



## *Arnebia purpurea*: a new member of formerly monotypic genus *Huynhia* (Boraginaceae-Lithospermeae)

ANDREA COPPI<sup>1</sup>, LORENZO CECCHI<sup>2</sup>, DANIELE NOCENTINI<sup>3</sup> & FEDERICO SELVI<sup>4\*</sup>

<sup>1</sup>Università di Firenze, Dipartimento di Biologia, Via G. La Pira 4, 50121, Florence, Italy

<sup>2</sup>Università di Firenze, Museo di Storia Naturale, sezione botanica “Filippo Parlatore”, Via G. La Pira 4, 50121, Florence, Italy

<sup>3</sup>Università di Siena, Dipartimento di Scienze della Vita, Via P.A. Mattioli 4, 53100 Siena, Italy

<sup>4</sup>Università di Firenze, Dipartimento di Scienze delle Produzioni Agroalimentari e dell’Ambiente (DISPAA), Laboratorio di Botanica, P.le delle Cascine 28, 50144 Florence, Italy

\*corresponding author: [federico.selvi@unifi.it](mailto:federico.selvi@unifi.it)

### Abstract

The taxonomic position and affinities of the rare Turkish endemic *Arnebia purpurea* are analyzed using nuclear and plastid DNA sequence data and morphological characters. Phylogenetic analysis of a wide sample of old-world Lithospermeae consistently retrieved a clade with this species sister to *Huynhia pulchra*, the only member of the genus *Huynhia*. All other members of *Arnebia* s.l. (including *Macrotomia*) formed a separate clade subdivided in two lineages corresponding to the groups of the annual and the perennial species. Consequently, *Arnebia* does not appear monophyletic. Floral and palynological characters support the affinity of *A. purpurea* to *Huynhia pulchra*, in especially the stamens inserted at different heights in the corolla tube and the pollen grains with a single row of endoapertures along the equatorial belt. We therefore advocate the placement of *A. purpurea* in *Huynhia* and propose a new combination, implying that the latter is no longer a monotypic genus but includes two species with a sharply allopatric range in the Middle-East. Further studies with additional markers and a wider taxon sampling will help to elucidate relationships in *Arnebia* s.l..

**Key words:** *Macrotomia*, molecular phylogeny, micromorphology, taxonomy, Turkish flora

### Introduction

When broadly circumscribed, *Arnebia* Forsskål (1775: 62) is a genus of Boraginaceae tribe Lithospermeae including about 30 annual or perennial herbs distributed in SW and C Asia, Himalaya, NE Africa and SE Mediterranean. Diagnostic morphological traits are the fruiting calyx, often hardening and tightly enclosing the nutlets, the corolla without faucal appendages, the usually di- or tetrastigmatic flowers, with the style once or twice forked, and the ovoidal-subglobose nutlets, often ventrally keeled and with ornamented surface. Also very characteristic are the pollen grains, which bear two rows of pores, one about each end of the oblong grain, often constricted at the equator and asymmetrical (Johnston 1954; Huynh 1971; Weigend *et al.* in press).

According to Johnston (1954), two sections are comprised in the genus, one (sect. “*Euarnebia*”) consisting of the only annual species *A. tinctoria* Forsskål (1775: 63; = *A. tetrastigma* Forsskål 1775: 62, the type species of the genus described from Egypt), and one (sect. *Strobilia* (Don 1838: 327) Johnston 1954: 55) subdivided in three subgroups based on the life-cycle (annual/perennial) and the presence of a pubescent annulus at the base of the corolla tube.

Previously, some of the perennial species that lack an annulus, such as *A. densiflora* (Ledeb. ex Nordmann 1837: 312) Ledebour (1847–1849: 140) and *A. benthamii* (Wall. ex Don 1838: 333) Johnston (1954: 56), have been placed in the separate genus *Macrotomia* DC. ex Meissner (1837–1842: 281), though more recently these have been included in *Arnebia* (Edmondson 1978; Strid & Tan 1993; Zhu 1982; Zhu *et al.* 1995), and *Macrotomia* is no longer recognized (see also Riedl 1964, 1971).

On the other hand, a single species, *A. pulchra* (Willd. ex Römer & Schultes 1819: 756) Edmondson (1977: 33; based on *Lycopsis pulchra* Willd. ex Roem. & Schult.) was considered outside *Arnebia* by most authors and already placed in the separate monotypic genus *Aipyanthus* Steven (1851: 599), with the misapplied name *Aipyanthus echioides* (Linnaeus 1762: 199) Steven (1851: 600; formally based on *Nonea echioides* (L.) Römer & Schultes 1819: 71), then

composed of these three genera (*Huynhia*+*Macrotomia*+*Arnebia*)” cannot be supported. In fact, grains of *Arnebia* (incl. *Macrotomia*) can have up to seven ectoapertures, hence up to 14 pores close to the polar regions, while those of *H. pulchra* have nine apertures provided each with a single pore along the equatorial belt (Huynh 1971). Therefore, no clear palynological synapomorphy is shared between *Arnebia*/*Macrotomia* and *Huynhia*. Here, we show that *A. purpurea* is closer to *Huynhia* than to *Arnebia* especially in the presence of a single row of endoapertures along the equatorial belt, though it differs in the number of apertures, 6(7) vs. 9 as in *H. pulchra*. Hence, there is a palynological confirmation of the molecular data, corroborating the phylogenetic signal of pollen characters in Lithospermeae (Johnston 1954; Díez *et al.* 1986; Cecchi & Selvi 2009; Weigend *et al.* 2009; Liu *et al.* 2010; Cohen 2011).

At the present state of knowledge, we believe therefore that *A. purpurea* should be placed in *Huynhia*, to make *Arnebia* (incl. *Macrotomia*) monophyletic. Hence, *Huynhia* is no longer a monotypic genus of Lithospermeae and its distribution range becomes considerably wider to the west and the south of Turkey. In fact, *A. purpurea* is endemic to a narrow area of the central section of the Taurus chain (Ekim 2009), implying a considerable disjunction with respect to *H. pulchra*, which is distributed in NE Anatolia and the Caucasian area (Fig. 6).

It is finally worth to add that our phylogenetic results are primarily based on ITS due to the low resolution power of IGS, and this may not reveal other processes that can be responsible for the homogenization of ITS after natural hybridization events, such as concerted evolution (Fuertes Aguilar *et al.* 1999). Since the *Arnebia*/*Macrotomia* group is not strongly supported, nor is the backbone of the combined ITS-IGS tree uniting it to *Huynhia*, the possibility that further analyses with more or different molecular markers reveal different topologies and retrieve these two lineages as sister clades, or nested into each other, cannot be ruled out. Such scenario would support a wider circumscription of *Arnebia*, including *Huynhia*, as in several former treatments. While at present morphology and molecules clearly indicate that *A. purpurea* is sister to the latter in a separate clade, our phylogenies may not be conclusive concerning the intergeneric relationships in the group. Further studies using additional markers and a wider taxon sampling would be useful to address this issue.

## Taxonomy

The placement of *A. purpurea* in the genus *Huynhia* is here proposed:

*Huynhia purpurea* (Erik & Sümbül) L.Cecchi & Selvi, *comb. nov.*

≡ *Arnebia purpurea* Erik & Sümbül in Notes Roy. Bot. Gard. Edinburgh 44: 151. 1986 (basionym).

Type: TURKEY. C4 Antalya, Gazipaşa: Sugözü köyü, Akçal tepesi, 1900–2000 m, 17 May 1983, *H. Sümbül* 3028 (holotype HUB, isotype E).

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