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The Stomatal Characteristics of *Cheirolepis* section of *Centaurea* Genus (Asteraceae) and Its Relatives in Turkey

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Abstract: The present study reveals the stomatal characteristics of *Centaurea* genus of *Cheirolepis* section and its relatives. The levels of stomatas of taxa were detected as mesomorphic and also the stomata type were determined as anomocytic or anizosytic in studied taxa. The epidermis were showed polygonal in shape and their anticlinal cell walls are straight or undulating. This arrangement was stable in the same taxon. However, it was different in point of adaxial and abaxial surface. The stomatal index were calculated and confirmed close values in taxa.

Key words: Centaurea, Cheirolepis, Stomata, Pseudoseridia, Plumosipappus, Pteracantha.

1. Introduction

An outstanding and relatively large genus in Flora of Turkey, *Centaurea* L., at the present time, is comprised of ca. 250 species distributed across Eurasia, particularly in the Irano-Turanian and Mediterranean region [1]. Recently, with the description of new taxa, the genus *Centaurea* has reached 200 taxa, of which 111 are endemic in Turkey [2, 3, 4, 5, 6, 7]. Hence, the endemism rate is about 56%. Section *Cheirolepis* of *Centaurea* is represented by 17 taxa, of which 6 are known only by their type locality in Turkey [4, 8, 9]. There are morphological and molecular studies about section *Cheirolepis*, in which sections *Pseudoseridia*, *Pteracantha*, and *Plumosipappus* have been merged, as they all have systematic problems. In a recent study, Garcia-Jacas et al. [10] had declared that the species of these 4 sections could be reunited in a single section, called *Cheirolepis-Pseudoseridia* complex [11]. They determined that the species have morphological differences but practically no genetic differences at a molecular level. On the basis of this work, we also selected 4 taxa (*C*.

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odyseii Wagenitz, C. kizildaghensis E. Uzunhisarcıklı, E. Doğan & H. Duman, C. paphlagonica (Bornm.) Wagenitz, and C. nallihanense Uysal & Hamzaoğlu) from these related sections and also determined their stomatal characteristics.

Because leaves are probably the most varied anatomical organ among the angiosperms, a large number of investigations have examined the diagnostic value of leaves [12]. Of these, the taxonomic value of features such as stomata structure, surface ornamentation and epidermal cell wall configuration have been repeatedly demonstrated [13]. However, the leaf epidermis of *Centaurea* species has been little examined with the light microscope [14, 15]. Therefore, in this study we will examine the epidermal features of 21 Turkish *Centaurea* species, to see if epidermal features can provide information of taxonomic value, and whether such information supports the discrimination of the members of *Cheirolepis* and its relatives.

With the exception of *C. odyssei* the stomatal characteristics of the remaining species were examined for the first time in the present study.

2. Materials and Methods

The specimens used for the present study were collected from several localities in Turkey and were deposited in KNYA herbarium (Selçuk University) and the capitula photos were given in Figure 1. Some of the specimens were put in 70% ethyl-alcohol during the field conditions. The superficial sections were taken with a razor blade and the sections were mounted with a cover slip in glycerol. To determine the length of stomata guard cells, at least 30 randomly selected stomata from five leaves per plant were measured with using the software of Kameram 21 programme. In a similar way, 30 randomly selected microscopic field areas from five leaves were counted per plant to obtain stomatal and epidermal cell frequency. Stomatal index (SI) was calculated according to the formula of Salisbury [16]:

$$SI = S/(E+S) \times 100$$
,

where S is the number of stomata per unit leaf area and E is the number of epidermal cells per unit leaf area.

