



## **How Many Species are there in the Holomycotrophic Genus *Didymoplexis* Griff. (Orchidaceae) in Madagascar?**

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# How many species are there in the holomycotrophic genus *Didymoplexis* Griff. (Orchidaceae) in Madagascar?

Phillip Cribb, Laurent Gautier & Louis Nusbaumer

## Abstract

CRIBB, P., L. GAUTIER & L. NUSBAUMER (2013). How many species are there in the holomycotrophic genus *Didymoplexis* Griff. (Orchidaceae) in Madagascar? *Candollea* 68: 41-49. In English, English and French abstract.

The holomycotrophic genus *Didymoplexis* Griff. (Orchidaceae) is revisited for Madagascar. Two species, *Didymoplexis avaratraensis* P. J. Cribb, Nusb. & L. Gaut. and *Didymoplexis recurvata* P. J. Cribb, Nusb. & L. Gaut. are newly described based on recently collected flowering specimens. The relationship between these two species, the recently observed *Didymoplexis verrucosa* J. Stewart & Hennessy and the cryptic *Didymoplexis madagascariensis* (H. Perrier) Summerh., only known from two fruiting specimens, is discussed. A key to species in the genus in Madagascar and Africa based on flowering material is provided.

## Key-words

ORCHIDACEAE – *Didymoplexis* – Madagascar – Taxonomy – Mycotrophy – Conservation

## Résumé

CRIBB, P., L. GAUTIER & L. NUSBAUMER (2013). Combien d'espèces y a-t-il dans le genre holomycotrophique *Didymoplexis* Griff. (Orchidaceae) à Madagascar? *Candollea* 68: 41-49. En anglais, résumé anglais et français.

Le genre holomycotrophique *Didymoplexis* Griff. (Orchidaceae) est révisé pour Madagascar. Deux espèces nouvelles, *Didymoplexis avaratraensis* P. J. Cribb, Nusb. & L. Gaut. et *Didymoplexis recurvata* P. J. Cribb, Nusb. & L. Gaut. sont décrites sur la base de spécimens en fleur récemment collectés. Elles sont comparées à *Didymoplexis verrucosa* J. Stewart & Hennessy une espèce récemment observée à Madagascar et à l'espèce cryptique, *Didymoplexis madagascariensis* (H. Perrier) Summerh., connue seulement de deux spécimens en fruit. Une clef des espèces du genre pour Madagascar et l'Afrique basée sur les caractères floraux est proposée.

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The holomycotrophic genus *Didymoplexis* Griff. (*Orchidaceae*) is represented by about 20 species in the Old World, mainly in Asia (Southeast Asia, India, Pacific Islands, North Australia, Ryukyu Islands and Taiwan; see HSU & CHUNG, 2007), but also including the Afro-Madagascan region with three known species (HERMANS & al., 2007; AFRICAN PLANTS DATABASE, 2012).

The species of *Didymoplexis* are whitish to brownish, achlorophyllous, terrestrial and leafless herbs reaching up to 40 cm and with the capsule borne on an erect stalk often elongating during fruit maturation (PRIDGEON & al., 2006). Published phylogenetic studies focus on species of the neighbouring holomycotrophic genus *Gastrodia* R. Br., particularly those used in traditional Chinese medicine and cuisine (WANG & al., 2012), but we have not found any phylogenetic study that includes *Didymoplexis*.

The three species occurring in the Afro-Madagascan region are *D. africana* Summerh. from continental tropical Africa, *D. verrucosa* J. Stewart & Hennessy, known from Southern Africa (N KwaZulu-Natal) but recently found also in Madagascar (CRIBB & al., 2011) and *D. madagascariensis* (H. Perrier) Summerh., endemic to Madagascar based upon our current knowledge of the genus.

