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Pollen morphology of some species of the genus *Veronica* (Scrophulariaceae) in Iran

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Summary: The pollen morphology of 17 species as representatives of five sections of *Veronica* distributed in Iran was examined by light microscopy and scanning electron microscopy. Detailed pollen morphological characteristics are given for these species. Based on the sculpture of pollen grains, three pollen types can be recognized among the studied species: rugulate-perforate, microreticulate and scabrate. Our studies show that sculpturing of exine provides valuable characters for separating sometimes even closely related species. Pollen morphological evidences are not completely congruent with the current classification for delimitation of existing groups; however, they provide some valuable information.

Keywords: exine sculpture, Iran, pollen morphology, scanning electron microscopy, subgeneric classification, *Veronica*

Veronica with about 300 species distributed worldwide (Willis 1980) has an important centre of diversity in Iran. This genus contains 60 species, among them are 18 endemics in the country (Saeidi, unpublished data). According to Fischer (1981) these species are traditionally arranged in five sections: *V.* sect. *Alsinebe*, *V.* sect. *Veronica*, *V.* sect. *Veronicastrum*, *V.* sect. *beccabunga* and *V.* sect. *Paederotoides*.

Several genera like *Synthyris*, *Besseya*, *Pseudolysimachion*, *Drewentia*, *Hebe*, *Veronicastrum*, and *Wulfenia* are closely related to *Veronica* or even cluster within the genus (Elenevsky 1977, 1978; Olmstead & Reeves 1995; Kampany & Dengler 1997), nevertheless specific relationships within the genus are still uncertain.

It has been confirmed that pollen morphology provides reliable characters useful in species delimitation and subgeneric classification (Erdtman 1969; Walker & Doyle 1975). A pollen-morphological study of the whole family Scrophulariaceae has been conducted by Varghese (1968). Hong (1984) studied the pollen morphology of 19 genera of tribe *Veroniceae* including 49 species using scanning electron microscopy (SEM) and light microscopy (LM). A detailed pollen morphological study of 13 taxa of *Veronica* in SW Spain has been reported by Fernandez et al. (1997). However, only few species considered in these studies are also present in Iran.

This study includes a detailed pollen morphological analysis on 17 species of *Veronica* which are distributed in Iran. Of these, 6 species have not been considered in former studies. We also will discuss the potential contribution of palynological characters in systematics of the genus. The aims of the work are: first to find palynological characters which are systematically important, and second, to determine whether pollen characters are useful in infrageneric taxonomy of the genus.

Materials and Methods

Pollen of 17 species, representatives of five sections of *Veronica* distributed in Iran were studied by means of light microscopy (LM) and scanning electron microscopy (SEM). Pollen samples were

Table 1: Characteristic features of pollen grains in Iranian representatives of the sections of the genus *Veronica*. Abbreviations: **P** polar axis length; **E** equatorial axis length; **M** mesocolpium width. All voucher's specimens are preserved at herbarium TARI.

