Informative title: Concurrent shifts in species distribution and pollination syndrome in east Asian Heloniadeae (Melanthiaceae)

Running title: Biogeography and pollination of Heloniadeae

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**Abstract**

The tribe Heloniadeae (Melanthiaceae) presents a typical East Asia–North America disjunct distribution. Different genetic markers and morphological data support the tribe, including *Helonias*, *Heloniopsis*, and *Ypsilandra*, as a monophyletic group, although its phylogenetic relationships and biogeographic history, together with the character evolution, are not clear. Therefore, we first built a Bayesian phylogenetic tree for the tribe using five chloroplast fragments and then reconstructed the historical biogeography and floral character evolution. The results revealed that Heloniadeae are distributed in high-latitude areas of East Asia and North America, originating at 5.1 mya. The East Asia clade migrated into southwest China, and subsequently colonized the Korean peninsula, Taiwan, and the Ryukyus, and finally spread northward to Japan and southern Sakhalin. The evolution of inflorescence type and of the number of flowers per inflorescence was consistent with the phylogeny and historical biogeography of Heloniadeae. Generally, the inflorescences transferred from raceme to sub-umbel, and the number of flowers decreased when *Heloniopsis* migrated from the continent to insular environments. Such a process might be acccompanied by a change of pollination syndrome. Besides, the anthesis period was linked more to distribution rather than phylogeny, and the flowering temperature of most species was below 20°C, except for *H. kawanoi*. Although Heloniadeae seem not to have a specific pollinator, the relatively low flowering temperature was adverse to pollinator activity, especially for the shaded habitat under forest. This disadvantage might be compensated by the long-lasting flowers. Together with aggregated flowers and a non-specialized pollinator, seed production could be maintained by such a mechanism. A new species, *Heloniopsis yongjhihii* C.T.Chao, is described here by its racemous inflorescences.

**Keywords:** character evolution, East Asia, Heloniadeae, historical biogeography, new species, pollination

**Introduction**

Heloniadeae, a tribe of Melanthiaceae, comprises three genera, namely *Helonias* L., *Heloniopsis* A.Gray, and *Ypsilandra* Franch. (APG 2016; Kim et al. 2016). These genera have a number of characters in common, such as being perennial herbs with rosette leaves, scapose inflorescences, tepals with various colors, and seeds with caudate appendages on both ends. They also inhabit a similar habitat of the understory, shady and moist places, except that *Heloniopsis kawanoi* (Koidz.) Hondahad been reported to be epiphytic on trunks (Chen et al. 2000; Fuse and Tamura 2016). *Helonias* has a compact raceme and three styluli; however, in contrast, *Heloniopsis* and *Ypsilandra* have relatively sparse racemes or umbels, and capitate or tri-lobed stigmas. The stamen of *Heloniopsis* is adnate to the opposite tepal, and the anther is dorsifixed with two locules or nearly so. On the other hand, the stamen of *Ypsilandra* is free from the tepals, and the anther is basifixed with one locule. The diagnostic characters for species delimitation rely on floral traits, such as inflorescence type, flower number per inflorescence, color of the tepals, stamen adnation, anther type, and style and stigma morphology (Tamura and Fuse 2016; Tanaka 2019; Chen et al. 2000).

Among the genera of Heloniadeae, *Helonias* is a monotypic genus with *He. bullata* L. distributed in eastern North America (Utech 2002); in contrast, *Heloniopsis* has ca. 6–8 taxa distributed in Japan, Korea, Taiwan, and south Sakhalin (Chen et al. 2000); *Ypsilandra* has five species in China and the Himalayas (Chen et al. 2000). Such a pattern is a typical East Asia–North America disjunction, which is found in many plant taxa, e.g. *Sassafras* J.Presl (Lauraceae) (Nie et al. 2007), *Pogonia* Juss. (Orchidaceae) (Cameron and Chase 1999), and *Chamaecyparis* Spach (Cupressaceae) (Liao et al. 2010).