The first herbarium specimen of *Didymoplexis* for Madagascar was a fruiting specimen collected by H. Perrier de la Bâthie in September 1912 at Fandrarazana on the mainland coast of north-east Madagascar opposite the island of Sainte-Marie. It was initially described as *Gastrodia madagascariensis* H. Perrier. SUMMERHAYES (1953) transferred it to *Didymoplexis* as *D. madagascariensis* (H. Perrier) Summerh. However, it is extremely difficult to distinguish these two genera when the plants are in fruit since both are mycotrophic orchids with a similar habit and many species of both genera produce erect ellipsoidal capsules borne on elongated stalks (PRIDGEON & al., 2006).

A second known herbarium specimen of *Didymoplexis* was collected 83 years later by F. Rasoavimbahoaka in June 1995 at Belaoko Lokoho on the Ambatosoratra Mountain in the Integral Natural Reserve of Marojejy close to Andapa (*Rasoavimbahoaka* 748 [TAN, MO, P and three more unlocalized duplicates]). This was also a fruiting specimen and it was identified by J. Bosser in November 2001 as *D. madagascariensis*, the only known species of this genus in Madagascar at this time.

A number of field photographs of flowering and fruiting specimens of *Didymoplexis* have subsequently been made:

1. One fruiting population on 26.X.2005, in Marojejy National Park, at 471 m elevation (photographed by Jean-Michel Hervouet).
2. Two flowering populations on 18.XI.2006, also in Marojejy National Park, between entrance, Mantella camp and Humbert View Waterfall, at 437 to 500 m elevation (photographed by Mark Clements & Anne Mackenzie).
3. One fruiting population on 19.XI.2006, near the “Marojejy camp 2” of the Marojejy National Park, at 760 m elevation (photographed by Mark Clements and Anne Mackenzie, see photographs in CRIBB & HERMANS, 2009).
4. Four to five flowering individuals on 8.XII.2007, in the Verezanantsoro circuit in the National Park of Northern Mananara, at 231 m elevation (photographed by Jean-Michel Hervouet, see BOSSER & LECOUFLE, 2011).
5. One flowering individual on 17.VII.2010, along Manambolo river near Bekopaka in the National Park of Bemaraha, at 150 m elevation (photographed by Jean-Philippe Castillon).

The specimen photographed by Castillon (5) has been recently identified as *D. verrucosa* J. Stewart & Hennessy, hitherto known from N KwaZulu-Natal in Southern Africa (CRIBB & al., 2011). The two other above mentioned flowering population's photographs (2 and 4) have been associated with *D. madagascariensis* by CRIBB & HERMANS (2009) and BOSSER & LECOUFLE (2011) also based on the assumption that it was the only species found on the island. Unfortunately, no herbarium specimens were collected of any of the plants from these populations.

Two recent flowering herbarium collections (*Nusbaumer & Ranirison 1763*, *Nusbaumer & Ranirison 1763bis*) have been made in December 2005 at 818 and 985 m in the Loky-Manambato region (Daraina) in northern Madagascar and were accompanied by high quality photographs. The specimens of these two collections were originally collected under the same number but were later separated since they proved to represent two different taxa. These recent herbarium collections and photographs bring new elements that allow a revision of the delimitation of the species of *Didymoplexis* in Madagascar.

For most tropical orchids, fruiting specimens are difficult to identify at species level. Identification is even more problematic in holomycotrophic genera where leaves are lacking. A screening of 30 articles dealing with the genus *Didymoplexis* revealed that fruiting characters have never been used to distinguish species (SMITH, 1905; HOLTUM, 1953; BACKER & BAKHUIZEN VAN DEN BRINK, 1968; COMBER, 1990; for a review, see PRIDGEON & al., 2006). Fruiting collections are thus impossible to identify to species level based on morphology. As the type of *D. madagascariensis* is a fruiting collection, its relation with any other species will remain cryptic as it could potentially be conspecific with any flowering specimen in the genus.

Among the specimens observed or collected with flowers, the distinction is however very clear. The main criteria used for identification are the absence or presence of the arms at the apex of the column and their shape when present; the lateral sepals adnate to a third or to half their length; the division of the apex of the callus, located at the base of the lip, into two or three lobes; the presence or absence of two lines of verrucose warts along lip length; and the petal and sepal colour and length.