Species	Voucher	P (µm)	E (µm)	M (µm)	P/E ratio	Pollen type
V. acrotheca Bornm. & Gauba	Saeidi 24226	30.0(32.2 ± 1.1)34.0	18.0(21.7 ± 1.3)23.0	8.0(9.9 ± 1.4)12.0	1.3(1.5 ± 0.10)1.7	rugulate-perforate
V. anagalis-aquatica L.	Saeidi 1505	23.0(27.4 ± 1.6)29.0	16.0(20.0 ± 2.1)23.0	?	1.2(1.4 ± 0.10)1.5	rugulate-perforate
V. beccabunga L.	Saeidi 24072	27.0(29.6 ± 1.7)33.0	17.0(19.4 ± 1.0)21.0	6.0(7.4 ± 1.0)10.0	1.3(1.5 ± 0.10)1.7	microreticulate
V. campylopoda Boiss.	Saeidi & Kaviani 1311	28.0(28.6 ± 0.7)30.5	18.0(19.7 ± 0.8)21.0	?	1.2(1.3 ± 0.06)1.4	rugulate-perforate
V. capillipes Nevski	Saeidi 1301	29.0(29.7 ± 0.7)31.0	20.0(20.7 ± 0.8)22.0	?	1.3(1.4 ± 0.06)1.5	rugulate-perforate
V. ceratocarpa C. A. Mey.	Saeidi 24021	27.0(29.3 ± 1.4)32.0	17.0(19.6 ± 1.1)21.0	7.0(8.7 ± 1.1)10.0	1.4(1.5 ± 0.10)1.6	rugulate-perforate
V. kurdica Benth.	Saeidi & Assadi 24068	28.0(31.0 ± 1.5)34.0	18.0(20.1 ± 1.2)22.0	8.0(9.2 ± 1.2)12.0	1.4(1.5 ± 0.09)1.7	rugulate-perforate
V. francispetae M. A. Fischer	Saeidi 24025	30.0(34.3 ± 2.2)39.0	17.0(19.0 ± 1.5)22.0	5.0(6.5 ± 0.81)8.0	1.5(1.8 ± 0.16)2.1	rugulate-perforate
V. gentianoides Vahl	Saeidi 22062	40.0(42.6 ± 1.4)45.0	28.0(30.0 ± 1.6)33.0	10.0(11.9 ± 1.1)13.0	1.3(1.4 ± 0.06)1.5	rugulate-perforate
V. hederifolia L.	Saeidi 1310	34.0(39.1 ± 2.4)42.0	25.0(28.1 ± 2.3)31.0	6.0(7.9 ± 1.5)10.0	1.2(1.4 ± 0.10)1.6	scabrate
V. khorassanica Czernjak.	Saeidi 22053	23.0(24.7 ± 1.4)28.0	15.0(17.1 ± 1.1)20.0	5.0(6.6 ± 1.0)8.0	1.3(1.4 ± 0.08)1.6	rugulate-perforate
V. mirabilis Wendelbo	Mozaf. & Moham. 49270	24.0(26.2 ± 1.6)29.0	16.0(17.3 ± 1.1)19.0	6.0(7.4 ± 0.97)9.0	1.3(1.5 ± 0.12)1.7	rugulate-perforate
V. orientalis Miller	Saeidi 24064	37.0(39.2 ± 1.3)41.0	23.0(24.5 ± 1.1)26.0	7.0(8.9 ± 1.3)11.0	1.5(1.6 ± 0.09)1.8	rugulate-perforate
V. persica Poir.	Jamzad & Asri 71766	32.0(35.2 ± 1.8)40.0	25.0(27.3 ± 1.7)30.0	12.0(14.0 ± 1.0)15.0	1.2(1.3 ± 0.08)1.4	rugulate-perforate
V. polita Fries	Saeidi 1315	25.0(27.3 ± 1.8)30.0	19.0(22.2 ± 2.1)25.0	8.0(10.1 ± 0.9)11.0	1.2(1.2 ± 0.10)1.3	rugulate-perforate
V. rechingeri M. A. Fischer	Saeidi & Gholipour 24023	36.0(38.6 ± 1.7)41.0	22.0(24.4 ± 1.4)27.0	8.0(10.4 ± 1.6)14.0	1.4(1.6 ± 0.09)1.7	rugulate-perforate
V. siaretensis Lehmann	Saeidi & Kaviani 1248	28.0(30.6 ± 1.7)34.0	18.0(20.1 ± 1.3)23.0	8.0(10.0 ± 1.4)13.0	1.3(1.5 ± 0.10)1.7	rugulate-perforate

obtained from herbarium specimens collected in the year of sampling. The voucher specimens were deposited in herbarium TARI. Voucher specimens along with selected pollen morphological characters are listed in table 1. Detailed information of voucher specimens is available from the authors on request.

Pollen samples were acetolyzed following the standard method described by Erdtman (1969). After that pollen was mounted in glycerol jelly on glass slides and sealed. Prepared slides were studied and photographed using an Olympus microscope model BX-50. Length of equatorial and polar axis were measured with aid of a ×100 eyepiece. Measurement of grains is based on 30 grains per sample.

