Thismia nigricoronata, a new species of Burmanniaceae (Thismieae, Dioscoreales) from Vang Vieng, Vientiane Province, Laos, and a key to subgeneric classification

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Abstract

Thismia nigricoronata is described as a new species in family Burmanniaceae. Both morphological and phylogenetic analyses indicate that this new Lao endemic is allied to T. taiwanensis in section Glaziocharis, and it can be differentiated on the basis of its longer vestigial stem leaves, reflexed free outer perianth lobes and ornamented, vibrantly coloured outer surface of the perianth tube. The infrageneric taxonomy of Thismia is reviewed, the genera Geomitra and Scaphiophora are officially reduced to sectional status in Thismia, and all species are enumerated in systematic order. A key to all currently accepted subgenera, sections and subsections is presented to facilitate further examination of their phylogenetic integrity in light of apparent conflict between the traditional morphology-based system and the emerging DNA-based classification.

Key words: achlorophyllous plants, Dioscoreaceae, holomycoheterotrophs, Laotian flora, Thismiaceae

INTRODUCTION

Burmanniaceae Miers (1847: 129) are a widely recognised family of mycoheterotrophic flowering plants (Chase et al. 1995, Chase et al. 2000, Woodward et al. 2007, Merckx 2013, Merckx & Smets 2014, Truòng et al. 2014). DNA analyses have indicated that the five genera typically included in tribe Thismieae, namely, Thismia Griffith (1845: 221), Afrothismia Schlechter (1906: 138), Oxygyne Schlechter (1906: 140), Haplothismia Airy Shaw (1952: 277) and Tiputinia Berry & Woodward (2007: 158), all fall outside Burmanniaceae (Merckx & Smets 2014), but their specific placement within the order Dioscoreales remains unclear (Merckx et al. 2009, Merckx & Smets 2014). In particular, tree topologies recovered in recent analyses revealed Tacca Forster & Forster (1776: 69) to be sister to core Thismieae species representing the genera Thismia, Oxygyne, Haplothismia and Tiputinia, but with Afrothismia sister to both (Merckx et al. 2009, Merckx & Smets 2014). Despite this apparent polyphyly, however, Thismiae are readily distinguished from Tacca on the basis of pollen morphology (Cranwell 1953, Chakrapani & Raj 1971), and the two have hence been treated as separate from Burmanniaceae within Dioscoreales by APG IV, in the hope that this taxonomic issue will be resolved by further study (APG, 2016).

Thismia comprises small mycoheterotrophic, ground dwelling herbs that are for the most part poorly studied (Mar & Saunders 2015). The genus is the largest of the tribe, being represented by c. 56 species (Merckx & Smets 2014, Govaerts et al. 2007) distributed from warm temperate and tropical Asia, eastern and southeastern Australia, New Zealand, southern North America and South America (Govaerts et al. 2007). Of these, 37 species are found in tropical and temperate Asia, including 12 species in the Indo-Burma Biodiversity Hotspot (Govaerts et al. 2007). Thismia is characterised by the presence of an urceolate perianth tube that is formed by the fusion of the tepals that bear two whorls with apically free lobes (Caddick et al. 2000, Mar & Saunders 2015). Anther morphology, presence of a mitre (a hat-like structure formed from overlapping of the inner perianth lobes that covers the annulus) and morphology of mitral appendages and perianth lobes have been used as diagnostic characters in species delimitation, although Merckx & Smets (2014) suggested that the perianth appendages may carry little phylogenetic significance in accurately assessing species relationships within the genus.
According to the latest generic classification proposed by Merckx & Smets (2014), *Thismia* is divided into two subgenera: *Ophiomeris* (Miers 1847: 328) Maas & Maas (1986: 144), which is characterised by connate anther thecae, and *Thismia*, which is characterised by free anther thecae. Subgenus *Ophiomeris* is further divided into three sections, namely, *Myostoma* (Miers 1866: 57), *Ophiomeris* (Miers 1847: 328), and *Pyramidalis* (Miers 1847: 328) Maas & Maas (1986: 145) and *Pyramidalis* Maas & Maas (1986: 161), whereas subgenus *Thismia* is further divided into six sections, namely, *Thismia* (Euthismia) Schlechter, 1921: 34, which cannot be used under Article 21.3 of the Melbourne code, *Sarcosiphon* Blume (1850: 65), *Glaziocharis* (Taubert ex Warming 1901: 75) Hatusima (1976: 4), *Rodwaya* (Schlechter 1921: 38), *Geomitra* and *Scaphiophora*; however, sections *Geomitra* and *Scaphiophora* have never been formally published. Section *Thismia* is further divided into subsections *Odoaroda* Schlechter (1921: 34) and *Brunonithismia* Jonker (1938: 242).


