

## Systematic Significance of Seed Morphology in *Veronica* (Plantaginaceae): A Phylogenetic Perspective

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• **Background and Aims** A new infrageneric rearrangement for *Veronica* has been proposed based on the most recent evidence from DNA sequence data, morphological evidence, and biogeographical considerations. Looking for morphological synapomorphies for each monophyletic subgenus has been problematic, due to difficulties arising from widespread homoplasy (mainly parallel evolution). In an attempt to overcome these difficulties, previously underexplored morphological characters are starting to be studied in more depth.

• **Methods** A molecular phylogenetic hypothesis was used based on sequences of ITS (nuclear ribosomal DNA) and plastid *trnL-F* regions, as a framework to test the use of seed coat ultrastructure (studied under scanning electron microscope) in the systematics of the genus. A sample of 132 taxa representing ten of the 13 subgenera in *Veronica*, excluding the species of the southern hemisphere *Hebe* complex and the exclusively North American subgenus *Synthyris*, was studied.

• **Key Results and Conclusions** The results demonstrate that, in many cases, the ultrastructure of the testa can be employed to assess relationships of taxa within the genus, and the character provides additional support for molecular trees. Further characters relevant for the classification of *Veronica*, i.e. base chromosome number, iridoid chemical data, life cycle, inflorescence position, have been taken into consideration in a discussion where an attempt is made to highlight the best traits to characterize each subgenus investigated.

**Key words:** Plantaginaceae, *Veronica*, seed coat, ITS, *trnL-F*, phylogeny, systematics.

### INTRODUCTION

One common result of recent DNA investigations has been the discovery of many, large and grossly paraphyletic genera within which several smaller genera are nested. An example of this is *Veronica* and tribe Veroneae [formerly part of Scrophulariaceae and now, following APG (1998, 2003), within an enlarged Plantaginaceae]. Based on the most recent evidence from DNA sequence data (three DNA regions, both nuclear and plastid), karyological and morphological evidence, as well as biogeographical considerations, a new infratribal and infrageneric rearrangement for the Veroneae and for *Veronica* has been proposed (Albach *et al.*, 2004a). Nine monophyletic genera are recognized in the tribe and 13, also monophyletic, subgenera within a broadly circumscribed genus *Veronica*, in which up to ten genera have been found to be nested.

Morphological synapomorphies, especially for each subgenus (or clade) in *Veronica s.l.*, are difficult to find, due to difficulties arising from widespread homoplasy, mainly parallel evolution (Albach *et al.*, 2004a–c). In an attempt to overcome these difficulties, previously underexplored characters are being studied in more depth. That is the case of phytochemical characters whose importance had been already emphasized by the previous works of Grayer-Barkmeijer (1973, 1978, 1979) and Taskova *et al.* (1998, 2002). Taskova *et al.* (2004) have demonstrated the correlation between nine clades in

*Veronica* and specific patterns of iridoid glucosides. Flavonoid compounds (Albach *et al.*, 2005a, b) and verbascoside-like compounds (Jensen *et al.*, 2005) have also been shown to be chemotaxonically useful in the delimitation of major infrageneric clades in *Veronica*.

Similarly, both macro- and micromorphological characters of the seed were shown to be of essential systematic importance within *Veronica* and, therefore, have been studied by several authors: Riek-Häußermann (1943), Thieret (1955), Yamazaki (1957), Kulpa (1968), Hong (1984) and Juan *et al.* (1994). Later, Martínez-Ortega (1999) and Martínez-Ortega and Rico (2001), working with the perennial species of *Veronica*, demonstrated a correlation between specific patterns of seed coat ornamentation, base chromosome number and the traditional subsections recognized in the genus [so called ‘natural groups’ by some authors or, in German, and especially after Römpf (1928), ‘Verwandschaftsgruppen’]. Nevertheless, no correspondence was found between such characters and the sections in *Veronica* (mainly based on life cycle, position of the inflorescence, i.e. stem ending in an inflorescence or in a vegetative shoot, and general shape of the seed).

The aim of the present study is to investigate whether the ultrastructure of the testa [as seen under a scanning electron microscope (SEM)] can be used as an additional support for molecular trees, i.e. to extend the previous study of Martínez-Ortega and Rico (2001), in order to check whether fine seed sculpture, combined with base chromosome number, phytochemical characters and life

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cycle among other relevant characters, can provide additional phylogenetic information in *Veronica*. The intention is to look for additional characters that can be used to describe subgenera and support clades, as well as to test whether the classification proposed by Albach *et al.* (2004)—mainly based on DNA-sequence data—is sustainable once more taxa and more characters are studied.

## MATERIALS AND METHODS

### Seed morphology

Mature seeds from 132 *Veronica* belonging to ten of the 13 subgenera in *Veronica* have been studied. *Veronica* subgen. *Hebe*, *V.* subgen. *Derwentia* and *V.* subgen. *Synthyris* are excluded: the first two were not formally included in the classification of Albach *et al.* (2004), pending publication of a forthcoming monograph by M. Bayly and A. V. Kellow; and the data for *Synthyris* and *Besseyia* (both merged into *Veronica* in *V.* subgen. *Synthyris*) by Hufford & McMahon (2004) are, unfortunately, not comparable with the present data.

All seeds were either collected in the field or obtained from herbarium specimens. Of the studies of the seeds mentioned in the Introduction plus two additional modern ones (Aseyeva, 2002; Hufford and McMahon, 2004), only four (Hong, 1984; Juan *et al.*, 1994; Martínez-Ortega, 1999; Martínez-Ortega and Rico, 2001) provide comparable SEM data, that can be used in the present study. The list of the material studied, with information about voucher specimens or data source is shown in Appendix 1.

Macromorphological observations were carried out under a stereoscopic microscope (SM). For SEM, dry seeds were treated in two different ways. Some of them were soaked in a 1 : 1 solution of chloroform and methanol for 48 h, dehydrated through an ethanol series (70, 90 and 100 %) and finally treated with xylene for 3 d with the object of detecting and removing wax deposits. The rest of the dry seeds were just directly mounted on stubs using double-sided adhesive tape. The samples were coated with gold palladium in a BALZER SCD040 ion-sputter and then observed by standard techniques using a JSM T 330 A (JEOL) and a DSM 940 (ZEISS) or a LEO 1440 (LEO GmbH, Oberkochen, Germany) microscope.

The terminology used to describe seed coat surface sculpturing follows mainly Font Quer (1953), Stearn (1992), Juan *et al.* (1994) and Martínez-Ortega and Rico (2001).

Taxonomic concepts for species or subspecies follow current floras (Kemularia-Nathadze, 1952; Borissova, 1955; Hartl, 1966; Grossheim, 1967; Walters and Webb, 1972; Fischer, 1978; Fischer, 1981, 1991; Hong and Fischer, 1998; Martínez-Ortega *et al.*, 2007; among others). Circumscription of subgenera follows the system by Albach *et al.* (2004a). Assignment of species to subgenera is indicated (by means of different colours) in Fig. 1.

Voucher specimens are deposited in the following herbaria: B, BC, BM, BONN, GOET, K, MA, P, RNG, SALA, SEV, TI, VANF, W and WU.

### Molecular analyses

From the sample of 132 taxa of *Veronica*, which have been sampled for seed SM and SEM, 86 taxa have been sequenced for either ITS (16 taxa) or the *trnL-F* region (eight taxa) or both (62 taxa) (Appendix 2). Of these sequences, 22 ITS-sequences and 14 *trnL-F* sequences are reported here for the first time. DNA from the same specimen was used for both DNA regions, except for *V. abyssinica* and *V. glauca*. Voucher specimens for all plants and GenBank accession numbers for all sequences used in this study are presented in Appendix 2.

**DNA extraction, amplification and sequencing.** The *trnL* intron, 3'exon, and *trnL-F* spacer (hereafter *trnL-F*) were amplified with primers c and f of Taberlet *et al.* (1991). ITS sequences were amplified and sequenced using the primers 17SE (Sun *et al.*, 1994) and ITS4 (White *et al.*, 1991). PCR products were run on a 1.0 % TBE-agarose gel, cut out of the gel, and cleaned using QIAquick™ PCR purification and gel extraction kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocols. Sequencing reactions (10 µL) were carried out using 1 µL of the Taq DyeDeoxy Terminator Cycle Sequencing mix (Applied Biosystems Inc.). Reactions were run on a Prism 377 automated sequencer (Applied Biosystems Inc.), and both strands were sequenced.

All sequences were assembled and edited using Sequence Navigator™ (Applied Biosystems Inc.). Assembled sequences were manually aligned prior to analysis following the recommendations of Kelchner (2000). Aligned sequence matrices are available from D. C. Albach upon request.

**Sequence analysis.** Insertions and deletions are frequent in both DNA regions. In a conservative approach, gaps were scored as missing data, thus removing them from the analysis. Visual inspection of the data matrix revealed only a few potentially informative gap characters that did not support clades supported by base substitutions. Both molecular datasets have been combined without further incongruence testing, which has been done in previous analyses showing that the results for all molecular datasets within *Veronica* are generally congruent (Albach and Chase, 2004; Albach *et al.*, 2004b). Those analyses showed that incongruence between analyses of ITS and *cpDNA* regions in *Veronica* is due to the lack of phylogenetic signal rather than different underlying phylogenetic history of the two DNA regions. *Wulfenia carinthiaca* Jacq. was used as an outgroup.

Matrices were analysed with PAUP\* 4.0b10 (Swofford, 1998) using heuristic parsimony methods. Five runs of random taxon addition (ten replicates, starting from random trees) using tree bisection reconnection (TBR) were conducted with MulTrees (keeping multiple shortest trees) in effect and a tree limit of 200 trees. Trees from these analyses were saved and used as starting trees in a second round of analyses with the same conditions but no tree limits. Bootstrap percentages were assessed using 500 replicates and SRP-branch swapping with a maximum of 50 trees per replicate.

