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 (#30777)

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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 the 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-diversity 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.

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Abstract

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 the 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-diversity 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.

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Introduction

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

Asteraceae is a young family originating in the Paleocene and have an age about 60–75 Ma



(Barreda et al., 2015; Panero & Crozier, 2016). It is also the largest family of plants, containing near 30,000 species (Heywood, 2009; Funk et al., 2009). Members of this mega-diversity family occur in a variety of habitats, thus providing an excellent opportunity to study convergent evolution (Heywood, 2009).

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

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



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

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

Materials and Methods

Taxon sampling

Seventy-three taxa were used for phylogenetic analysis, representing 19 related genera and the major clades of *Aster* and one outgroup. Sequences of 71 of these species were downloaded from GenBank. Most species with narrowly lanceolate leaf in *Aster* were sampled. Five samples of the potential new taxon and six samples of the superficially similar species *Aster dolichophyllus* were newly added in this study. The taxonomic treatment in the *Flora of China* and the definitions of *Aster* and "core *Aster*" in our previous study (Zhang et al., 2015) were followed. In the phylogenetic analysis, *Chrysanthemum indicum* L. was designated as outgroup based on previous



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

DNA extraction, amplification, and sequencing

Leaf tissues were collected in the field and dried using silica gel. DNA extraction, purification, and sequencing followed the methods described by Zhang et al. (2015). Methods of PCR amplification of ITS and ETS sequences followed Linder et al. (2000), *trnL-F* sequence followed Zhang et al. (2015). The ITS primers of Linder et al. (2000), ETS primers "Ast-8" and "18S-IGS" and *trnL-F* primers "c" and "f" of Taberlet et al. (1991) were used.

Phylogenetic analysis

DNA sequences alignment was fulfilled using MAFFT online version (Katoh et al., 2017), and then was manually adjusted using BioEdit v7.0.8.0 (Hall, 1999). jModelTest 2.1.4 (Darriba et al., 2012) was used to select DNA substations module based on the Akaike information criterion (AIC). The GTR + G model was fit for ETS and ITS, and TVM + G model for *trnL-F*. Phylogenetic analyses were then conducted for two individual data sets, one therein consist of ITS and ETS sequences, another consist of *trnL-F*, and a combinative dataset. Phylogenetic trees were reconstructed using Maximum Likelihood methods and Bayesian Inference. Bootstrap support



value (BS) for ML tree was calculated using 1000 bootstrap replicates. Bayes inference was performed with 20 million generations, tree sampled every 1000 generations. Bayesian posterior probabilities were calculated after omitting the first 500 trees (burn-in = 0.25). Analyses was done using RAxML 8.0.24 (Stamatakis, 2014) and MrBayes 3.2.4 (Ronguist et al., 2012) on the CIPRES science gateway portal (Miller, Pfeiffer, & Schwartz, 2010). The parameter settings in Zhang et al. (2015) were followed.

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Correlation evolution analysis

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



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ten specimens for each species. In this study, the leaves with leaf shape index less than 0.15 were defined as narrowly lanceolate. This criterion is near to the traditionally recognized index (Stearn, 1985).

For the character correlation analysis, the DISCRETE module of BayesTraits v 2.0 (Meade & Pagel, 2014) was used to analyse the correlation of the two binary characters above (the first character is habitat, state 0 means non-riparian and state 1 means riparian; the second character is leaf shape index, state 0 means the index is more than 0.15 and state 1 means less than (including) 1.5; see Fig. 2). Two models were provided in this module, i.e. the dependent model and the independent model. We checked which model fitted our data by comparing the maximum likelihood value obtained via the Maximum Likelihood (ML) method and the marginal likelihood value obtained via the Markov Chain Monte Carlo (MCMC) method. In the ML analysis, searching times for the maximum likelihood value of each calculation was set at 1000 and each calculation was repeated 10 times. In MCMC method analysis, priors were set as Gamma hyper-prior (Pagel, Meade, & Barker, 2004) with default parameters. Marginal likelihood values were obtained by the stepping stones method (Xie et al., 2011). Based on the results of our preliminary analysis, the number of iterations was set as ten million with 100 stepping stones. Each calculation was repeated 10 times and then the final marginal likelihood value was obtained from the mean of ten marginal likelihood values. The parameter restriction test (Pagel, 1994) was used to determine the order of trait evolution. Each parameter (Fig. 2) was respectively set as zero in different runs to determine if any trait transitions could be excluded from the process of trait evolution. Contingent change and temporal order test (Pagel, 1994) was employed to determine the dependence between the two traits and the acquisition order. The likelihood values of different analysis were compared using likelihood ratio test (LRT, for ML results) and Bayes Factors (BF, for MCMC results) test (Gilks, Richardson, & Spiegelhalter, 1996) following the procedure recommended in the manual of BayesTraits v2 (Meade & Pagel, 2014).

