

## TAXONOMIC SIGNIFICANCE OF NUTLET AND LEAF CHARACTERS IN *HYMENOCRATER*, *NEPETA* SECT. *PSILONEPETA* AND *LOPHANTHUS* (NEPETINAE, NEPETOIDEAE: LAMIACEAE)

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Received 2013. 06. 30. Accepted for publication 2013. 12. 08

Serpooshan, F., Jamzad, Z., Nejdassattari, T. & Mehregan, I. 2014. 06. 31: Taxonomic significance of nutlet and leaf characters in *Hymenocrater*, *Nepeta* sect. *Psilonepeta* and *Lophanthus* (*Nepetinae*, *Nepetoideae*: Lamiaceae).- *Iran. J. Bot.* 20 (1): 80-95. Tehran.

*Hymenocrater* (Lamiaceae) in Iran was studied using morphological characters of nutlets and leaves. The species of the genus *Nepeta* sect. *Psilonepeta* and a few species of the genus *Lophanthus* were also examined for comparison. Scanning electron micrographs showed the surface of the nutlets and trichome types on leaves in detail. Two types of nutlets including smooth and sculptured were recognized. Among the species with smooth nutlets *H. incanus* is very characteristic having an absolutely smooth nutlet surface. Sculptures may be prominently tuberculate e.g. in *H. bituminosus* and *H. calycinus* or verrucose e.g. in *H. sessilifolius* and *N. sessilifolia*. Most species have constant features in nutlet surface, but minor differences could be identified within a few species, i.e. *H. elegans* and *H. yazdianus*. Leaf surfaces in studied group are covered with dense or laxe trichomes. Different trichome types are observed including glandular and non-glandular trichomes. Two different glandular trichomes were identified: peltate or sub-sessile glands and capitate or stalked glands. Non-glandular trichomes consist of short or long trichomes with (1)2-8(11) cells. Relationship among the species of the three genera was investigated based on data provided from morphological features, using cluster and PCA analysis. Three species groups are provided by the cluster analysis. Sculptured nutlets and peltate glands with two- or multi-celled head are characteristic features of most species grouped in the first cluster. Most species of the second and third clusters have smooth nutlets. Micro-papillate trichomes and capitate glands with a long, one- or multi-celled stalk are significant respectively in species of second and third clusters. Characters with the most variation were identified using FA based on PCA. Closely placement of *Hymenocrater* species together with *Nepeta* and *Lophanthus* species in obtained phenogram and ordination supports the affinity of these genera. It also reveals that the morphological features are not significant for defining the boundaries of the studied genera but raised the proposal of very close relationships among the studied species and the possibility of re-circumscribing the genera within *Nepetinae*.

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**Key words:** *Hymenocrater*; *Nepeta*; *Lophanthus*; micromorphology; trichome, nutlet

ارزش تاکسونومیک صفات مورفولوژیکی فندقه و برگ در جنس‌های *Hymenocrater*, *Nepeta* بخش *Psilonepeta* و *Lophanthus*  
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گونه‌های جنس *Hymenocrater* در ایران همراه با گونه‌های جنس *Nepeta* بخش *Psilonepeta* و سه گونه از جنس *Lophanthus* از نظر صفات ماکرو- و میکرومورفولوژیکی فندقه و برگ مورد مطالعه قرار گرفتند. تصاویر میکروسکوپ الکترونی از آراستار سطح فندقه حالت‌های گوناگونی از دو تیپ صاف و دارای تزیینات را نشان داده است. صاف‌ترین سطح فندقه با بافت سلولی مشبک در گونه

*H. incanus* دیده می‌شود و در بین فندقه‌های دارای تزئینات انواع مختلفی مانند تزئینات برجسته در *H. calycinus* و *H. bituminosus* و تزئینات با مرکز فرو رفته در *H. sessilifolius* و *N. sessilifolia* وجود دارد. اگر چه در اکثر موارد آراستار فندقه صفت ثابتی در سطح گونه است اما در گونه‌های *H. yazdianus* و *H. elegans* این صفت تنوع کوچکی نشان می‌دهد. بر اساس تصاویر میکروسکوپ الکترونی و میکروسکوپ نوری در سطح برگ این گونه‌ها غده‌های یک یا چند سلولی بدون پایه یا پایه کوتاه، غده‌هایی با پایه یک یا چند سلولی بلند و کرک‌های غیر غده‌ای (۱۱)۸-۲ (۱) سلولی وجود دارد. به‌منظور تعیین خویشاوندی گونه‌های این سه جنس، داده‌های به‌دست آمده با روش خوشه‌ای و رسته‌بندی مورد آنالیز قرار گرفتند. بر اساس فنوگرام به‌دست آمده گونه‌ها در سه خوشه اصلی جای می‌گیرند که در اکثر گونه‌های خوشه اول فندقه دارای تزئینات است و بیشتر گونه‌هایی که در دو خوشه دیگر قرار گرفته‌اند فندقه‌هایی با سطح صاف دارند. وجود اشکال خاصی از کرک‌ها نیز در گونه‌های هر یک از خوشه‌ها قابل توجه می‌باشد. حضور نزدیک گونه‌های جنس *Hymenocrater* با گونه‌های دو جنس دیگر در این فنوگرام قرابت این سه جنس را تایید می‌کند. طبق این نتایج ترکیب صفات فندقه و برگ در تفکیک جنس‌های فوق از یکدیگر قابل استفاده نمی‌باشد ولی این فرضیه را تقویت می‌کند که مرزهای تاکسونومیکی جنس‌های زیرطایفه *Nepetinae* می‌بایستی تعریف مجدد بشوند.

## INTRODUCTION

The genera considered in *Nepetinae* Coss. & Germ. (*Mentheae*, *Nepetoideae*, *Lamiaceae*) are characterized with 15-nerved calyx, strongly 2-lipped corolla, having the posterior pair of stamens longer than the anterior pair and pericarp structure (Wagstaff 1992). Twelve genera are classified within this subtribe (Harley & al. 2004), among which *Nepeta* L., *Lophanthus* Adans., *Hymenocrater* Fisch. & C. A. Mey. and *Marmoritis* Benth. are phylogenetically closely related (Budantsev & Lobova 1997 and Drew & Systma 2012). *Hymenocrater* with 12 species is mainly distributed in Iran and Afghanistan (Rechinger 1982 and Pojarkova 1954). The western limit of its geographical distribution is Turkey where it is represented with one species in east Turkey. In Iran the genus is present with nine species from which four are endemics (Rechinger 1982; Budantsev 1992; Harley & al. 2004 and Jamzad 2012). The genus is characterized by large, broad, membranous and mostly colored calyx teeth and resupinate corolla in most species. The genus *Lophanthus* has c. 22 species in the alpine regions of central Asia, Afghanistan, Mongolia, China and Turkey (Dirmenci & al. 2010). In *Lophanthus* calyx is 15-nerved with a hairy annulus in throat, similar to *Hymenocrater* and *Nepeta* sect. *Psilonepeta* Benth. and corolla is resupinate, similarly in *Hymenocrater* (Pojarkova 1954; Rechinger 1982 and Dirmenci & al. 2010). The similarities between *Nepeta* species sect. *Psilonepeta* and species belonging to the genus *Lophanthus* has been discussed by different authors. Levin (1941) included the species belonging to *Nepeta* sect. *Psilonepeta* in the genus *Lophanthus* and classified them as sect. *Psilonepeta*. Budantsev (1992) divided the species of *Lophanthus* into two sections (*Lophanthus* and *Psilonepeta*).

The usefulness of nutlet and trichome morphological characters for different taxonomic levels in family *Lamiaceae* has been proved by different authors i.e. Hedge 1992; Marin & al. 1996; Budantsev & Lobova 1997; Jamzad & al. 2000; Navarro & Oualidi 2000; Padure 2003; Abbas-Azimi & al. 2006; Moon & Hong 2006; Kaya & Dirmenci 2008; Dinc & al. 2009; Moon & al. 2009; Salmaki & al. 2009; Ryding 2010 and Eshratifar & al. 2011. Budantsev & Lobova (1997) admitted that the surface ornamentation of nutlets in *Hymenocrater* is quite similar to species of *Lophanthus* and *Nepeta* but is distinguished from these by its lack of myxocarpy.

