1   **Style polymorphism in *Linum* (Linaceae): a case of Mediterranean parallel**
2   **evolution?**

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13   Running title: convergence of heterostyly in *Linum*

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Abstract

29 • Heterostyly is a sex polymorphism which has challenged evolutionary biologists
30 ever since Darwin. One of the lineages where heterostyly, and related stylar
31 conditions, appears more frequently is the family Linaceae and its most diverse
32 and widespread genus, *Linum*. Thus, this group is particularly suitable for testing
33 competing hypotheses about ancestral and transitional stages on the
34 evolutionary building up of heterostyly.

35 • We generated a well-resolved phylogeny of *Linum* based on extensive sampling
36 and plastid and nuclear DNA sequences, and used it to trace the evolution of
37 character states of style polymorphism and its association with traits related to
38 pollination and breeding systems, obtained from our samples and the literature.

39 • Our results supported former phylogenetic hypotheses: the paraphyly of *Linum*
40 and the non-monophyly of current taxonomic sections. Heterostyly was common
41 in the genus, but appeared concentrated in the Mediterranean basin and, to a
42 lesser extent, in the South African Cape. Ancestral character state reconstruction
43 failed to determine a unique state as the most probable condition for style
44 polymorphism in the genus. In contrast, approach herkogamy was resolved as
45 ancestral state in some clades, in agreement with recent hypotheses on the
46 evolution of heterostyly. Some traits putatively related with heterostyly, such as
47 life-history and polyploidy, did show marginal or non significant phylogenetic
48 correlation respectively. Although pollinator data are limited, the available
49 evidence suggests that beeflies are associated with specific cases of heterostyly.

50 • The consistent association between style polymorphism and heteromorphic
51 incompatibility points out to ecological factors as drivers of the multiple
52 evolution of style-polymorphism in *Linum*. Albeit based on limited evidence, we
53 hypothesized that specialized pollinators and lack of mating opportunities drive
54 evolution of style polymorphism and loss of the polymorphism, respectively.

55

Introduction

57

The great variation of flowers across lineages has inspired modern plant classification since Linneaus (1735), as well as the formulation of hypotheses about the causes of extreme angiosperm diversification, otherwise known as the Darwin's abominable mystery (Grant & Grant 1965; Stebbins 1970, 1974; see Friedman 2009 and references therein for an historical account of Darwin's views). This floral variation also occurs within species and populations, can be continuous or discontinuous, and often appears associated with geographical variation, which has been important to bring insights on the biotic and abiotic causes of such variation (Herrera *et al*., 2006; Strauss & Whittall 2006, Gómez *et al*. 2009). Discontinuous variation at the population level, that is, presence of discrete and modal phenotypes, has been interpreted in the context of population divergence through disruptive selection (Ortiz *et al.* 2015). However, discontinuous variation sometimes results from negative frequency dependent selection, as the fitness of one phenotype strongly depends on the abundance of alternative phenotypes. At equilibrium, it is expected to find all phenotypes at the same proportion in the population. Discontinuous variation is better understood when accompanied by gender differentiation. With negative frequency selection, the success of the uncommon gender is larger than the common gender, as mate availability for the latter is lower (McCauley & Taylor 1997; Dufay *et al.* 2009). A similar situation can be achieved without gender differentiation (Pannell *et al.* 2005). Such is the case of reciprocal style polymorphisms, present in some hermaphroditic plants, where floral morphs display styles and stamens in a reciprocal position (Fig. 1), in a way that pollination and mating occurs more often between morphs rather than within morphs, maintaining the frequency of morphs at balance (Barrett 2002).

The most common style polymorphism is heterostyly (Barrett & Shore 2008), for which flowers in populations present two (distyly) or three (tristyly) morphs. This polymorphism called the attention of evolutionists ever since Darwin (1877), and early geneticists, who soon discovered its apparently simple genetic basis (Bateson & Gregory 1905). Yet, in those early times, it was recognized that most heterostylous species showed the so-called heteromorphic incompatibility system (only crosses between different morphs are compatible, whereas self-fertilization and within-morph cross-

88 fertilization is impeded, Darwin 1877, Dulberger 1992). During most of 20th century,

89 heterostyly was used as model system to study the evolution of inbreeding avoidance.

90 Specifically, most of the studies interpreted the evolutionary pathways of heterostyly

91 following the proposals of Mather & de Winton (1941), with important modifications by

92 Baker (1966), ultimately leading to the quantitative model of Charlesworth &

93 Charlesworth (1979). In short, these models predict that reciprocal style polymorphism

94 evolved after the appearance of the incompatibility system, with an ancestral state of

95 non-herkogamous (homostylous) flowers showing high selfing rates and inbreeding

96 depression. These models were challenged by that of Lloyd & Webb (1992 a, b), who

97 suggested that the main driving force for the establishment of the polymorphism was

98 the promotion of compatible cross pollination and the decrease pollen discount

99 (enhanced male fitness, as Darwin himself proposed in 1877). The latter model

100 presumed (i) an independent evolution of sex organ reciprocity and an heteromorphic

101 incompatibility system, and (ii) an ancestral condition of an outcrosser with approach

102 herkogamous flowers (i.e., with the stigma protruding the anthers). This model strongly

103 emphasized the ecological context of pollination: specialized pollinators select for and

104 maintain the style morphs if they are able to place pollen grains on different parts of the

105 body, and legitimately deliver pollen to the opposite stigmas, with minimal pollen loss.

106   The model of Lloyd & Webb (1992 a, b) has progressively gained more support

107 from both micro- and macroevolutionary studies. Microevolutionary analyses have

108 mostly examined the relative rates of pollination and mating between and within

109 morphs in populations (Lau & Bosque 2003, reviewed in Costa 2017). In contrast,

110 macroevolutionary models to study how the heterostylous floral syndrome evolved

111 have been relatively scarce compared to population level studies. To this respect,

112 macroevolutionary studies in some plant groups, such as *Narcissus*, *Lithodora* and

113 related genera, Pontederiaceae, *Exochaenium*, *Amsinckia*, or *Primula* (Kohn *et al.* 1996;

114 Schoen *et al.* 1997; Guggisberg *et al.* 2006; Pérez-Barrales *et al.* 2006; Ferrero *et al.*

115 2009; Kissling & Barrett 2013; Santos-Gally *et al.* 2013) have provided strong support to

116 Lloyd & Webb's (1992 a, b) ideas. Given that heterostyly is well represented both among

117 lineages of Angiosperms (28 families across many orders in both monocots and dicots;

118 Barrett & Shore 2008) and biomes, these studies offer good opportunities to explore the

119   ecological and biogeographical correlates of heterostyly in order to infer the conditions

120   that favour this polymorphism to arise and be maintained. For example, heterostyly

121   should be common in plants with specialised pollination, or should be disadvantageous

122   where outcrossing is at risk, as expected when pollinators are scarce, or in highly

123   disturbed environments (Piper *et al.* 1986). Likewise, it would be unlikely to find

124   heterostyly associated with hybridization and polyploidy (both associated with self-

125   fertilization as by-product, Ramsey & Schemske 1998), or with short-lived plants,

126   particularly in annuals, as these typically present higher selfing rates and occur more

127   frequently in disturbed places compared to perennial plants (Barrett 2002).

128   Heterostyly in Linaceae was first reported in the seminal works of Darwin (1864,

129   1877) and Hildebrand (1864). In particular, Darwin's experimental and observational

130   work on *Linum grandiflorum* and *L. perenne* was influential in determining the function

131   of the polymorphism. Later, it was suggested that other genera in the family could

132   include distylous and tristylous species (Lloyd *et al.* 1990; Thompson *et al.* 1996). After

133   Darwin´s work, geneticists used species of *Linum* to study the inheritance of heterostyly,

134   and showed that style polymorphism and heteromorphic incompatibility appear linked

135   (Lewis 1943; Dulberger 1992; Lewis & Jones 1992; Ushijima *et al.* 2012). Furthermore,

136   the stability of heterostyly as a trait has been valuable for taxonomists, who used it as a

137   binary character ("heterostylous" vs "homostylous") in identification keys and

138   diagnoses (e.g., Ockendon & Walters 1968; Ockendon 1971; Martínez-Labarga & Muñoz-

139   Garmendia 2015; Ruiz-Martín *et al.* 2015). Thus, taxonomic descriptions have been

140   valuable to characterize species and conduct evolutionary reconstructions of the trait

141   (McDill *et al.* 2009). However, *Linum* is a highly diverse genus with a wide geographic

142   distribution, in which the diversity of stylar conditions is much greater than previously

143   reported (Ruiz-Martín, unpublished data; Darwin 1877; Heitz 1980; Armbruster *et al.*

144   2006). Most of the taxonomic diversity appears in the Mediterranean and, surprisingly,

145   the morphological variation on the types of polymorphism and other associated traits

146   remains to be explored. Thus, *Linum* represents an excellent study system for testing

147   macroevolutionary hypotheses and correlates with heterostyly.

148   The specific aims of our study were: (1) to generate an updated phylogeny of

149   *Linum*, including lineages and infrageneric taxa recognized in taxonomic studies, (2) to

150   estimate divergence times in order to date events of evolutionary significance for the

151   polymorphism, (3) to reconstruct ancestral states for stylar condition and other related

152   traits, (4) to estimate the significance of correlated evolution between style

153   polymorphism and those other traits across the phylogeny, and (5) to integrate all these

154   results in a geographical and ecological context , in order to infer the conditions under

155   which heterostyly most likely evolved. Ultimately, we wished to validate current

156   evolutionary models of heterostyly.

157

158   **Material and methods**

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160   **Floral measurements and categorization**

161   Previous work reported that style polymorphism in *Linum* concentrates mostly in the

162   Mediterranean basin and South Africa (McDill *et al.* 2009). Thus, we concentrated our

163   field sampling efforts in these regions (although other regions were also explored), and

164   also extracted information from published sources. We collected up to 100 flowers from

165   50 populations from 50 taxa of *Linum* (Table S1), and preserved flowers in 70% ethanol

166   for morphological measurement in the laboratory. *Linum* flowers have five styles and

167   five stamens, reaching each of five similar heights (we conducted a pilot study to assess

168   within flower variation in the position of anthers and stigmas, and found that variation

169   within flower was nearly negligible, results not shown). Anther and stigma heights were

170   measured as the distance from base of the ovary to the top of the organ. All

171   measurements were taken from digital images of the lateral view of flowers with petals

172   removed, using ImageJ (Rasband 2008). Images were previously taken using a

173   stereomicroscope (Zeiss Stemi-2000) with attached digital camera (Zeiss Axiocam).

174   Data for the remaining *Linum* species and outgroups were collected from the literature

175   (see Table S1 in Suppl. Material).

176   We classified flowers of style polymorphic species as L-morph when the stigmas

177   were positioned above the anther whorl, and S-morph when the stigmas were below the

178   anther whorl. Style polymorphism includes two morphs (distyly and stigma height

179   dimorphism) or three morphs (tristyly and stigma height trimorphism); and here we

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180   refer to stigma height polymorphism as the discrete variation in stigma height but not in

181   anther height, a condition related with heterostyly (Barrett *et al.* 2000). Species with

182   populations with only one floral morph were named monomorphic and classified as

183   follows: homostylous (no apparent separation between sexual organs), approach or

184   reverse herkogamous (stigmas placed above or below the anther whorl respectively),

185   and horizontal herkogamous (anther-stigma separation along the horizontal plane of the

186   flower). This classification was based on extensive flower measurements and the

187   frequency distribution of sex organ heights among population (Ruiz-Martín,

188   unpublished data). It is important to highlight that most of taxonomic references classify

189   style polymorphism as heterostylous (sometimes discriminating distyly from tristyly) or

190   homostylous; the latter referring to any style monomorphic condition, regardless the

191   relative position of anthers and stigmas (see description above). This distinction is

192   critical for testing models of evolution of heterostyly in relation to the ancestral stylar

193   condition (true non-herkogamous homostyly in Charlesworth & Charlesworth 1979 vs.

194   approach herkogamy in Lloyd & Webb 1992a). Hence, the species that could not be

195   sampled in the field were we characterised using the quantitative information provided

196   in taxonomic descriptions (e.g. approach or reverse herkogamous when no overlap was

197   reported between stamen and style length, otherwise homostylous).

198   We included other biological traits of species putatively related with style

199   polymorphism, and gathered information from the literature on life-history,

200   chromosome number, breeding system, pollinators, ancillary traits (polymorphism in

201   size and form of pollen grains and/or stigma papillae) and genetic control of

202   polymorphism (see Table S1 Suppl. Material, for references).

203   Given the lack of a comprehensive monograph for species identification on

204   *Linum*, we followed the most recent and comprehensive taxonomic treatment for

205   regions with high species diversity in the genus: Yusepchuk (1949), Davis (1967),

206   Ockendon & Walters (1968), Rogers (1981), Greuter *et al.* (1984), Yilmaz & Kaynak

207   (2008) and McDill *et al.* (2009).

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209   **Phylogeny and divergence times**

210   **Sampling.** 103 samples from 93 species or subspecies of *Linum* were included as

211   ingroup, representing the five taxonomic sections. Two or three samples from different

212   localities were included for nine *Linum* species with taxonomical doubts to test for

213   monophyly. In addition, samples from eight species representing closely related genera

214   (*Anisadenia*, *Cliococca*, *Hesperolinon*, *Hugonia, Radiola*, *Reinwardtia*, *Sclerolinon* and

215   *Tirpitzia*, McDill *et al.* 2009) were included to evaluate if *Linum* is a monophyletic genus.

216   Three species from closely related families (*Hypericum perforatum* from Hypericaceae,

217   *Viola pubescens* from Violaceae, and *Humiria balsamifera* from Humiriaceae) were also

218   included as outgroup (Table S1).

219   Fifty-five leave samples from 48 species or subespecies of *Linum* were collected

220   in field trips (vouchers stored at SEV herbarium; Table S1), whereas leaves from

221   additional 18 taxa were obtained from herbaria collections (SEV, MA and E, Table S1).

222   The DNA sequences from the remaining 29 species of *Linum*, eight of Linaceae and three

223   from other families were directly downloaded from GenBank data base and previously

224   published (see Table S1 for species and references). Two taxa were sampled in the field

225   and obtained from herbaria.

226   **DNA extraction, PCR and sequencing.** Total genomic DNA was extracted using DNEasy

227   Plant Minikit (QIAGEN Inc., BIO Laboratories Inc., Carlsbad, CA, USA). One nuclear DNA

228   region, ITS (internal transcribed spacer), and three plastid DNA regions, NADH

229   dehydrogenase subunit F (*ndhF)* gene*,* maturase K (*mat*K) gene and *trn*L-F spacer were

230   amplified, purified and sequenced. PCR amplification was performed following McDill *et*

231   *al.* (2009), with minor modifications. Products were purified using ExoSAP-IT (USB,

232   Cleveland, Ohio, USA). Sequencing reactions were performed using the ABI BigDye®

233   Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific Inc., Massachusetts,

234   U.S.A.) in Macrogene Europe Laboratory (Amsterdam, The Netherlands).

235   **Phylogenetic analyses.** Sequences from the four DNA regions were aligned separately

236   using MaffT 6.0 FFT-NS-I (Katoh & Toh 2008) as implemented in Geneious Pro™ 5.3

237   (Kearse *et al.* 2012). The resulting alignments were manually revised. Putative

238   homoplasic regions were detected and removed from the alignments using GBlock

239   v0.91b (Castresana 2000). Incongruence between DNA regions was discarded and the

240   four DNA regions were combined in a single matrix (2,900 bp).

