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Afrothismia kupensis sp. nov. (*Thismiaceae*), Critically Endangered, with observations on its pollination and notes on the endemics of Mt Kupe, Cameroon

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Key words

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Abstract The achlorophyllous mycotroph *Afrothismia kupensis* (*Thismiaceae*), formerly misidentified either as *A. pachyantha* or as *A. gesnerioides*, is described from Mt Kupe in South West Region, Cameroon and assessed as Critically Endangered using the IUCN (2012) categories and criteria. It is threatened by forest clearance due to small-holder agriculture. Mt Kupe, with four of the 16 described species of *Afrothismia*, three of which are site endemics, is now the most species-diverse location known globally for the genus. Observations of floral visitors over seven days resulted in the identification of the likely pollinator as being females of an unknown species of *Phoridae* (scuttle fly) probably of the genus *Megaselia*. This is the first record of pollination known in the *Thismiaceae*, and may represent a mutualism between plant and animal partners.

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INTRODUCTION

Achlorophyllous mycotrophic plants, often known as saprophytes, are remarkable for lacking all chlorophyll and being completely dependent on fungi for their survival. In continental Africa, individual species or entire genera which are achlorophyllous mycotrophs occur in the families *Orchidaceae*, *Gentianaceae* and *Burmanniaceae*, while all members of *Triuridaceae* and *Thismiaceae* are achlorophyllous mycotrophs (Cheek & Ndam 1996, Cheek & Williams 2000, Cheek 2006, Sainge et al. 2010).

Although some earlier authors (Jonker 1938, Maas-van de Kamer 1998) placed *Afrothismia* Schltr. and its allies as a tribe *Thismieae* within the family *Burmanniaceae* s.lat., recent molecular phylogenetic data (Merckx et al. 2006) strongly indicate that *Thismiaceae* are best placed as a separate family. These relatively well-sampled analyses place *Thismiaceae* as sister to *Taccaceae*, in a different subclade of *Dioscoreales* from *Burmanniaceae* s.str. Furthermore, the two families are separated from each other by numerous morphological characters:

Burmanniaceae — Perianth long-cylindrical, only the upper portion detaching in fruit; stigma and stamens positioned at throat of tube; stamens 3, sessile, anther cells lateral on broad central connective; stigmas often cupular; annulus absent; ovary septal nectaries present; ovary 3-locular, or 1-locular.

Thismiaceae — Perianth cup-like or campanulate, rarely shortly cylindrical, detaching in fruit at base of perianth tube; stigmas inserted in basal 1/4 to 1/2 of perianth tube; stamens inserted at mouth of tube, or deep inside tube; stamens 6 (3 in *Oxygyne* Schltr.), on long filaments; anther cells collateral, not separated by connective; stigmas lobed; annulus present at mouth of tube or deep inside (except *Tiputinia* P.E.Berry & C.L.Woodw. and *Haplothismia* Airy Shaw); ovary septal nectaries absent; ovary 1-locular.

Thismiaceae, with five genera and c. 55 species are pantropical, with a few species in temperate zones. A key to the genera of *Thismiaceae* is given in Cheek et al. (2018b).

Two genera of *Thismiaceae* occur in continental Africa (not a single species is known from Madagascar and the Mascarenes). *Afrothismia* differs from other *Thismiaceae* genera by the annulus inserted deep inside the perianth tube; stamens inserted below the annulus; anthers adnate to stigma; rhizomes with clusters of spherical tubers, being confined to tropical equatorial central and eastern Africa (Cheek et al. 2018b). Fifteen species have been described, but there may be as many as four additional undescribed species (Sainge et al. 2017).

Oxygyne Schltr. the second genus of African *Thismiaceae*, is unique in the family in having three, not six stamens and is disjunct, restricted to W-C Africa and southern Japan, with six species (Cheek et al. 2018b). *Afrothismia* is additionally distinguished from *Oxygyne* by having zygomorphic flowers.

The genus *Afrothismia* was erected by Schlechter (1906). He transferred to it *Thismia winkleri* Engl. and described a new species, *A. pachyantha* Schltr., that he had collected in the then German colony Kamerun, now Cameroon. The range of the genus was extended to E Africa by Cowley (1988), with *A. insignis* Cowley from Tanzania. By 2003, the original two species known for the genus had doubled in number to four, with a third species from Cameroon, *A. gesnerioides* H.Maas (Maas-van de Kamer 2003).

In the ensuing 15 years the publication of new species increased and with this paper will reach 16, quadrupling the number of *Afrothismia* species known to science. New species have been discovered in Kenya (Cheek 2004a), Malawi (Cheek 2009) and Tanzania (Cheek & Jannerup 2006), but the largest number of discoveries by far has been made in Cameroon and adjoining Nigeria, within the Cross-Sanaga interval which holds the highest flowering plant species diversity per degree square in tropical Africa (Cheek et al. 2001). Here seven new species of *Afrothismia* have been published (Franke 2004, Franke et al. 2004, Sainge & Franke 2005, Sainge et al. 2005, Cheek 2007, Sainge et al. 2013).

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Lacking any green tissue, *Afrothismia* depend on fungi for sustenance. The genus *Rhizophagus* (*Glomales*, *Glomeromycota*) has been implicated as the fungal partner of the genus (Franke et al. 2006). Delayed co-speciation between *Afrothismia* and the fungal partner has been demonstrated (Merckx 2008, Merckx & Bidartondo 2008).

Pollination has not previously been reported in *Thismiaceae*, nor in *Burmanniaceae*. For this reason the authors sought to seek for potential pollinators.