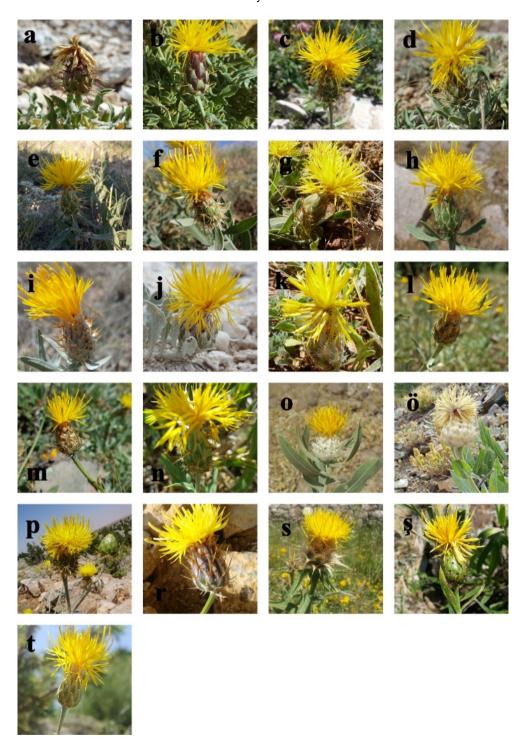


Figure 1. The photos of capitula of Cheirolepis and its relatives

a. Centaurea drabifolia subsp. drabifolia, b. C. drabifolia subsp. austro-occidentalis, c. C. drabifolia subsp. floccosa, d. C. drabifolia subsp. cappadocica, e. C. kotschyi var. kotschyi, f. C. kotschyi var. persica, g. C. kotschyi var. decumbens, h. C. kotschyi var. floccosa, i. C. derderiifolia, j. C. nivea, k. C. deflexa, l. C. sericea, m. C. cankiriense, n. C. glabro-auriculata, o. C. saligna, ö. C. ensiformis, p. C. isaurica, r. C. odyssei, s. C. paphlagonica, ş. C. kizildaghensis, t. C. nallihanense

Table 1. The localities of studied taxa

Serial	Taxa		Voucher number		
no.	Cheriolepis	Location			
C1	Centaurea drabifolia subsp. drabifolia	A2 Bursa: Uludağ, 2100-2200 m., 20 August 2016	B. Çıtak 250		
C2	Centaurea drabifolia subsp. austro-occidentalis	20.1.1.2016			
С3	Centaurea drabifolia subsp. floccosa Boissier	B5 Kayseri: Pinarbaşı, Şirvan Mountain, 2210 m., 5 August 2015 C3 Konya: Derebucak, Çamlık Town, 1350 m., 15 June 2016	B. Çıtak 177 H. Dural 3566-B.Çıtak		
C4	Centaurea drabifolia subsp. cappadocica	 B6 Sivas: Gürün, between Gürün-Kangal road, 1844 m., 14 July 2015 C3 Konya: Beyşehir, Doğanhisar-Hüyük road, 1800 m., 20 June 2016 	B. Çıtak 171 H. Dural 3573-B.Çıtak		
C5	Centaurea kotschyi var. kotschyi	H. Dural 3570 B. Çıtak			
C6	Centaurea kotschyi var. persica	m.,14 July 2015 C4 Konya: 8 km from Hadim to Korualan, 1850 m., steppe, 21 July 2015			
C8	Centaurea kotschyi var. floccosa	B5 Kayseri: Pinarbaşı, Şirvan Mountain, 2210 m., 05 August 2015	B. Çıtak 176		
С9	Centaurea derderiifolia	B6 Kayseri: Gürün-Divriği road, 1500-1600 m., 27 July 2003.	B. Çıtak 172		
C10	Centaurea deflexa	C4 Konya: Hadim-Taşkent-Alanya road, 1750-1800 m., 21 July 2015 C4 Konya: Taşkent-Ermenek road, 1800 m., 19 July 2012	K. Ertuğrul 5011 H. Dural K. Ertuğrul-4699-H. Dural		
C11	Centaurea nivea B3 Eskişehir: 20 km from Mihaliçik to Alpu, 940 m., 13 June 2016		B. Çıtak 182		
C12	C3 Eskişehir: Bozüyük-Kütahya road, 1140 m., 14 July 2016.		B. Çıtak 207		
C13	Centaurea cankiriense A4 Çankırı: Atkaracalar-Kalfat road m., 13 July 2016		B. Çıtak 201		
C14	Centaurea saligna	C9 Hakkari: Hakkari-Van road, 1800 m., 10 July 2015	B. Çıtak 168		
C15	Centaurea glabro-auriculata	B. Çıtak 225-H.			

