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Evidence from micromorphology and gross morphology of the genus *Loranthus* (Loranthaceae) in Iran

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Abstract: The genus *Loranthus* L. (Loranthaceae) is represented in Iran by 2 species: *Loranthus europaeus* Jacq. and *L. grewingkii* Boiss. & Buhse; the latter species is endemic to the area of *Flora Iranica*. The plants of *Loranthus* are hemiparasites growing on various host trees such as *Quercus infectoria* Oliv., *Acer monspessulanum* L., and *Armeniaca vulgaris* Lam. In order to investigate the range of morphological variation in different populations of these species, an inclusive gross morphological and micromorphological study was conducted. For this purpose, the anatomical characteristics of the stem, leaf, petiole, and fruit were also investigated. The most prominent features were the presence or absence of calcium oxalate crystals, their types, and distribution. In general, 3 types of wax crystalloid structures are identified on the leaf surface, including irregular glandular as well as platelet in *L. grewingkii* and smooth in *L. europaeus*. The crystalloid structures of the wax allow differentiation of both species. Seed surface ornamentation demonstrates little difference among these taxa. The seed surface is deeply wrinkled in *L. europaeus* and shallowly wrinkled in *L. grewingkii*. Finally, 62 qualitative characters were scored and analysed by both cluster analysis and principle component analysis (PCA), resulting in 2 distinct groups.

Key words: Loranthaceae, gross morphology, anatomy, micromorphology, Iran

Introduction

Loranthaceae as a family of Santalales comprises about 73 genera and 900 species (Vidal-Russell & Nickrent, 2008; APG III, 2009). *Loranthus* L. contains between 450 and 500 species (Hegi, 1981), but most of the historic species have been transferred to other genera and 10 species are now present considered within the genus (Nickrent et al., 2010). This genus consists of hemiparasitic shrubs on other seed plants, with leaves opposite or subopposite and pinnately veined, and inflorescences axillary or terminal with spikes sessile. The flowers are bisexual or unisexual (dioecious plants), 5- or 6-merous, actinomorphic. Calyx is usually persistent. Corolla is greenish, yellowish, or white, petals free, small, shorter than 10 mm. Stamens only are inserted on the petals. Berry is ovoid or subglobose. In addition to the embryo and the endosperm, each berry contains a mucilaginous tissue, called viscin, which consists of cellulose in a mixture of acidic and neutral polysaccharides (Sallé, 1983; Gedalovich et al., 1988).

The viscin tissue consists of a thick layer of highly viscous substance. It is attached to the berry mesocarp next the peduncle on both sides of the seed. The seed consists of white smooth endosperm on which there are 2 (sometimes 1 or 3) brown protuberances corresponding to the hypocotyls that extend to the inner embryo. The *Loranthus* seed is enclosed in sac-

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like compact tissue that is smooth on the endosperm side, but covered with hairy cells on the other side (Azuma et al., 2000).

The genus *Loranthus* occurs in temperate and subtropical regions of Asia and Europe (Huaxing et al., 2003). Of the 2 species distributed in Iran, *L. grewingkii* Boiss. & Buhse is endemic to the area of *Flora Iranica* and *L. europaeus* Jacq. is distributed in south-west Europe, south Russia, Anatolia, Iraq, and Iran. On the basis of the honey pollen analysis from Croatia, pollen grains of *L. europaeus* Jacq. were present (0.5%) in the honey's sediment (Sabo et al., 2011).

Morphological and anatomical studies are limited to *Loranthus*. Demuth and Weber (1987) investigated the leaf anatomy of the family Loranthaceae. There is no detailed taxonomic study on Loranthaceae in Iran except for that presented in *Flora Iranica* (Rechinger, 1976), which lacks taxonomic description of the species, but encompasses a diagnostic key and notes on the distribution of the species.

The ultrastructure of epicuticular waxes on the leaves of *Loranthus* has not yet been investigated. Barthlott et al. (1998) discussed the classification, terminology, and taxonomic value of plant epicuticular waxes in about 13,000 plant species. The epicuticular waxes often form complex 3-dimensional crystalline microstructures such as platelets, rods, and tubules (Koch & Barthlott, 2006). These structures are studied here for the first time in both selected species of *Loranthus*.

The present paper provides a detailed description of micromorphological as well as gross anatomical features of the stem, leaf, and fruit in *Loranthus* species growing in Iran, and evaluates their systematic significance.

Material and methods

Taxonomic and gross morphological studies

For the morphological studies, at least 10 individuals of each species were investigated. From each population, 20 specimens were collected directly in the field. Voucher specimens are deposited in the Herbarium of Guilan University. Description of both species was made using both fresh material and herbarium specimens. For each specimen, 25 distinctive morphological characters were documented and measured. Species chorology was determined according to Zohary (1973).

Anatomical studies

The materials for anatomical study were conserved in a solution of alcohol-water-glycerine. Anatomical observations were performed on transverse sections of the stem, leaf, petiole, and fruit (2-3 samples for each species from 2-3 localities). The cross sections were stained with methylene blue and congo red and mounted with glycerine jelly to make permanent slides (Vardar, 1987). Well-stained sections were photographed with an Olympus BX-51 light microscope (LM).

