New or Noteworthy Plant Collections from Myanmar (9)
Agapetes (Ericaceae) from Northwestern Myanmar

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As the result of field explorations to northwestern Myanmar, a total of five species of the genus \textit{Agapetes} (Ericaceae) were collected. Of these, two species were hitherto undescribed taxa. They were recorded with citation of voucher specimens and a photograph of each species was provided. Two new species were described as \textit{Agapetes oxycoccoides} and \textit{A. pentastigma}. \textit{Agapetes oxycoccoides} is similar to \textit{A. bracteata}, but distinguished by the corymbose inflorescence without distinct bract, and more strongly reflexed longer corolla lobes. \textit{Agapetes pentastigma} is morphologically similar to \textit{A. hillii}, but differs in having a greenish-yellow collora with ladder-like red stripes, and a short peduncle.


\textbf{Key words:} \textit{Agapetes oxycoccoides}, \textit{Agapetes pentastigma}, Myanmar, new species, phylogeny.

The genus \textit{Agapetes} D. Don ex G. Don is assignable to the tribe \textit{Vaccinieae} Rchb. of the subfamily \textit{Vaccinioideae} Arn. (Ericaceae). The past classification treated the species from New Guinea and the SW Pacific as \textit{Agapetes} (Airy Shaw 1958). The species, however, except \textit{A. scorchtchini} (King & Gamble) Sleumer, of which taxonomic status has been in question, are currently placed in \textit{Paphia} Seeman (Kron et al. 1999, Stevens 2004). \textit{Agapetes}, holding about 80 species at present, is distributed from the Himalayas to China, and Indo-China to Southeast Asia (Fang and Stevens 2005, Watthana 2012, Tong and Xia 2014). In morphological and anatomical characters, \textit{Agapetes} is closely related to \textit{Vaccinium} from SE Asia (Stevens 1985). The molecular phylogeny indicated that \textit{Agapetes} species nested among \textit{Vaccinium} species, cf. \textit{V. gaultheriifolium} (Griff.) Hook. f. and \textit{V. nummularia} Hook. f. & Thomson, although only three \textit{Agapetes} species, \textit{A. buxifolia} Nutt. ex Hook. f., \textit{A. hosseana} Diels, and \textit{A. serpens} (Wight) Sleumer, were analyzed (Kron et al. 2002). Currently it is thought that many species of \textit{Agapetes} are part of the same lineage as many SE Asian-Malesian \textit{Vaccinium}
Field expeditions to Kachin State, northwestern Myanmar to update the flora of Myanmar were carried out. Northwestern Myanmar is known for its wealth of plant species diversity. Our previous works revealed many new records and also new taxa based on these northwestern excursions (Tanaka et al. 2006, Tanaka and Hughes 2007, Tanaka and Hayami 2011, Tanaka et al. 2016).

Differences in the amount of rainfall in the dry and rainy seasons are remarkable in Myanmar, and it is presumed that plants there are adapted to the environment by having a characteristic life cycle. The genus Agapetes with its characteristic storage rootstock may be one of such a kind of plant, and it has apparently diversified in Myanmar. Including twenty new species that were previously described from Myanmar by Airy Shaw (1935, 1948, 1959, 1960a, 1960b), Kress et al. (2003) listed 54 species of Agapetes from Myanmar. Recently Tong and Xia (2014) added two new taxa, A. putaoensis Y. H. Tong & N. H. Xia and A. wardii W. W. Smith var. heterotricha Y. H. Tong & N. H. Xia to the flora of Myanmar, for a total of 56 taxa to date. However, many of them were known from fragmentary information based on the type and few additional herbarium specimens. Observation on living plants in the field is desirable for a taxonomic and phylogenetic revision of Agapetes and related species. The identifications of the specimens collected by our fieldworks led us to recognize five species of Agapetes (Ericaceae) from the field of northwestern Myanmar. Based on morphological and phylogenetic investigations two of them were recognized as new to science. We describe these two species as A. oxycoccodes and A. pentastigma in this study.

