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Abstract

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The Levant in the E Mediterranean presents a region of remarkable species diversity due to the conjunction of four biogeographic provinces, a remarkable diversity of habitats and long human history in the area. However, little is known about the evolutionary history of plants in the Levant. Here, we analyse plastid DNA data for members of *Veronica* subg. *Pentasepalae* (*Plantaginaceae*) from the Levant and adjacent areas. The data support the recognition of *V. orientalis*, *V. leiocarpa* and *V. polifolia* as separate species. Flow cytometric analyses demonstrate the former two to be octoploid but *V. polifolia* as tetraploid. The Levantine lineage of *V. orientalis* is clearly distinct from more northern lineages of the species, as found in many animal species. However, no affinities to specific other taxa from Anatolia, whether of Mediterranean or Irano-Turanian origin, can be made at present.

Additional key words: Mediterranean, Turkey, Anatolia, Syria, Israel, Jordan, phylogeny, plastid DNA, ploidy level, *Veronica orientalis*, *Veronica leiocarpa*, *Veronica polifolia*, *Plantaginaceae*

Introduction

The Levant historically encompasses the whole E Mediterranean, but nowadays comprises the region of Cyprus, Israel, Jordan, Lebanon, Palestine, Syria, and part of S Turkey. With more than 3000 species, it constitutes one of the hotspots within the Mediterranean global biodiversity hotspot (Danin 1992; Médail & Quézel 1997). The species richness of the region is only to a minor extent explained by endemism, but more so by its biogeographic location at the crossroads of four regions, the Mediterranean, Irano-Turanian, Saharo-Arabian and Sudano-Zambesian

biogeographic regions (Danin 1992; Danin & al. 1975; Zohary 1973) as well as its diversity of habitats and its long history of anthropogenic changes (Danin 1999a). Thus, Israel and Jordan record both about 2800 species (Al-Qura'n 2009; Danin 1999a). However, the number of endemics is rather low (6.5% for Israel; Shmida 1984) and many species have their margin of distribution in that region. Nevertheless, Zohary (1973) suggested a high speciation rate in this region, especially for Irano-Turanian elements, a rate that could be concealed by insufficient differentiation of these taxa from their closest relatives further north and east.

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Veronica subg. *Pentasepalae* (Benth.) M. M. Mart. Ort., Albach & M. A. Fisch. comprises the majority of perennial species of the genus *Veronica* L. (*Plantaginaceae*) from SW Asia. Currently there are 70 species recognized in the subgenus (Albach & al. 2008) ranging from Morocco to the Altai Mountains in C Asia. More than half of these occur in Turkey and about one-quarter occur further west and east, respectively, but only six occur S of Turkey (Fischer 1984). Of these, *V. macrostachya* Vahl (= *V. aleppica* Boiss.) reaches just barely into N Syria. Two are endemic cushion-forming taxa restricted to alpine habitats of the Lebanon and Anti-Lebanon Mountains: the entirely glabrous *V. caespitosa* subsp. *leiophylla* (Boiss.) M. A. Fisch. and the woolly-tomentose *V. bombycina* Boiss. & Kotschy subsp. *bombycina*. The more widespread species are *V. orientalis* Mill., *V. leiocarpa* Boiss. (= *V. stenobotrys* Boiss. & C. I. Blanche) and *V. polifolia* Benth., which spread south to montane habitats in Israel and Jordan. Thus, these latter three are the only perennial species of *Veronica* reaching the southernmost tip of the Irano-Turanian biogeographic region. The relationship of these three species has been confusing in the past, with various authors differing in opinion as to whether these plants constitute one, two or three good species.

Veronica orientalis was described by Miller (1768) likely based on material cultivated in botanical gardens, although it was stated to grow “naturally in the Levant” (Miller 1768). Although the main distribution area is found in E Turkey, the presence of *V. orientalis* in the Levant was recognized by Post (1896) and Thiebaut (1953) based on differences in capsule base (cuneate at base in *V. orientalis*, rounded in *V. leiocarpa*) and a shorter inflorescence (not quantified). Whereas Post (1896) recorded *V. orientalis* from Mount Gilead (Mt Jalad) in NW Jordan, the species was not mentioned in Flora palaestina (Feinbrun-Dothan 1978), which covers this area. Also, the checklist of Jordanian flora, updated in 2012 (Royal Botanic Garden, Jordan, unpublished), did not mention the occurrence of *V. orientalis* in Jordan. Flora of Israel online (Danin 2006+) gives the species for N Israel (Mt Hermon).