The phylogeny of Heloniadeae has been reported in Tanaka (1997, 2019), Fuse and Tamura (2000), Fuse et al. (2004), and Kim et al. (2016, 2019). Among them, Tanaka (1997, 2019), Fuse et al. (2004), and Kim et al. (2019) also discussed the biogeographic history. However, these biogeographic studies were merely based on morphological and phenetic aspects (Tamura 1997, 2019), considered Heloniadeae lumped in the higher rank of Melanthiaceae (Kim et al. 2019), or just had limited discussions (Fuse et al. 2004). Therefore, the present study aims to reconstruct the ancestral area of Heloniadeae from a phylogenetic perspective, concentrating especially on inferring the origin and spreading of *Heloniopsis*, by model-based Bayesian evolutionary analysis. The results will give rise to a hypothesis for the formation of the disjunct distribution. Furthermore, reconstructing the ancestral state and character evolution of taxonomic traits, especially for floral parts, will also be considered.

**Materials and methods**

*Plant materials*

Material of *H. umbellata* Baker and *Heloniopsis* sp. was collected from Taiwan, and young leaves were preserved in silica gel for DNA extraction. Voucher specimens were deposited in the herbarium of the Taiwan Forestry Research Institute (TAIF) [herbarium acronym according to Thiers (2019, continuously updated)]. All taxa of Heloniadeae were included in this study; for the scientific name of each taxon Fuse and Tamura (2016) was followed. *Chamaelirium luteum* (L.) A.Gray, *Chionographis chinensis* K.Krause, and *Chionographis japonica* (Willd.) Maxim. were selected as outgroups following the studies of Kim et al. (2016), and Fuse and Tamura (2016). Fresh young leaves were dried in silica gel for molecular experiments.

*DNA extraction and PCR protocols*

Most sequences used in this study were adopted from Fuse and Tamura (2016) and downloaded from GenBank. In addition, samples of *H. umbellata* and *H.* sp. from Taiwan were included. Accession numbers of each sequences were listed in appendix 1. Total genomic DNA was extracted by the modified CTAB method (Doyle and Doyle 1987). Five cpDNA fragments (atpB-rbcL, matK, trnG, trnK, and trnL-F) were applied for phylogeny reconstruction. The primers of matK were newly designed for this study: matK-F (5’-CTAATACCCTATCCCATCCATC-3’), matK-R (5’-CAAAGTTCTAGCACACGAAAGTC-3’). For trnL-F and trnG, the primers of Taberlet et al. (1991) and Fuse and Tamura (2016), respectively, were used. Polymerase chain reaction (PCR) amplification was conducted by the following protocol: 3 mins at 94°C for enzyme activation, and 35 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 1 min, followed by a final extension at 72°C for 5 min. Quality and quantity of the PCR products were checked by gel electrophoresis and then they were sequenced using an ABI PRISMH3730XL DNA Sequencer (Perkin-Elmer, Foster City, CA, USA).

*Phylogeny reconstruction*

The sequences were aligned in BioEdit 7.2.5 (Hall 1999). Substitution models for the five cpDNA fragments were selected with MEGA 7.0 (Kumar et al. 2016); the models with the lowest Akaike and Bayesian Information Criterion (AIC and BIC) values were applied in the following analyses.

The species tree was reconstructed using BEAST v.1.8.0 (Drummond et al. 2012). The *xml* file was generated in BEAUti v.1.8.0 (Drummond et al. 2012), and the substitution models selected as mentioned above were applied. The clock model was set as a strict clock with a constant rate of 2 × 10−9 per site per year (Wolfe et al. 1987). The length of the MCMC chain reaction was set as 5 × 108 generations sampled every 50,000 generations; thus, a total of 10,000 trees were kept. Tracer v.1.6 (Rambaut et al. 2018) was used to check that the values of mean and ESS in the log file were over 200. TreeAnnotator v.1.8.0 (Drummond et al. 2012) was applied to construct the consensus tree and it was visualized using FigTree v.1.4.0 (Rambaut 2012).

