



## NOTE

## *Euploca formosana* (Heliotropiaceae): A new combination from Taiwan

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**ABSTRACT:** In Taiwan, six species of *Heliotropium* (Heliotropiaceae) have been documented, including *Heliotropium strigosum* Willd., a species now considered synonymous with *Euploca strigosa*, in Kinmen. The genus *Euploca* Nutt. was recognized as an independent genus from *Heliotropium* L. in 2003, based on molecular phylogenetic analysis using *trnL* and ITS1 sequence data, and the key morphological trait—the presence of pits on the endocarpid. In *Euploca*, the fruit separates into four nutlets, with pits on the endocarpid, whereas in *Heliotropium*, the fruit separates into two nutlets and lacks pits on the endocarpid. As a result of these diagnostic differences, *H. procumbens* var. *depressum* and *H. strigosa* were transferred to the genus *Euploca*. In this study, both morphological characteristics and molecular evidence demonstrate that *H. formosanum* aligns with species within the genus *Euploca*. Thus, we propose a new combination for *Heliotropium formosanum* as *Euploca formosana* (I.M.Johnst.) P.H.Chen & A.C.Chung.

**KEY WORDS:** *Euploca*, *Euploca procumbens*, *Euploca strigosa*, *Heliotropium*, Heliotropiaceae, new combination.

## INTRODUCTION

The order Boraginales, according to The Angiosperm Phylogeny Group (2016), is considered a single family, Boraginaceae *s.l.*, which encompasses Boraginaceae *s.str.*, Codonaceae, Cordiaceae, Ehretiaceae, Lennoaceae, Wellstediaceae, Heliotropiaceae, Hydrophyllaceae, and the *Nama* L. clade. However, based on recent molecular phylogenetic studies, morphological characters, and the need to maintain nomenclatural stability, Luebert *et al.* (2016) divided the order Boraginales into eleven families, namely Boraginaceae *s.str.*, Codonaceae, Coldeniaceae, Cordiaceae, Ehretiaceae, Heliotropiaceae, Hoplostigmataceae, Hydrophyllaceae, Lennoaceae, Namaceae, and Wellstediaceae. In Taiwan, the classification system of Luebert *et al.* (2016) is adopted (Lin and Chung, 2017; Editorial Committee of the Red List of Taiwan Plants, 2017; Chung, 2018), and recognizing five families within Boraginales: Boraginaceae, Coldeniaceae, Cordiaceae, Ehretiaceae, and Heliotropiaceae. The family Heliotropiaceae globally comprises four genera: *Heliotropium* L. (ca. 325 species), *Euploca* Nutt. (ca. 100 species), *Ixorhea* Fenzl (only one species), and *Myriopus* Small. (ca. 25 species) (Luebert *et al.*, 2016).

According to Hilger and Diane (2003), the genus *Euploca* Nutt. was distinguished from *Heliotropium* L. based on *trnL* and ITS1 sequence data and the presence of pits on its endocarpid. Luebert *et al.* (2016) also accepted this treatment of the genus. *Heliotropium* and *Euploca* can be distinguished according to Panwar (2022), where *Euploca* is characterized as having nutlets with pits on the endocarpid and a curved embryo, whereas

*Heliotropium* is characterized as having nutlets without pits on the endocarpid and a non-curved embryo. Furthermore, Rueangsawang and Chantaranonthai (2023) noted that *Euploca* can be identified by its bracteate inflorescence and fruit that separates into four nutlets, whereas *Heliotropium* can be recognized by its ebracteate inflorescence and fruit that separates into two nutlets.

