

## NOTES ON *CYRTANDRA* (GESNERIACEAE) FROM JAPAN, TAIWAN AND BATAN ISLAND (PHILIPPINES)

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As part of ongoing molecular phylogenetic work on the large Gesneriaceae genus *Cyrtandra*, new insights into the taxonomy and relationships of the *Cyrtandra* of Japan, Taiwan and Batan Island in the northern Philippines have emerged. *Cyrtandra umbellifera* is confirmed as a species with a distribution that includes both Taiwan and Batan Island. *Cyrtandra yaeyamae* is found to be distinct from the widespread *C. cumingii*, with a distribution that includes both the Ryukyu Islands in Japan and Batan Island.

*Keywords.* Batan Island, *Cyrtandra*, Japan, lectotypification, Philippines, Taiwan, taxonomy.

### INTRODUCTION

*Cyrtandra* J.R.Forst. & G.Forst. is the largest genus in the Gesneriaceae, with more than 800 species (Atkins *et al.*, 2013). Its distribution extends from the Nicobar Islands in the west, across the islands of Malesia to New Guinea, and across the Pacific to Hawaii. In continental Asia, the genus is distributed as far north as Phetchaburi in Thailand and reaches its most northern limit in Asia in Taiwan (Lanyu Island) and the Ryukyu Archipelago (hereafter the Ryukyus) of Japan, where it is represented by two species; in Taiwan by *Cyrtandra umbellifera* Merr. (Li & Kao, 1998), and in Japan by the widespread *C. cumingii* C.B.Clarke (Yamazaki, 1993) (Fig. 1).

### TAXONOMIC HISTORY OF *CYRTANDRA* IN JAPAN AND TAIWAN

#### *Taiwan*

*Cyrtandra umbellifera* was first described by Merrill in 1908 from near the summit of Mount Iraya in Batan Island, Philippines (Merrill, 1908). In 1935, Hosokawa described *Cyrtandra kotoensis* Hosok. from Mount Hontou-shan (formerly Mount Koto-yama) on Lanyu Island in Taiwan and referred to it as being distinct from *C. umbellifera* by having leaves with oblique bases and much shorter subulate calyx lobes (Hosokawa, 1935). This was considered to be a synonym of *Cyrtandra umbellifera* (Kao & DeVol, 1972), and this was maintained in the most recent *Flora of Taiwan* (Li & Kao, 1998).

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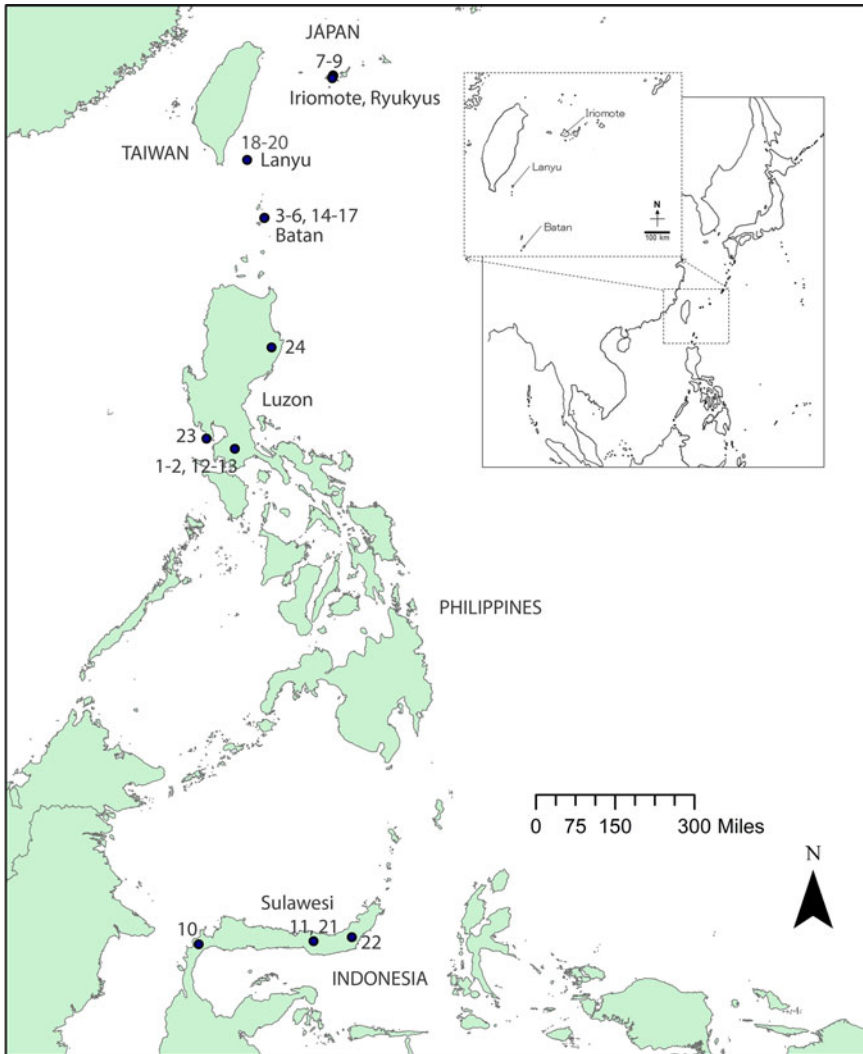


