

# Convergent origin of the narrowly lanceolate leaf in the genus *Aster*—with special reference to an unexpected discovery of a new *Aster* species from East China

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Narrowly lanceolate leaves occur frequently in the genus *Aster*. It was often employed as a distinguishing character in the taxonomy of this genus. The origin of this particular leaf shape, however, has never been investigated using comparative methods. In this study, we reconstructed a comprehensive phylogeny that includes most species of *Aster* with narrowly lanceolate leaf. We then gathered data on riparian habitats and the presence or absence of narrowly lanceolate leaves, and investigated the evolutionary association between them in a phylogenetic context. Our analysis indicated that the species with narrowly lanceolate leaves are nested in unrelated lineages of the genus *Aster*, implying that they originated independently several times. Using Pagel's comparative method of discrete data, we demonstrated a significant correlation between riparian habitats and narrowly lanceolate leaves. We further inferred the sequence of transition of the two characters. This analysis indicated that the sequence of evolution of riparian habitat and narrowly lanceolate leaf form was usually uncertain, but some positive results showed that the occurrence of riparian habitats may not precede the evolution of narrowly lanceolate leaf form. This study provided new insights into the adaptive evolution in a mega-diverse family. In addition, *Aster tonglingensis*, an unexpected new species with narrowly lanceolate leaves, was discovered and established based on the evidence from morphology, micromorphology and molecular phylogeny.

1 **Convergent origin of the narrowly lanceolate leaf in the genus *Aster* —with special reference**  
2 **to an unexpected discovery of a new *Aster* species from East China**

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## 28 **Abstract**

29 Narrowly lanceolate leaves occur frequently in the genus *Aster*. It was often employed as a  
30 distinguishing character in the taxonomy of this genus. The origin of this particular leaf shape,  
31 however, has never been investigated using comparative methods. In this study, we reconstructed  
32 a comprehensive phylogeny that includes most species of *Aster* with narrowly lanceolate leaf. We  
33 then gathered data on riparian habitats and the presence or absence of narrowly lanceolate leaves,  
34 and investigated the evolutionary association between them in a phylogenetic context. Our analysis  
35 indicated that the species with narrowly lanceolate leaves are nested in unrelated lineages of the  
36 genus *Aster*, implying that they originated independently several times. Using Pagel's comparative  
37 method of discrete data, we demonstrated a significant correlation between riparian habitats and  
38 narrowly lanceolate leaves. We further inferred the sequence of transition of the two characters.  
39 This analysis indicated that the sequence of evolution of riparian habitat and narrowly lanceolate  
40 leaf form was usually uncertain, but some positive results showed that the occurrence of riparian  
41 habitats may not precede the evolution of narrowly lanceolate leaf form. This study provided new  
42 insights into the adaptive evolution in a mega-diverse family. In addition, *Aster tonglingensis*, an  
43 unexpected new species with narrowly lanceolate leaves, was discovered and established based on  
44 the evidence from morphology, micromorphology and molecular phylogeny.

45

## 46 **Introduction**

47 How the environments modify morphology is one of the key questions in evolutionary  
48 biology (Grant & Grant, 2002; Lamichhaney et al., 2015; Malinsky & Salzburger, 2016; Meier et  
49 al., 2017). One focus is convergent evolution. Convergent evolution means that organisms from  
50 different lineages develop similar structures or forms in similar environments (Washburn et al.,  
51 2016). This phenomenon is widespread in plants, such as the lotus and water lilies, or the xeric  
52 highly succulent species of *Euphorbia*, Cactaceae and some species of Apocynaceae (McGhee,  
53 2011; Alvarado-Cárdenas, 2013).

54 Asteraceae is a young family that originated at ca. 69–85 Ma (Barreda et al., 2015; Panero &

55 Crozier, 2016). It is also the largest family of plants, containing nearly 30,000 species (Heywood,  
56 2009; Funk et al., 2009). Members of this mega-diverse family occur in a variety of habitats, thus  
57 providing an excellent opportunity to study convergent evolution (Heywood, 2009).

58 *Aster*, the type genus of Asteraceae, contains ca. 150 species mainly distributed in Eurasia  
59 (Nesom, 1994; Nesom & Robinson, 2007; Chen, Brouillet, & Semple 2011). Its diversity centre is  
60 from East Asia to Himalaya (Chen, Brouillet, & Semple, 2011). It occupies diverse habitats  
61 ranging from the Frigid Zone (e.g. *Aster alpinus*) to the Tropical Zone (e.g. *A. philipinensis*), from  
62 alpine talus (e.g. *A. prainii*) or alpine meadows (e.g. *A. flaccidus*) to forests (e.g. *A. ageratoides*)  
63 and coastal zones (e.g. *A. spathulifolius*). Some species occur in extreme dry hot valleys (e.g. *A.*  
64 *poliothamnus*) and others grow in wet places along the Yangtze River (e.g. *A. moupinensis*). The  
65 broad range and diversified habitats provide multiple niches and space for convergent evolution.  
66 For example, the short pappus, a character widely used in the taxonomy of *Aster*, was shown to be  
67 convergent (Ito et al., 1995). It has evolved several times within the genus *Aster*. Other characters  
68 with more plasticity, like leaf shape, however, have never been investigated in the genus.

69 Previous studies of the character of leaf shape in *Ainsliaea* (Asteraceae: Pertyeae) indicated  
70 that species growing in forests tend to have wide leaves which is of benefit to absorb sunlight  
71 (Mitsui et al., 2011; Mitsui & Setoguchi, 2012). In contrast, species growing along streams tend  
72 to have narrow leaves that can minimise any harm caused by water flow (Mitsui et al., 2011; Mitsui  
73 & Setoguchi, 2012). This narrow leaf shape of riparian species is an adaption to stream or river  
74 bank habitat. In the genus *Aster*, there are a few species with narrow leaves (e.g. *Aster*  
75 *dolichophyllus* Y. Ling, occurring as two small populations in Guangxi, China). To revise the  
76 genus *Aster*, we have conducted extensive field collections and observations in the field in Eurasia.  
77 During an expedition to south-eastern China in 2013, we encountered a distinctive species of *Aster*  
78 in Mt. Tongling National Forest Park in Wencheng county of Zhejiang province. It grew on rocks  
79 near a stream and had narrow leaves. In external morphology, it was very similar to *A.*  
80 *dolichophyllus* in having narrowly lanceolate, glabrous leaves and reflexed phyllaries. Similar  
81 morphology and habitat initially made us think that this plant might be conspecific with *A.*

82 *dolichophyllus*. However, the distance between them was more than 1000 km and there are many  
83 high mountains and big rivers separating the two places. So, our first question was: are these two  
84 *Aster* entities from these two distant places different populations of one species or are they two  
85 totally different species with similar morphology resulting from convergent evolution in similar  
86 habitats? There are also other species in *Aster* with similar narrowly lanceolate leaves and/or  
87 growing in riparian habitats, such as *Aster moupinensis* (Franch.) Hand.-Mazz., *A. rugulosus*  
88 Maxim., and *Turczaninovia fastigiata* (Fisch.) DC. (i.e. *A. fastigiatus* Fisch.). Our second question  
89 is whether the correlation between narrowly lanceolate leaves and stream habitats in *Aster* is  
90 significant statistically?

91 In this study, we aim to 1) use three molecular markers to reconstruct the phylogeny of *Aster*  
92 to resolve the phylogenetic positions of the *Aster* species with narrowly lanceolate leaf and test the  
93 monophyly of the two similar species of *Aster*; 2) use Pagel's trait evolution analysis methods  
94 (Pagel, 1994) to test whether the narrowly lanceolate leaf is significantly correlated with the  
95 riparian habitat, and if so to test the transformation ratio among four potential character  
96 combinations, and then to determine whether the riparian habitat drives the evolution of narrowly  
97 lanceolate leaf; and 3) examine the morphological and micro-morphological characters of the  
98 distinctive *Aster* species from Mt. Tongling and *A. dolichophyllus* to determine if the Tongling  
99 plant is a new species.