In a phylogenetic study of *Nepeta* (Jamzad & al. 2003), species of the section *Psilonepeta* were grouped in a clade within the genus *Nepeta*, furthermore a few species of *Hymenocrater* were examined and added to the analysis matrix, they were nested in *Nepeta* sect. *Psilonepeta* clade (Jamzad unpublished). Yet a few of *Hymenocrater* species have been included in morphological, anatomical, palynological and phytochemical studies (Satil & al. 2007; Jafari & Jafarzadeh 2008; Moon & al. 2008a; Moon & al. 2008b; Moon & al. 2009; Gohari & al. 2010 and Ryding 2010).

In this study morphological examination of nutlets and leaves of 9 species of *Hymenocrater*, 7 species of *Nepeta* sect. *Psilonepeta* and 3 species of *Lophanthus* is represented and taxonomic significance of these characters in defining the generic boundaries is discussed. It is part of a Ph.D. thesis undertaken by F. Serpooshan.

## MATERIALS AND METHODS

Most specimens examined in this study were from TARI herbarium that include some new collections from northern and north-eastern parts of Iran. *Lophanthus* species are dedicated duplicates to TARI and materials of two species were taken from IRAN herbarium (Tab. 1). Macro-morphological characters were studied using an OLYMPUS stereomicroscope and for micro-morphological studies nutlets and dissected middle part of the leaves were fixed on stubs using a double adhesive tape. Coating were done by platinum or gold and scanning electron micrographs were supplied respectively using Cambridge LEO 440i or VEGA\\ TESCAN SEM. Leaf trichomes were studied also using LEICA DM500 Light Microscope (LM). Terminology and description of nutlet micro-morphology is based on Budantsev & Lobova (1997) and general classification and typology of trichomes is based on Roe (1971) and Cantino (1990). Twenty two macro- and micro-morphological characters of nutlets and leaves were chosen, quantitative characters were measured and the state of qualitative ones were determined (Tabs. 2-3). Variables were standardized (range 0 to 1), and then taxa were clustered using WARD method with Squared Euclidean distance. Ordination of taxa based on Principal Component Analysis (PCA) was performed with Varimax rotation. Factor analysis based on PCA was performed to determine the most influential variable characters of nutlet and leaf among the taxa (Tab. 4). SPSS version 21 software was used for analysis.

## RESULTS

The nutlet and trichome characters of the studied species are described below. Micrographs of nutlets and leaf surfaces are illustrated in details (Figs. 1-4). The comparison of characters among the studied taxa is given (Tabs. 2-3). Cluster analysis and ordination of the species were achieved (Fig. 5).

### Nutlet

**Hymenocrater.** Nutlets of nine species were examined (Tab. 1). They are elliptic, ovate or oblong, mostly trigonous in shape with the size of  $2\text{-}3.65 \times 1.1\text{-}1.9$  mm. The nutlet apex is rounded and the base is truncate to attenuate. *Hymenocrater incanus* and *H. longiflorus* have the smallest and largest nutlets respectively (Figs. 1A & C). Usually on the dorsal side of nutlets 3-5 nerves are observed. Areole is whitish, lateral and bilobed. Attachment scar has a granular texture. Two types of ornamentation, smooth and sculptured are recognized on the surface of nutlets.

**Smooth nutlets:** *H. incanus* and *H. longiflorus* are

characterized by smooth nutlets (Fig. 1A, C). In *H. incanus* absolutely smooth surface is consisting of reticulate-cellular texture with oblong or polygonal cells. The anticlinal walls (AW) are straight; the external periclinal walls (EPW) are flat or convex and smooth (Fig. 1B) or wrinkled. In *H. longiflorus* surface texture is reticulate-cellular, consisting of rounded to polygonal cells, with prominent AW and depressed EPW (Fig. 1D).

**Sculptured nutlets:** *H. bituminosus*, *H. calycinus*, *H. oxyodontus*, *H. platystegius* and *H. sessilifolius* are characterized with sculptured nutlets (Figs. 1G, I, K). The following structures can be recognized within this group: *Hymenocrater bituminosus*, *H. calycinus*, *H. oxyodontus* and *H. platystegius* have tuberculate nutlets; tubercles have a truncate apex in *H. oxyodontus* and *H. bituminosus* (Fig. 1H). In *H. calycinus* tubercles are truncate (Fig. 1J) similar to *H. bituminosus*, or have convex apex. Tubercles are less prominent in *H. platystegius* (Fig. 1L). *Hymenocrater sessilifolius* has verrucose nutlets. These sculptures consist of a ring of radial cells with a depression on their center.

In *H. elegans* nutlet surface is sculptured with an undulate reticulate-cellular texture (Fig. 1E, F) or sculptured with pressed and flattened ornamentations. Within *H. yazdianus* two different nutlet types were found, smooth nutlet with reticulate-cellular in which anticlinal wall (AW) is prominent and external periclinal wall (EPW) is depressed, similar to *H. longiflorus*, or sculptured nutlets with verrucose consisting of radial cells and a depression on their center, similar to *H. sessilifolius*.

**Nepeta.** The nutlets of six species of *Nepeta* sect. *Psilonepeta* were studied (Tab. 1). They are oblong or obovate, trigonous and rounded at the apex. Their sizes varies between  $1.75\text{-}2.55 \times 0.85\text{-}1.2(1.65)$  mm. Areole is whitish, lateral, bilobed and has a granular texture the same as *Hymenocrater*. Nutlet surface is smooth or sculptured.

**Smooth nutlets:** Nutlet surface is smooth in *N. dschuparensis*, *N. depauperata* and *N. makuensis* (Fig. 2A). It is characterized by ridged cellular texture consisting of irregular cells with prominent AW in *N. dschuparensis*. Nutlet surface of *N. depauperata* consist of rounded or polygonal cells with convex EPW. Rounded or polygonal cells with straight AW and depressed EPW were observed in *N. makuensis* (Fig. 2B).

**Sculptured nutlets:** *N. laxiflora*, *N. oxyodonta* and *N. sessilifolia* are characterized by verrucose nutlets (Fig. 2C). Similar to some *Hymenocrater* species, these are forming by a ring of convex radial cells and have a depression on their center (Fig. 2D).

