



## Flora of Nam Kading National Protected Area VII: a new species of *Diospyros* (Ebenaceae), *D. laoensis*

SHUICHIRO TAGANE<sup>1,2,5\*</sup>, PHETLASOY SOULADETH<sup>3,6</sup>, CHEN-JUI YANG<sup>4,7</sup> & TETSUKAZU YAHARA<sup>1,8</sup>

<sup>1</sup>Center for Asian Conservation Ecology, Kyushu University, Fukuoka, Japan

<sup>2</sup>The Kagoshima University Museum, Kagoshima University, Kagoshima, Japan

<sup>3</sup>Faculty of Forest Science, National University of Laos, Vientiane, Laos

<sup>4</sup>Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, Taiwan

<sup>5</sup>✉ [stagane29@gmail.com](mailto:stagane29@gmail.com); <https://orcid.org/0000-0002-1974-7329>

<sup>6</sup>✉ [p.souladeth@nuol.edu.la](mailto:p.souladeth@nuol.edu.la); <https://orcid.org/0000-0001-8563-1773>

<sup>7</sup>✉ [hitlefinal@gmail.com](mailto:hitlefinal@gmail.com); <https://orcid.org/0000-0002-7364-1218>

<sup>8</sup>✉ [tet.yahara@gmail.com](mailto:tet.yahara@gmail.com); <https://orcid.org/0000-0001-5105-7152>

\*Corresponding: [stagane29@gmail.com](mailto:stagane29@gmail.com)

### Abstract

A new species of *Diospyros*, *D. laoensis*, from Nam Kading National Protected Area, central Laos, is described and illustrated. It morphologically resembles *D. longipilosa* of Thailand, *D. martabanica* of India and Southeast Asia, and *D. xishuangbannaensis* of China, but is distinguished by its adaxially glabrous midrib, shorter calyx lobes and shorter fruiting pedicel. DNA barcodes of the *rbcL* and *matK* regions, a vernacular name and preliminary conservation assessment are also provided. As the species is considered to be endemic to a restricted area which is under threat from deforestation, it is here suggested for assessment under the category of Endangered (EN) within the IUCN Red List of Threatened Species.

**Keywords:** DNA barcoding, Ericales, flora, Indochina, Laos, taxonomy

### Introduction

The genus *Diospyros* (Ebenaceae), with over 600 species, is widely distributed in Asia, Africa, the Americas, Australia and the South Pacific Islands, in mostly tropical and subtropical regions (Wallnöfer 2001; Duangjai *et al.* 2006, 2009, 2018). In tropical Asia, species of *Diospyros* are found from coastal sandy or rocky shores up to lower montane mossy forests, including limestone hills and peat swamps, and are ecologically important components in the forests (Ng, 2002). In Laos, 31 species with four varieties of *Diospyros* have been recorded (Newman *et al.* 2007; Zhu 2017; Prosperi *et al.* 2018; Tagane *et al.* 2018).

During our floristic inventories of the evergreen forests of the Nam Kading National Protected Area (Souladeth *et al.* 2017; Tagane *et al.* 2017 & 2018; Yang *et al.* 2018) (Fig. 1), we collected an unknown species of *Diospyros*. Further studies based on specimens and literature of related species (Phengkhai 1981, 2005; Li *et al.* 1996; Hô 1999; Ng, 2002; Gardner *et al.* 2015; Duangjai *et al.* 2018) showed that this taxon was morphologically distinct from previously known taxa. Here, we describe this new species as *Diospyros laoensis* Tagane & Soulad., and provide illustrations, a vernacular name and preliminary conservation assessment.

In addition to the morphological examination, DNA sequences are often helpful for delimiting species (Hebert and Gregory 2005, Dick and Webb 2012). We sequenced two DNA barcode regions, *rbcL* and *matK*, following the recommendation of CBOL Plant Working Group (2009).

### Material & Methods

#### *Morphological observations*

Specimens were seen in herbaria BKF, FOF, FU, HNL, KAG, KYO, RUPP, SNP, TNS and VNM, while digital images were seen on websites of JSTOR Global Plants (<https://plants.jstor.org/>), Muséum National d'Histoire Naturelle

(<https://www.mnhn.fr/>) and Chinese Virtual Herbarium (<http://www.cvh.ac.cn/>). The measurements of the description below are based on the herbarium specimens we collected in our field surveys.