Molecular phylogenetic analysis was also applied to understand the relationship of these species among the related taxa. It suggested that A. oxycoccodes was related to A. setigera D. Don ex G. Don, distributed in India and Myanmar, and A. pentastigma was close to A. odontocera (Wight) Hook. f., distributed in E India.

Materials and Methods

Plant materials

Field excursions to the Hukaung Valley Tiger Reserve, Kachin State, northwestern Myanmar have been conducted twice, in 2005 and 2007. The Hukaung Valley is one of the largest conservation sites in the country, but it remains floristically poorly known. The valley is a flat alluvial plain measuring about 80 km north–south by 50 km east–west, surrounded on all sides by hills (Cruickshank and KoKo 2003).

A total of five species of Agapetes were collected in the evergreen tropical lowland forest in Shinbweyang, northwest of Tanaing, Hukaung Valley, and they were morphologically investigated. Voucher specimens were deposited in TI, TNS and RAF.

DNA sequencing and phylogenetic analysis

For molecular phylogenetic analyses, 24 species and one variety of Agapetes, including A. scortechinii, eight representative species of Vaccinium including species that are morphologically and phylogenetically related to Agapetes (V. gaultheriifolium and V. nummularia; Kron et al. 2002), and two species of Paphia were collected (Appendix 1). Considering the phylogenetic relationship of Vaccinieae, Andromeda polifolia L. and Dimorphantha kempteriana Schltr. were used as outgroups. Total genomic DNA was extracted from silica-gel-dried leaf tissue, using the modified HEPES-CTAB method described by Ohi-Toma et al. (2010).

Nucleotide sequences of the chloroplast trnK intron (including the matK gene) were determined using polymerase chain reaction (PCR) and direct bi-directional sequencing. PCR amplification was conducted using TaKaRa ExTaq (TaKaRa Bio, Shiga, Japan), with the following cycling conditions:
denaturation at 96°C for 45 sec, followed by 33 cycles at 96°C for 45 sec, annealing at 50°C for 45 sec, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. For amplifying and sequencing the trnK intron, we used newly designed primers in addition to published universal primers (Table 1). Amplification products were purified using illustra ExoProStar (GE Healthcare UK Ltd., Buckinghamshire, England, UK). The purified PCR fragments were amplified using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA). Cycle-sequencing reactions were performed using the same primers for the PCR amplification and newly designed primers for sequencing. Complementary strands of the sequenced regions were assembled and edited using ATGC v. 4.2 (GENETX Co., Tokyo, Japan). All newly generated nucleotide sequences were deposited in the DNA Data Bank of Japan (DDBJ) and their accession numbers are shown with sample information in Appendix.

The nucleotide sequences were manually aligned and gap coding was employed as described in Ohi-Toma et al. (2010). The coded gap-states were used as a fifth character; however, the length polymorphisms caused by mononucleotide repeats [poly(A) or poly(T)] and other unalignable portions were excluded from gap coding. In the data matrix, when substitutions or overlapping gaps occurred at gap positions, the differences were indicated by adding ‘n’ to the position of the substitution and/or gap-state. In order to use coded gaps, Maximum Parsimony analysis was applied using PAUP* v. 4.0a149 (Swofford 2002). Nucleotide substitutions and coded gaps were given equal weights, and the tree search was performed using the branch-and-bound search option with MulTrees in effect. The strict consensus tree of the most parsimonious trees was generated, and changes of substitutions and gaps were reconstructed on the tree using ACCTRAN character optimization. The MP bootstrap support (Bs) values were estimated using a heuristic search of 10,000 replicates, with simple sequence addition, TBR branch swapping, MulTrees in effect, and no limit of MaxTree.

