



Cremastra saprophytica (Orchidaceae: Epidendroideae), a new leafless autonomously self-pollinating orchid species from Gifu Prefecture, Japan

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Abstract

A new species of *Cremastra* (Orchidaceae), *C. saprophytica*, is described from Gifu Prefecture, Japan. The new species is similar to *C. aphylla* in having a leafless and mycoheterotrophic habit. However, it is distinguishable from *C. aphylla* by its green stem, more closed perianth tube, smaller lateral lobes of lip, smaller callus of lip positioned at base of the midlobe and absence of a rostellum and viscidium. An illustration and ecological information on the new species are provided. A key to the *Cremastra* species is also provided.

Keywords: Calypsoinae, Japanese flora, mycorrhiza, mycoheterotrophy, new species, self-pollination, taxonomy

Introduction

Cremastra Lindley (1833: 172; Orchidaceae) is a small genus of five species consisting of both leafless and leafy species distributed from Nepal to Japan (Hu *et al.* 2013). *Cremastra* is characterized by an underground pseudobulb, a racemose inflorescence arising directly from the apex of the pseudobulb, flowers with a clawed lip and lip callus (Lund 1988, Hu *et al.* 2013, Watthana *et al.* 2015). Based on molecular phylogenetic classification, *Cremastra* has been placed in subtribe Calypsoinae, tribe Epidendreae (Epidendroideae; Chase *et al.* 2015). Lund (1988) revised *Cremastra* and recognised two species, *C. appendiculata* (Don 1825: 36) Makino (1904: 24) and *C. unguiculata* (Finet 1896: 698) Finet (1897: 235); in *C. appendiculata*, he also recognized two varieties, var. *appendiculata* and var. *variabilis* (Blume 1849: 48) Lund (1988: 201). After this, three new species, *C. aphylla* Yukawa (1999: 59), *C. guizhouensis* Chen & Chen (2003) and *C. malipoensis* Hu in Hu *et al.* (2013: 64) have been described. Given that recent floral surveys have revealed several new species and new records in the genus (Chen & Chen 2003, Hu *et al.* 2013, Watthana *et al.* 2015), it is likely that the diversity of *Cremastra* species remains underestimated. As expected, a leafless but green *Cremastra* species with significantly different morphology compared to the other species was discovered during recent botanical surveys on Gifu Prefecture, Japan.

Taxonomic treatment

Cremastra saprophytica Suetsugu, *sp. nov.* (Figs 1, 2)

Type:—JAPAN. Gifu Pref.: Ibi County, Ibigawa Town, Kasugakawai, 5 Jun 2021, *Suetsugu Sa52* (holotype: KYO!, spirit collection).

Cremastra saprophytica is similar to *C. aphylla* but differs by its more closed perianth tube, smaller lateral lip lobes, smaller callus positioned at base of the midlobe and lack of rostellum and viscidium.

Terrestrial, leafless herbs, 28–48 cm tall with subterranean tuberous pseudobulbs, creeping rhizomes and sometimes coralloid mycorrhizal rhizomes. Roots fibrous, whitish, densely hairy, spreading from the base of the pseudobulb. Inflorescence erect from the upper part of pseudobulb, 25–45 cm long, slightly purplish green with 2–3