241   Bayesian inference analysis was performed using Markov chain Monte Carlo

242   (MCMC) as implemented in MrBayes3.0b4 (Huelsenbeck & Ronquist 2001). The best-

243   fitted model of DNA evolution for each DNA region was selected from the analysis in

244   ModelTest 3.06 (Posada & Crandall 1998). GTR +G +I was selected for *ndh*F and *mat*K

245   regions and GTR +G for trn*L-F* and ITS regions. To avoid overparameterization, we

246   combined the three plastid regions in a matrix and analyzed it together using GTR +G +I

247   model. Two independent analyses of four Metropolis-coupled Markov chains were run

248   for 10 million generations. After a burn-in of 25%, the remaining trees (15,000) were

249   used to construct a majority-rule consensus tree using posterior probability values as a

250   measure of clade support. Phylogenetic analyses were performed using CIPRES Science

251   Gateway V. 3.3 portal (Miller *et al.* 2010).

252   **Analyses of divergence times.** The four DNA regions were combined in a single

253   partition (using GTR+G+I as DNA model of evolution). Analyses were conducted using

254   three independent MCMC runs of 120 million generations each, using Yule process as

255   tree model and relaxed clock log normal as clock model, as implemented in BEAST

256   v1.4.8 (Drummond & Rambaut 2007). Run convergence and burn-in were assessed in

257   Tracer 1.6 (Rambaut & Drummond 2007). Trees from the three independent runs were

258   combined using LogCombiner 1.4.8 (10% of burn-in). Maximum clade credibility trees

259   were calculated with TreeAnnotator 2.3.2 using a posterior probability limit of 0.95,

260   maximum clade credibility tree and the mean heights options.

261   Two calibration points were used: 1) a secondary calibration base on the age of

262   the stem node of Linaceae which is the Malpighiales crown node (Bell et al. 2010).

263   Specifically, a normal distribution with a mean of 93.5 Ma (95% CI 88-97 Ma) was used

264   as recommended for secondary calibrations. And, 2) a log-normal distribution with

265   mean = 0, standard deviation = 1.0 and zero offset = 33.9 for the crown node of genus

266   *Linum* (which includes genera *Cliococca*, *Hesperolinon*, *Radiola* and *Sclerolinon*). This last

267   calibration point accounts for the oldest *Linum* fossil. This is a pollen grain from Ebro

268   River Basin (33.9-37.2 Ma, Late Eocene, Cavagnetto & Anadón 1996). Analyses of times

269   of divergence were performed using CIPRES Science Gateway V. 3.3 portal (Miller *et al.*

270   2010) and the cluster located in Andalusian Scientific Information Technology Center

271   (CICA, Seville, Spain).

272   **Ancestral state reconstruction.** We used maximum likelihood approaches to

273   reconstruct the ancestral states of the stylar polymorphism in *Linum*, implemented in R

274   (R Core Team 2015). We performed the analyses on the BEAST Bayesian phylogenetic

275   tree obtained from ITS and chloroplast DNA regions. This tree was pruned to remove

276   tips when the information of character state was unavailable. Because we included more

277   than one sample for eight species, we also pruned the additional samples for the same

278   species in the case of monophyly. Outgroup species and *Hugonia busseana* (Linaceae)

279   were also pruned. Character ancestral state was estimated for each internal node of the

280   tree using the re-rooting method of Yang *et al.* (1995) provided as a function in the

281   package "phytools" (Revell 2012), where conditional probabilities are calculated for the

282   root node (which is the same as the marginal state reconstruction for that node) and

283   consecutively moves the root to each node in the tree. First, just to compare results with

284   former studies based on a simple binary codification (McDill *et al.* 2009), we

285   reconstructed ancestral states to understand the evolution of monomorphic vs.

286   polymorphic states. The former included any of the states without within-population

287   differentiation in morphs, with or without herkogamy; the latter include any of the style

288   polymorphisms found. Second, we considered for the analysis of ancestral state

289   reconstruction only relevant states to the two competing hypotheses of the evolution of

290   heterostyly (Charlesworth & Charlesworth 1979; Lloyd & Webb 1992a). Thus, we

291   formed five state groups: 1) monomorphic homostyly (ancestral state proposed by

292   Charlesworth & Charlesworth 1979); 2) monomorphic approach herkogamy (ancestral

293   state proposed by Lloyd & Webb 1992a); 3) monomorphic reverse herkogamy, which is

294   the alternative state to monomorphic approach herkogamy; 4) style polymorphism -

295   including conventional distyly, three-dimensional distyly, stigma-height dimorphism

296   and trimorphism; and 5) monomorphic horizontal herkogamy. The latter is not

297   considered in any of the models, but it was found in some species and we were

298   interested in determining its evolutionary pathway. Finally, because the most common

299   ancestor of *Linum* (genus *Tirpitzia)* presents two monomorphic and one heterostylous

300   species (Suksathan & Larsen 2006), we reconstructed ancestral states for *Linum*

301   codifying the genus *Tirpitzia* first as monomorphic and second as heterostylous.

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303   **Phylogenetic correlations.** To test the evolutionary correlations between stylar

304   polymorphism and life history, and stylar polymorphism and polyploidy in *Linum*, we

305   performed Pagel's (1994) binary character correlation test implemented in the package

306   "phytools" (Revell 2012) in R (R Core Team 2015). We performed the analyses on the

307   same tree used for ancestral reconstruction analysis. The tree was pruned to include

308   species for which information on stylar morph (monomorphic vs. polymorphic), life

309   history (perennial vs. annual), and polyploidy (diploid vs. polyploid) was available. The

310   method applies a continuous-time Markov model of trait evolution that calculates the

311   likelihood of discrete trait data under two models of evolution, one in which the traits

312   are allowed to evolve independently of one another on the phylogenetic tree and one in

313   which they evolve in a correlated fashion (dependent model). The independent and

314   dependent models can be compared by means of a likelihood ratio test, calculated as

315   2(log[likelihood (dependent model)] – log[likelihood (independent model)]).

316   Significance of the difference in log likelihoods is based on a χ^2 distribution with 4

317   degrees of freedom (4 parameters are estimated in the independent model and 8 are

318   estimated in the dependent model). The parameters of the model of trait evolution are

319   the values of the transition rates between the four possible character state combinations

320   in a model of correlated evolution.

321

322

323   **Results**

324

325   **Style polymorphism and other traits**. Table S1 includes detailed information on traits

326   from species. From field sampling or from bibliographic sources, we obtained

327   information for 85 *Linum* species or subspecies, and 11 outgroup species. Our data

328   includes 60% of species number (141) of *Linum,* as recorded at The Plant List (2013).

329   Detailed quantitative data of flower measurements are still unpublished, and here we

330   summarize the main results (see Table S1). Within *Linum*, 44 (47.3%) species presented

331   some kind of style polymorphism, 41 (44.1%) were monomorphic, and eight (8.6%)

332   lacked sufficient information to ascertain the stylar condition. Style polymorphic species

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333   were mostly distylous (two morphs), but we identified deviations from typical distyly in

334   some species, which we describe here. Armbruster et al (2006) reported a new type of

335   distyly in the western Mediterranean endemic *L. suffruticosum,* showing high

336   reciprocity, in three dimensions: on the vertical axis of the flower (flowers are from

337   either L- or S-morph), on the radial axes (flowers have either outer stamens and inner

338   styles or vice-versa) and on the longitudinal axis of each sex organ (anthers and stigmas

339   are twisted to inner or outer side of the flower, Fig. 1). Information provided by Darwin

340   (1877) in *L. grandiflorum* indicates that the species displays stigma-height dimorphism,

341   that is, styles are either long or short, but stamens are not perfectly in a reciprocal

342   position to stigmas. In the literature, we also found that Heitz (1980) mentioned some

343   populations of *L. perenne* as having similar stigma-height dimorphism as in *L.*

344   *grandiflorum*. Finally, *L. hirsutum* represents an interesting case resembling

345   trimorphism. In our survey, we observed two anther levels and three style lengths in

346   three populations sampled, but our sample size was limited as to completely ascertain it

347   (Ruiz-Martín, unpublished data). Given the paucity of these unconventional cases of

348   polymorphism, all of them were pooled as style polymorphism for the analysis of

349   ancestral state reconstruction and correlated evolution, and their particular position

350   along the tree is discussed below.

351   Monomorphic species or subspecies of *Linum* were also variable: non-

352   herkogamous homostyly was observed in 16 species, approach herkogamy in 19

353   species, reverse herkogamy in three species and horizontal herkogamy also in three

354   species

355   We found information on breeding system in only 19 species. Twelve species

356   were reported as self-incompatible and seven species as self-compatible; the former

357   were all style polymorphic whereas the later were all monomorphic. All self-

358   incompatible species presented a typical heteromorphic incompatibility system. We

359   found data on ancillary traits (any heteromorphism on pollen size or colour, exine

360   sculpturing, stigma width, stigmatic papillae) for eight taxa, all of them being distylous.

361   With regards life-form, 27% *Linum* species in our sample were annual and 73%

362   perennial (Table S1). We found reports on chromosome numbers in 50 taxa, with 23

363   being style polymorphic and 27 monomorphic. Ten out the former and three out the

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364   latter showed variation in the level of polyploidy (different counts of the whole

365   chromosome set, Table S1). A particularly noteworthy case is that of *L. suffruticosum*,

366   with a polyploid series from diploid to decaploid (Nicholls 1986; Ana Afonso, personal

367   communication).

368   The current information on the pollination biology of *Linum* species is scarce.

369   Beeflies from the genus *Usia* (Bombyliidae) seem important pollinators in some

370   distylous species from the Mediterranean basin. Distylous *L. pubescens* was almost

371   exclusively pollinated by *U. bicolor* in eastern Mediterranean (Johnson & Dafni 1998;

372   Gibbs 2014). Armbruster *et al.* (2006) observed that *L suffruticosum* was also almost

373   exclusively pollinated by several *Usia* beeflies, whereas other flies and bees visited

374   flowers but did not function as effective pollinators. Our own observations in additional

375   populations of *L. suffruticosum* confirmed that *Usia* beeflies are the main pollinators, as

376   well as in the distylous western Mediterranean *L. tenue*, and to a lesser extent *L.*

377   *viscosum,* and *L. narbonense* (unpublished data). In contrast, monomorphic European-

378   Mediterranean *L. tenuifolium* was visited by a wide array of pollinators, including mostly

379   bees and to a lesser extent flies (but not beeflies) of different size (see Fig 1).

380   Monomorphic *L. bienne* was reported to be visited by large *Bombylius* spp. beeflies

381   (Boesi *et al.* 2009), which often hover over flowers to collect nectar, rather than crawl

382   down to the bottom of the flower, as observed in smaller *Usia* (Johnson & Dafni 1998;

383   Armbruster *et al.* 2006)*.* Its close relative, the monomorphic *L. usitatissimum* (cultivated

384   flax), appeared visited mostly by bees (Ssymank *et al.* 2009). Finally, Kearns & Inouye

385   (1994) reported that North American monomorphic *L. lewisii* received visits by 25

386   species of nine families of flies and 19 species of four families of different orders, with

387   very different body size, pollination efficiency, visit rate, and frequency across

388   populations.

389

390   **Phylogenetic reconstruction based on Bayesian inference.** The analyses of the three

391   plastid (*rbc*L, *mat*K and *trn*L-F) and nuclear (ITS) regions recovered congruent

392   topologies under Bayesian criteria (data not shown), thus a consensus tree is shown

393   (Fig. S1). Inferred trees were partially congruent with taxonomical subgeneric

394   classification of *Linum* (sections) as already shown by McDill *et al.* (2009). Whereas the

395   genus *Linum* was paraphyletic, as core *Linum* included the genera *Cliococca*,

396   *Hesperolinon*, *Screrolinom* and *Radiola*, the family Linaceae was monophyletic. The

397   topology recovered by MrBayes (Fig S1) showed two main clades, similar to what was

398   found by McDill *et al.* (2009). The first clade, Clade A, was mainly formed by sects. *Linum*

399   and *Dasylinum*, mainly from Eurasia. Specifically, a species from sect. *Linum*, *L.*

400   *stelleroides* from China, is sister to two main clades, Clade A1, the one formed by most of

401   the species from sect. *Dasylinum* and the second clade, Clade A2, formed by most of the

402   species from sect. *Linum* (including also some species from sect. *Dasylinum*). The second

403   main clade, clade B, was formed by the other genera included in core *Linum* and the

404   remaining sections (*Linopsis, Syllinum* and *Cathartolinum*). Specifically, *Radiola* is sister

405   to two main clades, Clade B1, the one formed by genera *Cliococca, Hesperolinon* and

406   *Scleronlinon* and sect. *Linopsis* from North and South America and South Africa, and the

407   second clade, Clade B2, formed by sects. *Linopsis* (excluding the species from America

408   and South Africa), *Syllinum* and *Cathartolinum*, and with a distribution mainly in Europe,

409   Mediterranean basin, and western Asia*.*

410

411   **Times of diversification.** The topology of the maximum credibility tree inferred from

412   BEAST (Fig. 2) analyses was highly congruent with the majority rule consensus tree

413   inferred from MrBayes. The divergence time for crown node of Linaceae was 61.35

414   (MYA) (95% CI: 44.48 - 84.62) (Fig 2). The crown node of core *Linum* was dated back to

415   35.37 MYA (95% CI: 33.95 - 43.31). The crown node of Clade A was dated back to 30.38

416   (95% CI: 23.65 - 38.59). The crown node of clade A1 was about 10.62 MYA (95% CI: 5.62

417   – 17.42) and the crown node of clade A2 was about 21.89 MYA (95% CI: 15.26 – 28.67).

418   The crown node for clade B was dated back to 19.7 MYA (95% CI: 11.48 - 29.49). Finally,

419   the crown node of clade B1 was about 9.02 MYA (95% CI: 5.58 - 29.49) and the crown

420   node of clade B2 was about 14.67 MYA (95% CI: 8.95 - 22.06).

421

422   **Evolutionary pathways of style polymorphism and phylogenetic correlations.**

423   **Binary reconstruction (monomorphism vs. polymorphism).** There were no

424   significant differences when *Tirpitzia* was coded as polymorphic or monomorphic.

425   Equivocal ancestral state reconstruction of the most common ancestor of *Linum* and

426   core *Linum* (Clade A, Clade A1, Clade A2, Clade B and Clade B2, Fig. 3) precludes

427   inference whether the evolution of heterostyly derived from monomorphic or

428   polymorphic condition. However, within particular clades in the genus it is possible to

429   infer some trends. In Clade A1, there is a transition from polymorphism to

430   monomorphism, although this is not significant (see *L. seljukorum*). Within Clade A2,

431   three clear and significant transitions from polymorphism to monomorphism were

432   inferred (see *L. leonii, L. pallescens* and *L. lewisii*). The transitions from monomorphic to

433   polymorphic state are also inferred in this clade (see *L. grandiflorum* and *L. narbonense*)

434   but they were not significant. The most recent common ancestor of Clade B1 is clearly

435   inferred as monomorphic with two significant transitions to polymorphism (see South

436   African *L. comptonii* and *L. heterostylum*). Within Clade B2, transitions from

437   polymorphism to monomorphism and from monomorphic to polymorphic states were

438   not clear.

