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# Taxonomic description and pollination ecology of *Cyrtorchis okuensis* (Orchidaceae, Angraecinae), a new species endemic to the Cameroon Volcanic Line

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**Background and aims** – A recent taxonomic and molecular study of the genus *Cyrtorchis* revealed three new species. Here, we describe one of these new species, endemic to the Cameroon Volcanic Line (CVL), and compare it to its closest relatives. Its conservation status is provided along with detailed information on its ecology and reproductive biology.

**Material and methods** – A detailed examination of 21 herbarium specimens was performed using standard practices of herbarium taxonomy. The conservation status of the new species was assessed using the IUCN Red List Categories and Criteria. Furthermore, we investigated the reproductive biology of the new species in an easily accessible subpopulation in Cameroon and tested the hypothesis of hawkmoth pollination in the genus *Cyrtorchis*.

**Key results** – The newly discovered species, here named *C. okuensis*, is restricted to the montane forest (1600 to 2500 m elevation) of the CVL and is currently assessed as Near Threatened (NT). Among angraecoid orchids in which hawkmoth pollination is recorded (~16 species), *C. okuensis* has the shortest nectar spur (19–40 mm). In situ observations revealed hawkmoths with short proboscis of the genus *Hippotion* (*H. celorio*, *H. eson*, and *H. osiris*) as the main pollinators. The rate of pollination and fruiting under natural conditions was low and among the 448 surveyed flowers only 38.8% (174/448 flowers) had their pollinia removed, 19% produced fruits (87/448 flowers), and 16% (70/448 flowers) set mature fruits.

**Conclusion** – *Cyrtorchis okuensis* is clearly differentiated from *C. submontana* by its longer and wider floral bracts, longer anther caps, and wider triangular dorsal sepal.

**Keywords** – Angraecoid orchids; epiphyte; hawkmoth; IUCN Red List Categories and Criteria; pollination; systematics.

## INTRODUCTION

A recent study combining morphological and molecular data on the African angraecoid genus *Cyrtorchis* tested the monophyly of its two sections and re-assessed the circumscription of each described species (Azandi et al.

2021). This study revealed three novelties, and among these, one is endemic to the Cameroon Volcanic Line (CVL). Some of the specimens ascribable to this taxon have been wrongly identified as *C. guillaumetii* (Pérez-Vera) R.Rice, *C. brownii* (Rolfe) Schltr., or *C. submontana* Stévert, Droissart

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& Azandi because of their similarities in leaf shape and size and in inflorescence and spur lengths.

African angraecoid orchids include a high proportion of species characterised by white, long-spurred, nectariferous, and night-fragrant flowers, features which are typical of hawkmoth pollination or sphingophily (Martins & Johnson 2007; Micheneau et al. 2009). These pollination syndromes or combination of floral traits are known to have independently evolved to attract hawkmoths (Ashworth et al. 2015). In tropical Africa, several studies on this pollination syndrome have been performed in its eastern part (see Martins & Johnson 2007, 2013), its southern part (see Luyt & Johnson 2001; Johnson & Raguso 2016; Johnson et al. 2017), and in Madagascar (see Nilsson et al. 1985, 1987, 1992; Nilsson 1992, 1998; Wasserthal 1997); yet such studies are missing for Central Africa. Most of these previous studies on angraecoid orchids pollination have demonstrated that the long nectar spurs of orchids and the proboscis of pollinators are involved in evolutionary processes resulting in the remarkable angraecoid floral specialization. The reproductive success of these orchids is known to be pollinator-mediated and generally results in low fruit set due to low visiting frequency of hawkmoths (Micheneau et al. 2009; Amorim et al. 2014). In-depth knowledge about the pollination ecology of threatened species would enable the identification and description of the factors limiting their reproduction and allow proposing adequate conservation measures for both plants and their pollinators (Senapathi et al. 2015).

During our field trips in Cameroon, we discovered a large and accessible subpopulation of the new species in Oku National Park (North-West Region). That subpopulation provided us with the opportunity to test Cribb's (1989) suggestion that the considerable resemblance of the flowers in the genus *Cyrtorchis* derives from a common hawkmoth pollination syndrome (i.e. sphingophily), a hypothesis that remains unverified to date.

The present contribution intends to describe the new *Cyrtorchis* species revealed by the recent study combining morphological and phylogenetic data on *Cyrtorchis* spp. and to describe, for the first time in Central Africa, aspects of reproductive biology of an angraecoid orchid genus.

## MATERIAL AND METHODS

### Taxonomy

**Material and morphological examination** – Taxonomic work is based on a detailed examination of 21 specimens of the novelty, which have been collected in different locations in Cameroon, Bioko (Equatorial Guinea), and Nigeria, 15 of which were collected since 1997 by our team (see Stévert et al. 2020). Specimens include 10 dried and 11 spirit-preserved samples and are deposited in the following herbaria: BRLU, FHI, K, MA, P, WAG, and YA (acronyms according to Thiers continuously updated). For stereomicroscopic observations, flowers from dried materials were initially boiled in water. Morphological investigations were performed using a Zeiss stereomicroscope Stemi SV11, both for spirit-preserved and herbarium materials. Morphology of seeds was observed

under a light microscope Olympus BX51; quantitative parameters (length, width, and shape of viable seeds and embryos, and number of testa cells) were measured in  $\mu\text{m}$  with the image acquisition and processing software ArchiMed microvision v.6.1.3. Measurements, colours, and other details given in the description below are based on living material, spirit-preserved, herbarium specimens, and data derived from field notes.

**Description, geographical distribution, and conservation status assessment** – The terminology used to describe structures and shapes follows the Systematics Association Committee for Descriptive Biological Terminology (1962a, 1962b) and the Kew Plant Glossary (Beentje 2010). The geographical distribution was determined from data given on the herbarium sheets. The distribution map was prepared using the software ArcGIS v.10.5.1 (ESRI 2017). The risk of extinction of the novelty was assessed by applying the International Union for Conservation of Nature (IUCN) Red List Categories and Criteria (IUCN 2012, 2019) and using GeoCAT (Bachman et al. 2011) to calculate the area of occupancy (AOO) and extent of occurrence (EOO).

### Ecology and reproductive biology

**Study site** – Flowering phenology and floral visitors of the novelty were investigated in the Mount Oku National Park in May 2017. Mount Oku, the second highest mountain on the West African mainland, with an elevation of about 3,011 m a.s.l. (Asanga 2002), is part of the Bamenda Highlands, in the North-West Region of Cameroon ( $6^{\circ}12'N$ ,  $10^{\circ}32'E$ ). Mount Oku is located within the Kilum-Ijim forest massif, which is the largest remnant of West African upper montane forests (Maisels et al. 2000). The vegetation includes a mosaic of montane forest, montane grassland, and sub-alpine habitats (Cheek et al. 2000; Asanga 2002).

Our ecological survey was conducted in 2017 in an area of wooded savannah, at the upper montane forest-grassland edge, located on the north-western part of Mount Oku crater lake, which rises to about 2,400 m elevation (fig. 1A). This site was chosen because of the abundance of mature individuals of the novelty within reach, to explore their ecology and reproductive biology. We planned to return to complete our data with more and improved camera traps. However, since mid-2017, troubles in this part of Cameroon prevented all access to the study area. No improvement of the situation seems conceivable in the short- and medium-term.