Veronica orientalis is a highly polymorphic species and was recently shown to be polyphyletic in Turkey (Sonibare & al. 2014). If the same pattern is found on the S range margin, it is likely that other taxa can be split from traditional *V. orientalis*. The only name available for such a taxon in the Levant is *V. billardierei* Vahl (Vahl 1804), generally considered to be synonymous to *V. orientalis* (e.g. Boissier 1879; Fischer 1980; Post 1896). It is described as a prostrate, grey-haired form with ovate-lanceolate leaves with an entire margin. A possible type in the Willdenow Herbarium in Berlin (B-W 00215-01 0, <http://herbarium.bgbm.org/object/BW00215010>) resembles more *V. polifolia*, thus demonstrating difficulties distinguishing *V. orientalis* from *V. polifolia*.

Veronica leiocarpa was described by Boissier (1853) from plants in what is now the Turkish province of Hatay.

It was differentiated from *V. latifolia* L. (= *V. teucrium* L.), a European plant, which resembles *V. leiocarpa* in habit and leaf shape but not other species despite the description fitting into the broad concept of *V. orientalis*. Fischer (1980) differentiated between *V. leiocarpa* and *V. orientalis* based on leaf length (20–45 mm vs 5–20 mm) and on apical leaf margin (0–2 teeth vs 10–25 teeth per side), respectively. Three years after the description of *V. leiocarpa*, Boissier (1856) published the name *V. stenobotrys* based on plants from the Syrian province of Homs. According to Boissier (1856), *V. stenobotrys* differs from *V. leiocarpa* in its more strongly ascending habit, in being densely pubescent with crisped hairs rather than glabrous to puberulent, in having leaves shortly petiolate rather than sessile, leaf blade elliptic rather than oblong, inflorescence denser, and capsule ovate-elliptic vs obcordate. The species were later reduced to varietal rank by Boissier (1879) himself and most subsequent authors (e.g. Feinbrun-Dothan 1978) or not differentiated at all (e.g. Fischer 1980).

Veronica polifolia was described by Bentham (1846) based on material from NW, S and E Turkey as well as Syria (collected by Labillardiere). Later, Post (1896) regarded it as a small variety of *V. orientalis* with revolute leaf margin from the Lebanon and Anti-Lebanon Mountains only and transferred specimens from other regions to other species. Fischer (1978) reported the species in the Flora of Turkey only from a few localities in C Anatolia today. It is not given in Flora palaestina (Feinbrun-Dothan 1978) but was recently added (Danin 1997) for the area. It differs from the two other species by the presence of glandular hairs, stems creeping to ascending and flowers purple and white (Fischer 1980).

The view that these are three different species is currently prevailing but is untested. Recently, Sonibare & al. (2014) analysed relationships of *Veronica orientalis* and relatives in Turkey using plastid DNA sequences but did not include specimens from the Levant. In their analyses, different ploidy levels and hybridization or introgression with other species made definite taxonomic conclusions impossible but nevertheless revealed some biogeographic patterns among Anatolian plants and offered insight into which species are closely related to *V. orientalis* and which are not. Here, we expand the dataset analysed by Sonibare & al. (2014) by including samples of *V. orientalis*, *V. leiocarpa* and *V. polifolia* from the S range of distribution. Remarkably, the *Veronica* species collected from Jordan for the present study are deemed rare and at increasing risk from destruction of their habitats and overgrazing. *Veronica leiocarpa*, a newly recorded species for S Jordan, is represented by a sample from its southernmost distribution. It occurs there in just one population, protected by the presence of large flint stone piles in cropland, and consists of only three mature individuals. Analysing its relationship is therefore of importance for its future conservation. Furthermore, we provide first genome size estimates of these three species from that



Fig. 1. Map of the Levant region with origin of material indicated.

area in order to reveal a likely scenario of evolution of these samples.

Material and methods

Plant material and ploidy level estimation

We expanded the sampling by Sonibare & al. (2014) by adding nine samples of *Veronica leiocarpa*, *V. orientalis* and *V. polifolia* from Jordan and Israel including the southernmost populations known for these species (Fig. 1; Table 1). Owing to logistical and political problems, we were not able to include more samples.