During a field survey in Laos in April 2012, the first two authors came across a population of a terrestrial herb. After a thorough literature survey, it was found to be an undescribed species in section *Glaziocharis*, described here as *T. nigricoronata*.

**MATERIALS AND METHODS**

**Morphological data**

Morphological study was conducted on living as well as preserved specimens collected in the field in Laos. Voucher specimens were deposited at Herbier National du Laos (HNL). A detailed taxonomic description was prepared, and critical comparison with allied species was undertaken. In order to clarify morphological features used to define sections of *Thismiaceae* and facilitate subsequent systematic studies of *Thismiaceae*, a unified key to the subgenera, sections and subsections of *Thismia* was compiled and is presented here.

**Phylogenetic analysis**

**Sampling:**—Sequence data (nuclear ribosomal 18S DNA and mitochondrial *atp1*) for 13 accessions representing ten species of *Thismiaceae* (Table 1). Given recent phylogenetic evidence to suggest that *Afrothismia* is an independent clade sister to the other four genera typically included in *Thismiaceae* (*Thismia*, *Oxygyne*, *Haplothiisma* and *Tiputinia*) plus *Tacca* (Merckx & Smets 2014), *Afrothismia* was excluded from our analysis. The plants discovered in Laos were sequenced using protocols as described below. Two species, *Tacca leontopotataloides* (Linneè 1753: 313) Kuntze (1891: 704) and *Tacca parkeri* Seemann (1866: 102), were chosen as outgroups following Merckx & Smets (2014).

**DNA extraction and phylogenetic analysis:**—Total DNA was extracted from a dried flower of the collection from Laos using the QIAGEN DNeasy® plant DNA kit (Hilden, Germany) according to the manufacturer’s instructions. Using the primers NS1, NS2, NS3, NS4, NS5 and NS8 (White et al. 1990), 18S rDNA was amplified, and mitochondrial *atp1* was amplified with the primers and conditions described by Eyre-Walker & Gaut (1997). PCR was performed in a total reaction mixture of 25 μl containing 1 μl of template DNA (2–10 ng), 5 μl of 5 × Phire® reaction buffer with MgCl2, 0.5 μl 10 mM of dNTP mix, 0.5 μl of Phire® hot start II DNA polymerase (Finnzymes, Finland) and 10 pmol of each primer (Beijing Genomics Institute). The thermal cycler programme consisted of an initial denaturation step of 30 s at 98°C, followed by 35 cycles of 5 s at 98°C, 10 s at 60°C for 18S rDNA and 10 s at 55°C for *atp1*, 20 s at 72°C, and a final extension of 1 min at 72°C. Amplification products were purified using a DNA purification Kit (Beijing Genomics Institute). Purified PCR products were sequenced using an ABI 3730 DNA Sequencer (Beijing Genomics Institute, Hong Kong).

Alignment was conducted using the MAFFT multiple alignment plugin in Geneious v6.1.6 (Drummond et al. 2011), with subsequent adjustment by eye. An incongruence length difference (ILD) test (Farris et al. 1995) was performed in PAUP* v4.0b10 (Swofford 2003) to assess whether the 18S rDNA and *atp1* data sets reflect similar potential trees; 1,000 replicates, each with 1,000 random addition sequence replicates and TBR branch swapping, were performed in each test, and a *P* value of < 0.05 was considered significant (Sullivan 1996, Darlu & Lecointre 2002).
A “hard” incongruence test was also performed by directly comparing respective topologies and resolution for each of the clades generated in the separate analyses, with bootstrap percentages (BP) of ≥ 85 (Chase et al. 2000) and posterior probabilities (PP) of ≥ 0.95 (Martínez-Azorín et al. 2011) being taken as evidence of strong support.

Phylogenetic analysis of individual and multilocus alignments were carried out using maximum parsimony (MP) in PAUP* v4.0b10 and Bayesian inference (BI) in MrBayes v3.2 (Huelsenbeck & Ronquist 2003). For MP analyses, heuristic searches were conducted with 1,000 random addition replicates followed by tree bisection-reconnection branch swapping. All characters were unordered and equally weighted with gaps (including unavailable sequences) treated as missing data. Topological robustness was assessed using 1,000 bootstrap replicates. For BI analyses, each DNA region was assigned its own model of nucleotide substitution, as determined by the Akaike information criterion (AIC) in Modeltest v3.06 (Posada & Crandall 1998). Four simultaneous Monte Carlo Markov Chains (MCMC) were run, with sampling one tree every 1,000 generations for 3,000,000 generations, starting with a randomly generated tree. Majority rule (> 50%) consensus trees were constructed after removing the first 25% of sampled trees as burn-in.