For calculating the stomatal index, dried leaves were used. The leaves were placed in a tube filled with 70% lactic acid for 4-5 days. Lactic acid softens the leaf and so it was possible to scrape the leaf surface with a sharp scalpel or by hand.

The length and width of the stomata were measured with a LM. The stomatal index was calculated according to the method described by Meidner and Mansfield (1968).

For numerical analysis, 62 characters (25 morphological as well as 37 anatomical characters) were measured. The characters are listed in Table 1.

All numerical analyses were performed using NTSYS ver. 1.6. A standardised data matrix was subjected to cluster analysis using the unweighted pair-group method with arithmetic averages (UPGMA) and principal components analysis (PCA).

For UPGMA, the similarity matrix was calculated from the standardised data matrix, using Gower's coefficient of resemblance for mixed data sets (Sneath & Sokal, 1973). For PCA, the standardised data were used to create a correlation matrix, and 2 eigenvectors were extracted, providing 2 axes onto which the standardised data were projected to give a 2-dimensional plot of the taxa and characters.

Ultimately, UPMGA trees and PCA plots obtained from the 2 analyses (i.e. morphological and anatomical characters) were compared with each other.

Table 1. Morphological and anatomical characters of *Loranthus* species and their coding.

Stem		Leaf		Petiole		Fruit		Seed	
Colour:	dark brown 0 light brown 1	Length (cm) (a)		Length (cm)		**Colour:**	yellow 0 red 1	**Shape:**	ovate 0 non-ovate 1
Branches type		Width (cm) (b)		Epidermis diameter (µm)		**Shape:**	ovate 0 obovate 1	Length (cm) (a)	
Internodes type		a/b		Cuticle diameter (µm)		Length (cm) (a)		Width (cm) (b)	
Internodes length (cm)		**Marginal shape:**	entire 0 non-entire 1	**Epidermal cell shape:**	circle 0 circle-elliptic 1	Width (cm) (b)		a/b	
Bract number	without bract 0 four bracts 1	**Blade shape:**	oblong-obovate 0 oblanceolate 1	**Cross section shape:**	elliptic 0 non-elliptic 1	a/b			
Cross section shape	circle 0 quadrangular 1	**Apex shape:**	obtuse 0 mucronate 1	Lower cortex diameter (µm)					
Cuticle diameter (µm)		**Base shape:**	cuneate 0 attenuate 1	Upper cortex diameter (µm)					
Epidermal cell shape:	elliptic 0 elliptic-quadrangular 1	**Colour:**	light green 0 dark green 1	**Parenchyma cells shape:**	irregular 0 regular 1				
Epidermis diameter (µm)		**Texture:**	deciduous 0 evergreen 1	**Intercellular space amount:**	little 0 large 1				
Parenchyma layer number		Phyllotaxy		**Crystal type:**	without crystal 0 prismatic 1				
Crystal type:	without crystal 0 cubical 1	Cuticle diameter (µm)		**Vascular fibre:**	on xylem 0 on xylem & phloem 1				
Vascular fibre		**Epidermal cell shape:**	elliptic 0 non-elliptic 1	Vascular bundle diameter (µm)					
Periderm:	absent 0 present 1	Epidermis diameter (µm)							
Cortex diameter (µm)		Stomata type							
Vascular bundle diameter (µm)		Lower cortex diameter (µm)							
Parenchyma cells shape:	regular 0 irregular 1	Upper cortex diameter (µm)							
Intercellular space amount:	little 0 large 1	**Crystal type:**	without crystal 0 prismatic-cubical 1						
Pith:	absent 0 presence 1	**Intercellular space amount:**	little 0 large 1						
		Lower surface stomatal index (mm^2)							
		Upper surface stomatal index (mm^2)							
		Stomata length to width ratio							
		Lower to upper stomata number ratio							
		Vascular fibre:	cellulose 0 sclerenchyma 1						
		Vascular bundle diameter (µm)							

Micromorphological studies

Leaves, seeds, and fruits were removed from herbarium specimens and the authors' own collection from wild populations. For ultrastructural characterisation of seed, viscin tissue was carefully dissected out from the seed surface; the seeds were then directly placed on aluminium stubs and covered with gold for scanning electron microscope (SEM) studies. The leaves and fruits were put into water for 12 h and 1 h, respectively, and were dried subsequently with a freeze dryer for 3 h at –34 °C and in an oven for 1 h at 30 °C. Photographs were taken with a SEM (LEO 1430 VP). Seed and fruit terminology followed Bojnansky and Fargasova (2007), while the terminology of leaf epicuticular waxes was according to Barthlott et al. (1998).