439   **Five-state reconstruction**. There were no significant differences when *Tirpitzia* was

440   coded as polymorphic or monomorphic. Again, equivocal ancestral state reconstruction

441   of the most common ancestor of *Linum* precludes sound inference (Fig. 4). The most

442   recent common ancestor of core *Linum*, Clade A, Clade A1, Clade A2, Clade B and Clade

443   B2 is equally likely to have presented homostyly or polymorphic state. Within Clade A,

444   clear and significant transitions from polymorphism to homostyly (see *L. leonii* and *L.*

445   *pallescens*; also see *L. seljukorum* although it was not significant) and from

446   polymorphism to approach herkogamy (see *L. lewisii*) were inferred. Also within Clade

447   A, transitions from homostyly to polymorphic state (see *L. grandiflorum* and *L.*

448   *narbonense*) and, to approach herkogamy (see *L. hologynum*) were inferred, although

449   they were not significant. The most recent common ancestor of Clade B1 is approach

450   herkogamy with four possible transitions inferred: to horizontal herkogamy (see *He.*

451   *micrantum* and *L. tenuifolium*), to polymorphism (see *L. comptonii* and *L. heterostylum*),

452   to reverse herkogamy (see *L. littorale* and *L. prostratum*) and to homostyly (see *S.*

453   *digynum*). Reconstruction of shallower nodes of Clade B2 inferred clear and significant

454   transitions from polymorphic state to reverse herkogamy (see *L. nodiflorum*), to

455   horizontal herkogamy (see *L. tenuifolium*) and to homostyly (see *L. corymbulosum- L.*

456   *trigynum* clade; only marginally significant). Also within Clade B2 a transition from

457   homostyly or from polymorphic state to approach herkogamy was inferred (*see L.*

458   *volkensii*).

459

460   **Trait correlations**. There was marginal support for the correlation between presence

461   of stylar polymorphism and perennial life-history of species. Our results indicated that a

462   dependent model of evolution between life history and stylar polymorphism provided a

463   marginally significant better fit to the data than an independent model (difference

464   between likelihood–ratio = 9.136, *p*=0.057). For the set of 50 species where we were

465   able to obtain data on chromosome number, there was no significant correlation

466   between presence of stylar polymorphism and polyploidy (difference between

467   likelihood-ratio= 3.646, *p*= 0.456).

468

469   **Discussion**

470

471   Linaceae is a family that includes some of the largest morphological diversity of style

472   polymorphisms, with homostyly and different types of herkogamy, stigma-height

473   dimorphism and trimorphism, distyly, and tristyly, and *Linum* seems to display most of

474   this diversity. This allows testing evolutionary models for those traits where specific

475   transitions are predicted, as proposed by Charlesworth & Charlesworth (1979) and

476   Lloyd & Webb (1992a). Particularly, Lloyd & Webb's (1992a) model challenged the

477   formerly prevalent ideas represented by Charlesworth & Charlesworth (1979), and

478   proposed an alternative ancestral condition (approach herkogamy, instead of

479   homostyly) to heterostyly. Interestingly, *Hugonia* within Linaceae was one of the study

480   cases that inspired the new model (Lloyd *et al.* 1990), which was later confirmed as

481   tristylous (Thompson *et al.* 1996; Meeus *et al.* 2011). Although the variation in *Linum*

482   inspired Darwin to interpret the adaptive significance of heterostyly (Darwin 1877), it is

483   surprising that the variation of stylar conditions in the genus has rarely been explored

484   (but see Armbruster *et al.* 2006 and McDill *et al.* 2009). In our study, we wished to

485   validate current evolutionary models, for which we generated an updated phylogeny,

486   incorporated the wide variety of stylar conditions, and explored trait correlates to throw

487   light on the plausibility of the alternative models. As discussed below, our results failed

488   to ascertain clearly the ancestral condition in the genus, which precluded supporting

489   any of the competing models, with the exception perhaps of the South African clade,

490   which supported the Darwinian model of Lloyd & Webb (1992a). The information that

491   we gathered in addition to the stylar condition was limited, and precluded statistical

492   analyses to incorporate the evolutionary significance of breeding systems, pollination

493   biology and biogeography of species for this purpose. However, life-history and

494   polyploidy provided plausible explanations for the presence of style polymorphism. Our

495   main result is that, with the data available, both models could explain parts of the

496   evolution of heterostyly in *Linum*.

497

498   **Phylogeny, divergence times and geographic ranges.** We confirmed taxonomic

499   aspects that deserve further work (e.g., the inclusion of four Linaceae genera resulted in

500   the paraphyly of *Linum,* and the non-monophyly of some sections, see McDill *et al.* 2009

501   and McDill & Simpson 2011). Despite our sampling efforts almost duplicated sampling in

502   previous systematic work (McDill *et al.* 2009) and included a larger proportion of *Linum*

503   species, and that some of the DNA regions used were different, we obtained similar

504   results to those previously reported by McDill *et al.* (2009) and McDill & Simpson

505   (2011), making the phylogeny reported here more plausible and valuable for testing

506   evolutionary hypotheses.

507   In our study we found that, unlike species from other geographic regions, the

508   South African species, which all belong to the sect. *Linopsis,* formed a well-supported

509   monophyletic clade. In addition, the South African clade turned to be closely related to

510   the American clades, rather than the Euroasiatic clades from the same section. This

511   result has important implications for evolutionary interpretations because none of the

512   surveyed American *Linum* species present stylar polymorphisms, while species in sect.

513   *Linopsis* in Eurasia do. In our analyses, we were interested to estimate the sequence of

514   divergence dates leading to clades present in the Mediterranean Basin and South Africa,

515   the latter being the only region with style polymorphic *Linum* species outside the

516   Mediterranean basin. Thus, it is remarkable that the South African clade separated from

517   its monomorphic sister American clade in the late Miocene, about 9 MYA. In contrast, its

518   closest Mediterranean clade, which includes members of sect. *Linopsis* and sect. *Syllinum*

519   (with mostly western and eastern Mediterranean species respectively), diverged much

520   earlier (in middle Miocene, more than 14 MYA). Unlike the American clade,

521   Mediterranean clades include many style polymorphic species. By the time the clades

522   split, continents were already separated, particularly Africa and the Americas. Thus,

523   episodes of long distance dispersal should be invoked or, alternatively, massive

524   extinctions of connecting clades in Africa, which would not have left a living or fossil

525   trace. These episodes are coincident with last Antarctic glaciation and sharp decrease in

526   temperature in southern Africa (Linder 2005). Regardless the specific events, it is

527   remarkable that the American clades did not include any style polymorphic lineage. A

528   proper biogeographical analysis incorporating explicit palaeogeographic settings would

529   be necessary to ascertain the most likely scenario.

530

531   **Evolution of style polymorphism in *Linum* (models test)**. Previous work in *Linum*

532   (McDill *et al.* 2009) provided a plausible reconstruction of pathways of heterostyly and

533   "homostyly" (including all types of monomorphic conditions). Despite differences in

534   sampling and molecular markers, our findings were similar to those previously reported

535   (Fig. 3). Specifically, we were unable to determine the most likely ancestral stylar

536   condition in the genus, which could be either style polymorphic and monomorphic (our

537   terms). The variability of stylar conditions in Linaceae and in *Linum* (Ganders 1979;

538   Lloyd *et al.* 1990; Thompson *et al.* 1996; Suksathan & Larsen 2006; McDill & Simpson

539   2011) combined with the inferred high transition rates among character states, and

540   long-branches arising from the root of the phylogeny may explain this lack of resolution.

541   An analysis at the family level would probably throw more light and allow better

542   resolution of the ancestral condition. Despite lack of resolution at the basal stage, we

543   detected several events of independent evolution of the polymorphism along the

544   evolutionary history of *Linum*. Although some clades are integrated by mostly

545   monomorphic or polymorphic species, any of these conditions appears secondarily lost,

546   even in pairs of sister species. For example, loss of polymorphism was detected in *L.*

547   *seljukorum-L. pubescens*, *L. leoni-L. punctatum*, *L. lewisii-L pallescens*, *L. tenuifolum-L.*

548   *suffruticossm*, *L. corymbulosum-L. trigynum*. In addition, polymorphic species evolved in

549   most of monomorphic clades, as shown by the species pairs *L. grandiflorum-L.*

550   *decumbens, L. comptoni-L. pungens; L. heterostylum-L. esterhuysenae*. Particularly

551   dynamic in evolutionary grounds was clade B2 (Fig. 3), especially most of the Western

552   Mediterranean subclade, including species from *L. virgatum to L. setaceum*. This clade

553   includes *L. suffruticosum* s.l., (López-González 1979; Martínez-Labarga & Muñoz-

554   Garmendia 2015) with a special case of three-dimensional reciprocity (Armbruster *et al.*

555   2006), *L. tenue*, a polyphyletic species with substantial morphological variation in NW

556   Africa (J. Arroyo and J. Ruiz-Martín, pers. observ.), as well as a recently named new

557   distylous species, *L. flos-carmini* (Ruiz-Martín *et al.* 2015), different from its sister

558   species, the homostylous *L. setaceum*. All this variation clearly reflects that further work

559   is required in these taxa and geographic range.

560   Perhaps one of the most remarkable outcomes is the independent evolution of

561   heterostyly in two South African species within a clade integrated by 14 species. In his

562   taxonomic review, Rogers (1981) suggested that heterostyly appeared in South Africa

563   independently from its occurrence in the Mediterranean basin and nearby regions,

564   which was later supported by McDill *et al.* (2009), and here we confirmed. Although

565   limited, our population sampling allowed us to confirm the presence of distyly in *L.*

566   *comptonii* and *L. heterostylum*. Because the South African *Linum* clade is monophyletic

567   and closely related to the monomorphic clade of American *Linum* species, the

568   independent evolution of the polymorphism is thus fully supported. Unlike American

569   species, all South African *Linum* species, except *L. thurnbergi*, are restricted to

570   Mediterranean type climate of the Cape Floristic Region (Rogers 1981). Thus, the

571   presence of style polymorphism restricted to Mediterranean climates (the Cape and the

572   Mediterranean basin) points out to an apparent case of parallel evolution linked directly

573   or indirectly to climate. In other Mediterranean climate regions of the world the number

574   of *Linum* species is much lower.

575   The characterisation of monomorphism as homostyly and different types of

576   herkogamy (Fig. 4) depicted a complex picture with regards the evolutionary

577   reconstruction of pathways, but allowed us to explicitly test competing hypotheses of

578   ancestral stylar state. Whereas the ancestral state at the genus level was unresolved, the

579   only clade within *Linum* with certainty in the ancestral condition was the South African

580   clade. Here, the Lloyd & Webb (1992a) model was fully supported, with approach

581   herkogamy as ancestral condition. Interestingly, approach herkogamy is widespread in

582   this clade. In contrast, approach herkogamy is uncommon in other clades (e.g. *L.*

583   *hologynum, L. lewisii*, *L.* and *volkensii*) whereas homostyly appears frequently. This

584   homostyly is secondary, derived from a polymorphic condition, and probably associated

585   with shifts towards selfing to increase reproductive assurance (see for instance *L.*

586   *corymbulosum* and *L. trigynum*, or *L. leonii*). Such shifts have been reported in other style

587   polymorphic groups (Schoen *et al.* 1997; Guggisberg *et al.* 2006; Mast *et al.* 2006; Pérez-

588   Barrales *et al.* 2006; Kissling & Barrett 2013; Santos-Gally *et al.* 2013). More detailed

589   information on the breeding system of the species would confirm this hypothesis.

590   Other stylar conditions are scarcer. Reverse herkogamy, a necessary phenotype

591   in an intermediate step for the establishment of style polymorphism in any model, was

592   detected in the Mediterranean *L. nodiflorum* and the two South American sister species

593   *L. littorale* and *L. prostratum*. Surprisingly, reverse herkogamy appeared in these species

594   as derived monomorphic condition. This transition has been reported in *Exochaenium* in

595   the Gentianaceae (Kissling & Barrett 2013), although it remains unclear the mechanisms

596   that favours the selection of monomorphic reverse herkogamy. Horizontal

597   monomorphic herkogamy was detected in two *Linum* species, *L. kingii* and *L.*

598   *tenuifolium*, and in two closely related genera, *Hesperolinum* and *Radiola*, which are

599   placed within *Linum.*   This condition might result from selection to avoid self-

600   pollination, as in the self-compatible *L. tenuifolium* (Nicholls 1986) (see Fig. 1). Finally, it

601   was not possible to include an evolutionary reconstruction of stigma height dimorphism,

602   as it is an unusual condition in *Linum*, only present in *L. grandiflorum* and perhaps *L.*

603   *perenne* (Heitz 1980). This condition has been reported as an intermediate and unstable

604   state towards heterostyly (Lloyd & Webb 1992b, but see Barrett & Harder 2005), which

605   is consistent with its unclear ancestral/derived condition. This evolutionary lability has

606   been reported for stigma-height dimorphism in some Boraginaceae (Ferrero *et al.*

607   2009).

608

20

609   **Correlated evolution and trait associations.** Few studies have attempted to

610   investigate correlations between style polymorphisms and other traits in an explicit

611   phylogenetic context, and these have focused on associations with other floral traits (e.g.

612   corolla size and form: Santos-Gally *et al.* 2013; Kissling & Barrett 2013). In our study, we

613   were interested to investigate the association between style polymorphism and life

614   history (annual vs perennial). This association is expected (Dulberger 1992) because

615   pollination of style polymorphic plants is often specialized (Darwin 1877; Lloyd & Webb

616   1992a; Lau & Bosque 2003), and short-lived plants, especially annuals, are more

617   sensitive to loss of these pollinators or pollinator uncertainty, and shifts to selfing are

618   more likely to occur. Our results showed that style polymorphism occurs more

619   frequently among perennial than annual species, although the association was only

620   marginally significant. However, we only gathered data for a subset of species, and data

621   on breeding systems from more species would be particularly valuable here. Despite the

622   limitations, this result suggests that reproductive assurance is probably important in

623   annual species, and most likely plays a role against maintaining style polymorphism.

624   An important trait associated with breeding system and thus with style

625   polymorphism is polyploidy. The available evidence shows variation in the correlation

626   between heterostyly and polyploidy, ranging from lack of association to heterostyly

627   being frequent among diploids (Naiki 2012). Across families, a phylogenetic account of

628   these studies suggests that this may stand only for Rubiaceae and Primulaceae (Naiki

629   2012). At least for *Primula*, it has been demonstrated that heterostyly is not present

630   among allopolyploid taxa (Guggisberg *et al.* 2006), which has been also suggested for

631   *Turnera* (Shore *et al.* 2006). This is in agreement with the mechanism of breakdown of

632   heterostylous supergenes by recombination linked to hybridization (Lewis & Jones

633   1992). Although hybridization between some *Linum* species has been reported, the

634   species involved displayed similar chromosome numbers (Seetharam 1972; Muravenko

635   *et al*. 2003; Yurkevich *et al.* 2013), which does not promote breakdown of heterostyly.

636   We were unable to detect a significant correlation between polyploidy and heterostyly

637   in our data set of 50 species of *Linum*. It could be possible that our data includes mostly

638   polyploidy series of autopolyploids. This is well illustrated by the closely related *L.*

639   *tenuifolium* and *L. suffruticosum*. *Linum tenuifolium* is monomorphic, self-compatible and

640   diploid across its wide range in Europe and western Asia (Nicholls 1986). In contrast, *L.*

641   *suffruticosum,* with three-dimensional reciprocity (Fig. 1, Armbruster *et al.* 2006),

642   displays a polyploid series from diploidy to decaploidy (Nicholls 1986; Ana Afonso,

643   unpublished data) across its western Mediterranean range whilst maintaining the style

644   polymorphism and heteromorphic incompatibility (Ruiz-Martín, unpublished). Despite

645   the information on incompatibility systems in *Linum* is limited to only few species, all

646   self-incompatible species display heteromorphic incompatibility, whereas self-

647   compatible species are monomorphic, with no intermediate cases being reported. Thus,

648   the independent evolution of presence and type of self-incompatibility and style

649   polymorphism proposed by Lloyd & Webb (1992a) is not supported. Interestingly, in

650   eight style-polymorphic ancillary traits (dimorphism on pollen grains and stigmas)

651   seemed to be linked to specific floral morphs, reinforcing the cohesiveness of the

652   heterostylous syndrome in *Linum*.

