

Morpho-anatomical and palynological properties of *Acanthoprasium integrifolium* (Benth.) Ryding (Lamiaceae), endemic to Cyprus

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Received: 01 September 2022 / Revised: 11 October 2022 / Accepted: 12 October 2022

ABSTRACT: This study aimed to describe the morphological, anatomical and palynological properties of *Acanthoprasium integrifolium* (Benth.) Ryding, which is endemic in Cyprus. Plant samples were collected from Northern Cyprus. Morphological observations were made using a binocular stereo microscope with a drawing tube. For anatomical studies, the samples were conserved in 70% alcohol. The transverse and surface sections were taken by hand with a help razor blade. The nutlet micromorphologies and pollen properties were examined using scanning electron microscopy. In morphological studies, the description of *A. integrifolium* was given in detail for the first time and the general appearance of plant and leaf, bract, bracteole, flower, calyx, corolla and nutlet shapes were supported by drawings. The stem and leaf characteristics of the plant were examined in anatomical studies. The stem is almost tetragonal-rounded in shape. The vascular bundles are well-developed in the corners. The pith area covers a large area and includes simple crystals. The leaf is bifacial type and hypostomatic. The pollen grains are tricolpate and prolate shaped. While the surface of pollen grains is reticulate, the nutlets have scalariform ornamentation.

KEYWORDS: *Acanthoprasium integrifolium*; morphology; anatomy; nutlet; palynology.

1. INTRODUCTION

The island of Cyprus is one of the Mediterranean regions with rich flora with many woody and herbaceous beneficial plants [1, 2]. As the third largest Mediterranean island, Cyprus is among the islands with the highest percentage of endemism of plants in Europe [3]. The Flora of Cyprus consists of 1649 indigenous taxa (species and subspecies), 141 endemics in the whole island; 20 endemics to Northern Cyprus [4].

The Lamiaceae family is one of the major families of the world. Species of this family are cosmopolitans due to their worldwide distribution. This family is typically shrubby and herbaceous, with trees being extremely rare [5]. This family has great importance due to its economic value and being rich in species number. The family of Lamiaceae is represented by 27 genera and 24 endemic species in Cyprus [2, 4].

In recent studies, the genus *Ballota* L. was determined to be polyphyletic and *Acanthoprasium* was accepted as a new genus. *Ballota frutescens* (L.) Woods and *B. integrifolia* Benth. form a clade separate from other species of *Ballota*. The two species differ from other species of *Ballota* in having an herbaceous habit and herbaceous bracteoles versus a woody habit and spiny bracteoles, also differ from most other *Ballota* in lacking branched hairs and having the calyces internally glabrous. For this reason, it's considered and recognized as *B. sec. Acanthoprasium* [6]. The new genus is represented by 2 species in the world. While *A. frutescens* (L.) Spenn. grows in the Maritime Alps, *A. integrifolium* (Benth.) Ryding grows in Cyprus.

Up to now some limited studies of *A. integrifolium* have been conducted. In a study conducted in Egypt, seed micromorphology was examined [7]. In a study conducted in Germany, it was tried to be grown in an ex-situ environment using seed samples of the plant [8]. In another study, the palynological features of the species were determined [9].

How to cite this article: Findik A, Kaya A. Morpho-anatomical and palynological properties of *Acanthoprasium integrifolium* (Benth.) Ryding (Lamiaceae), endemic to Cyprus. J Res Pharm. 2023; 27(1): 196-206.

In this study, it is aimed to contribute to the Flora of Cyprus by examining the morphological, anatomical and palynological features of endemic *A. integrifolium*, naturally distributed in Cyprus, in detail for the first time.

2. RESULTS

2.1. Morphological properties



Figure 1. General appearance of *A. integrifolium*; **a.** plant, **b.** flowering branch.

As presented in Figures 1-2, *A. integrifolium* plants are slender sprawling shrubs to 100-140 cm. The stem is erect, slightly tetragonal-rounded, short with retrorsely adpressed pubescent, lower branches dull brown and thick, upper branches green or sometimes purplish. Stems covered by glandular and eglandular hair.

Leaves are decussate, simple, oblong, ovate-obovate or suborbicular, green in color, 11-28 x 8-21 mm, apex obtuse, sometimes shortly apiculate, margins entire, base cuneate, sparse short strigillose and sessile glandular hair is present on both surface of leaves. Petiole 2.5-7 x 0.2-1 mm.

Inflorescence is very lax, running almost the full length of the branches, 6-35.5 cm. Verticillasters are opposite 2 flowered.

Bracts are foliaceous and petiolate, petiole 1-8.1 x 0.2-1.1 mm long, blade 4.8-29 x 2.2-15 mm, simple, oblong, ovate-obovate, apex obtuse, sometimes shortly apiculate, margins entire, base cuneate.

Bracteoles in pairs at the base of the pedicel, hard, spinose, blackish-brownish, sometimes purplish, 5-7.5 x 0.5-1.1 mm, recurved and strigillose.

