A New Variety of the Mycoheterotrophic Plant *Sciaphila yakushimensis* from Okinawa and Ishigaki Islands, Japan

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*Sciaphila yakushimensis* var. *okinawensis* from Okinawa and Ishigaki Islands, Japan, is described as new. It can be distinguished from *S. yakushimensis* var. *yakushimensis* by the morphology of the staminate and carpellate flowers.

Key words: mycoheterotroph, new locality, *Sciaphila*, taxonomy, Triuridaceae

Member of the Triuridaceae Gardner are fully mycoheterotrophic plants that grow in deep shade in the understory of ever-wet forests in the tropics and subtropics worldwide. They reach their northernmost limit of distribution in temperate Japan (van de Meerendonk 1984, Averyanov 2007). *Sciaphila* Blume, which contains ca. 40 species, is the largest genus in the family (van de Meerendonk 1984).

As with most other mycoheterotrophs (e.g., Suetsugu 2016, 2017), *Sciaphila* usually occurs in small populations, is small, up to 40 cm in height, and recognizable only during its reproductive season (Suetsugu *et al.* 2016, Suetsugu & Nishioka 2017). Consequently, collections are scarce and the morphological traits rarely described in detail. Additionally, key characteristics of the staminate flowers, which are crucial for precise identification, have not been documented in some species, particularly in individuals too young at the time of collection (Tsukaya & Okada 2013, Tsukaya & Suetsugu 2014, Suetsugu *et al.* 2017). Given the difficulty of precise identification, the taxonomy of *Sciaphila* remains unclear.

Because Japan is known for its great diversity of *Sciaphila*, harboring eight species (Suetsugu *et al.* 2016, Suetsugu & Nishioka 2017, Suetsugu & Sugimoto 2018), it is likely that further investigations will yield additional collections from which more precise data regarding diversity and distribution can be obtained. During our botanical surveys on Okinawa and Ishigaki Islands in the Ryukyu Archipelago, we collected plants of *Sciaphila* that we could not identify. The plants resembled *S. yakushimensis* but differed in the morphology of the staminate and carpellate flowers. To determine their identity, we analyzed their morphological characters and DNA barcoding.

**Materials and Methods**

**Morphological observations**

To compare the morphology of the unknown plants of *Sciaphila*, we undertook a thorough literature review, conducted field sampling throughout Japan and consulted specimens from the herbaria TI, TNS, KYO, MAK, and KAG, as well as online digital images of plant specimens on JSTOR Global Plants (http://plants.jstor.org/).
Table 1. Materials used for DNA sequencing.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Locality</th>
<th>Collection date</th>
<th>Collection no.</th>
<th>DDBJ accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sciaphila yakushimensis var. okinawensis</td>
<td>Mt. Omoto, Ishigaki Isl.</td>
<td>2016.6.27</td>
<td>A. Kinoshita KS133 (TNS)</td>
<td>LC371047</td>
</tr>
<tr>
<td>Sciaphila yakushimensis var. okinawensis</td>
<td>Mt. Omoto, Ishigaki Isl.</td>
<td>2016.10.17</td>
<td>T. Sugimoto KS131 (TNS)</td>
<td>LC371045</td>
</tr>
<tr>
<td>Sciaphila yakushimensis var. okinawensis</td>
<td>Mt. Omoto, Ishigaki Isl.</td>
<td>2016.10.18</td>
<td>T. Sugimoto KS132 (TNS)</td>
<td>LC371046</td>
</tr>
<tr>
<td>Sciaphila yakushimensis var. okinawensis</td>
<td>Mt. Yonaha, Okinawa Isl.</td>
<td>2016.8.25</td>
<td>K. Suetsugu et al. KS135 (TNS)</td>
<td>LC371048</td>
</tr>
<tr>
<td>Sciaphila yakushimensis var. okinawensis</td>
<td>Along the Hanaage River, Yakushima Isl.</td>
<td>2016.11.27</td>
<td>K. Suetsugu KSI154 (TNS)</td>
<td>LC371049</td>
</tr>
<tr>
<td>Sciaphila nana</td>
<td>Sudogawa, Fuji City, Shizuoka Pref.</td>
<td>2017.9.20</td>
<td>K. Suetsugu KSI198 (TNS)</td>
<td>LC371050</td>
</tr>
</tbody>
</table>

DNA barcoding

For DNA isolation, we collected the aboveground parts of the unknown Sciaphila, S. yakushimensis var. yakushimensis, and the closely related S. nana and placed them in plastic bags with silica gel in the field (Table 1). Total DNA was extracted from the desiccated plant material using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). The internal transcribed spacer (ITS) of nuclear ribosomal DNA was amplified by TaKaRa EX Taq polymerase (TaKaRa, Otsu, Japan) using the forward primer ITS5 or ITS-p5 in combination with the reverse primer ITS4 or ITS-u4 (White et al. 1990, Cheng et al. 2016). PCR products were purified using ExoSAP-IT (Thermo-Fisher Scientific, USA) and sequenced using BigDye Terminator Cycle Sequencing Kit v.3.1 (Applied Biosystems, Foster City, CA, USA) with an ABI3500 capillary sequencer (Applied Biosystems, Foster City, CA, USA). Chromatograms of the sequences were compared to correct for base-calling errors by using ATGC v.4.3 (Genetyx Co., Tokyo, Japan). The full ITS sequences (ca. 700–750 bp including partial 18S and 28S regions) were submitted to the DNA Data Bank of Japan (DDBJ; Table 1).

