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Micromorphological comparison of *Nepeta viscida*, *N. nuda* subsp. *nuda* and their putative hybrids *N.* × *tmolea*

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Abstract

In this study, two Nepeta species, Nepeta viscida, N. nuda subsp. nuda, and their putative hybrids N. × tmolea were investigated for their trichome structure and density. For this purpose, fresh or herbarium specimens were used. SEM photographs were taken with the standard techniques in Balıkesir University. While N. × tmolea individuals are similar to N. nuda subsp. nuda in some respects (for example, absence long glandular trichomes on the stem, the absence of glandular hairs on the corolla), it shows some transition between N. viscida and N. nuda subp. nuda in some respects (for example, while the corolla of N. viscida does not contain long glandular hair, presence in a high density in N. nuda subsp. nuda and low density in N. × tmolea individuals).

Keywords: *Nepeta viscida, Nepeta nuda, N. × tmolea, Trichome, Turkey.*

Nepeta viscida, *N. nuda* subsp. *nuda* ve muhtemel hibritleri *N.* × *tmolea*'nın mikromorfolojik olarak karşılaştırılması

Özet

Bu çalışmada, iki Nepeta türü, Nepeta viscida, N. nuda subsp. nuda ve bunların muhtemel hibritleri N. × tmolea, tüy yapıları ve yoğunlukları açısından incelenmiştir. Bu amaçla taze veya herbaryum örnekleri kullanılmıştır. Tüy tipleri ve dağılışları istatistiki olarak değerlendirilmiştir. N. × tmolea bireyleri bazı açıdan N. nuda subsp. nuda'ya benzerlik gösterirken (Örneğin; gövdede uzun salgı tüyü olmaması, korollada sapsız salgı tüylerinin (peltat) bulunması), bazı açıdan ise N. viscida ile N. nuda subp. nuda arasında geçiş özelliği göstermektedir (Örneğin; N. viscida korollası uzun salgı

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tüyü içermezken, N. nuda subsp. nuda türünde yoğun şekilde bulunması ve N. × tmolea bireylerinde düşük yoğunlukta olması).

Anahtar kelimeler: *Nepeta viscida, Nepeta nuda, N. × tmolea, Tüy, Türkiye.*

1. Introduction

Lamiaceae has significant medicinal and aromatic plants for the pharmaceutical industry. The genus *Nepeta* consists of approximately 250 species all over the World in the various climatic-geographical zones: Europe, Western Siberia, Far East, and North Asia. Also, some species of this genus are cultivated in Western Europe, America, CIS countries as an aromatic plant [1-3]. It is represented in Turkey by 44 species, 22 of them endemic [4-6].

Nepeta nuda L. is one of the most well-known and widespread species of the genus *Nepeta* in Turkey. *N. nuda* is divided into four distinct subspecies mainly differing in their morphology and geographic distribution: subsp. *nuda*, subsp. *albiflora* (Boiss.) Gams, subsp. *glandulifera* Hub.-Mor. & P.H.Davis, and subsp. *lydiae* P.H.Davis. According to the Flora of Turkey, *N. nuda* subsp. *nuda*, which was studied in this paper, is distinguished from the other subspecies with its violet-blue calyx and corolla. However, according to [6], some specimens of subsp. *nuda* had some variation especially regarding calyx and corolla color. Also, *N. nuda* subsp. *nuda* is a cosmopolitan subspecies of *N. nuda* in Turkey [4].

N. viscida Boiss. belongs to Group A just like *N. nuda* subsp. *nuda* [4, 6]. *N. viscida* has very characteristic and viscous glandular indumentum that allows to stick leaves and stems each other when it was gripped. According to Flora of Turkey, its stem is villous with non-glandular and glandular trichomes, its leaves and calyces are glandular-villous with many sessile glands. There is no information about corolla indumentum. Also, it is mentioned that this species hybridizes with *N. nuda*, in describing *N. × tmolea* Boiss. which is an endemic taxon for Turkey[4].

According to previous studies, the type and density of trichomes vary in different taxa, and the trichomes have significant taxonomic values and could be considered for identification of species [7]. On the other hand, trichomes type and density may differ on organs of the same plant [8-15].

Essentially all Lamiaceae species bear glandular trichomes on their surfaces. These trichomes have many important roles in plants [16, 17]. According to a hypothesis, glandular hairs produce active agents, which are related to photoperiodic induction of flowering, and are involved in the synthesis of steroid hormones and gibberellins [18, 19]. The essential oil produced by glandular trichomes may act to protect the aerial parts of the plant against herbivores and pathogens [20], and the biological activity of the secondary metabolites in the secreted products is of interest to the pesticide, pharmaceutical, flavouring, and fragrance industries [20-22]. Moreover, several studies [21, 23] have shown that these hairs are also connected to pollinator animals' attraction or accumulation of salt. Also, non-glandular hairs are also thought to decrease the heat load of plants, maximize freezing tolerance, participate in seed dispersal, hold the

balance of water in plant leaves, deflect intense radiation of the sun, and offer protection from herbivores [24].

