Phytotaxa 207 (3): 265–272
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.207.3.4

Two new species of *Aspidistra* (Asparagaceae, Nolinoideae) from northern Vietnam: *A. clausa* and *A. triradiata*

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Abstract

Aspidistra clausa and *Aspidistra triradiata* are described and illustrated as new species from northern Vietnam (Vinh Phuc province, Tam Dao National Park). *Aspidistra clausa* is similar to *A. crassifila*, but has narrower leaves (lamina 2.8–4.5 cm vs. 6–12 cm), perigone tube wide tubular vs. campanulate, appendages longer, stigma conical vs. mushroom-shaped. *Aspidistra triradiata* is similar to *A. hainanensis*, but flowers larger (1.8–2.4 cm long, Ø 1.8–2.8 cm vs. 1–1.2 cm long, Ø 1.3–1.5 cm), perigone urceolate, lobes with 4 verrucose keels, stigma with three purple radial lines.

Key words: DNA barcoding, Tam Dao, taxonomy

Introduction

Aspidistra Ker Gawler (1822: 628) is a large genus of herb plants growing in tropical forests of SE Asia. The number of species in genus rapidly increased during a few last decades (Tillich 2005). Currently the genus comprises more than 130 species (Averyanov & Tillich 2014b) inhabiting China, India, Japan, Laos, Malaysia, Thailand (Blume 1834, Hooker 1892, De Wilde & Vogel 2005, Tillich 2005, Phonsena & De Wilde 2010, Averyanov & Tillich 2014b, Vislobokov *et al.* 2014b), and at least 48 species in Vietnam (Averyanov & Tillich 2014a, 2014b, Tillich 2014, Vislobokov *et al.* 2014c). Also a few species known from Tam Dao National Park in northern Vietnam (Tillich 2005). So *A. bicolor* Tillich (2005: 317) and *A. subrotata* Wan & Huang (1987: 223) var. *crassinervis* Tillich (2005: 322) Phonsena in Phonsena & De Wilde (2010: 53) were described by H.-J. Tillich from specimens collected by J. Bogner from northeastern side of Tam Dao ridge (Thai Nguyen province). N.N. Arnautov (2002) found in the same part of Tam Dao (Thai Nguyen province) plants which he recognized as *A. hainanensis* Chun & How (1977: 533), but later H.-J. Tillich (2005) considered it as *A. carnosa* Tillich (2005: 318).

Considering the extremely high diversity of flower morphology, *Aspidistra* is interesting for investigation of flowering biology and pollination system (Tillich 2005, Vislobokov *et al.* 2013, Vislobokov 2014). Some studies show that flowers of *Aspidistra* are pollinated by tiny soil invertebrates (Kato 1995, Conran & Bradbury 2007). According to recent investigations myiophily occurs in some species of *Aspidistra* (Vislobokov *et al.* 2013, 2014a).

Application of methods of molecular phylogeny is still unsuccessful for *Aspidistra* (Kocyan & Renner 2007), despite high flower diversity within the genus. Otherwise a potentially useful tool for identification of plant material is molecular barcoding (e.g., Filipowicz 2012, González Gutiérrez *et al.* 2013). Two DNA regions (plastid *psb*A-*trn*H region and the nuclear 5S-NTS region) were tested for barcoding in *Aspidistra* (Vislobokov *et al.* 2014b, 2014c). These regions were found useful at a specific level in other angiosperms (e.g. Pornpongrungrueng *et al.* 2009, Degtjareva *et al.* 2012).

In the present paper two new species of *Aspidistra* are described from northern Vietnam and molecular diagnoses of new species are provided in comparison with other species of *Aspidistra* for which molecular data are available.

