

**COMPARATIVE MICROMORPHOLOGY AND ANATOMY OF
CHRYSOCHAMELA (FENZL) BOISS. (CRUCIFERAE)
GROWING IN TURKEY**

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Abstract

The micromorphological and anatomical characteristics of three species of *Chrysochamela* genus have been comparatively presented by using light microscopy (LM) and scanning electron microscopy (SEM). The micromorphological studies are related to the epidermal and seed surface. In anatomical studies, cross sections of the stems and leaves were examined and biometric measurement of cell and tissues were made. The stem and leaf anatomy of the genus were described. In addition, the stomatal index rate and stomatal index of these species were calculated. The leaf is dorsiventral in mesophyll-type. Except its *C. noeana* taxon, stellate trichomas are coincided in other taxon. Stoma can be observed on both sides of the leaf. Whereas stoma index rates of this genus were found in the range of 0.7 - 1.2, stoma types were observed as anicytic and anomocytic. Seed surface of the genus can be found in reticulate, verrucae or in verrucae-rugose types. In conclusion, the presence/absence of trichome over stem and leaves, the presence/absence of collenchyma in middle the vascular region, the seed shape, epidermal cell shape, and the characteristics of anticlinal boundaries and periclinal cell walls were found to be the important characters for identification of the *Chrysochamela* genus.

Introduction

The Cruciferae (Brassicaceae) is one of the largest Angiosperm families, comprising 338 genera and 3709 species distributed throughout the world, mainly in temperate regions of the Northern Hemisphere (Al-Shehbaz 1984, Warwick *et al.* 2006, Kasem *et al.* 2011). Turkey is one of the richest countries in the world in terms of the number of species of the Cruciferae. In Turkey, Cruciferae is represented by about 571 species 65, subspecies, 24 varieties and 660 taxa belonging to 91 genera (Al-Shehbaz *et al.* 2007). The genus *Chrysochamela* (Fenzl) Boiss., is one of the genus of Cruciferae which includes 4 species, is distributed across Turkey, Russia, Lebanon and Syria (Appel and Al-Shehbaz 2003). In the flora of Turkey, there are 3 species of *Chrysochamela* genus *viz.*, (*C. elliptica* (Boiss.) Boiss., *C. velutina* (DC.) Boiss. and *C. noeana* (Boiss.) Boiss.), (Hedge 1965).

Metcalf and Chalk (1957) studied the anatomy of Cruciferae and determined the diagnostic anatomical characteristics which were epidermal cell type, stoma type, and the arrangement of the sclerenchymatic cells around the vascular bundles of the leaves. The number of morphological and taxonomic studies in Cruciferae has increased in recent years (Pinar and Oybak 2000, Khatun *et al.* 2011, Erdoğan *et al.* 2012, Kaya and Dirmenci 2012, Bayirli *et al.* 2014, Selvi *et al.* 2014, Hayta *et al.* 2014, 2015, Çavuşoğlu and Karaferyeli, 2015, Satil *et al.* 2015).

But the micromorphological and anatomical properties of *Chrysochamela* genus have not been studied to date. Therefore, the purpose of this paper was to investigate the micromorphological and anatomical properties of this genus.

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Materials and Methods

Plant specimens were collected from different locations in Turkey and stored in the Tunceli University Herbarium. The locations of collection for the specimens are given in Table 1. Anatomical studies were carried out on specimens kept in 70% alcohol. Whole cross-sections which were taken from the stem and leaves were stained with Phloroglucinol-HCl solutions (Yakar-Tan 1982) and chlorophyll in leaves was cleared with chloral hydrate solution.

Stomatal density on the abaxial and adaxial sides of the leaf was measured under a light microscope. The stomatal index was calculated according to the method of Meidner and Mansfield (1968). Stomatal terminology was based on the classification proposed by Dilcher (1974) and the leaf epidermal terminology was based on the classification by Wilkinson (1979). Measurements and photographs were taken using Olympus BX 51 and Nikon Eclipse E600 binocular light microscopes. The epidermal surfaces of stems, leaves and seeds were studied by Tabletop scanning electron microscopy (SEM). For SEM, small pieces of leaves and stem with seed were fixed on aluminum stubs using double-sided adhesive. The SEM micrographs were taken in a NeoScope JCM-5000 at an accelerating voltage of 10 kV.

Table 1. Species used for anatomical studies and their collected localities.