Conservation status
A conservation assessment was carried out using the criteria of IUCN (2012).

**TABLE 1. A list of samples.**

<table>
<thead>
<tr>
<th>Species</th>
<th>GenBank accession numbers</th>
<th>18S nrDNA</th>
<th>atp1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thismia aseroe</td>
<td>AF309404</td>
<td></td>
<td>EU421048</td>
</tr>
<tr>
<td>Thismia clavarioides</td>
<td>KF692533</td>
<td></td>
<td>KF692539</td>
</tr>
<tr>
<td>Thismia clavigera</td>
<td>AF309405</td>
<td></td>
<td>EU421049</td>
</tr>
<tr>
<td>Thismia huangii</td>
<td>KF692534</td>
<td></td>
<td>KF692543</td>
</tr>
<tr>
<td>Thismia javanica</td>
<td>KF692535</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Thismia hillii (accessioned as)</td>
<td>AF309403</td>
<td></td>
<td>AY299849</td>
</tr>
<tr>
<td>Thismia rodwayi*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thismia rodwayi (TAS1)</td>
<td>KF692536</td>
<td></td>
<td>KF692540</td>
</tr>
<tr>
<td>Thismia rodwayi (TAS2)</td>
<td>KF692537</td>
<td></td>
<td>KF692542</td>
</tr>
<tr>
<td>Thismia rodwayi (VIC)</td>
<td>KF692538</td>
<td></td>
<td>KF692541</td>
</tr>
<tr>
<td>Thismia taiwanensis</td>
<td>DQ786080</td>
<td></td>
<td>EU421051</td>
</tr>
<tr>
<td>Thismia nigricoronata sp. nov.</td>
<td>MF589340</td>
<td>MF589341</td>
<td></td>
</tr>
<tr>
<td>Haplothirschis exannulata</td>
<td>DQ786082</td>
<td></td>
<td>EU421037</td>
</tr>
<tr>
<td>Tiputinia foetida</td>
<td>FJ215764</td>
<td></td>
<td>FJ215770</td>
</tr>
<tr>
<td>Tacca leontopetaloides</td>
<td>EU420999</td>
<td></td>
<td>AF039252</td>
</tr>
<tr>
<td>Tacca parkeri</td>
<td>EU421001</td>
<td></td>
<td>JN850562</td>
</tr>
</tbody>
</table>

*Merckx & Smets (2014) explain use of this name.

**RESULTS**

**Morphological analysis**
Examination of the plants collected from Vang Vieng, Laos, revealed them to possess free anther thecae, placing them in subgenus *Thismia*, and an urceolate perianth tube bearing a mitre with prominent outer perianth lobes and long filiform appendages arising from the free part of the inner perianth lobe, suggesting they belong to section *Glaziocharis*. The same combination of characters closely allies it to *Thismia taiwanensis* (Yang et al. 2002: 485), which also belongs to section *Glaziocharis*. However, the plants are distinct from *T. taiwanensis* in having longer vestigial stem leaves, reflexed free outer perianth lobes and an ornamented, vibrantly coloured outer surface of the perianth tube.

**Phylogenetic analysis**
Statistics relating to the phylogenetic analysis for 18S rDNA and *atp1* sequence data were newly generated for the Lao *Thismia* and the combined data set are given in Table 2. Tree topologies generated for the 18S rDNA data partition using BI (Fig. 1) were approximately congruent with those using MP. Given this, the 13 samples of family Thismiaceae included here formed a strongly supported clade (BP 100, PP 1.00) within which the genus *Thismia*, represented by 11 samples, formed a strongly supported clade (BP 81, PP 1.00). The 11 *Thismia* samples were clearly resolved into two distinct clades: one comprising *T. hillii*, *T. clavarioides*, *T. rodwayi*, *T. clavigera*, *T. javanica* and *T. aseroe* that received moderate BP (82) and strong PP (1.00) support, and another comprising *T. taiwanensis*, *T. huangii* and the collection
from Laos, which was also strongly supported (BP 99, PP 1.00). Within this clade, the collection from Laos fell sister to *T. huangii*, although support for this relationship was very weak (BP 53, PP 0.53).

