Fruit morphological and micro-morphological study of the *Anchusa* species (*Boraginaceae*) in Iran

Fatemeh Nasrollahi[1], Maryam Keshavarzi[1] & Masoud Sheidai[2]

[1] Biology Dept., Faculty of Science, Alzahra University, Vanak, Tehran, Iran, e-mail: neshat112000@yahoo.com (corresponding author)

[2] Faculty of Biological Sciences, Shahid Beheshti University, Evin, Tehran, Iran.

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Abstract. With its four species, *Anchusa* is one of the weedy genera of *Boraginaceae* in Iran. Its species are distributed in different habitats. In this study, the fruit morphological and micro-morphological features of the *Anchusa* taxa have been considered, so as to evaluate their diagnostic value. Eight qualitative and quantitative fruit features from 12 accessions of *A. italica, A. strigosa, A. arvensis* and *A. aegyptica* have been studied by stereomicroscope and SEM. Statistical results have shown that this set of characters provides an efficient identification key. The most important factors comprise such characters as nutlet length and width, nutlet length-to-width ratio, nutlet shape and position (eliosome condition) which exercise a major effect on the observed variations. This is the first study of the *Anchusa* species of Iran.

Keywords: *Anchusa*, cluster analysis, Iran, nutlet micro-morphology

Inroduction

Anchusa L. (*Boraginaceae*) is a large genus with about 170 annual and perennial species in the temperate and subtemperate zone of the Old World. The Balkan Peninsula is the main center of diversification of this genus (Selvi & Bigazzi 2003). The species are distributed in Europe, North and South Africa and West Asia (Akçin & al. 2010).

The plants of *Anchusa* species are covered with robust hairs and have ornamental, medicinal and edible uses in different parts of Europe and Asia (Akçin & al. 2010). In Iran these species are used as a substitution for the famous medicinal Borago plant.

Many micro features of the mericarps (Akçin & Ulu 2008; Bigazzi & Selvi 2000; and Selvi & Bigazzi 2003) and leaves (Selvi & Bigazzi 2001) have been studied in the *Anchusa* taxa across the world and their diagnostic importance have been proved. The morphological and anatomical studies of this genus are not so extensive (Bigazzi & Selvi 2000; Selvi & Bigazzi 2000) and have mainly focused on the leaves features (Nyauwame & Gill 1990; Selvi & Bigazzi 2001).

Inter-specific and intra-specific variations of the species have been studied by different authors (Selvi & Bigazzi 1998; Akçin & al. 2010), but there has been no such study in Iran. In this study, *Anchusa* accessions are gathered from different parts of Iran to study the species relationship by means of the fruit micro-morphological features. A tetraploid species, such as *Anchusa italica* Retz., has a vast distribution pattern in Iran and shows some polymorphisms. The fruit surface features are of taxonomic importance in this family but in many taxonomic revisions they have been ignored or not mentioned as key features. Considering the fact that these characters are constant, the mericarp of the *Anchusa* taxa in Iran has been studied in the present study .

Material and methods

In the present study, 12 populations (5 to 10 individuals per each accession) from four *Anchusa* species were collected from different parts of Iran and studied. Fresh materials of the *Anchusa* species and herbarium specimen

were used (Table 1). Voucher specimens were deposited in the Herbarium of Alzahra University (AUH). Measured and evaluated features were extracted from literature (Riedl 1967; Chamberlain 1979; Chater 1972) and the authors' filed observations. A total of eight qualitative and quantitative features were adopted for fruit comparisons (Table 2).

Table 1. Collection data for populations used in this study.

Species	Origin	Voucher No	Collector
A. italica var.*italica*	Tehran, Vanak village	AUH 13910	Nasrollahi
A. italica var. *italica*	Tehran, Sadat Abad	AUH 13913	Nasrollahi
A. italica var. *italica*	Tehran, Evin, Darake	AUH 139050	Ganjivahed
A. italica var. *kurdica*	Kurdistan, Sanandaj to Saghez	AUH 139051	Abassian
A. italica var. *kurdica*	Kurdistan, Sanandaj, Mt Abidar	AUH 13902	Nasrollahi
A. arvensis subsp. *orientalis*	Alborz, SW Karaj, Mardabad	AUH 13671	Soudi
A. arvensis subsp. *orientalis*	Tehran, West of Tehran	AUH 13681	Labadi
A. arvensis subsp. *orientalis*	Tehran, Vanak	AUH 13672	Khalili
A. strigosa	Alborz, Mt Baghestan	AUH 13903	Mosaferi
A. strigosa	Alborz, Rejaii Shahr, Baghestan	AUH 13904	Mosaferi
A. strigosa	Quazvin, Mohamadiyeh 15 km to Quazvin	AUH 13905	Nasrollahi
A. aegyptiaca	Bushehr, Genaveh Port	AUH 13901	Nasrollahi

