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Authors: Dančák, Martin, Hroneš, Michal, and Sochor, Michal

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MARTIN DANČÁK[1*,4], MICHAL HRONEŠ[2] & MICHAL SOCHOR[3]

Thismia ornata and *T. coronata* (*Thismiaceae*), two new species from Sarawak, Borneo

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Abstract: *Thismia ornata* and *T. coronata* (*Thismiaceae*), two new species from Sarawak (Malaysian Borneo) are described and illustrated. *Thismia ornata* belongs to *T.* sect. *Thismia* subsect. *Odoardoa* and occurs at several localities in western Sarawak around the city of Kuching. This species has been misidentified as *T. aseroe* until now, although the two species differ considerably. The species is assigned a preliminary conservation status of VU according to IUCN criteria. *Thismia coronata* belongs to *T.* sect. *Sarcosiphon* and has been found at only one site near the village of Long Tuyo in Lawas district of northern Sarawak. It is related to *T. goodii* and *T. kelabitiana*. The species is assigned a preliminary conservation status of CR.

Key words: Borneo, Malesia, mycoheterotrophy, new species, Sarawak, *Thismia*, *Thismia aseroe*, *Thismiaceae*, tropical rain forest

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Introduction

Thismia Griff. (*Thismiaceae*) is a genus of monocotyledonous plants that typically inhabit the understorey of tropical and subtropical forests. The genus comprises about 85 species (Dančák & al. 2018; Suetsugu & al. 2018b) and is distributed mostly in the tropical regions of Asia, Australia and South America and extending into subtropical and temperate regions of Japan, New Zealand, Australia and the USA (Merckx & al. 2013). Two main centres of *Thismia* diversity in Southeast Asia are located on the Malay Peninsula and in Borneo, with 16 and 22 recognized species, respectively (Jonker 1948; Chantanaorrapint 2018; Dančák & al. 2018; Nishioka & al. 2018; Siti-Munirah 2018; Tanaka & al. 2018; Siti-Munirah & Dome 2019; Dančák & al. 2020). The number of described species has been increasing rapidly over the last few years, which is probably one of the highest rates among angiosperms (Stevens 2001+). For example, 14 species were described in 2018 alone (Chantanaorrapint & Suddee 2018; Dančák & al. 2018; Hroneš & al. 2018; Nishioka & al. 2018; Siti Munirah 2018; Sochor & al. 2018; Suetsugu & al. 2018a, 2018b, 2018c; Tanaka & al. 2018).

During our field trip to Sarawak (Malaysian Borneo) in January and February 2019 we found two new species of *Thismia* and describe them in this article, which brings the number of Bornean species to 24.

1 Department of Ecology and Environmental Sciences, Palacký University, Šlechtitelů 27, Olomouc, CZ-78371, Czech Republic; *e-mail: martin.dancak@upol.cz (author for correspondence).
2 Department of Botany, Palacký University, Šlechtitelů 27, Olomouc, CZ-78371, Czech Republic.
3 Centre of the Region Haná for Biotechnological and Agricultural Research, Crop Research Institute, Šlechtitelů 29, Olomouc, CZ-78371, Czech Republic.
4 Faculty of Forestry and Wood Technology, Mendel University, Zemědělská 3, Brno CZ-61300, Czech Republic.

Material and methods

This study is based on material collected during January and February 2019 in Limbang and Kuching Divisions of Sarawak. Morphological characters were studied using a hand lens (30–60× magnification), stereo microscope and macro photography. Collected specimens were thoroughly compared with original drawings and descriptions given in protologues of representatives of *Thismia* sect. *Thismia* and *T.* sect. *Sarcosiphon* (Blume) Jonker. Herbarium vouchers for this study are deposited in SAR and OL. Because *Thismia* populations are sometimes composed of a few or even a single reproductive individual, voucher specimens were not always collected to prevent unnecessary harm to the population. In these cases only DNA samples were taken because for DNA analysis substantially less material is needed than for useful herbarium specimens. Also, herbarium vouchers were not collected if the only plants available were not in full bloom. In both cases plants were documented by photographs. Additionally, herbarium material deposited in K and SAR was studied (herbarium codes according to Thiers 2019+).

DNA was extracted by the CTAB method (Doyle & Doyle 1987) from a silica gel-dried piece of tissue from one individual per population. Sequence data were generated for three nuclear and two mitochondrial loci. The small subunit of ribosomal DNA (SSU rDNA) was amplified and sequenced with primers NS1 and NS6, internal transcribed spacers of ribosomal DNA (ITS) with primers ITS1 and ITS4 (White & al. 1990) and large subunit of ribosomal DNA (LSU rDNA) by primers N-nc26S6 and 2134rev (Kuzoff & al. 1998). The mitochondrial genes *atpA* and *matR* were amplified and sequenced with primers developed by Eyre-Walker & Gaut (1997) and primers 26F and 1002R (Meng & al. 2002), respectively. All PCRs were performed with Kapa polymerase (Kapa Biosystems) following a standard protocol with 37 to 40 cycles and annealing temperature of 56°C (rDNA, *atpA*) or 47 °C (*matR*). The PCR products were purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25 M NaCl in the precipitation mixture) and sequenced in both directions by Sanger method at Macrogene Europe. The most variable locus, ITS, was sequenced in all collections to screen variation, whereas the other loci were only analysed in selected specimens.