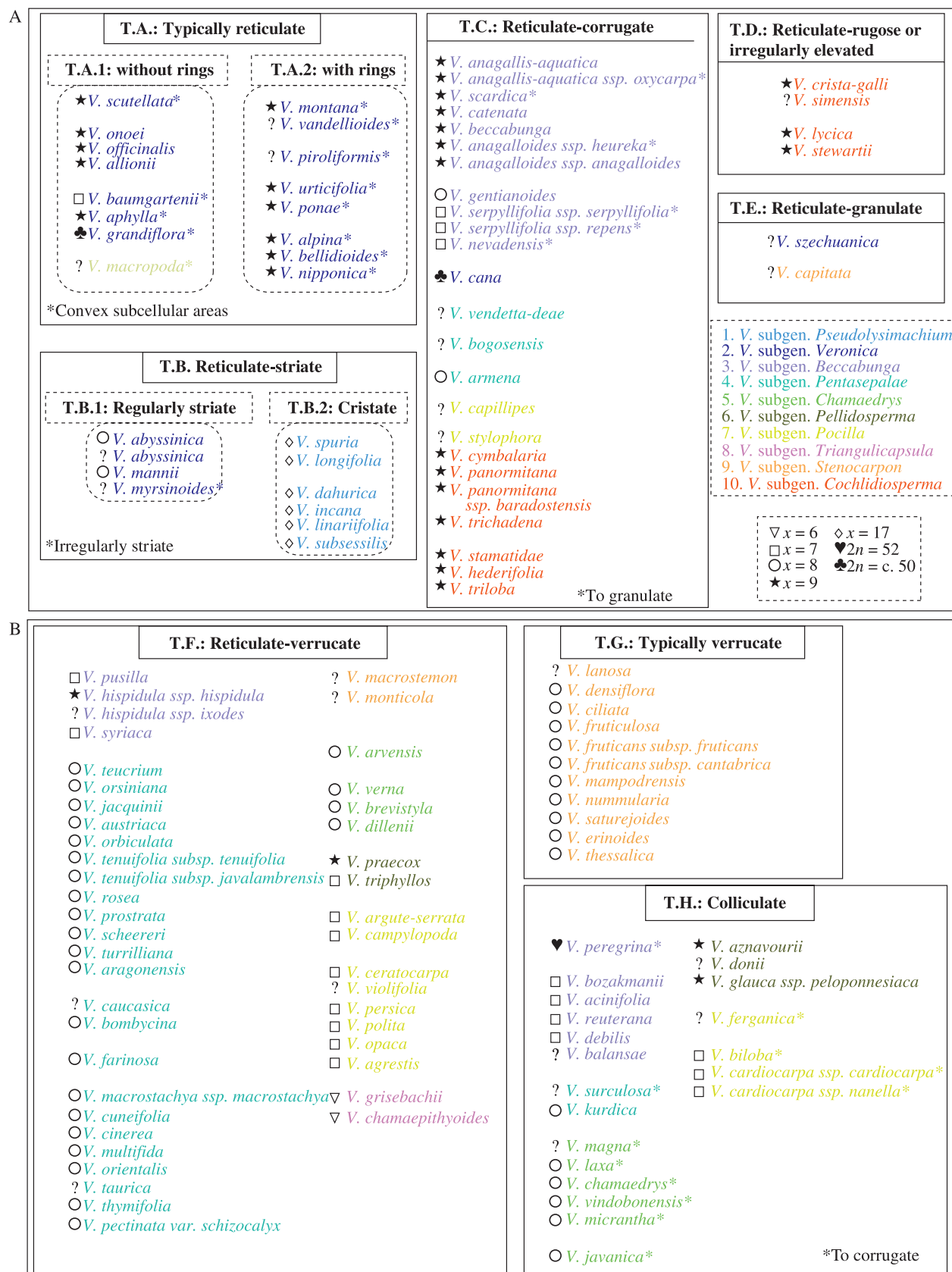


FIG. 1. Seed coat patterns in *Veronica*. Colours indicate adscription of species to subgenera. T.A.1, Typically reticulate, without rings; T.A.2, typically reticulate, with rings; T.B.1, reticulate-striate, regularly striate; T.B.2, reticulate striate, cristate; T.C., reticulate-corrugate; T.D., reticulate-rugose or irregularly elevated; T.E., reticulate-granulate; T.F., reticulate-verrucate; T.G., typically verrucate; T.H., colliculate.

For the comparison of seed coat types with a DNA sequence-derived phylogenetic hypothesis, taxa sampled for seed characters but not sequenced for either the ITS or the *trnL-F* region have been added to the tree (Fig. 2), based on their phenotypic affinity in those cases in which closely related taxa have been sampled in analyses of molecular data. Nevertheless, there are several enigmatic species, which the authors currently feel unable to place in a phylogeny based on their present knowledge of these species. This applies to *V. simensis*, *V. javanica* and *V. violifolia* in the present study.

Seed coat types have been mapped on the resulting tree using MacClade 4.0 (Maddison and Maddison, 2001) and a distinction between annuals and perennials has been made. Also, an indication of the chromosome base number for each subgenus has been included.

## RESULTS

Results from studies under SM are presented (transection and ornamentation; only original data) in Appendix 1.

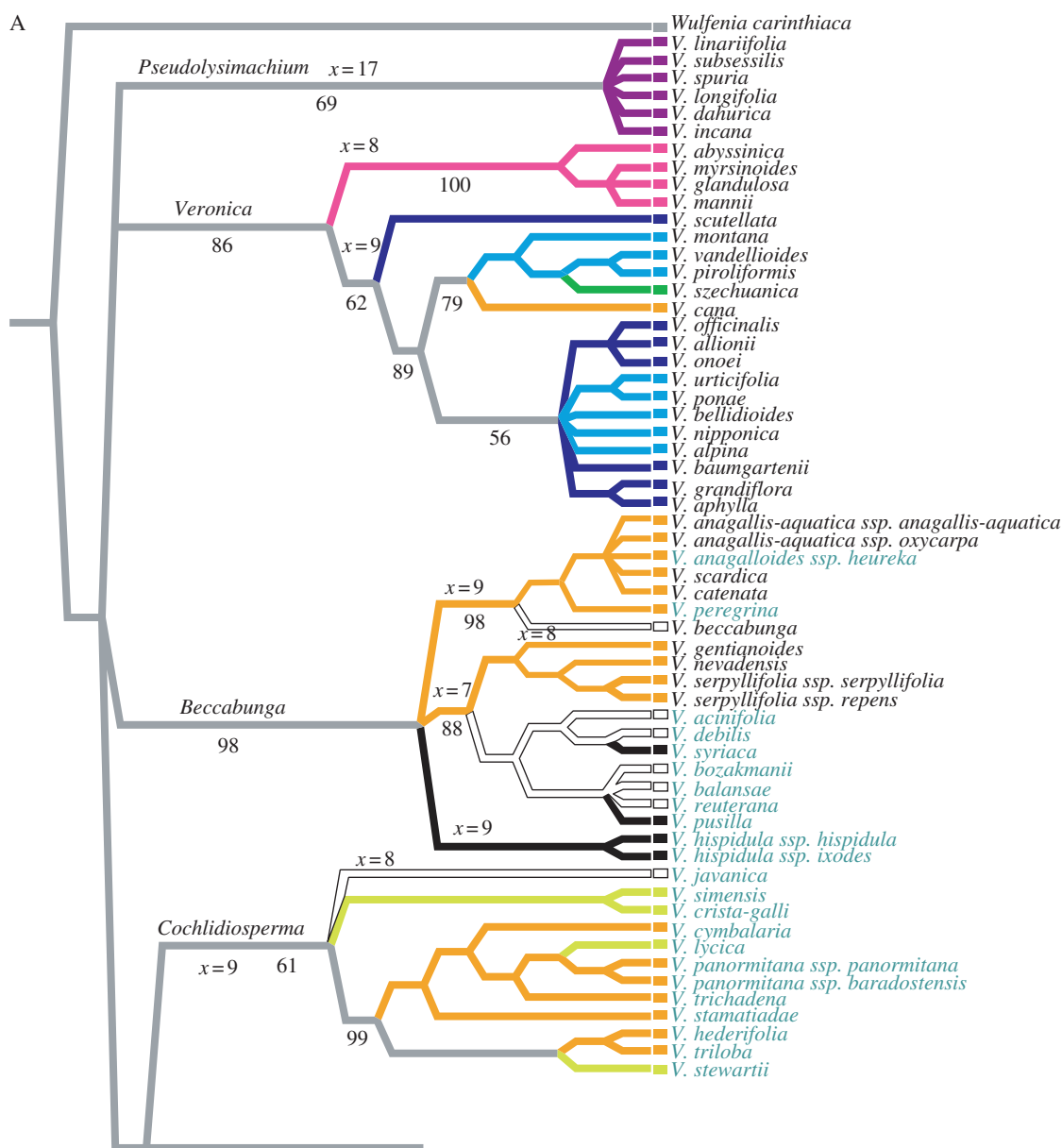


FIG. 2. Seed coat types mapped on the DNA sequence-derived phylogenetic tree (strict consensus), with an indication of chromosome base number for each subgenus. Names corresponding to annual taxa are written in blue, perennials in black. Numbers below the branches indicate bootstrap percentages for each major clade. (A1) Typically reticulate without rings; (A2) Typically reticulate with rings; (B2) reticulate-striate, regularly striate; (B1) reticulate-striate, irregularly striate; (C) reticulate-corrugate; (D) reticulate-rugose or irregularly elevated; (E) reticulate-granulate; (F) reticulate-verrucate; (G) typically verrucate; (H) colliculate. ■ A1; ■ A2; ■ B1; ■ B2; ■ C; ■ D; ■ E; ■ F; ■ G; ■ H; ■ Equivocal.

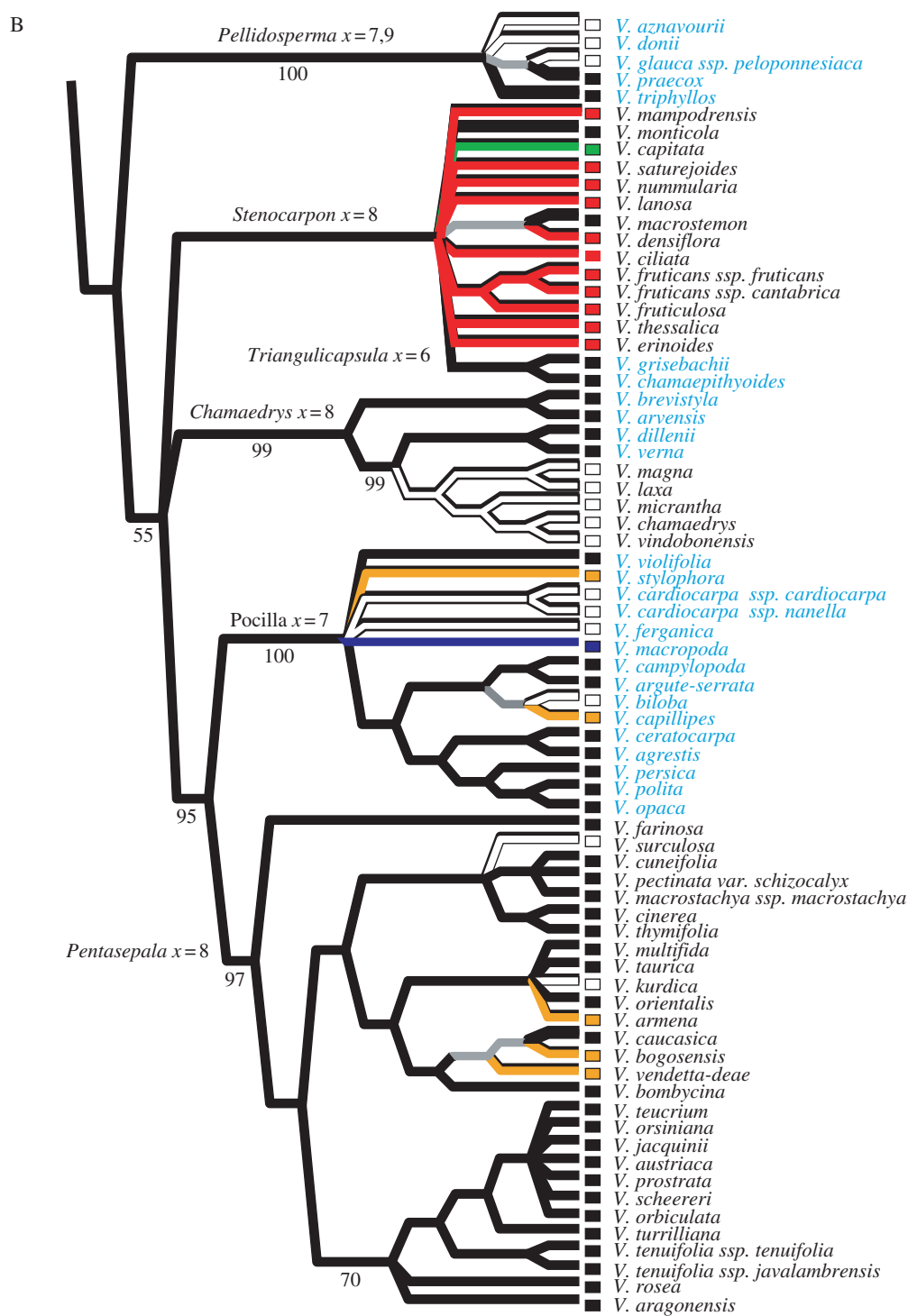


FIG. 2. Continued.