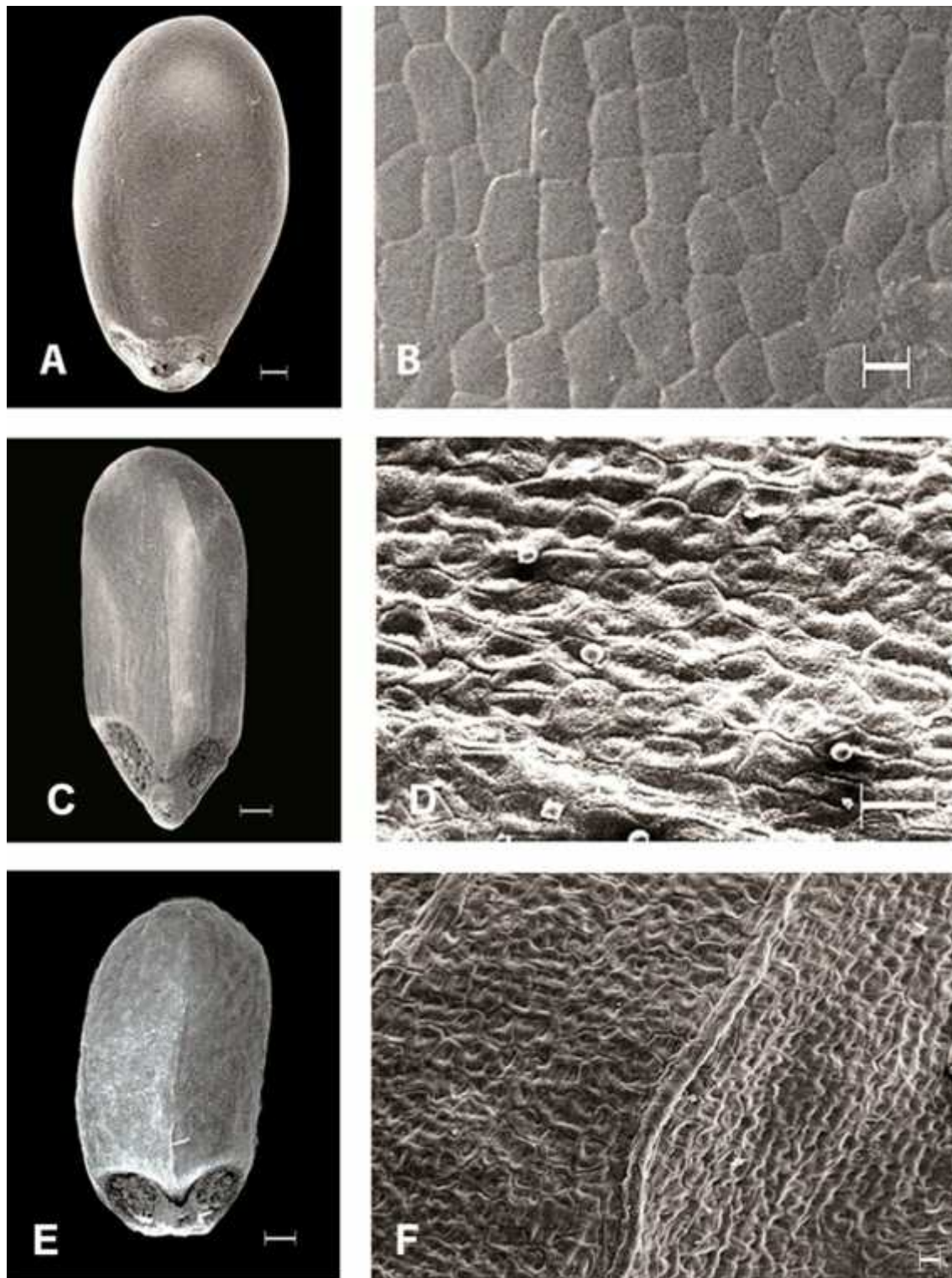


Fig. 1. SEM micrographs of nutlet in *Hymenocrater*: A, B, *H. incanus*; C, D, *H. longiflorus*; E, F, *H. elegans*. Scale bar: A=200  $\mu$ ; C, E=300  $\mu$ ; B, F=20  $\mu$ ; D=30  $\mu$ .

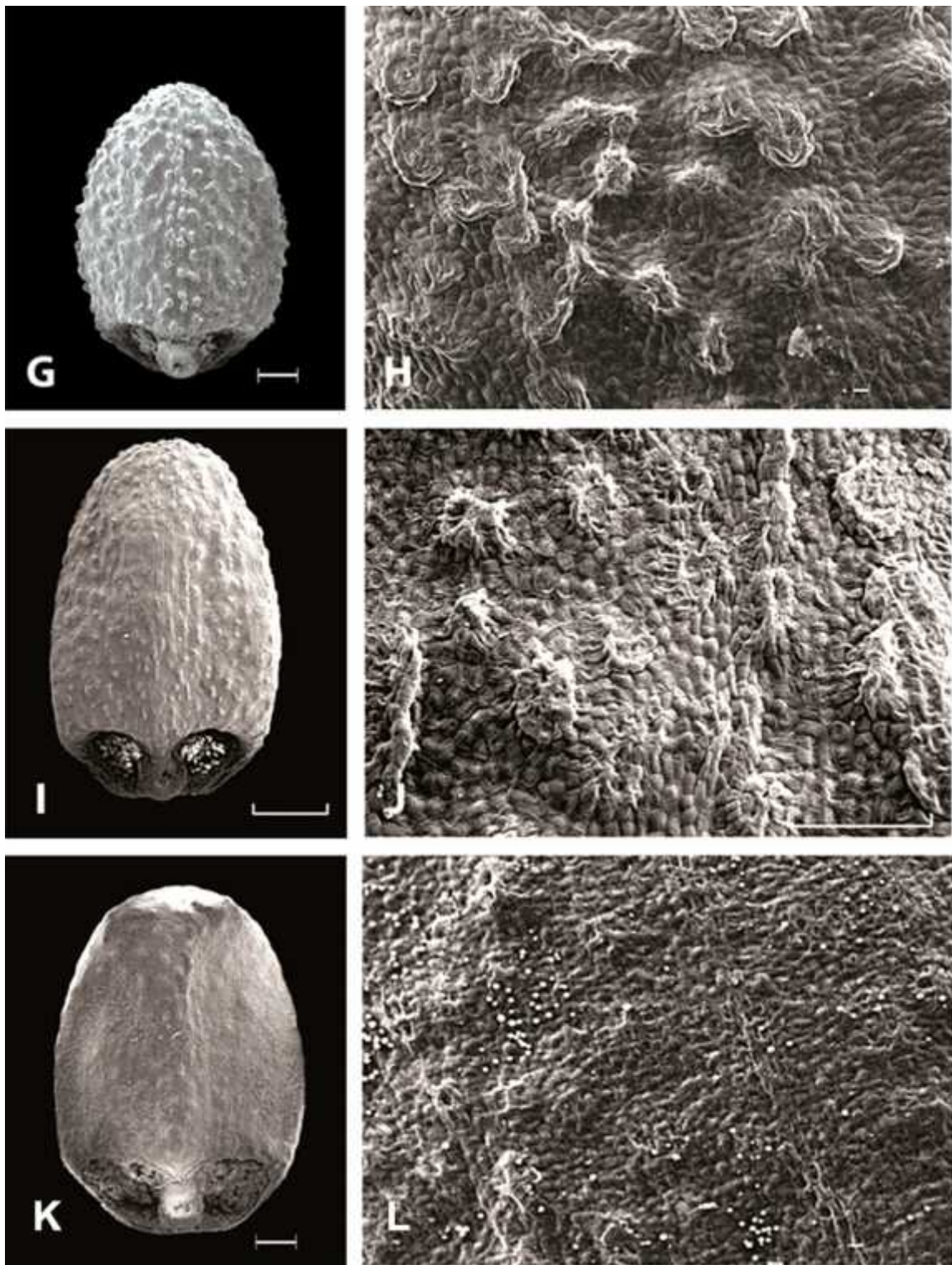


Fig. 1. Continued: G, H, *H. bituminosus*; I, J, *H. calycinus*; K, L, *H. platystegius* Scale bar: G, K=300 μ; I=500 μ; H, L=20 μ; J=100 μ.