100

## 101 **Materials and Methods**

### 102 **Taxon sampling**

103 Seventy-three taxa were used for phylogenetic analysis, representing 19 related genera, the  
104 major clades of *Aster*, and one outgroup. Sequences of 71 of these species were downloaded from  
105 GenBank. Most species with narrowly lanceolate leaves in *Aster* were sampled. Five samples of  
106 the potential new taxon and six of the superficially similar *Aster dolichophyllus* were added in this  
107 study. The taxonomic treatment in the *Flora of China* and the definitions of *Aster* and "core *Aster*"  
108 in our previous study (Zhang et al., 2015) were followed. In the phylogenetic analysis,

109 *Chrysanthemum indicum* L. was designated as outgroup as in previous studies (Li et al., 2012;  
110 Zhang et al., 2015). The ITS, ETS and *trnL-F* sequences were selected as molecular markers to  
111 generate the datasets. According to previous studies (Li et al., 2012; Zhang et al., 2015), the  
112 systematic position of shrub and alpine *Aster* groups are distinct from the core *Aster* (sensu Zhang  
113 et al., 2015; including the type of genus, *Aster amellus*) and may represent separate genera.  
114 Additionally, the habit of these two groups are significantly different from the core *Aster*.  
115 Therefore, in the character correlation analysis, a reduced data set was built to reconstruct the  
116 phylogeny of *Aster*. It was composed of the species above the clade of *Aster nitidus* Y. Ling and  
117 *A. hersileoides* Schneid. in Fig. 1. Two species, *Aster nitidus* and *A. hersileoides*, were set as roots  
118 according to our phylogeny and previous results (Li et al., 2012; Zhang et al., 2015). Voucher  
119 specimens for newly sequenced samples were deposited in PE. Voucher information and GenBank  
120 accession numbers are listed in Table S1.

121

### 122 **DNA extraction, amplification, and sequencing**

123 Leaf tissues were collected in the field and dried using silica gel. DNA extraction,  
124 purification, and sequencing followed the methods described by Zhang et al. (2015). Methods of  
125 PCR amplification of ITS and ETS sequences followed Linder et al. (2000), *trnL-F* sequence  
126 followed Zhang et al. (2015). The ITS primers of Linder et al. (2000), ETS primers “Ast-8”  
127 (Markos and Baldwin, 2001) and “18S-IGS” (Baldwin & Markos, 1998) and *trnL-F* primers “c”  
128 and “f” of Taberlet et al. (1991) were used.

129

### 130 **Phylogenetic analysis**

131 DNA sequences alignment was fulfilled using MAFFT online version (Katoh et al., 2017),  
132 and then was manually adjusted using BioEdit v7.0.8.0 (Hall, 1999). jModelTest 2.1.4 (Darriba et  
133 al., 2012) was used to select DNA substations module based on the Akaike information criterion  
134 (AIC). The GTR + G model was fit for ETS and ITS, and TVM + G model for *trnL-F*. Phylogenetic  
135 analyses were then conducted for two individual datasets, one consisting of ITS and ETS

136 sequences, another consisting of *trnL-F*, and a combinative dataset. Phylogenetic trees were  
137 reconstructed using Maximum Likelihood methods and Bayesian Inference. Bootstrap support  
138 values (BS) for ML tree were calculated using 1000 bootstrap replicates. Bayes inference was  
139 performed with 20 million generations, tree sampled every 1000 generations. Bayesian posterior  
140 probabilities were calculated after omitting the first 500 trees (burn-in = 0.25). Analyses were done  
141 using RAxML 8.0.24 (Stamatakis, 2014) and MrBayes 3.2.4 (Ronquist et al., 2012) on the CIPRES  
142 science gateway portal (Miller, Pfeiffer, & Schwartz, 2010). The parameter settings in Zhang et  
143 al. (2015) were followed.

144

#### 145 **Correlation evolution analysis**

146 The Maximum likelihood and Bayesian methods for discrete character analyses (Pagel, 1994)  
147 were used. These analyses were accomplished in the program BayesTraits v 2.0 (Meade & Pagel,  
148 2014). In order to reveal whether the leaf shape evolution and habitat are correlated, two traits  
149 including habitat (riparian versus non-riparian) and leaf shape (narrowly lanceolate (width/length  
150 < 0.15, see below) versus not narrowly lanceolate) were used to make the dataset. Habitat data  
151 were collected from floras (Chen, Brouillet, & Semple, 2011), herbarium specimens (kept in PE),  
152 and our long-termed field observations in Eurasia. Due to the complexity of plant habitats, two  
153 definitions of riparian were used in the analysis (Table S2). One is a narrowly riparian habitat. All  
154 or the vast majority of individuals of species associated with narrowly riparian habitats occur only  
155 on the banks of rivers or streams. The other is a broadly riparian habitat. Species associated with  
156 this habitat occur not only on river or stream banks but also in other wet habitats (such as swamps  
157 and pool margins). The definition of leaf shape is based on the leaf shape index. It was obtained  
158 by dividing the length of each leaf by the width and then calculating the arithmetic mean of all  
159 specimens of each species. We measured the length and width of three middle cauline leaves of  
160 each specimen and for each species we measured ten specimens. These specimens were from PE,  
161 K, E, BM, and PRC herbaria. For the shrubby species, we measured the middle leaves of the first  
162 branch, as the main stem was usually leafless. For the species with a solitary capitulum, we

163 measured lower leaves, as the middle part of the stem is leafless. In this study, leaves with a leaf  
164 shape index less than 0.15 were defined as narrowly lanceolate. This criterion approaches the  
165 traditionally recognized index (Stearn, 1985). Besides the narrowly lanceolate leaf shape, there  
166 were many other types of leaf shape that could be shaped by various factors. To eliminate the  
167 influence of these factors and to focus on the correlation between narrowly lanceolate leaf shape  
168 and riparian habitat, leaf index and habitat were treated as binary characters.

169 For the character correlation analysis, the DISCRETE module of BayesTraits v 2.0 (Meade &  
170 Pagel, 2014) that support binary characters was used to analyse the correlation of the two binary  
171 characters above (the first character is habitat, state 0 means non-riparian and state 1 means  
172 riparian; the second character is leaf shape index, state 0 means the index is more than 0.15 and  
173 state 1 means less than (including) 0.15; see Fig. 2). Two models were provided in this module,  
174 i.e. the dependent model and the independent model. We checked which model best fitted our data  
175 by comparing the maximum likelihood value obtained via the Maximum Likelihood (ML) method  
176 and the marginal likelihood value obtained via the Markov Chain Monte Carlo (MCMC) method.  
177 In the ML analyses, searching times for the maximum likelihood value of each calculation was set  
178 at 1000 and each calculation was repeated 10 times. In MCMC method analyses, priors were set  
179 as Gamma hyper-prior (Pagel, Meade, & Barker, 2004) with default parameters. Marginal  
180 likelihood values were obtained by the stepping stones method (Xie et al., 2011). Based on the  
181 results of our preliminary analyses, the number of iterations was set at ten million with 100  
182 stepping stones. Each calculation was repeated 10 times and then the final marginal likelihood  
183 value was obtained from the mean of ten marginal likelihood values. The parameter restriction test  
184 (Pagel, 1994) was used to determine the order of trait evolution. Each parameter (Fig. 2) was  
185 respectively set as zero in different runs to determine if any trait transition could be excluded from  
186 the process of trait evolution. Contingent change and temporal order test (Pagel, 1994) were  
187 employed to determine the dependence between the two traits and the acquisition order. The  
188 likelihood values of different analyses were compared using the likelihood ratio test (LRT, for ML  
189 results) and the Bayes Factors (BF, for MCMC results) test (Gilks, Richardson, & Spiegelhalter,



190 1996) following the procedure recommended in the manual of BayesTraits v2 (Meade & Pagel,  
191 2014).