**Results and Discussion**

**Morphological characters**
As the result of the morphological investigation, five species were recognized. Three were identified as *A. burmanica* W. E. Evans, *A. hillii* Brandis (Fig. 4) and *A. setigera* D. Don ex G. Don (Fig. 5), however, the other two taxa did not match any other species known from Myanmar and neighboring regions according to studies of related previous literatures (Airy Shaw 1935, 1948, 1959, 1960a and 1960b, Stevens 1985, Fang and Stevens 2005, Watthana 2012). These species are newly described as *A. oxycocoides* and *A. pentastigma*. 

![Fig. 1](image-url) Strict consensus tree of 20 most parsimonious trees, based on chloroplast DNA sequences of *trnK* intron including the *matK* gene (tree length = 477, CI = 0.8532, RC = 0.7508). Branch lengths were estimated based on ACCTRAN character optimization and a scale bar (five site change) is shown. Numbers on branches are bootstrap values (over 50%). Generic names of *Agapetes* and *Vaccinium* are abbreviated as AGA and VAC, respectively. Two new species are indicated in bold. Thick branch is the AGA-VAC clade.
Fig. 2. *Agapetes oxyccocoides* J. Murata, Nob. Tanaka & Ohi-Toma. A. Habit. B. Vertical section of flower showing style, stigma, and ovary. C. Side view of flower. D. Front view of flower. E. Stamens with pubescent flat filaments and connate anther tubules. Scale bar: 2 cm for A, 5 mm for B–E.
in the taxonomic treatment.

Morphology of the stigma may be one of the important characters for Agapetes identification based on our extensive observations on the Myanmar Agapetes specimens. As previously recognized (Fang and Stevens 2005), the stigma shape of Agapetes are divided into two types: 1) small, punctate, inconspicuous stigmas, and 2) thickened capitate, conspicuous stigmas, and they could be a useful character for identification. In addition to our new species, Agapetes pentastigma, A. sikkimensis Airy Shaw, A. setigera, A. hillii, and A. odontocera also have the latter type of stigma.

**Phylogenetic relationship**

The aligned sequence length of trnK intron (including the matK gene) for all samples was 2,564 bp, including 11 coded gaps. The maximum parsimony analysis resulted in a strict consensus tree from 20 most-parsimonious trees with tree length of 477 (CI = 0.8532, RC = 0.7508). Their strict consensus tree with branch lengths was generated (Fig. 1).

The phylogenetic tree indicated that Agapetes is not monophyletic because a part of Vaccinium species were nested in the same clade. Most species of Agapetes belonged to the clade with several Vaccinium (the AGA-VAC clade), but A. scortechinii, the only species of Agapetes section Pseudoagapetes (Airy Shaw 1958), is outside of the clade. As it has already been pointed out (Stevens 2004), this species should be treated under different genus.

In the AGA-VAC clade, A. pentastigma is most closely related to A. odontocera and the two species formed a clade with A. sikkimensis. These species share thickened capitate, conspicuous stigmas. Agapetes oxyccoides forms a clade with A. setigera, but the nucleotide variations between them are large. Agapetes oxyccoides is morphologically similar to A. bracteata Hook. f. ex C. B. Clarke but differs in the corymbose inflorescence without distinct bracts, a much more strongly reflexed corolla, and pseudoverticillate leaves.

Epiphytic shrub up to ca. 1 m tall with subcylindrical woody tubers, adnate to the tree trunk; branches terete, slightly reticulately fissured when old, pubescent. Leaves spirally aggregated at the top of branches (pseudoverticillate); petiole 3–4 mm long, glabrous; blades ovate-elliptic, 4–6 cm long, 2.0–2.7 cm wide, glabrous, veins brochidodromous, midveins raised on lower surface, secondary veins 3–5 pairs, with fine veins conspicuous on both surfaces, base attenuate, margin entire, revolute, apex obtuse. Inflorescences axillary or on leafless stem, 1–6-flowered; peduncle 3.5–4.5 mm long; bracts small, inconspicuous; pedicels spreading, slender, 2–2.5 cm long, pubescent. Flowers nodding, 1–1.2 cm long, 1 cm wide. Calyx tube reddish, widely campanulate, 3.5–4.0 mm across; lobes whitish, triangular, ca. 1.5 mm long, apex acute. Corolla white, lobed more than half its length; lobes triangular-lanceolate, 6–7.5 mm long, strongly reflexed at anthesis. Stamens 10, 1–1.2 cm long; filaments flat, ca. 1 mm long, white, pubescent; thecae maroon, ca. 3 mm long.
papillate; tubules brownish yellow, 6–6.5 mm long; spurs undeveloped. Style white, glabrous, ca. 7.5 mm long, base greenish, slightly exerted from connate anther tubules; stigma punctate. Fruit unknown.

