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## Taxonomic clarification in W Mediterranean *Androcymbium* (*Colchicaceae*): *A. wyssianum* sunk in the synonymy of *A. gramineum* and *A. europaeum* restored

### Abstract

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Capsule dehiscence has been used as a diagnostic character for W Mediterranean species of *Androcymbium*. Depending on the state of capsule maturity, the character, however, can be ambiguous in herbarium material. Based on morphological, phenological and cpDNA characters it is shown that misinterpretation of the capsule as indehiscent in the type material of *A. gramineum* has led to serious taxonomic confusion. The combined analyses produced evidence that *A. gramineum* of the population from the type locality at Essaouira, Morocco, is conspecific with *A. wyssianum*. *A. gramineum* is therefore the correct name for the species with dehiscent capsules, whereas the populations with indehiscent capsules at the Atlantic coast north of Essaouira and in SE Spain represent a second species, which is correctly named *A. europaeum*.

Additional key words: taxonomy, morphology, phenology, cpDNA, Morocco, Spain

### Introduction

The predominantly African genus *Androcymbium* comprises, according to current knowledge, 57 species (Hoyo & Pedrola-Monfort 2008). Since the description of *Androcymbium* by Willdenow in 1808 diverse taxonomic arrangements have been made in this genus, with a last revision published by Müller-Doblies & Müller-Doblies (2002). These authors re-organised *Androcymbium* into five sections and eight series.

*Androcymbium* sect. *Erythrostictus*, typified by the NW Mediterranean *A. gramineum* (Cav.) J. F. Macbr., includes six N African-S Mediterranean and two SW African species according to recent molecular phylogenetic analyses by Hoyo & al. (2009). They are characterised by having plane tepals without wings at the base of the blade, a small, green nectary located at the apex of the claw and bracts and leaves with idioblasts (Schlechtendal 1826).

Rather subtle morphological differences exist between the taxa of this section, making correct identification sometimes difficult (Pedrola-Monfort & Caujapé-Castells 1996). The Spanish botanist Antonio José Cavanilles described *A. gramineum*, under its basionym *Melanthium gramineum*, and *M. punctatum* from material collected by the French botanist Auguste Broussonet around Mogador (now Essaouira) in Morocco (Cavanilles 1801). Schlechtendal (1826) transferred Cavanilles's species in his genus *Erythrostictus* and Lange (1860) added a newly described third species, *E. europaeus* Lange, from Almería (southeastern Iberian Peninsula), which is very similar to the two species described by Cavanilles. Baker (1879), in his revision of the *Colchicaceae*, transferred *M. punctatum* Cav. ( $\equiv$  *E. punctatus* (Cav.) Schldl.) to *Androcymbium* as *A. punctatum* (Cav.) Baker and syno-

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nymised under this name also *E. europaeus* Lange and *M. gramineum* Cav. ( $\equiv$  *E. gramineus* (Cav.) Schldtl.). Baker's name *A. punctatum* of 1879 is illegitimate, being a younger homonym of *A. punctatum* Baker of 1874 (a synonym of *A. capense* (L.) Druce). Macbride (1918) therefore formed the combination *A. gramineum*, in the synonymy of which he placed both the illegitimate *A. punctatum* and *E. europaeus*. Later, *A. wyssianum* Beauverd & Turrett. was described as a new species (in Beauverd 1938), distributed from Morocco and Mauritania through Algeria to Tunisia. Greuter (1967), in a taxonomic revision of the northern African species, restored *A. europaeum* (Lange) K. Richt. as a separate species distinct from *A. gramineum* by its smaller capsule size. Later, additional populations of *Androcymbium* were discovered north of Essaouira, following the Atlantic coast of Morocco, and were assigned to *A. gramineum*.

Pedrola-Monfort & Caujapé-Castells (1994) carried out morphometrical and allozymatic studies with populations of *Androcymbium europaeum* and *A. gramineum*. These authors concluded that *A. europaeum* and *A. gramineum* should be re-united. They did not analyse individuals of the population from the type locality of *A. gramineum* at Essaouira but a later morphometric analysis of Cavanilles's type material from Essaouira seemed to corroborate this conclusion (Pedrola-Monfort & Caujapé-Castells 1998).