In Taiwan, the family Heliotropiaceae is represented solely by the genus *Heliotropium* (Lu, 2011; Lin and Chung, 2017; Editorial Committee of the Red List of Taiwan Plants, 2017; Chung, 2018), encompassing six species. These include one endemic species, *H. formosanum* I.M. Johnst., and three native species, *H. foertherianum* Diane & Hilger and *H. sarmentosum* (Lam.) Craven, both distributed in Taiwan, and *H. strigosum* Willd., distributed in Kinmen. Additionally, there are two naturalized species, *H. indicum* L. and *H. procumbens* var. *depressum* (Cham.) Fosberg & Sachet. Although Taiwan's classification system follows Luebert *et al.* (2016), *Euploca* was omitted. Nevertheless, Hilger and Diane (2003) had already reclassified *Heliotropium procumbens* var. *depressum* as *Euploca procumbens* (Mill.) Diane & Hilger, and *Heliotropium strigosum* Willd. as *Euploca strigosa* (Willd.) Diane & Hilger, based on molecular and morphological evidence.

In recent years, new taxa and combinations of *Euploca* have continued to be proposed (Melo, 2022; Silva and Melo, 2022). Currently, the list on Plants of the World Online includes 168 accepted species (POWO, 2024). The endemic Taiwanese species, *H. formosanum*, is characterized by its bracteate inflorescence, fruit that separates into four nutlets, and nutlets with pits on the endocarpid (Johnston, 1951). Based on previous studies

**Table 1.** Taxa and GenBank accession numbers applied in the present study.

Taxa	ITS1	trnL
<b>Outgroups</b>		
<i>Ehretia acuminata</i> R.Br.	AF385798	AY376167
<i>Ixorhea tschudiana</i> Fenzl	HQ286134	HQ286166
<i>Myriopus volubilis</i> (L.) Small	AF396882	AY376233
<i>Nama demissum</i> A.Gray	AF402590	AY376168
<b>Genus <i>Euploca</i></b>		
<i>Euploca convolvulacea</i> Nutt.	AY377797	AY376187
<i>Euploca formosana</i> (I.M.Johnst.) P.H.Chen & A.C.Chung	PQ037107*	PQ030941*
<i>Euploca humifusa</i> (Kunth) Diane & Hilger	AF396890	AY376191
<i>Euploca procumbens</i> (Mill.) Diane & Hilger	AF396885	AY376207
<i>Euploca rariflora</i> (Stocks) Diane & Hilger	AF396889	AY376209
<i>Euploca strigosa</i> (Willd.) Diane & Hilger	AY377814	MK261620
<b>Genus <i>Heliotropium</i></b>		
<i>Heliotropium adenogynum</i> I.M.Johnst.	AY377792	AY376172
<i>Heliotropium arbainense</i> Fresen.	AF396916	AY376176
<i>Heliotropium curassavicum</i> L. var. <i>argentinum</i> I.M.Johnst.	AF396898	AY376185
<i>Heliotropium erosum</i> Lehm.	AY377801	AY376189
<i>Heliotropium indicum</i> L.	AY377802	MK187084
<i>Heliotropium veronicifolium</i> Griseb.	AY377818	AY376215

**Note:** Asterisks (\*) indicate the accession numbers that were newly generated in the present study. The voucher was collected by P.-H. Chen (#3890) and has been deposited at the TAIF herbarium.

(Hilger and Diane, 2003; Panwar, 2022; Rueangsawang and Chantaranonthai, 2023), *H. formosanum* should be classified under *Euploca*. Therefore, this study aims to clarify the taxonomic status of *H. formosanum*.

## MATERIALS AND METHODS

**Morphological method:** We collected fresh specimens of *H. formosanum* from Fonchueisha, Pingtung, Taiwan, for the observation and measurement of key morphological features such as the bract, fruit, and nutlet. The specimens were photographed using a digital camera (OLYMPUS Tough TG-6), and voucher specimens were prepared and subsequently deposited in the TAIF herbarium. These measurements were then compared with the descriptions provided by Johnston (1951). In addition, we consulted materials from the following herbaria or their respective websites: HAST, NY, PPI, TAIE, and TAIF. Herbarium acronyms follow Thiers (2024).