The new species of *Afrothismia* reported in this paper was discovered as a result of a survey of the Kupe-Bakossi area in SW Region, Cameroon. Study was conducted as part of the long-term survey of plants in Cameroon led by botanists from Royal Botanic Gardens, Kew and the National Herbarium of Cameroon supported by the Earthwatch programme. This study has focussed on the Cross-Sanaga interval (Cheek et al. 2001) which contains the area with the highest species diversity per degree square in tropical Africa (Barthlott et al. 1996), including several endemic genera. Until recently the family *Medusandraceae* s.str. (*Medusandra* Brenan) was considered endemic to the Cross-Sanaga interval (Heywood 2007) and is present in Kupe-Bakossi together with *Afrothismia kupensis* but recent phylogenetic data shows that *Medusandra* together with *Soyauxia* Oliv., is confamilial with the rare American family *Peridiscaceae* (Soltis et al. 2007, Breteler et al. 2015).

The herbarium specimens collected from Mt Kupe formed the primary data for the 'Conservation Checklist of the Plants of Mt Kupe, the Bakossi Mts and Mwanenguba Forest' (Cheek et al. 2004), in the series that began with Mt Cameroon (Cable & Cheek 1998) and continued with Mt Oku and the Ijim Ridge (Cheek et al. 2000), Bali-Ngemba (Harvey et al. 2004), Dom (Cheek et al. 2010) and Lebialem (Harvey et al. 2010).

The number of flowering plant species known to science is disputed (Nic Lughadha et al. 2017), but a reasonable estimate is 369 000 (Nic Lughadha et al. 2016), while the number of species described as new to science each year regularly exceeds 2000 (Kew 2017).

About 5 % of plant species have been assessed for their conservation status using the IUCN (2012) standard. This number rises to 21–26 % when additional evidence-based assessments are considered, and 30–44 % of these rate the species assessed as threatened (Bachman et al. 2018). Newly discovered species such as that reported in this paper, are likely to be threatened, since widespread species tend to have been already discovered. There are notable exceptions to this rule (e.g., Cheek & Etuge (2009) described a species widespread in the Cameroon Highlands). Generally, it is the more localised, rarer species that remain undiscovered. This makes it all the more urgent to discover, document and protect such species before they become extinct as is *Oxygyne triandra* Schltr. (Cheek & Cable 2000, Cheek & Onana 2011, Cheek et al. 2018b). Most of the Cameroonian species are at risk of extinction due to habitat clearance, mainly for small-holder and plantation agriculture following logging (Onana & Cheek 2011). Efforts are now being made to delimit the highest priority areas in Cameroon for plant conservation as Tropical Important Plant Areas (TIPAs) using the revised IPA criteria set out in Darbyshire et al. (2017). This is intended to help avoid the global extinction of narrowly endemic species such as *Afrothismia kupensis*.

MATERIALS AND METHODS

The methodology for the surveys in which this species was discovered is recorded in Cheek & Cable (1997). Names of species and authors follow IPNI (continuously updated). Herbarium material was examined with a Leica Wild M8 dissecting binocular microscope fitted with an eyepiece graticule measuring in units of 0.025 mm at maximum magnification. The drawing was made with the same equipment with a Leica 308700 camera lucida attachment. Specimens were inspected from the following herbaria: B, BM, K, SRGH, YA, WAG. The format of the description follows those in other papers describing new species in *Afrothismia*, e.g., Cheek et al. (2004), Cheek & Jannerup (2006). All specimens cited have been seen unless indicated 'n.v.'. The conservation assessment follows the IUCN (2012) categories and criteria. Herbarium codes follow Index Herbariorum (Thiers, continuously updated).

To observe visitors to the flowers of *Afrothismia kupensis*, one of us (SAW) spent 23.5 hrs over seven days during the period 7 to 16th November (at the end of the wet season) of 1995, seated in the forest near Kupe village (*Williams s.n.*, K, YA; Cheek & Williams 2000) near a flowering plant of the species *in situ* within a plot of c. 25 by 25 m within which six flowering plants of the species occurred. All visitors that landed on the flowers, and also whether or not they entered the flower, were noted. If they did enter the flower, the length of time they spent within was recorded. Finally, attempts were made to catch the insect after flower visitation. Only those visitors that both entered and departed the flowers, and so might be effecting pollination, were targeted for collection.

The time and duration that such visitors spent within the flowers was recorded using a Casio F-91W wristwatch. After departure from the flower, floral visitors (all appeared to be the same species of dipteran), were caught where possible inside glassine paper packets and killed by compression to the head and thorax. Subsequently, at the Palynology Unit, R.B.G., Kew, the insects were washed with solvent to detect any pollen grains that might be adhering to their surface. The washings were collected and evaporated to concentrate any pollen grains that might be present. The concentrated washings were then examined using light microscopy and a Hitatchi 54700 scanning electron microscope to detect any pollen grains that might be present and to identify them using the palynological reference slide collection and literature of the Palynology Unit.

The insects were then sent to the Insect Identification Service of the Natural History Museum, South Kensington, London for identification. The insect samples were retained at the Natural History Museum under the code NHM IIS Enq. 2008-446.

