19 mm and *L. dubia* – 0,31-0,39 X 0,14-0,17 mm. The spermoderma cells are 4-5-angular, quite different in shape and size. The anticlinal walls are straight or arched and the edges are often rounded. The periclinal walls are concave or almost horizontal, densely covered with microvirucoses, with slightly protruding edges.

## CONCLUSIONS

The conducted horological analysis resulted in establishing the localities of *L. procumbens* unconfirmed with herbarium deposits, which needed confirmation. The anatomic structure and the submicroscopic organization of the spermoderm are very close. Microscopic sunk down 4-celled glandular trichomes are to be found in the

leaves, stems and the flower stalks of both species. Variability of the studied characteristics is within close parameters. The structural differences observed refer to the greater density of the basal epidermal cells and of the stomata on the lower and upper leaf surfaces in *L. procumbens* and the bigger number of cavities and medullary rays in the stem of *L. dubia*.

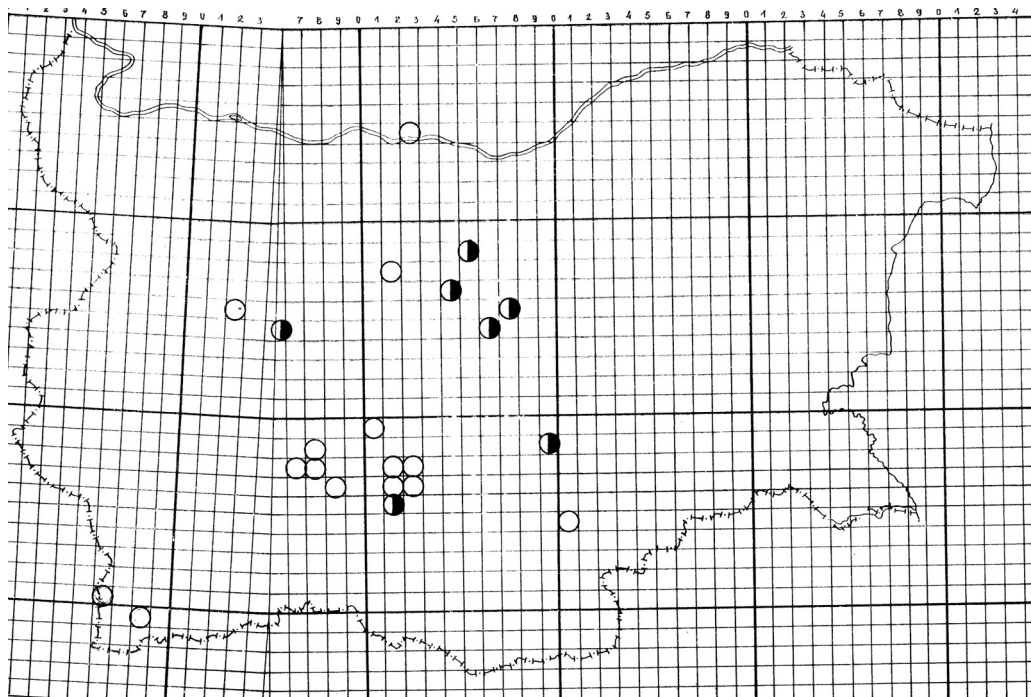
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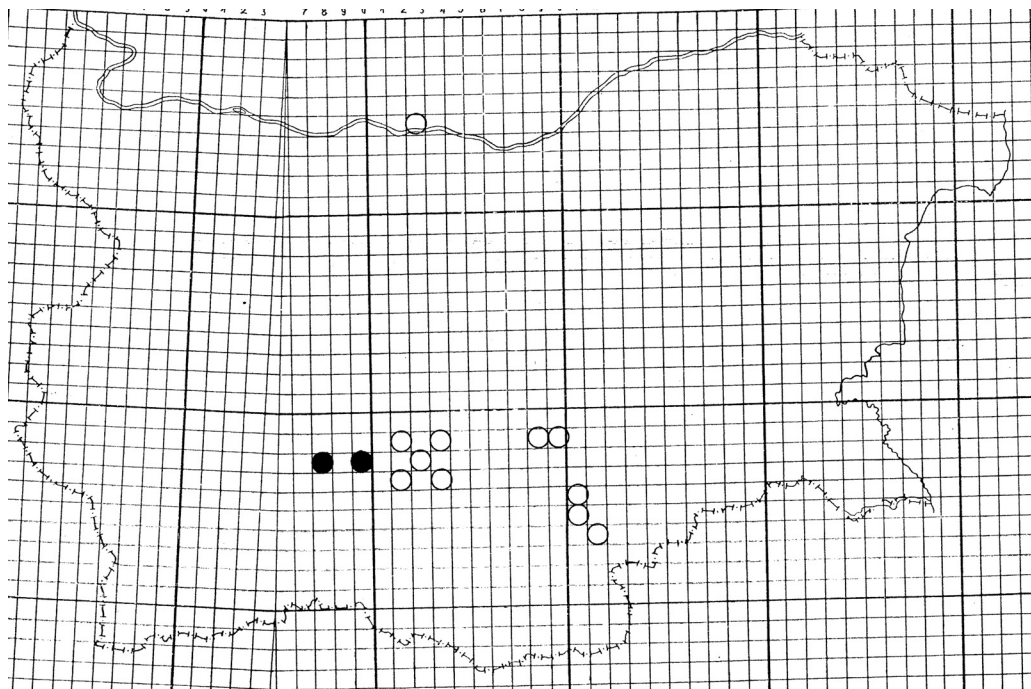
Leaf anatomical characters of Lindernia All. (in numbers and  $\mu\text{m}$ )