**TABLE 2. Statistics relating to the DNA data sets used in this study.**

<table>
<thead>
<tr>
<th>Information</th>
<th>18S rDNA</th>
<th><em>atp1</em></th>
<th>Combined 18S rDNA and <em>atp1</em> data sets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aligned length</td>
<td>1,620</td>
<td>1,186</td>
<td>2,806</td>
</tr>
<tr>
<td>No. of variable characters</td>
<td>111</td>
<td>53</td>
<td>164</td>
</tr>
<tr>
<td>Parsimony-informative characters</td>
<td>172</td>
<td>63</td>
<td>235</td>
</tr>
<tr>
<td>Tree length</td>
<td>510</td>
<td>149</td>
<td>661</td>
</tr>
<tr>
<td>Consistency index</td>
<td>0.68</td>
<td>0.89</td>
<td>0.73</td>
</tr>
<tr>
<td>Retention index</td>
<td>0.69</td>
<td>0.90</td>
<td>0.74</td>
</tr>
<tr>
<td>Best-fit model determined by AIC in Modeltest</td>
<td>GTR+I+G</td>
<td>TIM+I+G</td>
<td>GTR+I+G</td>
</tr>
</tbody>
</table>

**FIGURE 1.** Bayesian inference (BI) tree from analysis of 18s rDNA data. Numbers at the nodes indicate MP bootstrap percentages and Bayesian posterior probabilities, respectively.

The topology of the *atp1* tree (Fig. 2) was mostly concordant with the 18s rDNA phylogram. The 12 Thismiaceae samples formed a strongly supported clade (BP 100, PP 1.00), within which the ten samples representing genus *Thismia* again formed a strongly supported clade (BP 99, PP 1.00) composed of two discrete subclades, each comprising the same samples as in the 18s rDNA analysis, except that *T. javanica* was not included in this analysis because *atp1* sequence data were not available for this taxon. A clade comprising *T. taiwanensis*, *T. huangii* and the Lao material was again strongly supported (BP 98, PP 1.00), with a sister relationship between the latter two receiving moderate support (BP 64, PP 0.89).

A homogeneity test for the 18s rDNA and *atp1* data sets gave a *P* value of 0.76, indicating that there was no significant incongruence between the two regions. Moreover, visual node-by-node comparisons of trees generated for each region individually revealed no major disparity in topology for nodes of BP ≥ 85 or PP ≥ 0.95. Given this, we combined the two regions into a single analysis. Tree topologies generated by BI (Fig. 3) and MP analyses were consistent. This combined analysis resulted in greater resolution as compared with the separate analyses.

Thismiaceae formed a strongly supported clade (BP 100, PP 1.00) with the 11 *Thismia* samples forming two distinct clades composed of samples consistent with the individual 18s rDNA and *atp1* trees. Within the strongly supported clade (BP 100, PP 1.00) comprising *T. taiwanensis*, *T. huangii* and the collection from Laos, the latter two again fell sister to one another with weak support (BP 72, PP 0.77).
DISCUSSION

A specific combination of floral characters places the Lao plants unequivocally in *Thismia* section *Glaziocharis*. Given the significance of the characters that then distinguish them from *T. taiwanensis*, morphologically the most similar, it appears justified to regard them as an undescribed new species.

Molecular phylogenetic reconstruction also retrieves strong support for the placement of this taxon in a clade containing *T. taiwanensis*. However, within this clade, the new species falls sister to the morphologically distinct *T. huangii*, which has previously been assigned to section *Rodwaya* on account of its free anther thecae and its outer perianth lobes that lack filiform appendages. Furthermore, *T. clavarioides*, the other member of section *Glaziocharis* included in this study, does not associate with either *T. taiwanensis* or the Lao collection in our trees. This outcome corresponds with Merckx & Smets (2014) and corroborates their conclusion that the presence of perianth appendages may not be a phylogenetically informative character. The basis for incongruence between the morphology-based classification and the relationships inferred through molecular phylogenetic analysis requires further study. These appendages may be tracking pollinator relationships rather than phylogenetic relationships.

At present, sampling within tribe Thismieae for phylogenetic reconstruction remains low, limiting the taxonomic conclusions that can be drawn. The emerging picture suggests that the floral characters traditionally emphasised in defining infrageneric divisions may not reflect phylogeny, but it is also plausible that the two regions currently sequenced simply do not provide adequate resolution. To facilitate reappraisal of the current classification as more evidence becomes available, below we present a key to the subgenera and sections of *Thismia*, highlighting the critical morphological characters previously used to distinguish them and underscoring the need for broader sampling in molecular studies with which to evaluate infrageneric relationships.
FIGURE 3. Bayesian inference (BI) tree from analysis of the combined 18s rDNA and atp1 data. Numbers at the nodes indicate bootstrap percentages and Bayesian posterior probabilities, respectively.

TAXONOMIC TREATMENT

**Thismia** Griffith (1845: 221).


Type:—*Thismia brunonis* Griffith (1845: 221).