FIG. 1. Map of localities of all samples included in the analysis. Numbers relate to the sample list in Table 1.

### *Japan*

*Cyrtandra cumingii* is a large-leaved species with distinctive white bracts that was first described by Clarke in 1883 (Clarke, 1883) from two collections from Luzon in the Philippines. This species was later mentioned in a number of accounts of the Philippine flora by the American botanists Elmer and Merrill, in which it was described as being “widely distributed in the Philippines” (Merrill, 1907) and its distribution expanded to include Batan (Merrill, 1908), Luzon, Mindanao and Negros (Elmer, 1908); it was mentioned as being “especially common on Mt Banahao, the type locality” (Merrill, 1923).

In May 1937, a very similar-looking species was described from Iriomote Island, the southern Japanese island of the Ryukyus, and given the name *Cyrtandra yaeyamae* Ohwi and listed as being endemic to this island (Ohwi, 1937). No reference was made by Ohwi to the widespread Philippine species *Cyrtandra cumingii*. Just one month later, Masamune described another very similar-looking species from Iriomote in the Ryukyus and named it *Cyrtandra iriomotensis* Masam. (Masamune, 1937) and remarked that this was the first record of this widespread genus in Japan. This has subsequently always been treated as a synonym of *Cyrtandra yaeyamae* (Hatusima, 1956; Walker, 1976). In 1956, Hatusima listed *Cyrtandra yaeyamae* as a synonym of *C. cumingii*, but in 1971 he resurrected it as a variety of *C. cumingii* (Hatusima, 1956, 1971). Not long after, in 1973, Gillett, in his treatment of the *Cyrtandra* of the Caroline Islands and Ryukyus (Gillett, 1973), treated *C. yaeyamae* as a distinct species known only from Iriomote Island. Similarly, in 1976, in the *Flora of Okinawa and the Southern Ryukyu Islands*, Walker (1976) used the name *Cyrtandra yaeyamae* and stated that the Japanese species could be distinguished from the similar Philippine one by “having much smaller, narrower inflorescence-bracts and smaller indumentum-hairs, appearing less pubescent” and listed it as a Japanese endemic. Most recently, in the *Flora of Japan* (Yamazaki, 1993), *Cyrtandra yaeyamae* was sunk back into *C. cumingii* and listed as a widespread species from the Southern Ryukyus and the Philippines.

In this study, a stable phylogeny of the genus across Japan, Taiwan and the Philippines was generated, including the samples recently collected as *Cyrtandra cumingii* from the Ryukyus (Japan), Batan Island and Luzon (both from the Philippines) and *C. umbellifera* from Lanyu Island (Taiwan) and Batan Island (the Philippines), and we were able to test the relationships of these two species with their closest congeners to clarify their taxonomic status.

