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Systematic study of *Ceratocephala* (Ranunculaceae) in Iran

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Abstract: *Ceratocephala* Moench (Ranunculaceae, Ranunculeae) is a weedy and medicinal plant with 4-12 accepted taxa in the world from which two are present in Iran: *C. falcata*, *C. testiculata*. These are wide spread weedy plants in Iran. These two species are sympatric. *C. falcata* is very polymorphic. There is confusion between dwarf specimens of *C. falcata* with *C. testiculata.* In present study, the morphological (macro- and micro-) and anatomical properties of *Ceratocephala* species of Iran have been studied. Totally 27 populations are collected from different localities of Iran. Qualitative and quantitative morphological and anatomical features were evaluated and measured and were used to compare species. Stem and leaf cross sections were considered. Pollen and fruit surface were studied by Scanning Electron Microscopy. Although both species are very similar but the main differences are observed in their fruit surface indumentums and pollen exine sculpture. Species differences are discussed.

Keywords: *Ceratocephala*, micromorphology, macromorphology, anatomical structure, Iran.

Introduction

The genus *Ceratocephala* Moench (Ranunculaceae, Ranunculeae) is a small genus related to *Ranunculus* L. (MÁJEKOVÁ et al. 2013). The name of the genus is derived from Greek and means “horn on head”.

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Ceratocephala species are weeds in cereal and small grain crop with a high degree of adaptability. As a medicinal plant, the genus is used in traditional medicine of Central Asia to cure wounds, injuries and skin problems (KHALMATOV 1964, ZAUROV et al. 2013). The genus contains ranunculin, changing into protoanemonin which is a toxic substance. Sheep are poisoned after consuming shoots. The *Ceratocephala* sap can also cause irritations to human skin (NACHMAN & OLSEN 1983).

Ceratocephala includes 4 to 12 species in the world (KLOKOV 1978, THE PLANT LIST 2013). This genus is represented by two species in Iran: *C. falcata* (L.) Pers. and *C. testiculata* (Crantz) Besser (IRANSHAHR et al. 1992).

The genus occupies meadows and roadsides in large numbers, where it is an indicator of land disturbance. These are wide-spread plants in south and center of Europe, North West Africa and South West Asia (BENSON 1948, FISCHER et al. 2008) although genus is believed to have originated in central Asia (FISCHER et al. 2008). *Ceratocephala* was introduced to USA and Canada, where it behaves as an invasive weed (TUMA & WEEDON 1989). One species of *Ceratocephala* (*C. pungens*) is endemic to New Zealand (GARNOCK-JONES 1984). According to records in the Flora Iranica (IRANSHAHR et al. 1992), *Ceratocephala* species are distributed in all parts of Iran.

Micromorphological features are important characters in classification and delimitation of taxa. Ranunculaceae showed several forms of pollen grains which can be used in taxa delimitation (WODEHOUSE 1936). The basic form of pollen in family is the tricolpate class. Pollen shape, numbers of apertures and ornamentation can be pointed out as diagnostic features for delimitation of intra- and intergeneric levels within Ranunculaceae (WODEHOUSE 1936, NOWICKE & SKVARLA 1995).

Achene morphology and micromorphology have led to segregation of genera within family (WIEGAND 1895). Using achene and petals features, TAMURA (1995) identified several genera from *Ranunculus*. Moreover, previous studies showed that it has taxonomic value in species delimitation (EMADZADE et al. 2010, DEVYATOV et al. 2013).

The anatomical structure of these species has not been studied yet. *C. falcata* is very polymorphic and shows a lot of variation in indumentum, size and degree of up-curving of the fruit beak. Stem anatomical studies in Ranunculaceae have provided diagnostic features in some taxa (KÖKDIL et al. 2006, GHORBANI NOHOOJEI et al. 2011, KAYA & KIRIMER 2015, NOVIKOFF & MITKA 2015).

There is not any comprehensive systematic study on *Ceratocephala* species in Iran. Moreover there is confusion between dwarf specimens of *C. falcata* and *C. testiculata.* Therefore, the objectives of present study are (1) to find diagnostic morphological characters (macro- and micro-) and (2) to investigate anatomical properties of *Ceratocephala* in Iran. Interspecific variation has been considered.