*Character coding*

For the biogeographic and character evolutionary analysis, distribution and selected morphological characters were coded manually. The distributional range was divided into four areas, namely North America (A), Taiwan and the Ryukyus (B), Korea and China (C), and Japan (excluding the Ryukyus) (D). These areas were selected according to the patterns of endemism and the distribution of each taxon.

Regarding the morphological analysis, we mainly chose characters, especially of the floral parts, that were commonly used in the species diagnosis of Heloniadeae. Character states of each taxon were determined from herbarium specimens, living plants, and the studies of Hsu et al. (2011), Tanaka (2000, 2019), Utech (2002), Fuse et al. (2004), and Fuse and Tamura (2016). The following characters were applied in this study: inflorescence type, flower color, anthesis period, flower number, and stigma type. The final list of characters is presented in Table 1, together with their distribution. The anthesis period was classified into three categories according to the season of anthesis, namely spring to summer, summer to autumn, and winter to spring.

*Biogeographic inference*

RASP 4.2 (Yu et al. 2020) was used for historical biogeographic inference. The biogeographic models, including jump dispersal events (+J), were selected using the R package BioGeoBEARS (Matzke 2014). The maximum distribution range was set to two areas, and the following combination of areas was excluded from the analysis, i.e. North America + Taiwan + Ryukyus (AB). The model with the highest AICc\_wt was regarded as the best one for our dataset.

*Character evolution*

Identification of the ancestral states of discrete characters was done with BayesTraits (Meade and Pagel 2017) of RASP 4.2 (Yu et al. 2020) with default settings. Because non-discrete data cannot be applied in Bayes Traits, the ancestral state of flower number was determined using the package *phytools* of R (R core team 2019).

*Anthesis temperature analysis*

One of the characters we were interested in was the anthesis period. The records revealed that most Heloniadeae taxa bloom between late winter and early summer and are typical cases of vernal flowering plants. The anthesis of these species is often related to vernalization (Schemske 1978; Sheldon et al. 2000). Thus, we collected and organized the distribution and flowering period of each species of Heloniadeae from the Global Biodiversity Information Facility (GBIF), iNaturalist, naturing.net, and related references (Chen et al. 2000; Fuse et al. 2004; Fuse and Tamura 2016; Tanaka 2019). Further, we used the CHELSA Timeseries data, a high-resolution(~1 km) climate database for 1979 to 2013 (Karger et al., 2017a,b), to extract the long-term mean monthly temperature of habitats during each species' flowering period. One-way ANOVA was used to test the similarity of the mean monthly temperature of each species' flowering period, and the Scheffe test was employed to determine significant differences (α = 0.05) among groups.

**Results**

*Phylogenetic analysis*

The five chloroplast fragments, viz. atpB-rbcL, matK, trnG, trnK, and trnL-F, were concatenated to reconstruct the species tree. The substitution models selected for the phylogenetic analysis were HKY for atpB-rbcL and trnK, and HKY+G for the other sequences. With *Chionographis* (*C. chinensis* and *C. japonica*) and *Chamaelirium luteum* as the outgroups, the species tree confirms the monophyly of Heloniadeae that comprises a total of 11 taxa and one putative species from Taiwan. In this phylogenetic tree, the genus *Helonias* diverged first from the monophyletic group of *Ypsilandra* and *Heloniopsis*. Each genus forms a highly supported monophyletic group [posterior probability (pp) = 1] (Fig. 1), as does the clade of two *Ypsilandra* species. *Heloniopsis* consisted of three highly to moderately supported clades. Among them, two Korean species (*H. koreana* Fuse, N.S.Lee & M.N.Tamura and *H. tubiflora* Fuse, N.S.Lee & M.N.Tamura) formed a highly supported clade (pp = 1) sister to the others. Among the remaining species, *H. umbellata*, *H. leucantha* (Koidz.) Honda, and *Heloniopsis* sp. formed a robust clade (pp = 1), and *Heloniopsis* sp. was sister to the others. The other taxa formed a moderately supported clade (pp = 0.85), which consists of two subclades: one is comprised of two varieties of *H. orientalis* (Thunb.) Tanaka [var. *braviscapa* (Maxim.) Ohwi and var. *flavida* (Nakai) Ohwi], and another is composed of its autonym and *H. kawanoi*. However, the latter had only moderate support (pp = 0.73).