## MATERIALS AND METHODS

### *Taxon sampling*

We sampled 13 recent collections of the focus taxa *Cyrtandra cumingii* and *C. umbellifera*, which had been made by one of the authors (G.K.), and one by S. Scott (Royal Botanic Garden Edinburgh), from the key localities of Batan Island in the Philippines, Iriomote Island in Japan, and Lanyu Island in Taiwan, and an earlier collection of *C. cumingii* from near the type locality in Luzon, the Philippines. These were added to a matrix containing a further five samples of closely related taxa from the Philippines selected from a large phylogeny of the genus across Southeast Asia (Atkins *et al.*, unpublished data). Four additional samples of *Cyrtandra* from Sulawesi in Indonesia were included as outgroup taxa based on unpublished results of work by Atkins and colleagues, giving a total of 24 accessions (Table 1; see Fig. 1).

### *DNA extraction*

Total genomic DNA was extracted from fresh leaf material or silica-dried material using a modified CTAB procedure (Doyle & Doyle, 1987) or using the QIAextractor (Qiagen,

TABLE 1. Taxon list for samples of *Cyrtandra* included in the present analysis

No.	Species	Origin	Collector and number	Voucher deposition
1	<i>C. cf. roseo-alba</i>	Philippines, Luzon	QCB Cronk MAK1	E
2	<i>C. cumingii</i>	Philippines, Luzon	QCB Cronk MAK5	E
3	<i>C. cumingii</i>	Philippines, Batan	G Kokubugata GK15782	TNS
4	<i>C. cumingii</i>	Philippines, Batan	G Kokubugata GK15794	TNS
5	<i>C. cumingii</i>	Philippines, Batan	G Kokubugata GK15861	TNS
6	<i>C. cumingii</i>	Philippines, Batan	G Kokubugata GK15867	TNS
7	<i>C. cumingii</i>	Japan, Ryukyus	S. Scott 500	E
8	<i>C. cumingii</i>	Japan, Ryukyus	G Kokubugata GK8701	TNS
9	<i>C. cumingii</i>	Japan, Ryukyus	G Kokubugata GK18916	TNS
10	<i>C. fasciata</i> H.J. Atkins	Indonesia, Sulawesi	G Argent et al. 198	E
11	<i>C. fasciata</i>	Indonesia, Sulawesi	H Atkins et al. 54	E
12	<i>C. incisa</i> C.B. Clarke	Philippines, Luzon	QCB Cronk MAK4	E
13	<i>C. lagunae</i> Kraenzl.	Philippines, Luzon	QCB Cronk MAK3	E
14	<i>C. umbellifera</i>	Philippines, Batan	G Kokubugata GK15792	TNS
15	<i>C. umbellifera</i>	Philippines, Batan	G Kokubugata GK15857	TNS
16	<i>C. umbellifera</i>	Philippines, Batan	G Kokubugata GK15859	TNS
17	<i>C. umbellifera</i>	Philippines, Batan	G Kokubugata GK15858	TNS
18	<i>C. umbellifera</i>	Taiwan, Lanyu Island	G Kokubugata GK6016	TNS
19	<i>C. umbellifera</i>	Taiwan, Lanyu Island	G Kokubugata GK6025	TNS
20	<i>C. umbellifera</i>	Taiwan, Lanyu Island	G Kokubugata GK6031	TNS
21	<i>C. serratifolia</i> H.J. Atkins	Indonesia, Sulawesi	H Atkins et al. 93	E
22	<i>C. serratifolia</i>	Indonesia, Sulawesi	S Barber et al. BAKK 44	E
23	<i>C. sp.</i>	Philippines, Luzon	S Scott 501	E
24	<i>C. sp.</i>	Philippines, Luzon	M Mendum et al. 29009	E

Hilden, Germany). The primers used in this study are listed in Table 2. PCR was carried out with Biotaq DNA polymerase (Bioline, London) and with CES PCR enhancer (Ralsler *et al.*, 2006) with the following chemical concentrations: 10× buffer, 1 µL; 20 mM dNTP, 1 µL; 50 mM MgCl<sub>2</sub>, 0.3 µL; forward and reverse primers, 0.4 µL each; CES, 2 µL; Biotaq, 0.2 µL; DNA, 1–2 µL; with distilled H<sub>2</sub>O added to make up 10 µL. PCR amplifications of internal transcribed spacer (ITS) and *matK* were carried out with 94°C for 4 min, 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min, followed by 72°C for 10 min; for *trnL-F* with 94°C for 4 min, 35 cycles of 94°C for 30 s, 57°C for 30 s and 72°C for 1 min, followed by 72°C for 10 min; for *psbA-trnH* and *rpl32-trnL* with 94°C for 4 min, 35 cycles of 94°C for 1 min, 52°C for 1 min and 72°C for 1.5 min, followed by 72°C for 10 min.