For scanning electron microscopy studies, we used the protocol of Davies (1999) with some modifications (Zarrei & Zarre 2005). After preparation, specimens were mounted on 12.5 mm diameter stubs and attached with sticky tabs and then coated in a sputter coater with approximately 25 nm of Gold-Paladium. The specimens were examined and photographed with a Zeiss scanning electron microscope DSM 960A at an accelerating voltage of 10–15 kV.

The terminology follows in general Faegri & Iversen (1989), Moore et al. (1991), Punt et al. (1994, 1999), and Walker & Doyle (1975).

Results

Selected pollen morphological characters of all studied species are listed in table 1.

Pollen grains are shed as monads. They are tricolpate (fig. 1 e) with three, equidistant, furrow-like apertures which are perpendicular to the meridional equator, or tetracolpate (fig. 1 f), isopolar and perprolate (fig. 1 g) to spheroidal (fig. 1 h) in equatorial view. They are also euprolate and radio-symmetric (according to Walker & Doyle 1975). Exine is tectate-columellate (fig. 2 a).

The pollen grains are of medium size (according Walker & Doyle 1975) ranging from 24.7 ± 1.4 × 17.1 ± 1.1 µm in *V. khorassanica* Czernjak. to 42.6 ± 1.4 × 30.0 ± 1.6 µm in *V. gentianoides* Vahl. P/E ratio ranges from 1.2 (1.2 ± 0.10) 1.3 in *V. capillipes* Nevski to 1.5 (1.8 ± 0.16) 2.0 in *V. francispetae* M. A. Fischer. The mesocolpium varies in width from 5.0 (6.5 ± 0.81) 8.0 µm in *V. francispetae* M. A. Fischer to 12.0 (14.0 ± 1.0) 15.0 µm in *V. persica* Poir.

The apertures are simple: equatorial elongated, they reach the poles of the pollen with rounded or acute ends (fig. 1 e).

Based on exine sculpturing, three main pollen types can be recognized and are described below.

Rugulate-perforate

This type is more frequent among the examined species and occurs in 14 species: *V. acrotheca* Bornm. & Gauba (fig. 2 b), *V. anagalis-aquatica* L., *V. capillipes* Nevski, *V. ceratocarpa* C. A. Mey., *V. kurdica* Benth., *V. francispetae* M. A. Fischer, *V. gentianoides* Vahl (fig. 2 c, d), *V. khorassanica* Czernjak., *V. mirabilis* Wendelbo, *V. orientalis* Miller, *V. persica* Poir., *V. polita* Fries, *V. rechingeri* M. A. Fischer and *V. siaretensis* Lehmann. The largest pollen is classified under this type and occurs in *V. gentianoides* (40.0–45.0 × 28.0–33.0 [= 42.6 ± 1.4 × 30.0 ± 1.6] µm). The smallest pollen is observed in *V. khorassanica* (23.0–28.0 × 15.0–20.0 [= 24.7 ± 1.4 × 17.1 ± 1.1] µm) which also belongs to this type. There are some holes in the tectum which are less than 1 µm in