Additionally, we added the third marker (*ndhF-rpl32*) to *Veronica leiocarpa* from Turkey that was missing in the previous analysis. For ease of analysis, we deleted three individuals (*V. farinosa* Hausskn., *V. oltensis* Woronow ex Elenevsky, *V. peduncularis* M. Bieb.) shown to be only distantly related to our study group according to Sonibare & al. (2014).

We attempted to estimate ploidy level by flow cytometry from silica-gel-dried or herbarium material, which succeeded in four instances (Table 1). Nuclei were isolated using a modified woody plant buffer (0.2 M TrisHCl, 4 mM MgCl₂·6H₂O, 2 mM EDTA Na₂·2H₂O, 86 mM NaCl, 10 mM ascorbic acid, 1% PVP-25, 1% Triton X-100 at pH 7.5; based on Loureiro & al. 2007). Samples were run on a CyFlow SL (Partec GmbH, Münster, Germany) equipped with a green laser (532 nm) using *Hedychium gardnerianum* (1C = 2.01pg; Meudt & al., in prep.) for *Veronica leiocarpa* or *Solanum pseudocapsicum* (1C = 1.2964 pg; Tensch & al. 2010) for the other

species as internal standards. Propidium iodide staining was used following the protocol of Baranyi & Greilhuber (1996) with 15 minutes staining.

DNA sequencing

DNA was isolated from herbarium or silica-gel-dried leaves using the DNeasy™ Plant Mini kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions. The quality of the extracted DNA was checked on 0.8% TBE-agarose gels and the concentration was measured spectrophotometrically with a GeneQuant RNA/DNA calculator (Pharmacia, Cambridge, U.K.). Following the protocol in Sonibare & al. (2014) we sequenced

the *psbA-trnH^{GUG}* spacer using primers *psbA* (Sang & al. 1997) and *trnH* (Tate & Simpson 2003), the *ndhF-rpl32* spacer using primers *ndhF* and *rpl32F* (Shaw & al. 2007) and the *rps16-trnK* spacer using primers *rps16x2F2* and *trnK(UUU)x1* (Shaw & al. 2007).

The products were purified using QIA quick PCR purification and gel extraction kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocols. The same primers used for PCR amplification were also used for the sequencing reactions by commercial sequencing companies. Assembled sequences were manually edited using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, Michigan, U.S.A.).

Phylogenetic analysis

To account for the presence of indels, gap positions were coded according to the simple indel coding as defined by Simmons & Ochoterena (2000) using SeqState v.1.4.1 (Müller 2005). The resulting data matrix was analysed using parsimony in PAUP* 4.0a136 (Swofford 2002) starting from random trees and 10 replicates of random taxon addition and TBR branch-swapping. Bootstrap analyses were conducted for matrices with and without gap characters coded with the same settings with 1000 replicates and keeping only 50 optimal trees per replicate. In a second approach, a Median-joining network was conducted in SplitsTree version 4 (Huson 1998; Huson & Bryant 2006). The median-joining network algorithm (Bandelt & al. 1999) is a method to visualize alternative topologies and has been shown to be superior to other network methods when many node haplotypes are missing (Cas-

Table 1. Voucher and sequence information for accessions not used in Sonibare & al. (2014). Specimens marked with an asterisk were analysed flow cytometrically for genome size estimate.