Table 2. Studied fruit features in the *Anchusa* species of Iran.

Characters	State of character
Nutlet length	–
Nutlet width	–
Nutlet length-to-width ratio	–
Angle of nutlet apex	–
Nutlet shape	Erect (1), curved ovate (2), suberect ovate (3)
Nutlet position	Erect (1), curved (2)
Nutlet color	Green (1), pale-green to yellow (2), green to brown (3), brown (4)
Eliosome condition	Exerted (1), not exerted (2)

Mature nutlets were studied by digital stereo microscope (Dino-Lite, AM413T) and SEM without any treatment. Coating with gold by means of the physical vapor deposition method (PVD) was restricted to 100 A (Hacking & al. 2007). The SEM examination was carried out on a Philips XL30 microscope. The measurements were based on 10–20 readings for each specimen (five samples for each SEM observation were studied). For nutlet sculptures, the terminology of Buchner & Weber (2000) was followed.

In order to detect significant differences in the studied characters among the various studied species, an analysis of variance (ANOVA) was performed. To reveal the species relationships, we have used cluster analysis and principal component analysis (PCA) (Ingrouille 1986). For multivariate analysis, the mean of the quantitative characters was used, while qualitative characters were coded as binary/multi-state characters. Standardized variables were used for multivariate statistical analysis. The average taxonomic distances and squared Euclidean distances were applied as dissimilarity coefficient in the cluster analysis of morphological data. In order to determine the most variable micromorphological characters among the studied species, factor analysis based on principal components analysis was performed by using SPSS ver. 19 (2010).

Results

To evaluate the species relationships, the methods of numerical taxonomy were used. Cluster analysis based on morphological features has revealed relationships between the accessions. A dendrogram plotted by the WARD method has shown two main clusters (Fig. 1). The first cluster comprises two subclusters containing populations of *A. strigosa* Labill. and both varieties of *A. italica*. The main second cluster comprises two subsets: the first with populations of *A. arvensis* (L.) Nordh. and the second with populations of *A. aegyptiaca*. These two are related at Level 8.

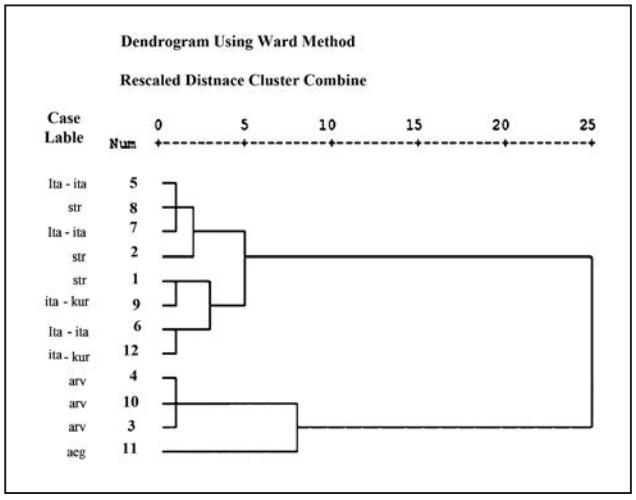

Fig. 1. A WARD dendrogram based on the nutlet morphological traits of four *Anchusa* species. **Species label: Ita-ita** = *A. italica* var. *italica*; **str** = *A. strigosa*; **ita-kur** = *A. italica* var. *kurdica*; **arv** = *A. arvensis* subsp. *orientalis*; **aeg** = *A. aegyptiaca*.