### Ecology, phenology, and pollination reproductive success

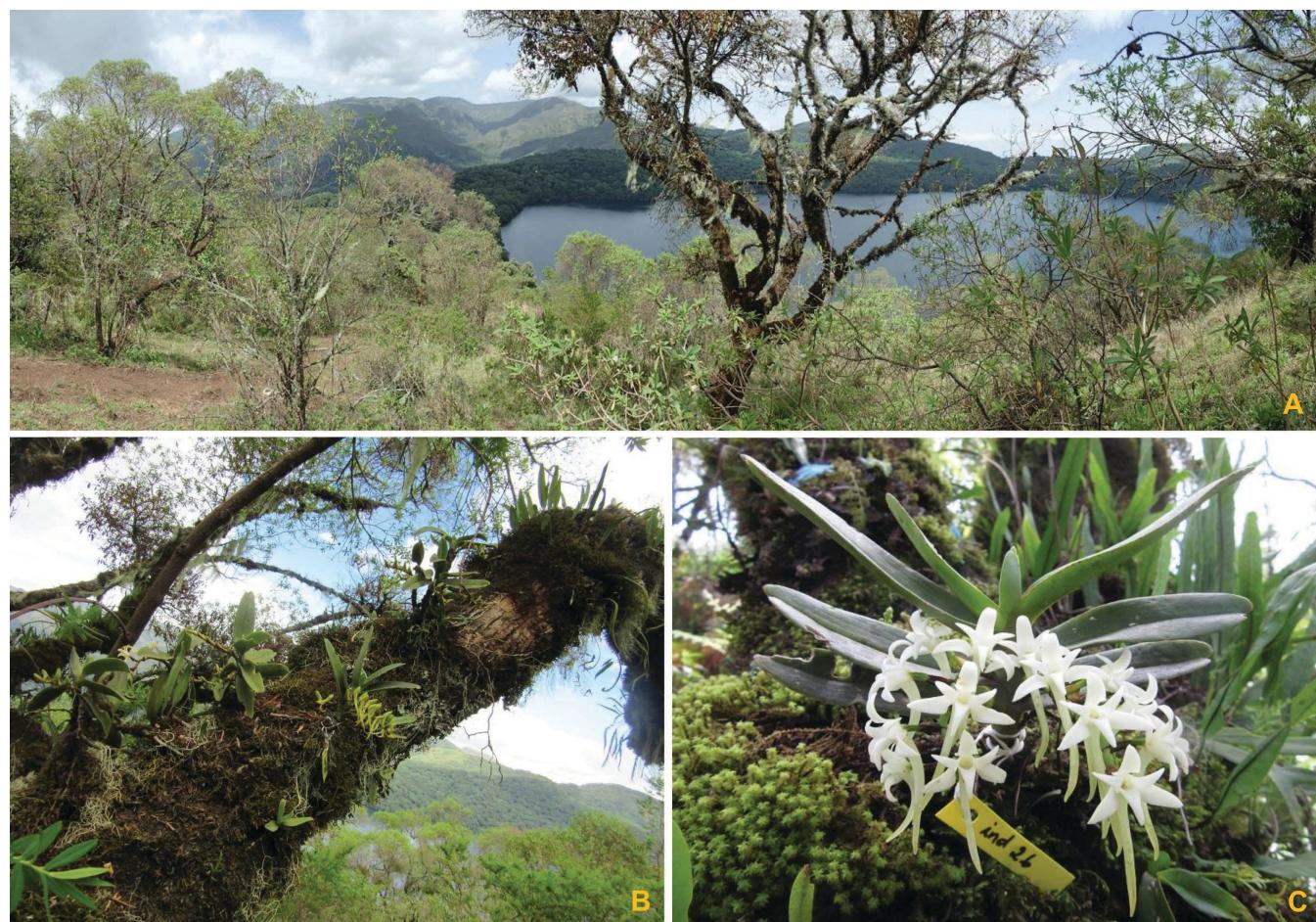
– We studied the ecology of the new *Cyrtorchis* species in about one hectare of natural vegetation. Phytosociological surveys of 2 m length each (see Stévert 2003) were made on branches of labelled host trees of 33 mature *Cyrtorchis* individuals bearing one to four inflorescences (one individual per survey) (supplementary file 1). Herbarium vouchers were collected for each of the host trees along with the epiphytes growing with the new species for subsequent identification. To study its reproductive biology, monitoring of flowering from anthesis (opening of the first flower on an individual) was conducted in situ from 5 to 29 May 2017 (25 days). We daily recorded (a) the proportion of individuals with open

flowers, (b) the number of open flowers and their opening date, and (c) the proportion of flowers visited by insects and fruit set (pollinia removal from the gynostemium for male fitness and pollinia load on stigmas or fruit set for female fitness).

**Pollinator observation and syndromes** – To identify the pollinators of this new species, we used the interval-programming function of a waterproof digital camera (Camera Bushnell Natureview HD MAX), using the settings to take pictures at 1-minute intervals, and also by motion detection in the field of vision on five individuals located at least 10 m apart from each other (see details in supplementary file 1). Furthermore, during two nights (on 6–7 May 2017 from 6 pm to midnight, and on 8–9 May 2017 from 7 pm to 2 am), light traps with mercury vapor lamps (Sylvania HQL bulb, 250 W), installed near the surveyed population, were used to investigate and collect potential pollinators (i.e. hawkmoths and settling moths). Captured moth specimens were examined to detect pollinia on their body. A subsequent analysis relating the pictures recorded with the cameras and the daily survey of each flower was used to determine whether a specific species is a passive visitor or an effective pollinator of the novelty. To further confirm

the potential pollinator identity, the relationship between the proboscis and spur length was assessed for hawkmoths seen on the camera footage foraging the flowers. The proboscis length of individual hawkmoths was measured to the nearest millimetre (from the base near the head to the tip) with a ruler (Martins & Johnson 2007; Peter & Venter 2017). The spur length of fully developed flowers was measured from BRLU herbarium specimens ( $n = 9$ ). The captured hawkmoths specimens were sent to Rodolphe Rougerie, a sphingid specialist at the Muséum national d'histoire naturelle in Paris for identification.

**Breeding system** – To test the ability of the new species to reproduce by autonomous self-pollination, we bagged three individuals (with a total of five inflorescences and with 20 closed flower buds) with fine nets prior to flower anthesis and we monitored their development in natural condition until the end of flowering. Hand pollinations were set up ex situ in the Elak village, to investigate the new species' breeding system, by performing 114 hand-pollinations including 58 cross-pollinations (20 individuals; 58 flowers) and 56 self-pollinations (19 individuals; 56 flowers) on 39 cultivated specimens. The Shapiro-Wilk Normality test (Royston 1982; Dytham 2011) was performed prior to statistical difference



**Figure 1** – *Cyrtorchis okuensis* in its natural habitat, the shrubby savannah around the volcanic lake Oku. **A.** Panoramic view of *C. okuensis* habitat in the survey area. **B.** Individuals of *C. okuensis* growing on a 2 meter-long branch of *Gnidia glauca*. **C.** A flowering plant of *C. okuensis* growing with mosses *Leptodontium viticulosoides*, and the ferns *Lepisorus excavatus* and *Asplenium theciferum*. Photographs by Vincent Droissart (A) and Laura Azandi (B, C).

testing to evaluate if fruit set followed a normal distribution pattern. To statistically evaluate the difference between the fruit set according to the pollination treatment (self- or cross-pollination) a Wilcoxon rank-sum test was performed, which is recommended for the comparison of two groups of non-parametric datasets (fruit set for self- and cross-pollination treatments), as observed here (e.g. McDonald 2014). These analyses were performed using R v.3.4.4 (R Core Team 2020).

## RESULTS

### Taxonomy

***Cyrtorchis okuensis*** Droissart, Azandi & M.Simo, sp. nov. (fig. 2) – *Cyrtorchis* aff. *guillaumetii* “Oku” (Azandi et al. 2021). – Type: CAMEROON • Région du Nord-ouest, arrondissement d’Oku. Savane boisée sur le flanc est du lac Oku; 6°12'18.667"N, 10°27'1.306"E; 28 May 2017; Azandi L. 26; holotype: BRLU; isotype: YA.