nodes, each node with a tubular sheathing scale, the sheath 2.5–6.0 cm long, rachis 7–15 cm long, 5–22-flowered, secund, floral bracts narrowly elliptic to lanceolate, obtuse, 0.5–1.0 cm long, green. Pedicel and ovary ca. 2 times longer than floral bract, up to 24 mm, dark purple. Flowers pendulous, hardly opening, rose-purple to orange-brown, narrowly campanulate. Dorsal sepal oblanceolate-spathulate, 26.0–30.0 × 3.1–3.9 mm at the widest part, apex acute to acuminate. Lateral sepals oblanceolate-spathulate, slightly oblique, 26.0–30.0 × 3.1–3.9 mm at the widest part, apex acute to acuminate. Petals oblanceolate-spathulate 24.5–27.0 × 2.7–3.2 mm at the widest part, apex obtuse. Lip 26.0–30.0 mm long, divided into epichile and hypochile, hypochile linear, shallowly saccate at base involute and furrowed, 18–21 mm long, epichile trilobed from base, lateral lobes extending from inrolled margins of hypochile, 2.5–3.5 mm long, apex never exceeding column, midlobe ovate to narrowly oblong, 8.0–9.0 mm long, apex acute or obtuse; base of epichile with a smooth callus, 2.0–3.0 mm long, 0.5–1.0 mm in diameter. Column straight, 24.0–26.5 mm long, without narrow ventral wings below anther, dilated at apex, light purple, purple surrounding stigma cavity and along midrib on ventral surface, stigma orbicular, rostellum absent, anther cap joined with column, triangular, yellow, apex thickened, pollinia 4, yellow, compressed, in 2 groups, viscidium absent. Capsules pendent, ellipsoid-cylindric, 30–33 mm long.

These measurements above are based on several specimens from the type locality and may not entirely represent the diversity present in the species when more specimens are found in the future.

Additional specimens examined:—JAPAN. Gifu Pref.: Ibi County, Ibigawa Town, Kasugakawai, 18 Dec 2020, *Nishida 20201218* (Lake Biwa Museum!)

Distribution and phenology:—*Cremastra saprophytica* is only known from the type locality. Flowering occurred from late May to early June, and fruiting from late June to early October.

Taxonomic notes:—*Cremastra saprophytica* is the second completely leafless species in the genus. It is superficially similar to *C. aphylla* due to their leafless and mycoheterotrophic habit (Yukawa 1999). However, *C. saprophytica* can be easily distinguished from *C. aphylla* by its green stem, more closed perianth tube, smaller lateral lobes of lip, smaller callus of lip positioned at base of the midlobe, and absence of a rostellum and viscidium (Figs 1–3 and Table 1).

TABLE 1. Morphological comparison of *Cremastra saprophytica* and *C. aphylla*.

characters	<i>C. saprophytica</i>	<i>C. aphylla</i>
stem colour	green	dark brownish purple
flower colour	rose-purple to orange-brown	brownish black
floral condition	almost closed	weakly open
length of lateral sepals	26–30 mm	32–36 mm
length of lateral lip lobes	2.5–3.5 mm long, never exceeding column	more than 5.0 mm long, consistently exceeding column
lip callus condition	linear or acute, smooth, 2.0–3.0 mm long, 0.5–1.0 mm in diameter and height	clavate, verruculose, ca. 5.0 mm long, 2.5 mm in diameter and height
anther cap condition	joined with column	free
rostellum condition	absent	flat, deeply cleft after removing pollinarium
viscidium condition	absent	large, folded

In *Cremastra*, except in the two leafless species, the leaf emerges in the autumn and persists through the winter, withering at flowering (Freudenstein *et al.* 2017). Therefore, because leafy *Cremastra* species often lack leaves when flowering, *C. saprophytica* may be confused with two varieties of *C. appendiculata* with relatively similar floral morphology. However, apart from its leafless habit, *C. saprophytica* can be distinguished from *C. appendiculata* var. *appendiculata* by the floral morphological characters mentioned above because *C. aphylla* and *C. appendiculata* var. *appendiculata* have identical floral structures (Lund 1988, Yukawa 1999). In addition, although *C. saprophytica* is somewhat similar to *C. appendiculata* var. *variabilis* in having a small smooth lip callus on the base of midlobe (Lund

1988), *C. saprophytica* can be distinguished by the other floral morphological characters mentioned above as well as its column wing condition (absent vs. a narrow ventral wing below anther).



FIGURE 1. *Cremastra saprophytica* from the type locality. A–C. Flowering plant. D. Flower, dorsal view. E. Flower, lateral view. F. Flower, front view. Central arrow points to a small smooth callus of lip positioned at the base of midlobe, whereas the other arrows point to the inconspicuous lateral lobes. G. Fruiting plants. H. Fruiting body of *Coprinellus disseminates*, one of the associated fungi of *C. saprophytica*.

