

**Phylogeny and taxonomic reexamination of the genus *Dioscorea*
(Dioscoreaceae)**

ヤマノイモ属（ヤマノイモ科）の系統と分類学的再検討

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Summary

ヤマノイモ属 (*Dioscorea* L.) は、熱帯を中心に、温帯にまで分布域を広げる単子葉植物ヤマノイモ科の多年生草本で、世界に 600 種以上が知られている。根茎または塊根をもち、花は単性で小さく、雌雄異株、殆どの種はつる性の茎をもつ。多くの種で果実はさく果となり、種子に翼が発達する。腋芽がムカゴをつくり、栄養繁殖する種もある。

ヤマノイモ属の分類は、これまで形態形質に基づいて Uline (1898)、Knuth (1924)、Burkill (1960)、Huber (1998)らによって提案されてきたが、特に属内分類群の認識は、見解の間で大きく異なっていた。分子系統解析については、まず、Wilkin ら(2005)が葉緑体 DNA (*matK*, *rbcL*) の塩基配列に基づいてアフリカの種を中心とした 67 種の分子系統樹を構築した。その後、Gao ら(2008)やHsu ら(2013)は中国の種を中心に解析し、Viruel ら(2016)は葉緑体 DNA (*matK*, *rbcL*, *trnL-F*, *atpB*) の塩基配列を用いてそれまでのデータにアメリカの種を加えて 135 種の比較的大規模な分子系統樹を構築した。その後、Maurin ら(2016)は他の葉緑体領域を用いてアフリカの種を解析、Couto ら(2018)は Viruel ら(2016)のデータを使って分岐年代を推定、Viruel ら(2018)は核 DNA (*Xdh*) の塩基配列を用いて解析し、Soto-Gomes ら(2019)は核の 264 遺伝子のデータを用いて 34 種の系統解析を行った。しかし、これまでは、日本を含む東アジアや東南アジアの種があまり解析には含められて来ず、ヤマノイモ属の系統関係の全体像は明らかではなかった。それゆえ、分子系統樹を用いて混乱している属内分類群の認識を再検討する試みはなされて来なかった。

また、日本産ヤマノイモ属に関しては、網羅的な分子系統解析は行われた

ことがない。葉の形の種内変異が大きく、複数種が同所的に生育することもしばしばあるにもかかわらず、雑種形成についても分子の側面から検討されたことがなかった。種認識に関しては、Yamashita and Tamura (2016)の見解はあるものの、特に南西諸島の植物を中心に、種認識は混乱している。

本研究の目的は、まず、東アジアや東南アジアの種を含めた世界のヤマノイモ属の分子系統樹を構築すること（第一章）、そして、その分子系統樹に基づいて、これまで分子の観点から再検討されたことがなかった属内分類群を見直し、旧世界の新しいヤマノイモ属の属内分類群レベルの分類体系を設立することによって、旧世界のヤマノイモ属の多様性を明確にすること（第二章）、次に、これまでやはり分子の観点から再検討されて来なかった日本産ヤマノイモ属の分類を分子系統解学的に整理し、特に混乱を極めていた南西諸島産植物の種認識を明確にすること（第三章）にある。

第一章・第二章では、葉緑体領域（*matK*, *rbcL*, *atpB*, *trnL-F* : 3,754bp）に基づいて、ヤマノイモ属 183 種 273 個体の大規模分子系統樹を最尤法と最節約法で構築した。また、染色体基本数を含む 10 種類の形態形質の情報を、野外調査時の観察、さく葉標本の検討、文献調査などにより収集し、Mesquite を使って形質進化を推定した（第一章）。そして、分子系統樹の各クレードの基部で新たに獲得されたと推定できる形質を見出して、分子系統樹と形態等の形質に基づいて、旧世界のヤマノイモ属の属内分類群レベルの新しい分類体系を設立した。

第三章では、まず、葉緑体領域（*matK* を含む *trnK*, *rbcL*, *atpB*, *trnL-F* : 6,288 bp）に基づいて、日本を中心としたヤマノイモ属 26 種 2 変種 123 個体の詳しい分子系統樹を最尤法と最節約法で構築した。さらに、*Enantiophyllum* 節と *Dioscorea* 節については、核領域（*PHYC*, 18S rDNA）の分子系統樹を構築し、葉緑体系統樹と比較した。葉緑体の *trnK*、核の *PHYC* と 18SrDNA を用いてヤマノイモ属の

分子系統樹を構築する試みは、本研究が初めてである。その後、ILD テストを経て、葉緑体データと核データの結合データセットを作成し（合計 8,624-8,647 bp）、最尤法と最節約法で詳細な分子系統樹を構築した。そして、その分子系統樹を軸にして、併せて雄蕊形態の観察なども行うことにより、日本産ヤマノイモ属の分類を再検討した。

葉緑体の大規模分子系統樹を構築した結果、ヤマノイモ属に 27 の主要なクレードが形成された。そのうち 20 の主要クレードは、旧世界の植物によって構成されていた。そして、染色体基本数、倍数性、地下茎、花粉、種子などの形質進化を調べて、現在見られるヤマノイモ属の多様性の成立過程を推定した。また、ヤマノイモ属にヤマノイモ亜属と *Helmia* 亜属の 2 亜属を認めることを提案した（第一章）。

次に、大規模分子系統樹に基づいて、旧世界のヤマノイモ属に 20 節を認めることを提案した。このうち、*Dioscorea* sect. *Afroborderia*, *D.* sect. *Perennia*, *D.* sect. *Pseudoshannicorea*, *D.* sect. *Pseudostenophora* を新しく記載し、*D.* sect. *Tamus* を新ランクとした。また、12 のレクトタイプを指定した（第二章）。

日本産のヤマノイモ属については、葉緑体 DNA と核 DNA に基づく系統樹を比較した結果、ILD テストの値が 0.1 より大きくなり、両系統樹のトポロジーは大きくは変わらないことが判明し、網状進化の痕跡は認められなかった。そして、沖縄県大東島のルズンヤマノイモと考えられてきた植物はキールンヤマノイモであること、イズドコロは *D. collettii* の変種とするのが妥当なことなどを見出した。その結果、日本には 16 種 3 変種のヤマノイモ属が分布すると結論付けた（第三章）。

General introduction

ヤマノイモ属 (*Dioscorea* L.) は、熱帯～温帯に分布する単子葉植物ヤマノイモ科の多年生草本であるが、分布中心は熱帯域にある。世界に 600 種以上が知られている。これは、2002 年時点で、植物の中で 43 番目に種数の多い属である (Frodin 2004)。ヤマノイモ属は根茎または塊根をもち、花は単性で小さく、雌雄異株、ほとんどの種はつる性の茎をもつ。多くの種で果実はさく果となり、種子に翼が発達する。腋芽がムカゴをつくり、栄養繁殖する種もある。

ヤマノイモ属の分類は、これまで形態形質に基づいて Uline (1898)、Knuth (1924)、Burkill (1960)、Huber (1998)らによって提案されてきたが、特に属内分類群の認識は、見解の間で大きく異なっている。多いものでは、属内に 58 節を認めているものもある (Knuth 1924)。最新の Huber (1998)の見解によると、ここで取扱うヤマノイモ属の中に、29 分類群 (5 属と 24 の属相当の節 "genus-equivalent sections") が認められている。

分子系統解析については、まず、Wilkin ら(2005)が葉緑体 DNA (*matK*, *rbcL*) の塩基配列に基づいてアフリカの種を中心とした 67 種の分子系統樹を構築した。その後、Gao ら(2008)や Hsu ら(2013)は中国の種を中心に解析し、Viruel ら(2016)は葉緑体 DNA (*matK*, *rbcL*, *trnL-F*, *atpB*) の塩基配列を用いてそれまでのデータにアメリカの種を加えて 135 種の比較的大規模な分子系統樹を構築した。その後、Maurin ら(2016)は他の葉緑体領域を用いてアフリカの種を解析、Couto ら(2018)は Viruel ら(2016)のデータを使って分岐年代を推定、Viruel ら(2018)は核 DNA (*Xdh*) の塩基配列を用いて解析し、Soto-Gomes ら(2019)は核の 264 遺伝子のデータを用いて 34 種の系統解析を行った。しかし、これまでは、日本を含む東アジアや東南アジアの種があまり解析には含まれて来ず、ヤマノイモ属の

系統関係の全体像は明らかではなかった。それゆえ、分子系統樹を用いて混乱している属内分類群の認識を再検討する試みはなされて来なかった。

また、日本産ヤマノイモ属に関しては、網羅的な分子系統解析は行われたことがない。葉の形の種内変異が大きく、複数種が同所的に生育することもしばしばあるにもかかわらず、これまで雑種形成についても分子の側面から検討されたことがなかった。種認識に関しては、Yamashita and Tamura (2016)の見解はあるものの、特に南西諸島の植物を中心に、種認識は混乱している。

例えば、ヤマノイモ類似群に関して、奄美大島にはヤマノイモのみが分布する (Masamune 1957)、キールンヤマノイモのみが分布する (Hatusima 1961)、キールンヤマノイモとユワンドコロが分布する (Hatusima 1986, Yamashita and Tamura 2016)、ナガイモのみが分布する (Hatusima and Amano 1994) など、見解が異なっている。奄美大島固有のアマミタチドコロを九州のツクシタチドコロに含めるかどうかも見解の別れるところである。日本では大東島のみから報告されてきたルゾンヤマノイモも、花を咲かせたことがなく（少なくとも私はその報告を見聞いたことがなく）、実体は謎である。伊豆半島固有種のイズドコロも、遠く離れた台湾以西の *Dioscorea collettii* に類縁が近いとされているが (赤堀 1963)、そのような分布パターンを示す植物は、私の知る限り、他に知られていない。

本研究の目的は、まず、東アジアや東南アジアの種を含めた世界のヤマノイモ属の分子系統樹を構築すること (第一章)、そして、その分子系統樹に基づいて、これまで分子の観点から再検討されたことがなかった属内分類群を見直し、旧世界の新しいヤマノイモ属の属内分類群レベルの分類体系を設立することによって、旧世界のヤマノイモ属の多様性を明確にすること (第二章)、次に、これまでやはり分子の観点から再検討されて来なかった日本産ヤマノイモ属の

分類を分子系統解学的に整理し、特に混乱を極めていた南西諸島産植物の種認識を明確にすること（第三章）にある。

Chapter I

A large-scale phylogenetic analysis of *Dioscorea* (Dioscoreaceae), with reference to
character evolution and subgeneric recognition

ヤマノイモ属（ヤマノイモ科）の大規模分子系統解析
—形質進化と亜属認識を中心にして—

Abstract

Dioscorea, a large genus consisting of more than 600 species, is greatly diversified. To understand relationships and character evolution within the genus, we conducted phylogenetic analyses of 273 samples from 183 species (including 28 newly sequenced species) based on four cpDNA regions. A phylogenetic tree obtained comprised eleven well-supported major clades; most of which further consisted of more than two subclades. Comparisons with previously proposed infrageneric taxa (23 to 58 sections and associated “genera”) show that some genera/sections are monophyletic and others polyphyletic. As in the previous studies, “*D. sect. Stenophora*” is sister to the rest of the genus. Analyses of character-state distribution on the tree confirms that “*D. sect. Stenophora*” is characterized by having rhizomes, monosulcate pollen, and diploid with the basic chromosome number of $x = 10$ (plesiomorphies), whereas the rest of the genus have tubers and bisulcate pollen (apomorphies), and are diversified with regard to characters including chromosome number, stem twining direction, fruit types, and seed wing morphology. Based on molecular and morphological evidence, we propose the recognition of two subgenera, *Dioscorea* (= “*D. sect. Stenophora*”) and *Helmia*. As for the subgenus *Helmia*, a revision is required for infrageneric classification, especially for the species in the Old World.

Introduction

Dioscorea, constituting Dioscoreaceae in monocots along with *Stenomeris* Planch., *Tacca* J.R. Forst. & G. Forster, and *Trichopus* Gaertn., is a yam genus consisting of about 630 species distributed worldwide (WCSP 2019). The species mostly have twining stems (Fig. 1a), and are dioecious with either staminate or pistillate

flowers (Fig. 1b, c). Since the large number of species represents the great morphological diversity, *Dioscorea* has often been divided into infrageneric taxa (for review see Caddick *et al.* 2002a). In short, Uline (1898) divided *Dioscorea* into 51 sections in three subgenera *Eudioscorea* Pax, *Helmia* (Kunth & Gatt.) Gris. and *Testudinaria* (Salisb. & Gatt.) Uline. Knuth (1924) recognized four subgenera *Eudioscorea*, *Helmia*, *Stenophora* (Uline) R. Knuth. and *Testudinaria* and placed 58 sections in the former three subgenera. Burkill (1960) recognized 23 sections for the Old World species, and Huber (1998) proposed five “genera” and 24 “genus-equivalent sections.” Huber (1998) further recognized at least three more “genus-equivalent sections” that have no descriptions. In all of these classification systems, a few *Dioscorea* species were assigned to separate genera *Borderea* Miégev., *Epipetrum* Phil., *Rajania* L., *Tamus* L., and *Testudinaria* Salisb. because of their unique morphological features. As stated below, however, molecular phylogenetic analyses have consistently indicated that all of those species are nested within a large clade composed of ordinary *Dioscorea* species (Caddick *et al.* 2002a; Wilkin *et al.* 2005; Viruel *et al.* 2016, 2018; Couto *et al.* 2018). Accordingly, *Dioscorea* is now circumscribed as a taxon that contains much more morphological diversity than had been previously considered.

In the previous systems of infrageneric classification of *Dioscorea* (including the aforementioned associated genera) (Uline 1898; Knuth 1924; Burkill 1960; Huber 1998), diverse morphological characters were used to distinguish from one section (subgenus/genus) from another. These characters include: fruit type (capsule, samara or berry), seed morphology (winged or unwinged, and if winged, wing types), underground stem (rhizome or tuber), the direction of stem twining (right or left), and phyllotaxis (alternate or opposite). Diagnostic keys to the sections (subgenera/genera)

differed greatly between authors, and no consensus has emerged with regard to the recognition and/or circumscription of many of the infrageneric taxa.

For the past two decades, molecular phylogenetic analyses have been conducted in order to for better understand relationships within Dioscoreaceae or *Dioscorea* (Caddick *et al.* 2002a; Wilkin *et al.* 2005; Gao *et al.* 2008; Hsu *et al.* 2013; Maurin *et al.* 2016; Viruel *et al.* 2018; Soto Gomez *et al.* 2019), and to elucidate the biogeographic origin of *Dioscorea* and subsequent species migrations (Viruel *et al.* 2016; Couto *et al.* 2018). Wilkin *et al.* (2005) performed phylogenetic analyses of 67 *Dioscorea* species using sequences of two cpDNA regions (*matK* and *rbcL*). They identified ten major clades in a phylogenetic tree, and suggested that the previous systems of infrageneric classification could be greatly simplified. Gao *et al.* (2008) performed phylogenetic analyses of 17 species assigned to *D.* sect. *Stenophora* using sequences of three cpDNA regions (*matK*, *rbcL*, and *trnL-F*). Hsu *et al.* (2013) analyzed relationships of 44 Asian *Dioscorea* species using sequences of four cpDNA regions (*matK*, *rbcL*, *atpB-rbcL*, and *trnL-F*), and discussed the relationships between several sections consisting of Asian species. More recently, Viruel *et al.* (2016) performed phylogenetic analyses of 135 *Dioscorea* species using sequences of four cpDNA regions (*matK*, *rbcL*, *atpB*, and *trnL-F*) and discussed the geographic origin of the genus and potential evolutionary processes leading to the current species distribution. Maurin *et al.* (2016) provided phylogenetic studies of *Dioscorea* with focus on African species using six cpDNA regions including *matK*, *rbcL*, and *trnL-F*. They newly sequenced six African species. Viruel *et al.* (2018) further obtained phylogenetic trees of 53 *Dioscorea* species based on a low copy nuclear gene *Xdh*, and showed that the divergence order of the main *Dioscorea* clades are congruent with those of the

plastid-based trees. Couto *et al.* (2018) also performed phylogenetic analyses of 161 *Dioscorea* species with focus on Neotropical species, and obtained new sequences of two chloroplast genes (*matK* and *rbcL*) from 34 species. Besides discussing a biogeographic history of *Dioscorea*, Couto *et al.* (2018) discussed how phylogeny supported the systems of infrageneric classification proposed by Uline (1897) and Knuth (1924). They also concluded that although some of the sections proposed by Uline (1897) may be supported, the infrageneric classifications of Uline (1897) and Knuth (1924) do not generally represent natural lineages.

The aforementioned molecular phylogenetic studies of *Dioscorea* (Wilkin *et al.* 2005; Viruel *et al.* 2016, 2018; Couto *et al.* 2018) have obtained a similar tree topology consisting of ten major clades, which were referred to as Stenophora, NWI (New World I), NWII (New World II), African, Mediterranean, NWIII (New World III) (including “*Rajania*”), compound leafed, Malagasy, Shannicorea, and Enantiophyllum clades. While the first three clades successively diverged, the divergent orders of the remaining seven were allocated differently by each of the authors. Recently Soto Gomez *et al.* (2019) analyzed genomic variations of 34 *Dioscorea* species which represent all major clades obtained by cpDNA and nuclear *Xdh* sequence analyses (Viruel *et al.* 2016, 2018). They showed robust relationships of the major clades using a concatenated matrix of 264 genes recovered with target enrichment methods. However, because of meager taxon sampling, a phylogenetic tree obtained do not contain subclades which likely represent infrageneric taxa, nor does it allow us to pursue the evolution of most of morphological characters.

As regards the evolution of morphological characters, Wilkin *et al.* (2005) first attempted an analysis of evolution of the five following morphological characters using

their own tree (of 67 species): (1) underground parts, namely, rhizomatous or perennial or annual tuber; (2) flowers bisexual (hermaphrodite) or unisexual (plants dioecious); (3) stem twining direction left or right; (4) fruits capsular, berry, or samara; (5) seeds winged all round margin, basal only, apical only, or only at base and apex. Schols *et al.* (2005) further discussed pollen character (size, aperture number, sexine ornamentation, perforation density) evolution using a tree identical to that obtained by Wilkin *et al.* (2005). After the character-state analyses of Wilkin *et al.* (2005) and Schols *et al.* (2005), sequence data of cpDNA regions from more *Dioscorea* species have become available (e.g., Gao *et al.* 2008; Hsu *et al.* 2013; Maurin *et al.* 2016; Viruel *et al.* 2016; Couto *et al.* 2018). Since a phylogenetic tree containing a much larger number of *Dioscorea* species than that available to Wilkin *et al.* (2005) can now be obtained, we can discuss character evolution (and in particular of Old World species groups) more extensively and precisely.

In the present paper, by combining all existing cpDNA sequence data with our original data, we present a large-scale phylogenetic analysis of 183 *Dioscorea* species (about 29% of the current total *Dioscorea* species). We compare major clades/subclades in the resultant tree with the previously proposed infrageneric taxa and then delineate the evolution of morphological characters to map out their diversification within the genus. The morphological characters we discuss include not only those used for the previous systems of infrageneric classification (Uline 1897, 1898; Knuth 1924; Burkill 1960; Huber 1998), but also pollen and chromosome characters whose systematic and evolutionary implications have been suggested for the past 40 years (Pei *et al.* 1979; Chin *et al.* 1985; Schols *et al.* 2005; Viruel *et al.* 2010). Our work provides additional knowledge of the evolution and systematics of the yam genus, and

suggests which species and/or characters should be the focus of future studies.

Materials and Methods

Taxon sampling

We sequenced 35 samples from 28 species of *Dioscorea* (Table 1) for four cpDNA regions (*matK*, *rbcL*, *atpB* and *trnL-F*); sequence data of the *matK* region were registered as those of the *trnK* region including the former. The samples were mostly collected from Japan, as there was a lack of sequence data from this country in previous studies. In order to enlarge taxon sampling, we further used the sequence data from *Dioscorea* species that were analyzed in previous studies (Caddick *et al.* 2002a; Wilkin *et al.* 2005; Gao *et al.* 2008; Hsu *et al.* 2013; Viruel *et al.* 2016; Maurin *et al.* 2016; Couto *et al.* 2018) (Appendix 1). In total, we obtained a dataset consisting of 273 samples derived from 183 *Dioscorea* species. Of the 183 species, 67 are New World, and 116 are Old World. Among them, 101 species are fully sequenced for the four markers (*matK*, *rbcL*, *atpB* and *trnL-F*), 43 are sequenced for three markers (*matK*, *rbcL*, and *trnL-F*), 12 are sequenced for a different combination of three markers (*rbcL*, *atpB* and *trnL-F*), and the remaining 27 are sequenced for two markers (*matK* and *rbcL*). The incomplete sequences were coded as missing data. We added samples of two other dioscoreaceous genera *Stenomeris* and *Tacca* in the analysis (*S. borneensis* Oliv. and seven species of *Tacca*) and we used *Nartheccium asiaticum* Maxim. as an outgroup (Table 1). Among these samples, two *Tacca* species and *N. asiaticum* were de novo sequenced in the current study. The four cpDNA markers, consisting of three coding genes (*matK*, *rbcL*, *atpB*) and the *trnL-F* (*trnL* intron–*trnL* exon–*trnL/trnF* spacer) region, were used as recommended by Viruel *et al.* (2016). Specifically, analyses

based on these markers are expected to result in phylogenetic resolution from family to species level.

DNA sequencing

Total genomic DNA was extracted from fresh, silica-gel dried leaves, or leaves of herbarium specimens, primarily using a modified CTAB method (Tamura *et al.* 2010). In the case of herbarium specimens, DNA was extracted by keeping a mixture of the CTAB and isopropanol (3: 2) solution in a refrigerator for over two weeks (Azuma personal communication). In the case of older herbarium specimens, DNA was extracted using the NucleoSpin Plant II kit (TaKaRa Bio). Regions of the four aforementioned DNA markers were amplified by PCR using the GeneAmp PCR System 2720 or TaKaRa PCR Thermal Cycler Dice. The amplification reaction mixture was prepared using TaKaRa EX Taq DNA polymerase following the manufacturer's instructions. The Primers used for amplification are listed in Appendix 2. PCR reaction conditions were as follows: initial denaturation at 94-95 °C for 5.0 min, followed by a 35-cycle reaction comprising denaturation at 94-95 °C for 10-30 s, annealing at 50-64 °C for 30 s, and extension at 72 °C for 0.5-1.5 min, and a final extension at 72 °C for 7.0 min. The PCR products were purified using Illustra MicroSpin S-300 HR Columns (GE Healthcare) or Exonuclease I with calf intestine alkaline phosphatase (TOYOBO). We used the purified double-stranded PCR products as templates for direct sequencing by the dideoxy method using an ABI PRISM 310 Genetic Analyzer or an ABI PRISM 3130xl Genetic Analyzer auto-sequencer, according to the manufacturer's instructions. For DNA sequencing in the case of an old herbarium specimen (*Hatsusima & Sato 29467*, TI) of *Dioscorea*

luzonensis Schauer that was collected from the Philippines in 1964, we used primers listed in Appendix 3 with the following reaction profile: initial denaturation at 96 C for 1.0 min, followed by a 25-35cycle reaction (10 s at 96 C, 5 sec at 50 C, and 4.0 min at 60 C). Either sense or antisense strands or both were sequenced in *D. luzonensis*.

Finally, we determined DNA sequences of the four regions in 35 new samples of 28 *Dioscorea* species. Species identification followed Yamashita and Tamura (2016) for the plants from Japan, Huang and Hsiao (2000) for the plants from Taiwan, and Wilkin and Thapayai (2009) for the plants from Thailand. Voucher specimens were deposited at the herbaria of The University of Tokyo (TI), Kyoto University (KYO), or Institute of Plant Science and Resources, Okayama University.

Phylogenetic analysis

The DNA sequences for the four-region analysis were initially aligned using MUSCLE alignment software (Edgar 2004), and then adjusted manually. When aligned manually, sequence data of *Dioscorea japonica* that were used as template were obtained from *matK* (from 430th to 1296th), *rbcL* (from 1st to 676th), *atpB* (from 126th to 1389th), and *trnL-F* (from 12th to 94th, 113rd to 602nd, and 618th to 717th).

Among the 28 species that we sequenced in the present study, eleven are new records, and the remaining 17 were duplicates of the species sequenced by Viruel *et al.* (2016). However, our collections of the 17 latter species had different sequences from those used by Viruel *et al.* (2016). Therefore, we included sequence data from 35 collections derived from 28 species in the analyses. For five of the 28 species, we sequenced two or three collections each; this was to account for a sequence variation within these species.

We employed the maximum-likelihood (ML) methods for phylogeny reconstruction using RAxML-NG v. 0.7.0git BETA (Kozlov *et al.* 2019). For the ML analysis, the best model of nucleotide evolution was estimated using jModelTest-2.1.10 (Darriba *et al.* 2012). The model selected by the Akaike's Information Criterion (AIC), Akaike Information Criterion Corrected (AICC), Bayesian Information Criterion (BIC) and a decision theory method (DT) was GTR+I+G. In order to assess the robustness of individual clades on the obtained phylogenetic trees, we used transfer bootstrap expectation (TBE). TBE has recently been proposed for application to phylogenetic analyses of large datasets, especially when the tree is inferred from multigenes, as it provides stronger supports than Felsenstein's bootstrap proportion (FBP) for deep branches (Lemoine *et al.* 2018).

For the maximum parsimony (MP) analysis, the following settings were adopted: heuristic search option of simple additional sequence, tree bisection reconnection (TBR) swapping algorithm and MULPARS. The consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated using PAUP4.0a (build 164) with the inclusion of uninformative characters. Besides TBEs, FBPs were obtained by an MP analysis with 1,000 bootstrap replications (heuristic; simple; TBR).

Inference of morphological character evolution

To identify morphological characters that support species relationships within *Dioscorea*, we mapped each of a few selected characters under a parsimony criterion onto the phylogenetic tree using the program Mesquite version 3.03 (Maddison and Maddison 2015) (see Fig. 4). Except for those of chromosome numbers and seed

wings, character-states of the other characters were determined by comparisons using *Stenomeris* as outgroup, because *Stenomeris* is sister to *Dioscorea* as stated below.

Observations of pollen aperture

Pollen aperture number is reportedly a character that can facilitate the understanding of evolution and relationships within *Dioscorea* (Schols *et al.* 2005). We therefore examined pollen aperture number in six species including *D. prazeri* Prain & Burkill and *D. biformifolia* S.J. Pei & C.T. Ting, which are positioned most basally in the Stenophora clade and previously not annotated with information on pollen. Their pollen grains were observed using a light microscope and a scanning electron microscope (SEM). For SEM-observations, specimens dehydrated through an ethanol series were critical-point dried in CO₂ and coated with platinum. Observations were made using a Hitachi Miniscope TM-1000.

Results

The data matrix of sequences of four cpDNA regions (*atpB*, *matK*, *rbcL*, and *trnL-F*) of a total of 273 samples from 183 *Dioscorea* species, one sample of *Stenomeris*, seven samples of *Tacca*, and one sample of *Nartheceium* (outgroup) was composed of 3,754 characters after alignment. Among these, 1,249 (33.3%) were variable sites [of which 781 (20.8%) characters were parsimony-informative] within the ingroup. The RAxML analysis generated one most parsimonious tree (Fig. 2a, b). The consistency index was 0.580, the retention index 0.881, and the rescaled consistency index 0.511.

Figure 2 shows that *Dioscorea* is monophyletic with *Stenomeris* as a sister group. The *Dioscorea* clade is supported by 100% TBE and 100% FBP (hereafter,

indicated like 100/100). *Dioscorea* comprises eleven major clades, which were tentatively labelled from basal to top Stenophora (100/100), NWI (New World I) (85/<50), NWII (New World II) (99/81), Mediterranean (93/81), African (96/69), *Dioscorea tentaculigera*, CL (Compound Leaved) (84/<50), NWIII–MG (a clade of New World III and Malagasy) (70/<50), Birmanica (Shannicorea) (80/-), *Dioscorea birmanica*, and *Enantiophyllum* (96/-) (each clade, labelled following Wilkin *et al.* 2005; Viruel *et al.* 2016, 2018; Couto *et al.* 2018). They are moderately to strongly supported, and each clade tends to have stronger TBE support than FBP support (for instance, 85/<50 in NWI). Relationships among the eleven major clades are clear, and a sister group relationship is strongly supported between each major clade and the rest, which consist of subsequently diverging clade(s). The tree topology is similar to that obtained in the previous studies (Wilkin *et al.* 2005; Viruel *et al.* 2016, 2018; Couto *et al.* 2018), particularly with respect to basally diverging clades. The Stenophora clade diverges first, followed successively by NWI, NWII, Mediterranean, and African clades (Fig. 2a).

Subsequently, the six other major clades, i.e., *Dioscorea tentaculigera*, CL, NWIII–MG, Birmanica (Shannicorea), *Dioscorea birmanica* and *Enantiophyllum* clades, diverged successively (Fig. 2b). The sequence data of *Dioscorea tentaculigera* Prain & Burkill were confirmed by three samples, one from Viruel *et al.* (2016) and two from samples sourced in the current study. The CL (Compound Leaved) clade used by Wilkin *et al.* (2005) and Viruel *et al.* (2016, 2018) includes not only compound-leaved species but also a few simple-leaved ones, i.e., *D. mollis* Knuth, *D. antaly* Jum. & H. Perrier, and *D. bulbifera* L., although Couto *et al.* (2018) restricted the Compound Leaved clade only to the compound-leaved species. The Birmanica clade used by

Wilkin *et al* (2005) and Viruel *et al.* (2016, 2018) contains the Shannicorea clade used by Couto *et al.* (2018), but it includes four more species (*D. daunaea* Prain & Burkill, *D. esculenta* (Lour.) Burkill, *D. preussii* Pax, and *D. petelotii* Prain & Burkill) that are external to the Shannicorea clade, although *D. birmanica* is positioned outside the Birmanica (Shannicorea) clade. The Dioscorea birmanica clade comprises *D. birmanica* Prain & Burkill alone, and is sister to the Enantiophyllum clade.

Except for the Dioscorea tentaculigera and Dioscorea birmanica clades, every major clade consists of more than two subclades. Although the subclades are not always strongly supported, a few of them represent particular infrageneric taxa (sections) proposed in the previous systems of classification as will be discussed later. Our analyses also confirmed that some species that had been separated as belonging to separate genera, i.e., *Borderea*, *Epipetrum*, *Rajania*, *Tamus*, and *Testudinaria*, were all nested within *Dioscorea*. Noticeably, the species (*D. biloba* [Phil.] Caddick & Wilkin and *D. humilis* Bertero ex Colla) of *Epipetrum* form a distinct subclade basally positioned within the NWI clade. Likewise, the species of *Rajania*, *Tamus*, and *Testudinaria* form distinct subclades within the NWIII, Mediterranean, and African clade, respectively. Only the species of *Borderea* were included separately in the African and Mediterranean clades.

Discussion

Infrageneric relationships of Dioscorea and classification

Molecular phylogenetic analyses of 183 *Dioscorea* species showed that all the species formed a single robust clade, confirming that *Dioscorea* is a well-distinguished, monophyletic genus in Dioscoreaceae as shown by the previous studies (Wilkin *et al.*

2005; Viruel *et al.* 2016, 2018; Couto *et al.* 2018). *Dioscorea* consisted of the eleven major clades. The previous molecular studies have distinguished ten major clades, which formed a similar topology to the ones obtained in our study. However, we found an additional clade (i.e., *Dioscorea birmanica*), because it has a distinctive morphology as discussed later. All of the major clades in our tree were supported strongly (i.e., with a high TBE).

The eleven major clades, which diverged successively, were referred to as the Stenophora, NWI, NWII, Mediterranean, African, *Dioscorea tentaculigera*, CL, NWIII–MG, Birmanica (Shannicorea), *Dioscorea birmanica*, and Enantiophyllum clades. Except for the two clades consisting of a single species, all of the nine other clades show high resolution for intra-clade species relationships. Among the eleven clades, the Stenophora clade is sister to a clade consisting of the rest of the genus. Such a sister group relationship was first reported by Wilkin *et al.* (2005), and thereafter was confirmed by all the subsequent molecular studies (Viruel *et al.* 2016, 2018; Couto *et al.* 2018). Our analyses also provided 100% TBE and 100% FBP for the distinctness of the Stenophora clade and the clade composed of the rest of the genus, respectively, and for their sister-group relationships. The distinct positions of the NWI and NWII clades have also been reported in previous studies, and in particular by Viruel *et al.* (2016, 2018) and Couto *et al.* (2018). As for the Mediterranean and African clades, Couto *et al.* (2018) suggested that they formed a weakly supported sister group relationship. However, our analyses showed that the Mediterranean clade (93/81) is sister to the whole of the seven remaining major clades including the African clade (94/-); this relationship is strongly supported (97/86). Five of the seven remaining major clades (i.e., excluding *Dioscorea tentaculigera* and *Dioscorea birmanica*) were

roughly distinguished in previous studies (Viruel *et al.* 2016, 2018; Couto *et al.* 2018), but the existence of mutual relationships between them has remained unclear. Our current analysis has clarified these relationships.

One of the major issues associated with *Dioscorea* systematics is whether or not — and how — the previously proposed infrageneric taxa receive phylogenetic support. The genus has been divided into 23–58 sections or “genus-equivalent sections” (Uline 1898; Knuth 1924; Burkill 1960; Huber 1998). In order to see which clades coincide with the previously proposed infrageneric taxa, in other words, to see which infrageneric taxa are supported by molecular evidence, we compared the clades/subclades in the tree with the genera/sections proposed by Knuth (1924), Burkill (1960) and Huber (1998) (Fig. 3a, b). Species assignment to individual genera/sections in Fig. 3a and b was made following the three authors or their section descriptions. In the case where a certain section coincided with a certain clade supported by more than 90% TBE, both the section and clade are indicated by a square shaded dark-grey. This is also the case, in which a section consists of a single species, or only a single species was examined for the section. In the case in which the clade is not strongly supported by molecular evidence but supported morphologically, both the section and clade are indicated by a square shaded light-grey.

The *Stenophora* clade coincides with *Dioscorea* section *Stenophora* Uline circumscribed by Burkill (1960). Our analyses showed that the species of the *Stenophora* clade belong to six and three sections in the systems of Knuth (1924) and Huber (1998), respectively. In the case of the Huber’s system, all of the three sections *Illigerastrum*, *Macropoda*, and *Stenophora* are polyphyletic.

The *Dioscorea tentaculigera* and *Dioscorea birmanica* clades consist of a single

species. *Dioscorea tentaculigera* is assigned by Knuth (1924) to *D.* section *Macropoda* and by Burkill (1960) to *D.* sect. *Shannicorea*. However, other species of *D.* sect. *Macropoda* sensu Knuth are scattered within the *Stenophora* clade; other species of *D.* sect. *Shannicorea* were all assigned to the Birmanica (*Shannicorea*) clade (Fig. 3a, b). On the other hand, *D. birmanica* was assigned by Knuth (1924) to *D.* sect. *Combilium*, by Burkill (1960) to *D.* sect. *Stenophora*, and by Huber (1998) to *D.* sect. *Macropoda*. However, in all of these sections the species other than *D. birmanica* are also scattered in other distantly related major clades (Fig. 3a, b). Both *D. tentaculigera* and *D. birmanica* require distinct sections for their own.

As for the eight other major clades, any of them does not coincide with a particular section. However, every major clade consists of more than two strongly supported subclades, a part of which may coincide with certain sections (or “genera”). For instance, within the NWI clade, “*Epipetrum*” is strongly supported. Other strongly supported subclades represent *Dioscorea* sect. *Trigonobasis* sensu Knuth in the NWII clade; “*Tamus*” in the Mediterranean clade; “*Testudinaria*”, *D.* sect. *Perennia* (nom. nud.) and *D.* sect. *Rhacodophyllum* in the African clade; *D.* sect. *Lasiophyton* sensu Burkill, *D.* sect. *Lasiophyton* sensu Huber and *D.* sect. *Opsophyton* sensu Huber in the CL clade; “*Rajania*” and *D.* sect. *Brachyandra* sensu Huber in the NWIII-MG clade; *D.* sect. *Shannicorea* in the Birmanica (*Shannicorea*) clade; and *D.* sect. *Enantiophyllum* in the Enantiophyllum clade (see subclades shaded dark in Fig. 3a, b).