The most important variations detected by SEM are shown in Fig. 3. These are summarized as the following types: flat, plano-convex with or without a keel, cymbiform (several variations within it, i.e. smooth, cristate, brain-like), subcyathiform smooth and cyathiform, either smooth or cristate.

Measurements are not reported, as most of them can be found in Kulpa (1968) and Martínez-Ortega and Rico (2001) and, as far as is known, they do not provide phylogenetic information.

Several patterns of seed coat sculpture have been described in accordance with differences in outer cell



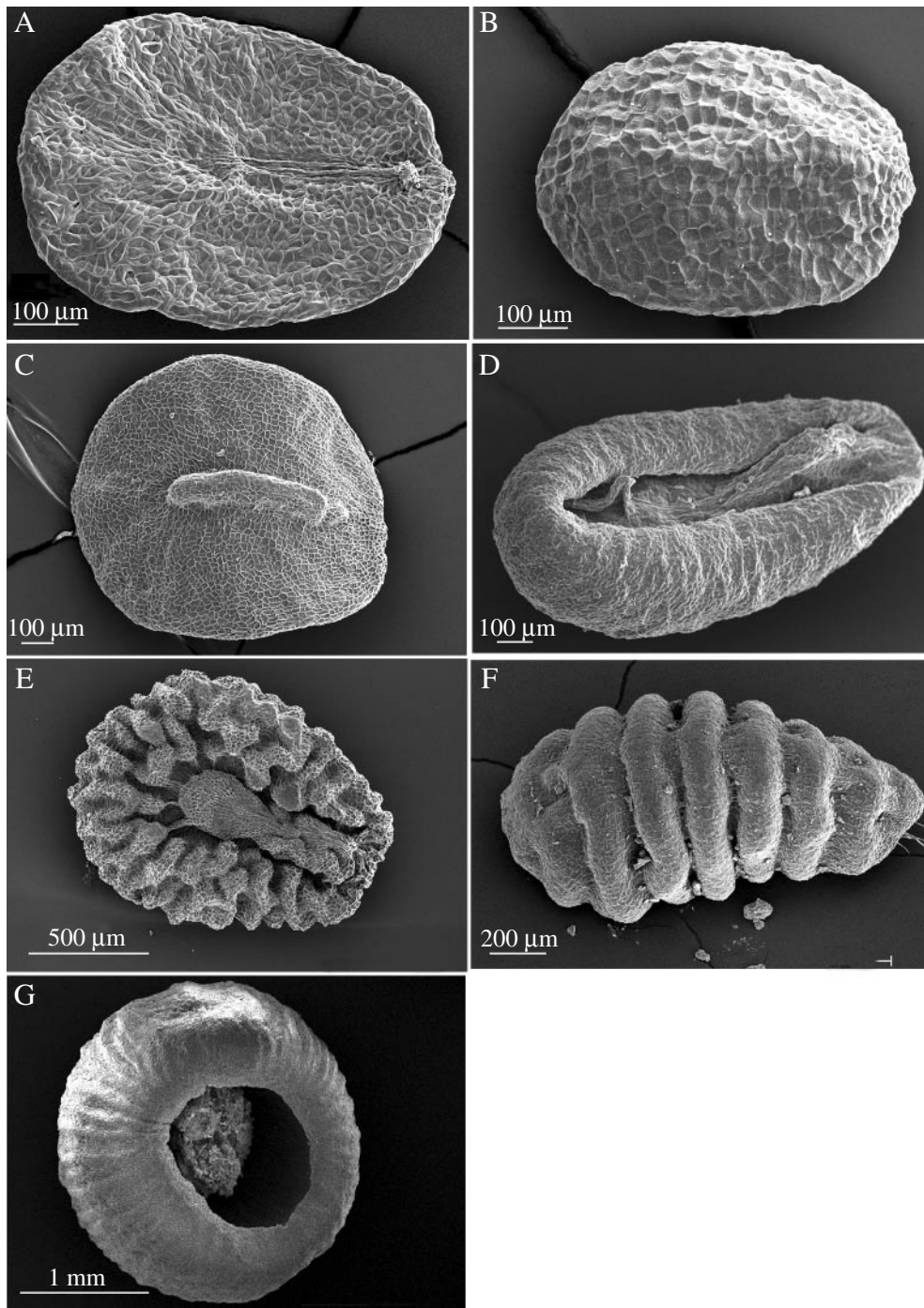


FIG. 3. Seeds in *Veronica*. General shape: (A) *V. alpina*, flat,  $\pm$  smooth; (B) *V. anagallis-aquatica* ssp. *oxycarpa*, plano-convex;  $\pm$  smooth; (C) *V. linariifolia*, plano-convex, with keel; (D) *V. vendetta-deae*, cymbiform; (E) *V. simensis*, cymbiform, brain-like; (F) *V. campylopoda*, cymbiform, cristate; (G) *V. stamatidae*, cyathiform, cristate.

morphology under SEM. The results are summarized in Fig. 1, along with basic chromosome numbers and the subgeneric assignments (following Albach *et al.*, 2004a). Some variation in the depth, thickness and ornamentation of radial and tangential walls exist. According to Juan *et al.* (1994) and also the present observations, these structures in the tangential walls are apparently elaborations of the wall lignifications rather than wax deposits.

Eight general sculpturing patterns, each of them sometimes divided into subtypes have been described as follows.

*Type A: Typically reticulate seed coat pattern*

Shallow to medium depth radial walls. Tangential walls commonly smooth, but sometimes a little rugose,

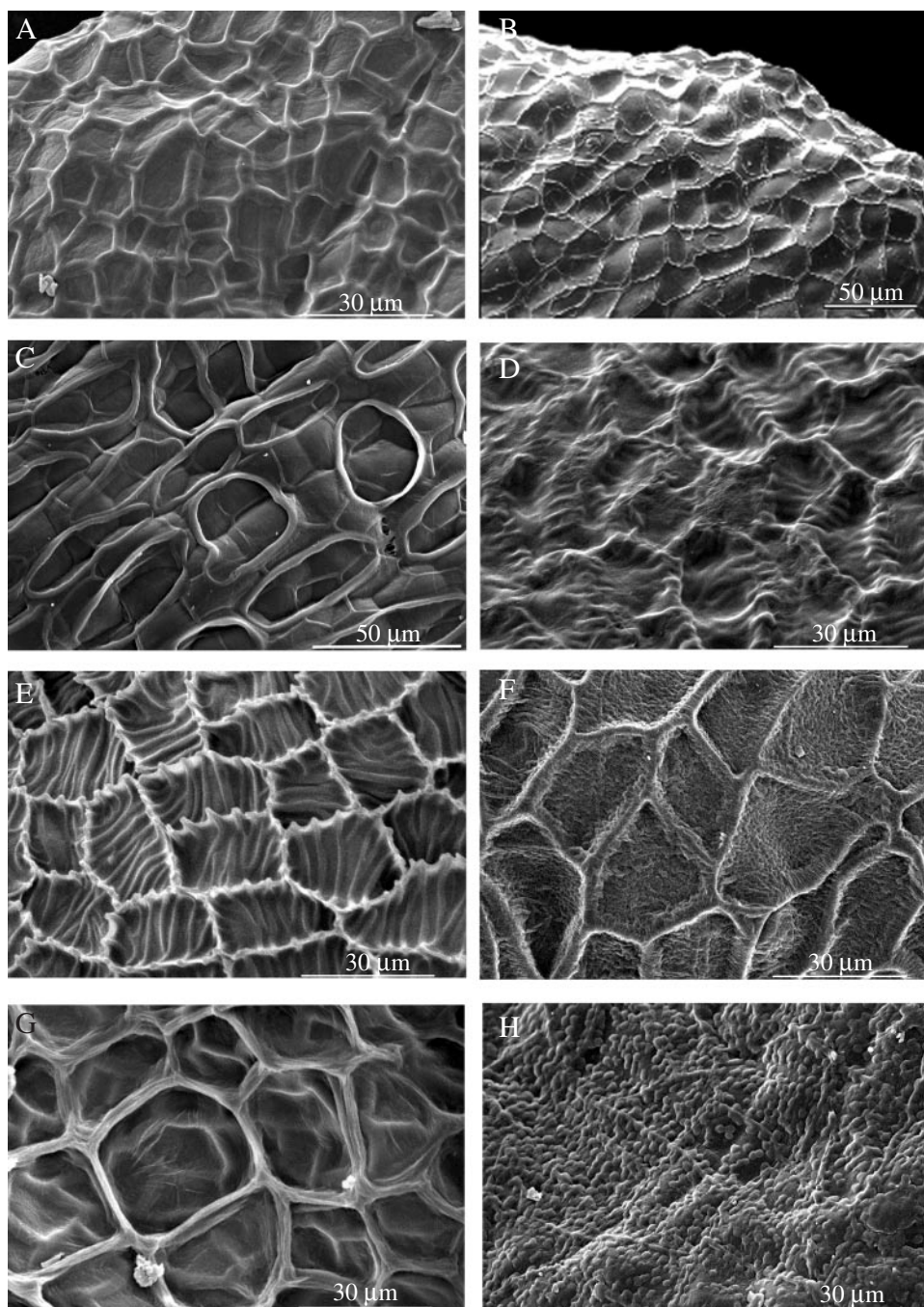


FIG. 4. Seeds in *Veronica*. Testa surface: (A) *V. onoei*; (B) *V. ponae*; (C) *V. alpina*; (D) *V. glandulosa*; (E) *V. spuria*; (F) *V. gentianoides*; (G) *V. simensis*; (H) *V. capitata*.

sometimes (marked with an asterisk after the species name in Fig. 1) with subcellular areas convex. Type A is found mostly in species of *V.* subgen. *Veronica* with the chromosome base number  $x = 9$  (except for *V. baumgartenii*), plus *V. macropoda* from *V.* subgen. *Pocilla*. Two subtypes have been distinguished:

*Subtype A.1.* Typically reticulate (Fig. 4A), as described in the previous paragraph.