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		Tekneliyatak region, 1800-1900 m., 07 August 2016.	Dural-H. Demirelma
C16	Centaurea isaurica	C4 Konya: Seydişehir, Maden Village, 1500 m.,	B. Çıtak 215
C17	Centaurea ensiformis	C2 Muğla: Köyceğiz, Sandras Mountain, 1700 m., 25 July 2016	B. Çıtak 193
	Pteracantha		
C18	Centaurea odyssei	B1 Çanakkale: Bayramiç, Kazdağ, Çıplak Hill, 1530 m., 05 June 2003	E. Karabacak 2495
	Plumosipappus		
C19	Centaurea paphlagonica	B5 Yozgat: Çayıralan, Elçi Village, 1666 m., 17 July 2015	B. Çıtak 174
_	Pseudoseridia		
C20	Centaurea kizildaghensis	C3 Konya: Derebucak, Kızıldağ, Çamlık Village, 1400 m., 16 June 2015	H. Dural 3575- B. Çıtak
	Cheirolepis-Pseudoseridia complex		
C21	Centaurea nallihanense	B2 Ankara: Nallıhan, Osmanköy-Yenice road, 10 July 2017	B. Çıtak 319

3. Results and Discussion

The results are summarized in Tables 2-4 and Figs. 2-5. In current study, the stomata were classified based on the criteria of Metcalfe and Chalk [17]. Also, Aydin et al [14] and Özcan et al. [15] were used for classification. The stomatal apparatus for all species was of anizocytic or anomocytic type (Figs. 2-5). Morover, the leaves of investigated taxa are amphistomatic. Leaf epidermal cells are usually irregular or polygonal in shape. The anticlinal walls are straight, undulating or both straight and undulating in studied taxa (Figs. 2-5). The stomatal dimensions were changed 20.21-39.9 µm on the adaxial epidermis and 19.68-42.41 µm on the abaxial epidermis (Tables 2-3). The stomatal density of studied taxa varied from 7.67 to 15.78 per square millimeter on the adaxial epidermis and from 8.60 to 15.82 per square millimeter on the abaxial epidermis (Table 4). The stomatal size of the investigated taxa varied from species to species and was higher on the abaxial surface than on the adaxial surface for all species.