Sequences were edited and aligned in Geneious 8 (Biomatters) and deposited in NCBI GenBank under accession numbers MN067232–MN067237, MN067250–MN067259, MN067281–MN067283, MN067288, MN067290, MN067300, MN067302, MN067303, MN067307, MN067309, MN067318–MN067320, MN067327 and MN067328. The newly generated sequences were added to the dataset from Sochor & al. (2018) and Dančák & al. (2020), which included nine species whose sequences were downloaded from NCBI GenBank. The alignments are provided as supplemental content online. Bayesian phylogeny inference from concatenated data from the five loci (or four in the GenBank accessions) was computed in MrBayes (ver. 3.2.4; Ronquist & al. 2012) with 2×10^7 generations, sampling every 3000th generation, in two independent runs, each with four chains; the first 10^7 generations (50%) were excluded as burn-in. The substitution model for each locus was used as determined by Sochor & al. (2018; ITS1, ITS2 and 5.8S rDNA were treated as separate partitions).

Results and Discussion

Thismia ornata Dančák, Hroneš & Sochor, **sp. nov.** – Fig. 1 & 2.

Holotype: Malaysia, Sarawak, Kubah National Park, 0.8 km NE of Matang Wildlife Centre, WGS 84: 01°36'49"N, 110°09'57"E, elevation 60 m a.s.l., 7 Feb 2019, *Sochor, Hroneš & Dančák BOR51/19* (SAR! [in spirit]; isotype: OL! [pressed specimen]).

Diagnosis — *Thismia ornata* differs from the most similar known species, *T. filiformis* Chantanaorr., by flower size (to c. 10 cm in diam. vs to c. 3 cm including tepal appendages), inner surface of floral tube (with very fine bright orange reticulum inside vs lacking any reticulum), length of tepal appendages (to 35 mm vs to 8 mm), appendages on apical margin of connective (five of three different shapes vs three of two different shapes) and shape of lateral appendage (with small horn-shaped projection arising from each side of the lateral appendage vs lacking any horn-shaped projections).

Description — Achlorophyllous herb, 7–11 cm tall. *Roots* creeping, vermiform, ± horizontal, poorly branched, pale brown. *Stem* white to pale cream-brown, 4.5–8 cm tall, erect, ribbed, verrucose, bearing 1 or 2 (or 3) flowers. *Leaves* spirally arranged, appressed, scale-like, narrowly triangular, acute, entire, 4–6 mm long, light brown to pinkish. *Bracts* 3, similar to leaves, surrounding base of ovary, lanceolate-triangular, acute, entire, verrucose, to 10 mm long, light brown to pinkish. *Flowers* (sub)sessile, actinomorphic, 1.7–2.2 cm long; *floral tube* funnel-shaped toward base, slightly urceolate at apex, widest (0.9–1.1 cm) in upper quarter, with 12 orange longitudinal ribs and conspicuous bright orange reticulum on inner surface, outer surface pinkish with 12 thin reddish brown longitudinal stripes, verrucose. *Annulus* circular, outer margin markedly raised and brownish yellow, inner margin bright yellow, middle part brownish orange. *Tepals* 6, all equal in shape and size, narrowly triangular, 6–10 mm long, c. 4 mm wide at base, yellow with 3 longitudinal red stripes, tapering into a white filiform appendage 20–35 mm long. *Stamens* 6, connate and forming a tube, pendent from floral tube aperture, translucent; *filaments* free, short, curved downward; *connectives* broad and flattened, each connective with 5 appendages at apical margin and a large box-shaped lateral append-

