

# Phylogenetic relationships of *Erysimum* (Brassicaceae) from the Baetic Mountains (SE Iberian Peninsula)

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## Abstract

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The Baetic mountains, located in the southern Iberian Peninsula, is a major hotspot of biodiversity in the Mediterranean Basin, constituting one of the most important glacial refugia for vascular plants in Europe. Despite their relatively limited extension, the Baetic Mountains contain almost 50% of the total endemic *Erysimum* species in the Iberian Peninsula. The broadly distributed *Erysimum* genus has diversified profusely in the Mediterranean region, with more than a hundred species described in the area, out of a total of c. 200 species included in the genus. We used two plastid DNA regions (*ndhF* and *trnT-L*) and one nuclear DNA region (ITS1-5.8S rDNA-ITS2), with 3,556 bp total length, to carry out phylogenetic analysis by Bayesian inference, maximum likelihood and maximum parsimony, in order to explore the evolutionary relationships between the *Erysimum* species inhabiting these ranges. Analyses of concatenated sequences from the two genomes identified two main clades with no overlap in species composition so that samples from the same species fell within the same major clade. The phylogenetic relationships depicted by those two clades do not give support to the *E. nevadense* group, previously proposed on taxonomic grounds. In addition, our results indicated recurrent changes in flower colour in the Baetic *Erysimum* species although, alternatively, reticulate evolution, which is suggested by incongruent position of taxa in the different trees, may have also affected this trait.

**Keywords:** cpDNA, flower colour, nDNA, *Erysimum nevadense* group, secondary contact.

## INTRODUCTION

*Erysimum* L. is one of the largest genera of the Brassicaceae, comprising more than 200 species, recently grouped in the unigeneric tribe *Erysimeae* (Couvreur & al., 2010; Al-Shehbaz, 2012). The evolutionary history of this genus is complex, with recurrent events of interspecific hybridization and polyploidization (Clot, 1992; Ancev, 2006; Marhold & Lihová, 2006). In fact, *Erysimum* is one of the few crucifer polybasic genera (i.e., characterized by multiple base chromosome numbers; Warwick & al., 2006). Reticulate evolution is likely to be behind

## Resumen

Abdelaziz, M., Muñoz-Pajares, A.J., Lorite J., Herrador, M.B., Perfectti, F. & Gómez, J.M. 2014. Relaciones filogenéticas en *Erysimum* (Brassicaceae) de las Cordilleras Béticas (SE Península Ibérica). *Anales Jard. Bot. Madrid* 71(1): e005.

Las cordilleras Béticas, localizadas en el sudeste de la Península Ibérica, representan una importante zona para la biodiversidad de la cuenca mediterránea, constituyendo uno de los refugios glaciares más destacados de plantas vasculares en Europa. A pesar de su extensión relativamente limitada, las cordilleras Béticas albergan casi el 50% del total de las especies endémicas de *Erysimum* de la Península Ibérica. *Erysimum* es un género ampliamente distribuido, que se ha diversificado profusamente en la región mediterránea, con más de un centenar de especies descritas en dicha área, del total de las alrededor de 200 especies incluidas en el género. Usamos dos regiones de ADN plastidial (*ndhF* y *trnT-L*) y una región de ADN nuclear (ITS1-5.8S rDNA-ITS2), con una longitud total de 3.556 pb, para llevar a cabo análisis filogenéticos mediante inferencia bayesiana, máxima verosimilitud y máxima parsimonia, con el fin de explorar las relaciones evolutivas entre las especies de *Erysimum* que habitan en éstas cordilleras. El análisis de secuencias concatenadas de los dos genomas identifica dos cladogramas principales y las muestras de la misma especie aparecen siempre en un clado o en el otro pero no en ambos. Las relaciones filogenéticas que indican esos dos cladogramas no apoyan la circunscripción del grupo taxonómico *E. nevadense*, previamente definido en dicho género. Además, los análisis indican cambios recurrentes en el color floral de las especies de *Erysimum* que habitan los Sistemas Béticos aunque la evolución reticulada, que sugiere la posición de algunos taxones en uno y otro árbol, puede haber también afectado a este rasgo, lo que sería una hipótesis alternativa.

**Palabras clave:** ADN cloroplastidial, color floral, ADN ribosómico nuclear, grupo *Erysimum nevadense*, contacto secundario.

the existence of species complexes and cryptic species which are common in *Erysimum* (Ancev, 2006; Turner, 2006; Abdelaziz & al., 2011), and have caused many taxonomic conflicts (Favarger 1978; Nieto Feliner 1991). As a consequence of this taxonomic complexity, the number of species included in the genus ranges from 180 to 223, depending on the taxonomic approach used (Al-Shehbaz & al., 2006; Warwick & al., 2006; Koch & Al-Shehbaz, 2008).

The genus *Erysimum* is primarily distributed in Eurasia, although some species occur in North and Central America, North Africa and the Macaronesian Islands (Al-Shehbaz

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& al., 2006; Warwick & al., 2006; Koch & Al-Shehbaz, 2008) and has diversified profusely in the Mediterranean region. More than 100 *Erysimum* species have been recognized in this area (Greuter & al., 1986), 22 of them in the Iberian Peninsula (Nieto Feliner, 1993). Molecular evidence suggests that many of these species are inter-fertile and have hybridized in the past (Abdelaziz, 2013; Muñoz-Pajares, 2013).