**Phylogenetical method:** The genomic DNA of a *H. formosanum* sample was extracted from a silica gel dried leaf material of a newly collected voucher (P.-H. Chen 3890, TAIF). The DNA extraction process followed the protocol given by the Plant Genomic DNA Extraction Miniprep System (Viogene, Taipei, Taiwan). A 1.0% agarose gel electrophoresis was then conducted to validate the quality and quantity of the extracted genomic DNA. Later, the *trnL* and ITS1 regions were amplified by applying ITS\_4/ITS\_5 (White *et al.*, 1990) and *trnLF\_c/trnLF\_f* (Taberlet *et al.*, 1991) primer pairs respectively. The amplification conditions followed

Hilger and Diane (2003)'s work. A 1.0% agarose gel electrophoresis was performed again to inspect the products of the amplifications. The successfully amplified products were purified with the QIAquick PCR Purification kit (Qiagen, Hilden, Germany) and then sequenced on both ends commercially using an ABI 3730XL platform with Genomics BioSci & Tech Co., Ltd., Taipei, Taiwan, with primer pairs mentioned above.

DNA reads were assembled utilizing the De Novo Assemble tool implemented in Geneious Prime 2024.0.5 (<https://www.geneious.com>). The newly assembled contigs were then deposited to GenBank (Sayers *et al.*, 2019) for further studies. To authenticate our morphological investigation with molecular data, we selected and included five *Euploca* taxa, six *Heliotropium* taxa, and four closely related outgroups from genera *Ehretia* P.Browne, *Ixorhea* Fenzl, *Myriopus* Small, and *Nama* L. in our analyses. Details of the sampled taxa for molecular analysis, along with their GenBank (Sayers *et al.*, 2019) accession numbers, are shown in Table 1. Assembled contigs were then aligned with the downloaded sequences using MUSCLE 5.1 (Edgar, 2022). The best evolutionary model for each alignment was estimated by jModelTest 2.1.10 (Darriba *et al.*, 2012). The maximum likelihood (ML) trees based on the *trnL* and ITS1 alignments were reconstructed separately using RAxML 8 (Stamatakis, 2014) with 1,000 bootstrap replicates. Before concatenating the *trnL* and ITS alignments into one, we performed the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) and Kishino-Hasegawa (KH) test (Kishino and Hasegawa, 1989) implemented in PAUP\* 4.0a168 (Swofford, 2002)