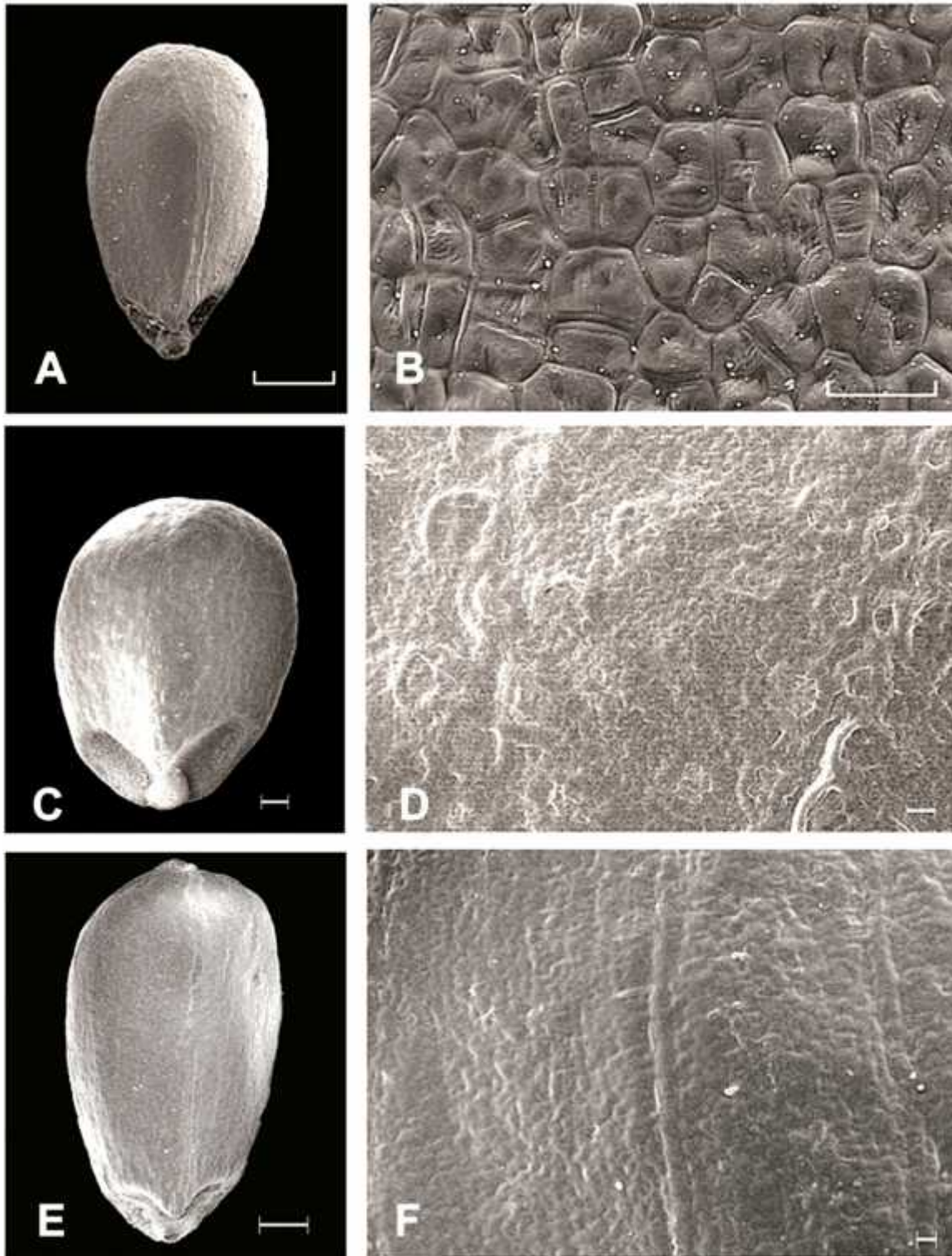


Fig. 2. SEM micrographs of nutlet in *Nepeta* sect. *Psilonepeta* and *Lophanthus*: A, B, *N. makuensis*; C, D, *N. sessilifolia*; E, F, *L. tschimganicus*. Scale bar: A=500 μ; C=200 μ; E=300 μ; B, F=20 μ; D=30 μ.

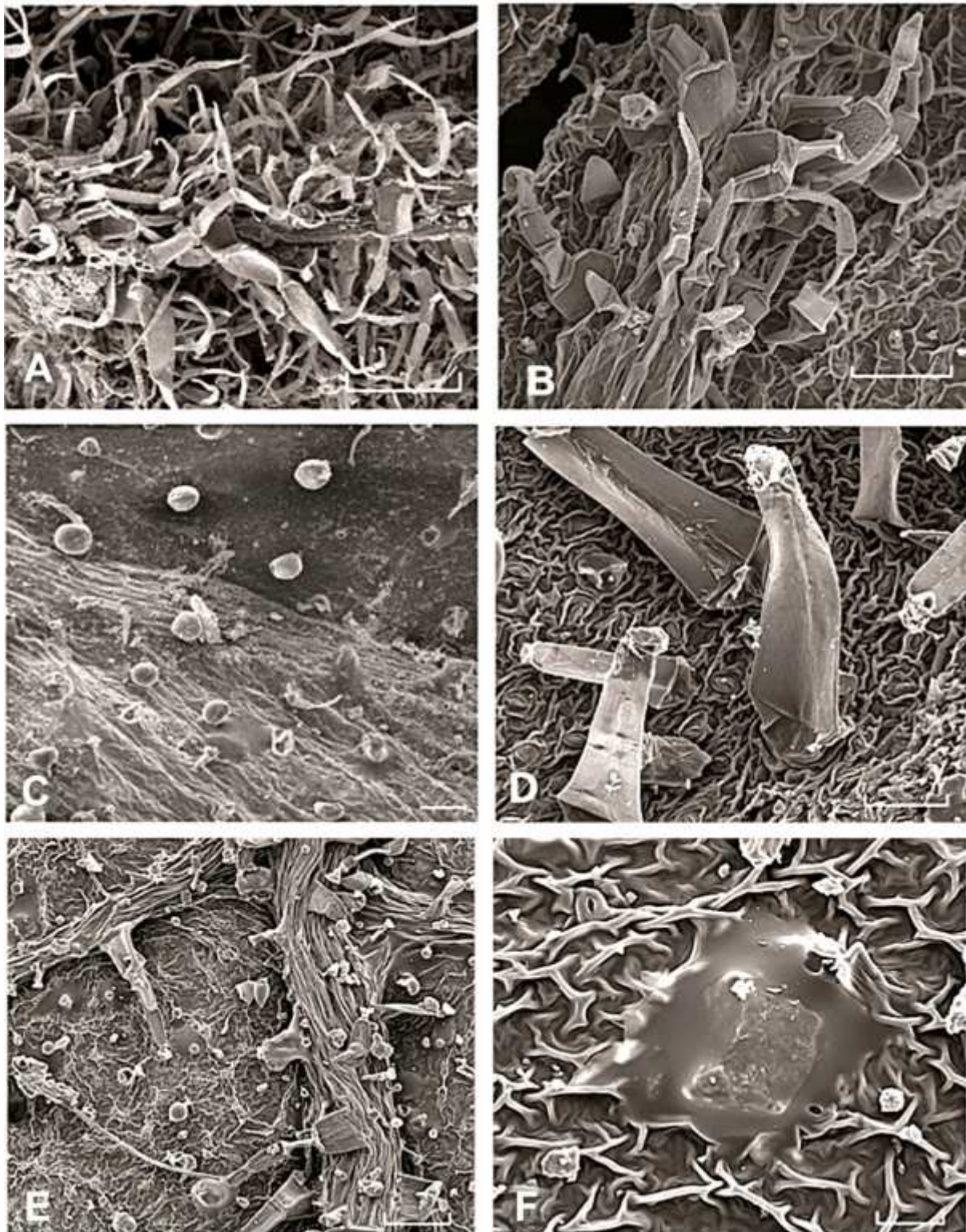


Fig. 3. SEM micrographs of non-glandular and glandular trichomes in studied species: A, *N. oxyodonta*; B, *N. depauperata*; C, *H. platystegius*; D, *N. allotria*; E, F, *N. makuensis*. Scale bar: A, C, E=100  $\mu$ ; B, D=50  $\mu$ ; F=20  $\mu$ .