The shape of the calyx is infundibular, 8-16 x 2-4.2 mm and is colored as green to greenish yellow. Calyx tube with prominently 10-nerved, somewhat reticulate, teeth 5 at apex, 0.8-3.5 mm long, 1.8-6.8 mm wide including acute-apiculate of 0.5-1.1 mm, tube strigillose externally, especially on the veins, pilose on the inner surface only towards the base. Calyx tube covered by dense sessile glandular hair outside.

The corolla is 9-14.8 x 1.7-5 mm, white, bilabiate; adaxial lip (upper lip) pinkish, 3.8-6.2 x 3.2-4.8 mm, dense pubescent externally; abaxial lip (lower lip) 3-lobed, the median lob is larger than the lateral lobes, wide, cordate, pink stripes, 2.5-4.5 x 3.1-5 mm, the lateral lobes oblong, 1.7-2.8 x 1.3-2.2 mm. Corolla tube is annular hairy on the inside.

Stamens 4, didynamous, long stamens below the lower lip, short stamens below the upper lip. Filaments are white, 3.7-8 x 0.2-0.7 mm, and densely hairy at the base. Anthers are pinkish, 0.5-1.8 x 1.2-2 mm, dorsifixed, divergent, with yellow glandular hairy.

Ovary hypogynous, 4 ovules, light brown or yellowish, 0.8-1.8 x 0.8-1.2 mm. Style is gynobasic, yellow, 11.2-12 x 0.1-0.5 mm, stigma whitish-yellow, bifurcate, 0.1-0.2 x 0.3 mm.

Nutlets are smooth, trigonous, obovoid, 3-4.5 x 2-2.5 mm, front side brown and half of the back side is usually brown-white in color. The nutlet surface ornamentation is reticulate-scalariform, the attachment area small and round, sparsely eglandular and peltate hair were observed (Figure 3).

2.2. Anatomical properties

2.2.1. Stem anatomy

Transverse sections taken from the middle part of the stem were observed as in Figure 4-8. The stem is slightly tetragonal-rounded in shape. The epidermis consists of oval-square cells and is single layered. The upper and lower epidermal walls are thicker than the lateral ones.

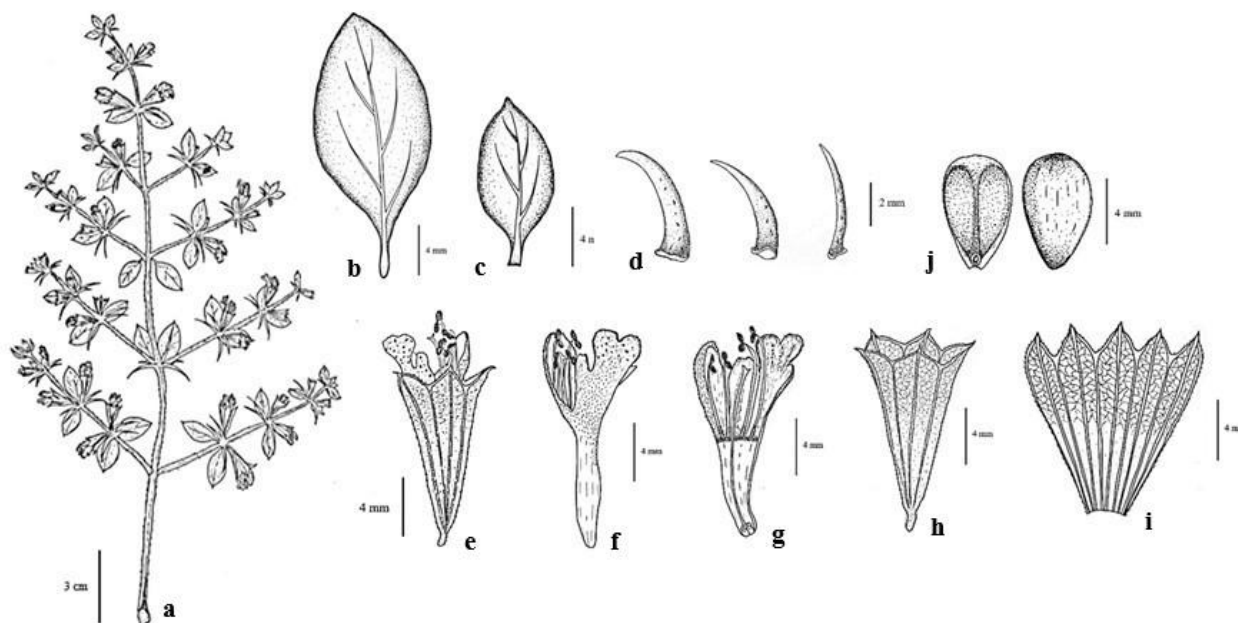


Figure 2. *A. integrifolium*; **a.** general appearance, **b.** leaf, **c.** bracte, **d.** bracteole, **e.** flower, **f.** corolla, **g.** dissected corolla, **h.** calyx, **i.** dissected calyx, **j.** nutlet (drawing b A. Findik).