The Baetic mountains, located in the southern Iberian Peninsula (Fig. 1), are a major hotspot of biodiversity in the Mediterranean Basin (Sainz-Ollero & Hernández Bermejo 1985; Domínguez & al., 1996; Blanca & al., 1998; Médail & Quézel, 1999; Quézel & Médail, 1995). The geological history of the Baetic Mountains differs from the rest of the Iberian Peninsula since they share a common geological origin with the Rif Mountains in northern Morocco, and together form the Baetic-Rifean Arc (Lonergan & White, 1997). More than 3,000 vascular plant species occur in the 45,000 km<sup>2</sup> occupied by the Baetic mountains, and 40% of them are endemic to the Iberian Peninsula (Medina-Cazorla & al. 2010). Such a high plant diversity has several non-exclusive causes. On the one hand, this area has acted as one of the most important glacial refugia for vascular plants in the Mediterranean region (Medail & Diadema, 2009); on the other, local diversification sometimes leading to allopatric and peripatric speciation has been favoured by its complex topography (Lavergne & al., 2012). Furthermore, its biogeographical connection with the North African Rif has facilitated migration from the Maghreb, enhancing plant diversity with African lineages (Lavergne & al., 2012), as previously showed for other plant genera, as e.g.: *Saxifraga*, *Hypochaeris*, *Cakile*, *Crithmum*, *Eryngium*,

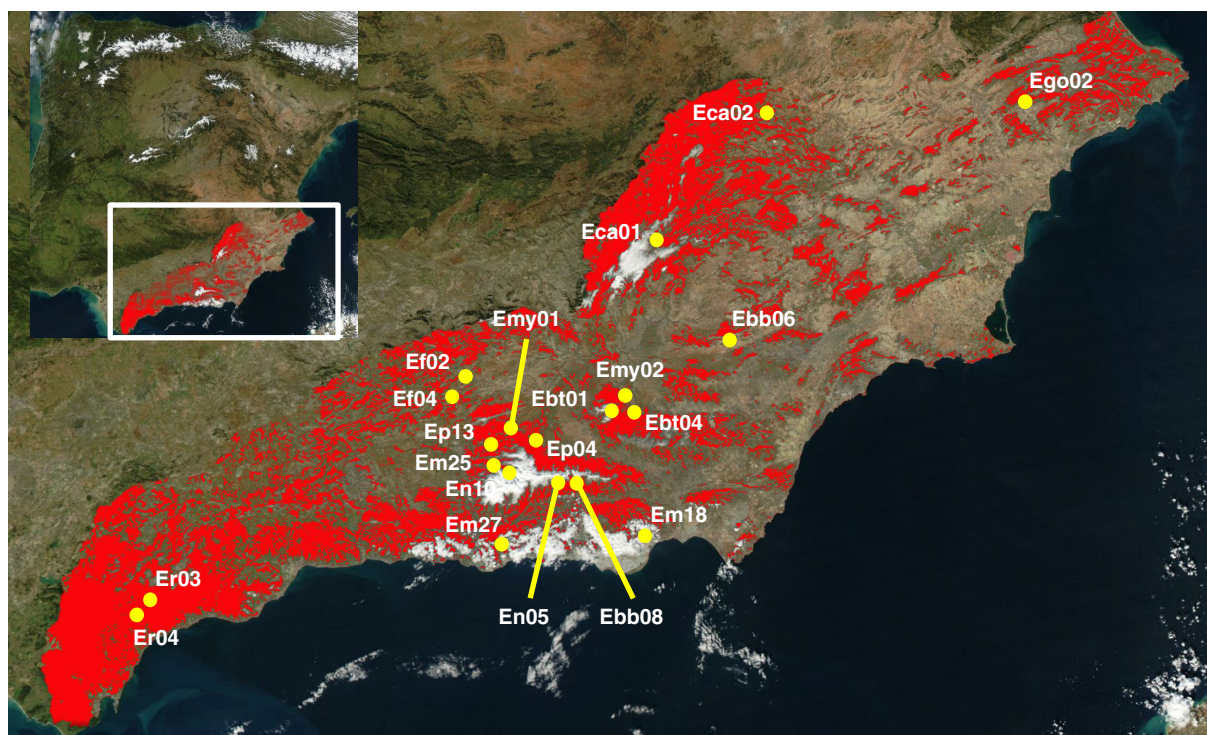
*Halimione*, *Salsola* and *Cistus* (Rodríguez-Sánchez & al., 2008, and references therein).

Ten *Erysimum* species inhabit the Baetic Mountains, seven of them endemic to the area (Blanca & al., 2009). The genus presents a range of life forms from perennial polycarpic (e.g., *E. nevadense*) to monocarpic annuals (e.g., *E. incanum*) that occur from the mountain tops (e.g., *E. nevadense*) to the lowlands (e.g., *E. mediobispanicum* or *E. gomezcampoi*) and are associated to different type of substrates (Table 1). The genus *Erysimum* also exhibits two flower colours in the species inhabiting the Baetic mountains (Table 1). In this study we explore the phylogenetic relationships among these wall-flower species. There is a recently published phylogeny of the genus based on nuclear ribosomal DNA ITS sequence data that is mainly focused on Asian species and only covers a small representation of the Baetic and even the Iberian taxa (Moazzeni & al. 2014).

## MATERIAL AND METHODS

### Study system

According to *Flora Iberica* (Nieto Feliner, 1993), nine outcrossing species of *Erysimum* are native to the Baetic Cordillera: *E. baeticum* Polatschek, *E. cazorlense* (Heywood) Holub, *E. fitzii* Polatschek, *E. gomezcampoi* Polatschek, *E. mediobispanicum* Polatschek, *E. myriophyllum* Lange, *E. nevadense* Reut., *E. popovii* Rothm., and *E. rondae* Polatschek. All these species belong to the section *Erysimum* L., along with the autogamous annual species *E. incanum* Kunze also growing in the area (see Fig. 1 and Table 1). *Erysimum gomezcampoi*, *E. mediobispanicum*, *E. nevadense* and *E. rondae*, together with *E. merxmulleri* and *E. ruscionense*



**Fig. 1.** Geographic location of the Baetic Mountains (in red) in South-East Spain and the populations sampled in the present study. Species, subspecies and population codes follow Tables 1 and 2.