To summarize, comparisons between the major clades/subclades in our tree and the three previously reported systems of classification, sections show that, Huber’s classification is better supported by molecular evidence. In fact, among the 35 “genera” or “genus-equivalent sections” proposed by Huber (1998), 19 were strongly

supported. In particular, of 22 Old World “genera” and “genus-equivalent sections”, 16 were strongly supported and many of them remained as they were initially classified (see below and Taxonomy at the end of this article). In contrast, very few (“*Epipetrum*”, *Dioscorea* sect. *Lasiogyne*, “*Rajania*”) of 13 New World “genera” and “genus-equivalent sections” (of which seven were analyzed in the present study) were supported. Both a broader taxon sampling and a subsequent revision are needed for the infrageneric taxa of the New World species (Couto *et al.* 2018).

Character evolution

Our analyses based on about 29% of the whole species provide an overall picture of the relationships within *Dioscorea*. We identified eleven major clades, among which nine have more than two weakly or strongly supported subclades (Fig. 2a, b). Using this tree framework, we traced evolution of some morphological characters to evaluate their respective systematic value. We examined the morphology of as many *Dioscorea* species as possible by compiling our own dataset and combining it with the information in published articles mainly from Uline (1898), Knuth (1924), Prain and Burkill (1927, 1933, 1936), Burkill (1951, 1960), Huber 1998, Ting and Gilbert (2000), Thapayai *et al.* (2004, 2005a, 2005b), Haigh *et al.* (2005), Wilkin *et al.* (2008, 2017), Wilkin and Thapayai (2009, 2011), and Yamashita and Tamura (2016). Pollen data were obtained from Zavada (1983), Furness and Rudall (1999), Schols *et al.* (2001, 2003, 2005), Wilkin *et al.* (2009) and Viruel *et al.* (2010), and chromosome data from Martin and Ortiz (1966), Takeuchi *et al.* (1970), Fedorov (1974), Orndoff (1967–1968), Moore (1973–1977), Goldblatt (1981–1988), Goldblatt and Johnson (1990–2010), and Viruel *et al.* (2008). Concerning many of the characters, there was

extensive homoplasy throughout the genus. We ultimately selected the following ten characters to understand the evolution in *Dioscorea*: (1) underground stem rhizome or tuber; (2) pollen aperture number one or two; (3) chromosome number diploid with $x = 10$, or decreasing toward different base numbers or multiplied of $x = 10$ to become polyploids; (4) fruit type capsule, berry or samara; (5) leaf simple or compound; (6) stem twining to left or right; (7) phyllotaxis alternate or opposite; (8) capsule wider than its length or longer than its width; (9) stem/leaf indumentum present or not; (10) seeds variously winged or unwinged. Polarities of their respective character states were determined using *Stenomeris* as the outgroup (for data matrix see Tables 2 and 3), and the distribution of the character states were mapped on a phylogenetic tree using parsimony in Mesquite version 3.03 (Maddison and Maddison 2015) (Figs. 4–6). Besides, some characters provided autapomorphies for a few subclades. They include: number of fertile stamens three; stem not twining; the tuber becoming annual; stem lignified; bulbils formed; tuber horizontal or globose; leaf lobed; and stems bearing spines. Although these characters are not presented in the figures, they distinguish certain section from one another.

Figure 4 shows the distribution of character-states relating to the underground stem and pollen aperture number on the tree indicated in Fig. 2. As discussed above, molecular analyses have consistently shown that *Dioscorea* section *Stenophora* is sister to the rest of the genus. A characteristic features of *D. sect. Stenophora* is the presence of a rhizomatous underground stem (a plesiomorphy). Irrespective of whether it is perennial or annual, the tuber occurs as a synapomorphy for the rest of the genus (Fig. 4a). However, an annual tuber occurs at least twice as a homoplasy in species of derived clades. As for pollen characters, Schols *et al.* (2005) showed that aperture

number increased from one in *D. sect. Stenophora* to two in the rest of the genus. Our analyses confirm this same evolutionary trend (Fig. 4b). However, pollen data from many other species, and in particular from species of the NWI, are still required in order to unequivocally confirm this evolutionary change. Thus, like molecular evidence, morphological evidence relating to the underground stem and pollen aperture number indicated that the genus should be divided into two major infrageneric taxa: one representing *D. sect. Stenophora* with the rhizome, and the other representing the rest of the genus that has the tuber. Hereafter, we tentatively refer to the *Stenophora* clade as the rhizome clade and the clade of the rest of the genus as the tuber clade (Fig. 5).

As for the eight other characters, their respective evolutionary changes occur only in infrageneric taxa or species groups of the tuber clade. They are presented in Figs. 5 and 6, which show a tree modified from Fig. 2, consisting of 27 major clades and/or final major subclades. Among the 27 major clades and/or final major subclades, the following 20 represent Old World sections or species groups: Birmanica group, *Borderea**, *Brachyandra**, Burchellii group, *Cardiocapsa*, *Combilium**, *Dioscorea** (= *D. sect. Stenophora* Uline circumscribed by Burkill [1960]), *Enantiophyllum*, Gillettii group, *Lasiophyton**, *Macrocarpaea**, *Macrourea**, *Opsophyton**, *Paramecocarpa**, *Rhacodophyllum**, *Shannicorea**, *Stenocorea**, *Tamus**, Tentaculigera group, and *Testudinaria*. Of these 20 Old World sections or species groups, 13 (indicated by a single asterisk) have already been proposed or circumscribed, mostly by Huber (1998) as “genera” or “genus-equivalent sections”; four are newly recognized Birmanica-group, Burchellii-group, Gillettii-group, and Tentaculigera-group; the three sections (*Cardiocapsa*, *Enantiophyllum*, and *Testudinaria*) are more broadly circumscribed than before. The remaining seven represent New World section or species groups: *D. sect.*

*Apodostemon*1, *D. sect. Apodostemon*2, *Epipetrum*, *D. sect. Lasiogyne*, NWI (except *Epipetrum*), NWII, and *Rajania*. Soto Gomez *et al.* (2019) recently provided a well-resolved phylogenetic tree of *Dioscorea*, but the tree lacks six of the 20 aforementioned Old World sections or species groups.

Figure 5 shows the evolution of the characters 3–9, along with the evolution of the underground stem and pollen aperture number. It also shows not only synapomorphies (twining stems and the presence of orbicules [Schols *et al.* 2005]) supporting a sister-group relationship between *Dioscorea* and *Stenomeris*, but also the synapomorphies (dioecy and $x = 10$) supporting the monophyly of *Dioscorea*. As for the chromosome numbers (character 3), we postulated diploid with $x = 10$ as the ancestral number in *Dioscorea*. *Stenomeris*, a sister group to *Dioscorea*, has no chromosome information. Within *Dioscorea*, diploid with $x = 10$ is characteristic of the rhizome clade, whereas in the tuber clade a variety of basic chromosome numbers are recorded including $x = 6, 7, 9$ and 10 . Because of its common occurrence in both the rhizome and tuber clades, $x = 10$ can be regarded as a (sym-)plesiomorphy, and is probably a synapomorphy of *Dioscorea*. Reductions from $x = 10$ to $x = 9$, accompanied with polyploidization of $x=9$ from diploid to tetraploid ($2n=36$), occur as a homoplasy at NWII and NWI groups (with the exception of the *Epipetrum* group), respectively. Likewise, reduction from $x = 10$ to $x = 6$, followed by polyploidization of $x=6$ from $2x$ to $4x$ occurs at base of a clade of *D.* sections *Borderea* and *Tamus*. Reduction from $x = 10$ to $x = 7$ occurs at the *Epipetrum* group, but without any polyploidization. Additionally, sporadic reductions from $x=10$ to $x=9$ occur within a clade of NWIII. The polyploidization of $x=10$ from diploid ($2x$) to tetraploid ($4x$), hexaploid ($6x$), octoploid ($8x$), and above occurs in a large clade consisting of many

derived sections and species groups.

Evolutionary change of fruit type (character 4) occurs from capsular fruits to berries in *Dioscorea* section *Tamus*, and from capsular fruits to samaras in the *Rajania* group. Evolutionary change from simple to compound leaves (character 5) occurs in *D.* sect. *Lasiophyton*. As for stem twining (character 6), *Stenomeris* has the left-twining stem which is prevalent in *Dioscorea*. However, stem twining to the right occurs as a homoplasy in the NWI group *pro parte* (not shown in Fig. 5), the NWII group, and a large derived *D.* sect. *Enantiophyllum*. According to Schols *et al.* (2003), pollen morphology strongly supports a monophyly of *D.* sect. *Enantiophyllum* sensu Burkill because it differs from the other *Dioscorea* in having small pollen grains with small perforations and a high perforation density. Further research is required to confirm that species of *D.* sects. *Asterotricha* and *Cotinifoliae* we included in *D.* sect. *Enantiophyllum* also have such small perforations. As for phyllotaxis (character 7), most species have alternate leaves. However, opposite leaves occur as homoplasies in both *D.* sects. *Macrourea* and *Enantiophyllum*. Fruit (mostly capsule) wider than its length instead of longer than its width (character 8) occurs in at least five times as homoplasies in a clade of *D.* sects. *Enantiophyllum*/Birmanica group, *Stenocorea*, *Cardiocapsa*, *Lasiogyne*, and a part of the NWI group (with the exception of the *Epipetrum* group; data not shown in Fig. 5). Indumentum (character 9) once developed on stems and leaves in *D.* sect. *Lasiophyton* and in a large clade consisting of *D.* sects. *Enantiophyllum*/Birmanica group, *Shannicorea*, *Paramecocarpa* (only on inflorescence axes), *Macrocarpaea*, *Combilium* and *Stenocorea*, but was lost as a reversal in *D.* sect. *Stenocorea*.

As for seed wing (character 10), *Stenomeris* sister to *Dioscorea* has seeds with

an apical wing. However, because such seeds occur in a few derived *D.* sections, *Enantiophyllum pro parte*, *Shannicorea/Paramecocarpa* and *Testudinaria* (Fig. 6), they are unlikely to represent a plesiomorphy in *Dioscorea*. A distribution of winged and unwinged seeds shows that those surrounded by the wing are widespread in *Dioscorea* and occur in eleven sections or species groups including *D.* sect. *Dioscorea* of the rhizome clade and a basally diverging NWI group of the tuber clade (Fig. 6). Likewise, seeds with the wing at the base only also occur in seven sections or species groups including *D.* sect. *Dioscorea* of the rhizome clade and a basally diverging NWI group of the tuber clade. However, if we regard seeds with basal wings as ancestral in the genus, more numerous steps (eight times homoplasies) are required than would be the case if we regard seeds completely surrounded by the wing as ancestral. We propose that the distribution of seed wing morphologies in *Dioscorea* indicates that ‘seeds completely surrounded by the wing’ represents a plesiomorphic character state. An evolution seems to have occurred from such plesiomorphic seeds to seeds partially losing the wing, resulting in wings appearing basally, apically, or in both positions (as in *D.* sects. *Macrocarpaea* and *Macrourea*, and *Tentaculigera* group); in some cases, the wing is completely absent as in *D.* sects. *Borderea*, *Tamus*, and the *Epipetrum*, *Gilletii*, and *Rajania* groups (Fig. 6).

Conclusion

Molecular analyses showed that the genus *Dioscorea* consists of the rhizome clade and tuber clade, the former comprising *D.* section *Dioscorea* (= *D.* sect. *Stenophora* Uline circumscribed by Burkill 1960), and the latter comprising the rest of the genus. The tuber clade is further characterized by having the bisulcate pollen instead of the

monosulcate pollen as in the rhizome clade. Based on molecular and morphological evidence, we propose dividing the genus *Dioscorea* into two subgenera *Dioscorea* and *Helmia* (Kunth) Benth.

Molecular analyses further showed that subg. *Helmia* consists of the ten major clades. While some of these clades reflected a geographical species distribution (for example, NWI and NWII), others roughly represent a species group that is defined morphologically (such as *Dioscorea* sections. *Shannicorea* and *Enantiophyllum*) (Fig. 2a, b). In future, these ten major clades may be treated as infrageneric taxa at the highest rank like sections within the genus, but half of them have no morphological synapomorphy(-ies) at the present stage. On the other hand, comparisons with the previously proposed infrageneric taxa or sections (Fig. 3a, b) showed that Huber's system of classification is better supported than classifications proposed by others.

The analysis of character-state distribution on the simplified tree (Figs. 5, 6) allowed us to distinguish 27 sections or species groups, among which 20 sections or species groups represent the Old World species. Our large-scale analysis contained 116 Old World species which cover all of the 22 "genera" or "genus-equivalent sections" Huber (1998) proposed for the Old World species. While a broader taxon sampling is needed from New World species in future analyses, a revision is needed for infrageneric classification of the Old World species of the subgenus *Helmia* in the light of phylogenetic and character-state distribution analyses.

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Table 1. Taxa examined of *Dioscorea*, *Tacca* and *Nartheicum*.

Taxon	Voucher	Acc. no. (<i>trnK</i>)	Acc. no. (<i>rbcL</i>)	Acc. no. (<i>atpB</i>)	Acc. no. (<i>trnL-F</i>)
<i>Dioscorea alata</i> L.	Cultivated, Kisaichi Bot. Gard., Osaka City Univ., <i>M. N. Tamura and S. Fuse 10019</i> (KYO).	LC327666	LC327795	LC327924	LC328053
<i>D. asclepiadea</i> Prain & Burkill	Japan: Amakusa, Pref. Kumamoto, <i>M. N. Tamura 20188</i> (KYO).	LC327668	LC327797	LC327926	LC328055
<i>D. bulbifera</i> L.	Japan: Shimoda-shi, Pref. Shizuoka, <i>M. N. Tamura and Noda 6</i> (KYO)	LC327671	LC327800	LC327929	LC328058
<i>D. bulbifera</i> L.	Thailand: Doi Phu Wae, Prov. Nan, Prov. Nan, <i>M. N. Tamura et al. T-30040</i> (KYO)	LC327683	LC327812	LC327941	LC328070
<i>D. bulbifera</i> L.	Thailand: Doi Phu Wae, Prov. Nan, Prov. Nan, <i>M. N. Tamura et al. T-30041</i> (KYO)	LC327684	LC327813	LC327942	LC328071
<i>D. cirrhosa</i> Lour.	Thailand:Khao Yai National Park, Prov. Nakhon Ratchasima, <i>M. N. Tamura et al. T-31329</i> (KYO)	LC327688	LC327817	LC327946	LC328075
<i>D. colletii</i> Hook. f.	Thailand: Doi Phu Wae, Prov. Nan, Prov. Nan, <i>M. N. Tamura et al. T-30029</i> (KYO)	LC327690	LC327819	LC327948	LC328077
<i>D. esculenta</i> (Lour.) Burkill.	Japan: Ishigaki Isl. Pref. Okinawa, <i>M. Togashi not numbering 3 Aug. 1977</i> (KYO)	LC327692	LC327821	LC327950	LC328079
<i>D. glabra</i> Roxb.	Thailand: Doi Chiang Dao, Prov. Chiang Mai, Prov. Chiang Mai, <i>M. N. Tamura et al. 9602</i> (KYO)	LC327693	LC327822	LC327951	LC328080
<i>D. glabra</i> Roxb	Thailand: Doi Phu Kha, Prov. Nan, <i>M. N. Tamura et al. 20156</i> (KYO)	LC327694	LC327823	LC327952	LC328081
<i>D. glabra</i> Roxb	Thailand: Doi Phu Wae, Prov. Nan, <i>M. N. Tamura et al. T-30042</i> (KYO)	LC327698	LC327827	LC327956	LC328085
<i>D. gracillima</i> Miq.	Japan: Kawakami-gun, Pref. Okayama, <i>N. Kurosaki 23741</i> (KYO)	LC327699	LC327828	LC327957	LC328086
<i>D. izuensis</i> Akahori	Japan: Shimoda-shi, Pref. Shizuoka, <i>S. Fuse and H. Noda 4646</i> (HYO)	LC327706	LC327835	LC327964	LC328093
<i>D. japonica</i> Thunb.	Japan: Izu Peninsula, Pref. Shizuoka, <i>M. N. Tamura & H. Noda 19470</i> (KYO)	LC327709	LC327838	LC327967	LC328096
<i>D. kratika</i> Prain & Burkill	Thailand:Khao Yai National Park, Prov. Nakhon Nayok, <i>M. N. Tamura et al. T-31029</i> (KYO)	LC327727	LC327856	LC327985	LC328114
<i>D. luzonensis</i> Schauer	The Philippines: Babuyan Isl, <i>S. Hatusima and M. Sato 29467</i> (TI)	LC327729	LC327858	LC327987	LC328116
<i>D. matsudae</i> Hayata	Taiwan: Taipei city, <i>Jose L. Panero 6396</i> (KYO)	LC327730	LC327859	LC327988	LC328117
<i>D. membranacea</i> Pierre ex Prain & Burkill	Thailand: Doi Ang Khang. Prov. Chiang Mai, <i>M. N. Tamura et al. T-30059</i> (KYO)	LC327732	LC327861	LC327990	LC328119
<i>D. nipponica</i> Makino	Japan: Ohmati-shi, Pref. Nagano, <i>Tamura and Noda 18</i> (KYO)	LC327735	LC327864	LC327993	LC328122
<i>D. pentaphylla</i> L.	Kingdom of Tonga, <i>Minori Suzuki & F. Toicelau T00423</i> (KYO)	LC327736	LC327865	LC327994	LC328123
<i>D. petelotii</i> Prain & Burkill	Thailand: Doi Phu Kha, Prov. Nan, <i>M. N. Tamura et al. 20155</i> (KYO)	LC327737	LC327866	LC327995	LC328124
<i>D. polystachya</i> Turcz.	Japan: Osaka-shi, Pref. Osaka, <i>M. N. Tamura et al. 5</i> (KYO)	LC327738	LC327867	LC327996	LC328125
<i>D. pseudojaponica</i> Hayata	Japan: Nakagusuku, Pref. Okinawa, <i>M. N. Tamura 20196</i> (KYO)	LC327745	LC327874	LC328003	LC328132
<i>D. pseudonitens</i> Prain & Burkill	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>Tamura No. 9614</i> (KYO)	LC327747	LC327876	LC328005	LC328134
<i>D. pseudonitens</i> Prain & Burkill	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M. N. Tamura et al. T-30072</i> (KYO)	LC327748	LC327877	LC328006	LC328135
<i>D. quinquelobata</i> Thunb.	Japan: Katano-shi, Pref. Osaka, <i>M. N. Tamura and Noda 16</i> (KYO)	LC327757	LC327886	LC328015	LC328144
<i>D. septemloba</i> var. <i>septemloba</i> Thunb.	Japan: Mt. Tanzawa, Pref. Kanagawa, <i>M. N. Tamura et al. 20126</i> (KYO)	LC327761	LC327890	LC328019	LC328148
<i>D. septemloba</i> Thunb. var. <i>sititoana</i> Ohwi	Japan: Izu-ohshima Isl., Pref. Shizuoka, <i>M. N. Tamura and H. Noda 20004</i> (KYO)	LC327764	LC327893	LC328022	LC328151
<i>D. tabatae</i> Hats. Ex Yamashita & Minoru N. Tamura	Japan: Amami-ohshima Isl., Pref. Kagoshima, <i>M. N. Tamura and H. Noda 20110</i> (KYO)	LC327767	LC327896	LC328025	LC328154
<i>D. tentaculigera</i> Prain & Burkill	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M. N. Tamura et al. T-30088</i> (KYO)	LC327770	LC327899	LC328028	LC328157
<i>D. tentaculigera</i> Prain & Burkill	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M. N. Tamura et al. T-30109</i> (KYO)	LC327771	LC327900	LC328029	LC328158
<i>D. tenuipes</i> Franc. & Sav.	Japan: Katano-shi, Pref. Osaka, <i>J. Yamashita 7448</i> (Okayama University)	LC327773	LC327902	LC328031	LC328160
<i>D. tokoro</i> Makino	Japan: Atami-shi, Pref. Shizuoka, <i>Fuse and Noda 13</i> (KYO)	LC327778	LC327907	LC328036	LC328165
<i>D. velutipes</i> Prain & Burkill	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M. N. Tamura et al. No. 9606</i> (KYO)	LC327783	LC327912	LC328041	LC328170
<i>D. zentaroana</i> Koidu.	Japan: Amami-ohshima Isl. Pref. Kagoshima, <i>M. N. Tamura 20352</i> (KYO)	LC327790	LC327919	LC328048	LC328177
<i>Tacca</i> sp.	Cultivated, Kisaichi Bot. Gard., Osaka City Univ., <i>M. N. Tamura & Noda 23</i> (KYO)	LC327793	LC327922	LC328051	LC328180
<i>Tacca</i> sp.	Cultivated, Kisaichi Bot. Gard., Osaka City Univ., <i>M. N. Tamura & Noda 20180525</i> (KYO)	LC327794	LC327923	LC328052	LC328181
<i>Nartheicum asiaticum</i> Maxim.	Japan: Mt. Sanpoiwadake, Pref. Gifu, <i>M. N. Tamura & Fuse 15335, 20 Oct. 2003</i> (KYO)	LC327792	LC327921	LC328050	LC328179

Table 2. Data matrix of underground stem and pollen aperture in Mesquite of *Dioscorea*.

Taxon	Underground stem	Number of pollen apertures
<i>Dioscorea acanthogene</i>	1	?
<i>D. alata</i>	1	?
<i>D. alatipes</i>	1	1
<i>D. althaeoides</i>	0	0
<i>D. amaranthoides</i>	1	?
<i>D. amazonum</i>	1	1
<i>D. antaly</i>	1	1
<i>D. arachidna</i>	1	?
<i>D. arcuatinervis</i>	1	1
<i>D. aristolochiifolia</i>	1	?
<i>D. asclepiadea</i>	0	0
<i>D. aspersa</i>	1	?
<i>D. asperula</i>	1	?
<i>D. banzhuana</i>	0	?
<i>D. baracoensis</i>	1	?
<i>D. bemarivensis</i>	1	1
<i>D. benthamii</i>	1	?
<i>D. besseriana</i>	1	?
<i>D. biformifolia</i>	0	0
<i>D. biloba</i> subsp. <i>biloba</i>	1	1
<i>D. biloba</i> subsp. <i>coquimbana</i>	1	?
<i>D. birmanica</i>	0	1
<i>D. brachybotrya</i>	1	1
<i>D. bradei</i>	1	?
<i>D. brevipetiolata</i>	1	?
<i>D. brownii</i>	1	1
<i>D. buchananii</i>	1	1
<i>D. bulbifera</i>	1	1
<i>D. burchellii</i>	1	1
<i>D. calcicola</i>	1	?
<i>D. campestris</i>	1	0
<i>D. campos-portoi</i>	1	?
<i>D. caucasica</i>	0	0
<i>D. chouardii</i>	1	?
<i>D. cienegensis</i>	1	?
<i>D. cirrhosa</i>	1	1
<i>D. cirrhosa</i> var. <i>cylindrica</i>	1	?
<i>D. cochleariopiculata</i>	1	?
<i>D. collettii</i>	0	0
<i>D. collettii</i> var. <i>hypoglaucia</i>	0	?
<i>D. communis</i>	1	1
<i>D. composita</i>	1	?
<i>D. convolvulacea</i>	1	?
<i>D. cordata</i>	1	?
<i>D. coriacea</i>	1	1
<i>D. coronata</i>	1	?
<i>D. cotinifolia</i>	1	1
<i>D. cumingii</i>	1	?
<i>D. cynanchiolia</i>	?	?
<i>D. daunaea</i>	1	1
<i>D. decipiens</i>	1	1
<i>D. delicata</i>	1	?
<i>D. deltoidea</i>	0	0
<i>D. dodecaneura</i>	1	1
<i>D. doryphore</i>	1	?
<i>D. dregeana</i>	1	1
<i>D. dumetorum</i>	1	1
<i>D. elegans</i>	1	?
<i>D. elephantipes</i>	1	1
<i>D. esculenta</i>	1	1
<i>D. esquirolii</i>	1	?
<i>D. exalata</i>	1	?
<i>D. fandra</i>	1	1
<i>D. fastuguata</i>	1	?
<i>D. fordii</i>	1	?

Tabel 2. (continued)

<i>Taxon</i>	Underground stem	Number of pollen apertures
<i>D. formosana</i>	1	?
<i>D. futschauensis</i>	0	?
<i>D. galeottiana</i>	1	1
<i>D. gillettii</i>	1	1
<i>D. glabra</i>	1	1
<i>D. glomerulata</i>	1	?
<i>D. gracilis</i>	1	?
<i>D. gracillima</i>	0	?
<i>D. grandiflora</i>	1	?
<i>D. hamiltonii</i>	1	1
<i>D. hassleriana</i>	1	?
<i>D. hastifolia</i>	1	1
<i>D. hemicrypta</i>	1	?
<i>D. hemsleyi</i>	1	?
<i>D. hexagona</i>	1	1
<i>D. hispida</i>	1	?
<i>D. homboka</i>	1	?
<i>D. humifusa</i>	1	?
<i>D. humilis</i> subsp. <i>humilis</i>	1	1
<i>D. humilis</i> subsp. <i>polyanthes</i>	1	1
<i>D. hyalinomarginata</i>	1	?
<i>D. inopinata</i>	1	?
<i>D. itatiensis</i>	?	?
<i>D. izuensis</i>	0	?
<i>D. japonica</i>	1	?
<i>D. juxtlahuacensis</i>	1	?
<i>D. kamoensis</i>	1	?
<i>D. kituiensis</i>	1	?
<i>D. kratika</i>	1	?
<i>D. lagoa-santa</i>	1	0
<i>D. lanata</i>	1	?
<i>D. larecajensis</i>	1	?
<i>D. laxiflora</i>	1	?
<i>D. lepcharum</i>	1	?
<i>D. leptostachya</i>	1	?
<i>D. longirhiza</i>	1	?
<i>D. luzonensis</i>	1	?
<i>D. maciba</i>	1	1
<i>D. macvaughii</i>	1	?
<i>D. madecassa</i>	1	1
<i>D. maianthemoides</i>	1	?
<i>D. marginata</i>	1	?
<i>D. martinii</i>	1	?
<i>D. matsudae</i>	1	?
<i>D. mayottensis</i>	1	?
<i>D. melanophyma</i>	1	?
<i>D. membranacea</i>	0	0
<i>D. minima</i>	1	1
<i>D. minutiflora</i>	1	?
<i>D. modesta</i>	1	?
<i>D. mollis</i>	1	?
<i>D. monadelpha</i>	1	?
<i>D. monandra</i>	1	?
<i>D. multiflora</i>	1	?
<i>D. multiloba</i>	1	?
<i>D. multinervis</i>	1	1
<i>D. mundtii</i>	1	?
<i>D. namorokensis</i>	1	1
<i>D. nipponica</i>	0	0
<i>D. nitens</i>	1	?
<i>D. nummularia</i>	1	1
<i>D. offersiana</i>	1	1
<i>D. orientalis</i>	1	1
<i>D. oryzetorum</i>	1	?
<i>D. ovata</i>	1	?

Tabel 2. (continued)

Taxon	Underground stem	Number of pollen apertures
<i>D. ovinala</i>	1	1
<i>D. pedalis</i>	1	0
<i>D. pedicellata</i>	1	?
<i>D. pentaphylla</i>	1	1
<i>D. perdicum</i>	1	?
<i>D. petelotii</i>	1	?
<i>D. pierreii</i>	1	?
<i>D. piperifolia</i>	1	1
<i>D. pohlii</i>	1	?
<i>D. polygonoides</i>	1	1
<i>D. polystachya</i>	1	?
<i>D. potaninii</i>	1	?
<i>D. prazeri</i>	0	0
<i>D. preussii</i>	1	1
<i>D. pseudojaponica</i>	1	?
<i>D. pseudomacrocapsa</i>	?	?
<i>D. pseudonitens</i>	1	1
<i>D. putisensis</i>	1	?
<i>D. pyrenaica</i>	1	0
<i>D. quinquelobata</i>	0	0
<i>D. rockii</i>	0	?
<i>D. rupicola</i>	1	1
<i>D. sansibarensis</i>	1	1
<i>D. saxatilis</i>	1	?
<i>D. schimperiana</i>	1	1
<i>D. scorpioidea</i>	1	?
<i>D. scortechinii</i>	1	?
<i>D. septemloba</i> var. <i>septemloba</i>	0	?
<i>D. septemloba</i> var. <i>sitiitana</i>	0	?
<i>D. simulans</i>	0	?
<i>D. sinoparviflora</i>	0	?
<i>D. sinuata</i>	1	1
<i>D. soso</i>	1	1
<i>D. sphaeroidea</i>	?	?
<i>D. spongiosa</i>	0	?
<i>D. stegelmanniana</i>	1	?
<i>D. stenophylla</i>	1	?
<i>D. stipulosa</i>	1	?
<i>D. strydomiana</i>	1	?
<i>D. subcalva</i>	1	?
<i>D. subhastata</i>	1	?
<i>D. sylvatica</i>	1	1
<i>D. tabatae</i>	1	?
<i>D. tentaculigera</i>	1	?
<i>D. tenuipes</i>	0	?
<i>D. therezopolensis</i>	1	?
<i>D. tokoro</i>	0	0
<i>D. tomentosa</i>	1	?
<i>D. trichantha</i>	1	?
<i>D. trifida</i>	1	1
<i>D. trisecta</i>	1	?
<i>D. variifolia</i>	1	?
<i>D. velutipes</i>	1	1
<i>D. wallichii</i>	1	1
<i>D. yunnanensis</i>	1	?
<i>D. zentaroana</i>	0	0
<i>D. zingiberensis</i>	0	0
<i>Stenomeris bornensis</i>	0	0
<i>Tamus edulis</i>	1	?

Characters and character-states: underground stem rhizome (0), tuber (1); pollen aperture monosulcate (0), bisulcate (1).

Table 3. Datamatrix of eight morphological characters in Mesquite of sections and species groups of *Dioscorea*.

	1	2	3	4	5	6	7	8
<i>Dioscorea</i>	0	0	0(&1)	0	0	0&1	0&1	1&0
NW I (<i>Epipetrum</i>)	2	0	0	2	0	0	0	4
NW I (expect <i>Epipetrum</i>)	1	0	0(&1)	0	0	0	0	1&0
NW II	1	0	0	1	0	0	0	1
<i>Borderea</i>	3	0	0	2	0	0	0	4
<i>Tamus</i>	3	1	0	0	0	?	0	4
Gillettii group	?	0	0	0	0	0	0	4
<i>Rhacodophyllum</i>	?	0	0	0	0	0	0	0
Burchellii group	?	0	0	0	0	0	0	1
<i>Testudinaria</i>	0	0	0	2(&0)	0	0	0	2
Tentaculigera group	4	0	0	0	0	0	0	3
<i>Opsophyton</i>	4	0	0	0	0	0	0	1
<i>Lasiophyton</i>	4	0	1	0	0	0	1	1
NWIII (<i>Apodostemon</i> 2)	?	0	0	0	0	0	0	0
NWIII (<i>Lasiogyne</i>)	4	0	0	0	0	1	0	0
NWIII (<i>Apodostemon</i> 1)	4(&1)	0	0	0	0	0	0	0
NWIII (<i>Rajania</i>)	4(&1)	2	0	0	0	?	0	4
<i>Macrourea</i>	4	0	0	0	1	0	0	3
<i>Cardiocarpa</i>	?	0	0(&1)	0	0	1	0	0
<i>Brachyandra</i>	?	0	0	0	0	0	0	1
<i>Stenocorea</i>	?	0	0	0	0	1	0	0
<i>Combilium</i>	4	0	0	0	0	0	1	0
<i>Shannicorea</i>	4	0	0	0	0	0	1	2
<i>Paramecocarpa</i>	?	0	0	0	0	0	1	2
<i>Macrocarpaea</i>	4	0	0	0	0	0	1	3
Birmanica group	?	0	0	0	0	1	1	0
<i>Enantiophyllum</i>	4	0	0	1	1	1	0&1	0&2

Characters and character-states.

1. chromosome: $x = 10$ and diploid (0), $x = 9$ (1), $x = 7$ (2), $x = 6$ (3), polyploid of $x = 10$ (4); 2. fruit: capsule (0), berry (1), samara (2); 3. leaf: simple (0), compound (1); 4. stem: twining to left (0), twining to right (1) or not twinned (2); 5. phyllotaxis alternate (0), opposite (1); 6. capsule longer than its width (0) or wider than its length (1); 7. stem/leaf indumentum absent (0) or present (1). 8. seed wings all around the seed entity (0), basally (1), apically (2), or both basally and apically (3), absence of a wing (4)

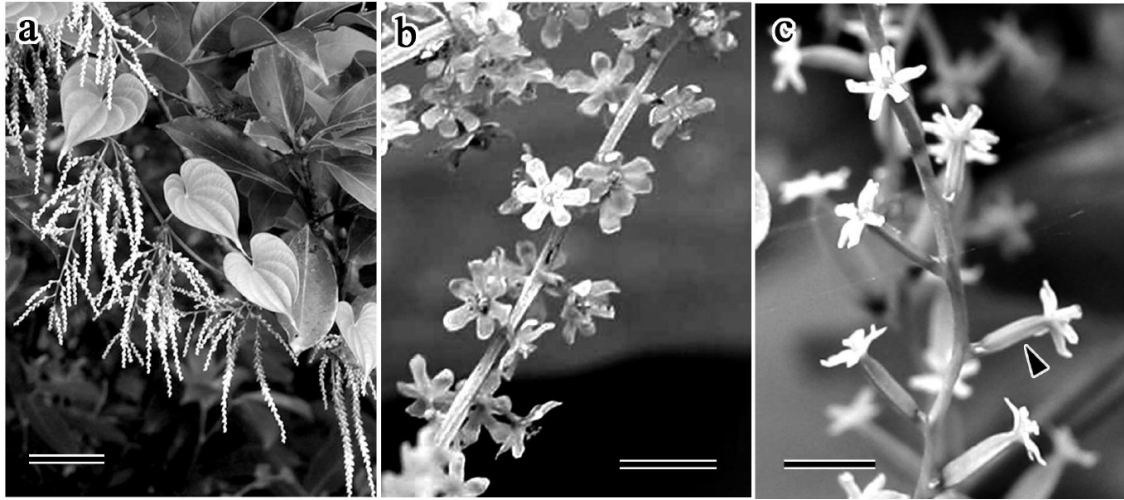


Fig. 1. Terrestrial stems and flowers of *Dioscorea*. a Twining stem (*D. bulbifera*). b Staminate flower (*D. tokoro*). c Pistillate flower (*D. tokoro*) with a distinct inferior ovary (arrowhead). Scale bars = 10 cm in a; 5mm in b, c.