192

### 193 **Morphological and micro-morphological observations**

194 For the description and the line drawings of the new species, living plants and herbarium  
195 specimens were examined by naked eye and under stereomicroscope. Living plants as well as FAA  
196 fixed materials were measured. The morphological comparison with other species of *Aster* was  
197 based on the study of herbarium specimens from PE (Chinese National Herbarium, Institute of  
198 Botany, Chinese Academy of Sciences).

199 The micro-morphological characters of the new species and its superficially similar species  
200 *Aster dolichophyllus* were examined. Voucher information of the materials is listed in Table S1.  
201 Anderson's sectioning method (Anderson, 1954) was followed. For herbarium specimens, the  
202 capitula were stored in FAA solution for 24 hours to soften tissues. The materials were then  
203 cleaned in a supersonic generator for 5 minutes at a frequency of 100 Hz. They were then  
204 transferred into a 5% NaOH solution and kept for 12 hours for the study of anthers and 6 hours for  
205 corolla and style. After cleaning with distilled water, the samples were transferred into a drop of  
206 Hoyer's solution on microscope slides, and observed and photographed using a Leica DM5000B  
207 microscope. The corolla, filament collar, base and tip appendages of anthers, endothelial tissue,  
208 the stylopodium, stigmatic lines, and tip appendages of style were observed and measured under  
209 the light microscopy.

210

### 211 **New taxon and the LSID statement**

212 The electronic version of this article in Portable Document Format (PDF) will represent a  
213 published work according to the International Code of Nomenclature for algae, fungi, and plants  
214 (ICN), and hence the new names contained in the electronic version are effectively published under  
215 that Code from the electronic edition alone. In addition, new names contained in this work which  
216 have been issued with identifiers by IPNI will eventually be made available to the Global Names

217 Index. The IPNI LSIDs can be resolved and the associated information viewed through any  
218 standard web browser by appending the LSID contained in this publication to the prefix  
219 "http://ipni.org/". The online version of this work is archived and available from the following  
220 digital repositories: PeerJ, PubMed Central, and CLOCKSS.

221

## 222 **Results**

### 223 **Phylogenetic results**

224 When we compared the phylogenetic trees separately reconstructed based on the chloroplast  
225 and nuclear matrices, no obvious topology conflict was found. The two matrices therefore were  
226 combined in the following analyses. Consensus tree from BI analyses had nearly identical  
227 topologies with the ML tree. The best ML tree (-lnL = 22036.02) is presented in Fig. 1. The  
228 topologies of our phylogenetic tree were largely consistent with previous studies (Li et al., 2012;  
229 Zhang et al., 2015). The species with narrowly lanceolate leaf were nested in different lineages on  
230 the tree (Fig. 1). Among them, *A. moupinensis* formed a well-supported clade with two species  
231 without narrowly lanceolate leaves (*A. smithianus* Hand.-Mazz. and *A. heterolepis* Hand.-Mazz.)  
232 (Fig. 1, BS = 85, PP = 1.00) nested in the core *Aster* clade; *A. sinoangustifolius* Brouillet, Sempé  
233 et Y.L. Chen lay at the base of the core *Aster* clade with strong support (Fig. 1, BS = 99, PP =  
234 1.00); *A. rugulosus* was resolved as sister to *A. scaber* Thunb. (Fig. 1, BS = 100, PP = 1.00);  
235 *Sheareria*, the monotypic semi-aquatic genus, was placed in the core *Aster* clade, and resolved as  
236 sister to a clade consisting of the taxa from *A. fanjingshanicus* Y.L. Chen & D.J. Liu to *A. souliei*  
237 Franch. with moderate support (Fig. 1, BS = 78, PP < 0.90); *Turczaninowia fastigiata* formed a  
238 weakly supported clade (Fig. 1, BS = 67, PP < 0.90) with *Aster procerus* Hemsl.; *Arctogeron*  
239 *gramineum* (L.) DC. was resolved as sister to a clade consisting of the taxa from *Asterothamnus*  
240 *fruticosus* (C. Winkl.) Novopokr. to *Aster poliothamnus* Diels with moderate support (Fig. 1, BS  
241 = 67, PP = 0.94). Besides these species, the distinctive *Aster* species from Mt. Tongling (formally  
242 described as *Aster tonglingensis* below) and the similar species *Aster dolichophyllus* had similar  
243 narrowly lanceolate leaves. The phylogenetic results show that all individuals of *Aster*

244 *tonglingensis* formed a strongly supported monophyletic clade (Fig. 1, BS = 100, PP = 1.00). It  
245 was nested in the strongly supported core *Aster* clade (Fig. 1, BS = 98, PP = 1), weakly resolved  
246 as sister to the subclade containing *Aster tianmenshanensis* G.J. Zhang and *A. verticillatus*  
247 (Reinw.) Brouillet, Semple & Y.L. Chen (Fig. 1, BS < 50, PP < 0.90). All individuals of *A.*  
248 *dolichophyllus*, formed another strongly supported clade nested outside of the core *Aster* (Fig. 1,  
249 BS = 100, PP = 1.00).

250

### 251 **Correlation analysis of characters**

252 Leaf shape indexes and the habitat information were kept in Table S2. For correlation analysis  
253 between leaf shape and broadly riparian habitat using ML method, the mean of the maximum  
254 likelihood value of the independent model was -33.85, that of the dependent model was -19.92,  
255 the likelihood ratio (LR) was 27.86, and the p-value of likelihood ratio (LRT) was smaller than  
256 0.00001. For MCMC method, the mean of the log marginal likelihood value of the independent  
257 model was -36.85, the mean marginal likelihood value of the dependent model was -29.15, and  
258 the Log Bayes Factor was 15.40. For the correlation analysis between leaf shape and narrowly  
259 riparian habitat of ML method, the mean of the maximum likelihood value of the independent  
260 model was -29.70, that of the dependent model was -22.45, LR was 14.48, and the p-value of LRT  
261 was 0.0059. For MCMC method, the mean of the log marginal likelihood value of the independent  
262 model was -32.73, that of the dependent model was -28.84, and Log BF was 7.78. The detailed  
263 results of the analyses and the test of parameter restrictions are listed in Table S3.

264

### 265 **Morphological and micro-morphological observation**

266 *Aster tonglingensis* is similar to *A. dolichophyllus* in external morphology. They have similar  
267 narrowly lanceolate and leathery cauline leaves, and reflexed phyllaries (Fig. 3 & 4). But they are  
268 different in the shape of the basal leaves, leaf indument, bracteal leaves, and number of phyllaries  
269 series (Table 1). *Aster tonglingensis* has long petiolate and lanceolate basal leaves (Fig. 3F & 4A),  
270 a puberulent adaxial leaf surface (Fig. 4A), more than 30 capitula, single or several in terminal and

271 axillary corymbs (Fig. 3E & 4A), whereas *Aster dolichophyllus* has spatulate and sessile basal  
272 leaves, a glabrous adaxial leaf surface, less than 10 capitula in a loose terminal corymb, and  
273 capitula never axillary. *Aster tonglingensis* has phyllary-like bracteal leaves and 5–7-seriate  
274 phyllaries (Fig. 3C), whereas *A. dolichophyllus* has bracteal leaves that are not phyllary-like and  
275 2–3-seriate phyllaries. *Aster tonglingensis* differs from the closely related *A. tianmenshanensis*  
276 (Table 1) by its greater height (70–100 cm versus ca. 10 cm in *A. tianmenshanensis*), narrowly  
277 lanceolate leaves (versus spatulate) and more capitula (more than 30 versus only one), and differs  
278 from *A. verticillatus* (Table 1) by having large capitula (20–25 mm in diameter versus ca. 10 mm  
279 in *A. verticillatus*), beakless achenes (versus beaked in *A. verticillatus*), and a robust pappus  
280 (versus a readily caducous pappus in *A. verticillatus*).