All PCR amplifications were carried out on a Bio-Rad Tetrad DNA Engine (Bio-Rad, Hercules, California). The PCR products were visualised under ultraviolet light after electrophoretic separation on a 1% agarose gel stained with SYBR Safe gel stain (Invitrogen, Carlsbad, California). PCR products were subsequently purified using Exo-SAP-IT (Thermo Fisher Scientific, Waltham, Massachusetts) following the manufacturer's protocol. Sequencing PCRs were carried out with BigDye Terminator version 3.1 Cycle

TABLE 2. Primers used for PCR and sequencing

Region	Name	Direction	Primer sequence	Reference
ITS	ITS_5P	Forward	GGA AGG AGA AGT CGT AAC AAG	Möller & Cronk (1997)
ITS	ITS_8P	Reverse	CAC GCT TCT CCA GAC TAC A	Möller & Cronk (1997)
<i>trnL</i> F	<i>trnL</i> cG	Forward	GTG AAG ACT TCT AAA TTC AGA GAA AC	The present study
<i>trnL</i> F	<i>trnL</i> f	Reverse	ATT TGA ACT GGT GAC ACG AG	Taberlet <i>et al.</i> (1991)
<i>psbA-trnH</i>	<i>psbA</i> f	Forward	GTT ATG CAT GAA CGT AAT GCT C	Sang <i>et al.</i> (1997)
<i>psbA-trnH</i>	<i>trnH</i> r	Reverse	CGC GCA TGG TGG ATT CAC AAA TC	Sang <i>et al.</i> (1997)
<i>rpl32-trnL</i>	<i>rpl32</i> -F	Forward	CAG TTC CAA AAA AAC GTA CTT C	Shaw <i>et al.</i> (2007)
<i>rpl32-trnL</i>	<i>trnL</i> <sup>(UAG)</sup>	Reverse	CTG CTT CCT AAG AGC AGC GT	Shaw <i>et al.</i> (2007)
<i>matK</i>	<i>matK</i> .206F	Forward	CCG GGT TAT GAC AAT AAA TCC AGT	Luna <i>et al.</i> (in press)
<i>matK</i>	<i>matK</i> .946R	Reverse	ATA AAT CCT TCT TGG ATG AAA CCA C	Luna <i>et al.</i> (in press)
<i>matK</i>	<i>matK</i> .cy2F	Forward	TGG CAA TGG CAT TTT TCG CT	The present study
<i>matK</i>	<i>matK</i> .1734R	Reverse	CCG TGC TTG CAT TTT TCA TTG C	Luna <i>et al.</i> (in press)

ITS, internal transcribed spacer.

Sequencing Kit (Applied Biosystems, Foster City, California) following the manufacturer's protocol, and the same primers used for PCR amplification. Sequencing was carried out by Edinburgh Genomics at the University of Edinburgh. The resulting electropherograms were combined and edited in Sequencher version 5.1 (Gene Codes Corporation, Ann Arbor, Michigan) and a matrix assembled, aligned and manually adjusted in Bioedit version 7.1.11 (Hall, 1999).

#### *Phylogenetic analyses*

Maximum parsimony (MP) analyses were initially conducted on individual regions to visually assess congruence, with areas of conflict determined by examining the placement of individual taxa on each gene tree. Relationships were considered incongruent if the placement of taxa varied among the individual gene trees and exhibited MP-BS values > 80%. No significant incongruence was present, so all further phylogenetic analyses were carried out on the combined data set.

Maximum parsimony analyses were carried out using PAUP version 4.0a163 (Swofford, 2002) on unweighted and unordered characters. Alignment gaps were treated as missing data.