Accepted by Lorenzo Peruzzi: 18 Apr. 2015; published: 11 May 2015

Material and Methods

The new species were collected during field work in Vinh Phuc province of Vietnam. Living plants, herbarium, and liquid material were collected in November 2014. Fixed material of reproductive structures was collected in 70% ethanol. Living plants from type locality were also studied in cultivation (*N.A. Vislobokov TD14AC01* for *A. clausa*; *N.A. Vislobokov TD14AH01* for *A. triradiata*). In the morphological description, mean values are given in square brackets after ranges of variation of quantitative characters. These data are based on 7–12 measurements of leaf characters, 3–11 measurements of floral and fruit characters.

The *psb*A-*trn*H and 5S-NTS sequences of two new species were generated (see Appendix) and added to the already existing alignment of Vislobokov *et al.* (2014b, 2014c). The methods used to obtain the *psb*A-*trn*H and 5S-NTS regions have been described previously (Vislobokov *et al.* 2014b).

Description of the new species

***Aspidistra clausa* N.Vislobokov *sp. nov.* (Fig. 1)**

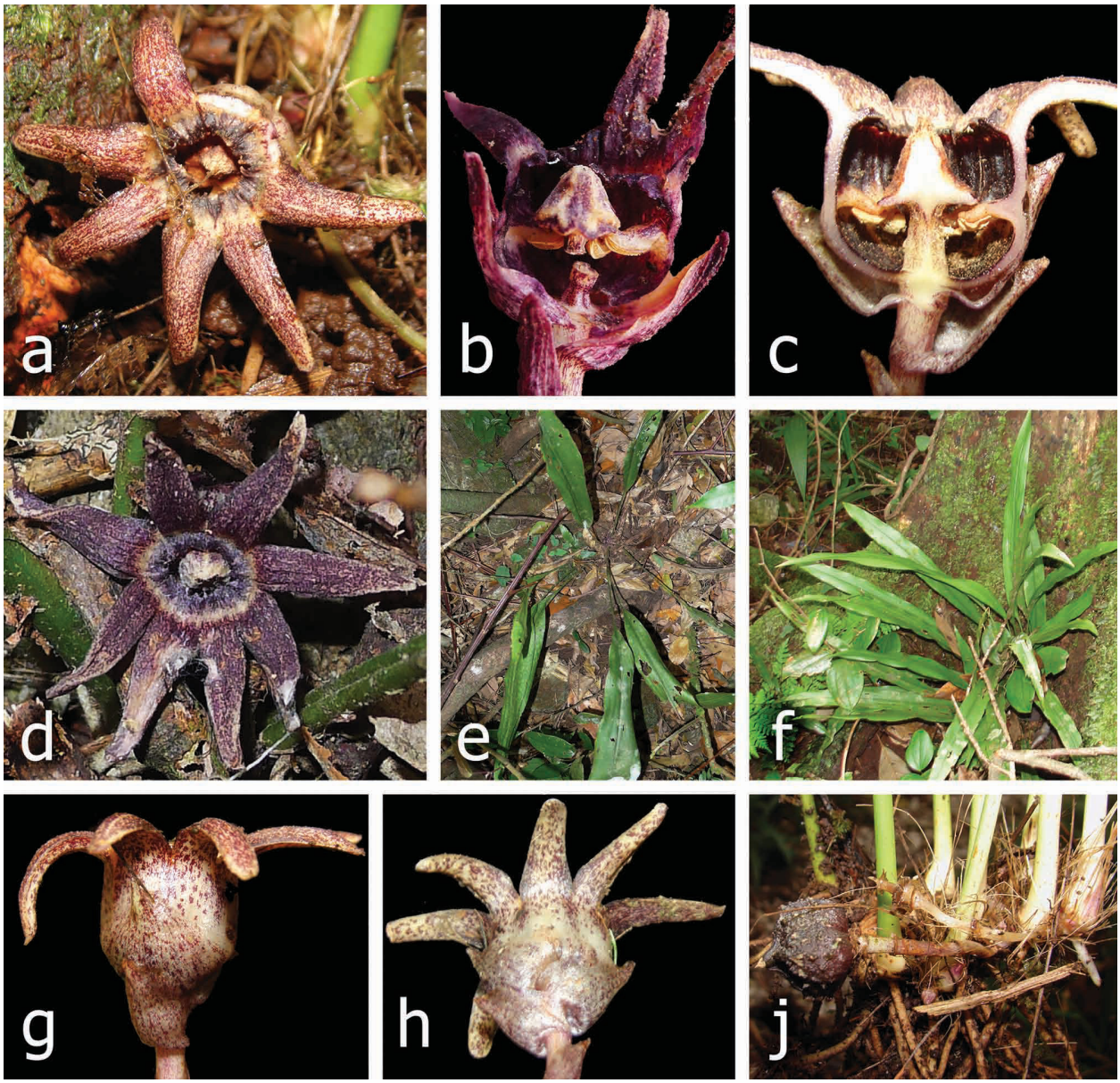

FIGURE 1. *Aspidistra clausa*. a, d. flowers, top view; b. longitudinal section of flower, style cross-dissected; c. longitudinal section of flower; e, f. flowering plant; g. flower, side view; h. flower, view from below; j. rhizome with fruit. (Images b, d, e provided by Olivier Colin).

Similar to *A. crassifila*, but leaves narrower (lamina 2.8–4.5 cm vs. 6–12 cm), perigone tube wide tubular vs. campanulate, appendages longer, stigma conical vs. mushroom-shaped.

Type:—VIETNAM, Vinh Phuc province, Tam Dao district, Tam Dao National Park, about 3 km NW from Tam Dao City. 21° 28,405' N, 105˚ 38,351' E, alt. 978 m, rainforest, 19 November 2014, *N.A. Vislobokov 14097* (holotype MW!, including reproductive organs in liquid collection at Moscow University).

TABLE 1. Differences between investigated species of *Aspidistra* found in the alignment of sequences of *psb*A-*trn*H region. For details on species other than *A. clausa* and *A. triradiata*, see Vislobokov *et al.* (2014b, 2014c).

Taxon	Positions in the alignment			
	90–106	108	120–125	281
A. clausa	-	C	indel 2	G
A. triradiata	-	C	indel 2	G
Aspidistra sp. 1	-	C	indel 2	G
A. xuansonensis	-	C	indel 2	A