This research aimed to study the trichomes of *Nepeta viscida*, *N. nuda* subsp. *nuda* and their hybrids to determine whether there are any differences in trichome types.

2. Material and methods

2.1. Plant materials

Samples were picked during the flowering stage (2016-2018) from natural habitats in Balıkesir (Dursunbey-Çamlık), İzmir (Ödemiş-Bozdağ) and Muğla (Fethiye-Babadağ). *N.* × *tmolea* and *N. nuda* subsp. *nuda* were collected from Çamlık and Bozdağ. Also, *N. viscida* was collected from all the given locations above. Voucher specimens are deposited in the Herbarium of Necatibey Education Faculty of Balıkesir University in Balıkeir, Turkey.

2.2. Scanning electron microscopy (SEM)

In this study, scanning electron microscopy (SEM) was used to determine the morphology and distribution of the glandular and non-glandular trichomes of *Nepeta viscida*, *N. nuda* subsp. *nuda* and their putative hybrids *N.* × *tmolea*. Stems, leaves, calyces, corollas, and nutlets were investigated and photographed using a NeoScope JCM. SEM studies took place in the Basic Sciences Research and Applied Center of Balıkesir University.

2.3. Statistical analyses

In order to simplify the data and make it easier to visualize, we used PCA analysis. We created 36 characters using the density of trichomes and presence/absence of trichomes types. All analyses were carried out with PAleontoSTatistics (PAST) [25]. Data were standardized (absence=0, presence=1) for analyses.

3. Results

Investigations into the trichome features (stems, leaves, calyx, and corolla) of *Nepeta viscida*, *N. nuda* subsp. *nuda* and *N.* × *tmolea* were carried out using SEM analysis. Diacytic type stomata are present on the epidermis of the stem, on both surfaces leaf (amphistomatic leaves) and calyx. All the specimens have characteristics indumentum with sessile glands, glandular and non-glandular trichomes. We found these types of glandular trichomes on the three species: Capitate glandular trichomes were of two types: made up of a short stalk cell with the head (A1) and made up of a long stalk with several cells with the head (A2). Sessile glandular trichomes (B) and capitate trichomes. The non-glandular trichomes were of two types: Fingerlike trichome, 1-3 celled with the verrucose surface (C1), and simple multicellular trichome, long, uniseriate, hooked, straight or cranked with pointed terminal cell and verrucose surface (C2) (Table 1).

N. viscida has some characteristics differing it from *N. nuda* subsp. *nuda* and *N.* × *tmolea* (Figure 1). These followings are some observed features of *N. viscida*: stems villous with rarely non-glandular and intensely glandular trichomes. Glandular ones

consist of short and long capitate trichomes, and glandular trichomes are more intense than non-glandular ones (Table 1, Figure 3A, D); leaves amphistomatic, glandular-villous and with many sessile glands (peltate) and capitate glandular trichomes are on both sides of the leaves, indumentum of the abaxial side is denser than the adaxial side, and short glandular trichomes are denser on the abaxial side, non-glandular trichomes are very rare on both sides (Figure 4A, D), it apparently gives a viscous feeling when touched (Figure 2C, F, I); calyx glandular-villous; short glandular trichomes are denser than long ones (Figure 5A); corolla pale violet to white, indumentum of the corolla lips are denser than the corolla tube, the most frequent one is A2 type of glandular trichomes (Table 1; Figure 5D); nutlets are glabrous and has verrucate structure, 2.0-2.2 × 0.9-1.1 (Table 1; Figure 6A, D, G).

Table 1. Detailed trichome types and intensity of *N. viscida*, *N.* × *tmolea* and *Nepeta nuda* subsp. *nuda*

Taxa	**Type**	**Stem**	**Leaves**		**Calyx**	**Corolla**	**Nutlet**
			Adaxial	**Abaxial**			
Nepeta viscida	A1	++	+	+++	+++	+	2.0-2.2 × 0.9-1.1 glabrous Type III
	A2	++	++	++	++	++	
	B	+	+	+	-	-	
	C1	very rare	very rare		-	-	
	C2	++	very rare		-	-	
Nepeta × *tmolea*	A1	+	-	-	+	+	1.6 × 1.0 glabrous Type III
	A2	-	-	-	-	+	
	B	+	+	+	++	++	
	C1	++	+	+	+	++	
	C2	+++	++	++	+++	+	
Nepeta nuda subsp. *nuda*	A1	+	-	-	+	+	1.9 × 1.2 glabrous Type III
	A2	-	-	-	+	-	
	B	++	+	++	+	++	
	C1	+	+	+	+	++	
	C2	++	+	+	++	+	

A. Capitate trichomes. A1: head unicellular, stalk unicellular; A2: head unicellular, stalk bicellular or multicellular;
B. Peltate trichomes: short stalk cell and four secretory cells;
C. Non-glandular trichomes. C1: short; C2: long;
Symbols: (−) absence of hairs, (+) few hairs, (++, +++) increasing the presence of hairs.

