Annals of Botany **114**: 61–83, 2014
doi:10.1093/aob/mcu081, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

Why Africa matters: evolution of Old World *Salvia* (Lamiaceae) in Africa

Maria Will* and Regine Claßen-Bockhoff

Institut für Spezielle Botanik und Botanischer Garten, Johannes Gutenberg Universität, Mainz, Germany
** For correspondence. E-mail willm@uni-mainz.de*

Received: 19 September 2013 Returned for revision: 10 January 2014 Accepted: 21 March 2014

• **Background and Aims** *Salvia* is the largest genus in Lamiaceae and it has recently been found to be non-monophyletic. Molecular data on Old World *Salvia* are largely lacking. In this study, we present data concerning *Salvia* in Africa. The focus is on the colonization of the continent, character evolution and the switch of pollination systems in the genus.
• **Methods** Maximum likelihood and Bayesian inference were used for phylogenetic reconstruction. Analyses were based on two nuclear markers [internal transcribed spacer (ITS) and external transcribed spacer (ETS)] and one plastid marker (*rpl32-trnL*). Sequence data were generated for 41 of the 62 African taxa (66 %). Mesquite was used to reconstruct ancestral character states for distribution, life form, calyx shape, stamen type and pollination syndrome.
• **Key Results** *Salvia* in Africa is non-monophyletic. Each of the five major regions in Africa, except Madagascar, was colonized at least twice, and floristic links between North African, south-west Asian and European species are strongly supported. The large radiation in Sub-Saharan Africa (23 species) can be traced back to dispersal from North Africa via East Africa to the Cape Region. Adaptation to bird pollination in southern Africa and Madagascar reflects parallel evolution.
• **Conclusions** The phenotypic diversity in African *Salvia* is associated with repeated introductions to the continent. Many important evolutionary processes, such as colonization, adaptation, parallelism and character transformation, are reflected in this comparatively small group. The data presented in this study can help to understand the evolution of *Salvia sensu lato* and other large genera.

Key words: *Salvia*, Lamiaceae, Canary Islands, character evolution, ITS, ETS, Madagascar, ornithophily, pollination, *rpl32-trnL*, Sub-Saharan Africa.

INTRODUCTION

Throughout the world, many *Salvia* spp. (Lamiaceae) are known as ornamental (e.g. *S. coccinea*, *S. patens*, *S. viridis*), medicinal (*S. officinalis*, *S. miltiorhiza*) and even hallucinogenic plants (*S. divinorum*) (Clebsch, 2008; Froissart, 2008). *Salvia* is the largest genus in the mint family, with 900–1000 species distributed worldwide (Alziar, 1988–1993; Harley *et al*., 2004). Molecular studies have shown many large genera to be non-monophyletic, and this is also true for *Salvia* (Walker *et al*., 2004), with respect to *Dorystaechas*, *Meriandra*, *Perovskia*, *Rosmarinus* and *Zhumeria* (Walker and Sytsma, 2007). Major clades containing *Salvia* spp. were named *Salvia* Clade I, II and 'III' *Salvia* (*s.l.*). So far, previous molecular studies have focused on New World (NW) *Salvia*. In contrast, Old World (OW) species have been largely disregarded (Walker *et al*., 2004; Sudarmono, 2007, 2008; Walker and Sytsma, 2007; Jenks *et al*., 2011, 2012; Li *et al*., 2013). Only seven African species were included in the genus-wide study of Walker and Sytsma (2007), which revealed that they are members of two of the three major clades proposed by the authors (Fig. 1; Clade I, 'Clade III'). In this study, *Salvia* 'Clade III' is paraphyletic with respect to *Zhumeria majdae*. Thus, we accept the south-west (SW) Asian species and *Zhumeria* as one clade (*S. trichocalycina* group; Clade III) and the East Asian species as the fourth independent evolutionary lineage (*S. miltiorhiza* group; Clade IV). According to Walker and Sytsma (2007), *Salvia* Clade I is monophyletic and covers the type species of the genus (*S. officinalis*; Jarvis, 2007). We therefore refer to it as *Salvia sensu stricto* (*s.s.*).

Sixty-two *Salvia* spp. occur in Africa and adjacent areas (Fig. 2; Hedge, 1974; Santos and Fernández, 1986; Van Jaarsveld, 1999). Most are endemics distributed in North Africa (25 species including the Canary Island endemics), southern Africa (23 species), Madagascar (six species), East Africa and on the Arabian Peninsula (eight species). Similar distribution ranges are known for other plant genera such as *Androcymbium*, *Senecio* and *Zygophyllum* (Caujapé-Castells, 2001; Colemann *et al*., 2003; Bellstedt *et al*., 2008; Del Hoyo *et al*., 2009) and for animals, e.g. Diptera (Kirk-Spriggs and McGregor, 2009). *Salvia* thus appears to be another genus adapted to the similar climatic conditions occurring in North (Mediterranean area), East (East African mountains) and southern (Cape Region) Africa.

African *Salvia* is of special interest for addressing evolutionary questions. Species are highly diverse in habitat preferences (Fig. 3), floral morphology (shape, size, colour and stamen construction) and pollination (Fig. 4). The only bird-pollinated species known from the Old World evolved in Sub-Saharan Africa (SSA) (Scott-Elliot, 1890; Van Jaarsveld, 1999; Wester and Claßen-Bockhoff, 2006, 2007). The most recent classification of African *Salvia* was presented by Hedge (1974). Based

© The Author 2014. Published by Oxford University Press on behalf of the Annals of Botany Company. All rights reserved.
For Permissions, please email: journals.permissions@oup.com

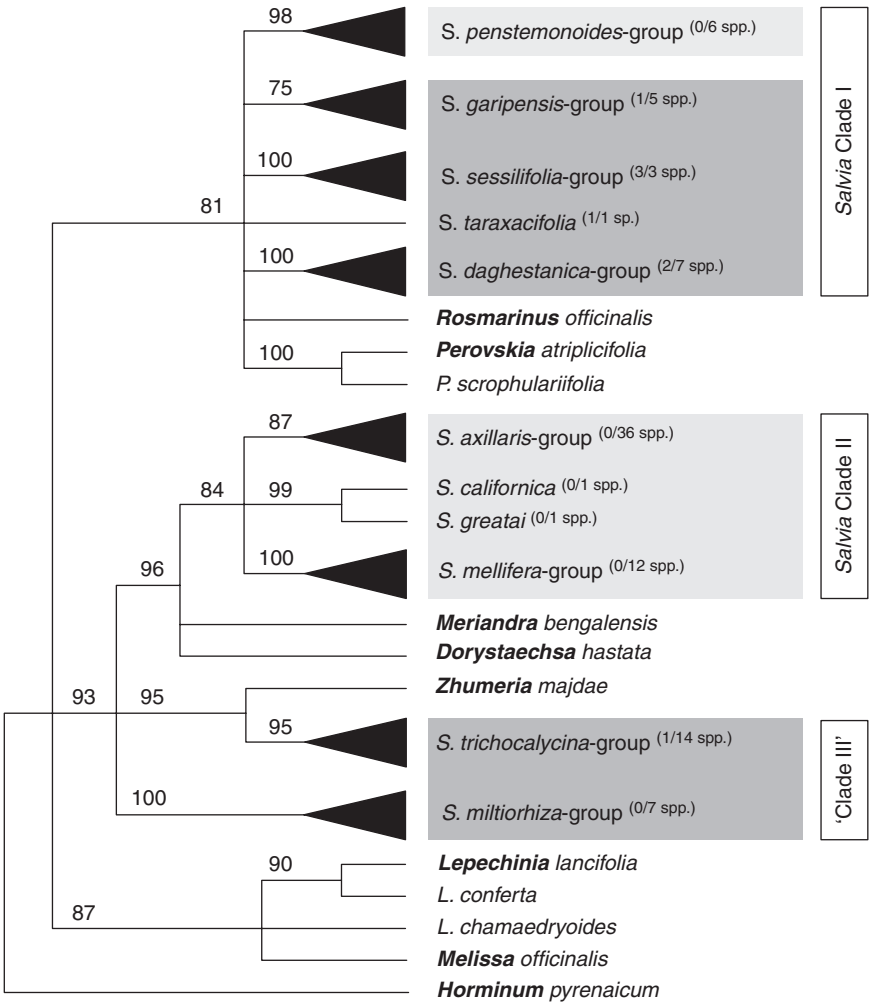


FIG. 1. Phylogenetic tree of *Salvia s.l.* defining the major clades (Walker and Sytsma, 2007, simplified, i.e. nodes with bootstrap values <75 % were collapsed). Strict consensus tree based on the combined analysis of *trnL-F* and ITS data; MP. Non-*Salvia* genera are highlighted (bold); NW *Salvia* is highlighted in light grey and OW taxa in dark grey. Numbers in parentheses, separated by slashes, indicate the number of African species and the total number of taxa in the corresponding clade in their analyses.

on distribution and morphology, he arranged 59 species in 23 species groups. However, it is not known to which degree these species groups represent natural lineages.

In the present study, we examine the monophyly of Hedge's (1974) species groups based on a phylogenetic analysis that includes two-thirds of the African species. We intend to determine the number of independent origins of African *Salvia*. Furthermore, the colonization of the African continent and the evolution of African *Salvia*, e.g. the origin of bird pollination, are reconstructed.

MATERIALS AND METHODS

Plant material

Our analyses include 84 *Salvia* spp., 46 of them sequenced for the first time. We cover 41 (66 %) of the 62 African species: two endemics from Madagascar, all endemics from the Canary Islands, eight species from eastern Africa and the Arabian Peninsula, 18 southern African species and 12 species restricted to North Africa, the circum-Mediterranean area and SW Asia. Accessions derived from GenBank were used to complement the data set. Voucher information and GenBank accession numbers are provided in the Appendix. Due to the lack of suitable plant material and/or successful PCR, some species are only represented by a sub-set of the three molecular markers.

Our sampling covers all major lineages previously identified in *Salvia* (Walker and Sytsma, 2007; Fig. 1). Well-supported clades that are not the focus of this study (Clades II and IV; see Walker and Sytsma, 2007; Will, 2013) are represented by a sub-set of species only. Independent accessions are included, especially for taxonomically critical and polymorphic species. *Hyptis lanifolia* was used as the outgroup in all analyses. Nomenclature is in accordance with Alziar (1988–1993) and with the International Plant Names Index (http://www.ipni.org/ipni/, accessed 30 April 2013). The term Sub-Saharan Africa is used in the sense of Linder (2001) but additionally includes the Namib–Kalahari region.

FIG. 2. Distribution of *Salvia s.l.* on the African continent. Five regions on the continent and adjacent areas are recognized with 62 *Salvia* spp.: (1) the Canary Islands; (2) southern Europe/North Africa to south-west Asia; (3) East Africa/Arabian Peninsula; (4) southern Africa; and (5) Madagascar. Distributions are based on: Hedge (1974), Codd (1985), Santos and Fernández (1986), Thulin (1993, 2009) and Van Jaarsveld (1999).

DNA extraction, amplification and sequencing

For *Salvia*, new sequence data are presented for the internal transcribed spacer (ITS; 39 species), the external transcribed spacer (ETS; 38 species) and the plastid marker *rpl32-trnL*^{UGA} (57 species). The latter was selected based on the results of previous primer screening (*trnL-F* and *rpl32-ndhF*). Total genomic DNA was obtained from silica-dried or herbarium leaf material. DNA was extracted according to the manufacturer's protocol for the NucleoSpin® plant DNA extraction kit (Macherey-Nagel, Düren, Germany). The standard 25 μL PCR mix consisted of 2 mM $MgCl_2$, 200 μM dNTPs, 1 pM primer, 0·025 U μL^{-1} *Taq* polymerase and 0·5–1·0 μL of DNA extract in the reaction buffer provided by the manufacturer of the polymerase.

The PCRs were carried out in a Biometra T3 or a PTC 100 MJ Research thermocycler using the following program: 60 s at 94 °C; followed by 35 cycles of 20 s at 94 °C, 30 s at 55 °C and 60 s at 72 °C; and a post-treatment of 80 s at 55 °C and 8 min at 72 °C for each marker. The whole ITS region was sequenced as a single piece using the ITS-A (Noyes and Rieseberg, 1999) and ITS-4 primers (White *et al.*, 1990). The ETS region was sequenced using 18S-E (Baldwin and Markos, 1998) and ETS-B (Beardsley and Olmstead, 2002). For plastid sequences, we used the *rpl32* and *trnL*^{UGA} primers (Shaw *et al.*, 2007). PCR products were purified according to the manufacturers' protocols using ExoSAP-IT PCR Product Clean-up (Affymetrix UK Ltd, Wooburn Green, UK) or NucleoSpin®Extract II-kit (Macherey-Nagel).

Cycle sequencing was performed using ABI Prism Big DyeReady Reaction Mix (Perkin Elmer/Applied Biosystems, Foster City, CA, USA) using the primers listed above and following the manufacturer's protocol. Products were purified with Sephadex™ G50 (VWR International GmbH, Darmstadt, Germany) and sequenced on a 16-capillary ABI 3130 *xl* automated sequencer (Life Technologies GmbH, Darmstadt, Germany).

DNA sequence alignment and phylogenetic analyses

Sequencing was straightforward for each marker. Forward and reverse sequences were edited manually, merged into consensus sequences using Sequencer™ 4.1.2. (GeneCodeCorp., Ann Arbor, MI, USA) and aligned manually in McClade4.1 (Maddison and Maddison, 2000). Ambiguously alignable regions (identified manually) were excluded from analyses. The three data sets were analysed separately. In order to increase resolution, we combined nuclear and plastid data (combined data set). Partitions were defined for the combined data set before the best-fit models of nucleotide substitution were selected with jModeltest 2.1.1 (Darriba *et al.*, 2012). Under the Akaike information criterion (AIC), the GTR+I+G model was selected for the ITS data set and TVM+G for ETS and the plastid marker. Two tree searches, one under maximum likelihood (ML) with bootstrapping (BS; RAxML-HPC BlackBox v.7.4.4; Stamatakis, 2006; Stamatakis *et al.*, 2008) and one under Bayesian inference (BI; MrBayes v.3.1.2 on XSEDE; Ronquist and Huelsenbeck, 2003), were performed on the CIPRES Science Gateway v.3.3 server (Miller *et al.*, 2010). Since MrBayes does not allow nst = 5, required for TVM+G, we chose the more complex model (nst = 6). For BI, we ran four Markov chains simultaneously for 10 million generations analysing the plastid and ETS data sets. Two independent runs of 40 million generations were performed for the ITS and combined data sets. Every thousandth generation was sampled. The burn-in was determined with Tracer v.1.5 implemented in BEAST. We generated 50 % majority rule consensus trees with posterior probabilities (PPs) using MrBayes v.3.1.2.

The ITS, ETS and plastid data were analysed separately to identify incongruences. To combine data without conflict, strongly supported (PP = 1·00 or ≥92 % ML BS) incongruences were dealt with by the duplication of the corresponding individuals, with one duplicate having only ITS and ETS sequences, and one only having plastid sequences (Pirie *et al.*, 2008). The absent sequences were coded as missing data ('?'). Sequences from the same or different species that were completely identical were reduced to one haplotype. In the text or figures, sequence identity is indicated by a slash separating the corresponding accessions. The existing concept of clades *sensu* Walker and Sytsma (2007) was adopted, except for 'Clade III'. The latter was split into two independent clades, i.e. the *S. trichocalycina* group (Clade III) and the *S. miltiorrhiza* group (Clade IV) (Fig. 1).

Ancestral character state reconstruction

Ancestral character states were reconstructed using the MESQUITE software package v.2.75 with Fitch parsimony optimization (Maddison and Maddison, 2011). Five characters, i.e. distribution area, life form, calyx morphology, stamen type and pollination system, were coded (Supplementary Data Table S1). Character states are based on literature research and observations.