| Country | Species | Locality | Voucher | Coordinates | Accession number <i>psbA-trnH</i> | Accession number <i>ndhF-rpl32</i> | Accession number <i>rps16-trnK</i> |
|---------|-----------------------------|---|--|--------------------|--------------------------------------|---------------------------------------|---------------------------------------|
| Jordan | <i>Veronica leiocarpa</i> * | Rajef, 1961 m | 15 Jun 2013, Al-Gharaibeh (OLD) | 30.08°N 35.26°E | KP122306 | KP122315 | KP052933 |
| Israel | <i>Veronica leiocarpa</i> | Upper Galilee, Mt Meiron, 990 m | 11 May 2014, Danin (OLD) | 33.00°N 35.24°E | KP122305 | KP122314 | KP052932 |
| Turkey | <i>Veronica leiocarpa</i> | Turkey, Mersin province, Cehennem Dere | 27 Jun 1971, T. Uslu 967 (ANK) | 37.13°N 34.51°E | JX440738 | KP342255 | JX440781 |
| Israel | <i>Veronica orientalis</i> | Mt Hermon, 1900 m | 1 May 2014, Danin (OLD) | 33.18°N 35.45°E | KP122309 | KP122318 | KP052936 |
| Israel | <i>Veronica orientalis</i> | Mt Hermon, Nahal Arar, 1600 m | 24 May 1985, Liston & Lev-Ari, 7-85-368/3 (HUJ) | 33.17°N 35.46°E | KP122308 | KP122317 | KP052935 |
| Israel | <i>Veronica orientalis</i> | N Mt Hermon, 2000 m | 19 Jun 1969, H. Wood 126 (HUJ) | 33.18°N 35.45°E | KP122307 | KP122316 | KP052934 |
| Israel | <i>Veronica polifolia</i> * | Mt Hermon, 2000 m | 7 May 2014, Danin (OLD) | 33.18°N 35.45°E | KP122303 | KP122312 | KP052930 |
| Israel | <i>Veronica polifolia</i> | Mann valley, S Mt Hermon, 1500–1600 m | 24 May 1985, Liston & Lev-Ari 7-85-366/6 (HUJ) | 33.18°N 35.36°E | KP122301 | KP122310 | KP052928 |
| Jordan | <i>Veronica polifolia</i> * | Queen Alia Forest, Shobak, 1522 m | 15 Jun 2013, Al-Gharaibeh (OLD) | 30.33°N 35.31°E | KP122304 | KP122313 | KP052931 |
| Jordan | <i>Veronica polifolia</i> | Edom, N section of Dana Reserve | 15 May 1996, A. Danin (HUJ) | 30.47°N 35.35°E | KP122302 | KP122311 | KP052929 |

sens & al. 2005). Finally, a partitioned Bayesian analysis using MrBayes 3.2 (Ronquist & al. 2012) was conducted using the GTR+ Γ +I-model for the nucleotide characters and a model with two different rates and gamma rate variation for the gap characters. Analyses were run twice for ten million generations, sampling every 1000th generation and discarding the first 10 % as burn-in. Stationarity was checked in MrBayes using the likelihood value plot and only reporting results from the runs with higher likelihood and the potential scale reduction factor (PSRF) being 1.000 for all parameters.

Results

Our genome size estimates for *Veronica polifolia* resulted in a 1C-value of 0.64pg (CV 5.5–6) for the sample from Shobak (Jordan) and 0.60pg (CV 5.1–7.3) for the sample from Mt Hermon (Israel). Estimates for *V. orientalis* from Mt Hermon from 2014 are 1.79pg (CV 2.2–2.5) and 1.50pg (CV 4.1–4.6) for *V. leiocarpa* from Jordan. This corresponds to the tetraploid for *V. polifolia* and the octoploid level for *V. leiocarpa* and *V. orientalis*, respectively, when compared with previous estimates of related *Veronica* species (Sonibare & al. 2014; Meudt & al., in prep.).

The DNA sequence data matrix included 1885 nucleotide characters and 89 gap characters for 56 taxa. Parsimony analysis with gap characters resulted in more than 1000 equally most parsimonious trees of 513 steps (consistency index (CI) 0.55; retention index (RI) 0.83). Gap characters had a notable lower CI and RI (0.37, 0.66) than nucleotide characters (0.68, 0.90) on these trees. Bootstrap support (BS) did not differ for clades with more than 75 % BS except for three clades. The outgroup, *Veronica prostrata* L. and *V. minuta* C. A. Mey., received higher support with gaps (83 vs 61 BS). Further, the clade of *V. orientalis* 5–21–23 received higher (90 vs 74 BS), whereas that clade with these three individuals plus *V. orientalis* 14 and *V. multifida* L. Eastern Anatolia received lower (70 vs 85 BS) support with gaps. All of the clades with more than 75 BS reached a posterior probability of more than 0.97 in the Bayesian analysis and, therefore only the latter is depicted in Fig. 2. In that phylogenetic tree we can broadly define four general lineages of *V. orientalis*: a Levantine lineage (strongly supported as sister of *V. armena* Boiss. & A. Huet), a Central Anatolian lineage (including *V. pectinata* L.), a Taurus lineage (strongly supported sister to *V. thymifolia* Sm.) and an Eastern Anatolian lineage (including *V. fridericae* M. A. Fisch., *V. kurdica* Benth., *V. fuhsii* Freyn & Sint. and *V. multifida* in part). In addition, the median-joining network is shown to display alternative topologies (Fig. 3). Although the members of the general lineages group close again, many do not form monophyletic lineages. The most noteworthy difference is the close association of the Taurus lineage to the Levantine lineage of *V. orientalis*.