N. nuda subsp. *nuda* has a pilose stem. Any A2 type capitate trichomes did not be observed on the stem. On the other hand, short capitate trichomes are lower than the other trichomes. Because of the lackness of long capitate trichomes, there were no viscoid structure (Table 1; Figure 2G; Figure 3C, F) B type peltate glandular and C2 type non-glandular trichomes are the most intense ones; leaves amphistomatic, sparsely pilose with many sessile glands; non-glandular, peltate and capitate glandular trichomes are on both sides of the leaves, indumentum of the abaxial side is denser than the adaxial side, and peltate trichomes are denser on the abaxial side (Table 1; Figure 4C, F); calyx sparsely villous, indumentum consists of A1, B, C1 and C2 trichomes, C2 trichomes are denser than C1 type, and B trichomes are the lowest ratio, C1 ve C2 trichomes are obviously papillate (Figure 5C); corolla white, indumentum of the corolla lips are longer than the tube, A1 type glandular trichomes have the minimum density, B type peltate trichomes as almost dense as C2 type non-glandular trichomes, C2 type

trichomes are denser on the lips and C1 type trichomes are denser on the tube, (Figure 5F); nutlets are glabrous and has verrucate structure, 1.7-1.9 × 1.0-1.2 (Table 1; Figure 6C, F, I).

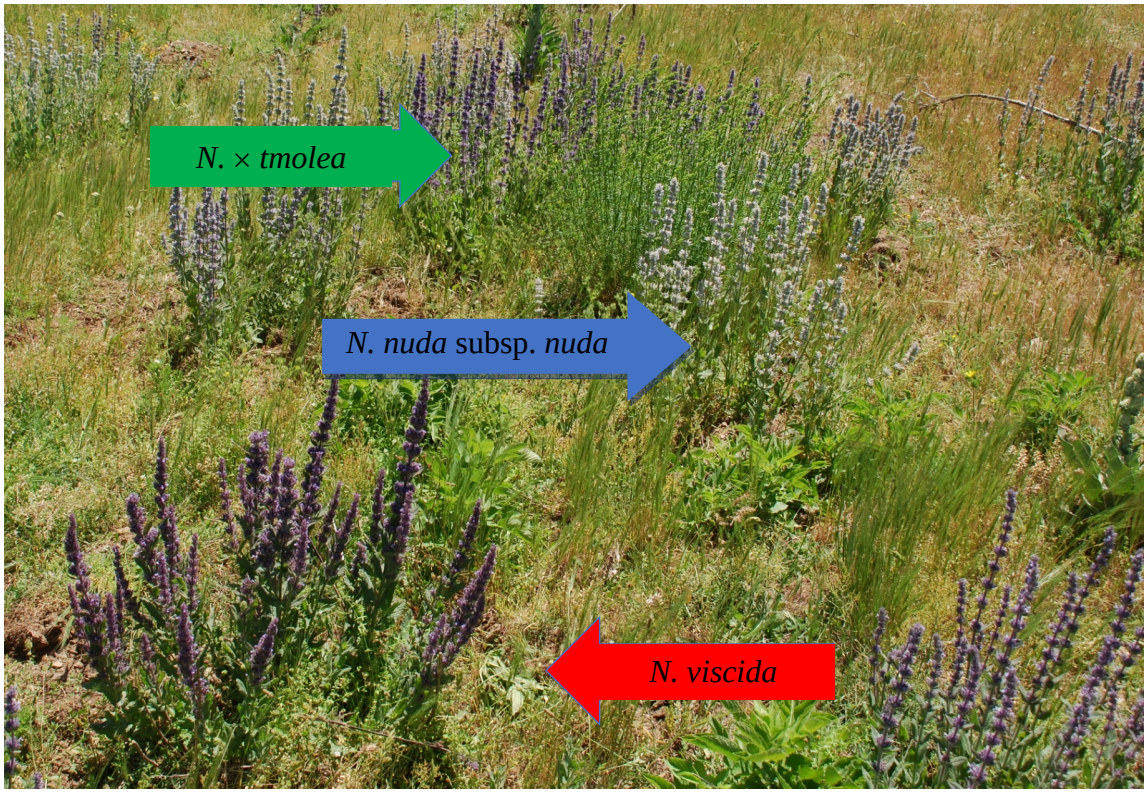

Figure 1. Mixed populations of *N. viscida*, *N.* × *tmolea* and *N. nuda* subsp. *nuda* (Dursunbey)