281 In micro-morphological characters (Fig. 5 & 6), both *Aster tonglingensis* and *A.*  
282 *dolichophyllus* have lanceolate style branches (Fig. 5A & 6A), triangular style appendages (Fig.  
283 5A & 6A), constricted style base (Fig. 5B & 6B), thickened filament collar (Fig. 5E & 6E), and  
284 obtuse and untailed anther base (Fig. 5E & 6E). However, *A. tonglingensis* differs from the latter  
285 (Table 1) by having long stigmatic lines equal to the length of the sterile style tip appendages (Fig.  
286 5A) (versus shorter than the sterile style tip appendages in *A. dolichophyllus*, Fig. 6A), disc corolla  
287 lobes split to two thirds or three fourths of the limb of the disc floret corolla (Fig. 5C) (versus split  
288 to one third in *A. dolichophyllus*, Fig. 6C), narrowly triangular anther tip appendages with length-  
289 width ratio ca. 2 (Fig. 5D) (versus triangular with length-width ratio ca. 1.5 in *A. dolichophyllus*,  
290 Fig. 6D), and a majority of anther endothelial cells polarized thickened (Fig. 5F) (versus radially  
291 thickened in *A. dolichophyllus*, Fig. 6F).

292 *Aster tonglingensis* is also different from its related species morphologically (Table 1). It  
293 differs from *A. tianmenshanensis* (Zhang et al., 2015) by having stigmatic lines as long as the  
294 sterile style tip appendages (Fig. 5A) (versus only one third as long as the appendages in *A.*  
295 *tianmenshanensis*), disc corolla lobes split to two thirds or three quarters of the limb of the disc  
296 floret corolla (Fig. 5C) (versus half way in *A. tianmenshanensis*), narrowly triangular anther tip  
297 appendages with length-width ratio ca. 2 (Fig. 5D) (versus triangular with length-width ratio ca.

298 1.5 in *A. tianmenshanensis*), and a majority of anther endothelial cells polarized thickened (Fig.  
299 5F) (versus radially thickened in *A. tianmenshanensis*). It differs from *A. verticillatus* by the latter  
300 having stigmatic lines two time longer than appendages, disc lobes split for half the length of the  
301 limb, and the majority of anther endothelial cells radially thickened (Zhang et al., 2015).

302

## 303 **Discussion**

### 304 **Convergent evolution of the narrowly lanceolate leaf in the genus *Aster***

305 In our study, the traditionally defined *Aster* (Ling, Chen, & Shih, 1985; Nesom, 1994; Chen,  
306 Brouillet, & Semple, 2011) was not a monophyletic group. Some genera like *Asterothamnus*,  
307 *Rhinactinidia*, *Arctogeron*, and *Myriactis* were nested within different clades of *Aster* and formed  
308 a weakly supported clade (BS < 50, PP < 0.9) with members of the traditionally defined *Aster*.  
309 This result was congruent with previous studies (Li et al., 2012; Zhang et al., 2015). Our molecular  
310 phylogenetic analysis indicated that the species with narrowly lanceolate leaves were nested in  
311 distantly related lineages of the genus *Aster*, implying that they originated independently at least  
312 eight times (Fig. 1). Narrowly lanceolate leaves are the results of convergent evolution in the genus  
313 *Aster*.

314

### 315 **Correlation evolution between leaf shape and habitat**

316 In our analysis, for broadly riparian habitat, p-value of LRT of ML method between two models  
317 was smaller than 0.01 and Bayes Factor of MCMC method was 15.40. These results suggested that  
318 the riparian habitat and narrowly lanceolate leaf shape was strongly correlated. The results of ML  
319 method parameter restriction tests showed all parameter could be exclusive (no significant  
320 difference with zero). This means every character state transitions were possible. When we set the  
321 opposite transition rates as equal, there was no significant difference comparing to unequal. This  
322 results showed that the order of character state transition was not clear. The single parameter test  
323 with MCMC method showed that parameter q24 was strongly supported to differ from zero (BF =  
324 5.34) and parameters q21 (BF = 2.05) and q34 (BF = 3.08) were positively supported to differ

325 from zero. Other parameters did not differ from 0 in these test. To set the opposite parameters as  
326 equal, the results positively supported q13 is not equal to q24 (BF = 3.01). The dependent model  
327 test showed q13 (7.08) was much smaller than q24 (62.90). These results indicated that, compared  
328 to the plants with wide leaves, the plants with narrow leaves tended to transfer more frequently to  
329 a riparian habitat. But our temporal order test showed that no significant order could be recognized.  
330 Therefore, we could not determine whether the riparian habitat or narrowly lanceolate leaves came  
331 first in this adaptive process.

332         Some broadly riparian species are not strictly growing on stream banks. So we did the same  
333 test for narrowly riparian habitat species. Most results are similar to the analysis for broadly  
334 riparian habitat. In ML method analysis, the likelihood ratio of two models is 14.48 with p-value  
335 less than 0.01. The Bayesian factor of MCMC method is 7.78. These results showed that the  
336 riparian habitat was strongly correlated with narrowly lanceolate leaves. However, the single  
337 parameter test with ML method showed that no parameter was strongly supported to differ from  
338 zero. The order of character states could not be fixed. The MCMC method parameter restriction  
339 tests showed that q12 (BF = 7.11) and q24 (BF = 6.25) were strongly supported to differ from zero,  
340 q21 (BF = 2.41) and q34 (BF = 2.56) are positively supported to differ from zero. Other parameters  
341 were not supported as different from zero. These results showed that the transitions from wide  
342 leaves to narrowly lanceolate leaves in non-riparian habitat and from non-riparian habitat to  
343 riparian habitat with narrow leaves cannot be ignored. These transitions indicated the potential  
344 path from non-riparian with wide leaves to riparian with narrow leaves in genus *Aster*.  
345 Furthermore, when setting  $q13 = q24$ , the negative result was supported. This result showed that  
346 the habitat changed from non-riparian to riparian likely depending on the narrowly lanceolate leaf  
347 shape. The test positively supported the q34 differs from zero. We also could find that q34 (=   
348 54.63) were much large than q12 (= 12.06). These results suggested that the habitat was more  
349 likely to change from non-riparian to riparian when the plants have narrowly lanceolate leaves.  
350 Our directivity test showed that the q12 was not significantly different from q13. But q12 (12.06)  
351 was larger than q13 (7.08). Furthermore, q12 was proved significantly different from zero whereas

352 q13 was not. This showed the rate of transition from wide leaf in non-riparian habit to narrowly  
353 lanceolate leaf in non-riparian habitat was higher than the rate of transition from wide leaf in non-  
354 riparian habitat to wide leaf in riparian habitat. Based on the results above, we propose that the  
355 narrowly lanceolate leaves trait acquisition was likely earlier than the riparian habitat acquisition  
356 in these riparian species with narrowly lanceolate leaves.