**Table 1.** Population codes (Pop), distribution, altitude range, main substrate, life form, plant height and flower colour of the *Erysimum* species and subspecies inhabiting the Baetic Mountains. Endemic species are marked with † whereas those belonging to the *nevadense* group are marked with asterisk.

Taxon	Pop	Distribution	substrate	Altitude Range (m)	Life history	Plant height (cm)	Flower color
<i>E. baeticum baeticum</i> †	Ebb (08)	Sierra Nevada and Filabres	siliceous	1600-2600	perennial polycarpic	25-60(70)	purple
<i>E. baeticum bastetanum</i> †	Ebt (01,04,06)	Sierra de Baza, Maria and Jureña	calcareous	1000-2000	perennial polycarpic	25-60(70)	purple
<i>E. cazorlense</i> †	Eca (01,02)	Sierra of Cazorla, Segura and Alcaraz.	calcareous	1200-1900	perennial monocarpic	25-80(15)	purple
<i>E. fitzii</i> †	Ef (02,04)	Sierra de la Pandera	calcareous	1200-1800	perennial monocarpic	15-35	yellow
<i>E. gomezcampoi</i> *	Ego (02)	Eastern mountain ranges of Spain	calcareous	600-1400	perennial polycarpic	15-30(50)	yellow
<i>E. incanum</i>	Ei (03)	S of Spain and N of África	Indiferent	900-1500	Anual	2-25	yellow
<i>E. incanum mairei</i>	Eim (04)	Spain and N of Africa	calcareous	1000-1600	Anual	10-40	yellow
<i>E. mediodispanicum</i> *	Em (18,21,25,27)	Center and South of Spain and Baetic Mountains	calcareous	700-2100	perennial monocarpic	25-50(70)	yellow
<i>E. myriophyllum</i> †	Emy (01,02)	Central and Eastern Baetic Mountains	dolomitic	700-1900	perennial monocarpic	14-40	yellow
<i>E. nevadense</i> †*	En (05,10)	Sierra Nevada	siliceous	1700-2800	perennial monocarpic	5-25	yellow
<i>E. popovii</i> †	Ep (04,13)	Central Baetic Mountains	calcareous	700-2000	perennial monocarpic	20-40(50)	purple
<i>E. rondae</i> †*	Er (03,04)	Western Baetic Mountains	calcareous	700-1700	perennial monocarpic	10-40	yellow

(from outside Baetic mountains), are considered micro-species that form a natural group called *nevadense* (Nieto Feliner, 1993 and Table 1).

### Phylogenetic analysis

Fresh leaf tissue material was collected from at least two populations from each Baetic *Erysimum* species (except for *E. gomezcampoi*, which inhabits only one locality on the Baetic Mountains; and for *E. baeticum* subsp. *bastetanum* from Sierra de María, which was sampled from seeds of the germplasm bank of the Real Jardín Botánico de Madrid (Table 2). In total, 23 populations were sampled. This material was dried and preserved in silica gel until DNA extraction. We extracted DNA by using GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, Missouri, USA) with at least 60 mg of plant material crushed in liquid nitrogen.

We amplified three different DNA regions: two plastidial (*ndbF*, ~2000 bp and *trnT-L*, ~1300 bp) and one nuclear (ITS1-5.8S-ITS2, ~710 bp). We used the following primers: *ndhF5* (5' ATGGAACATACATATCAATATTCATGG 3') and *ndhF2100* (5'CAAAGAACTYGTAACACSTACTCC 3') (Olmstead & Sweere, 1994) to amplify *ndbF*; *tabA* (5' CATTACAAATGCGATGCTCT 3') and *tabD* (5' GGGGATAGAGGGACTTGAAC 3') (Taberlet & al., 1991) for *trnT-L*; ITS1 (5' TCCGTAGGTGAACCTGCGG 3'), ITS2 (5' GCTGCGTTCTTCATCGATGC 3'), ITS3 (5' GCATCGATGAAGAACGCAGC 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') primers for the ITS1-5.8s-ITS2 region (White & al., 1990). PCR reactions were performed in a total volume of 50 µL, with the following composition: 5 µL 10× buffer containing MgCl<sub>2</sub> at 1.5 mmol/L (New England Biolabs), 0.1 mmol/L each dNTP, 0.2 µmol/L each primer and 0.02 U *Taq* DNA polymerase (New England Biolabs). PCRs were performed in a

Gradient Master Cycler Pro S (Eppendorf) using an initial denaturing step of 3 min at 94°C and a final extension step of 3 min at 72°C in all the reactions. Reactions for *ndbF* included 35 cycles of 94°C for 15 s, 47°C for 30 s, and 72°C for 90 s. Reactions for *trnT-3'trnL* included 35 cycles (94°C 15 s, 53°C 30 s, and 72°C 90 s). Reactions for ITS1 also included 35 cycles (94°C 15 s, 64°C 30 s, and 72°C 45 s). For ITS2, reactions included 35 cycles of 94°C 15 s, 53°C 30 s, and 72°C 45 s).

PCR products were mixed with 0.15 volume of 3 M sodium acetate, pH 4.6 and 3 volumes 95% (v/v) ethanol and subsequently purified by centrifuging at 4°C. Amplicons were then sent to Macrogen (Geumchun-gu, Seoul, Korea; <http://www.macrogen.com>) to be sequenced, using the respective PCR primers and additional internal primers for *ndbF* (*ndhF599*: 5' TAGGTCTTTATTGGATAAC 3'; *ndhF989-R*: 5' TGATGTTAGCTCTAGGATGTATGGG 3'; and *ndhF1354*: 5' AAATGTCCTTCAAAAGTAAG 3'; Olmstead and Sweere, 1994) as well as for *trnT-L* regions (*tabB*: 5'TCTACCGATTTCCGCATATC 3'; and *tabC*: 5' CGAAATCGGTAGACGCTACG 3'; Taberlet & al., 1991).

