

The Chromosome Numbers of Four Gesneriaceae Species Native to Japan

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Abstract Chromosome numbers at mitotic metaphase in four Gesneriaceae species native to Japan were investigated using the aceto-orcein squash method in the present study. The chromosome number was determined as $2n = 32$ in *Aeschynanthus acuminatus* and this is the first chromosome number report in this species. In the other three species, the chromosome number was $2n = 20$ in *Rhynchochelys discolor*; $2n = 34$ in *Opithandra primuloides*; and $2n = 40$ in *Titanotrichum oldhamii*.

Key words : chromosome number, Gesneriaceae, Japan, Ryukyu.

Introduction

Gesneriaceae is a middle to large sized plant family comprising approximately 150–160 genera, and over 3200 species. Its members are distributed primarily in the tropical regions, with some members extending to the temperate regions including Honshu of Japan (Weber and Skog, 2007 onward).

According to Yamazaki (1994), nine species from eight genera of Gesneriaceae are distributed in Japan, and these species are the northernmost species in each of the genera. Several taxonomic and molecular phylogenetic studies have been conducted for these Gesneriaceae species in Japan (Yamazaki, 1994; Kokubugata, 2002; Kokubugata and Peng, 2004; Kokubugata *et al.*, 2011, 2014). However, cytological data on the Gesneriaceae species in Japan is still limited; there are only two reports on *Conandron ramondiioides*, including *C. ramondiioides* var. *taiwanensis* (Kokubugata and Peng, 2004) and *Cytandra cumingii* (Kokubugata and Madulid, 2000).

The aim of this study is to investigate chromosome numbers of four Japanese Gesneriaceae species: *Aeschynanthus acuminatus* Wall. ex DC, *Opithandra primuloides* (Miq.) B. L. Burtt, *Rhynchochelys discolor* (Maxim.) B. L. Burtt var. *discolor* and *Titanotrichum oldhamii* (Hemsl.) Soler. Resulting chromosome counts will be compared with previous reports on the Gesneriaceae Species from different areas, and taxonomic issues will be discussed.

Materials and Methods

Plant materials

Plants materials of each species were collected by the authors, and were cultivated in the greenhouse of Tsukuba Botanical Garden, National Museum of Nature and Science for cytological experiments. Voucher specimens were deposited in the herbarium of National Museum of Nature and Science (TNS) (Table 1).

The genus *Aeschynanthus* Jack consists of about 160 species, mainly distributed from India,

Table 1. Collection localities and voucher specimens of Gesneriaceae species in the present cytological study

Species	Collection locality	Collection number (Herbarium)
<i>Aeschynanthus acuminatus</i>	Japan, Ryukyus, Okinawa, Yaeyama Group, Iriomote Island.	<i>G.Kokubugata11644</i> (TNS)
<i>Opithandra primuloides</i>	Japan, Shikoku, Kochi, Shimanto.	<i>G.Kokubugata9463</i> (TNS)
<i>Rhynchocheum discolor</i>	Japan, Ryukyus, Okinawa, Yaeyama Group, Ishigaki Island.	<i>G.Kokubugata214</i> (TNS)
<i>Titanotrichum oldhamii</i>	Japan, Ryukyus, Okinawa, Yaeyama Group, Iriomote Island.	<i>G.Kokubugata1346</i> (TNS)

the southern part of China, throughout Malaysia to New Guinea and the Solomon Islands (Middleton, 2007). *Aeschynanthus acuminatus* is widely distributed from India to Japan (Li and Kao, 1998). In Japan, this species was first found in 1973 in Iriomote Island of the Ryukyus Archipelago (hereafter the Ryukyus) (Hatusima, 1974). Thereafter, the species had not been found for decades, and Kobayashi and Kokubugata (2005) rediscovered the species in a locality near the original locality reported by Hatusima (1974). To date, no other population of this species has been found in Japan, and thus the species is protected as a critically endangered species (CR) in Japan (Japanese Ministry of Environment, 2015).

The genus *Opithandra* B. L. Burtt consists of 11 species, and is distributed in the southern part of China (10 species) and Japan (one species) (Wang, 2004). *Opithandra primuloides* is endemic to Japan proper (Honshu, Shikoku and Kyushu), and treated as a threatened species (VU) because of its rare occurrence (Japanese Ministry of the Environment, 2015).