653

654   **A possible role of pollinators in the evolution of style plymorphisms in *Linum*?** One

655   of the most insightful predictions made by Lloyd & Webb (1992a) stated that pollinators

656   are critical for the selection of style polymorphisms. Pollinators need to fit tightly with

657   flowers and contact anthers and stigmas in specific body parts to legitimately transfer

658   pollen between morphs. This involves precise shape of flowers and behaviour of

659   pollinators. At present, the scarcity of pollinator data on *Linum* precludes explicitly

660   testing this hypothesis across the genus. However, studies on the pollination ecology of

661   some species offer interesting insights. Specifically, flower morphology in *Linum* is

662   relatively consistent in shape across species (funnel-like corolla of limited variation in

663   tube width and length, Fig. 1), thus pollinator behaviour becomes crucial. This has been

664   studied in *L. pubescens* (eastern Mediterranean range, sect. *Dasylinum*, clade A1 in Fig. 4;

665   Johnson & Dafni 1998) and *L. suffruticosum* (western Mediterranean, sect. *Linopsis*, B2 in

666   Fig. 4; Armbruster *et al.* 2006), both almost exclusively pollinated by *Usia* beeflies

667   (Bombyliidae), with *U. bicolor* in *L. pubescens* and two species of different size in *L.*

668   *suffruticosum*. In these two *Linum* species, the behaviour of *Usia* was similar and typical

669   of these beeflies (Orueta 2002): they land on flowers and crawl to the bottom of the

670   flower tube searching for nectar. Armbruster *et al.* (2006) described that the three

671   dimensional reciprocity in *L. suffruticosum* allows separation of the placement of pollen

672   from L and S flowers on the ventral and dorsal parts of the *Usia* body respectively. Those

673   authors interpreted that the combination of the *Usia* behaviour with the three

674   dimensional reciprocity probably increased legitimate pollinations between style-

675   morphs (Fig. 1). *Usia* species seem to commonly visit other Mediterranean distylous

676   *Linum* species (Du Merle & Mazet 1978; and personal observations). Interestingly, *Usia*

677   is a truly Mediterranean genus, with its highest species diversity in southern Iberian

678   Peninsula, northwestern Africa, and Anatolia (Gibbs 2011; 2014), also with the highest

679   diversity in *Linum* species. Whether heterostyly in *Linum* is restricted in the Northern

680   Hemisphere to the Mediterranean basin due to its tight association with *Usia* flies, is a

681   challenging hypothesis that deserves further insight.

682   The examples of specialized pollination by *Usia* provide some support to the

683   Darwinian model of Lloyd & Webb (1992a), particularly in *L. suffruticosum*. This species

684   possess a heteromorphic incompatibility system, which prevents all illegitimate crosses

685   bewteen- and within morphs (Nicholls 1986; Ruiz-Martín, unpublished data). Why then

686   has the sophisticated three-dimensional reciprocal distyly, including reciprocal torsion

687   of stamens and styles, evolved apart from increasing efficiency of between-morph

688   pollination and thus avoiding pollen discounting? Torsion of sex organs was first

689   observed by Darwin in *L. grandiflorum* (Darwin 1877), and latter reported in the

690   monomorphic *L. usitatissimum* (Schewe *et al.* 2011). Unfortunately, we lack information

691   on the pollination ecology of heterostylous *Linum* species in the Cape Floristic Region

692   (CFR) of South Africa , which prevents us to make strong inferences about the causes of

693   the independent evolution of heterostyly there. Although *Usia* is not present in the CFR,

694   fly pollination in South Africa is common (Johnson 2010), and it would not be surprising

695   that other Bombyliidae or other fly families behave similarly to *Usia.* Interestingly, the

696   recent description of three-dimensional reciprocity in a group of tristylous CFR *Oxalis*

697   species (Oxalidaceae) (Turketti *et al.* 2012), with similar arrangement of stamens and

698   styles to that described in *L. suffruticosum* and similar flower morphology (i.e. funnel-

699   like corollas) confirms the suggestion of Armbruster *et al.* (2006) that perhaps this kind

700   of polymorphism is not so unusual, and closer examinations of sexual whorl

701 arrangement and pollinator fit can help identifying new examples, providing additional

702 support to the Darwinian view on the function and evolution of heterostyly.

703

704 **Conclusions**

705 *Linum* is a good model system for studying the evolution of heterostyly, both at macro

706 and microevolutionary levels. Our data revealed that *Linum* includes a wide range of

707 morphological variation related to the heterostylous floral syndrome. In contrast,

708 genetic systems linked to heterostyly seems to be rather invariant, for which it could be

709 assumed, as working hypothesis, that pollinators have moulded current floral

710 morphological variation on sex organs. Phylogenetic relationships have been reasonably

711 well resolved, allowing testing specific hypotheses about the evolutionary pathway that

712 allow the acquisition of the style polymorphism. While our analyses precluded inferring

713 the ancestral condition to style polymorphisms in the genus, some of its clades showed

714 that approach herkogamy appears to be the most likely ancestral condition, as Lloyd &

715 Webb (1992a) proposed. Interestingly, species with similar floral trait assemblages in

716 independent clades and in different areas of the Mediterranean basin and South Africa

717 are found. This suggests that ecological adaptations, perhaps mediated by pollinators,

718 rather than phylogenetic conservatism is probably the main driver for the evolution of

719 the stylar polymorphism. Future research to underpin the function of pollinators in the

720 promotion of disassortative pollen transfer in different conditions and regions is

721 necessary to provide further support to the Darwinian pollinator hypothesis for the

722 evolution of heterostyly.

723

724 **Acknowledgements**

739

740

741   **References**

742   Armbruster W.S., Pérez-Barrales R., Arroyo J., Edwards M.E., Vargas P. (2006) Three-

743   dimensional reciprocity of floral morphs in wild flax (*Linum suffruticosum*): a new

744   twist on heterostyly. *New Phytologist* **171**, 581-590.

745   Baker H.G. (1966) The evolution, functioning and breakdown of heteromorphic

746   incompatibility systems. I. The Plumbaginaceae . *Evolution* **20**, 349-368

747   Barrett S.C.H. (2002) The evolution of plant sexual diversity. *Nature Reviews Genetics* **3**,

748   274-284.

749   Barrett S.C.H., Jesson L.K., Baker A.M. (2000) The evolution and function of stylar

750   polymorphisms in flowering plants. *Annals of Botany* **85** (Supplement A): 253-265.

751   Barrett S.C.H., Harder L.D. (2005) The evolution of polymorphic sexual systems in

752   daffodils (*Narcissus*). *New Phytologist* **165**, 45-53.

753   Barrett S.C.H., Shore J.S. (2008). New insights on heterostyly: comparative biology,

754   ecology and genetics. In: V.E. Franklin-Tong (Ed.) *Self-incompatibility in flowering*

755   *plants* (pp. 3-32). Springer, Berlin Heidelberg.

756   Bateson W., Gregory R.P. (1905) On the inheritance of heterostylism in *Primula*.

757   *Proceedings of the Royal Society Series B* **76**, 581-586.

758   Bell C.D., Soltis D.E., Soltis P.S. (2010) The age and diversification of the angiosperms re-

759   revisited. *American Journal of Botany* **97**, 1296-1303

760   Boesi R., Polidori C., Andrietti F. (2009) Searching for the right target: oviposition and
761   feeding behavior in *Bombylius* bee flies (Diptera: Bombyliidae). *Zoological Studies* **48**,
762   141-150.

763   Castresana J. (2000) Selection of conserved blocks from multiple alignments for their
764   use in phylogenetic analysis. *Molecular Biology and Evolution* **17**, 540-552.

765   Cavagnetto C., Anadón P. (1996) Preliminary palynological data on floristic and climatic
766   changes during the Middle Eocene–Early Oligocene of the eastern Ebro Basin,
767   northeast Spain. *Review of Paleobotany and Palynology* **92**, 281– 305.

768   Charlesworth B., Charlesworth D. (1979) The maintenance and breakdown of distyly.
769   *American Naturalist* **114**, 499-513.

770   Costa J. (2017) *The function and adaptive significance of the floral polymorphism
771   heterostyly*. PhD Dissertation, University of Coimbra.

772   Darwin C. (1864) On the existence of two forms, and of their reciprocal sexual relation,
773   in several species of the genus *Linum. Journal of the Linnean Society, Botany* **7**, 69-83.

774   Darwin C. (1877). *The different forms of flowers on plants of the same species*. John
775   Murray, London.

776   Davis P.H. (1967) *Linum* L. In: P.H. Davis (Ed.) *Flora of Turkey and the East Aegean
777   Islands.*: Edinburgh University Press, Edinburgh, 2: 425-450

778   Drummond A.J., Rambaut A. (2007) BEAST: Bayesian evolutionary analysis by sampling
779   trees. *BMC Evolutionary Biology* **7**, 214

780   Dufay M., Cuguen J., Arnaud J. F., Touzet P. (2009) Sex ratio variation among
781   gynodioecious populations of sea beet: can it be explained by negative frequency-
782   dependent selection? *Evolution* **63**, 1483-1497.

783   Dulberger R. (1992) Floral polymorphisms and their functional significance in the
784   heterostylous syndrome. In: Barrett S.C.H. (ed.) *Evolution and function of heterostyly*
785   (pp. 41-84). Springer, Berlin Heidelberg.

786   Du Merle P., Mazet R. (1978) Données complémentaires sur la biologie de deux espècies
787   du genre *Usia* Latreille [Dipt. Bombyliidae]. *Bulletin de la Société Entomologique de
788   France* **83**, 115–122

789   Ferrero V., Arroyo J., Vargas P., Thompson J.D., Navarro L. (2009). Evolutionary

790   transitions of style polymorphisms in *Lithodora* (Boraginaceae). *Perspectives in Plant*

791   *Ecology, Evolution and Systematics* **11**, 111-125.

792   Friedman W.E. (2009) The meaning of Darwin ' s " abominable mystery ". *American*

793   *Journal of Botany* **96**, 5–21.

794   Ganders F.R. (1979) The biology of heterostyly. *New Zealand Journal of Botany* **17**, 607-

795   635.

796   Gibbs D. (2011) A world revision of the bee fly tribe Usiinae (Diptera, Bombyliidae)—

797   Part 1: *Usia* subgenus *Micrusia*, *U. versicolor* (Fabricius)(= black-haired species) and

798   *Usia martini* François. *Zootaxa* **2960**, 1-77.

799   Gibbs D. (2014) A world revision of the bee fly tribe Usiini (Diptera, Bombyliidae) Part 2:

800   *Usia* sensu stricto. *Zootaxa* **3799**, 001-085.

801   Gómez J.M., Perfectti F., Bosch J., Camacho J.P.M. (2009) A geographic selection mosaic in

802   a generalized plant–pollinator–herbivore system. *Ecological Monographs* **79**, 245-

803   263.

804   Grant V., Grant K.A. (1965). *Flower pollination in the Phlox family*. Columbia University

805   Press New York, NY, USA.

806   Greuter W., Burdet H.M., Long G. (Eds) (1984) *Med-Checklist : a Critical Inventory of*

807   *Vascular Plants of the Circum-Mediterranean Countries*. Conservatoire et Jardin

808   Botaniques de la Ville de Genève; Secrétariat Med-checklist, Botanischer Garten &

809   Botanisches Museum Berlin-Dahlem. (accessed at http://ww2.bgbm.org/mcl/home.asp at

810   December 2016).

811   Guggisberg A., Mansion G., Kelso S., Conti E. (2006) Evolution of biogeographic patterns,

812   ploidy levels, and breeding systems in a diploid–polyploid species complex of

813   *Primula*. *New Phytologist* **171**, 617-632.

814   Heitz B. (1980) La pollinisation des Lins heterostyles du groupe *Linum perenne* L.

815   (Linaceae). *Comptes Rendues de l'Académie des Sciences*, Paris **290**, 811-814.

816   Herrera C.M., Castellanos M.C., Medrano M. (2006) Geographical context of floral

817   evolution: towards an improved research programme in floral diversification. In:

818   Harder L.D., Barrett S.C.H. (Eds.) *Ecology and evolution of flowers*. Oxford University,.

819   Oxford, UK, pp. 278–294

820   Hildebrand F. (1864) Experimente uber Dimorphismus von *Linum perenne* und *Primula*

821   *sinensis. Botanische Zeitung* **22**, 1-5.

822   Huelsenbeck J.P., Ronquist F. (2001) MRBAYES: Bayesian inference of phylogenetic

823   trees. *Bioinformatics* **17**, 754-755.

824   Johnson S.D. (2010) The pollination niche and its role in the diversification and

825   maintenance of the southern African flora. *Philosophical Transactions of the Royal*

826   *Society of London B: Biological Sciences* **365**, 499-516.

827   Johnson S.D., Dafni A. (1998) Response of bee-flies to the shape and pattern of model

828   flowers: implications for floral evolution in a Mediterranean herb. *Functional Ecology*

829   **12**, 289-297.

830   Katoh K., Toh H. (2008) Recent developments in the MAFFT multiple sequence

831   alignment program. *Briefings in Bioinformatics* **9**, 286-298.

832   Kearns C.A., Inouye D.W. (1994) Fly pollination of *Linum lewisii* (Linaceae). *American*

833   *Journal of Botany* **81**, 1091-1095.

834   Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper

835   A., Markowitz S., Duran C., Thierer T., Ashton B., Mentjies P., Drummond A. (2012)

836   Geneious Basic: an integrated and extendable desktop software platform for the

837   organization and analysis of sequence data. *Bioinformatics* **28**, 1647-1649.

838   Kissling J., Barrett, S.C.H. (2013) Variation and evolution of herkogamy in *Exochaenium*

839   (Gentianaceae): implications for the evolution of distyly. *Annals of Botany* **112**, 95-

840   102.

841   Kohn J.R., Graham S.W., Morton B., Doyle J.J., Barrett, S.C.H. (1996) Reconstruction of the

842   evolution of reproductive characters in Pontederiaceae using phylogenetic evidence

843   from chloroplast DNA restriction-site variation. *Evolution* **50**, 1454-1469.

844   Lau P., Bosque C. (2003) Pollen flow in the distylous *Palicourea fendleri* (Rubiaceae): an

845   experimental test of the disassortative pollen flow hypothesis. *Oecologia* **135**, 593-

846   600.

847   Lewis D (1943) The physiology of incompatibility in plants . 2. *Linum grandiflorum.*
848   *Annals of Botany* **7**, 115-122.

849   Lewis D., Jones D.A. (1992) The genetics of heterostyly. In: Barrett, S.C.H. (Ed.) *Evolution*
850   *and function of heterostyly*. Springer, Berlin Heidelberg, pp. 129-150.

851   Linder H.P. (2005) Evolution of diversity: the Cape flora. *Trends in Plant Science* **10**, 536-
852   541.

853   Linnaeus C. (1735) *Systema naturæ sistens regna tria naturæ, in classes et ordines, genera*
854   *et species redacta tabulisque æneis illustrata*, editio sexta emendata et aucta
855   Stockholmiæ, impensis Godofr. Kiesewetter publ.

856   Lloyd D.G., Webb C.J. (1992a) The evolution of heterostyly. In: Barrett S.C.H. (Ed.)
857   *Evolution and function of heterostyly*. Springer, Berlin Heidelberg. pp. 151-178.

858   Lloyd D.G., Webb C. J. (1992b) The selection of heterostyly. In: Barrett S.C.H. (Ed.)
859   *Evolution and function of heterostyly*. Springer, Berlin Heidelberg. pp. 179-207.