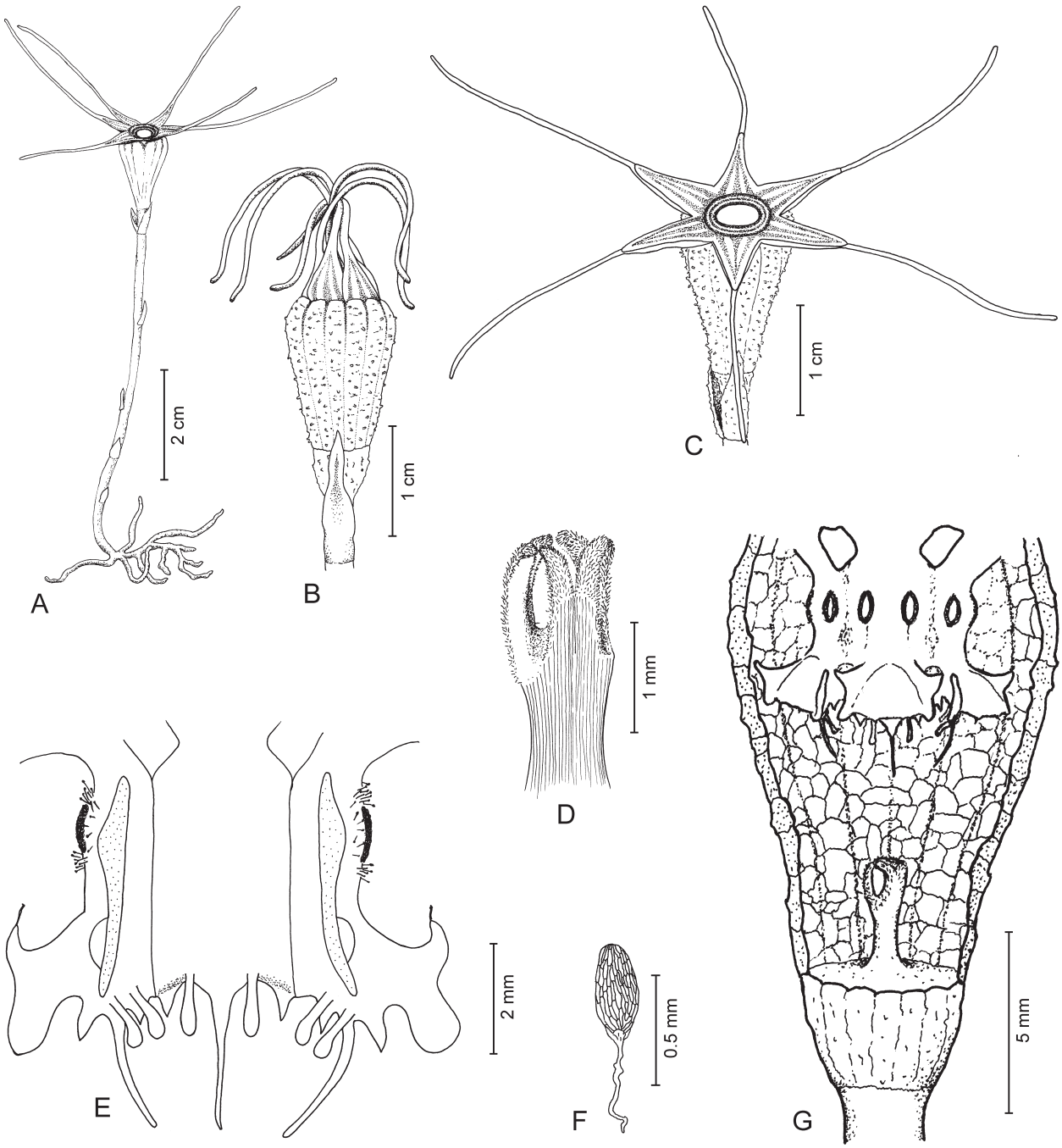

Fig. 1. *Thismia ornata* – A: habit of flowering plant; B: flower bud; C: detail of flower, apical view; D: stigma; E: inner view of stamens,; F: seed; G: outer view of stamens and inside of floral tube. – From *Sochor & al. BOR 51/19* (A, C–E, G), *BOR54/19* (B), *BOR 56/19* (F). – Drawn by Kateřina Janošíková.

age on distal part protruding toward floral tube with an interstaminal gland inserted on line of fusion between connectives; lateral appendage dark brown, with small horn-shaped projection arising from both sides, appendages on apical margin arranged as follows: outer pair of appendages directed outward, triangular, c. 0.8 mm long, apex acute; inner pair inserted above margin and directed inward, cylindric, c. 1 mm long, ± straight, apex domed; central solitary appendage caudate, c. 1.8 mm long, ± pendent. *Ovary* inferior, obconic, indistinctly ribbed, verrucose, whitish to pale brown, darker on apex; *style* c. 1.5 mm long; *stigma* 3-lobed, lobes bifid, hairy, c. 1.2 mm long. *Fruit* an obconical to cup-shaped pale brown ribbed capsule, 4–5 mm long, borne on very short fruit stalk. *Seeds* ellipsoid, beige with fine brown reticulum, 0.38–0.45 mm long, 0.17–0.21 mm wide.

Distribution — *Thismia ornata* occurs in western Sarawak in a number of locations surrounding Kuching, including Kubah National Park, Santubong National Park, Dered Krian National Park and Fairy Cave Nature Reserve (Fig. 3).

Fig. 2. *Thismia ornata* – A, B: overall appearance; C: detail of flower, apical view; D: ovary, stigma and inner surface of floral tube (background grid spacing = 1 mm); E: seeds; F: inner view of stamens; G: lateral view of connective after cutting off neighbouring connective; H: outer view of stamen. – From *Sochor & al. BOR53/19* (A), *BOR51/19* (B–D, F–H) and *BOR56/19* (E).