Material and methods

A total of 27 *Ceratocephala* populations were collected from different localities of Iran (Tab. 1, Fig. 1). Ten individuals were collected at each sampling site. Specimens are deposited in the ALUH (Herbarium of Alzahra University). Plant

samples were identified according to IRANSHAHR et al. (1992). Nine quantitative and three qualitative morphological features were evaluated and measured (Tab. 2). These features were sepal length, petal length, fruit length, fruit width, mericarp length, mericarp width, petiole length, pedicle length, width of leaf segments, fruitlet shape, sepal shape and petal shape. ANOVA (Analysis of Variance) test was performed to show significant morphological differences. In order to show variable morphological characters, PCA (Principle Component Analysis) was done among species. These analyses were done by PAST software v.2.17 (HAMMER et al. 2001).

Tab. 1. Population details of studied *Ceratocephala* populations in Iran.

Species	Locality	Voucher and specimen code
C. falcata	Khorasan Razavi, Gonabad, 1015 m.a.s.l	M. Keshavarzi R201(ALUH)
C. falcata	Hamedan, Hamedan cemetery, 1750 m. a.s.l	M. Keshavarzi R202 (ALUH)
C. falcata	Qazvin, beginning of Qazvin to Takestan Road, 1100 m. a.s.l	M. Keshavarzi R203 (ALUH)
C. falcata	Isfahan, Ghalamestan, 1500 m. a.s.l	M. Keshavarzi R204 (ALUH)
C. falcata	Hamedan to Razan, Jamishlu, 1800 m. a.s.l	M. Keshavarzi R205 (ALUH)
C. falcata	Buin Zahra to Hamedan, Sagzabad, 1050 m. a.s.l	M. Keshavarzi R206 (ALUH)
C. falcata	West Azerbaijan, Urmia, 1300 m. a.s.l	M. Keshavarzi R207 (ALUH)
C. falcata	Alborz, Karaj, Mardabad,1600 m. a.s.l	M. Keshavarzi R208 (ALUH)
C. falcata	Tehran, Vanak, Bagheno, 1500 m. a.s.l	M. Keshavarzi R209 (ALUH)
C. falcata	East Azerbaijan, Marand, 1300 m. a.s.l	M. Keshavarzi R210 (ALUH)
C. falcata	Fars, Kazerun, 855 m. a.s.l	M. Keshavarzi R211 (ALUH)
C. falcata	Fars, Maharlu Lake, 1400 m. a.s.l	M. Keshavarzi R212 (ALUH)
C. falcata	East Azerbaijan, Tabriz, 1300 m. a.s.l	G. Zare R213 (ALUH)
C. falcata	Tehran, Sorkhe hesar, 1500 m. a.s.l	M. Keshavarzi R214 (ALUH)
C. falcata	Tehran, Alzahra Campus, 1410 m. a.s.l	M. Keshavarzi R215 (ALUH)
C. falcata	East Azerbaijan, Tabriz, Basmenj, 1780 m. a.s.l	G. Zare R216 (ALUH)
C. falcata	Alborz, Bareghan, 1600 m. a.s.l	M. Keshavarzi R217 (ALUH)
C. falcata	Tehran, Bomehen, 1910 m. a.s.l	M. Keshavarzi R218 (ALUH)
C. falcata	Markazi, Arak, 1200 m. a.s.l	M. Keshavarzi R219 (ALUH)
C. falcata	Tehran, Darband, 1800 m. a.s.l	M. Keshavarzi R220 (ALUH)
C. falcata	East Azerbaijan, Maragheh, 1400 m. a.s.l	G. Zare R221 (ALUH)
C. testiculata	Buin Zahra to Hamedan, Sagzabad, 1050 m. a.s.l	M. Keshavarzi R222 (ALUH)
C. testiculata	East Azerbaijan, Tabriz, Basmenj, 1780 m. a.s.l	G. Zare R223 (ALUH)
C. testiculata	East Azerbaijan, Mianeh, 1000 m. a.s.l	G. Zare R224 (ALUH)
C. testiculata	Tehran, Vanak, 1500 m. a.s.l	M. Keshavarzi R225 (ALUH)
C. testiculata	East Azerbaijan, Shabestar, 1100 m. a.s.l	G. Zare R226 (ALUH)
C. testiculata	Khorasan Razavi, Mashhad, 980 m. a.s.l	M. Keshavarzi R227 (ALUH)

Tab. 2. Quantitative and qualitative morphological characters in *Ceratocephala* species.