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Morphological and micro-morphological observations



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

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

New taxon and the LSID statement

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants (ICN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. In addition, new names contained in this work which have been issued with identifiers by IPNI will eventually be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix "http://ipni.org/". The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central, and CLOCKSS.



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Results

Phylogenetic results

When compared the phylogenetic trees separately reconstructed based on the chloroplast and 220 nuclear matrix, no obvious topology conflict was found. Then the two matrices were combined in 221 the following analysis. Consensus trees from BI analyses had nearly identical topologies with the 222 ML tree. The best ML tree (-InL = 22036.02) was presented in Fig 1. The topologies of our 223 phylogenetic tree were largely consistent with previous studies (Li et al., 2012; Zhang et al., 2015). 224 The species with narrowly lanceolate leaf were nested in different lineages on the tree (Fig. 1). 225 Among them, A. moupinensis, formed a well-supported clade with two species without narrowly 226 lanceolate leaf (A. smithianus Hand.-Mazz. and A. heterolepis Hand.-Mazz.) (Fig. 1, BS = 85, PP 227 = 1.00) nested in the core Aster clade; A. sinoangustifolius Brouillet, Semple et Y.L. Chen lay at 228 the base of the core *Aster* clade with strong support (Fig. 1, BS = 99, PP = 1.00); *A. rugulosus* was 229 resolved as sister to A. scaber Thunb. (Fig. 1, BS = 100, PP = 1.00); Sheareria, the monotypic 230 semi-aquatic genus, was placed in the core Aster clade, and resolved as sister to a clade consisting 231 of the taxa from A. fanjingshanicus Y.L. Chen & D.J. Liu to A. souliei Franch. with moderate 232 support (Fig. 1, BS = 78, PP < 0.90); Turczaninowia fastigiata formed a weak support clade (Fig. 233 1, BS = 67, PP < 0.90) with Aster procerus Hemsl.; Arctogeron gramineum (L.) DC.was resolved 234 as sister to a clade consisting of the taxa from Asterothamnus fruticosus (C. Winkl.) Novopokr. to 235 Aster poliothamnus Diels with moderate support (Fig. 1, BS = 67, PP = 0.94). Besides these 236 237 species, the distinctive Aster species from Mt. Tongling (formally described as Aster tonglingensis below) and the similar species Aster dolichophyllus ad similar narrowly lanceolate leaves. The 238 phylogenetic results showed that all individuals of Aster tonglingensis formed a strongly supported 239 monophyletic clade (Fig. 1, BS = 100, PP = 1.00). It was nested in the strongly supported core 240 Aster clade (Fig. 1, BS = 98, PP = 1), weakly resolved as sister to the subclade containing Aster 241 tianmenshanensis G.J. Zhang and A. verticilatus (Reinw.) Brouillet, Semple & Y.L. Chen (Fig. 1, 242 BS < 50, PP < 0.90). All individuals of A. dolichophyllus, formed another strongly supported clade 243



nested outside of the core *Aster* (Fig. 1, BS = 100, PP = 1.00).

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Correlation analysis of characters

Leaf shape index and the habitat information were kept in Table S2. For correlation analysis 247 between leaf shape and broadly riparian habitat using ML methods, the mean of the maximum 248 likelihood value of the independent model was -33.85, mean of the maximum likelihood value of 249 250 dependent model was -19.92, the likelihood ratio (LR) was 27.86, and p-value of likelihood ratio (LRT) was smaller than 0.00001. For MCMC methods, the mean of log marginal likelihood value 251 of independent model was -36.85, the mean marginal likelihood value of dependent model was 252 29.15, and Log Bayes Factor was 15.40. For correlation analysis between leaf shape and narrowly 253 riparian habitat of ML methods, the mean of maximum likelihood value of independent model was 254 255 -29.70, mean of the maximum likelihood value of dependent model was -22.45, LR was 14.48, and p-value of LRT was 0.0059. For MCMC methods, the mean of log marginal likelihood value 256 of independent model was -32.73, the mean marginal likelihood value of dependent model was -257 28.84, and Log BF was 7.78. The detailed results of the analyses and the test of parameter 258 restrictions are listed in Table S3. 259

