

Oxygyne yamashitae, a New Species of Thismiaceae from Yaku Island, Japan

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A new species of *Oxygyne* (Thismiaceae), *O. yamashitae*, a saprophytic/mycoheterotrophic species from Yaku Island, Kagoshima Prefecture, Japan, is described and illustrated. The combination of characters such as three stamens attached to the perianth and the presence of lamellae at the mouth of the perianth tube indicate that it belongs to the genus *Oxygyne*. It is clearly distinct from other known species of *Oxygyne* in having an elliptic hole in the corona, in which stamens are inflexed, and in having three dichotomous appendages below the stigma. The discovery of *O. yamashitae* demonstrates that the lowland evergreen forest in Yaku Island is a hotspot of endemic plants and worthy to be conserved.

Keywords: Japan, *Oxygyne*, saprophyte, Thismiaceae, Yaku Island

Thismiaceae are a small group of saprophytic plants, comprising five genera (*Afrothismia*, *Haplothismia*, *Oxygyne*, *Thismia* and *Tipuntinia*) and approximately 50 species (Woodward *et al.* 2007). Until recently, the family has been treated as a tribe within the Burmanniaceae (Maas-van de Kamer 1998, APG 2003). Recent molecular phylogenetic studies, however, have shown that it is sister to Taccaceae, while other members of the Burmanniaceae are sister to Dioscoreaceae (Merckx *et al.* 2006). This finding implies that saprophytic habits evolved independently in Burmanniaceae *s. str.* and Thismiaceae. While some species of Burmanniaceae *s. str.* retain photosynthetic leaves with chlorophyll, all species of Thismiaceae are obligatory saprophytes lacking green tissue.

As in other saprophytic plants, Thismiaceae provide an interesting opportunity for studying the evolution of saprophytes. Most species of Thismiaceae, however, occur in the tropics and their populations are very sporadic and difficult to locate. An exceptional area is Japan where two species of *Thismia* and two species of *Oxygyne* have been described from several localities with a warm-temperate climate (Tsukaya in press). We here report a third species of *Oxygyne* from Yaku Island, Japan, that appears most similar to *O. synzatoi* (Hatus.) C. Abe & Akasawa native to Okinawa Island. The purpose of this paper is to describe this new species and to provide perspectives for further studies.

Materials and methods

Locality data

Yaku Island (Yaku-shima) is located 30 km south of the mainland of Kyushu, Japan, and harbors 46 endemic species, two endemic subspecies and 30 endemic varieties of plants (Yahara *et al.* 1987). *Oxygyne yamashitae* occurs in evergreen broadleaved forests on the southern slope of Yaku Island along the western branch of Futamata River at an altitude of 390 m. The exact location 30°15' 11.5"N, and 130°33' 11.4"E was determined using a GPS receiver and adjusted on a 1/25000 map from the Geographical Survey Institute, Japan. The evergreen forest is dominated by *Machilus thunbergii* Siebold & Zucc., *Distylium racemosum* Siebold & Zucc., *Eurya japonica* Thunb., and *Ardisia sieboldii* Miq. The topography is flat and approximately 50% of the ground surface is covered with ferns (*Arachniodes amabilis* (Blume) Tindale, *Ctenitis subglandulosa* (Hance) Ching and *Diplazium donianum* (Mett.) Tardieu), shrubs

(*Damnacanthus indicus* Gaertn. f. and *Maesa japonica* (Thunb.) Moritzi ex Zoll.), and saplings of trees (*Ardisia sieboldii* Miq. and *Myrsine seguinii* H. Lév.).

Samples

In early October 2000, Mr. Hiroaki Yamashita, a photographer living on Yaku Island, noticed an unfamiliar plant in the evergreen forest in Yudomari. In early October, 2006, he rediscovered it along the western branch of the Futamata River, where he photographed it. He showed the photo to the first author on Yaku Island on October 8, 2006. On October 4, 2007, Yahara and Mr. Yamashita revisited the habitat of *Oxygyne*, but could not find the plants. On October 24, 2007, Mr. Yamashita and Mr. Kengo Fuse again visited the area and found eleven flowering individuals and collected two individuals for a specimen. We dissected one of the plants to observe the floral morphology. The second plant serves as the holotype. On October 28, 2007, we visited the type locality with Mr. Fuse and found more than 30



FIG. 1. Flowering individuals of *Oxygyne yamashitae* in the type locality. A: a magnified view of a flower. B: a flower on the ground, after removing fallen leaves.

flowering individuals of *O. yamashitae*. Four individuals were collected for further observation.