A full heuristic search was carried out using stepwise random addition of 10,000 replicates, with TBR and Multrees on. Statistical branch support was obtained from 10,000 heuristic bootstrap replicates each starting with a random addition tree, optimised with TBR on and Multrees off. Bayesian inference (BI) phylogenetic analyses were carried out using Mr Bayes version 3.2.6 (Ronquist *et al.*, 2012). The data were divided into seven partitions (ITS spacers, 5.8S gene, *psbA-trnH*, *rpl32-trnL*, *trnL-F*, *matK* coding region, *matK* intron region), analysed under the best-fit model of nucleotide evolution for each genic region selected using the AIC criterion as implemented in MrModeltest version 2.4 (Nylander, 2004). These were GTR+G for the ITS spacers, *trnL-F*, *psbA-trnH* and the *matK* intron region, GTR+I+G for *rpl32-trnL* and the *matK* coding region, and SYM+I for the ITS 5.8S gene.

Two runs with four chains each were run for 1,000,000 generations with a tree sampled every 1000th generation, and convergence between the runs was checked in Tracer version 1.7 (Rambaut *et al.*, 2018). The first 10% of sampled trees were discarded as burn-in and the remainder summarised as a maximum clade credibility tree and posterior probabilities (PP) extracted. Maximum likelihood (ML) analyses were conducted with RAxML version 8 (Stamatakis, 2014) via the CIPRES Gateway (Miller *et al.*, 2010). The search for the optimal ML tree was performed using GTR+I+G and a rapid bootstrap analysis of 1000 replicates. For the ML and Bayesian analyses, tree topology and node support were examined in FigTree version 1.4.3 (Rambaut, 2007).

## RESULTS

Our results (Fig. 2) show that the three *Cyrtandra umbellifera* collections from Taiwan form a strongly supported clade (BI-PP = 1, ML-BS = 96%, MP-BS = 89%), in a maximally supported sister relationship to a clade of four samples of the same species from Batan Island in the Philippines (BI-PP = 1, ML-BS = 97%, MP-BS = 83%). These together were sister to an unidentified sample from Luzon with strong support (BI-PP = 1, ML-BS = 99%, MP-BS = 98%). The collections of *Cyrtandra cumingii* from Batan Island and Japan were mixed together in a clade that received maximum branch support (BI-PP = 1, ML-BS = 100%, MP-BS = 100%).

The single sample of *Cyrtandra cumingii* from Luzon was, however, in a well-supported sister relationship with *C. cf. roseo-alba*, also from Luzon, with maximum branch support (BI-PP = 1, ML-BS = 100%, MP-BS = 100%), that was weakly associated (BI-PP = 0.71, ML-BS = 56%, MP-BS = 52%) with another clade.

## DISCUSSION

### *Cyrtandra umbellifera*

The results for *Cyrtandra umbellifera* suggest that the species on Taiwan could be either separated as a distinct species from the Philippine collections or kept together as a species with a distribution that includes both the Philippines and Taiwan. Our observations of the morphology suggest that it is not possible to separate these two, and that the morphological

