

23 December 2021

10th APBON Webinar

Plant Diversity Assessments: Advanced Understanding by Connecting Indochina and Japan

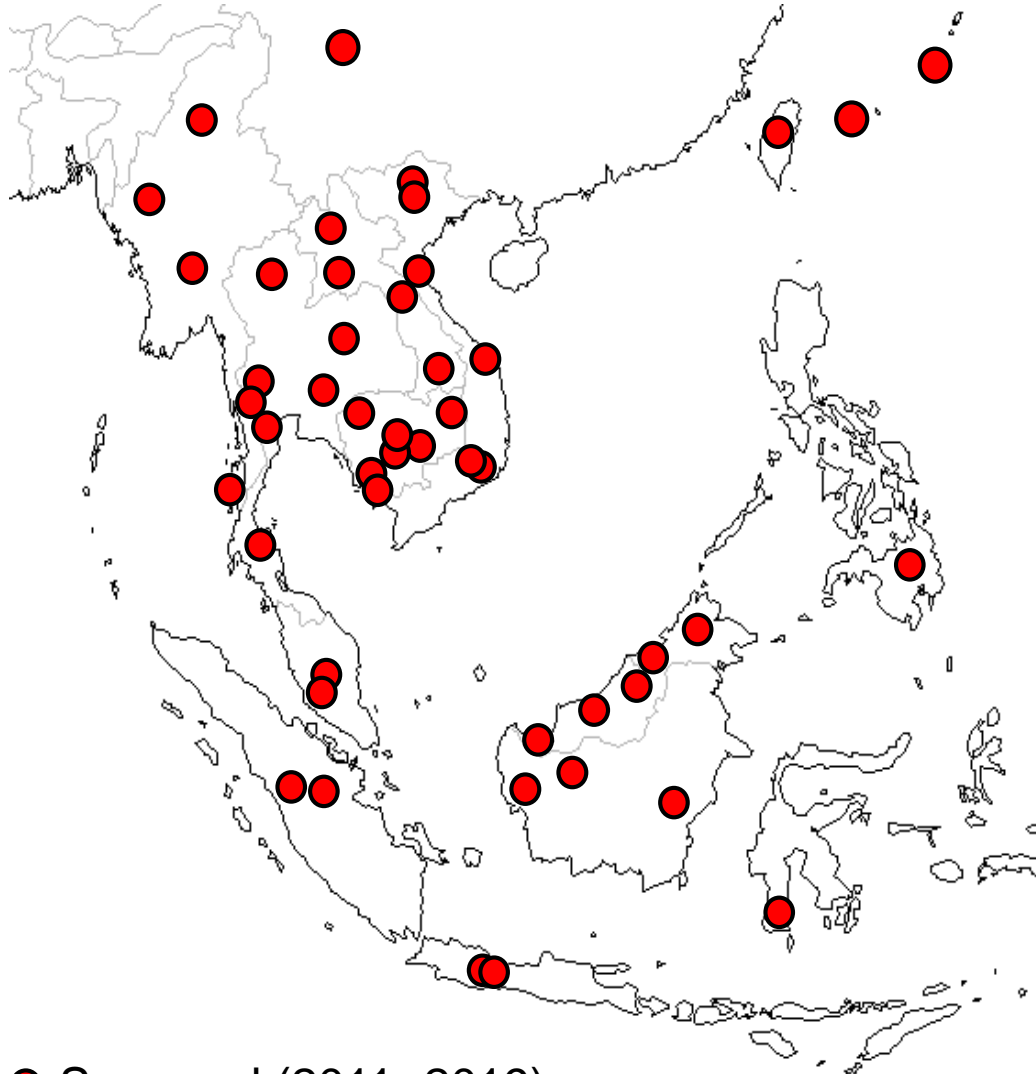
Tetsukazu Yahara

Kyushu Open University, Japan



Plant Diversity Assessments

167 plots at 56 locations
of 12 countries



● Surveyed (2011- 2019)

Brunei Darussalam (UBD)

Kuala Belalong FSC

Cambodia (FA)

Koh Kong, Bokor, Seima, Siem Reap, Kg Thom, Kg Chhnang

China (Guangxi Univ.)

Jiuwanshan NR

Laos (NUoL)

Nam Kading NPA, Nam Ha NPA, Phou Khao Khouay NPA, Dong Hua Sao NPA

Vietnam (ITB, Dalat University)

Ba Vi, Fan Si Pan, Vu Quang, Bach Ma, Ngoc Linh, Bidoup Nui Ba, Hon Ba

Thailand (BKF, KU)

Doi Inthanon, Phu Kradueng, Maeklong, Kaeng Krachan, Khao Soi Dao, Khao Luang, Khao Yai

Myanmar

Chin, Lampi Island, Indawgyi, Tanintharyi

Malaysia (FRIM, RDID, BRC)

Fraser's Hill, Pasoh; Batan Ai, Bintulu, Kubah, Lambir Hills; Kinabalu

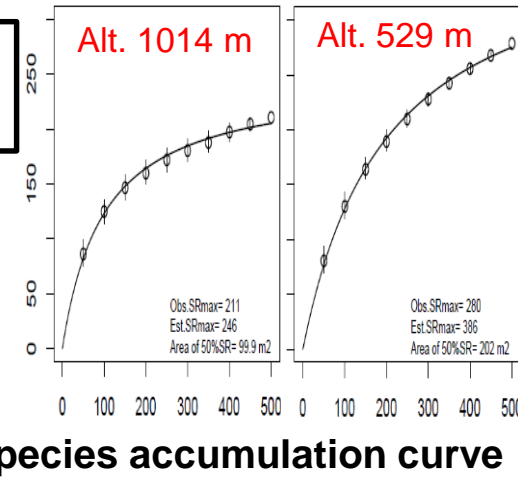
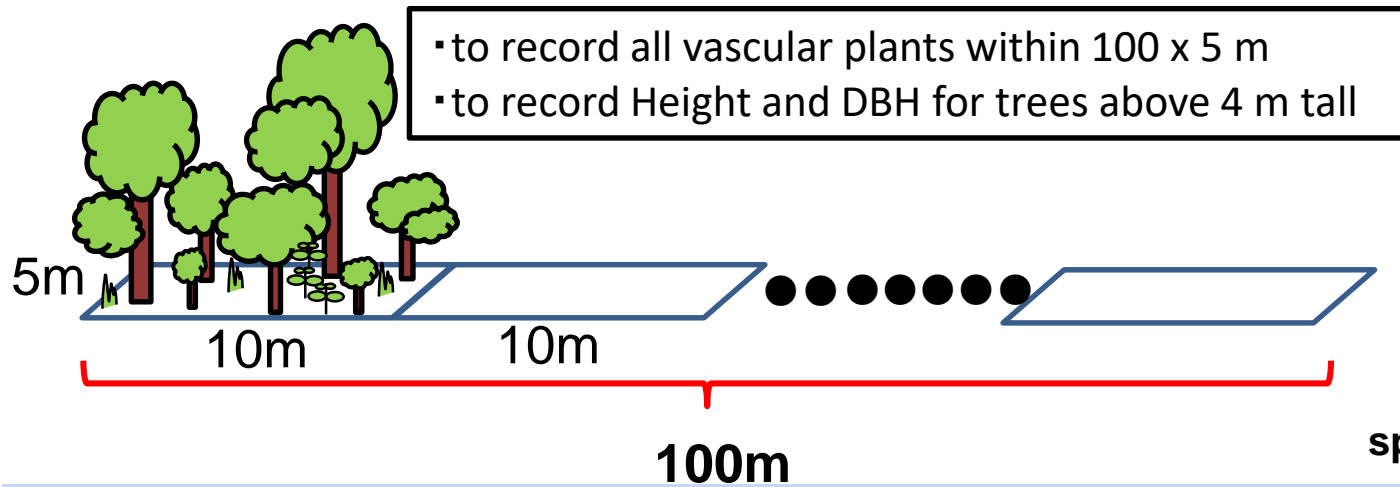
Indonesia (LIPI, Andalas Univ., Hasanudin Univ.)