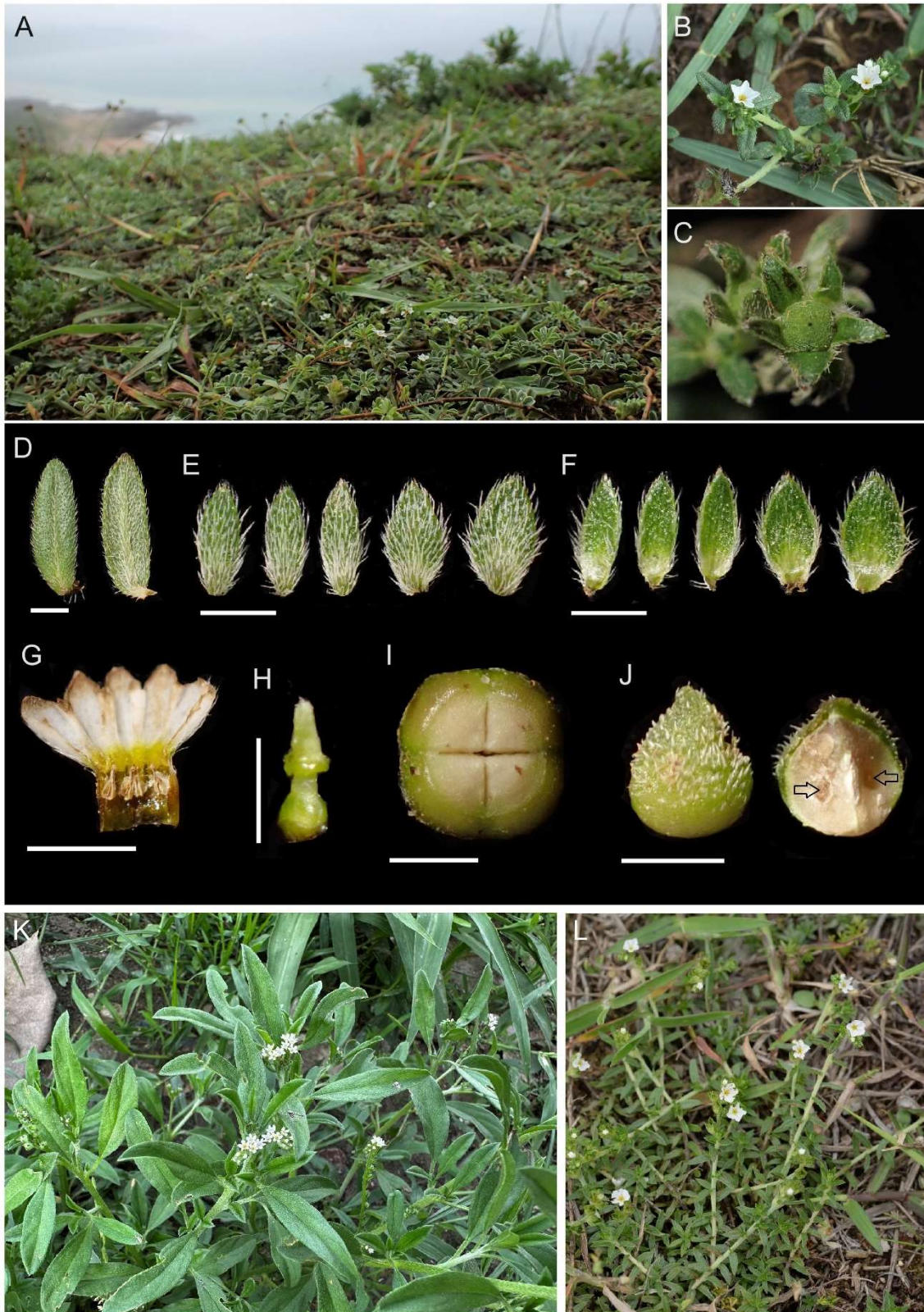
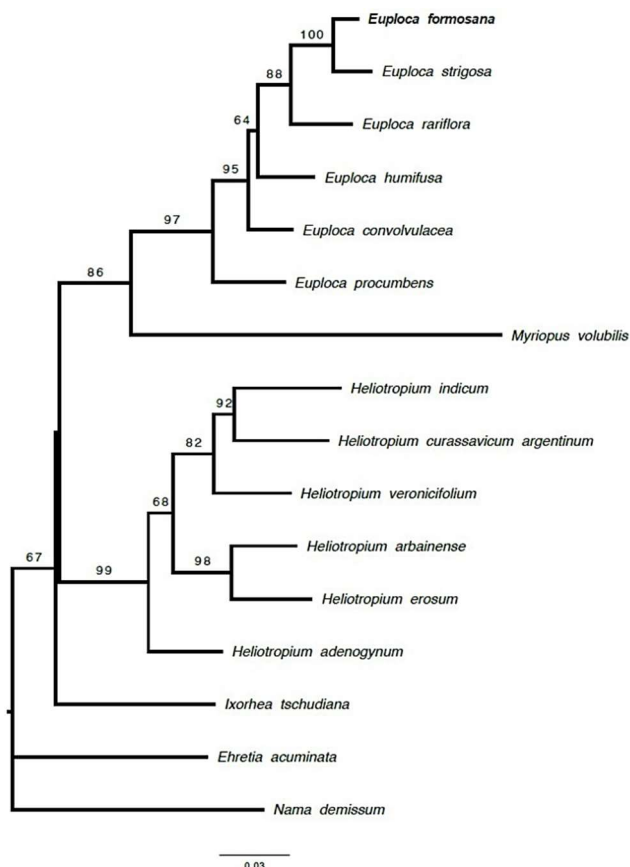