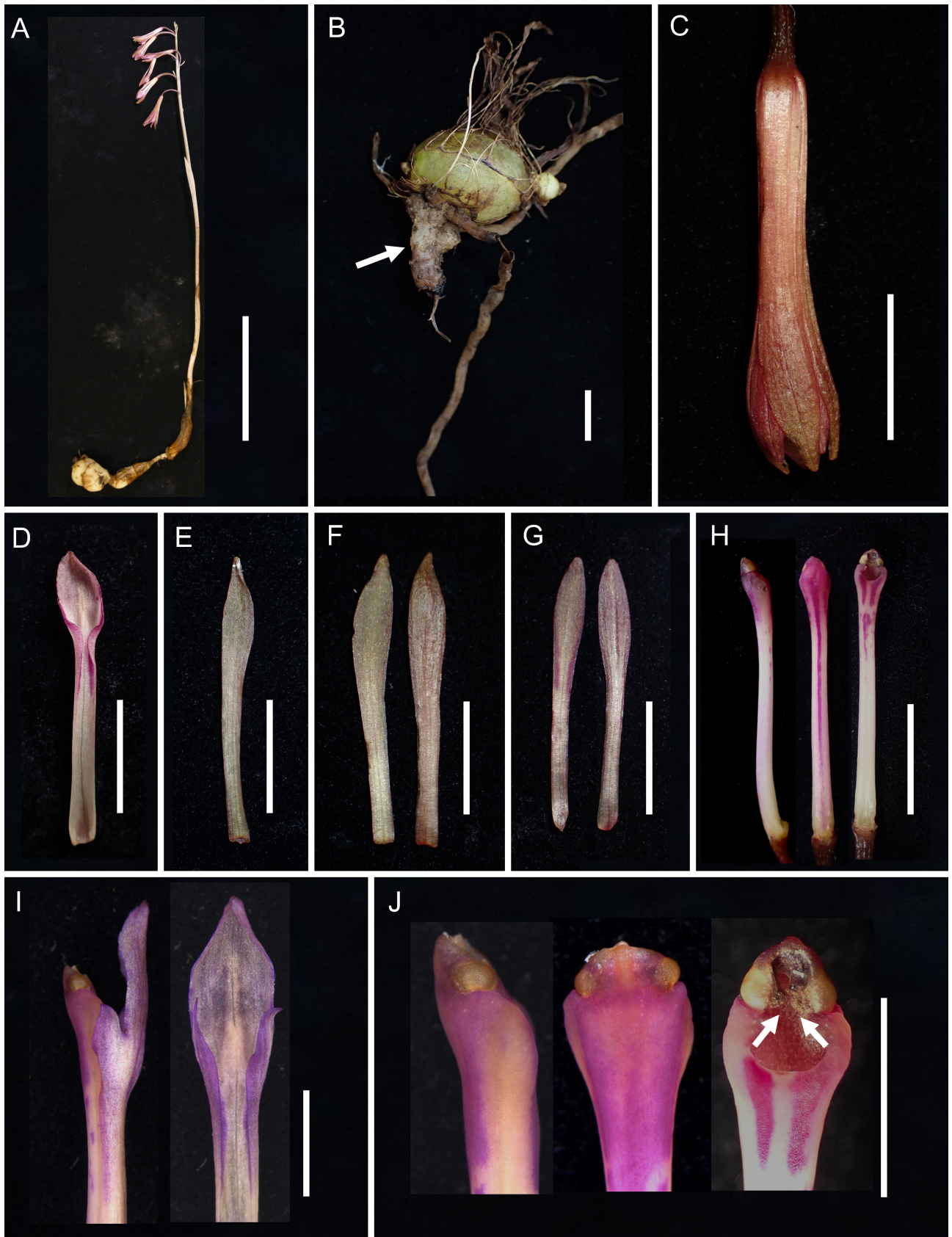


FIGURE 2. *Cremastra saprophytica* (holotype). A. Habit. B. Pseudobulb with roots and a coralloid mycorrhizal rhizome; arrow points to mycorrhizome. C. Flower, dorsal view. D. Lip. E. Dorsal sepal. F. Lateral sepals. H. Column (lateral, ventral and dorsal views). I. Close-up of the upper part of the lip (lateral and dorsal views). J. Close-up of the upper part of the column (lateral, ventral and dorsal views); arrow points to contact between pollinia and stigma. Scale bars: A = 10 cm; B–H = 1 cm; I–J = 5 mm.