Species	Collection areas and collector's number
<i>C. elliptica</i>	B4 Ankara: Between Ayas and Beypazarı, before 2 km to Beypazarı, gypsum slopes, 590 m, 01.05.2005, Paksoy 1446
<i>C. velutina</i>	C7 Şanlıurfa: Behind Evren auto industry site, Rock pit vicinity, limestone cliffs, 730 m, 05.04.2013, Paksoy 1400 C7 Şanlıurfa: Ceylanpınar, Hamitköy vicinity, rock cracks, 400 m, 06.04.2013, Paksoy 1401
<i>C. noeana</i>	B6 Sivas: Between Düzova and Dedeli village, Rock slopes, 1300 m, 08.06.2013, Paksoy 1482 B6 Sivas: Hafik, Tuzhisar village, rock slopes, 1000 m, 08.06.2013, Paksoy 1484

Results and Discussion

The comparative micromorphology and anatomy of *Chrysochamela* species growing in Turkey were thoroughly investigated. Micro-morphological and anatomic studies were conducted on *Chrysochamela* genus by the researchers so that a significant contribution was made into the systematical characteristics of the genus.

Epidermal surface characters of the taxa examined in the micromorphological studies are shown in Table 2, and biometric measurements of the anatomical characteristics of the species are presented in Table 3. Comparative anatomical characters of the stem and leaf of the *Chrysochamela* species are given in Tables 4 and 5, respectively. In addition to these, features of the seed morphological characters of these species are shown in Table 6.

Stem anatomy of Chrysochamela genus: In a cross section, the epidermis is found to have one layer on the outside. There is a very thin cuticle on the epidermis (0.5 - 1.5 µm). Its surface is covered with dentritic hairs. The epidermis also contains stomata of the anisocytic type. The cortex layer consists of cortex parenchyma and the endodermis. The cortex parenchyma is chloranchymatic and is made up of 2 - 6 layers, thin-walled, with regular oval or circular shaped cells. Underneath the cortex parenchyma is the endodermis which consist of 1 - 2 layers, usually rectangular-shaped, surrounding the stem. The pericycle is underneath the endodermis. It is usually sclerenchymatic and surrounding it are 1 - 3 layers of vascular bundles or cup-like phloem.

There are 5 - 9 vascular bundles. The interfascicular region is located between the vascular bundles and this region comprises sclerenchymatic cells. The phloem is 3 - 8 layers, and consist of irregular and squashed cells. The cambium cells are not clear. The pith region which is located in the center of the stem is composed of large orbicular or polygonal parenchymatous cells (Figs 1-2).

Table 2. Characteristics of epidermis surface of *Chrysochamela* species.

Characters	<i>C. elliptica</i>		<i>C. velutina</i>		<i>C. noeana</i>	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Anticlinal cell wall	Sinuate	Sinuate	Undulate	Sinuate	Undulate	Sinuate
Shape of epidermis cells	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
Stomata types	Anisocytic	Anisocytic	Anisocytic	Anisocytic	Anisocytic	Anisocytic
	Anomocytic	Anomocytic	Anomocytic	Anomocytic	Anomocytic	Anomocytic
Stomata length (μ)	21.8 \pm 3.4	24.8 \pm 3.8	20.1 \pm 1.7	19.2 \pm 1.9	27.4 \pm 3.3	24.3 \pm 3.9
Stomata width (μ)	13.3 \pm 1.5	14.5 \pm 2.5	15.2 \pm 1.4	13.2 \pm 1.1	17.4 \pm 3.1	14.7 \pm 2.7
Number of stomata (1 mm ²)	109 \pm 15	105 \pm 14	133 \pm 21.2	214 \pm 15.6	83.9 \pm 12.7	160.8 \pm 18.4
Number of epidermis cells	308 \pm 25	365 \pm 30	386 \pm 34.5	494.6 \pm 9.5	207.2 \pm 12.7	227.7 \pm 22.9
Stomata index	26.1	22.3	25.6	30.2	28.8	41.4
Stomata index ratio	1.2		0.85		0.7	