The genus *Rhynchocheum* Blume consists of 16 species and primarily occurs in tropical and subtropical Asia (Wang and Wang, 2000); in Japan one species and two varieties are known: *R. discolor* var. *discolor* and *R. discolor* var. *incisum* (Ohwi) E. Walker. *Rhynchocheum discolor* var. *discolor* is distributed in Kyushu and the Ryukyus of Japan, Taiwan, and the Philippines.

The genus *Titanotrichum* Soler is monotypic and consists of *T. oldhamii*, the species distributed in China, Japan and Taiwan (Weber and Skog, 2007 onward). In Japan, this species is rarely found on Ishigaki and Iriomote islands of the Ryukyus, and thus it is treated as a critically

endangered species (CR) in Japan (Japanese Ministry of Environment, 2015).

Chromosome observation

Root tips of cultivated accessions were pre-treated in 2mM 8-hydroxyquinoline at 20°C for 4h, and fixed in 45% acetic acid for 10sec. They were macerated in a mixture of 1 N hydrochloric acid and 45% acetic acid at 60°C for 5sec., and were stained in 2% aceto-orcein on glass slides at 20°C for 3hrs. The standard squash method was applied to make well-spread chromosomes at mitotic metaphase.

Results and Discussion

Aeschynanthus acuminatus Wall. ex DC (Fig. 1A)

One plant of *A. acuminatus* from Iriomote Island of the Ryukyus showed a chromosome number of $2n = 32$ (Fig. 1B). This is the first chromosome-number report in the species. Möller and Kiehn (2004) suggested three basic chromosome numbers of $x = 14, 15$ and 16 for the genus *Aeschynanthus*, and the present chromosome number in agreement with the idea that $x = 16$ is the most common in the genus *Aeschynanthus* (Möller and Kiehn, 2004).

Opithandra primuloides (Miq.) B.L.Burtt (Fig. 1C)

One plant of *Opithandra primuloides* was investigated and showed a chromosome number of $2n = 34$ (Fig. 1D). This agrees with the reports by Fussell (1958) and Ratter (1963). However, collection locality of plant materials was not indicated in their reports.

Concerning the taxonomy, the species was first described as *Boea primuloides* Miq. based on a type specimen collected from Japan (Miquel,