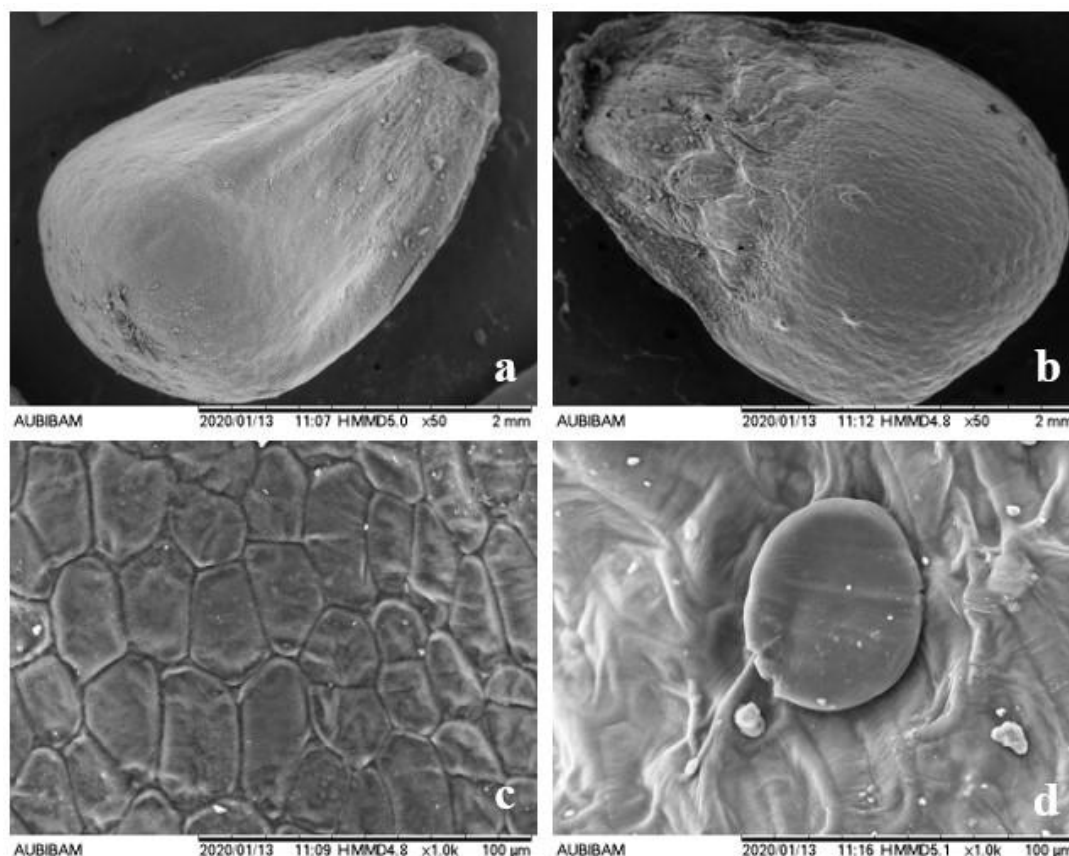


Figure 3. The nutlet of *A. integrifolium* in SEM; **a-b.** general appearance, **c.** surface ornamentation, **d.** peltate hair.

The upper surface is surrounded by a thin cuticle and contains sparse trichome. They are both of eglandular or glandular types. Eglandular hairs are usually recurved, 1-2 or rarely 3 cells and are covered by micro-papillae. Glandular hairs are capitate hair type consisting of unicellular head and unicellular stalk and it is rarely observed. Collenchyma tissue is located under the epidermis. It is composed of 5-7 layers in the corners and 1-2 layers in between the corners of the stem sections. The cortex tissue is formed by parenchymatous, rectangular flat cells. They are 2-3 layers in the corners while 4-5 layers are in between the corners. There are 1-2 layers of chlorenchymatous cortex tissue (=photosynthesizing tissue), below the collenchyma in the corners and 1-4 layers below the epidermis in between the corners, containing chloroplasts and a few crystals. The endodermis is partially interrupted. It is composed of oblong and ovoid-shaped cells. Underneath the endodermis, pericycle is located. The pericycle consists of 2-4 layers of thick-walled sclerenchymatous cells. Vascular tissue is well developed at the corners, spread in between the corners, and arranged in a ring. The phloem is formed by flattened cells and is 4-5 layered. The cambium is not distinguishable or consists of several cells. The xylem consists of trachea and tracheid cells occupying a large region. Trachea cells are rounded or oval in shape while tracheid cells are polygonal. Pith rays are usually one-rowed. The pith is large and is composed of rounded or polygonal parenchymatous cells with abundant intercellular spaces. The diameters of the parenchyma cells expand towards the center and generally include simple crystal.