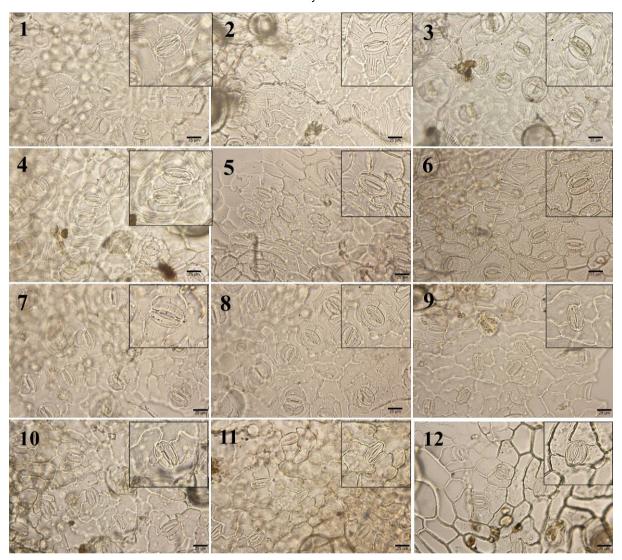


Figure 2. Stomata microphotographs of *Cheirolepis* section at light microscope. 1-*C. drabifolia* subsp. *drabifolia* (upper surface), 2-*C. drabifolia* subsp. *drabifolia* (lower surface), 3-*C. drabifolia* subsp. *austro-occidentalis* (upper surface), 4-*C. drabifolia* subsp. *austro-occidentalis* (lower surface), 5-*C. drabifolia* subsp. *floccosa* (upper surface), 6-*C. drabifolia* subsp. *floccosa* (lower surface), 7-*C. drabifolia* subsp. *cappadocica* (upper surface), 8-*C. drabifolia* subsp. *cappadocica* (lower surface), 9-*C.kotschyi* var. *kotschyi* (upper surface), 10-*C. kotschyi* var. *kotschyi* (lower surface), 11-*C. kotschyi* var. *persica* (upper surface), 12-*C. kotschyi* var. *persica* (lower surface)

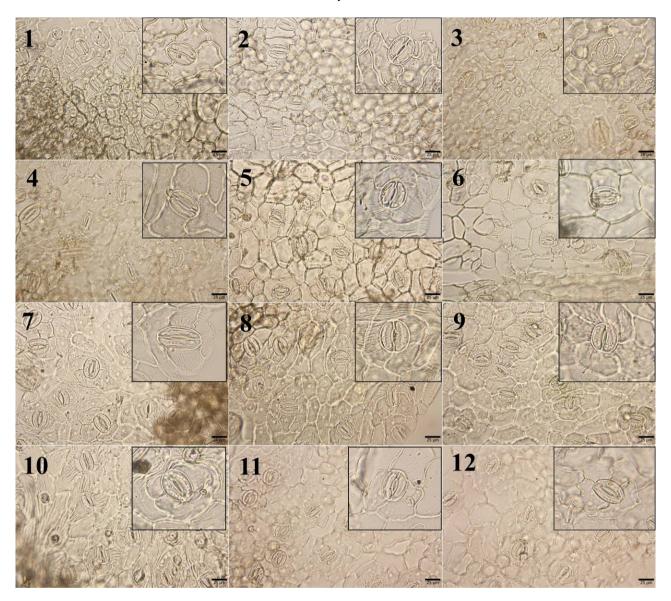


Figure 3. Stomata microphotographs of *Cheirolepis* section at light microscope. 1-*C. kotschyi* var. *decumbens* (upper surface), 2-*C. kotschyi* var. *decumbens* (lower surface), 3-*C. kotschyi* var. *floccosa* (upper surface), 4-*C. kotschyi* var. *floccosa* (lower surface), 5-*C. derderiifolia* (upper surface), 6-*C. derderiifolia* (lower surface), 7-*C. deflexa* (upper surface), 8-*C. deflexa* (lower surface), 9-*C.nivea* (upper surface), 10-*C. nivea* (lower surface), 11-*C. sericea* (upper surface), 12-*C. sericea* (lower surface)