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Morphological and micro-morphological observation

Aster tonglingensis is similar to A. dolichophyllus in external morphology. They have similar narrowly lanceolate and leathery cauline leaves, and reflexed phyllaries (Fig. 3 & 4). But they are totally different in the shape of the basal leaves, indument of leaves, bracteal leaves, and number of phyllaries series. A_E tonglingensis has long petiolate and lanceolate basal leaves (Fig. 3F & 4A), puberulent adaxial leaf surface (Fig. 4A), more than 30 capitula, single or several in terminal and axillary corymbs (Fig. 3E & 4A), whereas Aster dolichophyllus has spatulate and sessile basal leaves, glabrous adaxial leaf surface, less than 10 capitula in a loose terminal corymb, capitula never axillary. Aster tonglingensis has phyllary-like bracteal leaves and 5-7-seriate phyllaries (Fig. 3C), whereas A. dolichophyllus has bracteal leaves that are not phyllary-like and 2-3-seriate



phyllaries. Aster tonglingensis differs from its closely related species. A. tianmenshanensis, by its 271 greater height (70–100 cm versus ca. 10 cm in A. tianmenshanensis), narrowly lanceolate leaves 272 (versus spatulate leaves) and more capitula (more than 30 versus only one), and differs from A. 273 verticilatus by having large capitula (20–25 mm in diameter versus ca. 10 mm in A. verticilatus), 274 beakless achenes (versus beaked achenes in A. verticilatus), and a robust pappus (versus a readily 275 caducous pappus in A. verticilatus). 276 277 In micro-morphological characters (Fig. 5 & 6), both Aster tonglingensis and A. dolichophyllus have lanceolate style branches (Fig. 5A & 6A), triangular style appendages (Fig. 278 5A & 6A), constricted style base (Fig. 5B & 6B), thickened filament collar (Fig. 5E & 6E), and 279 obtuse and un-tailed anther base (Fig. 5E & 6E). However, A. tonglingensis differs from the latter 280 by having long stigmatic lines equal to the length of sterile style tip appendages (Fig. 5A) (versus 281 shorter than the sterile style tip appendages in A. dolichophyllus, Fig. 6A), disc corolla lobes split 282 to two third or three fourth of the limb of disc floret corolla (Fig. 5C) (versus split to one third in 283 A. dolichophyllus, Fig. 6C), narrowly triangular anther tip appendages with length-width ratio ca. 284 2 (Fig. 5D) (versus triangular with length-width ratio ca. 1.5 in A. dolichophyllus, Fig. 6D), and 285 majority of anther endothecial cells polarized thickened (Fig. 5F) (versus radially thickened in A. 286 dolichophyllus, Fig. 6F). 287 Aster tonglingensis is also different from its related species morphologically. It differs from 288 A. tianmenshanensis (Zhang et al., 2015) by having stigmatic lines as long as the sterile style tip 289 290 appendages (Fig. 5A) (versus only one third as long as the appendages in A. tianmenshanensis), disc corolla lobes split to two thirds or three quarters of the limb of the disc floret corolla (Fig. 5C) 291 (versus half way in A. tianmenshanensis), narrowly triangular anther tip appendages with length-292 293 width ratio ca. 2 (Fig. 5D) (versus triangular with length-width ratio ca. 1.5 in A. tianmenshanensis), and majority of anther endothecial cells polarized thickened (Fig. 5F) (versus 294 radially thickened in A. tianmenshanensis). It differs from A. verticilatus by the latter having long 295 stigmatic lines two time longer then appendages, disc lobes split for half the length of the limb, 296 and the majority of anther endothecial cells radially thickened (Zhang et al., 2015). 297



