ISSN 1346−7565 Acta Phytotax. Geobot. 59 (3): 183−193 (2008)

Two Species of *Entada* in Japan as Evidenced by cpDNA Phylogeny

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The phylogenetic relationships of *Entada* (Fabaceae) were examined to determine if two distinct species occur in Japan. Nucleotide sequences of the *trnK* and *trnL* introns and the intergenic spacer of *trnL–trnF* of cpDNA resolved 19 haplotypes among 39 widely collected samples of *E. phaseoloides*-like plants. In the resultant tree, two distinct clades corresponded to two groups also distinguished by diagnostic seed characters. The clades agree with the expected chorology of samples and with the diagnostic morphology from previous taxonomic work. One clade corresponds to *Entada phaseoloides* and the other to *E. tonkinensis*. A distinctive concentration of haplotypes was observed. Although seeds of *Entada phaseoloides* are renowned as sea drift seeds, our results do not suggest frequent gene flow caused by long distance seed dispersal.

Key words: *Entada koshunensis, Entada phaseoloides, Entada tonkinensis,* match-box bean, seed dispersal

Entada Adans. (Leguminosae: Mimosoideae) consists of about 30 species (Nielsen 1981, 1992, ILDIS 2005) distributed in the tropics and subtropics. While more than half of the species in *Entada* are shrubs or subshrubs with small pods, some species are lianas with gigantic pods that can be more than 1 m long. The large woody seeds of *Entada* are also known for drifting in seawater. These remarkable seeds are called "match-box beans" (the common name for *Entada phaseoloides* (L.) Merr. and *E. rheedii* Spreng.) or "sea hearts" (*E. gigas* (L.) Fawc. & Rendle), and are frequently found on beaches far from their origin.

Entada phaseoloides is widely distributed in tropical and subtropical areas of the Pacific, from eastern to southeastern continental Asia, the south Asian islands, Oceania, and Polynesia. The northeastern limit of the species' range is Japan (Nielsen 1992, Huang & Ohashi 1993). The wide distribution of *Entada phaseoloides* is thought to have been achieved by long distance dispersal of the seeds. Although *Entada phaseoloides* has some distinctive features in its fruits and seeds, considerable taxonomic confusion exists as to its classification in Japan and adjacent areas. While most studies treat the species of *Entada* in Japan as *E. phaseoloides*, some works have variously treated it as *E. rheedii* Spreng., *E. parvifolia* Merr., or *E.*

koshunensis Hayata & Kaneh. Some authors recognize two distinct species (Ho 1985, Huang & Ohashi 1993, Ohashi 2001), but the recognition of *E. rheedii* and *E. parvifolia* in the Ryukyu Islands (Okinawa), Japan, is apparently misleading and *E. koshunensis* cannot readily be separated from *E. phaseoloides* according to a detailed taxonomic study that focused on this taxonomic confusion (Tateishi *et al*. 2008).

Recently, Wakita *et al*. (2005) surveyed the number of leaflet pairs on the second pinnae, morphological characters of the seeds, and single strand conformation polymorphism (SSCP) band patterns of the *rps16* intron in chloroplast DNA (cpDNA) for species of *Entada* in the Ryukyu Islands. Although the number of pairs of leaflet on the second pinnae did not clearly divide the samples into two groups, the seeds and molecular markers clearly indicated the two groups of *Entada*. The first group has a small ('S'), convex seed type, 29.4–46.9 mm wide by 32.4–52.7 mm long with an angular margin. The second group has a large ('L'), compressed seed type, 39.5–59.1 mm wide by 45.8–64.7 mm long with a rounded margin (Wakita *et al*. 2005). Different SSCP band patterns, caused by a nucleotide substitution and some indels in the *rps16* intron, were also clearly observed in the two groups. Wakita *et al*. (2005) considered the first group as corresponding *Entada koshunensis* and the second to *E. phaseoloides* based on seed characteristics, following the criteria of Ho (1985) for distinguishing species of *Entada* in Taiwan. Wakita *et al*. (2005), however, did not resolve the taxonomic status of these two groups due to insufficient sampling limited only to the Ryukyu Islands. A molecular phylogenetic study using additional samples from a wider geographic area is necessary to clarify the presence of two species in Japan.