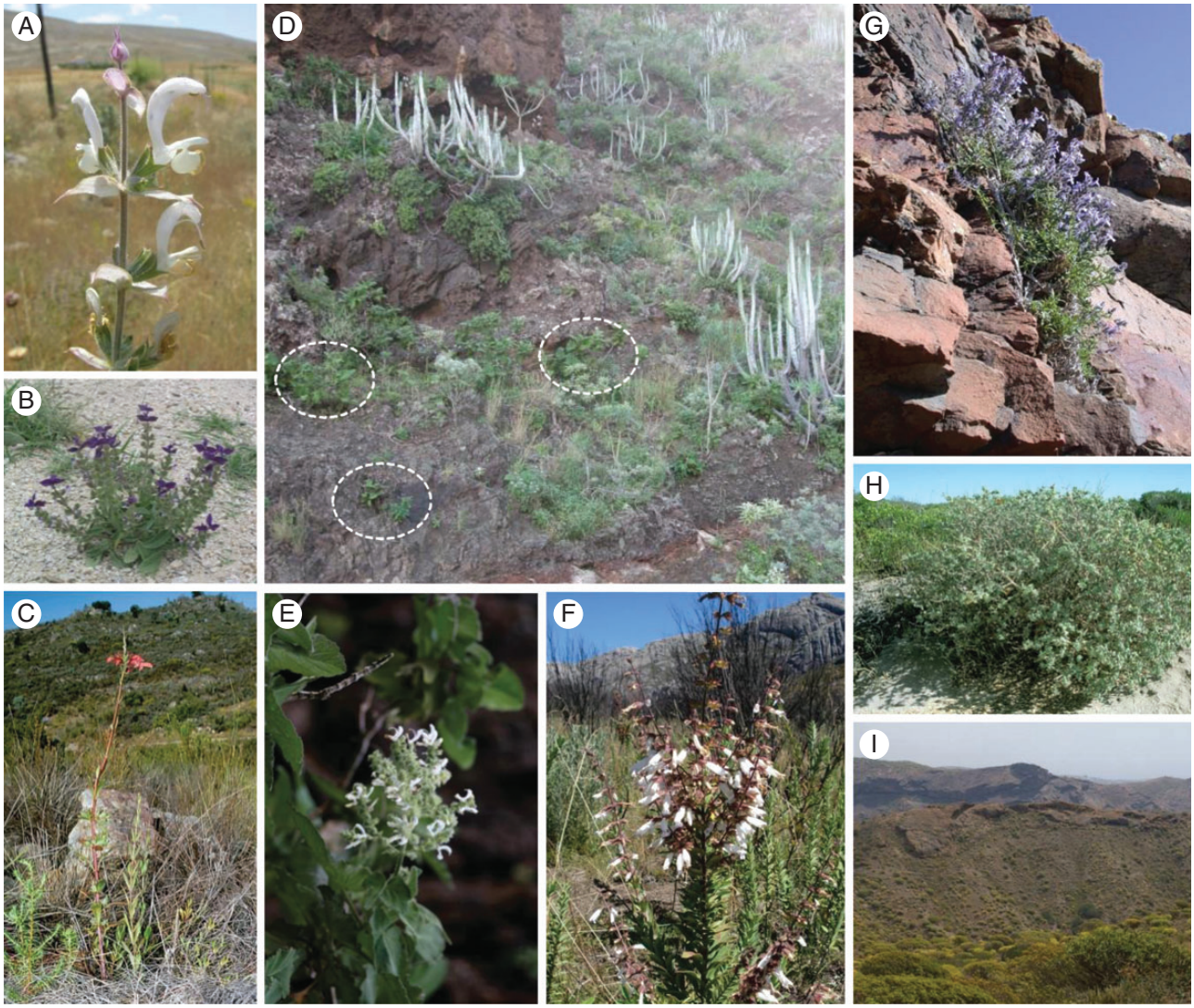


FIG. 3. Habitat diversity of *Salvia s.l.* in Africa. (A) *Salvia sclarea*, Anatolian steppe; (B) *S. viridis*, Anatolia; (C) *S. thermarum*, fynbos Western Cape, South Africa; (D, E) *S. broussonetii*, basalt maritime cliffs on Tenerife (D, dotted circles), flowering plant (E); (F) *S. leucodermis*, Madagascar; (G) *S. herbanica*, rocky habitat south-east Fuerteventura, Canary Islands; (H) *S. africana-lutea*, coastal sand dunes in the Western Cape; (I) *S. aegyptiaca*, semi-arid habitat on Gran Canaria, Canary Islands. Photographs: (A, B) F. Celep, (C, H) P. Wester, (D, E, G) R. Claßen-Bockhoff, (F) B. Bytebier, and (I) M. Thulin.

Stamen classification is based on stamen types and intermediate forms introduced by Hedge (1974, 1982): A, lower lever arm with fertile thecae; B, lower lever arm sterile; and C, lower lever arm reduced. Information on pollinators is based on the literature or personal communications, or was postulated according to character syndromes (e.g. Wester and Claßen-Bockhoff, 2011). In all cases, reconstructions are performed on 100 randomly sampled trees with branch lengths from the BI analyses, as well as the consensus tree (50 % majority rule) of the combined data BI analysis (Pirie *et al.*, 2009). Statistical support was calculated under maximum parsimony (MP).

RESULTS

Phylogenetic analyses: nrITS (Fig. 5)

The aligned length of the nuclear data set is 637 bp, 255 (40 %) of which are potentially parsimony informative. *Hyptis laniflora* is found in a polytomy with (1) *Collinsonia* and *Perilla* and (2) an unresolved, weakly supported clade. Within the last of these, nine lineages are strongly supported by BI: (1) *Horminum*; (2) *Melissa*; (3) *Lepechinia*; (4) *Perovskia*; (5) *Rosmarinus*; (6) Clade IV; (7) Clade III plus the genus *Zhumeria*; (8) a trichotomy including *Meriandra*, *Dorystaechas* and NW *Salvia* species (Clade II); and (9) Clade I (*Salvia s.s.*).

Clade IV is a well-supported clade including four East Asian and one European species (*S. glutinosa*). Clade III consists of a trichotomy composed of (1) *S. trichocalycina*, (2) *S. aristata* and (3) *S. aegyptiaca* plus *S. herbanica.* The American Clade II is strongly supported as part of a trichotomy with the two OW genera *Meriandra* and *Dorystaechas*. It is divided into two sub-clades, with *S. chionopeplica* and *S.* cf. *chionopeplica* in separate sub-clades. Clade I is only supported by BI (PP = 0·99). Sub-clade I-C forms a polytomy with Clade I-A, *S. judaica* and *S. taraxacifolia*. Sub-clade I-D is sister to this clade, and the American sub-clade I-B is in turn sister to the

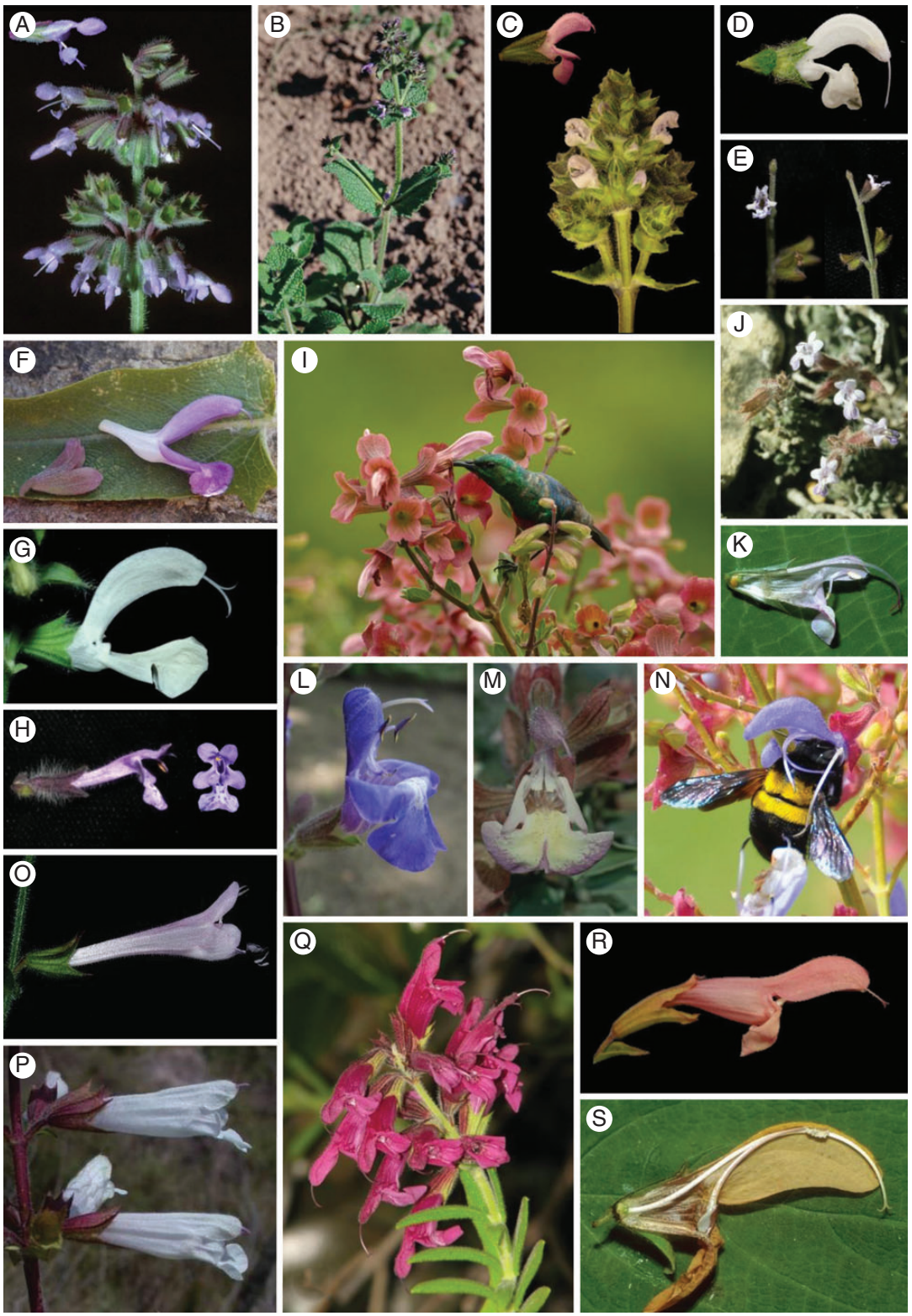


FIG. 4. Phenotypic diversity of *Salvia s.l.* in Africa. (A) *Salvia verticillata*; (B) *S. nilotica*; (C) *S. disermas*; (D) *S. argentea*; (E) *S. aegyptiaca*; (F) *S. canariensis*; (G) *S. broussonetii*; (H) *S. herbanica*; (I) *S. lanceolata* with *Cinnyris chalybeus* (southern double-collared sunbird; formerly *Nectarinia chalybeus*); (J) *S. geminata*; (K) *S. taraxacifolia*; (L) *S. interrupta*; (M) *S. dolomitica*; (N) *S. chamelaeagnea* with *Xylocopa caffra* (carpenter bee); (O) *S. scabra*; (P) *S. leucodermis*; (Q) *S. sessilifolia*; (R) *S. thermarum*; and (S) *S. africana-lutea*. Note the typical falcate upper corolla lip in bee-pollinated species (e.g. *S. argentea*, D) in contrast to the straight upper lip in bird-pollinated taxa (I, P–R) and the diverse floral morphologies in the Canary Islands endemics (F–H). Photographs: (A, B, C, D, F, L) M. Will, (E, H) R. Claßen-Bockhoff, (G, K, O, R, S) P. Wester, (I) R. Groneberg, (J) M. Thulin, (N) H. Technau, (P) P. B. Phillipson and (Q) D. Hannon.

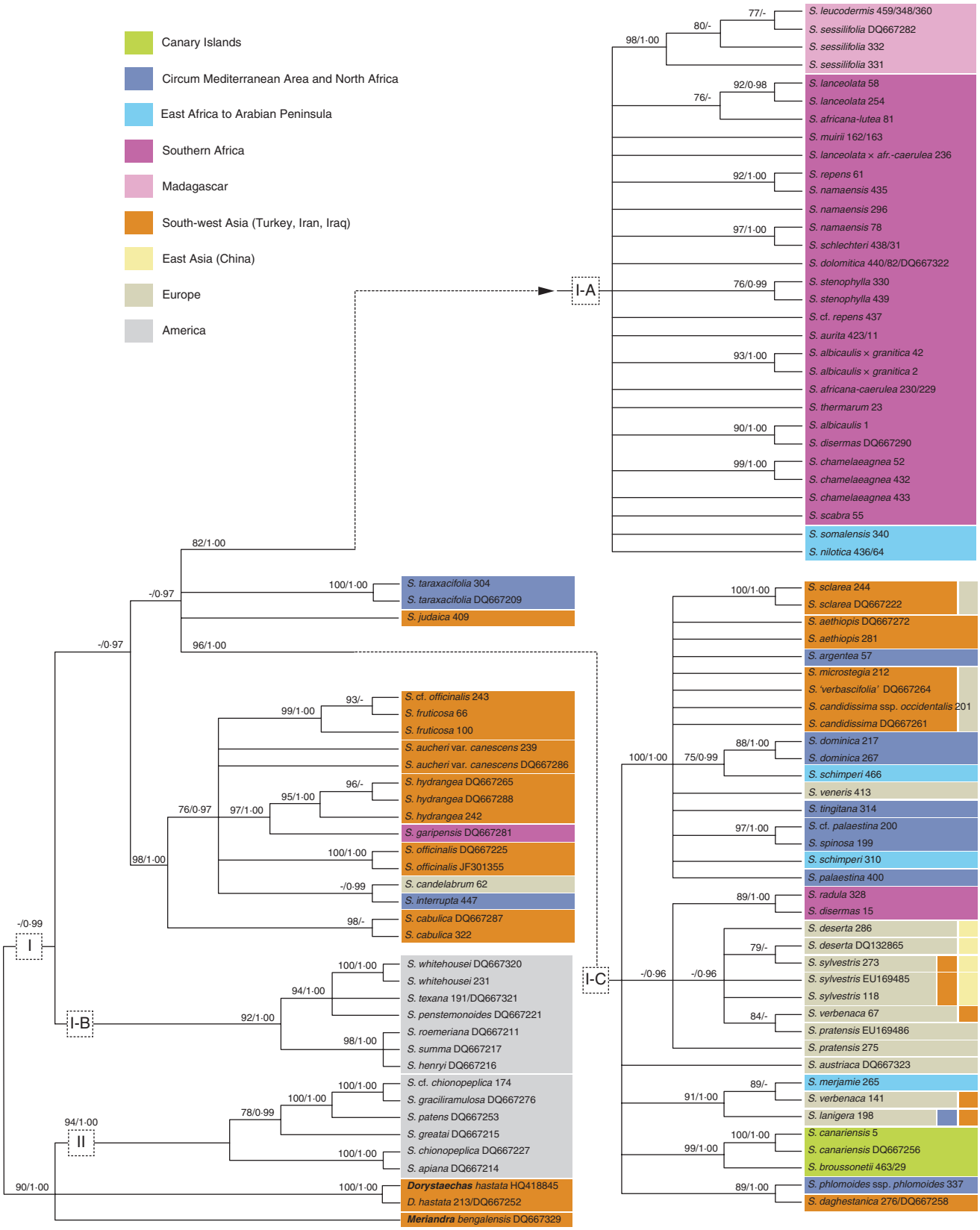


FIG. 5. Analyses of the nrITS data set. Non-*Salvia* genera are highlighted (bold); names of accessions with identical sequences are separated by slashes; only support values ≥75 % (BS) and ≥0·95 (PP) are illustrated. Species distribution is indicated by different colours.