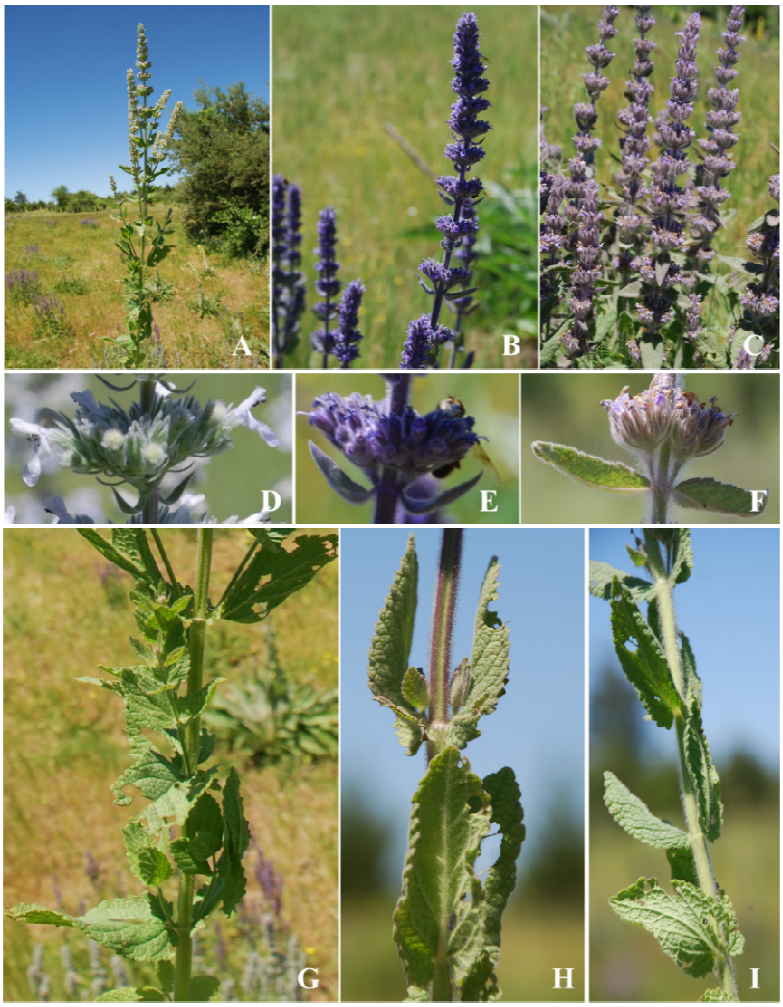

Figure 2. Morphological comparison of the general habitus: *N. nuda* subsp. *nuda* (A, D, G), *N.* × *tmolea* (B, E, H), *N. viscida* (C, F, I)

N. × *tmolea* has a sparsely villous stem. A1 and B type glandular trichomes were observed on the stem, the stem has not a viscoid structure, C2 type non-glandular trichomes are the most intense trichomes (Table 1; Figure 2H; Figure 3B, E); leaves amphistomatic, sparsely hairy with rare peltate glandular; non-glandular and peltate glandular trichomes are on both sides of the leaves, C2 type non-glandular trichomes are the most intense ones (Table 1; Figure 4B, E); calyx non-glandular villous with sparse B type trichomes; C2 trichomes are denser than B type (Figure 5B); corolla bluish, corolla bears A1 and B type glandular, and C1 and C2 type non-glandular trichomes, indumentum of the corolla lips are longer than the tube, C2 type trichomes are denser on the lips, and C1 type trichomes are denser on the tube, density of B type trichomes almost equals A1 type trichomes (Figure 5E); nutlets are glabrous and has verrucate structure, 1.7-1.9 × 1.0-1.2 (Table 1; Figure 6B, E, H).