Recent molecular phylogenetic studies in *Androcymbium*, however, surprisingly clustered the samples of the population from the type locality of *A. gramineum* at Essaouira together with *A. wyssianum* and separated them from the other populations of *A. gramineum* studied (Hoyo & Pedrola-Monfort 2008; Hoyo & al. 2009). These results triggered a closer investigation of the coastal populations at and north of Essaouira. The aim of the present paper is to revise the delimitation of the *Androcymbium* species in Morocco through an analysis of molecular, morphological and phenological characters.

## Material and methods

**Plant material.** — Individuals from various populations of *Androcymbium gramineum* from Almería and the Atlantic coast of Morocco and of *A. wyssianum* from Morocco, Algeria and Tunisia were collected in the field between 1989 and 1993 (Table 1, Fig. 1), incorporated in the living plant collection of the Marimurtra Botanic

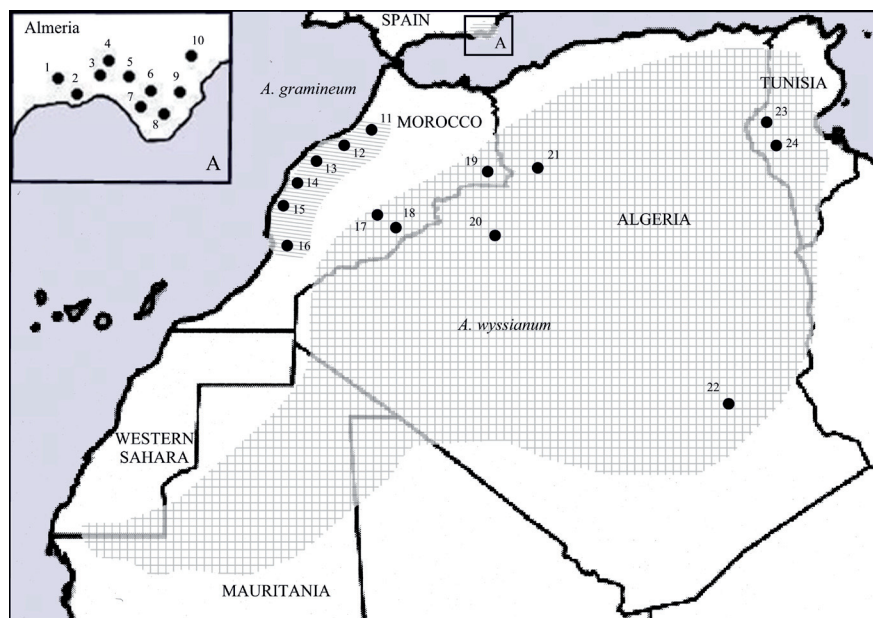


Fig. 1. Distribution of the two *Androcymbium* species under study according to data previously available.

Garden (MBG) in Blanes and cultivated in a greenhouse. Fresh leaves of *A. gramineum* were collected from Essaouira (Morocco) in January 2002 and dried in silica gel for molecular analyses. In May 2004 bulbs were collected again at Essaouira and cultivated at the MBG for morphological and phenological analyses.

All cultivated plants were grown under the same conditions regarding soil, light and watering in the research greenhouse at the MBG.

**DNA extraction, amplification and sequencing.** — DNA was extracted from fresh leaf tissue of all samples, previously dried in silica gel and snap-frozen in liquid nitrogen, using the CTAB buffer method (Doyle & Doyle 1987) with some modifications (Li & al. 2001). The isolated DNA was resuspended in T. E. buffer and stored at  $-20^{\circ}\text{C}$  until subsequent analyses.

Six chloroplast fragments (*trnL* intron, *trnL-trnF* IGS, *trnY-trnD* IGS, *trnH-psbA* IGS, *atpB-rbcL* IGS and *rps16* intron) and one nuclear fragment (PNA $\pi\omega\lambda_2$ \_i23) were PCR-amplified. Amplification and sequencing reactions were performed as described in Hoyo & Pedrola-Monfort (2006, 2008) and Hoyo & al. (2009). Sequence alignment of the seven DNA regions was performed manually using BioEdit (Hall 1999).

**Morphological and phenological analyses.** — In order to distinguish *Androcymbium gramineum* from *A. wyssianum*, the capsule dehiscence at maturity was applied as diagnostic character, because the capsules were described as indehiscent in *A. gramineum* and dehiscent in *A. wyssianum* (Pedrola-Monfort & Caujapé-Castells 1996), making determination unambiguous.