A. phanluongii	indel 1	T	indel 2	G
A. paucitepala	-	C	-	G

indel1: ACAAGGTTTCTCCCCCG; indel2: AGTATC

Herbaceous perennial, rhizomatous, evergreen plant. Rhizome creeping, with very short internodes, epigeous, Ø 5.4–7 [6.4] mm. Roots grey, Ø 1.1–1.4 [1.3] mm, with 1–2-layered velamen, root hairs deciduous, endoderm 1–2-layered, pericycle of cells with lignified walls. Rhizomes with regularly repeating units, each comprising ca. 4 distichously arranged cataphylls followed by a foliage leaf. Cataphylls oblong, up to 9 cm long, promptly withering and disintegrating. Foliage leaves distinctly divided into petiole and lamina. Petiole green, adaxially sulcate, 15–32 [23.8] cm long, Ø 2.2–3.2 [2.7] mm. Lamina light green, oblong, basally cuneate and distally acuminate, 26–41 [35.8] cm long, 2.8–4.5 [3.6] cm wide. The midvein somewhat prominent at lower surface. Peduncle (specialized reproductive shoot) white, purple spotted, 2.8–4.6 [3.7] cm long, Ø 2.4–2.8 [2.5] mm, with 5–7 purple mottled, widely ovate, acuminate, 11.8–13.4 [12.7] mm long, 8.9 mm wide distichously arranged scale leaves. Flower solitary at the end of peduncle. Perigone 18–18.3 [18.2] mm long, Ø 38.7–42.5 [40.5] mm; tube white with purple spots outside, purple inside, widely tubular, 12.9–14.6 [13.3] mm long, Ø 14.6–15.1 [14.8] mm; lobes 8, purple mottled to completely purple at both sides, triangular-lanceolate, with appendages at the base; appendages purple, protruding to mouth of perigone tube, fimbriate, 2.1–2.8 [2.5] mm long. Stamens 8 (in the same number as perigone lobes), inserted at the middle of the perigone tube lower than stigma, at the radii of tepals; filaments with purple spots, cylindrical, 4 mm long, Ø 1.3–1.5 [1.4] mm, anthers 2.4–2.5 [2.5] mm long, 1.4–1.8 [1.6] mm wide, introrse, pollen sacs positioned at lower side of connectives, connectives white. Pistil 11.7 mm high. Style white with purple spots, cylindrical, 4.8 mm long, Ø 2.9 mm. Stigma white with purple spots at lower surface, purple with four white radial bifurcated crests at upper surface, highly convex (conical) at center, 7 mm high, Ø 7–7.7 [7.4] mm, 4-lobed at margin, lobes emarginated at apex. Ovary inconspicuous, superior, 4-locular. Fruits grayish black, subspherical, 13–15.6 [14.7] mm long, Ø 14–16.6 [15.3] mm, with short protuberances. A carpophore is absent.

