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Comparative characteristics of anatomical and morphological adaptations of plants of two subgenera *Haworthia* Duval to arid environmental conditions

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Abstract

This paper presents the comparative anatomical and morphological characteristics of plants of two subgenera: *Haworthia* and *Hexangularis*. The study revealed two different strategies of adaptation to arid conditions of the growth of different subgenera of the genus *Haworthia*. Plants of the subgenus *Haworthia* adapted to arid conditions by increasing the accumulation of water, the presence of "windows", a smaller stoma size, and a thinner outer wall of the epidermis cells. On the other hand, plants of the subgenus *Hexangularis* adapted to arid conditions by reducing overheating and transpiration as well as by the presence of papillae and a thickened outer wall of the epidermis cells.

Keywords: *Haworthia*; *Hexangularis*; leaf; anatomy; morphology; arid conditions

Introduction

The French physician and expert of succulents Henri Auguste Duval isolated the genus *Haworthia* from the genus *Aloe* L. in 1809 and named it in honor of an English merchant, Adrian Haworth, who devoted most of his life to describing new species of the family Aizoaceae and Asphodelaceae, which came to Europe from South Africa [1,2].

According to the report of Jacobsen [3], almost 100 plant species belonged to the genus *Haworthia* and they were divided into 20 sections on the basis of their growth form and the morphological features of their leaves. A subdivision of the genus on the basis of the floral characters was suggested by Uitewaal [4]. Bayer reduced the number of species to 59 and divided the genus into three subgenera based the flower form: *Haworthia*, *Hexangulares*, and *Robustipedunculares* [5]. As the basis of his conclusions, he mostly used the shapes of the flowers, but the recognized subgenera also differed in leaf morphology. The subgenus *Haworthia* is the largest one within the genus in which Bayer included more than 40 species. The plants of this subgenus are characterized by a triangular or roundly-triangular base of the flower and an obovate-clavate curved tube of the perianth with free separate petals. The peduncle is simple, often few-flowered. The subgenus *Hexangularis* (*Hexangulares*; 16 species) is characterized by a hexagonal base of the flower. The perianth is back-capitate, tubular with ⅔ of petals fused, often curved. The peduncle is simple or branched, drooping, usually many-flowered [5].

The representatives of the genus *Haworthia* are mostly small herbaceous plants with a terrestrial rosette of leaves which with aging form more or less dense turf and small bushes of orthotropic or anisotropic directed leafy stems forming a loose tussock with aging. The leaves are from widely triangular to elongate triangular, from 1 to 10 cm long and from 5 mm to 2 cm wide at the base [6,7]. Most species are endemics of the provinces of Eastern and Western Cape (RSA), where they grow along the Indian Ocean and on the mountain slopes and hills, some of them at an altitude of 1500 m [8,9].

Haworthia are leaf succulents; the central part of the leaf contains a significant amount of water-storage tissue, surrounded on the abaxial and adaxial side with multi-layer chlorenchyma. However, the leaf shape and the features of the epidermal tissue vary in different species. The anatomical and morphological features of *H. reinwardtii* and *H. coarctata* were studied by P.E. Brandham and D.F. Culter [10] in a comparative ploidy of plants of different populations. The authors noted the presence of papillae on the leaf surface, domed epidermal cells among which are the stomata with 4 guard cells [5].

The aim of this work was to compare the anatomical and morphological characteristics of plants of two subgenera: *Haworthia* and *Hexangularis*, and to show different strategies that are characteristic for these plants in the arid climate environment.

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Material and methods

The genus *Haworthia* was included in the Asphodelaceae family [1,2]. According to the new taxonomy of this genus, it is now classified in the Xanthorrhoeaceae family [11]. 18 species from two subgenera of the genus *Haworthia* (according to the classification by Bayer [5]) are the objects of our research. (***i***) The subgenus *Haworthia*: *H. angustifolia* Haw., *H. blackburniae* W.F. Barker, *H. chloracantha* Haw., *H. cooperi* Baker, *H. cymbiformis* (Haw.) Duval, *H. marumiana* Uitewaal, *H. parksiana* v. Poelln., *H. pygmaea* v. Poelln., and *H. retusa* (L.) Duval; (***ii***) the subgenus *Hexangularis*: *H. attenuata* (Haw.) Haw., *H. coarctata* Haw., *H. fasciata* (Willd.) Haw., *H. glabrata* (Salm-Dyck) Baker, *H. glauca* Baker, *H. limifolia* Marloth, *H. pungens* M.B. Bayer, *H. reinwardtii* (Salm-Dyck) Haw., and *H. viscosa* (L.) Haw. Plants (5–6 of every species) from the collection of the O.V. Fomin Botanical Garden were used for the investigation [12]. Ten leaves from each species were studied. Leaf samples were taken in the middle part of the rosette or shoot. The anatomical data were obtained from the middle part of the leaf.

The samples were fixed according to Chamberlain [13]. They were embedded in gelatin by the standard method and leaf cross-sections were made (with a thickness of 10–15 μm) by a freezing microtome as well as the epidermis was skinned from macerated leaves. The sections were stained with safranin. The morphometric and anatomical measurements were carried out on the abaxial and adaxial side of the laminas: lengths and widths of stomata ($n = 100$ for species), number of stomata per 1 mm^2 ($n = 50$), number of epidermal cells per 1 mm^2 ($n = 50$), epidermal cell area ($n = 100$), thickness of the epidermal outer cell walls ($n = 100$), thickness of the epidermis ($n = 100$), numbersof chlorenchyma layers ($n = 20$). The evaluation was made of the presence of trichoma, epidermal protuberances and ridges, cuticular papillae and "leaf windows" (a translucent area where sunlight can enter into the interior surfaces of the plant's modified leaf where photosynthesis can occur) of different species. The structure of the epidermis was described following S.F. Zakharevich (projection and outline of the epidermal cells) [14] and M.A. Baranova (type of stomata) [15]. The following morphological features were described: leaf shape, rosette shape, leaf length and width [16]. Additionally, the ability to store water was evaluated (the weight of fresh leaves was 100% and it was compared to the measured weight of these leaves after complete drying). The microscopic measurements were carried out using an eyepiece-micrometer on an XSP-146TR microscope. The epidermal cell area was measured using ImageJ software. The obtained data were statistically processed with the program Statistica 6 at a $P \leq 0.05$ confidence level. The pictures were taken with a Canon Power Shot A630 digital camera.