860   Lloyd D.G., Webb C.J., Dulberger R. (1990) Heterostyly in species of *Narcissus*
861   (Amaryllidaceae) and *Hugonia* (Linaceae) and other disputed cases. *Plant*
862   *Systematics and Evolution* **172**, 215-227.

863   López González G. (1979) Algunas consideraciones sobre los linos del grupo *Linum*
864   *tenuifolium* L. en España. *Mémoires de la Société Botanique de Genève* **1**, 99-109.

865   Martínez-Labarga J.M., Muñoz-Garmendia (2015). In: Muñoz F., Navarro C., Quintanar
866   A., Buira, A. (Eds.) *Linum. Flora Iberica*, vol. IX. CSIC, Madrid.

867   Mast A.R., Kelso S., Conti E. (2006) Are any primroses (*Primula*) primitively
868   monomorphic? *New Phytologist* **171**, 605-616.

869   Mather K., de Winton D. (1941) Adaptation and counter-adaptation of the breeding
870   system in *Primula . Annals of Botany* **5**, 297-311.

871   McCauley D.E., Taylor D.R. (1997) Local population structure and sex ratio: evolution in
872   gynodioecious plants. *The American Naturalist* **150**, 406-419.

873   McDill J., Repplinger M., Simpson B.B., Kadereit J.W. (2009) The phylogeny of *Linum* and
874   Linaceae subfamily Linoideae, with implications for their systematics, biogeography,

875   and evolution of heterostyly. *Systematic Botany* **34**, 386-405.

876   McDill J.R., Simpson B.B. (2011) Molecular phylogenetics of Linaceae with complete

877   generic sampling and data from two plastid genes. *Botanical Journal of the Linnean*

878   *Society* **165**, 64-83.

879   Meeus S., Jacquemyn H., Honnay O., Pailler T. (2011). Self-incompatibility and pollen

880   limitation in the rare tristylous endemic *Hugonia serrata* on La Réunion Island. *Plant*

881   *Systematics and Evolution* **292**, 143-151.

882   Miller M., Pfeiffer W., Schwartz T. (2010) Creating the CIPRES Science Gateway for

883   inference of large phylogenetic trees. In: *Gateway Computing Environments Workshop*

884   (GCE), 2010 (pp. 1-8). IEEE (Institute of Electrical and Electronics Engineers).

885   Muravenko O.V., Lemesh V.A., Samatadze T.E., Amosova A.V., Grushetskaya Z.E., Popov

886   K.V., Semenova O.Y., Khotyuleva L.V., Zelenin, A.V. (2003) Genome comparisons with

887   chromosomal and molecular markers for three closely related flax species and their

888   hybrids. *Russian Journal of Genetics* **39**, 414-421.

889   Naiki A. (2012) Heterostyly and the possibility of its breakdown by polyploidization.

890   *Plant Species Biology* **27**, 3-29.

891   Nicholls M.S. (1986) Variation and evolution in *Linum tenuifolium* (Linaceae). *Plant*

892   *Systematics and Evolution* **153**, 243-258.

893   Ockendon D.J. (1971) Taxonomy of the *Linum perenne* group in Europe. *Watsonia* **8**,

894   205-235.

895   Ockendon D.J., Walters S.M. (1968) *Linum*. In: Tutin et al. (Eds.) *Flora Europaea*, vol. 2.

896   Cambridge University Press, Cambridge.

897   Ortiz P.L. , R. Berjano, M. Talavera, L. Rodríguez-Zayas, M. Arista (2015) Flower colour

898   polymorphism in *Lysimachia arvensis*: How is the red morph maintained in

899   Mediterranean environments? *Perspectives in Plant Ecology, Evolution and*

900   *Systematics* **17**, 142–150

901   Orueta D. (2002) Thermal relationships between *Calendula arvensis* inflorescences and

902   *Usia aurata* bombyliid flies. *Ecology* **83**, 3073-3085.

903   Pagel, M. (1994) Detecting correlated evolution on phylogenies - a general-method for

904   the comparative analysis of discrete characters. *Proceedings of the Royal Society of*
905   *London Series B-Biological Sciences* **255**, 37-45.

906   Pannell J.R., Dorken M.E., Eppley, S.M. (2005) 'Haldane's Sieve'in a metapopulation:
907   sifting through plant reproductive polymorphisms. *Trends in Ecology & Evolution* **20**,
908   374-379.

909   Pérez-Barrales R., Vargas P., Arroyo J. (2006) New evidence for the Darwinian
910   hypothesis of heterostyly: breeding systems and pollinators in *Narcissus* sect.
911   Apodanthi. *New Phytologist* **171**, 553-567.

912   Piper J.G., Charlesworth B., Charlesworth D. (1986) Breeding system evolution in
913   *Primula vulgaris* and the role of reproductive assurance. *Heredity* **56**, 207-217.

914   Posada D., Crandall K.A. (1998) Modeltest: testing the model of DNA substitution.
915   *Bioinformatics* **14**, 817-818.

916   R Development Core Team (2015). *R: A language and environment for statistical*
917   *computing.* R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-
918   07-0, URL http://www.R-project.org.

919   Rambaut A., Drummond A.J. (2007). *Tracer – MCMC Trace Analysis Tool Version v1.4.*
920   Available from: <http://beast.bio.ed.ac.uk/Tracer/>

921   Ramsey J., Schemske D.W. (1998) Pathways, mechanisms, and rates of polyploid
922   formation in flowering plants. *Annual Review of Ecology and Systematics* **29**, 467-501.

923   Rasband W. S. (2008) *ImageJ* 1997–2007. US National Institutes of Health: Bethesda,
924   Maryland, USA.

925   Revell L. J. (2012) Phytools: An R package for phylogenetic comparative biology (and
926   other things). *Methods in Ecology and Evolution* **3**, 217-223.

927   Rogers C.M. (1981) A revision of the genus *Linum* in southern Africa. *Nordic Journal of*
928   *Botany* **1**, 711-722.

929   Ruiz-Martín J., Jiménez-Mejías P., Martínez-Labarga J.M., Pérez-Barrales R. (2015) *Linum*
930   *flos-carmini* (Linaceae), a new species from northern Morocco. *Annales Botanici*
931   *Fennici* **52**, 383-395.

932   Santos-Gally R., Gonzalez-Voyer A., Arroyo, J. (2013) Deconstructing heterostyly: the
933   evolutionary role of incompatibility system, pollinators, and floral architecture.
934   *Evolution* **67**, 2072-2082.

935   Schewe L.C., Sawhney V.K., Davis A.R. (2011) Ontogeny of floral organs in flax (*Linum*
936   *usitatissimum;* Linaceae). *American Journal of Botany* **98**, 1077-1085.

937   Schoen D.J., Johnston M.O., L'Heureux A.M., Marsolais J.V. (1997) Evolutionary history of
938   the mating system in *Amsinckia* (Boraginaceae). *Evolution* **51**, 1090-1099.

939   Seetharam A. (1972) Interspecific hybridization in *Linum*. *Euphytica* **21**, 489-495.

940   Shore J.S., Arbo M.M., Fernández A. (2006) Breeding system variation, genetics and
941   evolution in the Turneraceae. *New Phytologist* **171**, 539-551.

942   Ssymank A., Hamm A., Vischer-Leopold M. (2009). *Caring for pollinators safeguarding*
943   *agro-biodiversity and wild plant diversity.* Federal Agency for Nature Conservation
944   (BfN) & Universität Bonn

945   Stebbins G.L. (1970) Adaptive radiation of reproductive characteristics in angiosperms.
946   I. Pollination mechanisms. *Annual Review of Ecology and Systematics* **1**, 307–326.

947   Stebbins G.L. (1974) *Flowering plants: evolution above the species level*. Belknap,
948   Cambridge, MA. USA.

949   Strauss S.Y., Whittall J.B. (2006) Non-pollinator agents of selection on floral traits. In:
950   Harder L.D., Barrett S.C.H. (Eds.) *Ecology and evolution of flowers.* Oxford University,.
951   Oxford, UK, pp. 120–138.

952   Suksathan P., K. Larsen (2006) A new species of *Tirpitzia* (Linaceae) from Thailand . *Thai*
953   *Forest Bulletin* **34**, 201 – 205.

954   The Plant List (2013). Version 1. Published on the Internet;
955   http://www.theplantlist.org/ (accessed 10th March 2017).

956   Thompson J.D., Pailler T., Strasberg D., Manicacci D. (1996). Tristyly in the endangered
957   Mascarene Island endemic *Hugonia serrata* (Linaceae). *American Journal of Botany*
958   **83**, 1160-1167.

32

959   Turketti S.S., Esler K.J., Dreyer L.L. (2012) Three-dimensional reciprocity: A new form of

960   tristyly in South African *Oxalis* (Oxalidaceae) species and its implications for

961   reproduction. *South African Journal of Botany* **78**, 195-202.

962   Ushijima K., Nakano R., Bando M., Shigezane Y., Ikeda K., Namba Y., Kume S., Kitabata T.,

963   Mori H., Kubo Y. (2012) Isolation of the floral morph-related genes in heterostylous

964   flax (*Linum grandiflorum*): the genetic polymorphism and the transcriptional and

965   post-transcriptional regulations of the S locus. *The Plant Journal* **69**, 317-331.

966   Yang Z., Kumar S., Nei M. (1995) A new method of inference of ancestral nucleotide and

967   amino acid sequences. *Genetics* **141**, 1641-1650.

968   Yılmaz Ö., Kaynak G. (2008) The check-list and chorology of the *Linum* L.(Linaceae) taxa

969   in the flora of Turkey. *Journal of Biological and Environmental Sciences* **2,** 5-43.

970   Yurkevich O.Y., Naumenko-Svetlova A.A., Bolsheva N.L., Samatadze T.E., Rachinskaya

971   O.A., Kudryavtseva A.V., Zelenina D.A., Volkow A.V., Zelenin A.V., Muravenko O.V.

972   (2013) Investigation of genome polymorphism and seed coat anatomy of species of

973   section *Adenolinum* from the genus *Linum*. *Genetic Resources and Crop Evolution* **60**,

974   661-676

975   Yusepchuk S.V. (1949) *Linaceae* In: B.K. Shishkin (Ed) *Flora of the USSR*, vol. XIV.

976   Akademii Nauk SSSR, Moscow, Leningrad.

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978

979   **Figure legends**

980

981   **Fig. 1.** Floral variation and pollinators in Mediterranean *Linum* species: a) *L. viscosum*

982   visited by an Halictidae bee, b*) L. narbonense* with *Usia pubera* beefly collecting nectar,

983   c) and d) *L. tenuifolium* visited by *Ceratina cucurbitina* and *Lasioglossum malachurum*

984   bees, respectively, e) three dimensional reciprocity in *L. suffruticosum*, f) *Usia* sp.

985   collecting nectar in *L. suffruticosum,* g) conventional distyly in *L. tenue.* h) *L. tenue* with

986   nectar collecting *U. pusilla*, i) *L. comptonii* visited by pollen collecting *Amegilla* in South

987   Africa, and j) *L. pubescens* with *Usia bicolor* in Israel. Photographs by Blanca Arroyo (c,

988   d), Ross Turner (i) and Yuval Sapir (j).

989

990   **Fig. 2**. Phylogenetic tree of Linaceae based on BEAST analysis of combined nuclear (ITS)

991   and plastid (*trn*L-F, *mat*K and *ndh*F) DNA regions. Numbers above each branch indicate

992   posterior probability support. Bars in each node indicate 95% CI of the age of each node.

993   Time scale on the horizontal axis is in millions of years.

994

995   **Fig. 3.** Maximum likelihood ancestral state reconstruction of stylar polymorphism in

996   *Linum*. Two ancestral states (blue = monomorphic, red = polymorphic) are considered

997   as the simplest way to understand the evolution of heterostyly (see Material and

998   methods for details). Letters above branches are referenced in main text.

999

1000   **Fig. 4.** Maximum likelihood ancestral state reconstruction of stylar polymorphism in

1001   *Linum*. Five relevant states to the two competing hypotheses of the evolution of

1002   heterostyly are considered (Charlesworth & Charlesworth 1979, Lloyd & Webb, 1992a;

1003   see Materials and Methods for details). Colours represent the different stylar conditions:

1004   blue = homostyly, red = style polymorphism, green = approach herkogamy, yellow =

1005   horizontal herkogamy, and orange = reverse herkogamy. Letters above branches are

1006   referenced in main text.

1007

34

1008   **Fig. S1**. Phylogenetic tree of Linaceae based on BEAST analysis of combined nuclear

1009   (ITS) and plastid (*trn*L-F, *mat*K and *ndh*F) DNA regions. Numbers above each branch

1010   indicate posterior probability support. Bars in each node indicate 95% CI of the age of

1011   each node. Time scale on horizontal axis is in millions of years. Tip labels include species

1012   name, section and distribution.

1013

1014   **Table S1.** Sources of plant material and traits considered in the study. Taxa are

1015   arranged alphabetically by section and family. Sampled populations refer to GenBank

1016   accession numbers when obtained from published references, to samples supplied by

1017   herbaria (E: Royal Botanic Garden Edinburgh, MA: Royal Botanic Garden Madrid, SEV:

1018   University of Seville; codes for specimens are given). NA, not available.
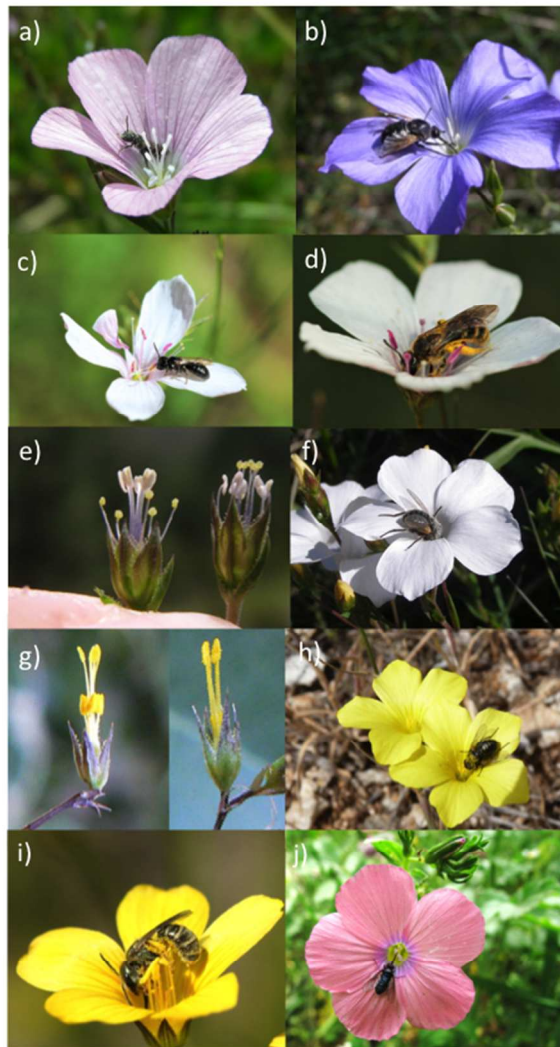
Fig. 1. Floral variation and pollinators in Mediterranean Linum species: a) L. viscosum visited by an Halictidae bee, b) L. narbonense with Usia pubera beefly collecting nectar, c) and d) L. tenuifolium visited by Ceratina cucurbitina and Lasioglossum malachurum bees, respectively, e) three dimensional reciprocity in L. suffruticosum, f) Usia sp. collecting nectar in L. suffruticosum, g) conventional distyly in L. tenue. h) L. tenue with nectar collecting U. pusilla, i) L. comptonii visited by pollen collecting Amegilla in South Africa, and j) L. pubescens with Usia bicolor in Israel. Photographs by Blanca Arroyo (c, d), Ross Turner (i) and Yuval Sapir (j).
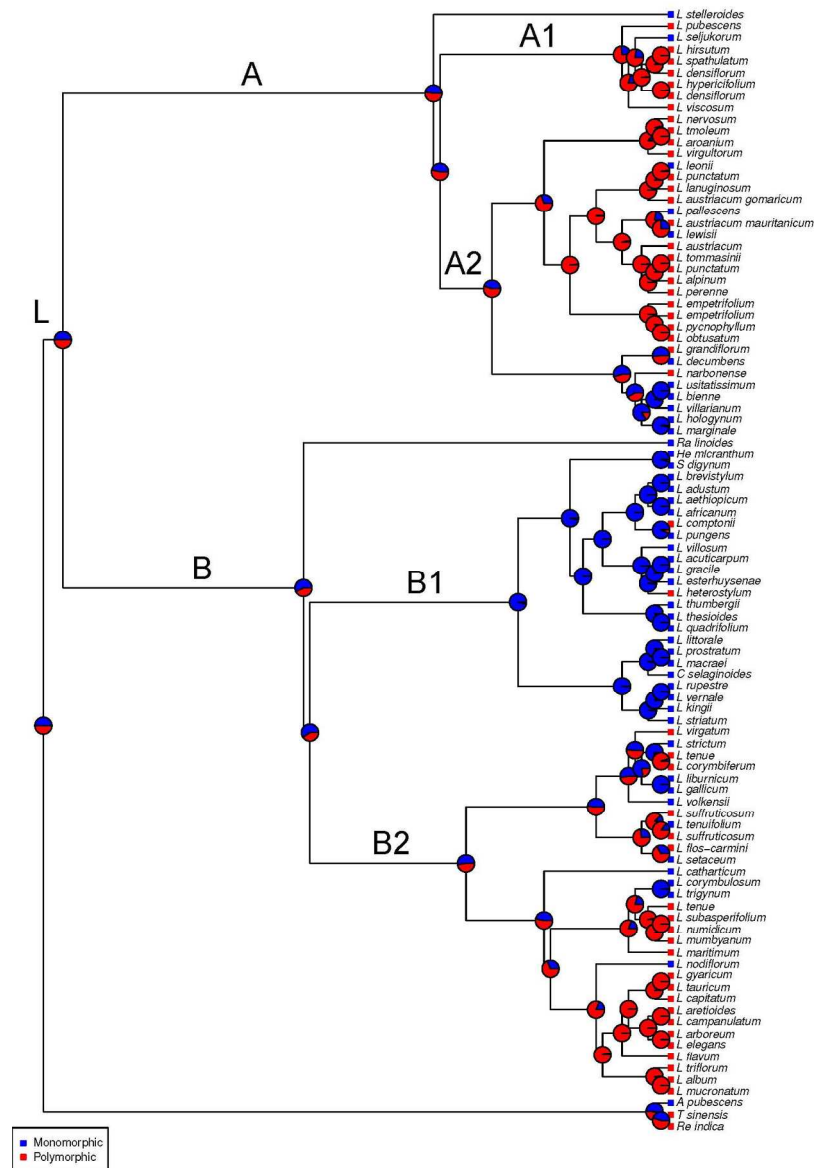
Fig. 2. Phylogenetic tree of Linaceae based on BEAST analysis of combined nuclear (ITS) and plastid (trnL-F, matK and ndhF) DNA regions. Numbers above each branch indicate posterior probability support. Bars in each node indicate 95% CI of the age of each node. Time scale on the horizontal axis is in millions of years.

405x958mm (200 x 200 DPI)

Fig. 3. Maximum likelihood ancestral state reconstruction of stylar polymorphism in Linum. Two ancestral states (blue = monomorphic, red = polymorphic) are considered as the simplest way to understand the evolution of heterostyly (see Material and methods for details). Letters above branches are referenced in main text.

210x297mm (200 x 200 DPI)
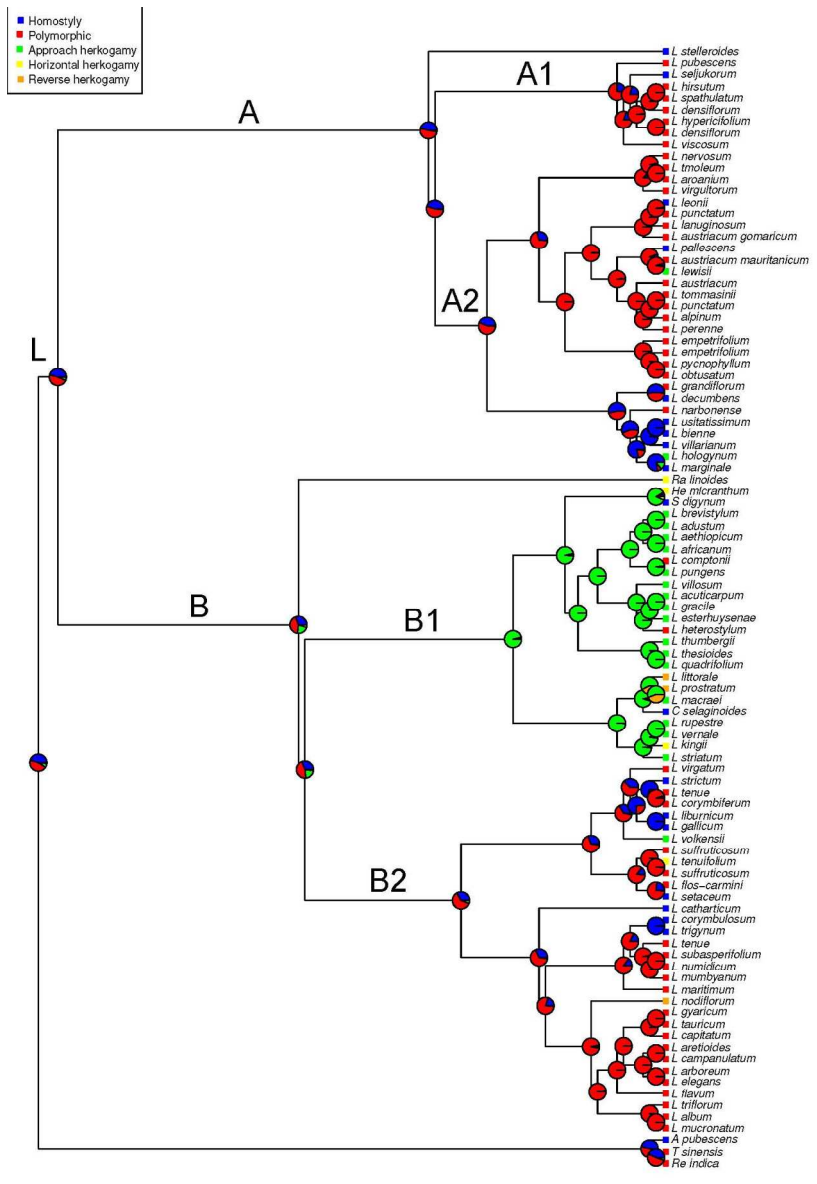
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210x297mm (200 x 200 DPI)
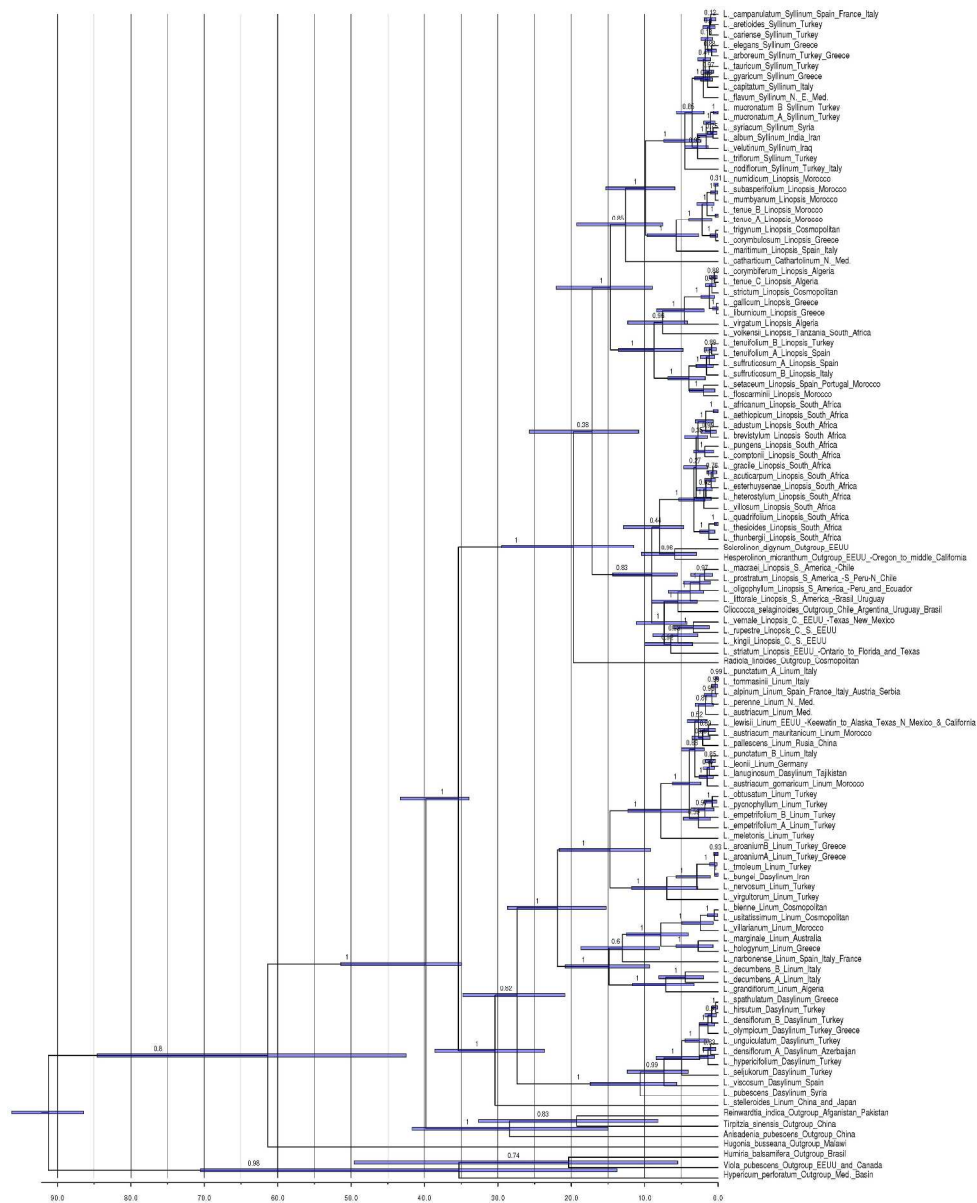
Fig. S1. Phylogenetic tree of Linaceae based on BEAST analysis of combined nuclear (ITS) and plastid (trnL-F, matK and ndhF) DNA regions. Numbers above each branch indicate posterior probability support. Bars in each node indicate 95% CI of the age of each node. Time scale on horizontal axis is in millions of years. Tip labels include species name, section and distribution.

464x574mm (200 x 200 DPI)

Table S1. Source of plant material and traits considered in the study. Taxa are arranged alphabetically by section and family. Sampled populations refer to GenBank accession numbers when obtained from published references, to samples supplied by herbaria (E: Royal Botanic Garden Edinburgh, MA: Royal Botanic Garden at Madrid, SEV: University of Seville; codes for specimens are given). NA, not available.

| 1. Taxon	2. Section or family	3. Distribution	4. Sampled populations	5. Coordinates	6. GenBank Accession no. ITS	7. GenBank Accession no. ndhF5-8	8. GenBank Accession no. trnL-F	9. GenBank Accession no. matK	10. Stylar condition (binary)	11. Stylar condition (five states)	12. Life-form	13. References for columns 10, 11, 12	14. Chromosome Number	15. References for column 14	16. Breeding system	17. References for column 16
Linum catharticum	Cathartolinum	N Medit.	Ref. 1	NA	FJ169533	FJ160796	FJ160880	HM544103	Monomorphic	Homostylous	Annual	2	n=8/2n=16	26	--	--
L. densiflorum A	Dasylinum	Azerbaijan	This study E00450740	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	--	--	--	--
L. densiflorum B	Dasylinum	Turkey	This study	40º06'57.8''N 32º36'17.8''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	--	--	--	--
L. hirsutum	Dasylinum	Turkey	Ref. 1	NA	FJ169520	FJ160788	FJ160872	HM544106	Polymorphic	Polymorphic	Perennial	3	n=8; 2n=16/n=16; 2n=32	27, 28, 29	SI	52
L. hypericifolium	Dasylinum	Turkey	Ref. 1	NA	FJ169519	FJ160789	FJ160873	HM544107	Polymorphic	Polymorphic	perennial	4	--	--	--	--
L. olympicum	Dasylinum	Turkey, Greece	This study E00450745	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	NA	NA	Perennial	5	--	--	--	--
L. pubescens	Dasylinum	Syria	Ref. 1	NA	FJ169518	FJ160790	FJ160874	NA	Polymorphic	Polymorphic	Annual	6	2n=18 ; 2n=16	30, 31	SI	53
L. seljukorum	Dasylinum	Turkey	This study E00450754	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Homostylous	Annual	3	n=8; 2n=16	--	--	--
L. spathulatum	Dasylinum	Greece	This study	40º04.9'N 22º22.7'E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	5	2n=16/2n=36	32;,28	--	--
L. unguiculatum	Dasylinum	Turkey	This study E00450741	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	NA	NA	Perennial	3	--	--	--	--
L. viscosum	Dasylinum	Spain	This study	NA	FJ169517	FJ160791	FJ160875	NA	Polymorphic	Polymorphic	Perennial	2	n=8; 2n=16	27	--	--
L. acuticarpum	Linopsis	South Africa	This study	33º59'55.6"S 20º26'33.7"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	n=15	33	--	--
L. adustum	Linopsis	South Africa	This study	34º33'02.1"S 19º25'37.8"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	--	--	--	--
L. aethiopicum	Linopsis	South Africa	This study	34º25'48.8"S 20º39'50.5"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	--	--	--	--
L. africanum	Linopsis	South Africa	This study	34º09'39.4"S 18º52'16.0"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	n=16/2n=30; 2n=29	30, 33	SC	54