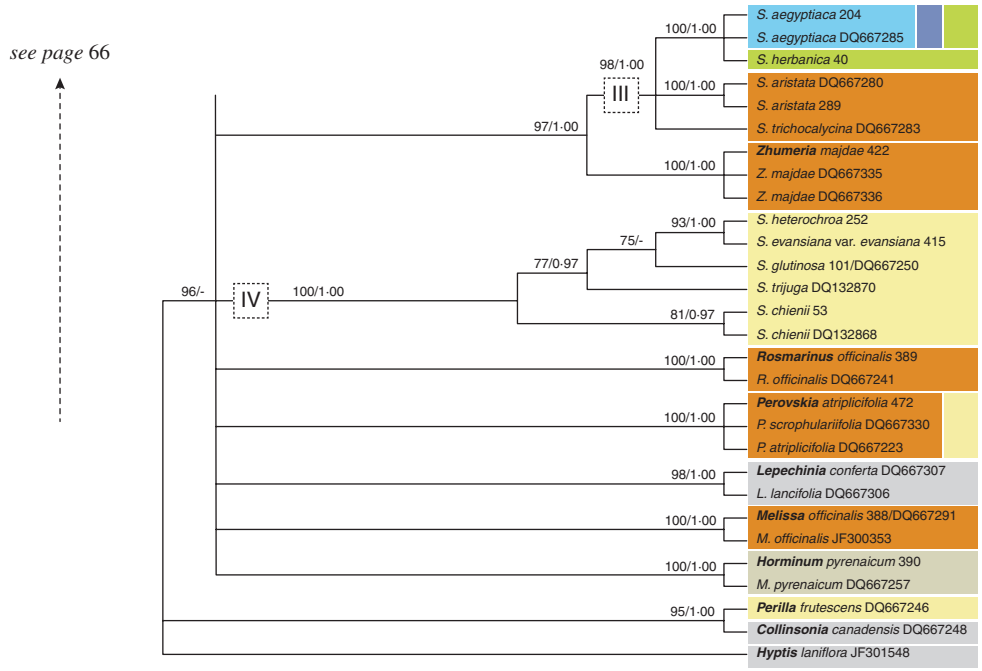


Fig. 5 *Continued*

remainder of Clade I. Within sub-clade I-D (seven species), *S. cabulica* is sister to a polytomy consisting of six lineages (Fig. 5: I-D). The two accessions of *S. fruticosa* do not cluster together. Six major lineages are recognized within sub-clade I-C. One lineage contains the two Canary Island endemics *S. broussonetii* and *S. canariensis* (Fig. 5: I-C; green). A second lineage includes the South African endemics *S. radula* and *S. disermas* (Fig. 5: I-C; magenta) along with four European species (Fig. 5: I-C; beige; monophyly for each of these species is not confirmed). Sub-clade I-A is only poorly resolved. It includes exclusively African taxa restricted to SSA. Within sub-clade I-A, one strongly supported clade includes all six Madagascan accessions (2 spp.) (Fig. 5: I-A; pink). The ML analysis weakly indicates that *S. sessilifolia* is paraphyletic with respect to *S. leucodermis*. Sequences of *S. leucodermis* and *S. sessilifolia* differ only in one position (A or C) which is ambiguous (IUPAC code: M) in *S. sessilifolia* accession DQ667282.

ETS (Supplementary Data Fig. S1)

The alignment contains 69 accessions (53 species), 60 (44 species) representing the genus *Salvia*. The aligned length of the data set is 466 bp, 230 (40·4 %) of which are potentially parsimony informative. There are few major conflicts with the ITS topology. Differences concern a clade which is moderately supported by BI (PP = 0·98) including: (1) Clade II plus *Meriandra* and *Dorystaechas*; (2) Clade III without *S. aristata* (here called Clade III-A); (3) *Zhumeria* plus *S. aristata*; and (4) *S. przewalskii* (Clade IV represented by only one species in this data set). This clade is not supported (but also not contradicted) in the ITS analyses. The incongruence between the ITS and ETS data sets is in the position of *Zhumeria*. However, this difference might be based on the slightly different sampling in Clade III. Furthermore, support for a monophyletic Clade II is lacking. Instead, the three lineages of NW *Salvia* spp. form a polytomy with *Meriandra* and *Dorystaechas* (Supplementary Data Fig. S1].

Compared with the ITS data set, support for Clade I (*Salvia s.s.*) is low (PP = 0·96). The four sub-clades (sub-clades I-A through I-D) form a polytomy. Differences in the topology of sub-clade I-C are mainly based on additional accessions in the ETS data set, e.g. *S. canariensis* 464. The latter renders *S. canariensis* paraphyletic with respect to *S. broussonetii*, but this relationship is not strongly supported. Similarly, adding *S. disermas* 454 causes *S. disermas* to be paraphyletic with respect to *S. radula*. Sub-clade I-A is better resolved in the ETS than in the ITS data set. The former supports sister relationships for (1) *S.* cf. *repens* 437 and *S. stenophylla* and (2) *S. aurita* and *S. dolomitica*. Furthermore, *S. africana-caerulea*, *S. albicaulis*, *S. chamelaeagnea* and *S. lanceolata* × *africana-caerulea* appear in a moderately supported clade not supported in ITS data (PP = 0·98).

rpl32-trnL (Fig. 6)

The aligned length of the plastid data set is 929 bp, with 246 (26·5 %) potentially informative nucleotide positions. *Hyptis* is again found in a trichotomy with *Collinsonia* and a strongly supported clade that includes all other accessions. The latter splits into two major lineages one only including non-*Salvia* samples (*Horminum*, *Glechoma*, *Thymus*, *Clinopodium* and *Mentha*). The second clade contains all *Salvia* samples and six additional genera. *Melissa* and *Lepechinia* are moderately supported (ML) as sister genera. They are found in a polytomy with two strongly supported clades.

The first includes Clade IV, sub-clades III-A, III-B, *Zhumeria*, Clade II and *Dorystaechas*, as found in the ETS data set. The

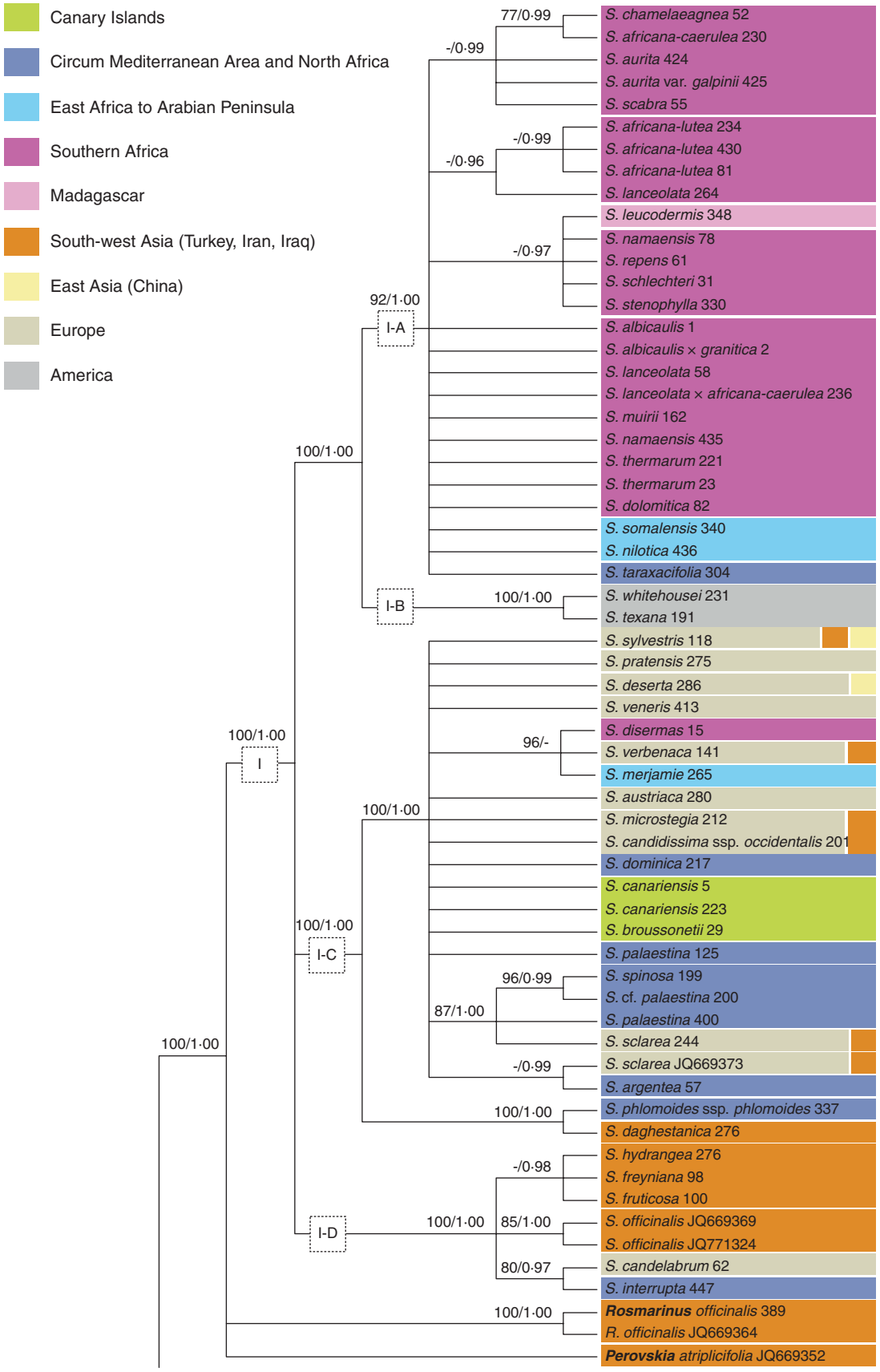


FIG. 6. Analyses of the *rpl32-trnL* data set. Non-*Salvia* genera are highlighted (bold); only support values ≥75 % (BS) and ≥0·95 (PP) are illustrated. Species distribution is indicated by different colours.

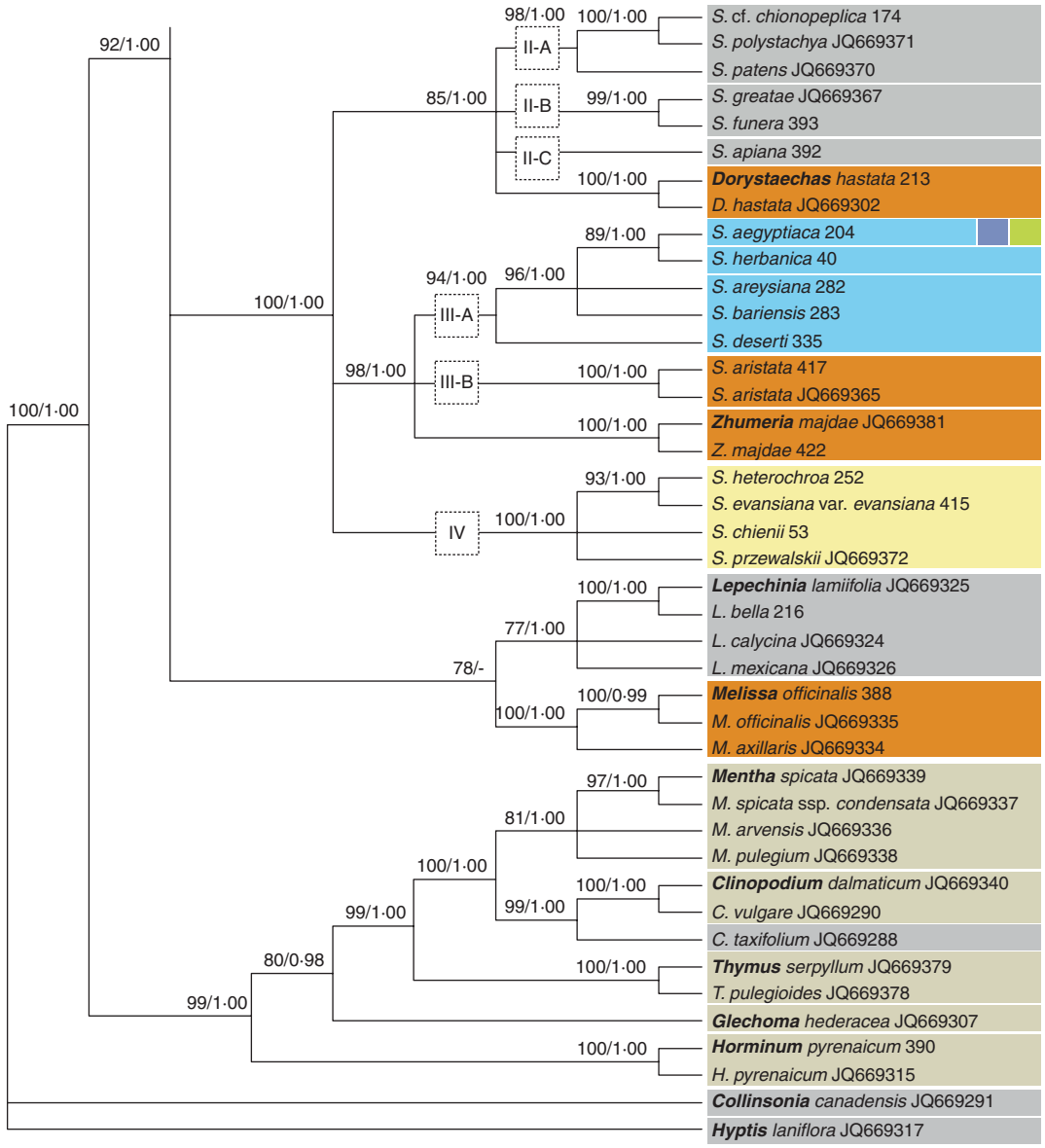
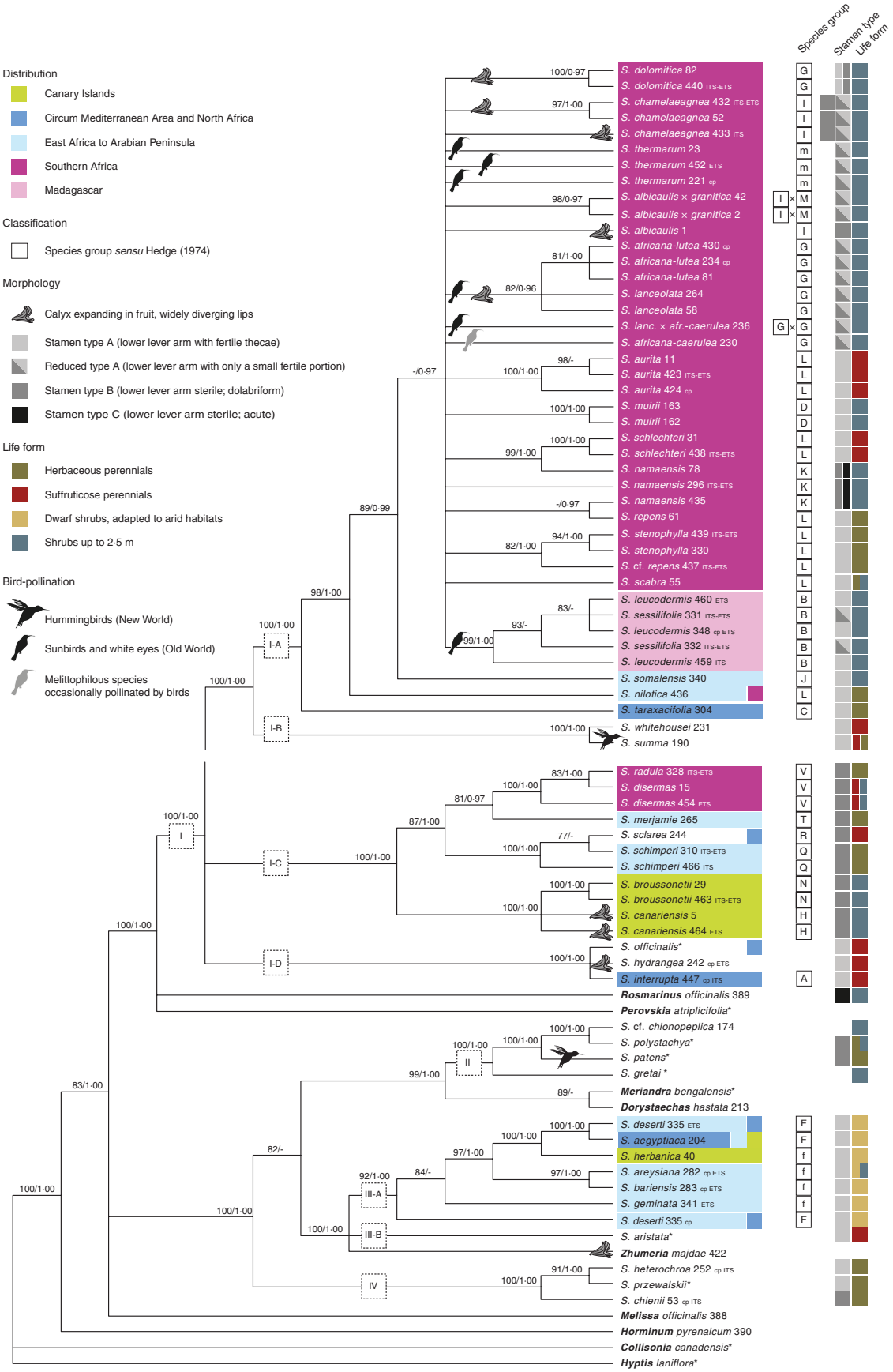


Fig. 6 *Continued*

position of *Zhumeria* differs from the ETS data; the genus is placed in a trichotomy with *S. aristata* (III-B) and the *S. aegyptiaca* group (III-A) based on plastid data. As in the ETS data set, the monophyly of Clade II is not supported. Instead, three sub-clades (II-A, II-B and II-C) are found in a polytomy with *Dorystaechas.* The most striking incongruence between nuclear and plastid data is the position of *S. deserti* (Fig. 6; III-A). It is sister to *S. aegyptiaca* based on ETS data (Supplementary Data Fig. S1) but strongly supported in a sister relationship to all species nesting in sub-clade III-A based on the plastid data set (Fig. 6). In the ITS data set, *S. deserti* is not represented.