The ITS dataset was aligned using MAFFT v.7 (Katoh & Standley 2013) under the –auto mode and modified manually. Nucleotide divergence and insertion/deletion among samples was compared using MEGA v.7 (Stecher & Tamura 2016).

Results and Discussion

Plants of Sciaphila on Okinawa and Ishigaki Islands are generally similar to S. nana in having the perianth of the staminate flowers with six segments, of which the larger three alternate with the smaller three, and stipitate globose to ellipsoid knobs lacking hairs at the apex of the three smaller segments. However, they can be easily distinguished by color (blackish purple plant body in our plants versus reddish purple in S. nana), angle between pedicel and rachis axis (60–90° in our plants versus 20–45° in S. nana), and style of the carpellate flower (ca. 0.4–0.6 mm long with cylindrical papillae in our plants and ca. 0.8–1.2 mm long with subulate papillae in S. nana; Figs. 1–3). The characteristics of the plants on Okinawa and Ishigaki Islands more closely match S. yakushimensis (Suetsugu et al. 2016, Figs. 1–3).

Despite their similarities, however, there are some differences between the plants on Okinawa and Ishigaki Islands and typical S. yakushimensis. The differences are summarized as follows: style of carpellate flowers from Okinawa and Ishigaki 0.4–0.6 mm long versus ca. 0.3 mm long; cylindrical papillae on style of Okinawa and Ishigaki plants less dense; connective is always extended into a long appendage in plants from Okinawa and Ishigaki, while connective of filaments of staminate flower often not extended in S. yakushimensis on Yakushima. Finally, plant of Sciaphila on Okinawa and Ishigaki Islands tend
Fig. 1. Flowering plants of *Sciaphila yakushimensis* var. *okinawensis* from Mt. Yonaha, Okinawa Prefecture (from the holotype). Bar = 5 cm.
to be taller than those on Yakushima (to 17 cm tall vs. to 9 cm tall, Figs. 1–3). However, we considered these differences to be relatively minor and representative of intraspecific variation. We therefore propose that plants from Okinawa and the Ishigaki islands as a new variety, *Sciaphila yakushimensis* var. *okinawensis*.

DNA barcoding also supports this distinction.
The DNA sequences of the ITS regions from plants of *Sciaphila yakushimensis* var. *yakushimensis* and *S. yakushimensis* var. *okinawaensis* were the same, while there were seven substitutions and three insertions/deletions for the same region between *S. yakushimensis* and *S. nana* (Table 1). The results endorse the conspecific status of *S. yakushimensis* var. *yakushimensis* and *S. yakushimensis* var. *okinawaensis* and the specific status of *S. yakushimensis* and *S. nana*.

We found that *S. yakushimensis* var. *okinawaensis* occurred in many localities on Okinawa and Ishigaki Islands, suggesting that *S. yakushimensis* has been mistaken for the more wide-
spread *Sciaphila nana*, which is similar in gross morphology. Indeed, Walker (1976) cited specimens of *S. yakushimensis* var. *okinawensis* at KAG as voucher specimens of *Andruris japonica* (a synonym of *S. nana*, Ohashi et al. 2008). Our field and herbarium surveys also indicated that *S. yakushimensis* var. *okinawensis* is more common than *S. nana* on Okinawa and Ishigaki Islands. Extensive surveys during the flowering season will be needed to elucidate the full range of distribution of *S. yakushimensis* var. *okinawensis*.

**Taxonomic Treatment**

*Sciaphila yakushimensis* Suetsugu, Tsukaya & H. Ohashi var. *okinawensis* Suetsugu, var. nov.—Figs. 1, 2A, B, 3A, B.

*Sciaphila yakushimensis* var. *okinawensis* is similar to *Sciaphila yakushimensis* var. *yakushimensis* but differs in having a longer style with relatively inconspicuous cylindrical papillae in the carpellate flowers and the connective of the filament extended into a long appendage in the staminate flowers.

**Typus.** JAPAN. Ryukyu. Okinawa Pref., Okinawa Island: Kunigami Village, Mt, Yonaha, 25 August 2016, K. Suetsugu, A. Abe, Y. Obara, T. Watanabe, H. Watanabe, T. Toma KS135 (holo- KYO!, dried plant on a herbarium sheet and liquid-preserved material in a bottle, labelled as the same specimen).

**Japanese name.** Okinawa-so, nom. nov.

Herbs, monoecious, mycoheterotrophic, perennial. Roots filiform, hairy. Stems erect, underground parts white, aerial parts blackish purple, simple or branched at base, ca. 4–17 cm tall, ca. 0.5 mm thick. Scale leaves linear, acute, ca. 2 mm long. Inflorescences racemose, rachis terminal, 2–8 cm long, with ca. 10–40 flowers spirally arranged; stamine flowers generally distal; pedicels ca. 3–6 mm long, straight, longer than flower, diverging at 60–90°; bracts linear, acute, ca. 1.5 mm long, appressed to pedicel. Stamine flowers ca. 1.5 mm across, perianth segments (5 or) 6, connate at base, segments narrowly ovate, glabrous, apex acute or acuminate, three segments slightly broader than alternating three segments; apex of broader segments acute; apex of narrower segments acuminate, with a globose knob. Stamens 3, sessile, connective of filament clearly extended. Anthers 4-lobed. Carpellate flowers ca. 1.5 mm across; perianth segments six, connate at base, ovate, apex acute. Carpels numerous, ellipsoid, ca. 0.4 mm long, apex rounded; style cylindrical, attached above middle on ventral surface of carpel, ca. 0.4–0.6 mm long, with cylindrical papillae.


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**References**


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