Distribution: Thus far known only from the type locality.

Etymology: The specific epithet is derived from the resemblance of flower shape to *Vaccinium oxycoccus* L. in having a strongly reflexed corolla.

Note: The phylogenetic analysis (Fig. 1) indicated that this species was related to *A. setigera* D. Don ex G. Don, but *A. setigera* is very much distinct in having racemose inflorescences with glandular hairs, a tubular and orange-red corolla, and a capitate stigma. Other similar species are compared in Table 2, although they were not available for our molecular phylogenetic analysis.

*Agapetes pentastigma* J. Murata, Nob. Tanaka & H. Murata, sp. nov. [Fig. 3]

**Type:** MYANMAR. Kachin State; along the Ledo Road, between Namyung and Shinbweyang, 5–7 miles from Shinbweyang toward Namyung, in border area of Sagaing Region, Hukaung Valley Tiger Reserve, 26°42'32"N, 96°11'55"–13'01"E, cultivated in the greenhouse of Medicinal Plant Garden, Setsunan University, H. Murata & J. Murata 1402 (TI–holotype, RAF, TNS–isotype).

*Agapetes pentastigma* is similar to *A. hillii* Brandis distributed in India and Myanmar but differs in having a greenish-yellow collora with ladder-like red stripes (vs. V-shaped pattern), and 1/2 divided calyx lobes (vs. deeply divided nearly to the base), shorter peduncle and longer slender pedicels (vs. thckend at apex end).

Epiphytic shrub up to 1.5 m high, with sub-cylindrical woody tubers, adnate to the tree trunk; branches suberete. Leaves spirally arranged on top of branches; leaf blade oblong-lanceolate, 15–18 cm long, 4–6.2 cm wide, dull yellow green on upper surface, whitish green beneath, apex acuminate, base attenuate, margin inconspicuously and distantly sinuous, glabrous on both surfaces, leathery, sessile; midrib prominent above, raised on lower surface; secondary veins 20–25 pairs. Inflorescence corymbose, 4–7 flowered, leaf-axillary; peduncle very short, glabrous; pedicel 2.5–3 cm long, articulate at both ends, grooved; bracts attached at the base of the pedicel, lanceolate,
Fig. 3. *Agapetes pentastigma* J. Murata, Nob. Tanaka & H. Murata. A. Habit. B. Close view and vertical section (right) of flowers showing stamens and stigma. C. Front view of flowers. D. Close view of thickened capitate stigma with pentagonal appearance at the top. Scale bar: 5 cm for A, 2 cm for B–C, 3 mm for D.
apex attenuate, 1.5–2 mm long, glandular hairs at margin. Calyx tube yellowish-green, tubular, 4.5–5 mm long, 5–7 mm wide, glabrous; calyx lobes 5, lobes 1/2 of the length, triangular, 5–6 mm long, reddish, apex attenuate. Corolla tubular, olive green with red ladder-like pattern consisting of longitudinal stripes and transverse bands, 4.5–5 cm long, 1–1.5 cm wide, glabrous; lobes olive green, triangular-lanceolate, 7–8 mm long, reflexed at anthesis. Stamens 10, 4–4.5 cm long; filaments flat, ca. 5.5 mm long, white, pubescent; thecae reddish brown, 6.5–7 mm long, papillate; tubules brownish yellow, 4–4.5 cm long. Style reddish-white, glabrous, 4–5.5 cm long. Style reddish-white, glabrous, 4–5.5 cm long.
### Table 3. Comparison of *Agapetes pentastigma* with its related species