### DNA barcoding

Leaf pieces were dried using silica-gel in the field (voucher specimen: *Tagane et al. L1037*, FU). DNA isolation was performed by the CTAB method (Doyle & Doyle 1987) with minor modifications as in Toyama *et al.* (2015). Two DNA barcode regions, the partial genes for the large subunit ribulose-1,5-bisphosphate carboxylase oxygenase (*rbcL*) and maturase K (*matK*) (CBOL Plant Working Group 2009), were sequenced following established protocols (Kress *et al.* 2009; Dunning and Savolainen 2010).

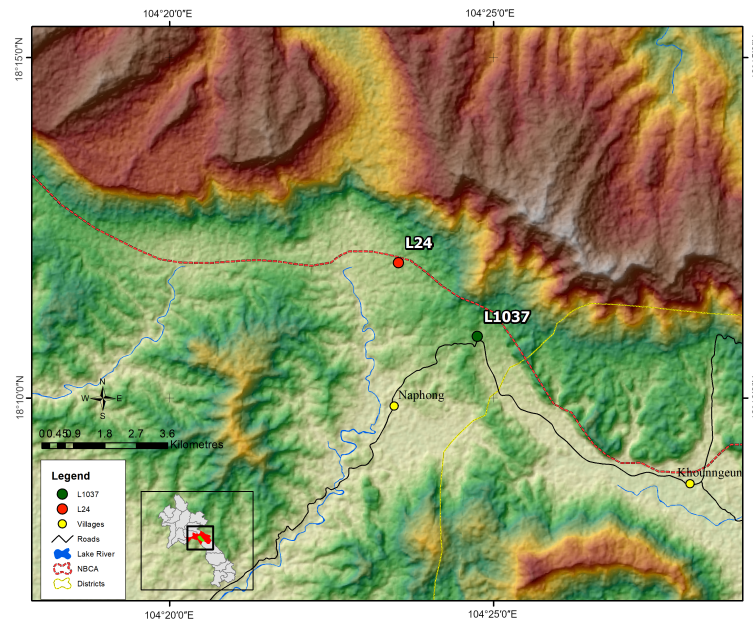


FIGURE 1. Collection localities of *Diospyros laeensis* Tagane & Soulad.

## Taxonomy

### *Diospyros laeensis* Tagane & Soulad., *sp. nov.* Figs. 2 & 3

**Type:**—LAOS. Bolikhamxay Province: Nam Kading National Protected Area, 18°11'59.1"N, 104°23'32.5"E, 272 m elev., 27 June 2017, *Tagane S., Souladeth P., Okabe N., Yang C.-J. L1037* [fr.] (holotype: FOF!, isotypes: BKF, E, HNL!, KAG!, KYO!, P).

*Diospyros laeensis* is distinct from all the other *Diospyros* species in the region by its young twigs, petioles and lower leaf surface of lamina densely covered with both short and long light brownish yellow hairs, glabrous midribs on the upper surface, oblong or oblong-lanceolate leaves with a cordate base, 2.5–4.5 mm long fruiting pedicels and 4-locular fruit.

Trees up to 8 m tall. Young twigs densely covered with short velvety hairs, mixed with long spreading hairs which are sparser than the shorter ones, hairs light brownish yellow, shorter ones ca. 0.8 mm long, longer ones 3.5–4.8 mm long, old twigs gray to grayish brown, glabrescent, lenticellate. Leaves alternate; petiole 2–3 mm long, indumentum same as young twigs; blade oblong to oblong-lanceolate, 4.6–12.6 × 1.3–3.5 cm, coriaceous, apex acute, base cordate, rarely rounded, margin entire, not undulate, recurved when dry, pale green to brownish, adaxial surface glabrous, abaxial surface covered with short yellowish brown, velvety hairs, obviously denser on the midrib, mixed with long, light brownish yellow soft hairs, midrib sunken adaxially, prominent abaxially, secondary veins (7–)10–12 on each side, slightly prominent adaxially, prominent abaxially, tertiary veins scalariform-reticulate, prominent abaxially. Flowers not seen. Mature fruit solitary, on 2.5–4.5 mm long stalk, subglobose to oblong-ovoid, 2.2–2.5 cm long, 1.6–2.4 cm in diam., dense with velvety-rusty hairs, 4-locular, 1–4-seeded. Fruiting calyx lobes 5, divided to the base, valvate, narrowly triangular, 4.2–5 × ca. 2 mm, chartaceous, hairy adaxially, glabrous abaxially. Seed ellipsoidal, 1.4–1.7 cm long, 0.6–0.8 cm in wide, dark reddish brown, glabrous; endosperm smooth, hard.