*thaliana* sequences from GenBank were used as outgroups, together with the Iranian species *Erysimum passgalense* Boiss. We tested for incongruence between the nuclear and plastid genes using Congruence Among Distance Matrices tests (CADM, Legendre & Lapointe, 2004), as implemented in APE (Paradis, 2004; R Development Core Team, 2011). The phylogenetic information resulting from the three analyzed regions was significantly congruent ( $W=0.708$ ,  $\chi^2=688.6$ ,  $P=0.001$ ). Sequences of different markers were thus concatenated on an individual basis and then aligned using the ClustalW (Thompson & al., 1994) tool in BioEdit (Hall, 1999; Larkin & al., 2007). The sequences reported in the present study have been deposited in GenBank (Table 2).



**Table 2.** Population origin of the samples used in the phylogenetic analyses and GenBank accession numbers for the nuclear and plastidial sequences.

Taxon	Pop	Sample origin	GenBank accession no.		
			ITS1-5.8s-ITS2	<i>ndhF</i>	<i>trnT-trnL</i>
<i>A. thaliana</i> Heynh.	-	GeneBank	X52322	AP000423	AP000423
<i>E. baeticum</i> subsp. <i>baeticum</i>	Ebb08	Spain: Almería, Sierra Nevada	KF445238	KF445274	KF445310
<i>E. baeticum</i> subsp. <i>bastetanum</i>	Ebt01	Spain: Granada, Sierra de Baza	KF849859	KF849843	KF849876
	Ebt04	Spain: Granada, Sierra de Baza	KF445237	KF445273	KF445309
	Ebt06	Spain: Almería, Sierra de María	KF849860	KF849844	KF849877
<i>E. cazorlense</i>	Eca01	Spain: Jaén, Sierra de Cazorla, Segura y las Villas	KF445242	KF445278	KF445314
	Eca02	Spain: Albacete, Sierra de Alcázar	KF849861	KF849845	KF849878
<i>E. fitzii</i>	Ef02	Spain: Jaén, Sierra de la Pandera	KF849862	KF849846	KF849879
	Ef04	Spain: Jaén, Sierra de la Pandera	KF445248	KF445284	KF445320
<i>E. gomezcampoii</i>	Ego02	Spain: Alicante, Font Roja	KF849863	KF849847	KF849880
<i>E. incanum</i>	Ei03	Morocco: Meknès-Tafilalet, Ifrane	KF849864	HM235747	HM235759
<i>E. incanum mairei</i>	Eim04	Spain: Cuenca, Altobuey	KF849865	KF849848	KF849881
<i>E. mediohispanicum</i>	Em18	Spain: Almería, Sierra de Gador	KF849866	KF849849	KF849882
	Em21	Spain: Granada, Sierra Nevada	KF849867	KF849850	KF849883
	Em25	Spain: Granada, Sierra Nevada	KF445254	KF445290	KF445326
	Em27	Spain: Granada, Sierra de Lújar	KF849868	KF849851	KF849884
<i>E. myriophyllum</i>	Emy01	Spain: Granada, Sierra de Huétor	KF849869	KF849852	KF849885
	Emy02	Spain: Granada, Sierra de Baza	KF849870	KF849853	KF849886
<i>E. nevadense</i>	En05	Spain: Almería, Sierra Nevada	KF849871	KF849854	KF849887
	En10	Spain: Granada, Sierra Nevada	KF849872	KF849855	KF849888
<i>E. passgalense</i>	Epa01	Iran: Karaj-Chalus road	KF445262	KF445298	KF445334
<i>E. popovii</i>	Ep04	Spain: Granada, La Peza	KF849873	KF849856	KF849889
	Ep13	Spain: Granada, Sierra de Huetor	KF445261	KF445297	KF445333
<i>E. rondae</i>	Er03	Spain: Cádiz, Sierra de Grazalema	KF445265	KF445301	KF445337
	Er04	Spain: Cádiz, Sierra de Grazalema	KF849874	KF849857	KF849890
<i>M. moricandioides</i>	-	Spain: Almería	KF849875	KF849858	KF849891

The alignments were manually reviewed, and a region of indels and a string of adenines in the *trnT-L* (positions 2880-3300 of the concatenated alignment) were deleted using the GBlocks Server (<http://molevol.cmima.csic.es/castresana/Gblocks.html>; Castresana, 2000) with the less stringent selection. In addition to estimating phylogenetic relationships over the concatenated matrix, we also analysed the nuclear sequences and plastidial sequences independently. For the three datasets we used maximum parsimony with PAUP v4.0 (Swofford, 2002), maximum likelihood (Felsenstein, 1973) with PhyML v2.4.4 (Guindon & Gascuel, 2003) and Bayesian Markov chain Monte Carlo (MCMC) inference (Yang & Rannala, 1997) with MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). The PhyML analysis was performed with default options, assuming a general time reversible (GTR) model. This was the best-fitting evolutionary model implemented in PhyML for the three concatenated regions as estimated by the program ModelTest v3.7, using the Akaike information criterion (Posada & Crandall, 1998). Base frequencies, the proportion of invariable sites, substitution rates and the alpha parameter of the gamma distribution were estimated by PhyML. Branch support was estimated by both the approximate Likelihood Ratio Test (SH-like supports option) and by bootstrap (Felsenstein,

1985) with 1000 replicates. For Bayesian analysis, we used MrBayes on the online Bioportal of the University of Oslo (<http://www.bioportal.uio.no/>), partitioning the data into three regions, one for each locus used, and we estimated the best-fitting evolutionary model for each region using MrModelTest v2.3 (Nylander, 2004). The best-fitting evolutionary model obtained for nDNA region was GTR+ $\Gamma$ , and for *ndhF* and *trnT-L* it was GTR+I and GTR+ $\Gamma$ , respectively (Table 3). The analysis lasted for 4 million MCMC generations, with a sample frequency of every 100 generations, and we removed the first 25% of trees as burn-in, after checking trace files with the program Tracer v1.4 (Rambaut & Drummond, 2007) to determine when the stationary phase was reached and the convergence of the two independent Bayesian MCMC runs. The consensus trees were visualized, edited, and exported using the program MEGA v4.0.2 (Tamura & al., 2007), which was also used for the characterization of the sequences (Table 3).