Molecular description:—Differences found in alignments of all currently available accessions of *Aspidistra* of investigated sequences of *psb*A-*trn*H and 5S-NTS regions are summarised in Tables 1–2. In *psb*A-*trn*H region (Table 1), the new species *A. clausa* is similar to *A. triradiata* and *Aspidistra* sp. 1, an unidentified species (see Vislobokov *et al.* 2014b). It seems that possibility of using *psb*A-*trn*H region for molecular barcoding within *Aspidistra* is limited. In 5S-NTS region, the most remarkable feature of the new species is a 9 bp deletion between nucleotides 188 and 119 (direct nucleotide positions; these are equivalent to positions 201–209 in the alignment, Table 2) within indel 8, which is common for *A. clausa*, *A. triradiata*, and *A. xuansonensis* Vislobokov in Vislobokov *et al.* (2014c: 227) (a species from northern Vietnam vs. other accessions from southern Vietnam). In addition, there are a few unique features distinguish 5S-NTS sequences of the new species from other accessions: Thymine not Guanine at direct nucleotide position 8 (within indel 1); Guanine not Adenine at direct nucleotide position 38; Cytosine not Guanine at direct nucleotide position 271; Guanine not Adenine at direct nucleotide position 303.

Etymology:—The specific epithet "*clausa*" means "closed", it explains the structure of flowers where stamens are hidden by tepal appendages. In flowers of the similar species *A. crassifila* Liu & Peng in Lin *et al.* (2013: 43) stamens are visible from above between adaxial surface of perigone tube and margin of stigma.

TABLE 2. Differences between investigated species of *Aspidistra* found in the alignment of sequences of 5S-NTS region. For details on species other than *A. clausa* and *A. triradiata*, see Vislobokov *et al.* (2014b, c).

Taxon	Positions in the alignment														
	8–21	23	26	27	28	29	31	38	43	44	48	57–129	131	132	133
A. clausa	indel1	T	T	T	C	A	T	T	G	G	-	-	A	-	-
A. triradiata	indel2	T	T	T	T	A	T	T	G	A	T	indel7	A	C	T
Aspidistra sp. 1	indel3	A	T	T	C	A	T	T	G	A	-	-	A	-	-
A. xuansonensis	indel4	T	T	T	C	A	C	T	G	A	-	-	A	C	T
A. phanluongii	indel5	T	T	T	C	A	T	C	A	A	-	-	A	-	-
A. paucitepala	indel6	T	G	-	C	G	T	-	G	A	-	-	T	-	-