In October 2006, the second author, Tsukaya, along with Prof. Masatsugu Yokota of Ryukyu University, Mr. S. Higa, Mr. K. Shinjo, and Ms. K. Ishii collected *O. shinzatoi* at its type locality on Okinawa Island (Kunigami-son, Okinawa Pref., Ryukyu University Yona Experimental Field site, Japan) (Tsukaya *et al.* 2007, Yokoyama *et al.* 2008) and fixed several flowers in alcohol (voucher specimens: *H. Tsukaya 061008* [TI], *M. Yokota s.n.* [RYU]). Those materials were used to compare the floral morphology of *O. shinzatoi* and *O. yamashitae*.

Results

Taxonomic description

***Oxygyne yamashitae* Yahara & Tsukaya, sp. nov.**

Oxygyne shinzatoi primo aspectu similis, sed stigmatibus trifidis, appendicibus majusculis dichotomis differt.

Typus. JAPAN, Kagoshima Pref.: along the western branch of Futamata River, Yaku Island, 24 October 2007, Kengo Fuse, Hiroaki Yamashita & Hiroko Ikeda *s.n.* (holo- FU [in glycerine-alcohol]; iso- TI [in 80% alcohol]).

Herbs, achlorophyllous, mycoheterotrophic. *Roots* 1–5, ca. 0.5–1 cm long. *Stem* simple or branched, erect, glabrous, less than 1 cm tall. *Peduncle* ca. 1 mm in diam. *Inflorescence* white, racemose, 1 or 2(or 3)-flowered. *Bracts* at base of flowers, 3 or more, scale-like, 1 mm long, lanceolate, white. *Flowers* in October, upright or inclined, pale blue, glabrous, ca. 5 mm long, 5 mm in diam.; *perianth segments* united, 6-lobed, tube campanulate, ca. 3 mm long; *perianth lobes* whitish blue, narrowly triangular, ca. 2 mm long, together appearing star-like; *perianth lamellae* at mouth convex, inflexed, united, forming an annular corona with a hole in center; *lamella of outer perianth* with hole in which a stamen is bent inward toward the stigma;

lamella of inner perianth 1 mm long, 0.7 mm wide, trapezoid, apex sharply bifurcate, 2-lobed, lobes 0.3 mm long. *Stamens* 3, attached to outer perianth; filaments inflexed, with 2 pairs of projections at base. *Anthers* basifixed, pale yellow, bithecal, introrse, 0.5 mm long. *Style* 1, 1.3 mm long, thick, ending in 3 short, triangular stigmatic branches, 0.25 mm long, 0.2 mm wide at base, surrounded by 3 dichotomous appendages. *Stalk of appendages* ca. 0.15 mm long, with 2 unequal-sized branches elongated opposite each other along longitudinal axis of stigma; upper branches longer, pointed, ca. 0.1 mm in diameter, ca. 0.5 mm long; lower branches club-shaped, ca. 0.15 mm in diameter, ca. 0.15 mm long. *Ovary* ca. 1 mm long, white. *Ovules* many.

Japanese name. Yaku-no-hina-hoshi.

Additional observations on O. yamashitae and O. shinzatoi

In appearance, *O. yamashitae* is similar to *O. shinzatoi* in having a characteristic star-like perianth (Fig. 1) formed by the six perianth lobes. The lobes are shorter and narrowly triangular in *O. yamashitae* (Fig. 2A) and filiform in *O. shinzatoi* (Fig. 2C). At the base of each filiform lobe of *O. shinzatoi* is a callus-like cluster of round cells on the adaxial surface (Fig. 3H). The triangular lobes of *O. yamashitae* are smooth. While *O. shinzatoi* has dark verdigris flowers, the flowers of *O. yamashitae* are whitish blue. A more definitive difference between *O. yamashitae* and *O. shinzatoi* is in the morphology of the pistils; the stigma is surrounded by 3 rounded appendages in *O. shinzatoi* and 3 dichotomous appendages in *O. yamashitae* (Fig. 2B vs 2D). Additionally, the stigma of *O. yamashitae* is larger and distinctly trichotomous (Fig. 3D vs 3G). Another distinctive feature of *O. yamashitae* is in the lamellae of the perianth; the lamella of the outer perianth has an elliptic hole in which a stamen is bent inward toward the stigma (Fig. 3E). In *O. shinzatoi*, the