1. Sepal length (mm)	7. Pedicel length (mm)
2. Petal length (mm)	8. Petiole length (mm)
3. Fruit length (mm)	9. Width of leaf segment (mm)
4. Fruit width (mm)	10. Shape of fruitlet (incurved beak 1, narrow straight beak 2)
5. Fruitlet length (mm)	11. Sepal shape (elliptic to ovate-oblong 1, long elliptic 2)
6. Fruitlet width (mm)	12. Petal shape (oblong-obovate 1, narrowly obovate or ligulate 2)

Fig. 1. Distribution map of studied species (•:*C. testiculata*, ■: *C. falcata*).

Samples were fixed in FAA for anatomical studies. Leaf cross sections and stem structure were considered. Eight quantitative and qualitative anatomical features were used to compare species. These characters were number of vascular bundles, stem cross section outline, number of collenchyma layers, number of parenchyma layers and status of pith in stem cross section and presence/absence of trichomes, shape of spongy cells and midrib outline in leaf cross section. Handmade cross sections were prepared for study by use of double coloration (Methylene green for 10 seconds and Congo red for 15-20 minutes). Sections were studied by use of Olympus DP12 light microscope and photography.

For Scanning Electron Microscopy (SEM) studies, the pollen grains suspended in a drop of water were directly transferred by a fine pipette to a metallic stub using double sided cello tape and coated with gold in a sputtering chamber (Sputter Coater BAL-TEC, SCDOOS). Achenes were studied by both digital stereomicroscope and Scanning Electron Microscopy without any treatment. Coating with gold by the physical vapor deposition method (PVD) was restricted to 100 Å. The SEM examination was carried out on a TESCAN microscope. The measurements were based on 10–20 readings for each specimen. The terminology of HESSE et al. (2009) was followed for pollen and fruit sculptures.

Results

Macro- and micromorphological study

The analysis of variance (ANOVA) test showed a significant difference ($p < 0.05$) for quantitative morphological characters among *Ceratocephala* species. PCA analysis revealed that the three first components comprised of about 78.61% of total variation. In the first PCA component with 38.73%, characters such as achene length, achene width, mericarp width, petiole length and petiole width showed the highest correlation (>0.7). Fruit shape, sepal shape and petal shape, and sepal length are the characters with highest correlation within second (25.90%) and third component respectively (13.98%). PCA ordination based on studied morphological characters separated species in to distinct groups (Fig. 2).

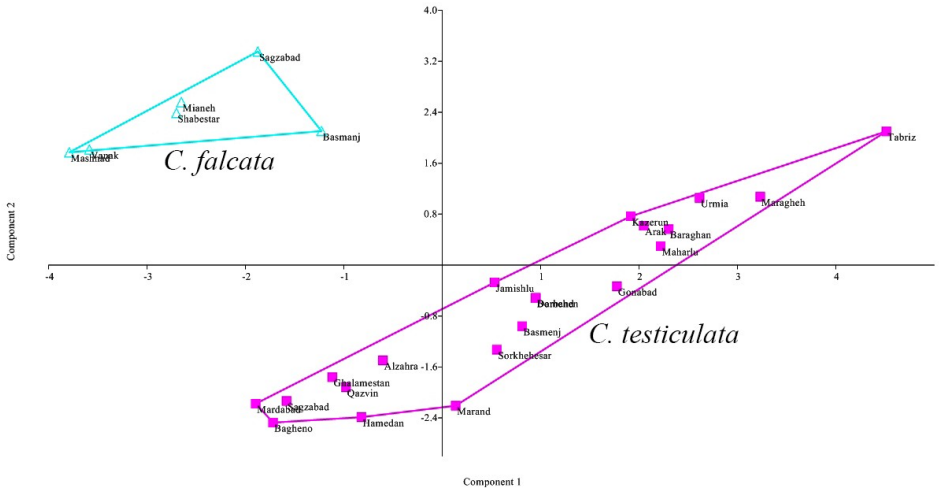

Fig. 2. PCA ordination based on morphological characters.