**Diagnosis** – *Cyrtorchis okuensis* is similar to *C. submontana* but differs in having wider floral bracts (7–12.5 × 8–13.7 mm vs 4–6.5 × 5–7 mm), longer anther caps (2.5–3 vs 1–2 mm), and wider triangular dorsal sepal (4–5 mm vs 2–3 mm). These species also differ by their habitat; *C. submontana* is found in submontane and evergreen moist forest (between 550 and 1,200 m), whereas *C. okuensis* is restricted to the upper montane vegetation (between 1,600 and 2,500 m) along the CVL.

**Description** – Epiphytic herb, erect, monopodial, with short to long unbranched stems, 3–18.5 cm long bearing 3–13 alternate leaves with a 16–21.5 cm leaf span. Roots 15.5–20.8 cm long and 0.3–0.4 cm diameter, pale green and covered by mosses. Leaves closely elliptic, distichous, subimbricate, fleshy, olive green, margins entire, 4.6–11.3 × 1.2–3 cm, unequally bilobed at the apex, with rounded lobes. Inflorescences up to 4, axillary or below the leaves, 4.4–9 cm long, 3–12-flowered, flowers 3.3–4.6 cm long with floral node ranging from 0.5–0.9 cm long. Bracts broadly ovate, 7–12.5 mm long and 8–13.7 mm apart, dark brown apiculate at the apex. Flowers white, fading to orange, 3–4.7 cm long and 1–1.8 cm in diameter. Dorsal sepal triangular, 8–13 × 4–5.5 mm. Lateral sepals lanceolate, acute at the apex, 8–15.5 × 3–5.2 mm. Petals similar to lateral sepal but shorter and usually acuminate at the apex, 8–13 × 3–4 mm. Lip triangular with acuminated fleshy apex, 8.5–12 × 5–6 mm; spur incurved sometime sigmoid, 1.9–4 cm long. Anther cap whitish, turning brown with senescence, obovate in the upper part and with narrowed lower part, slightly toothed in the margins and covering the base of the rostellum 2.5–3 × 1.5–2 mm. Pollinia 2, oblate from side rarely ovoid. Column stout and short, 1–2 mm long. Viscidium saddle-shaped, with a uniformly hyaline texture, bifid at the basal apex, 2–4 mm. Stipites spatulate or slightly clavate with acute apices pointing towards the middle side, insertion point at the rear third of the length of the viscidium, 1.5–2.5 mm long. Pedicel with ovary 10.5–19.5 mm long; ovary triquetrous. Rostellum trifid, 2.5–4 mm long, midlobe straight, acute, lateral lobes linear, about three times as long as the midlobe,

pendulous and parallel to each other with rounded apices. Fruits narrowly ellipsoid capsules, triquetrous with three smooth sides, narrowly ellipsoid, turning yellowish-green at maturity, 12.9–24.5 × 4.5–10.8 mm. Seeds fusiform, 149–194 × 70–93 µm, rarely ellipsoid with ovoid to ellipsoid embryo, 117–141 × 56–76 µm, with two testa cells in an individual seed coat along the longitudinal axis of seed.

**Position** – *Cyrtorchis okuensis* belongs to section *Homocolleticon* Summerh. based on the structure of its viscidium, uniformly hyaline in texture, and its thin apices of the stipites.

**Distribution** – Endemic to the Cameroon Volcanic Line: Nigeria, Cameroon, and Bioko (Equatorial Guinea) (fig. 3).

**Habitat and ecology** – Restricted to montane vegetation (moist forest, forest-grassland edge, and wooded savannah from 1,600 to 2,500 m), *Cyrtorchis okuensis* grows on mossy branches of *Gnidia glauca* (Fresen.) Gilg (Thymelaeaceae), *Albizia gummifera* (J.F.Gmel.) C.A.Sm. (Fabaceae), and *Prunus africana* (Hook.f.) Kalkman (Rosaceae). On these phorophytes, *C. okuensis* is usually found growing with mosses, lichens, ferns, and other orchid species (see details below in “Ecology and reproductive biology”).

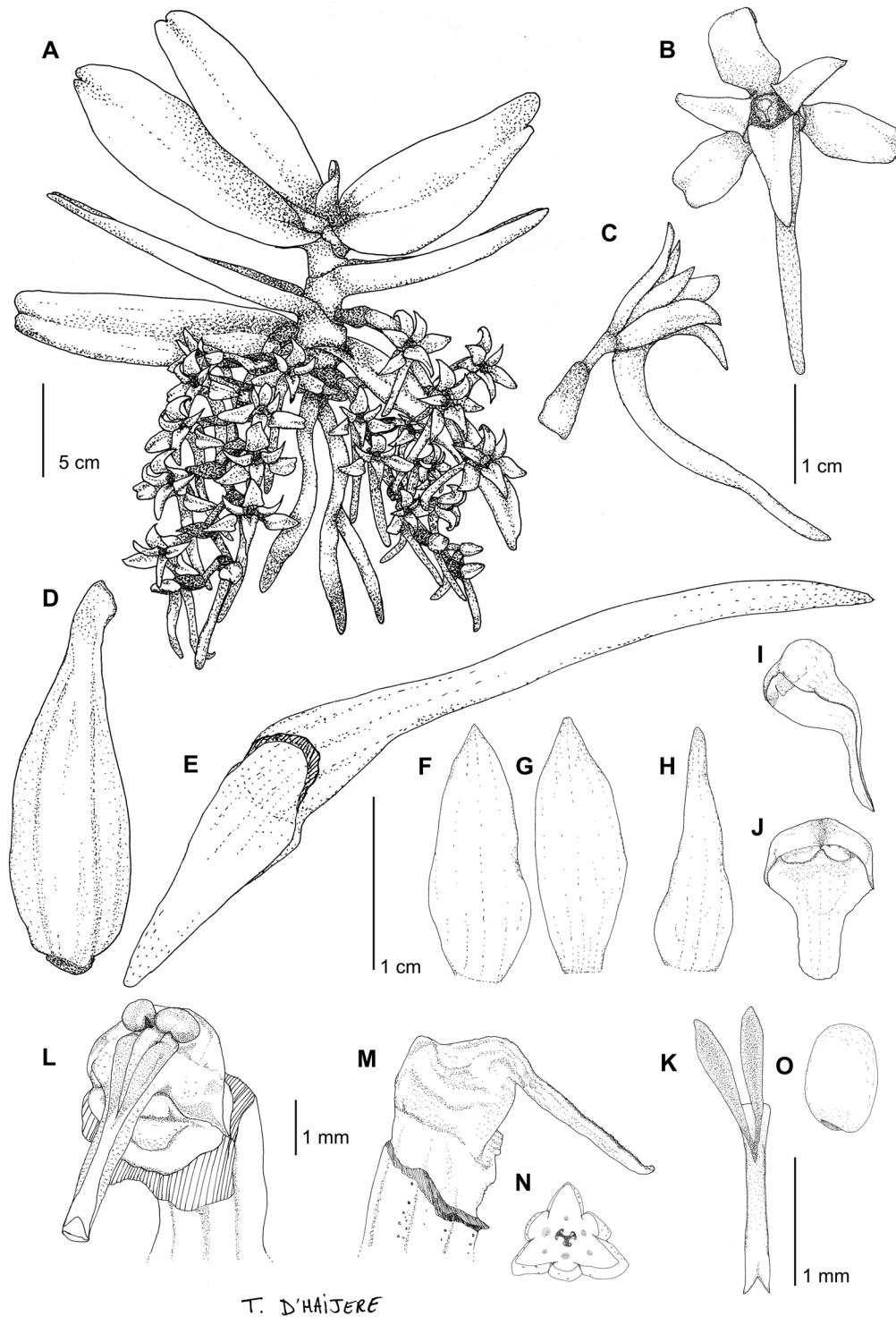
**Phenology** – The flowering period occurs between April and June with a flowering peak in May. Fruit maturation lasts about 230 days on average (from June to January).