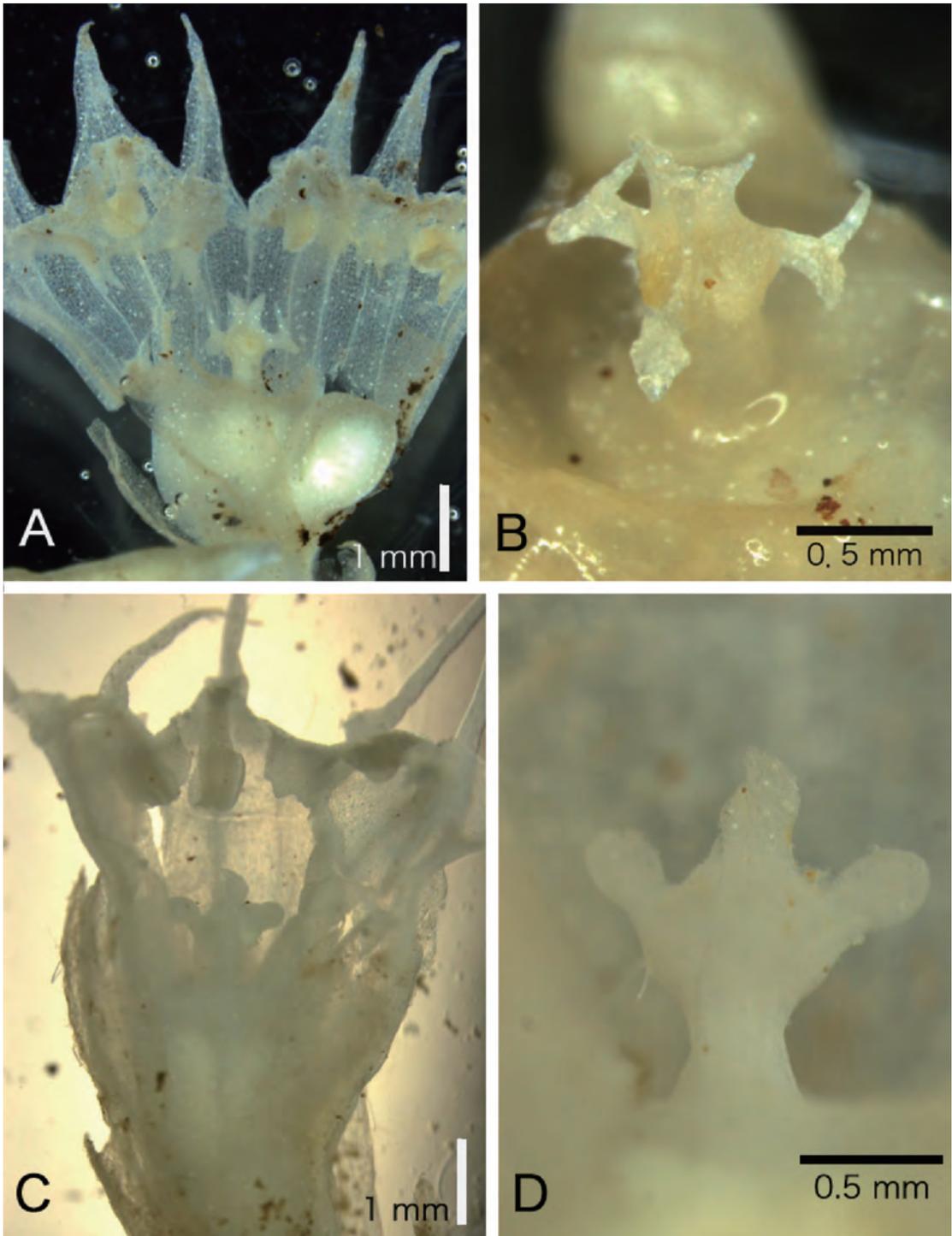


FIG. 2. Floral morphology of *Oxygyne yamashitae* (A–B) and *O. shinzatoi* (C–D). A and C: side view of a dissected flower tube; bar = 1 mm. B and D: pistil; bar = 0.5 mm.