Our observations indicated that the general shape of the plants is somehow similar in the species studied. Both species are small annual herbs with tiny parted leaves having linear segments. Plants are covered with woolly hairs. *C. testiculata* had more dense hair cover (subtomentose). Flowers are actinomorphic, hermaphrodite, solitary and terminal with 5 sepals and 5 yellow petals, narrowly obovate and clawed at base. Numerous stamens were observed. In *C. falcata*, peduncles are as long as or longer than the plant leaves, but in *C. testiculata* peduncles are as long as or shorter than the plant leaves (Fig. 3). Main differences between these two species are in their fruit features. Aggregate fruits are globular or cylindrical with 10 to numerous achenes, spirally attached to elongated receptacle. In *C. falcata*, the fruit has a long incurved and flattened beak with two hard hollow protuberances whereas in *C. testiculata*, achenes have a long narrow straight beak. The fruit indumentum is another difference in these species. *C. falcata* has woolly fruit ornamentations while *C. testiculata* shows a very dense subtomentose hair cover (Fig. 4).

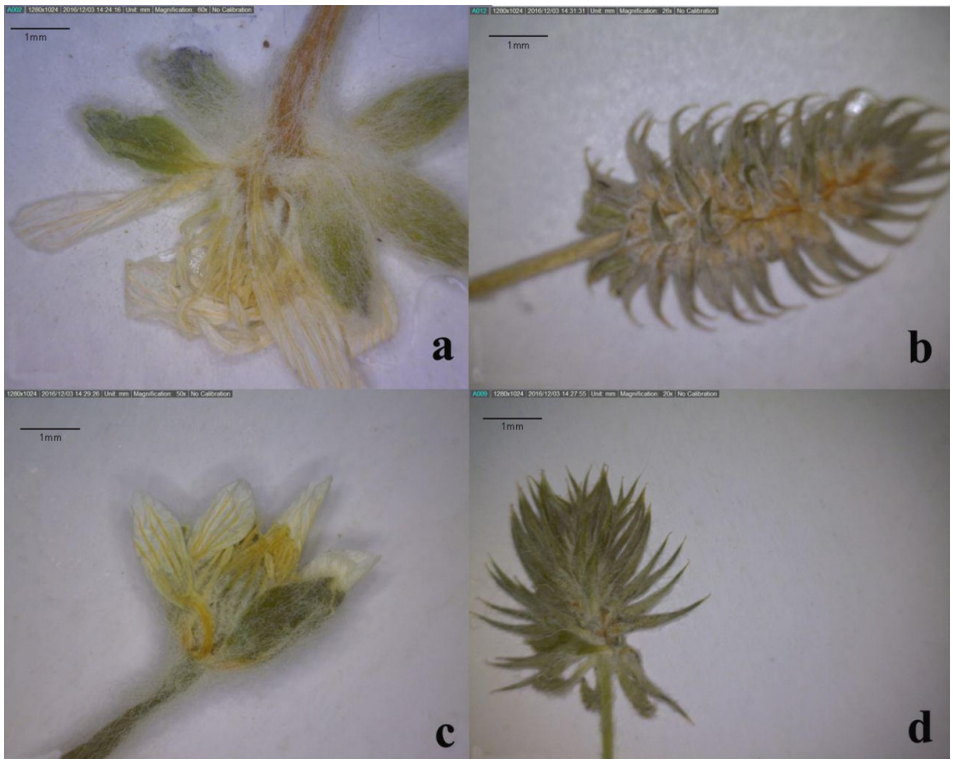

Fig. 3. General morphology of *Ceratocephala.* a & b) *C. falcata,* c & d) *C. testiculata.*

Pollen grains are monad, isopolar and colpate with medium size (26-50 µm). Polar outline is circular. In *C. testiculata*, exine ornamentation is scabrate and microechinate, perforate. *C. falcata* has identical features except for exine ornamentations (Fig. 5). In *C. falcata* a reticulate and heterobrochate reticulum was observed. Pollen grains were tricolpate. The pollen features are compared in Tab. 3.