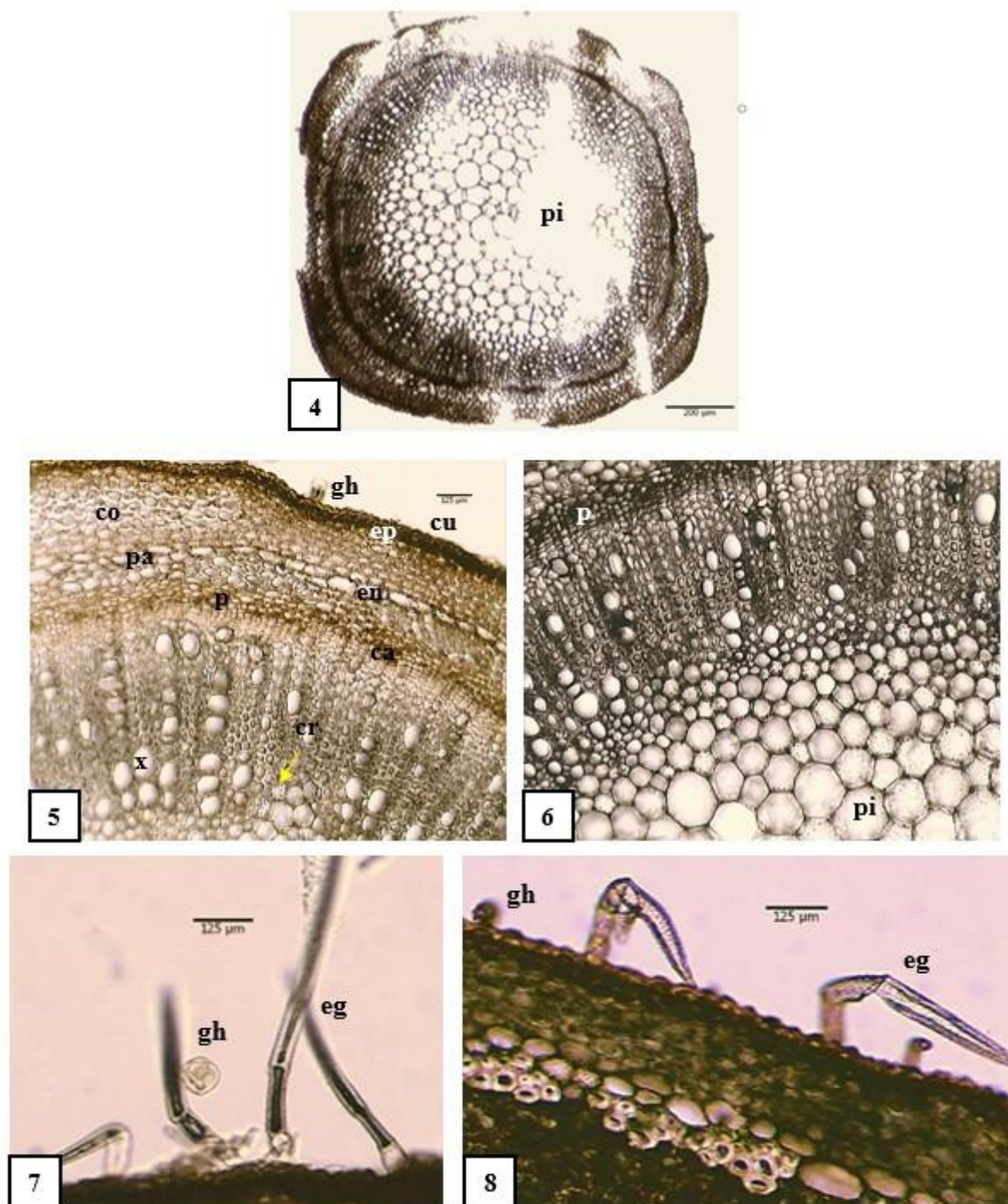


Figure 4-8. The transverse section of *A. integrifolium* stem; **cu.** cuticle, **ep.** epidermis, **co.** collenchyma, **pa.** parenchyma, **sc.** sclerenchyma, **ca.** cambium, **x.** xylem, **p.** phloem, **en.** endoderm, **pi.** pith region, **cr.** simple crystal, **eg.** eglandular hair, **gh.** glandular hair.

2.2.2. Leaf anatomy

In transverse section, the upper and lower epidermis consist of uniseriate, squarish and rectangular cells. (Figure 9,10). In the superficial sections taken from the leaf, the upper walls of the epidermis are straight or slightly wavy while the lower walls of the epidermis are prominent and wavy (Figure 11,12). Both epidermises are covered by a thin cuticle. The upper epidermal cells are bigger in size than the lower ones. The upper and lower walls of both epidermis cells are thicker than the lateral walls. Covering trichomes are of both eglandular and glandular types on both surfaces. Most of them are eglandular hairs which are 1-2

cellular. The glandular hairs are peltate type and are common on the lower surface (Figure 9,10). In superficial sections taken from the leaf, the stomata are ovoidal in shape and diacytic. The stomata cells are observed on only the lower epidermis (hypostomatic). The leaf type is bifacial. The mesophyll tissue comprises 1 layered palisade parenchyma and 4-5 layered spongy parenchyma (Figure 10). The vascular bundle is collateral, occurring over a large area in the midrib. The midrib region forms a projecting part in *A. integrifolium*. The phloem is in the direction of the lower epidermis, and the xylem is in the direction of the upper epidermis. Below the phloem, several seriate sclerenchyma cells were observed. 1-2 series collenchymatous cell layers and 2-3 seriate parenchymatous cell layers are located below the upper epidermis while 2-3 seriate collenchymatous cells and 3-6 seriate parenchymatous cells are located below the lower epidermis in the midrib region (Figure 9).