Discussion

Systematics and taxonomy of *Veronica* species in the Levant

Our results support the recognition of *Veronica leiocarpa* and *V. polifolia* as distinct to different degrees from *V. orientalis* in the Levant. Genetic distinctiveness is more pronounced for *V. polifolia* (Fig. 2 & 3), which also differs in being tetraploid, rather than octoploid as are the other two species in the area. Morphological differences of *V. polifolia* were described by Fischer (1980) as being the glandular (vs eglandular) inflorescence, the trailing (vs ascending to erect) habit and the purple-white (vs blue) flowers. Ecologically *V. polifolia* is restricted to drier habitat in rocky steppes. It needs to be emphasized that despite the clear genetic separation, *V. polifolia* may still be one of the ancestors of octoploid *V. orientalis* since we only studied plastid DNA and *V. polifolia* could well be the paternal parent of *V. orientalis*. This will need to be investigated further using nuclear DNA markers.

The distinction between *Veronica leiocarpa* and *V. orientalis* is more difficult. Both species are octoploid and seem to include two different haplotype lineages in the area with one overlap, which was *V. orientalis* from Mt Hermon (Fig. 2). However, based on the median-joining network, the samples of *V. leiocarpa* include a polyphyletic but closely related group of haplotypes in an intermediate position between the Eastern Anatolian and Taurus *V. orientalis* lineages. Ecologically the two species form part of a continuous variation, with *V. leiocarpa* occurring on rocky outcrops in lower-elevation forests and maquis and *V. orientalis* at higher elevations in more open habitats. According to Fischer (1980), morphological variation in *V. leiocarpa* ranges from 20–45 mm-long leaves (vs 5–20 mm long in *V. orientalis*) and 30–70 cm-tall plants (vs 3–30 cm tall in *V. orientalis*). *Veronica leiocarpa* is erect but *V. orientalis* ascending. Further differences are suggested to be in the leaf margins (Fischer 1980). However, more in-depth analysis of morphological variation is required, since the Jordanian population of *V. leiocarpa* differs from other *V. leiocarpa* in being ascending. The systematic significance of this is currently unclear, with adaptive introgression being a possibility. Furthermore, identical ploidy offers the possibility that gene flow occurs between both species in sympatry. Whether such gene flow is responsible for the position of *V. orientalis* from Mt Hermon collected in 2014 (Fig. 2) is unclear, since it is more distant from *V. leiocarpa* in the median-joining network (Fig. 3). A similar case is true for the Israeli sample of *V. leiocarpa*, which is distant from *V. leiocarpa* in the Bayesian analysis (Fig. 2) but close in the median-joining network (Fig. 3). An alternative to gene flow between taxa would be multiple origins of these octoploid species. With the given data we cannot currently decide between these alternatives.

However, our results of the phylogenetic analyses strengthen the conclusion by Sonibare & al. (2014) that