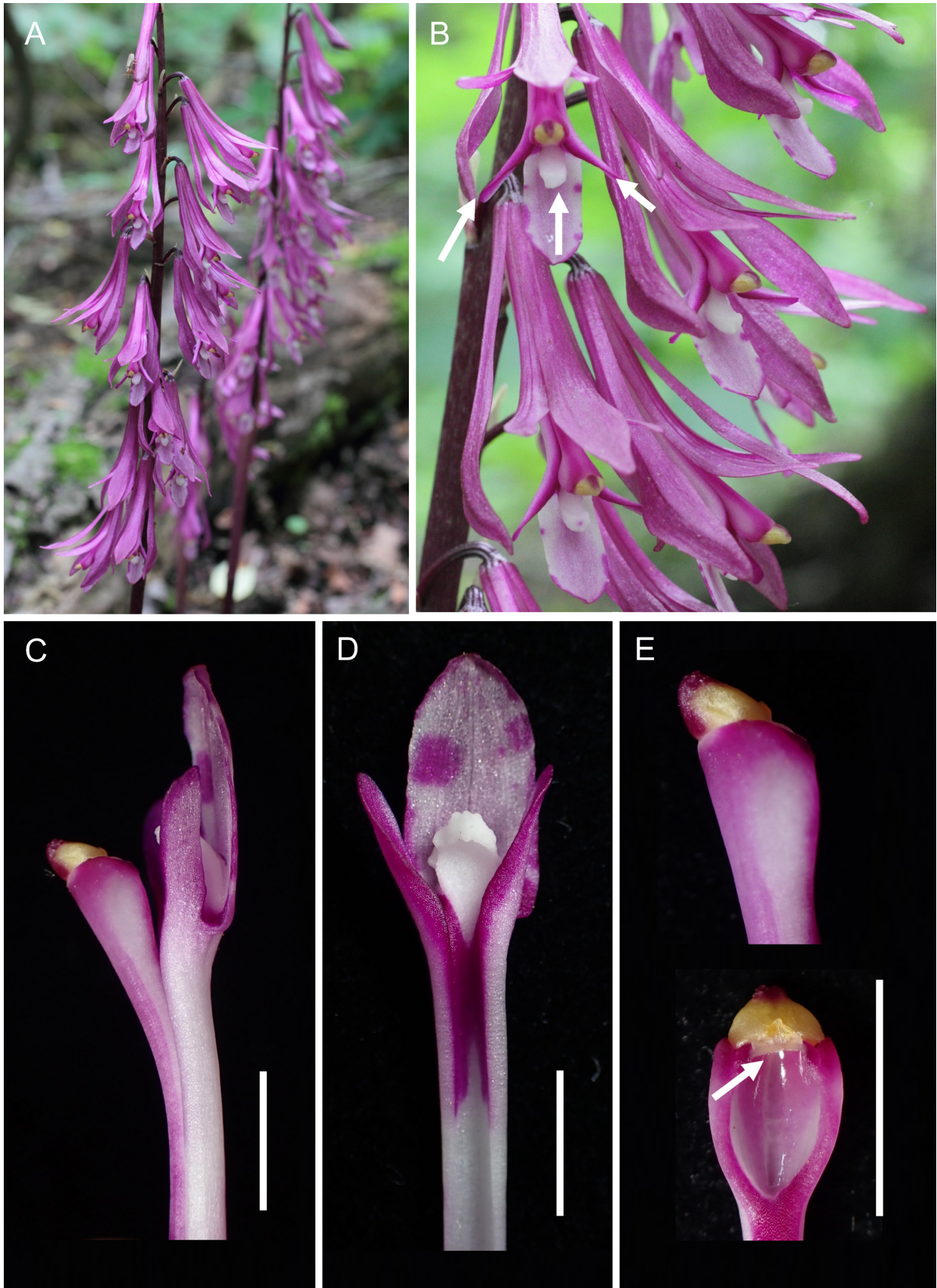


FIGURE 3. *Cremastra aphylla* from Hokkaido. A. Flowering plant. B. Flowers; central arrow points to a large verruculose callus of lip positioned at the base of midlobe, whereas the other arrows point to the conspicuous lateral lobes. C. Close-up of the upper part of the lip and column, lateral view. D. Close-up of the upper part of the lip, dorsal view. E. Close-up of the upper part of column and anther cap (lateral and ventral views); arrow points to a large, folded viscidium attached to pollinia. A–B: *Y. Sugawara* KS872 (KYO). C–D: *Suetsugu & Horie* KS411 (KYO). All scale bars = 5 mm.

Reproductive notes:—*Cremastra saprophytica* possesses an effective self-pollination system, and the fruit set is nearly 100% (Fig. 1G). In other *Cremastra* species, a well-developed rostellum/viscidium acts as a barrier to self-pollination (Fig. 3E; Chung & Chung 2003). In contrast, there is no rostellum and viscidium in *C. saprophytica*, resulting in contact between the pollinia and stigma (Fig. 2J) and allowing autonomous self-pollination. In addition, given that the viscidium acts as a glue to bind the pollinia to insect visitors, its lack must decrease the likelihood of insect pollination. Therefore, autogamy is likely the dominant, if not exclusive, reproductive strategy in *C. saprophytica*. As mentioned above, *C. saprophytica* can be distinguished from *C. aphylla* by not only column morphology but also smaller flower size and more closed perianth tube (Fig. 1, 3) probably associated with its autonomous self-pollination system.