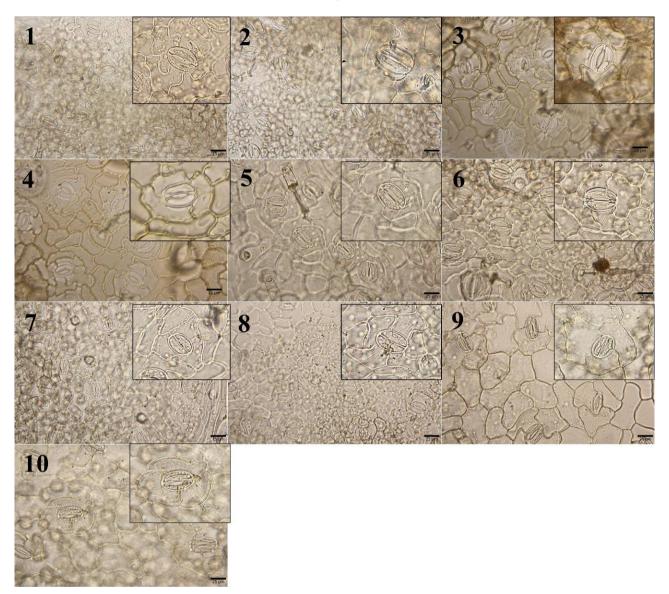


Figure 4. Stomata microphotographs of *Cheirolepis* section at light microscope. 1-*C. cankiriense* (upper surface), 2-*C. cankiriense* (lower surface), 3-*C. saligna* (upper surface), 4-*C. saligna* (lower surface), 5-*C. glabro-auriculata* (upper surface), 6-*C. glabro-auriculata* (lower surface), 7-*C. isaurica* (upper surface), 8-*C. isaurica* (lower surface), 9-*C. ensiformis* (upper surface), 10-*C. ensiformis* (lower surface)

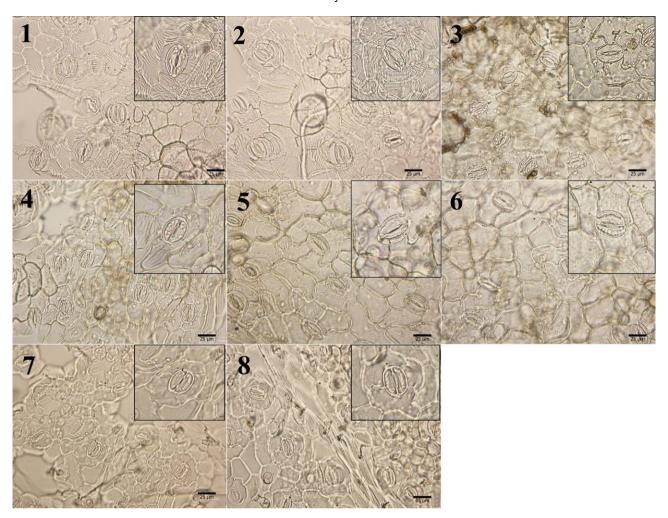


Figure 5. Stomata microphotographs of *Pseudoserida, Plumisopappus, Pteracantha and Plumisopappus-Cheirolepis* sections at light microscope. 1-*C. odyssei* (upper surface), 2-*C. odyssei* (lower surface), 3-*C. paphlagonica* (upper surface), 4-*C. paphlagonica* (lower surface), 5-*C. kizildaghensis* (upper surface), 6-*C. kizildaghensis* (lower surface), 7-*C. nallihanense* (upper surface), 8-*C. nallihanense* (lower surface)

Table 2. The stomatal characteristics of the studied taxa (ST: stomata type, SEC: shape of epidermal cells, AEW:

Anticlinal epidermal walls. See Table 1 for acronyms of taxa)

Taxa		Upper surfac	e		Lower surface			
	ST	SEC	AEW	ST	SEC	AEW		
C1	Anomocytic and	Polyganal	Straight	Anomocytic and	Polyganal	Straight		
	anizosytic			anizosytic				
C2	Anomocytic and	Polyganal	Straight	Anomocytic and	Polyganal	Straight,		
	anizosytic			anizosytic		undulating		
C3	Anomocytic and	Polyganal	Straight	Anomocytic and	Polyganal	Straight,		
	anizosytic			anizosytic		undulating		
C4	Anomocytic and	Polyganal	Undulating	Anomocytic and	Polyganal	Straight		
	anizosytic			anizosytic				
C5	Anomocytic and	Polyganal	Undulating	Anomocytic and	Polyganal	Straight,		
	anizosytic			anizosytic		undulating		
C6	Anomocytic and	Polyganal	Undulating	Anomocytic and	Polyganal	Straight,		
	anizosytic			anizosytic		undulating		
C7	Anomocytic and	Polyganal	Undulating	Anomocytic and	Polyganal	Straight,		
	anizosytic			anizosytic		undulating		
C8	Anomocytic and	Polyganal	Undulating	Anomocytic and	Polyganal	Straight,		
	anizosytic			anizosytic		undulating		
C9	Anomocytic and	Polygonal	Undulating	Anomocytic and	Polygonal	Straight		
	anizosytic			anizosytic				
C10	Anomocytic and	Polygonal	Undulating	Anomocytic and	Polygonal	Straight		
	anizosytic			anizosytic				
C11	Anomocytic and	Polygonal	Undulating	Anomocytic and	Polygonal	Straight		
	anizosytic			anizosytic				
C12	Anomocytic and	Polygonal	Undulating	Anomocytic and	Polygonal	Straight,		
	anizosytic			anizosytic		undulating		