## RESULTS

In the phylogenetic analyses of the three data sets out-group taxa, both external and congeneric, were maintained separate to the Baetic *Erysimum* species with the exception of

**Table 3.** Characterization of DNA regions included in the present study. Number of variable sites [(variable sites/total sites)\*100]; number of parsimony informative sites [(variable sites–singletons)/total sites]\*100; number of singleton sites [(singleton sites/variable sites)\*100]. Percentage of the different nucleotides in the sequences are also given, as well as the models used per sequence.

	ITS1-5.8S rDNA-ITS2	ndhF	trnT-3'trnL	Combined
Aligned base pairs	708	2004	844	3556
Number of variable sites (%)	20.19	5.73	10.90	9.84
Number of parsimony informative sites (%)	6.07	1.04	1.54	2.16
Number of singleton sites (%)	14.12	4.69	9.36	7.68
%A	24.8	29.5	39.0	30.8
%C	26.2	14.6	14.6	16.9
%G	26.3	15.7	16.0	17.9
%T	22.7	40.2	30.4	34.4
Used models	GTR+ $\Gamma$	GTR+I	GTR+ $\Gamma$	

the ITS tree in which the Asian species, *A. passgalense*, is sister to the outcrossing Baetic species and *E. incanum* is basal to all of them (Figs. 2, 3 and 4). In the inclusive analyses of nDNA and cpDNA, *E. gomezcampoi* is sister to the rest of the outcrossing Baetic *Erysimum* species, although this topology did not show a high support. The rest of the Baetic species were distributed in two main clades. Clade A included *E. baeticum* (both subspecies), *E. popovii*, *E. mediobispanicum* and *E. nevadense* with significant support, whereas Clade B included *E. fitzii*, *E. cazorlense*, *E. myriophyllum* and *E. rondae* (Fig. 2). Part of the populations belonging to the same species appeared as monophyletic, but some of the samples of *E. cazorlense*, *E. myriophyllum*, *E. baeticum* and *E. mediobispanicum* did not.

Despite the result of the test for congruence, the phylogenetic relationships shown by the nuclear region and plastidial regions were at variance. Comparing the nDNA tree and the combined tree, the composition of clade A and B holds but *E. gomezcampoi* is sister to Clade A in the nDNA tree instead of sister to the clade comprising A+B (Figs. 2, 3).

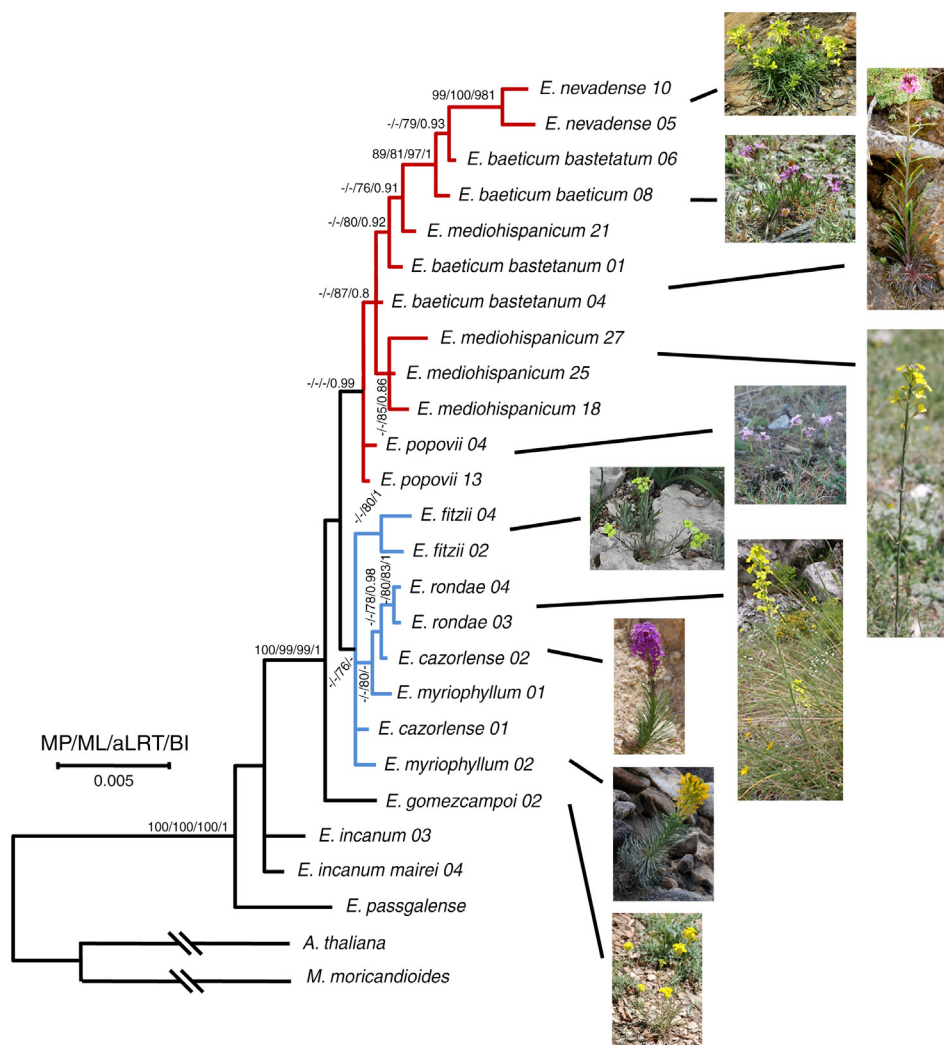
However, the cpDNA tree was less resolved, neither clade A nor B appear and the distribution of taxa is very discordant compared to the nDNA tree. For instance, one sample of *E. popovii* is sister to the rest of the outcrossing Baetic species and one of the samples of *E. fitzii* is sister to *E. nevadense* and *E. baeticum* p.p. (Fig. 4).