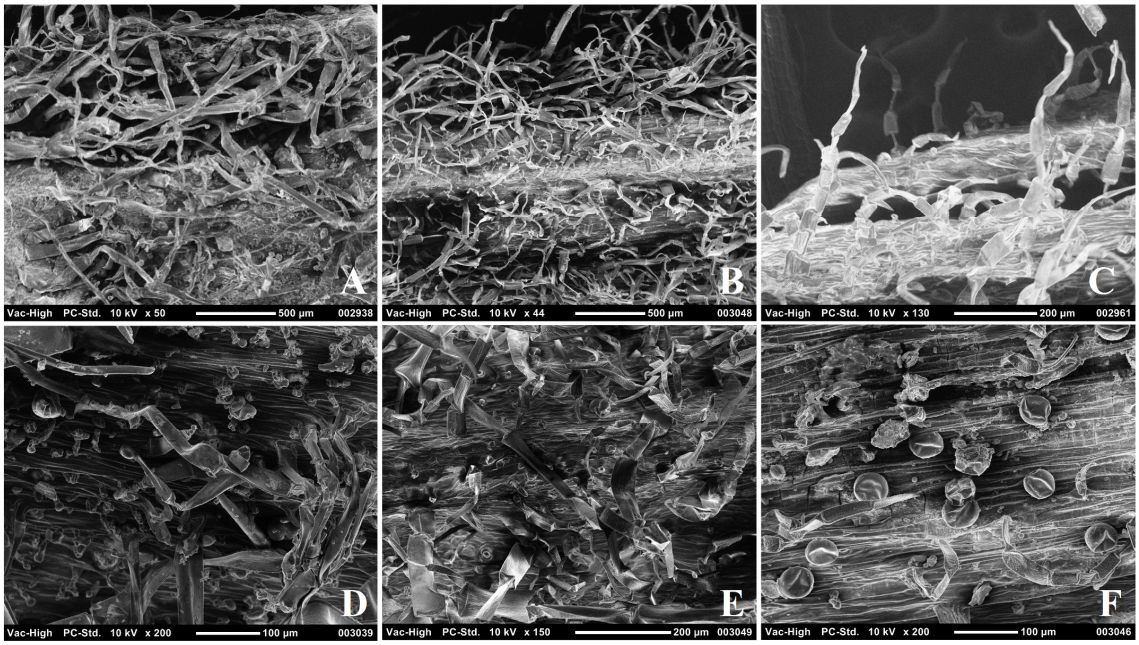

Figure 3. Comparison of the stem indumentum: *N. viscida* (A, D), *N.* × *tmolea* (B, E), *N. nuda* subsp. *nuda* (C, F)

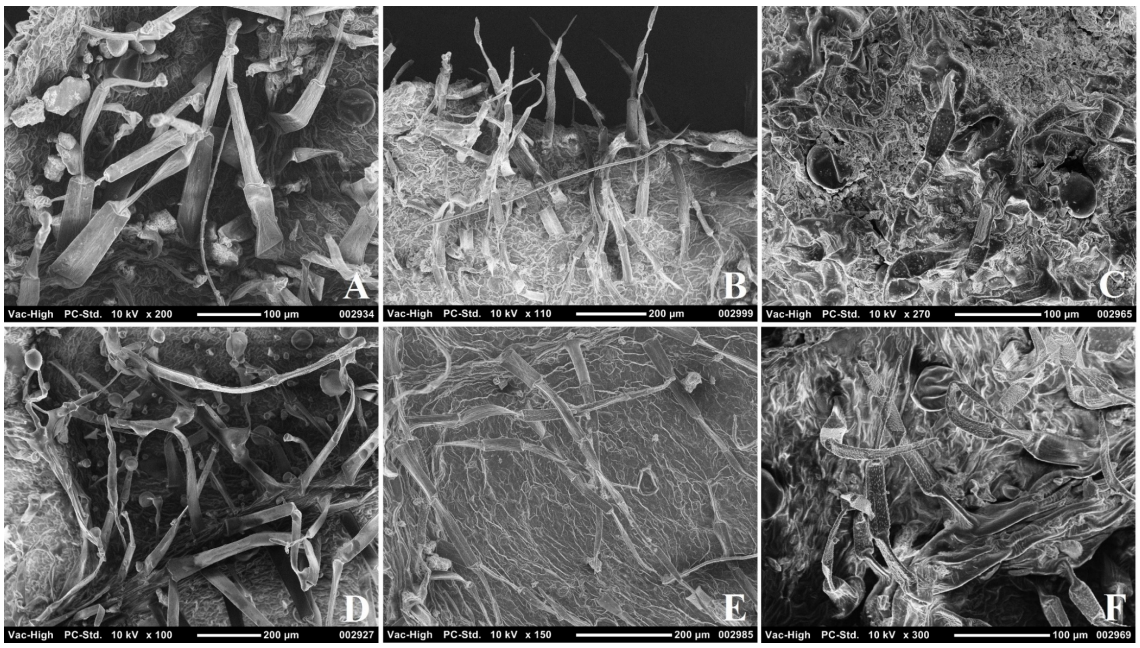

Figure 4. Comparison of the leaf indumentum of adaxial (A-C) and abaxial sides (D-F): *N. viscida* (A, D), *N.* × *tmolea* (B, E), *N. nuda* subsp. *nuda* (C, F)