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>A. pentastigma</em></th>
<th><em>A. odontocera</em></th>
<th><em>A. burmanica</em></th>
<th><em>A. hillii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>oblong-lanceolate</td>
<td>lanceolate</td>
<td>oblong-lanceolate</td>
<td>lanceolate</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>corymbose, axillary on young leafy stem</td>
<td>small corymbose, on leafless old stem</td>
<td>corymbose, on leafless old stem</td>
<td>corymbose, terminal</td>
</tr>
<tr>
<td>Peduncle</td>
<td>very short, glabrous</td>
<td>almost lacking</td>
<td>almost lacking, glabrous</td>
<td>glabrous</td>
</tr>
<tr>
<td>Pedicel</td>
<td>glabrous</td>
<td>puberulous</td>
<td>glabrous</td>
<td>glabrous</td>
</tr>
<tr>
<td>Calyx</td>
<td>divided to 1/2, lobes triangular, reddish green</td>
<td>divided to 1/2, lobes triangular</td>
<td>shortly lobed, lobes triangular, red</td>
<td>deeply divided nearly to the base, lobes narrowly triangular</td>
</tr>
<tr>
<td>Corolla</td>
<td>about 4 cm long, greenish yellow with red longitudinal stripes and red transverse (non zig-zag) bands</td>
<td>about 1.5 cm long when dry, (reddish yellow with red transverse zig-zag bands)</td>
<td>about 6 cm long, pink with red transverse zig-zag bands</td>
<td>about 5 cm long, greenish-yellow with red V-shaped patterns</td>
</tr>
<tr>
<td>Corolla lobes</td>
<td>green</td>
<td>green</td>
<td>green</td>
<td>green</td>
</tr>
<tr>
<td>Stigma</td>
<td>capitate, green</td>
<td>punctate, brownish yellow</td>
<td>punctate, green</td>
<td>capitate, green</td>
</tr>
</tbody>
</table>

108 cm long, exerted ca. 4 mm from the connate anther tubules; stigma capitate, 3–3.5 mm wide, green. Fruit unknown.

**Distribution:** Thus far known only from the type locality.

**Etymology:** The specific epithet is derived from its representative character, a large thickened capitate stigma with pentagonal appearance at the top.

**Note:** The phylogenetic analysis (Fig. 1) indicated that, among the species examined, this species was closest to *A. odontocera* Benth. & Hook. f. distributed in India. Morphologically, however, this species is easily distinguished from the latter by having much larger flowers and a distinctly capitate stigma. Other similar species are compared in Table 3, although some of them were not available for our molecular phylogenetic analysis.

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**Other collected taxa from northwestern Myanmar**


Specimen examined: MYANMAR. Kachin State. East of Shinbweyang, in border area of Sagaing Region, Hukaung Valley Tiger Reserve, J. Murata & al. 041355 (MBK), and H. Murata 1601 (TI, TNS, RAF).

**Distribution:** China and Myanmar.

**Note:** This species was described from N.E. Burma based on the Forrest specimen (Htawgaw, G. Forrest 24981, E). The plant collected in northwestern Myanmar is slightly different from the typical *A. burmanica* in having very short corolla lobes with rounded apex. To clarify the range of variation of calyx shape in *A. burmanica* further observations in the field are required.


Specimen examined: MYANMAR. along the Ledo Road, between Namyung and Shinbwyang, 5–7 miles from Shinbwyang toward Namyung, in border area of Sagaing Region, J. Murata & al. 041479 (MBK, TI).