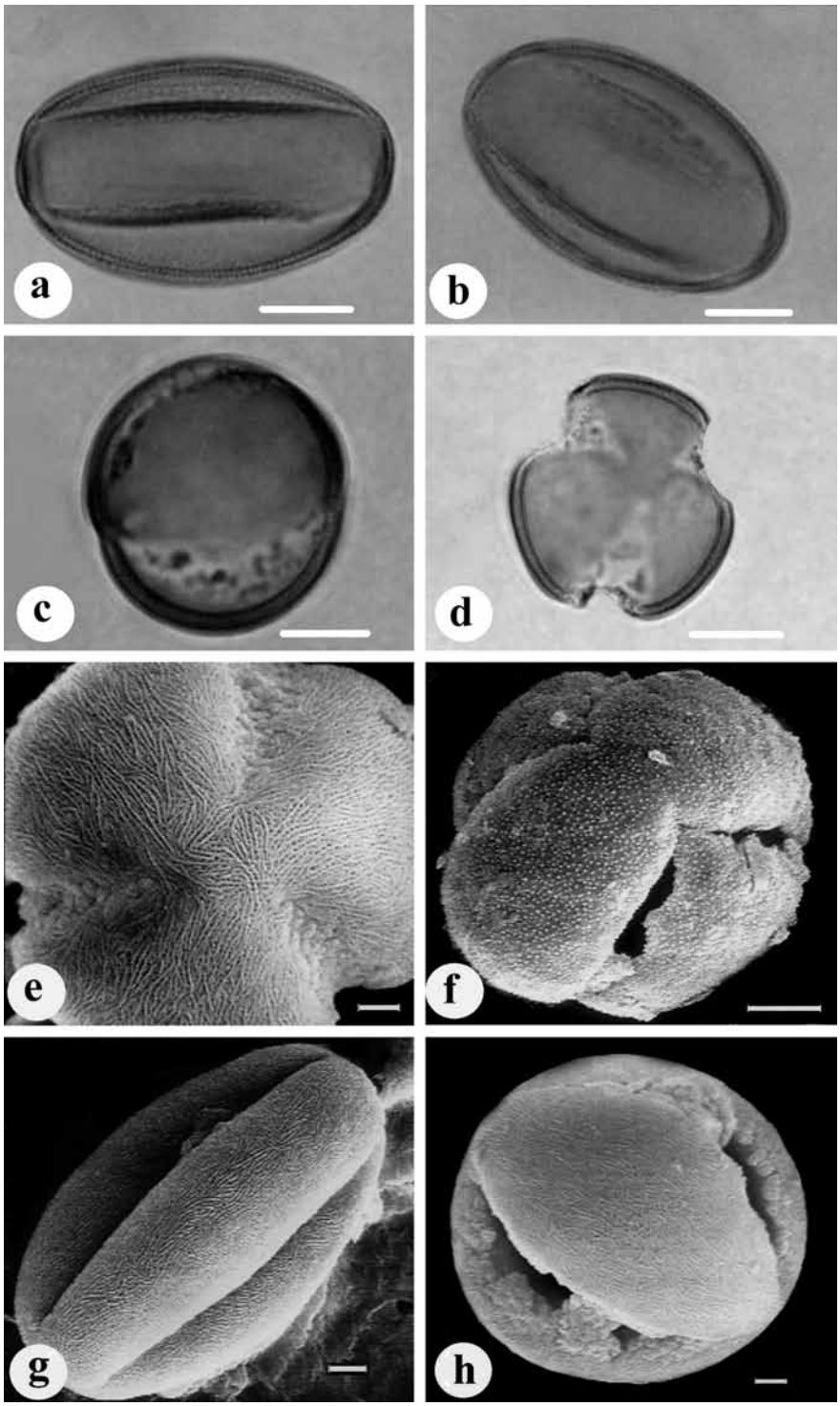

Figure 1: LM (a–d) and SEM (e–h) micrographs of pollen grains in *Veronica*: a) pollen of *V. fransicpetae* at equatorial view; b) pollen of *V. orientalis* at equatorial view; c) pollen of *V. polita* at equatorial view; d) pollen of *V. rechingeri* at polar view; e) pollen of *V. rechingeri* at polar view; f) pollen of *V. hederifolia* at polar-equatorial view; g) pollen of *V. khorasanica* at equatorial view; h) pollen of *V. polita* at equatorial view. a–d) scale bar = 10 μm; e, g, h) scale bar = 2 μm; f) scale bar = 5 μm.

diameter and the distance between holes is greater than their width. Upon the tectum, there is a type of ornamentation consisting of elongated sexine elements longer than 1 µm and arranged in an irregular pattern intermediate between striate and reticulate sculpturing.

Microreticulate

This type occurs only in *V. beccabunga* L. (fig. 2 e). The lumina are as wide as the muri or wider and smaller than 1 µm in diameter. The muri are solid and simplicolumellate.