Fig. 1. *Euploca* species in Taiwan. **A–J.** *Euploca formosana*. **K.** *E. procumbens*; **L.** *E. strigosa*. **A.** habitat; **B.** flowering stem; **C.** fruit; **D.** leaves, adaxial (left) and abaxial (right) sides; **E.** calyx lobes, abaxial sides; **F.** calyx lobes, adaxial sides; **G.** corolla (with stamens); **H.** gynoecium; **I.** base of the fruit, where could be observed that the fruit separates into four nutlets; **J.** nutlets, dorsal (left) and ventral (right) views, with pits (indicated with arrows) on the endocarpid in the former. Scale bars: D–G = 2 mm; H–J = 1 mm. Photographers: A–K: Po-Hao Chen; L: Tian-Chuan Hsu.



**Table 2.** The results of the Shimodaira-Hasegawa (SH) and Kishino-Hasegawa (KH) tests indicate that no significant topological conflicts were found between the *trnL* and ITS1 trees.

Tree	Likelihood score -ln L	P value	
		KH test	SH test
ITS1	2256.77181	Best tree	
<i>trnL</i>	2265.57312	0.3823	0.1863



**Fig. 2.** A maximum likelihood tree of the selected *Euploca*, *Heliotropium*, and closely relative taxa suggests that *H. formosanum* should be reclassified as ***E. formosana***, based on the *trnL* and ITS1 regions. The target taxon, ***E. formosana***, is highlighted in bold. Numbers on the branches show the bootstrap values.

to understand the topological conflicts between these two trees. If no significant conflict exists between the two trees, the *trnL* and ITS1 alignments would be concatenated into a combined matrix. A combined ML tree was reconstructed from the combined alignment by applying RAxML 8 (Stamatakis, 2014) again. Lastly, FigTree 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) was utilized to depict the combined ML tree.

## RESULTS AND DISCUSSION

**Morphological analyses:** Our specimens of *Heliotropium formosanum* collected from Fengchuisha exhibit key morphological features, including ovate to

elliptic bracts, fruit that separates into four distinct nutlets (Fig. 1I), and nutlets with characteristic pits on the endocarp (Fig. 1J). These traits are consistent with Johnston's (1951) description, confirming the identity of the species. Moreover, these morphological characteristics are also in agreement with those of the genus *Euploca* as described by Hilger and Diane (2003), Panwar (2022), and Rueangsawang and Chantaranonthai (2023), further supporting the taxonomic placement of this species within the genus.

**Molecular phylogenetic analyses:** Both the SH and KH tests suggest that there are no significant topological conflicts between the *trnL* and ITS1 trees ( $p$ -values > 0.05) (Table 2). Therefore, the combined alignment was applied for the following phylogenetic analysis. Our combined ML tree with GTR+G+I model agrees with Hilger and Diane (2003)'s work that *Euploca* and *Heliotropium* are well-supported monophyletic groups with bootstrapping values 97 and 99, respectively. This provides solid evidence that the target taxon, *H. formosanum*, clusters with other species within *Euploca* and is closely related to *E. strigosa* in our sampling (Fig. 2).

The accession numbers of newly generated *trnL* and ITS1 sequences are PQ030941 and PQ037107, respectively (Table 1). The alignment and tree files are accessible on figshare (doi: 10.6084/m9.figshare.26289781).

Combining both morphological and molecular data, *Heliotropium formosanum* should be transferred to the genus *Euploca*. With this taxonomic revision, the genus *Euploca* in Taiwan now comprises a total of three species.