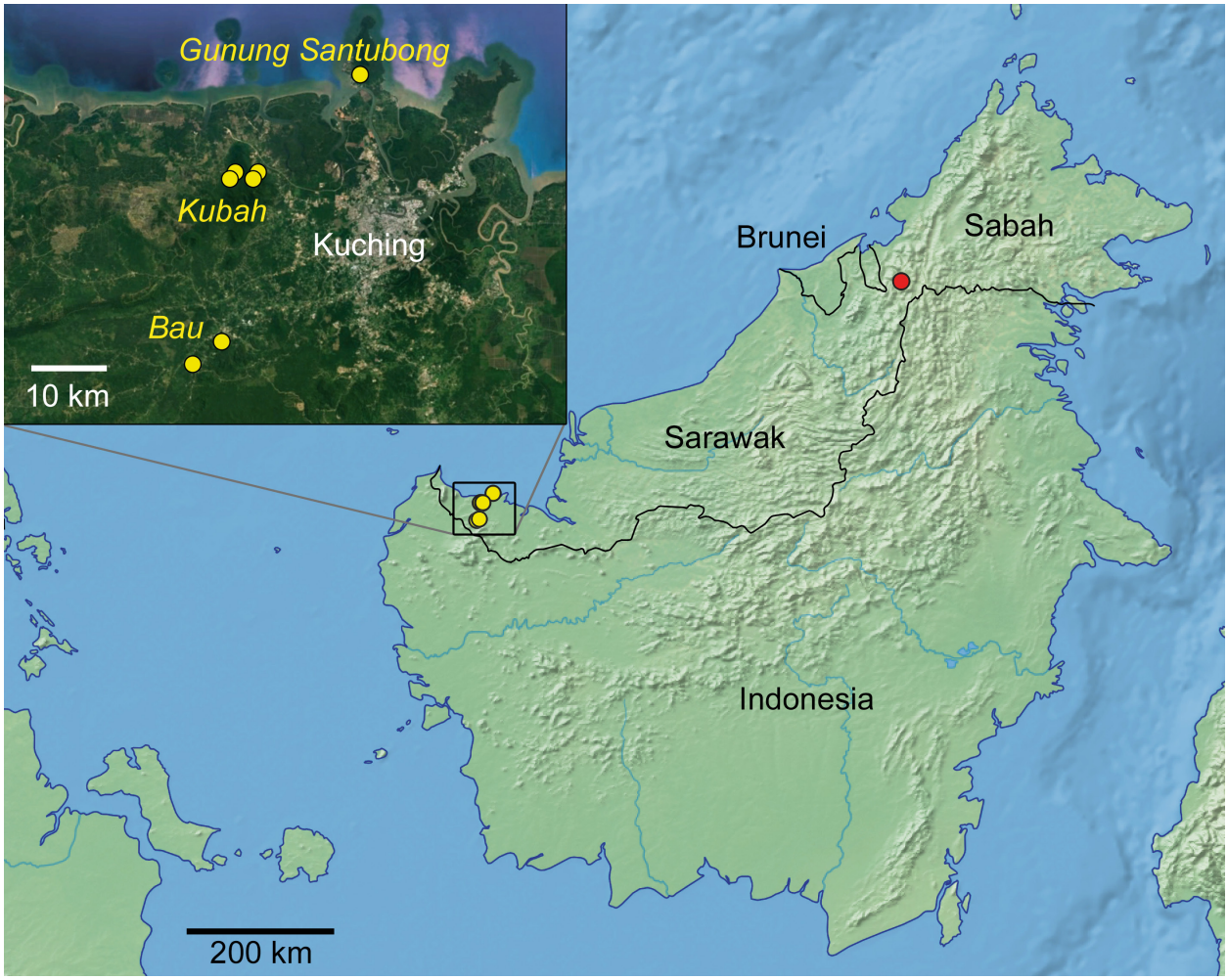

Fig. 3. Distribution of *Thismia ornata* (yellow circles) and *T. coronata* (red circle). – Aerial image modified from Google Earth (https://www.google.com/earth/).

Habitat — *Thismia ornata* inhabits a wide range of tropical lowland rain forest habitats with an altitudinal range from 40 m to c. 300 m a.s.l. It is known from rather dry limestone outcrops, lowland mixed dipterocarp forests, riverine forests including forests with some anthropogenic disturbance.

Conservation status — *Thismia ornata* is endemic to Borneo. Most, if not all, known populations occur within national parks and other protected areas. While the extent of occurrence (EOO) is c. 270 km^2, its minimal area of occupancy (AOO) could be estimated to be 30 km^2. It is known from several populations, which represent three locations (sensu IUCN 2012). *Thismia ornata* is therefore assigned a preliminary conservation status of VU (D1+2) according to the IUCN Red List categories and criteria (IUCN 2012).

Etymology — The specific epithet is the feminine form of the Latin adjective *ornatus* (ornate or decorated), which reflects the colourful flowers and very fine bright orange reticulum inside the floral tube that resembles lace.

Remarks — *Thismia ornata* is not completely unknown to the scientific community; it has been collected several times (see Additional material examined) and many of its photographs circulate on the internet, especially from limestone outcrops around Bau town (e.g. Fehland 2019). However, it has been misidentified as *T. aseroe* Becc., a superficially morphologically similar but not closely related species (unpublished data). *Thismia ornata* was also studied under the name of *T. aseroe* in an extensive study of floral morphology and development in *Dioscoreales* (Caddick & al. 2000) and is adequately described and illustrated in that work. Correspondingly, the DNA sequence entries in GenBank that are based on the specimen *Caddick 349* (K, SAR!) and stored under the name *T. aseroe* belong to *T. ornata* (see Fig. 4).

Thismia ornata belongs to *T.* sect. *Thismia* subsect. *Odoardoa* Schltr. on account of its having six free tepals of the same size and shape. Relationships of *T. ornata* within this section however are unclear. Its morphology is rather unique although it superficially resembles *T. filiformis* (and its relatives). The inner morphology of the flower is different. The bright orange reticulum in-