**Etymology** – The epithet *okuensis* refers to Oku, a subdivision in the North-West Region of Cameroon where the large, surveyed subpopulation was discovered around Lake Oku. The term Oku also refers to the people who live in this area and their native language.

**Red list conservation status** – The species is given a Red List status of Near Threatened [NT]. The species is known from 21 specimens in Nigeria, Cameroon, and Bioko (Equatorial Guinea), which represent 21 occurrences. The extent of occurrence (EOO) of *Cyrtorchis okuensis* is estimated to be 38,319.1 km<sup>2</sup>, far exceeding the upper limit of the Vulnerable category (20,000 km<sup>2</sup>) under the subcriterion B1, while its area of occupancy (AOO) is estimated to be 68 km<sup>2</sup>, which falls within the limits of the Endangered category under the subcriterion B2.

In Nigeria, *C. okuensis* has been collected in the Boshi Extension Forest Reserve. In Bioko, the species is known from the Pico Basilé National Park and the Reserva Científica de la Caldeira de San Carlos (at Lago Moka). In Cameroon, the species is recorded in three protected areas: the Mount Oku and the Mount Manengouba National Parks (where some occurrences are below the lower altitudinal limit of effective protection) and the Bafut Nguemba Forest Reserve. In unprotected sites, the species habitat is mainly subject to small-scale shifting agriculture, the logging activities on *Prunus africana*, and the fuelwood collection for small-scale subsistence.

Based on the most serious plausible threat, which is shifting agriculture, the 21 occurrences represent 16 locations (sensu IUCN 2019), which is more than 10, the upper limit of the Vulnerable category under the condition ‘a’ of subcriterion B2. *Cyrtorchis okuensis* is not severely fragmented and there are no extreme fluctuations



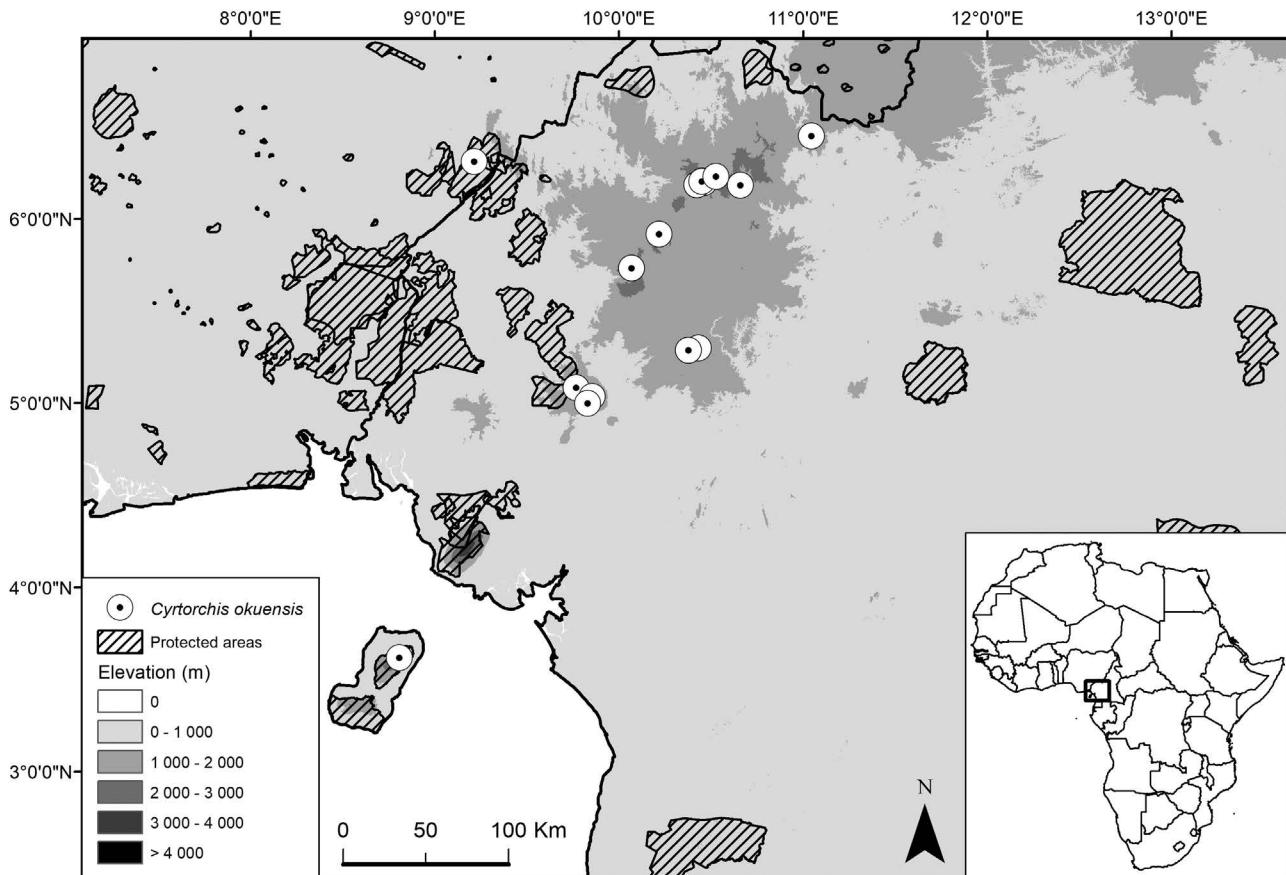
**Figure 2 –** *Cyrtorchis okuensis* (Azandi 26). **A.** Habit and inflorescence. **B.** Flower, frontal. **C.** Flower, side view. **D.** Fruit (cultivated plant Y 6377 BA, fruit ID 499). **E.** Labellum with spur frontal. **F.** Dorsal sepal. **G.** Lateral sepal. **H.** Petal. **I.** Anther cap, side view. **J.** Anther cap, frontal. **K.** Viscidium with stipites frontal (*Droissart et al. (Ombrière de Yaoundé) 5109*). **L.** Column, frontal. **M.** Column, side view. **N.** Ovary section. **O.** Pollinia. Drawing by Tania D'haijère.

in its parameters (EOO, AOO, number of locations or subpopulations, and number of mature individuals). The projected ongoing loss of its habitat leads us to predict a continuing decline in the number of subpopulations in four Regions in Cameroon (in Bangem near the Banyang-Mbo Wildlife Sanctuary in the South-West Region, in Bambuto in the West Region, in Mount Manengouba near Nkongsamba in the Littoral Region, in Nkambe and the Bafut Nguemba Forest Reserve in North-West Region), in mature individuals, in its AOO and its EOO. Since two (a and c) of the three conditions under subcriterion B2 are not met, *C. okuensis* cannot be regarded as a threatened species. Considering that shifting agriculture will lead to the loss of four locations in the near future, reducing the number of locations to 12, *Cyrtorchis okuensis* is assigned a IUCN Red List status of Near Threatened (NT).

**Additional specimens examined** – NIGERIA • Boshi Extension Forest Reserve; 6°18'38.16"N, 9°12'46.8"E; 1600 m; 23 May 1971; van Meer P.P.C. 1765; FHI web.