*Subtype A.2.* Subcellular or supracellular pattern formed by rings, irregular in outline and, respectively, included

inside the perimeter of one single cell (Fig. 4B), or taking in more than one cell (Fig. 4C).

#### *Type B: Reticulate-striate seed coat pattern*

The testa is here quite regularly reticulate (only exceptionally irregular), formed by more or less polygonal cells with radial walls of medium depth, irregular thickness and usually striate. Tangential walls are also



striate. The seeds are planoconvex. Two subtypes have been distinguished:

**Subtype B.1.** Typically reticulate-striate (Fig. 4D), as described above. In this case the striation is usually continuous between one cell and the adjacent, therefore, the intercellular boundaries are crossed by the striation. Exceptionally (only in *V. myrsinoides*, marked with an asterisk after species name in Fig. 1) the striation forms an irregular network superimposed on the basal reticulum; thus, the coat has an irregularly reticulate overall appearance. This pattern is exclusive for the African species of *V. subgen. Veronica*. The members of this group have a base chromosome number, if known, of  $x = 8$ .

**Subtype B.2.** Reticulate-striate, cristate (Fig. 4E). The radial walls are in this case longitudinally more deeply striate than in subtype B.1. Additionally, the striation is not continuous between one cell and the adjacent; therefore, the radial walls appear cristate on their upper (external) ends. This pattern is exclusive of *V. subgen. Pseudolysimachium*. The chromosome base number in this subgenus is also exclusively  $x = 17$ .

*Type C: Reticulate-corrugate seed coat pattern (Fig. 4F)*

Shallow to medium depth radial walls, not uniformly thickened, sometimes reinforced by a striation or network (rarely with irregular thickenings on cell angles). Tangential walls regular or irregularly corrugate, irregularly compressed or sometimes slightly granulated (marked with an asterisk after the name of the taxon in Fig. 1). This type is widely represented appearing in *V. subsect. Serpyllifoliae* (chromosome base number  $x = 7$ ) and *V. subsect. Beccabunga* (chromosome base number  $x = 9$ ), as well as *V. gentianoides* (chromosome base number  $x = 8$ ), all of *V. subgen. Beccabunga*, in the greater part of *V. subgen. Cochlidiosperma* (chromosome base number  $x = 9$ ), in *V. cana* an Asian representative of *V. subgen. Veronica*, in three representatives from *V. subgen. Pentasepalae* (*V. vendetta-deae*, *V. bogosensis* and *V. armena*) and in two species from *V. subgen. Pocilla*.

*Type D: Reticulate-rugose or irregularly elevated seed coat pattern (Fig. 4G)*

The pattern is basically a reticulate one, with the tangential cell walls rugose or, sometimes, irregularly elevated. Radial walls extend to a medium depth. This pattern is restricted to the remainder of *V. subgen. Cochlidiosperma* (chromosome base number  $x = 9$ ), and particularly to *V. subsect. Diplophyllum*, which is frequently segregated from *Veronica* as an independent genus, plus *V. lycica* and *V. stewartii* from the same subgenus.

*Type E: Reticulate-granulate seed coat pattern (Fig. 4H)*

With tangential walls granulated and shallow radial walls, frequently also granulated. As far as is known, this

particular pattern is restricted to two Asiatic species belonging to very different subgenera (*V. szechuanica* from *V. subgen. Veronica* and *V. capitata* from *V. subgen. Stenocarpon*). The chromosome number of both species is unknown.

*Type F: Reticulate-verrucate seed coat pattern (Fig. 5A)*

Polygonal cells of variable sizes, with radial walls irregularly thickened and from medium depth to shallow, defining a clear basal reticulum. Tangential walls with a central or sometimes excentric amorphous wart. As far as is known, this is the most common pattern in *Veronica*, present in seven subgenera. All representatives of *V. subgen. Pentasepalae* except for five (*V. vendetta-deae*, *V. bogosensis* and *V. armena* of seed type C; *V. surculosa* and *V. kurdica* of seed type H) have this type of seed coat. Additionally, all annual representatives of *V. subgen. Chamaedrys*, again with  $x = 8$ , have a seed coat of this type. The same type also occurs in representatives of *V. subgen. Triangulicapsula*, but these have a unique chromosome base number in the genus of  $x = 6$ . A seed with a testa of this type is also represented in the subgenera: *V. subgen. Stenocarpon* ( $x = 8$ ), *V. subgen. Pocilla* ( $x = 7$ ), *V. subgen. Beccabunga* ( $x = 7$  or  $9$ ) and *V. subgen. Pellidosperma* ( $x = 7, 9$ ).

*Type G: Typically verrucate seed coat pattern (Fig. 5B)*

Obscure radial walls or the walls shallow and regularly thickened. Tangential walls with an amorphous or irregularly striate wart. The pattern is exclusive for *V. subgen. Stenocarpon* with a base chromosome number  $x = 8$ . From the species in this subgenus investigated, only three (*V. capitata*, *V. macrostemon* and *V. monticola*) have seeds with other coat patterns.

*Type H: Colliculate seed coat pattern*

Obscure or sometimes shallow radial walls. This seed coat pattern is typically colliculate Fig. 5C, but sometimes a tendency to present corrugations exists (marked with an asterisk after taxon name; Fig. 5D). This type is also a widely represented one. All of the perennial representatives in *V. subgen. Chamaedrys* (chromosome base number  $x = 8$ ) have a seed coat of this type. It appears also in the following subgenera: *V. subgen. Pocilla* ( $x = 7$ ), *V. subgen. Beccabunga* (only in some taxa with a chromosome base number  $x = 7$ , plus *V. peregrina*  $2n = 52$ ) and *V. subgen. Pellidosperma* (only taxa with a chromosome base number  $x = 9$ ), plus in two species (*V. surculosa* and *V. kurdica*) from *V. subgen. Pentasepalae*.

As regards molecular data, the analysis of the two DNA regions included 1882 aligned characters (1114 from the *trnL-F* region, 768 from the ITS region), 491 potentially parsimony-informative (250 from the *trnL-F* region, 241 from the ITS region). Due to the large number of most-parsimonious trees, heuristic searches were run with



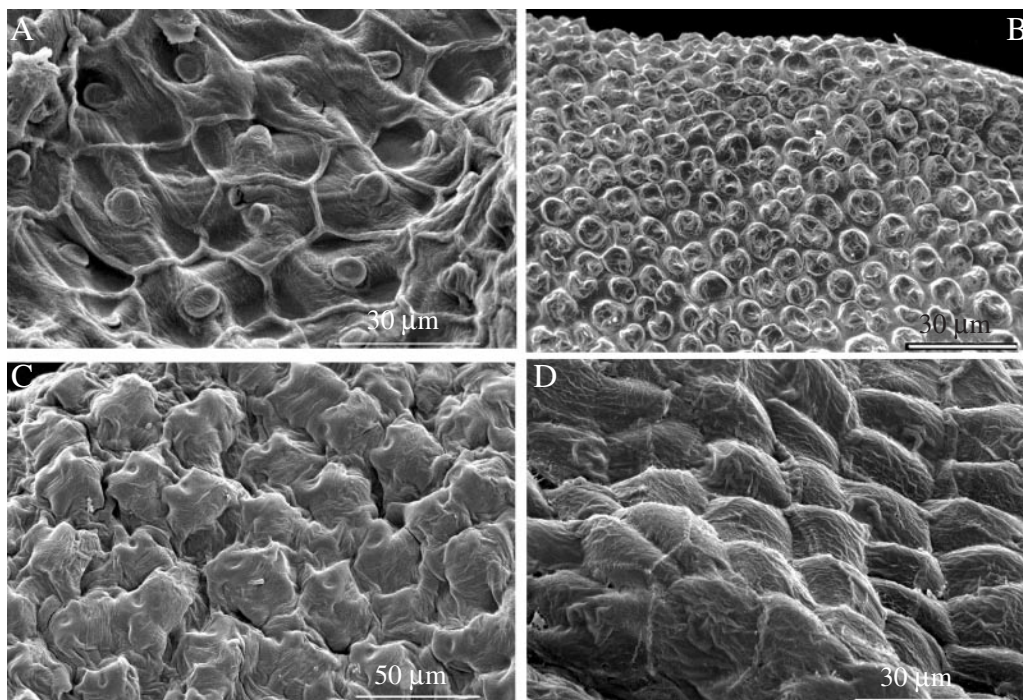


FIG. 5. Seeds in *Veronica*. Testa surface: (A) *V. ceratocarpa*; (B) *V. mampodrensis*; (C) *V. reuterana*; (D) *V. cardiocarpa*.

a tree maximum of 2000. Comparison of three separate runs revealed identical strict consensus trees, which are congruent with previous analyses (e.g. Albach *et al.*, 2004b, c, 2005b). These had a length of 2024 (CI = 0.51, RI = 0.79). The distribution of the ten main seed coat sculptural patterns in *Veronica* was then compared with the phylogenetic hypothesis, and each type represented by a different colour. The results can be seen in Fig. 2.

## DISCUSSION

The seeds of *Veronica* are flat or planoconvex, but further specializations occur in some groups. Seeds in subgenera *Veronica* and *Beccabunga* are flattest (<0.2 mm), but seeds from species in subgenus *Veronica* are flatter than those from all other species relative to its size (based on data from Kulpa, 1968). Margins of seeds in subgenera *Pocilla*, *Pellidosperma* and *Cochlidiosperma* [according to Hufford and McMahon (2004), there are also cochleariform seeds in two species in *V.* subgen. *Synthyris*—*V. californica*, i.e. *S. cordata* and *V. regina-nivalis*, i.e. *S. reniformis*—, while the remainder species of this subgenus have seeds that are dorsiventrally flat] turn inwards to form cymbiform or cyathiform seeds. Cyathiform seeds are usually correlated with a shift of the funicular attachment from terminal to ventral (and loss of the raphal line). Based on our phylogenetic hypothesis (Fig. 2), this character has independently evolved at least twice. It also occurs in some species or infraspecific taxa in species of subgenera *Stenocarpon* and *Pentasepalae* (see Appendix 1, Riek-Häußermann, 1943 and references therein; D. C. Albach, unpubl. res.). It is not clear

whether this indicates a predisposition for this character or whether it is an artefact of old and dried seeds. The most derived seeds can be found in *V. hederifolia* and relatives, in which the margins turn further inwards to form cochlidiospermous (cyathiform, i.e. urn-shaped) seeds.

