Time to split *Salvia* s.l. (Lamiaceae) – New insights from Old World *Salvia* phylogeny

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ABSTRACT

Aims: *Salvia* L. is widely known as the largest genus in the mint family. A morphological modification of the androecium (lever-like stamens) was used to support this genus. However, molecular data revealed that *Salvia* is polyphyletic. Since phylogenetic studies largely underrepresented Old World *Salvia* species, we filled this gap and combined new data with existing sequences. The aim of our study was the identification of well-supported clades that provide the basis for evolutionary and taxonomic conclusions.
Methods: We included ITS data (internal transcribed spacer) from 220 *Salvia* species, 86 of which were sequenced for the first time. Additionally, the highly variable plastid marker *rpl*32-*trn*L was sequenced, providing new data for 100 *Salvia* species. These sequences were combined with the accessions available from GenBank. Old World *Salvia* is represented herein with 57% of its species. The two datasets were analyzed separately using BI and ML approaches.
Results: Our data confirm that *Salvia* is polyphyletic with four distinct evolutionary lineages (Clade I-IV), including five additional genera. The clades strongly reflect the geographical distribution, i.e., Clade IV (East Asia), Clade III (Southwest Asia to Northern Africa), and Clade II (America). The origin of *Salvia* s. s. (Clade I) is most likely Southwest Asia. A high degree of parallel character evolution was identified in most of the Old World sections. Based on our results, we reconstructed the evolution and biogeography of *Salvia* s.l. and propose to split this large group into six genera, each supported by geographical distribution, morphology, and karyology.
Conclusion: *Salvia* s.l. is a polyphyletic group that was originally regarded as a genus because its species share a derived stamen structure. However, phylogenetic data clearly indicate that this floral trait and other morphological characters evolved in parallel. Our study illustrates that the combination of different data sets allows a comprehensive reconstruction of taxa and characteristic evolution, both of which are a precondition for future revision.

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1. Introduction

1.1. Salvia L. – a highly polyphyletic genus

Large genera, including *Astragalus*, *Euphorbia*, *Minuartia*, *Psychotria*, *Ranunculus* and *Solanum*, have repeatedly been found to be non-monophyletic (Bruyns et al., 2006; Dillenberger and Kadereit, 2014; Emadzade et al., 2010; Hörandl et al., 2005; Nepokroeff et al., 1999; Osaloo et al., 2003; Rastipishe et al.,

2011; Weese and Bohs, 2007; Zimmermann et al., 2010). In contrast to paraphyly (Hörandl, 2006; Hörandl and Stuessy, 2010; Zander, 2008), polyphyly is unacceptable to describe natural groups and demands taxonomic consequences. Because it is rather unpopular to split well-known genera, new concepts for classification should not be introduced lightly (e.g., Kress et al., 2005; Kučera et al., 2013; Mansion, 2004; Whitten et al., 2007) but also should not be delayed for practical or sentimental reasons.

Salvia is well-known for its ornamental, medicinal, hallucinogenic, or esculent plants (Clebsch, 2008; Froissart, 2008). To date, approximately 980 species have been recognized, most of which are restricted to the New World (NW[1]), the most important center of species diversity (Appendix A, see Wester and Claßen-Bockhoff, 2011; Bedolla-García et al., 2011; Martínez-Gordillo and Lozada-Pérez, 2011; Turner, 2011; Véliz Pérez and Quedensley, 2011;

Fernández Alonso, 2012; González-Gallegos and Castro-Castro, 2012; González-Gallegos et al., 2012a, 2012b; Iltis et al., 2012; Sagástegui Alva and Rodríguez Rodríguez, 2012; González-Gallegos, 2013; González-Gallegos and Castro-Castro, 2013; Gonzáles-Gallegos and Gama-Villanueva, 2013; González Gallegos et al., 2013; Fragoso-Martínez and Martínez-Gordillo, 2013; Turner, 2013; Fernández-Alonso, 2014; González-Gallegos, 2014; González-Gallegos and Aguilar-Santelises, 2014; Lara-Cabrera et al., 2014; Fragoso-Martínez et al., 2015; González-Gallegos, 2015; Bedolla-García and Zamudio, 2015). Further hotspots of species richness are located in the Old World (OW₂), where approximately 350 spp. are found (Appendix B, see Thulin, 1993; Vural and Adigüzel, 1996; Van Jaarsveld, 1999; Dönmez, 2001; Haber and Semaan, 2004; Hamazaoğlu et al., 2005; Yıldırımlı and Ertekin, 2008; Ilçim et al., 2009; Thulin, 2009; Celep and Dogan, 2010; Kahraman et al., 2011b; Zhu et al., 2011; Hu et al., 2013; Takano et al., 2014; Celep et al., 2015).

The major characteristic supporting *Salvia* as a genus is the peculiar modification of its stamens. The latter have a lever-like structure and function, a characteristic that has also been described as a key innovation for the genus (Claßen-Bockhoff et al., 2004b). Together with the calyx and corolla morphology, 'stamen types' have been consistently used to separate *Salvia* from other genera (Harley et al., 2004). However, despite this morphological support, the phenotypic diversity (Plate 1A–S) in *Salvia* has repeatedly caused conflicting opinions regarding classification (Table 1).

1.2. Taxonomic history

In the **early nineteenth century**, various attempts were made towards a classification of *Salvia* with the introduction of different subgenera and sections (Bentham, 1832–36, 1848, 1876; Boissier, 1879; Briquet, 1897; Bunge, 1873). A split of the genus into various genera was even proposed (Rafinesque, 1837). These early treatments were largely based on morphology and only partly on the species distribution (e.g., Bunge, 1873).