The second major clade consists of a trichotomy composed of *Rosmarinus*, *Perovskia* and a strongly supported Clade I (*Salvia s.s.*). Compared with nuclear data, the latter is better resolved, splitting into three major lineages (sub-clades): (1) I-D (topology corresponding to nuclear data); (2) I-C; and (3) I-B plus I-A. Within sub-clade I-C, neither *S. sclarea* (244 and JQ669373) nor *S. palaestina* (400 and 200) is supported as monophyletic. Sister grouping of sub-clades I-A (Africa) and I-B (America) is in conflict with ITS data (Fig. 5). Sub-clade I-A was strongly supported by the nuclear data, but the relationships among its species were largely unresolved. However, its topology slightly differs. Instead of being part of a basal polytomy, *S. chamelaeagnea* 52 and *S. africana-caerulea* 230 are sister species in the plastid data set, closely related to *S. aurita* and *S. scabra*. The ITS data do not suggest any relationships for *S. aurita*, whereas the ETS data weakly support a sister relationship to *S. dolomitica*. Furthermore, in contrast to the ITS topology, different accessions of *S. namaensis* (78 and 435) and *S. lanceolata* (264 and 58) are not supported to be monophyletic based on plastid data.


Species group
Stamen type
Life form
Distribution
Canary Islands
Circum Mediterranean Area and North Africa
East Africa to Arabian Peninsula
Southern Africa
Madagascar
Classification
Species group sensu Hedge (1974)
Morphology
Calyx expanding in fruit, widely diverging lips
Stamen type A (lower lever arm with fertile thecae)
Reduced type A (lower lever arm with only a small fertile portion)
Stamen type B (lower lever arm sterile; dolabriform)
Stamen type C (lower lever arm sterile; acute)
Life form
Herbaceous perennials
Suffruticose perennials
Dwarf shrubs, adapted to arid habitats
Shrubs up to 2·5 m
Bird-pollination
Hummingbirds (New World)
Sunbirds and white eyes (Old World)
Melittophilous species occasionally pollinated by birds
S. dolomitica 82
S. dolomitica 440 ITS-ETS
S. chamelaeagnea 432 ITS-ETS
S. chamelaeagnea 52
S. chamelaeagnea 433 ITS
S. thermarum 23
S. thermarum 452 ETS
S. thermarum 221 cp
S. albicaulis × granitica 42
S. albicaulis × granitica 2
S. albicaulis 1
S. africana-lutea 430 cp
S. africana-lutea 234 cp
S. africana-lutea 81
S. lanceolata 264
S. lanceolata 58
S. lanc. × afr.-caerulea 236
S. africana-caerulea 230
S. aurita 11
S. aurita 423 ITS-ETS
S. aurita 424 cp
S. muirii 163
S. muirii 162
S. schlechteri 31
S. schlechteri 438 ITS-ETS
S. namaensis 78
S. namaensis 296 ITS-ETS
S. namaensis 435
S. repens 61
S. stenophylla 439 ITS-ETS
S. stenophylla 330
S. cf. repens 437 ITS-ETS
S. scabra 55
S. leucodermis 460 ETS
S. sessilifolia 331 ITS-ETS
S. leucodermis 348 cp ETS
S. sessilifolia 332 ITS-ETS
S. leucodermis 459 ITS
S. somalensis 340
S. nilotica 436
S. taraxacifolia 304
S. whitehousei 231
S. summa 190
S. radula 328 ITS-ETS
S. disermas 15
S. disermas 454 ETS
S. merjamie 265
S. sclarea 244
S. schimperi 310 ITS-ETS
S. schimperi 466 ITS
S. broussonetii 29
S. broussonetii 463 ITS-ETS
S. canariensis 5
S. canariensis 464 ETS
S. officinalis*
S. hydrangea 242 cp ETS
S. interrupta 447 cp ITS
Rosmarinus officinalis 389
Perovskia atriplicifolia*
S. cf. chionopeplica 174
S. polystachya*
S. patens*
S. gretai *
Meriandra bengalensis*
Dorystaechas hastata 213
S. deserti 335 ETS
S. aegyptiaca 204
S. herbanica 40
S. areysiana 282 cp ETS
S. bariensis 283 cp ETS
S. geminata 341 ETS
S. deserti 335 cp
S. aristata*
Zhumeria majdae 422
S. heterochroa 252 cp ITS
S. przewalskii*
S. chienii 53 cp ITS
Melissa officinalis 388
Horminum pyrenaicum 390
Collisonia canadensis*
Hyptis laniflora*
I-A
I-B
I-C
I-D
I
II
III-A
III-B
IV

Combined analyses of the nuclear and plastid data sets (Fig. 7)

The aligned length of the combined data set is 1982bp, of which 582 (29·4 %) are potentially parsimony informative. The sequence duplication approach was not suitable to resolve the conflicting placement of sub-clade I-B within *Salvia s.s.*, which was placed either at the base of Clade I (ITS; Fig. 5) or as sister to sub-clade I-A (plastid; Fig. 6). Thus, we used the tree with the best topology for illustration, being aware of the unresolved conflict for the two clades.

The combined tree largely reflects the topology of the plastid data set (e.g. sister relationship of sub-clade I-A and I-B), but shows better resolution and higher support within sub-clade I-A. *Melissa* is in a trichotomy with two clades containing *Salvia* spp. The first covers (1) Clade II, *Dorystaechas* and *Meriandra*, and the latter two moderately supported as sister; (2) sub-clades III-A, III-B and *Zhumeria*; and (3) Clade IV. The second includes *Perovskia*, *Rosmarinus*, and *Salvia s.s.* (Clade I). The latter is strongly supported and falls into the same three major lineages as in the plastid data set. As to sub-clade I-A, *S. taraxacifolia* is sister to all remaining taxa. *Salvia nilotica* splits next, followed by *S. somalensis*, which is sister to a large clade including only taxa from southern Africa and Madagascar. Monophyly of *S. namaensis*, *S. repens*, *S. sessilifolia* and *S. leucodermis*, each of which was represented by more than one accession, is not confirmed.

Ancestral character state reconstruction (Supplementary Data Figs S2–S6; Fig. 7)

All African areas, except Madagascar, were colonized more than once (Supplementary Data Fig. S2). The ancestral area is reconstructed as East Africa and the Arabian Peninsula for sub-clades of *Salvia sensu lato* (*s.l.*) (III-A; MP 100 %) and *Salvia s.s.* (sub-clade of I-C covering *S. sclarea*, *S. schimperi*, *S. merjamie*, *S. disermas* and *S. radula*; MP 89 %). Within the latter, *S. disermas* and *S. radula* point to the colonization (migration and/or dispersal) of southern Africa. A second colonization is supported for the largest lineage of sub-clade I-A, which only contains accessions from southern Africa (MP 94 %). Madagascar was most probably colonized from southern Africa (MP 64 %).

Reconstruction of the ancestral life form (Supplementary Data Fig. S3) revealed that three of the four defined growth forms evolved several times in parallel. Only some crown groups can clearly be characterized by this character, e.g. Clade IV (MP 100 %: perennial herbs), Clade III-A (MP 100 %; dwarf shrubs) and the clade consisting of *S. canariensis* and *S. broussonetii* (sub-clade I-C; MP 100 %; shrubs). For the clade containing Clades II, III and IV, herbaceous growth most probably reflects the ancestral state (MP 72 %). Clade IV includes exclusively herbaceous perennials, while its sister group is characterized by shrubby growth (MP 94 %). Within this clade, dwarf shrubby growth is a synapomorphy for sub-clade III-A (Fig. 7). A character transformation from herbaceous to shrubby growth is also found in *Salvia s.s.* (sub-clade I-A), and a reversal back to herbaceous growth is found for *S. repens* and *S. stenophylla* nesting within the same clade. Clade II (including *Dorystaechas hastata* and *Meriandra bengalensis*) appears to be originally shrubby. However, our study underrepresents the diversity of growth forms in this large NW clade, which includes shrubs, perennials, annuals and trees. The SW Asian species (sub-clade III-A, *S. aristata* and *Zhumeria*) show two different trends, one towards a suffruticose life form (*S. aristata*) and one towards dwarf shrubby growth (III-A).

A thick-textured, non-expanding calyx represents the ancestral state in each of the major *Salvia* clades (MP 100 %) (Supplementary Data Fig. S4). Expanding calyxes with widely diverging lips, which are papery and often coloured, evolved several times in parallel, not only in *Salvia* (sub-clades I-A, I-C and I-D) but also in *Zhumeria* (Fig. 7).

For stamen construction, type A with a fertile theca at the lower lever arm is strongly supported as the ancestral state in sub-clade III-A and *Salvia s.s.* (Supplementary Data Fig. S5). Within the latter, stamen type B evolved in sub-clade I-C and in I-A. Furthermore stamen type C is found in *S. namaensis* in sub-clade I-A (Fig. 7). The same reduction of the lower lever arm is found in *Rosmarinus officinalis*, which is not closely related to *S. namaensis*.

Melittophily, bee pollination, is reconstructed as the ancestral pollination system for each clade, except for sub-clade I-B (Supplementary Data Fig. S6). The latter is represented by only two species, one of them assumed to be ornithophilous and the other psychophilous (Wester and Claßen-Bockhoff, 2011). Thus, its ancestral character state remains ambiguous. Bird-pollinated flowers evolved repeatedly in the NW (Clade II and sub-clade I-B) and in the OW sub-clade I-A (Fig. 7). Within the latter, at least two pollinator shifts are suggested (Supplementary Data Fig. S6; Fig. 7), one by the Madagascan sub-clade and another by two South African species forming a clade (*S. lanceolata* and *S. africana-lutea*). A third switch to bird pollination might be represented by *S. thermarum* from South Africa. Two of the sampled accessions are found in a derived position of a melittophilous lineage (MP 60 %; Fig. 7).

DISCUSSION

Our study confirms the non-monophyly of both *Salvia s.l.* and the African *Salvia* spp. (Walker *et al.*, 2004). We also confirm that all African species are restricted to two of the four major lineages of *Salvia* (Clades I and III; Fig. 7).

Interspecific relationships in African Salvia*: species groups* sensu *Hedge*

Subgeneric classification of the genus is based on morphology and distribution (Bentham, 1832–1836, 1848, 1876; Briquet,

FIG. 7. Analyses of the combined data set. Non-*Salvia* genera are highlighted (bold); GenBank accessions are marked with an asterisk (*); for taxa with only one or two markers, the corresponding marker is given after the taxon name and extraction number. Uncapitalized letters (m, f) indicate taxa described after the revision of African *Salvia* (Hedge, 1974); classification in the corresponding species group is based on morphology and the corresponding species description (Santos and Fernández, 1986; Thulin, 1993, 2009; Van Jaarsveld, 1999). For detailed information about ancestral character reconstruction see also Supplementary Data Table S1 and Figs S2–S6.

1897). Hedge (1974) established 23 'species groups' to address relationships among African *Salvia* spp. and their affinities beyond the continent. For four of these groups, molecular data can be used to discuss their monophyly.

The two taxa placed in species group V (*S. disermas* and *S. radula*) form a clade within sub-clade I-C (Fig. 7). Both species occur in southern Africa but do not overlap in their distribution (Fig. 9E: 26, 34). They have similar flower morphology but differ in flowering time and indumentum (Hedge, 1974). We confirm monophyly of this group and, based on ETS and combined data sets, find some support for Hedge's (1974) idea that *S. radula* could be a subspecies of *S. disermas* (Supplementary Data Table S1).

Species group F originally included three African species (Hedge, 1974). The two species included in this study (*S. aegyptiaca* and *S. deserti*) form a clade with four more recently described species (Fig. 7; III-A), all of which are adapted to arid or semi-arid habitats. Except for the widespread *S. aegyptiaca* (Fig. 10C: 10), they are all local endemics, e.g. in East Africa and the Arabian Peninsula (*S. areysiana*, *S. bariensis* and *S. geminata*) or Fuerteventura (*S. herbanica*) (Fig. 9B: 9; C: 12, 13, 15, D: 14). As the clade is well supported by synapomorphies, e.g. growth as dwarf shrubs with simple, revolute leaves, straight upper corolla lips with exposed stamens and minute flowers (Hedge, 1974; Santos and Fernández, 1986; Scholz, 1993; Thulin, 1993, 2009), we not only confirm monophyly of species group F but extend it to include at least the six sampled species. Based on the unique character syndrome, more species are likely to be included in this species group (Bokhari and Hedge, 1977; M. Will and R. Claßen-Bockhoff, unpubl. data). Furthermore, our data confirm the close relationship proposed for African and SW Asian *Salvia* (Davis and Hedge, 1971; Hedge, 1974).

In contrast, relationships for the monospecific species groups H and N were not predicted before. While Hedge (1974) supposed that the allies of *S. canariensis* (H) occur in southern Africa, he described *S. broussonetii* (N) as a relict species without any close ally. However, our data point to a close relationship between these two endemics from the Canary Islands (Fig. 7).

Morphological characters used for classification: stamen types

Stamen morphology was used by Walker and Sytsma (2007) to distinguish two major lineages within Clade I. However, our analysis clearly shows that stamen morphology is much more variable. Character state reconstruction revealed that stamen type A (Supplementary Data Fig. S5) is the ancestral state for Clade III-A and *Salvia s.s.* (Clade I). Consequently, the reduction of the lower lever arm evolved several times in parallel. In *Salvia s.s.* (Fig. 8), sub-clade I-A covers the whole range of stamen modifications described in African *Salvia* (Fig. 8; Hedge, 1974), including the rare stamen type C (*S. namaensis*). In the OW, this stamen modification is only known from the Eurasian *S. verticillata* group (four species), which is also part of *Salvia s.s.* (Clade I; Will, 2013), but not closely related to *S. namaensis* from SSA. A third species with stamen type C and the same ontogeny as *S. verticillata* is *Rosmarinus officinalis* (unpubl. res.). Parallel evolution is thus evident (Supplementary Data Fig. S5), restricting the use of stamen types to lower taxonomic levels.

Floristic links of Salvia *spp. in North Africa*

Salvia spp. distributed in North Africa are clearly members of two different clades (Fig. 7; I-A and III-A). Based on the molecular data, they show many floristic links to southern Europe, SW Asia, East and southern Africa (Fig. 5). One example is *S. taraxacifolia*, a relict species endemic to the High Atlas, Morocco (Hedge, 1974). It is most closely related to East (*S. nilotica* and *S. somalensis*) and southern African species (Fig. 7; I-A). Our data suggest dispersal from North to East Africa followed by a second dispersal to southern Africa. *Salvia taraxacifolia* and the two East African species are adapted to mesic habitats, whereas their southern African relatives prefer arid habitats (Fig. 9E–G). This indicates that the common ancestor of sub-clade I-A might have been adapted to mesic conditions and that within the large SSA radiation (sub-clade I-A), adaptation to arid localities evolved in southern Africa.