Taxon	Positions in the alignment														
	134	135	137	142	143	149	150	151	156	157	168	189–257	270	287	294–460
A. clausa	G	A	G	T	C	C	C	G	C	A	A	indel8	G	G	-
A. triradiata	C	A	T	T	C	C	C	G	C	A	A	indel8	G	C	-
Aspidistra sp. 1	G	A	G	G	T	C	C	G	C	G	C	indel9	T	T	-
A. xuansonensis	C	C	T	T	C	A	A	T	T	A	A	indel8	G	G	-
A. phanluongii	G	A	G	G	T	C	C	G	C	G	G	indel9	T	T	-
A. paucitepala	G	A	G	T	T	C	C	G	C	G	C	indel9	T	T	indel10

Taxon	Positions in the alignment																			
	461	471	482	488	504	508	509	511–636	638	641	655	663	668	672	687	688–731	733	741	750	756
A. clausa	A	A	A	G	C	G	T	-	A	A	C	A	G	G	G	indel12	C	C	T	A
A. triradiata	A	A	G	G	C	G	T	-	A	A	G	A	G	G	A	indel12	C	A	T	A
Aspidistra sp. 1	A	G	A	A	C	A	G	indel 11	T	A	G	A	C	G	A	indel12	C	C	C	A
A. xuansonensis	A	A	A	G	G	G	C	-	A	G	G	C	G	A	A	indel13	C	C	T	A
A. phanluongii	A	G	A	A	C	G	G	indel 11	T	A	G	A	G	G	A	indel12	G	C	C	A
A. paucitepala	T	G	A	A	C	A	G	indel 11	T	A	G	A	G	G	A	indel12	C	C	T	G

indel 1: GGTTCTTTTT; indel 2: TGTTCTTT; indel 3: GGTTCTTT; indel 4: GCTTCTTCTTTTTT; indel 5: NNNTTTTTTTTTTT; indel 6: TGTTT; indel 7: a 73 bp insertion; indel 8: a 69 bp sequence, but at position 201–209 deletion in *A. clausa*, at position 209 Cytosine in *A. triradiata* and Guanine in *A. xuansonensis*; indel 9: a 67 bp sequence, but at position 194 Guanine not Adenine in *A. paucitepala* Vislobokov *et al.* (2014: 272), at position 197 Adenine not Guanine in *A. paucitepala*, at position 216 Cytosine not Thymine in *A. paucitepala*, at position 217 Guanine not Adenine in *Aspidistra* sp. 1, at position 226 Adenine not Guanine in *A. paucitepala*, at position 229 Guanine not Thymine in *A. paucitepala*; indel 10: a 167 bp insertion; indel 11: a 126 bp insertion, but at position 513 Guanine not Adenine in *A. paucitepala*, at position 530 Adenine not Cytosine in *Aspidistra* sp. 1, at position 555 Adenine not Thymine in *Aspidistra* sp. 1, at position 574 Cytosine not Thymine in *A. phanluongii* Vislobokov in Vislobokov *et al.* (2011: 349), at position 584 Thymine not Adenine in *A. paucitepala*, at position 598 Guanine not Adenine in *A. paucitepala*, at position 607 Thymine not Guanine in *A. phanluongii*, at position 609 Cytosine not Thymine in *A. phanluongii*; indel 12: a 44 bp sequence, but at position 705 Thymine not Cytosine in *Aspidistra* sp. 1, at position 706 Thymine not Cytosine in *A. clausa* and *A. triradiata*, at position 715 Guanine not Cytosine in *A. paucitepala*, at position 721 Adenine not Guanine in *A. clausa* and *A. triradiata*, at position 722 Thymine not Cytosine in *Aspidistra* sp. 1 and *A. phanluongii*; indel 13: a 44 bp deletion.

Distribution:—The species is known only from the type locality.