RESULTS AND DISCUSSION

The species described in this paper as *Afrothismia kupensis* Cheek & S.A.Williams was first encountered in Nov. 1995 near Kupe village, Mt Kupe (*Williams s.n.*, K). It was initially suspected to be a new species to science until it was viewed at the fruiting stage. It was then mistakenly identified by the first author as *Afrothismia pachyantha* since it appeared identical to the illustration in the protologue of that species (Cheek et al. 2004: 188, 427, Cheek & Onana 2011: 358). Yet, while the globose structures of *Williams s.n.* are externally identical to those of *A. pachyantha*, they are not homologous. The internal structures of *A. pachyantha* (Schlechter 1906) clearly show the presence of the stamens and an annulus indicating that they are flowers, while these structures are lacking in the superficially identical fruit of *Williams s.n.*

Independently Merckx (2008) identified another collection, conspecific with *Williams s.n.* at Mt Kupe as *A. gesnerioides*, to which it is superficially similar in flower. These two species are alone in the genus in having horizontal, slightly sigmoidal perianth tubes and perianth lobes which are not filiform, but triangular to ovate. All other known species of the genus have filiform tepal lobes apart from the presumably extinct *Afrothismia pachyantha* which

Table 1 The characters separating *Afrothismia kupensis* from *Afrothismia gesnerioides*. Data for the second species from Maas-van de Kamer (2003).

	Afrothismia gesnerioides	*Afrothismia kupensis*
Tepals	Dimorphic; reflexed over perianth tube	Monomorphic; patent or ± forward-directed
Annulus	Symmetrical, reduced to a flange c. 0.5 mm wide	Strongly asymmetric, on dorsal side projecting into the lower perianth tube for 3 mm; on ventral side only 0.5 mm wide
Staminal flange	Absent	6-lobed, lobes alternating with staminal filaments
Diam of perianth tube at central constriction	5–6 mm	7.5 mm
Indumentum of inner surface of lower perianth tube below staminal insertion	Glabrous	Sparsely hairy
Stigma lobe number	6	0
Ovary placentation	Free basal (placenta attached only at centre of base of ovary)	Axile (placenta attached to both base and apex of ovary at centre)
Fruit dehiscence	Distal end of ovary (base of perianth tube) with 3 regular apertures developing in fruit	Distal end of ovary not developing apertures, but the whole lifting off to expose the seed mass
Geography	South Region, Cameroon (Nyanong)	South West Region, Cameroon (Mt Kupe)

was unique in its globose ovary-perianth tube. The identification of the Kupe plants as *A. gesnerioides* has been maintained until the present (Imhof et al. 2013, Onana 2013, Sainge et al. 2017). The two species are geographically separated by over 200 km and are easily separated morphologically using the characters indicated in Table 1 and in the diagnosis. *Afrothismia gesnerioides* is remarkable for being the only published species of the genus from Cameroon which is not known from the Cross- Sanaga interval (Cheek et al. 2001).

Afrothismia kupensis Cheek & S.A. Williams, *sp. nov.* — Fig. 1

Differing from *Afrothismia gesnerioides* H.Maas in the monomorphic, patent or forward-directed tepals (not dimorphic, reflexed), the annulus strongly asymmetric, 3 mm long on the dorsal side but reducing to a flange projecting 0.5 mm on the ventral side (not with an even flange projecting 0.5 mm the entire circumference), inner surface of lower perianth tube sparsely hairy (not glabrous). Differing from *Afrothismia pachyantha* Schltr. in the tubular, slightly sigmoid perianth tube (not globose), the straight, flat tepals held away from the mouth of the perianth tube (not incurved, occluding the mouth of the tube), stigma entire (not 6-lobed), flange present just above the filament insertion (vs staminal flange absent), upper perianth tube well-developed above an annulus, as long as lower perianth tube (vs upper perianth tube extremely contracted and inconspicuous). — Type: *Etuge 1831* (holo K, iso SCA, YA), Cameroon, South West Region, Kupe-Muanenguba, Tombel, Kupe village, 1200 m, fl. & fr., 28 Mar. 1996.

Afrothismia pachyantha sensu Cheek et al. (2004: 188, 429, f. 15B; Cheek & Onana 2011: 358), non Schltr. (1906).

Afrothismia gesnerioides sensu Merckx 2008: 123, 126, f. 8.1A, 137 f. 8.8; Onana 2013: 218 p.p.; Imhof et al. (2013); Sainge et al. (2017), non Maas-van de Kamer (2003).

Etymology. Named for both Mt Kupe and the people of Kupe village that hosted us during our several weeks of botanical surveys in their forest.