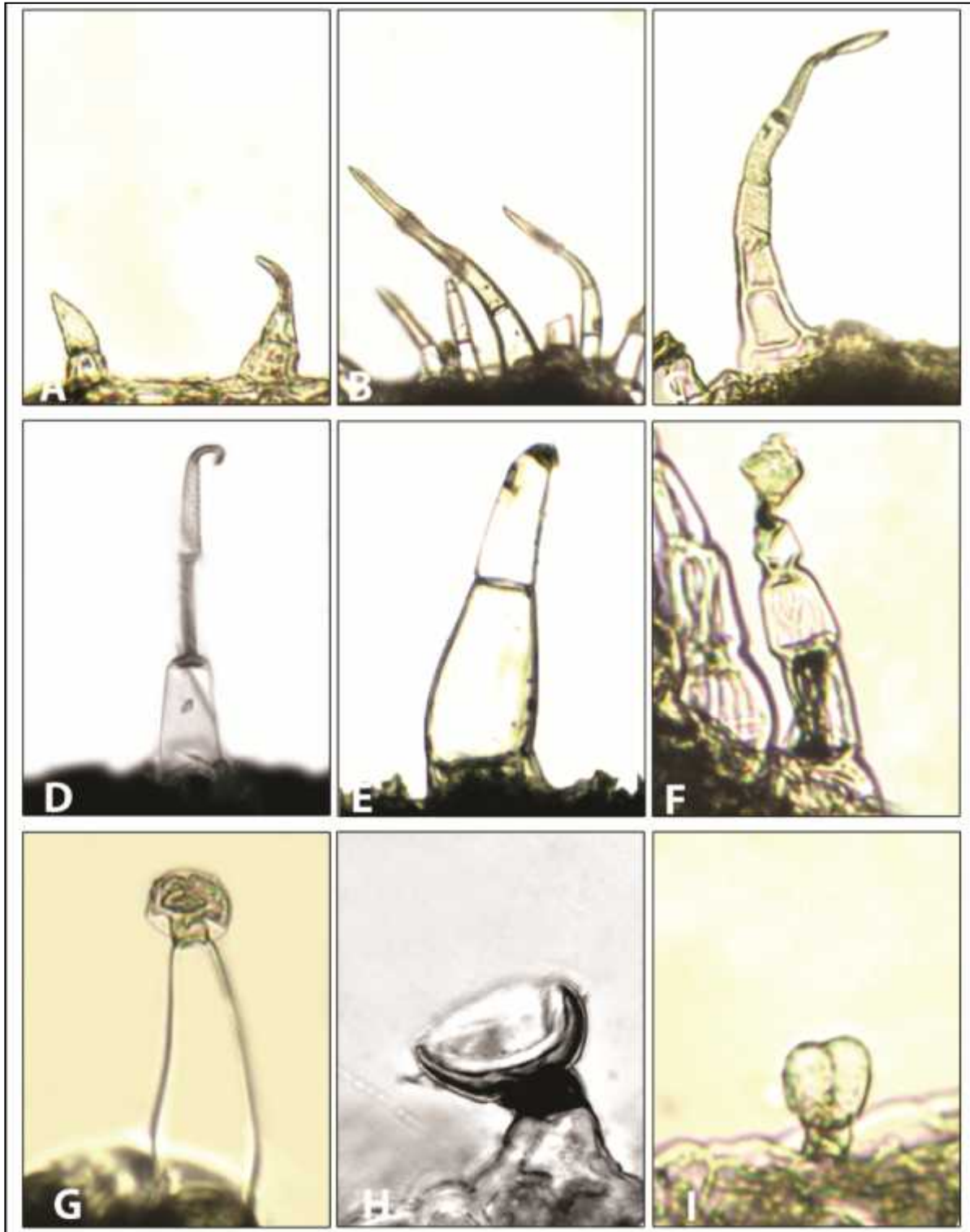


Fig. 4. LM micrographs of trichomes in studied species: A, *H. bituminosus*; B, *H. incanus*; C, *N. dschupSarensis*; D, E, *H. yazdianus*; F, *N. makuensis*; G, *N. sessilifolia*; H, I, *H. calycinus*.



Table 1. Voucher specimen of examined materials. (\*:species endemic to Iran)

Taxa	Collecting data	Nutlet	Leaf
<i>Hymenocrater</i>			
<i>H. bituminosus</i> Fisch. & C. A. Mey.	Iran, Mazandaran, Kandavan, Pol-e Zangule, after Mazid village, between Valashed & Takor, 1700 m, Jamzad & Serpooshan, 98737 (TARI).	Ñ	Ñ
	Iran, Azarbayejan, Kuh-e Sahand, between Lighvan and Isperekhan, 2200-2600 m, Assadi & Mozaffarian, 30629 (TARI).	Ñ	Ñ
	Iran, Esfahan, Natanz, Mazdeh, Kuh-e Karkas, 1800-2320 m, Shams & Feyzi, 10716 (TARI).		Ñ
	Iran, Tehran, Sorkhehesar, 1700 m, Dini & Arazm, 12850 (TARI).	Ñ	
	Iran, Tehran, Road of Qom, 1170 m, Babakhanlou & Amin, 12854 (TARI).	Ñ	Ñ
<i>H. calycinus</i> (Boiss.) Benth.	Iran, Isfahan, Golpayegan, Hende, 2400-2500 m, Feyzi & Shams, 12551 (TARI).	Ñ	Ñ
	Iran, Isfahan, Ghamsar, Kuh-e Kargaz, above Barazuk village, 2081 m, Asadi, 82731 (TARI).	Ñ	
	Semnan, Turan protected region, W. of Oshtoran Kuh, 1300-1500 m, Freitag & Mozaffarian, 28465 (TARI).	Ñ	Ñ
<i>H. elegans</i> Bunge	Iran, Mazandaran, Kandavan, Pol-e Zangule, road to Baladeh, after Mazid village, 1900 m, Jamzad & Serpooshan, 98729 (TARI).	Ñ	Ñ
	Iran, Tehran, Firuzkuh, Chehel Cheshme, Abbarik, 2350 m, Dini & Arazm, 13303 (TARI).	Ñ	Ñ
<i>H. incanus</i> * Bunge	Hamadan, 100 km N. Aq Bolaq, Aq Daque mts., 2050-2350 m, Safikhani, Kalvandi & Faramarzi, 2809 (TARI).	Ñ	Ñ
	Iran, Isfahan, 10 km Dehaghan to Borujen, Noruzi, 3980 (TARI).	Ñ	Ñ
<i>H. longiflorus</i> Benth.	Iran, Kermanshah, N.W. of Kermanshah, Shamshir village, Shahu mountains, 1760-1980 m, Assadi, 60748 & Hamzeh, 1277 (TARI).	Ñ	Ñ
<i>H. oxyodontus</i> * Rech. f.	Iran, Semnan, Shahrud, Turan protected region, Kuh-e Peyghambar, S. Zamanabad, 1300-1600 m, Iranshahr, 35661 (IRAN).		Ñ
	Iran, Khorasan, Shahrud, Biarjmand, Kuh-e Mollahado, Gharb Khane Khody, 1450 m, Maddah & Moradi, 3931 (TARI).	Ñ	Ñ
<i>H. platystegius</i> * Rech. f.	Iran, Khorasan, Mashad, Torghabe, Noghondar village, 1500 m, Serpooshan, 97852 (TARI).	Ñ	Ñ
	Iran, Khorasan, Dargaz, Laein-e No, Hezarmasjed, Khakestar village, 1600 m, Serpooshan, 97854 (TARI).	Ñ	Ñ
	Iran, Khorasan, 42 km to Birjand, on the road from Ghayen, 2000 m, Assadi & Amirabadi, 84719 (TARI).	Ñ	Ñ
<i>H. sessilifolius</i> Benth.	Iran, Khorasan, 14 km from Kashmar to Neyshabur, 1400-1500 m, Assadi & Mozaffarian, 35593 (TARI).	Ñ	Ñ
<i>H. yazdianus</i> * Rech. f.	Iran, Yazd, Nudushan, Geyluk, 2400 m, Mozaffarian, 77766 (TARI).	Ñ	Ñ
	Yazd, Taft, Deh Bala village, Shir Kuh, 3400 m, Mahmoodi & Noruzi, 98646 (TARI).	Ñ	Ñ
<i>Nepeta</i>			
<i>N. allotria</i> * Rech. f.	Iran, Mazandaran, Iлека, between Makloz and Dahla, 3800 m, Terme, 15184 (IRAN).		Ñ
<i>N. depauperata</i> * Benth.	Iran, Bandar-Abbas, N. slope of rocky mts. of Bokhon, N. of Fareghan, 1500-2000 m, Mozaffarian, 44723 (TARI).	Ñ	Ñ
<i>N. dschuparensis</i> * Bornm.	Iran, Kerman, Kuh-e Lalezar, Zarda valley, 3000 m, Foroughi & Assadi, 16289 (TARI).	Ñ	Ñ
<i>N. laxiflora</i> * Benth.	Iran, Chaharmahal-e Bakhtiari, Darr-e Bazoft, Mavarz, Kuh-e Sefid, from Talkhedan valleys, 1450-2200 m, Mozaffarian, 74565 (TARI).	Ñ	Ñ

Table. 1. Continued

Taxa	Collecting data	Nutlet	Leaf
<i>N. makuensis</i> * Jamzad & Mozaffarian	Iran, Azarbajegan, Maku, rocky mountain between Shut and Umeridash and Nieyaz to Dashfishel, 1700 m, Mozaffarian, 71140 (TARI).	Ñ	Ñ
<i>N. oxyodonta</i> * Boiss.	Iran, Chaharmahal-e Bakhtiari, Shahr-e Kord to Naghan, N. of Sulegan, Kuh-e Shahpurnaz, 2200-2700 m, Mozaffarian, 57425 (TARI).	Ñ	Ñ
<i>N. sessilifolia</i> * Bunge	Iran, Arak, Shazand, Hafteh-o Emarat, Anbarteh and Tajereh, Kuh-e Sero, 2150-2950 m, Mozaffarian, 63945 (TARI).	Ñ	Ñ
<i>Lophanthus</i>			
<i>L. lipskyanus</i> Ikonn.-Gal. & Nevski	Turkmenistan, Kugitang, without herbarium number (TARI).		Ñ
<i>L. tschimganicus</i> Lipsky	Uzbekistan, W. of Tian-Shan, Tschimgan, Baranov & Raikova, 6426 (TARI).	Ñ	Ñ
<i>L. turcicus</i> Dirmenci, Yildiz & Hedge	Turkey, Van, Dirmenci, Yildiz & Yildiz, 16959 (TARI).	Ñ	Ñ

**Lophanthus.** Two species of *Lophanthus* were examined (Tab. 1). They are obovate and trigonous with the size of 2.25-2.7 × 1.2-1.3 mm. The nutlet apex is acute and areole is lateral and bilobed with two short lobes (Fig. 2E). Attachment scar has a granular texture similar to *Hymenocrater* and *Nepeta*. Nutlet surface is smooth and has rounded or polygonal cells with convex EPW (Fig. 2F) the same kind as in *N. depauperata*.