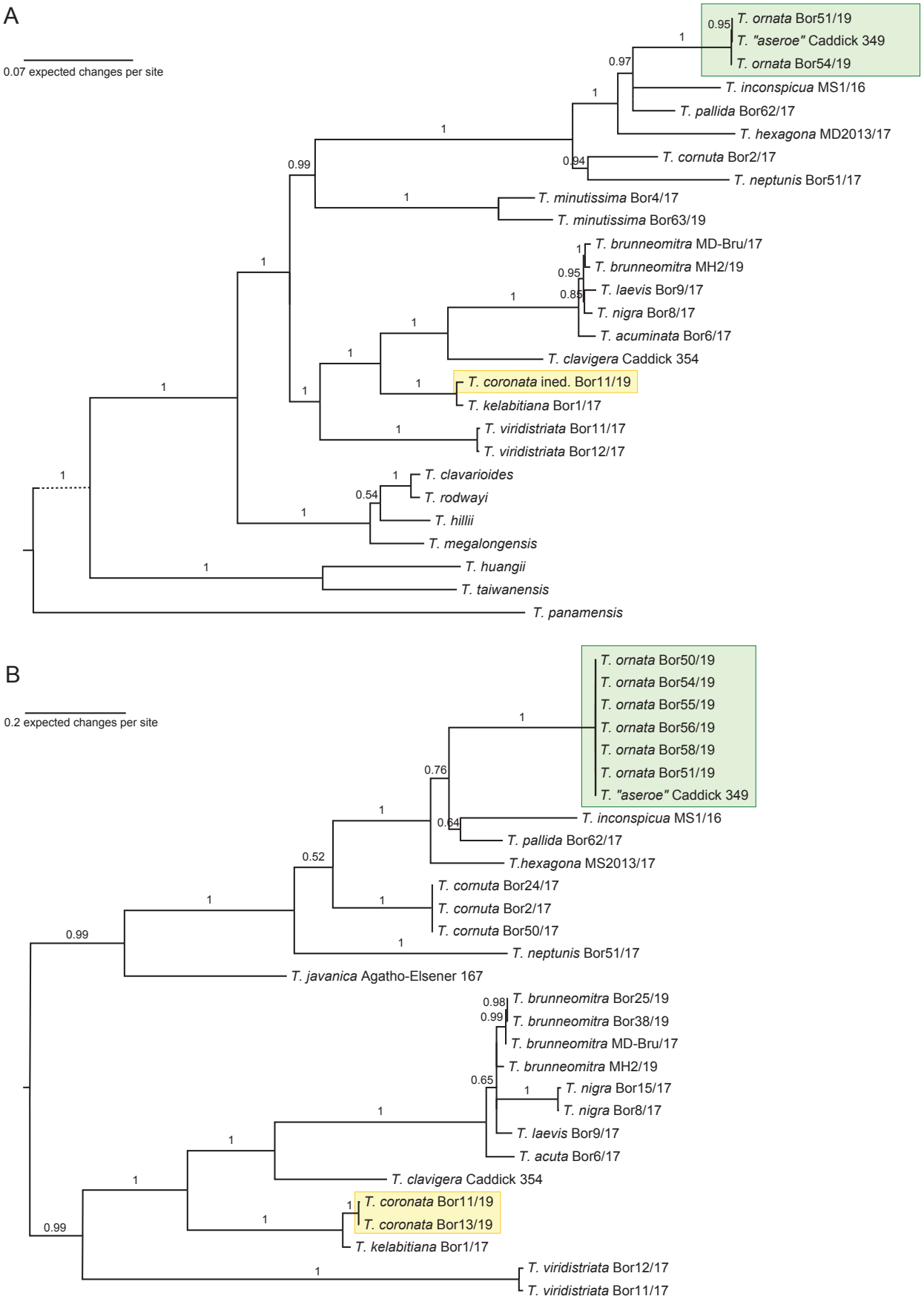

Fig. 4. A: Bayesian phylogeny tree based on five-locus analysis of a wider Old World species set. – B: Bayesian phylogeny tree of *Thismia* sect. *Thismia* and *T.* sect. *Sarcosiphon* based on ITS sequences. – Posterior probabilities are shown above the branches.

side the floral tube is absent in other Bornean species of *Thismia*, with the exception of *T. kelabitiana* Dančák, Hroneš & Sochor, which is somewhat reticulated. For *T. ornata*, the apical margin of the connective bears five appendages of three different sizes and shapes. The single central appendage is the longest while the remaining appendages are considerably shorter. One pair of these appendages sits directly on the apical margin of the connective while the other pair is shifted upward to the surface of the connective and resembles appendages known in *T. hexagona* Dančák, Hroneš, Kobrlová & Sochor and *T. bryndonii* Tsukaya, Suetsugu & Suleiman (Dančák & al. 2013; Tsukaya & al. 2017). Although it has been frequently misidentified as *T. aseroe*, the two species are different. *Thismia aseroe* has a prominently raised annulus and small teeth alternating with the tepals, which make it appear different to the naked eye. The structure of the connectives is also strikingly different between the two species (for comparison see Groom 1895 and Fig. 2).

Although no variation was detected among the seven studied specimens (six of them from Kubah) at the ITS locus (see also Fig. 4B), one SNP was detected at each of the SSU and *matR* loci in the Santubong population (Fig. 4A). Such a small difference, of course, cannot have consequences in taxonomy, but it implies that the species exhibits some small geographical genetic variation. Nevertheless, no significant variability in phenotype was observed.

Additional material examined — Borneo, Sarawak (Malaysia): 1st Division, Bau, Tai Ton, 2 Jan 1977, *P. J. Martin 38504* (SAR); 1st Division, Bau, limestone hill behind lake, 11 Nov 1989, *P. Cribb 89/16* (K); Bau, Seburan, 8 Jun 1966, *J. A. R. Anderson 25524* (SAR); Matang National Park, Sungai Rayu, 150 m a.s.l., 21 Oct 1998, *L. Caddick, V. B. Kasik, D. Jude, M. Kapi LRC 349* (SAR); Kubah National Park, Matang Wildlife Centre, near the crocodile cage, WGS 84: 01°36'31"N, 110°09'42"E, 45 m a.s.l. 9 Feb 2019, *Sochor, Hroneš & Dančák BOR55/19* (only DNA material, no voucher specimen); Kubah National Park, 0.8 km SW of headquarters, WGS 84: 01°36'25"N, 110°11'30"E, 235 m a.s.l., 9 Feb 2019, *Sochor, Hroneš & Dančák BOR56/19* (only DNA material, no voucher specimen); Kubah National Park, 30 m SW of Park hostel, WGS 84: 01°36'42"N, 110°11'43"E, 170 m a.s.l., 9 Feb 2019, *Sochor, Hroneš & Dančák BOR58/19* (only DNA material, no voucher specimen); Gunung Santubong, along contour trail, WGS 84: 01°43'55"N, 110°19'34"E, c. 300 m a.s.l., 8 Feb 2019, *Sochor, Hroneš & Dančák BOR54/19* (OL).