## DISCUSSION

Some cautionary words are necessary when discussing the present phylogenetic results, because more Iberian and West-Mediterranean species from outside the Baetic Mountains would need to be included to verify our results. However, there are some tentative conclusions that can be drawn. The result of the test for congruence, which does not find significant discordance, is striking in view of the topologies of the cpDNA, and the nDNA. This may be due to the low resolution and low support of clades appearing in the cpDNA tree. The disparate positions of specific taxa (e.g., *E. fitzii*, *E. popovii* or *E. mediobispanicum*, on the cpDNA tree compared to the other two, suggest the occurrence of introgressive hybridization or incomplete lineage sorting events (Linder & Rieseberg, 2004; Nieto Feliner & Roselló,

2007). The latter cause is more likely in species with recent origin in which reciprocal monophyly for sampled genes has not yet been reached (van Oppen & al. 2001), as many groups in the genus *Erysimum* apparently are (Moazzeni & al. 2014). However, patterns such as the placement of *E. mediobispanicum* in the ITS tree with some of the species with which it co-occurs in sympatry (*E. popovii*, *E. baeticum* and *E. nevadense*), suggest introgressive hybridization events between taxa (Nieto Feliner & Roselló, 2007). In spite of potential distorting events for the recovery of species phylogeny, our ITS data that is somehow reinforced in the combined analysis suggest that there are two main *Erysimum* clades in the Baetic Mountains, and all conspecific samples fall in one clade or the other, not in both. One clade (A) with significant support includes *E. baeticum*, *E. popovii*, *E. nevadense* and *E. mediobispanicum*, whereas the other one (B) includes *E. fitzii*, *E. cazorlense*, *E. myriophyllum* and *E. rondae* although with no support (Fig. 2). Previous phylogenetic studies like Moazzeni & al. (2014) or other works from our own group (Gómez & al. 2014a, 2014b, 2014c) are not conclusive to support or question the existence of those two clades since their representation of taxa from the Iberian Peninsula is scarce. The two populations of *E. incanum* from outside the Baetic Mountains that were included in the analyses fall outside the outcrossing Baetic taxa.

According to our results, the *E. nevadense* group as considered by some authors (e.g. Nieto Feliner, 1993; Blanca & al. 2009) does not have phylogenetic support, as the four (out of six species) that are studied here do not form a monophyletic group (Fig. 2). Thus, *E. nevadense* and *E. mediobispanicum* are located in the first clade, whereas *E. rondae*, is located in the other lineage; and the single sample of the fourth species, *E. gomezcampoi*, does not clear relationships in this study since it is sister to the outcrossing Baetic *Erysimum* in the combined tree but is sister to Clade A in the ITS tree (Figs. 2, 3). Nieto Feliner (1993) indicates that *E. rondae* sometimes displays an intermediate phenotype between the *nevadense* group and *E. myriophyllum*. In keeping with this notion, our analysis suggests a close evolutionary relationship between *E. rondae* and the latter species. In contrast, Blanca & al. (2009) consider *E. rondae* a subspecies of *E. mediobispanicum*, a taxonomical status that is not supported by our phylogenetic analysis.