CAMEROON • Province du Nord-Ouest, Lac à proximité du village d'Oku; 6°11'48.77"N, 10°27'38.74"E; 2239 m; 3 Sep. 2011; Droissart V. et al. 894; BRLU • Belo, Mont Oku, autour du lac; 6°11'8.2"N, 10°25'19.88"E; 2300 m; 21 Apr. 2014; Droissart V. et Simo M. 1693; BRLU, YA • ibid.; 5 May 2014; Droissart V. et al. (*Ombrière de Yaoundé*) 5046; BRLU • ibid.; 16 May 2014; Droissart V. et al. (*Ombrière*

*de Yaoundé*) 5074; P, YA • Belo, Mont Oku, savane boisée autour du lac; 6°12'14.4"N, 10°26'E; 2313 m; 28 Apr. 2017; Droissart V. et al. (*Ombrière de Yaoundé*) 7163; BRLU • Forêt du mont Oku; 6°13'51.6"N, 10°31'33.6"E; 15 May 2017; Droissart V. et al. (*Ombrière de Yaoundé*) 7174; BRLU • Entre Kumbo et Ndop; 6°11'1.32"N, 10°39'32.94"E; 9 Jun. 2011; Simo M. et al. (*Ombrière de Yaoundé*) 2848; BRLU • Près du point trigonométrique de Mbem (40 km ESE de Nkambe); 6°26'59.75"N, 11°2'40.92"E; 1800 m; 11 Jul. 1904; Letouzey R. 8860; P, YA • Bamenda district, along the footpath from the rest house to number 6 plot in Bafut Ngemba Reserve; 5°55'0"N, 10°13'E; 19 May 1959; Daramola B.O. FHI 41182; FHI, K • Région du Sud-Ouest, crête NW Muetan Aku, 9 km SW Bangem; 5°5'4.04"N, 9°45'59.93"E; 2 Jun. 1982; Villiers J.F. 1397; BRLU, P n.v. • Province de l'Ouest, Bayangam; 5°17'55.7"N, 10°25'50.52"E; 1650 m; 2 Jun. 2014; Droissart V. et al. (*Ombrière de Yaoundé*) 5117; BRLU • Bambuto; 5°44'N, 10°4'E; 2500 m; 21 Nov 1972; Jacques-Félix H. 5441; K n.v., P • ibid.; 2300 m; May 1940; Jacques-Félix H. 5483; P • Baham; 5°17'12.47"N, 10°22'32.98"E; 1920 m; 28 Apr. 2017; Droissart V. et al. (*Ombrière de Yaoundé*) 7164; BRLU • ibid., 15 Jun. 2017; Droissart V. et al. (*Ombrière de Yaoundé*) 7203; YA • Dschang district, Massif Manengouba, near Nkongsamba, village of Bouroakou to crater lakes; 5°0'0"N, 9°49'48"E; 2133 m; 18 Nov. 1968; Sanford W.W. 5553; K • Province du Littoral, Melong, Mont Manengouba;



**Figure 3** – Distribution of *Cyrtorchis okuensis* in Central Africa, along the Cameroon Volcanic Line. Map created with ArcGIS v.10.5.1 (ESRI 2017). © Esri and its licensors, all rights reserved.

**Identification key to *Cyrtorchis okuensis* and related species (amended from Azandi et al. 2016)**

1. Leaves thin and flexible, equally bilobed at the apex ..... *Cyrtorchis ringens* (Rchb.f.) Summerh.
- 1'. Leaves fleshy and rigid, unequally bilobed at the apex ..... 2
2. Floral bract imbricate, longer than the pedicel and ovary ..... *Cyrtorchis brownii* (Rolfe) Schltr.
- 2'. Floral bract subimbricate or well-space, shorter than the pedicel and ovary ..... 3
3. Inflorescence loose at the base and densely flowered near the apex ..... *Cyrtorchis guillaumetii* (Pérez-Vera) R.Rice
- 3'. Inflorescence not loose at the base with flowers distributed all along ..... 4
4. Anther cap 2.5–3 mm long, dorsal sepal triangular, 4–5 mm wide ..... *Cyrtorchis okuensis* Droissart, Azandi & M.Simo
- 4'. Anther cap 1–2 mm long, dorsal sepal narrowly triangular, 2–3 mm wide ..... *Cyrtorchis submontana* Stévert, Droissart & Azandi

5°2'14.3"N, 9°51'17.75"E; entre 1800 et 2200 m; 2 Jun. 2014; *Droissart V. et al (Ombrière de Yaoundé) 5109*; BRLU. EQUATORIAL GUINEA (BIOKO) • Malabo-Pico Basilé, estrada km 17–18, Bioko Norte; 3°37'7.5"N, 8°48'22.5"E; 2 Jul. 1989; *Carvalho M.F. 4004*; BRLU, K n.v., MA n.v., P n.v., WAG n.v. • ibid., Pico Basilé, bosquete ralo; 3°36'25.49"N, 8°46'55.49"E; 2310 m; 3 Jul. 1986; *Fernández Casas et al. 10166*; MA web • Bioko Sur, Lago Moka; 3°21'22.5"N, 8°37'43.5"E; 30 Jan. 1947; *Guinea E. 2236*; BRLU, MA web.

**Ecology and reproductive biology**

**Ecology, phenology and pollination reproductive success** – In the shrubby savannah surveyed around Lake Oku, the main host trees were *Gnidia glauca* with 32 host trees and *Prunus africana* with only one host tree. *Gnidia glauca*, a small bushy tree growing in a large, monodominant subpopulation in the study area, was always associated with a high number of individuals of *C. okuensis*, bearing up to 13 individuals (fig. 1B). Specimens of *C. okuensis* were found growing on branches of 4 to 30 cm in diameter, under the canopy of both host trees at 1 to 4 m above ground level. On the 33 phytosociological branch surveys, the species was found growing in association with mosses such as *Daltonia minuta* Thér. (Daltoniaceae) and *Leptodontium viticulosoides* (P.Beauv.) Wijk & Margad. (Pottiaceae), with the lichen *Usnea barbata* (L.) F.H.Wigg. (Parmeliaceae), with the ferns *Asplenium aethiopicum* (Burm.f.) Becherer, *A. theciferum* (Kunth) Mett., *Lepisorus excavatus* (Bory ex Willd.) Ching, and *Pleopeltis macrocarpa* (Bory ex Willd.) Kaulf., with *Kalanchoe crenata* (Andrews) Haw. (Crassulaceae), and with the orchids *Kylicanthe bueae* (Schltr.) Farminhão, Stévert & Droissart and *Polystachya alpina* Lindl.

The time between the opening of the first and the last flower on a given individual varied from two to 10 days for the 16 individuals bearing one to two inflorescences (with up to 19 flowers in total) and from 5 to 28 days for the 14 individuals bearing 3 to 4 inflorescences (with up to 31 flowers in total). This timing of flowering was not estimated

for the three individuals for which the anthesis had already passed at the beginning of the survey. The daily monitoring of each of the 33 labelled individual conducted during 25 days enabled us to measure the frequency of flowering and visiting events. Flowering peaks (i.e. the maximum number of flowers opened the same day) in the *C. okuensis* subpopulation surveyed were registered on the 16<sup>th</sup>, 17<sup>th</sup>, and 21<sup>st</sup> day (fig. 4A). More than 50% (17 individuals) of all the 33 surveyed individuals were already flowering on the 13<sup>th</sup> day and by the end of the survey all the 33 individuals had at least one open flower. Concerning the visitation rate, flower visits started on the 5<sup>th</sup> day till the end of the monitoring session with a maximum observed on the 11<sup>th</sup> and 19<sup>th</sup> day (see fig. 4B). Eight hawkmoth visits recorded with cameras on the 9<sup>th</sup>, 12<sup>th</sup>, 16<sup>th</sup>, 17<sup>th</sup>, and 23<sup>rd</sup> day matched daily observations of the number of visited flowers (fig. 4C).