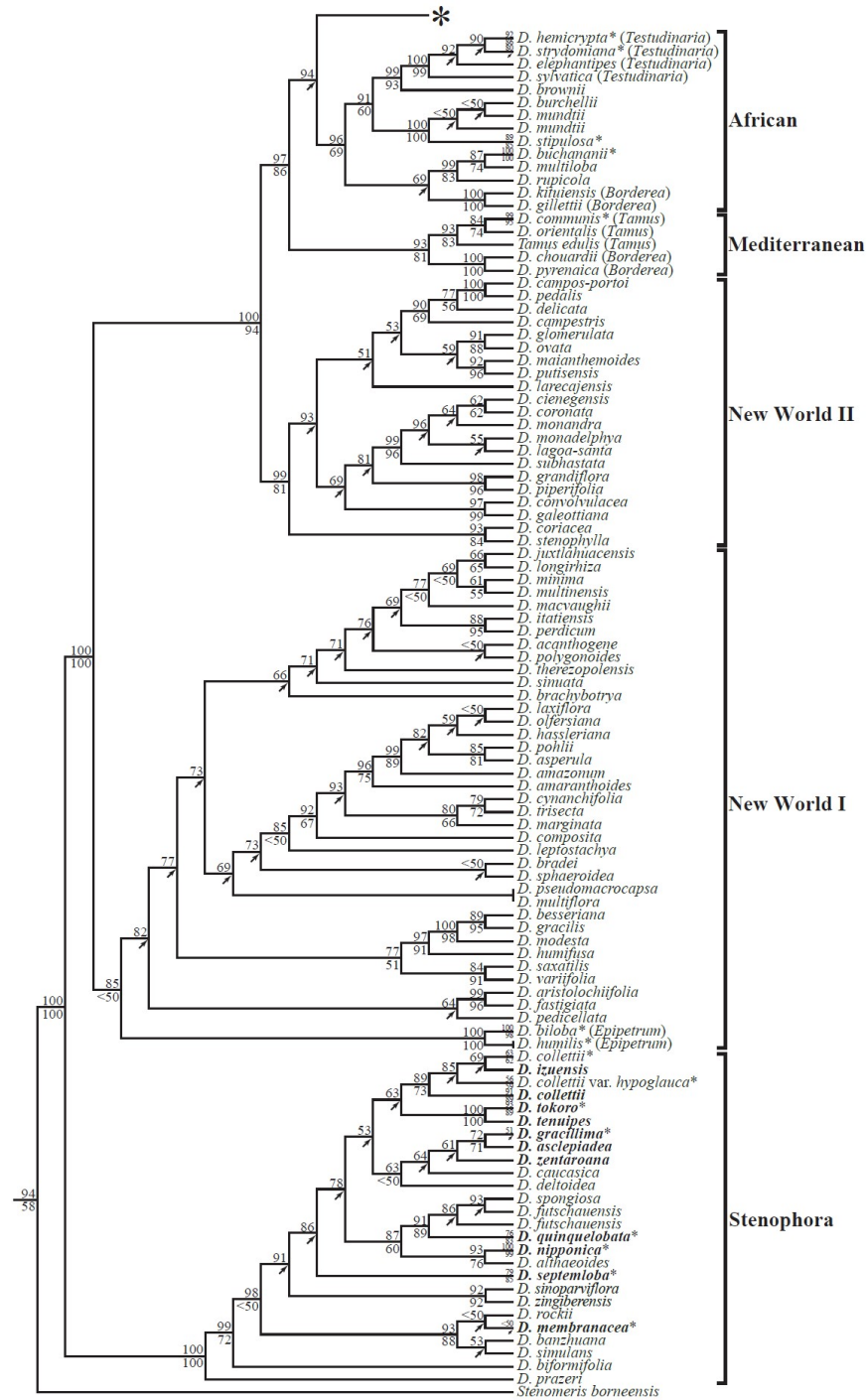


Fig. 2a. ML tree of 183 species of *Dioscorea*, produced by combined sequence data of four cpDNA regions (*rbcL*, *matK*, *trn L-F*, and *atpB*). *Narthecium asiaticum* (outgroup) and seven species of *Tacca* are not shown in the figure. Numbers above and below branches indicate Transfer bootstrap expectation (TBE) and Felsenstein's bootstrap proportion (FBP), respectively. Bold lines for the clade names indicate the ranges used by Couto *et al.* (2018), and narrow lines added to the bold ones indicate the expanded ranges used by Wilkin *et al.* (2005) and Viruel *et al.* (2016, 2018). Species sequenced by ourselves are indicated in bold. Asterisks indicate that there is sequence variation within a species. The nodes indicated by arrows are collapsed in the strict consensus tree of all equally parsimonious trees.

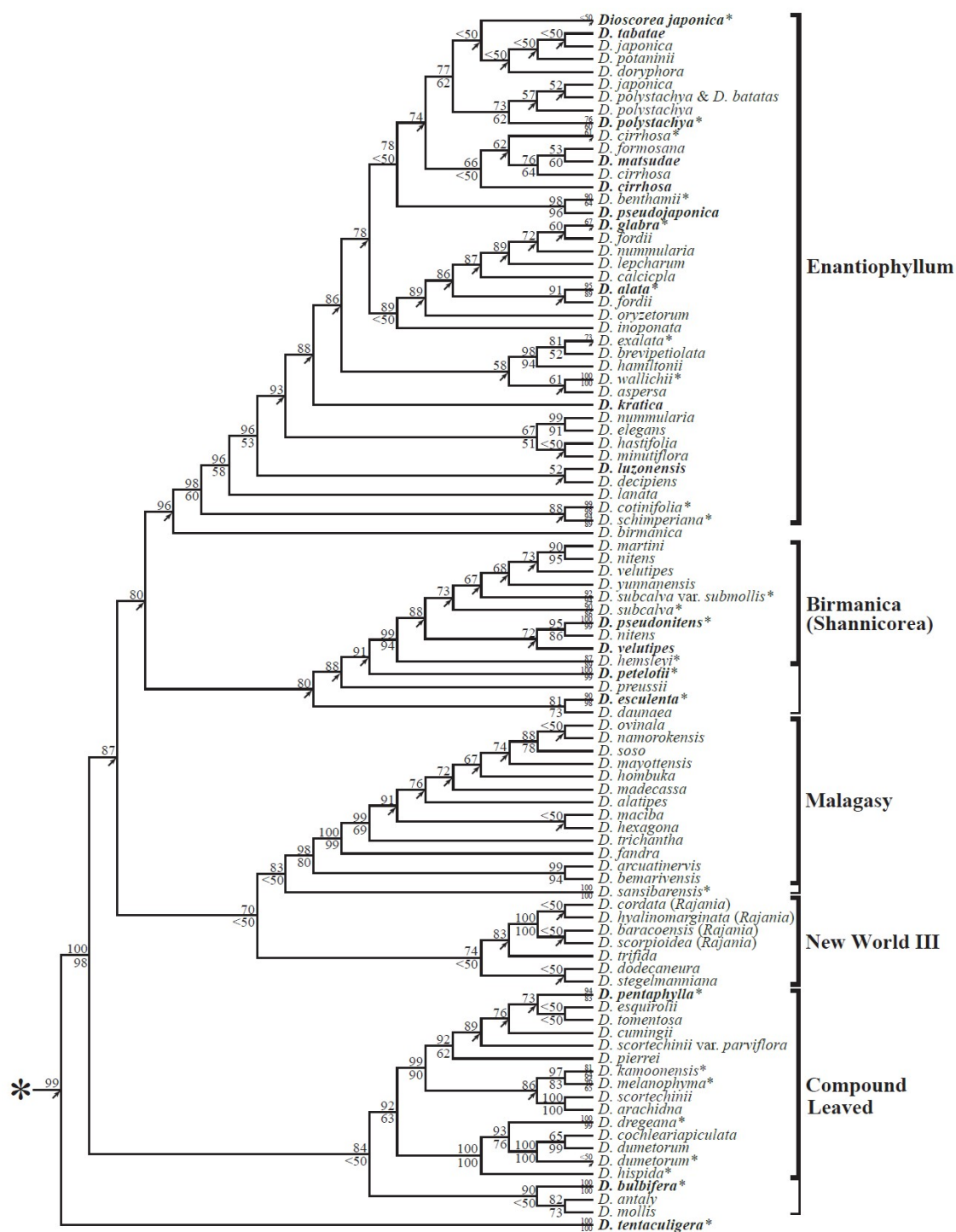


Fig. 2b. (continued)

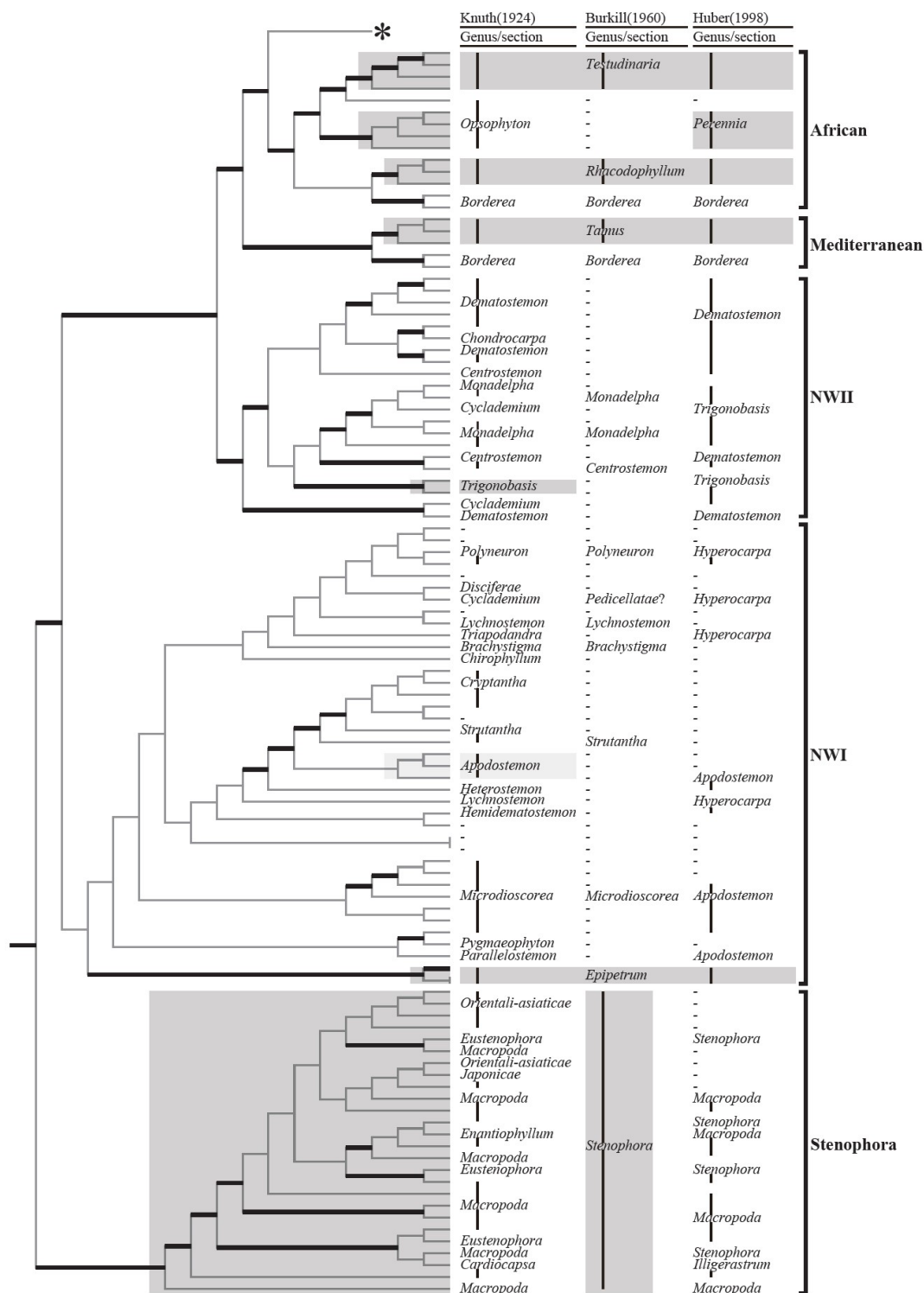
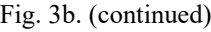


Fig. 3a. Comparisons between a *Dioscorea* tree topology of Fig. 2 and infrageneric taxa (genera/sections) proposed in three previous systems of classification (Knuth 1924; Burkill 1960; Huber 1998). Clades with 90-100% TBE support are marked with thick branches. NWI, NWII, CL, NWIII and MG indicate the New World I, New World II, Compound leaved, New World III and Malagasy clades, respectively. Bold lines and narrow ones for the clade names indicate the ranges as in Fig. 2. Among the clades that coincided with any of the infrageneric taxa, those with over 90% TBE support and those with less than 90% TBE support are shaded dark and shaded light dark, respectively.



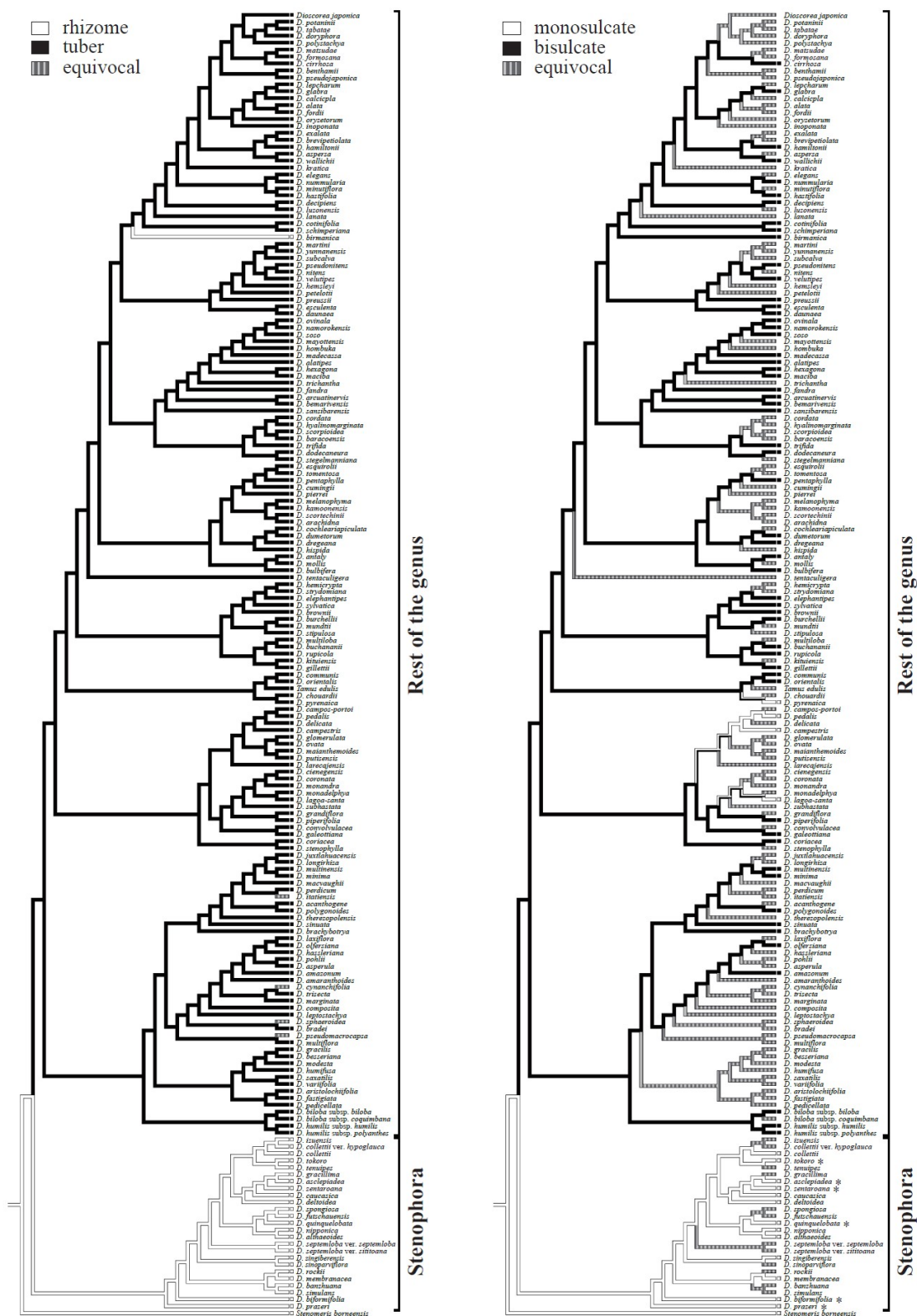


Fig. 4. Distributions of character states relating to underground stem and pollen aperture number on the *Dioscorea* tree given in Fig. 2. Asterisks on six species including *D. bifurcata* and *D. pruriens* indicate original data obtained by ourselves. Note a clear gap between the Stenophora clade and a clade consisting of the rest of the genus.

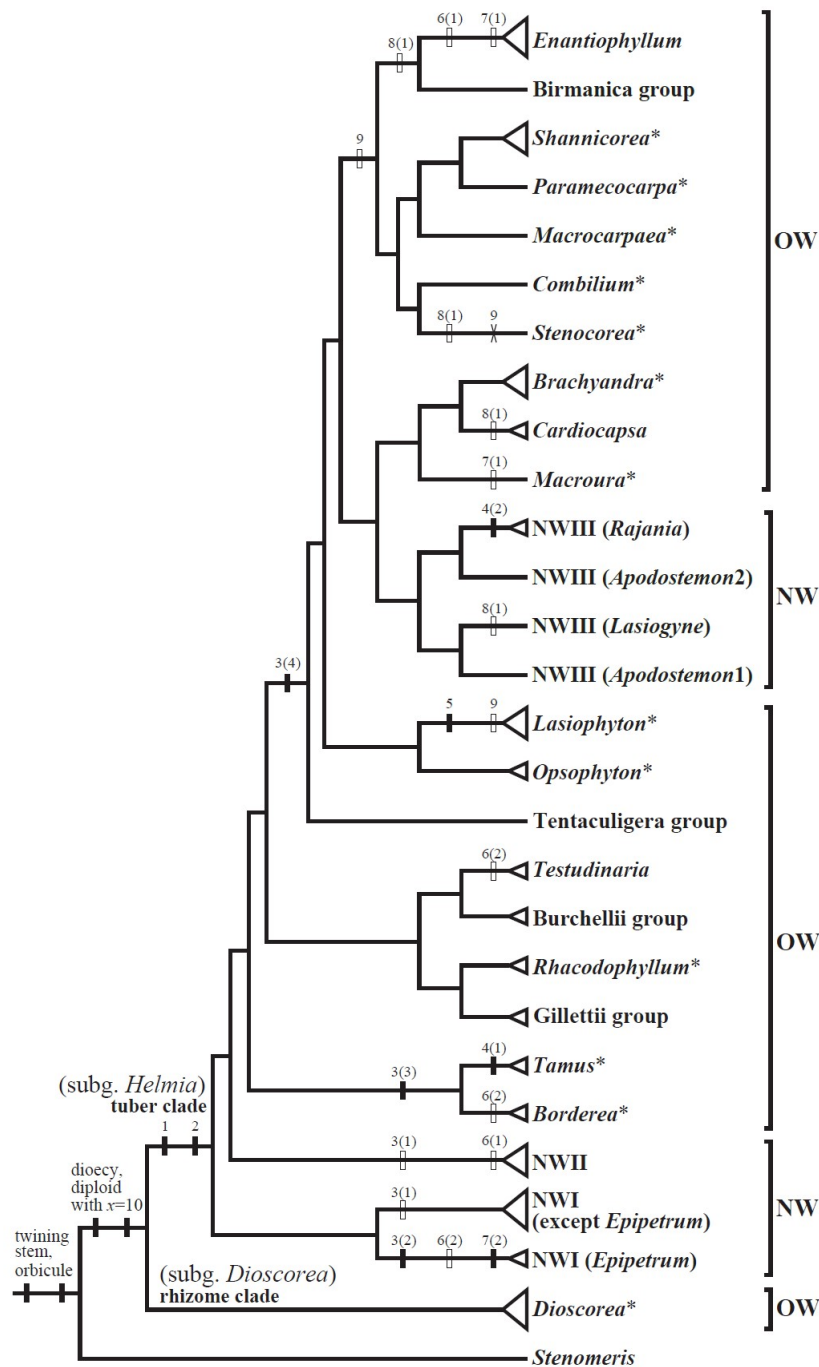


Fig. 5. Evolution of morphological characters in *Dioscorea*, shown on a tree modified from Fig. 2. The tree consists of 27 major clades and/or final major subclades, which represent 20 Old World sections and seven New World section or species groups. * indicates 13 already proposed or circumscribed sections mostly by Huber (1998). Characters (1 to 9) and character-state polarities are as follows. 1. underground stem: rhizome (0), tuber (1); 2. pollen aperture: monosulcate (0), bisulcate (1); 3. chromosome: $x = 10$ and diploid (0), $x = 9$ (1), $x = 7$ (2), $x = 6$ (3), polyploid of $x = 10$ (4); 4. fruit: capsule (0), berry (1), samara (2); 5. leaf: simple (0), compound (1); 6. stem: twining to left (0), twining to right (1) or not twinned (2); 7. phyllotaxis alternate (0), opposite (1); 8. capsule longer than its width (0) or wider than its length (1); 9. stem/leaf indumentum absent (0) or present (1). Solid square = apomorphy, open square = homoplasy, and cross bar = reversal. NW and OW indicate New World and Old World sections or species groups, respectively.

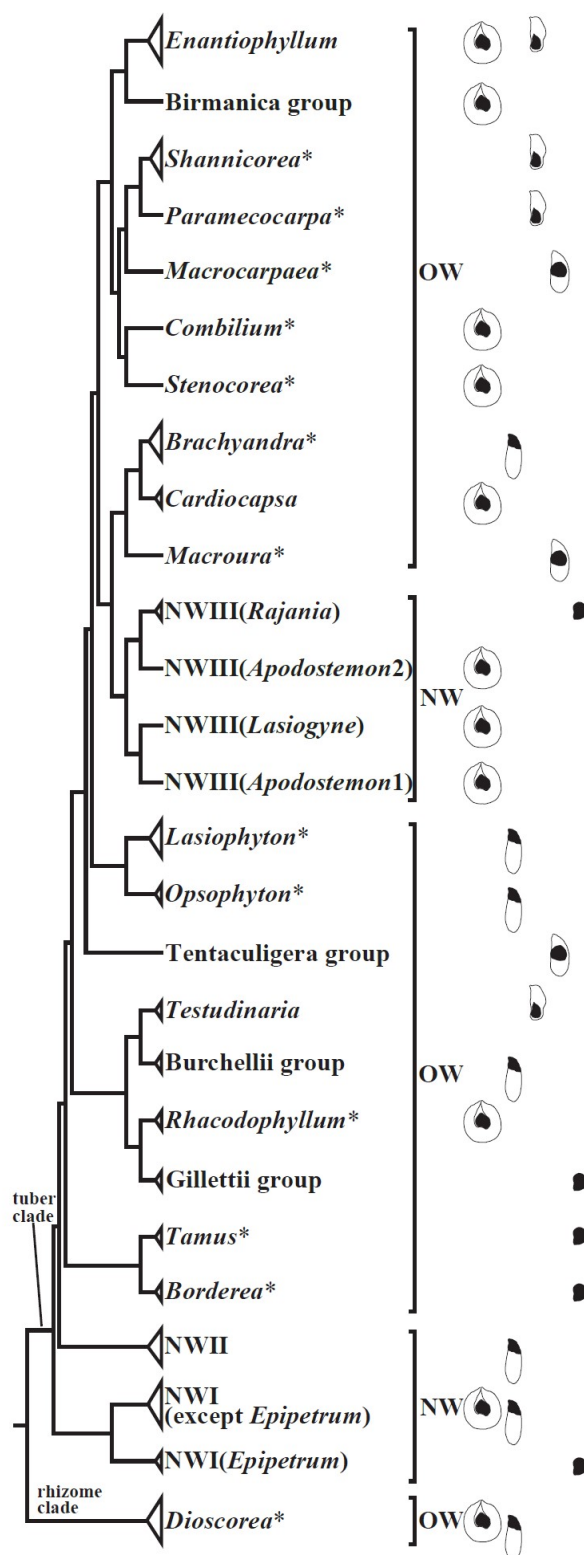


Fig. 6. Evolution of seed wings in *Dioscorea*, shown on a tree modified from Fig. 2. Note that *Dioscorea* seeds are diverse with regard to the presence or absence of a wing (right) and, if present, in the position where it occurs (completely surrounding the seed entity, at the basal or apical, or present at both basal and apical positions (arranged from left to right). *, NW and OW are used as in Fig. 5.

Appendix 1. Accession numbers for GenBank

The following items are arranged in the order of taxon name, *matK*, *rbcL*, *atpB* and *trnL-F*. “-” indicates data not available.

Dioscoreaceae R. Br. *Dioscorea* L. *D. acanthogene* Rusby, AY956477, AY667101, KM877941, KM878021, *D. alata* L., AB040208, AY667098, KM878003, KM878102, *D. alata* L., JQ733662, JQ733739, -, JQ733816, *D. alata* L., JQ733689, JQ733766, -, JQ733843, *D. alata* L., JQ733716, JQ733793, -, JQ733870, *D. alatipes* Burkill & H.Perrier, AY950687, AY667099, KM877977, KM878071, *D. althaeoides* R. Knuth, EU407548, EU407550, -, EU301741, *D. amaranthoides* C. Presl, AY950688, KM877873, -, KM878022, *D. amazonum* Griseb., KU308789, KU308744, -, -, *D. antaly* Jum. & H. Perrier, AY956476, AY667100, KM877991, KM878086, *D. arachidna* Prain & Burkill, AY956478, AF307468, AF308012, KM878087, *D. arcuatineris* Hochr., AY956479, AY667102, KM877978, KM878072, *D. aristolochifolia* Poepp., KM877834, KM877874, KM877942, KM878023, *D. aspersa* Prain & Burkill, EF614211, EF614213, -, DQ841304, *D. asperula* Pedralli, KU308790, KU308746, -, -, *D. banzhuana* S. J. Pei & C. T. Ting, DQ974182, DQ408174, -, DQ841301, *D. baracoensis* (R. Knuth) Raz (= *Rajania tenuiflora* R. Knuth) R., -, KM877926, KM877998, KM877943, *D. batatas* Decne., JQ733670, JQ733747, -, JQ733824, *D. bemarivensis* Jum. & H. Perrier, AY956480, AY667103, -, KM878073, *D. benthamii* Prain & Burkill, JQ733666, JQ733743, -, JQ733820, *D. benthamii* Prain & Burkill, JQ733695, JQ733772, -, JQ733849, *D. benthamii* Prain & Burkill, JQ733696, JQ733773, -, JQ733850, *D. besseriana* Kunth, KM877835, KM877875, KM877943, KM878024, *D. biformifolia* S. J. Pei & C. T. Ting, EU407549, EU301740, -, EU301742, *D. biloba* (Phil.) Caddick & Wilkin subsp. *biloba*, KM877836, KM877876, KM877944, KM878025, *D. biloba* (Phil.) Caddick & Wilkin subsp. *coquimbana* Viruel, Segarra-Moragues & Villar, KM877837, KM877877, KM877945, KM878026, *D. birmanica* Prain & Burkill, AY956481, AY667104, KM878004, KM878103, *D. brachybotrya* Poepp., AY956482, AF307469, AF308013, KM878027, *D. bradei* R. Knuth, KU308791, KU308747, -, -, *D. brevipedunculata* Prain & Burkill, AY956486, AY667105, KM878005, KM878104, *D. brownii* Schinz, KR086942, KR087028, -, KR070855, *D. buehneri* Benth., AY956487, AY904790, KM877968, KM878058, *D. buehneri* Benth., KR086943, KR087029, -, KR070856, *D. bulbifera* L., AY956488, AY904791, AF187059, KM878088, *D. bulbifera* L., JQ733667, JQ733744, -, JQ733821, *D. bulbifera* L., JQ733676, JQ733753, -, JQ733830, *D. bulbifera* L., JQ733680, JQ733757, -, JQ733834, *D. bulbifera* L., JQ733687, JQ733764, -, JQ733841, *D. bulbifera* L., JQ733713, JQ733790, -, JQ733867, *D. bulbifera* L., JQ733715, JQ733792, -, JQ733869, *D. bulbifera* L., KR086944, KR087030, -, KR070857, *D. burchellii* Baker, KM877853, KM877905, KM877969, KM878059, *D. calcicola* Prain & Burkill, AY956489, KM877930, KM878006, KM878105, *D. calderillensis* R. Knuth, KM877849, KM877891, KM877958, KM878043, *D. campestris* Griseb., KU308792, KU308748, -, -, *D. campos-portoi* R. Knuth, KU308793, KU308749, -, -, *D. caucasica* Lipsky, DQ974188, KM877870, KM877936, KM878015, *D. chouardii* Gausson, KM877855, KM877907, KM877971, KM878061, *D. cienegensis* R. Knuth, KU308794, KU308775, -, -, *D. cirrhosa* Lour., EF028329, AY904792, KM878007, KM878106, *D. cirrhosa* Lour., JQ733663, JQ733740, -, JQ733817, *D. cirrhosa* var. *cylindrica* C.T.Ting & M.C.Chang (= *D. cirrhosa* Lour.), DQ974189, DQ408184, -, DQ841314, *D. cirrhosa* var. *cylindrica* C.T.Ting & M.C.Chang, JQ733729, JQ733806, -, JQ733883, *D. cirrhosa* var. *cylindrica* C.T.Ting & M.C.Chang, JQ733730, JQ733807, -, JQ733884, *D. cochlearioides* De Wild., KM877862, KM877921, KM877992, KM878089, *D. collettii* Hook. f., DQ974178, DQ408173, -, DQ841300, *D. collettii* Hook. f., JQ733664, JQ733741, -, JQ733818, *D. collettii* Hook. f., JQ733728, JQ733805, -, JQ733882, *D. collettii* var. *hypoglaucia* (Palib.) S. J. Pei & C. T. Ting, JQ733720, JQ733797, -, JQ733874, *D. collettii* var. *hypoglaucia* (Palib.) S. J. Pei & C. T. Ting, DQ974176, EF614220, -, DQ841319, *D. communis* (L.) Caddick & Wilkin, KM877854, KM877906, KM877970, KM878060, *D. communis* (L.) Caddick & Wilkin, KR086947, KR087033, -, KR070871, *D. composita* Hemsl., DQ974180, DQ408172, -, DQ841330, *D. convolvulacea* Cham. & Schltdl., AY956491, KM877892, AJ235456, KM878044, *D. cordata* (L.) Raz (= *R. cordata* L.) R., AY973835, AF307472, KM877999, KM878099, *D. coriacea* Humb. & Bonpl. ex Willd., AY956492, KM877893, KM877959, KM878045, *D. coronata* Hauman, KU308797, KU308751, -, -, *D. cotinifolia* Kunth, DQ974185, EF614219, -, DQ841311, *D. cotinifolia* Kunth, KR086948, KR087034, -, KR070862, *D. cotinifolia* Kunth, KR086949, KR087035, -, KR070863, *D. cotinifolia* Kunth, KR086950, KR087036, -, KR070864, *D. cumingii* Prain & Burkill, JQ733668, JQ733745, -, JQ733822, *D. cynanchioides* Griseb., KU308798, KU308752, -, -, *D. daunea* Prain & Burkill, AY956493, AY904793, KM878008, KM878107, *D. decipiens* Hook. f., AY956494, AF307454, AF307998, KM878108, *D. delicata* R. Knuth, KU308799, KU308753, -, -, *D. deltoidea* Wall. ex Griseb., EF614207, EF614218, KM877937, KM878016, *D. dodecaneura* Vell., KU308800, KU308755, -, -, *D. doryphora* Hance, JQ733669, JQ733746, -, JQ733823, *D. doryphora* Hance, JQ733693, JQ733770, -, JQ733847, *D. dregeana* (Kunth) T. Durand & Schinz, KM877863, KM877922, KM877993, KM878090, *D. dregeana* (Kunth) T. Durand & Schinz, KR086952, KR087038, -, KR070866, *D. dregeana* (Kunth) T. Durand & Schinz, KR086953, KR087039, -, KR070868, *D. dregeana* (Kunth) T. Durand & Schinz, JQ024957, JQ025042, -, KR070867, *D. dregeana* (Kunth) T. Durand & Schinz, KR086951, KR087037, -, KR070865, *D. dumetorum* (Kunth) Pax, AY956495, AF307464, AF308008, KM878091, *D. dumetorum* (Kunth) Pax, KR086954, KR087040, -, KR070869, *D. dumetorum* (Kunth) Pax, KR086955, KR087041, -, KR070870, *D. elegans* Ridl. ex Prain & Burkill, KM877867, KM877931, -, KM878109, *D. elephantipes* (L'Hér) Engl., AY956496, KM877909, AF308005, KM878063, *D. esculenta* (Lour.) Burkill, JQ733724, JQ733801, -, JQ733872, *D. esculenta* (Lour.) Burkill, AY956497, AY904794, KM878009, KM878110, *D. esculenta* var. *spinosa* (Roxb. ex Prain & Burkill) R. Knuth, JQ733679, JQ733756, -, JQ733833, *D. esquirolii* Prain & Burkill, DQ974177, DQ408168, -, DQ841322, *D. exalata* C. T. Ting & M. C. Chang, EF028330, DQ408170, -, DQ841325, *D. exalata* C.T.Ting & M.C.Chang, JQ733681, JQ733758, -, JQ733835, *D. exalata* C.T.Ting & M.C.Chang, JQ733725, JQ733802, -, JQ733879, *D. fandra* H. Perrier, AY956498, AY904795, KM877979, KM878074, *D. fastigiata* Gay, KU308801, KU308756, -, -, *D. fordii* Prain & Burkill, EF028333, DQ974195, -, DQ841299, *D. fordii* Prain & Burkill, JQ733706, JQ733783, -, JQ733860, *D. formosana* Knuth, JQ733691, JQ733768, -, JQ733845, *D. futschauensis* Uline ex R. Knuth, DQ974175, DQ408166, -, DQ841316, *D. futschauensis* Uline ex R. Knuth, JQ733671, JQ733748, -, JQ733825, *D. galeottiana* Kunth, AY956499, AY904796, KM877960, KM878046, *D. gillettii* Milne-Redh., AY956500, AY904797, KM877972, KM878064, *D. glabra* Roxb., AY956501, AF307456, AF308000, KM878111, *D. glomerulata* Hauman, AY957586, KM877894, -, KM878047, *D. gracilis* Hook. ex Poepp., KM877838, KM877878, KM877946, KM878028, *D. gracillima* Miq., JQ733709, JQ733786, -, JQ733863, *D. gracillima* Miq., DQ974190, DQ408164, -, DQ841315, *D. grandiflora* Mart. ex Griseb., KM877850, KM877895, -, KM878048, *D. hamiltonii* Hook. f., AY957587, AF307465, AF308009, KM878112, *D. hamiltonii* Hook. f., JQ733678, JQ733755, -, JQ733832, *D. hassleriana* Chodat, KU308804, KU308759, -, -, *D. hastifolia* Nees., KM877868, KM877932, -, KM878113, *D. hemicypta* Burkill, KR086964, KR087050, -, KR070880, *D. hemicypta* Burkill, KR086963, KR087049, -, KR070879, *D. hemicypta* Burkill, KR086961, KR087047, -, KR070877, *D. hemicypta* Burkill, KR086962, KR087048, -, KR070878, *D. hemsleyi* Prain & Burkill, JQ733701, JQ733778, -, JQ733855, *D. hemsleyi* Prain & Burkill, JQ733721, JQ733798, -, JQ733875, *D. hexagona* Baker, AY957588, AY904798, KM877980, KM878075, *D. hispida* Dennst., AY957589, AF307463, AF308007, KM878092, *D. hispida* Dennst., JQ733672, JQ733749, -, JQ733826, *D. hispida* Dennst., JQ733718, JQ733795, -, JQ733872, *D. homboka* H. Perrier, -, KM877915, KM877981, KM878076, *D. humifusa* Poepp., KM877839, KM877879, KM877947, KM878029, *D. humilis* Colla subsp. *humilis*, KM877840, KM877880, KM877948, KM878030, *D. humilis* Colla subsp. *polyanthus* (F. Phil.) Viruel, Segarra-Moragues & Villar, KM877841, KM877881, KM877949, KM878031, *D. hyalinomarginata* Raz (= *R. spiculiflora* Uline ex R. Knuth) R., KM877865, KM877927, KM878000, KM878100, *D. inopinata* Prain & Burkill, AY957590, AY904799, KM878010, KM878114, *D. iatiensis* R. Knuth, KU308805, KU308760, -, -, *D. japonica* Thunb., DQ974183, AF307457, AF308001, DQ841307, *D. japonica* Thunb. 1, JQ733665, JQ733742, -, JQ733819, *D. japonica* Thunb. 2, JQ733683, JQ733760, -, JQ733837, *D. japonica* Thunb. 3, JQ733690, JQ733767, -, JQ733844, *D. juxtahuacensis* (O. Téllez & Dávila) Caddick & Wilkin, KM877842, KM877882, KM877950, KM878032, *D. kamoensis* Kunth, EF028332, DQ408175, KM877994, KM878093, *D. kamoensis* Kunth, JQ733684, -