Achlorophyllous mycotrophic herb with only the flower or fruit emerging above the leaf-litter. *Stem* (rhizome), succulent, concealed in substrate, vertical, unbranched, c. 45 mm long, 2 mm diam, scale-leaves ovate-triangular, c. 0.3 by 0.15 mm, axillary buds globose, internodes (0.3–)1.5 mm long. *Bulbil cluster* c. 15 by 17.5 mm, bulbils globose, each 1.5–2.5 mm diam, with an apical rootlet c. 2 mm long. *Flowers* developing in succession from the stems, 1-flower per stem open at a time, bract ovate, 7–8 by 3–4 mm, apex obtuse. *Perianth tube* directed horizontally, 16–17 mm long, 9 mm wide at midpoint, slightly S-shaped, constricted slightly between lower (proximal) and upper (distal) parts of the tube; outer surface white with 6 longitudinal purple lines on the lower tube, the upper tube purple in the proximal half, the distal half dull white, glabrous and lacking projections. *Lower perianth tube* horizontal or elevated < 45° above the horizontal, 9–10 mm along main axis from top of ovary/base of perianth tube to annulus, widening from 6.5 mm diam at junction with ovary, to 8.5–9 mm wide, but constricted to 7.5 mm wide at staminal filament insertion whorl, 6.5 mm from the ovary, inner surface with an internal flange immediately above the insertion of the staminal filaments, flange continuous, shallowly lobed, lobes 6, alternating with stamens, 0.5–1 by 3 mm (Fig. 1d), inner surface below the staminal flange minutely and sparsely hairy. *Annulus* inserted c. 4 mm above the free staminal filament and flange insertion, strongly asymmetric: on the dorsal side projecting 3 mm from the upper perianth into the lower perianth tube, on the ventral side narrowing gradually and evenly to < 0.5 mm wide. *Upper perianth tube* with axis at first ascending from that of the lower perianth tube, then curving to the horizontal at the mouth, c. 7.5 mm long from insertion of the annulus to insertion of the perianth lobes (dorsal surface) and 6 mm long (ventral surface), 7 mm wide above the annulus, narrowing to c. 6 mm wide at the mouth, the tube projecting beyond the insertion of the perianth lobes for c. 1 mm, inner surface glabrous. *Tepals* six, monomorphic (equal), patent or ± forward-directed, dull white, ovate-triangular, 3.5–4 by 2.5–3.5 mm, apex obtuse-apiculate, glabrous. *Stamens* 6, staminal filaments joined to perianth tube for c. 6 mm, appearing as purple lines on exterior tube surface, distal part free, terete c. 1.5 mm long, arching inward to stigma, anthers 0.9–1 mm long, 0.5 mm wide, the 2 thecae c. 0.01–0.02 mm wide, separated by connective (Fig. 1f); distal connective appendage triangular, 0.6–0.75 by 0.3 mm, joined to stigmatic surface (Fig. 1e). *Ovary* turbinate, 4.5 mm long, 6–6.5 mm wide at junction with perianth; placentation axile (placenta attached at base and apex), the ovules inserted on a recurved placental flange inserted in the centre of the ovary. *Style* cylindrical, 2 mm long, 1 mm diam, stigma obconical, c. 2.5 by 3.5 mm, lobes not detected, entire surface densely covered in 0.2 mm long patent hairs. *Fruit* turbinate, 8.5 mm long including lacerate perianth tube remains, 6.5 mm diam (Fig. 1g–h). Circumscissile dehiscence, the lid of fruit (perianth floor) detaching from the rest of the fruit, exposing the seed-covered placenta. *Seeds* numerous, narrowly obovoid, c. 1.5 by 0.5 mm, lacking appendages (Fig. 1i).

Distribution & Ecology — Lower submontane forest on SW and W slopes of Mt Kupe; 800–1200 m above sea-level. The following additional associated achlorophyllous species occurred intermingled with *Afrothismia kupensis* at the type locality near Kupe village site on the SW slopes of Mt Kupe: *Sebaea oligantha* Schinz (*Gentianaceae*), *Burmannia hexaptera* Schltr., *Burmannia congesta* (C.H.Wright) Jonker (also known as