*Pliocene origin with vicariant diversification of Heloniadeae*

The divergence time estimated in BEAST indicated an origin of Heloniadeae during the Pilocene, 5.1 mya [95% highest posterior density (HPD): 7.0–3.45 mya]. The age of *Ypsilandra* was estimated as 3.35 mya (95% HPD: 4.65–2.25 mya), and that of *Heloniopsis* was 2.0 mya (95% HPD: 2.9–1.3 mya). The two Korean endemic species, *H. koreana* and *H. tubiflora*, can be dated back to 0.95 mya (95% HPD: 1.65–0.35 mya). The subtending Taiwan + Ryukyu clades diversified at 0.1 mya (95% HPD: 0.3–0 mya) and 0.35 mya (95% HPD: 0.65–0.1 mya), respectively (Fig. 1).

The best model for our dataset selected by BioGeoBears was DIVALIKE + J (AICc\_wt = 0.54). The ancestral area reconstruction suggests a continuous distribution in the northern hemisphere of Heloniadeae, and the North America–East Asia disjunction might have been formed by a vicariance event. The East Asia lineage experienced in situ cladogenesis and vicariance, resulting in two lineages, *Ypsilandra* and *Heloniopsis*. *Heloniopsis* further dispersed into the Ryukyus and Taiwan and separated into two lineages (viz. China + Korea and the Ryukyus + Taiwan) due to a vicariance event. The southern lineage of the Ryukyus + Taiwan further spread northward to Japan and became separate lineages in the two areas (Japan and the Ryukyus). The former one underwent in situ cladogenesis and vicariance resulting in the three present taxa. The latter one experienced cladogenesis and colonized southward to the Ryukyus again. After the vicariance event, it diversified to *H. orientalis* in Japan, and *H. kawanoi* in the Ryukyus.

*Ancestral state reconstruction of selected characters*

Ancestral states of the floral and phenological characters were inferred on the reconstructed phylogeny. Different character revealed dissimilar state change patterns:

1. Inflorescence: The ancestral state of Heloniadeae and *Ypsilandra*+ *Heloniopsis* was raceme, changing to sub-umbel in *Heloniopsis*. However, this state changed to raceme again in the Ryukyus + Taiwan and China + Korea clade.

2. Flower color: The tate change of this character was more complex and with more ambiguous states than in inflorescence. The ancestral state of flower color in Heloniadeae and *Ypsilandra* + *Heloniopsis* was ambiguous, and in *Heloniopsis* the pink color had a 48.5% possibility of being ancestral. However, the ancestral state was white in the clade of Japan + the Ryukyus + Taiwan, and it was ambiguous in the Japan clade, and pink in the clade of *H. kawanoi* + *H. orientalis* var. *orientalis.*

3. Stigma: The ancestral state in Heloniadeae and *Ypsilandra* + *Heloniopsis* was reconstructed as capitate, and the trifid stigma of *He. bullata* was an autapomorphy. The state of *Ypsilandra* was trifid, and all clades of *Heloniopsis* were reconstructed as capitate.

4. Anthesis period: The ancestral state of Heloniadeae was spring to summer; however, this state became ambiguous in *Heloniopsis*. The state of the non-Korean species was reconstructed as summer to fall and switched to winter to spring in the Ryukyus + Taiwan clade, to spring to summer in the remaining species, and further reversely changed back to summer to fall in the clade of *H. kawanoi* + *H. orientalis* var. *orientalis*.