Scabrate

This type occurs only in *V. hederifolia* L. (fig. 2 f). This species is the only one having tetracolpate pollen. There are some elements of ornamentation on the tectum which are smaller than 1 µm in all directions.

Discussion

The taxonomy of *Veronica* is very difficult due to several reasons. Delimitation of species is in many cases problematic, because of the great variation in vegetative and generative characters, mostly promoted by different ecological conditions. Moreover, in respect with subgeneric grouping, there are controversial views.

Our results confirm the eurypalynous feature of the genus suggested by Hong (1984) and Fernandez et al. (1997). Pollen morphological evidences are not completely congruent with the current classification for delimitation of existing groups, and they provide some valuable information. Based on pollen morphological characters, the current subgeneric classification of the genus (Fischer 1981) is evaluated below:

I *Veronica* sect. *Alsinebe*

Veronica. sect. *Alsinebe* as defined by Römpp (1928) is the largest section of the genus. The representative species of this section in our study are: *V. capillipes* (fig. 2 a), *V. campylopoda, V. ceratocarpa, V. francispetae* (fig. 1 a), *V. persica, V. polita* (figs. 1 c, h), *V. siaretensis* and *V. hederifolia* (figs. 1 f, 2 f). Except for *V. hederifolia* with scabrate type (fig. 2 f), all other species show regulate-perforate exine. *V. capillipes* and *V. campylopoda* are closely related to each other according to seed morphology (Saeidi et al. 2001 a) and fruit morphology (Saeidi et al. 2001 b). *V. capillipes* is an Iranian endemic species with the chromosome number 2n=28, while the much wider distributed *V. campylopoda* occurring in Iran, Middle Asia, and Turkey has the chromosome number 2n=42 (Saeidi & Kharabian 2005). A rugulate-perforate exine confirms the close relationship between these species, although the P/E ratio in *V. capillipes* is slightly higher than in *V. campylopoda* which has been attributed to *V.* sect. *Alsinebe* subsect. *Biloba* by Römpp (1928). In our opinion, *V. capillipes* and *V. campylopoda* should be placed into *V.* sect. *Alsinebe* subsect. *Biloba* especially because the pollen features of these species are similar to *V. biloba* as suggested by Hong (1984).

Veronica ceratocarpa, V. persica, V. polita and *V. siaretensis* belong to *V.* sect. *Alsinebe* subsect. *Agrestis* (Römpp 1928). *V. siaretensis* and *V. francispetae* are endemic to North Iran, while *V. ceratocarpa* is native to sub humid deciduous forests of the Alborz mountain range in North Iran and Caucasus. *V. persica* is tetraploid (2n= 28), while the other species are diploid (2n= 14) (Saeidi & Kharabian 2005). From the seed micromorphological point of view, seed shape and surface of all mentioned species except *V. ceratocarpa*, are cyathiform and papillate (Juan et al. 1994; Saeidi et al. 2001),