The morphological differences and taxonomic affiliation of the known photographs and herbarium collections of *Didymoplexis* are presented in Table 1. Comparison of the flowering specimens of *Didymoplexis* photographed or collected in Madagascar brings to at least three the number of *Didymoplexis* species found in Madagascar among which two are new to science and described below. If *D. madagascariensis* proves to be distinct from these three, the number would increase to four. Although a flowering collection from the type locality would be an interesting find, only molecular data from the type and other specimens collected in Madagascar could possibly disentangle the application of this taxon. Until then, the name *D. madagascariensis* should be restricted to the type specimen.

***Didymoplexis avaratraensis*** P. J. Cribb, Nusb. & L. Gaut., spec. nova (Fig. 1-3).

**Typus:** MADAGASCAR. Prov. Diego-Suarez/Antsiranana: sous-préfecture de Vohemar, commune rurale de Daraina, forêt de Binara, 13°15'S 49°37'E, 818 m, fl., 12.XII.2005, Nusbauer & Ranirison 1763 (holo-: G [G00090146]!; iso-: P, TAN!, research herbarium of Daraina).

*Ab aliis speciebus Africanis Madagascariensibusque sepalis lateralibus ad medium connatis, petalis ac sepalo dorsali in parte inferius 1/5 adnatis, columna brachia apicalia breviter recurvata ad apicem acuta ferenti, labello sine seriebus verrucarum et callo ad apicem bilobato distinguendo.*

Holomycotrophic herb up to 15 cm tall, stem beige, brown to blackish when old, growing from a subterranean elongated brownish tuberous rhizome. Leaves scale like, very small. Inflorescence erect, racemose, laxly few-flowered, the flowers opening one at a time and successively; peduncle slender, erect, bearing a small ovate bract below inflorescence, white or pale buff-coloured; rachis terete, slender; pedicel with longitudinal ridges 1.5-2 cm during flowering period; bracts ovate, acuminate, 1-1.5 mm long. Flowers with pink or white sepals and petals, flushed with red-brown in the centre, the lip pink or white with reddish brown sides, the callus spotted with yellow. Dorsal sepal strongly curved forward, lanceolate, rounded, 15 × 3.-3.5 mm; lateral sepals somewhat recurved, fused in basal half, more or less paral-

lel and slightly falcate, ovate, rounded, 13 × 4 mm. Petals elliptic-lanceolate, acute, 13 × 4.5 mm, decurved at tip, adnate in basal fifth with sepals. Lip narrowly clawed at base, obovate-spatulate, truncate, 5-6 × 7-8 mm, the sides upcurved, bearing three patches on the apical lip lamina; callus at base shortly erect and bilobed at tip. Column 5-6 mm long, slender at base, with short recurved apical arms, triangular, broader than long and acute at the tips, 0.7 mm long. Capsule unknown.

**Etymology.** – The specific epithet refers to the North (“Avaratra” in Malagasy) because the species occurs in the northern part of Madagascar.

**Distribution.** – The species is known from Toamasina and Antsiranana provinces in North-East Madagascar. Four populations each of between (4)-15-20 individuals were observed, with a total of about 65-75 individuals observed in all sites.

**Habitat and ecology.** – *Didymoplexis avaratraensis* was recorded in evergreen forest on metamorphic rock; up to 820 m. This terrestrial herb occurs in forests with dense canopies reaching 10 to 15 m, with emergent trees reaching 18 to 20 m, with two woody strata in the canopy at 4 to 5 m and at 1 to 1.5 m high, and with an absent, sparse or very sparse ground flora, the soil densely covered with dry fallen leaves.

The most frequent species recorded together with *D. avaratraensis* (considering the Loky-Manambato population) in vegetation surveys include, in decreasing abundance: *Croton* sp., *Dypsis nodifera* Mart., *Ravenea sambiranensis* Jum. & H. Perrier, *Dypsis* aff. *madagascariensis* (Becc.) Beentje & J. Dransf. and *Noronhia* sp.