5. Anthesis temperature: The results indicated that except for *H. kawanoi*, the mean monthly temperature (mmt) of Heloniadeae was lower than 20°C, and the species could be classified into three groups. The first group (group a) comprised only *H. kawanoi*; the anthesis mmt of this group was higher than 20°C. In the second group (group c), comprising *H. tubiflora*, *H. umbellata*, and *H. yongjhihii* sp. n. (described below), the anthesis mmt was around or below 10°C; the third group (group b), which had an anthesis mmt between 10°C and 20°C, comprised *He. bullata*, *H. orientalis* and its varieties, *Y. thibetica* Franch., and *Y. yunnanensis* W.W.Sm. & Jeffrey. However, *H. koreana* and *H. leucantha* belonged to none of these groups, and formed transitions between groups b and c and a and b, respectively.

6. Flower number: The result showed that the flower number in Heloniadeae generally became smaller through time. A reversal event was only observed in the clade of the Ryukyus + Taiwan.

**Discussion**

*The biogeographic events and divergence time of Heloniadeae*

In the study of Tanaka (2019), Heloniadeae was suggested as a member of the “Arcto-Tertiary Geoflora,” a group of deciduous broad-leaved plant communities in northern circumpolar regions (Shotwell 1961; Grìmsson et al. 2015). The ancestor of Heloniadeae was widely distributed in the high latitudinal area of the Northern Hemisphere and dispersed southward to eastern Asia and eastern North America (Tanaka 2019). This inference was similar to our results. The North American taxon formed an independent species, *He. bullata*, and Tanaka (2019) regarded it as the most primitive species of Heloniadeae (= *Helonias* in Tanaka 2019).

Two diversification hypotheses for the East Asian *Heloniopsis* have been proposed: one presumes that *Heloniopsis* originated in Sakhalin and spread southward into Japan and Korea (Fuse et al. 2004); the alternative presumed it originated in the southern part of East Asia, i.e. Taiwan and the Ryukyus, and migrated into Japan, Korea, and Sakhalin (Tanaka 2019). Generally, these two hypotheses are similar. They presume *Heloniopsis* originated on the islands of East Asia, spreading unidirectionally among these islands and the Korean peninsula. However, our result does not support either the inference of Fuse et al. (2004) or that of Tanaka (2019). Our result shows that *Heloniopsis* possibly originated from southern China 3.35 mya, and experienced one vicariance event. Subsequently, one lineage spread into the Korean peninsula, and another colonized Taiwan and Ryukyus at approximately 2.0 mya. The colonization time roughly matched the formation of the islands of Taiwan and the Ryukyus ([Chi et al. 1981](#_ENREF_7); [Teng 1996](#_ENREF_72); [Wang et al. 2002](#_ENREF_76)). After that, dispersal was northward to Japan and Sakhalin starting 1.55 mya, in the Pleistocene. If the Korean species spread from Sakhalin or the Ryukyus as proposed by Fuse et al. (2004) and Tanaka (2019), respectively, we would suppose that *Heloniopsis* would inhabit areas surrounding the Korea Strait. However, populations of *Heloniopsis* are absent from Jeju Island, the southernmost part of the Korean peninsula, Shimayama island, and the Gotō archipelago. Besides, the dispersal of the island *Heloniopsis* may not be unidirectional. Despite a general trend of dispersal from the southern (Taiwan + the Ryukyus) to the northern part (Sakhalin) in *Heloniopsis*, one lineage was found to have colonized the Ryukyus from Japan, becoming *H. kawanoi* at approximately 1.05 mya.

For the dating of Heloniadeae, Kim et al. (2019) suggested that the origin of Heloniadeae could be traced back to 27.3 mya, which is different from our result (5 mya). Due to the lack of a fossil record of Melanthiaceae, Kim et al. (2019) calibrated the ages by applying fossil records of six outgroup clades. However, the reconstructed evolutionary rate might be an underestimate for the ingroup Heloniadeae beause of the relatively distant relationships among the outgroups. Here, we used a strategy that differed from the strict-clock model used by Kim et al. (2019). The evolutionary rate of the chloroplast genes was based on the study of Wolfe et al. (1987), and was faster than the rate usded by Kim et al. (2019), causing a relatively recent divergence time of the species of Heloniadeae. In our timeline, the divergence time of Heloniadeae between North America and Asia is approximately at the Miocene/Pliocene boundary (ca. 5 mya), which could be explained by the Beringia connection in the cooling Late Miocene (Holbourn et al. 2018) followed by its disconnection in the warming Early Pliocene (Montuire et al. 2006). Nonetheless, due to the vast difference from the time estimated by Kim et al. (2019), precise dating will still depend on the discovery of dated fossils of Heloniadeae or Melanthiaceae.