Results

The rosette size in the subgenus *Haworthia* rarely exceeds 5–6 cm in diameter. The leaves of species such as *H. parksiana* and *H. blackburniae* have an elongated triangular form and do not exceed 5 mm in width. The leaves of the rest of the studied species were oblanceolate or lanceolate with a more or less elongated apical part (Tab. 1). The leaves are often shiny, smooth, frequently with "windows" (Fig. 1a–c,e) and/or trichomes on the edge and in the apical part, whereas in some species in the central part of the leaf.

The leaves of plants of the subgenus *Hexangularis* are dark green, dull, often with appendages on the bottom or on both sides. Phyllotaxy is a rosette (many leaf rosettes) or regular (up to 25 cm high). The smallest leaf size is typical for *H. glauca*, *H. reinwardtii* and *H. viscosa*. The narrowest leaves are typical for *H. glauca*, *H. reinwardtii* and *H. pungens* (Fig. 2, Tab. 2). The leaf shape is more or less the same in all the studied species. The abaxial and adaxial side of the leaf of *H. coarctata*, *H. glauca* and *H. reinwardtii* differs because of the orientation of the leaves upward and increased exposure of the abaxial side.

Tab. 1 Traits characterizing the leaf rosette habit and leaf morphology of the studied species of the subgenus *Haworthia*.

Species	Rosette form / leaf direction	Leaf form / The widest part of the leaf (by Bayer B. [5])	Leaf length / width (cm)	No. of chlorenchyma-parenchyma layers (pcs)
H. angustifolia	Many leaves / upward and outward	Lanceolate with elongated apical part / in the bottom third leaf	2.7 ±1.1 / 0.5 ±0.2	3–4
H. blackburniae	Few leaves / upward	Elongated triangular / at the leaf base	8.2 ±2.0 / 0.8 ±0.1	3–4
H. cymbiformis	Many leaves / upward	Oblanceolate / upper third leaf	3.7 ±1.2 / 1.3 ±0.4	3–4
H. cooperi	Many leaves, dense / upward and outward	Oblanceolate / upper third leaf	2.4 ±0.5 / 1.3 ±0.2	4–6
H. chloracantha	Many leaves / upward and outward	Lanceolate with elongated apical part / in the bottom third leaf	3.0 ±0.5 / 0.7 ±0.2	4–5
H. marumiana	Many leaves / upward	Oblanceolate / in the center of the leaf	2.9 ±0.3 / 0.6 ±0.06	3–4
H. parksiana	Many leaves / outward	Elongated triangular with strongly elongated apical part / at the leaf base	2.7 ±0.6 / 0.6 ±0.2	7–8
H. pygmae	Few leaves, dense / outward	Oblanceolate / upper third leaf	3.2 ±0.7 / 1.4 ±0.3	5–6
H. retusa	Few leaves / outward	Oblanceolate / upper third leaf	3.2 ±0.4 / 1.3 ±0.2	5–7

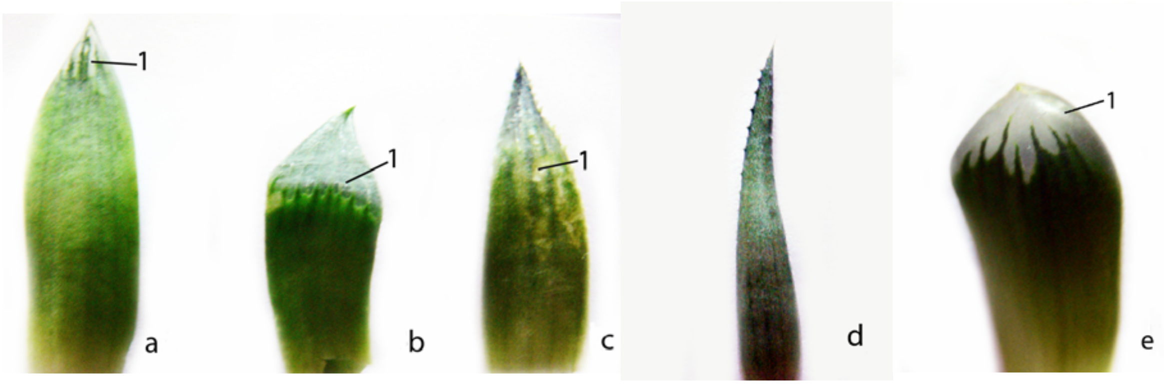

Fig. 1 Leaves of the subgenus *Haworthia*: *H. cymbiformis* (**a**), *H. retusa* (**b**), *H. chloracantha* (**c**), *H. angustifolia* (**d**), *H. cooperi* (**e**). 1 – "leaf windows".

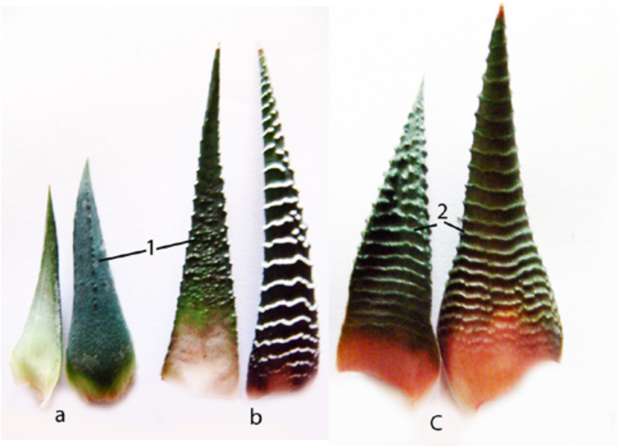

Fig. 2 Leaves of the subgenus *Hexangularis*: *H. glauca* (**a**), *H. fasciata* (**b**), *H. limifolia* (**c**). 1 – verrucae; 2 – ridges.