In order to determine the most variable characters among the studied species, a factor analysis based on PCA was performed, revealing that the first two factors comprise over 81 % of the total variation. In the first factor with about 61 % of the total variation features, such characters as nutlet length and width, length-to-width ratio, nutlet shape, erectness and color have shown more correlation. In the second factor with about 20 % of the total variation, the eliosome condition showed more correlation (Table 3).

Table 3. Results of the factor analysis based on the fruit morphological characteristics of the *Anchusa* populations in Iran.

Characters	1st factor	2nd factor
Nutlet length	0/96	–
Nutlet position	0/89	–
Nutlet color	0/88	–
Nutlet length-to-width ratio	0/82	–
Nutlet shape	0/77	–
Nutlet width	0/73	–
Eliosome condition	–	0/90
Angle of nutlet apex	–	–

A PCA graph based on two main factors (Fig. 2) reveals the relations between studied species and is in concordance with the cluster analysis.

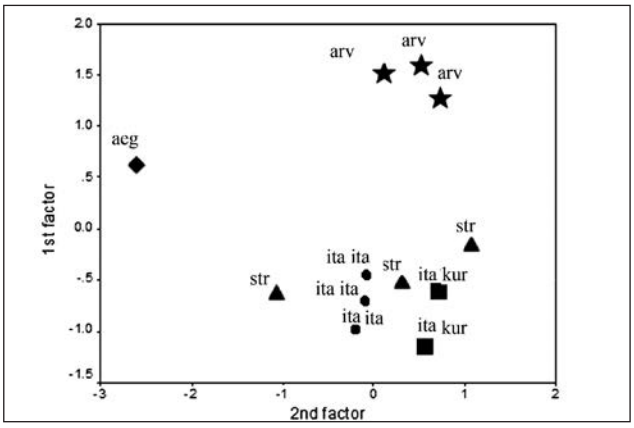

Fig. 2. PCA ordination of the *Anchusa* species based on the fruit morphological characteristics. **Species label: ita ita** = *A. italica* var. *italica*; **str** = *A. strigosa*; **ita kur** = *A. italica* var. *kurdica*; **arv** = *A. arvensis* subsp. *orientalis*; **aeg** = *A. aegyptiaca*.

Table 4. Diagnostic features of a SEM micrograph of the nutlet.

Species	*A. italica* var. *italica*	*A. italica* var. *kurdica*	*A. strigosa*	*A. arvensis* subsp. *orientalis*	*A. aegyptiaca*
Diagnostic features	spine like	spine like	spine like	tuberculate with wrinkled substrate	merely tuberculate

In order to find further diagnostic features of the nutlet, a SEM micrograph of the nutlet was studied. All samples were wrinkled, both varieties of *A. italica* (*kurdica* and *italica*) had spine-like ornamentations. *A. italica* var. *kurdica* has shown more pointed spines. In *A. strigosa,* the mericarp surface was smooth and flat, with identical spines. In *A. aegyptiaca,* the mericarp surface was tuberculate with a wrinkled substrate, while in *A. arvensis* the surface was merely tuberculate.