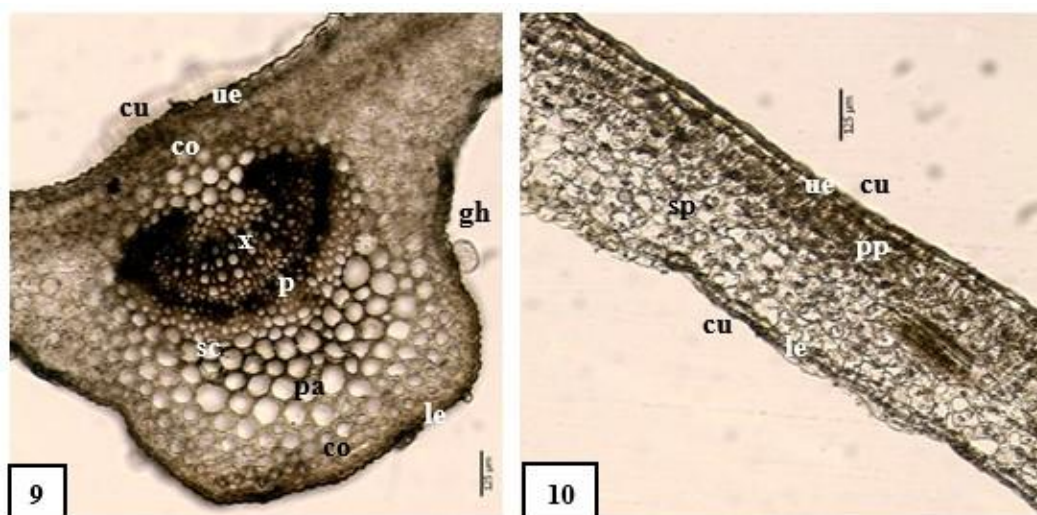


Figure 9-10. The transverse section of *A. integrifolium* leaf; **cu.** cuticle, **ue.** upper epidermis, **le.** lower epidermis, **co.** collenchyma, **pa.** parenchyma, **sc.** sclerenchyma, **x.** xylem, **p.** phloem, **pp.** palisade parenchyma, **sp.** spongy parenchyma, **gh.** glandular hair.

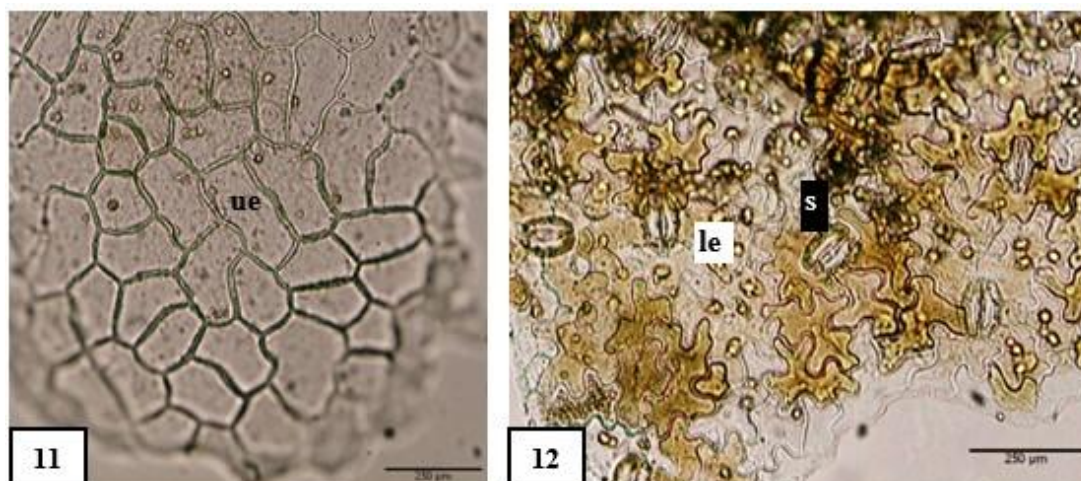


Figure 11-12. The superficial section of the *A. integrifolium* leaf; **ue.** upper epidermis, **le.** lower epidermis, **s.** stoma cell.