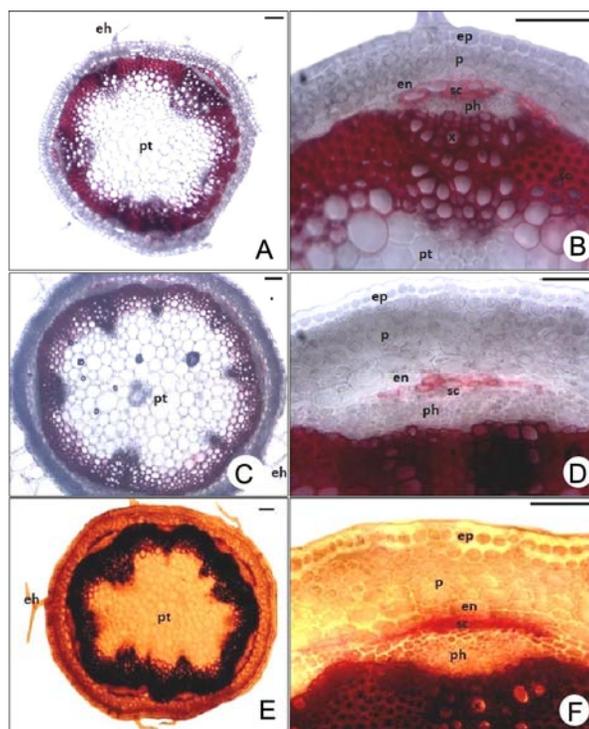


Fig. 1. Cross-section of stem of *Chrysochamela* species. A, B = *C. elliptica*; C, D = *C. velutina*; E, F = *C. noeana*. eh - eglandular hair; ep - epidermis; p - cortex parenchyma; sc - sclerenchyma; en - endodermis; ph - phloem; x - xylem; pt - pith parenchyma (Scale bar = 50 μ m).

Table 4. Stem anatomical characters of *Chrysochamela* genus.

Taxa	Presence/absence of trichomes	Cortex		Pericycle	Number of vascular bundles	Phloem
		Parenchyma	Endodermis			
<i>C. elliptica</i>	Present (densely)	2-3 layered	1	1	6-8	3-5
<i>C. velutina</i>	Present (densely)	4-6 layered	1-2	1-3	5-9	4-6
<i>C. noeana</i>	Present (sparsely)	3-5 layered	1-2	1-3	6-9	5-8

Table 5. Comparative leaf anatomical characters of *Chrysochamela* genus.

Taxa	Presence/absence of trichomes	Mesophyll type	Palisade layer	Spongy layer	Middle vascular bundle	
					Collenchyma on xylem	Collenchyma under phloem
<i>C. elliptica</i>	Present (densely)	Bifacial	2-3	2-4	Present (2-4 row)	Absent
<i>C. velutina</i>	Present (sparsely)	Bifacial	2-3	2-4	Absent	Present (1-3 row)
<i>C. noeana</i>	Absent	Bifacial	2-4	2-5	Present (1-2 row)	Present (1-3 row)

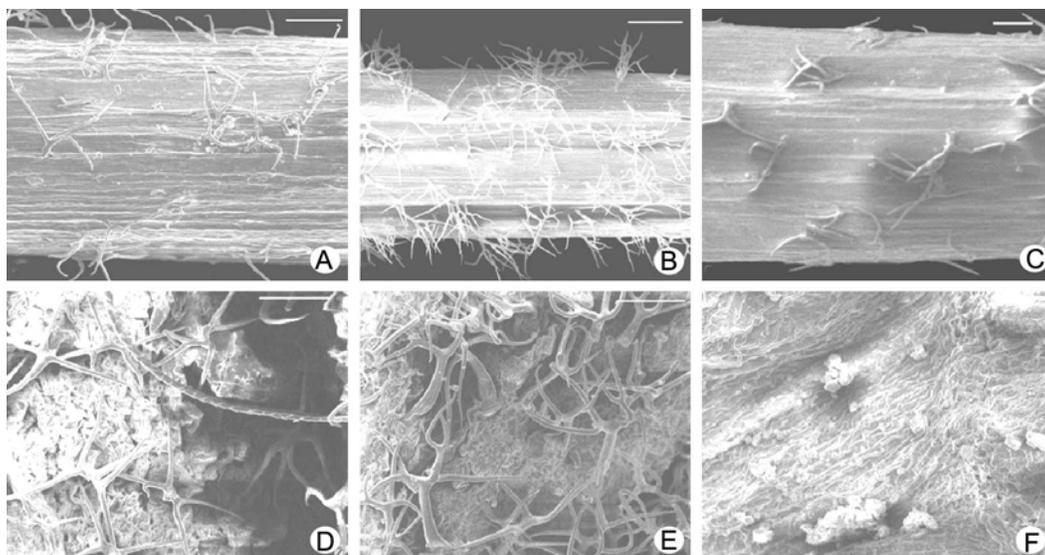


Fig. 2. Stem (A, B, C) and leaf (D, E, F) morphology of *Chrysochamela* species. A, D (*C. elliptica*); B, E (*C. velutina*); C, F (*C. noeana*) (Scale bar = 100 μ m).