Appendix 1 (continued)

JQ733761, -, JQ733838, *D. kamoonsensis* Kunth, JQ733712, JQ733789, -, JQ733866, *D. kituiensis* Wilkin & Muasya, KM877857, KM877910, KM877973, KM878065, *D. lagoa-santa* Uline ex R. Knuth, KM877851, KM877896, -, KM878049, *D. lanata* Bail, AY957593, AF307458, AF308002, KM878115, *D. larecajensis* Uline ex R. Knuth, AY957594, KM877897, -, KM878050, *D. laxiflora* Mart. ex Griseb., KU308806, KU308761, -, -, *D. lepcharum* Prain & Burkill, JQ733675, JQ733752, -, JQ733829, *D. leptostachya* Gardner, KU308807, KU308762, -, -, *D. longirhiza* Caddick & Wilkin, AY957595, AF307473, AF308016, KM878033, *D. maciba* Jum. & H. Perrier, AY957596, AY904800, KM877983, KM878078, *D. macvaughii* B. G. Schub., AY957597, AF307460, AF308004, KM878034, *D. madecassa* H. Perrier, Mem., AY957591, KM877916, KM877982, KM878077, *D. maianthemoides* Uline ex R. Knuth, -, KM877898, KM878051, *D. marginata* Griseb., KU308809, KU308764, -, -, *D. martinii* Prain & Burkill, JQ733710, JQ733787, -, JQ733864, *D. mayottensis* Wilkin, KM877861, KM877917, KM877984, KM878079, *D. melanophyllum* Prain & Burkill, EF614210, DQ408176, -, DQ841303, *D. melanophyllum* Prain & Burkill 1, JQ733688, JQ733765, -, JQ733842, *D. melanophyllum* Prain & Burkill 2, JQ733711, JQ733788, -, JQ733865, *D. membranacea* Pierre ex Prain & Burkill, AY957598, AF307467, AF308011, KM878017, *D. minima* C. B. Rob. & Seaton, KM877843, KM877883, AF308003, KM878035, *D. minutiflora* Engl., -, KM877933, KM878011, KM878116, *D. modesta* Phil., KM877844, KM877884, KM877951, KM878036, *D. mollis* Kunth, KU308810, KU308765, -, -, *D. monadelpha* (Kunth) Griseb., -, KM877899, KM877962, KM878052, *D. multiflora* Mart. ex Griseb., KU308812, KU308767, -, -, *D. multiloba* Kunth, KR086966, KR087052, -, KR070882, *D. multinervis* Benth., -, KM877885, KM877952, KM878037, *D. mundtii* Baker, KR086968, KR087054, -, KR070884, *D. mundtii* Baker, KR086967, KR087053, -, KR070883, *D. namorokensis* Wilkin, AY957599, KM877918, KM877985, KM878080, *D. nipponica* Makino, JQ733697, JQ733774, -, JQ733851, *D. nipponica* Makino, AY957600, AF307455, AF307999, KM878018, *D. nitens* Prain & Burkill, JQ733733, JQ733810, -, JQ733887, *D. nitens* Prain & Burkill, AY972487, KM877934, -, KM878117, *D. nummularia* Lam., KM877869, KM877935, -, KM878118, *D. nummularia* Roxb., JQ733723, JQ733800, -, JQ733877, *D. olfersiana* Klotzsch ex Griseb., KU308813, KU308768, -, -, *D. orientalis* (J. Thiebaut) Caddick & Wilkin, KM877858, KM877911, KM877974, KM878066, *D. oryzetorum* Prain & Burkill, AY972482, AY904801, KM878012, KM878119, *D. ovata* Vell., AY950686, KM877900, KM877963, KM878053, *D. ovalina* Baker, AY972481, KM877919, KM877986, KM878081, *D. pedalis* (R. Knuth) R.S. Couto & J.M.A. Braga, KU308814, KU308770, -, -, *D. pedicellata* Phil., KM877845, KM877886, KM877953, KM878038, *D. pentaphylla* L., AY972483, AF307470, AF308014, KM878094, *D. pentaphylla* L., JQ733677, JQ733754, -, JQ733831, *D. pentaphylla* L., JQ733704, JQ733781, -, JQ733858, *D. perdicum* Taubert, KU308815, KM877711, -, -, *D. petelotii* Prain & Burkill, AY972484, AY904802, KM878013, KM878120, *D. pierrei* Prain & Burkill, -, KM877923, KM877995, KM878095, *D. piperifolia* Humb. & Bonpl. ex Willd., KM877852, KM877901, KM877964, KM878054, *D. pohlii* Griseb., KM877846, KM877887, KM877954, KM878039, *D. polygonoides* Humb. & Bonpl. ex Willd., -, KM877888, KM877935, KM878040, *D. polystachya* Turcz., EF028331, DQ408181, -, DQ841313, *D. polystachya* Turcz., JQ733673, JQ733750, -, JQ733827, *D. polystachya* Turcz., JQ733699, JQ733776, -, JQ733853, *D. potaninii* Prain & Burkill, JQ733702, JQ733779, -, JQ733856, *D. prazeri* Prain & Burkill, AY973485, KM877871, KM877938, KM878019, *D. preussii* Pax, AY972486, KM877920, KM877987, KM878082, *D. pseudomacrocapsa* Barroso et al., KU308817, KU308774, -, -, *D. putisensis* R. Knuth, -, KM877902, KM877965, KM878055, *D. pyrenaica* Bubani & Bordère ex Gren., KM877859, KM877912, -, KM878067, *D. quinqueloba* Thunb, JQ733705, JQ733782, -, JQ733859, *D. rockii* Prain & Burkill, AY972488, AY939882, KM877939, KM878020, *D. rupicola* Kunth, KR086969, KR087055, -, KR070885, *D. sansibarensis* Pax, AY972489, AY939883, KM877988, KM878083, *D. sansibarensis* Pax, JQ733685, JQ733762, -, JQ733839, *D. sansibarensis* Pax, JQ733698, JQ733775, -, JQ733852, *D. sansibarensis* Pax, KR086970, KR087056, -, KR070886, *D. saxatilis* Poepp., KM877847, KM877889, KM877956, KM878041, *D. schimperiana* Hochst. ex Kunth, KR086972, KR087058, -, KR070888, *D. schimperiana* Hochst. ex Kunth, KR086971, KR087057, -, KR070887, *D. schimperiana* Hochst. ex Kunth, AY972490, AF307466, AF308010, KM878121, *D. scorpioidea* C. Wright (= *R. ekmanii* R. Knuth) R., KM877866, KM877928, KM878001, KM878101, *D. scortechinii* Prain & Burkill, KM877864, KM877924, KM877996, KM878096, *D. scortechinii* var. *parviflora* Prain & Burkill, JQ733714, JQ733791, -, JQ733868, *D. simulans* Prain & Burkill, EF614206, EF614217, -, DQ841320, *D. sinoparviflora* C. T. Ting, M. G. Gilbert & N. J. Turland, DQ974179, DQ408171, -, DQ841326, *D. sinuata* Vell., KU308818, KU308777, -, -, *D. soso* Jum. & H. Perrier, AY972491, AY939884, KM877989, KM878084, *D. sphaeroides* R.S. Couto & J.M.A. Braga, KU308820, KU308778, -, -, *D. spongiosa* J. Q. Xi, M. Mizuno & W. L. Zhao, DQ974191, DQ974194, -, DQ841317, *D. stegelmanniana* R. Knuth, KU308821, KU308779, -, -, *D. stenophylla* Uline, -, KM877903, KM877966, KM878056, *D. stipulosa* Uline ex R. Knuth, KR086974, KR087060, -, KR070890, *D. stipulosa* Uline ex R. Knuth, KR086973, KR087059, -, KR070889, *D. strydomiana* Wilkin, KM877860, KM877913, KM877975, KM878068, *D. strydomiana* Wilkin, KF147390, KF147467, -, KR070892, *D. strydomiana* Wilkin, KR086975, KR087061, -, KR070891, *D. subcalva* Prain & Burkill, EF614208, EF614214, -, EF614222, *D. subcalva* Prain & Burkill 1, JQ733682, JQ733759, -, JQ733836, *D. subcalva* Prain & Burkill 2, JQ733719, JQ733796, -, JQ733873, *D. subcalva* var. *submollis* C.T. Ting & P.P. Ling 1, JQ733694, JQ733771, -, JQ733848, *D. subcalva* var. *submollis* C.T. Ting & P.P. Ling 2, JQ733707, JQ733784, -, JQ733861, *D. subhastata* Vell., AY972492, KM877904, KM877967, KM878057, *D. sylvatica* Eckl., AY972826, KM877914, AF3080062, KM878069, *D. tentaculigera* Prain & Burkill, AY972828, AY939886, KM877976, KM878070, *D. therezopolensis* Uline ex R. Knuth, KU308823, KU308781, -, -, *D. tokoro* Makino, JQ733708, JQ733785, -, JQ733862, *D. tokoro* Makino ex Miyabe, DQ974186, KM877872, KM877940, DQ841312, *D. tomentosa* J. König ex Spreng., -, KM877925, KM877997, KM878097, *D. trichantha* Baker, AY973829, AY939887, KM877990, KM878085, *D. trifida* L.f., -, KM877929, KM878002, D89683&D89682, *D. trisecta* Griseb., KU308825, KU308783, -, -, *D. variifolia* Bertero, KM877848, KM877890, KM877957, KM878042, *D. velutipes* Prain & Burkill, JQ733732, JQ733809, -, JQ733886, *D. wallichii* Hook. f., AY973830, AY939888, KM878014, KM878122, *D. wallichii* Hook. f., JQ733674, JQ733751, -, JQ733828, *D. yunnanensis* Prain & Burkill, JQ733703, JQ733780, -, JQ733857, *D. zingiberensis* C. H. Wright, AY973831, AY939889, -, DQ841318, *Stenomeris* Planch. *S. borneensis* Oliv., AY973836, AF307475, AF308018, JQ733888, *Tacca* J.R. Forst & G. Forst, *T. integrifolia* Ker Gawl., AY973838, AF307478, AF308021, KM878124, *T. leontopetaloides* (L.) Kuntze, AY973839, AF307480, AF308023, KM878125, *T. palmata* Blume, AY973840, AF307479, AF308022, KM878126, *T. palmatifida* Baker, AY973841, AY939890, -, KM878127, *T. plantaginea* (Hance) Drenth, AY973842, AF307483, AF308026, KM878128, *Tamus* L. *T. edulis* Lowe, KM877856, KM877908, -, KM878062,

Appendix 2 All primers used for PCR amplification and DNA sequencing.

trnK-11 (Sense) (Liston and Kadereit 1995), trnK-670F (Sense) 5'-CCAAAGTCAAAAGAGCGATTGGGTTG-3', matK-AF (Sense) (Ooi et al. 1995), matK-1170F (Sense) 5'-AATTTACGATCTATTCATTC, matK-1300F (Sense) (Fuse and M. N. Tamura 2000), matK-1650F(mod-N1) (Sense) 5'-GGGAACTCGTCTTTTGATGA-3', matK-1923F (Sense) 5'-GGTAAGGAGTCAAATGCTAGA-3', matK-1940F (Sense) (Yamashita and Tamura 2000), matK-8F(mod-N1) (Sense) 5'-CGTGTTTCGTGTGCTAGAACTT-3', trnK-706R (Antisense) 5'-ATTATACATAGTGCAAATATGGTC modifide Johnson and Soltis(1995), matK-1000R(mod-N1) (Antisense) 5'-CACAAAAATCGAACCATTAAACC-3', matK-1235R (Antisense)(Johnson & Soltis 1995), matK-1470R(mod-N1) (Antisense) 5'-GAGGACCTTGATCGTAAATAAG-3', matK-1700R (Antisense)(Tamura et al. 2004), matK-8R (Antisense)(Ooi et al.1995), matK-2100R(mod-N1) (Antisense) 5'-GAATTCTTGTAATAATCCCGAACC-3', trnK-2300R(mod-N1) (Antisense) 5'-CATTTCCATTTTATTTGCACTC-3', trnK-2621 (Antisense)(Liston and Kadereit 1995), rbcL-N' (Sense)(Teruchi et al.1987), rbcL-200F(mod-N1) (Sense) 5'-ACTTACCAGTCTTGATCGTTAC-3', rbcL-360F (Sense) 5'-TTGGTTTCAAAGCCCTACGAGCC-3', rbcL-630F (Sense)(Teruchi et al. 1987), rbcL-840F (Sense)(Yamashita and Tamura 2000), rbcL-1100F(mod-N1) (Sense) 5'-TCAAGATTGGGTCTCTATGC-3', rbcL-250R(mod-N1) (Antisense) 5'-TATTGGTCTTCCTCCCCAAC-3', rbcL-400R(mod-N1) (Antisense) 5'-TAAGAAGTAGGAATTCGACG-3', rbcL-590R (Antisense) (Yamashita and Tamura 2000), rbcL-700R(mod-N1) (Antisense) 5'-CCTGTTTCGGCCTGTGCTTTA-3', rbcL-840R (Antisense) (Yamashita and Tamura 2000), rbcL-1150R(mod-N1) (Antisense) 5'-TATGCCAAACATGAATACCC-3', DBRBAS2 (Antisense)(Teruchi et al. 1987), trnLc (Sense)(Taberlet et al. 1991), trnL100F-modN4 (Sense) 5'-GGGCAATCCTGAGCCAAATC-3', trnL250F-modN4 (Sense) 5'-GGAAGCTGTCTAACGAATGG-3', trnL450F-modN3 (Sense) 5'-CCGAAGTTGAAGGAAGAATC-3', trnL610F-modN4 (Sense) 5'-GTCAATACTGACAACAATGAAATT-3', trnL200R-modN4 (Antisense) 5'-GAGTCTCTGCACCTATCCTT-3', trnL300R-modN4 (Antisense) 5'-TTGGGTATATAGAACTCCCC-3', trnL530R-modN3 (Antisense) 5'-ATTCTCGTCCGATTAATTCG-3', trnLd (Antisense)(Taberlet et al. 1991), trnF-R (Antisense) 5'-ATTTGAACTGGTGACACGAG (Taberlet et al. 1991), atpB-S2F (Sense) (Hoot et al. 1995), atpB-135F (Sense) 5'-AAAGGTCGAGCCGGTCAAC-3', atpB-240F (Sense) 5'-GAGAGGAATGGAAGTGATTG-3', atpB-345F (Sense) 5'-GCCTGTAGATACTCGCACAAAC-3', atpB-400F (Sense) 5'-CAGTTAGATACGAAATTATCAATC-3', atpB-450F (Sense) 5'-GATCTTTTAGCTCCTTATCG-3', atpB-505F (Sense) 5'-GTGGGTAAAACAGTCCTCATC-3', atpB-620F (Sense) 5'-ACATCGAAATGAAAGAATCTG-3', atpB-780F (Sense) 5'-CGTGCTTCTATTATTGACAA-3', atpB-900F (Sense) 5'-CTTTGCAAGAAAGAATTACTTC-3', atpB-1000F (Sense) 5'-CCACGACATTTGCACATTTAG-3', atpB-1050F (Sense) 5'-CCAAAGGTATTTATCCAGCG-3', atpB-1100F (Sense) 5'-GTTACAGCCTGGCATCGTTG-3', atpB-1160F (Sense) 5'-CTTACAACGTTACAAAGAAC-3', atpB-1215F (Sense) 5'-CGAATTATCCGAAGAAGATCG-3', atpB-1270F (Sense) 5'-CTTATCACAACCCTTCTTCG-3', atpB-215R (Antisense) 5'-CAGACCATCTGTAGCACTCAT-3',

Appendix 2 Continued

atpB-325R (Antisense) 5'-CCCTAAATTATCAACAGGTTC-3', atpB-440R (Antisense) 5'-GGAGCTAAAAGATCCACCAC-3', atpB-540R (Antisense) 5'-CATGAGCTTTGGCAATGTTG-3', atpB-650R (Antisense) 5'-CTTTTGATTCTGCAATATTTT-3', atpB-710R (Antisense) 5'-CCAACCTCTCATACGAGCTCC-3', atpB-825R (Antisense) 5'-TAAGGCGGAGACTTCTGATCC-3', atpB-990R (Antisense) 5'-CAAATGTCGTGGCAGGAGCAG-3', atpB-1090R (Antisense) 5'-GCTGTAACATAGTTGACGTTG-3', atpB-1195R (Antisense) 5'-CGTCCAACCCAAGGATAGCT-3', atpB-1255R (Antisense) 5'-GATAAGAAACGCTCAATTTTTCG-3', atpB-1300R (Antisense) 5'-CCTGGAGAACCCGTAAATAC-3', atpB-1370R (Antisense) 5'-CGGGAAGACTATCTAATTCTCC-3', atpB-1425R (Antisense) 5'-CATAGCTTTCGCGGTAGCTTC-3', atpB-S1494R (Antisense) (Hoot et al. 1995),

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Appendix 3

All primers used for PCR amplification and DNA sequencing. *Dioscorea luzonensis* used only.

trnK840F-modN7 (5'-AAGGATATTTAGAAAAAGATG-3'), matK920F-modN7 (5'-ACACTTGCTCACGATCATG-3'),
matK1020F-modN7 (5'-CATTTAATTATTCGAATGTATC-3'), matK1130F-modN7 (5'-TGATATCAGAAGGTTTTGC-3'),
matK1200F-modN7 (5'-AAGAAATACCTAAATCACAA-3'), matK1290F-modN7 (5'-TGTGTCAGATATATTAATACCC-3'),
matK1370F-modN7 (5'-TCCTCTTTGCATTTATTGC-3'), matK1450F-modN7 (5'-CGTGATTTCAATTTCAAAAG-3'),
matK1540F-modN7 (5'-TTTTCATAAACAATCCTCTTA-3'), matK1620F-modN7 (5'-GGCATTTTTTAGTAGTGTGT-3'),
matK1690F-modN7 (5'-GTTTCGATATCAAGGAAAAGC-3'), matK1770F-modN7 (5'-CCTTGTCCATTTTTGGCAA-3'),
matK1850F-modN7 (5'-TTTTCTGGGTATCTTTCA-3'), matK1940F-modN7 (5'-ACTAAGAAATTTGATACCAGAG-3'),
matK2000F-modN7 (5'-AATTTTGTACCGTATCCGG-3'), matK2080F-modN7 (5'-CGATTTGGTTGGATATGTA-3'),
matK2160F-modN7 (5'-TACTTCGTATTTCTGTGTC-3'), matK2240F-modN7 (5'-CGGGATTATTACAAGAATTC-3'),
trnK2320F-modN7 (5'-GGACGCATTTGGTATTTGG-3'), matK880R-modN7 (5'-TGAAAGAGAAGTGGATATAGG-3'),
matK990R-modN7 (5'-AATCCAAAATTTCCACAAA-3'), matK1080R-modN7 (5'-ATTTTGGTTTCAATCATCAG-3'),
matK1190R-modN7 (5'-CCTAATCGCAGTAAAAATGG-3'), matK1260R-modN7 (5'-CTCTAAGAAGGGAAATATTG-3'),
matK1340R-modN7 (5'-TTTGAACCAAGATTTCAG-3'), matK1420R-modN7 (5'-GAAACTATTCTGAATTATGATA-3'),
matK1510R-modN7 (5'-AAGAATTATATAGGAATCGAA-3'), matK1590R-modN7 (5'-TCAAGAAAGACTCCAGAGG-3'),
matK1670R-modN7 (5'-TTGAAGAACCATTGGGTCTT-3'), matK1750R-modN7 (5'-CCTCATCAAAAGACGAGTTC-3'),
matK1830R-modN7 (5'-GGTTTATATGAATCCTATGC-3'), matK1900R-modN7 (5'-CCTTACCACGGAAAAATTT-3'),
matK1990R-modN7 (5'-CAATGATCGAATAAGAGCA-3'), matK2060R-modN7 (5'-AATCTGATAAATTTGTCCATA-3'),
matK2130R-modN7 (5'-TTTTGAGGATCCACTGTGA-3'), matK2210R-modN7 (5'-GTACCCTACTTTTATGTTTACG-3'),
rbcLN^a (Terachi et al.1987), rbcL20F-modN7 (5'-ACTTATTATACTCTGACTACG-3'), rbcL100F-modN7
(5'-CCGAAGAAGCAGGAGCTGC-3'), rbcL180F-modN7 (5'-CTTGATCGTTACAAAGGACG-3'), rbcL260F-modN7
(5'-TATCCTTTAGACCTTTTTGAG-3'), rbcL340F-modN7 (5'-GCCCTACGTCTGGAGGAT-3'), rbcL410F-modN7
(5'-CCAAGTTGAAAGAGATAAG-3'), rbcL490F-modN7 (5'-ATCCGCAAAGAACTACGGC-3'), rbcL570F-modN7
(5'-CTCACAACCATTTATGCGT-3'), rbcL650F-modN7 (5'-CGAAATCAAGGGACATTAC-3'), rbcL730F-modN7
(5'-GGGAGTTCTTATCGTAATG-3'), rbcL810F-modN7 (5'-TAACGGCCTACTTCTTCAC-3'), rbcL880F-modN7
(5'-TTTCGTGTACTAGCTAAAGC-3'), rbcL960F-modN7 (5'-GAACGCGAGATGACTTTGG-3'), rbcL1040F-modN7
(5'-CAAGATTGGGTCTCTATGCC-3'), rbcL1130F-modN7 (5'-CTTTGGGGATGATTCCGTA-3'), rbcL1210F-modN7
(5'-ATCGGGTGGCTTTAGAAGC-3'), rbcL70R-modN7 (5'-TCGGAATGCCGCTAAGATAT-3'), rbcL150R-modN7
(5'-ATGTACCGGTGGACGATT-3'), rbcL230R-modN7 (5'-CTTCCTCCCCAACAACGCTC-3'), rbcL310R-modN7
(5'-CTACAATGGAAGTAAACATATTG-3'), rbcL400R-modN7 (5'-TTGAAAGTTTTGGAATAAG-3'), rbcL470R-modN7
(5'-AATAGTGCATCCCAATAGG-3'), rbcL540R-modN7 (5'-CAAGTCCACCACGTAGACA-3'), rbcL620R-modN7
(5'-AAAGTGCTTCGGCACAAAAT-3'), rbcL700R-modN7 (5'-ATCATTTCTTCACATGTACC-3'), rbcL780R-modN7
(5'-GTATTTGCGGTGAAGCCCC-3'), rbcL860R-modN7 (5'-CTATCAATAACTGCATGCA-3'), rbcL930R-modN7
(5'-CAGCGTGAATATGATCTCC-3'), rbcL1010R-modN7 (5'-CAATAAAATCATCACGTAAT-3'),

Appendix 3 (continued)

rbcL1100R-modN7 (5'-GAATACCCCCAGAAGCCAC-3'), rbcL1180R-modN7 (5'-CAAGGGTGCCCCAAAGTTC-3'),
 rbcL1260R-modN7 (5'-GGTCACGTCCCTCATTACG-3'), DBRBAS2 (Terachi et al. 1987), tmL-c(Taberlet et al. 1991),
 trnL40F-modN7 (5'-AAACCTGGAATTTGAAATG-3'), trnL110F-modN7 (5'-GGTTTATAAAAAACAAAACA-3'),
 trnL190F-modN7 (5'-CTGTTCTAACGAATGGAATT-3'), trnL270F-modN7 (5'-CTATATACCCAATACGTACG-3'),
 trnL325F-modN7 (5'-CCCGAATCCATTATTTTATT-3'), trnL395F-modN7 (5'-TATTTCAACCGAAGTTGAAG-3'),
 trnL490F-modN7 (5'-TGAAAAACGAATTAATCGG-3'), trnL570F-modN7 (5'-AGTAAGAAGAAAATCCGTC-3'),
 trnL630F-modN7 (5'-GTCCATTTTATTCCTAATT-3'), trnF980F-modN7 (5'-AAGAGTCCCCCTTAATTGAGA-3'),
 trnL90R-modN7 (5'-CAAAACAAATAAAGATTGG-3'), trnL160R-modN7 (5'-CCTTTTTGATTTTGTATC-3'),
 trnL240R-modN7 (5'-GTAGTTTTTCCAACCTACCA-3'), trnL320R-modN7 (5'-CGTGATTAATCCTTAATATG-3'),
 trnL390R-modN7 (5'-GATTCACAATATAACTTTCC-3'), trnL440R-modN7 (5'-CAATGATTTGATCATTAAT-3'),
 trnL540R-modN7 (5'-GTAGAGCAGGACTCTCTCT-3'), trnL620R-modN7 (5'-GGACTTGAACCCTCACGAT-3'),
 trnF1000R-modN7 (5'-CTTAAAAATCTTATCCAGTC-3'), trnF1050R-modN7 (5'-GCATCATCCTGCTAGAGTAC-3'), tmF-R
 (5'-Taberlet et al. 1991), atpB-S2F (5'-Hoot et al. 1995), atpB-40F (5'-CTTAGGACGTATCGTTCAAA-3'), atpB-90F
 (5'-CCCAGGAAAGATGCCTAATA-3'), atpB-135F (5'-AAAGGTCGAGCCGGTCAAC-3'), atpB-185F
 (5'-ATTAGGAAATAATCGAGTTAGAG-3'), atpB-240F (5'-GAGAGGAATGGAAGTGATTG-3'), atpB-295F
 (5'-CTACTCTCGGACGAATTTTC-3'), atpB-345F (5'-GCCTGTAGATACTCGCACAAAC-3'), atpB-400F
 (5'-CAGTTAGATACGAAATTATCAATC-3'), atpB-450F (5'-GATCTTTTAGCTCCTTATCG-3'), atpB-505F
 (5'-GTGGGTAAAACAGTCCTCATC-3'), atpB-560F (5'-GGGGTGTGTCTGTATTTGGC-3'), atpB-620F
 (5'-ACATCGAAATGAAAGAATCTG-3'), atpB-680F (5'-GCTCTAGTCTATGGTCAAATG-3'), atpB-730F
 (5'-GACTGCCCTAACCATGGCGG-3'), atpB-780F (5'-CGTGCTTCTATTCAATTGACAA-3'), atpB-850F
 (5'-GGGGAGAATGCCTTCTGCAG-3'), atpB-900F (5'-CTTTGCAAGAAAGAATTACTTC-3'), atpB-950F
 (5'-GATCCAAGCAGTTTATGTACC-3'), atpB-1000F (5'-CCACGACATTTGCACATTTAG-3'), atpB-1050F
 (5'-CCAAAGGTATTTATCCAGCG-3'), atpB-1100F (5'-GTTACAGCCTGGCATCGTTG-3'), atpB-1160F
 (5'-CTTCACAACGTTACAAAGAAC-3'), atpB-1215F (5'-CGAATTATCCGAAGAAGATCG-3'), atpB-70R
 (5'-AAAGGGCATCCAGTACTGGG-3'), atpB-130R (5'-CGGCTCGACCTTAACTACC-3'), atpB-170R
 (5'-CCTAATAATTGCTGTACCTCAC-3'), atpB-215R (5'-CAGACCATCTGTAGCACTCAT-3'), atpB-270R
 (5'-CCGCCAACCAGAACACTTAG-3'), atpB-325R (5'-CCCTAAATTATCAACAGGTTC-3'), atpB-385R
 (5'-CTAACTGTATAAAGGCAGGCGC-3'), atpB-440R (5'-GGAGCTAAAAGATCCACCAC-3'), atpB-480R
 (5'-CCCCAAATAGCCCCGATTT-3'), atpB-540R (5'-CATGAGCTTTGGCAATGTTG-3'), atpB-590R
 (5'-CATTTCCCTTCACGAGTACGCTC-3'), atpB-650R (5'-CTTTTGATTCTGCAATATTTT-3'), atpB-710R
 (5'-CCAATCTCATACGAGCTCC-3'), atpB-770R (5'-GAATAGAAGCACGTCTTGTC-3'), atpB-825R
 (5'-TAAGGCGGAGACTTCTGATCC-3'), atpB-880R (5'-CCATTTCTGTACTAAGGGTGG-3'), atpB-935R
 (5'-CTGCTTGGATCGAAGTTATAG-3'), atpB-990R (5'-CAAATGTCGTGGCAGGAGCAG-3'), atpB-1040R
 (5'-CTTTGGCAGCTAATCCTCTTG-3'), atpB-1090R (5'-GCTGTAACATAGTTGACGTTG-3'), atpB-1150R
 (5'-GTTGTGAAGTTTGCTTAACCTTT-3'), atpB-1195R (5'-CGTCCAACCCAAGGATAGCT-3'),

Appendix 3 (continued)

atpB-1255R (5'-GATAAGAAACGCTCAATTTTCG-3'), atpB-1300R (5'-CCTGGAGAACCCGTAAATAC-3'), atpB-1270F (5'-CTTATCACAACCCTTCTTCG-3'), atpB-1330F (5'-GGTCTCGCAGAAACAATTAGG-3'), atpB-1395F (5'-CAGGCCTTTTATTTGGTGGGTAAC-3'), atpB-1370R (5'-CGGGAAGACTATCTAATTCTCC-3'), atpB-1425R (5'-CATAGCTTTCGCGGTAGCTTC-3'), atpB-1494R (5'-Hoot et al. 1995),

Chapter II

A Revised Infrageneric Classification for Old World Species of the Genus

Dioscorea

(Dioscoreaceae)

旧世界産ヤマノイモ属（ヤマノイモ科）の属内分類の改訂

Abstract

Dioscorea is a morphologically diverse genus, consisting of more than 600 species distributed worldwide. Recent molecular and morphological analyses allowed us to recognize two subgenera *Dioscorea* and *Helmia*, and 19 Old World species groups (sections) in subgenus *Helmia*. In this paper we present a diagnostic key and taxonomic treatment for the subgenera and sections, including *D.* sect. *Tamus* (stat. nov.) and four new sections *Afroborderea*, *Perennia* (validated here), *Pseudoshannicorea* and *Pseudostenophora*. Twelve lectotypes were also determined.

Introduction

Dioscorea is known as a yam genus, and plants are mostly dioecious with twining stems (Fig. 1). Because of the great morphological diversity, about 630 species have been recognized from the world so far (WCSP 2019). For the *Dioscorea* species, previous authors proposed infrageneric taxa (23 to 58 sections and several subgenera) and several distinct “genera” (which were recently placed within *Dioscorea* by molecular evidence) (Uline 1898, Knuth 1924, Burkill 1960, Huber 1998; for review see Caddick *et al.* 2002a, Noda *et al.* 2020). However, diagnostic keys to the infrageneric taxa (“genera”/subgenera/sections) differed greatly between the authors, and no consensus has emerged with regard to the recognition and/or circumscription of many of the infrageneric taxa. In order to resolve this problem, we conducted phylogenetic analyses of 273 samples from 183 *Dioscorea* species based on four cpDNA regions matK, rbcL, atpB and trnL–F (Noda *et al.* 2020). For the phylogenetic analyses, 3,754 characters were used by combining all existing cpDNA sequence data obtained by previous studies (Caddick *et al.* 2002a; Wilkin *et al.* 2005; Gao *et al.* 2008; Hsu *et al.* 2013; Maurin *et al.* 2016; Viruel *et al.* 2016, Couto *et al.* 2018) with our original data.

A resultant phylogenetic tree consisted of eleven well-supported major clades (Fig. 2a), most of which further consisted of more than two weakly or strongly supported subclades (Noda *et al.* 2020). As in the previous studies (Wilkin *et al.* 2005, Viruel 2016, 2018, Couto *et al.* 2018), “*Dioscorea* section *Stenophora*” was sister to the rest of the genus. Using a tree (Fig. 2b) which was modified from Fig. 2a and consisted of 27 major or final major subclades including 20 Old World sections or species groups, we pursued evolution of ten selected morphological characters (Noda *et al.* 2020). The “Birmanica-group”, “Burchellii-group”, “Gillettii-group”, and “Tentaculigera-group” (Noda *et al.* 2020: Figs. 5, 6) are now replaced with four new sections *D.* sects. *Afroborderea*, *Perennia*, *Pseudoshannicorea*, and *Pseudostenophora*

(described later). The morphological characters we pursued include not only those used for the previous systems of infrageneric classification (Uline 1897, 1898, Knuth 1924, Burkill 1960, Huber 1998), but also pollen and chromosome characters whose systematic and evolutionary implications have been suggested for the past 40 years (Pei *et al.* 1979, Chin *et al.* 1985, Schols *et al.* 2005, Viruel *et al.* 2010). The ten characters and distribution of their respective character-states are shown in Table 1 (see also Tables 2 and 3 in Noda *et al.* 2020). Based on molecular and morphological evidence, we proposed the recognition of two subgenera *Dioscorea* (= “*D.* sect. *Stenophora*”) and *Helmia*, as well as the need of a revision of infrageneric classification for Old World species in the subgenus *Helmia* (Noda *et al.* 2020).

In this paper, we present a diagnostic key and taxonomic treatment for the two subgenera and the 20 Old World sections, of which 19 belong to the subgenus *Helmia*. Of the 20 Old World sections, the following 13 have already been proposed or circumscribed, mostly by Huber (1998) as “genera” or “genus-equivalent sections”. They are: *Borderea*, *Brachyandra*, *Combilium*, *Dioscorea* (= *D.* sect. *Stenophora* Uline circumscribed by Burkill [1960]), *Lasiophyton*, *Macrocarpaea*, *Macrourea*, *Opsophyton*, *Paramecocarpa*, *Rhacodophyllum*, *Shannicorea*, *Stenocorea*, and *Tamus*. The four sections *D.* sects. *Afroborderea*, *Perennia*, *Pseudoshannicorea*, and *Pseudostenophora* are new. The three remaining *D.* sects. *Cardiocapsa*, *Enantiophyllum*, and *Testudinaria* are more broadly circumscribed than before. Positions and relationships of those 20 Old World sections are presented on the tree of Fig. 3.

Taxonomy

Dioscorea L., Sp. Pl.: 1032 (1753); Kunth, Enum. Pl. 5: 325 (1850); Benth in Benth & Hook f., Gen. Pl. 3: 742 (1883); Pax in Engl., Nat. Pflanzenfam. 2 (5): 133 (1888); Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 80 (1897), in Bot. Jahrb. Syst. 25: 155 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 45 (1924); Prain & Burkill in Fl. Gén Indo-Chine 6: 699 (1934); Burkill in Fl. Males. I, 4: 299 (1951), in J. Linn. Soc. Bot. 56: 400 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 226 (1998); Ding & Gilbert in Wu & Raven, Fl. China 24: 276 (2000); Caddick *et al.* in Taxon 51: 108 (2002); Wilkin & Thapayai in Santisuk & Larsen, Fl. Thailand 10: 4 (2009); Yamashita & Tamura in Iwatsuki *et al.*, Fl. Japan 4b: 171 (2016). — *Lectotype* (McNeill *et al.* 1987): *Dioscorea villosa* L.

Merione Salisb., Gen. Pl. Fragm.: 12 (1866). — *Type*: *Dioscorea villosa* L. (cf. Caddick *et al.* 2002b).