Terrestrial, mycoheterotrophic herbs with tubers or coralloid roots. Stems unbranched. Leaves present or absent; if present, vestigial, scaly and colourless. Inflorescence terminal, solitary or more than 1, cymose or paniculate. Flowers actinomorphic or zygomorphic, often vibrantly coloured or colourless. Perianth campanulate, forming an urceolate, cylindrical or oboconical tube. Tepals 6, free or inner 3 fused to form a mitre above the floral tube. Stamens 6, rarely 3, epipetalous, suspended inside the perianth tube, forming a staminal tube or reflexed outside, thecae connate or fused, dehiscing longitudinally, connective adnate. Ovary inferior, unilocular with 3 parietal placentas, ovules numerous, style short, thick; stigma 3, free or fused. Fruit cupular or a dry capsule. Seeds numerous, attached to the 3 parietal placentae, dust-like. Embryo undivided, homogeneous.
Key to identification of the subgenera, sections and subsections of \textit{Thismia}

1. Anther thecae connate (subgenus \textit{Ophiomeris}) ............................................................... ................................. 2
- Anther thecae free (subgenus \textit{Thismia}) .............................................................................................................. 4
2. Rootstock vermiform, stem terete, vestigial stem leaves present, mitre present, stamen connective touching or fused to formstami-
nal tube ....................................................................................................................................................... section \textit{Pyramidalis}
- Rootstock tuberous, stem sulcate, stem leaves absent, mitre absent, stamen connective free ......................................................... 3
3. Anthers sagittate with single tip .............................................................................................................. section \textit{Myostoma}
- Anthers sagittate with double tip .............................................................................................................. section \textit{Ophiomeris}
4. Mitre present .............................................................................................................................................. 5
- Mitre absent (section \textit{Thismia}) .................................................................................................................. 9
5. Outer perianth lobes less than 2 mm long or absent ............................................................................. 6
- Outer perianth lobes more 2 mm long ........................................................................................................ 8
6. Mitre without filiform appendages ........................................................................................................ section \textit{Sarcosiphon}
- Mitre with filiform appendages ................................................................................................................ 7
7. Central mitral appendages free from each other ..................................................................................... 8
- Central mitral appendages fused into a column ........................................................................................ 7
8. Outer perianth lobes with filiform appendages ............................................................................ section \textit{Glaziocharis}
- Outer perianth lobes without filiform appendages ................................................................................ section \textit{Rodwaya}
9. Free perianth lobes of similar shape and size .................................................................................... 9
- Free perianth lobes distinctly dissimilar in shape and size ..................................................................... subsection \textit{Brunothismia}

\textbf{INFRAGENERIC CLASSIFICATION OF GENUS THISMIA}


Type—\textit{Thismia macahensis} (Miers) Mueller (1891: 232). [basionym: \textit{Ophiomeris macahensis} Miers (1847: 329)].


[basionym: \textit{Ophiomeris} Miers (1847: 328)].

Type—\textit{Thismia macahensis} (Miers) Mueller (1891: 232). [basionym: \textit{Ophiomeris macahensis} Miers (1847: 329)].

Species included:
\begin{itemize}
  \item \textit{T. espiritosantensis} Brade, Revista Brasil. Biol. 7: 286 (1947).
  \item \textit{T. glaziovii} Poulsen, Rev. Gén. Bot. 1: 549 (1889).
  \item \textit{T. luettelfurungi} Goebel & Suess., Flora 117: 56 (1924).
  \item \textit{T. panamensis} (Standl.) Jonker, \textit{Monogr. Burmann.} 234 (1938).
\end{itemize}


Type—\textit{Thismia hyalina} (Miers) Benth. & Hook.f. ex Mueller (1891: 234). [basionym: \textit{Myostoma hyalimum} Miers (1866: 25)].

Species included:
\begin{itemize}
\end{itemize}


Type—\textit{Thismia caudata} Maas & Maas (1986: 162).

Species included:
\begin{itemize}
\end{itemize}
Thismia subg. Thismia

Type:—Thismia brunonis Griffith (1845: 221).

Thismia subg. Thismia sect. Thismia

Type:—Thismia brunonis Griffith (1845: 221).

Note: Originally published as “Euthismia” (Schlechter 1921: 34), but the prefix “Eu-” is no longer permitted (McNeill et al. 2012, Article 21.3).


Type:—Thismia brunonis Griffith (1845: 221).

Species included:


Type:—Thismia aseroe Beccari (1878: 252), designated here.

Species included:

- T. alba Holttum ex Jonker, Fl. Males. 4: 23 (1948).

Notes:—Thismia aseroe Becc. is selected as the type in accordance with Article 10.2 of the Melbourne Code (McNeill et al., 2012).