### Trichome

Trichome of different plant parts in studied group of genera follows most Lamiaceae. Two basic types of trichomes, glandular and non-glandular, were observed on the leaf surfaces by SEM and LM.

**Type 1.** Non-glandular trichomes include uni-cellular and multi-cellular (uni-seriate) which are explained in more details below:

**Uni-cellular trichomes:** Uni-cellular trichomes were found very rarely in the studied species e.g. conical shape uni-cellular trichomes in *H. longiflorus* and *L. lipskyanus*.

**Multi-cellular trichomes:** Multi-cellular trichomes show a considerable variation based on number of consisting cells (2-11), trichome length (50-300 µm), shape of basal cell (inflated or not), shape of terminal cell (triangular, narrow or elongated) and presence or absence of micro-papillae.

**Short multi-cellular trichomes (<200 µm),** which contained 2-3(5) cells, with a rather wider basal cell and a narrower or triangular terminal cell were found for example in *H. calycinus*, *H. elegans*, *H. oxyodontus*, *H. bituminosus* and *N. depauperata* (Fig. 4A; 3B).

**Long multi-cellular trichomes** were observed in most species studied in which following features can be distinguished: long (>200 µm), 3-5(7) celled multi-

cellular trichomes were observed in *N. oxyodonta* and *H. incanus* on both abaxial and adaxial leaf surfaces (Fig. 3A, 4B). Very long (>500 µm), 4-8 celled multi-cellular trichomes with thin-walled and long basal cell were observed in *H. longiflorus*, *H. yazdianus* and *L. lipskyanus* (Fig. 4D), basal cell may be rather short and inflated as in *N. dschuparensis* (Fig. 4C). Based on the shape of consisting cells, multi-cellular trichomes are bead-like for example in *H. longiflorus*, *H. yazdianus* and *N. depauperata* (Fig. 3B), or have enlarged basal cell and subsequent more or less uniform cells terminating to a narrow terminal cell for example in *L. lipskyanus*.

**Non-glandular trichomes** were observed mostly on veins of abaxial leaf surface. Uni- and multi-cellular trichomes densely covered with micro-papillae were the common features in *N. depauperata* (Fig. 3B). Micro-papillate trichomes were also found in *N. dschuparensis*, *H. incanus*, *H. oxyodontus* and *H. platystegius*.

**Type 2.** Glandular trichomes include peltate (sessile or sub-sessile glands) and capitate or stipitate glands with short or long stalk.

**Peltate trichomes:** Peltate trichomes were observed in all studied species (see for example in *H. platystegius* Fig. 3C). Most of the glands have a one-celled head (Fig. 4H), but glands composed of two- or multi-celled head were also found (Fig. 4I).

**Capitate trichomes:** The length and cell number of stalk varied among the studied species. Capitate trichomes with a short/long thin-walled (ribbon-like) stalk cell are the common features in *L. turcicus*, *N. allotria* and *N. sessilifolia* (Fig. 3D; 4G). The capitate trichomes were found in some species including *H. longiflorus* and *N. dschuparensis*. The capitate glandular trichomes with 2-4(6) stalk cells occurred with two different forms, with smooth stalk cells e.g. in

Table 2. Quantitative characters of nutlet and leaf in studied taxa.

No.	Taxa	Code	Limit	Nutlet length (mm)	Nutlet width (mm)	Areole length ( $\mu$ m)	Angle of areole lobes (degree)	Leaf length (mm)	Leaf width (mm)	Basal petiole length (mm)	Stem petiole length (mm)
1	<i>H. bituminosus</i>	12854	Mean	2.60	1.45	500.00	133.00	10.60	7.75	4.50	3.00
			Min	2.45	1.35	460.00	127.00	10.10	7.00	4.00	2.50
			Max	2.70	1.55	530.00	137.00	11.00	8.50	5.00	3.30
2	<i>H. calycinus</i>	12551	Mean	2.35	1.50	653.00	121.00	16.00	10.25	4.00	3.25
			Min	2.33	1.48	651.00	120.00	15.50	9.50	3.80	3.00
			Max	2.36	1.52	655.00	122.00	16.50	10.75	4.20	3.75
3	<i>H. elegans</i>	13303	Mean	3.42	1.45	729.60	104.00	27.00	16.50	15.00	9.00
			Min	3.20	1.30	655.80	97.00	26.00	16.00	14.25	7.00
			Max	3.65	1.55	793.00	110.00	28.00	17.25	15.50	11.00
4	<i>H. incanus</i>	3980	Mean	2.95	1.37	480.00	125.00	15.00	7.00	7.00	4.50
			Min	2.92	1.35	475.00	120.00	14.00	6.00	6.75	3.75
			Max	2.97	1.40	485.00	130.00	17.00	8.00	7.50	5.00
5	<i>H. longiflorus</i>	1277	Mean	3.47	1.75	966.00	71.00	45.00	26.00	2.00	0.00
			Min	3.31	1.72	964.00	69.00	40.00	22.00	1.75	0.00
			Max	3.65	1.77	968.00	72.00	52.00	29.00	2.25	0.00
6	<i>H. oxyodontus</i>	3931	Mean	2.65	1.90	760.00	133.00	18.66	11.50	11.00	6.83
			Min	2.60	1.75	740.00	130.00	18.00	10.50	10.50	6.00
			Max	2.70	2.00	780.00	139.00	20.00	12.00	11.50	7.50
7	<i>H. platystegius</i>	84719	Mean	3.10	1.85	724.00	120.00	22.00	12.75	5.00	3.25
			Min	3.00	1.75	694.00	118.50	21.00	12.50	4.75	3.00
			Max	3.30	1.90	753.00	121.00	23.00	13.25	5.25	3.75
8	<i>H. sessilifolius</i>	35593	Mean	2.50	1.58	690.80	117.00	26.00	22.00	4.50	3.10
			Min	2.35	1.50	685.00	116.00	20.00	18.00	4.25	2.25
			Max	2.60	1.70	700.00	118.00	29.00	25.00	5.00	4.50
9	<i>H. yazdianus</i>	98646	Mean	2.35	1.25	780.00	70.00	12.00	8.25	7.00	7.25
			Min	2.32	1.22	760.00	68.00	11.00	8.00	6.50	6.25
			Max	2.37	1.27	800.00	71.00	12.50	8.50	7.50	8.50
10	<i>N. depauperata</i>	44723	Mean	1.77	1.00	475.00	91.00	8.25	4.50	6.25	5.75
			Min	1.75	0.80	470.00	88.00	7.75	4.25	6.00	5.25
			Max	1.80	1.20	480.00	93.00	9.00	4.75	6.50	6.75
11	<i>N. dschuparensis</i>	16289	Mean	1.77	.87	435.90	105.00	8.75	6.25	2.00	1.00
			Min	1.73	0.85	432.50	103.00	7.75	5.50	1.75	0.75
			Max	1.80	0.90	438.00	108.00	10.00	7.00	2.25	1.25
12	<i>N. laxiflora</i>	74565	Mean	2.47	1.10	666.70	85.00	14.30	8.10	6.80	5.60
			Min	2.40	1.00	665.00	80.00	11.00	6.50	5.30	5.00
			Max	2.56	1.20	669.00	89.00	16.00	9.75	8.50	6.00
13	<i>N. makuensis</i>	71140	Mean	1.95	1.12	476.20	85.00	26.00	22.00	8.00	2.00
			Min	1.85	1.10	465.00	81.00	24.00	21.00	7.50	1.70
			Max	2.10	1.16	483.00	90.00	27.00	23.00	8.50	2.20
14	<i>N. oxyodonta</i>	57425	Mean	2.50	1.15	616.70	102.00	15.00	11.50	8.50	3.75
			Min	2.45	1.10	612.00	101.00	14.50	10.00	7.00	3.50
			Max	2.55	1.20	622.00	103.00	15.30	13.00	10.00	3.90
15	<i>N. sessilifolia</i>	63945	Mean	2.35	1.65	766.70	105.00	25.60	19.00	1.25	0.00
			Min	2.30	1.64	762.00	102.00	24.00	18.00	1.00	0.00
			Max	2.38	1.66	770.00	107.00	27.00	20.00	1.50	0.00
16	<i>L. tschimganicus</i>	6426	Mean	2.47	1.27	415.40	98.00	16.25	13.50	8.00	4.75
			Min	2.25	1.25	411.00	95.00	15.75	13.00	7.75	4.50
			Max	2.70	1.30	420.00	100.00	17.00	14.25	8.25	5.00
17	<i>L. turcicus</i>	16959	Mean	2.35	1.20	402.00	113.00	27.00	15.60	15.00	9.00
			Min	2.31	1.18	401.00	111.00	26.00	15.30	14.75	8.75
			Max	2.39	1.21	403.00	116.00	28.00	16.00	15.25	9.50