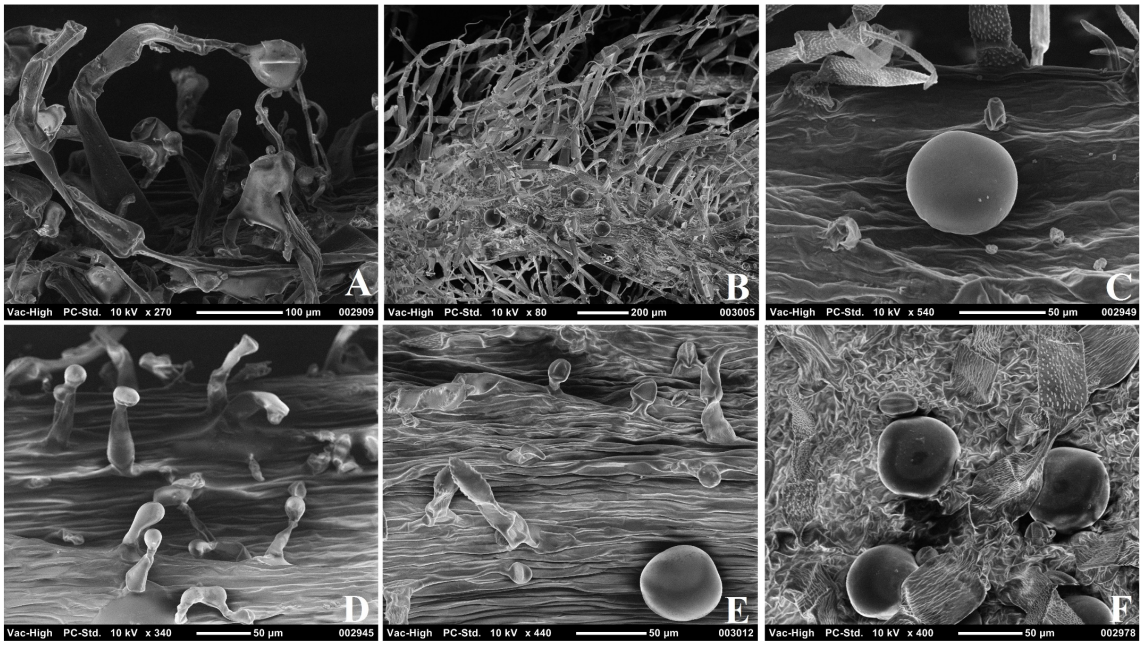

Figure 5. Comparison of the calyx (A-C) and corolla (D-F) indumenta: *N. viscida* (A, D), *N.* × *tmolea* (B, E), *N. nuda* subsp. *nuda* (C, F)