Type:—Thismia clandestina (Blume) Miquel 616 (1859: 616). [basionym: Sarcosiphon clandestinum Blume (1849: 65)].

Species included:


Species included:
- *T. nigricoronata* Kumar & S.W. Gale sp. nov.

**Notes:**—Warming (1901) described a single species, *Glaziocharis macahensis* Taub. ex Warm., in his genus *Glaziocharis*, but this was an illegitimate name [(Article 11.1, Melbourne Code (McNeill et al., 2012)] due to the prior existence of *T. macahensis* (Miers) Mueller (1891: 232) and is now considered a synonym of *Thismia caudata* Maas & Maas (1986: 162). The latter species possesses connate anther thecae, a character of subgenus *Ophiomeris*, and has hence been transferred to subgenus *Ophiomeris* as the type of section *Pyramidalis* (Maas & Maas 1986). When describing section *Glaziocharis*, Hatusima (1976) included three species, namely, *T. abei* (Akasawa) Hatus., *T. tuberculata* Hatus. and *T. macahensis* (Taub. ex Warm.) Hatus., of which two remain in the section and might therefore be considered as candidate types. In accordance with Article 10.2 of the Melbourne Code (McNeill et al., 2012), we designate *Thismia abei* (Akasawa) Hatus. as the type for this section because it clearly exhibits features characteristic of section *Glaziocharis* and due to the presence of a detailed description and illustration in the protologue.


Type:— *Thismia rodwayi* Mueller (1890: 115).

Species included:


Type:— *Thismia clavigera* (Becc.) Mueller (1891: 235) [basionym: *Geomitra clavigera* Beccari (1878: 251)], designated by Jonker (1938: 255).

Species included:

**Notes:**—Beccari established the genera *Bagnisia* (based on *B. crocea* Beccari (1878: 249)) and *Geomitra* (based on *G. episcopalis* Beccari (1878: 250) and *G. clavigera* Beccari (1878: 251)) in 1878, but Bentham & Hooker (1883) later suggested that the two were congeneric on account of both sharing a fleshy, leafless habit. Engler (1888) accordingly reduced *Geomitra* to sectional rank within *Bagnisia* but without designating a type, simultaneously making the new combination *B. episcopalis* (Beccari 1878: 250) Engler (1888: 48) and creating *B. sect. “Eubagnisia”* (not validly published: Art. 21.3) to accommodate *B. crocea*. Mueller (1891) subsequently transferred both *Geomitra* and *Bagnisia* to the genus *Thismia*, judging the difference in perianth lobe morphology that separates them (united in the former two, free in the latter) to be too minor for recognition at generic level. Jonker (1938) nevertheless treated *Geomitra* at genus level and selected *G. clavigera* as its type. Various authors have since either maintained *Geomitra* as a separate genus (Govaerts et al. 2007) or synonymised it under *Thismia* (Stone 1980; Merckx et al. 2006; Chantanaorrapint & Chantanaorrapint 2009; Hunt et al. 2014; Merckx & Smets 2014). Most recently, Merckx & Smets (2014) listed *Geomitra* as one of six sections within *Thismia* subg. *Thismia* but without formally making the combination. We therefore validate *Geomitra* as a section within *Thismia* here.
**Thismia** subg. **Thismia** sect. **Scaphiophora** (Schltr.) Kumar & S.W.Gale **comb. & stat. nov.** [basionym: **Scaphiophora** (Schlechter 1921: 39)].
Type:—**Thismia appendiculata** Schlechter (1918: 202) = **Scaphiophora appendiculata** (Schltr.) Schlechter (1921: 39).
Species included:

**Notes:** — Schlechter (1918) described the species *Thismia appendiculata* (Schlechter 1918: 203) from New Guinea. He later proposed the new genus *Scaphiophora* (Schlechter 1921: 39) along with the new combination *S. appendiculata* (Schlechter 1918: 202) Schlechter (1921: 39), highlighting the conlate appendages of the perianth lobes as one of the main characters to differentiate *Scaphiophora* from *Thismia* (in which the perianth lobe appendages are free). Jonker (1938) accepted the new genus and added one more species, *S. gigantea* (Jonker 1938: 257), which was later transferred to *Thismia* as *T. gigantea* (Jonker 1938: 257) Hroneš (2014: 55), following Merckx *et al.* (2013) and Merckx & Smets (2014). This new combination has been accepted and followed thereafter (Govaerts *et al.* 2007). Based on morphological and DNA analysis, Merckx & Smets (2014) informally reduced *Scaphiophora* to sectional rank within *Thismia* but without validly publishing the sectional name. It is therefore formally validated here.