Discussion

Convergent evolution of the narrowly lanceolate leaf in the genus Aster

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

Correlation evolution between leaf shape and habitat

In our analysis, for broadly riparian habitat, p-value of LRT of ML method between two models was smaller than 0.01 and Bayes Factor of MCMC method was 15.40. These results suggested that the riparian habitat and narrowly lanceolate leaf shape was strongly correlated. These two characters showed obvious correlated evolution. The results of ML method parameter restriction tests showed all parameter could be exclusive (no significant difference with zero). This means every character state transition was possible. When we set the opposite transition rate as equal, there was no significant difference comparing to unequal. This results showed that the order of character state transition was not clear. The single parameter test with MCMC method showed that parameter q24 was strongly supported to differ from zero (BF = 5.34) and parameters q21 (BF = 2.05) and q34 (BF = 3.08) were positively supported to differ from zero. Other parameters did not differ from 0 in these test. To set the opposite parameters as equal, the results positively supported q13 is not equal to q24 (BF = 3.01). The dependent model test showed q13 (7.08) was much smaller than q24 (62.90). These results indicated that, compared to the plants with wide leaves, the plants



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with narrow leaves tended to transfer more frequently to a riparian habitat. But our temporal order test showed that no significant order could be recognized. Therefore, we could not determine whether the riparian habitat or narrowly lanceolate leaves came first in this adaptive process.

Some broadly riparian species are not strictly growing on stream banks. So we did the same test for narrowly riparian habitat species. Most results are similar to the analysis for broadly riparian habitat. In ML method analysis, the likelihood ratio of two models is 14.48 with p-value less than 0.01. The Bayesian factor of MCMC method is 7.78. These results showed that the riparian habitat was strongly correlated with narrowly lanceolate leaves. However, the single parameter test with ML method showed that no parameter was strongly supported to differ from zero. The order of character states could not be fixed. The MCMC method parameter restriction tests showed that q12 (BF = 7.11) and q24 (BF = 6.25) were strongly supported to differ from zero, g21 (BF = 2.41) and g34 (BF = 2.56) are positively supported to differ from zero. Other parameters were not supported as different from zero. These results showed that the transitions from wide leaves to narrowly lanceolate leaves in non-riparian habitat and from non-riparian habitat to riparian habitat with narrowly leaves cannot be ignored. These transitions indicated the potential path from non-riparian with wide leaves to riparian with narrow leaves in genus Aster. Furthermore, when setting q13 = q24, the negative result was supported. This result showed that the habitat changed from non-riparian to riparian likely depending on the narrowly lanceolate leaf shape. The test positively supported the q34 differs from zero. We also could find that q34 (= 54.63) were much large than q12 (= 12.06). These results suggested that the habitat would more likely to change from non-riparian to riparian when the plants have narrowly lanceolate leaves. Our directivity test showed that the q12 was not significantly different from q13. But q12 (12.06) was larger than q13 (7.08). Furthermore, q12 was proved significantly different from zero whereas g13 was not. This showed the rate of transition from wide leaf in non-riparian habit to narrowly lanceolate leaf in non-riparian habitat was higher than the rate of transition from wide leaf in nonriparian habit to wide leaf in riparian habitat. Based on the results above, we proposed that the narrowly lanceolate leaves trait acquisition was likely earlier than the riparian habitat acquisition



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in these riparian species with narrowly lanceolate leaves.

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

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

375 habitat.