In this paper, we report the results of molecular phylogenetic analyses using samples obtained from a broad geographical range, including the presumed type locality of *Entada phaseoloides* in Indonesia. Key seed shape characters used in Wakita *et al*. (2005) were also examined to determine the correspondence between seed character groups and molecular phylogenetic groups. The goal of this study was to reveal the phylogenetic relationships of *Entada phaseoloides* and closely related species, and to determine if indeed two species of *Entada* occur in Japan.

Materials and Methods

Entada phaseoloides-like plants were collected from eastern to southeastern Asia and the South Pacific (Table 1). Samples from Indonesia and Vanuatu were obtained from individuals cultivated in the Bogor Botanical Garden (Bogor, Indonesia) and the Tsukuba Botanical Garden (Ibaraki, Japan), respectively (Table 1). Since plants of *Entada* are generally very large, spreading over the forest canopy, distinguishing individuals was difficult. We collected more than one sample per population only when individuals could be clearly distinguished. Seed samples were not obtained from all the populations. Key seed shape characters of all available seeds were observed, and seed type (S or L) was determined according to Wakita *et al*. (2005).

Leaf samples of the species of *Entada* obtained from eastern and southeastern Asia, Oceania, and the South Pacific were used for a molecular phylogenetic study (Table 1). *Entada gigas* (L.) Fawc. & Rendle, *E. rheedii*, and *E. spiralis* Ridley, which belong to the same subsection (*Entada* subsect. *Entada*) as *E. phaseoloides*, were also included in the phylogenetic analysis. Outgroups were *Entada abyssinica* Steud. (from Tanzania) and *E. polystachya* DC. (from Mexico). Those species were confirmed as not being in the ingroup by our preliminary analysis (N. Wakita et al., unpublished data) using published *trnL–trnF* sequences of Mimosoideae (Luckow *et al*. 2000,

2003). A total of 47 samples, including outgroups, was used for the sequence analysis. Voucher specimens of the samples used in this study were deposited in QCNE, TI, TNS, or URO (Table 1).

Prior to genomic DNA extraction, crushed leaf tissue was washed using HEPES buffer following the methods of Setoguchi & Ohba (1995). Total genomic DNA was extracted from washed leaf pellets using the methods of Doyle & Doyle (1987). The two regions of cpDNA, the *trnL* intron including the *trnL–trnF* intergenic spacer (IGS; hereafter referred to as *trnL–trnF*) and the *trnK* intron (including *matK*; hereafter referred to as *trnK*), were amplified by PCR. The following oligonucleotide primers were used for PCR amplification and sequencing: c, d, e, and f for *trnL–trnF*, designed by Taberlet *et al*. (1991); *trnK*-F (forward: 5′-TGGGTTGCTAACTCAACGG-3′) and *trnK*-R (reverse: 5′-GGAACTAGTCGGATGGAGT-3′) designed according to the complete cpDNA sequence data of tobacco (GenBank accession numbers Z00044 and S54304), matK-10 (Kato *et al*. 1998), matK-AF (Takayama *et al*. 2005), matK-1777L, and matK-1932R (Hu *et al*. 2000) for trnK. The PCR reaction mixture contained 2.0 μL of 0.2 mmol/L dNTP solution, 1.0 μL of 5 mmol/L of each primer, 0.5 unit of ExTaq DNA polymerase (TaKaRa Bio, Ohtsu, Shiga, Japan), 2.5 μL of 10× ExTaq buffer, and 10–30 ng of genomic DNA in a total volume of 25 μL. PCR amplification was performed in a PCR Thermal cycler Dice (TaKaRa Bio). The PCR cycle for *trnL–trnF* was 95°C for 1 min; followed by 25 cycles at 95°C for 45 s, 51°C for 1.5 min, and 72° C for 1.5 min; and a final extension at 72°C for 10 min. For *trnK*, the settings were 96°C for 1 min; followed by 35 cycles at 96°C for 45 s, 48°C for 1 min, and 72°C for 1.5 min; and a final extension at 72°C for 15 min. The extension time in both PCR programs was increased by 2 s every cycle. Amplified DNA was purified using a GeneClean III DNA purification Kit (BIO 101, Carlsbad, CA, USA). Purified DNA was directly sequenced using an ABI PRISM Big Dye Terminator version 3.1 Cycle Sequencing Ready Reaction kit and an ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Multiple sequence alignment was performed with ClustalX ver. 1.83 (Thompson *et al*. 1994) using the default setting, and the obtained alignment was manually modified using Se-Al Sequence Alignment Editor version 2.0a11 (Rambaut 2002). Combined nucleotide sequences were used for further analyses because the incongruence length difference (ILD) test implemented in PAUP* ver4.0. detected no significant incongruence between the two genetic regions for phylogenetic analyses.