The morphometric changes on the adaxial and abaxial surfaces are in general species-specific (Tab. 3, Tab. 4). There are only a few differences compared with the opposite side: a thickening of the outer cell wall of the epidermis as well as an increase in the number of epidermal cells per unit area and, respectively, a decrease in their size can be observed on the abaxial epidermis. Therefore, both epidermises are further described together. Most plants of both subgenera have the following common characters: strongly developed water-storage parenchyma, poorly developed vascular tissue, and practically absent mechanical tissue; deeply sunken stomata covered with the rim of emergencies of epidermal cells, tetracytic stomatal complexes, 1-layered epidermis of polygonal straight-walled cells; in most of the species, longitudinally directed raphides of calcium oxalate are deposited under the chlorenchyma. The xeromorphic features are more evident on the abaxial side of the lamina, which is caused by the leaf disposition.

Tab. 2 Traits characterizing the leaf rosette habit and leaf morphology of the studied species of the subgenus *Hexangularis*.

Species	Rosette form / leaf direction	Leaf form / the widest part of the leaf (by Bayer B. [5])	Leaf length / width (cm)	No. of chlorenchyma-parenchyma layers (pcs)
H. attenuata	Many leaves / upward and outward	Elongated triangular / at the base leaf	4.6 ±0.7 / 1.3 ±0.4	5–7
H.coarctata	Sprout to 20 cm tall. Leaves are alternate, directed upwards	Elongated triangular / at the base leaf	3.9 ±0.6 / 1.0 ±0.2	2–3
H.fasciata	Many leaves / upward and outward	Elongated triangular / at the base leaf	6.3 ±0.9 / 1.1 ±0.3	7–8
H.glabrata	Many leaves / upward and outward	Elongated triangular / at the base leaf	5.7 ±0.7 / 1.3 ±0.3	6–7
H.glauca	Sprout to 25 cm tall with alternate leaf arrangement / upward	Elongated triangular / at the base leaf	3.5 ±0.4 / 1.1 ±0.2	4–5
H.limifolia	Dense rosette / outward	Broadly triangular / at the base leaf	4.9 ±0.5 / 1.5 ±0.4	7–8
H.pungens	Dense rosette / outward	Elongated triangular / at the base leaf	5.1 ±0.4 / 0.8 ±0.2	2–3
H. reinwardtii	Sprout to 20 cm tall with alternate leaf arrangement / upward	Elongated triangular / at the base leaf	3.2 ±0.6 / 0.7 ±0.1	5–7
H.viscosa	Sprout to 20 cm tall with alternate leaf arrangement / outward	Elongated triangular / in the lower third of leaf	2.3 ±0.2 / 0.9 ±0.1	4–6

Tab. 3 The comparative statistical analysis of the results for each species of the genus *Haworthia*.

	H. glauca	***H. attenuate***	***H. coarctata***	***H. pungens***	***H. reinwardtii***	***H. limifolia***	***H. glabrata***	***H. fasciata***	***H. viscosa***	***H. cymbiformis***	***H. cooperi***	***H. angustifolia***	***H. parksiana***	***H. retusa***	***H. blackburniae***	***H. marumiana***	***H. pygmaea***	***H. chloracantha***
H. glauca		134567	123467	124567	123467	123467	123467	123467	123467	123456	123467	123456	123467	123467	123467	124567	123457	123467
H. attenuata	123467		12456	1234567	123467	124567	2467	27	1234567	123467	1234567	12347	123457	123467	1234567	123467	123467	12347
H. coarctata	12345	123467		1234567	123467	1234567	12467	123467	2347	123467	1234567	12467	12567	1234567	123467	1234567	1234567	1246
H. pungens	1234567	145	1234567		123456	1234567	1234567	1234567	1234567	23457	1234567	1234567	2346	123457	23457	124567	234567	1234567
H. reinwardtii	1234567	123567	1234567	13456		1234567	34567	34567	1567	1234567	123457	1234567	123456	123457	12345	1234567	1234567	1234567
H. limifolia	123457	124567	123457	14567	134567		3457	12457	134567	123456	1234567	246	1234567	123456	124567	1234567	12345	123457
H. glabrata	123456	134567	124567	1234567	234567	1234567		3456	134657	123456	1234567	12456	124567	123456	124567	1235467	12345	124567
H. fasciata	1234567	2467	124567	124567	1367	14567	124567		13467	123467	12346	124	123467	123456	12467	123467	123457	123457
H. viscosa	123467	23467	1234567	1234567	1467	13467	123467	13467		12467	12367	123467	1234567	123467	123467	1234567	12467	1234567
H. cymbiformis	123467	1234567	12567	4567	1234567	124567	1267	12346	123467		13467	1234	2347	123456	23467	123467	1246	12467
H. cooperi	123467	1234567	123467	3467	1234567	1234567	1234567	123467	123467	346		123567	13467	123467	123457	12347	1234567	123467
H. angustifolia	12347	1234567	1234567	213467	1234567	1234567	12467	12347	123467	123467	12367		123457	123456	12467	123467	123456	1347
H. parksiana	123467	123467	123467	123456	1246	1234567	123467	12367	1267	123467	123467	12467		1234567	3467	13467	1234567	12467
H. retusa	1234567	1234567	1234567	234567	123457	123456	1234567	12345677	1234567	23456	23457	1234567	1234567		123567	1234567	1234567	1234567
H. blackburniae	123467	123457	12367	123457	1234	123467	123567	1234567	12367	12346	13457	3467	1234567	12437		13456	23467	123467
H.marumiana	12356	1234567	123467	123467	1234567	123467	1234567	1234677	1234567	12346	134567	3467	1234567	123467	23467		123467	23467
H. pygmaea	12467	1234567	123456	1234567	123467	123467	123467	12347	123467	23467	2456	12347	123456	234567	1234567	123467		1234567
H. chloracantha	124567	1234567	12456	14567	1234567	234567	12457	12456	1234567	124567	1234567	1234567	1234567	1234567	1234567	1234567	14567	

$P \leq 0.05$: 1 – length of stomata; 2 – width of stomata, 3 – amount of stomata, 4 – No. of epidermal cells, 5- area of epidermal cells; 6 – thickness of outer cell wall of epidermis; 7 – thickness of epidermis. The white cells – adaxial side, the grey cells – abaxial side.