According to Pedrola-Monfort (1993) other distinguishing features between *Androcymbium gramineum*

Table 1. Locality data of the sampled *Androcymbium* populations.

Species	Population	Latitude	Longitude
1. <i>A. gramineum</i>	El Solanillo, Almería, Spain	36°44'12"N	2°39'21"W
2. <i>A. gramineum</i>	Zona de áridos, Almería, Spain	36°51'01"N	2°28'24"W
3. <i>A. gramineum</i>	Cerro de San Cristóbal, Almería, Spain	36°50'35"N	2°28'12"W
4. <i>A. gramineum</i>	Los Molinos, Almería, Spain	36°51'42"N	2°26'01"W
5. <i>A. gramineum</i>	Cerro de los Peligros, Almería, Spain	36°51'02"N	2°17'47"W
6. <i>A. gramineum</i>	Barranco de Curriá, Almería, Spain	36°48'58"N	2°14'59"W
7. <i>A. gramineum</i>	El Barranquete, Almería, Spain	36°51'25"N	2°13'38"W
8. <i>A. gramineum</i>	Playas de Monsul, Almería, Spain	36°43'53"N	2°08'46"W
9. <i>A. gramineum</i>	Cerro de los Lobos, Almería, Spain	36°50'31"N	2°00'37"W
10. <i>A. gramineum</i>	Charco del Lobo, Almería, Spain	36°57'04"N	2°00'37"W
11. <i>A. gramineum</i>	Ain Harrouda, Morocco	33°38'49"N	7°27'46"W
12. <i>A. gramineum</i>	Casablanca, Morocco	33°31'41"N	7°39'44"W
13. <i>A. gramineum</i>	Qualidia, Morocco	32°44'23"N	9°02'09"W
14. <i>A. gramineum</i>	Cap Beddouza, Morocco	32°33'13"N	9°12'43"W
15. <i>A. gramineum</i>	Safi, Morocco	32°19'59"N	9°13'22"W
16. <i>A. gramineum</i>	Essaouira, Morocco	31°29'21"N	9°46'25"W
17. <i>A. wyssianum</i>	Fonts Bleus of Meski, Morocco	31°51'27"N	4°17'09"W
18. <i>A. wyssianum</i>	Er Foud, Morocco	31°30'31"N	4°11'27"W
19. <i>A. wyssianum</i>	Figuig, Morocco	32°06'32"N	1°15'30"W
20. <i>A. wyssianum</i>	Taghit, Algeria	30°54'54"N	2°02'04"W
21. <i>A. wyssianum</i>	Ain Ouarka, Algeria	32°44'13"N	0°05'44"W
22. <i>A. wyssianum</i>	Tassili, Algeria	25°09'39"N	8°10'13"E
23. <i>A. wyssianum</i>	Nefta 1, Tunisia	33°51'11"N	7°54'36"E
24. <i>A. wyssianum</i>	Nefta 2, Tunisia	33°50'40"N	7°55'15"E

and *A. wyssianum* are: (i) the earlier emergence, blooming and senescence periods of *A. gramineum* compared to *A. wyssianum*, and (ii) biennial corm dormancy present in *A. wyssianum* but absent in *A. gramineum*.

Observations were made on the individuals cultivated at the MBG under the same conditions since 2004 of the Essaouira population of *Androcymbium gramineum* and since 1993 of *A. gramineum* and *A. wyssianum* populations.

## Results

**Genetic analysis.** — Because of the close phylogenetic relationship between the eight species of *Androcymbium* sect. *Erythrostickus* (Hoyo & Pedrola-Monfort 2008; Hoyo & al. 2009), very few variable positions were found between the two taxa under study. Four of the seven analysed regions were useful for discriminating *A. gramineum* from *A. wyssianum*: (i) *psbA-trnH* IGS, (ii) *trnL-trnF* IGS, (iii) *rps* 16 intron and (iv) *trnY-trnD* IGS (Table 2). Four useful variable positions were found in the *psbA-trnH* IGS: two transversions and two indels. Three additional useful variable positions were found in the three remaining regions: two transversions and one transition. According to our molecular data, individuals of the Essaouira population share seven autapomorphic

positions with individuals described as *A. wyssianum*. Nevertheless, four variable positions and two indels were exclusive for the Essaouira individuals in four of the seven analysed regions (*psbA-trnH* IGS, *trnL-trnF* IGS, *trnL* intron and *trnY-trnD* IGS).