The seeds of *Veronica* species range from ellipsoidal to suborbicular in outline. The trend seems to be from ellipsoidal seeds in species of the subgenera *Beccabunga* and *Pseudolysimachion* to the suborbicular seeds of subgenus *Pentasepalae* (Kulpa, 1968; Martínez Ortega and Rico, 2001).

Mapping seed sculpture on the phylogenetic tree reveals some evolutionary trends but, unfortunately, no conclusive answer can be given to the question about the ancestral seed coat sculpturing in *Veronica*. The most significant evolutionary change is maybe the fixation of the reticulate-verrucate ultrastructure of the testa in the clades corresponding to the subgenera *Pellidosperma*, *Stenocarpon* (incl. *V.* subgen. *Triangulicapsula*), *Chamaedrys*, *Pocilla* and *Pentasepalae*.

The colliculate testa is a confusing case. The pattern is found in representatives of all subgenera, except for subgenera *Veronica*, *Pseudolysimachium* and *Stenocarpon*, and was inferred to have evolved independently at least nine times. It may, therefore, represent a convergent reduction of the structure of the testa. Nevertheless, on the basis of the present phylogenetic hypothesis alone, it is impossible to assess whether an alternative explanation—this character state could be plesiomorphic in the genus and was conserved by many taxa except those of subgenera *Pseudolysimachium*, *Stenocarpon*, and *Veronica*—is more plausible or not.

Some exclusive characters supporting monophyly of clades, especially subgenera retrieved in phylogenetic analyses, have been found. Members of *Veronica* subgen. *Pseudolysimachium* share a reticulate-striate testa with crests on the upper (external) ends of the radial walls, and by a unique chromosome base number  $x = 17$  within *Veronica* s.l. (also found in other genera of the Veroniceae such *Veronicastrum* and *Picrorrhiza*) probably of polyploid origin (Albach *et al.*, 2004a; Taskova *et al.*, 2004). Moreover, the iridoid pattern is both extremely rich and very homogeneous, as all the species studied contain aucubin, catalpol and catalpol esters with both benzoic and cinnamic acid derivatives (Taskova *et al.*, 2004). Further putative synapomorphies for the clade are specific floral morphology and corolla development (Kampny and Dengler, 1997), as well as endosperm development (Yamazaki, 1957; Afanasyeva, 1971). Spicoside-type flavonoids, 6-OH-flavones acylated with phenolic acids, are found exclusively in this subgenus but may be derived within this clade (Albach *et al.*, 2006). This subgenus comprises only perennials and has been many times treated as an independent genus, although DNA sequence results show that it is derived from within *Veronica* (Albach and Chase, 2001).

On its side, *V.* subgen. *Stenocarpon* ( $x = 8$ , perennials and mostly with terminal inflorescence as a plesiomorphic character, cf. Albach *et al.*, 2004a) is characterized by an exclusive typically verrucate pattern with a progression in *V. capitata* to the rare reticulate-granulate pattern (found in only two species of the 132 studied *Veronica*, i.e. *V. capitata* and *V. szechuanica*, possess this probably autapomorphic character state), and two isolated species of the subgenus, *V. monticola* and *V. macrostemon*, maintaining or reversing to a character state that is likely to be plesiomorphic in the subgenus, a reticulate-verrucate pattern. Also taxonomically isolated is *V.* subgen. *Triangulicapsula*, a remarkable example of parallel evolution in annual habit despite considerable divergence in other characters, such as an exclusive (except for *V.* subgen. *Synthyris*) base chromosome number ( $x = 6$ ) or a particular capsule shape, that in the present phylogenetic hypothesis (Fig. 1) appears as a sister group of *V.* subgen. *Stenocarpon*. Regarding the ultrastructure of the seed coat, the subgenus also maintains the putative plesiomorphic state for the clade (reticulate-verrucate).

A unique character is also found in the seeds of the African representatives of *V.* subgen. *Veronica*, *V.* subsect. *Glandulosae*. They have a reticulate-striate seed coat pattern and a base chromosome number of  $x = 8$ , while the remaining representatives of this subgenus share a reticulate sculpturing of the seed coat, that probably represents a synapomorphy for the group, and  $x = 9$  (except *V. baumgartenii* with  $x = 7$ ). There are only two variants, in *V. cana* a reticulate-corrugate seed coat, a pattern well represented in the genus; and *V. szechuanica* with the very rare pattern reticulate-granulate that may represent an exclusive autapomorphy for this taxon (see above). Other than in *V.* subgen. *Veronica*, only *V. macropoda* (from *V.* subgen. *Pocilla*) shows a typically reticulate sculpturing of the seed coat. A further

specialization in the African *V. glandulosa* is found in its specific iridoid composition (Taskova *et al.*, 2004), as the species does not contain mussaenoside or any of the catalpol esters typical for *Veronica*, while this compound is present in the remainder of the taxa of *V.* subgen. *Veronica* studied. Moreover, there is a general coincidence of the iridoid spectra in those taxa from this subgenus *V.* subgen. *Veronica* whose phytochemical composition has been investigated, which supports the phylogenetic closeness of its members. Therefore, the characters of the seed greatly contribute, together with chemical and karyological data (number of chromosomes based on  $x = 8$ ), to support the autonomy of the African endemic *V.* subsect. *Glandulosae* within *V.* subgen. *Veronica*.

Regarding *V.* subgen. *Chamaedrys*, the perennial taxa within it are characterized by a derivation to a colliculate seed coat surface, while the annuals maintain the character in the putative plesiomorphic state for the subgenus, i.e. reticulate-verrucate. The progression from perennial to annual life history in the subgenus is remarkable and paralleled by a change in the sculpturing of the seed coat. As already stated in Albach *et al.* (2004a) the annual species superficially resemble annuals of other subgenera but, concerning testa surface the opposite seems to be true (i.e. a colliculate testa is linked, in most cases, to annuals, *V. peregrina*, *V. javanica*, *V.* subsect. *Acinifolia*, some representatives of *V.* subgen. *Pocilla* and *V.* subgen. *Pellidosperma*, while only in *V. surculosa* and *V. kurdica* is a perennial life habit paralleled by a colliculate testa). Apart from molecular characters, the phytochemical data also support the monophyly of this subgenus, as they are the only species in the genus that contain cornoside almost to the exclusion of iridoids which are predominant in the other species of *Veronica* (Jensen *et al.*, 2005).

Within *V.* subgen. *Beccabunga*, those species with a chromosome number based on  $x = 9$  show a reticulate-corrugate pattern, except for *V. hispidula* (the latter species belongs to *V.* subsect. *Acinifolia*, while the remaining ones belong to *V.* subsect. *Beccabunga*). According to Albach *et al.* (2004a), the clade shows a progression in reduction of the number of inflorescences in favour of a single terminal one seemingly connected with a shift from perennial to annual life form. A conspicuous reduction of indumentum (glabrous or with only glandular hairs) connected with an aquatic habitat is also observed. Concerning seeds, the reticulate-corrugate pattern is shared by the perennial representatives with  $x = 7$  or 8, but the annuals whose chromosome number is based on  $x = 7$  present different patterns of seed coat ornamentation (colliculate and reticulate-verrucate). Thus, the character does not seem to be fixed in the species belonging to that part of the subgenus. The primitive members of the subgenus, *V.* subsect. *Beccabunga*, are perennials found in aquatic habitats, so the shift to an annual life form is probably associated with progressions that have also affected to the seed coat. Regarding phytochemical characters, the study by Taskova *et al.* (2004) did not include annual species with a chromosome number based on  $x = 9$ , i.e. *V. hispidula* and related

taxa, with a reticulate-verrucate seed sculpturing, so no additional information is available for the species. Nevertheless, the whole subgenus seems to be characterized by the absence of 6-*O*-veratroylcatalpol, a character shared with most members of *V.* subgen. *Veronica*. Taskova and co-authors distinguished two groups: the aquatic species from *V.* subsect. *Beccabunga* ( $x = 9$ ) plus *V. peregrina*, showing a very homogeneous iridoid spectra, and sharing minecoside, while this compound is lacking in the remaining representatives of the subgenus. The presence of verminoside is shared by the perennial taxa with  $x = 7$  and 8, but both compounds, verminoside and minecoside, are absent in the annuals with the base chromosome number  $x = 7$ .

*Veronica peregrina* constitutes a remarkable case. Its chromosome number is  $2n = 52$ , and it is probably a hexaploid member of the group with  $x = 9$  within subgenus *Beccabunga*, which subsequently lost two chromosomes (cf. Albach *et al.*, 2004b). Its derived chromosome number is in correspondence with a derived seed coat pattern.

The small clade corresponding to *V.* subgen. *Cochlidiosperma* ( $x = 9$ ) comprises a few annual species only, and is characterized by comparatively uniform morphology (specific capsule and seed shape), habit (frondose inflorescence, also shared by some representatives of *V.* subgen. *Pocilla*) and, according to Taskova *et al.* (2004), it seems that the majority of the species lack the catalpol ester called veronicoside, but this happens also in species from other subgenera. With regard to seed characters, the ornamentation of the coat is predominantly reticulate-corrugate, with a derivation in some taxa to a reticulate-rugose or irregularly elevated pattern, a putative apomorphic character state shared only by some members of this subgenus, including those species that have been sometimes segregated from *Veronica* in a separate genus named *Oligospermum* (*V. simensis* and *V. crista-galli*).

The systematic position of *V. javanica* is really confusing, as seed characters, general morphology and karyology ( $x = 8$ ) support affinities with the perennial species of *V.* subgen. *Chamaedrys* (although *V. javanica* is an annual), but molecular characters favour a position closer to subgenus *Cochlidiosperma* (Fig. 2; see also Albach *et al.*, 2005b).

Subgenus *Pellidosperma* is another small clade comprising annuals characterized by cymbiform or subcylindrical seeds and lacking frondose inflorescences. The chromosome number is based either on  $x = 7$  or 9. The lack of catalpol esters with cinnamic acid derivatives (verminoside and minecoside) constitutes a clear phytochemical synapomorphy for this subgenus. The seed coat, on the contrary, provides no additional information in this respect.

*Veronica* subgen. *Pentasepalae* (perennials,  $x = 8$ ) and *V.* subgen. *Pocilla* (annuals,  $x = 7$ ), mainly show a reticulate-verrucate seed coat. Nevertheless, a parallel derivation to a reticulate-corrugate type, widely extended in other clades, such as those corresponding to the subgenera *Beccabunga* and *Cochlidiosperma*, can be observed in some isolated species (*V. stylophora*,

*V. capillipes*, *V. armena*, *V. bogosensis* and *V. vendetta-deae*). An alternative explanation is that the ancestral character state in *V.* subgen. *Beccabunga* and *V.* subgen. *Cochlidiosperma* is the reticulate-corrugate one, and a reversal has occurred in the previously mentioned species of *Pentasepalae* and *Pocilla*. A similar interpretation is possible for the colliculate state in the subgenera (*V. cardiocarpa*, *V. ferganica*, *V. biloba*, *V. surculosa* and *V. kurdica*).