Taxonomic relationships:—The new species resembles and probably closely relates to *A. crassifila* but differs in width of leaf blade (2.8–4.5 cm vs. 6–12 cm), perigone tube wide tubular vs. campanulate, appendages longer, filaments and connectives white with purple spots vs. completely purple, stigma conical vs. mushroom-shaped. Also flowers of *A. clausa* are somewhat similar to flowers of *A. patentiloba* Wan & Lu (1989: 99), but strongly differ in stigma shape and should not be considered as closely related species.

Aspidistra triradiata **N.Vislobokov** ***sp.nov.*** **(Fig. 2)**

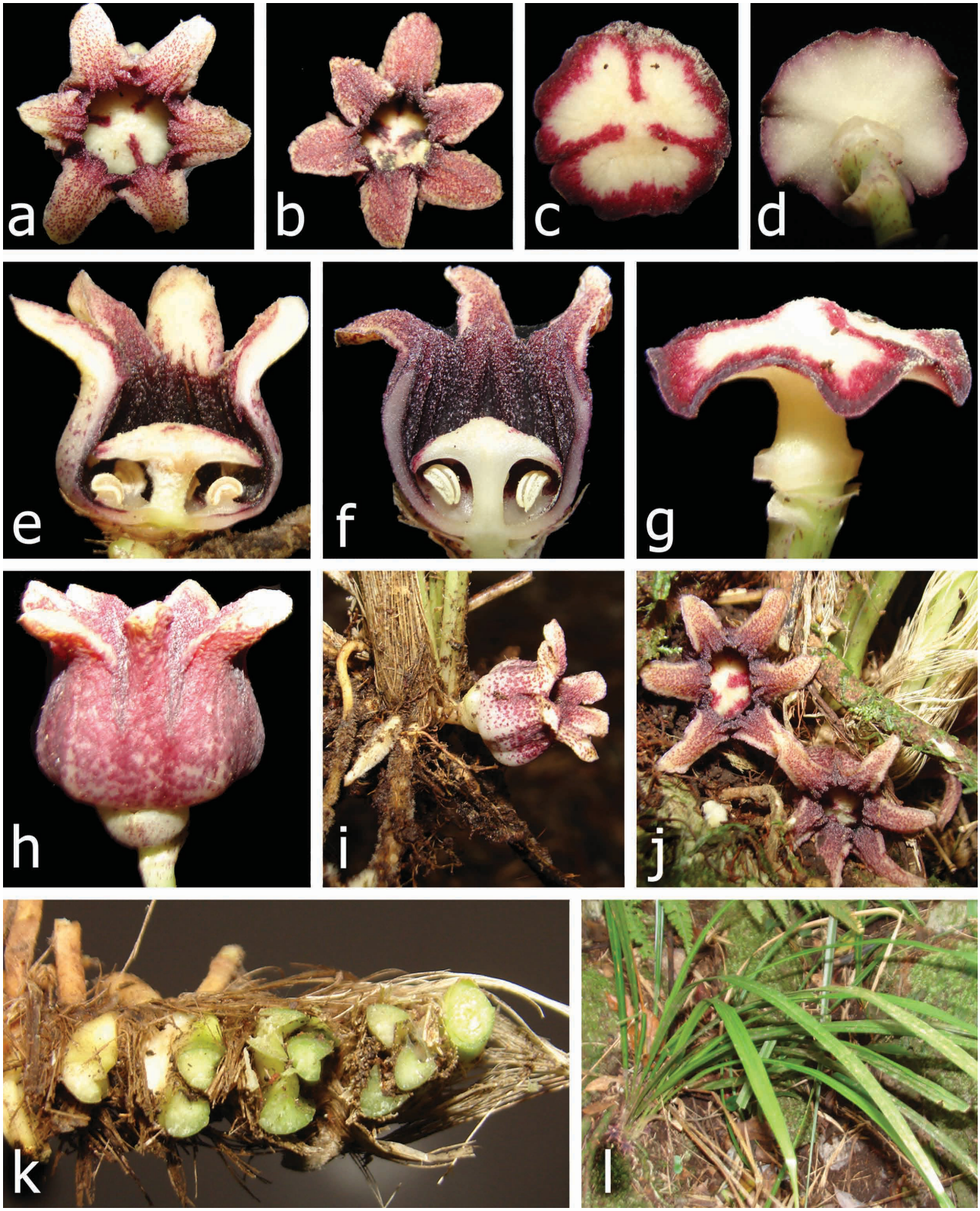

FIGURE 2. *Aspidistra triradiata*. a, b. flowers, top view; c, d. stigma, top view (c) and view from below (d); e, f. longitudinal section of flower; g. pistil, side view, perigone removed; h. flower, side view; i, j. flowers on rhizome of flowering plant; k. rhizome with bases of leaves, leaves removed; l. flowering plant.

Similar to *A. hainanensis*, but flowers larger (1.8–2.4 cm long, Ø 1.8–2.8 cm vs. 1–1.2 cm long, Ø 1.3–1.5 cm), perigone urceolate, lobes with 4 verrucose keels, stigma with three purple radial lines.

Type:—VIETNAM, Vinh Phuc province, Tam Dao district, Tam Dao National Park, about 6 km NW from Tam Dao City. 21° 29,942' N, 105˚ 36,973' E, alt. 1208 m, rainforest, 19 November 2014, *N.A. Vislobokov 14098* (holotype, MW!), including reproductive organs in liquid collection at Moscow University.

Herbaceous perennial, rhizomatous, evergreen plant. Rhizome creeping, with very short internodes, epigeous, Ø 8.6–9.0 [8.8] mm. Roots grey, Ø 2.4 mm, with 2–3-layered velamen and dense hairs persistent up to root base, endoderm 2–3-layered, pericycle of cells with not lignified walls. Rhizomes with regularly repeating units, each comprising distichously arranged phylloms: 4–8 