Key to the subgenera of the genus *Dioscorea*

1. Underground stem rhizomatous; pollen monosulcate.....I. subg. *Dioscorea*
1. Underground stem tuberous; pollen bisulcate.....II. subg. *Helmia*

I. *Dioscorea* subg. *Dioscorea*

Dioscorea subg. *Eudioscorea* Pax in Engl., Nat. Pflanzenfam. 2 (5): 134 (1888); Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 84 (1897), in Bot. Jahrb. Syst. 25: 157 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 157 (1924), nom. invalid.

About 35 species are recognized.

1. *Dioscorea* sect. *Dioscorea*

Dioscorea sect. *Stenophora* Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 84 (1897), in Bot. Jahrb. Syst. 25: 157 (1898); Prain & Burkill in Fl. Gén. Indo-Chine 6: 700 (1934); Burkill in Fl. Males. I, 4: 303 (1951), in J. Linn. Soc. Bot. 56: 402 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 229 (1998); Ding & Gilbert in Wu & Raven, Fl. China 24: 277 (2000); Yamashita & Tamura in Iwatsuki *et al.*, Fl. Japan 4b: 176 (2016). — *Dioscorea* subg. *Stenophora* (Uline) R. Knuth in Engl., Pflanzenr. 4 (43): 313 (1924). — *Lectotype (hic designatus): Dioscorea quinquelobata* Thunb., 'quinqueloba'.

Dioscorea sect. *Macropoda* Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 85 (1897), in Bot. Jahrb. Syst. 25: 157 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 171 (1924); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 231 (1998). — *Lectotype (hic designatus): Dioscorea villosa* L. (cf. Al-Shehbaz & Schubert 1989).

Dioscorea sect. *Orientali-asiaticae* R. Knuth in Engl., Pflanzenr. 4 (43): 252 (1924). — *Lectotype (hic designatus): Dioscorea gracillima* Miq.

Dioscorea sect. *Japonicae* R. Knuth in Engl., Pflanzenr. 4 (43): 254 (1924). — *Type: Dioscorea asclepiadea* Prain & Burkill.

Dioscorea sect. *Eustenophora* R. Knuth in Engl., Pflanzenr. 4 (43): 313 (1924), nom. invalid.

Distribution. Temperate Asia, extending to Malaysia, Caucasus and N America.

Representative species: *D. althaeoides*, *D. asclepiadea*, *D. banzhuana*, *D. biformifolia*, *D. caucasica*, *D. collettii*, *D. deltoidea*, *D. futschauensis*, *D. gracillima*, *D.*

izuensis, *D. membranacea*, *D. nipponica*, *D. prazeri*, *D. quinquelobata*, *D. rockii*, *D. septemloba*, *D. simulans*, *D. sinoparviflora*, *D. spongiosa*, *D. tenuipes*, *D. tokoro* (Fig. 1a), *D. villosa* (not analyzed here), *D. zentaroana* and *D. zingiberensis*.

II. *Dioscorea* subg. *Helmia* (Kunth) Benth. in Benth & Hook f., Gen. Pl. 3: 743 (1883); Pax in Engl., Nat. Pflanzenfam. 2 (5): 133 (1888); Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 83 (1897), in Bot. Jahrb. Syst. 25: 156 (1898); R. Knuth in Eng., Pflanzenr. 4 (43): 50 (1924). — *Helmia* Kunth, Enum. Pl. 5: 414 (1850). — *Lectotype* (Caddick *et al.* 2002b): *Helmia dregeana* Kunth [$\equiv$ *Dioscorea dregeana* (Kunth) T. Durand & Schinz.].

About 595 species are recognized.

Key to the Old World sections of the *Dioscorea* subgenus *Helmia*

1. Seeds wingless all round.....2
1. Seeds winged.....4
2. Fruits baccate..... sect. *Tamus*
2. Fruits capsular.....3
3. Perfect stamens 3, staminodes 3; stem twining to left; capsules ca. 1.5 times as long as wide.....sect. *Afroborderea*
3. Perfect stamens 6; stem not twining; capsules ca. 1.1 times as long as wide.....sect. *Borderea*
4. Stem woody, lignified.....sect. *Testudinaria*
4. Stem herbaceous, not lignified.....5
5. Stem twining to right.....sect. *Enantiophyllum*
5. Stem twining to left.....6
6. Leaves opposite.....sect. *Macrourea*
6. Leaves alternate.....7
7. Capsules wider than long.....8
7. Capsules longer than wide.....10
8. Plants totally pubescent.....sect. *Pseudostenophora*
8. Plants totally glabrous.....9
9. Tuber annual (solid tuber only one, connected with empty tubers); bulbils present; exine ornamentation of pollen perforate.....sect. *Stenocorea*
9. Tuber perennial (solid tubers several, connected with one another); bulbils absent;

exine ornamentation of pollen striate.....	sect. <i>Cardiocapsa</i>
10. Seeds winged all round margin.....	11
10. Seeds lacking wing on the both sides.....	12
11. Leaf blade margin lobed.....	sect. <i>Rhacodophyllum</i>
11. Leaf blade margin entire or obscurely undulate.....	sect. <i>Combilium</i>
12. Seeds with wing pointing toward the both (apex and base) ends of capsule.....	13
12. Seeds with wing pointing toward one (either apex or base) end of capsule.....	14
13. Abaxial surface of leaves woolly-hairy.....	sect. <i>Macrocarpaea</i>
13. Abaxial surface of leaves glabrous.....	sect. <i>Pseudoshannicorea</i>
14. Seeds with wing pointing toward capsule apex (Noda <i>et al.</i> 2020: Fig. 6).....	15
14. Seeds with wing pointing toward capsule base (Noda <i>et al.</i> 2020: Fig. 6).....	16
15. Stem prickly; tuber perennial.....	sect. <i>Paramecocarpa</i>
15. Stem hairy; tuber annual.....	sect. <i>Shannicorea</i>
16. Leaves compound.....	sect. <i>Lasiophyton</i>
16. Leaves simple.....	17
17. Tuber horizontal or globose; bulbils present; leaf blade oval.....	sect. <i>Opsophyton</i>
17. Tuber vertical; bulbils absent; leaf blade lanceolate to ovate.....	18
18. Tuber annual (solid tuber only one, connected with empty tubers); stem smooth.....	sect. <i>Brachyandra</i>
18. Tuber perennial (solid tubers several, connected with one another); stem usually verruculose.....	sect. <i>Perennia</i>

2. *Dioscorea* sect. **Tamus** (L.) Noda & M. N. Tamura, **stat. nov.**

Basionym. *Tamus* L., Sp. Pl.: 1028 (1753); Kunth Enum. Pl. 5: 453 (1850); Benth in Benth & Hook f., Gen. Pl. 3: 744 (1883); Pax in Engl., Nat. Pflanzenfam. 2 (5): 136 (1888); Uline in Bot. Jahrb. Syst. 25: 157 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 340 (1924); Burkill in J. Linn. Soc. Bot. 56: 400 (1960); Heywood in Tutin *et al.*, Fl. Europaea 5: 85 (1980); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 228 (1998). — *Type:* *Tamus communis* L. [= *Dioscorea communis* (L.) Caddick & Wilkin].

Distribution. Europe to Macaronesia.

Representative species: *D. communis*, *D. edulis* and *D. orientalis*.

3. *Dioscorea* sect. *Borderea* (Miégev.) Prain & Burkill in J. Proc. Asiat. Soc. Bengal 10: 5 (1914); Burkill in J. Linn. Soc. Bot. 56: 403 (1960). — *Borderea* Miégev., Bull. Soc.

Bot. France 13: 374 (1866); Pax in Engl., Nat. Pflanzenfam. 2 (5): 133 (1888); Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 80 (1897), in Bot. Jahrb. Syst. 25: 155 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 327 (1924); Heywood in Tutin *et al.*, Fl. Europaea 5: 85 (1980); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 230 (1998). — *Dioscorea* subg. *Borderea* (Miégev.) Benth. in Benth & Hook f., Gen. Pl. 3: 743 (1883). — *Type: Dioscorea pyrenaica* Bubani & Bordère ex Gren.

Distribution. Central Pyrenees.

Representative species: *D. chouardii* and *D. pyrenaica*.

4. *Dioscorea* sect. **Afroborderia** Noda & M. N. Tamura, **sect. nov.**

Dioscorea sect. *Afroborderia* is similar to *D.* sect. *Borderea*, but differs from it in having 3 fertile stamens with 3 sterile staminodes, and stem twining to left.

Type: Dioscorea gillettii Milne-Redh.

Distribution. W Africa (S Ethiopia to Kenya).

Representative species: *D. gillettii* and *D. kituiensis*.

5. *Dioscorea* sect. *Rhacodophyllum* Uline ex R. Knuth in Engl., Pflanzenr. 4 (43): 183 (1924); von Teichman *et al.* in Boissiera 24: 219 (1975); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 229 (1998). — *Lectotype (hic designatus): Dioscorea buchananii* Benth.

Distribution. S Africa.

Representative species: *D. buchananii*, *D. multiloba* and *D. rupicola*.

6. *Dioscorea* sect. *Testudinaria* (Salisb. ex Burch.) Burkill in J. S. African Bot. 18: 185 (1952), in J. Linn. Soc. Bot. 56: 403 (1960); von Teichman *et al.* in Boissiera 24: 222 (1975). — *Testudinaria* Salisb. ex Burch., Trav. S. Africa. 2: 147 (1824); Kunth Enum. Pl. 5: 440 (1850); Benth in Benth & Hook f., Gen. Pl. 3: 744 (1883); Pax in Engl., Nat. Pflanzenfam. 2 (5): 135 (1888); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 228 (1998). — *Dioscorea* subg. *Testudinaria* (Salisb. ex Burch.) Uline in Bot. Jahrb. Syst. 25: 157

(1898); R. Knuth in Engl., Pflanzenr. 4 (43): 321 (1924). — *Type: Dioscorea elephantipes* (L'Hér.) Engl.

Dioscorea sect. *Eutestudinaria* Uline in Bot. Jahrb. Syst. 25: 157 (1898), nom. invalid.

Distribution. S Africa.

Representative species: *D. brownii*, *D. elephantipes*, *D. hemicrypta*, *D. strydomiana* and *D. sylvatica*.

7. ***Dioscorea* sect. *Perennia*** Archibald ex Noda & M. N. Tamura, **sect. nov.**

Dioscorea sect. *Perennia* Archibald in J. S. African Bot. 33: 14 (1967); von Teichman *et al.* in Boissiera 24: 222 (1975); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 232 (1998), nom. nud.

Dioscorea sect. *Perennia* is similar to *D.* sect. *Brachyandra*, but differs from it in having perennial tuber.

Type: Dioscorea mundtii Baker

Distribution. S Africa.

Representative species: *D. burchellii*, *D. mundtii* and *D. stipulosa*.

8. ***Dioscorea* sect. *Pseudoshannicorea*** Noda & M. N. Tamura, **sect. nov.**

Dioscorea sect. *Pseudoshannicorea* is similar to *D.* sect. *Shannicorea*, but differs from it in having seeds with wing pointing toward both capsule apex and capsule base.

Type: Dioscorea tentaculigera Prain & Burkill

Distribution. China (SW Yunnan), Myanmar and Thailand.

Representative species: *D. tentaculigera* (Fig. 1b).

9. *Dioscorea* sect. *Opsophyton* Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 84 (1897), in Bot. Jahrb. Syst. 25: 157 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 85 (1924); Prain & Burkill in Fl. Gén. Indo-Chine 6: 703 (1934); Burkill & H. Perr. in Fl. Madag. 44e Fam.: 24 (1950); Burkill in Fl. Males. I, 4: 311 (1951), in J. Linn. Soc. Bot. 56: 403 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 233 (1998); Ding & Gilbert in Wu & Raven, Fl. China 24: 287 (2000); Yamashita & Tamura in Iwatsuki *et al.*, Fl. Japan 4b: 175 (2016). — *Lectotype* (Huber 1998): *Dioscorea bulbifera* L.

Distribution. Widespread in the Old World (except *D. mollis* from Brasil).

Representative species: *D. antaly*, *D. bulbifera* (Fig. 1c) and *D. mollis*.

10. *Dioscorea* sect. *Lasiophyton* Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 84 (1897), in Bot. Jahrb. Syst. 25: 157 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 131 (1924); Prain & Burkill in Fl. Gén. Indo-Chine 6: 702 (1934); Burkill & H. Perr. in Fl. Madag. 44e Fam.: 20 (1950); Burkill in Fl. Males. I, 4: 313 (1951), in J. Linn. Soc. Bot. 56: 404 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 233 (1998); Ding & Gilbert in Wu & Raven, Fl. China 24: 290 (2000). — *Lectotype* (**hic designatus**): *Dioscorea dumetorum* (Kunth) Pax.

Dioscorea sect. *Trieuphorostemon* Uline in Engl. & Prantl. Nat. Pflanzenfam. Nachtr. 2 (5): 84 (1897), in Bot. Jahrb. Syst. 25: 157 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 137 (1924). — *Type:* *Dioscorea pentaphylla* L.

Dioscorea sect. *Botryosicyos* (Hochst.) Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 84 (1897), in Bot. Jahrb. Syst. 25: 157 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 150 (1924); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 233 (1998); Ding & Gilbert in Wu & Raven, Fl. China 24: 287 (2000); Yamashita & Tamura in Iwatsuki *et al.*, Fl. Japan 4b: 174 (2016). — *Botryosicyos* Hochst. in Fl. 27, Bes. Beil. 3 (1844). — *Type:* *Botryosicyos pentaphyllus* Hochst. (= *Dioscorea quartiniana* A. Rich.).

Dioscorea sect. *Illigerastrum* Prain & Burkill in Kew Bull. 1933: 241 (1933), in Fl. Gén. Indo-Chine 6: 701 (1934); Burkill in J. Linn. Soc. Bot. 56: 404 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 233 (1998). — *Lectotype* (**hic designatus**): *Dioscorea dissimulans* Prain & Burkill.

Distribution. Widespread in the Old World.

Representative species: *D. arachidna*, *D. cochleariapiculata*, *D. cumingii*, *D. dregeana*, *D. dumetorum*, *D. esquirolii* (Fig. 1d), *D. hispida*, *D. kamoonsensis*, *D. melanophyma*, *D. pentaphylla*, *D. pierrei*, *D. scortechinii* and *D. tomentosa*.

11. *Dioscorea* sect. *Macrourea* Burkill in Blumea, Suppl. 1: 232 (1937); Burkill & H. Perr. in Fl. Madag. 44e Fam.: 4 (1950); Burkill in J. Linn. Soc. Bot. 56: 403 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 229 (1998). — *Type:* *Dioscorea sansibarensis* Pax.

Distribution. Tropical Africa.

Representative species: *D. sansibarensis*.

12. *Dioscorea* sect. *Brachyandra* Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 83 (1897), in Bot. Jahrb. Syst. 25: 156 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 68 (1924); Burkill & H. Perr. in Fl. Madag. 44e Fam.: 35 (1950); Burkill in J. Linn. Soc. Bot. 56: 404 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 232 (1998). — *Type:* *Dioscorea heteropoda* Baker.

Distribution. Madagascar.

Representative species: *D. alatipes*, *D. fandra*, *D. hexagona*, *D. hambuka*, *D. karatana*, *D. maciba*, *D. mayottensis*, *D. namorokensis*, *D. ovinala*, *D. soso* and *D. trichantha*.

13. *Dioscorea* sect. *Cardiocapsa* Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 87 (1897), in Bot. Jahrb. Syst. 25: 159 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 311 (1924), '*Cardiocarpa*'; Burkill & H. Perr. in Fl. Madag. 44e Fam.: 12 (1950); Burkill in J. Linn. Soc. Bot. 56: 403 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 230 (1998). — *Type:* *Dioscorea bemarivensis* Jum. & H. Perrier (= *D. lucida* Scott Elliot, nom. illeg.).

Dioscorea sect. *Madagascarienses* R. Knuth in Engl., Pflanzenr. 4 (43): 70 (1924); Burkill & H. Perr. in Fl. Madag. 44e Fam.: 9 (1950); Burkill in J. Linn. Soc. Bot. 56: 403 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 230 (1998). — *Lectotype (hic designatus):* *Dioscorea arcuatinervis* Hochr.

Distribution. Madagascar.

Representative species: *D. arcuatinervis* and *D. bemarivensis*.

14. *Dioscorea* sect. *Combilium* Prain & Burkill in J. Proc. Asiat. Soc. Bengal 10: 7 (1914); R. Knuth in Engl., Pflanzenr. 4 (43): 186 (1924); Prain & Burkill in Fl. Gén. Indo-Chine 6: 701 (1934); Burkill & H. Perr. in Fl. Madag. 44e Fam.: 7 (1950); Burkill in Fl. Males. I, 4: 307 (1951), in J. Linn. Soc. Bot. 56: 403 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 228 (1998); Ding & Gilbert in Wu & Raven, Fl. China 24: 284 (2000); Yamashita & Tamura in Iwatsuki *et al.*, Fl. Japan 4b: 175 (2016). — *Type:* *Dioscorea esculenta* (Lour.) Burkill (= *D. aculeata* Roxb., nom. illeg.).

Distribution. Malesia to India.

Representative species: *D. esculenta*.

15. *Dioscorea* sect. *Stenocorea* Prain & Burkill in J. Proc. Asiat. Soc. Bengal 10: 40 (1914); R. Knuth in Engl., Pflanzenr. 4 (43): 310 (1924); Prain & Burkill in Fl. Gén. Indo-Chine 6: 701 (1934); Burkill in Fl. Males. I, 4: 305 (1951), in J. Linn. Soc. Bot. 56: 403 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 230 (1998); Ding & Gilbert in Wu & Raven, Fl. China 24: 284 (2000). — *Type:* *Dioscorea stenomeriflora* Prain & Burkill.

Distribution. China to India.

Representative species: *D. daunaea*.

16. *Dioscorea* sect. *Macrocarpaea* Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 86 (1897), in Bot. Jahrb. Syst. 25: 158 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 221 (1924); Burkill in J. Linn. Soc. Bot. 56: 403 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 229 (1998). — *Lectotype (hic designatus):* *Dioscorea hylophila* Harms.

Distribution. Tropical Africa.

Representative species: *D. preussii*.

17. *Dioscorea* sect. *Paramecocarpa* Burkill in Gard. Bull. Str. Settl. 3: 123 (1924), *in nota*, R. Knuth in Engl., Pflanzenr. 4 (43): 355 (1924); Prain & Burkill in Fl. Gén. Indo-Chine 6: 702 (1934), in Ann. R. Bot. Gard. Calc. 14: 93 (1936); Burkill in Fl. Males. I, 4: 309 (1951), in J. Linn. Soc. Bot. 56: 403 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 229 (1998). — *Lectotype (hic designatus): Dioscorea piscatorum* Prain & Burkill.

Distribution. Indochina Pen.

Representative species: *D. petelotii*.

18. *Dioscorea* sect. *Shannicorea* Prain & Burkill in J. Proc. Asiat. Soc. Bengal 10: 7 (1914); R. Knuth in Engl., Pflanzenr. 4 (43): 317 (1924); Prain & Burkill in Fl. Gén. Indo-Chine 6: 702 (1934); Burkill in J. Linn. Soc. Bot. 56: 404 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 229 (1998); Ding & Gilbert in Wu & Raven, Fl. China 24: 285 (2000). — *Lectotype (hic designatus): Dioscorea velutipes* Prain & Burkill.

Distribution. China to SE Asia.

Representative species: *D. hemsleyi*, *D. martinii*, *D. nitens*, *D. pseudonitens*, *D. subcalva*, *D. velutipes* (Fig. 1e) and *D. yunnanensis*.

19. ***Dioscorea* sect. *Pseudostenophora* Noda & M. N. Tamura, sect. nov.**

Dioscorea sect. *Pseudostenophora* is similar to *D. sect. Dioscorea* (= *D. sect. Stenophora*), but differs from it in having bisulcate pollen grains.

Type: *Dioscorea birmanica* Prain & Burkill.

Distribution. China to SE Asia.

Representative species: *D. birmanica*.

20. *Dioscorea* sect. *Enantiophyllum* Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 87 (1897), in Bot. Jahrb. Syst. 25: 159 (1898); R. Knuth in Engl., Pflanzenr. 4

(43): 257 (1924); Prain & Burkill in Fl. Gén. Indo-Chine 6: 703 (1934); Burkill & H. Perr. in Fl. Madag. 44e Fam.: 69 (1950); Burkill in Fl. Males. I, 4: 320 (1951), in J. Linn. Soc. Bot. 56: 403 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 231 (1998); Ding & Gilbert in Wu & Raven, Fl. China 24: 291 (2000); Yamashita & Tamura in Iwatsuki *et al.*, Fl. Japan 4b: 172 (2016). — *Lectotype (hic designatus)*: *Dioscorea alata* L.

Dioscorea sect. *Asterotricha* Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 87 (1897), in Bot. Jahrb. Syst. 25: 159 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 255 (1924); Burkill in J. Linn. Soc. Bot. 56: 403 (1960); Huber in Kubitzki, Fam. Gen. Vasc. Pl. 3: 231 (1998). — *Type*: *Dioscorea schimperiana* Hochst.

Dioscorea sect. *Syntepaleia* Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 87 (1897), in Bot. Jahrb. Syst. 25: 159 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 307 (1924). — *Lectotype (hic designatus)*: *Dioscorea semperflorens* Uline.

Dioscorea sect. *Stenophyllidium* Uline in Engl. & Prantl., Nat. Pflanzenfam. Nachtr. 2 (5): 87 (1897), in Bot. Jahrb. Syst. 25: 159 (1898); R. Knuth in Engl., Pflanzenr. 4 (43): 309 (1924). — *Type*: *Dioscorea hastifolia* Nees.

Distribution. Widespread in the Old World.

Representative species: *D. alata*, *D. aspersa*, *D. benthamii*, *D. brevipetiolata*, *D. calcicola*, *D. cirrhosa*, *D. cotinifolia*, *D. decipiens*, *D. doryphore*, *D. elegans*, *D. exalata*, *D. fordii*, *D. formosana*, *D. glabra*, *D. hamiltonii*, *D. hastifolia*, *D. inopinata*, *D. japonica* (Fig. 1f), *D. kratika*, *D. lanata*, *D. lepcharum*, *D. luzonensis*, *D. matsudae*, *D. minutiflora*, *D. nummularia*, *D. oryzetorum*, *D. polystachya*, *D. potaninii*, *D. pseudojaponica*, *D. schimperiana*, *D. tabatae* and *D. wallichii*.

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Table 1. Datamatrix of ten morphological characters in Mesquite of sections and species groups of *Dioscorea*.

	1	2	3	4	5	6	7	8	9	10
<i>Dioscorea</i>	0	0	0	0	0&1	0	0	0&1	0&1	1&0
NW I (<i>Epipetrum</i>)	1	1	2	0	0	2	0	0	0	4
NW I (expect <i>Epipetrum</i>)	1	1	1	0	0&1	0	0	0	0	1&0
NW II	1	0&1	1	0	0	1	0	0	0	1
<i>Borderea</i>	1	0	3	0	0	2	0	0	0	4
<i>Tamus</i>	1	1	3	1	0	0	0	?	0	4
<i>Afoborderea</i>	1	1	?	0	0	0	0	0	0	4
<i>Rhacodophyllum</i>	1	1	?	0	0	0	0	0	0	0
<i>Perennia</i>	1	1	?	0	0	0	0	0	0	1
<i>Testudinaria</i>	1	1	0	0	0	0&2	0	0	0	2
<i>Pseudoshannicorea</i>	1	?	4	0	0	0	0	0	0	3
<i>Opsophyton</i>	1	1	4	0	0	0	0	0	0	1
<i>Lasiophyton</i>	1	1	4	0	1	0	0	0	1	1
NWIII (<i>Apodostemon</i> 2)	1	?	?	0	0	0	0	0	0	0
NWIII (<i>Lasiogyne</i>)	1	1	4(&1)	0	0	0	0	1	0	0
NWIII (<i>Apodostemon</i> 1)	1	1	4(&1)	0	0	0	0	0	0	0
NWIII (<i>Rajania</i>)	1	?	?	2	0	0	0	?	0	4
<i>Macrourea</i>	1	1	4	0	0	0	1	0	0	3
<i>Cardiocarpa</i>	1	1	?	0	0&1	0	0	1	0	0
<i>Brachyandra</i>	1	1	?	0	0	0	0	0	0	1
<i>Stenocorea</i>	1	1	?	0	0	0	0	1	0	0
<i>Combilium</i>	1	1	4	0	0	0	0	0	1	0
<i>Shannicorea</i>	1	1	4	0	0	0	0	0	1	2
<i>Paramecocarpa</i>	1	?	?	0	0	0	0	0	1	2
<i>Macrocarpaea</i>	1	1	4	0	0	0	0	0	1	3
<i>Pseudostenophora</i>	0	1	?	0	0	0	0	1	1	0
<i>Enantiophyllum</i>	1	1	4	0	0	1	1	1	0&1	0&2

Characters and character-states.

1. Underground stem rhizome (0), tuber (1); 2. number of pollen aperture monosulcate (0), bisulcate (1); 3. chromosome: $x = 10$ and diploid (0), $x = 9$ (1), $x = 7$ (2), $x = 6$ (3), polyploid of $x = 10$ (4); 4. fruit: capsule (0), berry (1), samara (2); 5. leaf: simple (0), compound (1); 6. stem: twining to left (0), twining to right (1) or not twinned (2); 7. phyllotaxis alternate (0), opposite (1); 8. capsule longer than its width (0) or wider than its length (1); 9. stem/leaf indumentum absent (0) or present (1); 10. seed wings all around the seed entity (0), basally (1), apically (2), or both basally and apically (3), absence of a wing (4).

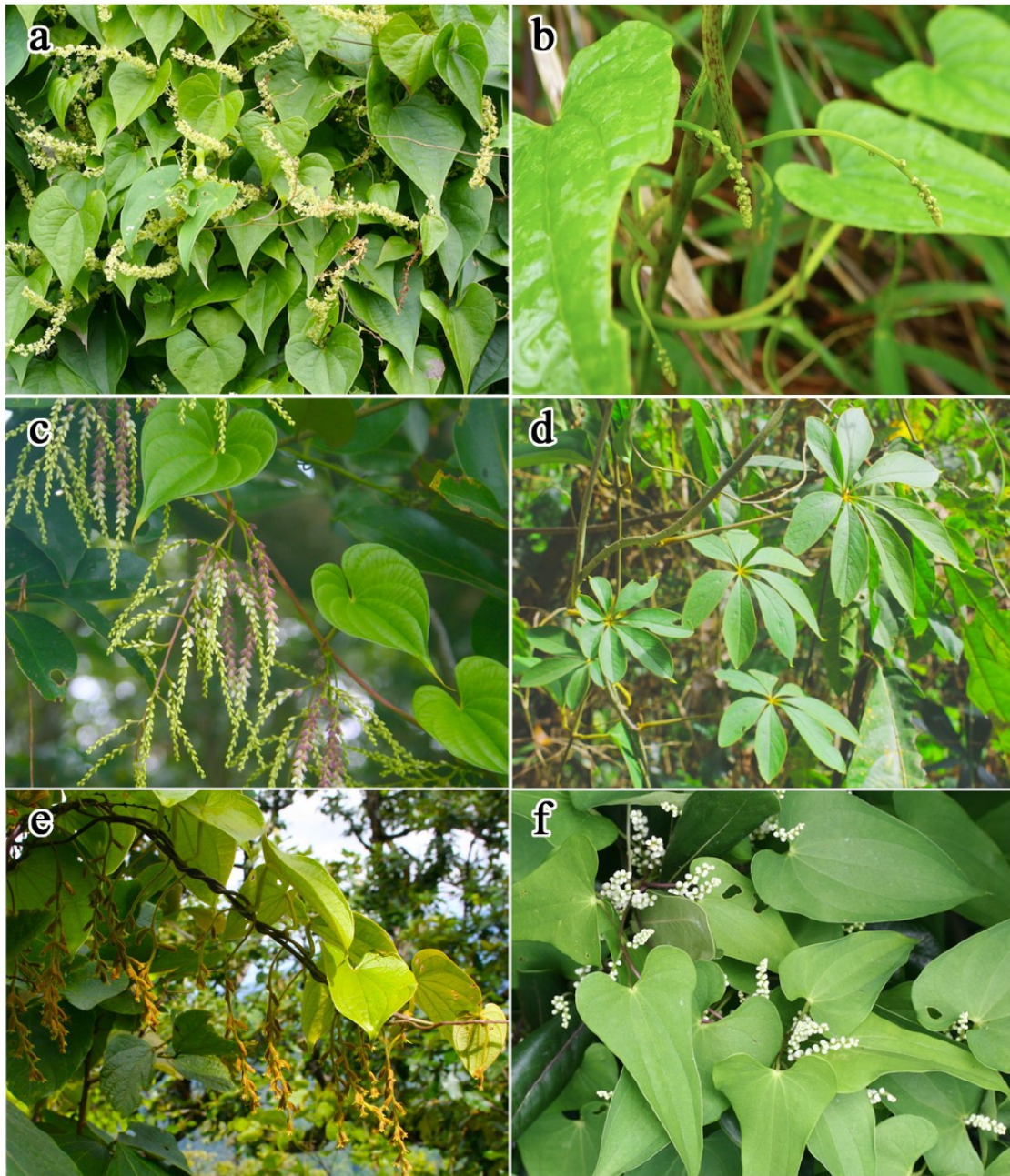


Fig. 1. Habit of *Dioscorea* species. a. *D. tokoro* (male); b. *D. tentaculigera* (male). c. *D. bulbifera* (male); d. *D. esquirolii* (sterile); e. *D. velutipes* (female); f. *D. japonica* (male).

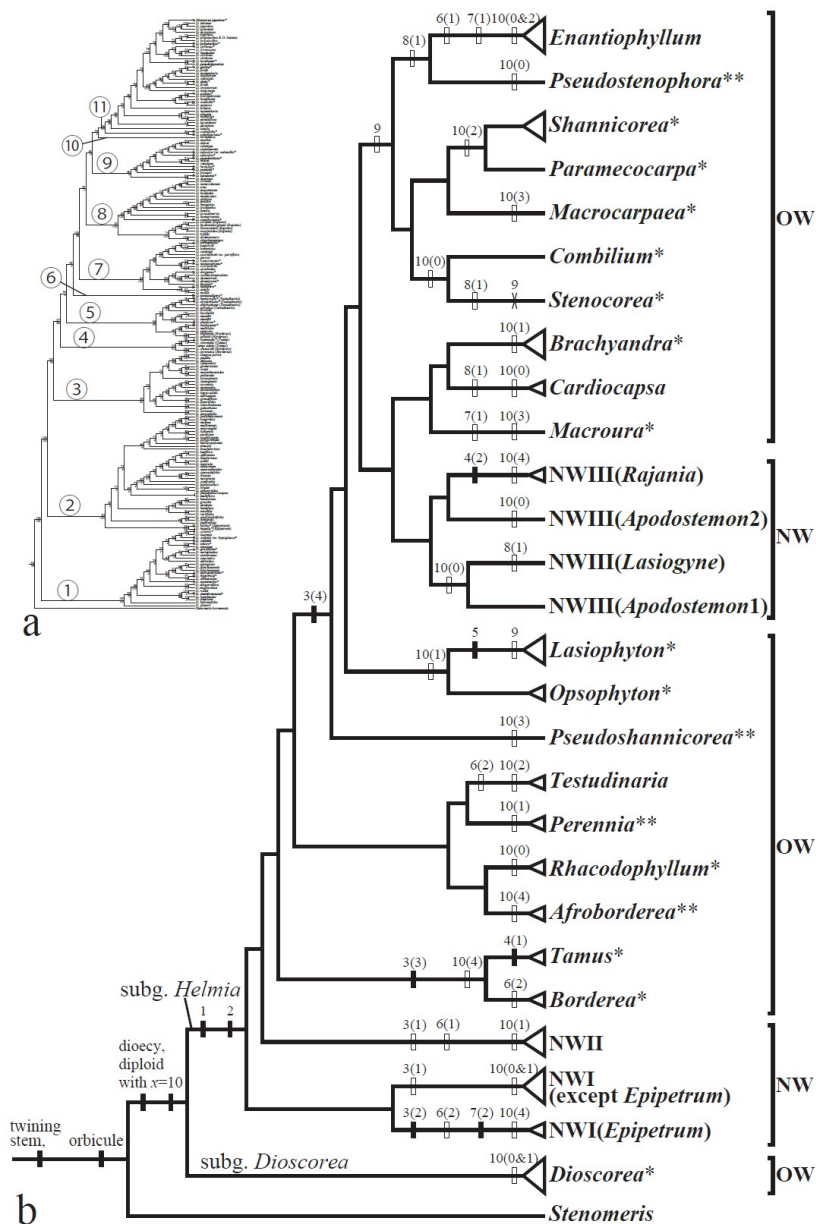


Fig. 2a. Phylogenetic tree of *Dioscorea*. b. Evolution of morphological characters on a tree modified from Fig. 2a. The tree consists of 27 major clades and/or final major subclades, which represent 20 Old World sections and seven New World section or species groups. ①~⑪ represent 11 major clades. * indicates 13 already proposed or circumscribed sections mostly by Huber (1998). ** indicates four new sections (for detail see "Taxonomy" in the text). Characters (1 to 10) and character-state polarities are as follows. 1. Underground stem rhizome (0), tuber (1); 2. number of pollen aperture monosulcate (0), bisulcate (1); 3. chromosome: $x = 10$ and diploid (0), $x = 9$ (1), $x = 7$ (2), $x = 6$ (3), polyploid of $x = 10$ (4); 4. fruit: capsule (0), berry (1), samara (2); 5. leaf: simple (0), compound (1); 6. stem: twining to left (0), twining to right (1) or not twinned (2); 7. phyllotaxis alternate (0), opposite (1); 8. capsule longer than its width (0) or wider than its length (1); 9. stem/leaf indumentum absent (0) or present (1); 10. seed wings all around the seed entity (0), basally (1), apically (2), or both basally and apically (3), absence of a wing (4). Solid square = apomorphy, open square = homoplasy, and cross bar = reversal. Data matrix showing character-state distributions are presented in Table 1. NW and OW indicate New World and Old World sections or species groups, respectively.