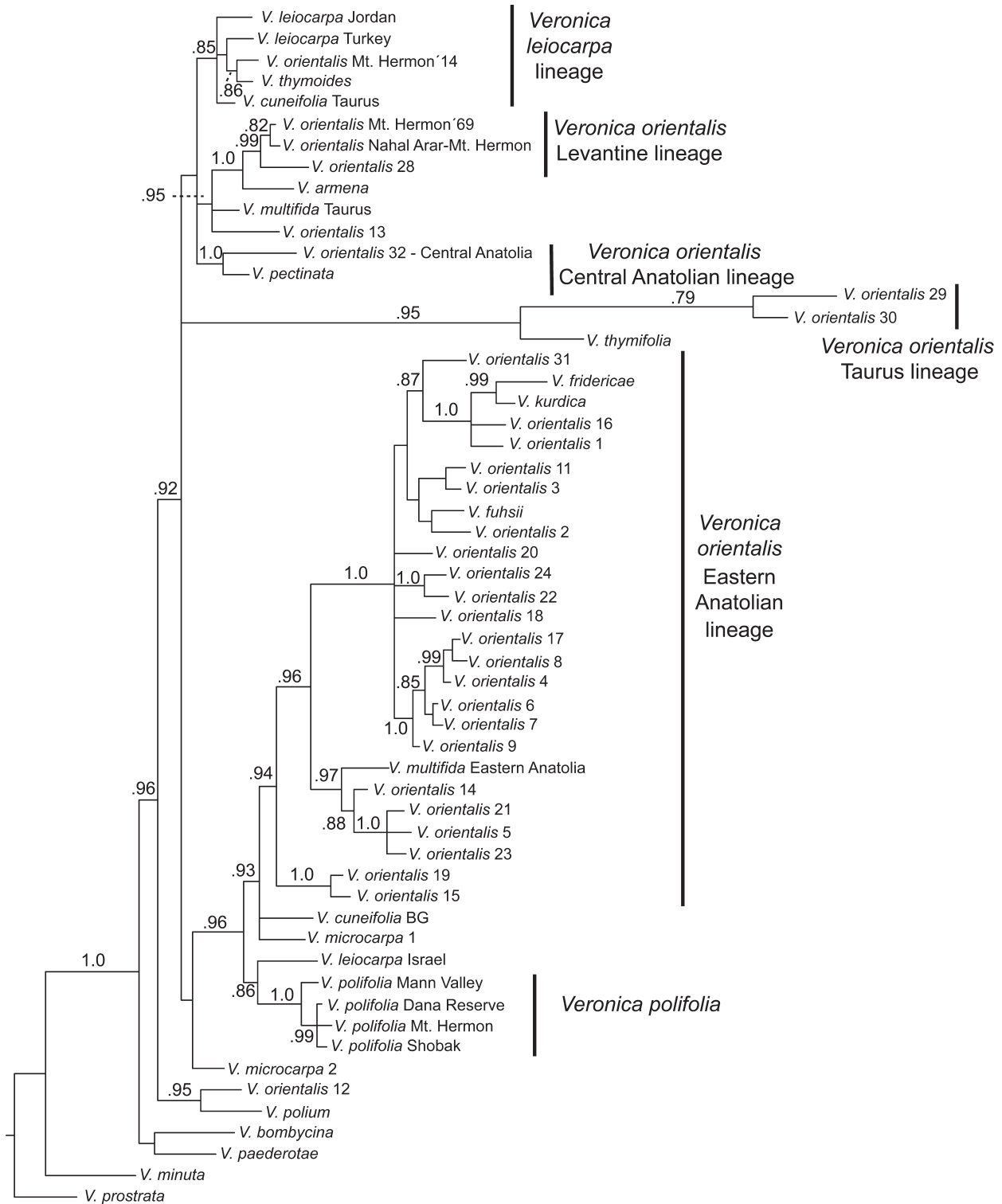


Fig. 2. Strict consensus of post-burn-in Bayesian trees. Posterior probabilities of above 0.8 are given on the branches. Simple numbers following *Veronica orientalis* refer to sample numbers used in Sonibare & al. (2014).

Veronica orientalis is polyphyletic on the broad scale. While the Bayesian analysis suggests at least six different haplotype lineages (Fig. 2), the median-joining network suggests five different lineages (Fig. 3). However, closer examination suggests that *V. orientalis* 15, 19, *V. cuneifolia* D. Don BG and *V. microcarpa* Boiss. are in-

termediate between *V. polifolia* and the Eastern Anatolian lineage (Fig. 2 & 3), and therefore may still form part of that lineage based on the strong support in the Bayesian analysis. Specimen *V. orientalis* 12 is closely related to *V. polium* P. H. Davis in the Bayesian analysis but is part of the Eastern Anatolian lineage in the network. In con-