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



In the phylogenetic tree, all individuals of A. tonglingensis formed a strongly supported clade (Fig. 379 1) nested in the core Aster clade. All the accessions of the similar species A. dolichophyllus formed 380 381 a strongly supported clade outside this clade (Fig. 1). In summary, our molecular analysis indicated that A. tonglingensis was a strongly supported monophyletic group and a unique lineage quite 382 distinct from the lineage including A. dolichophyllus (Fig. 1). 383 Although they look similar, Aster tonglingensis and A. dolichophyllus are different in many 384 385 characters. Both of them do have similarly shaped narrowly lanceolate, leathery cauline leaves, reflexed phyllaries, and almost glabrous leaf surfaces (Fig. 3 & 4). However, the shape of their 386 basal leaves is totally different. The basal leaves of A. tonglingensis are lanceolate with a long 387 petiole (Fig. 3F & 4A), whereas those of A. dolichophyllus are spatulate and sessile. In the previous 388 studies of Aster (Ling, Chen & Shih, 1985; Nesom, 1994; Chen, Brouillet, and Semple, 2011) 389 based on herbarium specimens, many lacked descriptions of the basal leaves because in many 390 species these were withered by the time of flowering. The present study showed that the character 391 of the basal leaves can be taxonomically important. In addition, many floral characters of these 392 two species are different. For instance, A. tonglingensis differs from A. dolichophyllus by having 393 terminal and axillary corymbs and more than 30 capitula (Fig. 3 & 4), whereas the latter species 394 has a lax terminal corymb with usually fewer than 10 capitula. Axillary corymbs (Fig. 3E) are rare 395 in Eurasia, Aster, such as A. turbinatus and A. verticilatus. Both Aster tonglingensis and A. 396 dolichophyllus have reflexed phyllaries, but the 5-7-seriate phyllaries and the enormous linear 397 bracteal leaves below the involucres of A. tonglingensis snow the obvious differences (Fig. 3C) in 398 comparison with the 2–3-seriate phyllaries and few lanceolate bracteal leaves of A. dolichophyllus. 399 Aster tonglingensis has longer disc corolla lobes about two thirds as long as the limb of the floret 400 (Fig. 5C), whereas the lobes of A. dolichophyllus are only one third as long as the limb. 401 Furthermore, the disc florets of A. tonglingensis (ca. 5-7 mm in length, Fig. 4G) are significantly 402 smaller than those of A. dolichophyllus (ca. 9-11 mm in length). These differences of floret 403 characters may be related to their pollination. 404 Our molecular analysis shows that the most closely related species of Aster tonglingensis are 405



A. tianmenshanensis and A. verticilatus. However, both of them differ from A. tonglingensis in their gross morphology: A. tianmenshanensis is a small herb with a solitary capitulum and spatulate leaf blades growing on limestone cliffs. A. verticilatus has tiny capitula (involucre ca. 2–7 mm in diameter), and beaked achenes with an easily caducous pappus.

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

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

Taxonomic treatment

- 427 Aster tonglingensis G.J. Zhang & T.G. Gao, sp. nov. Fig. 4.
- 428 Type: CHINA. Zhejiang Province, Wencheng County, Mt. Tongling, elev. 640 m, 119° 52′ E,
- 429 27° 49′ N, 2nd Sept. 2013, *H. H. Hu 331-1* (holotype PE!, isotype PE!).
- **Diagnosis:** The new species superficially resembles *Aster dolichophyllus* Ling. Both species have
- arrowly lanceolate cauline leaves, recurved phyllary tips, and occur near streams. However, the
- phyllaries of Aster tonglingensis were 5–7-seriate, green (vs. 2–3-seriate, green with purple tip in



A. dolichophyllus), capitula usually more than 30, both terminal and axillary (vs. less than 10, only 433 terminal), adaxial surface of all leaves puberulent (vs. glabrous), basal leaves lanceolate, apex 434 rounded or obtuse (vs. spatulate, apex acute), corolla of disk floret 5-7 mm, lobes half to two thirds 435 as long as limb (vs. corolla 9–11mm, lobes one third as long as limb), pappus whitish (vs. slightly 436 brown). 437 Perennial herb. Rhizomes thin, transverse, slightly woody, 3 - 15 cm long, ca. 0.3 - 0.5 cm in 438 diameter, sometimes expanded near the base of stem becoming a 2-4 cm hard node. Stem solitary 439 or two to three together, erect, 70 - 100 cm high (including inflorescence), unbranched except for 440 inflorescence, lower part glabrous, upper part puberulous, leafy. Leaves of rosette lanceolate, 4 – 441 $18 \times 0.8 - 2.5$ cm, base gradually narrowing, margin serrately four to eight toothed, petiole 3 - 10442 cm long, apex acute; lower cauline leaves similar to rosette leaves, sessile or petiole obscure, 443 narrowly lanceolate, $4 - 13 \times 0.4 - 1$ cm, margin entire or serrately 3-5-toothed, base gradually 444 narrowing, apex acute; all leaves thinly leathery, abaxially glabrous and light green, main vein and 445 lateral veins prominent, adaxially puberulent, dark green and glossy. Capitula usually more than 446 30. in one to five terminal and axillary corymbs, peduncle puberulous, with dense bracteal leaves, 447 bracteal leaves ciliate, abaxially glabrous, adaxially densely puberulous; involucre campanulate, 448 ca. 8-10 mm long, 5-8 mm in diameter, phyllaries in 5-7 imbricate series, green, lanceolate, 449 $5-7 \times 1-1.5$ mm, hardened at their bases, herbaceous above, the outer shorter than the inner, 450 ciliate, upper part of abaxial surface densely puberulous, with a revolute acute apex, ca. 1 mm 451 long, both surfaces densely puberulous; Ray florets ca. 15, female, with a greenish, glabrous tube 452 ca. 3 mm long; ligules whitish, lanceolate $7 - 10 \times \text{ca.}\ 2$ mm, with four nerves, apex with two or 453 three teeth; Disc florets many, hermaphrodite, corolla greenish white to yellow, tube greenish and 454 puberulent at the top, ca. 3 mm long, thin but expended at base, lobes five, lanceolate, unequal, 455 two thirds as long as limb; Achenes of both florets identical, narrowly oblong, four-ribbed, ca. 2 456 mm long, puberulous, lower part densely so; pappus 1-seriate, whitish, bristles barbellate, ca. 7 457 mm long, nearly as long as disc corolla at anthesis. Flowering in July. 458 Etymology: The species is named after its type locality, Mt. Tongling, Wencheng County, 459