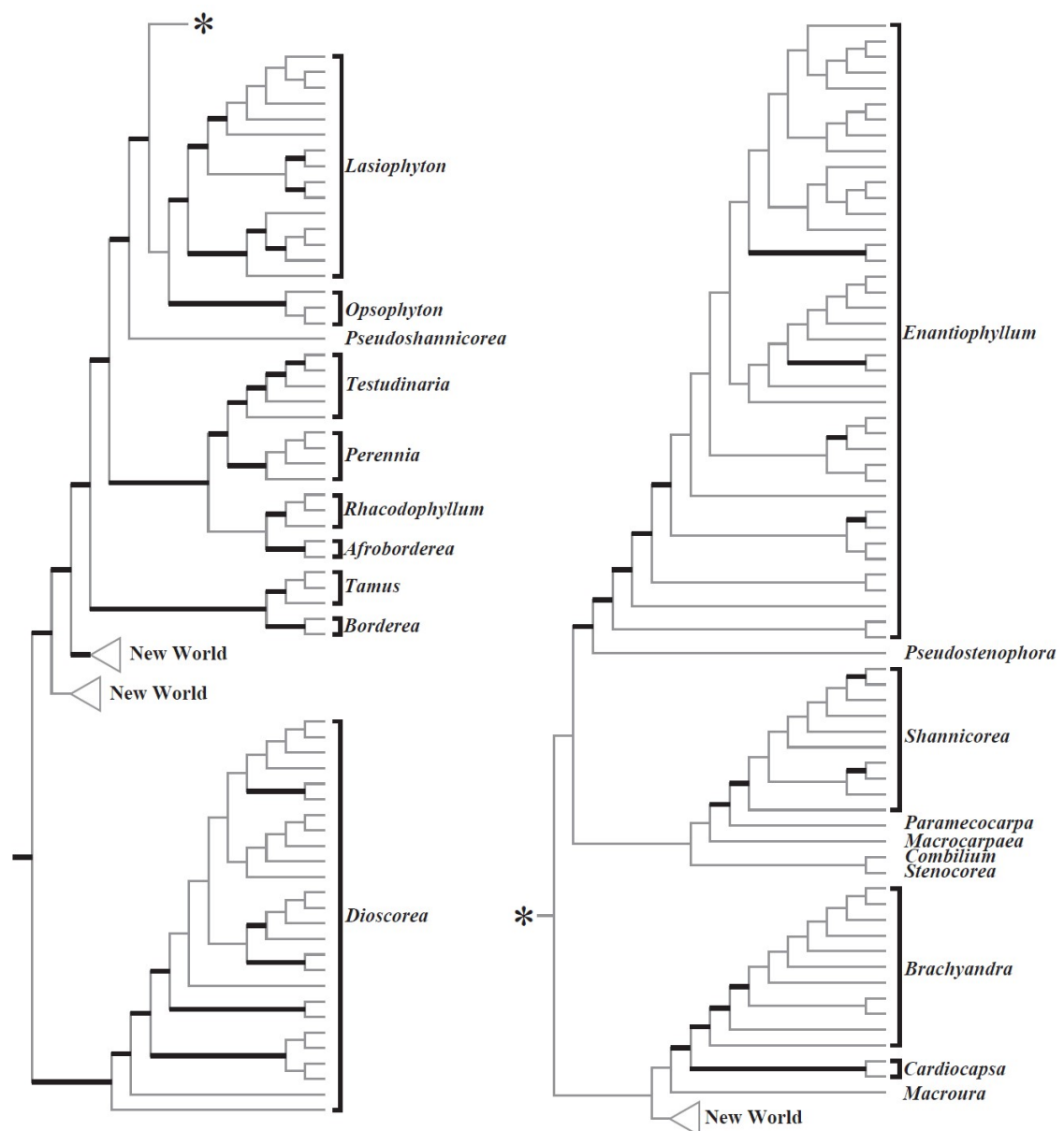


Fig. 3. A phylogenetic tree of *Dioscorea* showing the relationships of 20 Old World sections. Strongly supported clades (90-100% TBE support; see Noda *et al.* 2020) are marked with thick branches.

Chapter III

Phylogenetic analysis of species of *Dioscorea* (Dioscoreaceae) from Japan and other Asiatic regions based on chloroplast and nuclear (*PHYC* and 18S ribosomal)

DNA sequences with special reference to their taxonomic recognition

日本及び他のアジア地域産ヤマノイモ属（ヤマノイモ科）各種の
葉緑体と核（*PHYC* と 18S リボソーム）の DNA 塩基配列に基づく系統解析
—各種の分類学的認識に着目して—

Abstract

To clarify detailed phylogenetic relationships of Japanese *Dioscorea* taxa and to reexamine the taxonomy based on the relationships, we conducted molecular phylogenetic analyses of four chloroplast regions (*trnK* including *matK*, *rbcL*, *atpB* and *trnL-F*) and two nuclear regions (*PHYC* and 18S rDNA) covering all Japanese species and varieties in addition to some other Asiatic species. The results showed that the Japanese *Dioscorea* appears not to include hybrid species, although the different species often grow sympatrically and the variation range of leaf shape within the same species is often extremely high without any clear gap between the variation ranges of different species. Further, the results solved several taxonomic issues on Japanese species, e.g. (1) *Dioscorea japonica*, *D. polystachya*, *D. pseudojaponica* and *D. tabatae*, which highly resemble one another morphologically, turned out to be different species; (2) The plants identified *D. luzonensis* from Daito Islands, whose flowers have never been reported as far as we know, proved to be *D. pseudojaponica* instead of *D. luzonensis*; (3) *Dioscorea zentaroana*, which is often considered to be conspecific with *D. asclepiadea*, is distinct from the latter; (4) *Dioscorea izuensis* should be treated as *D. collettii* var. *izuensis*. In conclusion, 16 species and three varieties of native *Dioscorea* are distributed in Japan.

Introduction

Dioscorea L. includes more than 600 species, widely distributed in tropical to temperate regions of the world (Govaerts *et al.* 2007). In Japan, 17 species and two varieties were recorded as natives (Yamashita and Tamura 2016). Noda *et al.* (2020b) constructed a large-scale phylogenetic tree of 183 *Dioscorea* species of the world based on

chloroplast DNA regions of *matK*, *rbcL*, *atpB* and *trnL-F*, but number of the Asiatic samples and resolution of the phylogenetic tree were still insufficient to solve taxonomic issues especially on Japanese *Dioscorea* species.

Species and varieties of Japanese *Dioscorea* were taxonomically treated based on the morphology by Yamashita and Tamura (2016). Curiously enough, no hybrid has never been reported from Japanese *Dioscorea*, although the different species often grow sympatrically (Fig. 1) and the variation range of leaf shape within the same species is often extremely high (Fig. 2) without clear gap between the variation ranges of different species. With the taxonomic treatment of Yamashita and Tamura (2016), the four questions of the recognition of extant species have once again been brought to the fore: (1) a species complex of *D. japonica* Thunb., *D. polystachya* Turcz., *D. pseudojaponica* Hayata and *D. tabatae* Hatus. ex Yamashita & M. N. Tamura, (2) *D. luzonensis* Schauer, (3) *D. zentaroana* Koidz. and (4) *D. izuensis* Akahori.

As *Dioscorea japonica*, *D. polystachya*, *D. pseudojaponica* and *D. tabatae* are very similar in morphology, the identification of *Dioscorea* of Ryukyu (southwestern Japan) is quite controversial (Fig. 3). For example, the plants of Central Ryukyu (Fig. 3: southernmost part of Tokara Islands to Okinawa Island) were once identified as *D. japonica* by Masamune (1957), subsequently as *D. pseudojaponica* by Hatusima (1961), and then as *D. polystachya* (= *D. batatas*) by Hatusima and Amano (1994). Further, *D. tabatae* was recognized by Hatusima (1986), but ignored by Hatusima and Amano (1994). Yamashita and Tamura (2016) recognized all of the four species except *D. polystachya* in Central Ryukyu. Walker (1976) doubted the distinction between *D. japonica* and *D. pseudojaponica* in Central and South Ryukyu. Thus, the recognition / identification should be reexamined.

Dioscorea of Daito Islands., Pref. Okinawa, Japan, which is located ca. 300km east of Okinawa Island, was first identified as *D. japonica* by Masamune (1957), and subsequently considered *D. pseudojaponica* by Hatusima (1961). Later, Hatusima and Amano (1967) changed the recognition from *D. pseudojaponica* to *D. luzonensis*. Since then, it has been consistently identified as *D. luzonensis* (Hatusima 1971, Shiroma 2011, Yamashita and Tamura 2016), although no certain report on the flowers of *Dioscorea* in Daito Islands is known. As the flowers have never been checked, the identification should be reexamined with molecular methods.

Dioscorea zentaroana was described by Koidzumi (1928) from Amami-ohshima Island, Pref. Kagoshima, Japan, located ca. 300km south-southwest of Kyushu, Japan, but reduced to *D. asclepiadea* of Kyushu by Hatusima (1961). Since Hatusima (1961), *D. zentaroana* has never been recognized except Yamashita and Tamura (2016), although the leaf shape and pedicel length differ from those of *D. asclepiadea* to a high degree (Fig. 4). Thus, the recognition will be reexamined with molecular methods in this study.

Dioscorea izuensis endemic to Izu Peninsula, Pref. Shizuoka, Japan, is morphologically almost identical with *D. collettii* (Akahori 1963) distributed in China, Taiwan to India, although even Taiwan, which is the nearest to the Izu Peninsula, is located ca. 2000km southwest of the Izu Peninsula. The reason of the morphological similarity should be considered for the exact recognition of *D. izuensis*: homologous or analogous.

The purpose of this study is to construct a highly resolved phylogenetic tree of *Dioscorea* species from Japan and other Asiatic regions by using chloroplast DNA regions including the highly variable *trnK* and nuclear *PHYC* and 18S ribosomal DNA

regions. The *trnK*, *PHYC* and 18S rDNA regions are first applied for phylogenetic analysis of *Dioscorea* in this study. And, based on the phylogenetic results, we try to check whether the reticulate evolution occurred among the highly variable Japanese *Dioscorea* species and to solve the aforementioned taxonomic issues on Asiatic *Dioscorea* species, especially on Japanese ones.

Materials and Methods

[Plant materials]

The sources of plant materials used in this study are listed in Table 1. All of the DNA sequence data (Table 1) were determined by ourselves: 123 samples for chloroplast regions of *trnK* (including *matK*), *rbcL*, *atpB* and *trnL-F*, and 83-84 samples for nuclear regions of *PHYC* and 18S rDNA. We followed the most recent classifications for species identification of *Dioscorea*: Yamashita and Tamura (2016) for the species from Japan, Huang and Hsiao (2000) for the species from Taiwan, Wilkin and Thapayai (2009) for the species from Thailand, and Burkill (1951) for the species from the Philippines. We identified a sample of JA19 from Amami-ohshima Isl. as *D. japonica* (Table 1) based on "Key to the species" of Yamashita and Tamura (2016), although they did not include Amami-ohshima Isl. in its distribution range. And, we followed Noda *et al.* (2020a) for the infrageneric classification. All of the sequence data are deposited in the DNA Data Bank of Japan (DDBJ) database. The voucher specimens are preserved at the herbaria of Museum of Nature and Human Activities, Hyogo (HYO), Kagoshima University (KAG), Kyoto University (KYO), The University of Tokyo (TI), and Institute of Plant Science and Resources, Okayama University.

[DNA extraction, polymerase chain reaction (PCR) amplification, and DNA sequencing]

Total genomic DNA was extracted from fresh or silica-gel dried leaves, or leaves of herbarium specimens. DNA extraction, PCR amplification, DNA sequencing followed Noda *et al.* (2020b). However, in case of *PHYC* and 18S rDNA, we amplified DNA by adding 5 % DMSO to the reaction mixture. The PCR and sequence primers used in this study, except those for *Dioscorea luzonensis* from the Philippines, are listed in Appendix 1. In case of *D. luzonensis* from the Philippines, we used 177 primers for sequencing only chloroplast regions (*trnK*, *rbcL*, *atpB* and *trnL-F*) of one sample (Appendix 2), because we used the very old herbarium specimen that was collected more than half a century ago (in 1964) for DNA extraction. As nuclear markers for *Dioscorea* species, we tried to sequence not only *PHYC* and 18S rDNA, but also ITS, *waxy* and *CHS* (chalcone synthase). However, we failed to sequence the latter three regions.

[Phylogenetic analysis]

We made a manual alignment of the DNA sequences obtained. When several samples have the identical DNA sequences of chloroplast (cp) regions (*trnK*, *rbcL*, *atpB* and *trnL-F*) / *PHYC* / 18S rDNA, we included only one of the samples in our analyses. We employed the maximum-parsimony (MP) and maximum-likelihood (ML) methods for phylogeny reconstruction using PAUP* version 4.0a166 (accessed in 2019), an update of PAUP* version 4 (Swofford 2003).

For the MP analysis, the following settings were used: heuristic search option of 10,000 replicates of random sequence addition, tree bisection reconnection (TBR)

swapping algorithm and MulTrees. Only the base substitutions were treated as characters for phylogenetic reconstruction. The consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated using PAUP, including uninformative characters. A MP bootstrap analysis with 1000 replications (heuristic; simple; TBR) was performed using PAUP.

For the ML analysis, the best model of nucleotide evolution was estimated using jModelTest 2 (Darriba *et al.* 2012). The models selected by the Akaike's Information Criterion (AIC) were listed in Table 2. The models were implemented in PAUP, using a heuristic search option of as-is additional sequence and a TBR swapping algorithm, according to the parameters specified by the models. We conducted a ML bootstrap analysis with 1000 replications (heuristic; as-is; TBR), using RAxML-NG v. 0.7.0git BETA (Kozlov *et al.* 2019) because PAUP takes much longer time for the calculation.

As 15/17 native Japanese species are included in either *Dioscorea* sect. *Dioscorea* or *D.* sect. *Enantiophyllum*, we conducted partition-homogeneity tests for the two sections, respectively, to know appropriateness of the combined analysis, which usually yields more highly resolved phylogenetic trees than a single gene analysis. Congruence among the three data sets, i.e. (1) chloroplast regions (*trnK*, *rbcL*, *atpB* and *trnL-F*), (2) nuclear *PHYC* region, and (3) nuclear 18S rDNA region, were examined with the incongruence length difference (ILD) test (Farris et al. 1994), implemented in PAUP, as the partition-homogeneity test. The tests were performed using a heuristic search (200 replicates) with a simple addition sequence option, TBR swapping, and MulTrees. Using an incongruence threshold of 0.05, we concluded whether the three (one chloroplast and two nuclear) data sets were combinable or not.

[Observation of stamens of *Dioscorea izuensis* and *D. collettii*]

The herbarium specimens of *Dioscorea izuensis* and *D. collettii* preserved in KYO and TI were used for observation of the stamens. First, male flowers were taken from the herbarium specimens, and boiled in hot water to restore the original flower shape. And, the stamens were sketched under a binocular microscope. The observed flowers were later put in paper bags attached on the corresponding sheets of the herbarium specimens, respectively.

Results

[An phylogenetic analysis of the chloroplast DNA regions (*trnK*, *rbcL*, *atpB* and *trnL-F*) for the genus *Dioscorea*]

Phylogenetic information is listed in Table 2. The maximum likelihood (ML) tree is shown in Fig. 5. The topology of MP strict consensus tree is identical to that of ML tree, except the two branches (arrowed in Fig. 5) that collapse in the former tree (Fig. 5).

According to the ML tree (Fig. 5), all samples of the genus *Dioscorea* are monophyletic with 100 % bootstrap support, and the genus comprises two major clades: the former major clade (100%; *D. sect. Dioscorea*) and the latter major clade (100%), where a clade of *D. sect. Pseudoshannicorea* diverges as the next branch, followed successively by a clade of *D. sects. Lasiophyton + Opsophyton*, a clade of *D. sects. Combilium + Paramecocarpa + Shannicorea*, and a clade of *D. sect. Enantiophyllum*. Further, clades corresponding to these eight sections, unless containing only one sample, receive 100 % bootstrap supports, respectively. This branching order of the sections agrees to that of Noda *et al.* (2020b).

Among the 19 Japanese species including two cultivated ones, nine species, i.e. *D.*

tenuipes, *D. tokoro*, *D. quinquelobata*, *D. septemloba*, *D. zentaroana*, *D. asclepiadea*, *D. gracillima*, *D. nipponica* and *D. izuensis*, are included in *D. sect. Dioscorea*, and seven species, i.e. *D. alata* (cult.), *D. cirrhosa*, *D. luzonensis*, *D. pseudojaponica*, *D. polystachya*, *D. tabatae* and *D. japonica*, are in *D. sect. Enantiophyllum*. The remaining three species, i.e. *D. pentaphylla*, *D. bulbifera* and *D. esculenta* (cult.), are included in *D. sects. Lasiophyton*, *Opsophyton* and *Combilium*, respectively.

[Phylogenetic analyses of the chloroplast DNA regions (*trnK*, *rbcL*, *atpB* and *trnL-F*) and the nuclear DNA regions (*PHYC* and 18S rDNA) for *Dioscorea* sect. *Dioscorea*]

Phylogenetic information is listed in Table 2. The maximum likelihood (ML) trees based on chloroplast (cp) DNA regions, nuclear *PHYC* region and nuclear 18S rDNA region, respectively, are shown in Fig. 6. They are identical to the MP strict consensus trees in terms of topology, respectively (Fig. 6). We selected *Dioscorea bulbifera* (sect. *Opsophyton*), *D. japonica* and *D. alata* (sect. *Enantiophyllum*) as an outgroup, based on the aforementioned cp DNA tree for the whole genus (Fig. 5).

Apart from resolution, the topologies of cp regions and nuclear *PHYC* region do not contradict each other except a phylogenetic position of *Dioscorea collettii* from Taiwan as well as relationships within the same species (Fig. 6). Likewise, apart from resolution, the topologies of nuclear *PHYC* and 18S rDNA regions do not contradict each other except that two individuals of *D. collettii* from Thailand have a different DNA sequence of 18S rDNA from that of *D. collettii* from Taiwan, *D. izuensis*, *D. nipponica*, *D. septemloba* and *D. quinquelobata* (Fig. 6). Thus, as for the Japanese species (*D. collettii* is not distributed in Japan), the relationships between species inferred from cp regions,

PHYC and 18S rDNA do not contradict one another, and hybrid formation and reticulate evolution do not seem to be plausible.

Then, to know whether the three datasets of *Dioscorea* sect. *Dioscorea*, i.e. cp regions, *PHYC* and 18S rDNA, are combinable or not, we conducted the incongruence length difference (ILD) test among the three datasets, which found a P value of 0.11. Thus, we concluded that the three datasets were combinable, and we constructed a highly resolved molecular phylogenetic tree of *D.* sect. *Dioscorea* based on the combined dataset (Fig. 7).

Apart from resolution, the topology of this combined tree (Fig. 7) agrees to the cp tree topology of either *Dioscorea* sect. *Dioscorea* (Fig. 6) or the whole genus (Fig. 5). According to the combined tree (Fig. 7), all samples of *D.* sect. *Dioscorea* are monophyletic (100 %), and the section comprises two major clades: the former of *D. tokoro* and *D. tenuipes* (100%) and the latter of the remainings (89% in MP, 54% in ML). The former major clade consists of a clade of *D. tokoro* (100%) and a clade of *D. tenuipes* (99-100%). The latter major clade includes five clades: (1) *D. izuensis* + *D. collettii* (100%), (2) *D. nipponica* (100%), (3) *D. gracillima* + *D. asclepiadea* + *D. zentaroana* (100%), (4) *D. septemloba* (100%) and (5) *D. quinquelobata* (100%).

Further, the following relationships were revealed. Three individuals of *Dioscorea collettii* are paraphyletic, because *D. collettii* from Taiwan is sister to *D. izuensis* (66-67%), not to *D. collettii* from Thailand. *Dioscorea asclepiadea* is sister to *D. gracillima* (96-99%), not to *D. zentaroana*. *Dioscorea septemloba* var. *sititoana* is excluded from a clade of *D. septemloba* var. *septemloba* (99%).

[Phylogenetic analyses of the chloroplast DNA regions (*trnK*, *rbcL*, *atpB* and

***trnL-F*) and the nuclear DNA regions (*PHYC* and 18S rDNA) for *Dioscorea* sect. *Enantiophyllum*]**

Phylogenetic information is listed in Table 2. The ML trees based on cp DNA regions, nuclear *PHYC* region and nuclear 18S rDNA region, respectively, are shown in Fig. 8. They are identical to the MP strict consensus trees in terms of topology, respectively, except the four branches (arrowed in Fig. 8) that collapse in the MP strict consensus trees (Fig. 8). We used *Dioscorea pentaphylla* (sect. *Lasiophyton*) and *D. velutipes* (sect. *Shannicorea*) as an outgroup, based on the aforementioned cp DNA tree for the whole genus (Fig. 5).

Apart from resolution, the topologies of cp regions and nuclear *PHYC* region agree to each other except a phylogenetic position of one individual (PO6) of *Dioscorea polystachya* (Fig. 8). As for 18S rDNA, only *D. alata* has a different DNA sequence from that of the other species analyzed in this study (Fig. 8). Thus, the relationships between species inferred from cp regions, *PHYC* and 18S rDNA do not contradict one another, and hybrid formation and reticulate evolution do not seem to be plausible.

Then, to know whether the three datasets of *Dioscorea* sect. *Enantiophyllum*, i.e. cp regions, *PHYC* and 18S rDNA, are combinable or not, we conducted ILD test among the three datasets, which found a P value of 0.72. Thus, we concluded that the three datasets were combinable, and constructed a highly resolved molecular phylogenetic tree of *D. sect. Enantiophyllum* based on the combined dataset (Fig. 9).

Apart from resolution and a relationship within *Dioscorea polystachya*, this combined tree (Fig. 9) agrees to the cp trees of either *Dioscorea* sect. *Enantiophyllum* (Fig. 8) or the whole genus (Fig. 5) and also the *PHYC* tree of *D. sect. Enantiophyllum* (Fig. 8) in terms of topology. According to the combined tree (Fig. 9), all samples of *D.*

sect. *Enantiophyllum* are monophyletic (100 %). And, only *D. luzonensis* from the Philippines (LU2) is excluded from the remaining *D. sect. Enantiophyllum* (<65%), which comprises two major clades: the former of *D. alata* and *D. glabra* (89-92%) and the latter of the others (84% in MP, 58% in ML). The former major clade consists of a clade of *D. alata* (100%) and a clade of *D. glabra* (99-100%). The latter major clade includes three clades: (1) *D. cirrhosa* + *D. matsudae* (74-92%), (2) *D. pseudojaponica* + *D. luzonensis* (LU1) from Daito Isl., Japan (88-92%) and (3) *D. polystachya* + *D. tabatae* + *D. japonica* (87-92%). This clade of (3) consists of a subclade of *D. polystachya* (85-90%) and a subclade of *D. tabatae* and *D. japonica* (83-92%).

Further, the following relationships were revealed. *Dioscorea cirrhosa* from Japan (C11) is sister to *D. matsudae* from Taiwan (66-73%), not to *D. cirrhosa* from Thailand (C12). As for the samples of Pref. Okinawa, Japan, *Dioscorea pseudojaponica* from Ishigaki Isl. (PJ2) is sister to *D. luzonensis* (LU1) from Daito Isl. (92%) in the ML tree, not to *D. pseudojaponica* from Okinawa Isl. (PJ1). Three samples of *D. tabatae* (94-97%) and 19 samples of *D. japonica* (85-87%) are grouped, respectively.

[Observation of stamens of *Dioscorea izuensis* and *D. collettii*]

Each male flower of both *Dioscorea izuensis* and *D. collettii* has three stamens and three staminodes. The sketches of stamens were shown in Fig. 10. The length of anthers is nearly constant among the observed *D. izuensis* and *D. collettii* including var. *hypoglauca*, i.e. from 0.25 mm to 0.3 mm. On the other hand, the length of their filaments is not constant: 0.41 mm for *D. izuensis*, but 0.19-0.26 mm for *D. collettii* (0.23 mm for var. *hypoglauca*). Thus, the filaments of *D. izuensis* are much longer than those of *D. collettii*.

Discussion

[Detailed chloroplast phylogeny of the Japanese *Dioscorea*]

Noda *et al.* (2020a) constructed a chloroplast phylogenetic tree of the genus *Dioscorea* based on *atpB*, *matK*, *rbcL* and *trnL-F* (3754 bp in total) using 183 species covering all over the world. This time, we constructed a more highly resolved chloroplast phylogenetic tree (based on 6288 bp in total) using the entire *trnK*, instead of *matK*, although our materials are restricted mostly within Japanese species. The present results elucidated the detailed chloroplast phylogeny of the Japanese *Dioscorea* (Fig. 5), and enabled us to solve the aforementioned taxonomic issues on Asiatic *Dioscorea* species, especially on Japanese ones.

[Possibility of the existence of hybrid species in the Japanese *Dioscorea*]

Further, we also constructed nuclear phylogenetic trees of the Japanese species of *Dioscorea* sections *Enantiophyllum* and *Dioscorea* (= *Stenophora*) using *PHYC* and 18S rDNA. Using the aforementioned highly resolved chloroplast tree in addition to the nuclear trees, we tried to investigate whether the Japanese *Dioscorea* often yielded hybrid species in the past and evolved reticulately, because the different species often grow sympatrically in Japan (Fig. 1) and the variation range of leaf shape within the same species is often extremely high (Fig. 2) without any clear gap between the variation ranges of different species. Based on the comparison between the chloroplast tree and the nuclear ones, however, their topologies turned out to be highly congruent with ILD values of 0.72 for sect. *Enantiophyllum* and 0.11 for sect. *Dioscorea*, and the existence of hybrid species and the past reticulate evolution among the Japanese

Dioscorea species are not much plausible (Judd *et al.* 2016). Thus, the difficulty in identifying the Japanese *Dioscorea* species might not be due to the existence of hybrids.

[A species complex of *Dioscorea japonica*, *D. polystachya*, *D. pseudojaponica* and *D. tabatae*]

The species concepts of Yamashita and Tamura (2016) for the Japanese *Dioscorea* proved to be primarily concordant with the present molecular phylogenetic results except a few species including *D. japonica* complex. The *D. japonica* complex consists of *D. japonica*, *D. polystachya*, *D. pseudojaponica* and *D. tabatae*, and their species recognition is quite controversial especially in Central Ryukyu, Japan. The *D. japonica* complex there was once considered as one species, *D. japonica*, by Masamune (1957). Hatusima (1961) also considered it as one species, but identified it as *D. pseudojaponica* instead of *D. japonica*. Hatusima (1986) recognized *D. tabatae* in addition to *D. pseudojaponica*, although Hatusima and Amano (1994) regarded all of the four taxa as *D. polystachya*, ignoring *D. tabatae*. Yamashita and Tamura (2016) considered the four taxa as independent species.

For the present molecular analysis, we widely sampled *D. japonica* (from Prefs. Tochigi to Kagoshima, Japan) and *D. polystachya* (from Osaka, Japan, to Taiwan). Further, in the analysis we included samples of *D. japonica* and *D. tabatae*, which grew in a distance less than only ten meters in Amami-ohshima Island, Pref. Kagoshima. Nevertheless, the present molecular phylogeny indicated that all of the four taxa are monophyletic, respectively. Thus, we agree to the species concept of Yamashita and Tamura (2016), where *D. japonica*, *D. polystachya*, *D. pseudojaponica* and *D. tabatae* are treated as independent species, except the distribution range of *D. japonica*.

Yamashita and Tamura (2016) considered the southernmost edge of distribution range of *D. japonica* being in Tokara Islands, Pref. Kagoshima (Fig. 3), while this study revealed that *D. japonica* grows also in Amami-ohshima Island, representing a southern range extension (from Tokara Islands to Amami-ohshima Island).

[*Dioscorea luzonensis* from Daito Isls., Japan]

Dioscorea luzonensis was considered to be endemic to the Philippines Burkill (1951) before Hatusima (1971) identified *Dioscorea* from Daito Isls., Japan, to be *D. luzonensis*. However, the identification was somewhat doubtful because Daito Isls. are located far from the Philippines, and he seemed to identify it only by its vegetative organs. Although the flowers of *Dioscorea* from Daito Isls. have never been observed to this day as far as we know, all recent Japanese floras including Yamashita and Tamura (2016) accepted the Hatusima's identification. However, the present molecular phylogeny indicates that *Dioscorea* from Daito Isls. should not be identified as *D. luzonensis* but *D. pseudojaponica*. It's a fact that it was identified as *D. pseudojaponica* before Hatusima (1971), and Yamashita and Tamura (2016) mentioned that their key distinguishing *D. luzonensis* of Daito Isls. from *D. pseudojaponica* is not clear-cut. Thus, *Dioscorea* from Daito Isls. should be identified as *D. pseudojaponica*, and *D. luzonensis* should be considered again to be endemic to the Philippines.

[*Dioscorea zentaroana*]

Although *Dioscorea zentaroana* was described from Amami-ohshima Island by Koidzumi (1928), Hatusima (1961) reduced it to *D. asclepiadea* from Kyushu, Japan. Since then, *D. zentaroana* has been considered to be a synonym of *D. asclepiadea* in all

floras of Japan except Yamashita and Tamura (2016). We confirmed, however, the large difference in morphology between *D. asclepiadea* and *D. zentaroana*, i.e. in leaf shape and presence/absence of pedicels (Fig. 4). Further, our molecular phylogeny based on the combined chloroplast and nuclear sequences revealed that *D. asclepiadea* is sister to *D. gracillima* (96-99%), not to *D. zentaroana* (Fig. 7). Thus, we can safely conclude that *D. zentaroana* is a different species from *D. asclepiadea*.

[*Dioscorea izuensis*]

Since Akahori (1963) noted that *Dioscorea izuensis* is morphologically similar to *D. collettii* in his original description for *D. izuensis*, the two species have been considered to be closely related. However, as the distribution of *D. izuensis* is restricted to a limited area of the eastern coast of Izu Peninsula, East Japan, which is located far northeastwards from the east edge of the distribution area of *D. collettii*, Taiwan, the two have been considered to be different species. The present molecular phylogeny, however, indicated that *D. izuensis* was embedded in the clade of *D. collettii*, and *D. izuensis* is considered to be conspecific to *D. collettii*. As *D. izuensis* slightly differs from *D. collettii* including *D. collettii* var. *hypoglauca* in having long staminal filaments of male flowers (Fig. 10), we recognize the “*izuensis*” as a variety of *D. collettii*.

[*Dioscorea* species and varieties in Japan]

Yamashita and Tamura (2016) reported 17 species and two varieties of native *Dioscorea* from Japan. Based on this study, the plants that were believed to be *D. luzonensis* in Daito Isls. were turned out not to be *D. luzonensis* actually, but *D. pseudojaponica*. And, we considered that *D. izuensis* in Izu Peninsula should be treated

as *D. collettii* var. *izuensis*. Thus, we recognize 16 species and three varieties of native *Dioscorea* from Japan.

Dioscorea collettii* J. D. Hook. var. *izuensis* (Akahori) Noda & M. N. Tamura, **stat. nov.*

Basionym: *Dioscorea izuensis* Akahori in Acta Phytotax. Geobot. 19: 161 (1963). --

Typus: JAPAN, Pref. Shizuoka, Izu Peninsula, Yawatano, 20 Jul. 1962, A. Akahori SHIONOGI-002357 (holo-KYO!).

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Table 1. Sources of materials of *Dioscorea* and outgroup. “-” indicates data not available.

Taxon	Code	Locality and Voucher	Acc. no. (<i>trnK</i>)	Acc. no. (<i>rbcL</i>)	Acc. no. (<i>atpB</i>)	Acc. no. (<i>trnL-F</i>)	Acc. No. (<i>PHYC</i>)	Acc. No. (18s rDNA)
<i>Dioscorea alata</i> L.	AL1	Cultivated, Kisaichi Bot. Gard., Osaka City Univ., <i>M.N.Tamura & S.Fuse 10019</i> (KYO).	LC327666	LC327795	LC327924	LC328053	LC514752	LC514836
	AL2	Thailand: Doi Phu Kha, Prov. Nan, <i>M.N.Tamura et al. 20159</i> (KYO)	LC327667	LC327796	LC328054	LC327925	LC514753	LC514837
<i>D. asclepiadea</i> Prain & Burkill	AS1	Japan: Amakusa, Pref. Kumamoto, <i>M.N.Tamura 20188</i> (KYO).	LC327668	LC327797	LC327926	LC328055	LC514754	LC514838
	AS2	Japan: Amakusa, Pref. Kumamoto, <i>M.N.Tamura 20189</i> (KYO)	LC327669	LC327798	LC328056	LC327927	LC514755	LC514839
<i>D. bulbifera</i> L.	BL1	Japan: Shimoda-shi, Pref. Shizuoka, <i>M.N.Tamura & H.Noda 6</i> (KYO)	LC327671	LC327800	LC327929	LC328058	-	-
	BL2	Japan: Okayama-shi, Pref. Okayama, <i>J.Yamashita 6565</i> (Okayama University)	LC327682	LC327811	LC328069	LC327940	LC514756	LC514840
	BL3	Japan: Okayama-shi, Pref. Okayama, <i>Yamashita & Kobatake 7562</i> (Okayama University)	LC327670	LC327799	LC328057	LC327928	-	-
	BL4	Japan: Amami-Ohshima Isl., Pref. Kagoshima, <i>M.N.Tamura & H.Noda 20107</i> (KYO)	LC327674	LC327803	LC328061	LC327932	-	-
	BL5	Japan: Amami-Ohshima Isl., Pref. Kagoshima, <i>M.N.Tamura & H.Noda 20117</i> (KYO)	LC327675	LC327804	LC328062	LC327933	-	-
	BL6	Japan: Amami-Ohshima Isl., Pref. Kagoshima, <i>M.N.Tamura & H.Noda 20120</i> (KYO)	LC327676	LC327805	LC328063	LC327934	-	-
	BL7	Japan: Amami-Ohshima Isl., Pref. Kagoshima, <i>M.N.Tamura & H.Noda 20122</i> (KYO)	LC327677	LC327806	LC328064	LC327935	-	-
	BL8	Japan: Amami-Ohshima Isl., Pref. Kagoshima, <i>M.N.Tamura & H.Noda 20100</i> (KYO)	LC327678	LC327807	LC328065	LC327936	-	-
	BL9	Japan: Amami-Ohshima Isl., Pref. Kagoshima, <i>M.N.Tamura 20348</i> (KYO)	LC327680	LC327809	LC328067	LC327938	-	-
	BL10	Japan: Amami-Ohshima Isl., Pref. Kagoshima, <i>M.N.Tamura 20354</i> (KYO)	LC327681	LC327810	LC328068	LC327939	-	-
	BL11	Japan: Mt. Nago, Pref. Okinawa, <i>M.N.Tamura 20202</i> (KYO)	LC327679	LC327808	LC328066	LC327937	-	-
	BL12	Thailand: Doi Phu Wae, Prov. Nan, <i>M.N.Tamura et al. T-30040</i> (KYO)	LC327683	LC327812	LC327941	LC328070	-	-
	BL13	Thailand: Doi Phu Wae, Prov. Nan, <i>M.N.Tamura et al. T-30041</i> (KYO)	LC327684	LC327813	LC327942	LC328071	-	-
	BL14	Thailand: Doi Ang Khang, Prov. Chiang Mai, <i>M.N.Tamura et al. T-30060</i> (KYO)	LC327685	LC327814	LC328072	LC327943	-	-
	BL15	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N.Tamura et al. 9601</i> (KYO)	LC327672	LC327801	LC328059	LC327930	-	-
	BL16	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N.Tamura et al. T-30090</i> (KYO)	LC327686	LC327815	LC328073	LC327944	-	-
	BL17	Indonesia: Waigeo Isl., <i>H.Noda 1009</i> (KYO)	LC327673	LC327802	LC328060	LC327931	-	-
<i>D. cirrhosa</i> Lour.	CI1	Japan: Ishigaki Isl., Pref. Okinawa, <i>S.Fujii s.n.</i> (KYO)	LC327687	LC327816	LC328074	LC327945	LC514757	-
	CI2	Thailand: Khao Yai National Park, Prov. Nakhon Ratchasima, <i>M.N.Tamura et al. T-31329</i> (KYO)	LC327688	LC327817	LC327946	LC328075	LC514758	LC514841
<i>D. collettii</i> HooK. f.	CO1	Taiwan: Coun. Hualien, <i>T.Yamasaki 912973</i> (KYO)	LC327689	LC327818	LC328076	LC327947	LC514759	LC514842
	CO2	Thailand: Doi Phu Wae, Prov. Nan, <i>M.N.Tamura et al. T-30029</i> (KYO)	LC327690	LC327819	LC327948	LC328077	LC514760	LC514843
	CO3	Thailand: Doi Phu Wae, Prov. Nan, <i>M.N.Tamura et al. T-30033</i> (KYO)	LC327691	LC327820	LC328078	LC327949	LC514761	LC514844
<i>D. esculenta</i> (Lour.) Burkill	-	Japan: Ishigaki Isl. Pref. Okinawa, <i>M. Togashi s.n. 3 Aug. 1977</i> (KYO)	LC327692	LC327821	LC327950	LC328079	-	-
<i>D. glabra</i> Roxb.	GL1	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N.Tamura et al. 9602</i> (KYO)	LC327693	LC327822	LC327951	LC328080	LC514767	LC514845
	GL2	Thailand: Doi Phu Kha, Prov. Nan, <i>M.N.Tamura et al. 20156</i> (KYO)	LC327694	LC327823	LC327952	LC328081	LC514762	LC514846
	GL3	Thailand: Doi Phu Kha, Prov. Nan, <i>M.N.Tamura et al. 20158</i> (KYO)	LC327695	LC327824	LC328082	LC327953	LC514763	LC514847
	GL4	Thailand: Doi Phu Kha, Prov. Nan, <i>M.N.Tamura et al. 20160</i> (KYO)	LC327696	LC327825	LC328083	LC327954	LC514764	LC514848
	GL5	Thailand: Doi Phu Wae, Prov. Nan, <i>M.N.Tamura et al. T-30042</i> (KYO)	LC327698	LC327827	LC327956	LC328085	LC514766	LC514849
	GL6	Thailand: Doi Phu Wae, Prov. Nan, <i>M.N.Tamura et al. T-30038</i> (KYO)	LC327697	LC327826	LC328084	LC327955	LC514765	LC514850
<i>D. gracillima</i> Miq.	GR1	Japan: Agatsuma-gun, Pref. Gunma, <i>K.Masuda s.n.</i> (KYO)	LC327702	LC327831	LC328089	LC327960	LC514771	LC514854