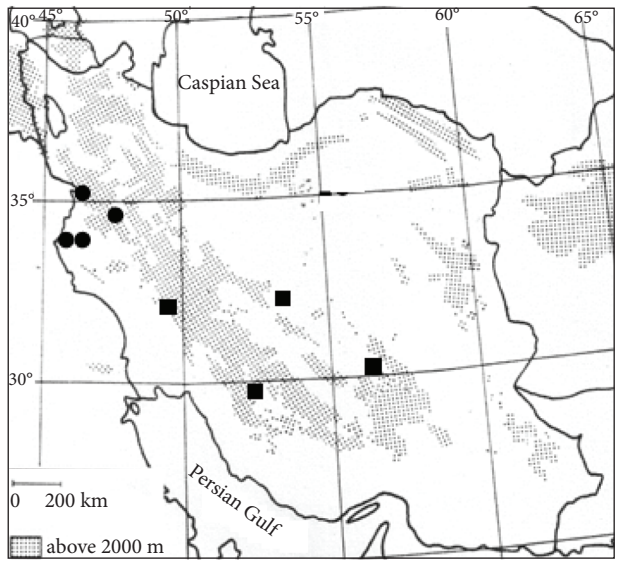

Figure 1. Map showing the distribution of the sampled population; (●): *Loranthus europaeus*, (■): *L. grewingkii*.

Results

Taxonomical account

Identification key for the species

1. Leaves deciduous; without bract; fruits yellow ... . **L. europaeus**
- Leaves evergreen; with 4 bracts; fruits red .... **L. grewingkii**

Loranthus europaeus Jacq., Enum. Stirp. Vindob. 230 (1762).

Types: Australia

Shrub 25-27 cm tall; light to dark brown; stem 1 cm diam. in lower part, up to 0.4 cm in upper part, with 2-3 branches; without any bract in branching site; internodes 1.5-4 cm long, nodes are swollen. Leaves decussate, leathery, deciduous; light green; 1.5-4 × 1-7 cm; leaf blade oblong-obovate, margins often entire, base cuneate, apex obtuse; petiole 0.2-0.8 cm. Inflorescences raceme or spike; flowers small, green white-creamy; perianth 6, linear. Fruit a berry, ovate, yellow, 1 × 0.6 cm. Seeds globose, dark brown, 0.5 × 0.3 cm. This species is distributed generally in west and south-west Iran (Figure 1).

Hosts: *Quercus infectoria* and *Quercus brantii*.

Fl. and Fr.: May-October

Habitat: Dry regions.

Loranthus grewingkii Boiss. & Buhse, Nouv. Mem. Soc. Nat. Mosc. 12: 106 (1860).

Types: Persia borealis in jugo Elburs prope Tschehordeh ad Pyrum commune parasiticus, BUHSE 1121.

Shrub 20-25 cm tall; light brown; stem to 1 cm diam. in lower part, up to 0.4 cm in upper part, with 2-3 branches; internodes 2-4 cm long, with 4 bracts in branching site, 0.1-0.2 cm long; nodes are swollen. Leaves decussate, leathery and evergreen; light green; 2-3.5 × 0.5-0.8 cm; leaf blade oblanceolate, margins entire, base attenuate, apex mucronate; petiole 0.2-0.8 cm. Inflorescences raceme or spike; flowers small, yellow greenish; perianth 6, oblong and succulent. Fruit a berry, obovate, red, 0.5 × 0.3 cm. Seeds ovate, brown darkish, 0.3 × 0.2 cm. This species is distributed generally in west, central and south Iran (Figure 1).

Hosts: *Amygdalus* sp., *Pyrus* sp., *Armeniaca vulgaris*, *Acer monspessulanum*, *Cotoneaster* sp., *Crataegus* sp., and *Quercus infectoria*.

Fl. and Fr.: May-October

Habitat: Sloping regions and lime soils and on stony rocks.

Anatomical studies

Stem: In cross sections the stem is circular (*Loranthus europaeus*) or quadrangular (*L. grewingkii*). Outermost layer is composed of epidermal cells in different sizes and shapes (e.g., elliptic in *L. europaeus* and elliptic-quadrangular in *L. grewingkii*). Epidermal cells are covered with a layer of cuticle. Periderm was observed in both taxa. Cortex was made up of irregular polygonal cells with few intercellular spaces in *L. europaeus* and regular elliptic in *L. grewingkii*. Cubical calcium oxalate crystals were observed in cortex of *L. europaeus*, but in *L. grewingkii* there was no crystal. In cortex, there are bundles of fibres just above the phloem of the vascular bundle. Stem shows secondary growth; a complete ring of cambium is formed among all studied taxa. In the central part there is rarely parenchymatous pith (Figure 2). The characteristics of the stem anatomy of both species of *Loranthus* are given in Table 2.