Gn. Gede Pangrango NP, Gn. Halimun (Java), Bantimulung Bulusarung (Sulawesi), Gn. Gadut, Pekambaru (Sumatra), Mandor, Serimbu (W. Kalimantan), Bukit Bangkirai (E. Kalimantan)

Phillipines (DENR)

Banahao

A standardized belt-transect method



(1) Collect plants and record data, (2) Taking photos, (3) Collect leaf pieces for DNA analysis and (4) Make voucher specimens.

(5) Identify the plant species based on herbarium specimens, literature and DNA barcoding

(6) Study on taxonomy, ecology, phylogeny and biogeography; Picture guide, Database, etc.

Collecting specimens of tall trees



Recording all species in 100m x 5m

An example of transect record: data from Nam Kading National Park, Laos

ID	no.	Family	Field name	0-10m	90-100m	girth[cm]	height[m]
73	56	Moraceae	Streblus	1		51	8
74		Fabaceae	Callerya	1		107.4	20
75	57	Urticaceae	Poikilospermum	1		6	15
76	58	Melastomataceae	Memecylon コバ	1		7.8	4
77	59	Ebenaceae	Diospyros ナガバ	1		19	7
78	60	Cannabaceae	Girronierrra ?	1		8.9	4
79	61	Fabaceae	Archidendron 微毛	1		14	6
80	62	Achariaceae	Hydnocarpus ilicifolius	1		62.9	12
					.		
					.		
					.		
450	212	Phyllanthaceae	Aporosa		1		
451	213	Polypodiaceae	Drynaria		1		
452		Asparagaceae	Pelliosanthes		1		
453		Annonaceae	Polyarthia シワシワ		1		
454		Opiliaceae	Urobotria siamensis		1		
455		Cannabaceae	Girroniera ?		1		
456		Anacardiaceae	Melanochyla 2		1		
457	214	Sapindaceae	Nephelium		1	14.8	7
458		Celastraceae	Lophopetalum		1	10.8	7

Scientific name: Moraceae *Streblus*

Local name:

No. L56

#

1st record

Laos Nam Kading Line 1
Evergreen forest (alt. 280 m)



Scientific name: Lauraceae *Machilus*

Local name:

No. L100

#

Laos Nam Kading Line 1
Evergreen forest (alt. 280 m)



Scientific name: Myrtaceae *Syzygium*

Local name:

No. L173

#

Laos Nam Kading Line 1
Evergreen forest (alt. 280 m)



Scientific name: Sapindaceae *Nephelium*

Local name:

No. L214

#

Last record

Laos Nam Kading Line 1
Evergreen forest (alt. 280 m)



Vascular Plant Species Richness / Transect (500 m²)

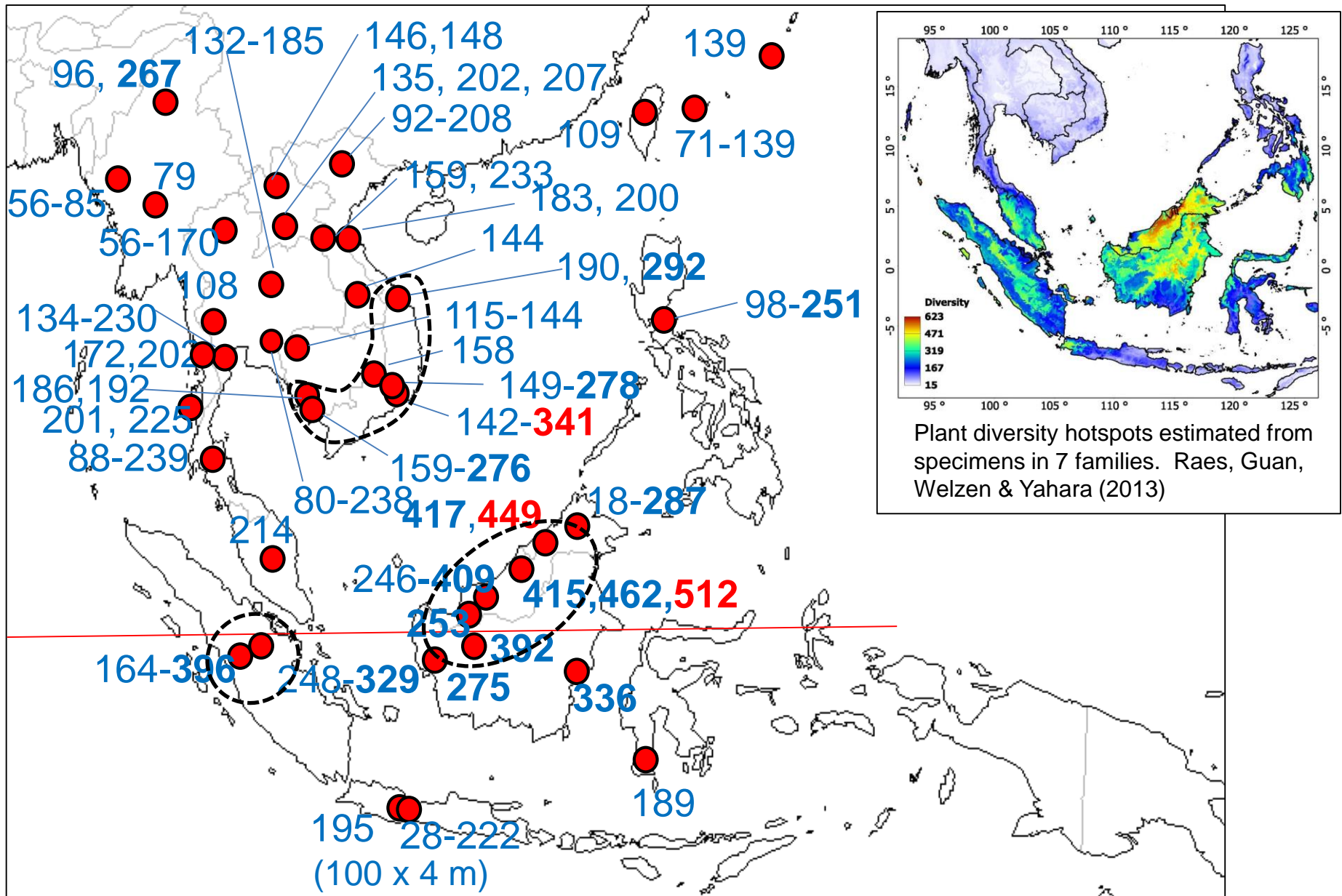


Fig. species richness observed in one transect line (500m²)

Next-generation sequencing of DNA samples

7th International Legumae Conference (29 Aug.-2 Sept. 2018, Sendai)



TOHOKU
UNIVERSITY

MIG-seq and multiplexed DNA barcoding : an efficient combination for molecular phylogenetic analysis

Yoshihisa Suyama^{a*}, Ayumi Matsuo^a, Shun Hirota^a, Chika Mitsuyuki^b, Tetsukazu Yahara^b

^aTohoku University, ^bKyushu University

MIG-seq: Multiplexed ISSR Genotyping by sequencing

A PCR-based procedure for SNP discovering and their genotyping using next-generation sequencing (NGS).

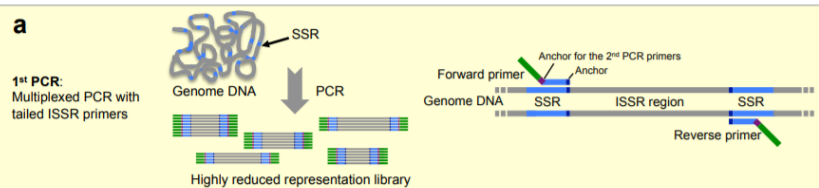
Overview

- **PCR-based (multi ISSR-PCR)**
=applicable to low quantity DNA
- **~1000< SNP discovery & typing**
=without prior genetic info.
- **Applicable to a wide range of species**
=without any optimization



Advantages

- **Quick:** 3 days for 192 or more samples
- **Simple:** 2 PCRs for library construction
- **Low cost:** ca. 10 USD/sample



Multiplexed DNA barcoding

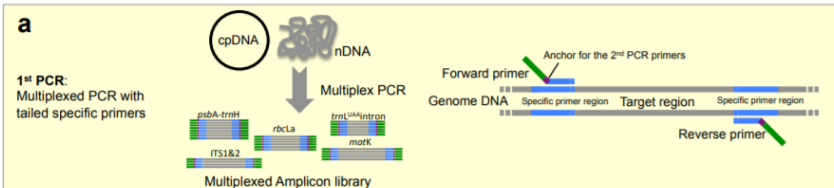
A simple and economical protocol to detect sequences of ITS and several cpDNA regions together using one run of NGS.