**NEW SPECIES**

**Thismia nigricoronata** Kumar & S.W.Gale, **sp. nov.** (Figs. 4, 5)
Type:—LAOS. Vientiane Province: Vang Vieng District, Nam Pae Village, elevation 544 m, 28 April 2012, Gale, Kumar, Santainsy & Phunthavong HNL-KFBG 0099 (holotype: HNL, spirit).

*Thismia nigricoronata* is morphologically similar to *Thismia taiwanensis* but can be differentiated on the basis of its longer vestigial stem leaves (more than 6 mm long in the former versus less than 6 mm in the latter); its reflexed free outer perianth lobes (versus erect and projecting upwards in the latter); the ornamented outer surface of its perianth tube, which is verrucose below and papillose above (versus smooth and glabrous in the latter); and its vibrantly coloured perianth tube (versus translucent white in the latter).

Terrestrial, mycoheterotrophic herbs, erect, ca. 5.5 cm tall. Roots clustered, prostrate, unbranched, pale yellow-brown, vermiform, 3.2–7.5 × 0.8–1.5 mm, tapering towards apex. Stem erect, 10.3–14.5 × 1.2–1.5 mm at the base, ca. 2.5–3.0 mm wide near the apex; unbranched, off-white, somewhat translucent, covered with the bases of sheathing vestigial leaves. Leaves entire, white, translucent, shiny, scattered along the stem, obovate, upper ones 6.0–7.5 × 1.0–1.5 mm at base, lower ones up to 5.0–5.3 mm long, acute, margin smooth. Pedicel white, ca. 0.50 × 1.75 mm, finely and irregularly verrucose. Flowers solitary, actinomorphic, 25–40 mm long, 7.0–7.5 mm wide; tepals fused to form an urceolate perianth tube with a whorl of 3 inner and 3 outer free apical perianth lobes. Perianth tube obovoid, clearly divisible into upper and lower parts by the point of placement of the free perianth lobes; lower part of perianth tube ca. 4.5 mm long, narrower towards the base (ca. 4.5 mm in diameter), wider above (ca. 5.5 mm in diameter), white tinged yellow-green, densely covered with longitudinally arranged irregularly sized verrucae, with 12 depressed green ribs of which 6 alternate ribs run from the base of the tube to the mouth of the annulus on the upper half of the perianth tube, inner wall smooth with a protruding ring-like structure just below the stigma; upper part of perianth tube 3.5–4.0 mm long, wider at the base (ca. 5.5 mm in diameter), narrower at the annular opening (ca. 2.7 mm in diameter), bright yellow-green with 6 dark green ribs terminating at the annulus, covered with densely arranged papillae on the outer surface except on the ribs, inner wall smooth. Free part of outer perianth lobes white, translucent, triangular, 2.5–3.0 mm long, reflexed, ca. 1 mm wide at the base, gradually tapering. Free part of inner perianth lobes distinctly divisible into 2 parts: lower obliquely spatulate bases and upper flagelliform appendages; obliquely spatulate bases 3, black, rough and irregularly carunculate, ca. 1 mm wide at the point of attachment to the perianth tube, broadening above (ca. 4 mm wide) and then fused with one another in a contorted aestivation to form a crown-like structure arching over the annulus with a concavity in the centre and an aperture directly above the annulus aperture, surrounded by flagellate appendages that arise at the base of the concavity; flagelliform appendages 3, erect, immobile, black and carunculate towards the base, becoming translucent white and smooth above, 7.2–17.7 mm long, ca. 0.8 mm wide towards the base but abruptly narrowing towards the apex to less than 0.2 mm wide. Stamens 6, 4.8–5.5 mm long, 0.7–0.9 mm wide, quadrangular, filaments partially fused, arranged radially and forming a staminal tube that originates from the opening of the annulus at the base (ca. 1 mm in diameter) and is continuous with the connective towards the apex; suspended...
FIGURE 4. Thismia nigricoronata. A. Plant in habitat. B. Habit. C. Close-up of the crown. D. Whole plant showing the structure of the crown. E. Whole plant showing the structure of the annulus. F. Transverse section of the perianth tube. G. Dorsal view of the crown. H. Stamens showing the anther locules. I. Stamens showing the connectives and staminal tube.
FIGURE 5. Thismia nigricoronata. A. Whole plant in longitudinal section showing the internal parts (fa, flagellate appendage; ipl, free part of inner perianth lobe; an, annulus; upt, upper part of perianth tube; al, anther locule; opl, free part of outer perianth lobe; co, connective; lpt, lower part of perianth tube; th, thecae; st, stigma). B. Whole plant showing the mitre. C. Whole plant showing the upper part of perianth tube. D. Staminal tube showing anther locules and connectives. E. Longitudinal section of the perianth tube showing stamen and ovary. F. Mitre. (Drawn by P. Kumar from preserved specimen HNL-KFBG 0099.)
above the stigma; connectives laterally connate and forming a narrow opening (0.4–0.5 mm in diameter); anthers ca. 2.5 × 0.5 mm wide, mounted on fused connectives, thecae free, positioned ca. 2.5 mm below the annulus, opening with longitudinal slits facing the inner wall of the perianth tube, covered with sparse glandular trichomes especially along the longitudinal slit; connectives protruding ca. 0.75 mm beyond the anther locules with the apical 1/3 bent at 90° towards the inner wall of the perianth tube, sparsely covered with multicellular cilia. Ovary inferior, unilocular, obconical, outer wall longitudinally verrucose, ca. 3.5 mm long; style cylindrical, ca. 0.75 mm long, ca. 0.70 mm wide; stigmas 3, fused together and forming a dome-like structure, ca. 1 mm long and wide.