Table 1

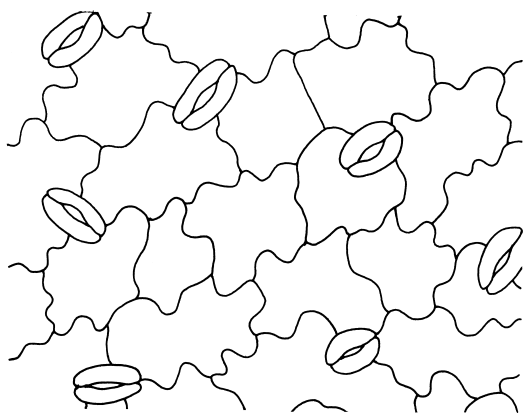
Localities	L. procumbens			L. dubia			L. dubia			L. dubia						
	Plovdiv			Plovdiv			Tsalapitsa			Pazardjik			Average			
	(min) $\bar{x} \pm S \bar{\sigma}$ (max)	S%	max: min	(min) $\bar{x} \pm S \bar{\sigma}$ (max)	S%	max: min	(min) $\bar{x} \pm S \bar{\sigma}$ (max)	S%	max: min	(min) $\bar{x} \pm S \bar{\sigma}$ (max)	S%	max: min	(min) $\bar{x} \pm S \bar{\sigma}$ (max)	S%	Max: min	
ad epidermis	per $\text{mm}^2$	(160)200 $\pm$ 9,42(260)	14,9	1,6	(100)128 $\pm$ 5,33(160)	13	1,6	(140)194 $\pm$ 10,34(240)	17	1,7	(80)114 $\pm$ 6,69(140)	18,6	1,8	80(145,3) $\pm$ 7,78(240)	29,34	3
	length stomata	(21,9)25,89 $\pm$ 0,38,(30)	8,16	1,4	(21,8)26,88 $\pm$ 0,44(32,2)	9	1,5	(22,8)24,7 $\pm$ 0,22(26,7)	4,9	1,2	((21,9)24,9 $\pm$ 0,34(27,8)	7,5	1,3	(21,8)25,3 $\pm$ 0,22(32,3)	8,27	1,5
	width	(13,4)15,4 $\pm$ 0,17(18,8)	6,34	1,4	(15)16,5 $\pm$ 0,23(18,6)	7,7	1,2	(14,25)14,9 $\pm$ 0,06(16,5)	2,4	1,1	(15)15,7 $\pm$ 0,14(17,1)	5,04	1,1	(14,3)15,7 $\pm$ 0,11(18,6)	6,92	1,3
ab epidermis	per $\text{mm}^2$	(600)700 $\pm$ 10,44(800)	8,17	2	(500)610 $\pm$ 12,77(800)	11	1,6	(600)813,3 $\pm$ 16,6(950)	11	1,6	(350)458,3 $\pm$ 12,24(550)	14,6	1,6	(350)627,2 $\pm$ 17,4(950)	26,26	2,7
	height	(10,2)15,84 $\pm$ 0,52(22)	18,2	2,2	(14)19,05 $\pm$ 0,62(25)	18	1,8	(16)19,1 $\pm$ 0,48(25,6)	14	1,6	(12)18,86 $\pm$ 0,77(29,4)	22,5	2,5	(12)19 $\pm$ 0,36(29,4)	18,19	2,5
	external walls	(2,7)3,8 $\pm$ 0,12(6)	18,6	2,2	(2)2,77 $\pm$ 0,06(3,4)	13	1,7	(1,5)2,75 $\pm$ 0,07(3,6)	14	2,4	(2)2,55 $\pm$ 0,06(3,5)	14,7	1,8	(1,5)2,69 $\pm$ 0,04(3,6)	14,37	2,4