Maximum-parsimony (MP) and neighbor-joining (NJ) analyses were conducted using PAUP* version 4.0 beta 10 (Swofford 2002). Branch support was assessed with 1,000 replicates for MP and 10,000 for NJ. A Bayesian phylogenetic analysis was performed in MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003) with the best-fit model (GTR+I+G) for both of *trnL–trnF* and *trnK* selected by Akaike's information criterion in MrModeltest 2.2 (Nylander *et al*. 2004). Metropolis-coupled Markov chain Monte Carlo sampling was performed with one cold chain and three heated chains that were run for 1,000,000 generations. Trees were sampled every 100 generations. In total, 10,000 trees were obtained and the first 2,500 samples from each run were discarded as burn-in to ensure the chains had become stationary. A majority rule consensus tree was generated to compute posterior probabilities. Insertion/deletion events (indels) were not included in the NJ and Bayesian analyses. Indels of more than two base pairs were treated as binary (0/1) characters for the MP analysis if not caused by a simple sequence repeat.

TABLE1. *Entada* samples used in this study. Clade and haplotype are designated as in Fig. 2. Seed type is according to Wakita *et al*. (2005). Accession numbers are shown on the first line for each haplotype.

Taxon	Locality		Voucher specimen	Clade	Haplotype	Seed type	Accession No.	
							trnK	*trnL–F*
Entada								
Entada phaseoloides-like samples								
JAPAN	Okinawa Isl.	Ibu, Okinawa Pref.	*N.W & Matsumura 210526-1,2*(URO)	I	P01	S	EU328402	EU328465
	Ishigaki Isl.	Uganzaki, Okinawa Pref.	*N.W 210722* (URO)	I	P01	—		
	Ishigaki Isl.	Nagura, Okinawa Pref.	*N.W & Matsumura 210722b* (URO)	I	P06	—	EU328407	EU328470
	Ishigaki Isl.	Motonagura, Okinawa Pref.	*N.W & Matsumura 210722c* (URO)	I	P05	—	EU328406	EU328469
	Ishigaki Isl.	Sokohara, Okinawa Pref.	*N.W 210614* (URO)	I	P06	—		
	Ishigaki Isl.	Ota, Okinawa Pref.	*N.W & Tateishi 231111* (URO)	I	P04	—	EU328405	EU328468
	Kohama Isl.	Kohama, Okinawa Pref.	*N.W 220613* (URO)	I	P01	—		
	Iriomote Isl.	Yutsun riv., Komi, Okinawa Pref.	*N.W 230917* (URO)	I	P02	S	EU328403	EU328466
	Iriomote Isl.	Funaura, Okinawa Pref.	*N.W 220612a* (URO)	I	P08	S	EU328409	EU328472
	Iriomote Isl.	Nakama riv., Ohara, Okinawa Pref.	*N.W 220612b* (URO)	I	P07	—	EU328408	EU328471
	Yonaguni Isl.	Higawa, Okinawa Pref.	*N.W 210610* (URO)	I	P09	S	EU328410	EU328473
	Yaku Isl.	Anbo, Kagoshima Pref.	*N.W & Y. Tateishi 210622* (URO)	II	T01	L	EU328419	EU328482
	Amami-Ohshima Isl.	Sumiyo, Kagoshima Pref.	*N.W & Y. Tateishi 210624-1,2* (URO)	II	T01	L		
TAIWAN	Southern part	Chiopeng, Pingtung Co.	*N.W 240922-1,2,3* (URO)	I	P01	S		
	Southern part	Nanjen-shan, Pingtung Co.	*N.W 240923* (URO)	I	P03	—	EU328404	EU328467
	Northern part	Juntou, I-lan Co.	*N.W 240925-A,B* (URO)	II	T01	—		
	Northern part	Tatung, I-lan Co.	*N.W 240926-A,B* (URO)	II	T01	L		
CHINA	Hong Kong	Tai Po Kau, Man Ping, Hoi Ha	*T. Ohi-Toma 20050504* (TI)	II	T01	—		
VIETNAM	Northern part	Vinh Yen Prov.	*Y. Tateishi 1283* (URO)	II	T02	L	EU328420	EU328483
	Northern part	Ninh Binh Prov. Cuc Phuang National park	*J. Murata et al. 20041218-39* (TI)	II	T02	L		
INDONESIA	Ambon Isl.	Cultivated in the Bogor Botanical Garden	*N.W 250510-E25* (TI)	I	P13	—	EU328414	EU328477
	Java Isl.	Cultivated in the Bogor Botanical Garden	*N.W 250509-B8* (TI)	I	P11	S	EU328412	EU328475
	Java Isl.	Cultivated in the Bogor Botanical Garden	*N.W 250509-E43a* (TI)	I	P14		EU328415	EU328478
	Kai Isls.	Cultivated in the Bogor Botanical Garden	*N.W 250509-E35* (TI)	I	P14	—		
	Sulawesi Isl.	Cultivated in the Bogor Botanical Garden	*N.W 250510-E18* (TI)	I	P12	—	EU328413	EU328476
	Ternate Isl.	Cultivated in the Bogor Botanical Garden	*N.W 250510-E05* (TI)	I	P12	—		
AUSTRALIA	Queensland	Daintree National Park	*T. Kajita & K. Takayama 04121301* (TI)	I	P15	—	EU328416	EU328479
	Queensland	Daintree National Park	*T. Kajita & K. Takayama 04121303* (TI)	I	P17	S	EU328418	EU328481
VANUATU	Efate Isl.	Cultivated in the Tsukuba Botanical Garden	*Konishi 1299* (TNS)	I	P10	S	EU328411	EU328474
SAMOA	Upolu Isl.	Southern coast, Safata	*T. Kajita et al. 02102605* (TI)	I	P16	S	EU328417	EU328480
	Upolu Isl.	Southern coast, Safata	*T. Kajita et al. 02102801* (TI)	I	P17	S		
E. rheedii Spreng.								
THAILAND		Chiang Mai, Doi Sutep	*Y. Tateishi 33040* (URO)		R01		EU328421	EU328484
		Khao Yai	*J. Murata et al. 18 Sept. 2001*(TI)		R02		EU328422	EU328485