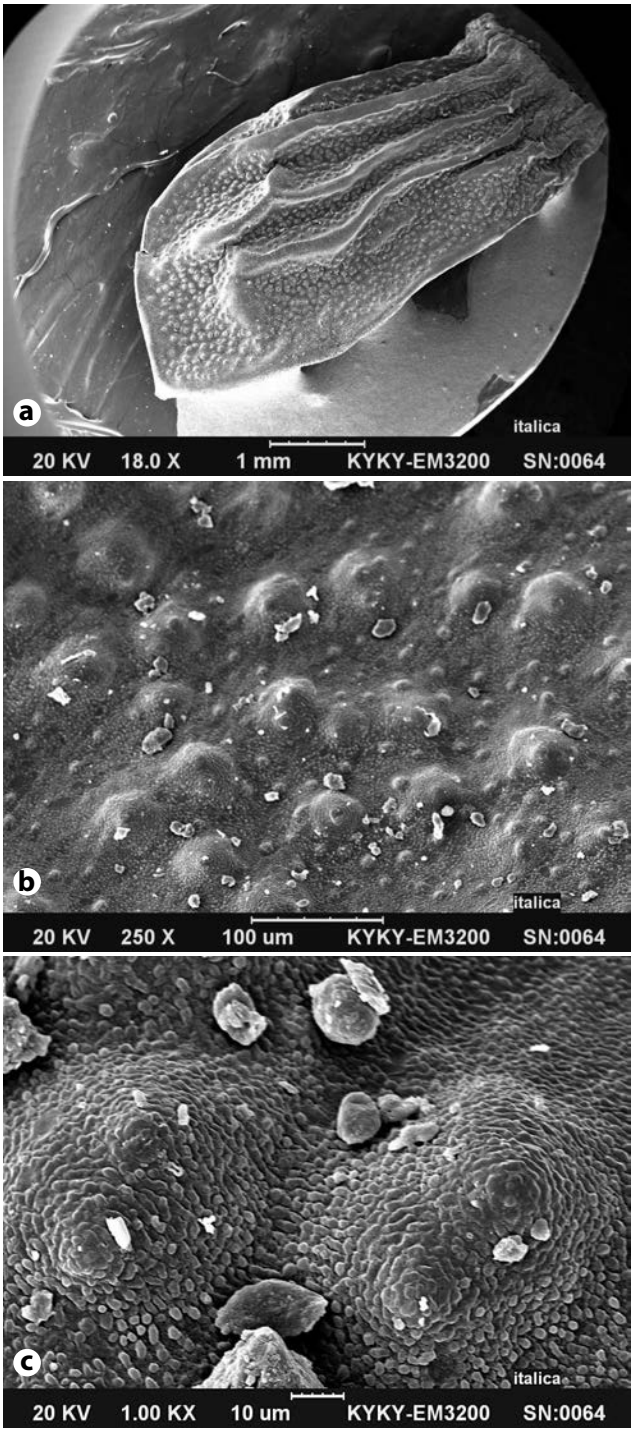

Fig. 3. SEM micrographs of the mericarps of *A. italica* var. *italica*. **a)** Lateral view. **b)** Ornamentation with 250×. **c)** Ornamentation with 1.00 KX.