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L. brevistylum	Linopsis	South Africa	This study	34°44'26.1"S 19°40'44.9"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	--	--	--	--
L. comptonii	Linopsis	South Africa	This study	32º37'51.2"S 19º09'05.8"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	7	n=15	33	--	--
L. corymbiferum	Linopsis	Algeria	This study	36°52'4.8"N 4°50'16.4"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial, biennial	8	n=15; 2n=18/2n=30	27, 28	--	--
L. corymbulosum	Linopsis	Greece	This study	38º00'28.9"N 22º16'30.4"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Homostylous	Annual	4	n=9; 2n=18	30	--	--
L. esterhuysenae	Linopsis	South Africa	This study	33º55'05.1"S 22º01'32.2"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	--	--	--	--
L. flos-carmini	Linopsis	Morocco	This study	34º54'33"N 5º 32' 12"W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphism	Polymorphic	Annual	11	--	--	--	--
L. gallicum	Linopsis	Greece	This study	37º59'32.3"N 22º27'47.0"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Homostylous	Annual	5	n=10; 2n=20	27	--	--
L. gracile	Linopsis	South Africa	This study	33º57'24.5"S 23º31'02.0"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	n=15	33	--	--
L. heterostylum	Linopsis	South Africa	This study	33º58'09.4"S 21º13'06.0"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	7	n=15	33	--	--
L. kingii	Linopsis	C, S USA	Ref. 1	NA	FJ169555	FJ160780	FJ160864	NA	Monomorphic	Horizontal herkogamous	Perennial, biennial	9	n=13; 2n=26	28	--	--
L. liburnicum	Linopsis	Greece	This study	38º04'06.6"N 22º23'00.3"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Homostylous	Annual	5	--	--	--	--
L. littorale	Linopsis	S America	Ref. 1	NA	FJ169543	FJ160781	FJ160865	NA	Monomorphic	Reverse herkogamous	Perennial	10	n=18; 2n=36/n=36; 2n=72	28	--	--
L. macraei	Linopsis	S America	Ref. 1	NA	FJ169544	FJ160782	FJ160866	NA	Monomorphic	Approach herkogamous	Perennial	10	n=36; 2n=72	28	--	--
L. maritimum	Linopsis	Spain, Italy	Ref. 1	NA	FJ169535	FJ160811	FJ160895	NA	Polymorphic	Polymorphic	Perennial	2	n=10; 2n=20	28	SI	54
L. mumbyanum	Linopsis	Morocco, Algeria	This study	34°50'59.1"N 1°21'24.4"W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Biennial, perennial	8	n=10; 2n=20	28	--	--
L. numidicum	Linopsis	Morocco, Algeria	This study	35º11'52.8"N 03º58'50.2"W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	8	--	--	--	--
L. oligophyllum	Linopsis	S America	Ref. 1	NA	FJ169546	FJ160783	FJ160867	HM544111	NA	NA	Perennial	10	n=18; 2n=36	28	--	--
L. prostratum	Linopsis	S America	Ref. 1	NA	FJ169545	FJ160784	FJ160868	NA	Monomorphic	Reverse herkogamous	Perennial, annual	10	n=18; 2n=36	28	--	--
L. pungens	Linopsis	South Africa	This study	32º22'26.1"S 19º03'48.3"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	--	--	--	--
L. quadrifolium	Linopsis	South Africa	This study	33º57'06.7"S 18º27'05.7"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	--	--	--	--
L. rupestre	Linopsis	C, S USA	Ref. 1	NA	FJ169553	FJ160785	FJ160869	HM544113	Monomorphic	Approach herkogamous	Perennial	79	n=18	26	--	--
L. setaceum	Linopsis	Spain, Portugal, Morocco	This study	36º47'39.0"N 4º59'24.4"W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Homostylous	Annual	2	n=9; 2n=18	27, 80	--	--
L. striatum	Linopsis	USA, Canada	Ref. 1	NA	FJ169554	FJ160786	FJ160870	NA	Monomorphic	Approach herkogamous	Perennial	12	n=18	26	--	--
L. strictum	Linopsis	Cosmopolitan	Ref. 1	NA	FJ169530	FJ160806	FJ160890	NA	Monomorphic	Homostylous	Annual	2	n=9; 2n=18/ 2n=32	27, 30	--	--
L. subasperifolium	Linopsis	Morocco	This study	34°46'28"N 3°47'43"W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Annual	8	--	--	--	--
L. suffruticosum A	Linopsis	Spain	This study	36°47'39"N 4°59'24.4"W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	2	n=18; n=36/2n=72	28, 34	SI	55, 56, 57, 58

Species	Section	Distribution	Source	Coordinates	Seq1	Seq2	Seq3	Seq4	Dimorphism	Herkogamy	Life form	Ref.	Chromosome	n	SI/SC	References
L. suffruticosum B	Linopsis	Italy	This study	44°12'30"N 8°23'33"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	2	n=18; n=36	28	SI	55, 56, 57, 58
L. tenue A	Linopsis	Morocco	This study	32º00'58.2"N 06º43'12.3'W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Annual	2	n=10; 2n=20/2n=30	28, 35	SI	52
L. tenue B	Linopsis	Morocco	This study	30º40'43"N 09º29'15''W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Annual	2	n=10; 2n=20/2n=31	28, 35	SI	52
L. tenue C	Linopsis	Algeria	This study	34°52'39.2"N 1°14'38.7"W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Annual	2	n=10; 2n=20/2n=32	28, 35	SI	52
L. tenuifolium A	Linopsis	Spain	This study	42º20'27.8"N 1º43'08.5''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Horizontal herkogamous	Perennial	2	n=9; 2n=18/2n=16	27, 36	SC	56, 57, 58
L. tenuifolium B	Linopsis	Turkey	This study	40º38'01.0''N 33º36'32.1''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Horizontal herkogamous	Perennial	2	n=9; 2n=18/2n=17	27, 36	SC	56, 57, 58
L. thesioides	Linopsis	South Africa	This study	33°57'06.7"S 18°27'05.7"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	--	--	--	--
L. thunbergii	Linopsis	South Africa	This study	28º41'09.9"S 28°53'57.1"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	--	--	--	--
L. trigynum	Linopsis	Cosmopolitan	Ref. 1	NA	FJ169536	FJ160810	FJ160894	NA	Monomorphic	Homostylous	Annual	3	n=10; 2n=20	28	--	--
L. vernale	Linopsis	USA	Ref. 1	NA	FJ169552	FJ160812	FJ160896	NA	Monomorphic	Approach herkogamous	Annual	10	n=15	26	--	--
L. villosum	Linopsis	South Africa	This study	33º51'57.2"S 22º48'04.4"E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	7	--	--	--	--
L. virgatum	Linopsis	Algeria	This study	34°47'16.4"N 0°15'27.7"W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Annual, biennial	Ruiz-Martín J., unpubl.	--	--	--	--
L. volkensii	Linopsis	Tanzania	Ref. 1	NA	FJ169531	FJ160813	FJ160897	HM544116	Monomorphic	Approach herkogamous	Perennial, annual	13	n=27	81	--	--
L. alpinum	Linum	W Medit., C Europe	This study	42º29'17.4"N 13º00'28.9''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	15	n=9; 2n=18/2n=36	27, 38	--	--
L. aroanium A	Linum	Turkey, Greece	This study	38º00'37.9''N 22º16'06.5''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	2n=36	5	--	--
L. aroanium B	Linum	Turkey, Greece	This study	40º06'57.8''N 32º36'17.8''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	2n=36	5	--	--
L. austriacum	Linum	Medit. Basin	Ref. 1	NA	FJ169522	FJ160799	FJ160883	NA	Polymorphic	Polymorphic	Perennial	8	n=9; 2n=18/2n=36	27, 35	SI	2
L. austriacum subsp. *gomaricum*	Linum	Morocco	This study SEV156580 SEV156577	35º08'N 05º08'W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	8	--	--	--	--
L. austriacum subsp. *mauritanicum*	Linum	Morocco	This study	33° 3'33.2"N 5° 2'14.3"W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	8	n=9	83	--	--
L. bienne	Linum	Cosmopolitan	Ref. 1	NA	FJ169527	FJ160797	FJ160881	HM544102	Monomorphic	Homostylous	Annual, biennial	8	n=15, 18/2n=30, 32	39, 2, 40, 41,	SC	54
L. bungei	Linum	Iran	This study E00450816	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	NA	NA	Perennial	Ruiz-Martín J., unpubl.	--	--	--	--
L. decumbens A	Linum	Italy	This study MA628332	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Homostylous	Annual, biennial	2	n=15,18; 2n=32	39, 42, 31	--	--
L. decumbens B	Linum	Italy	This study	37º50'42.0''N 13º25'58.9''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Homostylous	Annual, biennial	2	n=15, 18; 2n=32	39, 42, 31	--	--
L. empetrifolium A	Linum	Turkey	This study	38º24'09.5''N 34º01'41.4''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	--	--	--	--
L. empetrifolium B	Linum	Turkey	This study	40º02'19.1''N 40º29'08.2''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	--	--	--	--

L. grandiflorum	Linum	Algeria	Ref. 1	NA	FJ169525	FJ160798	FJ160882	NA	Polymorphic	Polymorphic	Annual	16	n=8; 2n=16	27, 43	SI	59, 60
L. hologynum	Linum	Greece	This study	39º50'46.7''N 21º12'31.0''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Approach herkogamous	Perennial	2	n=9; 2n=18/2n=42	27, 32	--	--
L. lanuginosum	Linum	Tajikistan	This study	38º38'06''N 70º42'36''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	4	--	--	--	--
L. leonii	Linum	Germany, France	This study	51º29'32''N 09º18'13''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Homostylous	Perennial	2	n=9; 2n=18; 2n=20	2, 44	--	--
L. lewisii	Linum	W of N America	Ref. 1	NA	FJ169523	FJ160800	FJ160884	NA	Monomorphic	Approach herkogamous	Perennial	2	n=9; 2n=18	26, 27	SC	61
L. marginale	Linum	Australia	Ref. 1	NA	FJ169528	FJ160804	FJ160888	NA	Monomorphic	Homostylous	Perennial, anual	14	2n=80	37	--	--
L. meletonis	Linum	Turkey	This study E00212261	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	NA	NA	Perennial	Ruiz-Martín J., unpublished	--	--	--	--
L. narbonense	Linum	Spain, Italy, France	This study	39°06'16.8"N 1°01'56.3"W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	2	n= 7, n=9, n=14/ 2n=28, 2n=20, 2n=18	27, 45, 30, 35	SI	52
L. nervosum	Linum	Turkey	This study	38º52'20.1''N 42º31'24.9''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	2n=30, 2n=18	82, 46	--	--
L. obtusatum	Linum	Turkey	This study E00450930	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	--	--	--	--
L. pallescens	Linum	Russia, China	Ref. 1	NA	FJ169521	FJ160801	FJ160885	NA	Monomorphic	Homostylous	Biennial, perennial	4	2n=18	2	--	--
L. perenne	Linum	Medit. Basin	Ref. 1	NA	FJ169524	FJ160802	FJ160886	NA	Polymorphic	Polymorphic	Perennial	2	n=9/ 2n=18; 2n=36	27, 30	SI	2, 60
L. punctatum A	Linum	Italy	This study MA646775	37º51'N 14º01'E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	17	n=18	27	--	--
L. punctatum B	Linum	Italy	This study	37º51'40.6''N 14º00'45.5''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	17	n=18	27	--	--
L. pycnophyllum	Linum	Turkey	This study E00450918	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	5	2n=18	5	--	--
L. stelleroides	Linum	China, Japan	Ref. 1	NA	FJ169516	FJ160805	FJ160889	NA	Monomorphic	Homostylous	Annual, biennial	4	n=10/ 2n=20 ,2n=18	39, 47	--	--
L. tmoleum	Linum	Turkey	This study	40º30'45.2''N 38º21'05.0''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Annual, biennial	3	--	--	--	--
L. tommasinii	Linum	Italy	This study	42º22'23.2''N 13º23'25.9''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	15	n=9; 2n=18	27	--	--
L. usitatissimum	Linum	Cosmopolitan	Ref. 1	NA	FJ169526	FJ160803	FJ160887	HM544115	Monomorphic	Homostylous	Annual	2	n=15; 2n=30	27	SC	54
L. villarianum	Linum	Morocco	This study	35º52'30.6''N 05º24'14.4'W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Monomorphic	Homostylous	Perennial	18	--	--	--	--
L. virgultorum	Linum	Turkey	This study E00289593	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Annual	2	--	--	--	--
L. album	Syllinum	India, Iran	Ref. 1	NA	FJ169547	FJ160792	FJ160876	NA	Polymorphic	Polymorphic	Perennial	19	2n=30; 2n=28	82, 48	--	--
L. arboreum	Syllinum	Turkey, Greece	Ref. 1	NA	FJ169537	FJ160793	FJ160877	HM544100	Polymorphic	Polymorphic	Perennial	3	2n=28	27	--	--
L. aretioides	Syllinum	Turkey	This study E00175938	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	20	--	--	--	--
L. campanulatum	Syllinum	Spain, France, Italy	This study	40°16'52"N 2°50'56"W	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	15	n=14; 2n=28	27	--	--
L. capitatum	Syllinum	Italy	This study MA698754	42º09'N 14º06'E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	2	n=12, n= 28/ 2n=28, 2n= 34	27, 30	--	--

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Species	Section	Locality	Source	Coordinates					Style 1	Style 2	Life history	No.	Chromosome	Ref	SI	Ref
L. cariense	Syllinum	Turkey	This study MA590884	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	NA	NA	Perennial	3	--	--	--	--
L. elegans	Syllinum	Greece	This study	37º51'03.7''N 22º14'47.5''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	2n=28, 2n=30	49, 73	--	--
L. flavum	Syllinum	NE Medit. Basin	Ref. 1	NA	FJ169538	FJ160794	FJ160878	HM544105	Polymorphic	Polymorphic	Perennial	4	n=15; 2n=30/n=14/2 n=28	27, 30, 39	SI	52
L. gyaricum	Syllinum	Greece	This study E00175803	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	21	n=30+1B	50	--	--
L. mucronatum A	Syllinum	Turkey	This study	38º04'34.6''N 36º44'05.1''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	n=14	48	SI	62
L. mucronatum B	Syllinum	Turkey	This study	38º11'20.8''N 36º49'43.9''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	n=14	48	--	--
L. nodiflorum	Syllinum	Turkey, Italy	Ref. 1	NA	FJ169539	FJ160795	FJ160879	NA	Monomorphic	Reverse herkogamous	Annual	3	n=13/ 2n=26, 2n=24	30, 39, 40	SI	54
L. syriacum	Syllinum	Syria	This study E00450664	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	NA	NA	Perennial	74	--	--	--	--
L. tauricum	Syllinum	Turkey	This study	40°52'3''N 26°46'13''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	2n=28	40	--	--
L. triflorum	Syllinum	Turkey	This study	38º34'59.8''N 42º16'10.7''E	Forthcoming	Forthcoming	Forthcoming	Forthcoming	Polymorphic	Polymorphic	Perennial	3	--	--	--	--
L. velutinum	Syllinum	Iraq	This study E00175935	NA	Forthcoming	Forthcoming	Forthcoming	Forthcoming	NA	NA	Perennial	75	--	--	--	--
Anisadenia pubescens	Outgroup (Linaceae)	China	Ref. 1	NA	FJ169513	FJ160772	FJ160856	NA	Monomorphic	Homostylous	Perennial	76	--	--	--	--
Cliococca selaginoides	Outgroup (Linaceae)	S America	Ref. 1	NA	FJ169540	FJ160774	FJ160858	NA	Monomorphic	Homostylous	Perennial	22	n=18; 2n=36	51	--	--
Hesperolinon micranthum	Outgroup (Linaceae)	W USA	Ref. 1	NA	FJ169542	FJ160775	FJ160859	NA	Monomorphic	Horizontal herkogamous	Annual	23	n=18; 2n=36	28	--	--
Hugonia busseana	Outgroup (Linaceae)	Malawi	Ref. 1	NA	FJ169512	FJ160773	FJ160857	NA	Monomorphic	Homostylous	Perennial	13	--	--	--	--
Radiola linoides	Outgroup (Linaceae)	Cosmopolitan	Ref. 1	NA	FJ169534	FJ160815	FJ160899	NA	Monomorphic	Horizontal herkogamous	Annual	2	2n=18	51	--	--
Reinwardtia indica	Outgroup (Linaceae)	Afganistan, Pakistan	Ref. 1	NA	FJ169514	FJ160814	FJ160898	NA	Polymorphic	Polymorphic	Perennial	12	2n=20,22	51	--	--
Sclerolinon digynum	Outgroup (Linaceae)	USA	Ref. 1	NA	FJ169541	FJ160787	FJ160871	NA	Monomorphic	Homostylous	Annual	9	n=6; 2n=12	28	--	--
Tirpitzia sinensis	Outgroup (Linaceae)	China	Ref. 1	NA	FJ169515	FJ160816	FJ160900	NA	Polymorphic	Polymorphic	Perennial	77	--	--	--	--
Humiria balsamifera	Outgroup (Humiriaceae)	Brasil	Ref. 70, 71, 72	NA	NA	EU002231	AF350941	AY935932	Monomorphic	Homostylous	Perennial	78	--	--	--	--
Hypericum perforatum	Outgroup (Hypericaceae)	Medit. Basin	Ref. 67, 68, 69	NA	EU796888	NA	KC709009	AB698447	Monomorphic	Homostylous	Perennial	24	--	--	--	--
Viola pubescens	Outgroup (Violaceae)	USA, Canada	Ref. 63, 64, 65, 66	NA	DQ006044	FJ670135	JF767162	JX661966	Monomorphic	Homostylous	Perennial	25	--	--	--	--

References:

1. McDill J., Repplinger M., Simpson B.B., Kadereit J.W. (2009) The phylogeny of *Linum* and Linaceae subfamily Linoideae, with implications for their systematics, biogeography, and evolution of heterostyly. *Systematic Botany* **34**, 386-405.