**Flora of Laos**  
 Faculty of Science, Kyushu University, Fukuoka (FU), Japan  
 Herbarium of Faculty of Forestry, National University of Laos (FOF), Laos

No.: L1037      **Family:** Ebenaceae  
**Name:** Diospyros  
**Det.:**

**Latitude:** 18°11'59.1"      **Longitude:** 104°23'32.5"  
**Altitude:** 272 m  
**Locality:** Bolikhamxay Province, Nam Kading National Protected Area; in semi-evergreen forest, along a loggin road  
**Date:** 27 June 2017  
**Coll.:** Tagane S., Souladeth P., Okabe N., Yang C.-J. [No.: L1037]  
**Note:**

**FIGURE 2.** Isotype of *Diospyros laevis* Tagane & Soulad., *Tagane et al.* L1037 (KAG).





**FIGURE 3.** *Diospyros laoensis* Tagane & Soulad. A. Leafy twig; B. Portion of lower leaf surface; C. Young twig; D. Old twig; E. Mature fruit; F. Fruiting calyx; G. Transverse section of 4-seeded fruit; H. Seeds. Materials from Tagane *et al.* L1037 (isotype, KAG). Scale bars D & H = 1 cm; F = 2 mm; G = 4 mm.

**Additional specimens examined:**—LAOS. Bolikhamxay Province: Nam Kading National Protected Area, 18°10'54.6"N, 104°24'45.9"E, 228 m elev., 23 Dec. 2016, Yahara T., Tagane S., Zhang M., Okabe N., Hyakumura K., Souladeth P., Sengthong A., Vorasane H., Chayer S. L24 [ster.] (FOF, KYO, TAI).

**Distribution and ecology:**—LAOS. At present, *Diospyros laoensis* is known only from the southern part of the Nam Kading National Protected Area (Fig. 1). The species was found in evergreen forest and its vicinities at elev. 220–280 m, and growing with *Hydnocarpus ilicifolius* King (Achariaceae), *Saraca declinata* (Jack) Miq. (Fabaceae), *Streblus crenatus* (Gagnep.) Corner (Moraceae), and *Tarenna hoensis* Pit. (Rubiaceae). Fruiting specimens were collected in late June.

**Etymology:**—This specific epithet *laoensis* refers to the country of the type locality.



**Vernacular name:**—ໝາກກວ້ອລາວ [Mark kua lao (“Mark Kua” means *Diospyros* species in general in Lao), suggested here].

**GenBank accession no.:**—*Tagane et al. L1037*: LC415119 (*rbcL*) and LC415120 (*matK*). The BLAST similarity search based on the partial *rbcL* sequence of *Diospyros laoensis* resulted in homology as high as 590/591 bp with the sequence of *D. hainanensis* Merrill (1923: 258)(GenBank accession no. MH778100) and *D. xishuangbannaensis* Wu & Zhu (1995: 296)(GU471703), 564/565 bp with that of *Diospyros* sp. JH-2017 (MF435487MF435499, MF435520, MF435534, MF435632 and MF435648) and 578/580 bp with the sequence of *D. ferox* Bakhuisen (1933: 170)(EU980681), *D. mindanaensis* Merrill (1903: 309)(EU980713), *D. pilosula* (Candolle 1844: 220) Hiern (1873: 188) (EU980731) and *D. rigida* Hiern (1873: 257)(EU980743) in the DNA database (GenBank: <https://www.ncbi.nlm.nih.gov/genbank/>). Those of *matK* sequence resulted in homology as high as 784/785 bp with the sequence of *D. pilosula* (DQ924048), 783/785 bp with those of *D. xishuangbannaensis* (GU471720, KU379125, KU379126), *D. howii* Merrill & Chun (1935: 299)(KU379003, KU379004, KU379005, KU379006, KU379007, KU379008) and *D. filipendula* Lecomte (1928: 101)(DQ924019).

**Preliminary conservation assessment:**—Endangered (EN). From our field observations through the surveys in 2016–2017 (*Tagane et al. 2018*), *Diospyros laoensis* is scattered in the evergreen forest in the lower elevations (220–280 m) of the southern part of Nam Kading National Protected Area and the area of occupancy (AOO) for this species is calculated as 8 km<sup>2</sup> (using a 2 km cell width) and estimated to be less than 500 km<sup>2</sup> based on our field observations. Although there are only two actually known sites for *D. laoensis*, the estimated area 500km<sup>2</sup> is the amount of potential habitat of this species, which could be regards as the extent of occupancy (EOO). The two localities where we collected the above specimens are along a recently constructed logging road, and are being gradually deforested. Given this situation, we suggest the category of this species as Endangered according to the IUCN criteria EN B2a & bii,iii (IUCN 2017). More accurate data on its population size and number of individuals is needed for developing a conservation plan.