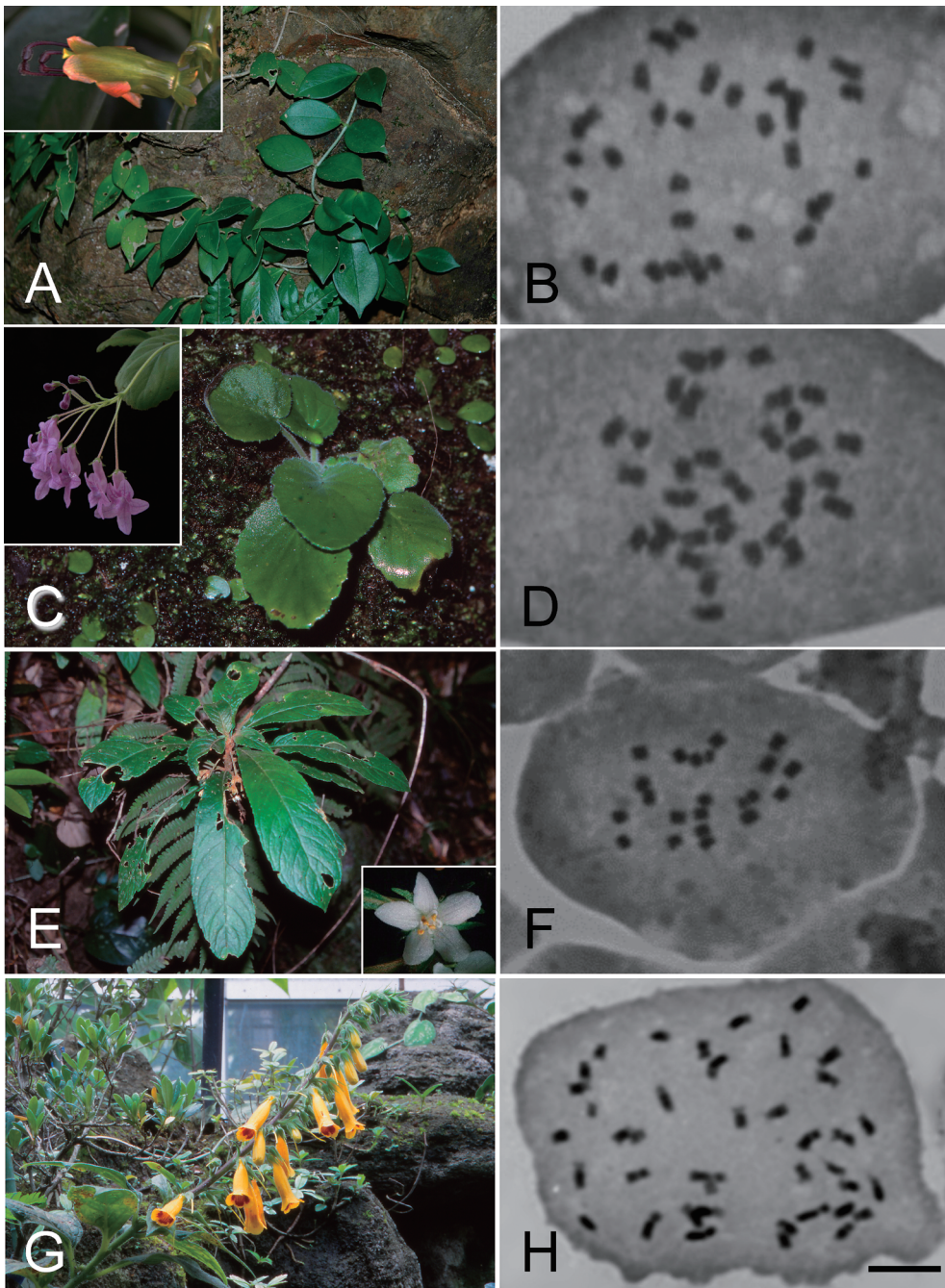


Fig. 1. Plants and chromosomes at mitotic metaphase of four Gesneriaceae species. A, C, E and G. Plants. B, D, F and H. Somatic chromosomes at mitotic metaphase. A and B. *Aeschynanthus acuminatus* (G.Kokubugata11644). C and D. *Opithandra primuloides* (G.Kokubugata9463). E and F. *Rhynchotechum discolor* var. *discolor* (G.Kokubugata214). G and H. *Titanotrichum oldhamii* (G.Kokubugata1346). In A, C and G, the flowers were photographed in Tsukuba Botanical Garden. Bar indicates 5 μ m for B, D, F and H.

1867). Thereafter, the species was transferred and named *Oreocharis primuloides* (Miq.) Benth. et Hook.f. ex Clarke (Clarke, 1883); and then *Opithandra primuloides* by Burt (1956) as the genus *Opithandra* was established. A recent molecular phylogenetic study revealed that *Opithandra primuloides* was included in a clade of *Oreocharis sensu lato*, and supported the taxonomic treatment of Clarke (1883) (Möller *et al.*, 2011). Previously, a chromosome number of $2n=34$ was also reported for two *Oreocharis* species from China (Lu *et al.*, 2002; Zhou *et al.*, 2003). Although it is premature to conclude whether *Opithandra* and *Oreocharis* are independent genera or not and the generic treatment of *O. primuloides*, the present cytological study suggests that these two genera sharing the same chromosome number are closely related, in agreement with the molecular phylogenetic result (Möller *et al.*, 2011).

Rhynchoechum discolor (Maxim.) B. L. Burt var. *discolor* (Fig. 1E)

One plant of *R. discolor* var. *discolor* from Ishigaki Island of the Ryukyus showed a chromosome number of $2n=20$ (Fig. 1F), agreeing with the chromosome count reported by Ratter (1963; no locality information); and that from Taiwan by Wang and Wang (2000).

Titanotrichum oldhamii (Hemsl.) Soler. (Fig. 1G)

One plant of *T. oldhamii* from Iriomote Island of the Ryukyus showed a chromosome number of $2n=40$ (Fig. 1H), agreeing with that reported by Ratter (1963).

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References

Burt, B. L. 1956. An independent genus for *Oreocharis primuloides*. *Baileya* 4: 161–162.
Clarke, C. B. 1883. *Cyrtandreae*. In: de Candolle, A. and