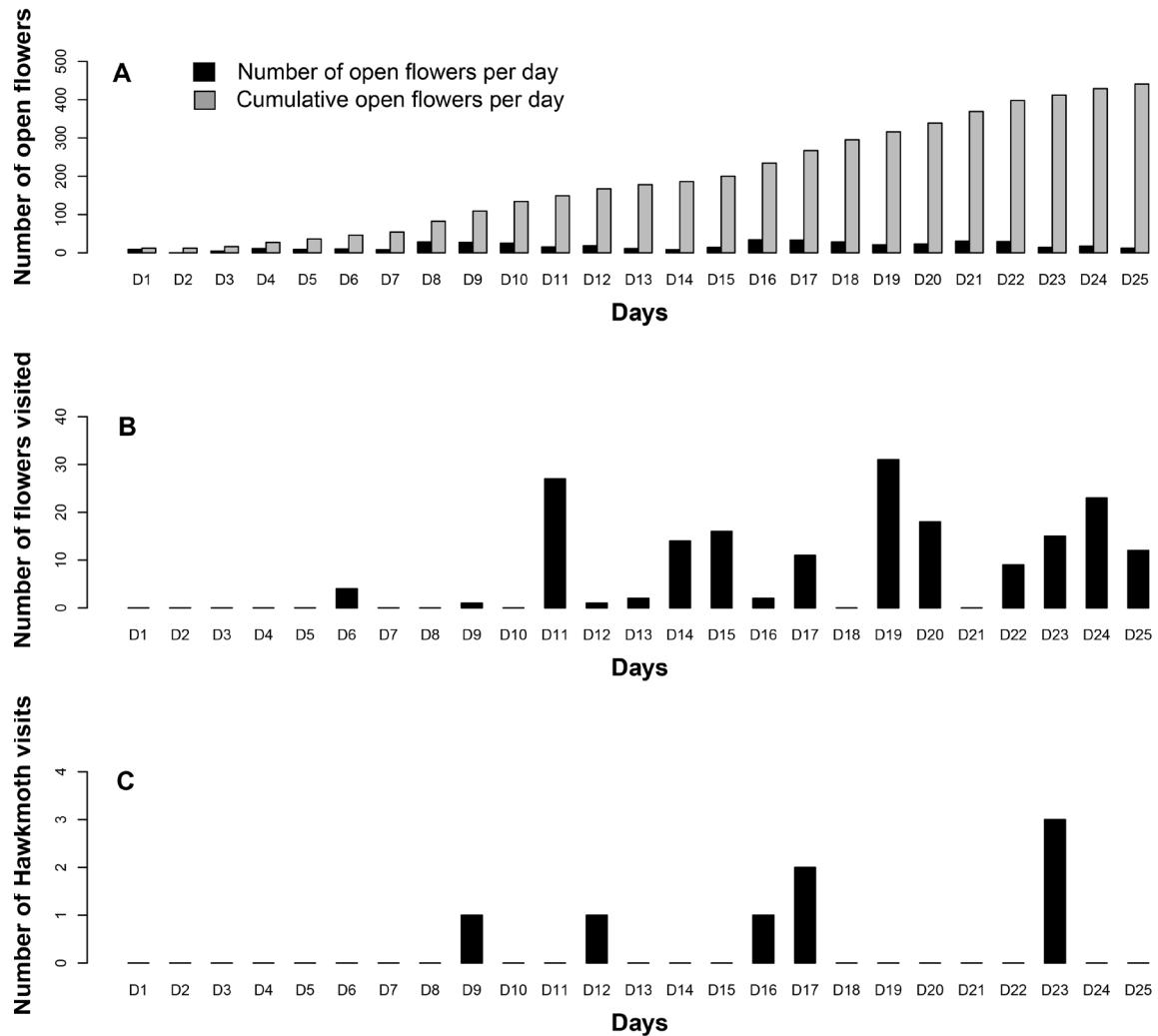
In natural conditions, the rate of pollinaria removal averaged 38.8% (174 flowers out of 448 flowers) for the 33 individuals. Successful pollen deposit on the stigma, indirectly measured by fruit set rate, was 19.4% (87 fruits/448 flowers), and 15.6% reached maturity (70 matured fruits/448 flowers) after an average of 230 days (table 1).

**Pollinator observation and syndromes** – A total of 72,476 pictures (more than 1207 hours of observation) were recorded with four camera traps, showing visits of at least seven different insects, one spider, and two bird species (the northern double-collared sunbird (*Cinnyris reichenowi* Sharpe, 1891) and the oriole finch (*Linurgus olivaceus* Fraser, 1843)). During daytime, flowers were mostly visited by birds and flies, while hawkmoths, settling moths, and grasshoppers were the main visitors at night. Apart from birds, which ate the perianth parts and with possible nectar robbery, and hawkmoths, which only foraged on flowers for nectar, the other insects were passive visitors.

The opening of the flowers occurred only at night. A slight floral scent was detected during daytime when getting near to the flowers. At dusk, from about 6 pm, a jasmine-like scent began to emanate from the flowers and was detectable a few meters away. Scent release began from about 7:30 pm and remained strong throughout the night. Hawkmoth

**Table 1** – Breeding system and reproductive success of *Cyrtorchis okuensis* under natural and controlled pollination experiments. Values in brackets represent the percentage of sample size (total number of flowers used or fruits produced) per treatment.

Treatments	Number of individuals	Number of flowers	Number of flowers with pollinia removed (%)	Number of fruits set (%)	Number of mature fruits (%)
<b>In situ</b>					
<b>Pollinators excluded (bagged flowers)</b>	3	20	–	–	–
<b>Natural pollinations</b>	33	448	174 (38.8)	87 (19.4)	70 (15.6)
<b>Ex situ (controlled pollinations)</b>					
<b>Manual self-pollinations</b>	19	56	–	36 (64.2)	21 (37.5)
<b>Manual cross-pollinations</b>	20	58	–	51 (88)	27 (46.5)

**Figure 4** – Reproductive phenology of *Cyrtorchis okuensis* per day in the studied population (total of 33 individuals) from 5 to 29 May 2017. A. Daily number of open flowers B. Daily number of flowers visited. C. Daily number of hawkmoth visits recorded with camera traps.

**Table 2** – Visitor’s activities on flowers of *Cyrtorchis okuensis*. Results are based on a 25-day monitoring session with four camera traps (May 2017). Column “Days” represent the day when the interaction was registered based on the first day of the follow-up session.

Visitors	Date	Visiting time	Days	Number of visits per session
Birds	12 May 2017	15:39	Day 8	1
Birds	13 May 2017	08:35 & 11:28	Day 9	2
<i>Hippotion</i> spp.	13 May 2017	23:22	Night 9	1
Birds	15 May 2017	05:17	Night 11	1
Flies	16 May 2017	10:41	Day 12	1
Grasshopper	16 May 2017	20:43	Night 12	1
<i>Hippotion</i> spp.	16 May 2017	01:33	Night 12	1
Settling moths	16 May 2017	18:29	Day 12	1
<i>Hippotion</i> spp.	17 May 2017	00:24 & 01:52	Night 13	2
Settling moths	18 May 2017	18:48	Night 14	1
Birds	19 May 2017	09:16	Day 15	1
Flies	19 May 2017	07:22	Day 15	1
Flies	20 May 2017	10:54	Day 16	1
<i>Hippotion</i> spp.	20 May 2017	18:30	Night 16	1
Wasps	20 May 2017	03:43	Night 16	1
Flies	21 May 2017	05:52	Day 17	1
Birds	22 May 2017	17:09 & 17:16	Day 18	2
Flies	22 May 2017	10:48	Day 18	1
Flies	23 May 2017	16:25	Day 19	1
Spider	26 May 2017	08:50	Day 22	1
Grasshopper	27 May 2017	19:08	Night 23	1
<i>Hippotion</i> spp.	27 May 2017	22:08 & 22:12	Night 23	2
<i>Hippotion</i> spp.	27 May 2017	18:17	Day 23	1
Ants	28 May 2017	07:24	Day 24	1
Flies	28 May 2017	12:44	Day 24	1
Spider	28 May 2017	00:03	Night 24	1