2. Ockendon D. J., Walters S.M. (1968). Linaceae. In: Tutin T.G., Heywood V.H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M., Webb D. A. (Eds.) *Flora Europaea vol. 2: Rosaceae to Umbelliferae.* Cambridge: University of Cambridge Press, Cambridge, pp. 589 – 603.

3. Davis, P. H. (1967). Linaceae. In: Davis P.H. (Eds.) Flora of Turkey and the Aegean Islands. Edinburgh University Press Edinburgh, pp. 425 – 450.

4. Yuzepchuk S. V. (1974). Linaceae. In:. Shishkin B.K., Bobrov E. G. (Eds.) *Flora of the U.S.S.R. vol. 14*. Botanical Institute of the Academy of Sciences of the USSR, Leningrad. Translated by the Israel Program for Scientific Translations, Jerusalem, pp. 67 – 112.

5. Strid A. (1986). *Mountain flora of Greece*. Cambridge University Press, Cambridge.

6. Wolfe L.M. (2001). Associations among multiple floral polymorphisms in *Linum pubescens* (Linaceae), a heterostylous plant. *International Journal of Plant Sciences* **162**, 335-342.

7. Rogers C.M. (1981) A revision of the genus *Linum* in southern Africa. *Nordic Journal of Botany* **1**, 711-722.

8. Fennane M., Ibn-Tattou M. (2007). *Flore pratique du Maroc. Vol. 2. Angiospermae (Leguminosae-Lentibulariaceae)*. Institut Scientifique, Université Mohammed V de Rabat

9. Rogers C. M. (1968) Yellow-flowered species of *Linum* in Central America and western North America. *Brittonia* **20**, 107-135

10. Mildner R.A., Rogers C.M. (1978) Revision of the native South American species of *Linum* (Linaceae). *Phytologia* **39**, 343 - 390

11. Ruiz-Martín J., Jiménez-Mejías P., Martínez-Labarga J.M., Pérez-Barrales R. (2015) *Linum flos-carmini* (Linaceae), a new species from northern Morocco. *Annales Botanici Fennici* **52**, 383-395.

12. Small, J. K. (1907) *North American Flora*. The New York Botanical Garden vol.25 p.87

13. Robson N.K.B. (1963) Linaceae. *Flora Zambesiaca* Vol. 2 part. 1.

14. Bentham G., Mueller F. (1863). *Flora australiensis: A description of the plants of the Australian territory, Vol. 1*. L. Reeve and Co., London.

15. Pignatti S. (1982) *Flora d'Italia, vol I-III*. Edagricole, Bologna.

16. Quezel P. & Santa S. (1963) *Nouvelle flore de l'Algerie: et des regions desertiques meridionales*. CNRS, Paris.

17. Giardina G., Raimondo F. M., Spadaro V. (2007) A catalogue of plants growing in Sicily. *Bocconea* **20**, 198-199

18. Valdés B., Rejdali M, Achhal el Kadmiri A., Jury J.L., Montserrat J.M. (Eds.) (2002) *Catalogue des plantes vasculaires du nord du Maroc, incluant des cles d'identification. Checklist of vascular plants of N Morocco with identification keys, 2 vols*. Consejo Superior de Investigacions Científicas Madrid.

19. Mohagheghzadeh A., Hemmati S., Alfermann A.W. (2006) Quantification of aryltetralin lignans in *Linum album* organs and in vitro cultures. *Iranian Journal of Pharmaceutical Sciences* **2**, 47-56

20. Güvensen A., Seçmen Ö., Şenol S.G. (2013) Heterostyly in *Linum aretioides*. *Turkish Journal of Botany* **37**, 122-129.

21. Christodoulaki, D. (1995) A new subspecies of *Linum gyaricum* (Linaceae) from Greece. *Nordic Journal of Botany* **15**, 145–147.

22. Rogers C. M., Mildner, R. (1971) The reevaluation of the genus *Cliococca* (Linaceae) of South America. *Rhodora* **73**, 560-565.

23. Abrams, L. (1951) *Illustrated Flora of the Pacific States Washington, Oregon and California: Vol. 3*. Stanford University Press. Stanford, CA.

24. Linnaeus C. (1753) *Species Plantarum, Vol. 2*. Stockholm.

25. Culley T. M., Wolfe A. D. (2001) Population genetic structure of the cleistogamous plant species *Viola pubescens* Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. *Heredity* **86**, 545-556.

26. Harris B.D. (1968) Chromosome numbers and evolution in North American species of *Linum*. *American Journal of Botany* **55**, 1197-1204.

27. Ray C. (1944). Cytological studies on the flax genus (*Linum*). *American Journal of Botany* **31**, 241-248.

28. Rogers C.M., Mildner R., Harris, B.D. (1972) Some additional chromosome numbers in the Linaceae. *Brittonia* **24**, 313-316.

29. Pogan E., Jankun A., Turasla-Szybowska K. (1987) Further studies in chromosome numbers of Polish angiosperms. Part XX. *Acta Biologica Cracoviensia*, *Ser. Bot.* **29**, 1–17.

30. Chennaveeraiah M.S., Joshi K. K. (1983) Karyotypes in cultivated and wild species of *Linum*. *Cytologia* **48**, 833-841.

31. Mohamed M.K. (1997) Chromosome counts in some flowering plants from Egypt. *Egyptian Journal of Botany* **37**, 129-156.

32. Petrova A.V. (1972) In: Löve (Ed.), IOPB chromosome number reports XXXV. *Taxon* **21**, 161-166.

33. Rogers C.M. (1983) Chromosome numbers in some South African species of *Linum* L. (Linaceae). *Journal of South African Botany* **49**, 181-184.

34. Elena-Rosselló J.A., Zapatero M.A.G., Andrés F. N (1987) Números cromosmáticos de plantas occidentales, 411-419. *Anales del Jardín Botánico de Madrid* **43**, 417–424.

35. Seetharam A. (1972) Interspecific hybridization in *Linum*. *Euphytica* **21**, 489-495.

36. Baksay L. (1956) Cytotaxonomical studies in the Flora of Hungary. - *Ann. Hist. Mus. Nat. Hung.*, n.s. **7**, 321-334.

37. Jhala A.J., Hall L.M., Hall J. C. (2008) Potential hybridization of flax with weedy and wild relatives: an avenue for movement of engineered genes?. *Crop science* **48**, 825-840.

38. Van Loon J., Kieft B. (1980) In: Love (Ed.), IOPB chromosome number reports LXVIII. *Taxon* **29**, 538-542.

39. Fu Y.B., Allaby R.G. (2010) Phylogenetic network of *Linum* species as revealed by non-coding chloroplast DNA sequences. *Genetic Resources and Crop Evolution* **57**, 667-677.

40. Mugnier C. (1981) In: Love (Ed.), IOPB chromosome number reports LXXIII. *Taxon* **30**, 829-861.

41. González-Zapatero M. A., Elena-Roselló J. A., Andrés F. N. (1988) Números cromosómicos para la flora Española. *Lagascalia* **15**, 112-119.

42. Chichiricco G., Tammaro F. (1980) Numeri cromosomici per la flora italiana: 742-751. *Informatore Botanico Italiano* **12**, 161-165.

43. Muravenko O.V., Amosova A.V., Samatadze T.E., Semenova O.Y., Nosova I.V., Popov K.V., Zelenin A.V. (2004) Chromosome localization of 5S and 45S ribosomal DNA in the genomes of *Linum* L. species of the section *Linum* (Syn. *Protolinum* and *Adenolinum*). *Russian Journal of Genetics* **40**, 193-196.

6

44. Gregor T., Hand R. (2006) Chromosomenzahlen von Farn-und Samenpflanzen aus Deutschland 2. *Kochia* **1**, 135-140.

45. Ruiz de Clavijo E. (1993) Números cromosómicos para la flora española, 664–690. *Lagascalia* **17**, 161-172.

46. Magulaev A.V. (1984) Cytotaxonomic study in some flowering plants of the North *Caucasus. Bot. Zhurn. SSSR* **69**, 511–517.

47. Sokolovskaia A.P., Probatova N.S. (1985) Chromosome numbers in the vascular plants from the Maritime Territory, Kamchatka Region, Amur Valley and Sakhalin. *Botanicheskii zhurnal* **70**, 997–999.

48. Ghaffari S.M. (1987) Chromosome counts of some angiosperms from Iran: 2. *Iranian Journal of Bot*any **3**, 183-188.

49. Franzen R., Gustavsson L.Å. (1983) Chromosome numbers in flowering plants from the high mountains of Sterea Ellas, Greece. *Willdenowia* **13**, 101-106.

50. Phitos D. (1988) Chromosome numbers in some species of the Greek flora. *Bot. Chronika* **8**, 45-50.

51. Dressler S., Repplinger M., Bayer C. (2014) *Linaceae. In Flowering Plants. Eudicots*. Springer Berlin Heidelberg, pp. 237-246.

52. Murray B.G. (1986) Floral biology and self-incompatibility in *Linum*. Botanical Gazette **147**, 327-333.

53. Dulberger R. (1967) *Pollination systems in plants of Israel: heterostyly*. PhD thesis, Hebrew University, Jerusalem.

54. Dulberger R. (1974) Structural dimorphism of stigmatic papillae in distylous *Linum* species. *American Journal of Botany* **61**, 238-243.

55. Rogers C.M. (1979) Distyly and pollen dimorphism in *Linum suffruticosum* (Linaceae). *Plant Systematics and Evolution* **131**, 127-132.

56. Nicholls M.S. (1985) A systematic study of the *Linum tenuifolium* group (Linaceae). *Botanical Journal of the Linnean Society* **91**, 473-490.

57. Nicholls M.S. (1985) Pollen flow, population composition, and the adaptive significance of distyly in *Linum tenuifolium* L. (Linaceae). *Biological Journal of the Linnean Society* **25**, 235-242.

58. Nicholls M.S. (1985) The evolutionary breakdown of distyly in *Linum tenuifolium* (Linaceae). *Plant Systematics and Evolution* **150**, 291-301.

59. Lewis D. (1943) The physiology of incompatibility in plants: II. *Linum grandiflorum*. *Annals of Botany* **7**, 115-122.

60. Darwin C. (1877) *The different forms of flowers on plants of the same species*. John Murray, London.

61. Kearns C.A., Inouye D.W. (1994) Fly pollination of *Linum lewisii* (Linaceae). *American Journal of Botany* **81**, 1091-1095.

62. Dulberger R. (1973) Distyly in *Linum pubescens* and *L. mucronatum*. *Botanical Journal of the Linnean Society* **66**, 117-126.

63. Kress W.J., Wurdack K.J., Zimmer E.A., Weigt L.A., Janzen D.H. (2005) Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 8369-8374.

64. Wurdack K., Davis C. (2009) Malpighiales phylogenetics: gaining ground on one of the most recalcitrant clades in the angiosperm tree of life. *American Journal of Botany* **96**, 1551-1570.

65. Marcussen T., Jakobsen K.S., Danihelka J., Ballard H.E., Blaxland K., Brysting A.K., Oxelman B. (2012) Inferring species networks from gene trees in high-polyploid North American and Hawaiian violets (*Viola*, Violaceae). *Systematic Biology* **61**, 107-26.

66. Xi Z., Ruhfel B.R., Schaefer H., Amorim A.M., Sugumaran M., Wurdack K.J., Endress P.K., Matthews M.L., Stevens P.F., Mathews S., Davis C.C. (2012) Phylogenomics and a posteriori data partitioning resolve the Cretaceous angiosperm radiation Malpighiales. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 17519-7524.

67. Kersten T., Knoess W. (2008) Development and validation of molecular biological methods for identification of medicinal plants in herbal substances, herbal preparations and finished product. (Unpublished).

68. Sánchez Meseguer A., Aldasoro J.J., Sanmartín I. (2013) Bayesian inference of phylogeny, morphology and range evolution reveals a complex evolutionary history in St. John's wort (*Hypericum*). *Molecular Phylogenetics and Evolution* **67**, 379-403.

69. Koi S., Kita Y., Hirayama Y., Rutishauser R., Huber K.A., Kato M. (2012) Molecular phylogenetic analysis of Podostemaceae: implications for taxonomy of major groups. *Botanical Journal of the Linnean Society* **169**, 461–492.

70. Hengchang W., Moore M.J., Soltis P.S., Bell C.D., Brockington S.F., Alexandre R., Davis C.C., Latvis M., Manchester S.R., Soltis D.E., Crane P. (2009) Rosid radiation and the rapid rise of angiosperm-dominated forests. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 3853-858.

71. Davis C.C., Anderson W.R, Donoghue M.J. (2001) Phylogeny of Malpighiaceae: Evidence from Chloroplast NdhF and TrnL-F Nucleotide Sequences. *American Journal of Botany* **88**, 1830-846.

72. Zhang L.B, Simmons M.P. (2006) Phylogeny and delimitation of the Celastrales inferred from nuclear and plastid genes. *Systematic Botany* **31**, 122-37.

73. Papanicolau K. (1984) Reports p.130-131. – Löve, A. (Ed.): IOBP Chromosome number reports LXXVII. – *Taxon* **33**, 126-134.

74. Post G.E. (1896) *Flora of Syria, Palestine, and Sinai*. Syrian Protestant college, Beirut.

75. Planchon J.E. (1847-48) Sur la famille des Linées. *The London Journal of Botany* 6 (1847) & vol 7 (1848).

76. Huang C.J., Huang B.X., Xu L.R. (1998) *Linum* L. In: *Flora Reipublicae Popularis Sinicae 43*. Science Press, Beijing, pp 98–106.

77. Hallier H. (1921) Beiträge zur Kenntnis der Linaceae (DC. 1819) Dumort *Beihefte zum Botanischen Centralblatt Abt. II*. **39**, 1–178.

78. Narayana L.L., Rao D. (1978) Systematic position of Humiriaceae, Linaceae and Erythroxylaceae in the light of their comparative floral morphology and embryology – a discussion. *Journal of the Indian Botanical Society* **57**, 258-266.

79. Stafford M.J. (2011). Linaceae. *Flora Mesoamericana* **3**, 1-7.

80. Rogers C.M. (1980). In: Löve (Ed.), Chromosome number reports LXVII. *Taxon* **29**, 347-367.
81. Rogers C.M. (1982) The systematics of *Linum* sect. Linopsis (Linaceae). *Plant Systematics and Evolution* **140**, 225.
82. Gill S. (1987). *Linseed*. Indian Council of Agricultural Research Krishi Anusandhan Bhavan Pusa, New Delhi.
83. Devesa J.A., Talavera S., Galiano E.F. (1984) In A. Löve (Ed.). Chromosome Number Reports LXXXII. *Taxon,* **33**, 126-134.