Molecular characters show two distinct groups within *V.* subgen. *Pentasepalae*: the European species from *V.* subsect. *Pentasepalae* on one side (subclade represented in the lower part of Fig. 2B), and the Asian representatives (mostly of *V.* subsect. *Orientales*) on the other. This is also supported by chemical characters (Taskova *et al.*, 2004), as the European species contain unesterified catalpol as a main compound and only traces of verproside, while the Asian ones have verproside and amphicoside as their major iridoid compounds and unesterified aucubin and catalpol found only in trace amounts. The seed coat of *V.* subsect. *Pentasepalae* is homogeneous showing a reticulate-verrucate pattern, while within the Asian species more variation is found.

On its side, *V.* subgen. *Pocilla* shows a variable iridoid profile (Taskova *et al.*, 2004). The subgenus consists solely of species with an annual life cycle (except for a remarkable reversal to perennial life form in *V. filiformis*) and fall into at least two clades according to the present phylogenetic hypothesis. They correspond to subsections *Biloba* and *Pocilla*. The first one is well defined by the presence of ajugol (Jensen *et al.*, 2005). The second, also well characterized from a phytochemical point of view (Jensen *et al.*, 2005), is rather polymorphic and displays several evolutionary trends such as enhancement of branching and reduction of the terminal racemes in favour of lateral branches probably connected with reduction of foliar leaves and compensatory enlargement of bracts and flat to cymbiform seeds.

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## APPENDIX 1

Taxon, transection and ornamentation of seeds under stereoscopic microscope (SM) and voucher specimen or data resource

1. *V. spuria* L.: Plano-convex;  $\pm$  smooth. China: Yun-Nan, Yunnansen, collines du Tchong chan, 23 July 1908, Ducloux, P.
2. *V. longifolia* L.: Plano-convex;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
3. *V. dahurica* Stev.: Flat to plano-convex?;  $\pm$  smooth. Ex-USSR: Planta dahuricae, 1892, Karo, P.
4. *V. incana* L.: Plano-convex;  $\pm$  smooth. Crimea: Iter tauricum, Abhänge des Elmeli in Karagatsch bei Sudak, Callier, 1896, P.
5. *V. linariifolia* Pall.: Plano-convex; with keel. China, Manchuria, Jehol, Nakai *et al.* (1993), TI.
6. *V. subsessilis* Furumi: Flat to plano-convex?;  $\pm$  smooth. Japan, Tokyo, (cult.), Yamazaki (1995), TI.
7. *V. anagallis-aquatica* L. ssp. *anagallis-aquatica*: Plano-convex;  $\pm$  smooth. Juan *et al.* (1994).
8. *V. anagallis-aquatica* L. ssp. *oxycarpa* (Boiss.) Elenevsky: Plano-convex;  $\pm$  smooth. Georgia: Terek valley near Kazbegi, Albach 329, WU.
9. *V. scardica* Griseb.: Plano-convex;  $\pm$  smooth. Greece: Distr. Joanina: Epirus, Montes Pindus, 12 August 1956, K. H. Rechinger, MA 496908.
10. *V. catenata* Pennell: Plano-convex;  $\pm$  smooth. Spain: Tarragona, San Carles de la Rápita, Delta de l'Ebre, 12 April 1951, O. de Bolós, BC 115171.
11. *V. beccabunga* L.: Plano-convex;  $\pm$  smooth. Hong (1984).
12. *V. anagalloides* ssp. *heureka* M. A. Fisch.: Plano-convex;  $\pm$  smooth. Georgia: Cross Pass, Albach 299, WU.
13. *V. peregrina* L.: Flat; with keel. Juan *et al.* (1994).
14. *V. gentianoides* Vahl: Flat;  $\pm$  smooth; Turkey: Anatolia, zwischen Ilgaz und Kastamonu, S. Karakol, 1920 m, 5 July 1984, Nydegger 19036, RNG.
15. *V. serpyllifolia* L. ssp. *serpyllifolia*: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
16. *V. serpyllifolia* ssp. *repens* (DC.) Hartl: Flat;  $\pm$  smooth. Martínez-Ortega (1999).
17. *V. nevadensis* (Pau) Pau: Flat;  $\pm$  smooth. Martínez-Ortega (1999).
18. *V. bozakmanii* M. A. Fisch.: Plano-convex;  $\pm$  smooth. Turkey: Bitlis, Kucuksu, Albach 611 WU and VANF.
19. *V. acinifolia* L.: Flat;  $\pm$  smooth. Spain: Badajoz, La Codosera, cruce hacia San Vicente de Alcántara, 28-03-99, J. A. Sánchez Agudo JA28 *et al.*, SALA 108481.
20. *V. reuterana* Boiss.: Plano-convex;  $\pm$  smooth. Syria: iter syriacum, circa Zebdaine, prope Damascus, 6 June 1855, Kotschy, P.
21. *V. pusilla* Kotschy: Plano-convex;  $\pm$  smooth. Turkey: prairies humides de la région alpine du Taurus, au-dessus de Boulgarmaden, 28 August 1855, Balansa, 689, P.
22. *V. hispidula* Boiss. & A. Huet ssp. *hispidula*: Plano-convex;  $\pm$  smooth. Turkey: Erzurum, circa Erzeroum, 06-1853, Huet du Pavillon, P.
23. *V. hispidula* ssp. *ixodes* (Boiss. & Bal.) M. A. Fisch.: Plano-convex;  $\pm$  smooth. Turkey: Nigde, Masmeneu-Dagh (= Masmili Da.), à 2 lieues au sud de Caesarei (= Kayseri), 8 August 1855, Balansa, P.
24. *V. syriaca* Roem. & Schult.: Plano-convex;  $\pm$  smooth. Syrie: champs cultivés, sur la lisière des jardins, à l'est de Saïda, 02-1853, Blanche, P.
25. *V. debilis* Freyn: Flat;  $\pm$  smooth. Turkey: Biredschik (Urfa, Birecik), Djebel Taken, 04-1889, Sintenis, P.
26. *V. balansae* Stroh: Flat;  $\pm$  smooth. Turkey: Adana, in montibus Kassan Oghlu ad pagum Gorumse lectae, in agris alpinis olim cultis loco Yayle dictis, 13 May 1859, Kotschy, P.
27. *V. scutellata* L.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
28. *V. montana* L.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
29. *V. vandelliioides* Maxim.: Flat;  $\pm$  smooth. China: Tibet Oriental, Princ. Kiala, Tongolo, champs cultivés, 20 July 1893, Soulié, P.
30. *V. onoei* Franch. & Sav.: Flat;  $\pm$  smooth. Japan: Honshu, Nagano, Mt Azumaya, Sugadaira, Sanada-cho, Chiisagata-gun, 25 August 1965, Murata and Tagawa, P.
31. *V. officinalis* L.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
32. *V. allionii* Vill.: Flat;  $\pm$  smooth. France: Lautaret, prairies de l'Hospice, 14 July 1869, Reverchon, R.
33. *V. cana* Wall.: Flat;  $\pm$  smooth. Japan: In tractu Hakone Lavatu?, P.
34. *V. piroliformis* Franch.: Flat;  $\pm$  smooth. China: Yun-Nan, Heè-Chang men, 16 August 1883, Delavay, P.
35. *V. szechuanica* Batalin: Flat;  $\pm$  smooth. Bhutan: 28°58'N, 93°13'E, Dickore 10451, GOET.
36. *V. urticifolia* Jacq.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
37. *V. ponae* Gouan: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
38. *V. baumgartenii* Roem. & Schult.: Flat;  $\pm$  smooth. Romania: Transsylvania, Mte Negoii, regione alpina, 12 August 1893, Barth, P.
39. *V. aphylla* L.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
40. *V. grandiflora* Gaertn.: Flat;  $\pm$  smooth. Ex-USSR: Peninsula Kamczatka, 4 August 1908, Komarov, P.
41. *V. abyssinica* Fresen.: Plano-convex;  $\pm$  smooth. Rwanda, Butare, domaine de l'I. N. R. S. savane du versant W et marais, 2°36'S-29°44'E, 9 June 1978, Raynal, P.
42. *V. glandulosa* Hochst. ex Benth.: Plano-convex;  $\pm$  smooth. Frontier Uganda- Rwanda: Volcan Muhavura, 3450 m, 06-1929, Humbert, P.
43. *V. mannii* Hook. f.: Plano-convex;  $\pm$  smooth. Cameroun: Near Johan-Albrechtshütte, 2 February 1962, Breteler *et al.*, P.
44. *V. myrsinoides* Oliv.: Plano-convex;  $\pm$  smooth. Tanzania: Tanganyika Territory, Shita Mts, W Kijlimajaro, 02-1928, Haarer, P.
45. *V. alpina* L.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
46. *V. bellidioides* L.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
47. *V. nipponica* Makino: Flat;  $\pm$  smooth. Japan: Supra Nerikura, 28 August 1905, (collector illegible), P.
48. *V. teucrium* L.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
49. *V. orsiniana* Ten.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
50. *V. jacquinii* Baumg.: Flat; brain-like. Martínez-Ortega and Rico (2001).
51. *V. austriaca* L.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
52. *V. orbiculata* A. Kern.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
53. *V. tenuifolia* Asso ssp. *tenuifolia*: Flat; brain-like. Martínez-Ortega and Rico (2001).