**Morphological analysis.** — Dehiscent mature capsules were observed in all individuals of *Androcymbium wyssianum*, whereas indehiscent mature capsule were found in all individuals of *A. gramineum* except for those from the Essaouira population. Both in the wild and in culture over five years, the individuals of the Essaouira population exclusively developed dehiscent capsules (Fig. 2).

**Phenological analysis.** — Biennial corm dormancy was found in both *Androcymbium gramineum* from Essaouira and *A. wyssianum*, but not in other Moroccan and Spanish *A. gramineum* individuals. Emergence, blooming and senescence periods were synchronous in *A. gramineum* from Essaouira and *A. wyssianum* but took place earlier in other *A. gramineum* individuals (Fig. 3).

## Discussion

The molecular, morphological and phenological findings strongly indicate that *Androcymbium gramineum* from



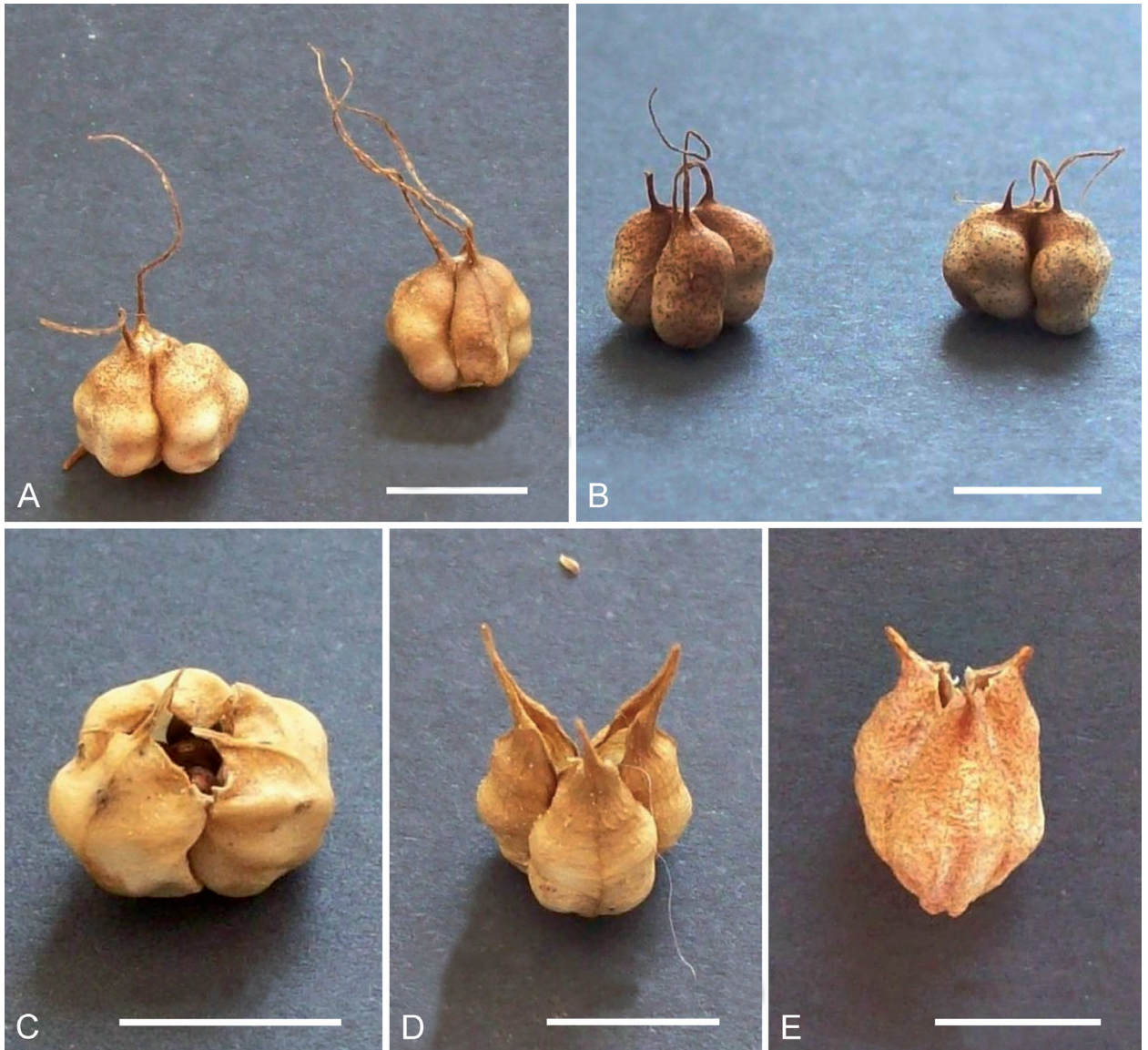


Fig. 2A-B: Mature indehiscent capsules of *Androcymbium* “*gramineum*” from Almería and Morocco, respectively; C-D: mature dehiscent capsules of *A. wyssianum* from Morocco; E: mature dehiscent capsules of *A. gramineum* from the population of the type locality at Essaouira. – Scale bars: A-E = 5 mm.

Essaouira and *A. wyssianum* belong to a single species that is distinct from other “*A. gramineum*” populations. The solution for this apparent taxonomic conflict lies in the type specimen of *A. gramineum*. The plant material collected by Broussonet at Essaouira was used by Cavanilles for the description of both *Melanthium gramineum* and *M. punctatum*, which are today recognised as conspecific and treated as *A. gramineum*. As we know from our analyses of material from the population at the type locality, the material collected by Broussonet was still immature, therefore the capsules appeared indehiscent. Because the further *Androcymbium* populations subsequently discovered north of Essaouira along the Atlantic coast of Morocco all had indehiscent capsules, they were identified as *A. gramineum*.

In 1938 Beauverd & Turretini described *Androcymbium wyssianum*, based on plant material collected

in the Algerian desert between Ghardaïa and El-Golea (in Beauverd 1938). With the exception of cataphyll, hypsophyll and tepal biometrics, the species diagnosis is ambiguous: “this plant shows some intermediate characters between the two sections (*Erythrostictus* and *Cymbanthes*), with uniflorous inflorescence and a nectary whose exudate crystallises on the base of the blade showing a reddish brown aspect”. The authors described neither the corm nor the capsule. The corm is absent in the type specimen and the capsule is immature, representing a source of confusion until it was shown that *A. wyssianum* has dehiscent mature capsules (Pedrola-Monfort 1993).

Another source of error is the high morphological variability present within the *Androcymbium gramineum* population of Essaouira. Cavanilles described two species from the same locality, *Melanthium punc-*

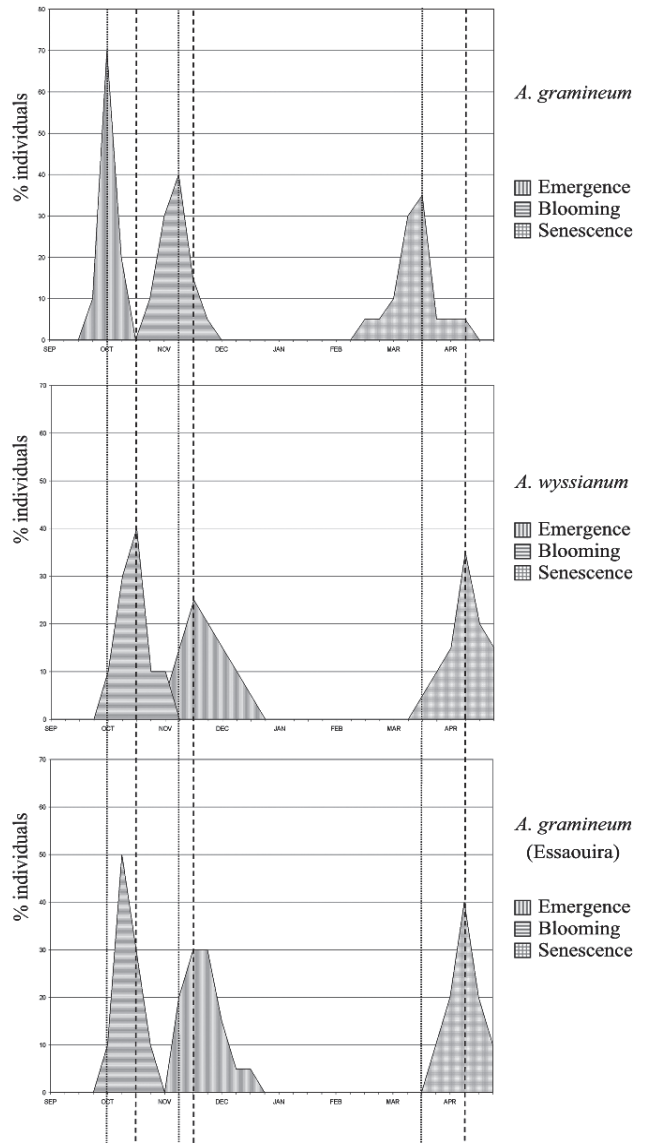
Table 2. Discriminating DNA positions for four chloroplast regions analysed among the two taxa of *Androcymbium*.