Stem cross sections

Transverse sections from the middle part of the stem revealed the following elements (Fig. 6). The stems are polygonal in both species. The epidermis is composed of single layered cells. Trichomes are absent on outer surface of stem cross sections. The collenchyma tissue is located under the epidermis of the stem. They are 4-5 layered in both species. The cortex tissue is composed of 4-5 layered parenchyma cells. A ring of sclerenchyma is present in both studied species. Vascular bundles are arranged in a single circle. There are 6 vascular bundles in *C. falcata* and 5 in *C. testiculata*. The xylem is surrounded by sclerenchymatous cells. Pith is composed of large orbicular parenchymatous cells with intercellular spaces. It is hollow in both studied species.

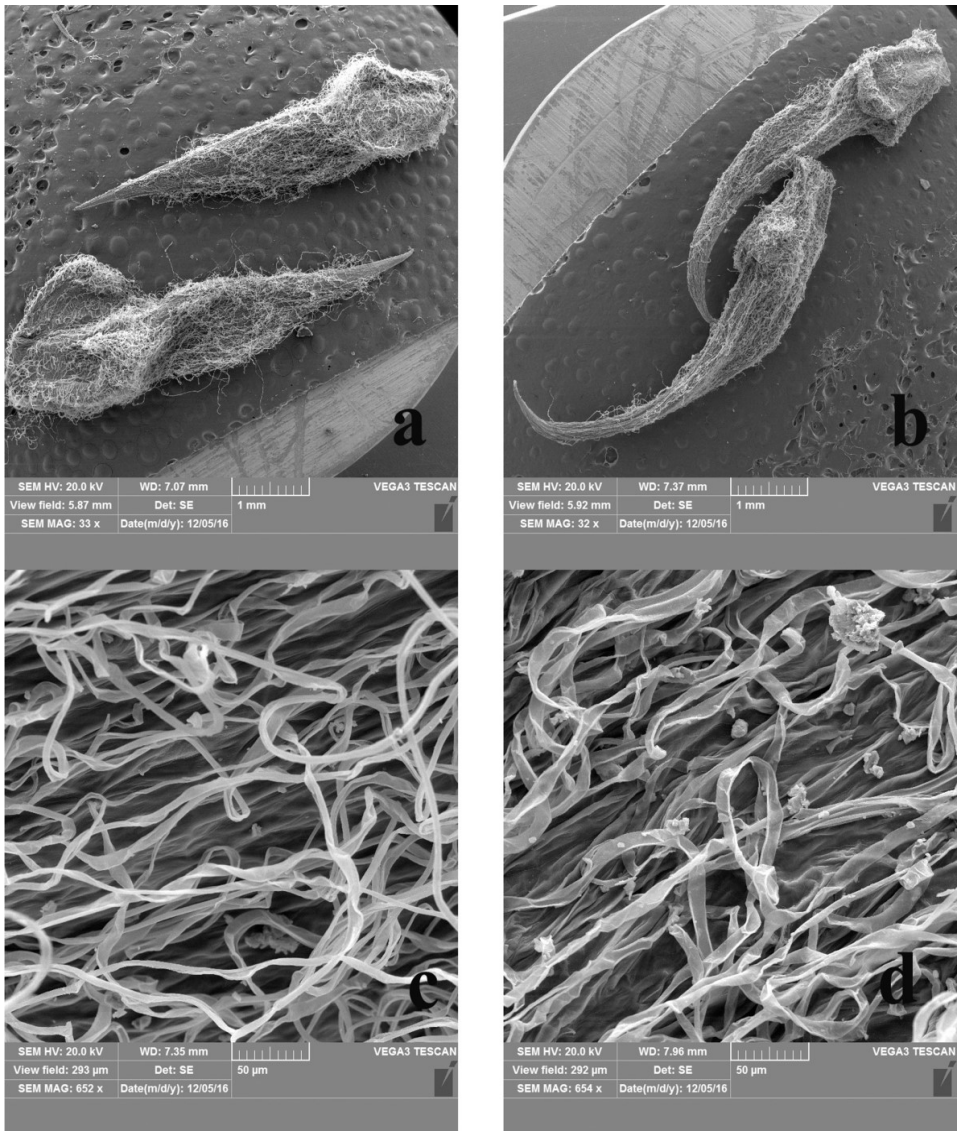

Fig. 4. Achene micrograph of *Ceratocephala.* a & c) *C. testiculata*, b & d) *C. falcata.*

Tab. 3. Comparison of pollen grains of *Ceratocephala* species in Iran.