AUSTRALIA	Queensland	York Cape Peninsula	*Y. Tateishi & Omine 53109* (URO)	R03	EU328423	EU328486
INDONESIA	Java Isl.	Cultivated in the Bogor Botanical Garden	*N.W 250510-G27* (TI)	R04	EU328424	EU328487
E. spiralis Ridley						
THAILAND		Chiang Mai, Doi Sutep	*Y. Tateishi 33009* (URO)	S01	EU328425	EU328488
E. gigas (L.) Fawc. & Rendle						
MEXICO		Veracruz	*N.W et al. 241125* (TI)		EU328426	EU328489
Outgroup						
Entada sect. *Neoentada* Harms, *pro parte*, *emend*. Brenan.						
E. abyssinica Steud. ex A. Rich.						
TANZANIA		Iringa	*Gereau 2720* (QCNE)		EU328427	EU328490
Entada sect. *Entadopsis* (Britton) Brenan						
E. polystachya DC.						
MEXICO		San Blas, Nayarit	*N.W et al. 241119A* (URO)		EU328428	EU328491

N.W; First author in this study.

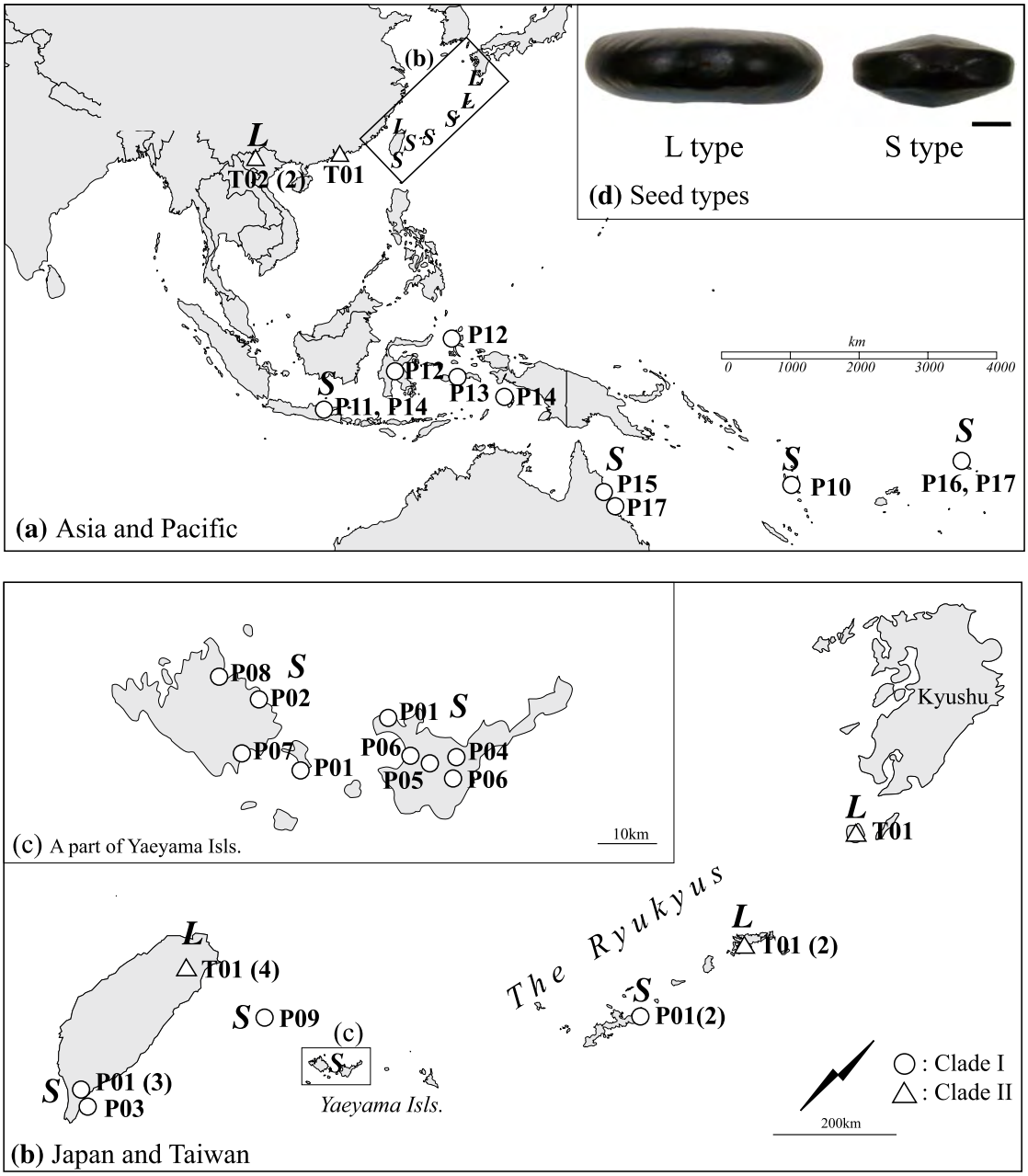

FIG. 1. Geographic distribution of *Entada* haplotypes. Haplotypes are designated by name as they appear in Table 1 and Fig. 2. Circles and triangles indicate the clade to which each haplotype belongs (see Fig. 2). Numbers in parentheses after haplotype names indicate numbers of individuals. Seed type is shown as *S* or *L* according to Wakita *et al*. (2005). (a)–(c): Distribution maps. (d): Pictures showing seed types. Scale bar = 1cm.