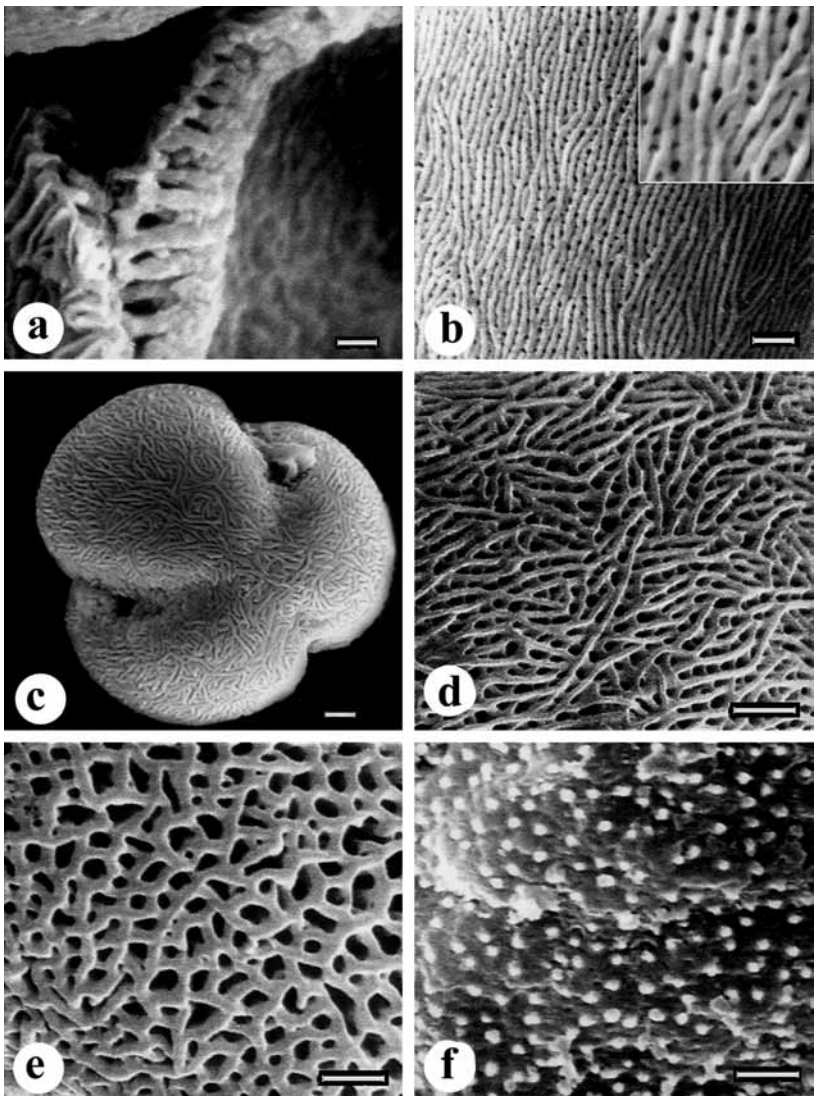

Figure 2: SEM micrographs of pollen grains in *Veronica*: a) cross section of the exine of *V. capillipes*; b) rugulate-Perforate tectum of *V. acrotheca*; c) pollen of *V. gentianoides* at polar view; d) rugulate-Perforate tectum of *V. gentianoides*; e) microreticulate tectum of *V. beccabunga*; f) scabrate tectum of *V. hederifolia*. a, b) scale bar = 500 nm; c) scale bar = 2 μm; d–f) scale bar = 1 μm.

respectively. The seed of *V. ceratocarpa* is plano-convex and reticulate. Interestingly, among the studied species of *V.* sect. *Alsinebe* subsect. *Agrestis, V. ceratocarpa* has the smallest pollen grains (mean P= 27 μm). Pollen characters connect *V. ceratocarpa* to *V. siaretensis*, *V. francispetae*, *V. persica*, and *V. polita*. All these species have been attributed to subsect. *Agrestis* (Römpp 1928). Among species of *V.* sect. *Alsinebe* subsect. *Agrestis,* the pollen of *V. persica* and *V. polita* are more or less rounded.

Veronica cymbalaria is palynologically very similar to *V. hederifolia* and its pollen type is scabrate as analyzed by Fernandez et al. (1997). They are tetraploid and hexaploid (2n= 36, 54) (Goldblatt & Johnson 1994). According to Römpp (1928) and Stroh (1942) *V. hederifolia* and *V. cymbalaria* belong to *V.* sect. *Alsinebe* subsect. *Megasperma* while they were attributed to sect. *Megasperma* by

Borrisova (1955). Both species were located in sect. *Cochlidiosperma* (Hong 1984). These species are also supported as belonging to separated clades in the cladogram presented on the phylogeny of *Veronica* by Saeidi & Zarre (2004).

II *Veronica* sect. *Paederotoides* and *V.* sect. *Veronicastrum*

Veronica sect. *Paederotoides* includes *V. paederotae* and *V. mirabilis*, while *V.* sect. *Veronicastrum* comprises *V. gentianoides*, *V. serpyllifolia*, and *V. davisii* in Iran.