Species	P/E	Length of polar axis	Length of equatorial axis	Exine ornamentation
C. falcata	Oblate-spheroidal	14.5±0.5	16±0.5	Reticulate
C. testiculata	Spheroidal	22.6±0.04	22.6±0.1	Scabrate- microechinate

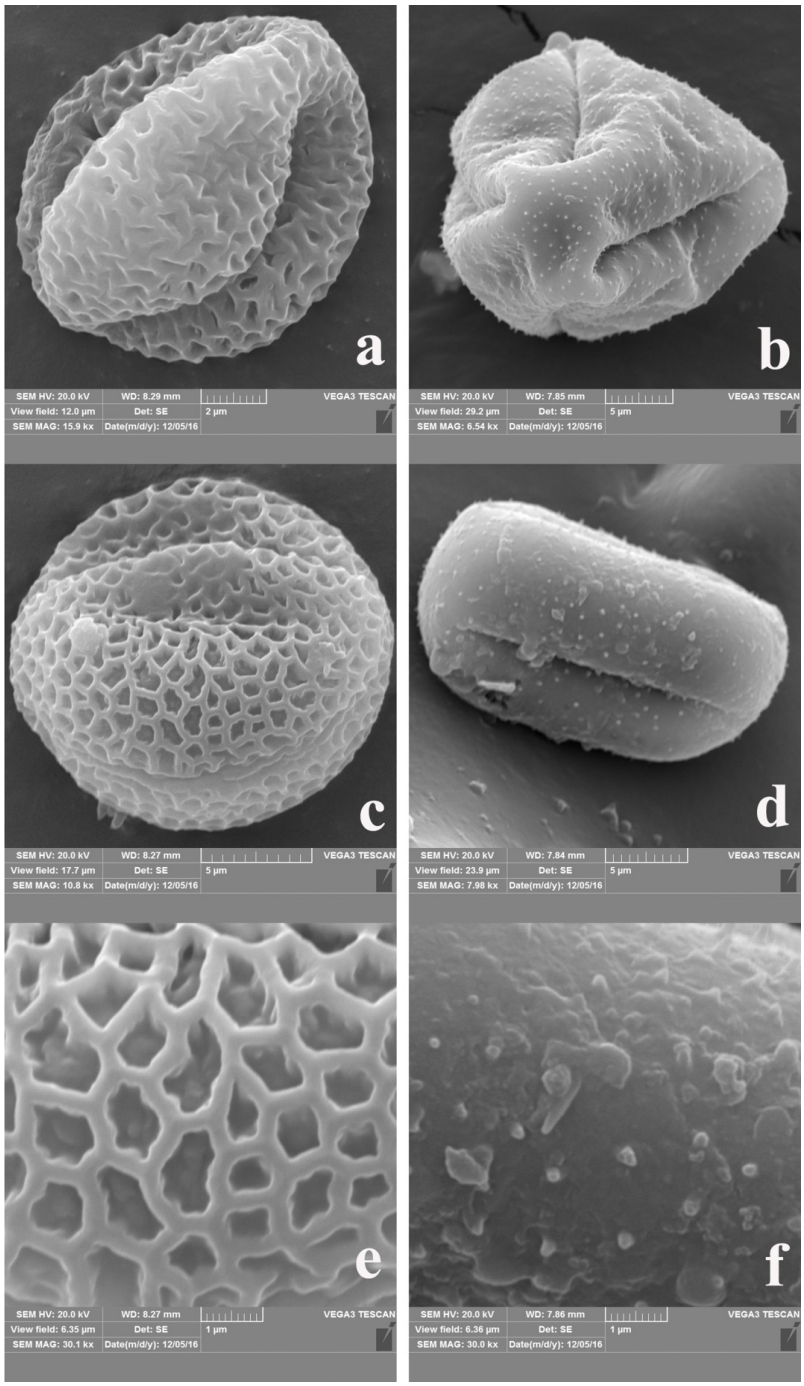

Fig. 5. Pollen micrographs of *Ceratocephala.* a, c & e) *C. falcata*, b, d & f) *C. testiculata.*