*Floral character evolution of Heloniadeae*

Most floral traits changed state, including reversals, multiple times, indicating a more complicated character evolution than the inference by Tanaka (2019). For the inflorescences, raceme was the primitive state, and sub-umbel was a derived one, which is consistent with the opinion of Tanaka (2019). The changing inflorescence types may be related to pollination syndrome: the transition from raceme to sub-umbel occurred when *Heloniopsis* spread from a continental to an insular environment, accompanied by shift of environment and pollinator. However, reversal of the inflorescence’s state was observed in the taxa of the Ryukyus and Taiwan (*H. leucantha* and *Heloniopsis* sp.). Consequently, whether the pollinator changes following this reversal needs further study.

Abundant flower-color variation is characteristic of the taxon of Heloniadeae (Tamura and Fuse 2016; Tanaka 2019). However, the evolution of the flower color of Heloniadeae was less discussed. Here we show that the probability of each flower-color state was less than 50% on all nodes, revealing ambiguous patterns of ancestral states (Supplement S1). Only in the Ryukyu + Taiwan clade (*H. leucantha*, *H. umbellata*, and *Heloniopsis* sp.) and the clade composed of *H. kawanoi* and *H. orientalis* var. *orientalis* white and pink were dominant , respectively. Takahashi (1988) reported that all parts of the flowers of *H. orientalis* showed strong UV absorption with no special pattern for insect vision. Therefore, the various flower colors may not correspond to a co-evolutionary relationship between Heloniadeae and their pollinator.

Compared to the other characters, the change of stigma states was relatively simple, both for Heloniadeae and *Heloniopsis*. The ancestral state of these taxa is capitate, whereas the tri-lobed one is a derived state. Except for the case of *Y. thibetica*, which is the only *Ypsilandra* species bearing a capitate or discoid stigma (Tanaka 2019), within each genus there was consensus about the stigma type. Although the evolution of floral organs is often tied with pollination syndrome, this seems not to be the case for the stigma of Heloniadeae.

The anthesis period reflects the flowering schedule, in which three anthesis periods were defined, viz. late spring to summer, late summer to autumn, and late winter to spring. The first period was suggested as the primitive state in Heloniadeae in this study, and the other two periods were derived ones. The spring–summer species (Heloniadeae excluding *H. leucantha*, *H. kawanoi*, *H. umbellata*, and *H. yongjhihii*) were all distributed in temperate or high-altitude areas; in contrast, the others are distributed in the subtropics. Therefore, the evolution of this character might be related to adaptation to the local climate instead of phylogenetic constraints. The late winter to summer anthesis period of most Heloniadeae taxa implied most members were vernal flowering species, and the flowering phenology was sensitive to temperature (Schemske 1978; Jackson 1966).

Our analysis of the mmt of the anthesis period indicated all species except *H. kawanoi* had an anthesis mmt below 20°C. Furthermore, species could be classified into three groups according to their mmt, and most temperate species were in group b, except for *H. tubiflora* and *H. koreana*. These two closely related speciesare endemic to the Korean peninsula and distributed sympatrically, but have a different anthesis mmt. Such differences in phenological characteristics might affect pollinator behavior and hence maintain reproductive isolation. However, the anthesis mmt of the subtropical species, viz. *H. kawanoi*, *H. leucantha*, *H. umbellata*, and *Heloniopsis* sp., classified them into different groups. In the monophyletic group of *H. leucantha*, *H. umbellata*, and *Heloniopsis* sp., mmt of *H. leucantha* was 15–20°C, and that of the others was around 10°C. Such differences might be due to the distributional differences in altitude: *H. leucantha* is distributed in the Ryukyus, while *H. umbellata* and *Heloniopsis* sp. are endemic to the medium-altitude mountains (1500–2000 m) in Taiwan.