**Notes:**—*Diospyros laoensis* is morphologically similar to *D. longipilosa* Phengklai (1977: 342) of southern Thailand, *D. martabanica* C.B.Clarke (1882: 554) of India, Myanmar, Thailand, and *D. xishuangbannaensis* of Xishuangbanna, southwestern China in having densely hairy young twigs and fruit, oblong to oblong-lanceolate leaf shape with rounded to cordate leaf base and 4-locular fruit, but distinguished by its adaxially glabrous midrib (vs. more or less pubescent) and shorter fruiting pedicels (2.5–4.5 mm long vs. 2–3 cm in *D. longipilosa*; 1–2.3 cm long in *D. martabanica*; 1–1.6 cm long in *D. xishuangbannaensis*) (Table 1). In addition, fruiting calyx lobes of *D. laoensis* are shorter than those of *D. longipilosa* and *D. xishuangbannaensis* (4.2–5 mm long vs. longer than 9 mm long).

**Table 1.** Morphological comparison between *Diospyros laoensis*, *D. longipilosa*, *D. martabanica* and *D. xishuangbannaensis*.

Characters	<i>D. laoensis</i>	<i>D. longipilosa</i> <sup>1</sup>	<i>D. martabanica</i> <sup>2</sup>	<i>D. xishuangbannaensis</i> <sup>3</sup>
Indumentum on young twigs	densely covered with short hairs, mixed with long soft hairs	densely covered with long soft hairs	densely covered with short hairs, mixed with long soft hairs	densely covered with short hairs
Petiole length	2–3 mm long	4–6 mm long	ca. 3.2 mm long	2–3 mm long
Leaf size	4.6–12.6 × 1.3–3.5 cm	10–15 × 2.5–4 cm	7–18 × 2–6 cm	8–18 × 3–4.5 cm
Leaf base	cordate to rounded	rounded to (sub)cordate	rounded to subcordate	rounded to subcordate
Leaf texture	coriaceous	chartaceous	chartaceous to thinly coriaceous	stiffly chartaceous to subcoriaceous
Hairiness on adaxial midrib	glabrous	densely covered with long hairs	densely covered with short hairs	densely covered with short hairs
Fruiting pedicel length	2.5–4.5 mm long	20–30 mm long	10–23 mm long	10–16 mm long
Fruiting calyx shape and size	narrowly triangular, 4.2–5 × ca. 2 mm	narrowly lanceolate, ca. 14 × 3 mm	narrowly lanceolate, no information on size	narrowly lanceolate, ca. 9 mm long
Fruit shape and size	subglobose to oblong-ovoid, 2.2–2.5 cm long, 1.6–2.4 cm in diam.	ellipsoid or ovoid, 3–4 cm long, 2.5–3 cm in diam.	globose to ovoid, 1.5–2.5 cm in diam.	globose, 2–2.5 cm in diam.

<sup>1</sup>Phengklai (1977), Gardner *et al.* (2015) and Larsen K & Larsen S. 33348 (type, BKF, image!).

<sup>2</sup>Clarke (1882), Gardner *et al.* (2015) and Kurz S. 989 (K, image!).

<sup>3</sup>Li *et al.* (1996), Zhu & Wu 2451 (isotype) and Zou & Wu 2452 (paratype) (HITBC, images!).

## Acknowledgements

The authors are grateful to the managers and staff of the Nam Kading National Protected Area for supporting our botanical inventory in the protected area. We also thank Saengmany Boutthavong (National University of Laos) for producing a map, Keiko Mase (Kyushu University) for her help with DNA barcoding, Jian-Wu Li (Xishuangbanna Tropical Botanical Garden) for information of *D. xishuangbannaensis*, and the curators and staff of the following herbaria: BKF, FOF, FU, HNL, KAG, RUPP, SNP, TNS and VNM for allowing us to examine their collections. This study was partly supported by the Environment Research and Technology Development Fund (4–1601) of the Ministry of the Environment, Japan, and MEXT/JSPS KAKENHI (Grant Number JP15H02640).