lamellae of the perianth are free (Fig. 3H). The elliptic hole of *O. yamashitae* is interpreted as being the result of fusion of the lamellae. The free lamellae of *O. shinzatoi* have very low teeth (Fig. 3H) but the lamellae of the inner perianth of *O. yamashitae* are deeply bilobed (Fig. 3E). The basal part of a the filament has two pairs of projections, one being larger (Fig. 3F).

Discussion

Systematic position and distinctness

Among the five genera of Thismiaceae (*Afrothismia*, *Haplothismia*, *Oxygyne*, *Thismia* and *Tipuntinia*), *Haplothismia* and *Oxygyne* are characterized by having filamentous stamens with filaments basally broadened, attached to the mid-vein at the base of the perianth lobes, and curving inward at the apex to partially obstruct the mouth of the floral tube (Heywood 1978, Woodward *et al.* 2007). In stamen morphology, *Haplothismia* and *Oxygyne* are similar to Taccaceae, with which Thismiaceae are related (Merckx *et al.* 2006). Other genera of Thismiaceae have more reduced and/or specialized filaments and are probably more derived. A recent molecular phylogenetic study also showed that *Oxygyne* is relatively basal within Thismiaceae (Yokoyama *et al.* 2008). *Oxygyne* is, however, derived in having three stamens while all other genera including *Haplothismia* have six stamens (Abe & Akasawa 1989, Woodward *et al.* 2007). *Oxygyne yamashitae* has three stamens and the stamen morphology is identical with other species of the genus.

Oxygyne triandra Schltr., the first species described in the genus is in tropical forests of Mt. Cameroon in West Africa (Schlechter 1906). The second species described, as *Saionia shinzatoi* (Hatusima 1976), from Okinawa Island, Japan, was placed in *Oxygyne* by Abe & Akasawa (1989) when they described a third species, *O. hyodoi* C. Abe & Akasawa, from Ehime Prefecture, Japan.

All three Japanese species, including *O. yamashitae* have blue or green, cup-shaped flowers, while *O. triandra* has deep brown, bell-shaped flowers (Schlechter 1906). It is highly probable that the three Japanese species are closely related.

While the overall morphology and the flower color of *O. yamashitae* resemble the other two species from Japan, *O. yamashitae* is clearly distinct from *O. shinzatoi*, as described above. *Oxygyne yamashitae* is also distinct from *O. hyodoi* based on the clearly illustrated floral features published with the figures associated with the original description (Abe & Akasawa 1989). *Oxygyne hyodoi* is similar to *O. yamashitae* in having all lamellae of the perianth fused to form an annular corona with a central hole at the mouth of the perianth tube. *Oxygyne yamashitae* has a more specialized corona having three narrow holes in which staminal filaments are inflexed (Fig. 3B, E). Second, *O. hyodoi* has three clavate appendages below the stigma, but *O. yamashitae* has three unequally dichotomous appendages (Fig. 3C, D). Thirdly, filaments of *O. hyodoi* are simple at the base, but those of *O. yamashitae* are broadened and toothed at the base (Fig. 3F). From those morphological observations, it remains uncertain whether *O. yamashitae* is sister to either of the other two species. Molecular phylogenetic studies are needed to elucidate the relationship among the three Japanese species.

A key to the species of *Oxygyne* is provided below.