Self-pollination is thought to be an adaptive response that provides reproductive assurance under conditions of pollinator limitation (Suetsugu 2013b), which is reported to be widespread among orchids (Tremblay *et al.* 2004). The fruit set of entomophilous *Cremastra* species is far lower than average in Orchidaceae (ca. 30%; Tremblay *et al.* 2004), with *C. appendiculata* in a Korean population exhibiting only 1.3–2.0% fruit set under natural conditions, even though artificial self- and cross-pollination both resulted in nearly 100% fruit set (Chung & Chung 2003). Therefore, it is likely that pollinator limitation severely affects the reproductive success of *Cremastra* species. It is also noteworthy that autonomous self-pollination has been suggested to be favourable for mycoheterotrophic plants because they are restricted to dark shaded forest understory with few pollinators (Leake 1994, Zhou *et al.* 2012, Suetsugu 2013a, 2015).

Ecological notes and etymology:—Mycoheterotrophic plants have often been misrepresented by many botanists as a form of saprophytism (Leake 1994). However, despite their leafless nature, they do not directly obtain carbon from decaying organic matter. Instead, most mycoheterotrophic plants depend on the photosynthate of adjacent autotrophic plants through shared mycorrhizal networks (Martos *et al.* 2009, Suetsugu *et al.* 2020). However, recent studies have shown that several mycoheterotrophic orchids obtain carbon from dead wood via saprotrophic fungi (Martos *et al.* 2009, Suetsugu *et al.* 2020). Therefore, even though the term “mycoheterotroph” has replaced the formerly misapplied term “saprophyte”, some mycoheterotrophic plants are indirectly saprotrophic (Martos *et al.* 2009). The genus *Cremastra* is one of such examples exploiting wood-decaying Psathyrellaceae (Suetsugu *et al.* 2021). *Cremastra saprophytica* is also associated with Psathyrellaceae fungi, and the fruiting bodies of *Coprinellus disseminates*, one of the mycobionts, could be observed on decayed fallen trees near *C. saprophytica* plants (Fig. 1H). The new species is named after its indirectly saprophytic habit.