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The shape of epidermal cells and the pattern of anticlinal cell walls, which vary between taxa but are stable within a taxon and also within the same section, may represent good characters for taxonomy of Centaurea genus or others [15]. In some taxa the anticlinal wall patterns are different on both surfaces; polygonal cells with straight anticlinal cell walls on the adaxial epidermis and polygonal cells with undulating anticlinal cell walls on the abaxial sides. This different pattern condition occurs in C. drabifolia subsp. cappadocica, C. drabifolia subsp. austro-occidentalis, C. derderiifolia, C. deflexa and C. nivea (Figs. 1,2). Straight to undulate epidermal shape conditions have been also reported in several Centaurea or related taxa [14, 15, 16, 17]. Amount of undulation is greater on the abaxial sides in these five taxa. This is because the abaxial epidermis is exposed to conditions of greater shadow and humidity [19]. The shapes of cells of C.cheiranthifolia var. purpurascens and C. woronowii on the adaxial epidermises with irregular and sinuous anticlinal cell walls are similar to abaxial ones [15]. Stace [13] points out that epidermal cells with straight outlines are more common in xeromorphic plants than in mesomorphic plants, where they are typically undulate (repand). In addition, Fahn [20] asserts that the epidermal cells of most leaves of shade-loving dicotyledons have sinuous anticlinal walls. It is also reported that such sinuosity is probably due to the tensions that occur in the leaf and to cuticle hardening during cell differentiation [21]. Our results are in agreement with these suggestions.

All of the studied the taxa have anomocytic-anisocytic stomatal type. Stomata cells are more abundant on the lower epidermis, whereas it is a few in the upper parts of all taxa. Number of stomata per mm² is much dense in the lower parts of *Centaurea drabifolia subsp. austro-occidentalis*, *C. drabifolia subsp. floccosa*, *C. kotschyi var. persica*, *C. kotschyi var. decumbens*, *C. kotschyi var. floccosa*, *C. nivea*, *C. isaurica and C. ensiformis* than those of others thirteen taxa. In addition, stomata are generally in the same level with epidermises, except for *Centaurea saligna* (Fig. 3). In this species the stomata are positioned below abaxial epidermis level (Fig. 3). This characteristic is also reported in *C. calcitrapa* ssp. *cilicica* and *C. solstitialis* ssp. *carneola* by Kaya et al. [18].

C. drabifolia subsp. drabifolia, C. drabifolia subsp. austro-occidentalis, C. isaurica, C. ensiformis, C. nivea, C. deflexa and C. odyssei have wrinkled cuticular membrane ornamentation as not reported previously in Centaurea.

Table 3. The stomatal characteristics of the studied taxa

Taxa		Upper surface		Lower surface			
	ST	SEC	AEW	ST	SEC	AEW	
C13	Anomocytic and	Polygonal	Undulating	Anomocytic and	Polygonal	Straight,	
	anizosytic			anizosytic		undulating	
C14	Anomocytic and	Polygonal	Undulating	Anomocytic and	Polygonal	Straight,	
	anizosytic			anizosytic		undulating	
C15	Anomocytic and	Polygonal	Undulating	Anomocytic and	Polygonal	Straight,	
	anizosytic			anizosytic		undulating	
C16	Anomocytic and	Polygonal	Undulating	Anomocytic and	Polygonal	Straight,	
	anizosytic			anizosytic		undulating	
C17	Anomocytic and	Polygonal	Undulating	Anomocytic and	Polygonal	Straight,	
	anizosytic			anizosytic		undulating	
C18	Anomocytic and	Polygonal	Straight	Anomocytic and	Polygonal	Straight	
	anizosytic			anizosytic			
C19	Anomocytic and	Polygonal	Straight,	Anomocytic and	Polygonal	Straight,	
	anizosytic		undulating	anizosytic		undulating	
C20	Anomocytic and	Polygonal	Straight,	Anomocytic and	Polygonal	Straight,	
	anizosytic		undulating	anizosytic		undulating	
C21	Anomocytic and	Polygonal	Straight,	Anomocytic and	Anomocytic and Polygonal St		
	anizosytic		undulating	anizosytic		undulating	