Leaf anatomy of *Chrysochamela* genus: In a cross section of the leaf, there is a thin cuticle (1.1 - 3.1 μ m) on the upper and lower epidermis. Both epidermis cells are almost isodiametric and oval or cubic in shape. The surface of epidermis was sometimes covered with dendritic hairs sometimes not. The stomata are on both surfaces of the leaf (amphistomatic type). Stomata are either located at the same level with epidermis cells (mesomorphic stomata) or at the level above the epidermis cells (higomorphic stomata). In a surface section, the epidermal cell walls are formed in large waves (sinuate) and mild waves (undulate). The stomata type is the Cruciferous

type (anisocytic) or, rarely the Ranunculaceous (anomocytic) type. The stomatal index is 26.1 - 28.8 (upper surface) and 22.3 - 41.4 (lower surface), while the stomatal index ratio is between 0.7 and 1.2. The mesophyll layer consists of palisade and spongy parenchyma cells. Palisade parenchyma cells are in layers of 2-4 and cylindrical in shape whereas spongy parenchyma cells are in layers of 2 - 5 and circular or oval in shape. The mesophyll is bifacial (dorsiventral). Vascular bundles were embedded in mesophyll and of the collateral structure type. In the middle vascular, the lower and upper epidermal cells were bigger than epidermal cells in the mesophyll layer. Also, collenchyma is sometimes present in the vascular bundle is present or not. The xylem faces towards the upper surface while the phloem faces the lower epidermis. Sclerenchyma sometimes present or phloem tissue (Figs 3, 4).

Table 6. The seed morphological characters as seen by SEM.

Taxa	Seed Shape	Seed size (mm) (length × weight)	Radicle	Epidermal cell shape	Color	Anticlinal cell wall	Periclinal cell wall	Seed surface
<i>C. elliptica</i>	Oblong	0.3-0.5 × 0.2-0.4	Incumbent	Polygonal, isodiametric	Brown	Raised, straight to undulate	Straight to concave, Microreticulate	Reticulate
<i>C. velutina</i>	Elliptic	0.7-0.9 × 0.3-0.5	Incumbent	Irregular	Brown	Raised, undulate	Dome-shaped, convex	Verrucate
<i>C. noeana</i>	Elliptic	0.5-0.6 × 0.3-0.4	Incumbent	Irregular	Brown	Raised, undulate	Dome-shaped, convex	Verrucate, rugose

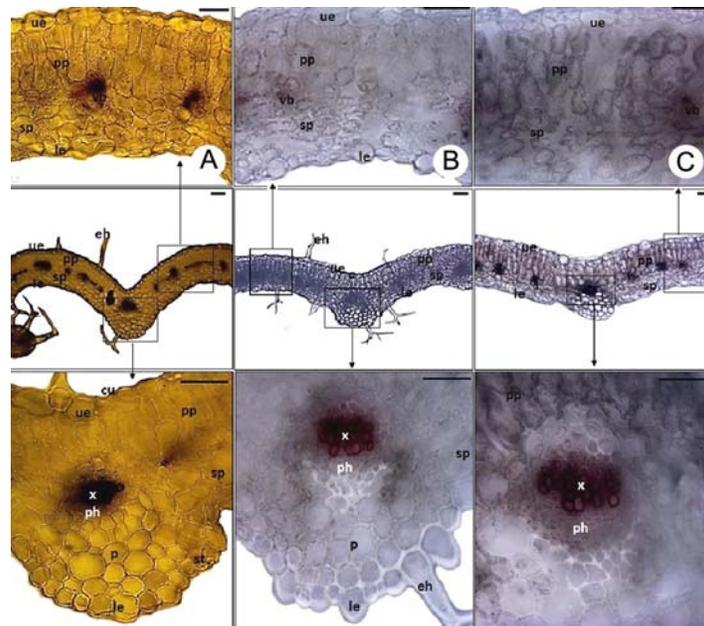


Fig. 3. Cross-section of leaves of *Chrysochamela* species. A = *C. elliptica*, B = *C. velutina*, C = *C. noeana*. cu - cuticle, eh - eglandular hair, ue - upper epidermis (adaxial surface), pp - palisade parenchyma, sp - spongy parenchyma, bs - bundle sheath, x - xylem, ph - phloem, sc - sclerenchyma, le - lower epidermis (abaxial surface) (Scale bar = 50 μ m).