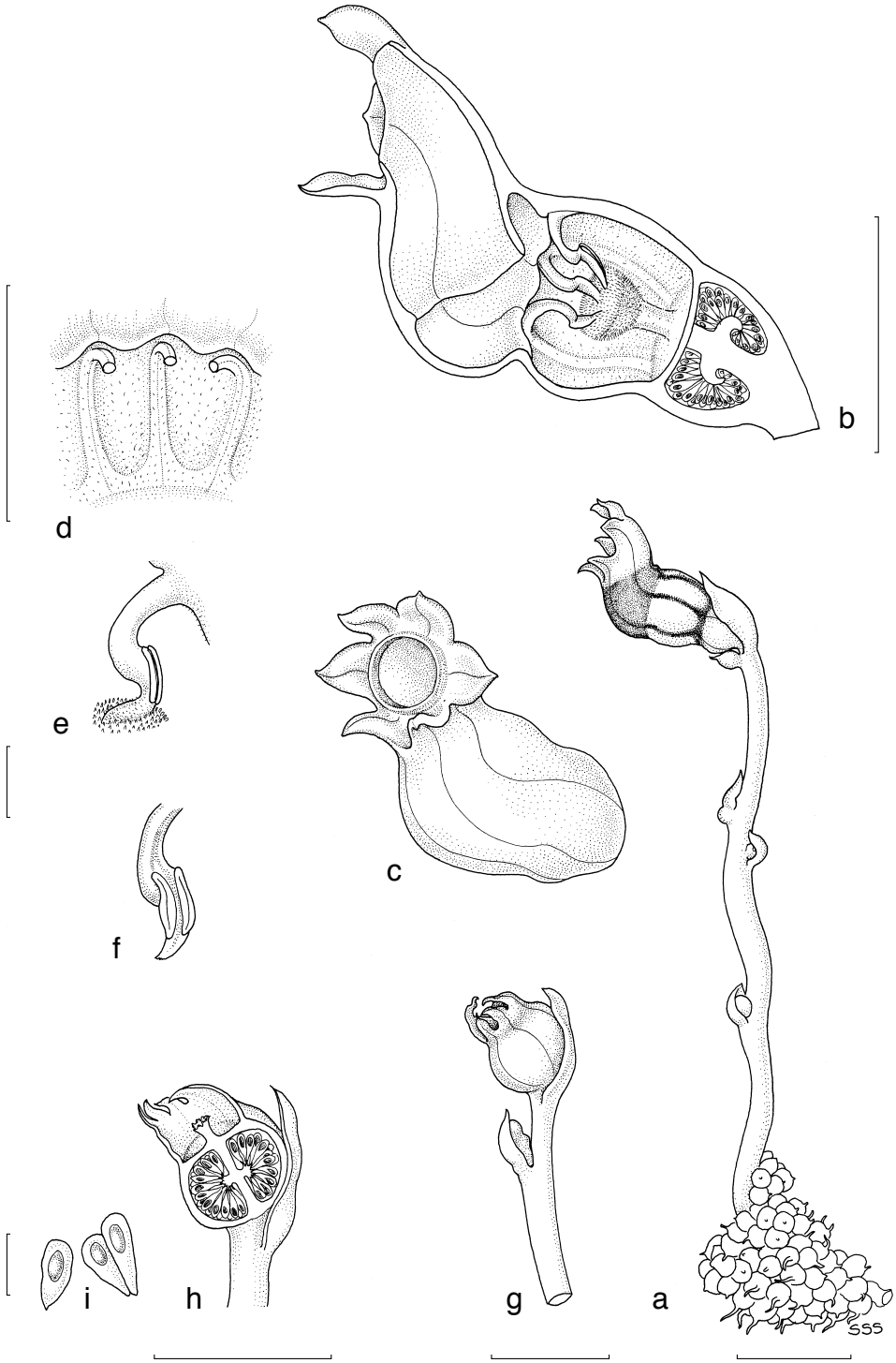

Fig. 1 *Afrothismia kupensis* Cheek. a. Habit, whole plant; b. subsagittal section of flower (style-stigma not sectioned); c. perianth tube and lobes, showing mouth of tube; d. detail of inner surface of lower perianth tube, showing three free staminal filaments (anthers removed) with the adjacent staminal flange; e. stamen showing attachment of connective to the stigmatic surface; f. stamen detached from stigma, showing anther thecae; g. fruiting stem; h. fruit in sagittal section, note absence of stamens and staminal flange; i. seeds (all from *Etuge 1831*, K). — Scale-bars: a–d, g–h = 10 mm; e–f, i = 1 mm. — Drawn by Susanna Stuart-Smith.

Campylosiphon congestus (C.H. Wright) Maas), *Gymnosiphon longistylus* (Benth.) Hutch. (all *Burmanniaceae*) and *Sciaphila ledermannii* Engl. (*Triuridaceae*). These species occurred together in an area a little larger than 25 by 25 m. The leaf-litter covered surface slopes steeply with an inclination of about 40 degrees from the horizontal.

Mt Kupe (also known as Mount Kupe or Mont Koupé) is part of the Cameroon line of highlands and rises to 2095 m above sea-level. Preliminary notes on the vegetation and species of the mountain can be found in Stoffelen et al. (1997) but a more comprehensive account is Cheek et al. (2004). 33 plant taxa were listed as being strictly endemic to the cloud forest of Mt Kupe (Cheek et al. 2004: 39), among which were listed *Afrothismia kupensis* (erroneously as *A. pachyantha*) and *Kupea martinetugei* Cheek & S.A.Williams (Cheek et al. 2003) another achlorophyllous mycotroph, which was shown to be a new tribe, *Kupeaeae*, sister to the rest of the *Triuridaceae* (Cheek 2004b). However, at that time, 23 of the 33 strictly endemic

taxa were still undescribed. Since that time a further ten new species have been described from those 23, leaving 13 still to be formally published. Most recently published are *Microcos magnifica* Cheek (Cheek 2017) and *Kupeantha kupensis* Cheek & Sonké (Cheek et al. 2018a).

Among the new species were two new genera to science: *Kupeantha* Cheek and *Kupea* Cheek & S.A.Williams (Cheek et al. 2003, 2018a).

Some of the original 33 strictly endemic taxa have since been found also at other locations such as at Mt Etinde, 70–80 km to the SW along the Cameroon line, e.g., *Dracaena kupensis* Mwachala et al. (Mwachala et al. 2007). Likewise it remains possible that some apparent site endemics at these other locations, such as *Impatiens etindensis* (Cheek & Fischer 1999) at Mt Etinde, might yet be found at Mt Kupe. Clearance of forest upslope for subsistence agriculture from the many small villages which surround the Mt Kupe is a concern, and has reached up to 1 500 m altitude on the eastern flank of the mountain.

Additional material. Cameroon, South West Region, Kupe-Muanenguba, Tombel, Kupe village, *Williams s.n.* (K, SCA, YA), 1200 m, fl. & fr., 28 Nov. 1995.

Conservation — The conservation status of *Afrothismia kupensis* was assessed under the erroneously applied name of *Afrothismia pachyantha* (Cheek 2004c, Cheek et al. 2004: 188). This assessment was maintained, under the same misapplied name, in Onana & Cheek (2011: 358). In those assessments, *Afrothismia pachyantha* was regarded as extinct at the type location of Moliwe near Mt Cameroon and so the assessment was based solely on what were then thought to be the only surviving plants of the species at Mt Kupe, shown in this paper to be a separate species, *A. kupensis. Afrothismia kupensis* was known from one site near Kupe village, measuring less than 50 by 50 m (Cheek et al. 2004). However, a second site at Mt Kupe was recently reported (as *A. gesnerioides*) by Sainge et al. (2017), from near the village of Mbulle. However, both sites face the same threat: clearance of their habitat for small-holder farming.

The area of occupation is estimated as 8 km^2 using the IUCN preferred 4 km^2 cell size and extent of occurrence as < 100 km^2. Therefore the two sites comprise a single threat-based location in the sense of IUCN (2012) and the assessment of Critically Endangered – CR B1+B2ab(iii); C2a(i); D1 is maintained here. However the assessment of A2c given in Onana & Cheek (2011) no longer applies since the Moliwe population is now known to be a separate species (*Afrothismia pachyantha* now presumed extinct), so the decline in population of 80 % signified by A2c can no longer be evidenced for *A. kupensis*.

At the Kupe village site the authors of this paper had engaged with the local community to protect the forest in which *A. kupensis* is found through a rental and monitoring programme. However, the insecurity since 2016 due to the struggle for independence of the Anglophone sector of Cameroon (https://www.today.ng/topic/ambazonia) means that such conservation activity is now difficult to continue.