visits mostly occurred at dusk between 6 pm and 1 am with a peak of visits being between 10 pm and 1 am (table 2). The combined camera survey and light trap design enabled us to capture nine individuals of potential effective pollinators of *C. okuensis* (fig. 5A), representing three species of the genus *Hippotion* Hübner, 1819, namely *H. celerio* (Linnaeus, 1758), *H. eson* (Cramer, 1779), and *H. osiris* (Dalman, 1823) (fig. 5B–D). Unfortunately, none of the recorded nor captured hawkmoth specimens were caught with the pollinia attached on any part of their bodies. Nevertheless, based on pollinia removal and/or deposit observed during daily monitoring, as well as fruit set after their visits, we infer that one or more of these hawkmoth species is the effective pollinator of *C. okuensis* in the Mount Oku area.

Morphologically, the mean spur length of *C. okuensis* was 28.2 mm ( $sd = 0.64$ ,  $n = 9$ ). This value matches the length of the proboscis of all nine captured *Hippotion* specimens (*H. celerio* (5 individuals), *H. eson* (3), and *H. osiris* (1)), which ranged between 20 and 42 mm, with a mean of 31 mm ( $sd = 0.86$ ,  $n = 9$ ). Based on these spur and tongue lengths, the majority of these species of hawkmoth are likely to forage on

the flowers in this subpopulation. There was no significant difference in the spur and proboscis length ( $p = 0.89$ ).

**Breeding system assessment** – None of the flowers tested for autonomous self-pollination (bagged inflorescence) produced fruits, suggesting that *C. okuensis* requires a pollinator to set fruit (table 1). The species is however self-compatible as the self-pollination treatments resulted in 64.2% fruit set. Nevertheless, cross-pollination shows a higher success rate with a fruit set of 88%. These two controlled pollination treatments did however not differ significantly for fruit set success ( $W = 138$ ,  $p > 0.05$ ).

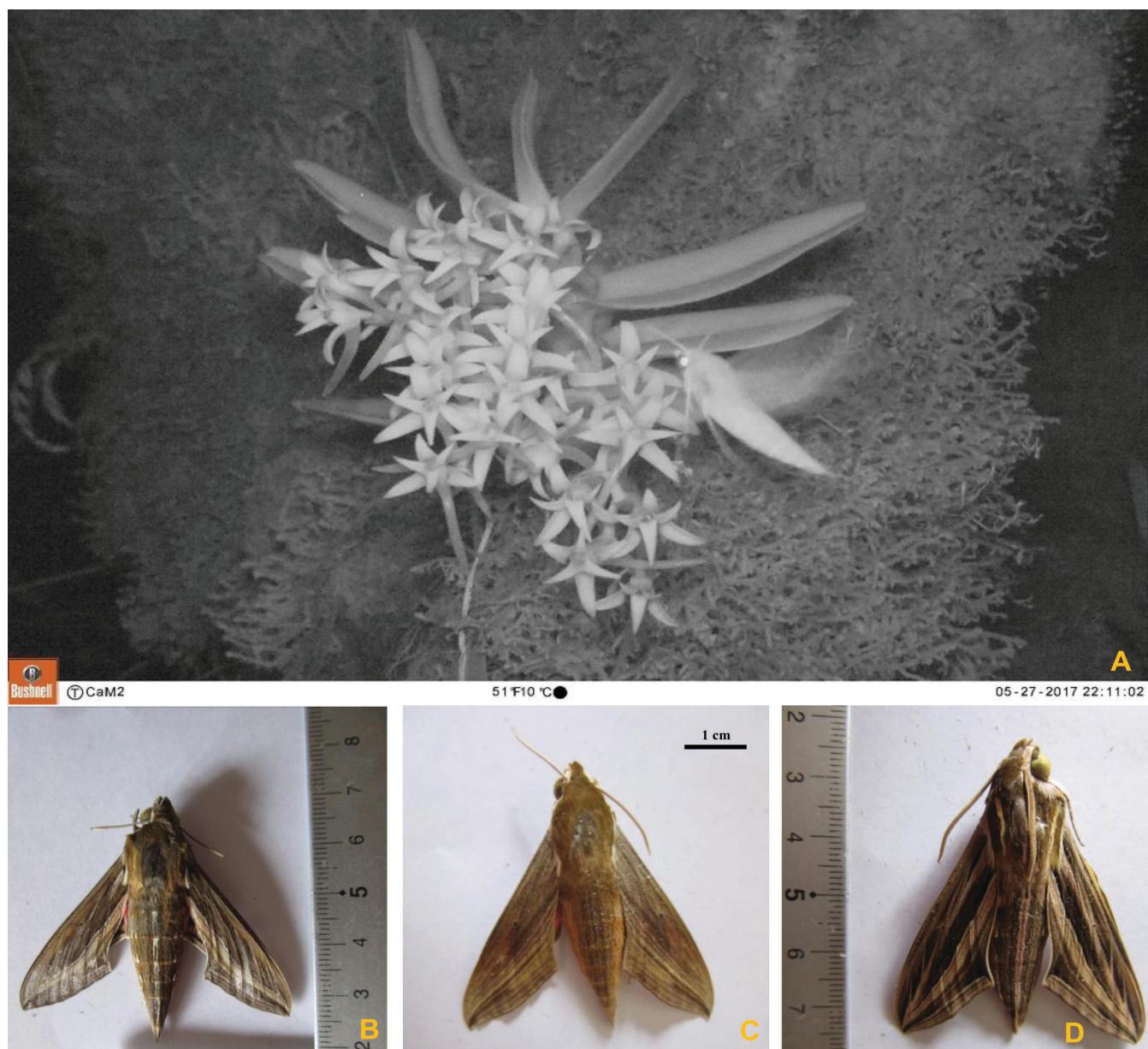
## DISCUSSION

### *Cyrtorchis okuensis* is the angraecoid orchid with the shortest spur that is pollinated by hawkmoths

Our formal description of *Cyrtorchis okuensis* constitutes an additional step towards a global revision of the genus *Cyrtorchis*. The current study not only confirms pollination by hawkmoths in the genus *Cyrtorchis*, as predicted by

Cribb (1989), but also brings down the lower limit of spur lengths associated with hawkmoth pollination within the angraecoid group (~800 species; see Simo-Droissart et al. 2018; Farminhão et al. 2021). *Cyrtorchis okuensis* exhibits clear floral adaptations to hawkmoth pollination, such as the white night-scented flowers with a “relatively” long nectar-rewarding spur (Martins & Johnson 2007, 2013; Vogel 2012; Johnson & Raguso 2016; Johnson et al. 2017). These white long-spurred orchids with night-scented flowers are common in Africa (Dressler 1981), with data supporting that about 50% of the African orchids are pollinated by hawkmoths, based on the high frequency of this floral syndrome (Martins & Johnson 2007, 2013; Johnson & Raguso 2016; Johnson et al. 2017). However, the spur length of *C. okuensis* appears to

be relatively short (1.9–4 cm vs 4.4–24 cm long) compared to most of the other known African continental angraecoids (~16 species) in which hawkmoth pollination syndromes are documented. Luyt & Johnson (2001) previously reported this pollination syndrome for *Mystacidium venosum* Harv. ex Rolfe, whose spur length ranges from 2.5 to 5.5 cm and Martins & Johnson (2007) later described it for *Aerangis confusa* J. Stewart with an average spur length of 4.5 cm. As observed for other short-spurred angraecoid species (i.e. *M. venosum* and *A. confusa*), there is a good match between the spur length in *C. okuensis* and the proboscis length of the pollinators. This might suggest that a strong selective pressure of the proboscis on spur length might have played a role.