The evolutionary trend in flower number was from more than 50 flowers in *He. bullata* to the solitary flower of *H. kawanoi*. The decreasing flower number might be related to inflorescence, in which the number of flowers per inflorescence decreased with the change of inflorescence from raceme to sub-umbel, i.e. taxa with a sub-umbel usually had fewer flowers than the ones with a raceme. Tanaka (2019) assumed that the aggregated flowers in sub-umbel inflorescences reflect higher pollination efficiency, which provides better opportunities for pollinators. Increased pollination efficiency might maintain seed production even when the number of flowers is decreased. Although increasing the number of flowers may increase attractiveness to pollinators, a high number of flowers also increases the possibility of geitonogamy (Klinkhamer and de Jong 1993). Therefore, we propose that decreasing flower numbers might be beneficial to avoid inbreeding in Heloniadeae.

*The taxonomic status of Heloniopsis sp. of Taiwan*

In Taiwan, only one species, *Helonias umbellata* (Baker) N. Tanaka (=*Heloniopsis umbellata* Baker) was recorded (Tanaka 2000). Later, Hsu et al. (2011) described *Ypsilandra thibetica* as a newly recorded species of Taiwan; the diagnostic characters of this species include rosette leaves, racemous inflorescences, and three-lobed capsules.

However, when we re-examined fresh material and herbarium sheets of *Y. thibetica* from Taiwan, we found this taxon was a member of *Heloniopsis* rather than *Ypsilandra*. The anther locules of of this taxon were not confluent apically, and the stamens were adnate to the opposite tepals, which are characters belonging to *Heloniopsis* rather than *Ypsilandra*. The taxonomic status of this taxon was still unresolved. This taxon was morphologically and genetically closest to *H. umbellata*. The difference between them was found in the type of inflorescence: *H. umbellata* has umbel inflorescences, but they are racemose in the new taxon. Based on the inflorescence character and the persistent populations in central Taiwan occur, this taxon was treated as a new species, namely *H. yongjhihii* C.T.Chao.

*Taxonomic treatment*

*Heloniopsis yongjhihii* C.T.Chao sp. nov. TYPE: Taiwan, Nantou County, Sun-link-sea vacation resorts, Sunglungyen, 27 Jan 2019, C. T. Chao 4742 (holotype: TAIF; isotype, HAST, TNM) 永鋕胡麻花

*Ypsilandra thibetica* sensu auct. Hsu et al. non Franch., Nouv. Arch. Mus. Hist. Nat., sér. 2, 10: 94, 1888.

Perennial herbs. Stems short, less than 1 cm long. Leaves in a rosette, oblanceolate, 3-9 cm long, 1–3 cm wide, chartaceous, base decurrent, margin entire, apex acuminate to acute, mucronate, sessile glabrous, venation parallel. Inflorescences a raceme, terminal, 2–4.5 cm long, with 4–20 flowers, scape with numerous bracts, the uppermost one sometimes bracteole-like, 1.5–2.2 cm long, 2–4 mm wide, peduncle 4–15 cm long, glabrous. Flowers actinomorphic, tepals 6, oblong to oblanceolate, 8–10 mm long, 1.5–2.5 mm wide, membranaceous, white to pink, becoming greenish when withered, glabrous, base saccate, apex obtuse, 3-veined. Stamens 6, antepetalous, inserted on the base of tepals, filaments filiform, 8–12 mm long; anthers elliptic, 1.2–1.6 mm long, ca. 0.5 mm wide, dorsifixed, latrorse and longitudinally dehiscent, purple. Ovary superior, spheroidal, ca. 0.4 mm in diam., grooved, style filiform, 7–10 mm long, stigma capitate to shallowly 3-fid. Fruit a capsule, seeds numerous, flattened, with white appendages on both ends. Chromosome number 2*n* = 34 (Hsu et al., 2007).