(1) <i>psbA-trnH</i> IGS				
	*	*	*	*
<i>A. gramineum</i> Almería	TGAAGTAGCAA	CCCTT - TTTCT	TTTCCATTT	AAAAAGAGA
<i>A. gramineum</i> Morocco	TGAAGTAGCAA	CCCTT - TTTCT	TTTCCATTT	AAAAAGAGA
<i>A. wyssianum</i>	TGAAGGAGCAA	CCCTTATTTCT	TTTCAATTT	AAAA - GAGA
<i>A. gramineum</i> Essaouira	TGAAGGAGCAA	CCCTTATTTCT	TTTCAATTT	AAAA - GAGA
(2) <i>trnL-trnF</i> IGS		(3) <i>rps16</i> intron		
	*		*	
<i>A. gramineum</i> Almería	TAATTTAAATT	<i>A. gramineum</i> Almería	TCTGATGTGGA	
<i>A. gramineum</i> Morocco	TAATTTAAATT	<i>A. gramineum</i> Morocco	TCTGATGTGGA	
<i>A. wyssianum</i>	TAATTGAAATT	<i>A. wyssianum</i>	TCTGACGTGGA	
<i>A. gramineum</i> Essaouira	TAATTGAAATT	<i>A. gramineum</i> Essaouira	TCTGACGTGGA	
(4) <i>trnY-trnD</i> IGS				
	*			
<i>A. gramineum</i> Almería	TAATTTAATTA			
<i>A. gramineum</i> Morocco	TAATTTAATTA			
<i>A. wyssianum</i>	TAATTTAATTA			
<i>A. gramineum</i> Essaouira	TAATTTAATTA			

*tatum* and *M. gramineum*. The first had a single bulb, white, purplish-dotted tepals with conspicuous venation and stamens with a dark dot at the base, whereas the second had two or three vertically arranged bulbs, narrower leaves, yellow anthers (erroneously stated) and white tepals with barely perceptible veins. We observed both forms within the same area: one in the *Tetraclinis articulata* underbrush on fissures of rocky terrains lacking soil, the other, closer to the sea, with wide leaves growing over fossil dunes, both on limestone. No chloroplast genetic differences were found between these two forms and we believe that this variation is induced by different soil conditions rather than genetic causes.

According to the actually dehiscent capsule and the other matches observed, and taking into account the high level of intraspecific morphological variation, *Androcymbium wyssianum* must be considered as conspecific with *A. gramineum*. For the reason of priority (McNeill & al. 2006), the correct name of the species is *A. gramineum*. For the *Androcymbium* populations from the Atlantic coast of Morocco north of Essaouira and in the Iberian Peninsula, with truly indehiscent mature capsules, the name published by Lange (1860) for plants from Almería in SW Spain can be taken up; the correct name for this species is thus *A. europaeum* (Lange) K. Richt. The distribution of the two species is shown in Fig. 4.

The high morphological variation found in the Essaouira population deserves further investigation since some individuals of this population show intermediate character states between *Androcymbium gramineum* and *A. europaeum*, namely striped petals, an abundance of idioblasts and deep green leaves (the easternmost *A. gramineum* individuals are light green). Because of that and hypotheses by Caujapé-Castells (2010) regarding the relationships between the Macaronesian and NW

Fig. 3. Phenology of the two analysed species of *Androcymbium*.



African floras, we hypothesise that some kind of introgression has occurred in this area between *A. europaeum*, *A. gramineum* and *A. psammophilum* (Fig. 4).