## References

- Bakhuizen, van den Brink, R.C. (1933) Enumeration of Malayan Ebenaceae. *The Gardens' Bulletin Straits Settlements* 7: 161–188.
- Candolle, A.P. de. (1844) *Prodromus systematis naturalis regni vegetabilis, sive, Enumeratio contracta ordinum generum specierumque plantarum huc usque cognitarium, juxta methodi naturalis, normas digesta*, vol. 8. Sumptibus Sociorum Treuttel et Würtz, Paris, 684 pp.
- CBOL Plant Working Group (2009) A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America* 106: 12794–12797.  
<https://doi.org/10.1073/pnas.0905845106>
- Clarke, C.B. (1882) Ebenaceae. In: Hooker, J.D. (Ed.) *The Flora of British India*. Vol. 3. L. Reeve & Co., London, pp. 549–572.
- Dick, C.W. & Webb, C.O. (2012) Plant DNA barcodes, taxonomic management, and species discovery in tropical forests. In: Kress, W.J. & Erickson, D.L. (Eds.) *DNA Barcodes*. Springer, New York, NY. *Methods and Protocols* 858: 379–393.  
[https://doi.org/10.1007/978-1-61779-591-6\\_18](https://doi.org/10.1007/978-1-61779-591-6_18)
- Duangjai, S., Wallnöfer, B., Samuel, R., Munzinger, J. & Chase, M.W. (2006) Generic delimitation and relationships in Ebenaceae *sensu lato*: evidence from six plastid DNA regions. *American Journal of Botany* 93: 1808–1827.  
<https://doi.org/10.3732/ajb.93.12.1808>
- Duangjai, S., Samuel, R., Munzinger, J., Forest, F., Wallnöfer, B., Barfuss, M.H.J., Fischer, G. & Chase, M.W. (2009) A multi-locus plastid phylogenetic analysis of the pantropical genus *Diospyros* (Ebenaceae), with an emphasis on the radiation and biogeographic origins of the New Caledonian endemic species. *Molecular Phylogenetics and Evolution* 52: 602–620.  
<https://doi.org/10.1016/j.ympev.2009.04.021>
- Duangjai, S., Sinbumroong, A. & Suddee, S. (2018) *Diospyros phengklaii* (Ebenaceae), a new species from south-western Thailand. *Thai Forest Bulletin (Botany)* 46: 34–39.  
<https://doi.org/10.20531/tfb.2018.46.1.05>
- Dunning, L.T. & Savolainen, V. (2010) Broad-scale amplification of *matK* for DNA barcoding plants, a technical note. *Botanical Journal of the Linnean Society* 164: 1–9.  
<https://doi.org/10.1111/j.1095-8339.2010.01071.x>
- Gardner, S., Sidisunthorn, P. & Chayamarit, K. (2015) *Forest Trees of Southern Thailand*, vol. 1. Kobfai Publishing Project, Bangkok, 768 pp.
- Hebert, P.D.N. & Gregory, T.R. (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology* 54: 852–859.  
<https://doi.org/10.1080/10635150500354886>
- Hiern, W.P. (1873) A monograph of Ebenaceae. *Transactions of the Cambridge Philosophical Society* 12: 27–300.
- Hô, P.H. (1999) *Cay Co Viet Nam: An Illustrated Flora of Vietnam*, vol. 1. Published by the author, Montreal, 1024 pp. [In Vietnamese]
- IUCN (2017) *The IUCN Red List of Threatened Species. Version 2017-3*. Available from: <http://www.iucnredlist.org> (accessed 25 April 2018)
- Kress, W.J., Erickson, D.L., Jones, F.A., Swenson, N.G., Perez, R., Sanjur, O. & Bermingham, E. (2009) Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proceedings of the National Academy of Sciences of the United States of America* 106 (44): 18621–18626.  
<https://doi.org/10.1073/pnas.0909820106>
- Lecomte, P.H. (1928) Ebénacées nouvelles de L'Indochine. *Notulae Systematicae* 4: 99–121.
- Li, S.-G., Gilbert, M.G. & White, F. (1996) Ebenaceae. In: Wu, Z.Y., Raven, P.H. & Hong D.Y. (Eds.) *Flora of China*, vol. 15. Missouri Botanical Garden Press, St. Louis and Science Press, Beijing, pp. 215–234. Available from: <http://www.efloras.org> (accessed 1 June 2018)