**Figure 5** – Pollinators of *Cyrtorchis okuensis* observed around the Lake Oku (North-West Cameroon). **A.** Pictures recorded with a camera trap showing a *Hippotion* sp. foraging on *C. okuensis* flowers. **B–D.** Sphinx moths captured during light trap sessions. **B.** *Hippotion celerio*. **C.** *H. eson*. **D.** *H. osiris*. Photographs by Laura Azandi.

Three different species of *Hippotion* (*H. celerio*, *H. eson*, and *H. osiris*) were identified as effective pollinators of *C. okuensis*. These results are in line with previous studies, as *H. eson* was also observed feeding on flowers of *Mystacidium venosum* (Luyt & Johnson 2001) and *H. celerio* was reported to be a pollinator of *Aerangis confusa* in Kenya (Martins & Johnson 2007). Although it was difficult to identify pollinators at the species level, the confirmed presence of the three captured *Hippotion* spp. and their quite similar proboscis length advocate in support of them being effective pollinators of *C. okuensis*. This was previously found in a study of Alexandersson & Johnson (2002) on *Gladiolus longicollis* Baker, in which moths with matching proboscis lengths were effective agents of pollen removal and deposition. Additionally, more recent studies suggested that hawkmoths are highly polyphagous and readily feed on flowers that have tubes much shorter than their proboscis lengths, while plants adapted to hawkmoths are more specialized, particularly when they are long tubed (Martins & Johnson 2013; Amorim et al. 2014). Given the slight difference in size between the average length of the pollinators' proboscis and the spur of *C. okuensis*, and the fact that pollinia in the genus *Cyrtorchis* are generally placed along the proboscis (Laura Azandi, pers. obs. on *Cyrtorchis letouzeyi* and *C. chailluana*), we can assume that there is a selection pattern for short spurs as described by Ellis & Johnson (2010) in their sex-specific selection model. This model states that if selection is made through the male, this would favour an evolution towards shorter spurs than proboscis because pollinia export is potentially highest in short-spurred species as pollinia are placed far from the base of the proboscis and could reach both the stigmas of long and short-spurred flowers. Our autonomous self-pollination experiment provides strong evidence that *C. okuensis* fruit production depends on external pollinators. Under natural conditions, hawkmoth visitation events on *C. okuensis* flowers seem relatively rare (see fig. 4 and table 2) and resulted in low reproductive success (pollen removal and fruit set) as only 19.4% of the 448 flowers we surveyed in May 2017 produced fruits. Amorim et al. (2014) and Micheneau et al. (2009) also reported low visitation and fruit set rates of orchid flowers when pollination is accomplished by hawkmoths.

### Importance and conservation of the Cameroon Volcanic Line

The CVL includes a chain of isolated volcanic or plutonic mountain peaks that covers ~40,877 km<sup>2</sup>, stretching from Annobón Island in the Gulf of Guinea to the Mandara Mountains in the Far North Region in Cameroon. It includes a broader continental part and a smaller oceanic portion in the form of four major islands (Ayonghe et al. 1999; Frodin 2001; Sainge et al. 2017). The CVL is known for its rich and diverse flora with high levels of endemism combined with many threats (Sosef et al. 2017; Droissart et al. 2018), which led to its classification of a biodiversity hotspot (Cheek et al. 2000, 2004; Myers et al. 2000; Barthlott et al. 2005). Plant diversity comprises about 4000 species (Droissart et al. 2018). The orchid family represents one of the most diverse plant groups of the CVL (Cable & Cheek 1998;

Cheek et al. 2000, 2004; Onana & Cheek 2011; Harvey et al. 2004) including 33 strictly endemic taxa, distributed in the submontane (17 taxa), montane (12 taxa), and both submontane and montane strata (4 taxa) (Droissart 2009). The new orchid described here further stresses the need for additional fieldwork in these mountains, specifically for sites that have so far been little or not explored. This seems particularly the case for the forests of the Cross River National Park, the Boshi Extension Forest Reserve, and the Gashaka-Gumti National Park in Nigeria, and for the Rumpi Hills area in Cameroon. Additional material from African herbaria (e.g. Forest Herbarium Ibadan (FHI)) should be carefully examined to identify putative unidentified or misidentified orchid material.

With over 200 plant species considered as threatened (Cheek et al. 2004; Onana & Cheek 2011) and with more than 80 endemic species (Cheek et al. 2004; Franke 2004; Sainge et al. 2005, 2010; Sainge 2012, 2016), the CVL is endangered by increasing threats due to human activities. Land use change and deforestation have resulted in degraded forest areas, fragmented populations, and the CVL is therefore impoverishment on all three levels of biodiversity (i.e. genetic, species, and ecosystem diversity). It has been estimated that over 96% of the original forest cover of the Bamenda highlands above 1,500 m elevation has been lost (Cheek et al. 2000). Hence, a regional synthesis, including recent species diversity checklists coupled with Important Plant Area assessments (Darbyshire et al. 2017), is urgently needed to better manage and conserve the remaining biodiversity of the CVL.

### Threats to the host trees of *Cyrtorchis okuensis*

Our results identified *Gnidia glauca* and *Prunus africana* as main host trees of *C. okuensis*. This disparity in the number of host trees identified in this region could be closely related to the fact that our study was conducted at a grassland edge of a natural and monospecific *G. glauca* forest (Momo et al. 2017), but also to the strong threat to *P. africana* over the past several years by unsustainable harvesting in many countries where it occurs. Indeed, this species is used by pharmaceutical companies to manufacture a drug used in treating benign prostatic hyperplasia and prostate gland hypertrophy (Stewart 2003). Cunningham (2005) reported that over the last 40 years, *P. africana* bark harvest for its medicinal properties has shifted from subsistence use to large-scale commercial use for international trade. Cameroon comprises large parts of the current species distribution, and in the Kilum-Ijim area, *P. africana* is traditionally used to treat malaria and stomach ache (Nsom & Dick 1992). Currently listed as Vulnerable on the IUCN Red List (IUCN 2020), the species is also included in Appendix II of the Convention on International Trade in Endangered Species of Flora and Fauna (CITES) (Betti 2011). Therefore, its overexploitation could have led to a drastic reduction of its populations.

Although *G. glauca* seems less threatened, it is not exempt of risks from future overexploitation as it is also widely used in traditional pharmacopoeia, in nanomedicine, in domestic service in the form of rope or yarn made from bark fibres,

and as an insecticide or piscicide (Ghosh et al. 2012; Avana-Tientcheu et al. 2018). A recent study (Avana-Tientcheu et al. 2018) conducted in the Oku area revealed that *G. glauca* was more vulnerable in forests than in savannah, because of the proximity of forest stands to neighbouring villages makes them more easily accessible than savannah stands. The IUCN conservation status of *G. glauca* has not yet been assessed and would help to propose effective conservation measures. As with *P. africana* (Avana 2006), domestication should be also considered for *G. glauca* to be able to meet potential high future demands (Avana-Tientcheu et al. 2018).

#### SUPPLEMENTARY FILE

**Supplementary file 1** – Detailed information about the inflorescences and flowers per surveyed individual and about the camera survey sessions. The IDs followed by a \* are those surveyed with cameras.

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