pallside cells	per $\text{mm}^2$	(140)182 $\pm$ 7,57(220)	13,2	1,6	(140)170 $\pm$ 8,56(220)	16	1,6	(140)182 $\pm$ 8,13(220)	14	1,6	(80)114 $\pm$ 7,33(140)	20,3	1,8	(80)155,3 $\pm$ 7,09(220)	25	2,8
	length	(22,5)26,29 $\pm$ 0,33(30)	6,92	1,3	(23,7)25,4 $\pm$ 0,23(28,8)	5,2	1,2	(21)23,02 $\pm$ 0,26(25,5)	6,3	1,2	(18,9)24,16 $\pm$ 0,44(27,9)	10,1	1,5	(18,9)24,2 $\pm$ 0,21(28,8)	8,38	1,5
	width	(15)17,64 $\pm$ 0,26(20,1)	8,3	1,3	(15)16,7 $\pm$ 0,26(18,6)	8,7	1,2	(11,85)14,2 $\pm$ 0,18(15)	7,1	1,3	(15)15,9 $\pm$ 0,18(17,4)	6,39	1,1	(11,8)15,6 $\pm$ 0,16(18,6)	10,11	1,6
pallside cells	per $\text{mm}^2$	(500)620 $\pm$ 13,26(750)	11,7	1,5	(400)546,7 $\pm$ 12,9(700)	13	1,8	(450)563,3 $\pm$ 10,96(650)	11	1,4	(300)433,3 $\pm$ 13,4(550)	17	1,8	(300)514,4 $\pm$ 9,4(700)	17,3	2,4
	height	(11,2)16,5 $\pm$ 0,52(25,2)	17,4	2,3	(11,6)17,09 $\pm$ 0,5(22,4)	16	1,9	(10,8)16,12 $\pm$ 0,44(20)	15	1,6	(10)16,5 $\pm$ 0,51(20)	17,2	2	(10)16,6 $\pm$ 0,28(22,4)	16,23	2,2
	external walls	(2,6)3,53 $\pm$ 0,11(4,8)	17,3	1,9	(1,9)2,63 $\pm$ 0,07(3,4)	15	1,8	(1,5)2,35 $\pm$ 0,06(2,9)	15	1,9	(1,7)2,14 $\pm$ 0,05(2,6)	13	1,5	(1,5)2,37 $\pm$ 0,04(3,4)	16,79	2,3
pallside cells	height	(39,6)45,7 $\pm$ 0,83(60)	10	1,5	(36)42,7 $\pm$ 0,79(52,8)	10	1,5	(34)41,29 $\pm$ 0,83(50,8)	11	1,5	(37,4)45,5 $\pm$ 0,99(62,6)	12	1,7	(34)43,2 $\pm$ 0,53(62,6)	11,8	1,8
	width	(18,4)24,03 $\pm$ 0,92(39)	21	2,1	(18)25,28 $\pm$ 0,64(34,4)	14	1,9	(16,8)25,62 $\pm$ 0,8(34,6)	17	2,1	(16,6)25,04 $\pm$ 0,66(34,4)	14,4	2,1	(16,6)25,3 $\pm$ 0,40(34,6)	15,13	2,1