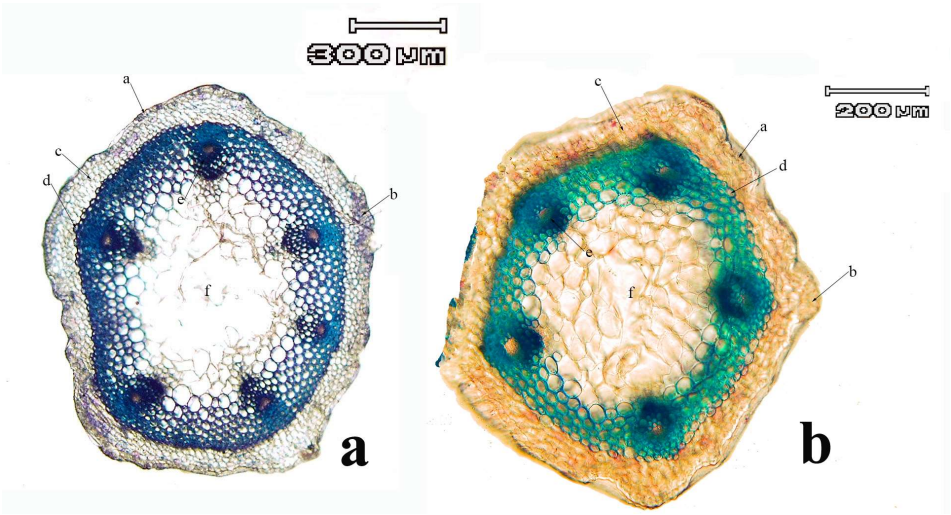

Fig. 6. Stem cross sections of *Ceratocephala.* a) *C. falcata*, b) *C. testiculata* (a: epidermis, b: collenchyma tissue, c: parenchyma tissue, d: sclerenchyma ring, e: vascular bundle, f: pith).

Leaf cross sections

Cross sections of the midrib and leaf blade presented the following features (Fig. 7): the upper and lower epidermis consist of a single cell layer with no significant difference in size. Covering trichomes are absent in *C. falcata* while fine long trichomes are observed in *C. testiculata*. Both species showed a bifacial leaf anatomical structure, so the mesophyll is differentiated into palisade and spongy tissues. The spongy cells are almost elongated in *C. falcata.* The shape of midrib showed some differences in *C. falcata* populations. The midrib region forms a more prominent projection in *C. falcata* while the midrib is somehow smooth, dome shaped in *C. testiculata*.

Discussion

Results of the study show that there are some macro- and micromorphological variations between *C. falcata* and *C. testiculata*. However, many features are identical. The pith is hollow and it is in concordance with previous records in other Ranunculaceae elements (METCALFE & CHALK 1965, GHORBANI NOHOOJEI et al. 2011) while the number of vascular bundles is of diagnostic importance in species studied.

Previous studies showed pantocolpate (CLARKE et al. 1991) and pericolpate pollen for *C. falcata* (EMADZADE et al. 2010) and pantocolpate for *C. testiculata* (= *C. orthoceras*; OBERSCHNEIDER 2016). Pollen grains in both species studied were tricolpate that was not in agreement with previous reports, although ornamentation of *C. testiculata* was in concordance with OBERSCHNEIDER (2016).