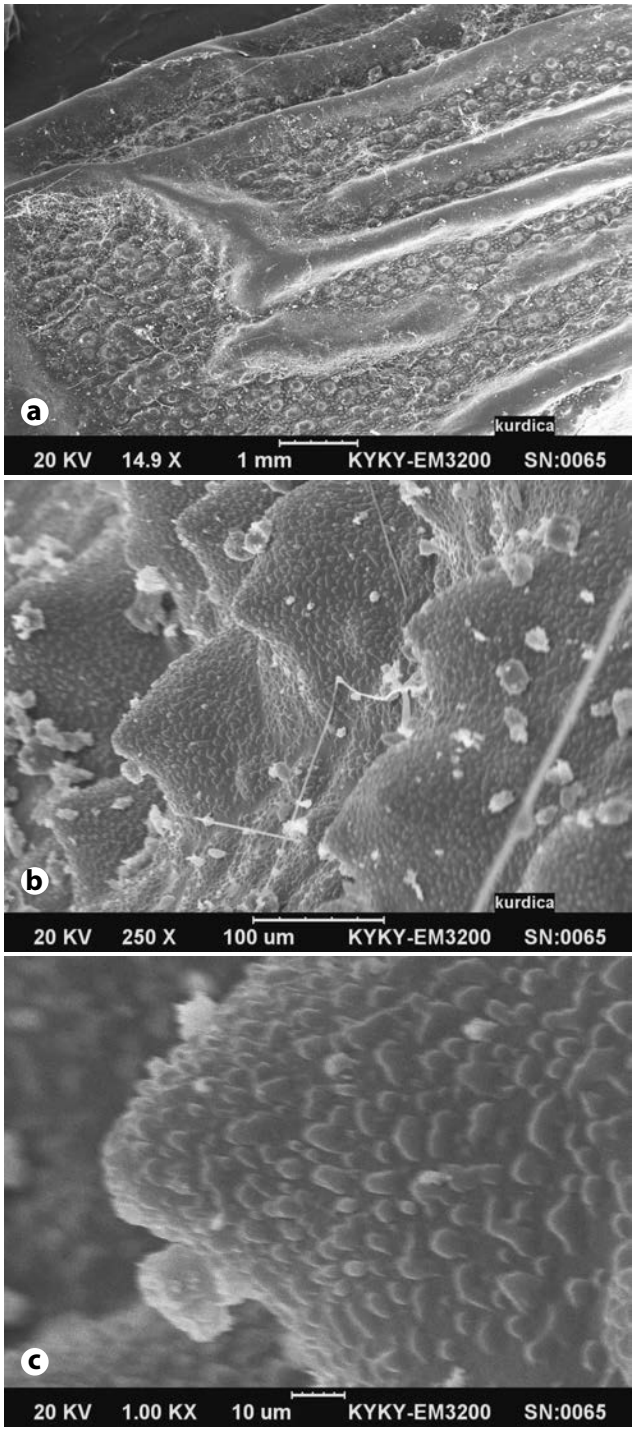

Fig. 4. SEM micrographs of the mericarps of *A. italica* var. *kurdica*. **a)** Lateral view. **b)** Ornamentation with 250×. **c)** Ornamentation with 1.00 KX.

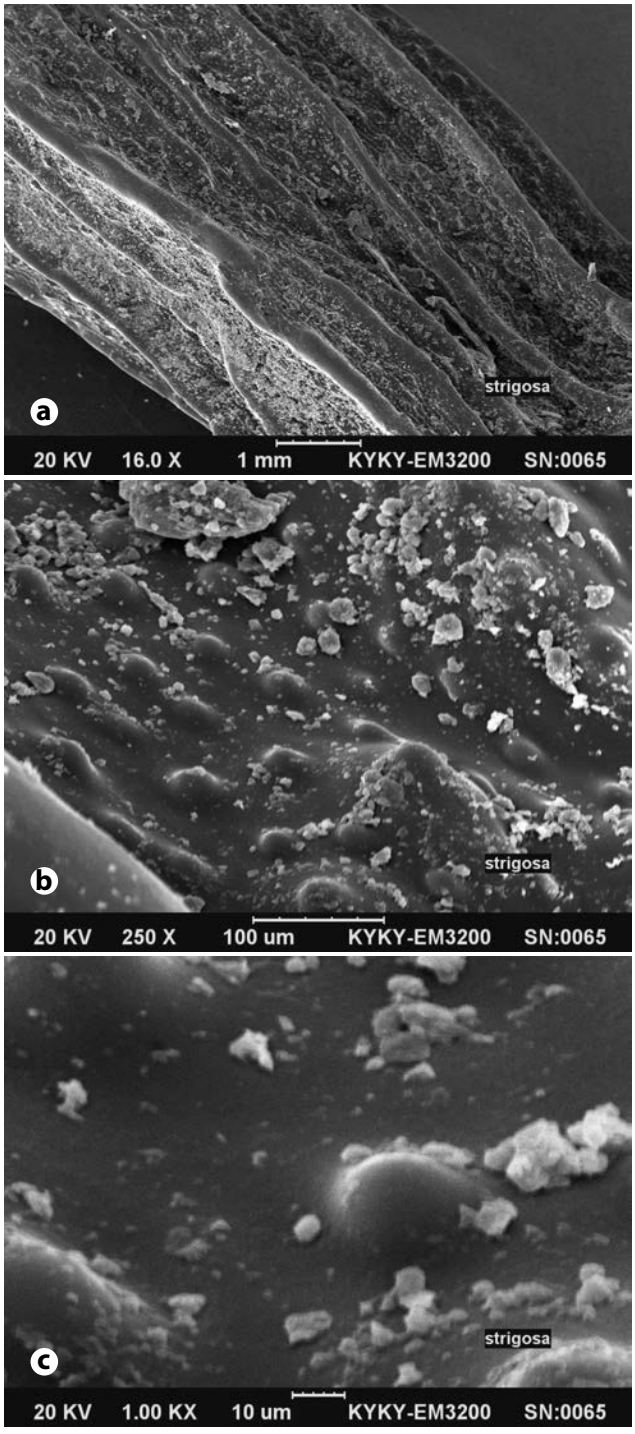

Fig. 5. SEM micrographs of the mericarps of *A. strigosa.* **a)** Lateral view. **b)** Ornamentation with 250×. **c)** Ornamentation with 1.00 KX.