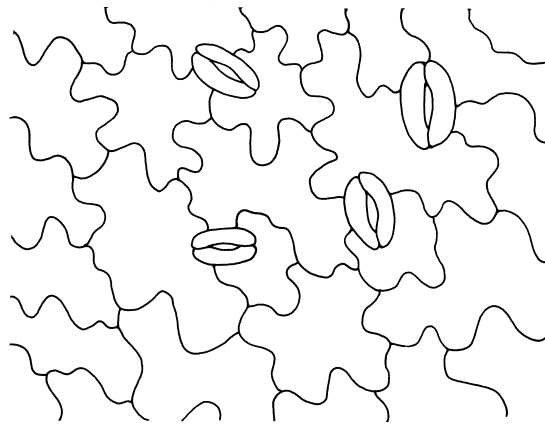
**Map.1.** Distribution of *Lindernia procumbens* in Bulgaria;  
O – localities after herbarium material; ● – data from literature.



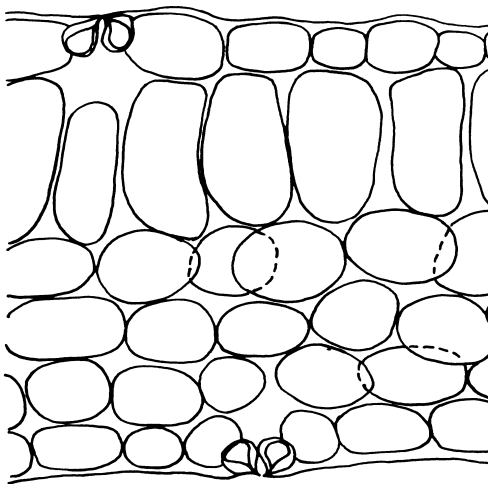
**Map. 2.** Distribution of *Lindernia dibia* in Bulgaria;  
O – localities after herbarium material; ● – new localities