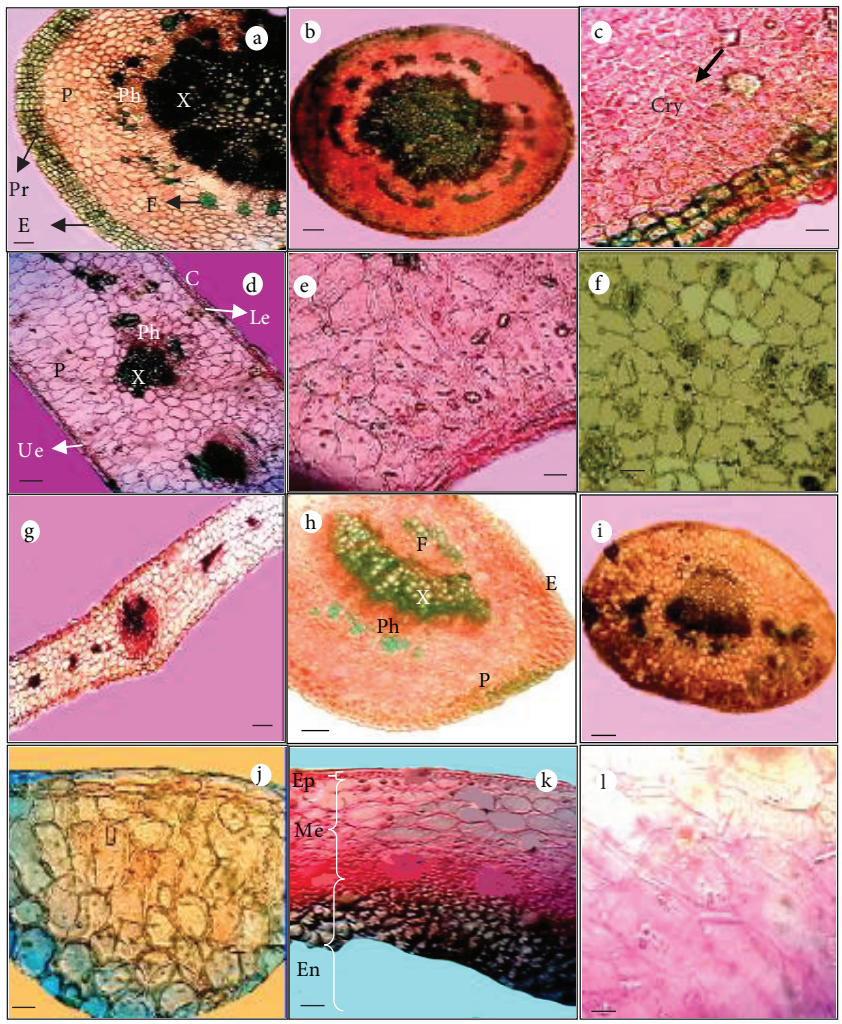

Figure 2. Transverse sections of stem: *Loranthus grewingkii*: a- a part of stem; *L. europaeus*: b- general aspect, c- a part of stem with cubical crystal (arrow). Transverse sections of leaf: *L. grewingkii*: d- a part of lamina, e- cubical and prismatic calcium oxalate crystals in the mesophyll, f- stomatal architecture; *L. europaeus*: g- general aspect. Transverse section of petiole: *L. europaeus*: h- general aspect, *L. grewingkii*: i- general aspect, j- detail of cortex. Transverse sections of fruit: *L. grewingkii*: k- a part of pericarp, l- a part of mesocarp.

Abbreviations: E: Epidermis; Ph: Phloem; X: Xylem; P: Parenchyma; Le: Lower epidermis; Ue: Upper epidermis; C: Cuticle; Pr: Periderm; Cry: Crystal (s); F: Fibre; Ep: Epicarp; Me: Mesocarp; En: Endocarp.- Scale bars: a, d, f, g, h, k = 0.04 mm; b, i = 0.1 mm; c, e, j, l = 0.01 mm.

Table 2. Anatomical and micromorphological comparison of *Loranthus europaeus* and *L. grewingkii*.

	Characters	***L. grewingkii***	***L. europaeus***
Stem	Cross section shape	quadrangular	circle
	Cuticle diameter (μm)	7.5	10
	Epidermal cell shape	elliptic- quadrangular	elliptic
	Epidermis diameter (μm)	12.5	22
	Parenchyma layer number	12	9
	Crystal type	-	cubical
	Vascular fibre	+	+
	Periderm	+	+
	Cortex diameter (μm)	480-500	275
	Vascular bundle diameter (μm)	1500-1750	1050-1085
Leaf	Cuticle diameter (μm)	12.5	7.5
	Epidermal cell shape	elliptic	elliptic
	Epidermis diameter (μm)	25	25
	Crystal type	prismatic and cubical	-
	Stomata type	paracytic	paracytic
	Vascular bundle diameter (μm)	375-400	300
	Lower surface stomatal index (mm^2)	89.3	69.2
	Upper surface stomatal index (mm^2)	76.7	79.3
	Stomata length to width ratio	1.7	1.3
	Cross section shape	elliptic	elliptic
Petiole	Cuticle diameter (μm)	7.5	5
	Epidermal cell shape	circular	elliptic
	Epidermis diameter (μm)	12	25
	Crystal type	prismatic	-
	Vascular fibre	primary phloem-xylem fibre	primary phloem fibre
	Vascular bundle diameter (μm)	300	600
Fruit	Colour	red	yellow
	Shape	obovate	ovate
	Surface sculpture	smooth	smooth
	Width × length (mm)	0.4-0.6 × 0.3-0.4	0.9-1 × 0.5-0.6
	Epicarp thickness (μm)	30-32.5	25-30
	Mesocarp thickness (μm)	250-350	250-300
	Endocarp thickness (μm)	1000-1250	1250-1500
	Crystal	prismatic	-
Seed	Shape	ovate	ovate
	Width × length (μm)	50-60 × 30-40	10-30 × 10-20
	Surface sculpture	superficial wrinkle	deep wrinkle