Note — With the addition of *Afrothismisa kupensis*, the Cross-Sanaga interval (SE Nigeria and western Cameroon) now holds ten of the 16 described species of the genus, all but two of which are confined to it. The species in this area show the greatest range of morphological diversity in the genus. These factors suggest that the genus may have originated in the Cross-Sanaga area.

RESULTS AND DISCUSSION – FLORAL VISITATION STUDIES

The 23.5 hour floral visitor observation period was spread over seven days. Between 1.5 and 7 hrs each day were spent observing, between the hours of 8am and 5pm (see Table 2).

Ten individual insects were observed to land on the flowers, all being judged by size, appearance and behaviour to be the same species, resembling a mosquito. Of these, seven entered the flower, each spending 3–9 seconds within the flower (median 5 seconds, average 6 seconds) before departing (Table 2). Six of the 10 visitors arrived between 9.30 am and 10.30 am, while eight of the ten visitors arrived between 9.30 am and 11 am. On two of the seven days no visitors were recorded, while at the other extreme three visitors per day were observed on two other days. The median was one insect visitor per day (Table 2). Two insects were retained for study.

From the insect body washings, Dr Hannah Banks of the Palynology Unit, Royal Botanic Gardens, Kew, reported that *Thismiaceae* type pollen predominated among the pollen grains captured. The results from the Natural History Museum Insect Identification Service were as follows: "Both samples are female scuttle Flies in the family *Phoridae* and are almost certainly the same species, possibly belonging to *Megaselia* which is the largest genus in the family, accounting for almost half the species in this large family. Over 200 genera and 2 500 species of *Phoridae* have been described and the actual total is likely to be significantly higher than this, with several species still undescribed. Their biology is highly variable, the larvae of many species being saprophagous but others are parasitic or fungivorous and others are associated with the nests of social insects such as bees, ants or termites. Adults of many species visit flowers and are likely to be significant pollinators of some species."

It seems probable that the scuttle Flies (*Phoridae*) sampled do act as pollinators to *Afrothismia kupensis* since they were found

Table 2 Observations of visitors to the flowers of *Afrothismia kupensis* in November 1995.

Date of observation	Time spent observing	Time insect observed	Time spent on flower (s = seconds)	Notes
06/11/95	Plant discovered			
07/11/95	3.30pm – 5.00pm	4.00pm insect entered	~9s	
09/11/95	12.00pm – 4.00pm	No insects observed		
11/11/95	11.30am – 5.00pm	No insects observed		
12/11/95	9.00am – 1.30pm	10.50am insect entered	~5s	Failed to catch
13/11/95	8.00am – 3.00pm	9.30am insect entered	~6s	Insect caught
15/11/95	9.00am – 3.00pm	9.30am insect entered	~7s	Insect caught
	9.54am insect on flower		~4s	Insect landed did not enter flower
	11.25am insect entered		~5s	Insect caught
16/11/95	9.00am – 1.00pm	10.25am insect entered	~3s	Failed to catch
	10.30am insect landed			2 x insect landed but did not enter flower
	10.41am insect entered		~8s	Insect caught

to be carrying pollen of *Thismiaceae* following visitation to the flowers. That these scuttle Flies were the only floral visitors during the study period and that both of those preserved were almost certainly the same species suggests that this one species of insect might be the only pollinator of *Afrothismia kupensis* present at this site and at this time. However, this study of floral visitors was far from comprehensive, being carried out only during the day over one week. Nonetheless, this appears to be the first reported observation of pollination in the *Thismiaceae*. Pollination by *Phoridae* has been shown in other plant families, such as in *Herrania* Goudot (*Byttneriaceae*) by Young (1984) and in *Aristolochia inflata* Kunth (*Aristolochiaceae*) by Sakai (2002). In both cases female flies are thought to be attracted to the flowers by scent, effect pollination and to lay eggs inside the flower. In the last case, characterised as a mutualism, the eggs hatch and feed on the inner tissue of the fallen flower for seven days before pupating, adults emerging 15 days after the eggs are laid. However in another species of the same genus, 96 % of the flies attracted were males (Hall & Brown 1993).

Further studies are recommended to discover if, as in *Aristolochia inflata*, the fallen flowers of *Afrothismia kupensis* provide the larval food for the *Phorideae* pollinator species, evidencing a mutualism. Surveying other species of *Afrothismia* in the field is suggested to determine if *Phorideae* are also pollinators or whether other groups of organisms effect pollination.

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REFERENCES

Bachman SP, Nic Lughadha EM, Rivers MC. 2018. Quantifying progress towards a conservation assessment for all plants. Conservation Biology. doi: https://doi.org/10.1111/cobi.13071.

Barthlott W, Lauer W, Placke A. 1996. Global distribution of species diversity in vascular plants: towards a world map of phytodiversity. Erdkunde 50: 317–328 (with supplement and figure).

Breteler FJ, Bakker, FT, Jongkind CCH. 2015. A synopsis of Soyauxia (Peridiscaceae, formerly Medusandraceae) with a new species from Liberia. Plant Ecology and Evolution 148(3): 409–419.

Cable S, Cheek M. 1998. The plants of Mount Cameroon. A conservation checklist. Royal Botanic Gardens, Kew, London.

Cheek M. 2004a. A new species of Afrothismia (Burmanniaceae) from Kenya. Kew Bulletin 58: 951–955. doi: https://doi.org/10.2307/4111208.

Cheek M. 2004b. Kupeaeae, a new tribe of Triuridaceae from Africa. Kew Bulletin 58: 939–949. doi: https://doi.org/10.2307/4111207.

Cheek M. 2004c. 'Afrothismia pachyantha'. The IUCN Red List of Threatened Species. IUCN. 2004:e.T39539A10246294. doi: https://doi:10.2305/IUCN.UK.2004.RLTS.T39539A10246294.en. Retrieved 10 Jan. 2018.