Rafinesque (1837) accepted 28 small genera instead of one large *Salvia* genus. Unfortunately, his specimens were not preserved, and little information on the characteristics supporting and separating these genera are currently available (Merrill, 1949; Von Hagen, 1947). Nonetheless, the names have been published and must be considered for future taxonomic revisions. As a first example, *Pleudia* Raf. has recently been resurrected (Will et al., 2015). Two further names proposed by Rafinesque are under consideration for resurrection, i.e., for the *Calosphace* and *Audibertia* clades, both of which are now a subject of taxonomic revision (Mark Porter, personal communication).

Bentham (1832–36), in contrast, proposed 14 sections based on morphology and distribution. Later, he arranged the species in 12 sections (Table 2; Bentham, 1848). In a third study, Bentham (1876) included *Salvia* in the tribe Monardeae, together with *Perovskia* Karel, *Dorystaechas* Boiss et. Heldr., *Meriandra* Benth., *Salviastrum* Scheele, *Audibertia*, *Rosmarinus* L., *Monarda* L., *Blephilia* Raf., and *Ziziphora* L. He established four subgenera, *Salvia*, *Sclarea*, *Calosphace* and *Leonia*, to further classify the 12 sections (Table 2). The geographical distribution and morphology of the calyx, corolla and stamens were the main arguments for his classification. Bentham's 1876 classification is still used today. Six species, originally placed in a separate genus, i.e., *Audibertia* Benth., were later accepted as *Salvia* sect. *Audibertia* Benth. (Epling, 1938).

Bunge (1873) basically revised the OW sections accepted by Bentham (1848), in particular the Southwest (SW₃) Asian ones. His classification is only partially comparable to the one of Bentham (1876) since he accepted different taxonomic groups (Table 2).

Boissier (1879) adopted the subsectional system of Bunge with a few changes (Table 2).

Briquet (1897) provided the most recent subgeneric classification and arranged approximately 500 *Salvia* species in 17 sections and eight subgenera (Table 2). He considered *Salviastrum*, *Polakia* Stapf and *Ramona* Greene (=*Audibertia* Benth. sensu Boissier, 1879) as genera distinct from, but closely related to, *Salvia* (Table 1). Together, these genera were included in the tribe Salvieae.

Many subsequent studies have addressed the **infrageneric classification** of *Salvia* s.l. (e.g., Dos Santos, 1991, 1995, 1996; Dos Santos et al., 2005; El-Gazzar et al., 1968; Emboden and Lewis, 1967; Epling, 1938, 1939; Espejo-Serna and Ramamoorthy, 1993; Fernández-Alonso, 2006; Fujita, 1970; Hedge, 1974a; Hrubý, 1962; Huang and Wu, 1975; Jenks et al., 2013; Kahraman et al., 2010, 2011a; Lippert, 1979; Neisess, 1983; Peter, 1936; Peterson, 1978; Pobedimova, 1954; Reales et al., 2004; Reisfield, 1987; Rosúa and Blanca, 1986, 1988; Stibal, 1934, 1935; Strachan, 1982; Torke, 2000; Walker and Elisens, 2001; Wang et al., 2013; Whitehouse, 1949).

Hrubý (1962) suggested the elevation of some subsections to the genus level, a taxonomic approach that was also proposed by Rafinesque (1837) more than one century earlier.

Hedge (1974a, 1982a, 1982b) revised Old World *Salvia*. In his study on African *Salvia* (Hedge, 1974a), he referred to Bentham's sections and introduced 'species groups'. The latter were based on morphology (e.g., floral and stamen morphology) and distribution. In his later treatments of SW Asian *Salvia* (Hedge, 1982a, 1982b) he focused on N African/SW Asian disjunctions and discussed their relationships (Davis and Hedge, 1971; Hedge, 1974a). While the corresponding treatments provided clear insights into the morphological relationships of *Salvia* in Africa, Turkey, and Iran (Hedge, 1974a, 1982a, 1982b), a synopsis of all OW species has remained difficult since the 'species groups' in Africa and SW Asia did not correspond to each other. Within the Flora of Turkey, 'species groups' were not explicitly named but indicated by horizontal dots (Hedge, 1982a, pp. 402–403).

Hedge's concept of 'species groups' not only contributed to a better understanding of relationships in OW *Salvia*, but it also provided the most up-to-date infrageneric classification for the corresponding local floras.

1.3. Molecular data: a new perspective on Salvia

The first **molecular studies** of *Salvia* rejected monophyly for the genus in its traditional circumscription (Walker et al., 2004). Indeed, the species were highly supported in four different clades that were closely related to *Dorystaechas*, *Meriandra*, *Perovskia*, *Rosmarinus*, and *Zhumeria* Rech. f. & Wendelbo. Walker et al. (2004) revealed three clades (I-III). Later, a slight increase in sampling placed the genus *Zhumeria* (Rechinger and Wendelbo, 1967) in a derived position within one of these clades, which was thus renamed *Salvia* 'clade III' (Walker and Sytsma, 2007). Will and Claßen-Bockhoff (2014) recognized the latter as two independent lineages and distinguished Clade III (SW Asian *Salvia* species and *Zhumeria*) and Clade IV (E Asian *Salvia*).

The existing molecular studies are either restricted to a small subset of species or reflect the flora of a certain region (Jenks et al., 2011, 2013; Li et al., 2013; Sudarmono, 2007; Sudarmono and Okada, 2008; Takano and Okada, 2011; Walker et al., 2015). Thereby, relationships between clades remain largely unresolved. The present study adds new sequences for OW *Salvia* and combines them with the existing molecular data for NW and OW *Salvia*. The aim of our study is the identification of well-supported clades providing the basis for evolutionary and taxonomic conclusions.