Leaf blade: In cross section, the leaf is quadrangular. *L. europaeus* has dorsiventral but *L. grewingkii* has isobilateral leaves. Epidermal cells are elliptic in both species. Cuticle is present on epidermis. Mesophyll is undifferentiated and encompasses few intercellular spaces. In *L. grewingkii* there are prismatic and cubical calcium oxalate crystals in mesophyll; these are absent in *L. europaeus*. Vascular bundles are collateral. Fibres are a made up of a type of sclerenchymatous cell with both celluloses and lignin in its walls; thus lignin fibres are absent in *L. europaeus*. Stomata are irregular in arrangement and of paracytic type (Figure 2).

Petiole: In cross section, the petiole is elliptic in both taxa. Epidermal cells in *L. europaeus* are elliptic, but in *L. grewingkii* the petiole cross-section is circular. There is a cuticle on both sides of the epidermis. The mesophyll is also undifferentiated with large intercellular spaces. In cortex of *L. grewingkii* prismatic calcium oxalate crystals were observed, but in *L. europaeus* there was no crystal. There is a main vascular bundle in the central part. As in the leaf, bundles are collateral. There were primary phloem fibres in *L. europaeus*, but *L. grewingkii* contains primary xylem fibres (Figure 2).

Fruit: Epicarp consists of one layer of epidermal cells. Mesocarp is parenchymatous with elliptic cells and few intercellular spaces. There are prismatic calcium oxalate crystals in mesocarp of *L. grewingkii*, but no calcium oxalate crystal was observed in *L. europaeus*. Both taxa consist of a viscin layer in endocarp (Figure 2).

Identification key based on anatomical characters

1. Stem cross section circular; cortex of stem with cubical crystals of calcium oxalate; leaf and petiole without any crystal ..... **L. europaeus**

- Stem cross-section quadrangular; cortex of stem without any crystal; leaf and petiole with cubical and prismatic crystals ..... **L. grewingkii**

Micromorphological studies

Leaf: Micromorphological studies are presented here for the first time in the genus. This part includes microstructural diversity of epicuticular waxes on adaxial and abaxial surfaces of leaves. Upper and lower surface of the leaf in *L. europaeus* is smooth and there is no ornamentation. In *L. grewingkii* granular and platelet crystalloids were observed with irregular orientations on both surfaces; however, the crystalloids were more condensed on the adaxial surface. Paracytic stomata were easily detectable on both surfaces (Figure 3).

Fruit: Fruit is a unilocular berry, with leathery surface and one seed. Two specific berry shapes and colours were recognised among the studied species: yellow and ovate in *L. europaeus* but red and obovate in *L. grewingkii*. The size of berries was 0.9-1 × 0.5-0.6 cm (*L. europaeus*) and 0.4-0.6 × 0.3-0.4 cm (*L. grewingkii*) (Table 2). The berry surface is generally smooth (Figure 3).

Seed: Morphological characters and SEM patterns of the seed coat in the examined taxa are presented in Table 2. The seeds were ovoid in both species. Seed size was similar in both species too. The patterns of sculpturing in both species were wrinkled, but there were minor differences between them; in *L. europaeus* the wrinkles were deeper than in *L. grewingkii* (Figure 3).

Numerical taxonomy

From numerical taxonomy point of view, some characters were important, e.g., leaf texture, length and width of leaf, blade shape, apex and base type, presence and absence of bract, internodes length, fruit and seed shape, presence and absence of calcium oxalate crystal, and its type were the characters showing high importance. The phenogram resulting from the UPGMA clustering of similarity matrix is presented in Figures 4 and 5. The dendrogram shows a cophenetic correlation of 0.98%-0.99%, suggesting that the dendrogram provides an accurate representation of the resemblances.

PCA results gained from morphological and anatomical characters are shown in Figures 6 and 7. The results showed that *L. europaeus* and *L. grewingkii* can be interpreted as separate species. Voucher specimens are given in Tables 3 and 4.

Discussion

The objective of this study was to find additional features for better delimitation of the examined *Loranthus* taxa using micro- and macromorphological characters.