Tab. 4 Characteristics of leaf epidermis of the studied species of the subgenus *Hexangularis*.

Species	Length of stomata (μm)	Width of stomata (μm)	Amount of stomata (pcs. / 1 mm²)	No. of epidermal cells (pcs. / 1 mm²)	Area of epidermal cells (μm²)	Thickness of outer cell wall of epidermis (μm)	Thickness of epidermis (μm)
Adaxial side							
H. cymbiformis	**38.7 ±2.8**	**30.7 ±4.2***	11.9 ±7.9*	*295.8 ±63.4*	*3506 ±620*	14.1 ±3.1*	56.3 ±4.9
H. cooperi	**35.6 ±4.2***	**31.2 ±4.1***	*18.1 ±5.9**	**496.7 ±52.9**	**2038 ±234**	*10.2 ±2.5*	*49.1 ±4.4**
H. angustifolia	**43.9 ±4.8***	39.6 ±4.8*	**6.4 ±4.2***	349.7 ±52.3*	2925 ±455	15.3 ±6	54.0 ±7.9
H. parksiana	**38.8 ±2.9***	**32.4 ±2.8***	**8.2 ±4.1***	339.4 ±60.7*	3038 ±537*	15.1 ±2.6	*41.7 ±4.2**
H. retusa	**33.4 ±2.6***	**27.3 ±4.9**	*23.7 ±9.1**	**614.0 ±82.1***	**1657 ±220**	*12.0 ±2.9*	53.8 ±7.8
H. blackburniae	**39.4 ±2.8**	**33.4 ±4.2**	**5.9 ±4.1***	*296.6 ±52.5*	*3488 ±683*	*9.7 ±2.59*	*36.8 ±7.1**
H. marumiana	**41.6 ±2.5***	**33.5 ±3.6**	**2.6 ±3.7**	305.4 ±31.4	3308 ±333*	*6.0 ±2.84*	*35.5 ±4.2**
H. pygmaea	**40.2 ±2.2**	37.6 ±3.3	11.5 ±4.2	**435.1 ±58.7***	**2342 ±337**	**19.1 ±3.3**	*56.6 ±6.3**
H. chloracantha	45.1 ±3.9*	38.2 ±3.9*	**2.6 ±3.5***	*191.7 ±41.5*	*5488 ±132*	13.4 ±2.7*	*44.9 ±5.2**
M ±m	39.6 ±3.2	33.8 ±4	10.1 ±5.2	369.4 ±55*	3088 ±395*	12.8 ±3.2	47.6 ±5.8
Abaxial side							
H. cymbiformis	**38.5 ±3**	**32.7 ±3.4**	**8.5 ±5.6**	*325.9 ±46.1*	*3128 ±438*	*7.6 ±2.5*	*47.6 ±7.5*
H. cooperi	**38.4 ±2.9**	**34.1 ±2.6**	*23.8 ±7.3*	**533.7 ±60.7**	**1897 ±215**	*12.5 ±4.7*	*50.1 ±6.6*
H. angustifolia	**41.3 ±2.5**	**35.8 ±2.6**	**10.9 ±5.1**	420.1 ±37.3	2399 ±212	17.9 ±6.3	59.3 ±6.7
H. parksiana	**35.7 ±2.6**	**26.7 ±3.9**	**12.0 ±6.3**	427.4 ±63.7	2389 ±345	14.8 ±2.5	*38.5 ±3.1*
H. retusa	**37.6 ±3.9**	**28.3 ±2.1**	*36.7 ±7.8*	**731.7 ±68.4**	**1379 ±129**	*12.6 ±4.3*	53.8 ±6.4
H. blackburniae	**41.4 ±2.5**	**34.8 ±4.5**	**3.9 ±4.2**	*315.9 ±41.8*	*3220 ±431*	*11.6 ±2.6*	*44.1 ±5.4*
H. marumiana	**41.9 ±2.9**	**34.6 ±3.5**	**2.3 ±3.7**	*269.4 ±32.2*	*3769 ±496*	*6.4 ±2.9*	*36.3 ±5.6*
H. pygmaea	**39.1 ±2.7**	38.3 ±4.1	*22.4 ±5.9*	**593.5 ±115**	**1760 ±404**	*17.9 ±5.9*	*51.1 ±5.6*
H. chloracantha	**40.7 ±4.2**	**33.8 ±6**	**5.3 ±5.1**	*210.8 ±49.7*	*5047 ±933*	*13.9 ±1.9*	*40.2 ±4.5*
M ±m	39.4 ±3	33.2 ±3.6	14.0 ±5.7	425.4 ±57.2	2776 ±400	12.8 ±3.7	46.8 ±5.7

All plants of the genus *Haworthia* are xeromorphic. The point of reference for comparing the degree of xeromorphic nature within the genus is the average value of the parameter for the genus. Bold – xeromorphic increase ($P \leq 0.05$) compared to the average of this parameter for the genus; italics – xeromorphic decrease ($P \leq 0.05$) compared to the average of this parameter for the genus. * $P \leq 0.05$ compared to this parameter on the abaxial side.