Table 3. Qualitative characters state of nutlet and leaf in studied taxa. (Sc, Sculptured; Sm, Smooth)

No.	Taxa	Herbarium code	Nutlet			Leaf			Leaf trich.			
			Shape	Surface	Structure	Shap	Base	Apex	Indumentum density	Short multi-cell trich.	Long multi-cell trich.	Micro-papillate trich.
1	<i>H. bituminosus</i>	12854	Elliptic	Sc	Truncate tubercle	Ovate-cordate	Sub-cordate	Acute	Laxe	Present	Absent	Absent
2	<i>H. calycinus</i>	12551	Elliptic	Sc	Convex tubercle	Ovate-cordate	Sub-cordate	Obtuse	Laxe	Present	Absent	Absent
3	<i>H. elegans</i>	13303	Oblong	Sc	Undulate	Ovate-cordate	Sub-cordate	Obtuse	Sub-dense	Present	Ribbon like	Absent
4	<i>H. incanus</i>	3980	Elliptic	Sm	Flattened EPW	Oblong-ovate	Truncate	Obtuse	Very dense	Present	Frequent	Present
5	<i>H. longiflorus</i>	1277	Oblong	Sm	Prominent AW	Oblong-ovate	Truncate	Acute	Dense	Absent	Ribbon like	Absent
6	<i>H. oxyodontus</i>	3931	Elliptic	Sc	Truncate tubercle	Oblong-ovate	Sub-cordate	Acuminate	Laxe	Present	Absent	Absent
7	<i>H. Platystegius</i>	84719	Oblong	Sc	Minute tubercle	Oblong-ovate	Sub-cordate	Acute	Sub-dense	Present	Rare	Present
8	<i>H. sessilifolius</i>	35593	Elliptic	Sc	Verrucose	Ovate-cordate	Cordate	Acute	Laxe	Present	Rare	Absent
9	<i>H. yazdianus</i>	98646	Elliptic	Sc	Verrucose	Ovate-cordate	Sub-cordate	Obtuse	Dense	Absent	Ribbon like	Absent
10	<i>N. depauperate</i>	44723	Oblong	Sm	Convex EPW	Triangular	Sub-cordate	Acute	Dense	Present	Absent	Present
11	<i>N. dschuparensis</i>	16289	Oblong	Sm	Irregular texture	Triangular	Cordate	Acuminate	Dense	Absent	Frequent	Present
12	<i>N. laxiflora</i>	74565	Oblong	Sc	Verrucose	Oblong	Sub-cordate	Acute	Laxe	Present	Absent	Absent
13	<i>N. makuensis</i>	71140	Obovate	Sm	Depressed EPW	Ovate-cordate	Cordate	Obtuse	Sub-dense	Absent	Absent	Absent
14	<i>N. oxyodonta</i>	57425	Oblong	Sc	Verrucose	Ovate-cordate	Sub-cordate	Obtuse	Very dense	Present	Frequent	Absent
15	<i>N. sessilifolia</i>	63945	Late obovate	Sc	Verrucose	Ovate-cordate	Sub-cordate	Acute	Sub-dense	Absent	Absent	Absent
16	<i>L. tschimganicus</i>	6426	Obovate	Sm	Convex EPW	Ovate-cordate	Sub-cordate	Obtuse	Sub-dense	Present	Ribbon like	Absent
17	<i>L. turcicus</i>	16959	Obovate	Sm	Convex EPW	Ovate-cordate	Sub-cordate	Acute	Very dense	Absent	Absent	Absent

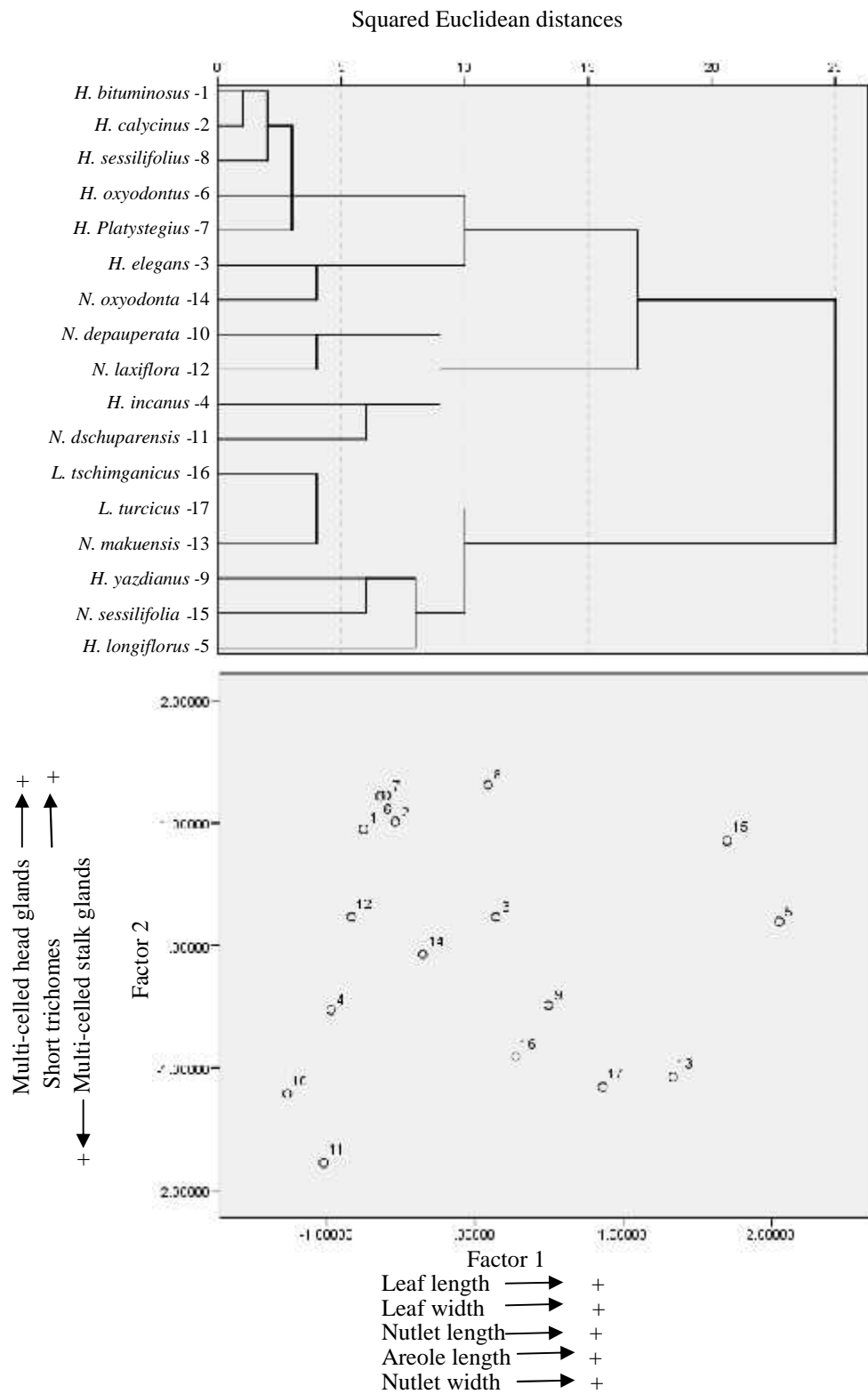


Fig. 5. Cluster analysis (WARD) and ordination of studied taxa. Species number as in tables. 2-3.

*L. turcicus* and *H. yazdianus* (Fig. 4E) and with striate stalk cells e.g. in *L. tschimganicus* and *N. makuensis* (Fig. 3E; 4F).