357 In our analysis, in both the broadly riparian species and the narrowly riparian species of *Aster*,  
358 habitat was strongly correlated with leaf shape (p-value < 0.00001 and BF = 15.40 for broadly  
359 riparian habitat; p-value < 0.01 and BF = 7.78 for narrowly riparian habitat). Our MCMC test  
360 supported that plants with narrowly lanceolate leaves were more likely to change to riparian habitat  
361 than those with wide leaves (q24 much large than q13, see Table S3). Our directivity analysis  
362 showed that some *Aster* species may have effectively employed a preadaptation strategy (Shelley,  
363 1999; Kangas, 2004; Losos, 2013), i.e. developing narrowly lanceolate leaves first, and then  
364 adapting to the riparian habitat. This pre-adaptation strategy could reduce the risk when plants  
365 encountered new habitats by chance (Shelley, 1999; Kangas, 2004; Losos, 2013). As shown in the  
366 case of *Ainsliaea* (Mitsui et al., 2011), individuals with broader leaves could be swept away under  
367 strong selection pressure within the species (e.g. damage to the broader leaves by strong water  
368 flow), while members with narrowly lanceolate leaves could survive. But the majority of our single  
369 parameter tests was not strongly supported (p-value > 0.01 and BF < 2), suggesting that the process  
370 of adaptation to the riparian habitat in genus *Aster* may be very complicated. Other factors could  
371 also contribute to the formation of narrowly lanceolate leaves. For instance, *Arctogeron*  
372 *gramineum*, a species having narrowly lanceolate leaves grows in extremely dry habitat instead of  
373 riparian habitats. Thus, although our results supported the strong correlation between narrowly  
374 lanceolate leaves and riparian habitat in the genus *Aster*, the details of the biological connections  
375 between them could be complicated.

376 To sum up, the riparian habitat and narrowly lanceolate leaf shape were strongly correlated in  
377 the genus *Aster* based on our comparative analysis. Some test results suggested that the pre-  
378 adaptation strategy could be an important factor in the adaptation of the *Aster* species to the riparian

379 habitat.

380

381 ***Aster tonglingensis* as a new species: evidence from morphology, micromorphology and**  
382 **molecular phylogeny**

383 In the phylogenetic tree, all individuals of *A. tonglingensis* formed a strongly supported clade  
384 nested in the core *Aster* clade (Fig. 1). All the accessions of the similar *A. dolichophyllus* formed  
385 a strongly supported clade outside this clade (Fig. 1). In summary, our molecular analysis indicated  
386 that *A. tonglingensis* was a strongly supported monophyletic group and a unique lineage quite  
387 distinct from the lineage including *A. dolichophyllus* (Fig. 1).

388 Although they look similar, *Aster tonglingensis* and *A. dolichophyllus* are different in many  
389 characters. Both of them do have similarly shaped narrowly lanceolate, leathery cauline leaves,  
390 reflexed phyllaries, and almost glabrous leaf surfaces (Fig. 3 & 4). However, the shape of their  
391 basal leaves is totally different. The basal leaves of *A. tonglingensis* are lanceolate with a long  
392 petiole (Fig. 3F & 4A), whereas those of *A. dolichophyllus* are spatulate and sessile. In the previous  
393 studies of *Aster* (Ling, Chen & Shih, 1985; Nesom, 1994; Chen, Brouillet, and Semple, 2011)  
394 based on herbarium specimens, many lacked descriptions of the basal leaves because in many  
395 species these were withered by the time of flowering. The present study showed that the character  
396 of the basal leaves can be taxonomically important. In addition, many floral characters of these  
397 two species are different. For instance, *A. tonglingensis* differs from *A. dolichophyllus* by having  
398 terminal and axillary corymbs and more than 30 capitula (Fig. 3 & 4), whereas the latter species  
399 has a lax terminal corymb with usually fewer than 10 capitula. Axillary corymbs (Fig. 3E) are rare  
400 in Eurasian *Aster*, such as *A. turbinatus* and *A. verticillatus*. Both *Aster tonglingensis* and *A.*  
401 *dolichophyllus* have reflexed phyllaries, but the 5–7-seriate phyllaries and the enormous linear  
402 bracteal leaves below the involucre of *A. tonglingensis* (Fig. 3C) show the obvious differences in  
403 comparison with the 2–3-seriate phyllaries and few lanceolate bracteal leaves of *A. dolichophyllus*.  
404 *Aster tonglingensis* has longer disc corolla lobes, about two thirds as long as the limb of the floret  
405 (Fig. 5C), whereas the lobes of *A. dolichophyllus* are only one third as long as the limb.



406 Furthermore, the disc florets of *A. tonglingensis* (ca. 5–7 mm in length, Fig. 4G) are significantly  
407 smaller than those of *A. dolichophyllus* (ca. 9–11 mm in length). These differences of floret  
408 characters may be related to their pollination.

409 Our molecular analysis shows that the most closely related species of *Aster tonglingensis* are  
410 *A. tianmenshanensis* and *A. verticillatus*. However, both of them differ from *A. tonglingensis* in  
411 their gross morphology: *A. tianmenshanensis* is a small herb with a solitary capitulum and spatulate  
412 leaf blades growing on limestone cliffs. *A. verticillatus* has tiny capitula (involucre ca. 2–7 mm in  
413 diameter), and beaked achenes with a readily caducous pappus.

414 Micro-morphological characters are useful in the taxonomy of *Aster*. For instance, Zhang et  
415 al. (2015) suggested the morphology of the stigmatic lines was an important character to  
416 distinguish different species. Similarly, the stigmatic lines could distinguish *A. tonglingensis* from  
417 the externally similar species as well as the closely related species. The stigmatic lines of *A.*  
418 *tonglingensis* are as long as the sterile style tip appendages (Fig. 5A), while those of *A.*  
419 *dolichophyllus* are two times longer than the sterile style tip appendages, those of *A.*  
420 *tianmenshanensis* are one third as long, and those of *A. verticillatus* are three times as long. This  
421 character is expected to be related to their pollination. In addition, the anther endothelial cells and  
422 shape of sterile anther tip appendages are also different in these species (Fig. 5) (also see Zhang et  
423 al., 2015).

424 To sum up, *Aster tonglingensis* is different from *A. dolichophyllus* in both gross  
425 morphological and micro-morphological characters. Therefore, *A. tonglingensis* is a unique new  
426 taxonomic entity. Moreover, our molecular phylogenetic analysis showed that *A. tonglingensis* is  
427 a unique lineage (Fig. 1). So we treated it as a new species. The similar narrowly lanceolate leaves  
428 shared by *A. tonglingensis* and *A. dolichophyllus* are probably the result of convergent evolution.

429

#### 430 **Taxonomic treatment**

431 *Aster tonglingensis* G.J. Zhang & T.G. Gao, *sp. nov.* Fig. 4.

432 **Type:** CHINA. Zhejiang Province, Wencheng County, Mt. Tongling, elev. 640 m, 119° 52' E,

433 27° 49' N, 2<sup>nd</sup> Sept. 2013, *H. H. Hu 331-1* (holotype PE!, isotype PE!).

434 **Diagnosis:** The new species superficially resembles *Aster dolichophyllus* Ling. Both species have  
435 narrowly lanceolate cauline leaves, recurved phyllary tips, and occur near streams. However, the  
436 phyllaries of *Aster tonglingensis* were 5–7-seriate, green (vs. 2–3-seriate, green with purple tip in  
437 *A. dolichophyllus*), capitula usually more than 30, both terminal and axillary (vs. less than 10, only  
438 terminal), adaxial surface of all leaves puberulent (vs. glabrous), basal leaves lanceolate, apex  
439 rounded or obtuse (vs. spatulate, apex acute), corolla of disk floret 5–7 mm, lobes half to two thirds  
440 as long as limb (vs. corolla 9–11 mm, lobes one third as long as limb), pappus whitish (vs. slightly  
441 brown).