54. *V. tenuifolia* ssp. *javalambrensis* (Pau) Molero & J. Pujadas: Flat; brain-like. Martínez-Ortega and Rico (2001).
55. *V. rosea* Desf.: Flat; brain-like. Martínez-Ortega and Rico (2001).
56. *V. prostrata* L.: Flat; brain-like. Martínez-Ortega and Rico (2001).
57. *V. scheereri* J.P. Brandt: Flat; brain-like. Martínez-Ortega and Rico (2001).
58. *V. turrilliana* Stoj. & Stef.: Flat; brain-like. Martínez-Ortega and Rico (2001).
59. *V. aragonensis* Stroh: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
60. *V. caucasica* M. Bieb.: Flat;  $\pm$  smooth. Caucasus, (no collection date), (no collector), P.
61. *V. vedetta-deae* Albach: Cymbiform. Georgia: Terek valley, near Kazbegi, Albach 327, WU.
62. *V. bombycina* Boiss. & Kotschy: Subcymbiform;  $\pm$  smooth. Libano: ad Bscherre et circa Cedretum in fissuris rupium summi Makmel, 23 July 1855, T. Kotschy, P.
63. *V. farinosa* Hausskn.: Subcymbiform;  $\pm$  smooth. (unlabelled material).
64. *V. macrostachya* ssp. *macrostachya* Vahl: Flat; brain-like. Syria: Syria bor., ex Amano prope Beilan, frequent versus Attyk pagum in graminosis, 25 June 1862, Kotschy, P.
65. *V. cuneifolia* D. Don: Flat; brain-like. Martínez-Ortega and Rico (2001).
66. *V. surculosa* Boiss. & Bal.: Cymbiform;  $\pm$  smooth. Turkey: Nigde, in regione alpina Tauri Orientalis supra Boulgarmaden (= Bolkar Maden), 16 September 1855, Balansa, P.
67. *V. cinerea* Boiss. & Bal.: Subcymbiform;  $\pm$  smooth. Turkey: Kayseri, région sous-alpine du Karamas-Dagh (Kuramaz Da.), à 5 lieues à l'Est da Césarée (Cappadoce), 3 August 1856, Balansa, P.
68. *V. multifida* L.: Flat; brain-like. Martínez-Ortega and Rico (2001).
69. *V. orientalis* Mill.: Flat; brain-like. Libani: In regione subalpina jugi Sanin, 1600–1700 m, 15 June 1897, Bornmüller, P.
70. *V. taurica* Willd.: Flat; brain-like. Martínez-Ortega and Rico (2001).
71. *V. kurdica* Benth.: Flat; brain-like. Iran: Persia borealis, in alpihus Demawend, 17 July 1902, Bornmüller 7818, P.
72. *V. thymifolia* Sibth. & Sm.: Flat; brain-like. Greece: Crete, in saxosis m. (illegible words), V-1846, Heldreich, P.
73. *V. bogosensis* Tumadz.: Cymbiform;  $\pm$  smooth. Caucasus orientalis: Czecheno-Inguschetia, ad fontes fl. Sharo-Argun, mons Chasenty, 5 August 1971, V. Prima, P.
74. *V. pectinata* var. *schizocalyx* (Frey & Sint.) Bornm.: Flat;  $\pm$  smooth. Turkey: Konya, Phrygia (Wilajet Konia), Sultandagh (= Sultan Da.), in saxosis et rupestribus ad Akscheher (= Aksehir), 14 June 1899, Bornmüller, 5381, P.
75. *V. armena* Boiss. & A. Huet: Cymbiform;  $\pm$  smooth. Armenia: Erzurum, in montibus Tech Dag (Palandöken Da.) supra Erzeroum, 06-1853, Huet du Pavillon, P.
76. *V. lanosa* Royle ex Benth.: Plano-convex?;  $\pm$  smooth. Himal. Bor. Occid. Regio temp., (no collection date), J. J., P.
77. *V. capitata* Royle: Flat;  $\pm$  smooth. China: Yun-Nan, Col de Yen-Tze-Hay, sur les bords du lac, 17 July 1889, Delavay, P.
78. *V. macrostemon* Bunge ex Ledeb.: Flat;  $\pm$  smooth. Ex-USSR-Mongolia: Flora altaica, rarior in lapidosis asperis summa (illegible words), Bunge 31, P.
79. *V. densiflora* Ledeb.: Plano-convex?;  $\pm$  smooth. In alpe ad fl. Gremiaezia prope Bay?zain, 1836, Fischer, P.
80. *V. ciliata* Fisch.: Plano-convex?;  $\pm$  smooth. China Septentrionale: Ka'won occidental, vers Chang Lian Hang, 3500 m, 31 August 1918, Licent, P.
81. *V. fruticulosa* L.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
82. *V. fruticans* Jacq. ssp. *fruticans*: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
83. *V. fruticans* ssp. *cantabrica* M. Laínz: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
84. *V. mampodrensis* Losa & P. Monts.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
85. *V. nummularia* Gouan: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
86. *V. satirejoides* Vis.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
87. *V. erinoides* Boiss. & Spruner: Flat;  $\pm$  smooth. Greece: In montis Kyllenes (Ziria hod.) Achaiae regione superiori, 25 June 1887, Heldreich, P.
88. *V. thessalica* Benth.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
89. *V. monticola* Trautv.: Flat;  $\pm$  smooth. Georgia: plantes de Transcaucasie, Mingrèlie, Mt Okare pâturages alpines, 30 August 1895, Alboff, P.
90. *V. magna* M. A. Fischer: Flat;  $\pm$  smooth. Turkey: Coruh, Bois des environs de Rohizé (Lazistan), 30 June 1866, Balansa, P.
91. *V. laxa* Benth.: Flat;  $\pm$  smooth. Japan: Montagnes de ?iobara, 30 June 1889, Faurie, P.
92. *V. chamaedrys* L.: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
93. *V. vindobonensis* (M. A. Fischer) M. A. Fischer: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
94. *V. micrantha* Hoffmanns. & Link: Flat;  $\pm$  smooth. Martínez-Ortega and Rico (2001).
95. *V. javanica* Blume: Plano-convex;  $\pm$  smooth. Vietnam: Tonkin, Laisses de la rivière Noire à Tu-Pahp, 08-03-1888, Balansa, P.
96. *V. arvensis* L.: Flat; brain-like. Juan *et al.* (1994).
97. *V. verna* L.: Flat;  $\pm$  smooth. Spain: Segovia: Aldeonsancho, 17 June 1984, T. Romero, SALA 38933.
98. *V. brevistyla* Moris: Flat;  $\pm$  smooth. France: Córcega, Asco, estación de esquí del Alto Asco hacia el monte Cinto, 22 July 1998, M. Martínez-Ortega MO483. SALA 108484.
99. *V. dillenii* Crantz: Flat;  $\pm$  smooth. Spain: Gerona: Montseny, cerca de Viladrau, 06 June 1948, A. y O. de Bolós, MA 152042.
100. *V. aznavourii* Dörfel.: Cymbiform;  $\pm$  smooth. Turkey: Constantinopel, in arvis prope Halkali, April 1901, G. V. Aznavour, P.
101. *V. donii* Römpf.: Subcymbiform;  $\pm$  smooth. Turkey: Kiale to Kozagaç (Milas-Yatagan), 13 April 1965, P. H. Davis, W.
102. *V. praecox* All.: Cymbiform; crestate. Juan *et al.* (1994).
103. *V. triphyllus* L.: Cymbiform; crestate. Juan *et al.* (1994).
104. *V. glauca* ssp. *peloponnesiaca* Boiss. & Orph.: Subcymbiform;  $\pm$  smooth. Greece: In Monte Malevö Lau?iae Supra Hajos Joannis, 2 May 1857, T. G. Orphanides, 716, P.
105. *V. macropoda* Boiss.: Cymbiform; crestate. Iran: Esfahan, Acher-Eloy, 5094, P.
106. *V. ferganica* Popov: Cymbiform;  $\pm$  smooth. Uzbekistan: Prov. Fergana, Distr. Kokand, in collibus siccissimis gypsaceis prope petroleas 'Santo', 18 April 1923, Popov and Vvedensky, P.



107. *V. argute-serrata* Regel & Schmalh.: Cymbiform; crestate. Anatoliae orientalis: Pontus Galaticus, in incultis ad Tafra-jaila in monte Gik-dagh, 22 May 1890, J. Bornmüller, P.
108. *V. campylopoda* Boiss.: Cymbiform; crestate. Iran: Persia borealis, Aucher-Eloy, P.
109. *V. biloba* L.: Cymbiform;  $\pm$  smooth. Turkey: Paphlagonia australis, 23 June 1929, Bornmüller, P.
110. *V. capillipes* Nevski: Cymbiform; strongly crestate. Iran: Iter persicum, Isfahan, 05-1859, Bunge, P.
111. *V. cardiocarpa* (Kar. & Kir.) Walp. ssp. *cardiocarpa*: Cymbiform;  $\pm$  smooth. Ex-USSR, Uzbekistan-Kirghistan, Syr-Darja, Distr. Taschkent, in arborum umbra in hortis urbis Taschkent, 24 April 1922, Vvedensky, P.
112. *V. cardiocarpa* (Vved.) M. A. Fisch. ssp. *nanella*: Cymbiform;  $\pm$  smooth. Afganistán: Vallée de Kuram, 1880, Aitchinson, P.
113. *V. stylophora* Popov: Cymbiform; strongly crestate. USSR: Uzbekistan, Prov. Samarkand, Distr. Katta-Kurgan, ad declivia argillosa in elevatione Zindan-tau haud procul a p. Mussa-kak, 6 May 1925, Popov, P.
114. *V. ceratocarpa* C. A. Mey.: Flat;  $\pm$  smooth. Austria: Salisburgia, ad margines viarum et in incultis Juvaviae, (no collection date), Eysn., P.
115. *V. violifolia* Hochst. ex Benth.: Plano-convex;  $\pm$  smooth. Ethiopia: in regione superiori montis Bachit fissuras rupium glarea repletas incolens, 25 June 1838, Schimper, P.
116. *V. persica* Poir.: Cymbiform; crestate. Juan *et al.* (1994).
117. *V. polita* Fr.: Cymbiform; crestate. Juan *et al.* (1994).
118. *V. opaca* Fr.: Cymbiform; crestate. Romania: Kojocna, à 30 km de Cluj-Napoca, 1 July 2000, X. Giráldez *et al.*, SALA 108480.
119. *V. agrestis* L.: Cymbiform; crestate. Juan *et al.* (1994).
120. *V. grisebachii* Walters: Flat;  $\pm$  smooth. Bulgary: distr. Kardzali, M. Rhodopae Orientalis, in arenosis loco dicto ZeleznáVrata, 24 April 1953, N. Stojanov and B. Kitanov, MA 182293.
121. *V. chamaepithyoides* Lam.: Flat;  $\pm$  smooth. Spain: Madrid, Altos de Amanuel (loc. class.), 10 April 1929, M. Martínez, MA 177321.
122. *V. crista-galli* Stev.: Cymbiform; brain-like. Iran: Iter persicum, pr. Siaret, 24 April 1858, Bunge, P.
123. *V. simensis* Fresen.: Cymbiform; brain-like. Ethiopia: Roba-Gébia-Seméne, 10 000', 19 August 1852, Schimper, P.
124. *V. cymbalaria* Bodard: Cyathiform; crestate. Juan *et al.* (1994).
125. *V. panormitana* Tineo: Cyathiform; crestate. Spain: Menorca, Ferrerías, Albranca Vell, barranco de la Cova., 20 April 2001, L. Delgado LD736, *et al.* SALA 105882
126. *V. panormitana* ssp. *baradostensis* (M. A. Fisch.) M. A. Fisch.: Cyathiform; crestate. Turkey: Pr. Hatay, 8 km SE Samandag, 2 April 1985, (no collector) 9412, WU.
127. *V. trichadena* Jord. & Fourr.: Cyathiform; crestate. Spain: Menorca, Ferrerías, Sta. Elena, cantera, 17 April 2001, L. Delgado LD674 *et al.*, SALA 108483.
128. *V. lycica* E. Lehm.: Cyathiform;  $\pm$  smooth. Turkey: Akdag, 24 June 1967 (no collector) 9427, WU.
129. *V. stamatidae* M. A. Fisch. & Greuter: Cyathiform; crestate. Greece: Dodekanes, Inselgruppe Kastellorizo, südlich des lykischen Küste, Insula Ro, SW der Bucht des Heiligen Gerg, 12 April 1974, W. Greuter, WU.
130. *V. hederifolia* L.: Cyathiform; crestate. Juan *et al.* (1994).
131. *V. triloba* Opiz: Cyathiform; crestate. Spain: Ciudad Real, Venta de Cárdenas, camino a la estación de Venta de Cárdenas, 11 April 1999, J. A. Sánchez Agudo JA42 *et al.*, SALA 100147.
132. *V. stewartii* Pennell: Cyathiform;  $\pm$  smooth. Pakistan: NW Pakistan, Swat, Ushu, 35°40'N–72°40'E prope Kalam, 28 January 1980, K. H. Rechinger 30780, W.