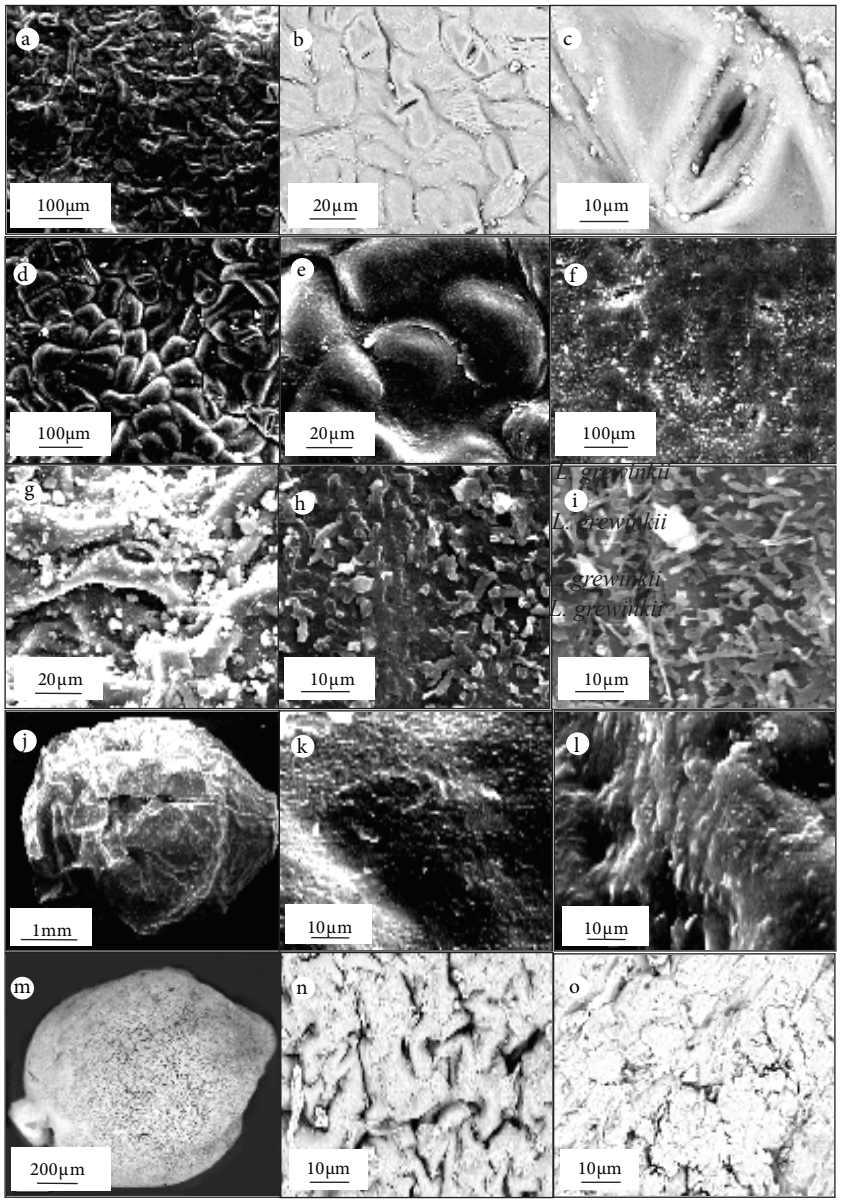

Figure 3. SEM photographs of *Loranthus* species. -Surface views of leaf cuticle: *L. europaeus*: a, b- abaxial cuticle, c- stoma on the abaxial cuticle, d, e- adaxial cuticle; *L. grewingkii*: f- abaxial cuticle, g- abaxial cuticle with granular layer, h- abaxial surface cuticle with platelet layer, i- adaxial cuticle with platelet layer. Fruit surface ornamentation: *L. europaeus*: j- pericarp surface, k- ultrastructure of pericarp surface; *L. grewingkii*: l- ultrastructure of pericarp surface. Views of seed: *L. europaeus*: m- seed shape, n- deep wrinkled; *L. grewingkii*: o- superficial wrinkled.

In previous taxonomic treatment of the genus *Loranthus* in Iran, characters such as the length of the leaf and internodes have been used for diagnosing the species (Rechinger, 1976). Although our results generally corroborate *Flora Iranica* with respect to occurrence of 2 species of *Loranthus* in Iran, some differences are recognised, which could contribute to a better determination of these species. The most important macromorphological characters are presence or absence of bracts and their numbers, fruit shape and colour, and phyllotaxy type.

In Iran, *L. europaeus* occurs only on oaks, confirming previous findings (Hegi, 1981), but *L. grewingkii* is found mostly on trees of the family

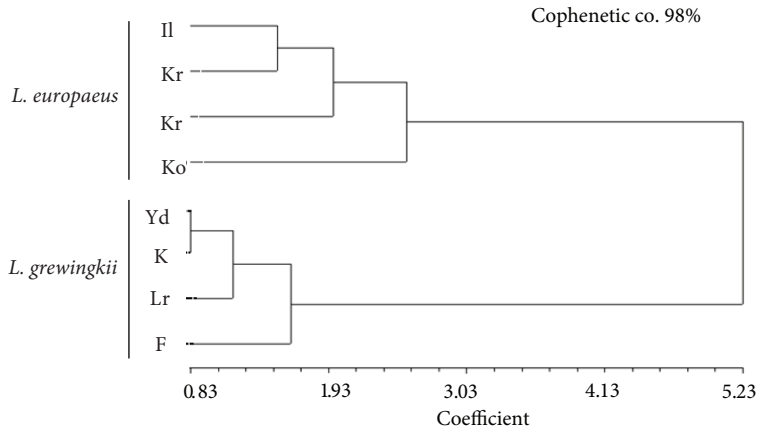

Figure 4. UPGMA dendrogram based on morphological characters. For abbreviations see caption of Tables 3 and 4.