**Distribution:** India and Myanmar.


Specimen examined: MYANMAR. Kachin State.
Along the Ledo Road, near Shinbweyang, in border area of Sagaing Region, Hukaung Valley Tiger Reserve, cultivated in the greenhouse of Koishikawa Botanical Garden, J. Murata & T. Ohi-Toma 1602 (TI, RAF).

Distribution: India and Myanmar.

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**Appendix**

List of samples used in the phylogenetic analysis (the species, source, voucher specimen, and DDBJ/GenBank accession number of trnK intron).

特徴である。その中で、Agapetes oxycoccoides

果、このうち

態比較および分子系統解析による近縁種の検討の結

田中伸幸、大井・東馬哲雄、邑田裕子、M. M. Aung、

・：マンマーレ植物についての新知見(9)

著者らが実施してきたマンマーレ部の植物調査
によりツツジ科Agapetes属植物5種が採集された。形
態比較および分子系統解析による近縁種の検討の結
果、このうち2種とは新縁であることが明らかとなった
ので記載・発表する。Agapetes oxyccocoides J. Murata、

邑田 & H. Murataはツツジ科 Vaccinium oxyccocoides

に似た花を苞が発達しない散房花序につける

ことが特徴である。Agapetes pentastigma J. Murata、 Nob.

A. sikkimensis Airy Shaw, Bhutan: Cultivated in Royal

Botanic Garden Edinburgh (19842032), Sinclair & Long

5778 (E), LC168869. A. smithiana Sleumer, Cultivated

in Sakuya Konohana Kan, J. Murata s.n. (TI), LC168870.

A. thailandica Watthana, Thailand: Cultivated in Royal

Botanic Garden Edinburgh (19991631), Argent 13 (E),

LC168871. A. variegata D. Don ex G. Don, cultivated in

Royal Botanic Garden Edinburgh (19696068), Conlon

C124 (E), LC168872. Andromeda polifolia L., Japan:

Cultivated in Koishikawa Botanical Garden, University of

Tokyo, T. Ohi-Toma s.n. (TI), LC168873. Dimorphantha

kemperiana Schltr., cultivated in Kyoto Botanical Garden,

J. Murata s.n. (TI), LC168874. Paphia meiniiana (F.

Muell.) Schltr., Australia: Cultivated in San Francisco

Botanical Garden (2001-0421), Bourell s.n. (CAS),

LC168875. P. stenantha Schltr., Papua New Guinea:

Milne Bay, cultivated in Royal Botanic Garden Edinburgh

(19762042), Woods 2521 (E), LC168876. Vaccinium

ammanianum Hatus., Japan: Amami, cultivated in

Koishikawa Botanical Garden, University of Tokyo, T. Ohi-

Toma s.n. (TI), LC168877. V. bulleyanum (Diels) Sleumer,

cultivated in South China Botanical Garden, Liao s.n.

(IBSC), LC168878. V. fragile Franch., China: Cultivated in

Koishikawa Botanical Garden, University of Tokyo, T. Ohi-

Toma s.n. (TI), LC168879. V. gaultheriifolium Hook. f. ex

C.B.Clarke, Nepal: Cultivated in University of California

Botanical Garden, Edinburgh Makalu Expedition (1991)

778 (E), LC168880. V. leucobotrys (Nutt.) G. Nicholson,
cultivated in South China Botanical Garden. Liao s.n.

(IBSC), LC168881. V. nummularia Hook. f. & Thomson

ex C. B. Clarke, cultivated in Sakuya Konohana Kan. T.

Ohi-Toma s.n. (TI), LC168882. V. oxyccocoides L., Japan:

Nagano, J. Murata s.n. (TI), LC168883. V. scopulorum W.

W. Sm., cultivated in South China Botanical Garden, Liao s.n.

(IBSC), LC168884.

田中伸幸*: 大井・東馬哲雄*, 邑田裕子*, M. M. Aung*
邑田 仁**: マンマーレ植物についての新知見(9)

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****マンマーレ・林業省野生生物保護局