Overview

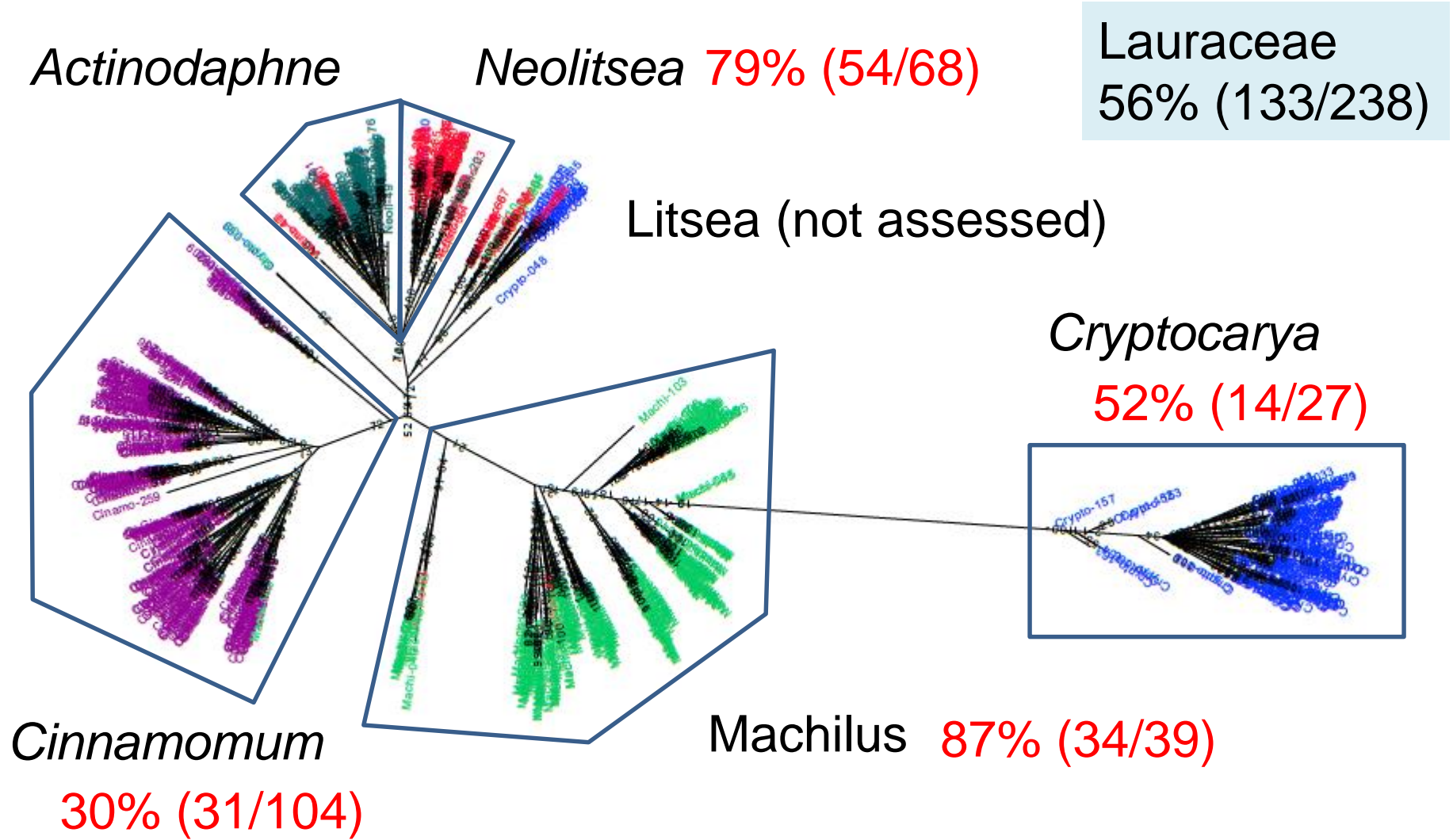
- **Multi PCR of ~5 or more regions**
=*psbA-trnH*, *rbcl*, *trnL^{UAA}*intron, (*matK*), and ITS1&2
- **~500 bp sequences for each region**
=~250 bp from both ends
- **Applicable to a wide range of plant species**
=without any optimization

Advantages

- **Quick:** 3 days for 384 or more samples
- **Simple:** 2 PCRs for library construction
- **Low cost:** ca. 2 USD/sample

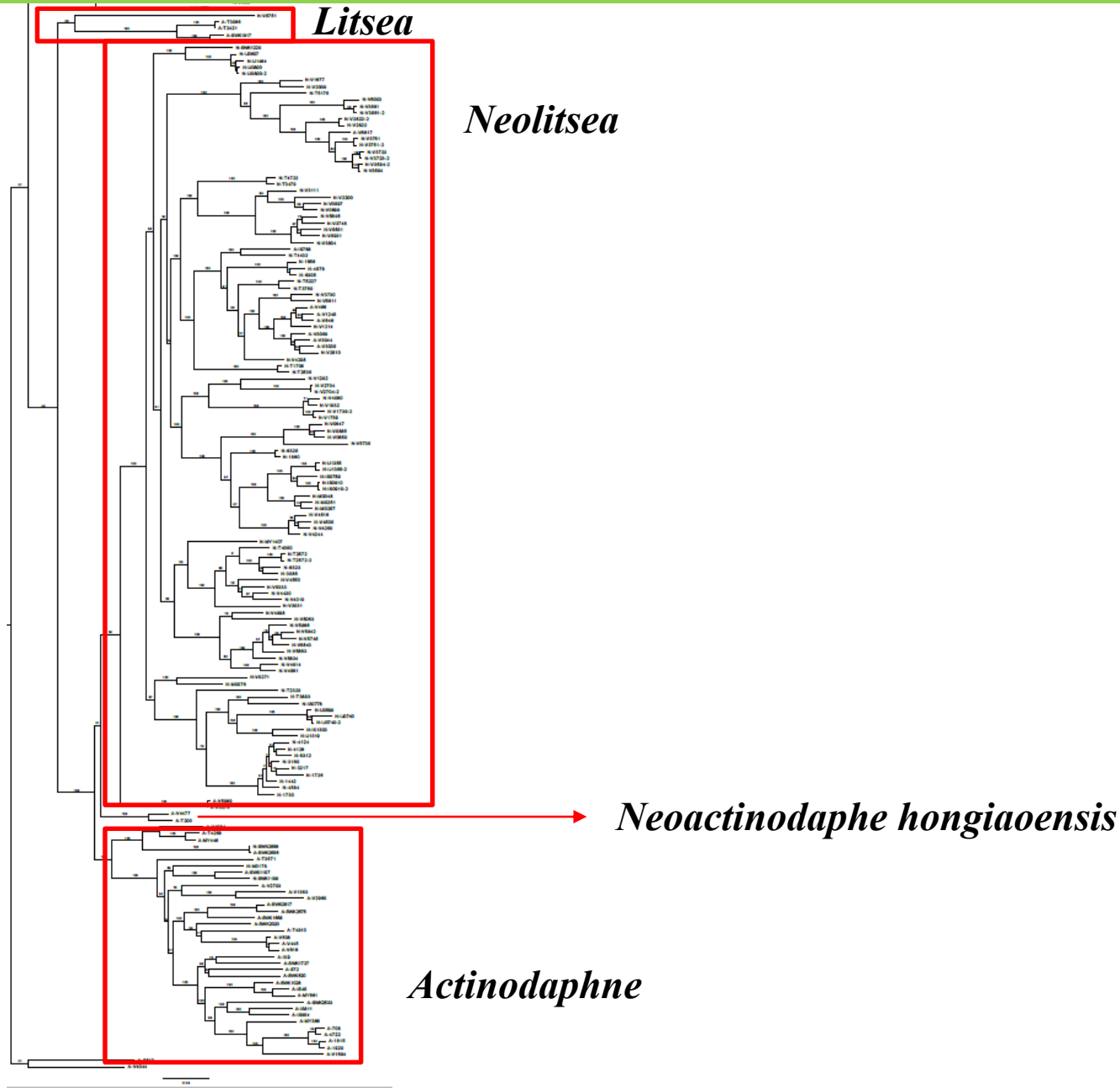


Proportion of new species in Lauraceae



Not assessed: *Alseodaphne*, *Beilschmiedia*, *Dehaasia*, *Nothaphoebe*, *Phoebe* etc.