**Flowering time.** – November to December.

**Conservation Status.** – With an extent of occurrence (EOO) of 2,022 km<sup>2</sup> and area of occupancy (AOO) of 300 km<sup>2</sup>, and only one subpopulation collected and three subpopulations observed, all four in protected areas (Station Forestière à Usage Multiple de Loky-Manambato, Marojejy National Park and Mananara Nord National Park), *Didymoplexis avaratraensis* is assigned a preliminary status of “Vulnerable” (VU D2) following the IUCN Red List Categories and Criteria (IUCN, 2012; calculation following CALLMANDER & al. (2007) and performed with MOAT (2012) tools.

**Notes.** – *Didymoplexis avaratraensis* is clearly distinguished from the other African and Malagasy species of *Didymoplexis* known with flowers (CRIBB, 1984; CRIBB & al., 2011) by its 2 lateral sepals fused to the mid-point while the remaining two petals and sepal are adnate together in the basal fifth, as well as by its column which has short recurved apical arms that are acute at the tips, and by its lip lacking rows of warts but with a bilobed callus at the apex. One observation of predation of flowering plants by some kind of invertebrate in one population is also interesting to note (Mark Clements, pers. comm.).

**Table 1.** – Known *Didymoplexis* Griff. herbarium collections (*D. avaratraensis* P. J. Cribb, Nusb. & L. Gaut., *D. madagascariensis* (H. Perrier) Summerh., *D. recurvata*

Characters used for discrimination (in decreasing order of importance)	«Perrier 11349»	«Rasoavimbahoaka 748»	Hervouet's fruiting photograph (26.X.2005)	Clement's and Mackenzie's fruiting photographs (19.XI.2006)	Hervouet's flowering photograph (8.XII.2007)
Arms at the apex of the column	unknown	unknown	unknown	unknown	present, triangular, acute at the tips, pink
Lateral sepal	unknown	unknown	unknown	unknown	fused to the mid point
Lip	unknown	unknown	unknown	unknown	lacking rows of warts, strongly upcurved sides
Lip basal callus	unknown	unknown	unknown	unknown	bilobed
Plant size [cm]			ca. 10	up to 5	ca. 10
Petal and sepal color	unknown	unknown	unknown	unknown	pale pink to pink
Petal and sepal length	unknown	unknown	unknown	unknown	unknown (a posteriori estimation of length of ca. 8 mm)
Capsule stalk	elongated, up to 40 cm	elongated, up to 32 cm	a posteriori estimation of about 15 cm	elongated, up to 30-35 cm	unknown
Final identification	<i>D. madagascariensis</i>	<i>D. sp.</i>	<i>D. sp.</i>	<i>D. sp.</i>	<i>D. avaratraensis</i>
Type of	<i>D. madagascariensis</i>	–	–	–	–

***Didymoplexis recurvata*** P. J. Cribb, Nusb. & L. Gaut., **spec. nova** (Fig. 1, 4).

**Typus:** MADAGASCAR. Prov. Diego-Suarez/Antsiranana : sous-préfecture de Vohehar, commune rurale de Daraina, forêt de Binara, 13°15'S 49°37'E, fl., 985 m, 12.XII.2005, Nusbauer & Ranirison 1763 bis (holo-: G [G00181978]!; iso-: TAN!, research herbarium of Daraina).