Table 1. (continued)

Taxon	Code	Locality and Voucher	Acc. no. (<i>trnK</i>)	Acc. no. (<i>rbcl</i>)	Acc. no. (<i>atpB</i>)	Acc. no. (<i>trnL-F</i>)	Acc. No. (<i>PHYC</i>)	Acc. No. (18s rDNA)
	GR2	Japan: Ena-gun, Pref. Gifu, <i>S.Tsugaru et.al 27657</i> (KYO)	LC327701	LC327830	LC328088	LC327959	LC514770	LC514853
	GR3	Japan: Kawakami-gun, Pref. Okayama, <i>N.Kurosaki 23741</i> (KYO)	LC327699	LC327828	LC327957	LC328086	LC514768	LC514851
	GR4	Japan: Kameoka-shi, Pref. Kyoto, <i>M. Inumochi 874</i> (KYO)	LC327704	LC327833	LC328091	LC327962	LC514773	LC514856
	GR5	Japan: Kobe-shi, Pref. Hyogo, <i>T.Kobayash 44607</i> (KYO)	LC327703	LC327832	LC328090	LC327961	LC514772	LC514855
	GR6	Japan: Takahashi-shi, Pref. Okayama, <i>J.Yamashita & H.Noda No. 25</i> (KYO)	LC327705	LC327834	LC328092	LC327963	LC514774	LC514857
	GR7	Japan: Ono-gun, Pref. Nara, <i>G.Murata 72632</i> (KYO)	LC327700	LC327829	LC328087	LC327958	LC514769	LC514852
<i>D. izuensis</i> Akahori	IZ1	Japan: Shimoda-shi, Pref. Shizuoka, <i>S.Fuse & H.Noda 4646</i> (HYO)	LC327706	LC327835	LC327964	LC328093	LC514775	LC514858
	IZ2	Japan: Shimoda-shi, Pref. Shizuoka, <i>S.Fuse & H.Noda 4647</i> (HYO)	LC327707	LC327836	LC328094	LC327965	LC514776	LC514859
<i>D. japonica</i> Thunb.	JA1	Japan: Kanuma-shi, Pref. Totigi, <i>M.N.Tamura 20137</i> (KYO)	LC327713	LC327842	LC328100	LC327971	LC514779	LC514864
	JA2	Japan: Kanuma-shi, Pref. Totigi, <i>M.N.Tamura 20140</i> (KYO)	LC327714	LC327843	LC328101	LC327972	LC514780	LC514865
	JA3	Japan: Komatu-shi, Pref. Ishikawa, <i>S.Tsugaru et.al 34947</i> (KYO)	LC327718	LC327847	LC328105	LC327976	LC514785	LC514869
	JA4	Japan: Kurashiki-shi, Pref. Okayama, <i>J.Yamashita 6141</i> (Okayama University)	LC327725	LC327854	LC328112	LC327983	LC514781	LC514876
	JA5	Japan: Takahashi-shi, Pref. Okayama, <i>J.Yamashita 7657</i> (Okayama University)	LC327710	LC327839	LC328097	LC327968	LC514794	LC514861
	JA6	Japan: Ohchi-gun, Pref. Shimane, <i>N.Kuroshaki 22341</i> (KYO)	LC327715	LC327844	LC328102	LC327973	LC514782	LC514866
	JA7	Japan: Izu Peninsula, Pref. Shizuoka, <i>M.N.Tamura & H.Noda 19470</i> (KYO)	LC327709	LC327838	LC327967	LC328096	LC514793	LC514860
	JA8	Japan: Shimoda-shi, Pref. Shizuoka, <i>S.Fuse & H.Noda 4649</i> (HYO)	LC327712	LC327841	LC328099	LC327970	LC514778	LC514863
	JA9	Japan: Nagaoka-gun, Pref. Kochi, <i>N.Inagaki, M.Matsumoto et.al. FOK-059185</i> (KYO)	LC327717	LC327846	LC328104	LC327975	LC514784	LC514868
	JA10	Japan: Mise-village, Pref. Saga, <i>M.N.Tamura 20292</i> (KYO)	LC327721	LC327850	LC328108	LC327979	LC514788	LC514872
	JA11	Japan: Amakusa, Pref. Kumamoto, <i>M.N.Tamura 20286</i> (KYO)	LC327719	LC327848	LC328106	LC327977	LC514786	LC514870
	JA12	Japan: Amakusa, Pref. Kumamoto, <i>M.N.Tamura 20287</i> (KYO)	LC327720	LC327849	LC328107	LC327978	LC514787	LC514871
	JA13	Japan: Aso, Pref. Kumamoto, <i>M.N.Tamura 20296</i> (KYO)	LC327726	LC327855	LC328113	LC327984	LC514789	LC514877
	JA14	Japan: Kimotuki-gun, Pref. Kagoshima, <i>N.Kurosaki & H.Nagamasu 2222</i> (KYO)	LC327716	LC327845	LC328103	LC327974	LC514783	LC514867
	JA15	Japan: Nakano-sima Isl., Pref. Kagoshima, <i>G.Ikeda 6624</i> (KAG)	LC327708	LC327837	LC328095	LC327966	-	-
	JA16	Japan: Kuchino-shima Isl., Pref. Kagoshima, <i>H.Noda 1001</i> (KYO)	LC327722	LC327851	LC328109	LC327980	LC514790	LC514873
	JA17	Japan: Kuchino-shima Isl., Pref. Kagoshima, <i>H.Noda 1002</i> (KYO)	LC327723	LC327852	LC328110	LC327981	LC514791	LC514874
	JA18	Japan: Kuchino-shima Isl., Pref. Kagoshima, <i>H.Noda 1003</i> (KYO)	LC327724	LC327853	LC328111	LC327982	LC514792	LC514875
	JA19	Japan: Amami-Ohshima Isl., Pref. Kagoshima, <i>M.N.Tamura & H.Noda 20111</i> (KYO)	LC327711	LC327840	LC328098	LC327969	LC514777	LC514862
<i>D. luzonensis</i> Schauer	LU1	Japan: Kitadaito-zima Isl., Pref. Okinawa, <i>T.Yamazaki 630</i> (TI)	LC327728	LC327857	LC328115	LC327986	-	-
	LU2	The Philippines: Babuyan Isl., <i>S.Hatusima & M.Sato 29467</i> (TI)	LC327729	LC327858	LC327987	LC328116	-	-
<i>D. matsudae</i> Hayata	MA1	Taiwan: Taipei city, <i>J.L.Panero 6396</i> (KYO)	LC327730	LC327859	LC327988	LC328117	LC514795	LC514878
	MA2	Taiwan: Taipei city, <i>C.-C.Liao 740</i> (KYO)	LC327731	LC327860	LC328118	LC327989	LC514796	LC514879
<i>D. nipponica</i> Makino	NI1	Japan: Ohmati-shi, Pref. Nagano, <i>M.N.Tamura & H.Noda 18</i> (KYO)	LC327735	LC327864	LC327993	LC328122	LC514799	LC514880
	NI2	Japan: Maniwa-gun, Pref. Okayama, <i>J.Yamashita 7583</i> (Okayama University)	LC327733	LC327862	LC328120	LC327991	LC514797	LC514881
	NI3	Japan: Maniwa-gun, Pref. Okayama, <i>J.Yamashita 7582</i> (Okayama University)	LC327734	LC327863	LC328121	LC327992	LC514798	LC514882
<i>D. pentaphylla</i> L.	-	Kingdom of Tonga, <i>M.Suzuki & F.Toicelau T00423</i> (KYO)	LC327736	LC327865	LC327994	LC328123	LC514800	LC514883
<i>D. petelotii</i> Prain & Burkill	-	Thailand: Doi Phu Kha, Prov. Nan, <i>M.N.Tamura et al. 20155</i> (KYO)	LC327737	LC327866	LC327995	LC328124	-	-

Table 1. (continued)

Taxon	Code	Locality and Voucher	Acc. no. (<i>trnK</i>)	Acc. no. (<i>rbcl</i>)	Acc. no. (<i>atpB</i>)	Acc. no. (<i>trnL-F</i>)	Acc. No. (<i>PHYC</i>)	Acc. No. (18s rDNA)
<i>D. polystachya</i> Turcz.	PO1	Japan: Kurashiki-shi, Pref. Okayama, <i>J. Yamashita 6140</i> (Okayama University)	LC327741	LC327870	LC328128	LC327999	LC514802	LC514887
	PO2	Japan: Osaka-shi, Pref. Osaka, <i>M.N. Tamura et al. 5</i> (KYO)	LC327738	LC327867	LC327996	LC328125	LC514805	LC514884
	PO3	Japan: Osaka-shi, Pref. Osaka, <i>M.N. Tamura et al. 1</i> (KYO)	LC327739	LC327868	LC328126	LC327997	LC514806	LC514885
	PO4	Japan: Katano-shi, Pref. Osaka, <i>J. Yamashita 7450</i> (Okayama University)	LC327740	LC327869	LC328127	LC327998	LC514801	LC514886
	PO5	Japan: Aso-shi, Pref. Kumamoto, <i>M.N. Tamura 20304</i> (KYO)	LC327742	LC327871	LC328129	LC328000	LC514803	LC514888
	PO6	Japan: Aso-shi, Pref. Kumamoto, <i>M.N. Tamura 20308</i> (KYO)	LC327743	LC327872	LC328130	LC328001	LC514804	LC514889
	PO7	Taiwan: Taipei city. <i>Namba et al 3553</i> (TI)	LC327744	LC327873	LC328131	LC328002	-	-
<i>D. pseudojaponica</i> Hayata	PJ1	Japan: Nakagusuku, Pref. Okinawa, <i>M.N. Tamura 20196</i> (KYO)	LC327745	LC327874	LC328003	LC328132	LC514807	LC514890
	PJ2	Japan: Ishigaki Isl., Pref. Okinawa, <i>T. Yamazaki s.n. 20 June 1971</i> (TI)	LC327746	LC327875	LC328133	LC328004	-	-
<i>D. pseudonitens</i> Prain & Burkill	PN1	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura No. 9614</i> (KYO)	LC327747	LC327876	LC328005	LC328134	-	-
	PN2	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30072</i> (KYO)	LC327748	LC327877	LC328006	LC328135	-	-
	PN3	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30075</i> (KYO)	LC327749	LC327878	LC328136	LC328007	-	-
	PN4	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30365</i> (KYO)	LC327750	LC327879	LC328137	LC328008	-	-
	PN5	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30914</i> (KYO)	LC327751	LC327880	LC328138	LC328009	-	-
	PN6	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30915</i> (KYO)	LC327752	LC327881	LC328139	LC328010	-	-
<i>D. quinquelobata</i> Thunb.	QU1	Japan: Kawabe-gun, Pref. Hyogo, <i>S. Fuse 4743</i> (HYO)	LC327755	LC327884	LC328142	LC328013	LC514809	LC514893
	QU2	Japan: Takahashi-shi, Pref. Okayama, <i>J. Yamashita 7478</i> (Okayama University)	LC327753	LC327882	LC328140	LC328011	LC514812	LC514891
	QU3	Japan: Katano-shi, Pref. Osaka, <i>M.N. Tamura & H. Noda 16</i> (KYO)	LC327757	LC327886	LC328015	LC328144	LC514811	LC514895
	QU4	Japan: Goshiki-shi, Pref. Nara, <i>M.N. Tamura 20147</i> (KYO)	LC327754	LC327883	LC328141	LC328012	LC514808	LC514892
	QU5	Japan: Amakusa, Pref. Kumamoto, <i>M.N. Tamura 20285</i> (KYO)	LC327756	LC327885	LC328143	LC328014	LC514810	LC514894
<i>D. septemloba</i> Thunb. var. <i>septemloba</i>	SE1	Japan: Mt. Tanzawa, Pref. Kanagawa, <i>M.N. Tamura et al. 20126</i> (KYO)	LC327761	LC327890	LC328019	LC328148	LC514815	LC514899
	SE2	Japan: Ashigarashimo-gun, Pref. Kanagawa, <i>M.N. Tamura et al. 20131</i> (KYO)	LC327762	LC327891	LC328149	LC328020	LC514816	LC514900
	SE3	Japan: Mt. Myoken, Pref. Gifu, <i>M.N. Tamura & S. Fuse 20192</i> (KYO)	LC327763	LC327892	LC328150	LC328021	LC514817	LC514901
	SE4	Japan: Mt. Rokkoku, Pref. Hyogo, <i>T. Fukuda & H. Noda 24</i> (KYO)	LC327758	LC327887	LC328145	LC328016	LC514819	LC514897
	SE5	Japan: Maniwa-gun, Pref. Okayama, <i>J. Yamashita 7520</i> (Okayama University)	LC327759	LC327888	LC328146	LC328017	LC514818	LC514896
	SE6	Japan: Maniwa-gun, Pref. Okayama, <i>J. Yamashita 7519</i> (Okayama University)	LC327760	LC327889	LC328147	LC328018	LC514820	LC514898
<i>D. septemloba</i> Thunb. var. <i>sititana</i> Ohwi	SI1	Japan: Izu-ohshima Isl., Pref. Shizuoka, <i>M.N. Tamura & H. Noda 20004</i> (KYO)	LC327764	LC327893	LC328022	LC328151	LC514813	LC514902
	SI2	Japan: Izu-ohshima Isl., Pref. Shizuoka, <i>M.N. Tamura & H. Noda 20005</i> (KYO)	LC327765	LC327894	LC328152	LC328023	LC514814	LC514903
<i>D. tabatae</i> Hatus. ex Yamashita & M.N. Tamura	TA1	Japan: Amami-ohshima Isl., Pref. Kagoshima, <i>M.N. Tamura & H. Noda 20110</i> (KYO)	LC327767	LC327896	LC328025	LC328154	LC514821	LC514904
	TA2	Japan: Amami-ohshima Isl., Pref. Kagoshima, <i>M.N. Tamura & H. Noda 20108</i> (KYO)	LC327768	LC327897	LC328155	LC328026	LC514822	LC514905
	TA3	Japan: Amami-ohshima Isl., Pref. Kagoshima, <i>T. Yamazaki 29 Tune 1999</i> (TI)	LC327769	LC327898	LC328156	LC328027	-	-
<i>D. tentaculigera</i> Prain & Burkill	TL1	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30088</i> (KYO)	LC327770	LC327899	LC328028	LC328157	-	-
	TL2	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30109</i> (KYO)	LC327771	LC327900	LC328029	LC328158	-	-
	TL3	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30111</i> (KYO)	LC327772	LC327901	LC328159	LC328030	-	-
<i>D. tenuipes</i> Franch. et Sav.	TP1	Japan: Mt. Tanzawa, Pref. Kanagawa, <i>M.N. Tamura et al. 20124</i> (KYO)	LC327775	LC327904	LC328162	LC328033	LC514824	LC514908
	TP2	Japan: Katano-shi, Pref. Osaka, <i>J. Yamashita 7448</i> (Okayama University)	LC327773	LC327902	LC328031	LC328160	LC514823	LC514906

Table 1. (continued)

Taxon	Code	Locality and Voucher	Acc. no. (<i>trnK</i>)	Acc. no. (<i>rbcL</i>)	Acc. no. (<i>atpB</i>)	Acc. no. (<i>trnL-F</i>)	Acc. No. (<i>PHYC</i>)	Acc. No. (18S rDNA)
<i>D. tokoro</i> Makino	TP3	Japan: Akaiwa-shi, Pref. Okayama, <i>J. Yamashita 7508</i> (Okayama University)	LC327774	LC327903	LC328161	LC328032	LC514825	LC514909
	TP4	Japan: Akaiwa-shi, Pref. Okayama, <i>J. Yamashita 7509</i> (Okayama University)	LC327776	LC327905	LC328163	LC328034	LC514826	LC514907
	TO1	Japan: Atami-shi, Pref. Shizuoka, <i>S. Fuse & H. Noda 13</i> (KYO)	LC327778	LC327907	LC328036	LC328165	LC514829	LC514911
	TO2	Japan: Izu Peninsula, Pref. Shizuoka, <i>M.N. Tamura & H. Noda 19471</i> (KYO)	LC327779	LC327908	LC328166	LC328037	LC514830	LC514912
	TO3	Japan: Shimoda-shi, Pref. Shizuoka, <i>S. Fuse & H. Noda 4648</i> (KYO)	LC327782	LC327911	LC328169	LC328040	LC514827	LC514915
	TO4	Japan: Takahashi-shi, Pref. Okayama, <i>J. Yamashita 6777</i> (Okayama University)	LC327777	LC327906	LC328164	LC328035	LC514828	LC514910
	TO5	Japan: Shiso-shi, Pref. Hyogo, <i>H. Noda et al. 8</i> (KYO)	LC327780	LC327909	LC328167	LC328038	LC514831	LC514913
	TO6	Japan: Yazu-gun, Pref. Tottori, <i>H. Noda et al. 9</i> (KYO)	LC327781	LC327910	LC328168	LC328039	LC514832	LC514914
<i>D. velutipes</i> Prain & Burkill	VE1	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. No. 9606</i> (KYO)	LC327783	LC327912	LC328041	LC328170	-	-
	VE2	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. No. 9607</i> (KYO)	LC327784	LC327913	LC328171	LC328042	LC514833	LC514916
	VE3	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30920</i> (KYO)	LC327785	LC327914	LC328172	LC328043	-	-
	VE4	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30944</i> (KYO)	LC327786	LC327915	LC328173	LC328044	-	-
	VE5	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30947</i> (KYO)	LC327787	LC327916	LC328174	LC328045	-	-
	VE6	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30948</i> (KYO)	LC327788	LC327917	LC328175	LC328046	-	-
	VE7	Thailand: Doi Chiang Dao, Prov. Chiang Mai, <i>M.N. Tamura et al. T-30949</i> (KYO)	LC327789	LC327918	LC328176	LC328047	-	-
<i>D. zentaroana</i> Koidz.	ZE1	Japan: Amami-ohshima Isl., Pref. Kagoshima, <i>M.N. Tamura 20352</i> (KYO)	LC327790	LC327919	LC328048	LC328177	LC514834	LC514917
	ZE2	Japan: Amami-ohshima Isl., Pref. Kagoshima, <i>M.N. Tamura 20353</i> (KYO)	LC327791	LC327920	LC328178	LC328049	LC514835	LC514918
<i>Tacca</i> sp.	TC1	Cultivated, Kisaichi Bot. Gard., Osaka City Univ., <i>M.N. Tamura & H. Noda 23</i> (KYO)	LC327793	LC327922	LC328051	LC328180	-	-
<i>Tacca</i> sp.	TC2	Cultivated, Kisaichi Bot. Gard., Osaka City Univ., <i>M.N. Tamura & H. Noda 20180525</i> (KYO)	LC327794	LC327923	LC328052	LC328181	-	-
<i>Nartheicum asiaticum</i> Maxim.	-	Japan: Mt. Sanpoiwadake, Pref. Gifu, <i>M.N. Tamura & S. Fuse 15335</i> (KYO)	LC327792	LC327921	LC328050	LC328179	-	-

Table 2. Phylogenetic information of the parsimony and likelihood analyses.

	Aligned length (bp)	Variable characters (bp)	Parsimony informative characters (bp)	Number of most parsimonious (MP) trees	Steps of MP trees	CI / RI / RC of MP trees	Model used for likelihood analysis
genus <i>Dioscorea</i>							
chloroplast	6288	1242	735	5607	1640	0.844 / 0.950 / 0.802	GTR+I+G
<i>D. sect. Dioscorea</i>							
chloroplast	6013	357	271	86	271	0.902 / 0.934 / 0.842	IM1+I+G
<i>PHYC</i>	998	148	111	65	179	0.911 / 0.951 / 0.866	TrN+G
18S rDNA	1636	17	11	4	20	0.950 / 0.950 / 0.902	TrN+I
combined	8647	522	411	6	614	0.896 / 0.947 / 0.848	TIM1+I+G
<i>D. sect. Enantiophyllum</i>							
chloroplast	5993	212	69	4	234	0.923 / 0.908 / 0.838	TIM1+I
<i>PHYC</i>	995	71	27	1000000	80	0.938 / 0.934 / 0.876	TrN+I
18S rDNA	1636	7	1	1	8	1.000 / 1.000 / 1.000	TrN+I+G
combined	8624	290	102	216	323	0.926 / 0.928 / 0.859	TIM1+I+G



Fig. 1. A habitat of *Dioscorea japonica* Thunb. (a, b) and *D. tokoro* Makino (c, d, e), which grow sympatrically in Izu Peninsula, Pref. Shizuoka, Japan; a, c and d, leaves; b and e, male inflorescences.

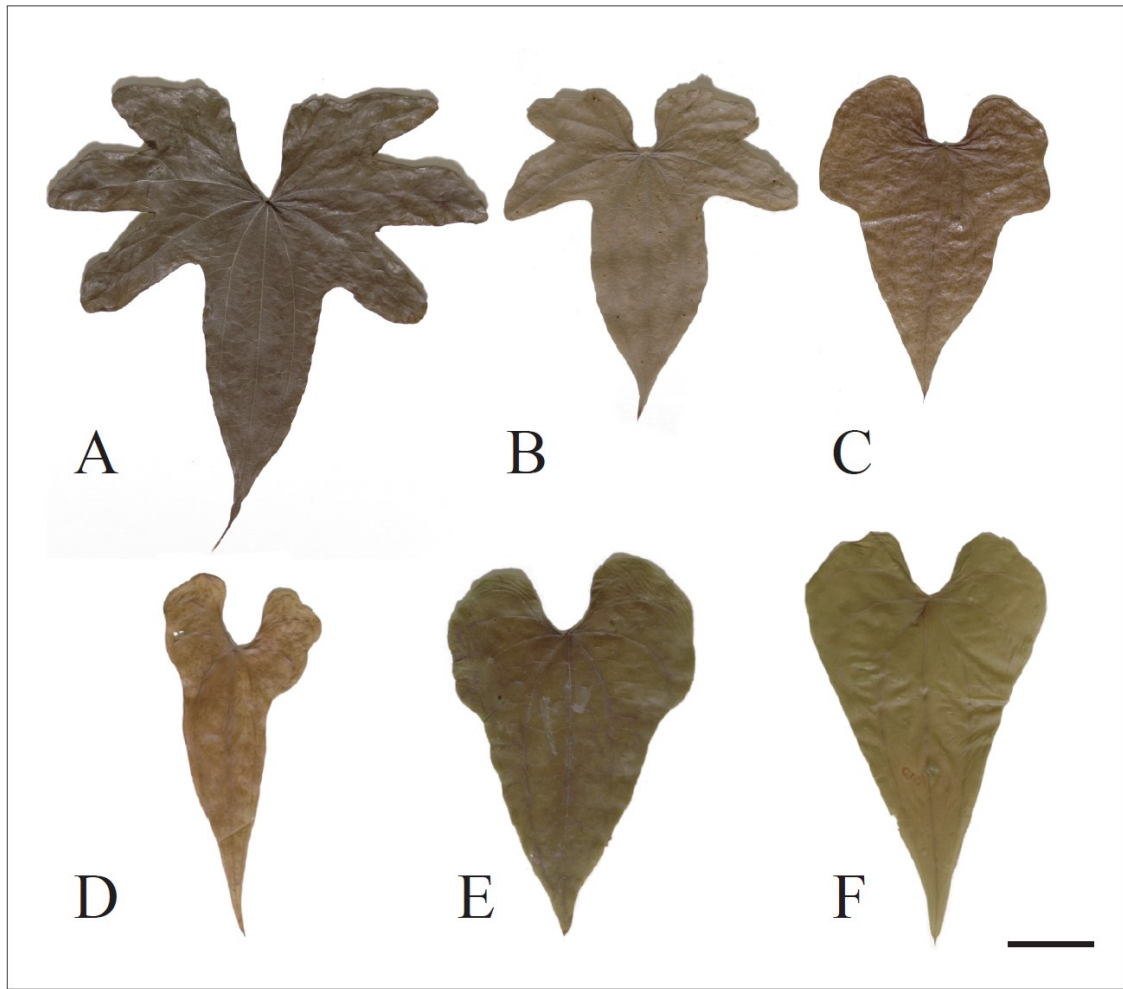


Fig. 2. Variation in leaf shape of *Dioscorea quinquelobata* Thunb. (A-C) and *D. tenuipes* Franch. et Sav. (D-F). C and E are very similar, although they are of different species. Scale bar = 2cm.

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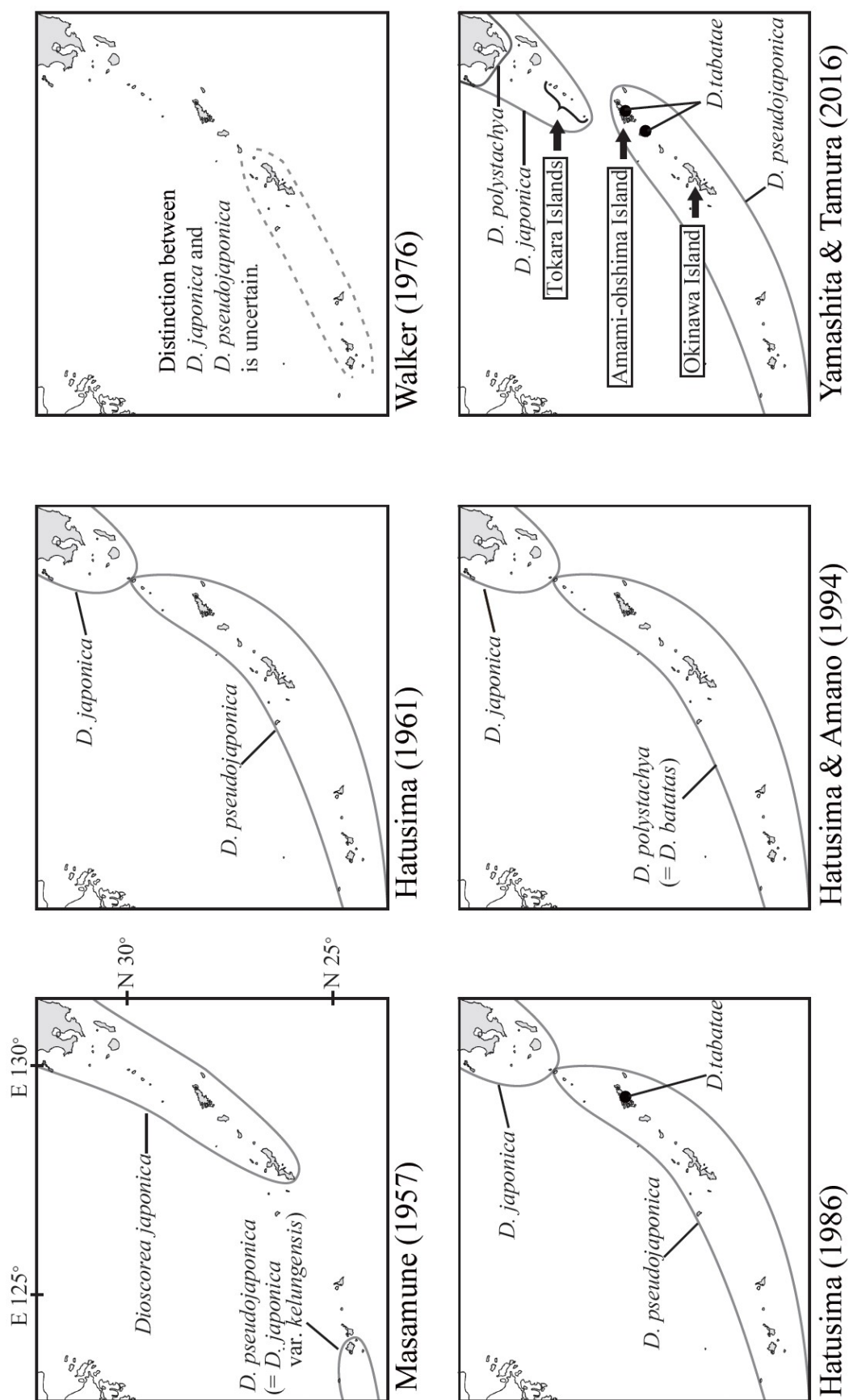


Fig. 3. Difference in identification of *Dioscorea* of Ryukyu (southwestern Japan) between six floras.

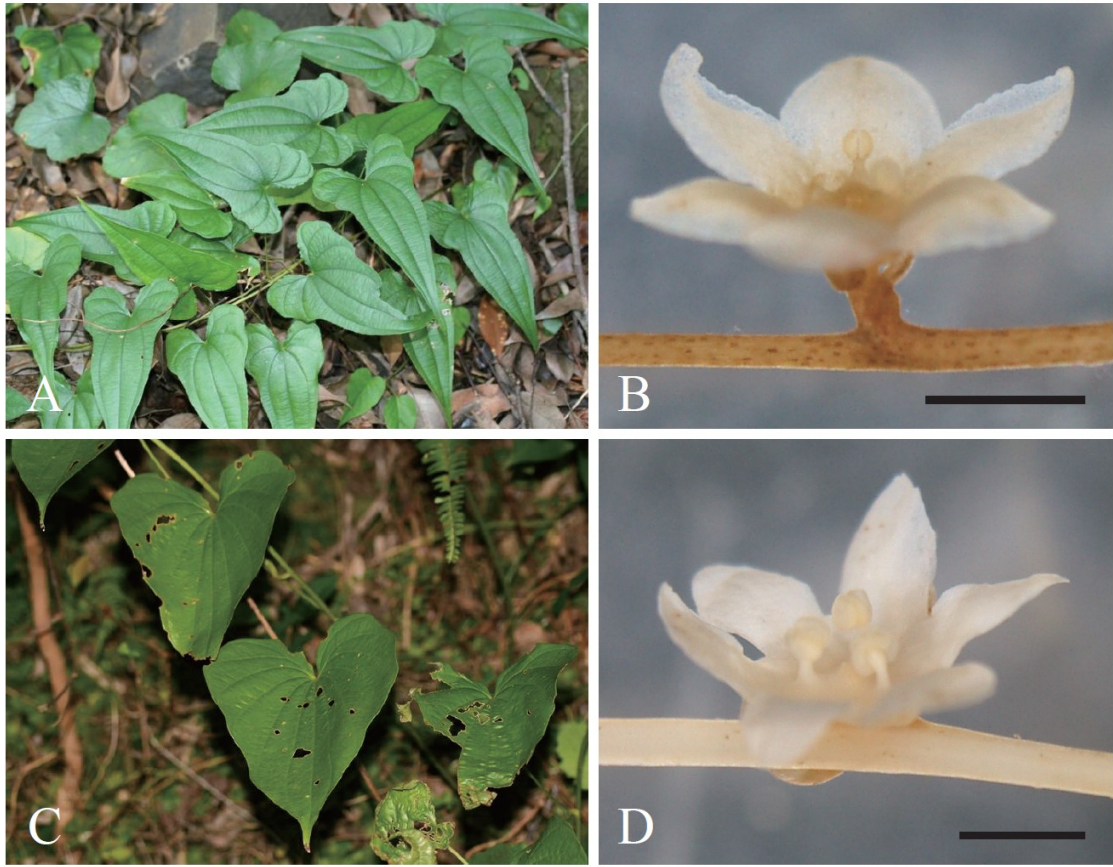


Fig. 4. *Dioscorea asclepiadea* (A, B) and *D. zentaroana* (C, D). A and C, leaves; B and D, male flower. Scale bars = 1mm.