2.3. Palynological properties

The pollen grains of *A. integrifolium* are single, radially symmetrical, isopolar and tricolpate. Their shape is prolate. The dimensions of polar axis and equatorial axis are 28.7-36.3 μm and 16.2-25.2 μm . The ratio of P/E is 1.44-1.77 μm . The outline is elliptic in the equatorial view and almost triangular in the polar view. Colpus are narrow and long. Its length is 24.6-33.0 μm and its width is 1.58-4.16 μm . Pollen ornamentation is reticulate, but some of them are reticulate-perforate. The shape of the lumina is irregularly polygonal, the holes are of different sizes and round. The surface of muri is almost smooth (Figure 13).

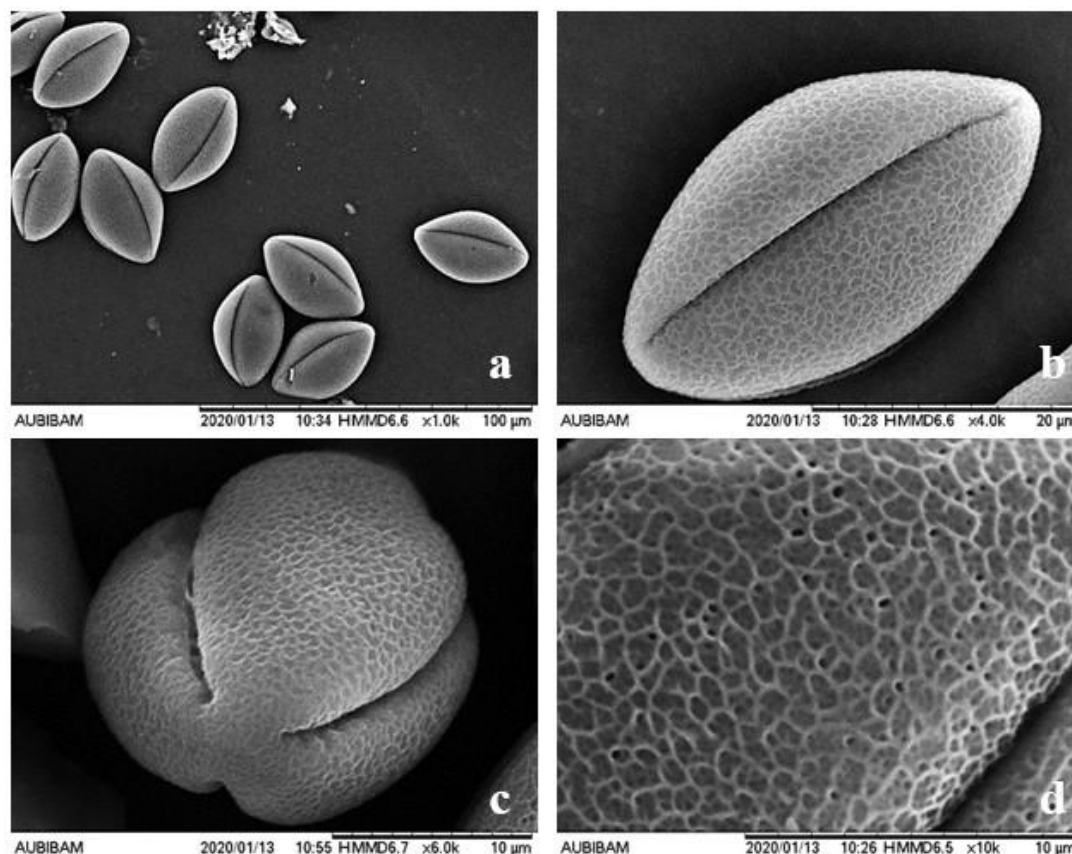


Figure 13. The pollen grains in SEM; a. general appearance, b. equatorial view, c. polar view, d. pollen ornamentation.

3. DISCUSSION

Morphological, anatomical and palynological characteristics of *A. integrifolium* endemic to Cyprus were investigated in this study and the findings obtained were compared with other studies and Flora of Cyprus [2].

Although the present results usually correspond with the description recorded in the Flora of Cyprus (Table 1), several differences were found here. According to our findings, the lengths of stem, calyx, corolla and nutlet were found higher than in Flora of Cyprus while the lengths of petiole and bracteole were longer in Flora of Cyprus. Furthermore, the length of verticillasters, the distance between verticillasters, the structure of bract, dimensions and shape, the color of calyx, and colors of stamen and pistil are reported here in detail for the first time. According to the result of this study, the variations borders of species were expanded.

Anatomical features typical of the Lamiaceae [11] such as the rectangular cross-section of the stem, well-developed collenchyma in each corner, sclerenchymatic tissue surrounding the vascular tissue and diacytic stomata were also observed and these characteristics are diagnostic value. A large group of collenchyma is observed at the corners of *A. integrifolium* stems. Furthermore, collenchyma tissue and vascular bundles are well developed in the corners. The stomata type is diacytic. Our observations show that *A. integrifolium* has the general anatomical characteristics of Lamiaceae with respect to leaf and stem.

Anatomical properties of the stem and leaf in *A. integrifolium* are observed similarly with studied *Ballota* species [12,13]. According to research in the literature, the anatomical features of the *B. nigra* subsp. *anatolica* P.H. Davis and *B. nigra* L. subsp. *nigra* are generally similar to the results of our study [12,13].