460 Zhejiang Province, China.

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

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

Conclusions

Leaf shape has been used as an important character in the taxonomy of *Aster* for a long time (Chen, Brouillet, & Semple, 2011). The relationship between it and the environment, however, has never been investigated. In the present study, a phylogeny including most species with narrowly lanceolate leaf in *Aster* was reconstruction based on three molecular markers. It was revealed that species with narrowly lanceolate leaves were placed in far related lineages of the genus *Aster* (Fig. 1). Thus, the narrowly lanceolate leaf shape originated independently several times in the genus *Aster*. It was the results of convergent evolution. Comparative analysis in the phylogenetic context revealed that narrowly lanceolate leaf shape and riparian habitat were strongly correlated. The transition order of riparian habitat and narrowly lanceolate leaf was shown to be usually uncertain. But the preadaptation of the narrowly lanceolate leaf was positively supported by some analysis (Fig. 2). In summary, convergent evolution and preadaptation may play important roles in the evolution of leaf shape in the genus *Aster*. Meanwhile, an unexpected new species with narrowly



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lanceolate leaves, Aster tonglingensis, was discovered and established based on the evidence of
molecular, morphology and micro-morphology. This new species was descripted and illustrated
here.

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

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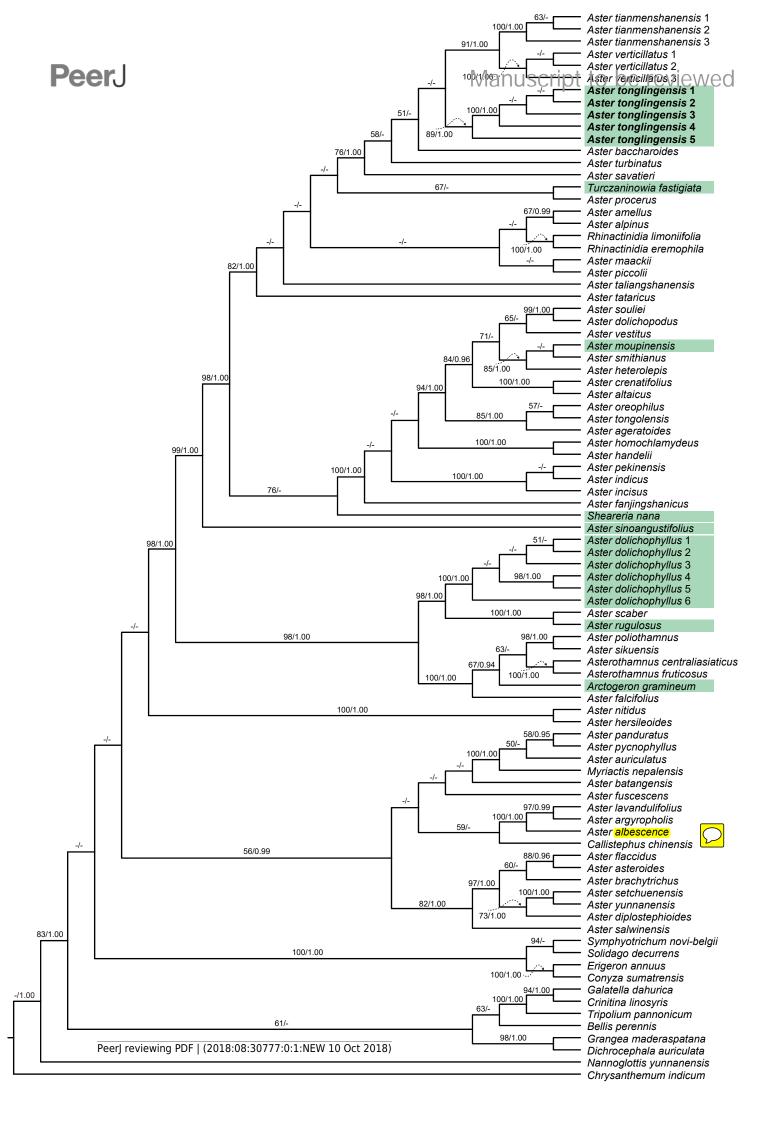
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Figure 1(on next page)