- A1. Flowers bell-shaped, brown *O. triandra*
- A2. Flowers cup-shaped, verdigris, emerald green, or whitish blue B
- B1. Perianth lobes filiform; base of lobes with callus-like cluster of round cells on adaxial surface; inner perianth with free lamellae at mouth of perianth tube; outer perianth lacking lamellae; pistil with 3 rounded appendages below stigma; filaments simple at base; flowers verdigris *O. shinzatoi*
- B2. Perianth lobes narrowly triangular; base of lobes smooth; inner and outer perianth lamellae fused

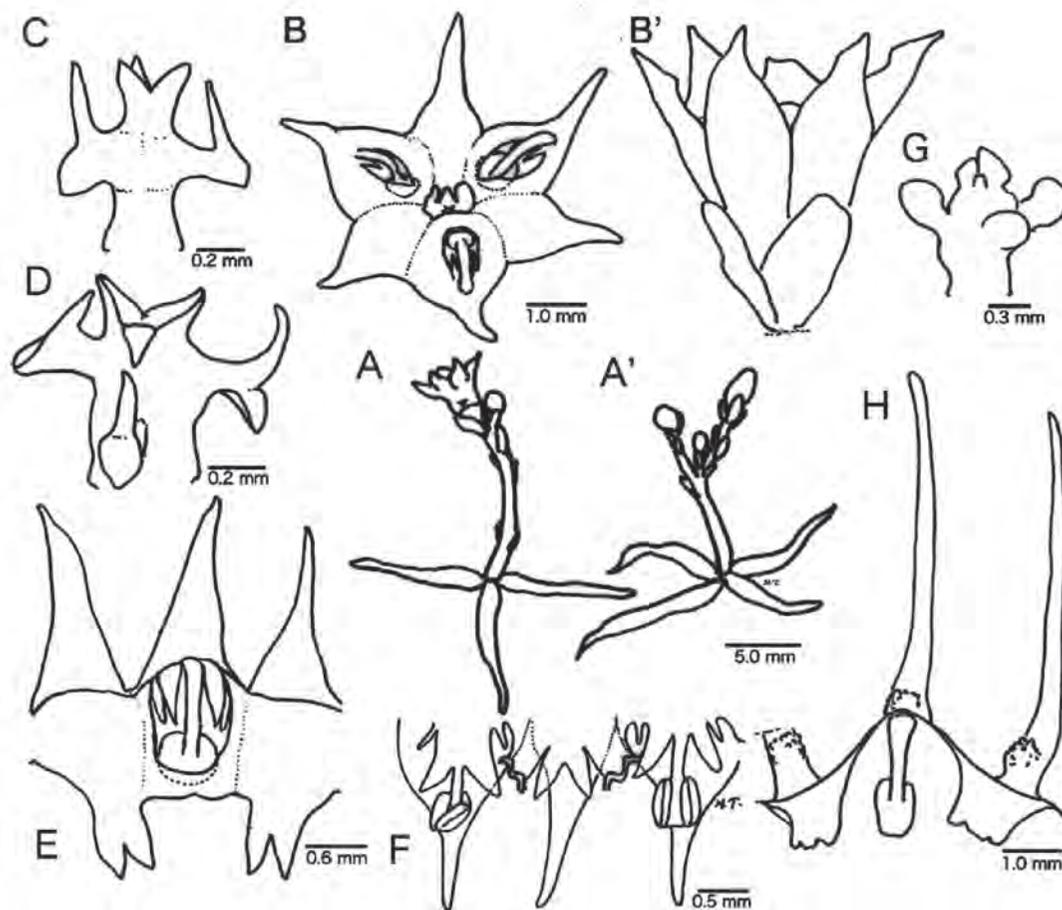


FIG. 3. Comparison of *Oxygyne yamashitae* and *O. shinzatai*. A to F: *O. yamashitae*. G and H: *O. shinzatai*. A and A': whole plant. B: front (B) and lateral (B') view of perianth tube and subtending bracts. C and D: stigma. E: perianth lobes, perianth lamellae, and a stamen viewed from inside of the corolla tube in *O. yamashitae*. F: two inner perianth lobes with stamens and a lamella of an outer perianth of *O. yamashitae*. The fused lamella was splitted at the junction (shown by wavy lines). G: Stigma. H: perianth lobes, perianth lamellae and a stamen viewed from inside of the perianth tube of *O. shinzatai*.