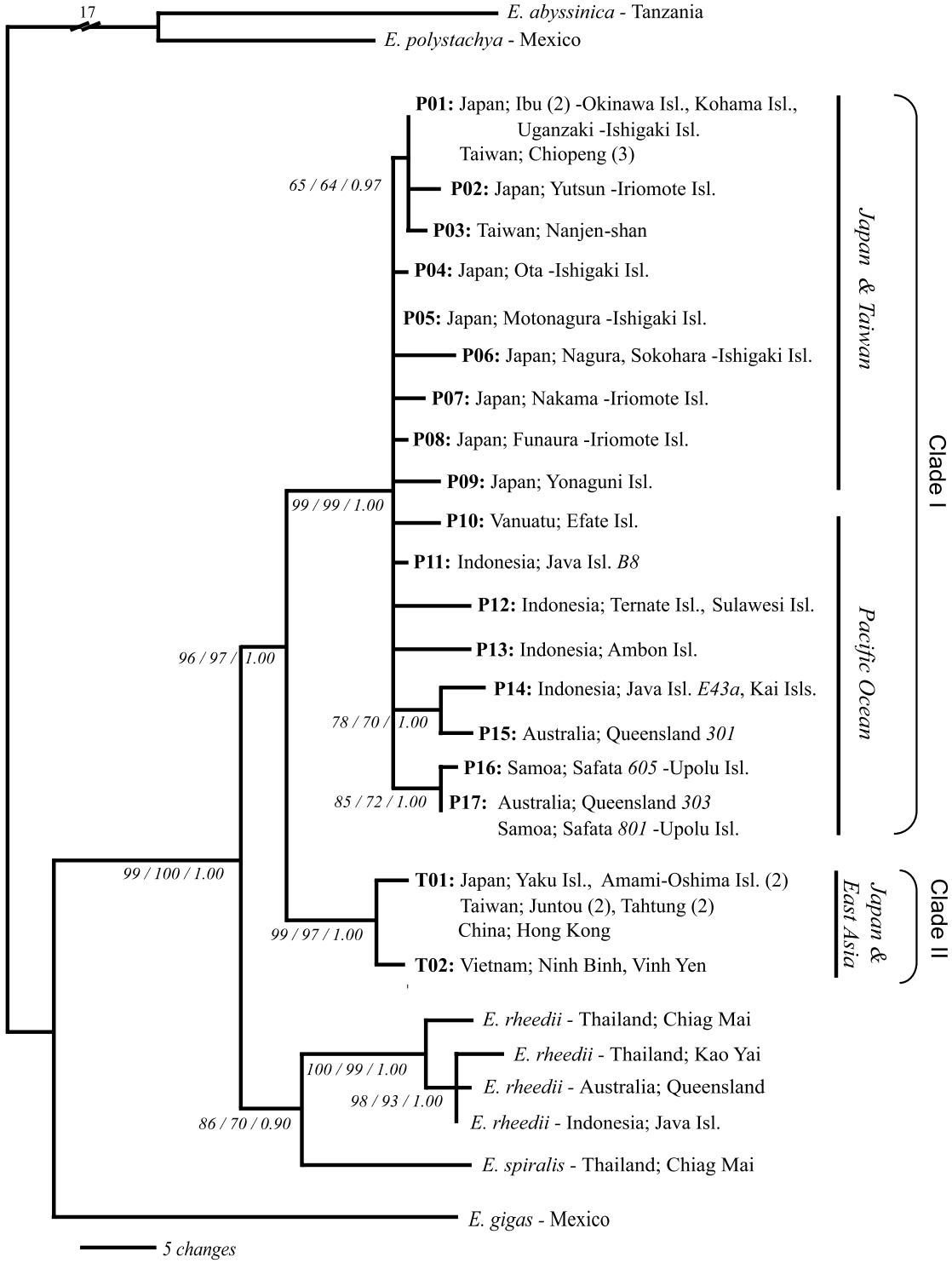

FIG. 2. Strict consensus tree of 123 most parsimonious trees of *E. phaseoloides*-like samples and their allies obtained from *trnL–trnF* and *trnK* sequences (length = 202 steps, CI = 0.861, RI = 0.8607, RC = 0.741). Measures of support are given at each node: NJ bootstrap support/MP bootstrap support/Bayesian posterior probability.

Results

Seed morphology

Using the key character of seed shape reported in Wakita *et al*. (2005), the seeds were sorted into one of two groups. The seed type assigned to all available seeds is shown in Table 1. Type S seeds were obtained from Japan (Okinawa), southern Taiwan, Indonesia, Australia, Vanuatu, and Samoa. Type L seeds were obtained from Japan (Kagoshima), northern Taiwan, China, and Vietnam. The geographic distribution of seed types is mapped in Fig. 1.

Molecular analyses

The *trnL–trnF* sequence was 1,050–1,057 bp long and the *trnK* sequence was 2,513–2,537 bp long. Using the combined sequences, 19 haplotypes were resolved among the 39 *Entada phaseoloides*-like samples. Twenty-four indels were obtained across the matrix of all haplotypes and three were coded as potentially phylogenetically informative for parsimony analyses. The phylogenetic analysis using *Entada abyssinica* and *E. polystachya* as outgroups produced 164 most parsimonious trees of 226 steps, with a consistency index of 0.876, retention index of 0.877, and rescaled consistency index of 0.769 (Fig. 2). The NJ and Bayesian tree topologies were nearly consistent with the MP tree presented here (Fig. 2). *Entada gigas* was sister to another clade, which was further divided into two major clades. One of those clades was composed of *Entada rheedii* and *E. spiralis* (branch support (BS) by bootstrap or posterior probability of 71 (MP analysis), 85 (NJ analysis), and 0.90 (Bayesian analysis); BS is presented in this order in the following text). The second clade was composed of all *Entada phaseoloides*-like samples (BS of 96, 96, and 1.00). This latter clade was further subdivided into two clades (Clades I and II), both of which were very well supported (BS of 99, 99, and 1.00 for Clade I and 98, 99, and 1.00 for Clade II). Clade I consisted of samples from southern Taiwan, Vanuatu, Samoa, Australia, and Indonesia, where S-type seeds were obtained. Clade II consisted of samples from northern Taiwan, Hong Kong, and Vietnam, where L-type seeds were obtained. Samples collected from the type locality of *Entada phaseoloides* (Ambon Island, Indonesia) were included in Clade I.