No prior micromorphological and anatomical studies of *Chrysochamela* species have been found. For this reason, it was not possible to compare the present results with others. However, *Chrysochamela* species have been shown to have the typical features of Cruciferae (Metcalf and Chalk 1957). Anatomically important characters for the identification of *Chrysochamela* species are presence/absence of trichome, the cortex parenchyma layer, presence/absence of collenchyma, and the epidermal surface.

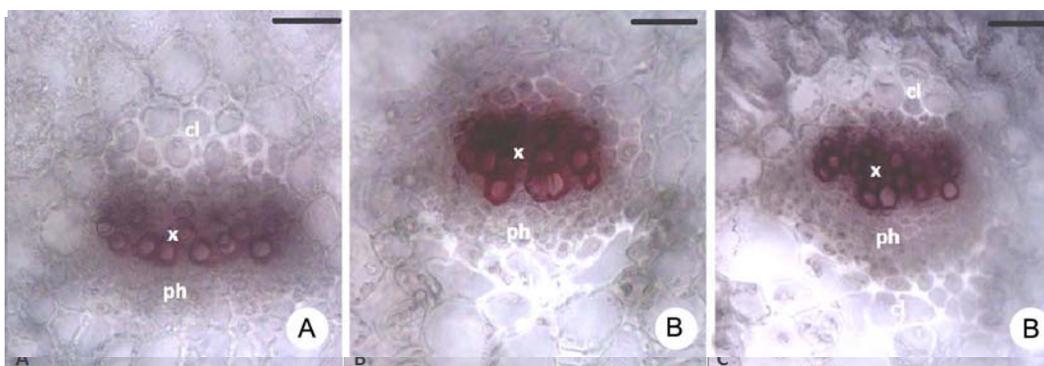


Fig. 4. Comparative leaf middle vascular of *Chrysochamela* species. A = *C. elliptica*; B = *C. velutina*; C = *C. noeana*. cl - collenchymas, x - xylem, ph - phloem (Scale bar = 20 μ m).

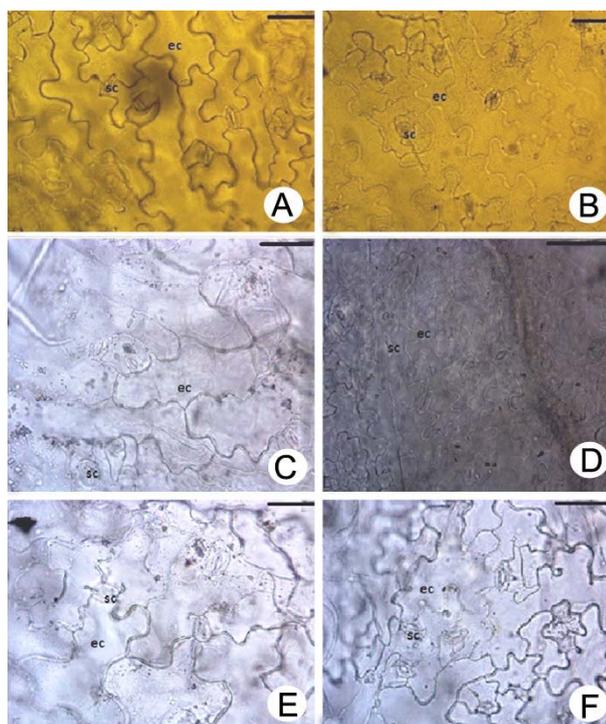


Fig. 5. Adaxial (A, C, E) and abaxial (B, D, F) epidermal surfaces of leaves of *Chrysochamela* species. A, B = *C. elliptica*. C, D = *C. velutina*; E, F = *C. noeana*. sc - stomata cell, ec - epidermis cell (Scale bar = 50 μ m).