Veronica mirabilis has rugulate-perforate pollen and Hong (1984) recognized a striate-reticulate type of exine in *V. paederotae*. Both, *V. paederotae* and *V. mirabilis* are endemic to Iran and the only important morphological character which connects these species to each other is the presence of an elongated corolla tube. Clarification of the systematic position of *V.* sect. *Paederotoides* needs more detailed studies.

Based on the classification presented by Römpp (1928) *V. gentianoides* and *V. serpyllifolia* belong to *V.* sect. *Veronicastrum* subsect. *Gouani* and *V.* sect. *Alsinebe*. subsect. *Serpyllifollia*. Borrisova (1955) separated all species with 5-partite calyces from those with 4-partite calyces for which she suggested the new sect. *Macrostemon*. The natural *Serpyllifolia* group was distinguished by Yamazaki (1957) as independent from sect. *Veronicastrum*. Based on pollen morphological features, Hong (1984) transferred *V. serpyllifolia* and its allies into sect. *Veronicastrum*.

Veronica gentianoides has the largest pollen grains among the studied species with rugulate-perforate exine (fig. 2c). Striate-reticulate exine has been recorded in *V. serpyllifolia* by Hong (1984). Seeds of *V. gentianoides* and *V. serpyllifolia* resemble each other: both show a reticulate surface (Saeidi et al. 2001a). Moreover, a close relationship between these species has been suggested by Albach & Chase (2001) based on nr DNA Internal Transcribed Spacer (ITS) sequences. This close relationship was supported in the cladistic analysis of the genus based on morphological characters (Saeidi & Zarre 2004). Pollen morphology is again not in accordance with data from other sources in the case of relationship between *V. gentianoides* and *V. serpyllifolia*.

III *Veronica* sect. *Beccabunga*

In contrast to other sections of *Veronica*, all species of *V.* sect. *Beccabunga* prefer very humid conditions. Two important species of this section shown here are *V. anagallis-aquatica* and *V. beccabunga*. The pollen type of *V. anagalloides*, also of this section, has been reported previously by Fernandez et al. (1997). The characteristics of pollen sculpturing in *V. anagalloides* are similar to *V. anagallis-aquatica*. The pollen type is rugulate-perforate in both species, confirming the evidences from other sources like seed morphology, inflorescence type, fruit morphology and anatomy on the close relationship between them (Juan et al. 1994). In addition, the similarity of endosperm formation pattern (Yamazaki 1957) and chromosome counts (Saeidi & Kharabian 2005) have been mentioned earlier. However, *V. beccabunga* (fig. 2e) shows a different pollen type in comparison with *V. anagallis-aquatica* and *V. anagalloides*: it belongs to the microreticulate pollen type. But a close relationship between *V. anagallis-aquatica* and *V. beccabunga* has been supported in the molecular systematic study presented by Albach & Chase (2001).

IV *Veronica* sect. *Veronica*

Representative taxa of this section are: *V. acrotheca* (fig. 2b), *V. khorassanica* (fig. 1g), *V. kurdica*, *V. orientalis* (fig. 1b), and *V. rechingeri* (fig. 1e). In Römpp's (1928) classification *V. kurdica* and

V. orientalis belong to *V.* sect. *Chamaedrys* subsect. *Orientales*, while *V. acrotheca* is attributed to *V.* sect. *Chamaedrys* subsect. *Euchamaedrys*.

There are several characteristic morphological features relating the species of *V.* sect. *Veronica* to each other, i.e. woody habit, seed morphology and inflorescence type (Saeidi, unpublished data). Similarity of pollen characters of selected species of *V.* sect. *Veronica* and *V.* sect. *Alsinebe* was suggested by Hong (1984). Our study confirms his point of view. The relationship between taxa of these sections is given in table 1. It seems that pollen morphology can not provide evidence for delimitation of these taxa.

Conclusions

All these facts as well as morphology strongly suggest the heterogeneity of *Veronica*. Only an extended study including the species distributed in other countries and comparing the data in all subgeneric groups known in the genus can lead to a better delimitation of the sections.

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