*Affinis Didymoplexis avaratraensis sed floribus albis vel roseis, sepalis petalisque latioribus, sepalis lateralibus tertio basali adnatis, labello late obovato emarginato, callo elongato ad apicem trilobato et brachiis dolabriformibus pendentibus ad apicem columnae satis distinguenda.*

Holomycotrophic herb up to 10 cm tall, growing from a subterranean elongated blackish tuberous rhizome. Leaves scale like, very small. Inflorescence erect, racemose, laxly up to 6-flowered, the flowers opening one at a time and successively; peduncle slender, erect, bearing a small ovate bract below inflorescence, white; rachis terete, slender; pedicel with longitudinal ridges; bracts ovate, acute, 1.5-2 mm long. Flowers with white sepals and petals and a white to rose-pink lip with a yellow callus, the column buff flushed. Dorsal sepal erect, lanceolate, subacute, 7-8 × 4-5 mm; lateral sepals adnate in basal third, ovate, rounded to subacute, 7-8 × 4-5 mm. Petals

oblanceolate, acute, 7-8 × 5-7.5 mm, adnate in basal third to the sepals. Lip shortly narrowly clawed at base, very broadly obovate-spatulate, emarginate, 3.5-4 × 5-6 mm, slightly decurved at apex; callus on claw erect, dilated and 3-lobed at tip. Column 3.5-5 mm long, slender at base, strongly winged at apex, the wings dependent, recurved, rounded at tip, 1 mm long. Fruit unknown.

*Etymology.* – The specific epithet refers to the column with recurved apical arms bilobed at the tips.

*Distribution.* – The species is only known from the Binara forests in the Loky-Manambato (Daraina) region, in Antsiranana Province in North-East Madagascar. Only 15-20 individuals were observed in one population in the Loky-Manambato region among the 54,000 plant occurrences recorded during the Loky-Manambato vegetation study (NUSBAUMER, 2011).

*Habitat and ecology.* – *Didymoplexis recurvata* was recorded in evergreen montane forest on metamorphic rock; at 985 m. This terrestrial herb occurs in forests with canopies reaching 11 to 15 m, emergent trees reaching 21 to 24 m and with a second aborescent stratum at 8 to 10 m, with two woody strata at 5 to 7 m and at 1 to 2 m high, and with a no herbaceous strata, the soil densely covered with dry leaves of trees.

P. J. Cribb, Nusb. & L. Gaut.) with observations (photographs), with the states of the main characters used for discrimination.

Clement's and Mackenzie's flowering photographs (18.XI.2006)	Castillon's photograph (17.VII.2010)	«Nusbaumer & Ranirison 1763»	«Nusbaumer & Ranirison 1763bis»
present, triangular, acute at the tips, white	absent	present, triangular, acute at the tips, white	present, bilobed at the tips, brown and white
fused to the mid point	free almost to the base	fused to the mid point	adnate in the basal third
lacking rows of warts, strongly upcurved sides	presence of two lines of verrucose warts along length, transversally triangular, 3-lobed at the tip	lacking rows of warts, strongly upcurved sides	lacking rows of warts, slightly emarginate, broadly obovate-spatulate, with sides only slightly upcurved
bilobed	callus of two lines of small warts from base to tip with a fleshy yellow ligule on the claw	bilobed	erect entire, trilobed at the tip
10-15	unknown	up to 15	up to 10
white with brown in the centre	white	white with brown in the centre	white
unknown (the whole flower was ca. 12-15 mm wide)	unknown	up to 15 mm	5-8 mm
unknown	unknown	unknown	unknown
<i>D. avaratraensis</i>	<i>D. verrucosa</i>	<i>D. avaratraensis</i>	<i>D. recurvata</i>
–	–	<i>D. avaratraensis</i>	<i>D. recurvata</i>

The most frequent species recorded together with *D. recurvata* in Loky-Manambato vegetation surveys include, in decreasing abundance: *Marojejya insignis* Humbert, *Ravenea sambiranensis* Jum. & H. Perrier, *Dypsis nodifera* Mart., *Weinmannia decora* Tul., *Syzygium condensatum* (Baker) Labat & G. E. Schatz, *Plagioscyphus* aff. *louvelii* Danguy & Choux and *Blechnum attenuatum* (Sw.) Mett. var. *attenuatum*.