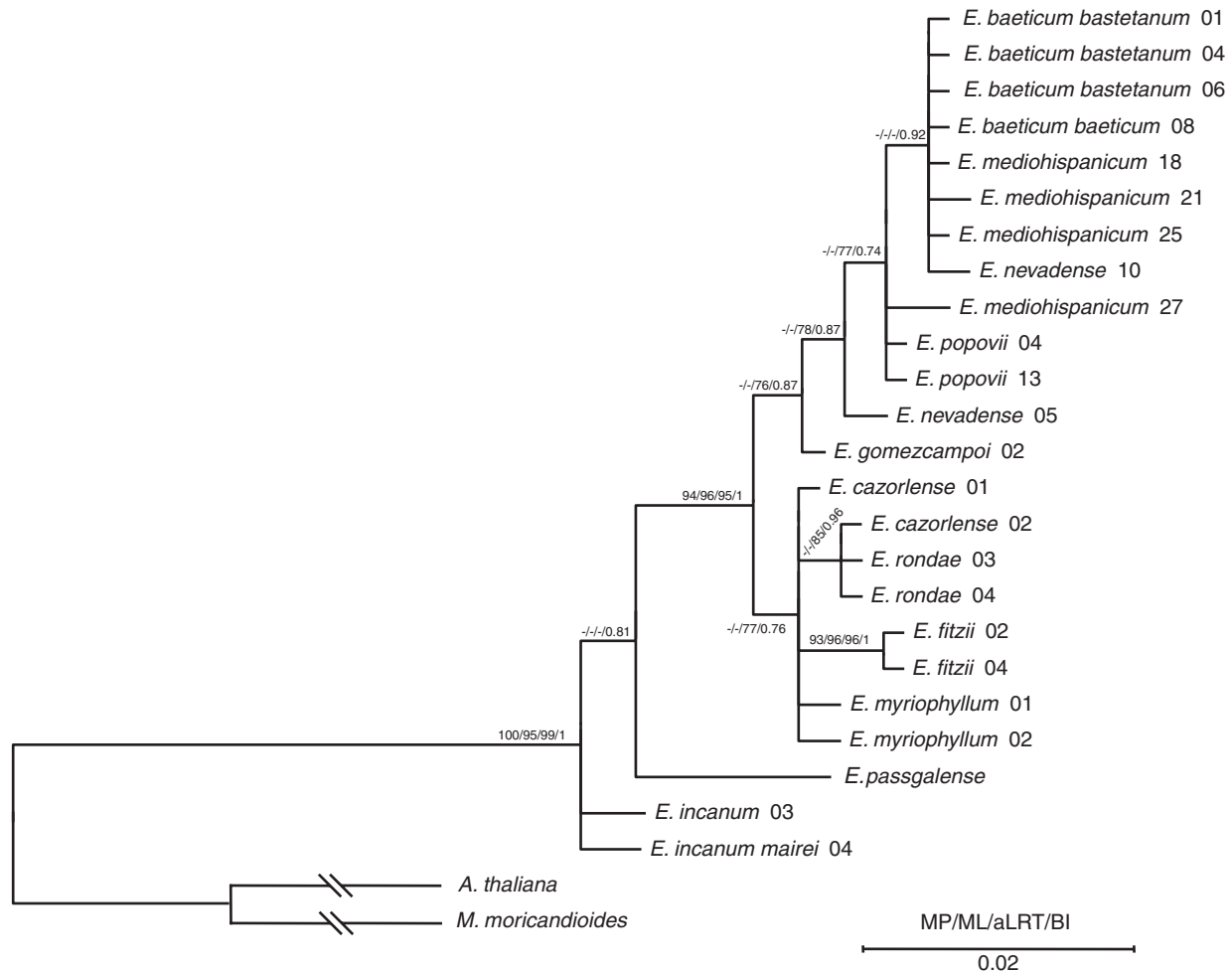


**Fig. 2.** Phylogenetic relationships of *Erysimum* taxa inhabiting the Baetic Mountains using the combined sequences from nuclear (ITS1-5.8S-ITS2) and plastidial (*ndhF* and *trnT-L*) DNA and three different methods. MP: branch support under Maximum Parsimony estimated by bootstrapping. / ML: branch support under Maximum likelihood estimated by bootstrapping. / aLRT: branch support under Maximum likelihood estimated by approximate likelihood ratio test. / BI: branch support values under Bayesian Inference are posterior probabilities. Only branch support values higher than 75% are shown. Clade A marked in red, clade B marked in blue.

The two subspecies of *E. baeticum* (subsp. *baeticum* and subsp. *bastetatum*), do not form a monophyletic group (Fig. 2). It is noteworthy that *E. baeticum* subsp. *bastetatum* from Sierra de María is grouped with the *E. baeticum* subsp. *baeticum* population from Sierra Nevada, even though Sierra de Baza (where the other two *E. baeticum* subsp. *bastetatum* populations were collected) is located between these two mountain ranges. Nieto Feliner (1992a) suggested that *E. baeticum* subsp. *baeticum* could have evolved as a consequence of hybridization between *E. nevadense* and the widespread subspecies *E. baeticum* subsp. *bastetatum*. This author invoked several circumstantial findings to support his hypothesis. First, *E. nevadense* and *E. baeticum* subsp. *baeticum* are phenotypically identical, apart from differing in petal colour (yellow and purple, respectively). Both taxa are short and polycarpic with multiple flowering stems arising directly from the rootstock rather than from the axillary leaflet fascicles (Nieto Feliner, 1992b). *Erysimum nevadense* and *E. baeticum* subsp. *baeticum* also share the

type of substrate where they grow (siliceous), which is different from that where *E. baeticum* subsp. *bastetatum* occurs (Table 1). Moreover, according to Blanca & al. (1992), *E. nevadense* is diploid ( $2n=14$ ), *E. baeticum* subsp. *bastetatum* is tetraploid ( $2n=28$ ), but *E. baeticum* subsp. *baeticum* can be tetraploid or octoploid ( $2n=56$  [ $n=28$ ]). Effectively, our phylogenetic analysis suggests a close relationship between *E. baeticum* subsp. *baeticum* and *E. nevadense*. We cannot rule out the hypothesis that *E. baeticum* subsp. *baeticum* evolved by hybridization, although further studies with additional populations and markers are needed to shed light on these questions.

Most *Erysimum* species have flowers with yellow petals. Specifically, purple-flowered species occur only in the Iberian Peninsula, the Macaronesian Islands, Near East (*E. purpureum* from Israel, Jordania, Lebanon, Syria and Turkey, *E. lilacinum* from Armenia, Azerbaijan and Iran, *E. oleifolium* from Irak, Iran, Jordania and Syria and *E. pseudopurpureum* from Turkey), Central Asia (*E. violascens* from