A new genus is discovered in Lauraceae



Scientific name: Lauraceae *Neoactinodaphne hongiaoensis*, **gen. & sp. nov.**

Local name:

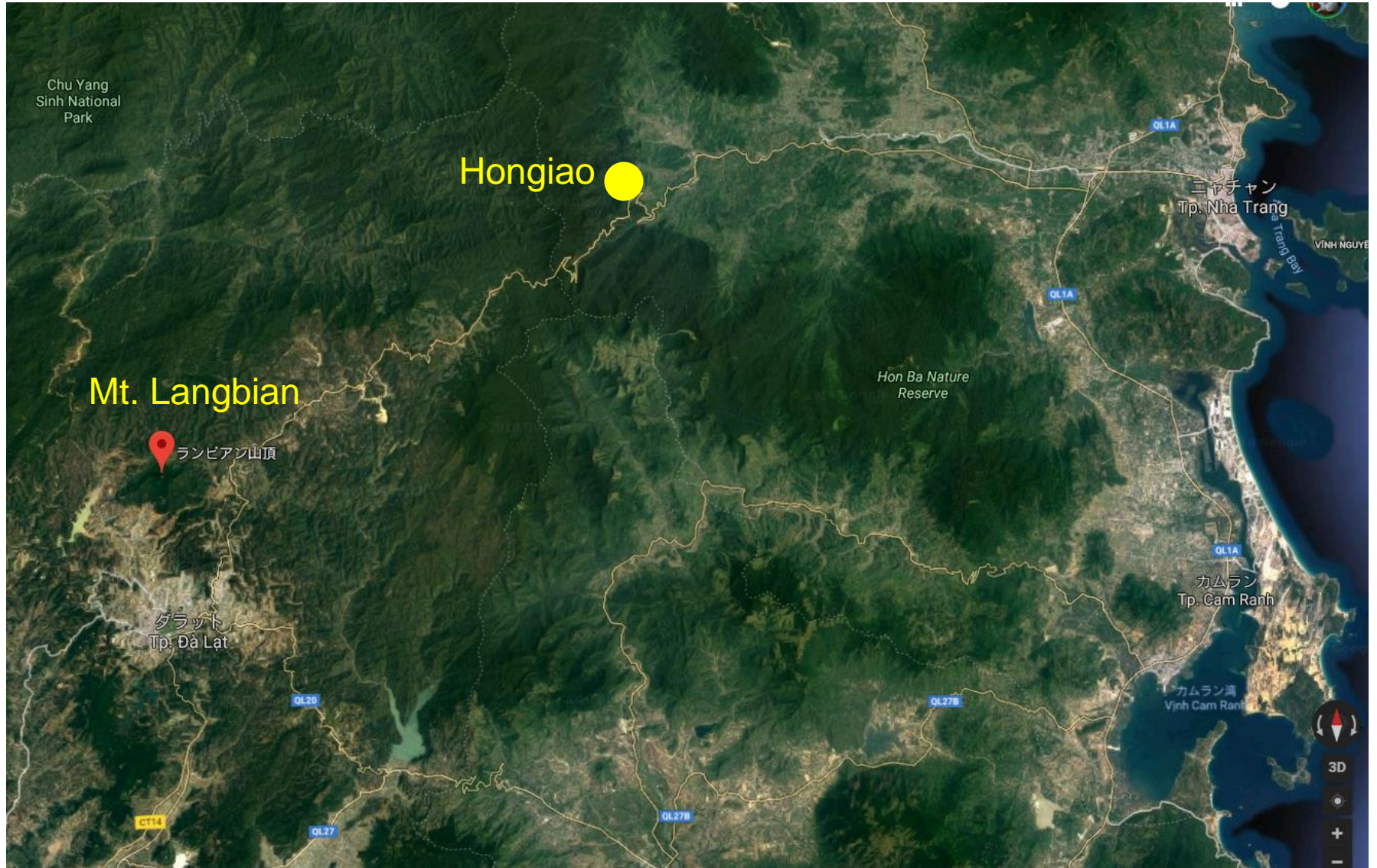
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Tagane photo



The area from Bidoup-Nui Ba NP to Hon Ba NR

This area has the highest plant species diversity in Indochina Peninsula



Plant diversity surveys in Bidoup-Nui Ba National Park

- Belt transects (100m x 5m); all life forms
 - Line 1 (1533m)
 - Line 2 (1807m)
 - Line 3 (1602m)
 - Line 4 (1905m; Mt. Langbian)
- Plots; only trees
 - Yellow Plot (1680m, Dinh Gia Rieng, 50x50, Y1-814)
 - Pink Plot (1666m, Hongiao, 100x10, P1-1000)
 - Blue Plot (1455m, Giang Ly, 30x50, B1-653)
 - Langbian Plot (1924m, Mt. Langbian, 30x50, L1-493)
 - Cong Troi Plot (1866m, 30x50, C1-703)

A total of 2528 specimens collected

Scientific name: Lamiaceae *Clinopodium* sp.

Local name:

No. V10079

#

Vietnam_Bidoup Nui Ba
Cong Troi (alt. 1884 m)



Scientific name: Lamiaceae *Clinopodium* sp.

Local name:

No. V4291

#

Bi Doup Nui Ba
Outside (alt. 1634 m)



Flora of Vietnam
S-S Project Collection 2011-2016
Department of Botany, Kyushu University, Fukuoka, Japan

No.: V4291 Family: Lamiaceae

Name: *Clinopodium*?

Det.:

Latitude: 12°09'23.51"N Longitude: 108°32'11.39"E
Altitude: 1634 m

Locality: Lam Dong Province; Bi Doup Nui Ba National Park; in
pine forest.

Date: February 24/2016

Coll.: Yagata S., Iiyama H., Nagamura H., Niiki A., Dung V.
Son, Nguyen V. Ngoc. & Wal J. [No.: V4291]

Note:



Scientific name: Lamiaceae *Clinopodium gracile* (Benth.) Kuntze

Local name:

No. V5363

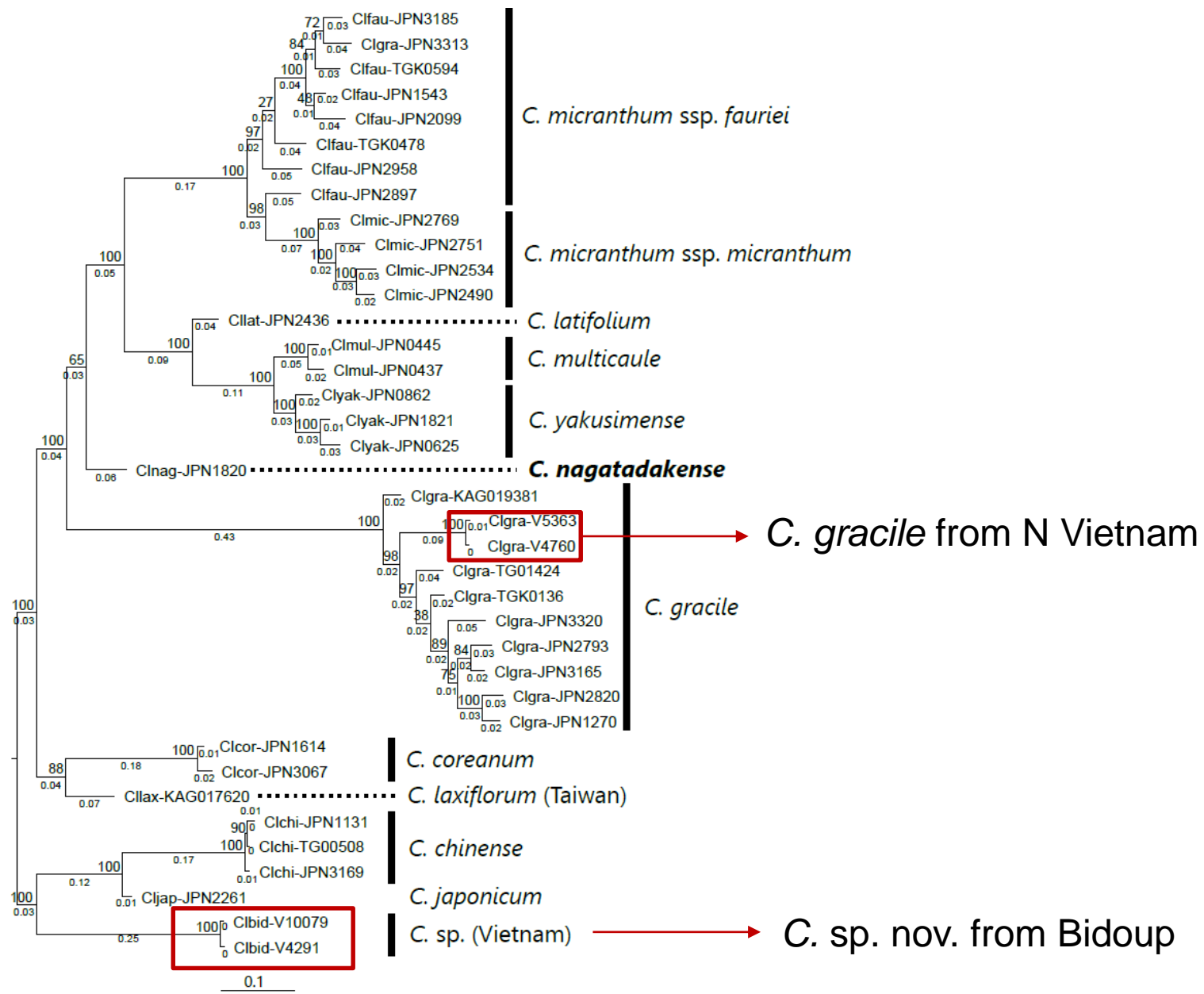
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Vietnam_Fansipan

Outside (alt. 1293 m)



MIG-seq tree of *Clinopodium*



Scientific name: Urticaceae *Pellionia* sp.

Local name:

No. V7178

#

Vietnam_Ba Vi NP
Roadside (alt. 700 m)



Scientific name: Urticaceae *Pellionia radicans* (Siebold et Zucc.) Wedd.

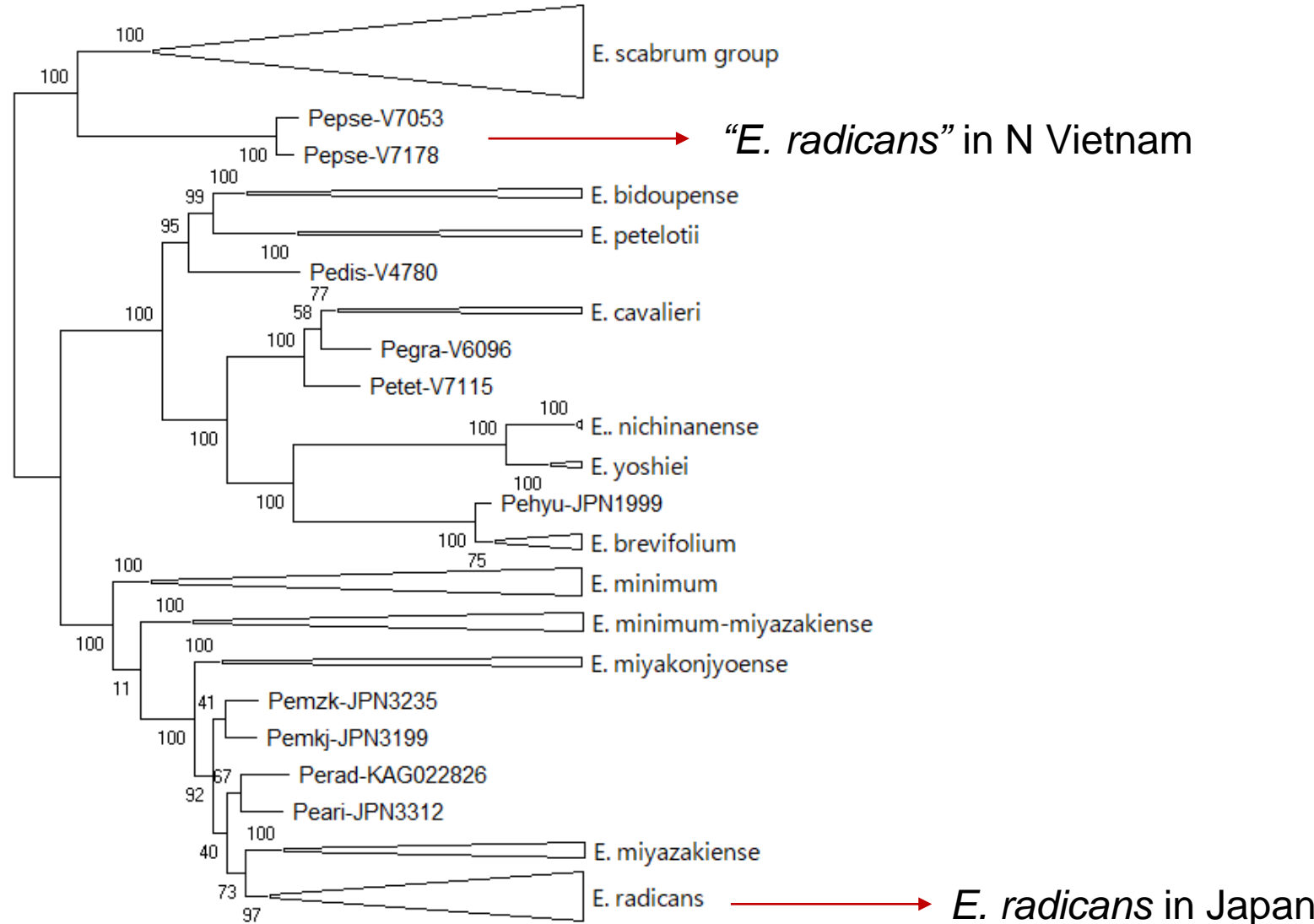
Local name: オオサンショウシヨウ

No. JPN3291

@ Kaeda Valley, Miyazaki City, Miyazaki, Japan



MIG-seq tree of *Elatostema* subgen. *Pellionia*



0.10

Scientific name: Urticaceae *Pellionia pseudoradicans* sp. nov.

Local name:

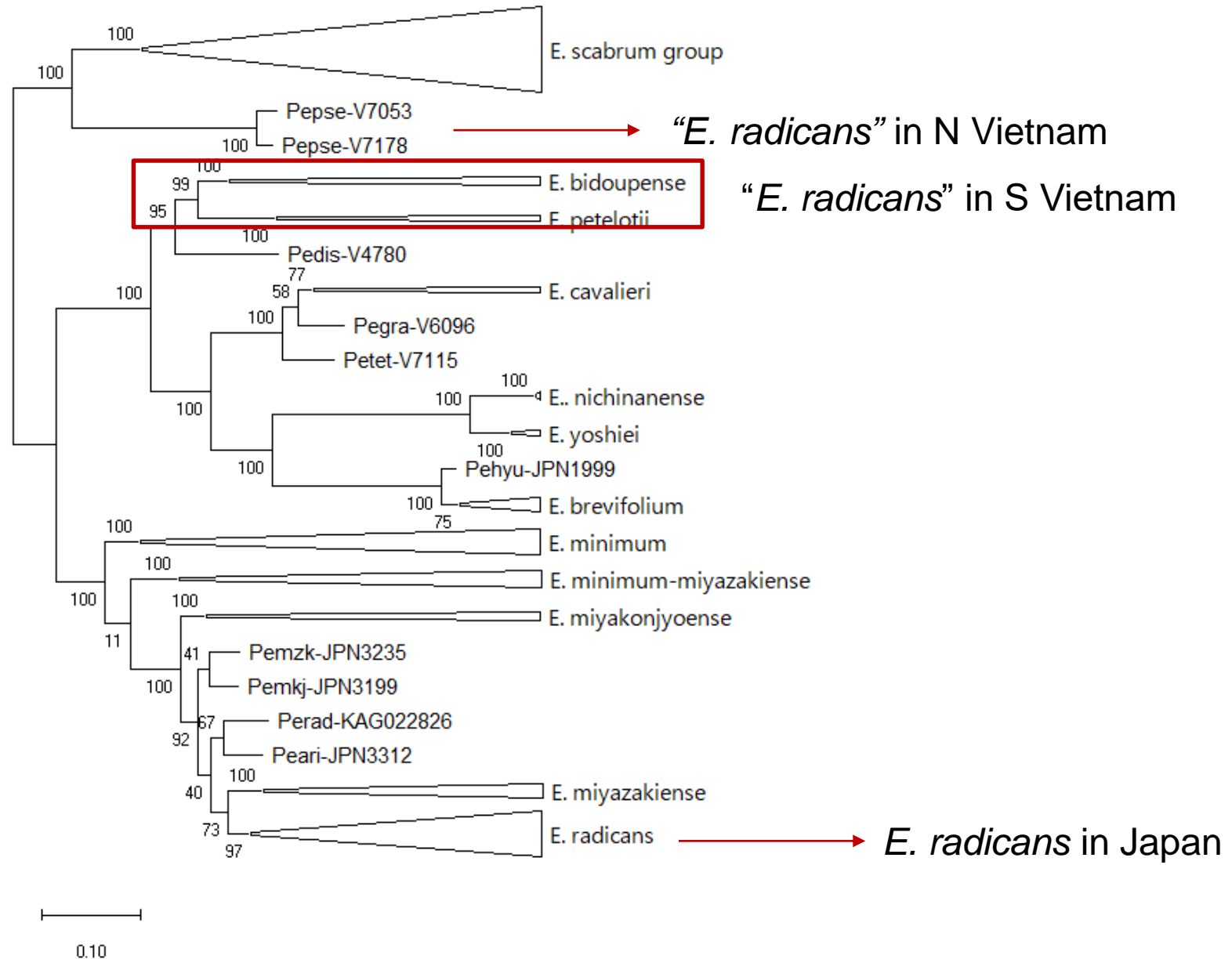
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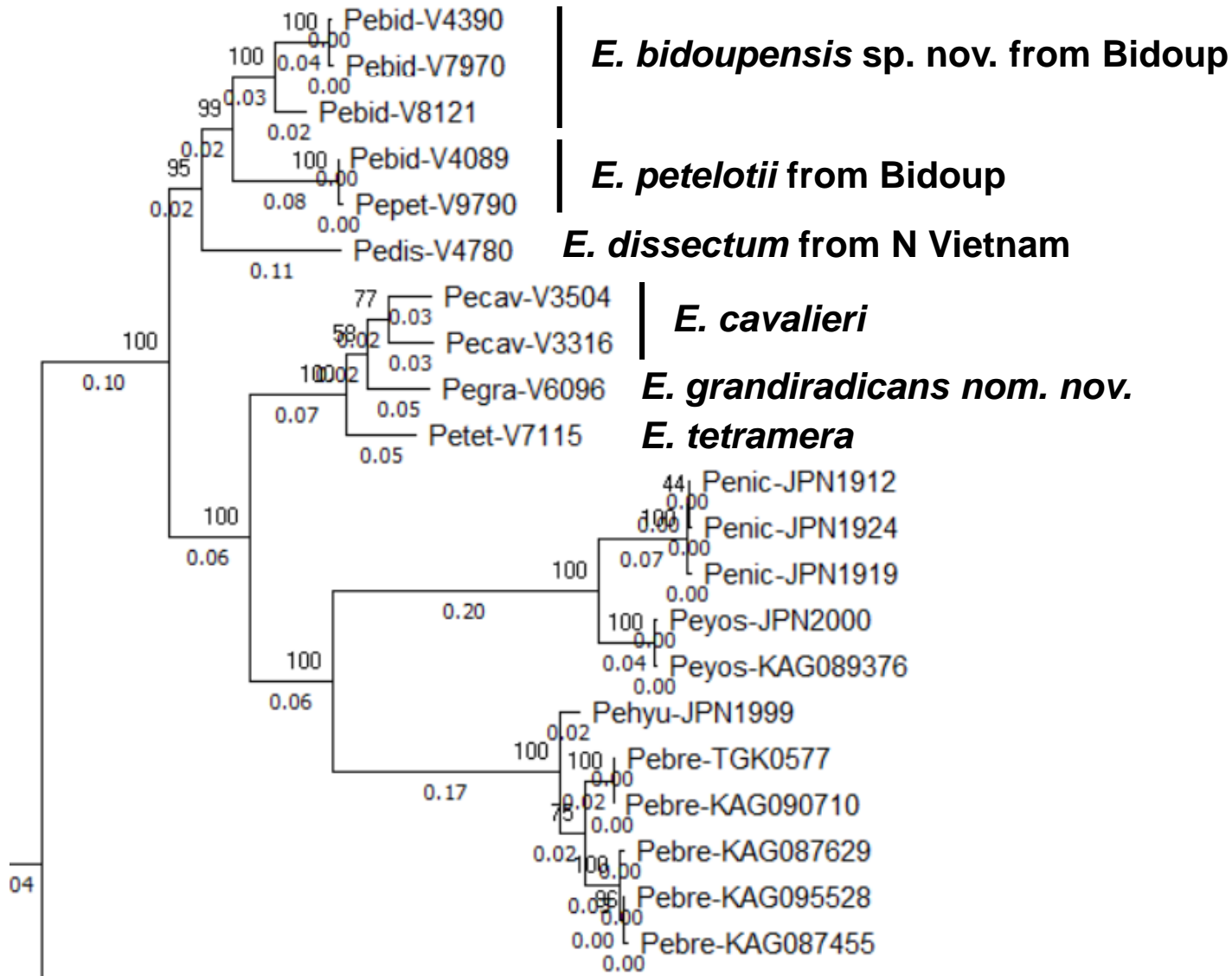
Vietnam_Ba Vi NP
Roadside (alt. 700 m)



MIG-seq tree of *Elatostema* subgen. *Pellionia*



Elatostema subgen. *Pellionia* in Bidoup



Scientific name: Urticaceae *Elatostema bidoupensis* sp. nov.

Local name:

No. V4390

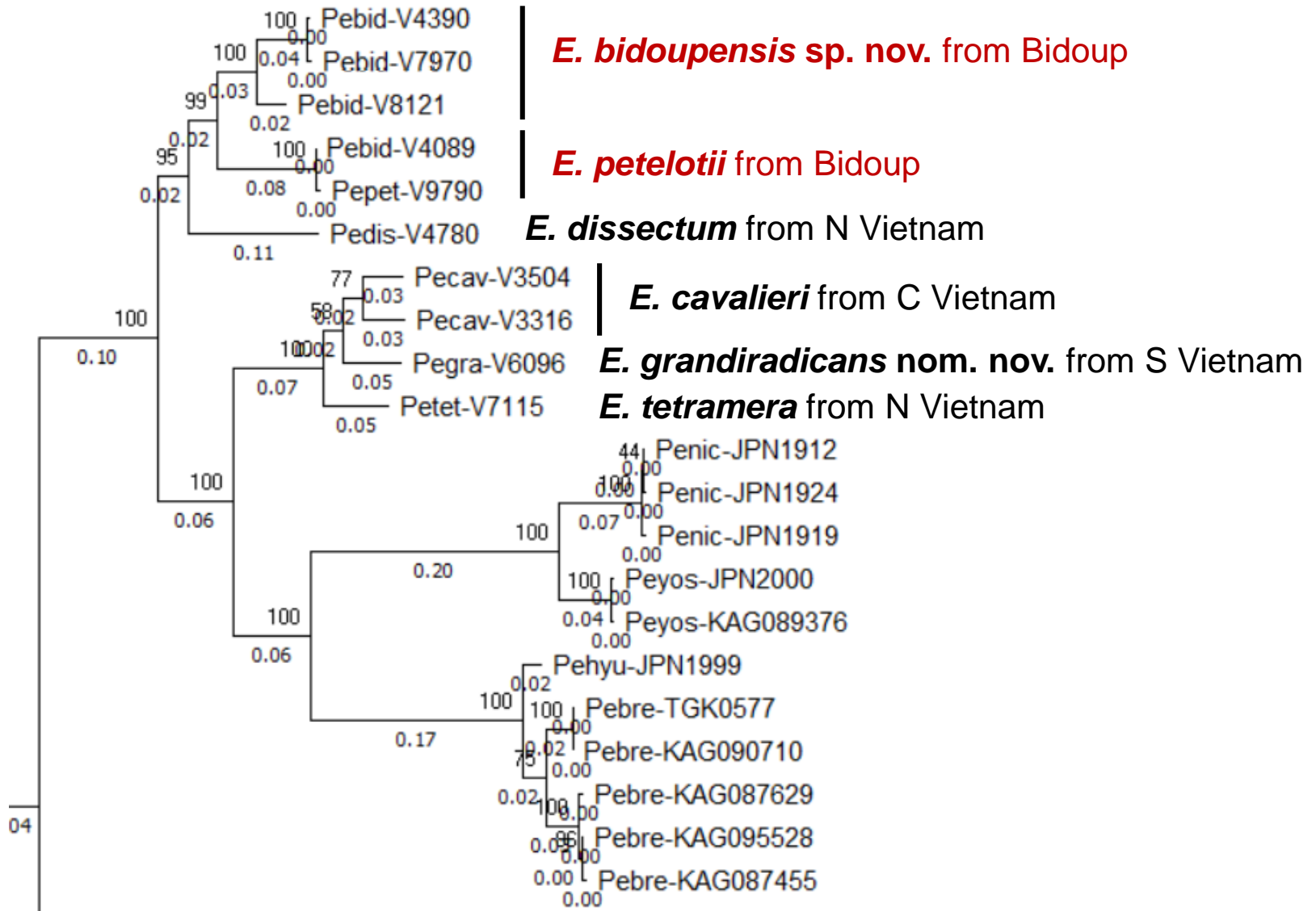
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Vietnam_Bi Doup-Nui Ba NP

Outside (alt. 1489 m)



Elatostema subgen. *Pellionia* in Bidoup



Scientific name: Urticaceae *Pellionia petelotii* Gagnep.

Local name:

No. V4089

#

Bi Doup-Nui Ba NP
(alt. 1533 m)



Scientific name: Urticaceae *Pellionia petelotii* Gagnep.

Local name:

No. V9790

#

Vietnam_Bidoup Nui Ba
Giang Ly (alt. 1460 m)