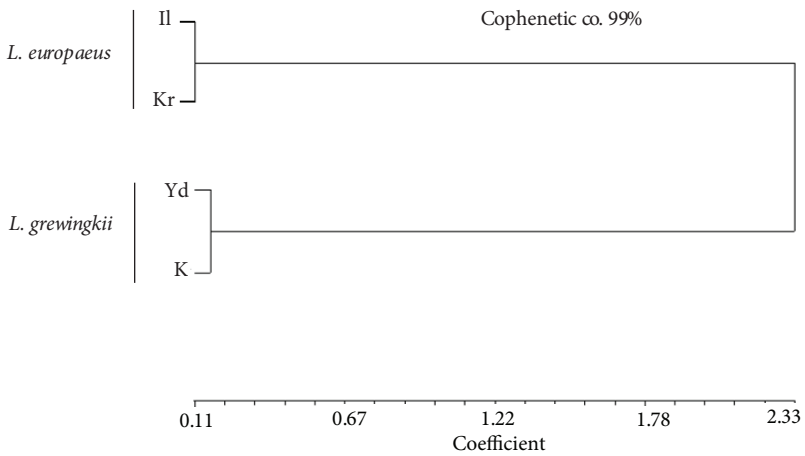

Figure 5. UPGMA dendrogram based on anatomical characters. For abbreviations see caption of Tables 3 and 4.

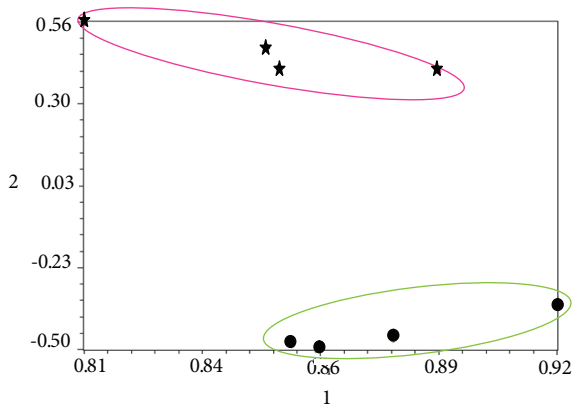

Figure 6. Principal component analysis of the 2 taxa based on morphological characters; (★): *L. europaeus*, (●): *L. grewingkii*.

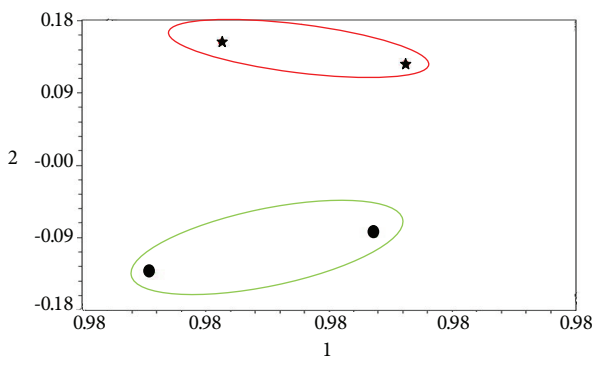

Figure 7. Principal component analysis of two taxa based on anatomical characters; (★): *L. europaeus*, (●): *L. grewingkii*.

Table 3. Voucher specimens of numerical analysis based on morphological characters.

Taxa	Code	Geo.	Collection data
L. europaeus	Il	West	Ilam: between Ghalajeh mountain and Ilam, 1700-1900 m, *Rechinger* 6836.
L. europaeus	Kr	West	Kermanshah: 8 km to Ivan Southwest, Sharazol mountain, 1500-1700 m, *Fathi, Tavakkoli* & *Mir abdoli* 74739 (TARI).
L. europaeus	Kr	West	Kermanshah: 110 km to Kermanshah West, Nura mountains, 2100 m, *Atashzar* & *Hatami*, *s.n.* (TARI).
L. europaeus	Ko	West	Khordestan: Marivan, Kani donar, 1350 m, *Fattahi* 76307 (TARI).
L. grewingkii	K	Centre	Kerman: Nasr mountain, 2800 m, *Ramezani* 3994 (GUH).
L. grewingkii	Yd	Centre	Yazd: 30 km to Southwest Marvest-Baghe shahi, 2148 m, *Asadi* & *Ranjbar* 57345 (TARI).
L. grewingkii	F	West	Fars: Dena Mountain, 2400-2600 m, *Asadi* & *Mozaffarian* 31274 (TARI).
L. grewingkii	Lr	West	Lorestan: Ghaher lake, 2100-2400, *Mozaffarian* & *Sardabi* 42269 (TARI).

Table 4. Voucher specimens of numerical analysis based on anatomical characters.