Most of the plants of the subgenus *Haworthia* have “windows”, except for *H. angustifolia* (Fig. 1d) and *H. blackburniae*. Also, all the plants with large “windows” have the leaves directed outwardly. All the studied species (except for *H. cymbiformis*) have trichomes on the margins and in the center of the abaxial side of the lamina (Fig. 3a). Some species (*H. pygmaea* v. Poelln, *H. marumiana* Uitew., etc) have trichomes also on the adaxial side of the lamina along its midrib. However, only *H. blackburniae* has a sufficiently high number of them on the abaxial side to be considered shady. In all the species, trichomes are non-glandular with a multicellular base and 1, 2 or 3 cells in the apical part (Fig. 3a). The species of the subgenus Haworthia have a thinner cuticle and less numerous papillae on only about half epidermal cells. *Haworthia cymbiformis* (Fig. 3b), *H. blackburniae* and *H. chloracantha* have a smooth cuticle and a straight outer cell wall of the epidermis. *Haworthia parksiana* is in the intermediate position, having pectinate cuticle (Fig. 3c).

The absence of the “windows” and trichomes is typical for all the studied species of the subgenus *Hexangularis*, except for *H. pungens* which has trichomes on the abaxial side of the leaf. The leaves of the subgenus *Hexangularis* are characterized by thicker epidermis and its outer cell walls as well as by copious epidermal verrucae and ridges (Fig. 2a–c, Fig. 4a–c). Most of them are observed in the epidermis of *H. limifolia* (Fig. 2c), in *H. glauca* and *H. coarctata* they are expressed moderately, while in *H. pungens* and *H. viscosa* they are absent altogether. However, *H. viscosa* has a very thick cuticle and a wavy outer wall of the epidermis (Fig. 4b). The cuticle of all the species forms relatively large papillae (which causes a greater thickening of the outer cell wall of the epidermis; Fig. 4c). The epidermal protuberances and ridges consist of multi-layered epidermis whose cells practically have no chlorophyll; together with the cuticular papillae (Fig. 5a), they provide natural shading that leads to the reduction in insolation and thus prevents plants from overheating and diminishes transpiration. The stomata are present only in the single-layered epidermis.

The statistical analysis of the results showed significant differences in the majority of traits for each species (Tab. 3). All plants of the genus *Haworthia* are xeromorphic. When studying the anatomical features of plants of the different subgenera, the increase or decrease in the measured character was compared with respect to the species within the genus. The point of reference for comparing the degree of xeromorphic nature within the genus is the average value of the parameter for the genus.

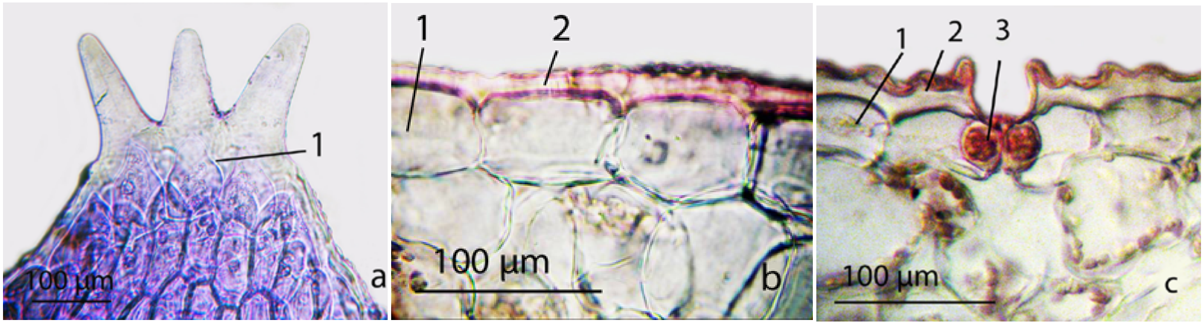

Fig. 3 Cross section of *Haworthia lamina*: trichome (1) of *H. marumiana* (**a**); upper epidermis, *H. cymbiformis* (1 – epidermis, 2 – cuticle; **b**); lower epidermis and stoma, *H. parksiana* (1 – epidermis, 2 – cuticle, 3 – stoma with two ledges; **c**).

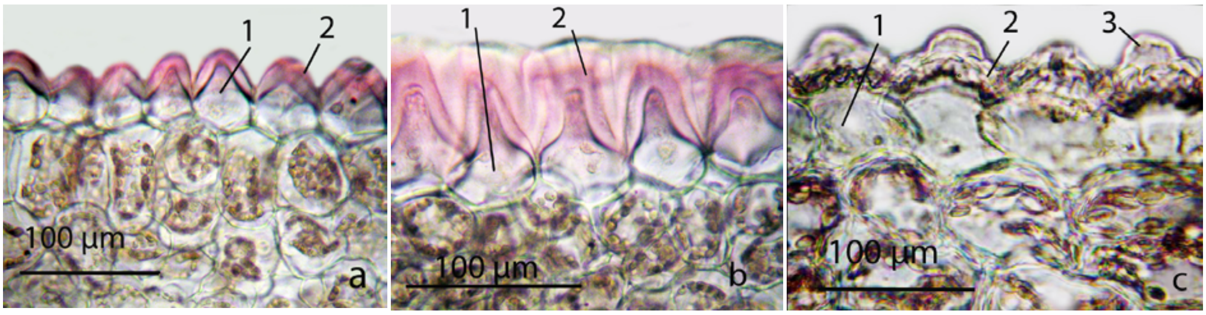

Fig. 4 Cross section of *Hexangularis* lamina (upper epidermis): *H. pungens* (**a**), *H. viscosa* (**b**), *H. glauca* (**c**). 1 – epidermis; 2 – cuticle; 3 – papillae.