Phenology: flowering in January and February.

Etymology: The species epithet “*yongjhihii*” was chosen as dedication to Mr. Yong-Jhih Yeh, a junior high-school student who had been bullied by his classmates due to his feminine gender expression for a long time, which finally caused his death. This tragic event prompted some people and the government to realize that human rights in Taiwan must be improved and facilitated the passing of the gender equity education act. From then on, more people chose to care about human rights for every kind of gender identity, gender expression, and sexual orientation. What happened to Mr. Yong-Jhih Yeh had become key to bringing a positive impact to human rights on this island. The author of this new species would in this way like to commemorate his important impact on our society.

Additional specimen examined: Nantou county, Hsinyi township, at ca. 17.9 km of Jenlun Forest Road, 12 Feb 2003, C. I Peng 19224 (HAST); Luku township, industrial road to Chugaowan tea garden, 27 Jan 2019, C. T. Chao 4743 (TAIF); same loc., 21 Feb 2009, C. T. Chao 412, 435, 436 (TCF); Hualien county, Hsiulin township, maintenance road of Taiwan power company, Chilai section 6–8.5 km, 29 Mar 2008, C. F. Chen et al. 3305 (TNM); maintenance road of Taiwan power company, Chilai section 4–6 km, 29 Mar 2008, C. F. Chen et al. 3309 (TNM).

**Conclusion**

This study built an evolutionary hypothesis for the origin and dispersal of Heloniadeae from molecular data. The migration route of this tribe in East Asia was generally from continental to insular areas, and then from south to north. Simultaneously, the inflorescence type became shorter and the number of flowers decreased during the dispersal process of *Heloniopsis* from the continent to the islands in East Asia, and the pollination syndrome altered synchronously. The low anthesis temperature was unfavorable for the pollinator. However, the long anthesis period of each single flower, the aggregated flowers, and an unspecialized pollinator still improved pollination efficiency, resulting in the abundant production of seeds. Along with the changes in the number of flowers, the evolutionary trend of these floral structures guarantees effective sexual reproduction. Our study provides a detailed biogeographic history of Heloniadeae in East Asia and proposes a new model for the anthesis phenology of vernal species. In this study, we also describe a new endemic species of Taiwan, *Heloniopsis yongjhihii*, characterized by its racemous inflorescence.

**Author contribution**

CTC and PCL designed the study and wrote the manuscript. CCK and JTC helped with data analysis and reviewed the draft.

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**Figure Legends**

Figure 1. Species tree of Heloniadeae reconstructed by chloroplast sequences. The value of each node represented supporting value (pp)/dating (mya).

Figure 2. Ancestral distribution of Heloniadeae reconstructed by RASP with probability on each node.

Figure 3. Dispersal map of Heloniadeae in East Asia.

Figure 4. Evolution of inflorescence state changing of Heloniadeae. A: raceme, B: solitary, C: sub-umbel, N: ambiguous.

Figure 5. Anthesis temperature of Heloniadeae.

Figure 6. Evolution of number of flowers per inflorescence of Heloniadeae.

Figure 7. Photos of *Heloniopsis jungjhihii* C. T. Chao. A: habitat; B: habit; C: leaf adaxial surface; D: leaf abaxial surface; E: flower (side view); F: flower (front view); G: flower (removed two tepals and stamens, showed the pistil); H: tepal adaxial surface; I: tepal abaxial surface; J: tepal with stamen; K: stamen; L: pistil; M: fruit; N: seed.

Figure 8: Line drawing of *Heloniopsis yungjhihii* C. T. Chao. A: habit; B: leaf adaxial surface; C: leaf abaxial surface; D: inflorescence; E: flower (front view); F: flower (side view); G: flower with two tepals and stamens removed; H: tepal with stamen; I: tepal adaxial surface; J: tepal abaxial surface; K: stamen; L: pistil; M: fruit; N: seed.

**Appendix**

Appendix 1. Accession numbers of sequences applied in this study.