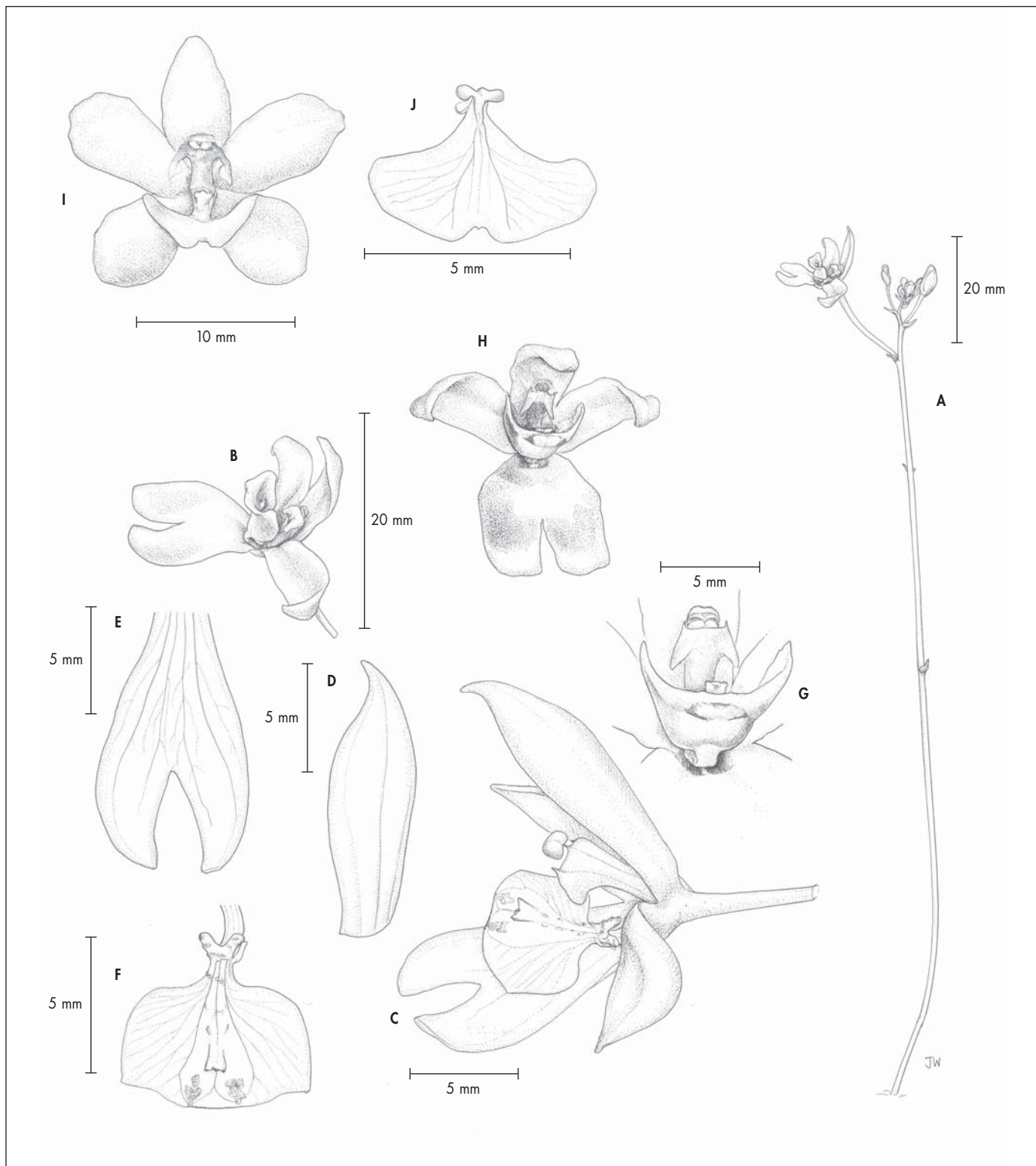
*Flowering time.* – December.

*Conservation Status.* – With only one small subpopulation, included in the Loky-Manambato protected area, *Didymoplexis recurvata* is assigned a preliminary status of “Critically Endangered” (CR D) following the IUCN Red List Categories and Criteria (IUCN, 2012).