- Merill, E.D. (1909) New or noteworthy Philippine plants, VII. *Philippine Journal of Science* 4: 247–330.
- Merill, E.D. (1923) Diagnosis of Hainan Plants, II. *Philippine Journal of Science* 23: 237–268.
- Merrill, E.D. & Chun, H.Y. (1935) Additions to our knowledge of the Hainan flora, II. *Sunyatsenia* 2: 203–332.
- Newman, M. F., Ketphanh, S., Svengsuka, B., Thomas, P., Sengdala, K., Lamxay, V. & Armstrong, K. (2007) *A checklist of the vascular plants of Lao PDR*. Royal Botanic Garden Edinburgh, Scotland, UK, 375 pp.
- Ng, F.S.P. (2002) Ebenaceae. In: Soepadmo, E., Saw, L.G. & Chung, R.C.K. (Eds.) *Tree flora of Sabah and Sarawak*, vol. 4. Forest Research Institute Malaysia (FRIM), Kepong, pp. 29–100.
- Phengkklai, C. (1977) New species of Ebenaceae from Thailand. *Natural History Bulletin of the Siam Society* 26: 342–344, Plate XII & XIII.
- Phengkklai, C. (1981) Ebenaceae. In: Smitinand, T. & Larsen, K. (Eds.) *Flora of Thailand*, vol. 2. The Forest Herbarium, National Park, Wildlife and Plant Conservation Department, Bangkok, pp. 281–392.
- Phengkklai, C. (2005) Two new species of *Diospyros* (Ebenaceae) from Thailand. *Thai Forest Bulletin (Botany)* 33: 157–160.
- Prosperi, J., Lamxay, V., Hallé, F., Bompard, J.-M., Blanc, P., Ramesh, B.R., Ayyappan, N. & Cardinal, S. (2018) New records in the flora checklist of Laos, resulting from a survey of Phou Hin Poun National Biodiversity Conservation Area. *Edinburgh Journal of Botany* 75: 91–106. <https://doi.org/10.1017/S096042861700035X>
- Souladeth, P., Tagane, S., Zhang M., Okabe, N. & Yahara, T. (2017) Flora of Nam Kading National Protected Area I: a new species of yellow-flowered *Strobilanthes* (Acanthaceae), *S. namkadingensis*. *PhytoKeys* 81: 11–17. <https://doi.org/10.3897/phytokeys.81.13203>
- Tagane, S., Souladeth, P., Rueangruea, S., Okabe, N., Zhang, M., Chayer, S., Yang, C.-J. & Yahara, T. (2017) Flora of Nam Kading National Protected Area II: 30 new records of angiosperms for Laos. *Edinburgh Journal of Botany* 75: 107–116. <https://doi.org/10.1017/S0960428617000361>
- Tagane, S., Souladeth, P., Zhang, M. & Yahara, T. (2018) Flora of Nam Kading National Protected Area IV: Two new species of Annonaceae, *Monoon namkadingense* and *Neo-uvaria laosensis*. *Phytotaxa* 336 (1): 82–88. <https://doi.org/10.11646/phytotaxa.336.1.6>
- Toyama, H., Kajisa, T., Tagane, S., Mase, K., Chhang, P., Samreth, V., Ma, V., Sokh, H., Ichihashi, R., Onoda, Y., Mizoue, N. & Yahara, T. (2015) Effects of logging and recruitment on community phylogenetic structure in 32 permanent forest plots of Kampong Thom, Cambodia. *Philosophical Transactions B: Biological Science* 370 (1662): 20140008. <http://dx.doi.org/10.1098/rstb.2014.0008>
- Yang, C.-J., Tagane, S., Souladeth, P., Okabe, N., Hu, J.-M. & Yahara, T. (2018) Flora of Nam Kading National Protected Area III: *Begonia namkadingensis* (Begoniaceae), a new species in limestone area. *Phytotaxa* 334 (2): 195–199. <https://doi.org/10.11646/phytotaxa.334.2.8>
- Wallnöfer, B. (2001) The biology and systematics of Ebenaceae: a review. *Annalen des Naturhistorischen Museums in Wien B* 103: 485–512.
- Wu, Z.Y. & Zhu, H. (1995) Validation of a new species of *Diospyros* (Ebenaceae) from Yunnan, China. *Novon* 5: 296.
- Zhu, H. (2017) Floristic characteristics and affinities in Lao PDR, with a reference to the biogeography of the Indochina peninsula. *PLOS ONE* 12 (6): e0179966. <https://doi.org/10.1371/journal.pone.0179966>