442 Perennial herb. Rhizomes thin, transverse, slightly woody, 3–15 cm long, ca. 0.3–0.5 cm in  
443 diameter, sometimes expanded near the base of stem becoming a 2–4 cm hard node. Stem solitary  
444 or two to three together, erect, 70–100 cm high (including inflorescence), unbranched except for  
445 inflorescence, lower part glabrous, upper part puberulous, leafy. Leaves of rosette lanceolate, 4–  
446 18 × 0.8–2.5 cm, base gradually narrowing, margin serrately four to eight toothed, petiole 3–10  
447 cm long, apex acute; lower cauline leaves similar to rosette leaves, sessile or petiole obscure,  
448 narrowly lanceolate, 4–13 × 0.4–1 cm, margin entire or serrately 3–5-toothed, base gradually  
449 narrowing, apex acute; all leaves thinly leathery, abaxially glabrous and light green, main vein and  
450 lateral veins prominent, adaxially puberulent, dark green and glossy. Capitula usually more than  
451 30, in one to five terminal and axillary corymbs, peduncle puberulous, with dense bracteal leaves,  
452 bracteal leaves ciliate, abaxially glabrous, adaxially densely puberulous; involucre campanulate,  
453 ca. 8–10 mm long, 5–8 mm in diameter, phyllaries in 5–7 imbricate series, green, lanceolate, 5–7  
454 × 1–1.5 mm, hardened at their bases, herbaceous above, the outer shorter than the inner, ciliate,  
455 upper part of abaxial surface densely puberulous, with a revolute acute apex, ca. 1 mm long, both  
456 surfaces densely puberulous. Ray florets ca. 15, female, with a greenish, glabrous tube ca. 3 mm  
457 long; ligules whitish, lanceolate 7–10 × ca. 2 mm, with four nerves, apex with two or three teeth.  
458 Disc florets many, hermaphrodite, corolla greenish white to yellow, tube greenish and puberulent  
459 at the top, ca. 3 mm long, thin but expanded at base, lobes five, lanceolate, unequal, two thirds as

460 long as limb. Achenes of both florets identical, narrowly oblong, four-ribbed, ca. 2 mm long,  
461 puberulous, lower part densely so; pappus uniseriate, whitish, bristles barbellate, ca. 7 mm long,  
462 nearly as long as disc corolla at anthesis. Flowering in July.

463 **Etymology:** The species is named after its type locality, Mt. Tongling, Wencheng County,  
464 Zhejiang Province, China.

465 **Conservation status:** *Aster tonglingensis* is a very narrowly distributed species and is currently  
466 known only from one stream in Mt. Tongling Natural Reserve. A population with ca. 100  
467 individuals was found along the stream. We scoured nearby places with similar habitats in this  
468 region but failed to find more populations. This part of the natural reserve currently is open to  
469 tourists. A footpath was built along this stream which passes through its location. The habitat of  
470 *A. tonglingensis* is easily disturbed or damaged. According to Criteria B2a of International Union  
471 for Conservation of Nature Red List Categories, this species should be treated as Critically  
472 Endangered. More attention and protection should be paid to this new but vulnerable species.

473 **Additional specimens examined (paratypes):** CHINA. Zhejiang province, Wencheng county,  
474 Mt. Tongling, elev. 640 m, 119° 52' E, 27° 49' N, 2<sup>nd</sup> Sept. 2013, *H. H. Hu* 331-2, 331-3, 331-4  
475 & 331-5 (PE !).

476

## 477 **Conclusions**

478 Leaf shape has been used as an important character in the taxonomy of *Aster* for a long time  
479 (Chen, Brouillet, & Semple, 2011). The relationship between it and the environment, however, has  
480 never been investigated. In the present study, a phylogeny including most species with narrowly  
481 lanceolate leaf in *Aster* was reconstruction based on three molecular markers. It was revealed that  
482 species with narrowly lanceolate leaves were placed in far related lineages of the genus *Aster* (Fig.  
483 1). Thus, the narrowly lanceolate leaf shape originated independently several times in the genus  
484 *Aster*. It was the results of convergent evolution. Comparative analysis in the phylogenetic context  
485 revealed that narrowly lanceolate leaf shape and riparian habitat were strongly correlated. The

486 transition order of riparian habitat and narrowly lanceolate leaf was shown to be usually uncertain.  
487 But the preadaptation of the narrowly lanceolate leaf was positively supported by some analysis  
488 (Fig. 2). In summary, convergent evolution and preadaptation may play important roles in the  
489 evolution of leaf shape in the genus *Aster*. Meanwhile, an unexpected new species with narrowly  
490 lanceolate leaves, *Aster tonglingensis*, was discovered and established based on the evidence of  
491 molecular, morphology and micro-morphology. This new species was described and illustrated  
492 here.

493 Asteraceae is the largest and relatively young plant family (Funk et al., 2009; Heywood,  
494 2009). Simultaneously, members of this mega-diverse family show abundant morphological  
495 diversity (Funk et al., 2009). They occur in almost every corner of the earth and occupy various  
496 habitats (Funk et al., 2009), thus providing an excellent opportunity to study convergent evolution  
497 (Heywood, 2009). The present study provided new insights into the process of convergent  
498 evolution of leaf form in a big genus of this mega-diverse family. In turn, understanding more  
499 details of the convergent evolution in this family helped to discover the cryptic biodiversity before  
500 they go extinct, as shown in the unexpected discovery of the new species *Aster tonglingensis* in  
501 this study.

502

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507

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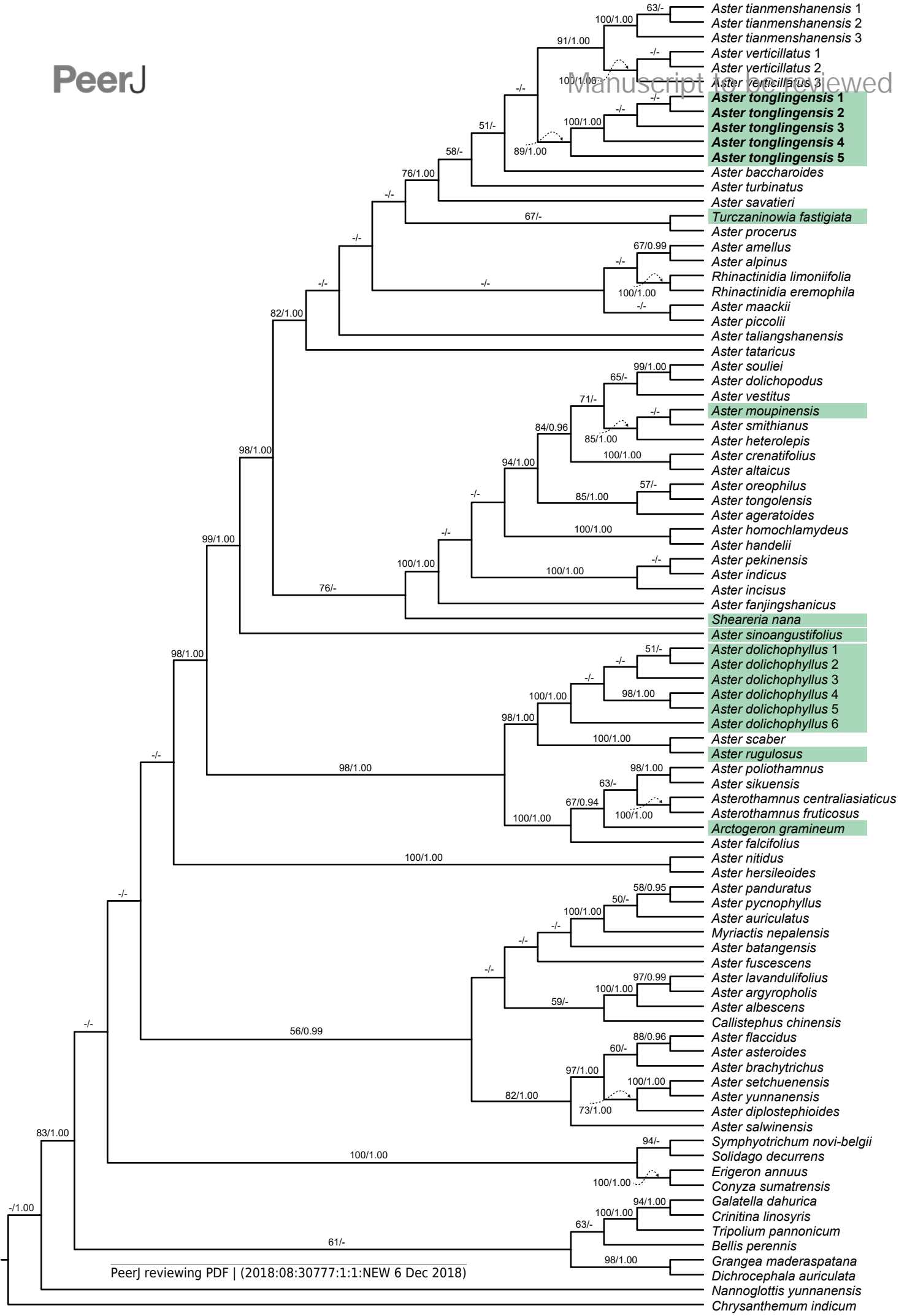
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- 610