Hydrangea acuminata ssp. *yakushimensis* ssp. nov. from Yaku Island, Japan

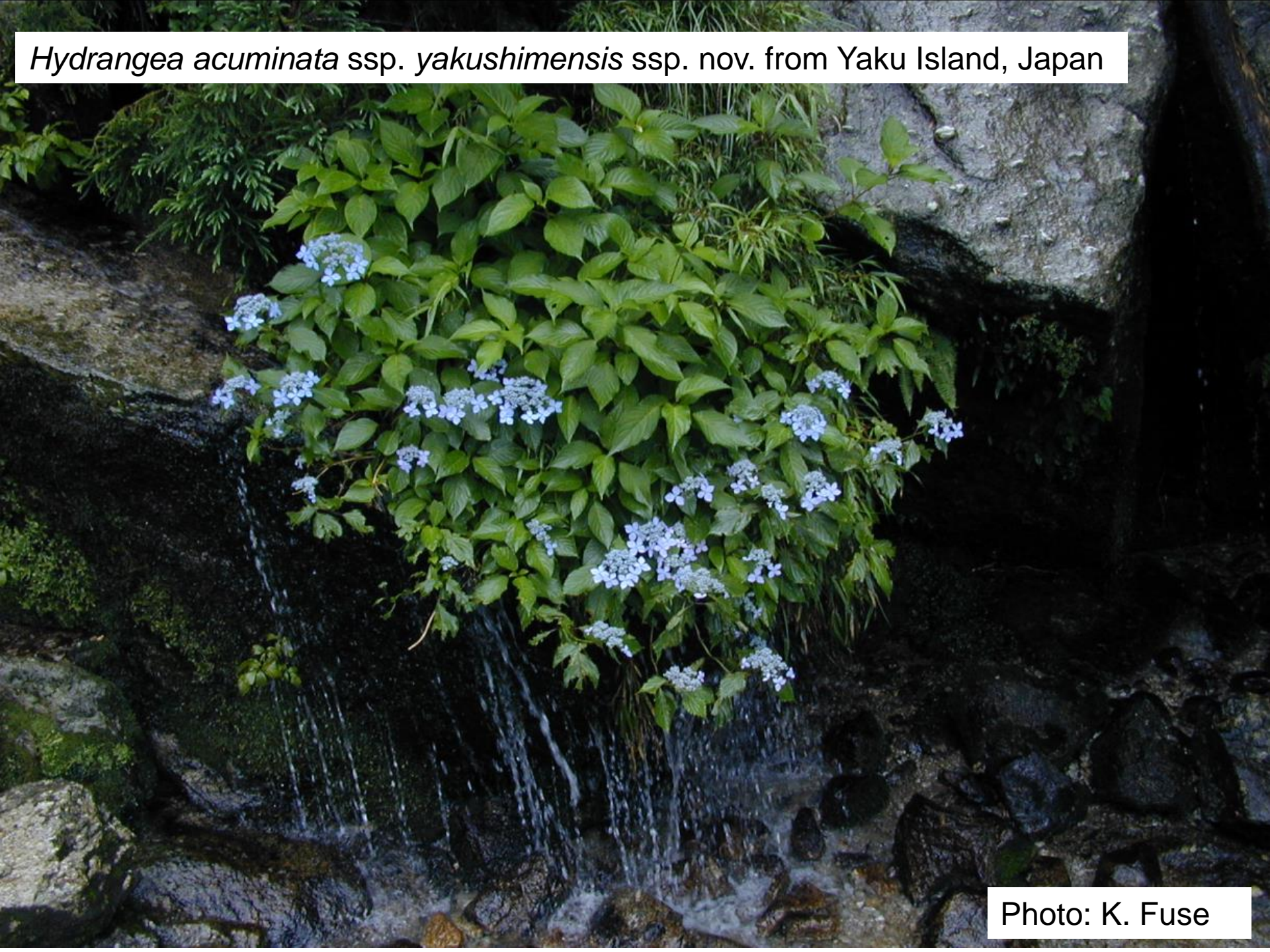
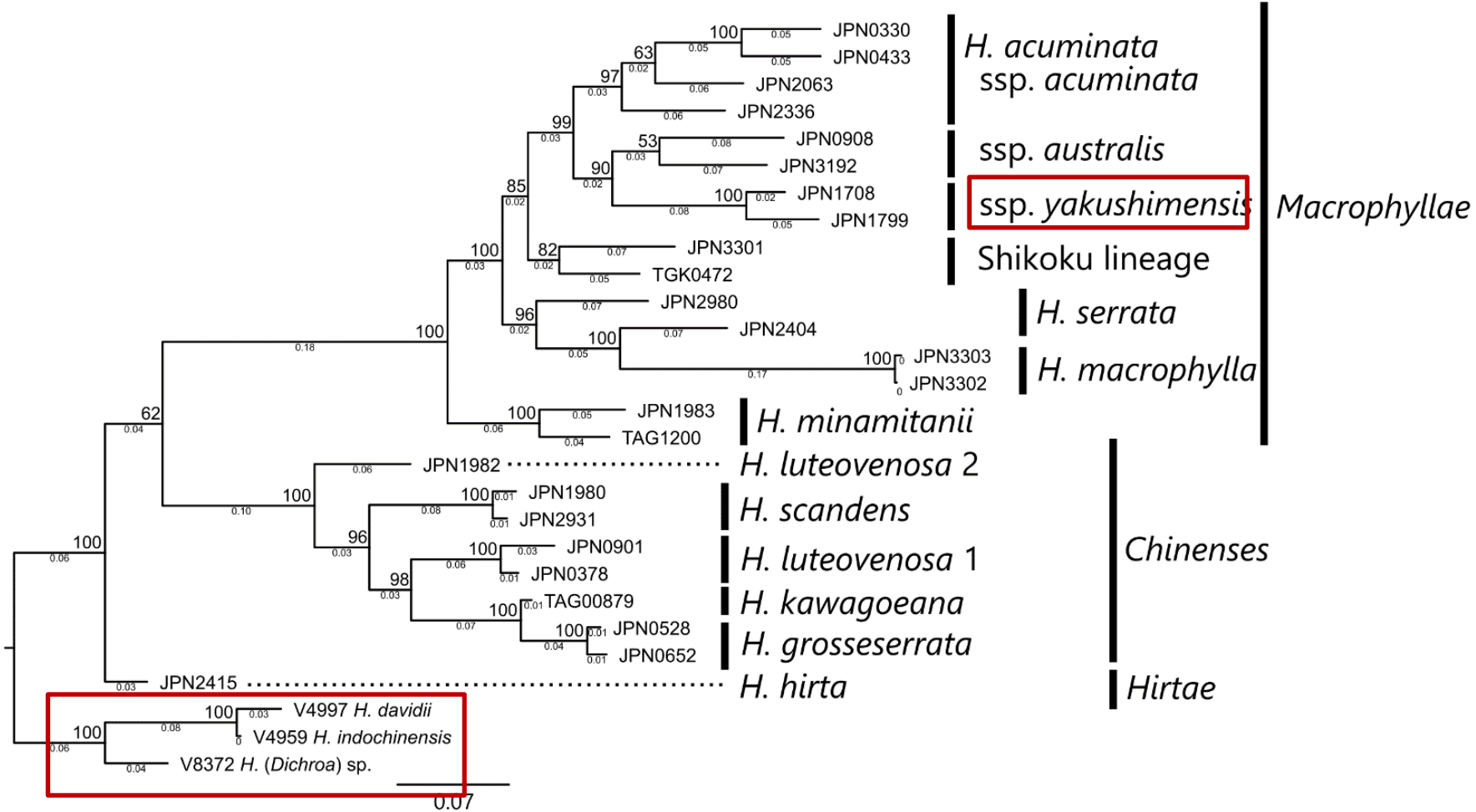
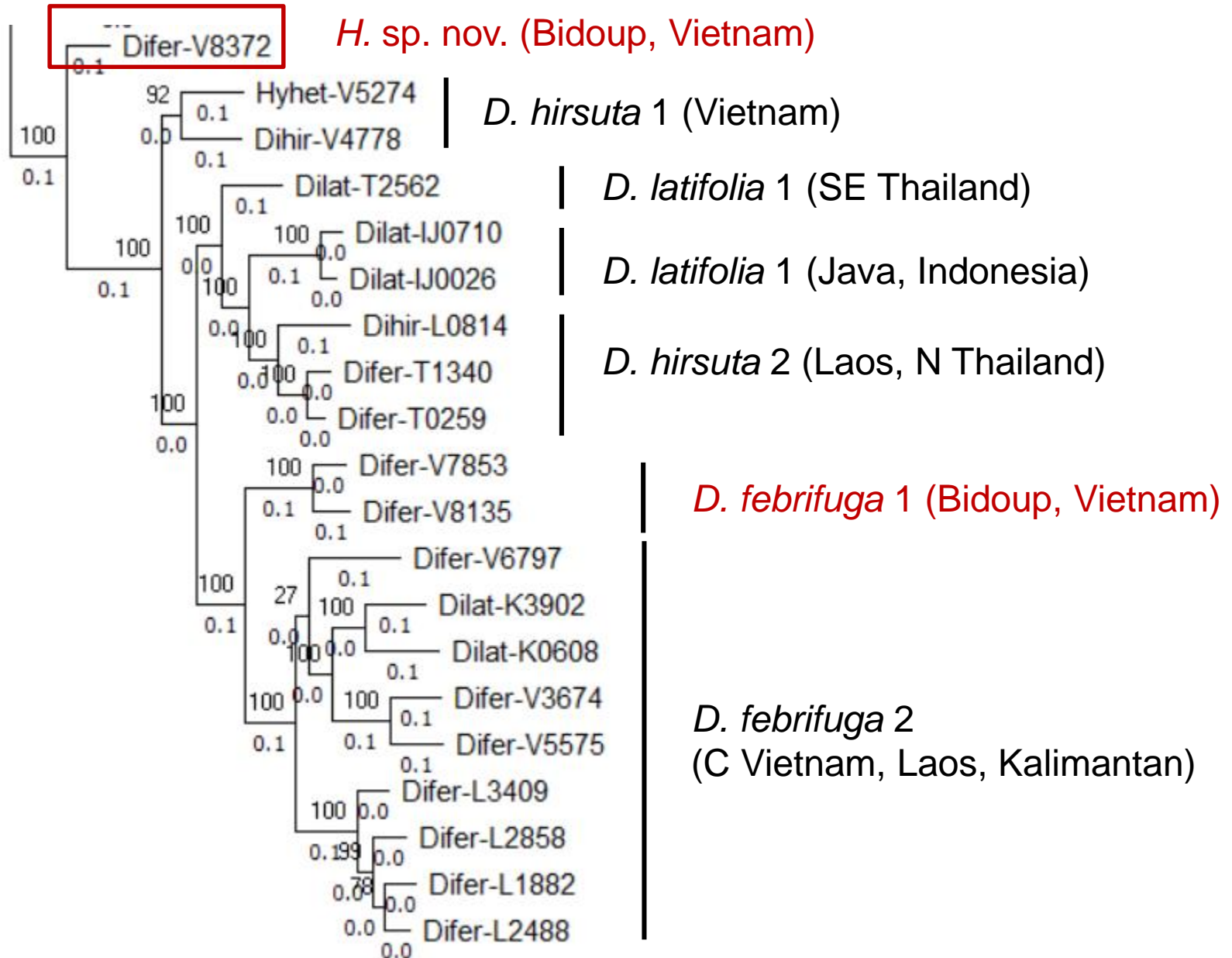


Photo: K. Fuse

MIG-seq tree of *Hydrangea*



MIG-seq tree of *Hydrangea* subgen. *Dichroa*



Scientific name: Hydrangeaceae *Hydrangea (Dichroa)* sp. nov.

Local name:

No. V8372

#

Vietnam Biduop Nui Ba
Yellow Plot (Alt. 1669 m)



Scientific name: Hydrangeaceae *Hydrangea (Dichroa)* sp. nov. [aff. *febrifuga*]

Local name:

No. V8135

#

Bi Doup Nui Ba

Mt. Langbian_(alt. 1962 m)



Scientific name: Hydrangeaceae *Dichloa febrifuga* Lour.

Local name:

No. V5575

#

Vietnam_Vu Quang
roadside (alt. 43 m)



Conclusion

- We can efficiently explore the flora of a particular area by **collecting all species including sterile plants** within plots of 100m x 5m placed along altitudinal gradients and identifying them with **MIG-seq**.
- MIG-seq analyses **using both Japanese and SE Asian samples** are promising, in the current situation where we cannot go out in the field.
- **Many new species of vascular plants** remain to be described from both Japan and SE Asia. Most of them are threatened. We should hurry to describe new species.

Acknowledgements

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Dokrak Marod, Kasesart Univ., Thailand

Mu Mu Aung, Forest Research Institution, Myanmar

Ferry Slik, University of Brunei Darussalam

Rimi Repin, Sabah Parks Malaysia

Mohizah Bt. Mohamad, Sarawak Herbarium, Malaysia

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Arief Hidayat, Research Center for Biology-LIPI, Indonesia

Anes Syamsuardi, Andalas Univ., Indonesia

Ngakan Putu Oka, Hasanudin Univ., Indonesia



Herbarium: ANDA, BK, BKF, BM, BO, BRUN, FOF, FU, HN, HNL, K, KAG, KEP, L, P, QBG, RAF, RUPP, SAR, SING, TI, TNS, VNM