Thismia coronata Hroneš, Dančák & Sochor, **sp. nov.** – Fig. 5, 6 & 7.

Holotype: Malaysia, Sarawak, Limbang Division, Lawas District, Long Tuyo village, primary forest between camps 1 and 2 on trail to Paya Maga mountain plateau, WGS 84: 04°26'37"N, 115°33'07"E, elevation 1305 m a.s.l., 29 Jan 2019 *Sochor, Hroneš & Dančák BOR11/19* (SAR! [in spirit]; isotype: OL! [pressed specimen]).

Diagnosis — *Thismia coronata* differs from *T. kelabitiana* by the shape of outer tepals (entire with a single tooth in the middle vs deeply divided into several acute lobes), mitre apex (without tetrahedral depressions on upper surface vs with tetrahedral depressions on upper surface), smaller size of flower (1.8–2.3 cm vs 2.6–2.8 cm long) and flower colour (dark yellow to orange across whole flower vs white floral tube and bright yellow upper parts).

Description — Achlorophyllous herb, 4.5–8 cm tall. *Roots* coralliform, short, clustered, light brown. *Stem* 2.4–4.2 cm long, erect (or sometimes ascending), unbranched or sparsely branched in upper part (branches developing after anthesis), indistinctly longitudinally ribbed, reddish brown to orange, bearing 1–6 flowers. *Leaves* 4–11, spirally arranged, appressed, scale-like, triangular, acute to acuminate, entire, 2–5 mm long, 1–2 mm wide at base, light brown to reddish. *Bracts* 3, lanceolate-triangular, entire to irregularly dentate, 4–8 × 2–3 mm, reddish to brown. *Flowers* sessile, actinomorphic, 1.8–2.3 cm long; *floral tube* funnel-shaped toward base, urceolate at apex, widest (0.7–1 cm) in upper quarter, dark yellow to orange throughout, with 6 brown-orange prominent longitudinal ribs alternating with 6 brown-orange longitudinal stripes on outer surface, inner surface with a weakly net-like pattern, especially toward apex. *Tepals* 6; outer 3 tepals falcate, c. 2 mm long, 6–7 mm wide, entire or slightly sinuate at margin, sometimes somewhat wavy, usually with a single tooth in centre, bright orange, arranged in one plane and together forming fringe around mouth of floral tube, 0.9–1.2 cm in diam.; inner 3 tepals orange, pillar-like and arched over floral tube, connate at apex and forming a rather flat triangular mitre 5–7 mm wide, proximal part of tepal 0.5–0.9 mm long, c. 1 mm wide, distal part of tepal ± flat with 3 joined ribs. *Annulus* absent. *Stamens* 6, pendent from floral tube aperture; *filaments* orange, curved downward, with bases slightly protruding above floral tube apex, not connate and forming 6 lateral apertures visible from upper side; *connectives* broad, laterally connate to form a tube, 5–6 mm long, each with prominent longitudinal rib extending along whole length of inner side of connective, apex of each connective white to bluish, with 1 central lobe (extension of rib) and 2 smaller lobes, each lobe bearing 1 very long transparent trichome; lateral appendage box-shaped, protruding toward floral tube, not reaching apex of connective, shallowly dentate and hairy on apical margin, with tufts of hairs on lateral margins, yellow-tinted; thecae creamy, surrounded by tufts of glandular hairs; interstaminal glands inserted on line of fusion between connectives. *Ovary* inferior, obconic, dark brown, covered by bracts, placentae 3, ovules nu-