**Figure 1**(on next page)

Cladogram of the maximum likelihood (ML) phylogenetic tree of *Aster*.

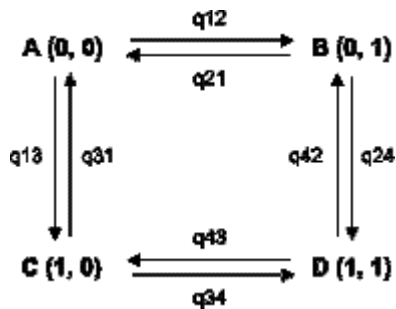
Phylogenetic tree based on combined data (ITS, ETS and *trnL-F*), showing the position of *Aster tonglingensis* (in bold) and the species with narrowly lanceolate leaf (with green background). Values above branch represent bootstrap values (BS) and Bayesian posterior probabilities (PP), respectively; the dash (-) indicates BS < 50% or PP < 0.90.



## Figure 2

Transitions among the four combinations of traits states.

The first trait is habitat, state 0 represents non-riparian habitat, state 1 represents riparian habitat; the second trait is leaf shape, state 0 represents leaf shape index  $> 0.15$ , state 1 represents leaf shape index  $\leq 0.15$ . (A) non-narrowly lanceolate leaf in non-riparian habitat; (B) narrowly lanceolate leaf in non-riparian habitat; (C) non-narrowly lanceolate leaf in riparian habitat; (D) narrowly lanceolate leaf in riparian habitat.



## Figure 3

Habitat and morphology of *Aster tonglingensis*.

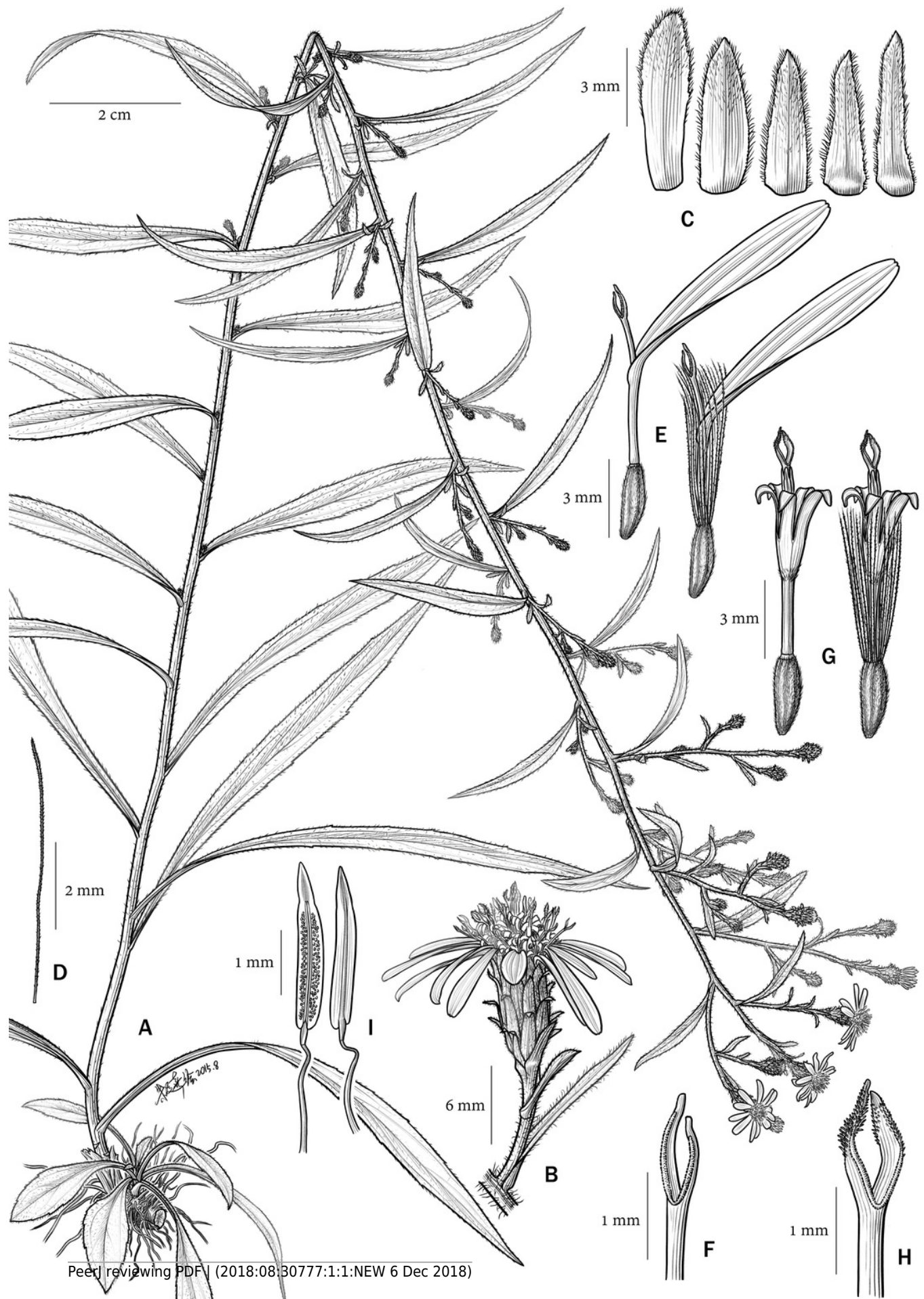
(A) *Aster tonglingensis* growing in its riparian habitat; (B) inflorescence; (C) capitula and phyllaries; (D) disc florets; (E) cauline leaves and axillary capitula; (F) seedling.



## Figure 4

*Aster tonglingensis*.

(A) habit; (B) capitula; (C) phyllaries; (D) bristle of pappus; (E) ray florets; (F) style branches of ray florets; (G) disc florets; (H) style branches of disc florets; (I) anthers.