### Epicuticular waxes

Epicuticular waxes are structural elements of leaf surface and of fundamental functional and ecological importance (Barthlott & al. 1998). Different types of waxes have been described in plants. Crystalloids are the local wax projections and are of crystalline nature. Crystalloids may arrange in locally restricted patterns in contrast to their usual orientation patterns that cover the whole epidermal surface. Locally restricted patterns are connected to a certain epidermal structure i.e. around stomata and at the base of trichome (Barthlott & al. 1998). In a few species of *Nepeta* studies in this work, locally restricted orientation pattern of crystalloid type of waxes were observed. Crystalloids of plate type with irregular shapes (amoeba shape) and dentate margins were observed around trichomes and stomata on abaxial and adaxial leaf surfaces in *N. sessilifolia* and *N. makuensis* (Fig. 3F). These structures were not observed in any studied *Hymenocrater* species.

### DISCUSSION

Cluster analysis and ordination among species of *Hymenocrater*, *Nepeta* sect. *Psilonepeta* and *Lophanthus* support the affinities among these genera. Three main clusters are produced and every cluster is enclosed by species of two or three genera. The first cluster with two sub-clusters consists of six species of *Hymenocrater* and one species of *Nepeta*. In the first sub-cluster *H. bituminosus*, *H. calycinus*, *H. sessilifolius*, *H. oxyodontus* and *H. platystegius* are placed very closely to each other. *Hymenocrater elegans* and *N. oxyodonta* form the second sub-cluster. As illustrated in the results, the studied species show two types based on nutlet surface, smooth and sculptured. All species placed in first cluster have sculptured nutlets. There are clear differences among *H. bituminosus*, *H. calycinus*, *H. elegans*, *H. oxyodontus* and *H. platystegius* with sculptured nutlets and other species which have smooth nutlets. This characteristic is congruent with their life form, the sculptured nutlet type occurs in species that are strongly lignose at the base. *Nepeta depauperata*, *N. laxiflora*, *H. incanus* and *N. dschuparensis* form the second cluster from which *H. incanus* has absolutely smooth surface. In the third cluster *L. tschimganicus*, *L. turcicus* and *N. makuensis* are placed closely in one sub-cluster and *H. yazdianus*, *N. sessilifolia* and *H. longiflorus* form another sub-cluster. All species of third cluster have smooth nutlets except *H. yazdianus*

(98646, TARI) and *N. sessilifolia*. Budantsev & Lobova (1997) in their studies on tribe *Nepeteae*, demonstrated taxonomic importance of fruit morphology. They have considered the species of *Nepeta* sect. *Psilonepeta* in the genus *Lophanthus* based on their morphological similarities. In this study nutlet surfaces show a constant feature within most species of *Hymenocrater* but intraspecific variation were also found in nutlets of *H. elegans* and *H. yazdianus*. The examination of trichomes on leaf surfaces of studied taxa shows different features among species groups that are congruent with nutlet surface patterns. The presence of multi-celled head glands and the absence or rarely presence of long stalked capitate glands is the characteristic features in species of the first cluster. Non-glandular trichomes covered with micro-papillae are common in most species placed in second cluster. In the third cluster species with capitate glandular trichomes with a long ribbon-like stalk cell or multi-cellular stalk are grouped. We did not have any nutlet of *N. allotria* and *L. lipskyanus* available, so they have not been included in our data set and analysis. As illustrated in results, *N. allotria* has significant long stalked capitate glands, similar to *N. sessilifolia* and *L. turcicus*, while *L. lipskyanus* has multi-cellular non-glandular trichomes with long and thin-walled basal cell, similar to *H. longiflorus* and *H. yazdianus*. According to our results different patterns of trichome types have taxonomic value and the group of species in each cluster have similar trichome types, so it seems that *N. allotria* and *L. lipskyanus* may be close to species formed the third cluster.

Factor analysis revealed the most influential variable characters among studied species (Tab. 4). The first 3 factors comprise about 46% and the first 7 factors comprise about 88% of total variation. The leaf length, leaf width, nutlet length, areole length, nutlet width, multi-celled head glands, short trichomes, multi-celled stalk glands, nutlet shape and the nutlet surface, with the highest correlation are the most variable characters in grouping the taxa in ordination (Fig. 5). The nutlet length, nutlet width, leaf length, leaf width and the length of areole are the most important and diagnostic characters in defining the group comprising *H. longiflorus* and *N. sessilifolia* and the group comprising *N. dschuparensis*, *N. depauperata* and *H. incanus*, besides the other characters which may be common within other species group. The group comprising of *H. bituminosus*, *H. calycinus*, *H. sessilifolius*, *H. oxyodontus*, *H. platystegius*, *H. elegans*, *N. oxyodonta* and *N. laxiflora* share the diagnostic characters including multi-celled head glands, short trichomes and sculptured nutlet surface. The group comprising of *L. tschimganicus*, *L. turcicus*, *N. makuensis* and *H.*

Table 4. Factor analysis of nutlet and leaf characters in studied taxa.

Characters	Component						
	1	2	3	4	5	6	7
Leaf length	0.922	-0.189	0.202	-0.140	-0.048	0.059	-0.083
Leaf width	0.846	-0.177	0.378	-0.070	-0.108	-0.003	0.191
Nutlet length	0.755	0.271	-0.220	0.082	0.164	-0.115	-0.443
Areole length	0.678	-0.102	-0.163	0.582	-0.158	0.031	-0.189
Nutlet width	0.667	0.490	0.101	0.287	-0.104	0.216	-0.220
Areole lobes angle	-0.146	0.924	0.099	-0.047	0.046	0.111	0.011
Multi-celled head glands	-0.176	-0.863	-0.092	-0.340	0.067	0.090	0.092
Short trichomes	0.088	-0.674	0.467	-0.133	-0.285	-0.098	-0.032
Multi-celled stalk glands	-0.259	0.636	-0.611	-0.010	0.015	-0.053	0.324
Nutlet shape	-0.041	0.021	0.928	-0.081	-0.007	0.184	0.066
Leaf shape	-0.248	-0.238	-0.745	-0.140	-0.211	0.295	0.016
Wax	-0.112	0.171	-0.665	0.079	0.367	-0.123	-0.314
Nutlet surface	0.022	0.066	-0.058	0.954	-0.083	0.026	0.102
Nutlet structure	0.079	-0.297	-0.041	-0.900	0.100	-0.216	-0.042
Micro-papillae	0.404	-0.173	0.442	0.449	0.407	0.027	0.244
Stem leaf petiole	-0.174	0.020	-0.119	-0.012	0.930	0.031	-0.122
Basal leaf petiole	0.045	0.065	0.065	-0.216	0.923	-0.122	0.018
Long trichomes	-0.006	0.051	0.263	0.107	0.137	0.861	0.304
Long capitate	-0.186	0.357	-0.283	0.062	-0.269	0.724	-0.039
Leaf apex	-0.225	0.171	-0.058	-0.094	0.070	-0.641	0.283
Leaf base	-0.251	-0.060	0.140	0.059	-0.082	-0.081	0.863
Indumentum density	-0.206	-0.330	0.076	-0.388	0.018	-0.443	-0.586

*yazdianus* share the multi-celled stalk glands as a significant character. The results of cluster analysis and ordination show almost the same species group (Fig. 5).

The morphological features of some genera in *Nepetinae* including *Lophanthus*, *Nepeta*, *Hymenocrater* and *Marmoritis* are very similar. We did not have access to specimens of the genus *Marmoritis* for our morphological studies but in our results the distribution of *Hymenocrater* species within all clusters together with species of *Nepeta* sect. *Psilonepeta* and *Lophanthus*, may be inferred as their close relationships and the possibility of inclusion of them in one genus. These results support previous idea about affinities between *Lophanthus* and *Nepeta* section *Psilonepeta* (Levin 1941 and Budantsev 1992) bringing the idea of the possible inclusion of some of these genera considering the nomenclatural rules. The phylogenetic relationships of the above mentioned genera have been elucidated by Drew & Systma (2012), but they examined one species from each genus, so in their result, the real relationship among the species of these four genera could not be inferred properly.

Although our results enhance the taxonomic significance of morphological characters, a comprehensive phylogenetic study is needed for defining systematic relationships in this group. We hope that it could be achieved by means of molecular study which will be done in continue of this project.

## ACKNOWLEDGEMENTS

We wish to thank the authorities of Research Institute of Forests and Rangelands for the permission of using the herbarium specimens of TARI. We are also thankful to the authorities of the IRAN herbarium for the permission of examining some type specimens. We acknowledge the Islamic Azad University, science and research Branch (Tehran) and Razi Metallurgical Research Center (Tehran) for their cooperatin in this project.

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