The floristic links between North Africa and southern Europe already proposed by Hedge (1974) (Fig. 7; sub-clade I-C) were confirmed by the close relationship of *S. interrupta* (SW Morocco; Fig. 10B: 56) and *S. candelabrum* (southern Spain; Fig. 10B). Both are thermophilic and partly overlap in their distribution (Fig. 10B; Rosúa and Blanca, 1986, 1990). They have a similar habit (divided leaves, most of them at the base of the stem), conspicuous, elongated inflorescences and the same chromosome number ($2n = 14$) (Hedge, 1974; Rosúa and Blanca, 1985, 1990). *Salvia interrupta* is considered as a Tertiary relict which was more widely distributed when the climate was more mesic (Rosúa and Blanca, 1990). Both species are obviously derived from a common, probably mesic-adapted, ancestor. Their relationship might reflect allopatric speciation probably triggered by different edaphic factors in the corresponding habitats.

The strongly supported sister relationship of *S. daghestanica* (Caucasus) and *S. phlomoides* subsp. *phlomoides* (North Africa and southern Europe; Fig. 10B: 47) reflects floristic links between the Mediterranean and SW Asia. Since contact between the African and Eurasia floras should have increased during the Messinian Salinity Crisis in the late Miocene [5·96–5·33 million years ago (Mya)], plant colonization across the Mediterranean is expected to have occurred often during this time frame (Caujapé-Castells and Jansen, 2003). We assume that this 'route' was also used repeatedly by *Salvia*. We thus support the hypothesis of Davis and Hedge (1971) that the SW Asian origin of some NW African species was triggered by a westward shift of Irano-Turanian elements.

Repeated colonization of the Canary Islands and long-distance dispersal in Salvia

The Macaronesian flora is composed of endemics derived from an ancient Tertiary relict flora and more recently introduced species (e.g. Helfgott *et al.*, 2000; Manen *et al.*, 2002; Carine *et al.*, 2004). This general pattern also appears to hold for the Macaronesian *S. canariensis*, *S. broussonetii* and *S. herbanica* (Fig. 9B: 7–9). Since the three species clearly differ in their morphology (Fig. 4F–H) and habitat preference (Fig. 3D, G), they were never expected to be closely related (Hedge, 1974; Carine *et al.*, 2004). However, *S. canariensis* and *S. broussonetii* are sister species forming one clade within

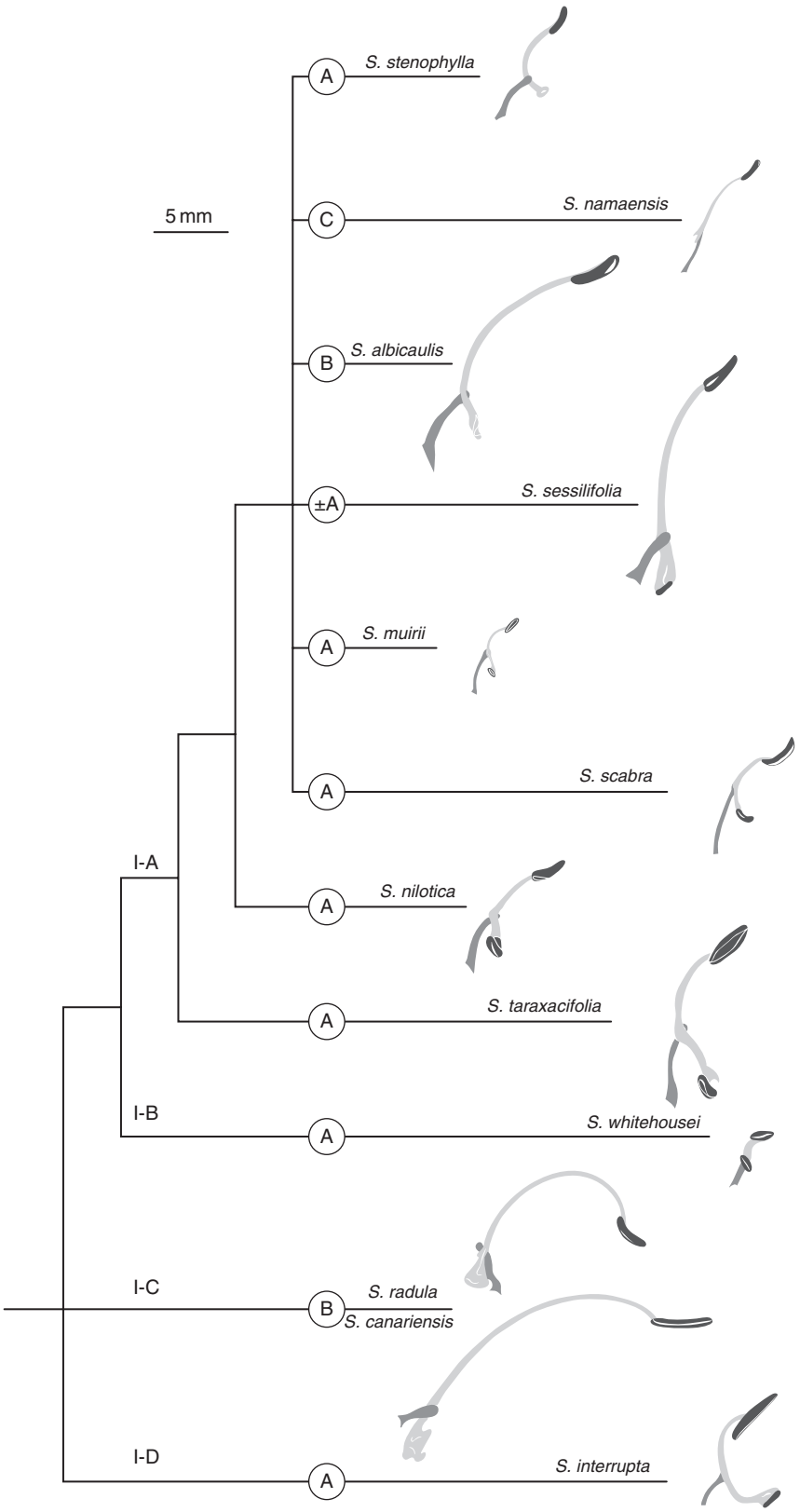


FIG. 8. Trends in the evolution of stamen types in African representatives of *Salvia s.s.* (Clade I). Proceeding from the ancestral stamen type A, the hypothetical stamen evolution is illustrated. Stamen of *S. whitehousei* modified after Whitehouse (1949), *S. interrupta* modified after Rosúa and Blanca (1986), schemata of all other stamens modified after Hedge (1974); filament (medium grey); connective (light grey); theca (dark grey); stamen types (in circles; ± A = reduced type A); and clades (I-A to I-D) represented by the species are given above the branches; scale bar = 5 mm.

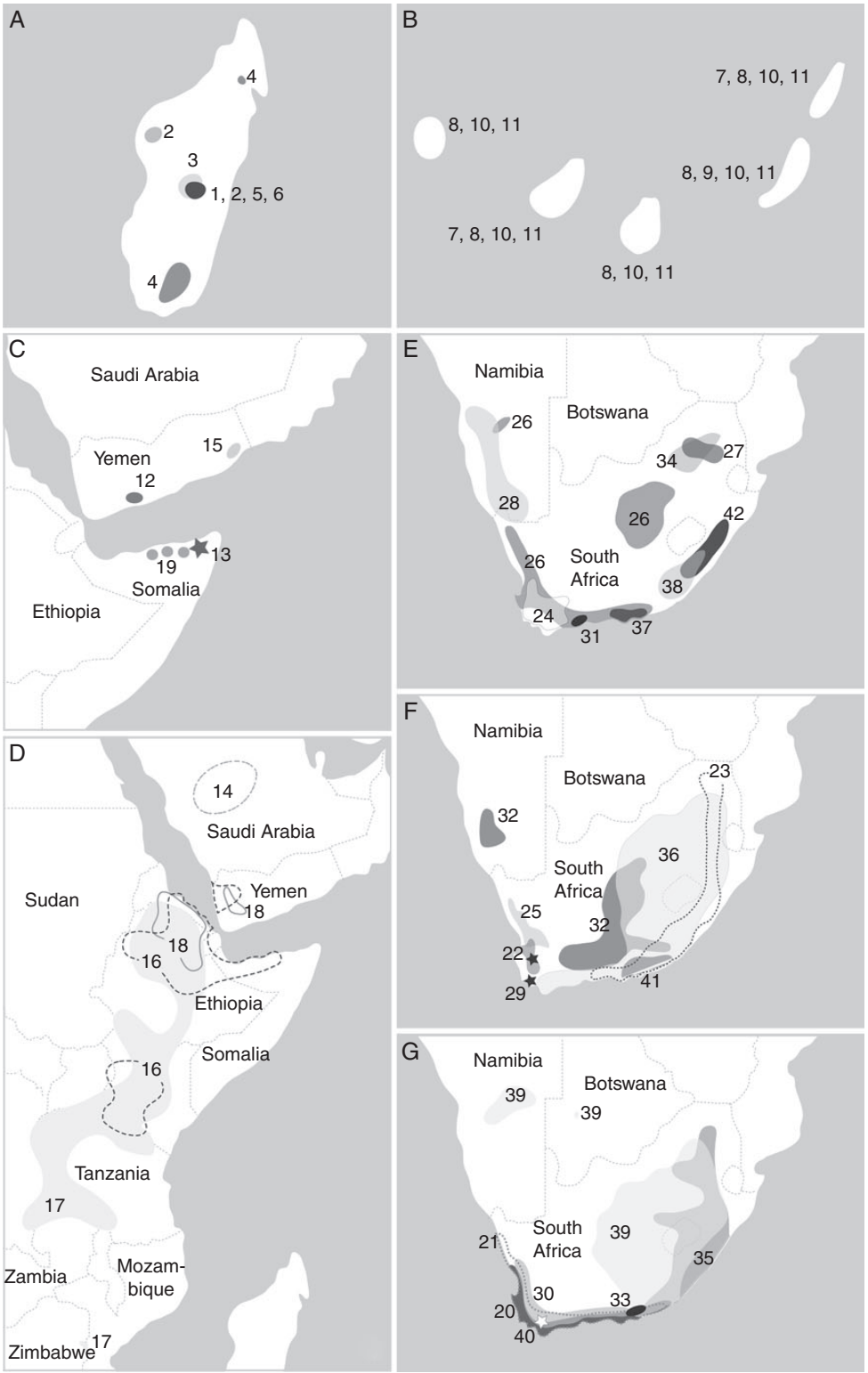


FIG. 9. Distribution of African *Salvia* spp. (except North Africa and circum-Mediterranean area). (A) Madagascar: (1) *S. cryptoclada*; (2) *S. leucodermis*; (3) *S. parvifolia*; (4) *S. perrieri*; (5) *S. porphyrocalyx*; (6) *S. sessilifolia*. (B) Canary Islands: (7) *S. broussonetii*; (8) *S. canariensis*; (9) *S. herbanica*; (10) *S. aegyptiaca*; (11) *S. verbenaca*. (C, D) East Africa and Arabian Peninsula: (12) *S. areysiana*; (13, black asterisk) *S. bariensis*; (14) *S. deserti*; (15) *S. geminata*; (16) *S. merjamie*; (17) *S. nilotica*; (18) *S. schimperi*; (19) *S. somalensis*. (E–G) southern Africa: (20) *S. africana-caerulea*; (21) *S. africana-lutea*; (22) *S. albicaulis*; (23) *S. aurita*; (24) *S. chamelaeagnea*; (25) *S. dentata*; (26) *S. disermas*; (27) *S. dolomitica*; (28) *S. garipensis*; (29, black asterisk) *S. granitica*; (30) *S. lanceolata*; (31) *S. muirii*; (32) *S. namaensis*; (33) *S. obtusata*; (34) *S. radula*; (35) *S. repens*; (36) *S. runcinata*; (37) *S. scabra*; (38) *S. schlechteri*; (39) *S. stenophylla*; (40, white asterisk) *S. thermarum*; (41) *S. triangularis*; (42) *S. tysonii*. Based on Codd (1985), Hedge (1974), Santos and Fernández (1986), Thulin (1993, 2009) and Van Jaarsveld (1999). Note the overlapping distributions of species in southern Africa (Fig. 7E–G) and the disjunct area of *S. stenophylla* (39) and *S. disermas* (26).

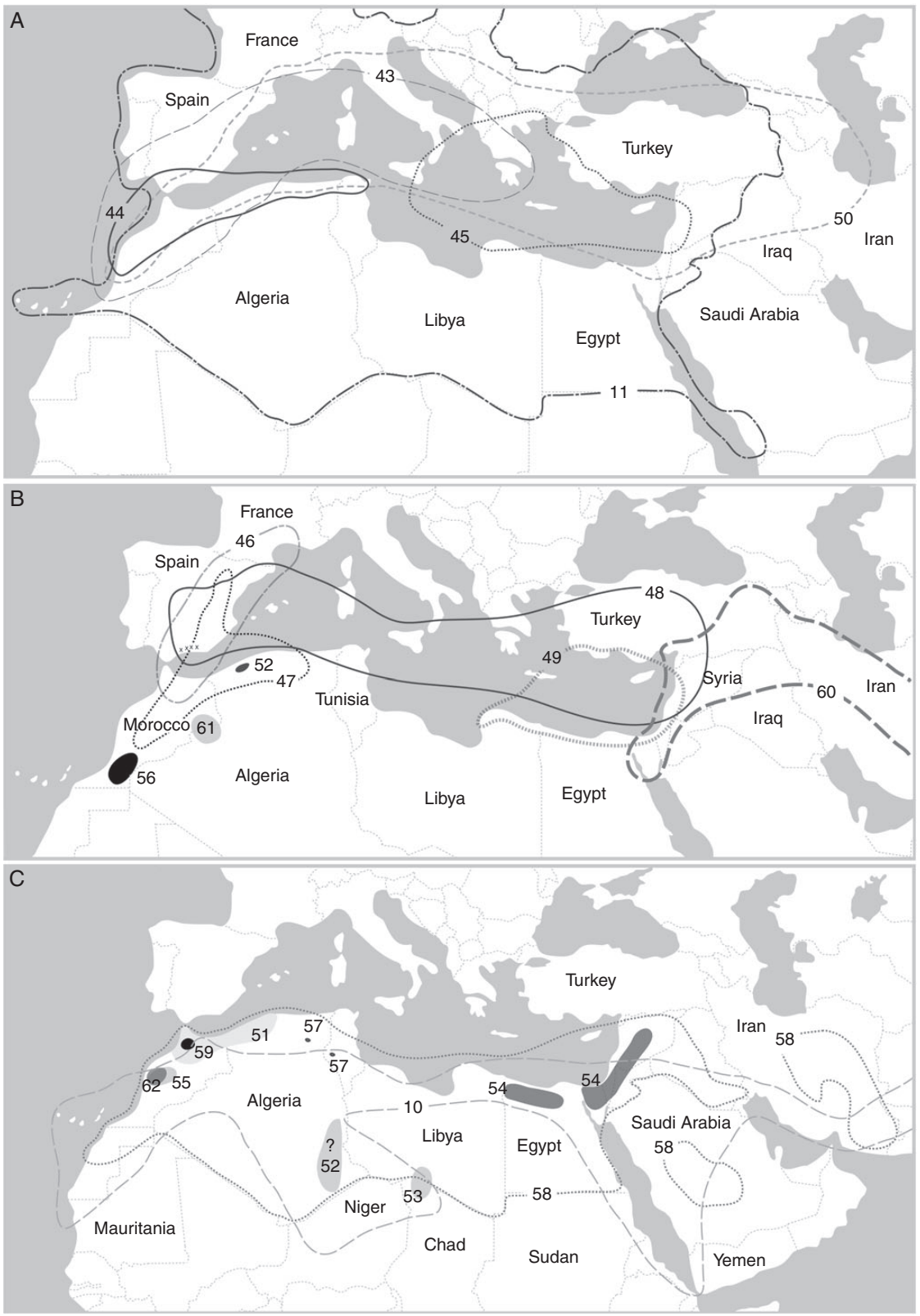


FIG. 10. Distribution of *Salvia* spp. from North Africa and the circum-Mediterranean area. (A–C) (10) *S. aegyptiaca*; (11) *S. verbenaca*; (43) *S. argentea*; (44) *S. barrelieri*; (45) *S. fruticosa*; (46) *S. lavandulifolia*; (47) *S. phlomoides*; (48) *S. sclarea*; (49) *S. spinosa*; (50) *S. viridis*; (51) *S. algeriensis*; (52) *S. balansae*; (53) *S. chudaei*; (54) *S. dominica*; (55) *S. gattefossei*; (56) *S. interrupta*; (57) *S. jaminiana*; (58) *S. lanigera*; (59) *S. mouretii*; (60) *S. palaestina*; (61) *S. pseudojaminiana*; (62) *S. taraxacifolia*. Based on Hedge (1974); the distribution of *S. sclarea* on the Iberian Peninsula is based on Rosúa and Blanca (1986). Note the overlapping distribution of *S. phlomoides* (47) and *S. candelabrum* (endemic to the Iberian Peninsula, indicated by 'x'; based on Rosúa and Blanca (1986).

sub-clade I-C (Figs 5 and 7). The proposed allies of *S. canariensis* (Hedge, 1974) are not closely related to these two species, thus contradicting Hedge's (1974) hypothesis of a link between the Canary Island and southern African *Salvia*.