Discussion

Presence of two species of Entada *in Japan*

The two well-defined clades (I and II) in our phylogenetic tree are clearly consistent with the two seed type groups (S or L) recognized by Wakita *et al*. (2005). Our results clearly indicate the presence of two species of *Entada* in Japan.

Entada phaseoloides was originally described by Merrill (1917). The type specimen (drawn as *Faba marina* in tab 4, Herbarium Amboinense 5: 5–8, Rumphius 1747) was obtained from Ambon, Indonesia. According to some floristic works that treated this species (Walker 1976, Nielsen 1992), the diagnostic characters of *Entada phaseoloides* are 1–3(or 4) pairs of asymmetric elliptic leaflets and seeds 3–6 cm long. *Entada phaseoloides* is distributed throughout subtropical to tropical areas from Southeast Asia to the Pacific islands. This general characterization is applicable to all samples considered to be *Entada phaseoloides*-like in this study. The wide distribution reported thus far may represent the sum of the two species revealed here. Our study, however, clearly suggests distinctive distribution areas for the two species. Samples included in Clade I were from Southeast Asia to the South Pacific, whereas samples in Clade II was collected only in areas extending from Vietnam to Japan (Fig. 1). Since the sample obtained from the type locality of *Entada phaseoloides* (Ambon Island, Indonesia) appears in Clade I,

we conclude that this clade corresponds to *E. phaseoloides*. All available seeds obtained from samples in Clade I were of the S type. Thus, the diagnostic seed character of *Entada phaseoloides* is a convex seed with an angular margin, and the range of *E. phaseoloides* encompasses the islands of eastern and southeastern Asia, a part of Oceania, and Polynesia (Fig. 1).

The second clade (Clade II) was composed of samples from Japan (Amami-Oshima and Yaku islands), northern Taiwan, Hong Kong, and Vietnam. All available seeds from these samples were type L (Table 1, Fig. 1). The samples comprising Clade II have generally been misidentified in Japan and Taiwan as *Entada phaseoloides*. After surveying various taxonomic works from Japan, Taiwan, China, and Vietnam, the seed morphology of *Entada tonkinensis* Gagnep. appears to be consistent with the samples in Clade II. *Entada tonkinensis* was originally reported from Vietnam (Gagnepain 1911), and its characteristically large seeds were recorded as 50 mm wide and 60 mm long. Clade II contained samples from Vietnam and the seeds obtained from this clade were 39.5 –59.1 mm wide and 45.8–64.7 mm long, which is consistent with the seed size reported for *E. tonkinensis*. Although the species has not been reported outside Vietnam, our study suggests a wider distribution extending to China, Taiwan, and Japan.

Unlike the above discussion, Wakita *et al*. (2005) assigned *Entada phaseoloides* to the group with type L seeds and *E. koshunensis* to the group with type S seeds, based on samples obtained from the Ryukyu Islands. Their assignment of *Entada phaseoloides* to the type L seed group is not likely correct, as discussed above. Regarding their assignment of *Entada koshunensis* to the type S seed group, our phylogenetic tree does not show clear evidence to distinguish this species. *Entada koshunensis* was originally described by Hayata (1921) based on a sample collected in the Henchun Peninsula, southern Taiwan. Some studies distinguish this species from *Entada phaseoloides* based on its smaller seeds (ca. 3 cm) and greater number of leaflet pairs (Hayata 1921, Ho 1985, Huang & Ohashi 1993), but other studies treat it as *E. phaseoloides* because of its morphological resemblance (Walker 1976, Huang & Ohashi 1977). In this study, four samples collected from the type locality of *Entada koshunensis* (Henchun Peninsula, southern Taiwan) had haplotypes P01 and P03. These haplotypes were included in Clade I and formed a clade having weak support with samples from the Ryukyu Islands (Fig. 2), but no clear morphological or biogeographical association with this clade was observed. Further taxonomic study also supports the difficulty in distinguishing *Entada koshunensis* as a separate species (Tateishi *et al*. 2008).