In our leaf anatomy study, we observed that *A. integrifolium* has bifacial leaves. The same result was seen in *B. nigra* subsp. *kurdica* P.H. Davis and *B. acetabulosa* (L.) Benth. [14-16]. In addition, *B. nigra* subsp. *anatolica* has crystals in the stem, which is consistent with our findings [17]. According to the results of the stem anatomical studies conducted by El-Deen Osman [18] with 5 *Ballota* taxa, the endoderm and cambium are not fully defined, the presence of the chlorenchyma layer and the presence of raphide crystals in the stem piths of the other 4 *Ballota* species (*B. damascena* Boiss., *B. kaiserii* Täckh., *B. pseudodictamnus* (L.) Benth., *B. undulata* (Sieber ex Fresen.) Benth.) except for *B. saxatilis* Sieber ex C. Presl, are similar to our results.

In a study by Siadati et al. [7] nutlet micromorphological characteristics of *A. integrifolium* were researched and the measurements were reported as follows: triangular nutlets and scalariform sculpturing, 2.75 x 1.39 mm in size, apex densely bearded, outer periclinal wall is a shallow concave and anticlinal wall is raised. However, these findings were found to be inconsistent with our study and features of *A. integrifolium* in Flora of Cyprus.

Nutlet samples used in this study were obtained from a Botanical Research Institute in Munich. In this case, the samples are probably mixed. Because in our study, densely bearded hairs were not observed in any of the nutlets. In addition, Meikle [2] determined that the nutlets are smooth and glabrous in Flora of Cyprus. The results of Siadati et al. [7] do not agree with our results. We can say that their study plant may belong to a different species.

Pollen characteristics are of taxonomical importance and are used for the classification of Labiatae family [19]. Erdtman [19] divided the Lamiaceae family into two subfamilies on the basis of palynological characteristics. The first group has tricolpate and comprises the subfamily Lamioideae. The second group has hexacolpate and contains the subfamily Nepetoideae. Abu-Asab and Cantino [9, 20] reported the palynological features of some species in the subfamily Lamioideae. The shape of pollen grains is suboblate to euprolate. The polar axis is 15-59 µm and equatorial axis is 13-52 µm in size. Pollen type is usually tricolpate. The exine sculpturing is generally scrobiculate, sometimes micro-reticulate or perforated. The surface ornamentation is usually subreticulate (it is called bireticulate in some sources) [21]. *A. integrifolium* has tricolpate pollen and belongs to the subfamily Lamioideae and Marrubieae tribe. In SEM, dimensions ranges are: polar axis 28.7-36.3 µm and equatorial axis 16.2-25.2 µm. The ratio of P/E is 1.44-1.77 µm. The shape of pollen grains is prolate. Pollen ornamentation is reticulate, but some of them are reticulate-perforate. The present results support the study of Abu-Asab and Cantino [20].

In the study of El-Deen Osman [18], the fact that the *Ballota kaiserii* Täckh. has reticulate-perforate pollen ornamentation is consistent with our results. Doaigey et al. [22] examined the pollen of the Lamiaceae family and defined the pollen ornamentation of *B. undulata* (Sieber ex Fresen.) Benth. as reticulate-perforate, which is consistent with the surface properties of *A. integrifolium* in our study.

Table 1. Morphological characters of *A. integrifolium* based on the present study and Flora of Cyprus.

		Present Data	Flora of Cyprus
Life form		erect, slender sprawling shrub	slender sprawling shrub
Plant size		100-140 cm	100 cm
Stem	shape	slightly tetragonal-rounded	tetragonal
	color	lower branches dull brown, upper branches green or sometimes purplish	old branches dull brown
	trichome	short, retrorsely adpressed pubescent	densely strigillose with retrorsely adpressed
Leaves	size	11-28 x 8-21 mm	0.7-2(-3) x 0.5-1.5(-2.5) cm
	shape	simple, oblong, ovate-obovate or suborbicular	oblong, obovate, suborbicular or occasionally ovate
	color	green	dull or lustrous green
	apex	obtuse, shortly apiculate	obtuse or subacute, sometimes shortly apiculate
	margins	entire	entire
	base	cuneate	cuneate or rounded