to form an annular structure with central hole at mouth C
 C1. Perianth lamellae equal in size at mouth, forming annular structure called annular taenia; stamens bent inward to central hole; pistil with 3 clavate appendages below stigma; filaments simple at base; flowers emerald-green *O. hyodoi*
 C2. Perianth lamellae unequal in size,, fused with each other to form an annular structure; 3 lamellae of inner perianth larger, with 3 holes in which stamens are bent inward; pistil with 3 three unequally dichotomous appendages below stigma; filaments toothed at base; flowers whitish blue *O. yamashitae*

Ecology and Conservation

Although the flowers of *O. yamashitae* are whitish blue, they are extremely inconspicuous in the forest. Most flowers we observed in the field were hidden among fallen leaves; the entire flower was visible only after the fallen leaves were removed (Fig. 1B). The flowers had neither recognizable nectar nor recognizable odor. We observed mites (Fig. 1A) and ants rarely visiting the flowers, but they showed no interest in the pollen. The flowers of *O. yamashitae* appear to be self-

fertilizing, irrespective of its highly specialized spatial arrangement of stamens and stigma.

The two populations of *O. yamashitae* discovered by Mr. Yamashita are on the lower southern slopes of Yaku Island in humid evergreen broad-leaved forests near streams. Unfortunately, the habitat of the first population at Yudomari, at an elevation of approximately 180 m, was destroyed by the construction of a forest road. Lowland evergreen forests remain in the vicinity of the first population, along a stream below Mt. Hasa. We expect that additional populations remain. Because the second population along the Futamata River occurs at 390 m, further efforts to find *O. yamashitae* between 200 and 400 m at the foot of Mt. Hasa should be undertaken.

At low altitude on the southern slope of Yaku Island, humid evergreen forests occur along Tainokoh River, Futamata River, Suzunokoh River, Yugo River and other smaller streams between those rivers. Two endemic ferns, *Pteris kawabatae* Sa. Kurata and *Lindsaea kawabatae* Sa. Kurata, are restricted to humid forests in that area. *Aster yakushimensis* (Kitam.) Soejima & Yahara and *Pertya yakushimensis* H. Koyama & Nagam. are also restricted to that area, inhabiting forest margins along streams. The discovery of *O. yamashitae* has provides additional evidence that the lowland evergreen forests in this area are a hotspot of endemic plants. Unfortunately, only a part of the area is preserved. On Yaku Island, 38,415 ha (76% of the 50,455 ha) is managed as a National Forest, 20,989ha (61%) is preserved as a National Park, and 10,747 ha (21%) is registered as a World Natural Heritage site. The Futamata River population of *O. yamashitae* is located within the National Forest area but outside of the National Park and the World Natural Heritage area. To conserve *O. yamashitae* and other endemic species restricted to the lower altitude of the southern slope of Yaku Island, further plans for restricting forest logging and construction are needed.

Perspectives for future research

Although Japanese species of *Oxygyne* provide an interesting opportunity for studying the evolution of achlorophyllous mycoheterotrophic plants, the populations are extremely difficult to find. In 2004, *O. shinzatoi*, which had not been collected since it was described (Hatusima 1976), was rediscovered at the type locality. The rediscovery triggered a series of studies, including a cytological study (Tsukaya *et al.* 2007), a molecular phylogenetic study (Yokoyama *et al.* 2008) and a molecular phylogenetic study of fungi associated with the roots of *O. shinzatoi*. The discovery of *O. yamashitae* has enriched our opportunity for elucidating the evolutionary history of *Oxygyne*. Studies using cytological and molecular phylogenetic techniques are now in progress. Further, more in-depth studies using *in vitro* culture and genomic analysis might help us to understand the mechanisms behind the evolution of mycoheterotrophic plants in general.

We express our deepest thanks to Mr. Hiroaki Yamashita who discovered *O. yamashitae* and provided us an extraordinary opportunity for studying this interesting plant. We also thank Mr. Kengo Fuse who helped us to find plants of *O. yamashitae* at the type locality, and Yoichi Arata, Ichiro Makise, Kenshi Tetuka and other members of the Yakushima Overall Conserving Association who invited Yahara to the YOCA meeting on 8 October 2006 where he was shown the photograph of *O. yamashitae* by Mr. Yamashita.

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