The cladogram of the maximum likelihood (ML) phylogeny tree of Aster.

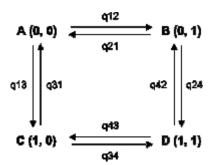
The phylogeny tree was reconstructed based on combined data (ITS, ETS and *trnL-F*), showing the position of *Aster tonglingensis* (in bold) and the species with narrowly lanceolate leaf (with green background). Values above branch represent bootstrap values (BS) and Bayesian posterior probabilities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





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 ≤ 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.





Habitat and morphology of Aster tonglingensis.

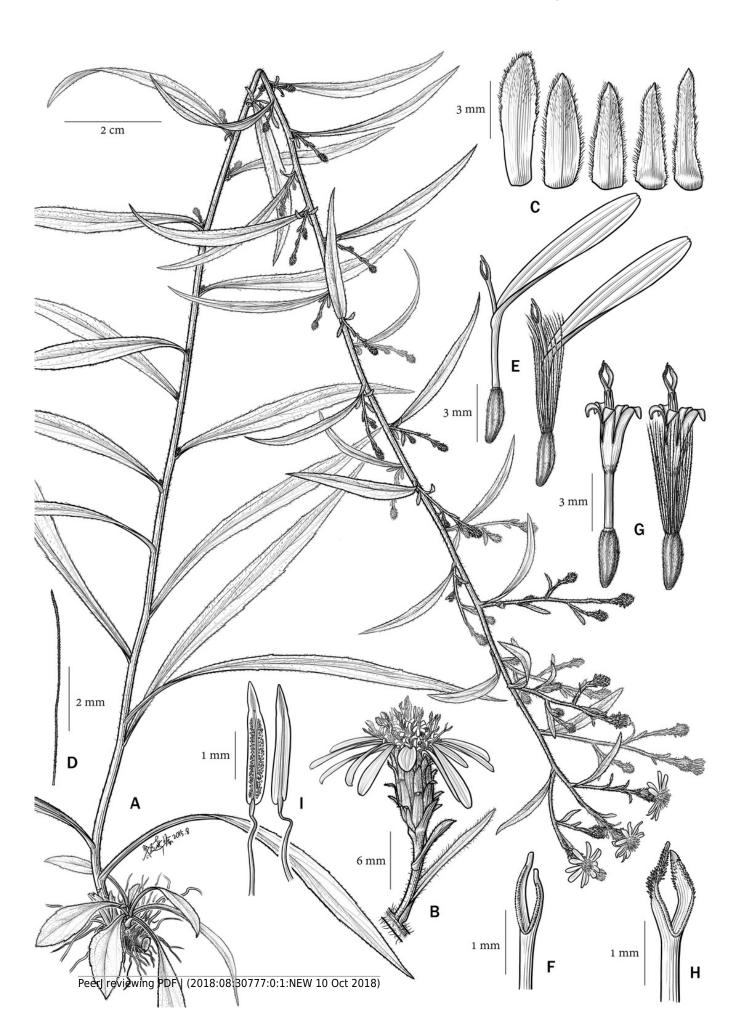
(A) Aster tonglingensis growing on the riparian habitat; (B) inflorescence; (C) capitula and phyllaries; (D) disc florets; (E) cauline leaves and axillary capitula; (F) seedling.