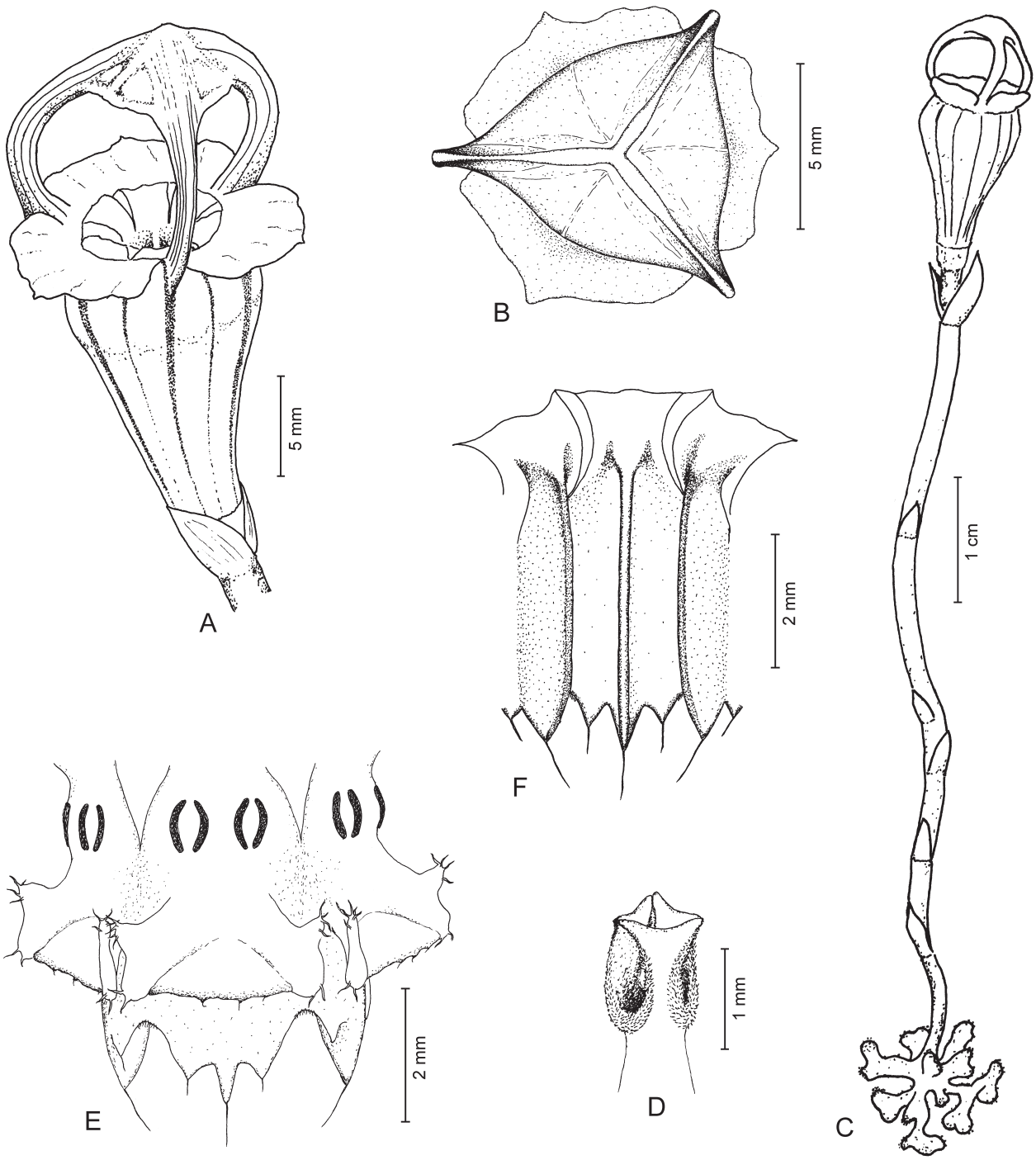

Fig. 5. *Thismia coronata* – A: lateral view of flower; B: apical view of flower; C: habit of flowering plant; D: stigma; E: outer view of stamens; F: inner view of stamens. – From *Sochor & al. BOR11/19* (A–H). – Drawn by Kateřina Janošíková.

merous, anatropous; *style* short; *stigma* 3-lobed, papillose, lobes ± rectangular, longitudinally furrowed. *Capsule* cup-shaped, 5–7 mm in diam., pale brown to reddish at maturity; fruiting pedicel 10–25 mm long. *Seeds* numerous, light brown, ellipsoid.

Distribution — Thismia coronata is known only from the type locality and its close surroundings in the Paya Maga mountain range in northern Sarawak (Fig. 3). At least 40 individuals were recorded at the locality.

Habitat — Thismia coronata occurs in lower montane primary tropical rainforest at an altitude around 1300 m a.s.l. It was found in relatively humid forest with dense understorey (Fig 7C). A variety of other mycoheterotrophic species were abundant at the type locality and surroundings, including *Burmannia lutescens* Becc. agg. (*Burmanniaceae*), *Cystorchis aphylla* Ridl. (*Orchidaceae*), *Epirixanthes kinabaluensis* T. Wendt, *E. pallida* T. Wendt (both *Polygalaceae*), *Exacum tenue* (Blume) Klack. (*Gentianaceae*), *Lecanorchis multiflora* J. J. Sm.

Fig. 6. *Thismia coronata* – A: flower prior to anthesis; B, C, D: overall appearance; E: apical view of flower; F: stigma; G: roots; H: outer bottom view of stamen. – Finest grid spacing in D and G = 1 mm. – From *Sochor & al. BOR11/19* (A–H).