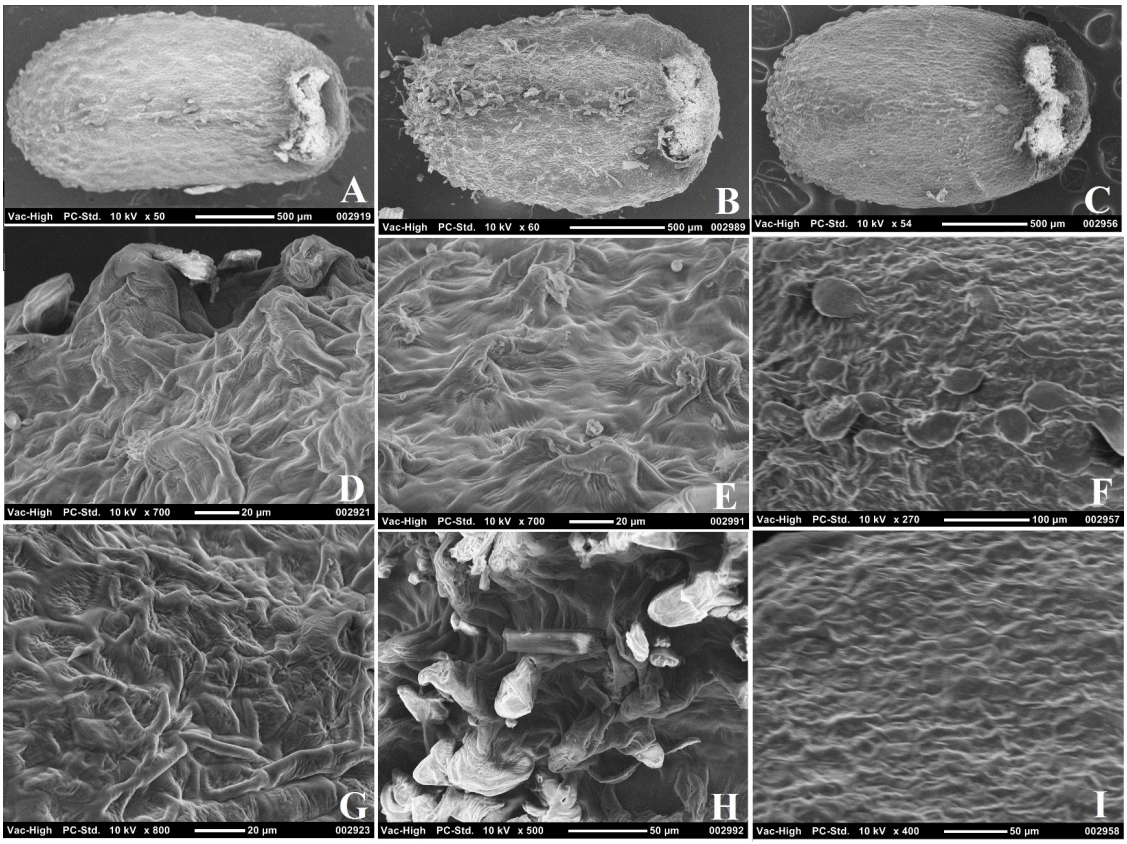

Figure 6. Comparison of the nutlet structure: *N. viscida* (A, D, G), *N.* × *tmolea* (B, E, H), *N. nuda* subsp. *nuda* (C, F, I)

According to our micromorphological statistical analysis, *N.* × *tmolea* is entirely different from its putative parents, and it is more similar to *N. nuda* subsp. *nuda* than *N. viscida* (Figure 7).

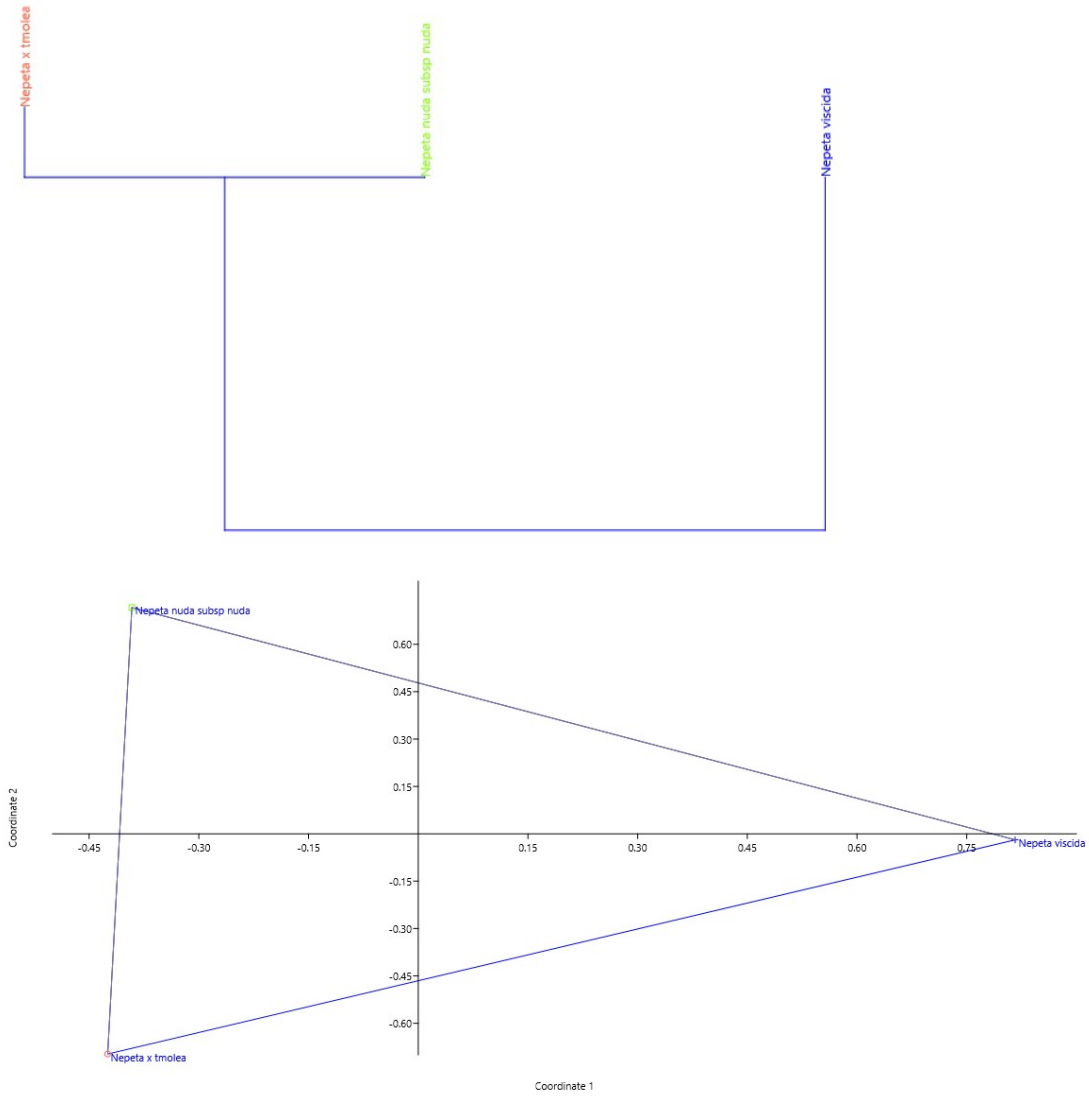

Figure 7. Neighbour-Joining Cluster Graphic (above side) and PCoA graphic (below side) of three specimens