cataphylls followed by 3–5 foliage leaves. Cataphylls oblong, 8.5–16.5 [12.5] cm long, 9 mm wide. Foliage leaves not divided into petiole and lamina, leaf gradually narrowing to base. Leaf base dark to light green, adaxially sulcate, 2.7–5.2 [3.9] mm wide. Leaf blade dark to light green, linear, distally narrowly acuminate, 56–82.5 [73.3] cm long, 1.2–2 [1.6] cm wide, with 1–2 secondary veins at both sides of the midvein. The midvein prominent at lower surface and the secondary veins somewhat prominent at upper surface. Peduncle (specialized reproductive shoot) pale green with purple spots, 0.7–3.7 [2.1] cm long, Ø 2.3–3.5 [3] mm, with 3–5 widely ovate, 7.5–10.1 [8.5] mm long, 5.6–6.5 [6.1] mm wide distichously arranged scale leaves. Flower solitary at the end of a peduncle. Perigone urceolate, 18.2–23.9 [20.4] mm long, Ø 17.5–28.1 [23.1] mm; tube white with purple spots to completely purple outside, purple and verrucose inside, 15.6–18.6 [14.6] mm long, Ø 14.2–19.8 [17.1] mm; lobes 6(–7), purple spotted to completely purple at both sides, verrucose at upper side, triangular-ovate, obtuse, 7.6–10.5 [9.3] mm long, 3.9–8.3 [5.6] mm wide, lobes basally with 4 prominent verrucose longitudinal keels, 2 median keels extending down inside perigone, each lateral keel fusing with adjacent one. Stamens 6(–7), in the same number as perigone lobes, inserted at the bottom of the perigone tube, at the radii of tepals; anthers sessile, 2.5–2.8 [2.7] mm long, 2.4 mm wide, introrse. Pistil table-shaped, 6.5–8.8 [7.9] mm high (Fig. 2g). Style white, 3.1–3.5 [3.3] mm long, Ø 1.9–2.9 [2.5] mm. Stigma white with three purple radial lines and purple edge at the upper side, white at lower side, disc-shaped, papillose at the upper surface, slightly convex, Ø 11.7–15 [13.2] mm. Ovary inconspicuous, superior, 3-locular. Fruits are unknown.