de Candolle, C. (eds.), *Monographie Phanerogamarum*, vol. 5, pp. 1–303 with 32 plates. G. Masson, Paris.
Fussell, C. P. 1958. Chromosome numbers in the Gesneriaceae. *Baileya* 6: 117–125.
Hatusima, S. 1974. New or noteworthy plants from the Ryukyus. In: Ikehara, S. (ed.), *Ecological Studies of Nature Conservation of the Ryukyu Islands*, vol. 1, pp. 37–45. University of the Ryukyus, Naha.
Japanese Ministry of the Environment. 2015. Red Data Book 2014—Threatened Wildlife of Japan—. Vol. 8, Vascular Plants. GYOSEI Corporation, Tokyo (in Japanese).
Kobayashi, S. and Kokubugata, G. 2005. Rediscover of *Aeschynanthus* in Japan. *Bunrui* 5: 43–44 (in Japanese).
Kokubugata, G. and Madulid, D. A. 2000. Comparative study of chromosome number and karyotype of *Cyrtandra cumingii* var. *yaeyamana* (Gesneriaceae) from Batan Island, the Philippines and Iriomote Island, Japan. *The Biological Magazine, Okinawa* 38: 1–7.
Kokubugata, G. 2002. Notes on species of Gesneriaceae common to both the Ryukyus and Taiwan. *Proc. Symposium on Collection Building and Natural History Studies in Asia. National Science Museum Monographs* 22: 137–146.
Kokubugata, G. and Peng, C.-I. 2004. Floral morphology and recognition of varieties in *Conandron ramondoides* (Gesneriaceae) from Japan and Taiwan. *Edinburgh Journal of Botany* 61: 21–31.
Kokubugata, G., Hirayama, Y., Peng, C.-I., Yokota, M. and Möller, M. 2011. Phylogeographic aspects of *Lysionotus pauciflorus sensu lato* (Gesneriaceae) in the China, Japan and Taiwan regions: phylogenetic and morphological relationships and taxonomic consequences. *Plant Systematics and Evolution* 292: 177–188.
Kokubugata, G., Nakamura, K., Saito, Y., Labuguen, M. L., Lopez-Feliciano, A. and Yokota, M. 2014. Molecular Phylogenetic distinction between Taiwan endemic *Rhynchoechum brevipedunculatum* (Gesneriaceae) and *R. discolor* widespread in subtropical and tropical Asia. *Bulletin of the National Museum of Nature and Science, Series B* 40: 47–53.
Li, Z.-Y. and Kao, M.-T. 1998. Gesneriaceae. In: Editorial Committee of the Flora of Taiwan (ed.), *Flora of Taiwan*, vol. 4, 2nd edn., pp. 688–712. Editorial Committee of the Flora of Taiwan, Taipei.
Lu, Y.-X., Sun, X.-F., Zhou, Q.-X. and Gu, Z.-J. 2002. Chromosome numbers in ten species in the Gesneriaceae from Yunnan. *Acta Botanica Yunnanica* 24: 377–382.
Middleton, D. J. 2007. A revision of *Aeschynanthus* (Gesneriaceae) in Thailand. *Edinburgh Journal of Botany* 64: 363–429.
Miquel, F. A. G. 1867. *Prolusio florum Japonicarum*. *Annales Musei Botanici Lugduno-Batavi* 3: 1–66.
Möller, M. and Kiehn, M. 2004. A synopsis of cytological

- studies in Gesneriaceae. *Edinburgh Journal of Botany* 60: 425–447.
- Möller, M., Middleton, D., Nishii, K., Wei, Y.-G., Sontag, S. and Weber, A. 2011. A new delineation for *Oreocharis* incorporating an additional ten genera of Chinese Gesneriaceae. *Phytotaxa* 23: 1–36.
- Ratter, J. A. 1963. Some chromosome numbers in the Gesneriaceae. Notes from Royal Botanic Garden, Edinburgh 24: 221–229.
- Wang, J.-C. and Wang, C.-C. 2000. The genus *Rhynchotechum* Blume (Gesneriaceae) in Taiwan. *Taiwaniana* 45: 355–365.
- Wang, Y.-Z. 2004. Genus *Opithandra*. In: Li, Z.-Y. and Wang, Y.-Z. (eds.), *Plants of Gesneriaceae in China*, pp. 113–118. Henan Science and Technology Publishing House, Zhengzhou (in Chinese).
- Weber, A. and Skog, L. E. 2007 onward. The genera of Gesneriaceae—Basic information with illustration of selected species—, 2nd edn. <http://www.genera-gesneriaceae.at/> (accessed on February 1, 2017).
- Yamazaki, T. 1994. Gesneriaceae. In: Iwatsuki, K., Yamazaki, T., Boufford, D. E. and Ohba H. (eds.), *Flora of Japan*, vol. IIIa, pp. 376–379. Kodansha, Tokyo.
- Zhou, P., Gu, Z.-J. and Möller, M. 2003. New chromosome counts and nuclear characteristics for some members of Gesneriaceae subfamily Cyrtandroideae from China and Vietnam. *Edinburgh Journal of Botany* 60: 449–466.