## TAXONOMIC TREATMENT

### Key to the species of *Euploca*

- 1a. Cymes unilateral ..... ***E. strigosa***  
 1b. Cymes 2-rowed scorpioid ..... 2  
 2a. Cymes slender, 2.5–10 cm; leaf blade linear-lanceolate to oblanceolate, 2–6 cm long ..... ***E. procumbens***  
 2b. Cymes stout, 0.8–1.5 cm; leaf blade elliptic to ovate, 3–4 mm long ..... ***E. formosana***

***Euploca formosana*** (I.M.Johnst.) P.H.Chen & A.C.Chung, *comb. nov.*

山豆根(臺灣天芹菜) Fig. 1A–J

*Heliotropium formosanum* I.M.Johnst., J. Arnold Arbor. 32:114. 1951; Hsiao & Liu, Fl. Taiwan ed. 2, 4: 396. 1998; Liu, Taiwania 43:127. 1998; Chung, Illustr. Fl. Taiwan 6:358. 2018. **Type:** TAIWAN. Pingtung County: South Cape, A. Henry 956 (holotype: NY00335460).

*Heliotropium strigosum* auct. non Willd., Henry, A List of Plants from Formosa 63. 1895; Hou, Taiwania 1: 211. 1950.

*Heliotropium strigosum* Willd. ssp. *brevifolium* auct. non (Wall.) Kazami, Hsiao, Fl. Taiwan 4: 403. 1978.

**Additional specimens examined:** TAIWAN. Hualien County: Hualien City, 22 May 1987, K.H. Yang s.n. (TAIF); Chian, 26 May 1993, S.F. Huang et al. 5226 (TAI). Pingtung County: Hsialiuchi, 5 Aug. 1930, T. Hosokawa 1775 (TAI); Nanshan Road, 4 Sep. 1977, T.C. Huang 7887 (TAI); Eluanbi, 18 Aug. 1981, W.L. Chiou 10292 (TAIF); 12 Sep. 1978, Ou and Kao 9008 (TAI); 22 Nov. 1942, Morimoto 496 (TAI); Fengchuisha, 14 Jun. 2008, P.F. Lu 16121 (TAIF);



14 Nov. 1987, *S.F. Huang and S.Y. Yang 3909* (TAI); 9 Sep. 2018, *P.-H. Chen 1708* (PPI); 28 May 2024, *P.-H. Chen 3890* (TAIE); 20 May 2002, *S.-H. Wu 14* (PPI); Maopitou, 26 May 1988, *T.C. Huang and S.F. Huang 13505* (TAI); 9 Jun. 2012, *S.W. Chung 10976* (TAIF); 20 Aug. 1968, *T.C. Huang 4784* (TAI); Hengchun Township, 25 Sep. 2008, *C.I. Huang 3535* (HAST); Lukun, 28 Aug. 2005, *G.P. Hsieh 2228* (PPI). Penghu County: Fongkuei, 25 Jul. 1972, *C.F. Hsieh 1085* (TAI); 25 Mar. 1972, *C.C. Hsu and C.S. Kuoh 14017* (TAD); Penghu Isl, 4 Aug. 1973, *T.C. Huang and M.T. Kao 6813* (TAI); Wangan, 26 Aug. 1995, *S.M. Liu 403* (HAST); Chimeiyu, 25 Aug. 2008, *M.J. Jung 3263* (TAIF); Feb. 1985, *S.F. Huang 28* (TAI); Siyu Island, 26 Aug. 2008, *M.J. Jung 3270, 3276* (TAIF); 6 Jun. 2001, *S.-M. Ku 1391* (PPI); Houliiao, 10 Jun. 1984, *Y.-L. Chung 349* (PPI); Jhuwan, 11 Jun. 1984, *Y.-L. Chung 399* (PPI). Taitung County: Donghe, 15 Mar. 2018, *T.W. Hsu 22119* (TAIE).

***Euploca procumbens* (Mill.) Diane & Hilger, Bot. Jahrb. Syst. 125: 48. 2003. 伏毛天芥菜 Fig. 1K**

*Heliotropium procumbens* Mill., Gard. Dict., ed. 8: no. 10. 1768.

**Type:** COLOMBIA. Bolívar: Cartagena, *W. Houstoun s.n.* (BM000953065).

*Heliotropium procumbens* Mill. var. *depressum* (Cham.) Fosberg & Sachet., Smithsonian Contr. Bot. 45: 24. 1980.