However, it should be noted that *C. saprophytica* accumulates more chlorophyll than *C. aphylla* in the shoot (Fig. 1A–C and 1G), although it is arguably at a late stage in the evolutionary development toward complete mycoheterotrophy due to its leafless habit. Given that recent studies have shown that the stems of these leafless orchids have been shown to provide some photosynthetic carbon to the plants (Zimmer *et al.* 2008, Suetsugu *et al.* 2018, Kobayashi *et al.* 2021), it is likely that *C. saprophytica* is a partially mycoheterotrophic species rather than fully mycoheterotrophic. In particular, because the green colour deepens during fruit maturation (Fig. 1G), its photosynthetic ability may significantly contribute to fruit and seed production. Notably, the underground parts of *C. aphylla* always consisted of pseudobulbs, roots and coralloid mycorrhizomes, whereas not all *C. saprophytica* plants formed coralloid mycorrhizomes. Because the main area of mycorrhizal colonization is coralloid rhizomes in *Cremastra* (Yagame *et al.* 2013, Suetsugu *et al.* 2021), occasional lack of coralloid rhizomes might reflect some autotrophic carbon gain. Thus, comparative studies in *C. saprophytica*, *C. aphylla* and *C. appendiculata* could be an ideal model to understand how the photosynthetic apparatus functions in chlorophyllous but highly mycoheterotrophic orchids.

Preliminary conservation status:—*Cremastra saprophytica* is currently known only from a single population. The population comprises roughly ten mature plants, and at present we are not aware of any other locality where this species persists.

Key to the species of *Cremastra* (based on Chen *et al.* 2013)

1. Mycoheterotrophic plants without foliage leaves.....2
- Apparently autotrophic plants with normal leaves.....3
2. Stem dark brownish purple; flower weakly open, dark brownish purple; lip lateral lobes consistently exceeding column; lip callus positioned at the base of midlobe, clavate, verruculose, ca. 5.0 mm long, 2.5 mm in diameter and height, rostellum flat, deeply cleft after removing pollinarium, viscidium large, folded..... *C. aphylla*
- Stem green; flower almost closed, rose-purple to orange-brown, lip lateral lobes never exceeding column, lip callus positioned at the base of midlobe, linear or acute, smooth, 2.0–3.0 mm long, 0.5–1.0 mm diameter, rostellum absent, viscidium absent..... *C. saprophytica*

3. Creeping rhizome between pseudobulbs 3–4 cm long, leaves green with purple blotches, flowers suberect, opening widely, lip 16–20 mm, midlobe strongly reflexed..... *C. unguiculata*
- Creeping rhizome between pseudobulbs ca. 1 cm long, leaves green without purple blotches; flowers pendulous or horizontal on rachis, not opening widely, lip 25–35 mm, mid-lobes straight to slightly reflexed 4
4. Pseudobulbs broadly cylindric, 10.0–14.0 cm, lip callus midway along midlobe *C. guizhouensis*
- Pseudobulbs corm-like, ovoid or subglobose, 1.5–3.0 cm, lip callus at base of midlobe 5
5. Inflorescence sparsely 4–7-flowered, flowers horizontal to slightly pendulous, lip lateral lobes geniculate and twisted 90° at the base, midlobe slightly reflexed, rhombic to broadly ovoid *C. malipoensis*
- Inflorescence compact to densely (5–)8–32-flowered, flowers pendulous, lip lateral lobes straight, midlobe ovate to narrowly oblong 6
6. Lip callus clavate, verruculose, ca. 5.0 mm long, 2.5 mm in diameter, column with a narrow ventral wing below anther *C. appendiculata* var. *appendiculata*
- Lip callus linear, smooth, 2.0–4.0 mm long, 1.0–2.0 mm in diameter, column wing absent *C. appendiculata* var. *variabilis*

Acknowledgements

I am grateful to Kenji Nishida, Shigeharu Fujita and Miki Ishida for their discovery of *Cremastra saprophytica* and their help with the field study. I also thank Yasuhiko Sugawara and Kenji Horie for providing specimens and pictures of *C. aphylla* for comparative study. This study was financially supported by the JSPS KAKENHI (17H05016).

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