The stomata of *Chrysochamela* species are surrounded by 3 subsidiary cells of which 1 is usually much smaller than the other 2, the so-called Cruciferous (anisocytic) type. Rarely the stoma is surrounded by a limited number of cells that are indistinguishable in size, shape, or form from those of the remainder of the epidermis, the so-called Ranunculaceae (anomocytic) type (Fig. 5). Also, the stomatal index of these species is 25.6 - 28.8 (upper surface) and 22.3 - 41.4 (lower surface), while the stomatal index ratio is between 0.7 and 1.2.

The presence of hairs on stem and leaf is a significant character for *Chrysochamela* species. *C. elliptica*, and *C. velutina*, have dentritic hairs, while *C. noeana* does not have hairs (exist stem) (Figs 2, 3).

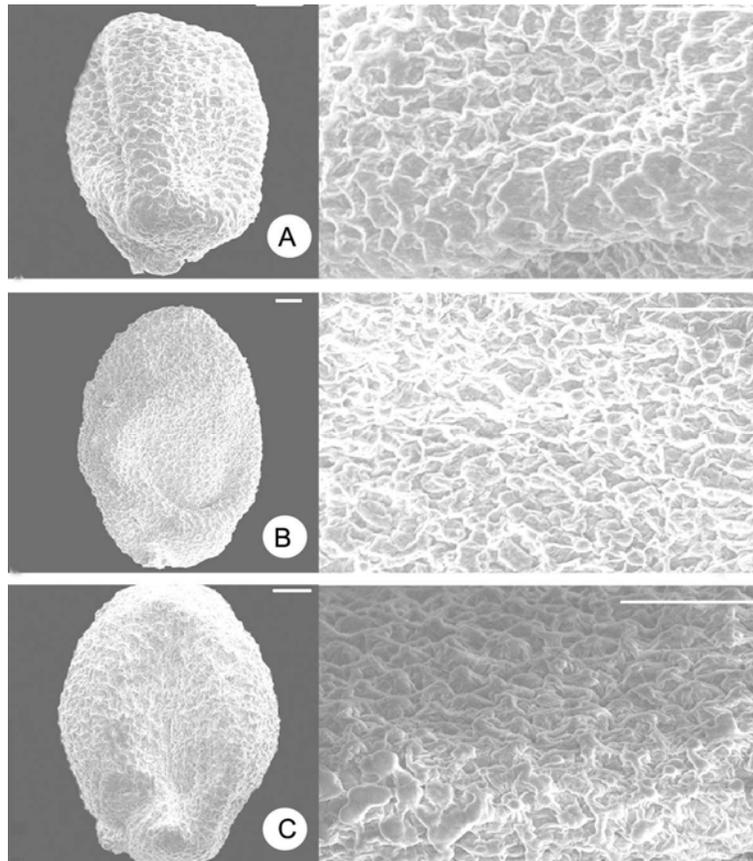


Fig. 6. Seed morphology of *Chrysochamela* species. A = *C. elliptica*; B = *C. velutina*; C = *C. noeana* (Scale bar = 50 μ m).

Seed characters are very important for differentiating between genera and species levels in Brassicaceae (Khalik and Van Der Maesen 2002). The results of this study showed that the seed sizes of the genus *Chrysochamela* are 0.3 - 0.9 \times 0.2 - 0.5 mm. Among them, *C. elliptica* has the smallest seed size at 0.3 - 0.5 \times 0.2 - 0.4 mm. The colour of seed in the *Chrysochamela* species is brown and the seed shape is oblong or elliptic. The radicle is incumbent. Epidermal cell shape varies from isodiametric, 4-5-6-polygonal to elongate in one direction or irregular. Anticlinal

boundaries were raised to channelled and anticlinal walls from straight to undulate or slightly sinuous; periclinal cell walls showed a large variation among genera and species level, and were concave, convex, or domate and microreticulate. Seed ornamentation exhibits difference among taxons. Observed ornamentation in *C. elliptica* was reticulate type; in *C. velutina* was verrucate type; and in *C. noeana* was verrucate-rugose type (Table 6, Fig. 6).

In conclusion, the presence/absence of trichomes on the stem and leaves, the presence/absence of collenchyma in the middle vascular region, the seed shape, epidermal cell shape, and the characters of anticlinal boundaries and periclinal cell walls were found to be important characters for identification of the *Chrysochamela* genus.

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