Geographic distribution of E. phaseoloides *and* E. tonkinensis *haplotypes*

This study revealed the approximate distributions of haplotypes of *Entada phaseoloides* and *E. tonkinensis* (Fig. 1). Both species have very large seeds that can drift in seawater, which might contribute to their population structure. Other sea-dispersed plants have recently been studied using molecular markers and evidence of long distance dispersal in haplotype structure has been reported (Takayama *et al*. 2006, Nettle & Dodd 2007). For example, *Hibiscus tiliaceus* L. (Malvaceae) has a wide distribution throughout the Old World tropics. Common haplotypes were distributed throughout the Pacific and Indian ocean areas, and genetic differentiation among populations was very low (Takayama *et al*. 2006). However, our results from the study of *Entada* in Asia and the Pacific region are somewhat contradictory. Based on the distribution of haplotypes (Fig. 1), few widespread haplotypes in *Entada phaseoloides* are observed and a geographic concentration of haplotypes appears to exist. Haplotypes P01–

P09 occur in Japan and Taiwan, haplotypes P11–P14 in Indonesia, and haplotypes P10 and P15–P17 in Australia and Samoa (Fig. 1). Although the number of samples surveyed was limited, no common haplotypes were found among these regions. The only case of long distance distribution of a common haplotype is in Australia and Samoa, separated by about 5,300 km, for haplotype P17.

The seeds of *Entada phaseoloides* are viable for at least 1 year in seawater (Nakanishi 1994); thus, seeds can be dispersed by ocean currents over very long distances. The distribution of each haplotype, however, was geographically limited (Fig. 1). One possible explanation for this is the low ability of seeds to establish in new localities after successful dispersal. *Entada phaseoloides* is rarely seen in littoral areas and is more common in the canopy of inland forest. Although seeds frequently reach open sandy beaches, subsequent colonization may be difficult. Other factors such as the direction of ocean currents may also result in the geographically limited haplotype distribution. Further studies using more samples from various localities are necessary to clarify this issue.

For *Entada tonkinensis*, Clade II included only two haplotypes, whereas Clade I of *E. phaseoloides* contained 17 haplotypes from 23 samples. This perhaps reflects the population size and distribution ranges of these two species. In Clade II, a common haplotype (T01) was found in eight populations in five regions (Fig. 2). The distribution of haplotype T01 might have been due to dispersal of seeds from continental Asia northward to Taiwan and Japan along the island chain, as the habitat of *Entada tonkinensis* in Japan and Hong Kong is on slopes exposed to the sea at 20–150 m. The occurrence in Taiwan, however, is inland about 15–30 km from the seashore. How this species has successfully colonized an inland forest from the seashore on which seeds washed ashore is still unknown. As the vines of this species grow very large, individual growth and seed production over many years might be one explanation for the colonization of an inland habitat.

In this study, a phylogenetic analysis using cpDNA sequences revealed that two distinct phylogenetic groups of *Entada* are present in Japan. Morphologically, one clade corresponds to *Entada phaseoloides* and the other to *E. tonkinensis*. Although seeds of *Entada phaseoloides* are remarkable examples of sea-drifted seeds, evidence of frequent long distance seed dispersal was not obtained. Even if the seeds can be dispersed by ocean currents across very long distances (Ridley 1930), the geographical concentration of haplotypes suggests that another factor, such as difficulty in establishment, prevents greater gene flow among regions.

We thank Drs B. Adje, K. Chayamarit, C. Nyomdham, T. Yukawa, Josph Yip, L. K. C. Chau, N. N. Thin, S. Matsumura, D. Neil, and Bogor Botanical Garden, MEXU, QCNE for their help in obtaining samples. We also thank Drs Y. Watano, T. Denda, T. Asakawa, K. Takayama for their valuable comments and useful suggestions during this study. We also express our great thanks to Drs G. Kenicer and D. Boufford for critically reading the manuscript. This work was mainly supported by JSPS KAKENHI No. 16370043 (to T.K.) and 14405015 (to Y.T.). Part of the study and sampling were supported by JSPS KAKENHI for foreign expeditions 14405015 and 12575011 (to Y.T.) and 17255004 to (J.M.). This work is part of the PhD dissertation of N.W.

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Received November 23, 2007; accepted April 24, 2008