Taxa	Code	Geo.	Collection data
L. europaeus	Il	West	Ilam: between Ghalajeh mountain and Ilam, 1700-1900 m, *Rechinger* 6836.
L. europaeus	Kr	West	Kermanshah: 8 km to Ivan south-west, Sharazol mountain, 1500-1700 m, *Fathi, Tavakkoli* & *Mir abdoli, s.n.* (TARI).
L. grewingkii	K	Centre	Kerman: Nasr mountain, 2800 m, *Ramezani* 3994 (GUH).
L. grewingkii	Yd	Centre	Yazd: 30 km to south-west Marvest-Baghe shahi, 2148 m, *Asadi* & *Ranjbar* 57345 (TARI).

Rosaceae. The distribution range of the *Loranthus* host and its distribution depends on the movement of birds as the main dispersal vectors, as well as local ecological conditions (Zebec & Idžojtić, 2006).

Metcalfe and Chalk (1957) gave information about the general anatomical characteristics of the family Loranthaceae, but there was little information about the anatomical structure of these species.

Leaf anatomy varies greatly among species and provides several systematically significant characters (Carlquist, 1961). Examined species are similar in general aspects of leaf anatomy. The leaf mesophyll had no differentiation of palisade and spongy cells; this confirms results obtained by Xue-Zhi et al. (2006) and Demuth and Weber (1987). Rao and Kelkar (1951) have reported the existence of both terminal and non-terminal sterosclereids in the leaves of various species of *Loranthus*; however, Kuijt and Lye (2005) have not reported any sclereid, which supports our observations on the leaves of the 2 species analysed here.

Our results show that the distribution and shape of the calcium oxalate crystals are of special importance. The use of calcium oxalate crystals in solving taxonomic problems has been suggested in previous studies in other plant groups (Edeoga & Okoli, 1992, 1995; Mbagwu & Edeoga, 2006).

Cubical calcium oxalate crystals in the leaves of *L. grewingkii* and their absence in *L. europaeus* is the most important character for distinguishing the 2 species. The occurrence of calcium oxalate crystals in leaves and stems is important due to their role in some plant physiological processes. According to Mbagwu and Edeoga (2006), Edeoga and Okoli (1992, 1995), and Edeoga and Ugbo (1997), these ergastic substances could have nutritional, mechanical, and transport roles in plants. These researchers pointed out that a close association of calcium oxalate crystals with the

site of photosynthesis suggests that these substances could be involved in the synthesis of sugars.

In the stem, the shape of cross sections and cortex thickness have taxonomical importance. Moreover, *L. europaeus* has cubical calcium oxalate crystals; whereas we observed no calcium oxalate crystals in the stem of *L. grewingkii*.

The size and shape of the stomata are taxonomically important characters (Tahir & Rajput, 2009); in all taxa, stomata type was paracytic; this is in agreement with observations reported by Demuth and Weber (1987). The stomatal index was similar; thus they did not provide additional diagnostic features in this genus.

The results of the petiole study show that some characters such as the diameter of midrib and shape of epidermal and parenchyma cells are important. Epidermal cell diameter in *L. europaeus* is double that in *L. grewingkii*. Cortex cells are irregular with large intercellular spaces in *L. europaeus*, while in *L. grewingkii* regular cells with few intercellular spaces were observed. Midrib diameter is an important diagnostic character; midrib diameter in *L. grewingkii* is double that in *L. europaeus*.

Epidermal characters have potential for taxonomic use and are helpful as additional taxonomic characters (Stace, 1965; Baronova, 1992). The results of the examination of epicuticular waxes showed variation between species; we observed smooth epidermal cells in *L. europaeus*, while those in *L. grewingkii* were glandular and platelet crystalloid. There is correlation between these features and the geographical distribution and environment of the plant. *L. grewingkii* grows in dryer regions and epicuticular waxes have an important role in preventing water loss.

Fruit shape and colour were different in the studied species, however, epicarp surface under SEM did not show any diagnostic feature and both taxa had smooth epicarps.

The importance of the ultrastructure of the seed surface as a reliable approach for solving taxonomic problems has been well recognized (Heywood, 1971). The mistletoe seed is enclosed in sac-like compact tissue that is smooth on the endosperm side but covered with hairy cells on the other side (Azuma et al., 2000). The general shape of the seeds in *L. europaeus* and *L. grewingkii* is similar in appearance, being ovate. There are some differences between *L. europaeus* and *L. grewingkii* in seed structure. Seeds of *L. europaeus* are deeply wrinkled on the surface, while those of *L. grewingkii* are superficially wrinkled. This result agrees with Bojnansky and Fargasova (2007).

The UPGMA and PCA analysis indicated that the 2 species could easily be separated. Furthermore, characters such as presence and absence of bract, internodes length, fruit and seed shape, presence and absence of calcium oxalate crystal, and its type were more important than other characters. These findings confirm that anatomical characters are particularly useful in distinguishing the 2 species.

The results of the present investigation with anatomical studies and SEM photographs depict leaf, fruit, and seed features of *Loranthus*, which will be considered as additional useful taxonomic characters.

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