## APPENDIX 2

Taxon, voucher specimen, GenBank accession number for ITS and GenBank accession number for the *trnL-F* region

- V. longifolia*: cult. BG Bonn; Albach 66, BONN; AF313021; AF486407.
- V. dahurica*: cult. BG Jena; Albach s.n, WU; AF313023; AF511479/80.
- V. incana*: cult. BG Bonn; Albach 155, BONN; no data for ITS; AY486449.
- V. beccabunga*: cult. RBG Kew; Albach 122, K; AF313015; AF486403.
- V. anagalloides* ssp. *heureka*: Georgia. Terek-Tal; Albach 307, WU; AF509797; AF486404.
- V. peregrina*: USA. Montana, Flathead Co.; Lesica 5668, NYBG; AF313016; AF513352.
- V. gentianoides*: cult. BG Bonn; Albach 72, BONN; AF313018; AF486401.
- V. serpyllifolia* ssp. *serpyllifolia*: Germany. Bonn; Albach 64, WU; AF313017; AF486400.
- V. acinifolia*: Greece. Lefkas; M. A. Fischer, WU; AF509798; AF486399.
- V. reuterana*: Turkey. north of lake Van; Albach 676, WU; AY540866; AY486447.
- V. pusilla*: Turkey; TRO 2/17, 12.6.2002, WU; AY540865; AY486446.
- V. hispidula* ssp. *hispidula*: Turkey. Erek Dagi; Albach 635, WU; AY741528; no data for *trnL-F* region.
- V. syriaca*: Turkey. Side; Albach 252, WU; no data for ITS; AF486398.
- V. scutellata*: Austria. Waldviertel; Dobes 7026, WU; AF509805; AF486393
- V. montana*: Germany. Oberkassel; Albach 151, WU; AF313014; AF486388.
- V. vandellioides*: China. Sichuan; Dickoree 8417, GOET; AY776287; AF509806.
- V. officinalis*: UK. Farthing Downs, cult. RBG Kew; Albach & Chase 114, K; AF313012; AF486391.
- V. allionii*: Italy. Prov. of Genoa, Liguria, Reserva della Navette, cult. RBG Kew; Chase s.n., K; AF509809; AF513348.
- V. piroliformis*: China. Yunnan 27°2'N 100°14'E 31 July 1995; Dickoree 14146, GOET; no data for ITS; AF486390.
- V. szechuanica*: Yu 7699, BM; AY741527; no data for *trnL-F* region.
- V. urticifolia*: cult. BG Bonn; Albach 73, BONN; AF313011; AF486389.
- V. baumgartenii*: Bulgaria. Mt Midjur; Albach 542, WU; AY144464; AY780808.
- V. aphylla*: Italy. Rollepess; Zhang s.n, WU; AF515211/2; AF513349.
- V. abyssinica*: Rwanda. E. Fischer 8060, BONN; no data for ITS; AF513350.

- V. glandulosa*: cult. BG Bonn; E. Fischer 713/98, WU; AF313008; AF486394.
- V. alpina*: Sweden. Abisko NR; Benson 1999.08 UPS; AF509810; AF511483/4.
- V. bellidioides*: Austria. Carinthia, Nockberge, Hoher Falkert, cult. RBG Kew; Albach 118, K; AF313010; AF513345.
- V. nipponica*: Japan. Hokkaido, Mt Kumagamine; Horii 20401, WU; no data for ITS; AY776286.
- V. teucrium*: cult. RBG Kew; Albach 119, K; AF312999; no data for *trnL-F* region.
- V. jacquini*: cult. BG Bonn; Albach 70, WU; AF313000; AF513341.
- V. tenuifolia*: Spain. Huesca, Arro, Martínez-Ortega MO1043; AY776286; no data for *trnL-F* region.
- V. rosea*: Morocco. Midelt, Martínez-Ortega MO1501; AY741519; no data for *trnL-F* region.
- V. turrilliana*: Turkey. Near Istanbul; Albach 278, WU; AF486360; AF486374.
- V. aragonensis*: Spain. Huesca, Turbón, Martínez-Ortega MO1045; AY741517; no data for *trnL-F* region.
- V. caucasica*: Georgia. Kazbegi; Albach 326, WU; AF486357; no data for *trnL-F* region.
- V. vedetta-deae*: Georgia. Kazbegi; Albach 327, WU; AF486361; no data for *trnL-F* region.
- V. bombycina*: cult. NYBG; Struwe 1403 WU; AF486353; AF486376.
- V. farinosa*: Alava 13656, TUR; AY741518; AY776283.
- V. cuneifolia* ssp. *isaurica*: cult. NYBG; Struwe 1409 WU; AF486354; AF486372.
- V. cinerea*: cult. RBG Kew; Albach & Chase 113, K; AY144458; no data for *trnL-F* region.
- V. orientalis*: Turkey. Van, Karabel pass; Albach 701, WU; AY741515; no data for *trnL-F* region.
- V. thymifolia*: Greece. Crete, Mt Iti; Stamatiadou 15 June 1971, SALA; AY741520; no data for *trnL-F* region.
- V. bogosensis*: Georgia. Prima s.n., MO; AF486359; no data for *trnL-F* region.
- V. armena*: cult. NYBG; Struwe 1404, WU; AF313040; no data for *trnL-F* region.
- V. lanosa*: Pakistan. Astore Valley, Schickhoff 1377, GOET; AY540868; AY486442.
- V. macrostemon*: Russia. Altai, Jushio-Tschujsij-Chrebet, 7 August 2002, Staudinger AL23-18, SALA; AY741522; AY486441.
- V. densiflora*: Russia. Altai, Jushio-Tschujsij-Chrebet, 7 August 2002, Staudinger AL22-8, SALA; AY741521; AY776282.
- V. ciliata*: China. Qinghai 34°45'N 100°14'E; Miehe *et al.*, 98-33313, GOET; AF515215; AF486385.
- V. fruticulosa*: cult. BG Bonn; Albach 71, BONN; AF313004; AF486383.
- V. fruticans* ssp. *fruticans*: UK. Scotland; Halcro VH030, K; AY144462; no data for *trnL-F* region.
- V. erinoides*: Greece. Gioua-Massiv; Hagemann, Scholz & Schmitz 461, SALA; AY741523; no data for *trnL-F* region.
- V. thessalica*: Greece. Thessalic Olymp; Raus & Rogl 5072, SALA; AF509792; AF513343.
- V. magna*: Georgia. Orbetia; Albach 360, WU; AY673615; AY673634.
- V. laxa*: Pakistan; Dickoree 13042, GOET; AY673613; AF486378.
- V. chamaedrys*: Norway. Horsland, Os, Hattvik, cult. RBG Kew; Albach 121, K; AF313003; AF486377.
- V. vindobonensis*: cult. BG Wien; M. A. Fischer, WU; AY673614; AF510426.
- V. javanica*: Ethiopia. Kaffa-Bonga, Catholic mission; Fries *et al.* 2016, BR; AY540867; AY540872.
- V. arvensis*: Germany. Stromberg bei Bockenau; Albach 147, WU; AF313002; AF486380.
- V. verna*: Germany. Bad Kreuznach; Albach 149, WU; AF509789; AF486379.
- V. donii*: Turkey. Cine to Yatagan; Albach 239, WU; AF509794; AF513351.
- V. praecox*: Spain. Cuenca, Sierra de Valdeminguete; Brummit, Gibbs, Ratter 539, K; no data for ITS; unpubl.
- V. triphyllos*: Turkey. Aphrodisias; Albach 244, WU; AF509795; AF486396.
- V. glauca* ssp. *peloponnesica*: Greece. Lefkas; M. A. Fischer 9, 7 April 1999, WU; no data for ITS; AF486395.
- V. glauca*: Greece. cult. in RBG Kew, collection number 1973-14554, Chase 8922, K; AF313006, no data for *trnL-F*.
- V. argute-serrata*: Afghanistan. Orozgan; Rechinger 35177, WU; AF509787; AF513337.
- V. campylopoda*: Jordan. Near ruin Shobek; Schönschwetter & Tribsch 4152, WU; AF486364; AF486370.
- V. biloba*: Turkey. Ereğ Dagi; Albach 636, WU; AY673606; AY673625.
- V. capillipes*: Iran. Prov. Khorasan; Rechinger 51188-c+b, WU; AY673607; AY673627.
- V. ceratocarpa*: Turkey. Öztürk 429, WU; AY741514; AY673626.
- V. persica*: UK. London, Manor Road allotments; Fay 175, K; AF509785; AF513336.
- V. polita*: Germany. Martinstein; Albach 146, WU; AF509818; AF486369.
- V. opaca*: Georgia. Dzuta; Albach 332, WU; AY673617; AY673630.
- V. agrestis*: Greece. Taygetos; Albach 386, WU; AF509784; AF513335.
- V. chamaepithyoides*: Spain. Segovia, San Miguel de Bernuy, SALA; AF509796; AF511477/8.
- V. crista-galli*: Dolmkanov 17 April 1983, TBS; AF509799; AF486367.
- V. cymbalaria*: Cyprus. Halla Tekke; Albach 130, WU; AF313007; AF486365.
- V. panormitana*: Greece. Lefkas; M. A. Fischer 9, 9 April 1999, WU; AF509800; AF513330.
- V. panormitana* ssp. *baradostensis*: Turkey. Hatay, Antakya; Sorger 85-34-33, WU; no data for ITS; AY776284.
- V. trichadena*: Greece. Lefkas; M. A. Fischer 22, 9 April 1999, WU; AF509801; AF513331.
- V. lycica*: Turkey. Near Kalkan; Albach 269, WU; AF509802; AF513332.
- V. stamatiadae*: Turkey, Kas; Buchner 30 March 1983, WU; no data for ITS; AY557598.
- V. hederifolia*: Cyprus. Olympus range; Albach 136, WU; AY741524; AF510425.
- V. triloba*: Turkey. Near Aphrodisias; Albach 242, WU; AF509804; AF513333.
- V. stewartii*: Pakistan. NW part; Rechinger 30780, WU; AY741525; AY540875.
- Wulfenia carinthiaca* Jacq.: cult. BG Bonn; Albach 74, WU; AF313025; AF486409.