In contrast, *S. herbanica* is found in a different clade (Fig. 7; III-A) and has clear links to species from East Africa and the Arabian Peninsula. Our findings indicate the non-monophyly of the Canary Island endemics and support the hypothesis of repeated dispersals to the archipelago from different mainland sources (Emerson *et al*., 2000; Arnedo *et al*., 2001; Fuertes-Aguilar *et al*., 2002; Carine *et al*., 2004, and references therein; Vargas, 2007).

The colonization of the Canary Islands raises the question of how dispersal might have taken place. The question of how (if at all) *Salvia* might be adapted to long-distance dispersal (LDD) is not yet answered. For the Canary Islands, the proximity to the African continent might have eased dispersal. The oldest islands, Lanzarote and Fuerteventura, are presently 100 km from the coast of North Africa (Francisco-Ortega *et al*., 2000; Acosta *et al*., 2005), but at some periods during the last 20 million years they were probably much closer (García-Talavera, 1997). García-Talavera (1997) suggested that the volcanic sea mounts served as 'stepping stones' when the sea level dropped during glacial periods. In addition, recent studies have assumed convective updraft to be the key mechanism for LDD of even heavy diaspores (Nathan *et al*., 2002; Tackenberg *et al*., 2003). Long-distance dispersal to the archipelago mediated by wind is conceivable for *S. herbanica*, *S. aegyptiaca* and their potential common ancestor. *Salvia aegyptiaca* is distributed within the area of the Saharan Air Layer, a westward-directed wind of comparably high velocity (Carlson and Prospero, 1972; Tackenberg *et al*., 2003)

East Africa and the Arabian Peninsula as 'melting pots' for Salvia

East African *Salvia* is found in three independent clades (Fig. 7; III-A, I-C and I-A) and consequently has various floristic links beyond the continent.

Salvia merjamie (sub-clade I-C; Fig. 9D: 16) is a frequent and extremely variable species in the montane forest belts from Ethiopia to Zimbabwe (Hedge, 1974). It is part of a strongly supported (ITS) clade with two widely distributed species from SW Asia. *Salvia merjamie* is moderately supported (ML) as the sister to *S. verbenaca* 141 from Turkey. Both are polymorphic, occasionally have cleistogamous flowers and share the chromosome number of $2n = 42$, which is uncommon in *Salvia* (Reese, 1957; Gadella *et al*., 1966; Hedge, 1974; Hedberg and Hedberg, 1977; Haque and Ghoshal, 1980; Codd, 1985; Vogt and Aparicio, 1999; Foley *et al*., 2008).

Salvia nilotica (sub-clade I-A; Fig. 9D: 17) has a broader distribution range than *S. merjamie*. Hedge (1974) assumed that *S. nilotica* was a distinct, taxonomically isolated species but also discussed its similarities with species restricted to the eastern Cape. With *S. taraxacifolia* and *S. somalensis*, *S. nilotica* is found in a basal position of sub-clade I-A, suggesting a dispersal from North to southern Africa via East Africa. The relationship of *S. nilotica* and the African members of section *Heterosphace* Benth. proposed by Hedge (1974) is confirmed, since all of these species are placed in the same sub-clade (I-A).

Salvia deserti (sub-clade III-A; Fig. 9D: 14) is an endemic of the Egyptian and Arabian deserts (Boulos, 2008; Hedge, 1974; Migahid, 1978). It is morphologically and genetically distinct from *Salvia s.s.* Incongruences detected in nuclear and plastid data support a hybrid origin of this species.

Pollinator diversity and evolution of bird pollination in Sub-Saharan Africa

Based on flower morphology, bees are seen as the most important pollinators in *Salvia* overall (Wester and Claßen-Bockhoff, 2006, 2011). This was also assumed for the African and in particular for the southern African species (Hedge, 1974). However, only a limited number of field observations confirm this view, e.g. the first report of small bees on *S. africana-caerulea* (Marloth, 1908), data on *Anthophora diversipes* (Goldblatt *et al*., 2000*a*, *b*), and observations of *Xylocopa caffra*, *Amegilla* spp., further solitary bees and honey-bees by P. Wester, R. Claßen-Bockhoff and H. Technau (pers. comm.).

Flowers with long tubes and freely accessible pollen were expected to be pollinated by long-tongued insects, e.g. flies. This pollinator guild is characteristic for South Africa, especially in the western Cape Region (Goldblatt and Manning, 2000). Potgieter and Edwards (2001, 2005) assumed that *S. scabra* and *S. repens* are pollinated by long-tongued flies (*Stenobasipteron wiedemanni* and *Prosoeca* spp.), but field observations are still lacking.

Although bird pollination is quite frequent in NW *Salvia* (Wester and Claßen-Bockhoff, 2007), the only known ornithophilous *Salvia* spp. in the OW appear in southern Africa (Wester and Claßen-Bockhoff, 2006). Three, *S. lanceolata*, *S. thermarum* and *S. africana-lutea*, are restricted to southern Africa (Fig. 4I, R, S). Probably two more (*S. leucodermis* and *S. sessilifolia*; Fig. 4P, Q) occur in Madagascar. Our data confirm that ornithophily evolved repeatedly in the NW (Fig. 7), but also indicate at least two pollinator shifts from bee to bird pollination in Africa (Supplementary Data Fig. S6). Most probably, this pollination system evolved three times in parallel within sub-clade I-A: (1) in the Madagascan sub-clade; (2) in the *S. africana-lutea* and *S. lanceolata* clade; and (3) in *S. thermarum*. Including species adapted to bee, bird and, most probably, long-tongued fly pollination, sub-clade I-A represents a further example of a monophyletic lineage having undergone pollinator-driven diversification in southern African (e.g. Van der Niet and Johnson, 2012; Sun *et al*., 2014; Van der Niet *et al*., 2014).

In bird-pollinated species, bees are largely excluded from nectar access but not from collecting pollen. They might therefore trigger hybridization between bird- and bee-pollinated species (Van Jaarsveld, 2002; P. Wester, University of Düsseldorf, Germany, pers. comm.), e.g. in *S. africana-caerulea*. The species is morphologically well adapted to bee pollination but is also occasionally pollinated by birds (Wester, 2013). It might be a species in which the exploitation of a food plant by pollinators (birds) can be observed even though both species are not yet perfectly adapted to each other (Thomson and Wilson, 2008). Thus, *S. africana-caerulea* might represent an example of a pollinator shift in progress (Rodríguez-Gironés and Santamaría, 2004).

Conclusions

African *Salvia* is non-monophyletic. Continental Africa, the Canary Islands, East Africa and the Arabian Peninsula were each colonized repeatedly. The morphological diversity of African sage results from independent dispersals from different mainland sources and diversification in the diverse African environment. Parallel evolution played an important role for the evolution of stamen types, calyx enlargement and pollination syndrome. These data can even be transferred to *Salvia s.l.* for which similar trends have been observed.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: character states coded for the ancestral character state reconstruction with references. Figure S1: analyses of the ETS data set (ML and BI). Figure S2: reconstruction of the ancestral distribution of Sub-Saharan African *Salvia*. Figure S3: ancestral character state reconstruction of the life form in Sub-Saharan African *Salvia*. Figure S4: ancestral character state reconstruction of the calyx morphology in Sub-Saharan African *Salvia*. Figure S5: ancestral character state reconstruction of the stamen morphology in Sub-Saharan African *Salvia*. Figure S6: ancestral character state reconstruction of the pollination syndrome in Sub-Saharan African *Salvia*.

ACKNOWLEDGEMENTS

We thank Dirk Albach (Oldenburg), Safi Bagherpour (Ankara), Benny Bytebier (KwaZulu-Natal), Ferhat Celep (Nevşehir), Ahmet El-Banhawy (Ismailia/Redding), Ahmed Kahraman (Ankara), Alexander P. Sukhorukov (Moscow), Mats Thulin (Uppsala) and Petra Wester (Düsseldorf) for sampling, and the following herbaria for offering plant material: ACECR (Iran), B, E, EA, GOET, HUH, M, MJG, MO, MPU and MW. Photographs were kindly provided by Ferhat Celep, Rafael N. B. Groneberg (Mainz), Dylan Hannon (San Marino, CA), Peter B. Phillipson (Paris), Hen Technau, Mats Thulin and Petra Wester. We thank Berit Gehrke and Michael D. Pirie (both Mainz) for assistance with data analyses, and Natalie Schmalz, Abigail J. Moore (Providence, RI) and two anonymous reviewers for helpful comments to improve the manuscript. This work was supported by the DFG (Deutsche Forschungsgemeinschaft; Cl 81/10–1) and the Fachbereich Biologie (Universität Mainz).

LITERATURE CITED

Acosta J, Uchupi E, Muñoz A, Herranz P, Palomo C, Ballesteros M. 2005. Geologic evolution of the Canarian Islands of Lanzarote, Fuerteventura, Gran Canaria and La Gomera, and comparison of landslides at these islands with those at Tenerife, La Palma and El Hierro. In: Clift P, Acosta J, eds. *Geophysics of the Canary Islands*. Dordrecht, The Netherlands: Springer, 1–40.

Alziar G. 1988–1993. Catalogue synonymique des *Salvia* L. du monde (Lamiaceae). *I à VI. Biocosme Mésogéen, Nice* 5: 87–136; 6: 79–115; 6: 163–204; 7: 59–109; 9: 413–497; **10**: 33–117.

Arnedo MA, Oromí P, Ribera C. 2001. Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: cladistic assessment based on multiple data sets. *Cladistics* **17**: 313–353.

Baldwin BG, Markos S. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* **10**: 449–463.

Beardsley PM, Olmstead RG. 2002. Redefining Phrymaceae: the placement of *Mimulus*, tribe Mimuleae, and *Phryma*. *American Journal of Botany* **89**: 1093–1102.

Bellstedt DU, van Zyl L, Marais EM, *et al*. 2008. Phylogenetic relationships, character evolution and biogeography of southern African members of *Zygophyllum* (Zygophyllaceae) based on three plastid regions. *Molecular Phylogenetics and Evolution* **47**: 932–949.

Bentham G. 1832–1836. Salvia. In: Bentham G, ed. *Labiatarum genera et species: or, a description of the genera and species of plants of the order Labiatae with their general history, characters, affinities, and geographical distribution.* London: J. Ridgway and Sons, 190–312.

Bentham G. 1848. Labiatae. In: De Candolle A, ed. *Prodromus systematis naturalis regni vegetabilis 12*. Paris: Treuttel and Würtz, 262–358.

Bentham G. 1876. Labiatae. In: Bentham G, Hooker JD, eds. *Genera plantarum* 2. London: Reeve and Co., 1160–1196.

Bokhari MH, Hedge IC. 1977. Anatomical observations on a desert group of *Salvia* species. *Notes from the Royal Botanical Garden, Edinburgh* **35**: 377–389.

Boulos L. 2008. Flora and vegetation of the deserts of Egypt. *Flora Mediterranea* **18**: 341–359.

Briquet J. 1897. Labiatae. In: Engler A, Prantl K, eds. *Die natürlichen Pflanzenfamilien nebst ihrer Gattungen und wichtigen Arten.* Leipzig: Engelmann, 183–380.

Carine MA, Russell SJ, Santos-Guerra A, Francisco-Ortega J. 2004. Relationships of the Macaronesian and Mediterranean floras: molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in *Convolvulus* (Convolvulaceae). *American Journal of Botany* **91**: 1070–1085.

Carlson TN, Prospero JM. 1972. The large-scale movement of Saharan Air outbreaks over the Northern Equatorial Atlantic. *Journal of Applied Meteorology* **11**: 283–297.

Caujapé-Castells J, Jansen RK. 2003. The influence of the Miocene Mediterranean desiccation on the geographical expansion and genetic variation of *Androcymbium gramineum* (Cav.) McBride (Colchicaceae). *Molecular Ecology* **12**: 1515–1525.

Caujapé-Castells J, Jansen RK, Membrives N, Pedrola-Monfort J, Montserrat JM, Ardanuy A. 2001. Historical biogeography of *Androcymbium* Willd. (Colchicaceae) in Africa: evidence from cpDNA RFLPs. *Botanical Journal of the Linnean Society* **136**: 379–392.

Clebsch B. 2008. *The new book of salvias: sages for every garden*. Cambridge: Timber Press.

Codd LE. 1985. *Salvia.* In: Leistner OA, ed. *Flora of Southern Africa. Lamiaceae.* Pretoria: Botanical Research Institute, Department of Agriculture and Water Supply, 79–101.

Coleman M, Liston A, Kadereit JW, Abbott RJ. 2003. Repeat intercontinental dispersal and Pleistocene speciation in disjunct Mediterranean and desert *Senecio* (Asteraceae). *American Journal of Botany* **90**: 1446–1454.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772–772.

Davis PH, Hedge IC. 1971. Floristic links between NW Africa and SW Asia. *Annalen des Naturhistorischen Museums in Wien* **75**: 43–57.

Del Hoyo A, García-Marín JL, Pedrola-Monfort J. 2009. Temporal and spatial diversification of the African disjunct genus *Androcymbium* (Colchicaceae). *Molecular Phylogenetics and Evolution* **53**: 848–861.

Emerson BC, Oromí P, Hewitt GM. 2000. Interpreting colonisation of the *Calathus* (Coleoptera: Carabidae) on the Canary Islands and Madeira through the application of the parametric bootstrap. *Evolution* **54**: 2081–2090.

Foley MJY, Hedge IC, Möller M. 2008. The enigmatic *Salvia tingitana* (Lamiaceae): a case study in history, taxonomy and cytology. *Willdenowia – Annals of the Botanic Garden and Botanical Museum Berlin-Dahlem* **38**: 41–59.

Francisco-Ortega J, Santos-Guerra A, Kim S-C, Crawford DJ. 2000. Plant genetic diversity in the Canary Islands: a conservation perspective. *American Journal of Botany* **87**: 909–919.

Froissart C. 2008. *La connaissance des sauges*. Aix-en-Provence, France: Edisud.

Fuertes-Aguilar J, Ray MF, Francisco-Ortega J, Santos-Guerra A, Jansen RK. 2002. Molecular evidence from chloroplast and nuclear markers for

multiple colonizations of *Lavatera* (Malvaceae) in the Canary Islands. *Systematic Botany* **27**: 74–83.

Gadella TWJ, Kliphuis E, Mennega EA. 1966. Chromosome numbers of some flowering plants of Spain and S-France. *Acta Botanica Neerlandica* **15**: 484–489.

García-Talavera F. 1997. Las Canarias orientales y la vecina costa Africana en el holoceno *Eres* **7**: 55–63.

Goldblatt P, Manning JC. 2000. The long-proboscid fly pollination system in Southern Africa. *Annals of the Missouri Botanical Garden* **87**: 146–170.