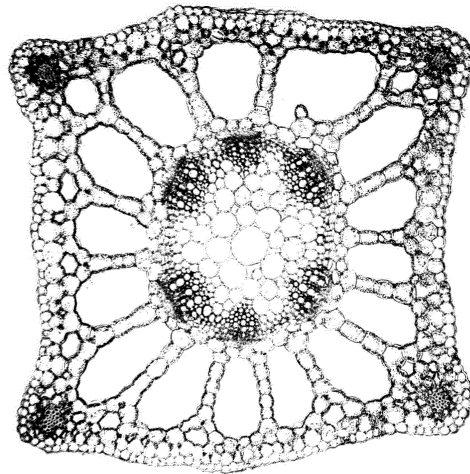
*a*



*b*



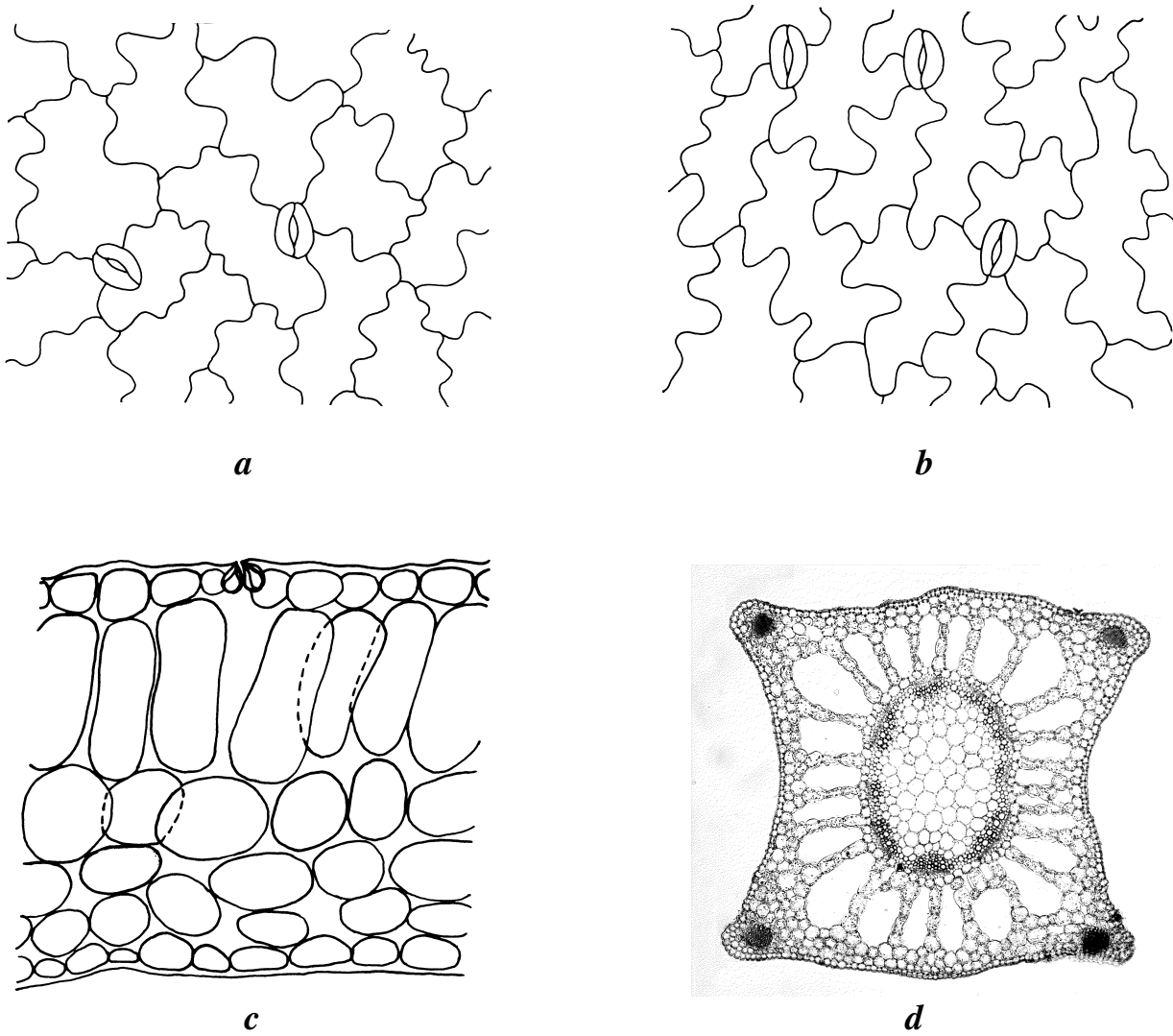
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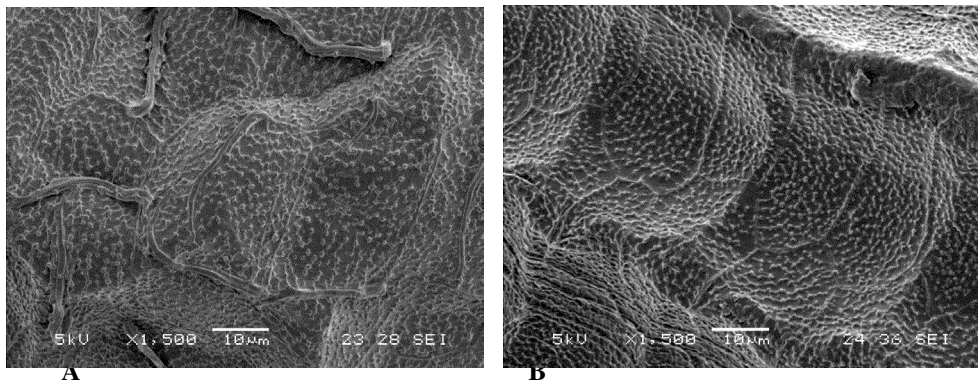
*d*

**Fig. 1** *Lindernia procumbens*; *a* – adaxial epidermis; *b* – abaxial epidermis; *c* – cross section of leaf; *d* – cross section of stem





**Fig. 2** *Lindernia dubia*; **a** – odaxial epidermis; **b** – abaxial epidermis; **c** - cross section of leaf; **d** – cross section of stem



**Fig. 3** Scanning electron micrographs of the spermoderm of *Lindernia procumbens* (A); and *Lindernia dubia* (B)