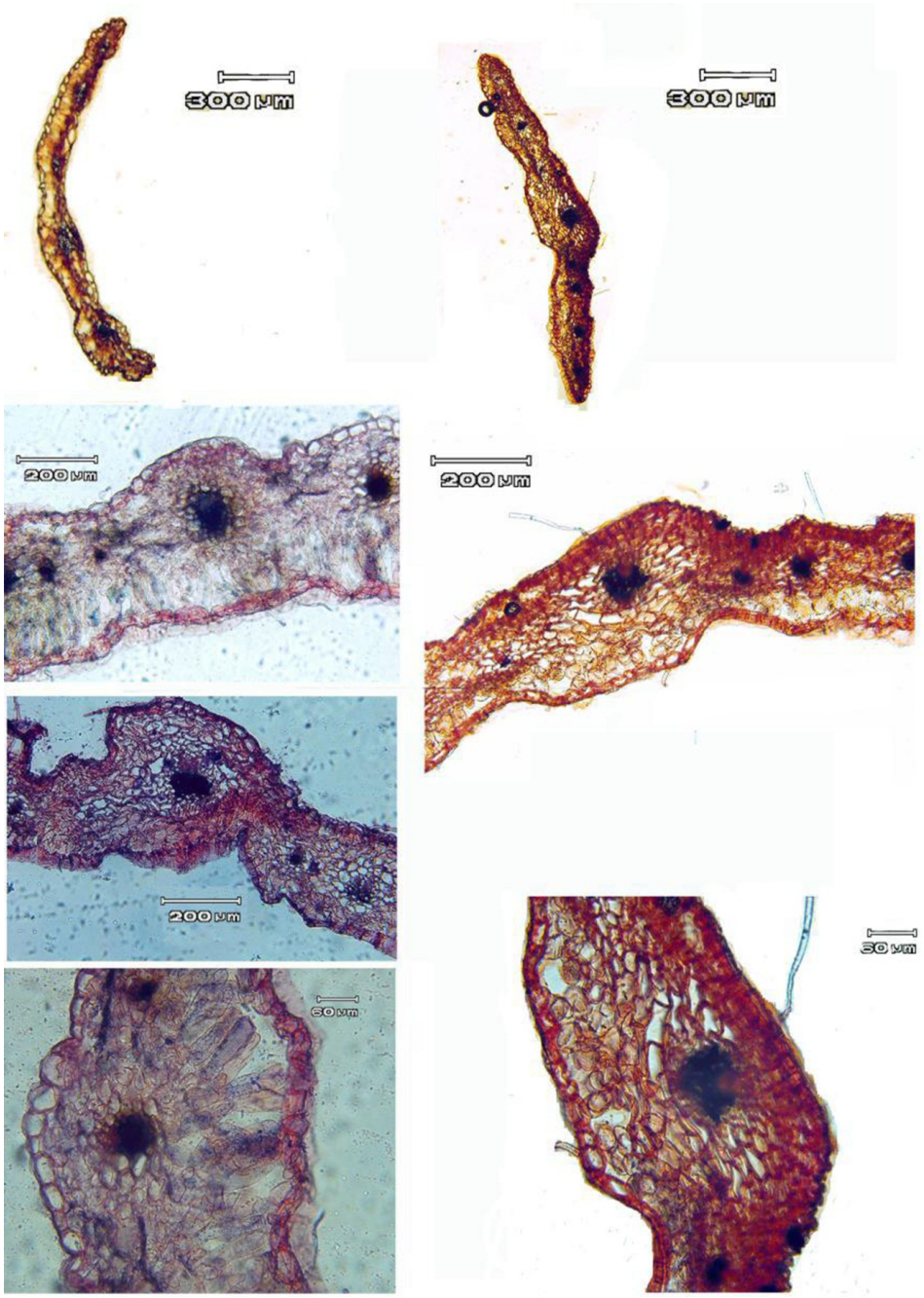

Fig. 6. Leaf cross section of *Ceratocephala.* (Left: *C. falcata*, right: *C. testiculata*).

Pollen grain characters, exine ornamentation and fruit shape and ornamentation showed differences between the two species, supporting the taxonomic value of these characters in this family (TATLIDIL et al. 2005, GHORBANI NOHOOJEI et al. 2008, EMADZADE et al. 2010, ZIMAN et al. 2011, XIE & LI 2012).

C. falcata and *C. testiculata* are sympatric species in Iran. Present study was carried out to provide additional evidence for taxonomists to help separate these two species. TAMURA (1995) believed that genera such as *Ceratocephala*, *Myosurus* and *Ficaria* seem to have extended their distribution rather recently. The high similarities in the two species studied can be due to recent divergence of these species (EMADZADE et al. 2010).

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