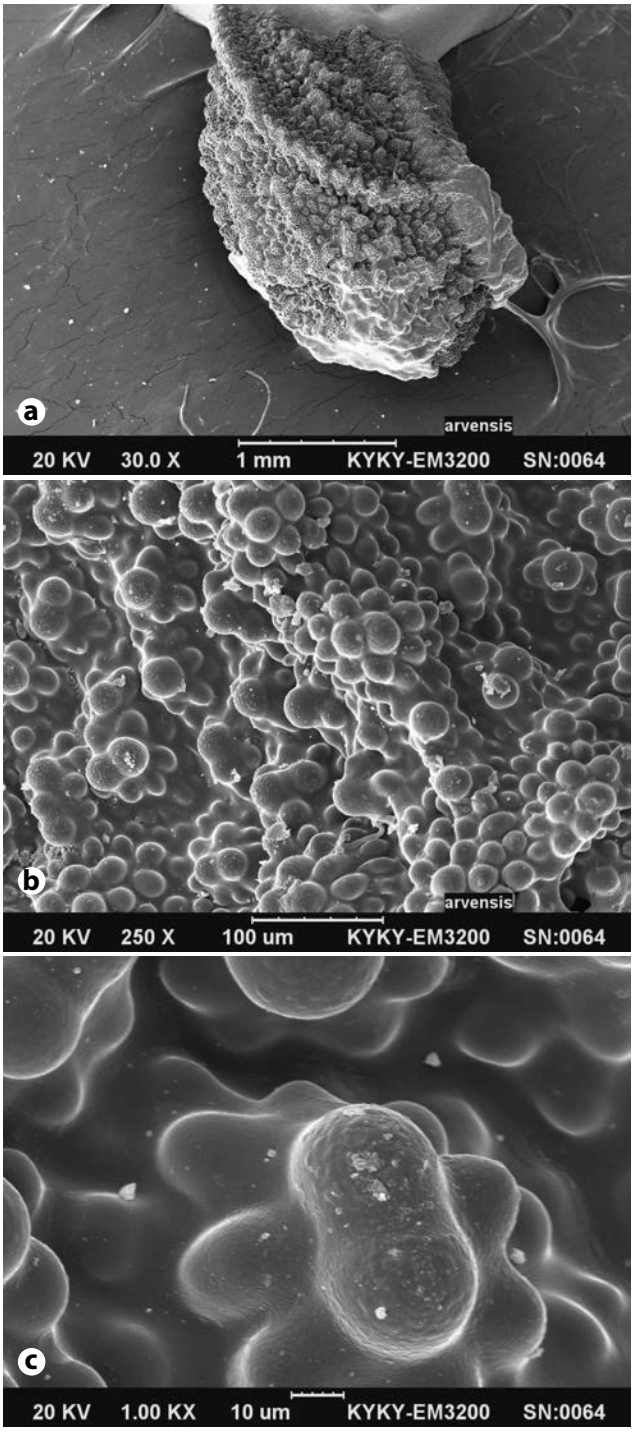

Fig. 6. SEM micrographs of the mericarps of *A. arvensis.* **a)** Lateral view. **b)** Ornamentation with 250×. **c)** Ornamentation with 1.00 KX.