Flowering:—April, fruits not seen.

Habitat:—Thismia nigricoronata was discovered on a steep slope of a limestone mountain, growing among leaf litter in clayey soils under a dense evergreen canopy.

Etymology:—The species epithet refers to the black, crown-like structure formed above the annulus by the fusion of the three inner perianth lobes. Hence the taxon may informally be referred to as ‘the black-crowned thismia’.

Specimens examined:—LAOS. Vientiane Province: Vang Vieng District, Nam Pae Village, elevation 544 m, 28 April 2012, Gale, Kumar, Santainsy & Phunthavong HNL-KFBG 0099 (HNL, spirit).

Taxonomic notes:—With diagnostic characters including the free anther thecae, the fused inner perianth lobes that form a mitre with prominent outer perianth lobes and long filiform appendages, there is little doubt that Thismia nigricoronata is presently best placed in section Glaziocharis. However, as noted above, the circumscription of this (and other sections) requires reappraisal, with phylogenetic analysis placing the morphologically distinct T. huangii, a member of section Rodwaya, as sister to our new species. Additionally, it is noteworthy that T. nigricoronata is the only species known so far in section Glaziocharis to have such a vibrantly coloured perianth tube, with that of all other previously described members of the section (namely, T. abei, T. clavarioides, T. taiwanensis and T. tuberculata) being whitish and somewhat translucent. Further study is required to assess whether or not this pigmentation is related to photosynthetic activity.

Conservation assessment:—Only ca. 10 individuals were observed at the site at a single locality on the lower slopes of a limestone mountain in Nam Pae Village of Vang Vieng District in central Laos. Thismia nigricoronata is an inconspicuous plant not easily observed in the field due to its small size and short flowering period. However, the area continues to be subjected to intense local pressures in the form of forest clearance for agriculture on the plains and selective logging on steeper slopes, cattle grazing and limestone mining for cement factories. Habitat destruction is therefore regarded as a very real threat to the long-term persistence of T. nigricoronata in this area. Although we recommend more surveys to confirm its occurrence in adjacent areas, we assess T. nigricoronata as critically endangered (B1+B2ab(iii); D) (IUCN 2012). The discovery of this apparently highly restricted endemic in the limestone karst landscape of central northern Laos underscores the need to ensure better protection of this biologically invaluable but highly threatened ecoregion.

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REFERENCES

https://doi.org/10.2307/4109280
https://doi.org/10.1111/boj.12385


**Thismia nigricoronata**, a new species of Burmanniaceae

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**quas in itinere ad insulas maris Australis: collegerunt, descripserunt, delinearunt, annis 1772-1775. White, Cadell & Elmsly, London, pp. 69–70.**

https://doi.org/10.5962/bhl.title.4448


https://doi.org/10.11646/phytotaxa.105.1.4


https://doi.org/10.1111/j.1759-6831.2009.00037.x


https://doi.org/10.3897/phytokeys.46.8963


https://doi.org/10.1093/bioinformatics/btg180


https://doi.org/10.7751/telopea20147809


https://doi.org/10.3897/phytokeys.46.8963


https://doi.org/10.1093/aob/mcq207


https://doi.org/10.1111/j.1091-0031.2008.00241.x


https://doi.org/10.1086/674315


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**THISMIA NIGRICORONATA, A NEW SPECIES OF BURMANNIACEAE**

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Seemann, B. (1866) *Tecca parkeri*. *Flora vitiensis: a description of the plants of the Viti or Fiji islands, with an account of their history, uses, and properties* 1: 102.