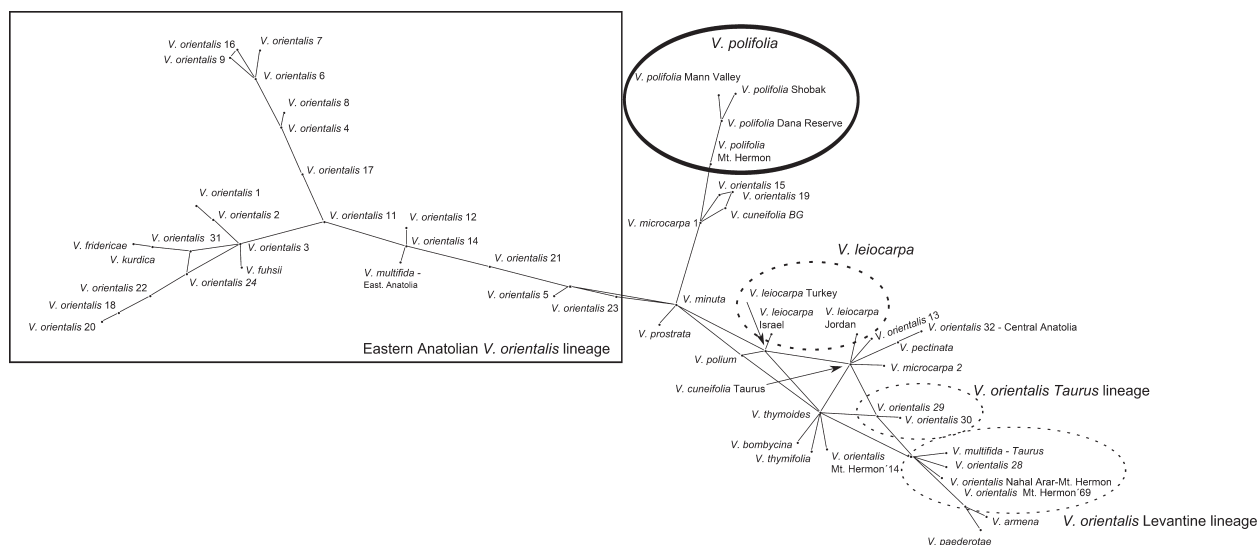


Fig. 3. Median-joining network of the plastid DNA dataset. Simple numbers following *Veronica orientalis* refer to sample numbers used in Sonibare & al. (2014).

trast, both analyses support the clear distinction between the remaining samples from further west and south and those in E Anatolia (Fig. 2 & 3). Within those, the sample from Ankara province in NC Anatolia (*V. orientalis* 13; Sonibare & al. 2014) is clearly distinct from the rest, in which those from the E Taurus Mountains are well separated from those further south in the Levant, in which the sample Mt Hermon '14 stands out from the rest. Thus, in a consensus, we can divide the *V. orientalis* haplotypes into four biogeographically defined lineages: a Central Anatolian, an Eastern Anatolian, a Taurus and a Levantine lineage. While we know that the Eastern Anatolian lineage includes multiple ploidy levels (Sonibare & al. 2014), only the octoploid level is known for the Levantine lineage, which has not been found from E Anatolia, yet. Since Pazy (2000) also investigated a specimen from Mt Hermon, our genome size estimate confirms the existence of an octoploid lineage of *V. orientalis* in the area but does not exclude other ploidy levels in other areas of the Levantine lineage.

The results have important taxonomic implications. In the description of *Veronica orientalis*, Miller (1768) states that *V. orientalis* occurs naturally in the Levant but without further details on the origin of its type. A photo of a syntype from BM has been published by Riek (1935; also in herbarium Edinburgh E00326070). It does not allow further identification on its origin. Nevertheless, the note by Miller suggests that the Levantine lineage should have priority for the name *V. orientalis* if further analyses strengthen the separation of the lineages at the species level.

Implications for biogeography of the Levant

Despite their genetic, morphological, ecological and cytological differences, the three taxa inhabit a roughly

similar distribution area when considering the phylogeographic results above, suggesting a possible similar recent history. *Veronica polifolia*, *V. leiocarpa* and the Levantine lineage of *V. orientalis* inhabit an area ranging from S Turkey to S Jordan in Mediterranean-type habitats along the SW margin of the Irano-Turanian region sensu Takhtajan (1986). The Seir/Sharah Mountains form the S range margin for a number of species of Mediterranean and Irano-Turanian origin (Al-Eisawi 1996). The vegetation type in the area is generally pine forest or Mediterranean vegetation with degraded *Pistacia atlantica-Juniperus phoenicea* woodland (Al-Eisawi 1996; Danin 1999b). The three species grow at their S range margin in rocky outcrops at sites usually drier than populations of the same species further north, which can be explained by either the higher water-carrying capacity of the underlying sandstone (Danin 1999a) or the higher run-off in crevices at these sites (Danin 2008). The Queen Alia forest population from Shobak, reported here for the first time, is the southernmost known locality of *V. polifolia* and is also the southernmost locality for other species (Al-Gharaibeh in Greuter & Raus 2011). The species was in recent years found about 30 km north in the Dana Reserve in dwarf shrub formations (Danin 1997), but was not known from Jordan before that time. It may occur more widely in the Seir/Sharah Mountains, but it is equally likely that it is only found in the most secluded parts of these highlands as remnants of a vegetation adapted to a wetter climate. Also, localities further north occur in places known to be rich in endemics (Mt Hermon, Zohary 1973; Dana reserve, Shmida 1984). Thus, it is likely that the three species occupy remnants of a Mediterranean vegetation more widespread in the Levant during moister periods of the Pleistocene (Danin 1999a, b). Such a wider distribution has also been suggested for Irano-Turanian elements in the Levant in gen-