Aster tonglingensis.

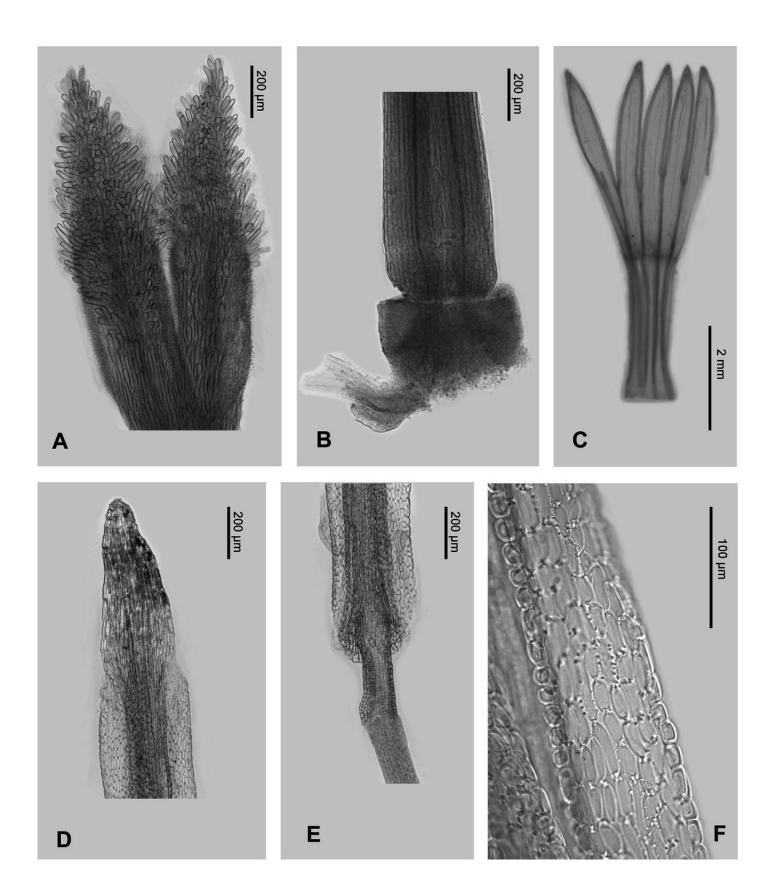
(A) habit; (B) capitula; (C) phyllaries; (D) bristle of pappus; (E) ray florets; (F) style branches of ray florets; (G) disc florets; (H) style branches of disc florets; (I) anthers.





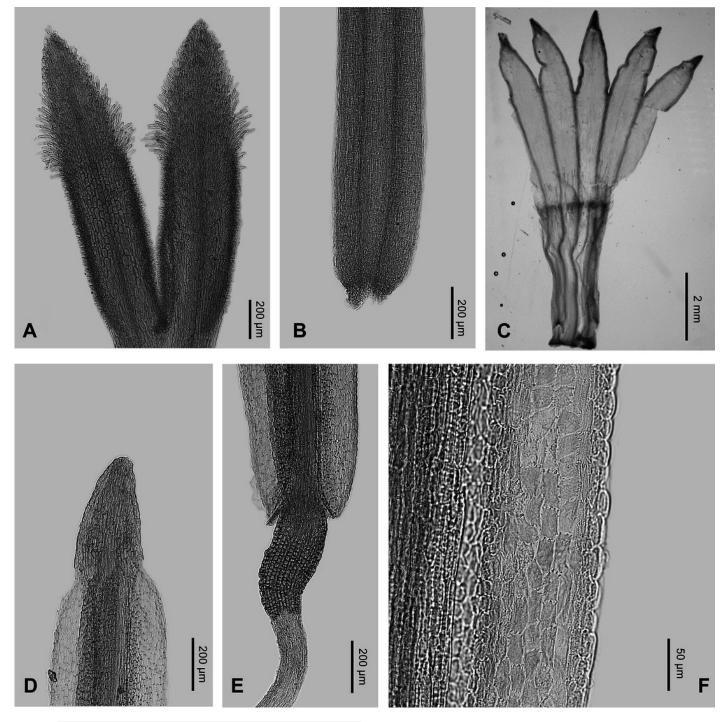
Micro-morphology of Aster tonglingensis.

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



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.



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