Goldblatt P, Bernhardt P, Manning JC. 2000*a*. Adaptive radiation of pollination mechanisms in *Ixia* (Iridaceae: Crocoideae). *Annals of the Missouri Botanical Garden* **87**: 564–577.

Goldblatt P, Manning JC, Bernhardt P. 2000*b*. Adaptive radiation of pollination mechanisms in *Sparaxis* (Iridaceae: Ixioideae). *Adansonia, série 3* **22**: 57–70.

Haque MS, Ghoshal KK. 1980. Karyotypes and chromosome morphology in the genus *Salvia* Linn. *Cytologia* **45**: 627–640.

Harley RM, Atkins S, Budantsev AL, *et al*. 2004. Labiatae. In: Kadereit JW, ed. *The families and genera of vascular plants 7, Lamiales*. Berlin: Springer, 167–282.

Hedberg I, Hedberg O. 1977. Chromosome numbers of afroalpine and afromontane angiosperms. *Botaniska Notiser* **130**: 1–24.

Hedge IC. 1974. A revision of *Salvia* in Africa including Madagascar and the Canary Islands. *Notes from the Royal Botanical Garden, Edinburgh* **33**: 1–121.

Hedge IC. 1982. *Salvia*. In: Davis PH, ed. *Flora of Turkey*. Edinburgh: Edinburgh University Press, 400–461.

Helfgott DM, Francisco-Ortega J, Santos-Guerra A, Jansen RK, Simpson BB. 2000. Biogeography and breeding system evolution of the woody *Bencomia* alliance (Rosaceae) in Macaronesia based on ITS sequence data. *Systematic Botany* **25**: 82–97.

Jarvis C. 2007. *Order out of chaos. Linnaean plant names and their types*. London: The Linnean Society of London in association with the Natural History Museum.

Jenks AA, Walker JB, Kim S-C. 2011. Evolution and origins of the Mazatec hallucinogenic sage, *Salvia divinorum* (Lamiaceae): a molecular phylogenetic approach. *Journal of Plant Research* **124**: 593–600.

Jenks A, Walker J, Kim S. 2012. Phylogeny of New World *Salvia* subgenus *Calosphace* (Lamiaceae) based on cpDNA (*psbA-trnH*) and *nr*DNA (ITS) sequence data. *Journal of Plant Research* **126**: 483–496.

Kirk-Spriggs AH, McGregor G. 2009. Disjunctions in the *Diptera* (Insecta) fauna of the Mediterranean province and southern Africa and a discussion of biogeographical considerations. *Transactions of the Royal Society of South Africa* **64**: 32–52.

Li QQ, Li MH, Yuan QJ, Cui ZH, Huang LQ, Gen XP. 2013. Phylogenetic relationships of *Salvia* (Lamiaceae) in China: evidence from DNA sequence datasets. *Journal of Systematics and Evolution* **51**: 184–195

Linder HP. 2001. Plant diversity and endemism in sub-Saharan tropical Africa. *Journal of Biogeography* **28**: 169–182.

Maddison WP, Maddison DR. 2000. *MacClade: analysis of phylogeny and character evolution, version 4.0*. Sunderland, MA: Sinauer Associates.

Maddison WP, Maddison DR. 2011. *Mesquite: a modular system for evolutionary analysis*. *Version 2.75*. http://mesquiteproject.org [accessed March 15th 2013].

Manen JF, Boulter MC, Naciri-Graven Y. 2002. The complex history of the genus *Ilex* L. (Aquifoliaceae): evidence from the comparison of plastid and nuclear DNA sequences and from fossil data. *Plant Systematics and Evolution* **235**: 79–98.

Marloth R. 1908. Some observations on entomophilous flowers. *South African Journal of Science* **4**: 110–113.

Migahid AM. 1978. Salvia. In: Migahid AM, ed. *Migahid and Hammouda's Flora of Saudi Arabia.* Riyadh: Riyadh University, 464–465.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop* (GCE), 14 November 2010. New Orleans, LA, 1–8. [available from http://www.phylo.org/portal2/login!input.action].

Nathan R, Katul GG, Horn HS, *et al*. 2002. Mechanisms of long-distance dispersal of seeds by wind. *Nature* **418**: 409–413.

Noyes RD, Rieseberg LH. 1999. ITS sequence data support a single origin for North American Astereae (Asteraceae) and reflect deep geographic divisions in *Aster s.l*. *American Journal of Botany* **86**: 398–412.

Pirie MD, Humphreys AM, Galley C, *et al*. 2008. A novel supermatrix approach improves resolution of phylogenetic relationships in a comprehensive sample of danthonioid grasses. *Molecular Phylogenetics and Evolution* **48**: 1106–1119.

Pirie MD, Humphreys AM, Barker NP, Linder HP. 2009. Reticulation, data combination, and inferring evolutionary history: an example from Danthonioideae (Poaceae). *Systematic Biology* **58**: 612–628.

Potgieter CJ, Edwards TJ. 2001. The occurrence of long, narrow corolla tubes in southern African Lamiaceae. *Systematics and Geography of Plants* **71**: 493–502.

Potgieter CJ, Edwards TJ. 2005. The *Stenobasipteron wiedemanni* (Diptera, Nemestrinidae) pollination guild in Eastern Southern Africa. *Annals of the Missouri Botanical Garden* **92**: 254–267.

Reese G. 1957. Über die Polyploidiespektren in der nordsaharischen Wüstenflora. *Flora* **144**: 608–634.

Rodríguez-Gironés MA, Santamaría L. 2004. Why are so many bird flowers red? *PLoS Biology* **2**: 1515–1519.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Rosúa JL, Blanca G. 1985. Notas carísosistemáticas de la sección *Salvia* del género *Salvia* L. (Lamiaceae). *Anales del Jardín Botánico de Madrid* **42**: 101–112.

Rosúa JL, Blanca G. 1986. Revisión del género *Salvia* L. (Lamiaceae) en el Mediterráneo occidental: la sección *Salvia*. *Acta Botanica Malacitana* **11**: 227–271.

Rosúa JL, Blanca G. 1990. Acerca de la distribucion de la seccion *Salvia* en la region mediterranea occidental y sus relaciones de vicarianza con el este del mediterraneo. *Lagascalia* **15**: 137–143.

Santos A, Fernández M. 1986. *Salvia herbanica* spec. nova (Labiatae) en la flora de Fuerteventura (I. Canarias). *Lazaroa* 9: 51–54.

Scholz S. 1993. Nuevos datos acerca de *Salvia herbanica* Santos et Fernandez (Lamiaceae). *Vieraea* **22**: 29–34.

Scott-Elliot GF. 1890. Ornithophilous flowers in South Africa. *Annals of Botany* **4**: 265–280.

Shaw J, Lickey EB, Schilling EE, Small RL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* **94**: 275–288.

Sudarmono HO. 2007. Speciation process of *Salvia isensis* (Lamiaceae), a species endemic to serpentine areas in the Ise-Tokai district, Japan, from the viewpoint of the contradictory phylogenetic trees generated from chloroplast and nuclear DNA. *Journal of Plant Research* **120**: 483–490.

Sudarmono HO. 2008. Genetic differentiations among the populations of *Salvia japonica* (Lamiaceae) and its related species. *Hayati Journal of Biosciences* **15**: 18–26.

Sun M, Gross K, Schiestl FP. 2014. Floral adaptation to local pollinator guilds in a terrestrial orchid. *Annals of Botany* **113**: 289–300.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

Stamatakis A, Hoover P, Rougemont J. 2008. Rapid bootstrap algorithm for the RAxML Web-Servers. *Systematic Biology* **75**: 758–771.

Tackenberg O, Poschlod P, Kahmen S. 2003. Dandelion seed dispersal: the horizontal wind speed does not matter for long-distance dispersal – it is updraft! *Plant Biology* **5**: 451–454.

Thulin M. 1993. *Salvia* (Labiatae) in the mountains of northern Somalia. *Opera Botanica* **121**: 145–148.

Thulin M. 2009. *Salvia geminata* sp. nov. with remarkable stamen arrangement from southern Yemen, with notes on *S. areysiana* (Lamiaceae). *Nordic Journal of Botany* **27**: 336–338.

Thomson JD, Wilson P. 2008. Explaining evolutionary shifts between bee and hummingbird pollination: convergence, divergence, and directionality. *International Journal of Plant Sciences* **169**: 23–38.

Van Jaarsveld EJ. 1999. *Salvia thermara*, a new species from the Western Cape, South Africa. *Bothalia* **29**: 100–102.

Van Jaarsveld EJ. 2002. South African sages. *Veld and Flora* **88**: 102–104.

Van der Niet T, Johnson SD. 2012. Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology and Evolution* **27**: 353–361.

Van der Niet T, Peakall R, Johnson SD. 2014. Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Annals of Botany* **113**: 199–212.

Vargas P. 2007. Are Macaronesian islands refugia of relict plant lineages?: a molecular survey. In: Weiss S, Ferrand N, eds. *Phylogeography of*

southern European refugia. Dordrecht, The Netherlands: Springer, 297–314.

Vogt R, Aparicio A. 1999. Chromosome numbers of plants collected during Iter Mediterraneum IV in Cyprus. *Bocconea* **11**: 117–169.

Walker JB, Sytsma KJ. 2007. Staminal evolution in the genus *Salvia* (Lamiaceae): molecular phylogenetic evidence for multiple origins of the staminal lever. *Annals of Botany* **100**: 375–391.

Walker JB, Sytsma KJ, Treutlein J, Wink M. 2004. *Salvia* (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Mentheae. *American Journal of Botany* **91**: 1115–1125.

Wester P. 2013. Sunbirds hover at flowers of *Salvia* and *Lycium*. *Ostrich* **84**: 27–32.

Wester P, Claßen-Bockhoff R. 2006. Bird pollination in South African *Salvia* species. *Flora – Morphology, Distribution, Functional Ecology of Plants* **201**: 396–406.

Wester P, Claßen-Bockhoff R. 2007. Floral diversity and pollen transfer mechanisms in bird- pollinated *Salvia* species. *Annals of Botany* **100**: 401–421.

Wester P, Claßen-Bockhoff R. 2011. Pollination syndromes of New World *Salvia* species with special reference to bird pollination. *Annals of the Missouri Botanical Garden* **98**: 101–155.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T, eds. *PCR protocols: a guide to methods and applications.* Orlando, FL: Academic Press, 315–322.

Whitehouse E. 1949. Revision of *Salvia* section *Salviastrum* Gray. *Field and Laboratory* **17**: 151–165.

Will M. 2013. *Old World Salvia – morphological and molecular evidence for its evolution and non-monophyly*. PhD thesis, Johannes Gutenberg University, Mainz, Germany.

APPENDIX: PLANT MATERIAL INCLUDED IN THIS STUDY

Taxon	Locality	Voucher collector with collection no. (herbarium)	DNA acc. no.	GenBank accession no.		
				rpl32-trnL	nrITS	ETS
Clinopodium *dalmaticum* (Benth.) Bräuchler & Heubl		M. Kintgen s.n.	–	JQ669340	–	–
Clinopodium taxifolium (Kunth) Govaerts		B. Drew 228	–	JQ669288	–	–
Clinopodium vulgare L.		Riina 1579	–	JQ669290	–	–
Collinsonia *canadensis* L.		JBW 958	–	–	DQ667248	–
		Raiche s.n. UCBG 1984.0696	–	JQ669291	–	JQ669157
Dorystaechas *hastata* Boiss. & Heldr. ex Benth.	Anatolia	Albach D6–4 (OLD)	213	KJ747319	KJ584248	KJ584275
		Cult. RBGE1972–0177D	–	JQ669302	DQ667252	–
			–	–	HQ418845	–
Glechoma *hederacea* L.		B. Drew 69	–	JQ669307	–	–
Horminum *pyrenaicum* L.		Cult. RBGE 1997–2109a	–	JQ669315	DQ667257	–
		M. Will 63 (MJG 003069)	390	KJ747327	KJ584247	KJ584279
Hyptis *laniflora* Benth.		B. Drew 41	–	JQ669317	–	–
		Isolate K36721	–	–	JF301548	JF404259
Lepechinia *bella* Epling	Bolivia	P. Wester 145 (MJG 009474)	216	KJ747326	–	–
Lepechinia calycina (Benth.) Epling ex Munz		Drew 197	–	JQ669324	–	–
Lepechinia conferta Epling		Alonso 8376 (F)	–	–	DQ667307	–
Lepechinia lamiifolia (Benth.) Epling		B. Drew 178	–	JQ669325	–	–
Lepechinia lancifolia (Rusby) Epling		Smith 444 (F)	–	–	DQ667306	–
Lepechinia mexicana (S.Schauer) Epling		B. Drew 164	–	JQ669326	–	–
Melissa *axillaris* (Benth.) Bakh.f.		D. E. Boufford *et al*. 24526	–	JQ669334	–	–
Melissa officinalis L.		M. Will 64 (MJG 003068)	388	KJ747325	KJ584249	KJ584285
		JBW 2575 (cult. USA/WIS)	–	–	DQ667291	–
		B. Drew 70	–	JQ669335	JF301353	–
Mentha *arvensis* L.		B. Drew 82	–	JQ669336	–	–
Mentha pulegium L.		Riina 1574	–	JQ669338	–	–
Mentha spicata L.		J. Walker 2566	–	JQ669339	–	–
Mentha spicata subsp. *condensata* (Briq.) Greuter & Burdet		Riina 1575	–	JQ669337	–	–
Meriandra *bengalensis* (Konig ex Roxb.) Benth.		Lavranus & Newton 15796 (MO 2633828)	–	–	DQ667329	–
Perilla *frutescens* (L.) Britton		JBW 1078 (cult. USA/WIS)	–	–	DQ667246	JF301326
Perovskia *atriplicifolia* Benth.		M. Will 65 (MJG 003070)	472	–	KJ584242	–
		JBW 2524 (cult. USA/WIS)	–	JQ669352	DQ667223	JF301328
Perovskia scrophulariifolia Bunge		Kinziraeva 6751 (MO 5201778)	–	–	DQ667330	–
Rosmarinus *officinalis* L.		M. Will 66 (MJG 003071)	389	KJ747310	KJ584197	KJ584296
		JBW 2558 (cult. USA/WIS)	–	JQ669364	DQ667241	–
Salvia *aegyptiaca* L.		M. Kuschewitz s.n. (cult. BG HH)	204	KJ747314	KJ584245	KJ584254
		McLeish 3728 (E)	–	–	DQ667285	–
S. aethiopis L.	Armenia	J. Hellwig s.n. 26/6/02 (MJG 009919)	281	–	KJ584163	–
	Armenia	J. Hellwig s.n. 26/6/02 (MJG 009919)	–	–	DQ667272	–
S. africana-caerulea L.	S Africa	P. Wester & R. Claßen-Bockhoff 319 (MJG 041401)	230	KJ747271	KJ584204	KJ584255
	S Africa	P. Wester & R. Claßen-Bockhoff 317 (MJG 041402)	229	–	KJ584203	–
S. africana-lutea L.	S Africa	P. Wester 342 (MJG 041393)	81	KJ747273	KJ584205	KJ584256
	S Africa	P. Wester 708 (MJG)	234	KJ747259	–	–
	S Africa	P. Wester 708 (MJG)	430	KJ747272	–	–
S. albicaulis Benth.	S Africa	P. Wester, R. Claßen-Bockhoff & E. v. Jaarsveld 340 (MJG 041403)	1	KJ747274	KJ584206	KJ584257
S. albicaulis × *granitica*	S Africa	P. Wester, R. Claßen-Bockhoff & E. v. Jaarsveld 341 (MJG 041404)	2	KJ747275	KJ584207	KJ584258
	S Africa	P. Wester, R. Claßen-Bockhoff & E. v. Jaarsveld 341 (MJG 041404)	42	–	KJ584215	KJ584277
S. apiana Jeps.	California	P. Wester 411 (MJG 041452)	392	KJ747321	–	–
		JBW 2509 USA (WIS)	–	–	DQ667214	–