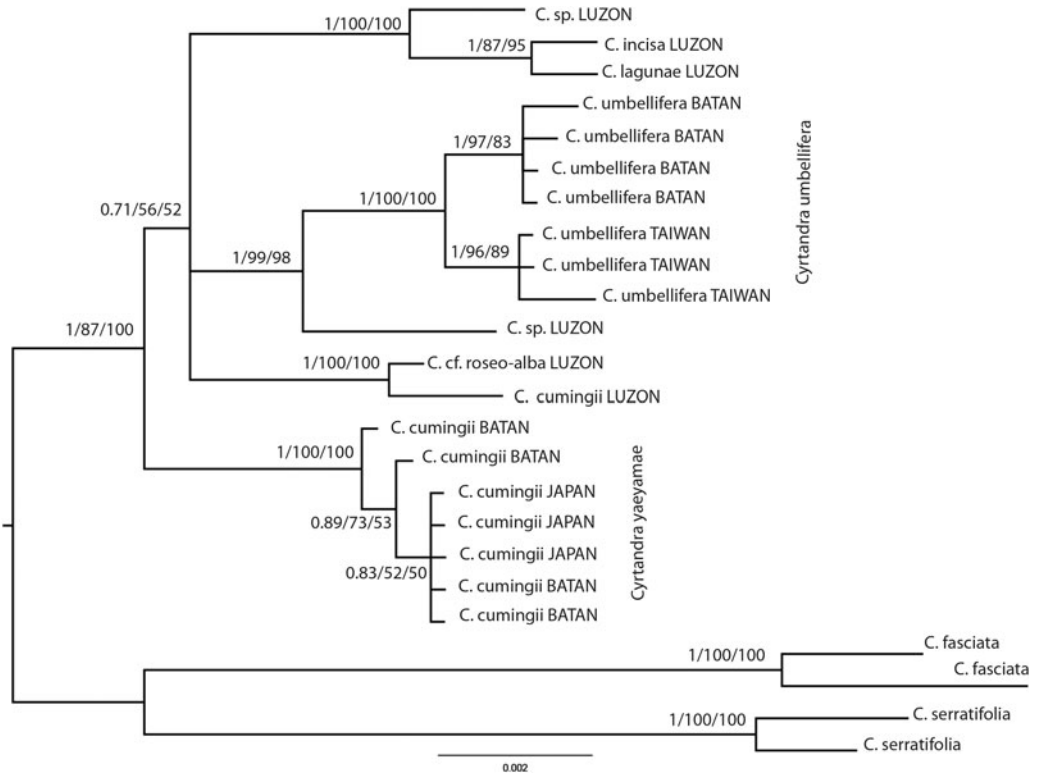


FIG. 2. Bayesian inference tree based on combined sequences of ITS, *psbA-trnH*, *rpl32-trnL*, *trnL-F* and *matK*. Node support is indicated as Bayesian posterior probabilities (BI-PP), maximum likelihood bootstrap support (ML-BS) and maximum parsimony bootstrap support (MP-BS).

differences suggested by Hosokawa (1935) to differentiate *Cyrtandra kotoensis* (i.e. leaves with oblique bases and much shorter subulate calyx lobes) are not consistent.

### *Cyrtandra cumingii*

Our phylogenetic results show that the collections from Japan are not *Cyrtandra cumingii*. This had been suggested previously by a number of authors (Ohwi, 1937; Gillett, 1973; Walker, 1976), although they had also stated that the species present on Iriomote Island of the Ryukyus was endemic. What these results show is that the species present in Iriomote Island of the Ryukyus is also present in the northern Philippines. This had not been suggested by earlier authors. On inspection of the specimens from the northern Philippines and Japan, there do appear to be good, consistent differences between these and the type of *Cyrtandra cumingii* from southern Luzon. These include differences in the number of lateral veins (15–18 in *Cyrtandra yaeyamae* and 12 in *C. cumingii*), calyx length (12–15 mm in *C. cumingii* and 5–6 mm in *C. yaeyamae*), and also leaf bases (often distinctly decurrent in *C. yaeyamae*, not in *C. cumingii*). There may be additional floral differences once a detailed revision of *Cyrtandra cumingii* in the Philippines can be carried

out, because no corolla was seen or described by Clarke (1883). The specimen from Batan Island cited as being *Cyrtandra cumingii* (Fenix 3787, US 00081311) (Merrill, 1908) and the justification for the expansion of its distribution to this island is, in fact, a close match for the other specimens included in this analysis from Batan Island and not a good match for the type collection of *C. cumingii* from Luzon.

In future studies, more intensive sampling across the rest of the Philippines would be useful, as well as a detailed study of morphology to determine the extent of the distribution of *Cyrtandra yaeyamae* and *C. cumingii* in the Philippines.

#### TAXONOMIC IMPLICATIONS

***Cyrtandra cumingii*** C.B.Clarke in A.DC. & C.DC., Monogr. Phan. 5(1): 263 (1883). – Type: Philippines, Luzon, 1841, *Cuming* 757 (lecto K [K000831609]; isolecto K [K000831610], P [P03884307], BM [BM000798277] hic. desig.).

*Distribution.* Philippines (Luzon, Mindanao, Mindoro, Panay).

This appears to be a fairly common and widespread species in Luzon, with a few records also from Mindanao, Panay and Mindoro. More intensive sampling and taxonomic work are required in the Philippines to determine the exact distribution and limits of this species. The name *Cyrtandra cumingii* has not been lectotypified, so we do that here, selecting the most complete specimen at Kew (K000831609) as the lectotype.