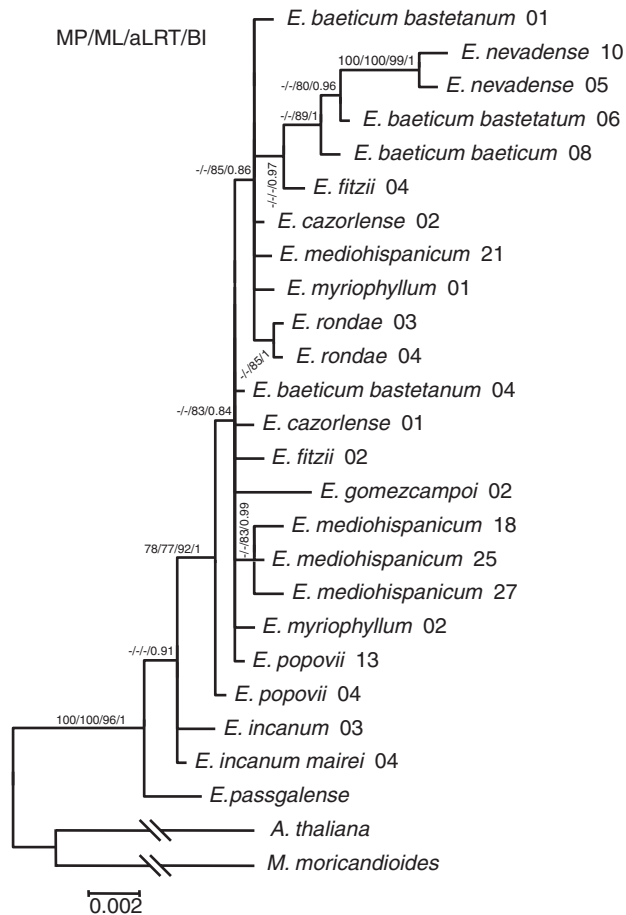
**Fig. 3.** Phylogenetic relationships based only on the nuclear region *ITS1-5.8S-ITS2* for the *Erysimum* species inhabiting the Baetic Mountains. MP: branch support under Maximum Parsimony estimated by bootstrapping. / ML: branch support under Maximum likelihood estimated by bootstrapping. / aLRT: branch support under Maximum likelihood estimated by approximate likelihood ratio test. / BI: branch support values under Bayesian Inference are posterior probabilities. Only branch support values higher than 75% are shown.

Uzbekistan, *E. samarkandicum* from Tajikistan, and *E. cyaneum* from Uzbekistan, Kyrgyzstan and Tajikistan), China (*E. funiculosum* and *E. roseum* from Tibet and *E. limprichtii* from West China) and East Siberia (*E. pallasi*) (Taiyan & al. 2001, Polatschek 2010, 2011). Despite the comparatively small geographical area of the Iberian Peninsula, it contains six purple-flowered taxa, four of them (*E. cazorlense*, *E. baeticum* subsp. *baeticum*, *E. baeticum* subsp. *bastetanum* and *E. popovii*) inhabiting the Baetic Mountains (Nieto Feliner, 1993). They have usually been considered a monophyletic group (Ball, 1990; Nieto Feliner, 1992c) or even a single species (Heywood, 1954). However, our phylogenetic analysis indicates that these species are not monophyletic and instead suggests that purple flowers appeared at least three times (Fig. 2). Since single or few mutations in the genes involved in the pigment biosynthetic pathways have been demonstrated to be responsible in petal colour shifts (Davies & al., 1998; Ono & al., 2006; Dick & al., 2011), these evolutionary transitions in nature may be common. However, such conclusion has two caveats. First, it is intriguing that in the Western Mediterranean region purple-flowered *Erysimum* appear only in the Iberian Peninsula, even though the genus

is frequent in other areas, such as Morocco (5 *Erysimum* species, Abdelaziz & al., 2011), or Italy (17 species and one subspecies, Polatschek, 1982). The scarcity of purple flowers in the region probably was an implicit argument for a single evolutionary event in the Iberian Peninsula. Second, introgression or hybridization might be involved the occurrence of purple flowers in five taxa in the Iberian Peninsula (see above), in which case the number of independent appearances of this flower trait would be reduced. It would be interesting to identify the role of the reticulate evolution together with the ecological factors that might promote recurrent evolution of purple flowers in Iberian *Erysimum* if this was the case.

Most *Erysimum* species inhabiting the Baetic Mountains are allopatric, since their distribution does not overlap at present (Blanca & al., 2009). However, several species co-occur in the same mountains and have some sympatric populations. *Erysimum baeticum* subsp. *bastetanum* and *E. myriophyllum* are sympatric in some localities on the Sierra de Baza. *Erysimum mediohispanicum* is sympatric with several species, namely with *E. popovii* in one locality on Sierra Nevada and in another locality on Sierra de Huétor; with





**Fig. 4.** Phylogenetic relationships based only on the plastidial DNA (*ndhF* and *trnT-L*) for the *Erysimum* species inhabiting the Baetic Mountains. MP: branch support under Maximum Parsimony estimated by bootstrapping. / ML: branch support under Maximum likelihood estimated by bootstrapping. / aLRT: branch support under Maximum likelihood estimated by approximate likelihood ratio test. / BI: branch support values under Bayesian Inference are posterior probabilities. Only branch support values higher than 75% are shown.

*E. baeticum* subsp. *bastetanum* in one locality of Sierra Jureña; with *E. myriophyllum* in one locality of Sierra de Cazorla and with *E. nevadense* in a narrow part of the Sierra Nevada. We can hypothesize that the sympatric *Erysimum* species in the Baetic Mountains evolved allopatrically, and probably made secondary contact as a result of the expansion of the geographic range of one or both members of the sympatric pair. The consequences of these hypothetical secondary contacts may have influenced the evolutionary history of the genus in the region but certainly to substantiate this statement each case has to be the subject of a detailed study.

With the caution imposed by the fact that we have not included in this study other non-Baetic related species, implying that some clades could not represent the complete evolutionary history of the genus in these mountains, we identify three relevant outcomes from the present work. First, with the exception of *E. incanum*, the *Erysimum* species inhabiting the Baetic Mountains are divided in at least two main clades. Second, there is no phylogenetic support for the *E. nevadense* taxonomic group, since some of the species included in this group are more related to species not included in it. Third, changes in flower colour in the

evolution of Baetic *Erysimum* species appear to have been recurrent and/or reticulate events may have significantly influenced this trait.

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