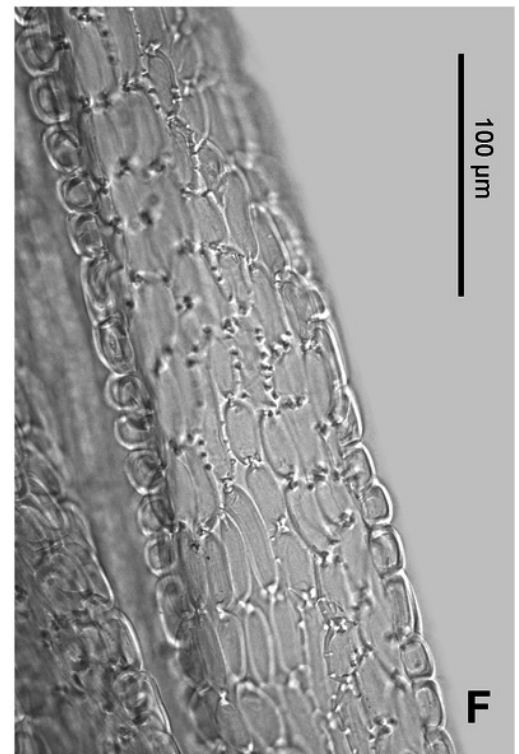
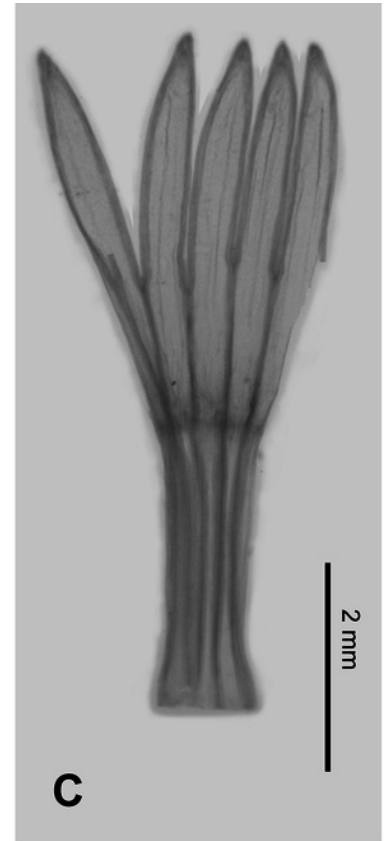


## Figure 5

Micro-morphology of *Aster tonglingensis*.

(A) style branches; (B) stylopodium; (C) corolla; (D) anther tip appendage; (E) anther base appendage, filament collar and anther endothecial tissue.

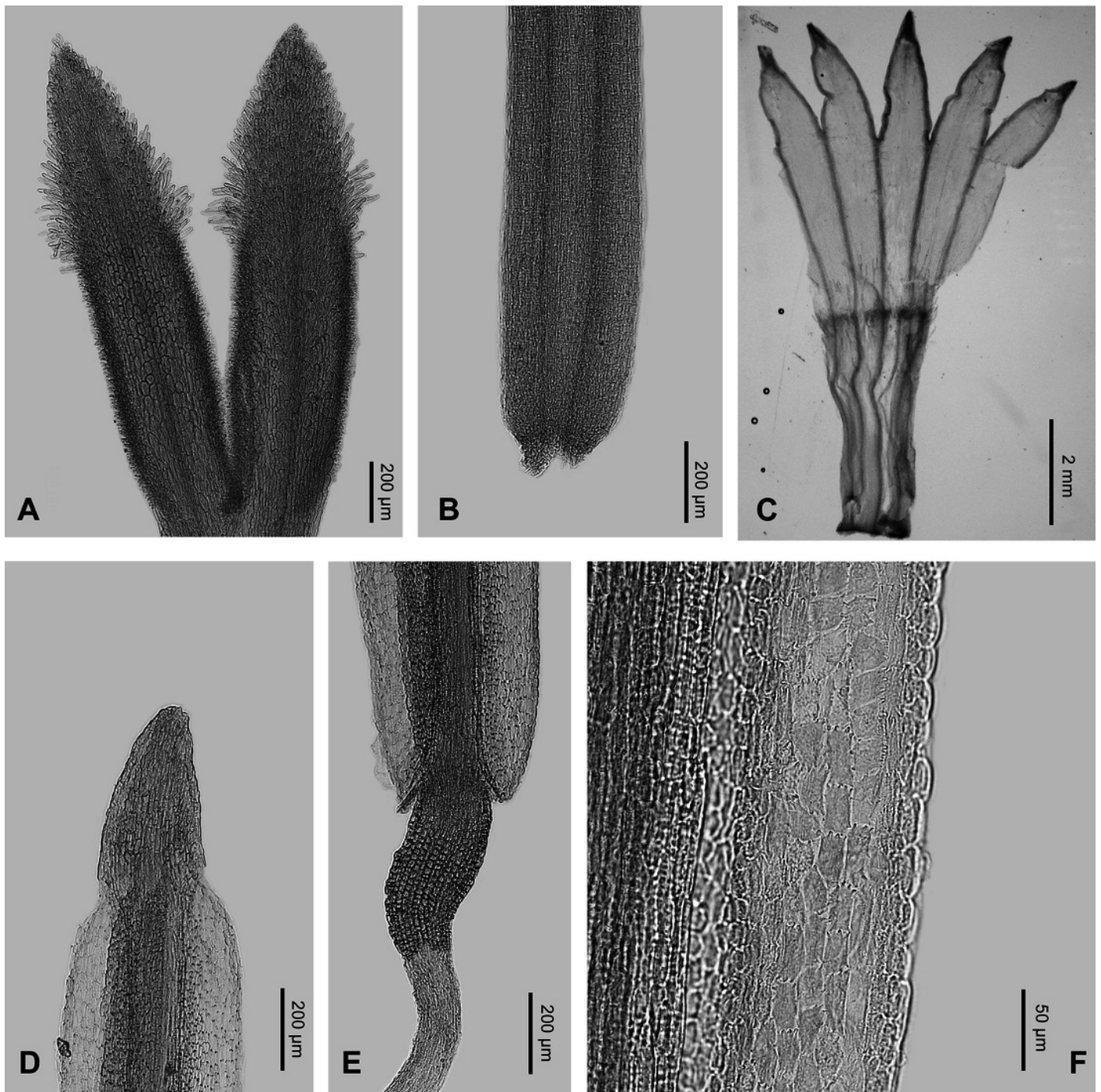




## Figure 6

Micro-morphology of *Aster dolichophyllus*.

(A) style branches; (B) stylopodium; (C) corolla; (D) anther tip appendage; (E) anther base appendage, filament collar and anther endothecial tissue.



**Table 1** (on next page)

Morphological and micro-morphological characters of four *Aster* species.

characters	<i>Aster tonglingensis</i>	<i>Aster dolichophyllus</i>	<i>Aster verticillatus</i>	<i>Aster tianmenshanensis</i>
height	70 to 100 cm	40 to 50 cm	25 to 100 cm	up to 10 cm
basal leaves	long petiolate, lanceolate	sessile, spatulate	petiolate, lanceolate	sessile, spatulate
adaxial surface of leaves	puberulent	glabrous	scabridulous	glabrous
capitula	more than 30, terminal and axillary, 20 to 25 mm in diameter	less than 10, terminal, 25 to 30 mm in diameter	more than 20, terminal and axillary, 10 mm in diameter	single, terminal, 15 to 20 mm in diameter
phyllaries	5 to 7 series	2 to 3 series	3 series	2 to 3 series
achenes	beakless	beakless	beaked	beakless
pappus	robust	robust	readily caducous	robust
stigmatic lines	equal to the sterile style tip appendages	shorter than the sterile style tip appendages	shorter than the sterile style tip appendages	longer than the sterile style tip appendages
disc corolla lobes	unequal, split to two thirds or three fourths of limb	equal, split to one third of limb	equal, split to one half of limb	unequal, split to one third of limb
anther endothelial cells	polarized thickened	radially thickened	radially thickened	radially thickened
anther tip appendages	narrowly triangular, length-width ratio ca. 2	triangular, length-width ratio ca. 1.5	triangular, length-width ratio ca. 1.5	narrowly triangular, length-width ratio ca. 2