Table 4. The length of stomata guard cells of the investigated taxa and stomata indexes (μm)

Taxa	Stomata index		The length of stomata guard cells						
1 4244				Upper surface			Lower surface		
	Upper	Lower	The ratio	Min	Max	Mean±SD	Min	Max	Mean±SD
	surface	surface	of						
			stomata						
			index						
C1	15.78	11.91	1.32	25.76	33.13	28.55±2.6	26.84	29.48	30.47±2.9
C2	14.40	15.82	0.91	25.53	31.11	27.41±1.97	24.68	29.76	28.64±1.84
C3	7.67	8.44	0.90	28.22	30.57	36.02±3.57	35.95	40.16	36.44±2.43
C4	11.21	8.60	1.30	22.6	34.52	26.22±2.42	22.31	32.22	27.61±2.3
C5	14.36	14.31	1.00	23.11	37.75	30.29±2.98	25.76	34.94	31.17± 1.9
C6	12.59	13.79	0.91	27.27	35.68	31.25±2.7	24	35.26	30.59±2.6
C7	14.07	14.48	0.97	24.46	36.8	30.36±2.9	25.03	41.02	34.1±3.52
C8	11.60	12.85	0.90	25.52	37.55	32.29±2.7	24.63	38.42	32.92±3.0
C9	12.87	11.01	1.16	22.78	35.15	26.42±2.73	21.24	38.24	28.58±3.63
C10	13.59	11.98	1.13	25.65	34.75	30.24±2.17	28.14	34.3	31.53±1.92
C11	11.94	14.12	0.84	22.41	31.48	27.00±1.99	20.05	35.54	27.68±4.49
C12	15.71	13.19	1.19	25.16	34.19	29.47±2.11	26.89	30.94	29.01±1.01
C13	14.02	13.04	1.07	23.86	30.44	27.71±1.81	20.89	29.15	26.61±2.26
C14	13.21	11.24	1.17	24.11	31.95	28.33±2.28	24.4	34.61	28.45±2.51
C15	13.73	12.76	1.07	26.33	33.94	31.02±1.75	28.64	38.21	32.81±2.54
C16	11.80	11.91	0.99	23.22	30.11	26.7±1.87	21.72	31.01	26.26±2.11
C17	14.02	14.49	0.96	30.87	39.9	34.76±2.11	32.22	42.41	38.37±2.34
C18	11.32	11.08	1.02	26.76	34.1	29.22±2.32	23.84	32.2	28.55±2.14
C19	12.79	11.36	1.12	22.72	31.63	27.27±2.09	20.94	31.77	27.12±2.29
C20	13.19	13.12	1.00	30.93	38.4	34.94±1.91	28.1	39.29	34.48±2.99
C21	14.33	11.34	1.26	20.21	29.48	24.15±2.11	19.68	29.58	24.4±2.05

4. Conclusion

The stomatal properties of 17 species of section *Cheirolepis* and 4 species of sections *Pseudoseridia*, *Pterecantha*, and *Plumisopappus* were observed via light microscopy, and the length of stomata guard cells and undulating on the epidermis cells were determined as the most useful characters of systematic value in *Cheirolepis* and relatives.

In conclusion, a detailed analysis of the stomatal characteristics is very useful for the classification of the taxa investigated. Moreover, changing the size of the stomata among the taxa could be associated with their different habitat types or microclimatic areas.

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