Cheek M. 2006. African Saprophytes: new discoveries. In: Ghazanfar SA, Beentje HJ (eds), Taxonomy and ecology of African plants, their conservation and sustainable use. Proceedings of the 17th AETFAT Congress, Addis Ababa, Ethiopia. Royal Botanic Gardens Kew, London.

Cheek M. 2007. Afrothismia amietii (Burmanniaceae), a new species from Cameroon. Kew Bulletin 61: 605–607.

Cheek M. 2009. Burmanniaceae in Flora Zambesiaca 12: 145–148.

Cheek M. 2017. Microcos magnifica (Sparrmanniaceae) a new species of cloudforest tree from Cameroon. PeerJ 5: e4137. doi: https://doi.org/10.7717/peerj.4137.

Cheek M, Alvarez-Aguirre MG, Grall A, et al. 2018a. Kupeantha (Coffeeae, Rubiaceae), a new genus from Cameroon and Equatorial Guinea. PLoS ONE 13(6): e0199324. doi: https://doi.org/10.1371/journal.pone.0199324.

Cheek M, Cable S. 1997. Plant inventory for conservation management: the Kew-Earthwatch programme in Western Cameroon, 1993–96. In: Doolan S (ed), African rainforests and the conservation of biodiversity: 29–38. Earthwatch Europe, Oxford.

Cheek M, Cable S. 2000. Oxygyne triandra. The IUCN Red List of Threatened Species 2000: e.T39556A10248327. doi: https://doi.org/10.2305/IUCN.UK.2000.RLTS.T39556A10248327.en. Downloaded on 30 July 2017.

Cheek M, Etuge M. 2009. Deinbollia oreophila (Sapindaceae), a new submontane species of Deinbollia (Sapindaceae) from Western Cameroon and adjoining Nigeria. Kew Bulletin 64: 503–508. doi: https://doi.org/10.1007/s12225-009-9132-4.

Cheek M, Fischer E. 1999. A tuberous and epiphytic new species of Impatiens (Balsaminaceae) from Southwest Cameroon. Kew Bulletin 54: 471–475. doi: https://doi.org/10.2307/4115828.

Cheek M, Harvey Y, Onana JM. 2010. The plants of Dom, Bamenda Highlands, Cameroon. A conservation checklist. Royal Botanic Gardens, Kew, London.

Cheek M, Jannerup P. 2006. A new species of Afrothismia (Burmanniaceae) from Tanzania. Kew Bulletin 60: 593–596.

Cheek M, Mackinder B, Gosline G, et al. 2001. The phytogeography and flora of western Cameroon and the Cross River-Sanaga River interval. Systematics and Geography of Plants 71: 1097–1100. doi: https://doi.org/10.2307/3668742.

Cheek M, Ndam N. 1996. Saprophytic flowering plants of Mount Cameroon. In: Van der Burgt XM, Van der Maesen J, Van Medenbach de Rooy JM (eds), The biodiversity of African plants. Proceedings XIV AETFAT Congress: 612–617. Kluwer. doi: https://doi.org/10.1007/978-94-009-0285-5_74.

Cheek M, Onana J-M. 2011. Extinct species in Cameroon: Oxygyne triandra. In: Red data species in Cameroon, a guide for secondary school teachers. Royal Botanic Gardens, Kew, London.

Cheek M, Onana J-M, Pollard BJ. 2000. The plants of Mount Oku and the Ijim Ridge, Cameroon. A conservation checklist. Royal Botanic Gardens, Kew, London.

Cheek M, Pollard BJ, Darbyshire I, et al. 2004. The plants of Kupe, Mwanenguba and the Bakossi Mts, Cameroon. A conservation checklist. Royal Botanic Gardens, Kew, London

Cheek M, Tsukaya H, Rudall PJ, et al. 2018b. Taxonomic monograph of Oxygyne (Thismiaceae), rare achlorophyllous mycoheterotrophs with strongly disjunct distribution. PeerJ 6:e4828. doi: https://doi.org/10.7717/peerj.4828.

Cheek M, Williams S. 2000. A review of African saprophytic flowering plants. In: Timberlake K (ed), African plants. Biodiversity, taxonomy & uses. Proceedings of the 15th AETFAT Congress at Harare, Zimbabwe: 39–49. Royal Botanic Gardens, Kew, London.

Cheek M, Williams S, Etuge M. 2003. Kupea martinetugei, a new genus and species of Triuridaceae from western Cameroon. Kew Bulletin 58: 225–228. doi: https://doi.org/10.2307/4119366.

Cowley EJ. 1988. Burmanniaceae. Flora of Tropical East Africa. Balkema, Rotterdam.

Darbyshire I, Anderson S, Asatryan A, et al. 2017. Important plant areas: revised selection criteria for a global approach to plant conservation. Biodiversity Conservation 26: 1767–1800. doi: https://doi.org/10.1007/s10531-017-1336-6.

Franke T. 2004. Afrothismia saingei (Burmanniaceae, Thismieae), a new myco-heterotrophic plant from Cameroon. Systematics and Geography of Plants 74: 27–33.

Franke T, Beenken L, Döring M, et al. 2006. Arbuscular mycorrhizal fungi of the Glomus-group A lineage (Glomerales' Glomeromycota) detected in myco-heterotrophic plants from tropical Africa. Mycological Progress 5: 24–31.

Franke T, Sainge M, Agerer R. 2004. A new species of Afrothismia (Burmanniaceae, tribe Thismieae) from the western foothills of Mt Cameroon. Blumea 49: 451–456.