### Taxonomic conclusions

#### *Androcymbium gramineum*

(Cav.) J. Macbr. in Contr. Gray Herb. 53: 6. 1918 ≡ *Melanthium gramineum* Cav. in Anales Ci. Nat. 3: 50. 1801. – Holotypus: [Morocco], “*Melanthium acaule*, foliis imbricatis, carinatis, lanceolato acuminatis, floribus sessilibus” / “Petalas sex, lanceolatas, longitudinaliter venosa, pollicem et amplius longa, duas lineas lata, stamniifera” / “Habitat in viciniis Mogador [= Essaouira]” Broussonet (MA!; isotype: G).

= *Melanthium punctatum* Cav. in Anales Ci. Nat. 3: 50. 1801 ≡ *Erythrostictus punctatus* (Cav.) Schtdl. in Linnaea 1: 90. 1826 ≡ *Androcymbium punctatum* (Schtdl.) Baker in J. Linn. Soc. London (Bot.) 17: 445. 1879, non Baker 1874. – Holotype: [Morocco], Mogador [= Essaouira], Broussonet (MA!; isotype: G).

= *Androcymbium punctatum* var. *saharae* Maire in Bull. Soc. Hist. Nat. Afrique N. 16: 96. 1925 ≡ *Androcymbium gramineum* var. *saharae* (Maire) Maire in Bull. Soc. Hist. Nat. Afrique N. 25: 320. 1934. – Holotype: [Algeria], “Sahara occidental”, 1923, D. Tripeau (MPU [digital image]!).

= *Androcymbium wyssianum* Beauverd & Turrett. in Candollea 7: 371. 1938. – Holotype: [Algeria], inter Ghar-daia et El Golea, 19.12.1937, Turrettini & Wyss (G!).

*Androcymbium europaeum* (Lange.) K. Richt., Pl. Eur. 1: 188. 1890 ≡ *Erythrostictus europaeus* Lange in Vidensk. Meddel. Dansk Naturhist. Foren. Kjøbenhavn, ser. 2, 1: 69. 1860. – Holotypus: [Spain] “Cabo de Gata et urben Almería, Lange 141, ... Decembre 1851 florigerum legi. E bulbis postea, benevole curante cl. D. José de Medina y Ximenez, missis in horto botanico nostro educatus est floresque editit” (C; isotype: G).

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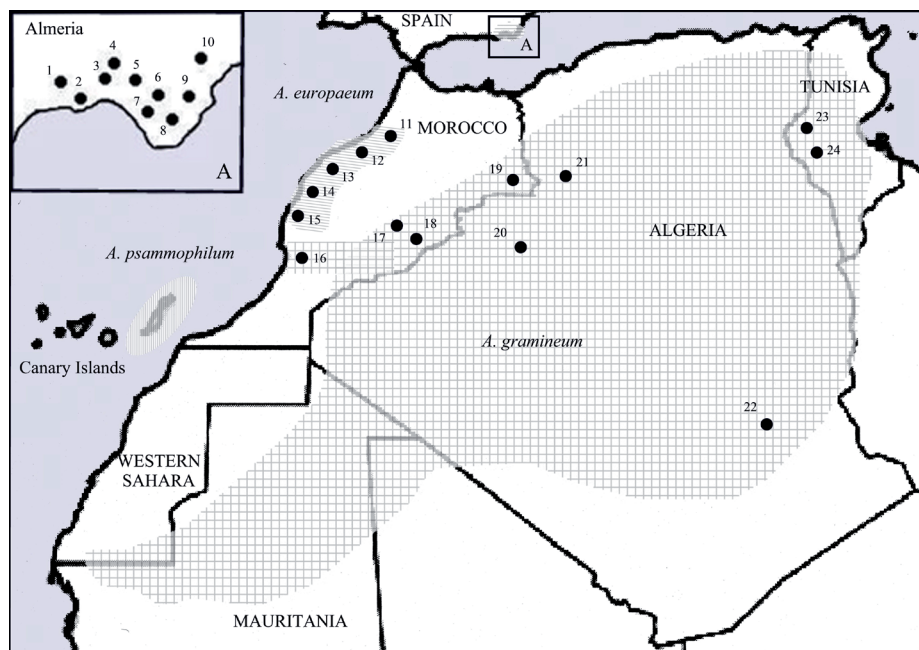


Fig. 4. Distribution of the two analysed species of W Mediterranean *Androcymbium* after taxonomic revision and of *A. psammophilum*.

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