S. areysiana Deflers	Yemen	Thulin, Eriksson, Gifri & Långström 8472 (UPS)	282	KJ747315	–	KJ584259
S. argentea L.	Italy	R. Claßen-Bockhoff s.n. Mai 2002 (MJG)	57	KJ747299	KJ584164	–
S. aristata Aucher	Iran	K.H. Rechinger s.n. 1974 (M)	289	–	KJ584244	–
	Iran	Y. Ajani 1569 (ACECR)	417	KJ747264	–	–
		Wedelbo & Assadi s.n. (E)	–	JQ669365	DQ667280	JF301336
S. aucheri var. *canescens* Boiss. & Heldr.	Anatolia	F. Celep 1245 (PSL METU)	239	–	KJ584193	–
		Archibald 7670 (E)	–	–	DQ667286	–
S. aurita L.f.		P. Wester & R. Claßen-Bockhoff 324 (MJG 041405)	11/424	KJ747276	KJ584218	KJ584261
		M. Will 26 (MJG 041563)	423	–	KJ584219	KJ584260
S. aurita var. *galpinii* (Skan) Hedge		P. Wester 472 (MJG)	425	KJ747269	–	–
S. austriaca Jacq.		R. Claßen-Bockhoff s.n. 2004 (cult. BG Mz)	–	–	DQ667323	–
	Austria	R. Claßen-Bockhoff s.n. 12.06.2003 (MJG)	280	KJ747261	–	–
S. bariensis Thulin	Somalia	M. Thulin, A. Dahir & A. Osman 9429 (UPS)	283	KJ747316	–	KJ584262
S. broussonetii Benth.		M. Will 33 (MJG 041537)	29	KJ747293	KJ584226	KJ584263
	Tenerife	R. Claßen-Bockhoff 2/10 (MJG 009887)	463	–	KJ584225	KJ584264
S. cabulica Benth.	Afghanistan	H. Freitag 4683 (MSB 137713)	322	–	KJ584189	–
		Ghafoor & Goodman 5148 (E)	–	–	DQ667287	–
S. canariensis L.		M. Will 46 (MJG 041565)	5	KJ747295	KJ584227	KJ584266
	Tenerife	R. Claßen-Bockhoff 3/10 (MJG)	464	–	–	KJ584265
	Tenerife	R. Claßen-Bockhoff 1/03 (MJG)	223	KJ747294	–	–
		Cult. RBGE 1986–0478	–	–	DQ667256	–
S. candelabrum Boiss.		M. Will 42 (MJG 041557)	62	KJ747255	KJ584190	–
S. candidissima subsp. *occidentalis* Hedge	Anatolia	F. Celep 1487 (PSL METU)	201	KJ747300	KJ584165	–
S. candidissima Vahl.		Cult. RBGE 1999–2202A	–	–	DQ667261	–
S. chamelaeagnea Berg.		P. Wester & R. Claßen-Bockhoff 314 (MJG 041407)	52	KJ747289	KJ584210	KJ584268
	S Africa	P. Wester, R. Claßen-Bockhoff & E. Van Jaarsveld 313 (MJG 041406)	432	–	KJ584211	KJ584267
		M. Will 47 (MJG 041541)	433	–	KJ584212	–
S. chienii E. Peter		M. Will 61 (MJG 003066)	53	KJ747322	KJ584250	–
		AnH0305–21	–	–	DQ132868	–
S. chionopeplica Epling		JBW 2545 (cult. USA/WIS)	–	–	DQ667227	–
S. cf. *chionopeplica* Epling		P. Wester 485 (MJG 041435)	174	KJ747318	KJ584188	KJ584269
S. daghestanica Sosn.		M. Will 34 (MJG 041551)	276	KJ747308	KJ584187	–
		Cult. RGB E 1988–2283A	–	–	DQ667258	–
S. deserta Schang		M. Will 96 (MJG 003100)	286	KJ747263	KJ584176	–
		XingJ0305–1	–	–	DQ132865	–
S. deserti Dcne.	Egypt	E. Gamal Eldin s.n. 3.5.1991 (GOET)	335	KJ747312	–	KJ584270
S. disermas L.		P. Wester & R. Claßen-Bockhoff 326 (MJG 041413)	15	KJ747296	KJ584179	KJ584271
		M. Will 80 (MJG 003116)	454	–	–	KJ584272
S. disermas L. (syn. *S. rugosa* in GenBank)		Goldblatt 7500 (E)	–	–	DQ667290	–
S. dolomitica Codd		P. Wester & R. Claßen-Bockhoff 321 (MJG 041411)	82	KJ747290	KJ584214	KJ584274
		F. Brusse 5610 (M)	440	–	KJ584213	KJ584273
		JBW 3200 (cult. USA/WIS)	–	–	DQ667322	–
S. dominica L.	Cyprus	A. Seregin, D. Sokoloff & M. Remizova A-211 (MW)	267	–	KJ584167	–
		M. Kuschewitz s.n. (MJG 009323)	217	KJ747262	KJ584166	–
S. evansiana Hand.-Mazz. var. *evansiana*		M. Will 55 (MJG 003060)	415	KJ747323	KJ584251	–
S. freyniana Bornm.	Anatolia	S. Bagherpour 493 (PSL METU)	98	KJ747266	–	–
S. fruticosa Miller	Anatolia	F. Celep 1373 (PSL METU)	100	KJ747256	KJ584195	–
		G. Hausner GR 31 (MJG 003078)	66	–	KJ584194	–
S. funerea Jones		P. Wester 490 (MJG 041430)	393	KJ747320	–	–
S. garipensis E.Meyer ex Benth.		Strohbach 149 (E)	–	–	DQ667281	–
S. geminata Thulin	Yemen	M. Thulin, Beier & M. Hussein 9629 (UPS)	341	–	–	KJ584276
S. glutinosa L.	Anatolia	F. Celep 1196 (PSL METU)	101	–	KJ584253	–
		JBW 2568 (cult. USA/WIS)	–	–	DQ667250	–
S. graciliramulosa Epling & Játiva	Bolivia	P. Wester 14 (MJG 041090)	–	–	DQ667276	–

Continued

Continued

Taxon	Locality	Voucher collector with collection no. (herbarium)	DNA acc. no.	GenBank accession no.		
				rpl32-trnL	nrITS	ETS
S. gretai Brandegee	USA	JBW 2511 (WIS)	–	JQ669367	DQ667215	JF301331
S. henryi Gray	USA	JBW 2516 (WIS)	–	–	DQ667216	–
S. herbanica A.Santos & M.Fernández	Fuerteventura	R. Claßen-Bockhoff 1/05 (MJG 009888)	40	KJ747313	KJ584246	KJ584278
S. heterochroa Fern.	Yunnan, China	D. E. Boufford, J. H. Chen, S. L. Kelley, R. H. Ree, H. Sun, B. Xü, J. P. Yue, L. L. Yue, D. C. Zhang & W. D. Zhu 35205 (HUH 286716)	252	KJ747324	KJ584252	–
S. hydrangea Benth.	Anatolia	A. Kahraman 1468 (PSL METU)	242	KJ747257	KJ584192	–
		Rechinger 47123 (E)	–	–	DQ667288	–
S. hydrangea Benth. (syn. *S. dracocepha-loides* Boiss. in GenBank)	Armenia	Hellwig s.n. (MJG 009920)	–	–	DQ667265	–
S. interrupta Schousb.		M. Will 30 (MJG 041550)	447	KJ747265	KJ584191	–
S. judaica Boiss.		M. Will 57 (MJG 003061)	409	–	KJ584241	–
S. lanceolata Lam.		P. Wester 316 (MJG 041396)	58	KJ747278	KJ584202	KJ584281
	S Africa	P. Wester 1129 (NBG)	264	KJ747277	KJ584201	KJ584280
S. lanceolata × *africana-caerulea*		P. Wester & R. Claßen-Bockhoff 315 (MJG 041400)	236	KJ747279	KJ584216	KJ584282
S. lanigera Poir.	Sinai	A. El-Banhawy 11 (University of Ismailia, Egypt)	198	–	KJ584185	–
S. leucodermis Baker	Madagascar	R. A. Clement, P. B. Phillipson & G. Rafamantanantsoa 2137 (E 00161484)	348	KJ747280	KJ584220	KJ584284
	Madagascar	P. Wester 1131 (TAN)	459	–	KJ584221	–
	Madagascar	B. Bytebier 3193 (TAN)	460	–	KJ584222	KJ584283
S. merjamie Forsk.		M. Will 83 (MJG 003113)	265	KJ747297	KJ584184	KJ584286
S. microstegia Boiss. & Bal.	Armenia	J. Hellwig s.n. (MJG 009884)	212	KJ747307	KJ584171	–
S. microstegia Boiss. & Bal. (*S. verbascifolia* M.Bieb.in GenBank)	Armenia	J. Hellwig s.n. (MJG 009884)	–	–	DQ667264	–
S. muirii L.Bolus		P. Wester & R. Claßen-Bockhoff 328 (MJG 041409)	162	KJ747283	KJ584208	KJ584287
		P. Wester & R. Claßen-Bockhoff 318 (MJG 041410)	163	–	KJ584209	KJ584288
S. namaensis Schinz		M. Will 28 (MJG 041552)	435	KJ747284	KJ584217	KJ584290
		P. Wester & R. Claßen-Bockhoff 330 (MJG 041415)	78	KJ747281	KJ584234	KJ584291
	SW Africa	W. Giess & M. Müller 14319 (M)	296	–	KJ584200	KJ584289
S. nilotica Juss. ex Jacq.		U. Hecker g3186 (MJG 003079)	64	–	KJ584230	–
		M. Will 49 (MJG 041538)	436	KJ747258	KJ584229	KJ584292
S. officinalis L.		JBW 2580 (cult. USA/WIS)	–	–	DQ667225	–
		Voucher 2160	–	JQ771324	–	–
		M. Palma s.n. UCBG 7·0083	–	JQ669369	JF301355	JF301332
	N Africa	A. El-Banhawy 16 (University of Ismailia, Egypt)	243	–	KJ584196	–
S. palaestina Benth.	Anatolia	F. Celep 1083 (PSL METU)	400	KJ747304	KJ584175	–
	Anatolia	A. Kahraman 1443 (PSL METU)	125	KJ747301	–	–
S. cf. *palaestina* Benth.	N Africa	A. El-Banhawy 6 (University of Ismailia, Egypt)	200	KJ747302	KJ584172	–
S. patens Cav.		Cult RBGE 1973–9197	–	JQ669370	DQ667253	JF301333
S. penstemonoides Kunth & Bouché		JBW 2578 (cult. USA/WIS)	–	–	DQ667221	–
S. phlomoides ssp. *phlomoides* Asso	Morocco	R. Vogt 10336 & Ch. Oberprieler 4784 (B 100145114)	337	KJ747309	KJ584186	–
S. polystachya Ort.		Breedlove & Mahoney 72286 (UC) cult. UCBG 92·052	–	JQ669371	–	JF301334
S. pratensis L.	C Russia	A. Suchorukow s.n. VII 2003 (MW)	275	KJ747291	KJ584178	–
		Isolate S0628	–	–	EU169486	–
S. przewalskii Maxim.		Cult. RBGE 1993–2067A	–	JQ669372	DQ667254	JF301339
S. radula Benth.	S Africa	Germishuizen 3950 (MO 4385830)	328	–	KJ584180	KJ584293
S. repens Burch. ex Benth.		M. Will 50 (MJG 041931)	61	KJ747282	KJ584232	KJ584295
S. cf. *repens* Burch. ex Benth.		P. Wester & R. Claßen-Bockhoff 325 (MJG 041412)	437	–	KJ584231	KJ584294
S. roemeriana Scheele	USA	JBW 2515 (WIS)	–	–	DQ667211	–
S. scabra L.		M. Will 37 (MJG 041549)	55	KJ747285	KJ584233	KJ584297
S. schimperi Benth.	Yemen	D. Podlech 36057 (M 55003)	310	–	KJ584174	KJ584298

		M. Will 72 (MJG 003099)	466	–	KJ584168	–
S. schlechteri Briq.		P. Wester & R. Claßen-Bockhoff 323a (MJG 041416)	31	KJ747286	KJ584235	KJ584299
		M. Will 51 (MJG 041539)	438	–	KJ584236	KJ584300
S. sclarea L.	Anatolia	F. Celep 1492 (PSL METU)	244	KJ747305	KJ584162	KJ584301
		JBW 2527 (cult. USA/WIS)	–	JQ669373	DQ667222	–
S. sessilifolia Baker	Madagascar	R. A. Clement, P. B. Phillipson & G. Rafamantanantsoa 2001 (MO 4328854)	331	–	KJ584223	KJ584302
	Madagascar	C. H. Jongkind & S. Rapanarivo 929 (MO 4870099)	332	–	KJ584224	KJ584303
	Madagascar	Jongkind & Rapanarivo 929 (E)	–	–	DQ667282	–
S. somalensis Vatke		M. Will 77 (MJG 003119)	340	KJ747311	KJ584240	KJ584304
S. spinosa L.	N Africa	A. El-Banhawy 14 (University of Ismailia, Egypt)	199	KJ747303	KJ584173	–
S. stenophylla Burch. ex Benth.	S Africa	Burgoyne & Snow 4805 (MO 5649981)	330	KJ747260	KJ584237	KJ584305
		Giess & Hübsch 11607 (M)	439	–	KJ584238	KJ584306
S. summa A. Nelson	Texas	JBW 1972 USA (WIS)	–	–	DQ667217	–
	Texas	P. Wester 373 (MJG 041338)	190	–	–	KJ584307
S. sylvestris L.	Anatolia	A. Kahraman 1568 (PSL METU)	118	KJ747292	KJ584181	–
(syn. *S. tesquicola* Kiok. & Pobed.)	C Russia	A. Suchorukow s.n. VIII 1994 (MW)	273	–	KJ584177	–
		Isolate S0626	–	–	EU169485	–
S. taraxacifolia Hook.f.	Morocco	W. Lippert 25355 (M)	304	KJ747270	KJ584228	KJ584308
		JBW 2521 (cult. USA/WIS)	–	–	DQ667209	–
S. texana (Scheele) Torr.	Texas	P. Wester 362 (MJG 041477)	191	KJ747267	KJ584199	–
	Texas	P. Wester 362 (MJG 041477)	–	–	DQ667321	–
S. thermarum Van Jaarsv.		P. Wester & R. Claßen-Bockhoff 336 (MJG 041398)	23	KJ747288	KJ584239	KJ584309
		M. Will 52 (MJG 041933)	452	–	–	KJ584310
	S Africa	P. Wester 312 (MJG 041397)	221	KJ747287	–	–
S. tingitana Etling	Morocco	D. Podlech 43384 (M 54979)	314	–	KJ584169	–
S. trichocalycina Benth.		Breckle 4963 (E)	–	–	DQ667283	–
S. trijuga Diels		YunN0309–5	–	–	DQ132870	–
S. veneris Hedge	Cyprus	M. J. Y. Foley 1701 (E 00147797)	413	KJ747306	KJ584170	–
S. verbenaca L.	Syria	W. Licht SYR 307 (MJG 003082)	67	–	KJ584182	–
	Anatolia	F. Celep 1408 (PSL METU)	141	KJ747298	KJ584183	–
S. whitehousei Alziar	Texas	P. Wester 352 (MJG 041389)	231	KJ747268	KJ584198	KJ584311
	Texas	P. Wester 352 (MJG 041389)	–	–	DQ667320	–
***Thymus** pulegioides* L.		Riina 1577	–	JQ669378	–	–
Thymus serpyllum L.		J. Walker 2564 (cult. USA/WIS)	–	JQ669379	–	–
***Zhumeria** majdae* Rech.f. & Wendelbo	Iran	F. Sharififar 1651 (ACECR)	422	KJ747317	KJ584243	KJ584312
		Wendelbo 15793 (V 21730)	–	–	DQ667336	–
		Ghazi s.n. (V 01176)	–	–	DQ667335	–

acc. no., accession number; locality: origin from natural habitats confirmed; ACECR, Iranian Academic Center for Education Culture and Research; BG HH, Botanical Garden Hamburg (Germany); cult., cultivated; PSL METU, Plant Systematics Lab. Department of Biological Sciences, Middle East Technical University, Ankara (Turkey); RBGE, Royal Botanical Garden Edinburgh (UK); herbarium acronyms according to Index Herbariorum.