Hall DW, Brown BV. 1993. Pollination of Aristolochia littoralis (Aristolochiales: Aristolochiaceae) by Males of Megaselia spp. (Diptera: Phoridae)**.** Annals of the Entomological Society of America 86(5): 609–613. doi: https://doi.org/10.1093/aesa/86.5.609.

Harvey YH, Pollard BJ, Darbyshire I, et al. 2004. The plants of Bali Ngemba Forest Reserve, Cameroon. A conservation checklist. Royal Botanic Gardens, Kew, London.

Harvey YH, Tchiengue B, Cheek M. 2010. The plants of the Lebialem Highlands. A conservation checklist. Royal Botanic Gardens, Kew, London.

Heywood VHH. 2007. Medusandraceae. In: Heywood VH, Brummitt RK, Culham A, et al. (eds), Flowering plant families of the world: 205. Royal Botanic Gardens, Kew, London.

Imhof S, Massicotte HB, Melville LH, et al. 2013. Subterranean morphology and mycorrhizal structures. In: Merckx VSFT (ed), Mycoheterotrophy: the biology of plants living on fungi: 157–213. Springer.

IPNI (International Plant Names Index). 2012. www.ipni.org/ipni/ [acc. Jan. 2018].

IUCN. 2012. IUCN Red List Categories: version 3.1. Prepared by the IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK.

Jonker FP. 1938. Monograph of the Burmanniaceae. Mededeelingen van het Botanisch Museum en Herbarium van de Rijks Universiteit te Utrecht.

Kew. 2017. The state of the world's plants report - 2017. Kew, Royal Botanic Gardens, London.

Maas-van de Kamer H. 1998. Burmanniaceae. In: Kubitzki K (ed), The family and genera of vascular plants III. Monocots, Lilianae (except Orchidaceae). Springer, Berlin.

Maas-van de Kamer H. 2003. Afrothismia gesnerioides, another new species of Afrothismia (Burmanniaceae) from tropical Africa. Blumea 48: 475–478.

Merckx VSFT. 2008. Mycoheterotrophy in Dioscoreales. PhD Thesis. Katholieke Universiteit Leuven.

Merckx VSFT, Bidartondo MI. 2008. Breakdown and delayed cospeciation in the arbuscular mycorrhizal mutualism. Proceedings of the Royal Society B. 275: 1029–1035.

Merckx VSFT, Schols P, Maas-van de Kamer H, et al. 2006. Phylogeny and evolution of Burmanniaceae (Dioscoreales) based on nuclear and mitochondrial data. American Journal of Botany 93: 1684–1698.

Mwachala G, Cheek M, Fischer E, et al. 2007. A new species of Dracaena L. (Ruscaceae) from Cameroon. Kew Bulletin 64: 613–616.

Nic Lughadha E, Bachman SP, Govaerts R. 2017. Plant states and fates: response to Pimm and Raven. Trends in Ecology & Evolution 32: 887–889. doi: https://doi.org/10.1016/j.tree.2017.09.005.

Nic Lughadha E, Govaerts R, Belyaeva I, et al. 2016. Counting counts: Revised estimates of numbers of accepted species of flowering plants, seed plants, vascular plants and land plants with a review of other recent estimates. Phytotaxa 272: 82–88. doi: https://doi.org/10.11646/phytotaxa.272.1.5.

Onana JM. 2013. Synopsis des Espèces Végétales Vasculaires Endémiques et rares du Cameroun. Flore du Cameroun 40. MINRESI, Yaoundé, Cameroun.

Onana JM, Cheek M. 2011. Red data book of the flowering plants of Cameroon: IUCN Global Assessments. Royal Botanic G, Kew, London.

Sainge MN, Chuyong GB, Peterson AT. 2017. Endemism and geographic distribution of African Thismiaceae. Plant Ecology and Evolution 150: 304–312. https://doi.org/10.5091/plecevo.2017.1196.

Sainge MN, Franke T. 2005. A new species of Afrothismia (Burmanniaceae) from Cameroon. Nordic Journal of Botany 23(3): 299–303.

Sainge MN, Franke T, Agerer R. 2005. A new species of Afrothismia (Burmanniaceae, tribe Thismieae) from Korup national park, Cameroon. Willdenowia 35: 287–291.

Sainge MN, Franke T, Merckx M, et al. 2010. Distribution of myco-heterotrophic (saprophytic) plants of Cameroon. In: Van der Burgt X, Van der Maesen J, Onana JM (eds), Systematics and conservation of African plants: 281–288. Royal Botanic Gardens, Kew, London.

Sainge MN, Kenfack D, Chuyong GB. 2013. Two new species of Afrothismia from Southern Cameroon. Kew Bulletin 68(4): 591–597.

Sakai S. 2002. Aristolochia spp. (Aristolochiaceae) pollinated by flies breeding on decomposing flowers in Panama. American Journal of Botany 89(3): 527–534.

Schlechter R. 1906. Burmanniaceae africanae. Botanischer Jahrbücher 38: 140–141.

Soltis DE, Clayton JW, Davis CC, et al. 2007. Monophyly and relationships of the enigmatic family Peridiscaceae. Taxon 56: 65–73.

Stoffelen P, Cheek M, Bridson D, et al. 1997. A new species of Coffea (Rubiaceae) and notes on Mt Kupe (Cameroon). Kew Bulletin 52: 989–994. doi: https://doi.org/10.2307/4117826.

Thiers B. continuously updated. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/ [acc. Jan. 2018].

Young AM. 1984. Mechanism of pollination by Phoridae (Diptera) in some Herrania species (Sterculiaceae) in Costa Rica. Proceedings of the Entomological Society of Washington 86: 503–518.