***Cyrtandra umbellifera*** Merr. in Philipp. J. Sci. 3: 435 (1909 [“1908”]). – Type: Batan Island, Philippines, 8 vi 1907, *Fenix* 3785 (lecto P [P03899661], isolecto US [US00126364] hic. desig.). **Fig. 3.**

*Cyrtandra kotoensis* Hosokawa in Trans. Nat. Hist. Soc. Formosa 25: 412 (1935). – Type: Taiwan, Island of Botel Tobago, Mt Koto, 7 xii 1935, *Hosokawa* 8129 (holo TAI).

*Distribution.* Taiwan and Batan Island, Philippines.

This distinctive species is characterised by its umbellate inflorescence and appears morphologically to be close to a number of species in the Philippines and Sulawesi that include *Cyrtandra longirostris* De Vriese, *C. callicarpifolia* Elmer and *C. bruteliana* Koord. from C.B.Clarke’s section *Cuneatae*.

Merrill did not specify a herbarium when designating *Fenix* 3785 as the type for this species (Merrill, 1908), and it has not been possible to find any subsequent lectotypification. The collection from Paris (P03899661) is selected here as the lectotype.

***Cyrtandra yaeyamae*** Ohwi in J. Jap. Bot. 13: 339 (1937). – “*Cyrtandra cumingii* var. *yaeyamae*” (Ohwi) Hatusima Fl. Ryukyus 557 (1971) (‘*yaeyamana*’) (not validly published; basionym cited only as “*C. yaeyamana* Ohwi (May 1937)”, cf. Art. 41.5). – Type: Japan, Iriomote Island, Nakara-gawa, x 1936, *Sonohara s.n.* (holo KYO [KYO00069674]). **Fig. 4.**

*Cyrtandra iriomotensis* Masam., Not. Syst. 6: 38 (1937). – Type: Japan, Iriomote Island, *Masamune s.n.* (holo TAI [Herbar. Taihoku Imp. University] n.v.).

*Cyrtandra cumingii* auct. non C.B.Clarke; Hatusima (1956); Yamazaki (1993).

*Distribution.* Iriomote Island of the Ryukyus, Japan and Batan Island, Philippines.





FIG. 3. *Cyrtandra umbellifera* Merr. A, Flower; B, habit; C, flower and fruits; D, habit. (Photographs: Goro Kokubugata; A and B, Lanyu, Taiwan; C and D, Batan Island, Philippines.)

*Chromosome number.*  $2n = 34$  (Kokubugata & Madulid, 2000).

This species is morphologically similar to the Philippine *Cyrtandra cumingii* but can be distinguished by the number of lateral veins (15–18 in *C. yaeyamae* and 12 in *C. cumingii*), calyx length (12–15 mm in *C. cumingii* and 5–6 mm in *C. yaeyamae*) and also often leaf bases (usually distinctly decurrent in *C. yaeyamae*, not in *C. cumingii*). The type of *Cyrtandra iriomotensis* has unfortunately not been seen. The type is cited as being at the Herbarium of Taihoku Imperial University in the protologue (Masamune, 1937). This became the Herbarium of the National Taiwan University (TAI) in 1945. Masamune was based in Taiwan throughout the Second World War and returned to Japan in 1945 (National Museum of Natural Sciences n.d.) with some of his collections, including a number of orchid types (Inoue *et al.*, 1998) and potentially other types, as a number of his specimens cited as being at TAI have not been found there (Inoue *et al.*, 1998). On returning to Japan, his collections were kept primarily at his home, and following his death, moved to the herbarium of Kanagawa Prefectural Museum of Natural History (KPM) (Inoue *et al.*, 1998).

Despite efforts made to locate the type of *Cyrtandra iriomotensis* at TAI and KPM and other likely herbaria in Taiwan and Japan (HAST, KANA, KYO, RYU, TAIF, TI and



FIG. 4. *Cyrtandra yaeyamae* Ohwi. A, Inflorescence; B, habit; C, inflorescence; D, habit. (Photographs: Goro Kokubugata; A and B, Batan Island, Philippines; C and D, Iriomote, Japan.)

TNS), we were unable to do so. Nonetheless, we are certain that this is conspecific with *Cyrtandra yaeyamae*, because the description of *C. iriomotensis* is a good match for the type of *C. yaeyamae* and, based on recent comprehensive fieldwork on Iriomote (area = 289.3 km<sup>2</sup>) by one of the authors (G.K.), we are confident that there is only one species of *Cyrtandra* present on the island.

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