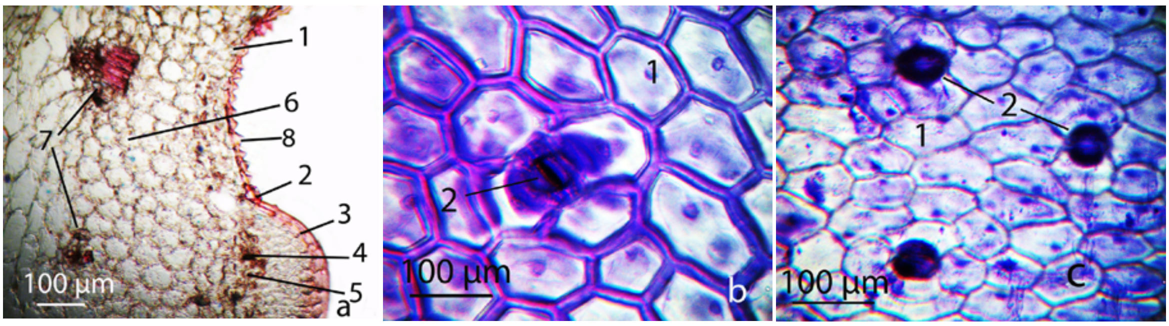

Fig. 5 Microphotography of *Haworthia* leaf: cross section of *H. fasciata* lamina (**a**), epidermis of *H. glauca* (**b**), epidermis of *H. pungens* (**c**). 1 – epidermal cell; 2 – stoma with two ledges; 3 – multi-layered epidermis on ridges; 4 – raphides of calcium oxalate; 5 – chlorenchyma; 6 – water-storage parenchyma; 7 – vascular tissue; 8 – cuticular papillae.

The data in Tab. 4 show that the size of stomata in the investigated species of the subgenus *Haworthia* is similar on the adaxial and abaxial leaf surface. The number of stomata is generally low. Their smallest amount is typical for *H. marumiana* (2 units per mm^2), while the highest one for *H. retusa* (from 24 on the abaxial surface to 37 on the adaxial side of the leaf). In general, for most of the species the number of stomata on the abaxial side of the leaf is higher than on the adaxial one. Only *H. cymbiformis*, *H. marumiana* and *H. blackburniae* (its leaves are directed upwards) have an equal number of stomata on both sides, or higher on the adaxial side of the leaf.

The number of epidermal cells per unit area varies from 191 to 731 (Tab. 4). The maximum number of epidermal cells per 1 mm^2 is typical for *H. cooperi*, *H. retusa* and *H. pygmae*. These species have a rosette with few leaves, each of them tightly pressed to other leaves, and with substantial "windows". At the same time, these species have the most numerous stomata. Most species of this subgenus have more epidermal cells on the abaxial side of the leaf than on the adaxial one, except for *H. marumiana*. It should be noted that the difference in the number of epidermal cells per unit of area on the abaxial and adaxial sides is not great for most of the species of the subgenus *Haworthia*.

The thickest outer wall of the epidermal cells on the abaxial side, but especially on the adaxial side of the leaf, is typical for *H. pygmae* which has a high number of stomata and epidermal cells per unit area. The thick outer wall of the epidermal cells on both sides is also characteristic of *H. angustifolia*, *H. chloracantha* and *H. parksiana*, which have leaves directed upward and outward.

Tab. 5 (subgenus *Hexangularis*) shows that the size of stomata within this group differs in length, from 37 to 67 µm, and in width, from 33 to 54 µm. The largest size of stomata is typical for *H. glauca* (Fig. 5b) and *H. coarctata*, while the smallest one for *H. pungens* (Fig. 5c). However, *H. reinwardtii* and *H. viscosa* are characterized by the greatest number of stomata.

The thickness of the outer epidermal cell wall on the adaxial leaf surface is generally higher than that of the subgenus *Haworthia* and ranges from 11 to 27 µm. The thickest outer epidermal cell wall is typical for *H. glauca*, *H. coarctata* and *H. viscosa* (Fig. 4b,c). The thicker outer epidermal cells wall on the abaxial side of the leaf is typical for *H. coarctata*, *H. limifolia*, *H. glabrata* and *H. viscosa*. A great number of large stomata per unit area is typical for *H. viscosa*. In this group of species, the number of epidermal cells per 1 mm^2 ranges from 131 to 497, which is generally also lower than for plants of the previous subgenus. The plants of *H. fasciata*, *H. attenuata*, *H. viscosa* and *H. reinwardtii* species have the greatest number of epidermal cells per unit area. Moreover, the number of epidermal cells is greater than on the abaxial side of the leaf, except for *H. viscosa* and *H. coarctata* whose leaves are directed outwards.

Our investigation of the ratio of dry weight and content of water in the leaves of the studied species of the subgenus *Haworthia* showed that in most of the species the amount of water exceeds 90%. The exception is only *H. parksiana* in which the amount of dry matter in the leaves is 13% (Fig. 6). This is the only one of the studied species which has a dark-green color of the leaf and a relatively small leaf thickness as well as, as indicated above, pectinate cuticle and the greatest amount of layers of chlorenchyma (Tab. 1).

The investigation of the ratio of dry weight and water content in the leaves of the studied species of the subgenus *Hexangularis* showed that the amount of water in the leaves of this subgenus varies from 87 to 89%, while in *H. glauca* does not exceed 84% (Fig. 7). This is generally lower than that for the previous subgenus, *Haworthia*.

Discussion

The succulent leaves of the genus *Haworthia* have numerous traits in their structure that help the plant adapt to xerothermic conditions. It is well-known that the epidermis

Tab. 5 Characteristics of leaf epidermis of the studied species of the subgenus *Hexangularis*.