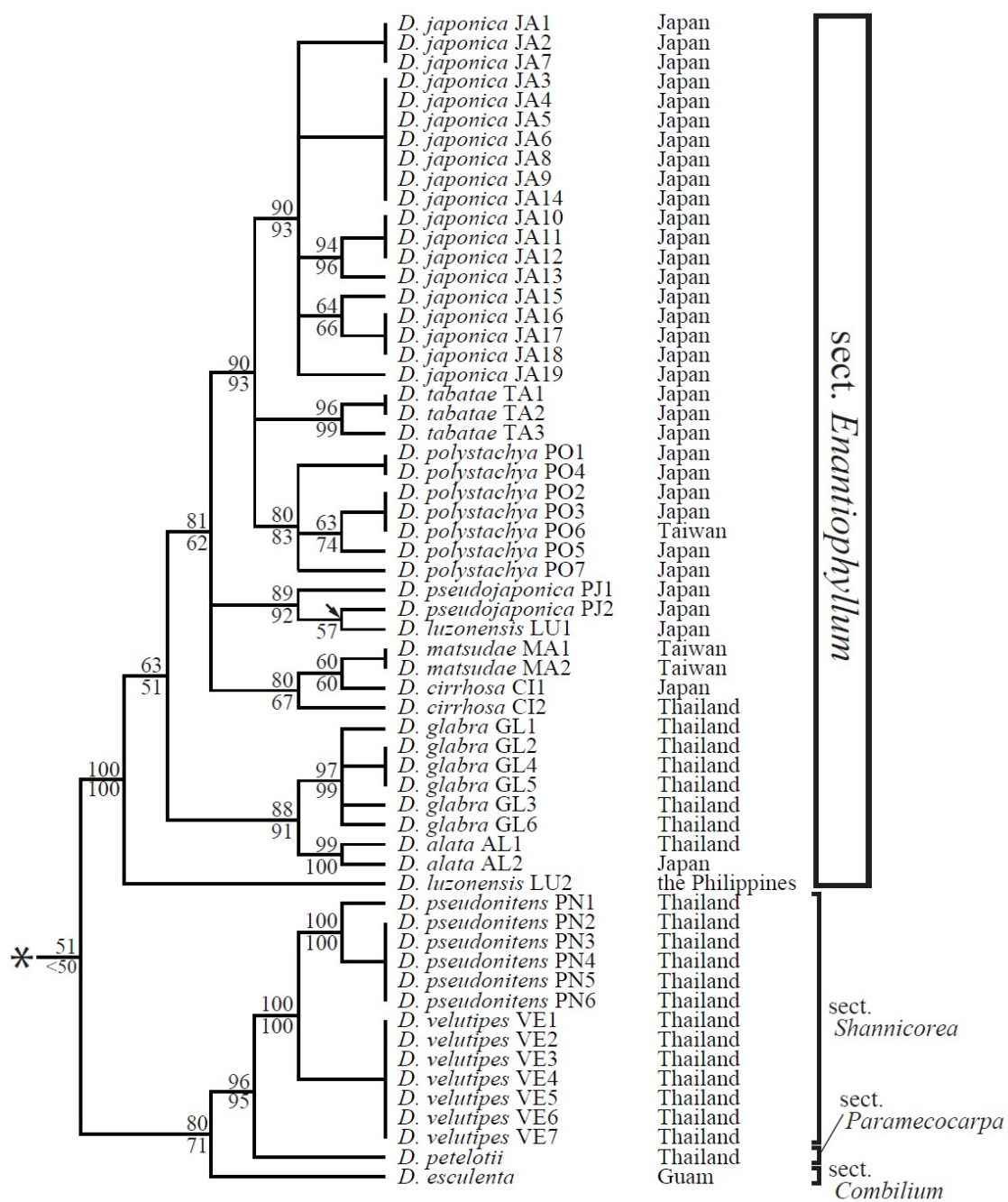


Fig. 5b. (continued)

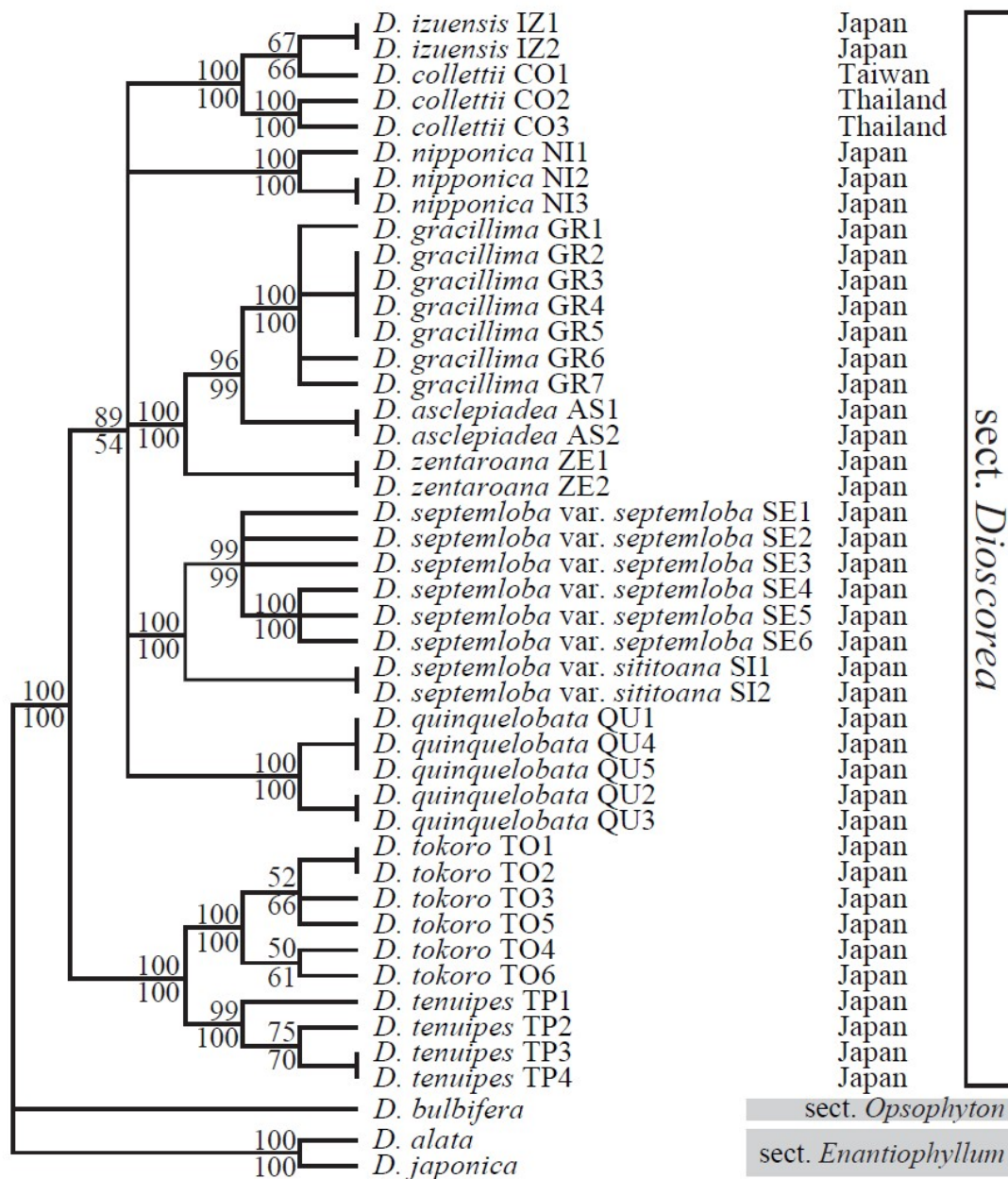


Fig. 7. Maximum likelihood (ML) tree of *Dioscorea* sect. *Dioscorea* and its outgroup in Japan and other Asiatic regions, using TIM1+I+G model selected by Akaike's Information Criterion (AIC), based on the combined data set of chloroplast regions of *trnK*, *rbcL*, *atpB* and *trnL-F*, nuclear *PHYC* region, and nuclear 18S rDNA region ($p = 0.11$ in ILD test). Topology of strict consensus of six equally most parsimonious (MP) trees is identical to topology of this figure. Numbers above and below branches indicate MP and ML bootstrap values, respectively. Samples under the same vertical line segment at the tree end share the same DNA sequence.

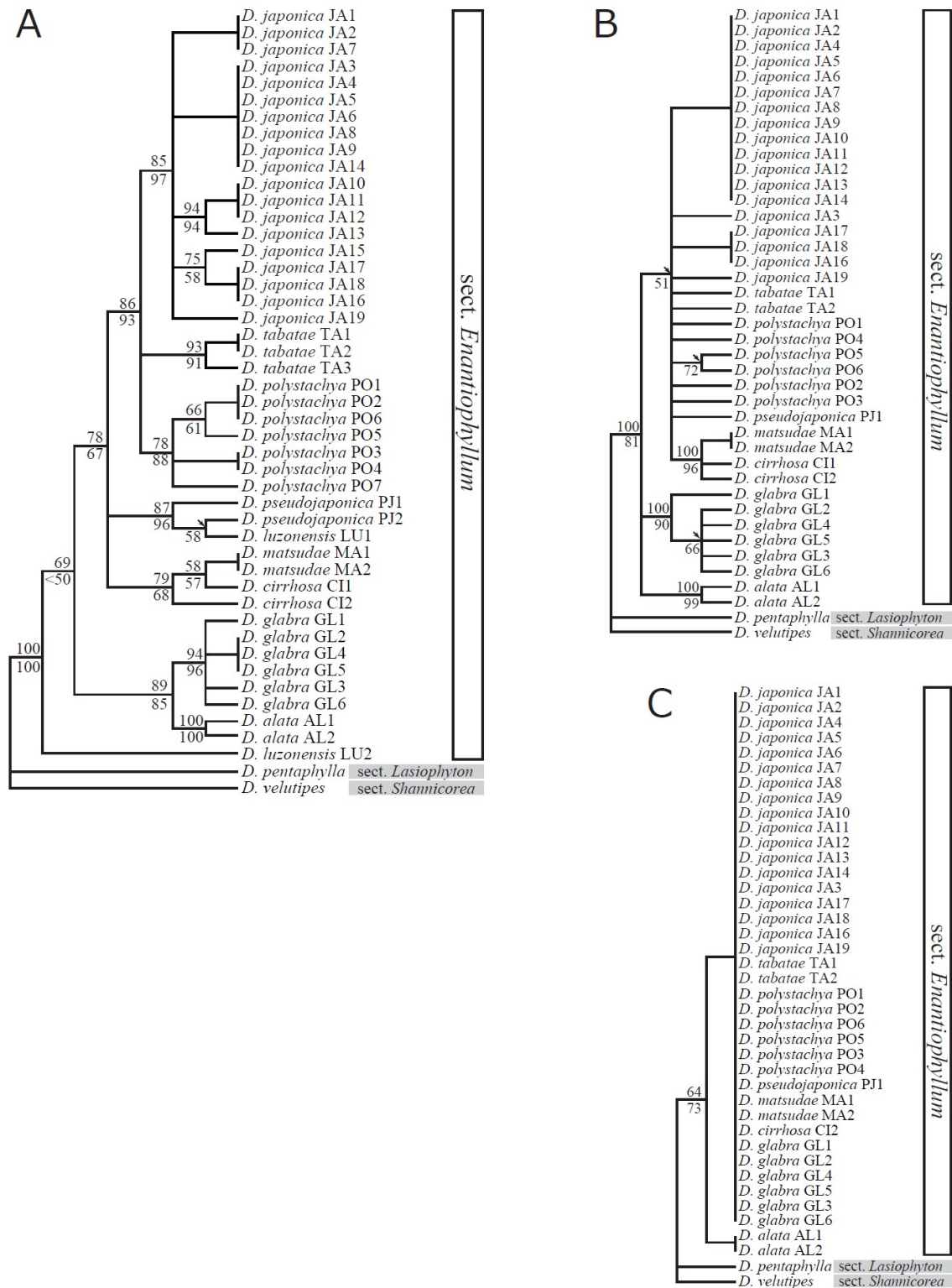


Fig. 8. Maximum likelihood (ML) trees of *Dioscorea* sect. *Enantiophyllum* and its outgroup in Japan and other Asiatic regions, based on chloroplast DNA regions (*trnK*, *rbcL*, *atpB* and *trnL-F*) (A), nuclear *PHYC* region (B) and nuclear 18S rDNA region (C). They are identical to the MP strict consensus trees in terms of topology, respectively, except four arrowed branches that are not present in the MP strict consensus trees. Numbers above and below branches indicate MP and ML bootstrap values, respectively. Samples under the same vertical line segment at the tree end share the same DNA sequence.

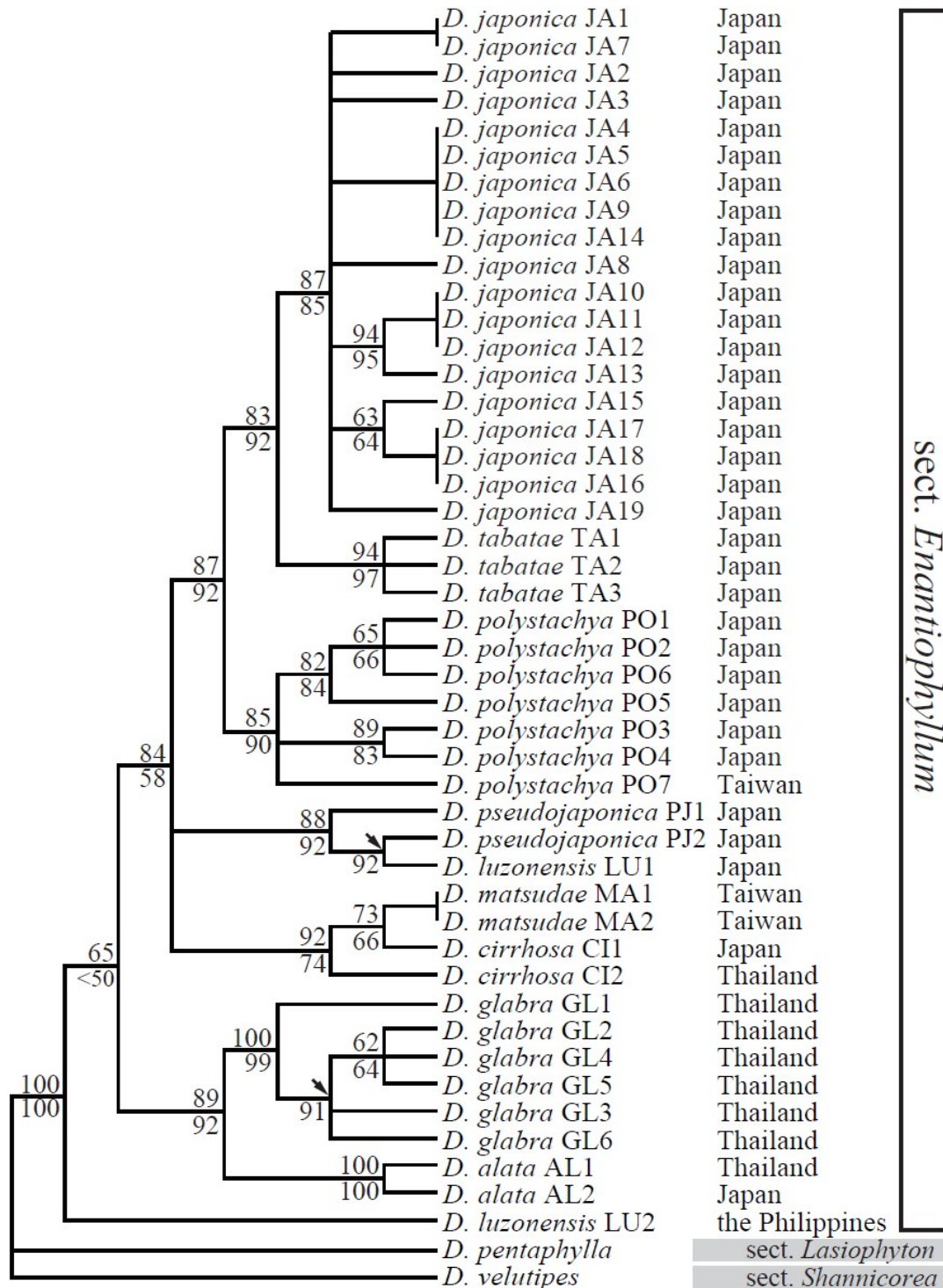


Fig. 9. Maximum likelihood (ML) tree of *Dioscorea* sect. *Enantiophyllum* and its outgroup in Japan and other Asiatic regions, using TIM1+I+G model selected by Akaike's Information Criterion (AIC), based on the combined data set of chloroplast regions of *trnK*, *rbcL*, *atpB* and *trnL-F*, nuclear *PHYC* region, and nuclear 18S rDNA region ($p = 0.72$ in ILD test). Topology of strict consensus of 216 equally most parsimonious (MP) trees is identical to topology of this figure, except two arrowed branches that are not present in the MP strict consensus tree. Numbers above and below branches indicate MP and ML bootstrap values, respectively. Samples under the same vertical line segment at the tree end share the same DNA sequence.

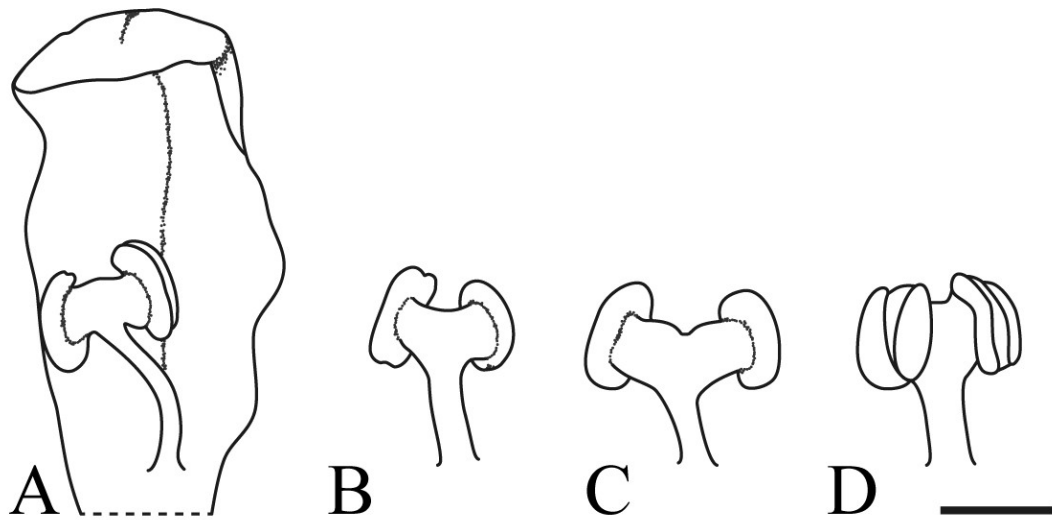


Fig. 10. Comparison of the outer stamens. A-C adaxial, D abaxial. A. *D. izuensis* (Japan: *Akahori 2404*, KYO); B. *D. collettii* var. *collettii* (Taiwan: *Panero 6402*, KYO); C. *D. collettii* var. *collettii* (Thailand: *Tamura et al. T-30029*, KYO); D. *D. collettii* var. *hypoglauc* (China: *Nie 92183*, TI). Scale bar = 0.3 mm.

Appendix 1. Name, direction, DNA sequence and reference for PCR and sequence primers used in this study.

[trnK (including matK) region] trnK-11 (sense) (Liston and Kadereit 1995), trnK-150F (sense) 5'-TATCCTTACCGGATCGTTAC-3', trnK-300F (sense) 5'-GCCCAATTCGGGTAAAATAA-3', trnK-670F (sense) (Noda et al. 2020b), matK-AF (sense) (Ooi et al. 1995), matK-1170F (sense) (Noda et al. 2020b), matK-1300F (sense) (Fuse and Tamura 2000), matK-1650F(mod-N1) (sense) (Noda et al. 2020b), matK-1923F (sense) (Noda et al. 2020b), matK-1940F (sense) (Yamashita and Tamura 2000), matK-8F(mod-N1) (sense) (Noda et al. 2020b), trnK-2250F (sense) 5'-CTGCGGAATCTCATGATTGA-3', trnK-350R (antisense) 5'-AACATCTAATTAGATCGGGTAATTG-3', trnK-700R (antisense) 5'-GTGCAATATGGTCAGAACAGG-3', trnK-706R (antisense) (Noda et al. 2020b), matK-1000R(mod-N1) (antisense) (Noda et al. 2020b), matK-1235R (antisense) (Johnson & Soltis 1995), matK-1470R(mod-N1) (antisense) (Noda et al. 2020b), matK-1700R (antisense) (Tamura et al. 2004), matK-8R (antisense) (Ooi et al. 1995), matK-2100R(mod-N1) (antisense) (Noda et al. 2020b), trnK-2300R(mod-N1) (antisense) (Noda et al. 2020b), trnK-2621 (antisense) (Liston and Kadereit 1995), **[rbcL region]** rbcL-N' (sense) (Teruchi et al. 1987), rbcL-200F(mod-N1) (sense) (Noda et al. 2020b), rbcL-360F (sense) (Noda et al. 2020b), rbcL-630F (sense) (Teruchi et al. 1987), rbcL-840F (sense) (Yamashita and Tamura 2000), rbcL-1100F(mod-N1) (sense) (Noda et al. 2020b), rbcL-250R(mod-N1) (antisense) (Noda et al. 2020b), rbcL-400R(mod-N1) (antisense) (Noda et al. 2020b), rbcL-590R (antisense) (Yamashita and Tamura 2000), rbcL-700R(mod-N1) (antisense) (Noda et al. 2020b), rbcL-840R (antisense) (Yamashita and Tamura 2000), rbcL-1150R(mod-N1) (antisense) (Noda et al. 2020b), DBRBAS2 (antisense) (Teruchi et al. 1987), **[trnL-trnL-trnF region]** trnL-c (sense) (Taberlet et al. 1991), trnL100F-modN4 (sense) (Noda et al. 2020b), trnL250F-modN4 (sense) (Noda et al. 2020b), trnL450F-modN3 (sense) (Noda et al. 2020b), trnL610F-modN4 (sense) (Noda et al. 2020b), trnL200R-modN4 (antisense) (Noda et al. 2020b), trnL300R-modN4 (antisense) (Noda et al. 2020b), trnL530R-modN3 (antisense) (Noda et al. 2020b), trnL-d (antisense) (Taberlet et al. 1991), trnF-f (antisense) (Taberlet et al. 1991), **[atpB region]** atpB-S2F (sense) (Hoot et al. 1995), atpB-135F (sense) (Noda et al. 2020b), atpB-240F (sense) (Noda et al. 2020b), atpB-345F (sense) (Noda et al. 2020b), atpB-400F (sense) (Noda et al. 2020b), atpB-450F (sense) (Noda et al. 2020b), atpB-505F (sense) (Noda et al. 2020b), atpB-620F (sense) (Noda et al. 2020b), atpB-780F (sense) (Noda et al. 2020b), atpB-900F (sense) (Noda et al. 2020b), atpB-1000F (sense) (Noda et al. 2020b), atpB-1050F (sense) (Noda et al. 2020b), atpB-1100F (sense) (Noda et al. 2020b), atpB-1160F (sense) (Noda et al. 2020b), atpB-1215F (sense) (Noda et al. 2020b), atpB-1270F (sense) (Noda et al. 2020b), atpB-215R (antisense) (Noda et al. 2020b), atpB-325R (antisense) (Noda et al. 2020b), atpB-440R (antisense) (Noda et al. 2020b), atpB-540R (antisense) (Noda et al. 2020b), atpB-650R (antisense) (Noda et al. 2020b), atpB-710R (antisense) (Noda et al. 2020b), atpB-825R (antisense) (Noda et al. 2020b), atpB-990R (antisense) (Noda et al. 2020b), atpB-1090R (antisense) (Noda et al. 2020b), atpB-1195R (antisense) (Noda et al. 2020b), atpB-1255R (antisense) (Noda et al. 2020b), atpB-1300R (antisense) (Noda et al. 2020b), atpB-1370R (antisense) (Noda et al. 2020b), atpB-1425R (antisense) (Noda et al. 2020b), atpB-S1494R (antisense) (Hoot et al. 1995), **[PHYC region]** PHYC70F (sense) 5'-GCTGATGTGCCAGTGAAGTGC-3', PHYC240F (sense) 5'-ATGGTGAAGTTATAGCTGAATGC-3', PHYC320F (sense) 5'-CAGGATAAGAGATTGCCACAGCC-3',

Appendix 1. (continued)

PHYC400F (sense) 5'-CAGGATAAGAGATTGCCACAGCC-3', PHYC550F (sense) 5'-GATATGCTTGTGAATTTTGTCTAC-3', PHYC560F (sense) 5'-GATGAAACTTTGGGGACTTG-3', PHYC730F (sense) 5'-GACTGTTCTTTGTGACATGCTTC-3', PHYC850F (sense) 5'-CACTGACAGCCTAACAGAAGCAG-3', PHYC940F (sense) 5'-CAGCCTAACAGAAGCAGGATATC-3', PHYC320R (antisense) 5'-CATAAACAGAAACCTTGAAGCC-3', PHYC480R (antisense) 5'-CCATGTTAGCCATGTACTGAGC-3', PHYC490R (antisense) 5'-CATCACAAGGGAAGCAATTGAAC-3', PHYC640R (antisense) 5'-CAAAGAACAGTCTGTGTTTCAAG-3', PHYC650R (antisense) 5'-CAATTGTATACCAAACACTTGTAGC-3', PHYC780R (antisense) 5'-CGTATCTGTGCCTCTGTTGGTG-3', PHYC820R (antisense) 5'-GGTTCTTATAATAAAGAGCAGCACC-3', PHYC980R (antisense) 5'-CATACCGCAAACGGCATCTCC-3', PHYC1200R (antisense) 5'-CCACCTCCAAGAAGGCCTTG-3', **[18S rDNA region]** NR18S-12F (sense) 5'-CATATGCTTGTCTCAAAGATTAAGCC-3', NR18S-500F (sense) 5'-GGGTCTGGTAATTGGAATGAG-3', NR18S-1115F (sense) 5'-GGAGTATGGTCGCAAGGCTG-3', NR18S-600R (antisense) 5'-CTGCAACAACCTTAAATATACGCT-3', NR18S-1200R (antisense) 5'-GCTATGTCTGGACCTGGTAAG-3', NR18S-1760R (antisense) 5'-CTTGTTACGACTTCTCCTTCCTC-3'.

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Appendix 2. Name, DNA sequence and reference for PCR and sequence primers used in this study for *Dioscorea luzonensis*.

[trnK (including matK) region] trnK11F (Liston and Kadereit 1995), trnK40F-modN7 5'-CAACGAATTCGTTTAGACT-3', trnK50F-modN7 5'-AAAACAGCATGTCGTAATA-3', trnK120F-modN7 5'-AGTAGAATTTTTGAATTTATC-3', trnK200F-modN7 5'-GAATTGATTCTCAATTTAGAG-3', trnK290F-modN7 5'-CGAACTTATGTTCTTAAATTTG-3', trnK390F-modN5 5'-GAACCATTGATTCCTTTT-3', trnK470F-modN7 5'-GTTGGAGACAGATGTGTAG-3', trnK570F-modN7 5'-GGATTTTCTACTAACGAA-3', trnK680F-modN7 5'-CGGGGTATATTTCTTACTG-3', trnK760F-modN7 5'-ACTATGTATCATTTTATAACCC-3', trnK840F-modN7 (Noda et al. 2020b), matK920F-modN7 (Noda et al. 2020b), matK1020F-modN7 (Noda et al. 2020b), matK1130F-modN7 (Noda et al. 2020b), matK1200F-modN7 (Noda et al. 2020b), matK1290F-modN7 (Noda et al. 2020b), matK1370F-modN7 (Noda et al. 2020b), matK1450F-modN7 (Noda et al. 2020b), matK1540F-modN7 (Noda et al. 2020b), matK1620F-modN7 (Noda et al. 2020b), matK1690F-modN7 (Noda et al. 2020b), matK1770F-modN7 (Noda et al. 2020b), matK1850F-modN7 (Noda et al. 2020b), matK1940F-modN7 (Noda et al. 2020b), matK2000F-modN7 (Noda et al. 2020b), matK2080F-modN7 (Noda et al. 2020b), matK2160F-modN7 (Noda et al. 2020b), matK2240F-modN7 (Noda et al. 2020b), trnK2320F-modN7 (Noda et al. 2020b), trnK2320F-modN7 5'-GGACGCATTTGGTATTTGG-3', trnK2410F-modN7 5'-CCCTATAATGAATGCAAATA-3', trnK2500F-modN7 5'-CATTCTGAAATGCTCATCTA-3', trnK2540F-modN7 5'-GTATAGTGGTTGAATCAACC-3', trnK20R-modN7 5'-CATTACCTTCAGGATCAG-3', trnK90R-modN7 5'-AAATGAAAATCCAAATTT-3', trnK170R-modN7 5'-CAATATATTTGTAACGATCC-3', trnK260R-modN7 5'-ATAAGACTCTATCCATTTAGTC-3', trnK340R-modN7 5'-AACATCTAATTAGATCGGG-3', trnK440R-modN7 5'-AGTTTATTATAGTTAGGATTCATT-3', trnK540R-modN5 5'-TTTGGGAATAAACTTTCTTTATC-3', trnK650R-modN7 5'-CTCTCCTTTCTATCCAAT-3', trnK760R-modN7 5'-ATGGTCAGAACAGGATATG-3', trnK820R-modN7 5'-TCATTTCTACTTAAACAGAAA-3', matK880R-modN7 5'-TGAAAGAGAAGTGGATATAGG-3', matK880R-modN7 (Noda et al. 2020b), matK990R-modN7 (Noda et al. 2020b), matK1080R-modN7 (Noda et al. 2020b), matK1190R-modN7 (Noda et al. 2020b), matK1260R-modN7 (Noda et al. 2020b), matK1340R-modN7 (Noda et al. 2020b), matK1420R-modN7 (Noda et al. 2020b), matK1510R-modN7 (Noda et al. 2020b), matK1590R-modN7 (Noda et al. 2020b), matK1670R-modN7 (Noda et al. 2020b), matK1750R-modN7 (Noda et al. 2020b), matK1830R-modN7 (Noda et al. 2020b), matK1900R-modN7 (Noda et al. 2020b), matK1990R-modN7 (Noda et al. 2020b), matK2060R-modN7 (Noda et al. 2020b), matK2130R-modN7 (Noda et al. 2020b), matK2210R-modN7 (Noda et al. 2020b), trnK2300R-modN7 5'-GCTTGAGGGAAGATCAAAG-3', trnK2380R-modN7 5'-GATTCCGCAGGTCATTGAT-3', trnK2490R-modN7 5'-GAATTTTCTTGATCAGTTAG-3', trnK2570R-modN7 5'-GTTAGTTTAGTTGGTTGATTC-3', trnK2650R-modN7 5'-TTCATTGCACACGACTTTC-3', **[rbcL region]** rbcLN' (Terachi et al.1987), rbcL20F-modN7 (Noda et al. 2020b), rbcL100F-modN7 (Noda et al. 2020b), rbcL180F-modN7 (Noda et al. 2020b), rbcL260F-modN7 (Noda et al. 2020b), rbcL340F-modN7 (Noda et al. 2020b), rbcL410F-modN7 (Noda et al. 2020b), rbcL490F-modN7 (Noda et al. 2020b), rbcL570F-modN7 (Noda et al. 2020b),

Appendix 2. (continued)

rbcL650F-modN7 (Noda et al. 2020b), rbcL730F-modN7 (Noda et al. 2020b), rbcL810F-modN7 (Noda et al. 2020b), rbcL880F-modN7 (Noda et al. 2020b), rbcL960F-modN7 (Noda et al. 2020b), rbcL1040F-modN7 (Noda et al. 2020b), rbcL1130F-modN7 (Noda et al. 2020b), rbcL1210F-modN7 (Noda et al. 2020b), rbcL70R-modN7 (Noda et al. 2020b), rbcL150R-modN7 (Noda et al. 2020b), rbcL230R-modN7 (Noda et al. 2020b), rbcL310R-modN7 (Noda et al. 2020b), rbcL400R-modN7 (Noda et al. 2020b), rbcL470R-modN7 (Noda et al. 2020b), rbcL540R-modN7 (Noda et al. 2020b), rbcL620R-modN7 (Noda et al. 2020b), rbcL700R-modN7 (Noda et al. 2020b), rbcL780R-modN7 (Noda et al. 2020b), rbcL860R-modN7 (Noda et al. 2020b), rbcL930R-modN7 (Noda et al. 2020b), rbcL1010R-modN7 (Noda et al. 2020b), rbcL1100R-modN7 (Noda et al. 2020b), rbcL1180R-modN7 (Noda et al. 2020b), rbcL1260R-modN7 (Noda et al. 2020b), DBRBAS2 (Terachi et al. 1987), [*trnL-trnL-trnF* region] trnL-c (Taberlet et al. 1991), trnL40F-modN7 (Noda et al. 2020b), trnL110F-modN7 (Noda et al. 2020b), trnL190F-modN7 (Noda et al. 2020b), trnL270F-modN7 (Noda et al. 2020b), trnL325F-modN7 (Noda et al. 2020b), trnL395F-modN7 (Noda et al. 2020b), trnL490F-modN7 (Noda et al. 2020b), trnL570F-modN7 (Noda et al. 2020b), trnL630F-modN7 (Noda et al. 2020b), trnF980F-modN7 (Noda et al. 2020b), trnL90R-modN7 (Noda et al. 2020b), trnL160R-modN7 (Noda et al. 2020b), trnL240R-modN7 (Noda et al. 2020b), trnL320R-modN7 (Noda et al. 2020b), trnL390R-modN7 (Noda et al. 2020b), trnL440R-modN7 (Noda et al. 2020b), trnL540R-modN7 (Noda et al. 2020b), trnL620R-modN7 (Noda et al. 2020b), trnF1000R-modN7 (Noda et al. 2020b), trnF1050R-modN7 (Noda et al. 2020b), trnF-f (Taberlet et al. 1991), [*atpB* region] atpB-S2F (Hoot et al. 1995), atpB-40F (Noda et al. 2020b), atpB-90F (Noda et al. 2020b), atpB-135F (Noda et al. 2020b), atpB-185F (Noda et al. 2020b), atpB-240F (Noda et al. 2020b), atpB-295F (Noda et al. 2020b), atpB-345F (Noda et al. 2020b), atpB-400F (Noda et al. 2020b), atpB-450F (Noda et al. 2020b), atpB-505F (Noda et al. 2020b), atpB-560F (Noda et al. 2020b), atpB-620F (Noda et al. 2020b), atpB-680F (Noda et al. 2020b), atpB-730F (Noda et al. 2020b), atpB-780F (Noda et al. 2020b), atpB-850F (Noda et al. 2020b), atpB-900F (Noda et al. 2020b), atpB-950F (Noda et al. 2020b), atpB-1000F (Noda et al. 2020b), atpB-1050F (Noda et al. 2020b), atpB-1100F (Noda et al. 2020b), atpB-1160F (Noda et al. 2020b), atpB-1215F (Noda et al. 2020b), atpB-70R (Noda et al. 2020b), atpB-130R (Noda et al. 2020b), atpB-170R (Noda et al. 2020b), atpB-215R (Noda et al. 2020b), atpB-270R (Noda et al. 2020b), atpB-325R (Noda et al. 2020b), atpB-385R (Noda et al. 2020b), atpB-440R (Noda et al. 2020b), atpB-480R (Noda et al. 2020b), atpB-540R (Noda et al. 2020b), atpB-590R (Noda et al. 2020b), atpB-650R (Noda et al. 2020b), atpB-710R (Noda et al. 2020b), atpB-770R (Noda et al. 2020b), atpB-825R (Noda et al. 2020b), atpB-880R (Noda et al. 2020b), atpB-935R (Noda et al. 2020b), atpB-990R (Noda et al. 2020b), atpB-1040R (Noda et al. 2020b), atpB-1090R (Noda et al. 2020b), atpB-1150R (Noda et al. 2020b), atpB-1195R (Noda et al. 2020b), atpB-1255R (Noda et al. 2020b), atpB-1300R (Noda et al. 2020b), atpB-1270F (Noda et al. 2020b), atpB-1330F (Noda et al. 2020b), atpB-1395F (Noda et al. 2020b), atpB-1370R (Noda et al. 2020b), atpB-1425R (Noda et al. 2020b), atpB-1494R-Hoot et al. 1995).

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Conclusion

本研究では、まず、葉緑体 DNA 塩基配列を用いて、ヤマノイモ属 183 種の大規模分子系統樹の構築に成功した。そして、ヤマノイモ属内で大きな変異幅を示し、しばしばヤマノイモ属の多様性を示す指標に使われてきた地下茎、茎の巻き方、葉序、葉形、茎や葉の毛、花粉の発芽孔、果実、種子、染色体基本数と倍数性の形質進化を、この系統樹に基づいて推定した。その結果、ヤマノイモ属の祖先は、根茎をもち、茎は左巻きで無毛、葉は互生・単葉・無毛、花粉は単溝粒型、縦長のさく果をもち、種子には翼があり、 $x=10$ の 2 倍体という特徴をもつ植物と推定された。

次に、ヤマノイモ属の多様性を詳細に示すために、この大規模分子系統樹に基づいて、旧世界のヤマノイモ属の属内分類群の認識を再検討した。その結果、属内に 2 亜属 20 節を認識するのが妥当であると考えた。それらは、*Dioscorea* subg. *Dioscorea*, *D.* subg. *Helmia*, *D.* sect. *Dioscorea*, *D.* sect. *Tamus*, *D.* sect. *Borderea*, *D.* sect. *Afroborderea*, *D.* sect. *Rhacodophyllum*, *D.* sect. *Testudinaria*, *D.* sect. *Perennia*, *D.* sect. *Pseudoshannicorea*, *D.* sect. *Opsophyton*, *D.* sect. *Lasiophyton*, *D.* sect. *Macrourea*, *D.* sect. *Brachyandra*, *D.* sect. *Cardiocapsa*, *D.* sect. *Combillium*, *D.* sect. *Stenocorea*, *D.* sect. *Macrocarpaea*, *D.* sect. *Paramecocarpa*, *D.* sect. *Shannicorea*, *D.* sect. *Pseudostenophora*, *D.* sect. *Enantiophyllum* で、このうち、*D.* sect. *Afroborderea*, *D.* sect. *Perennia*, *D.* sect. *Pseudoshannicorea*, *D.* sect. *Pseudostenophora* は今回新しく記載した節、*D.* sect. *Tamus* は新ランクである。

さらに、本研究では、日本産ヤマノイモ属各種の葉緑体 DNA 塩基配列に基づく高解像度かつ詳細な分子系統樹を構築し、加えて、ヤマノイモ属では初めて *PHYC* と 18SrDNA を用いることにより核 DNA に基づく分子系統樹を構築し

た。それらを比較すると、日本産ヤマノイモ属では網状進化の痕跡は特には見られないことが判明した。そして、ヤマノイモ類似群としては奄美大島にヤマノイモ、キールンヤマノイモ、ユワンドコロの3種が分布すること、大東島のルズンヤマノイモとされてきた植物はキールンヤマノイモであること、奄美大島のアミタチドコロは九州のツクシタチドコロとは別種で、独立種であること、イズドコロは *Dioscorea collettii* の変種に位置づけるのが妥当であることを見出した。その結果、日本にヤマノイモ属は、16 種 3 変種が分布すると結論付けた。

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