*Notes.* – *Didymoplexis recurvata* is clearly distinguished from the other African and Malagasy species of *Didymoplexis* known in flower by its column which has short recurved apical arms that are bilobed at the tips, as well as by its lip which lacks rows of warts but has at the base a tall apically trilobed entire callus and by its sepals and petals that are adnate in the basal third.

## Conservation implications

These two new species add to numerous other botanical and zoological taxa discovered in the Loky-Manambato area. Based on these discoveries, the NGO Fanamby succeeded in the establishment of a protected area (“station forestière à usages multiples”, SFUM) for eight important forest blocks of the Loky-Manambato region (RABOTOARISON, 2005), including the forests where the two new *Didymoplexis* species have been collected. Monitoring the populations is the next critical step for the conservation of these species but it is a difficult task since these plants do not conserve vegetative organs aboveground, making them impossible to observe except during the flowering time.



**Fig. 1.** – *Didymoplexis avaratraensis* P. J. Cribb, Nusb. & L. Gaut. **A.** Habit; **B.**, **C.** Flower, side view; **D.** Dorsal sepal abaxial view; **E.** Lateral sepals, front view; **F.** Lip (labellum), flattened, front view; **G.** Labellum and column details, front view; **H.** Flower, front view. *Didymoplexis recurvata* P. J. Cribb, Nusb. & L. Gaut. **I.** Flower, front view; **J.** Lip (labellum), flattened, front view.

[**A-H:** Nusbaumer & Ranirison 1763, **G;** **I-J:** Nusbaumer & Ranirison 1763bis, **G**] [Drawing: Juliet Beentje]



**Fig. 2.** – *Didymoplexis avaratraensis* P. J. Cribb, Nusb. & L. Gaut. **A.** Flower, front view; **B.** Labellum and column details, front view; **C.** Flower and pedicel, side view; **D.** Habit; **E.** Tuberous rhizome.

[Nusbaumer & Ranirison 1763] [Photo: L. Nusbaumer, 12.XII.2005]





**Fig. 3.** – *Didymoplexis avaratraensis* P. J. Cribb, Nusb. & L. Gaut. **A.** Habitus; **B.** Flower, front view; **C.** Habitus; **D.** Flower, front view. [Photos: **A-B:** Mark Clements and Anne Mackenzie, 18.XI.2006; **C-D:** Jean-Michel Hervouet, 8.XII.2007]

**Key to flowering *Didymoplexis* in Africa and Madagascar**

- 1. Two or more sepals or petals fused to the mid-point or more, petals and sepals 11-15 mm ..... 2
- 1a Sepals or petals adnate in the basal third or less but none of them fused to the mid-point, petals and sepals 5-8 mm long ..... 3
- 2. Lateral sepals fused to the mid-point, other sepal and petals free to the base or fused to a fifth of their length; column with short recurved triangular apical arms, broader than long and acute at the tips; sepals and petals white up to 15 mm long; lip with a low basal bilobed callus and strongly upcurved sides ..... *D. avaratraensis*
- 2a All sepals and petals fused to the mid-point; column lacking apical arms; sepals and petals white, up to 12 mm long; lip flabellate, orbicular, distinctly clawed, apex of claw bearing a reflexed or retrorsely placed ligule-like callus ..... *D. africana*
- 3 Column lacking apical arms; lip 3-lobed at the tip, with two lines of verrucose warts along length; sepals and petals white ..... *D. verrucosa*
- 3a Column with recurved apical arms that are bilobed at the tips; lip broadly obovate-spatulate, lacking rows of warts, with sides only slightly upcurved, and a tall apically trilobed callus at the base; sepals and petals white, pink or brown ..... *D. recurvata*

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**Fig. 4.** – *Didymoplexis recurvata* P. J. Cribb, Nusb. & L. Gaut. **A.** Flower, front view; **B.** Flower from below, bud and pedicel. [Nusbaumer & Ranirison 1763bis] [Photos: L. Nusbaumer, 12.XII.2005]

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