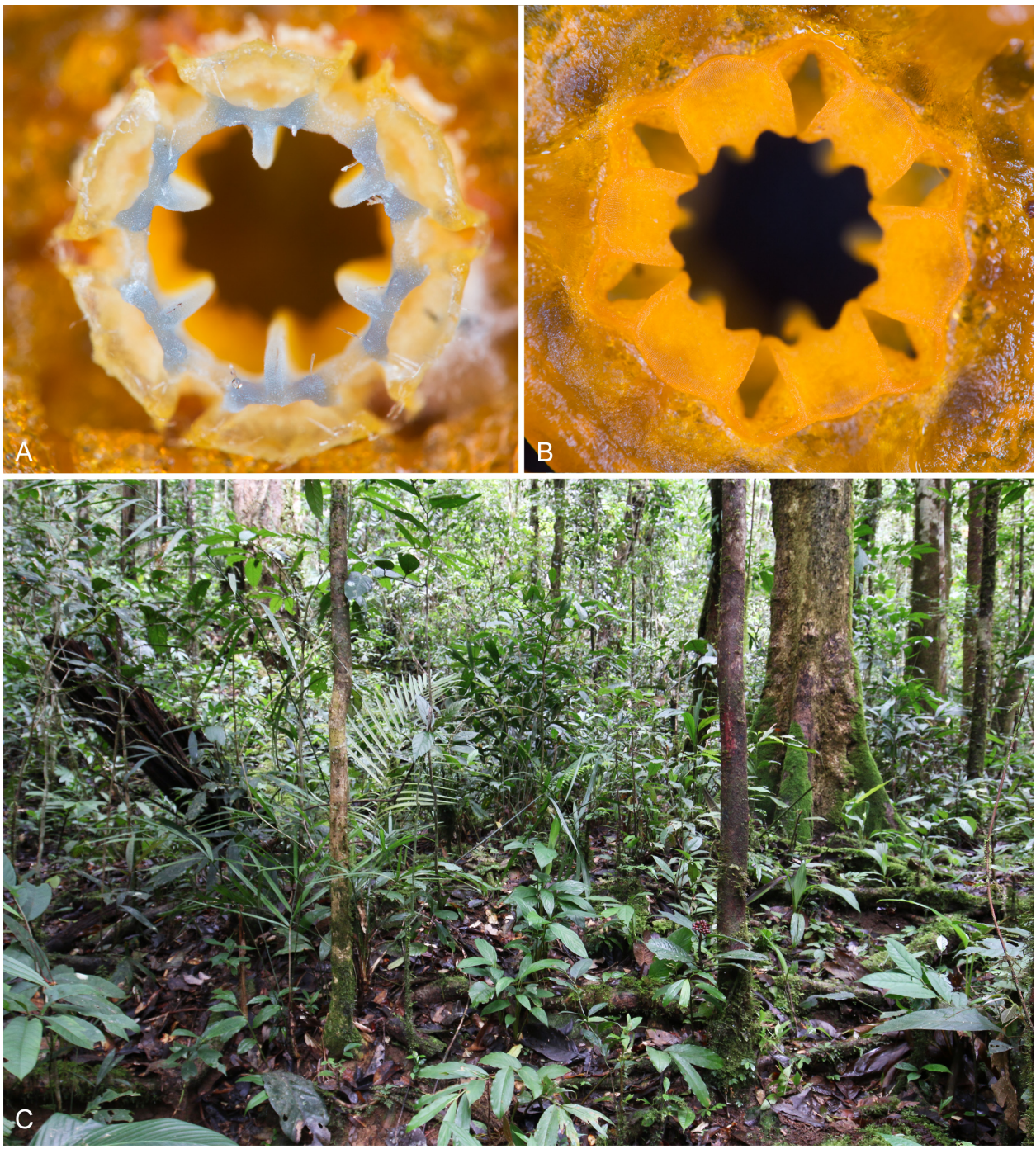

Fig. 7. *Thismia coronata* – A: connective tube viewed from lower side; B: floral tube apex and opening showing 6 lateral apertures between stamens; C: type locality, 29 Jan 2019, photograph by M. Sochor. – From *Sochor & al. BOR11/19* (A–C).

(*Orchidaceae*), *Sciaphila arfakiana* Becc., *Sciaphila* sp. (both *Triuridaceae*) and *Thismia viridistriata* Sochor, Hroneš & Dančák. Herbaceous vegetation was otherwise sparse.

Conservation status — *Thismia coronata* is endemic to Borneo and the only known population occurs outside national parks and other protected areas. The number of mature individuals observed is fewer than 50. The extent of occurrence cannot be estimated because the species is known only from the type locality and its area of occupancy (AOO) is estimated to be only 4 km^2. *Thismia coronata* is therefore assigned a preliminary status of CR (B2ab(iii); D) according to the IUCN Red List categories and criteria (IUCN 2012).

Etymology — The specific epithet is the feminine form of the Latin adjective *coronatus* (crowned), which reflects the shape and colour of the tepals resembling a royal crown.

Remarks — Thismia coronata superficially resembles *T. kelabitiana* mostly by its colour, which is, nevertheless, darker and nearly homogeneous across the flower. However, the flower shape is similar to *T. goodii* Kiew, which differs in colour (blue tepals and white floral tube with a green tinge), smaller mitre and hairless apex of the connective. These three species (*T. coronata*, *T. goodii* and *T. kelabitiana*) are clearly closely related (see also Fig. 4), forming a morphologically distinct group within *T.* sect. *Sarcosiphon*. They share the well-developed outer tepals that are wider than long, trilobed apex of the connective and a prominent central rib along the inner side of the connective. *Thismia goodii* is almost sympatric with *T. coronata*: it is known from a nearby site only 1.5 km away (Ampeng & al. 2013). All three species occur in the highlands of northern Borneo and have no apparent relatives outside this region. Interestingly, this group bears some similarities to the group of *T. clavigera* (Becc.) F. Muell. (see also Dančák & al. 2018). Although both groups are superficially rather different, primarily due to the three mitre appendages present in the *T. clavigera* group, the structure of the stamens is somewhat similar in both groups because the filaments reach the floral opening (which has no annulus) and the connectives have a prominent central rib along their inner side.

Additional material examined — Borneo, Sarawak (Malaysia): Limbang Division, Lawas District, Long Tuyo village: primary forest between camps 1 and 2 on trail to Paya Maga Mountain plateau, WGS 84: 04°26'36"N, 115°33'04"E, elevation c. 1280 m a.s.l., 29 Jan 2019 *Sochor, Hroneš & Dančák BOR13/19* (only DNA material, no herbarium specimen).

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