Species	Length of stomata (µm)	Width of stomata (µm)	Amount of stomata (pcs./ 1 mm^2)	No. of epidermal cells (pcs./ 1 mm^2)	Area of epidermal cells (μm^2)	Thickness of outer cell wall of epidermis (µm)	Thickness of epidermis (µm)
Adaxial side							
H. glauca	_63.7 ±5.1*_	_47.6 ±5_	__2.6 ±3.2__	_273.4 ±27.7_	_3694 ±365_	__20.9 ±4.1__	__80.6 ±6.4*__
H. attenuata	_50.0 ±4.9_	_49.5 ±4.3*_	__6.6 ±3.7*__	405.8 ±27.4*	2475 ±161	16.0 ±3.6	60.4 ±7.8
H. coarctata	_55.1 ±6.8_	_52.6 ±7.7_	__8.3 ±4.8__	328.6 ±27.3*	3063 ±244	__22.0 ±4.6__	60.6 ±8.4*
H. pungens	__39.4 ±3.7*__	36.2 ±4*	__3.6 ±3.4__	_131.8 ±36.2_	_8252 ±255_	13.0 ±2.9*	_42.2 ±7.4_
H. reinwardtii	47.6 ±3.3	_44.2 ±2.9*_	14.5 ±6.1*	__471.8 ±64.8__	__2157 ±283__	_11.0 ±4.2_	_40.7 ±6.6_
H. limifolia	__43.1 ±2.9*__	_42.7 ±2.8_	__5.6 ±2.9__	_164.4 ±15.5*_	_6137 ±602*_	__18.3 ±4.3*__	56.8 ±5.7
H. glabrata	48.2 ±5.8	_43.5 ±4.3*_	__8.2 ±5.2__	_255.5 ±34.8*_	_3993 ±604*_	__19.9 ±3.5*__	53.6 ±8.3
H. fasciata	48.9 ±2.6*	_44.1 ±3.4*_	__5.5 ±3.7*__	401.8 ±36.6*	2510 ±235*	17.4 ±3.9	52.1 ±9.3*
H. viscosa	_54.8 ±5.1*_	_43.3 ±2.7*_	12.5 ±4.8	__493.0 ±38.9*__	__2041 ±165__	__26.9 ±5.6*__	__75.9 ±12*__
M ±m	50.1 ±4.5^	44.8 ±4^	7.5 ±4.2^	325.1 ±34.4*^	3814 ±324*	18.4 ±4.1*^	58.1 ±8^
Abaxial side							
H. glauca	_67.7 ±3.8_	_48.9 ±5.6_	__3.6 ±3.9__	_276.9 ±28.9_	_3650 ±373_	19.1 ±3.7	__70.5 ±13__
H. attenuata	50.6 ±3.7	_46.2 ±3.3_	__7.8 ±3.8__	475.1 ±46.3	2125 ±219	10.6 ±3.6	__64.4 ±4.1__
H. coarctata	_58.4 ±7.8_	_54.6 ±6.1_	__8.6 ±3.9__	_311.9 ±22.1_	_3221 ±221_	__20.9 ±4.8__	__66.6 ±7.8__
H. pungens	__37.7 ±3.1__	__33.4 ±2.7__	__6.4 ±4.9__	_139.3 ±32.5_	_7563 ±171_	_10.2 ±2.5_	_40.2 ±6.1_
H. reinwardtii	48.6 ±3.8	42.1 ±2.9	__13.1 ±3.4__	482.0 ±34.1	2084 ±140	_12.4 ±5.1_	_41.2 ±10_
H. limifolia	__44.9 ±3.4__	41.9 ±4.2	__6.2 ±4.1__	_185.3 ±24.8_	_5496 ±764_	__20.4 ±3.3__	54.9 ±6.7
H. glabrata	47.9 ±3.2	_46.0 ±3.9_	__9.3 ±6.1__	_330.3 ±36.1_	_3066 ±357_	__28.1 ±8.4__	__72.7 ±12__
H. fasciata	50.9 ±5.9	42.2 ±4.8	__7.8 ±5.4__	497.1 ±54	2036 ±235	17.5 ±3.8	_46.9 ±7.8_
H. viscosa	51.0 ±3.9	41.2 ±2.4	__13.1 ±4.9__	447.9 ±29.1	2242 ±150	__53.0 ±5.5__	__100.1 ±22__
M ±m	50.9 ±4.3^	44.1 ±4^	8.4 ±4.5^	349.5 ±34^	3498 ±292^	21.4 ±6.8^	62.0 ±10^

Bold – xeromorphic increase ($P \leq 0.05$) compared to the average of this parameter for the genus); italics – xeromorphic decrease ($P \leq 0.05$) compared to the average of this parameter for the genus). * $P \leq 0.05$ compared to this parameter on the abaxial side. ^ $P \leq 0.05$ compared to this parameter for the subgenus *Haworthia*.

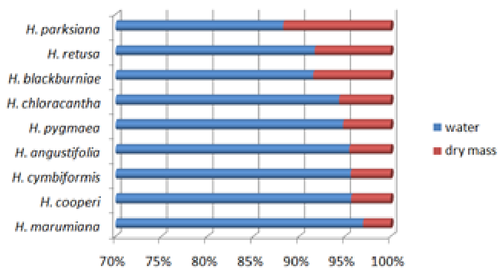

Fig. 6 Diagram of the ratio of water content and dry mass in the leaves of plants of the subgenus *Haworthia*.

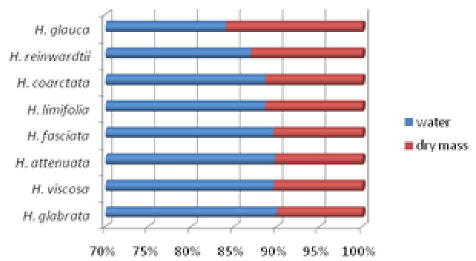

Fig. 7 Diagram of the ratio of water content and dry mass in the leaves of plants of the subgenus *Hexangularis*.

with a thick layer of cuticle fulfils various protective functions and displays numerous xeromorphic traits in its structure [17,18].

The presence of various types of appendages and cuticular sculptures produces stronger sunlight dispersion. A smooth surface of the cuticle in epidermal cells with flat outer walls releases water more quickly than an undulating surface [17]. Thus, we can say that the plants of the subgenus *Haworthia* with flat outer walls release water more quickly than the undulating surface in plants of the subgenus *Hexangularis*. Proliferation of the multiple epidermis produces highly succulent leaves in plants of the subgenus *Hexangularis* (like some species of *Peperomia* Ruiz & Pav. [19] which exhibit extraordinary capacity for water retention). The cuticle is the main barrier to water loss.

Small sized epidermal cells are typical for plants growing in dry regions [17]. The results of the present study have demonstrated that the abaxial epidermis of *Haworthia* leaves is composed of smaller cells than those on the adaxial surface. This is related to the number of epidermal cells in a unit of surface. The present results are confirmed by the data of other researchers on succulent plants of the genus *Orostachys* Fisch. [20] and *Kalanchoë* Adans. [21].