eral, based on their adaptation to tolerate wide diurnal and annual temperature fluctuations but not continuously warm climate (Danin 1999b). Zohary (1973) considered the immigration of Irano-Turanian elements into the area in the Pleistocene as most plausible, which would also give our scenario a reasonable timeline. Dated phylogeographic analyses suggest a range of ages, between 0.22–1 mya for pine processionary moths (Simonato & al. 2007) and around 2.5 mya in tree frogs (Gvoždík & al. 2010), for the separation of Levantine and Anatolian lineages.

Thus, we are left with the question whether our three species represent Mediterranean elements with affinities to plants in W Anatolia or Irano-Turanian elements with closer relationship to E Anatolian taxa. Support for either can be found in the phylogenetic analyses. Irano-Turanian elements such as the Eastern Anatolian lineage of *Veronica orientalis* show a closer relationship to *V. polifolia*, *V. thymoides* P. H. Davis to *V. leiocarpa* and *V. armena* to the Levantine lineage of *V. orientalis* (Fig. 2 & 3). However, similarly, Mediterranean elements such as *V. cuneifolia* show close affinities to *V. leiocarpa*, while *V. multifida* from the Taurus is allied to the Levantine lineage of *V. orientalis*.

Considering the relationship of the Levantine species to related species, the clear separation of these Levantine lineages from Anatolian *Veronica* suggests separation along some biogeographic border in former times. While many studies have been published for taxa occurring in either Anatolia or the Levant (Arafeh & al. 2002; Bilgin 2011; Comes & Abbott 1999), few studies of plants have investigated species across this area. More studies have been published on animals. Distinct Anatolian and Israeli lineages have been found, for example, in hedgehogs but sampling does not allow more fine-scale determination of the phylogeographic boundary (Seddon & al. 2002), and a similar pattern is found in mice (Macholán & al. 2007), snake-eyed lizards (Kyriazi & al. 2008) and terapins (Fritz & al. 2008), where a suture zone has been found near the Bay of Iskenderun. Separation of lineages in a N-to-E Anatolian and a S Anatolian-Levantine lineage was also detected in oriental lizards (Ahmadzadeh & al. 2013) with a similar border line between samples 120 km distant E of the Amanus Mountains and across the Amik plain. The exact barrier is difficult to detect and may be species-specific. In the pine processionary moth it seems to be the Amanus Mountain range separating two haplotype lineages over less than 40 km with suggested different refugia in the E Taurus Mountains and the Lebanon Mountains (Simonato & al. 2007). Such a border, however, may be gene-specific as seen in the analysis of tree frogs (Gvoždík & al. 2010), in which the border between a W Anatolian and a Levantine-C Anatolian lineage runs sharply near the Bay of Iskenderun in mitochondrial DNA but in different parts of Anatolia with nuclear genes. However, similar to our analysis, the suture zone runs in a north-south direction from the Bay

of Iskenderun, forming a distribution area that extends from the Levant S and E of the Taurus range and the Anatolian diagonal.

In conclusion, we find support here for the recognition of *Veronica orientalis*, *V. leiocarpa* and *V. polifolia* as distinct species in the Levant. The recognition is also supported by morphological, ecological and karyological characters. All three species appear to present Levantine lineages separated from close relatives to the north since the Pleistocene, which did not, yet, lead to lineage sorting. Alternatively, post-speciation gene flow or multiple origins prevented the monophyly of the species until now. Apart from the separation into these southern lineages, our results do not allow us to distinguish between a W Anatolian-Mediterranean and an E Anatolian-Irano-Turanian origin of the species. Finally, undoubtedly, the genetic material from the southernmost distribution area of *V. leiocarpa* and *V. polifolia* is a basic component for any further investigation on systematics and biogeography of *Veronica*. Here, we highly recommend the ex situ and in situ conservation of the remaining populations in Jordan.

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