4. Discussion

In the field trips during this study, we found some *N. nuda* subsp. *nuda* and *N. viscida* individuals that reflect their typical characters. However, some individuals had some intermediate morphological characters. These specimens looked like *N. viscida* as general, but they were not viscid, and their stem, leaf and corolla colors were quite different from *N. nuda* subsp. *nuda* and *N. viscida*. So, we detected these specimens as *N.* × *tmolea*. These *N.* × *tmolea* hybrid individuals were more similar to *N. viscida* regarding general habitus, calyx and leaf characters, on the other hand, the same

samples were more similar to *N. nuda* subsp. *nuda* regarding bluish color on the verticillasters and having no sticky glandular trichomes (Figure 1 and 2).

According to previous studies, glandular and non-glandular trichomes have some special roles in plant living and their interactions with biotic and abiotic factors. Trichome types and their density vary among the different populations due to climate, altitude, etc. change, and trichome variety can help plants to better adapt to various environmental conditions. Some valuable investigations about the interspecific variations in trichomes type and densities were seen in the genus *Nepeta*. These following studies were some of them: *N. cataria* [26], *N. heliotropifolia* [27], *N. nuda* [28], *N. congesta* var. *congesta* [29], *N. cataria, N. nuda* subsp. *nuda*, *N. parviflora*, *N. ucranica* subsp. *ucranica* [30], and nutlets of some *Nepeta* species [31].

While the stem of *N. viscida* bears long capitate glandular trichomes (A2), *N.* × *tmolea* and *N. nuda* subsp. *nuda* have not A2 type trichomes on their stem. On the other hand, A1 type glandular trichomes were sparsely seen on *N.* × *tmolea* and *N. nuda* subsp. *nuda* stems. Because of this, *N. viscida* has a viscoid structure, but the others are not. While *N. viscida* rarely has B type glands on its stem, *N. nuda* subsp. *nuda* has denser than *N.* × *tmolea*. All three species bear C1 type non-glandular trichomes. C2 type non-glandular trichomes are the most intense on the stems of *N.* × *tmolea* (Figure 3). N. viscida, *N. nuda* subsp. *nuda* and *N.* × *tmolea* bear stomata on both surfaces of the leaves. Generally, stomata occur in a higher number on the abaxial leaf surface than on the adaxial one. Leaves of these species also bear numerous epidermal non-glandular and glandular trichomes. Non-glandular ones are mostly 4-7-celled multicellular trichomes with the apical cell acute. Non-glandular trichomes are almost equal on the adaxial and abaxial leaf surfaces of *N. nuda* subsp. *nuda* and *N.* × *tmolea*. On the other hand, *N. viscida* has intensive glandular trichomes on both surfaces, and they are much denser on the abaxial leaf surface than the adaxial side. Peltate trichomes are present on the leaf surfaces of three species, but capitate trichomes cannot be observed on *N. nuda* subsp. *nuda* and *N.* × *tmolea*. Peltate trichomes are numerous on both leaf surfaces of *N. viscida* (Figure 4). *N. viscida* has glandular-villous calyces and *N.* × *tmolea* has non-glandular-villous calyces. Nevertheless, *N. viscida* has not non-glandular and B type peltate trichomes and *N.* × *tmolea* has not A2 type long capitate trichomes on their calyces, calyces of *N. nuda* subsp. *nuda* have all trichome types mentioned in this study (Figure 5). Corolla indumenta of *N.* × *tmolea* and *N. nuda* subsp. *nuda* is quite different from *N. viscida*. *N. viscida* has densely A2 and sparsely A1 type glandular trichomes, but B type glandular and C1, C2 type non-glandular trichomes were not seen on the corollas of *N. viscida*. On the other hand, *N.* × *tmolea* and *N. nuda* subsp. *nuda* densely have B and C1 types trichomes, and *N. nuda* subsp. *nuda* has not any A2 type trichomes on their corollas (Figure 5). All the nutlets of three species are glabrous and have a verrucate structure on their surfaces (Figure 6).

Although micromorphological characters are important and distinctive among species, there are no significant intermediate characters to distinguish *N.* × *tmolea* as a hybrid from its putative parents. According to some hybrid studies [32-38], especially morphological and molecular give some valuable intermediate information about the hybrid nature of some species. For example, some hybrids have polymorphic loci in their DNA sequences, and some have intermediate shapes and sizes on their leaves, calyces, corollas or stems. On the other hand, our micromorphological results

distinguished the three species, but we could not observe intermediate trichome size or shape on these species.

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