Stomatal density in the abaxial epidermis of the species of the genus *Haworthia* is higher than that in the adaxial epidermis. That is probably due to the fact that the leaves in rosettes are directed upwards and outwards and thus can be illuminated by the sun from all sides. The number of stomata in this unit of surface in *Kalanchoë* is bigger 3 times [21], while in mesophytes the number is 6 times bigger in the adaxial epidermis and 36 times bigger in the abaxial epidermis [22].

A small number of stomata is revealed in all the investigated species of the subgenus *Hexangularis*, while this is typical of only about ⅔ species of the subgenus *Haworthia*. In our opinion, the presence of epidermal emergencies and their components in the species of the subgenus *Hexangularis* as well as a more evident reduction in the number of stomata enable the stomata to be relatively large.

The ratio of the thickness of the outer epidermal cell wall and the thickness of the leaf epidermis of the studied species of the subgenus *Haworthia* is from ½.5 to ⅙. The greater is this ratio, the more place the epidermal outer cell wall with the cuticle occupies in the cell and then the leaf is probably better protected against the environment. *H. angustifolia*, *H. parksiana*, *H. pygmae* and *H. chloracantha* have the greatest ratio, which have average indices of sizes of epidermal cells per unit of area. The increase in the number of chlorenchyma layers (Tab. 1) in the species with large "leaf windows" (*H. parksiana*, *H. pygmaea*, *H. cooperi*, *H. retusa*) is probably due to the layers of inner parenchyma facing the "leaf windows",

The ratio of the thickness of the outer epidermal cell wall and the thickness of the whole epidermal cells of the leaf of the studied species of the subgenus *Hexangularis* is from ½ to ¼, which shows an increase in this value compared with plants of the subgenus *Haworthia*. The greatest ratio of the outer cell wall to the size of the entire epidermal cell is typical for *H. viscosa*. It is high enough for such species as *H. coarctata*, *H. reinwardtii*, *H. limifolia*, *H. glabrata* and *H. fasciata*.

The distinctive devices of plants of the subgenus *Haworthia* are characterized by a reduction in the size of stomata and a decrease in their number per unit of area, an increase in the number of epidermal cells, the presence of trichomes and "leaf windows" in the epidermis compared to *Hexangularis*. However, in *H. angustifolia*, *H. blackburniae*, and *H. chlorocanta* the "leaf windows" are absent. The literature data show that several genera of small succulents, *Haworthia*, *Bulbine*, *Fenestraria*, *Lithops*, *Conophytum*, *Peperomia*, *Senecio*, etc., have developed these epidermal windows to perfection [23,24]. The leaf rosettes of many plants of the subgenus Haworthia are partially sunken into the soil. Their leaves are very succulent and only the leaf tip protrudes from the soil surface. During evolution this leaf tip lost its pigmentation almost completely and the solar radiation incident on the window area is transmitted via the colorless water storage tissue to the assimilatory tissue [23].

The average number of layers of chlorenchyma in plants of the subgenus *Haworthia* is less (on average 3–5) than in plants of the subgenus *Hexangularis* (on average 5–7), which is probably due to the presence of "leaf windows" in the former one. Window leaves consist primarily of tissues specialized for water storage (window tissue) and photosynthesis (chlorenchyma). In mature leaves of *Peperomia columella* Rauh & Hutchison, the percentages of chlorenchyma and window tissue are approximately 20% and 58% [25]. The leaves of plants of the subgenus *Haworthia* do not have epidermal formations in the form of epidermal protuberances and ridges. At the same time, plants of *H. pungens* from the subgenus *Hexangularis* have signs like plants of the subgenus *Haworthia*.

On the average, the investigated species of the subgenus *Hexangularis* have a higher number of chlorenchyma layers, which is probably caused by the absence of "windows". Such species as *H. attenuata*, *H. fasciata*, *H. glabrata*, *H. limifolia*, and *H. reinwardtii* have more cell layers of chlorenchyma (Tab. 2), indicating their greater shading tolerance.

The central part of the leaf in succulents contains specialized water-transporting tissue with single chloroplasts [19,26]. Plants of the subgenus *Hexangularis* accumulate significantly less water than plants of the subgenus *Haworthia*.

Conclusions

Two different strategies of adaptation to arid conditions of the growth of different subgenera of the genus *Haworthia* were revealed. Plants of the subgenus *Haworthia* adapted to arid conditions by increasing the accumulation of water, the presence of "windows", a smaller stoma size, and a thinner outer wall of the epidermis cells. On the other hand, plants of the subgenus *Hexangularis* adapted to arid conditions by reducing overheating and transpiration as well as by the presence of papillae and a thickened outer wall of the epidermis cells. We suppose that the development of representatives of each of the subgenus was parallel.

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Authors' contributions
The following declarations about authors' contributions to the research have been made: research design: GM, NN; conducting anatomical experiments: NN; conducting morphological experiments: GM; writing the manuscript: NN, GM.

Competing interests
No competing interests have been declared.

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Charakterystyka porównawcza anatomicznych i morfologicznych przystosowań roślin dwóch podrodzajów *Haworthia* Duval do suchych warunków środowiska

Streszczenie

Niniejsza praca przedstawia porównawczą charakterystykę cech anatomicznych i morfologicznych roślin dwóch podrodzajów: *Haworthia* i *Hexangularis*. Badania pokazują dwie odmienne strategie przystosowania rozwoju różnych podrodzajów rodzaju *Haworthia* do suchych warunków. Rośliny z podrodzaju *Haworthia* przystosowały się do takich warunków poprzez zwiększenie akumulacji wody, obecność „okienek", mniejszą wielkość aparatów szparkowych oraz cieńszą ścianę zewnętrzną komórek epidermy, podczas gdy rośliny z podrodzaju *Hexangularis* przystosowały się do suchych warunków poprzez zmniejszenie przegrzewania i transpiracji jak również poprzez obecność papilli i pogrubienie ściany zewnętrznej komórek epidermy.