	trichome	sparse short strigillose	thinly adpressed-strigillose upper surface, subglabrous lower surface, or occasionally thinly lanuginose on both surfaces
Petiole	size	2.5-7 x 0.2-1 mm	1.5 cm long
Verticillasters	size	6-35.5 cm	-
		branched	branched
	flower	2-flowered	2-flowered
	inter verticillus distance	1-4 cm	-
Bracts	size	4.8-29 x 2.2-15 mm	-
	shape	foliaceous, simple, oblong, ovate-obovate	-
	apex	obtuse, shortly apiculate	
	margins	entire	
	base	cuneate	-
	petiole size	1-8.1 x 0.2-1.1 mm	
Brakteoles	size	5-7.5 x 0.5-1.1 mm	8-15 mm long
	shape	hard, spinose and recurved	hard, spinose, straight or recurved
	color	blakish-brownish, sometimes purplish	brownish
	trichome	sparsely strigillose	strigillose
Calyx	size	8-16 x 2-4.2 mm	11-12 x 7-9 mm
	shape	infundibular	infundibular
	color	green to greenish-yellow	-
	veins	10-nerved	10-nerved
	tooth	5-tooth	5-tooth
	tooth size	0.8-3.5 x 1.8-6.8 mm	2-3 x 4-5 mm
	tooth shape	acute, apiculate	apiculate
	trichome	strigillose towards above, pilose towards below	glabrous or pilose
Corolla	size	9-14.8 x 1.7-5 mm	9 x 3.5 mm
	shape	bilabiate	bilabiate
	color	white, upper lip pinkish, lower median lobe pink stripes	white, with purple or red stripes
	upper lips	3.8-6.2 x 3.2-4.8 mm	7 x 3.5 mm
	lateral lobes	1.7-2.8 x 1.3-2.2 mm	2 x 2 mm
	median lobe	2.5-4.5 x 3.1-5 mm	3.5 x 6-7 mm
Stamen	number	4-stamened	4-stamened
	shape	didynamous	didynamous
	position	long stamens below lower lip, short stamens below upper lip	inserted at about the same level, 2 mm down the corolla tube
Filaments	size	3.7-8 x 0.2-0.7 mm	6-8 mm long
	color	white	-
	trichome	densely hairy at the base	thinly hairy
Anther	size	0.5-1.8 x 1.2-2 mm	2.3 mm wide
	color	pinkish	-
Ovary	size	0.8-1.8 x 0.8-1.2 mm	1.8 mm diameter
	color	light brown or yellowish	
Style	size	11.2-12 x 0.1-0.5	12 mm long
	color	yellow	-
Stigma	size	0.1-0.3 x 0.1-0.2 mm	the adaxial less than 0.5 mm long, the abaxial a little longer

Nutlet	color	whitish-yellow	-
	size	3-4.5 x 2-2.5 mm	3.5 x 2.5 mm
	shape	trigonus, obovoid	trigonus, obovoid
	color	front side brown and half of the back side is usually brown-white	dark brown
	ornamentation	reticulate-scalariform	smooth or slightly granulose
	trichome	sparsely egandular and peltate hairy	-

4. CONCLUSION

In this study, the morphological, anatomical and palynological features of *A. integrifolium* were revealed for the first time. In morphological studies, in detail description of *A. integrifolium* was given were supported by drawings. The stem and leaf characteristics of the plant were examined in anatomical studies. The stem is almost tetragonal-rounded in shape. The vascular bundles are well-developed in the corners. The pith area covers a large area and includes simple crystals. The leaf is bifacial type and hypostomatic. The pollen grains are tricolpate and prolate shaped. While the surface of pollen grains is reticulate, the nutlets have scalariform ornamentation. The data obtained at this stage enrich the information about these features of the members of the Lamiaceae family and can be used in comparative studies, both between the species of the genus *Acanthoprasium* and also with molecularly related genera.

5. MATERIALS AND METHODS

The plant specimens for the research were collected during the flowering and fruiting stages from the Kyrenia (in the vicinity of Alevkayası 23° NW 35°16'58" N 33°31'50" E) district of the Turkish Republic of Northern Cyprus during the field studies carried out in April-June 2019 (Figure 14). Voucher samples are stored in the Herbarium of the Faculty of Pharmacy, Anadolu University (ESSE 15513, ESSE 15516). Each value is the average of at least 20 measurements from different samples. Macromorphological observations were made using a Olympus SZX12 binocular stereo microscope with a drawing tube.

Anatomical investigations were performed using fresh specimens kept in 70% alcohol. Manuel transverse sections were made from the middle part of the stem and leaf. All sections were covered by glycerin gelatin and examined with and light microscope with a camera. Nutlet and pollen micromorphology were studied by HITACHI TM 3030 Plus Tabletop Scanning Electron Microscopy. For the SEM, the nutlet and pollen of the plant were directly placed on aluminum stubs using double-sided adhesive tape and sputter-coated with gold using an SPC-900 Single Target Plasma Sputtering Thin Film gold-coating apparatus. The terms used for describing the pollen and nutlet surface have been adopted according to Stearn, 1978 [22].



Figure 14. The collected area of *A. integrifolium* [23].

Acknowledgements: The authors would like to thank Dr. Şennur Görgülü and Anadolu University (AUBIBAM) for SEM studies.

Author contributions: Concept – A.F., A.K.; Design – A.F.; Supervision – A.K.; Resources – A.F., A.K.; Materials –

A.F., A.K.; Data Collection and/or Processing – A.F., A.K.; Analysis and/or Interpretation – A.F., A.K.; Literature Search – A.F., A.K.; Writing – A.F., A.K.; Critical Reviews – A.F., A.K.

Conflict of interest statement: The authors declared no conflict of interest.

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