*Heliotropium procumbens* Mill. var. *depressum* (Cham.) H.Y. Liu, *Taiwania* 43:127. 1998. *nom. superfl.*; Hsiao & Liu, *Fl. Taiwan* ed. 2, 4:397. 1998; Chung, *Illustr. Fl. Taiwan*. 6:359. 2018.

*Heliotropium ovalifolium* Forssk. var. *depressum* (Cham.) Merr., *Philip. J. Sci. (Bot.)* 9: 134. 1914; Huang, *Fl. Taiwan* 6: 120. 1979.

**Additional specimens examined:** TAIWAN. Taichung City: Taichung Industrial Park, 25 Jul. 2009, *P.F. Lu 18590* (TAIF). Chiayi County: Zengwen Reservoir, 4 Mar. 2016, *S.W. Chung 12423* (TAIF). Tainan City: Liuchia Township, 15 Apr. 2012, *S.Y. Tsai TSY299* (TAIF); Anping, 10 Sep. 2012, *S.W. Chung 11194* (TAIF). Kaohsiung City: Sanmin District, 2 Feb. 1985, *T.H. Chung s.n.* (TAIF); Mt. Takang, 7 Feb. 1984, *K.C. Chen s.n.* (TAIF); Dongsha Island, 13 Mar. 2015, *C.F. Chen et al. 5701* (TAIF); Kuanyin Village, 2 May 2004, *C.F. Li s. n.* (TAIF); Meinong District, 3 Apr. 2005, *I.-C. Chang s. n.* (TAIF); Hsiwei, 18 Jan. 2013, *S.W. Chung 11348* (TAIF); Chishan, 11 Dec. 2003, *W.F. Ho 1515* (TAIF). Pingtung County: Shenshan Waterfall, 30 Mar. 2020, *P.F. Lu 32758* (TAIF); Eluanbi, 13 Feb. 2019, *S.W. Chung 13713* (TAIF); Taiwan Banana Research Institute, 27 Aug. 2001, *Y.S. Chao Chao14* (TAIF); Linpien, 3 Feb. 2003, *C.L. Tsai s.n.* (TAIF); Linluo, 18 Jun. 1988, *W.L. Chiou 13497* (TAIF); Hengchun Town, 19 Feb. 2009, *S.Y. Wu 120* (TAIF); Dapeng Bay, 1 Mar. 2008, *P.S. Chi s.n.* (TAIF). Taitung County: Luyeh, 1 Feb. 2002, *S.C. Hua and H.-P. Su s.n.* (TAIF); Lanyu Township, 10 May 2014, *W.Y. Wang 2113* (TAIF); Chihpen, 25 Jan. 2002, *H.C. Chen and P.I. Li s.n.* (TAIF); Pishan Hot Spring, 30 Jun. 2013, *P.F. Lu 25815* (TAIF). Ilan County: Luchiu Market, 15 Apr. 2003, *Y.C. Chou s.n.* (TAIF). Hualien County: Mataian Stream, 10 May 2003, *C. L. Yang s.n.* (TAIF); Hsuehtien Bridge, 12 Jul. 2002, *Y.T. Hsu and S.H. Su s. n.* (TAIF).

***Euploca strigosa* (Willd.) Diane & Hilger, Bot. Jahrb. Syst. 125: 49. 2003. 細葉天芥菜 Fig. 1L**

*Heliotropium strigosum* Willd., *Sp. Pl.* 1(2): 743. 1798; Lu, *Fl. Kinmen* (II), 210. 2011. **Type:** GHANA, Greater Accra Region: Ada, *P.E. Isert s.n.* (C10003972).

**Additional specimens examined:** TAIWAN. Kinmen County: Jinhu Township, 25 May 2017, *T.C. Hsu 9218* (TAIF); *S.W. Chung 12948* (TAIF). Fuguodun, 25 May 2017, *T.C. Hsu 9224* (TAIF). Jinsha Township, 3 Oct. 2015, *S.W. Chung 12306* (TAIF).

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