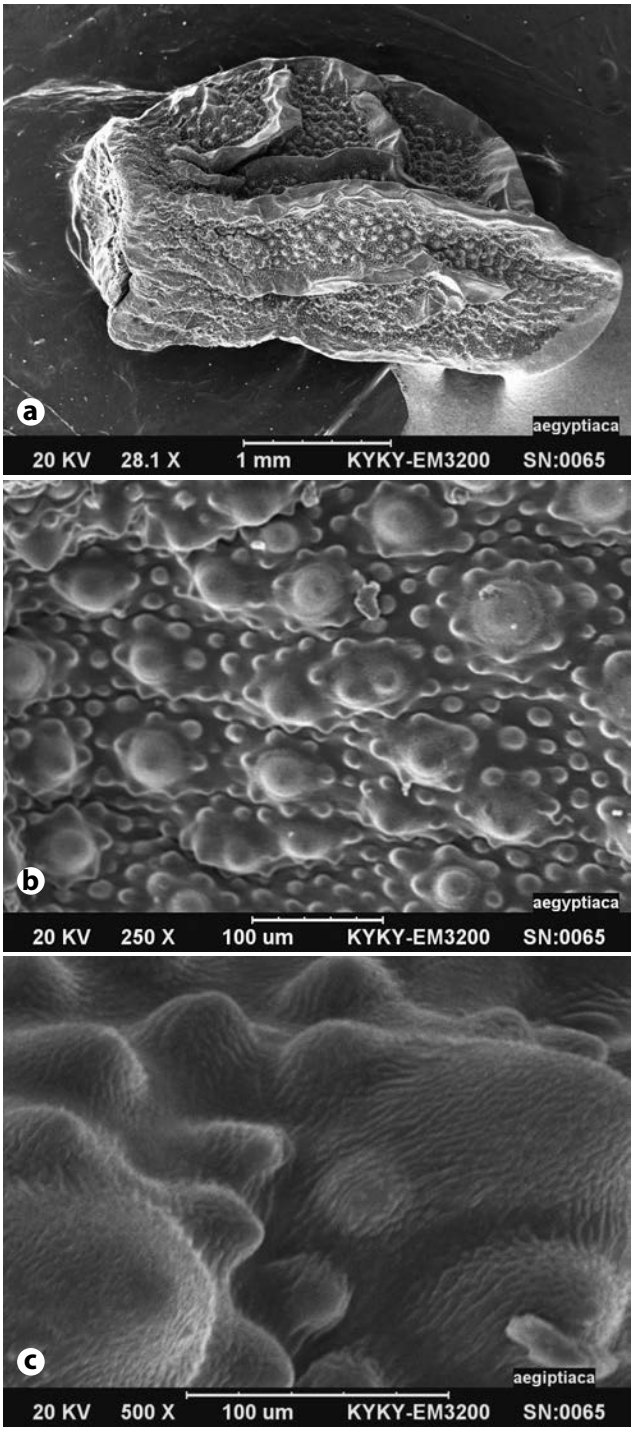

Fig. 7. SEM micrographs of the mericarps of *A. aegyptiaca.* **a)** Lateral view. **b)** Ornamentation with 250×. **c)** Ornamentation with 1.00 KX.

Discussion

Mericarp micro-morphological evaluation of the *Anchusa* species has shown the diagnostic value of these characters. Mericarp erectness is one of the diagnostic features. Pakravan & al. (2009) and Akçin & Binzet (2011) have pointed out the diagnostic value of such features in *Nonea medicus* and *Onosma* L. respectively. The mericarp has two shapes: erect and curved. In *A. strigosa* and both varieties of *A. italica,* the mericarp is erect and cylindrical. *A. aegyptiaca* has a subcurved mericarp. The mericarp is horizontal only in *A. arvensis*. So it is clearly distinguished.

Riedle (1967) and Khatamsaz (2002) used such features in their identification key. These authors considered three subgenera in the *Anchusa,* namely *Buglossoides* (Reichenb.) Guşuleac, *Anchusa* and *Lycopsis* L. The authors saw the fruit shape as very efficient in the separation of the *Anchusa* subgenera. Selvi & Bigazzi (2000a,b) showed that *A. italica* and *A. strigosa* belong to subgenus *Anchusa* and manifest close relations. They both have rectangular and erect mericarps. By the way, these two species are more distanced from *A. aegyptica* and *A. arvensis*. *A. aegyptiaca* belongs to *Buglossoides* and has an ovate, semierect mericarp. *A. arvensis* belongs to subgenus *Lycopsis* and has an oblique and ovate mericarp. Thus these subgenera were distingushed by these features.

These findings are incongruent with the results of Akçin & Ulu (2008) about the *Anchusa* species. By means of SEM they have found that shape and surface ornamentations are taxonomically important for these taxa. The obtained clustering pattern (Fig. 1) shows a close relationship between *A. italica* var. *italica*, *A. italica* var. *kurdica* and *A. strigosa,* with a spiny wrinkled substrate on the mericarp surface, while *A. arvensis* and *A. aegyptiaca* are tuberculate. These results support the macro-morphological similarities.

According to data obtained in the present study, it is evident that the mericarp characters are useful for species identification. They showed significant differences among the studied *Anchusa* species. Mericarp morphology could help in clarifying the systematics of the *Anchusa* species.

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