Molecular description:—Differences found in alignments of all currently available accessions of *Aspidistra* of investigated sequences of *psb*A-*trn*H and 5S-NTS regions are summarised in Tables 1–2. In *psb*A-*trn*H region (Table 1), the new species *A. triradiata* is similar to *A. clausa* and *Aspidistra* sp. 1. In 5S-NTS region, the most remarkable feature of the new species is a large insertion represented by nucleotides 53–125 (direct nucleotide positions; these are equivalent to positions 57–129 in the alignment, Table 2). In addition, the following features distinguish 5S-NTS sequences of the new species from other accessions (in all cases, direct nucleotide positions): Thymine not Cytosine at position 24; Thymine insertion at position 44; Cytosine not Guanine at position 205 (within indel 8); Cytosine not Guanine/Thymine at position 283; Guanine not Adenine at position 311; Adenine not Cytosine at position 444.

Additional specimen examined (paratype):—VIETNAM, Vinh Phuc province, Tam Dao district, Tam Dao National Park, about 6 km NW from Tam Dao City. 21° 29,942' N, 105˚ 36,973' E, alt. 1208 m, rainforest, 24 November 2014, *N.A. Vislobokov 14111a-e* (MW!).

Etymology:—The specific epithet refers to the color pattern of stigma which includes three radial bright purple lines.

Distribution:—The species is known only from the type locality.

Taxonomic relationships:—The new species resembles and closely relates to the *A. hainanensis* complex mentioned by Tillich & Averyanov (2012). The complex includes SE Asian *Aspidistra* species with oblanceolate to lineate, tufted leaves and trimerous flowers, e.g. *A. caespitosa* Pei (1939: 101), *.A. larutensis* De Wilde & Vogel (2005: 126), *A. linearifolia* Wan & Huang (1987: 220), *A. lingchuanensis* Lin & Guo in Guo *et al.* (2015: 86), *A. minutiflora* Stapf (1903: 113), *A. yingjiangensis* Peng (1989: 173). However, *A. triradiata* shows a number of peculiar character-states, i.e. clearly urceolate perigone tube, 4 verrucose keels, verrucose adaxial surfaces of tepals and tube, and peculiar color patterns of perigone and stigma.

Acknowledgements

Author is grateful to D.D. Sokoloff and D.F. Lyskov for providing expedition to Vietnam; to A.N. Kuznetsov and S.P. Kuznetsova (Joint Russian-Vietnamese Tropical Scientific and Technological Center) for providing field work in Vietnam; to G.V. Degtjareva and C.M. Valiejo-Roman for helpful advices in molecular study; to O. Colin for helpful comments and photos of *A. clausa*; to H.-J. Tillich and L.V. Averyanov for helpful comments. The study is supported by grant from Russian Scientific Fund (project No. 14-14-00250).

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APPENDIX 1. Accessions of *Aspidistra* used in molecular study and GenBank accession numbers.

Taxon	Locality and collection date	Collectors and collection number	GenBank accession number, 5S-NTS	GenBank accession number, psbA-trnH
A. clausa	Vietnam, Vinh Phuc province, Tam Dao district, Tam Dao National Park. 21° 28,405' N, 105˚ 38,351' E, alt. 978 m, rainforest, 19 Nov 2014	*N.A.Vislobokov 14097*	KR078332	KR078334
A. triradiata	Vietnam, Vinh Phuc province, Tam Dao district, Tam Dao National Park. 21° 29,942' N, 105˚ 36,973' E, alt. 1208 m, rainforest, 19 Nov 2014	*N.A.Vislobokov 14098*	KR063106	KR078333