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Cycas hoabinhensis, an endangered stenoendemic of Vietnam

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Summary: Cycas hoabinhensis is a typical local paleoendemic with a strictly limited distribution in the central part of northern Vietnam It g grows in evergreen, broad-leaved lowland limestone forests at elevations of 70–250 m a.s.l. and is usually associated with about 130 native species of vascular plants (110 genera and 47 families). Three of them were reported as new species for science and four as new records for the flora of Vietnam. The morphological variation of *C. hoabinhensis* has been studied in a series of individuals from different subpopulations. Updated global conservation status of *C. hoabinhensis* is assessed as Endangered A2cd; B1ab (ii, iv, v). The significant information of pollination, seed dispersal, climatic and edaphic elements and threats to the existence of this stenoendemic cycad are provided here for the first time.

Keywords: Cycas hoabinhensis, pollination, seed dispersal, plant ecology, plant geography, plant conservation, endangered species

Many species of *Cycas* L. are faced with extinction in Vietnam. Among them, 14 species are listed as Critically Endangered (CR), Endangered (EN) and Vulnerable (VU) in the IUCN Red List of Threatened Species (Osborne et al. 2007; 2012). Eleven species are included in the Red Data Book of Vietnam (Ministry of Science and Technology 2007) and all species of the genus are listed in the appendix of the official national nature protection governmental decision 32/2006/NĐ-CP: “Limit of exploitation and use for commercial purpose (IIA)” (The Government of Vietnam 2006). The northern part of Vietnam is a shelter for many cycads. Eleven species have been found here in different habitats. Three species are stenoendemics in northern Vietnam. They have been poorly studied and are quickly reducing their ranges, often approaching the verge of full extinction in nature (Tang 2004; Osborne et al. 2007).

Effective conservation actions require current detailed information about species (Böhm et al. 2013). Modern information on geographic distribution, ecological range, population sizes, breeding system, seed dispersal mechanism, mode of pollination and age structure of the populations is particularly valuable in assessments (Hoffmann et al. 2008). Local endemic species with very restricted areas are the most significant component in global conservation prioritizations (Swenson et al. 2012). Therefore, an assessment on biology, ecology and conservation of the endangered stenoendemic cycad *Cycas hoabinhensis* in northern Vietnam is an essential part of the effective strategic conservation for this unique, highly endangered cycad.

Materials and methods

The present investigation is based on numerous field surveys, personal field observations and collections of voucher materials, photographs, herbarium specimens, seeds, insect pollinators, rocks and soil samples stored at the Herbarium and Museum of the Institute of Ecology and Biological Resources of the Vietnam Academy of Sciences and Technology HN, the Herbarium of the Center for Plant Conservation (CPC), at IBSC and LE. Description of vegetation and plant communities are based on herbarium collections, comprising about 300 collecting numbers. Geographic positions of studied localities and model plots were identified by standard Global Positioning System (GPS). Rock and soil samples were characterized by measuring numerous variables in the Centre for Environmental Analysis and Technology Transfer (CEAT), Hanoi, Vietnam.

Plant specimens were identified by the study of type specimens and internet resources and by means of morphological analysis with use of the following literature: Smitinand (1972), Hiêp & Vidal (1996), Ho (1999), Lindstrom & Hill (2002), Hill et al. (2004), Osborne et al. (2007), Hill (2008), Bi et al. (2010), Li & Xia (2012) and Averyanov et al. (2014).

The assessment of conservation status was done following the recommendations of the last edition of the IUCN Red List (IUCN 2012) and inferring from the GeoCAT website (http://geocat.kew.org).

Results

Biological characteristics of *Cycas hoabinhensis* (Figs 1, 2, 3, 4)

Cycas hoabinhensis (*C.* sect. *Stangerioides* Smitinand) from Vietnam was described and illustrated in 2004 (Hill et al. 2004):

Cycas hoabinhensis P.K. Loc & T.H. Nguyen, Bot. Rev. 70(2): 153–155, fig. 5 (2004).

Types. Vietnam: Hoa Binh: Lac Thuy, Chi Ne, Jul 1976, *Phan K. Loc P3194a* [Holotype: HNU; Isotypes: HN, NSW].

Vernacular name. Thiên tuê hòa bình.

Description. Plant caulescent or acaulescent, aerial stem when present up to 0.6 m, (4)5–12(15) cm in diameter at the narrowest point, with 2–12 leaves in an apical rosette. Leaves bright green, glossy, (30)70–130(185) cm long, flat, with (19)40–70(113) pinnae, young white tomentose, rachis usually terminated by paired pinnae. Petiole (10)35–55(80) cm long, brown to dark-brown, pubescent when young, glabrous when old, with (8)30–50(70) spines, distance between two closest spines (0.5)1–2.5(4) cm. Pinnae abruptly reduced to spines basally. Pinnae (4)12–18(25) cm long; median pinnae simple, strongly discolorous, (10)17–28(40) cm long, (0.7)1.5–1.8(2.2) cm wide, decurrent for 2–4 mm, narrowed to 2.5–5 mm at base, 15–20 mm apart, section flat, margins straight or undulate, apex softly acuminate, not spinescent; midrib raised above and below. Cataphylls broadly to narrowly triangular, soft, pilose, (1.7)3–5(5.5) cm long, (0.6)1.5–1.8(2.2) cm wide. Pollen cones narrowly ovoid or fusiform, yellow-brown, (6)12–18(22) cm long, (1.5)3–5(6) cm in diameter. Microsporophyll lamina soft, yellow- or brown-tomentose, not thickened dorsiventrally, (6)14–16(18) mm long, (4)10–12(14) mm wide; sterile apex 1–2 mm long, raised, apical spine distinct or rudimentary, appressed, sharply upturned. Megasporophylls (5)8–10(12) cm long, brown-tomentose; ovules 2–4, glabrous; lamina orbicular, (1.6)2–3(3.5) cm long, (1.2)1.6–2(2.5) cm wide, deeply pectinate, with (6)10–15(18) soft spines, (12)15–30(40) mm long, (1.0)1.5–3.0(4.0) mm wide, apical spine not distinct or distinct from lateral spines. Seed ovoid or subglobose, (1.6)1.8–2.2(2.5) × (1.8)2.0–2.5(2.8) cm, sarcotesta yellow, not pruinose; fibrous layer absent; sclerotesta verrucose; spongy layer absent.

Figure 1. *Cycas hoabinhensis* in exposed habitat. A – habitat; B, C, D – plant growing on rock and soil; E – stem; F – herbarium sheet; G, H – petiole; I, J – part of leaf; K – leaflets; L – cataphylls; M – microsporangiate cone; N – megasporangiate cone; O, P – microsporophylls; Q, R – megasporophylls.

Note. The morphological characteristics of *C. hoabinhensis* are very variable in response to different ecological conditions. The number of leaves, cataphylls, microsporophylls and megasporophylls of cultivated plants is higher and they are all larger than those of wild plants. The stem in

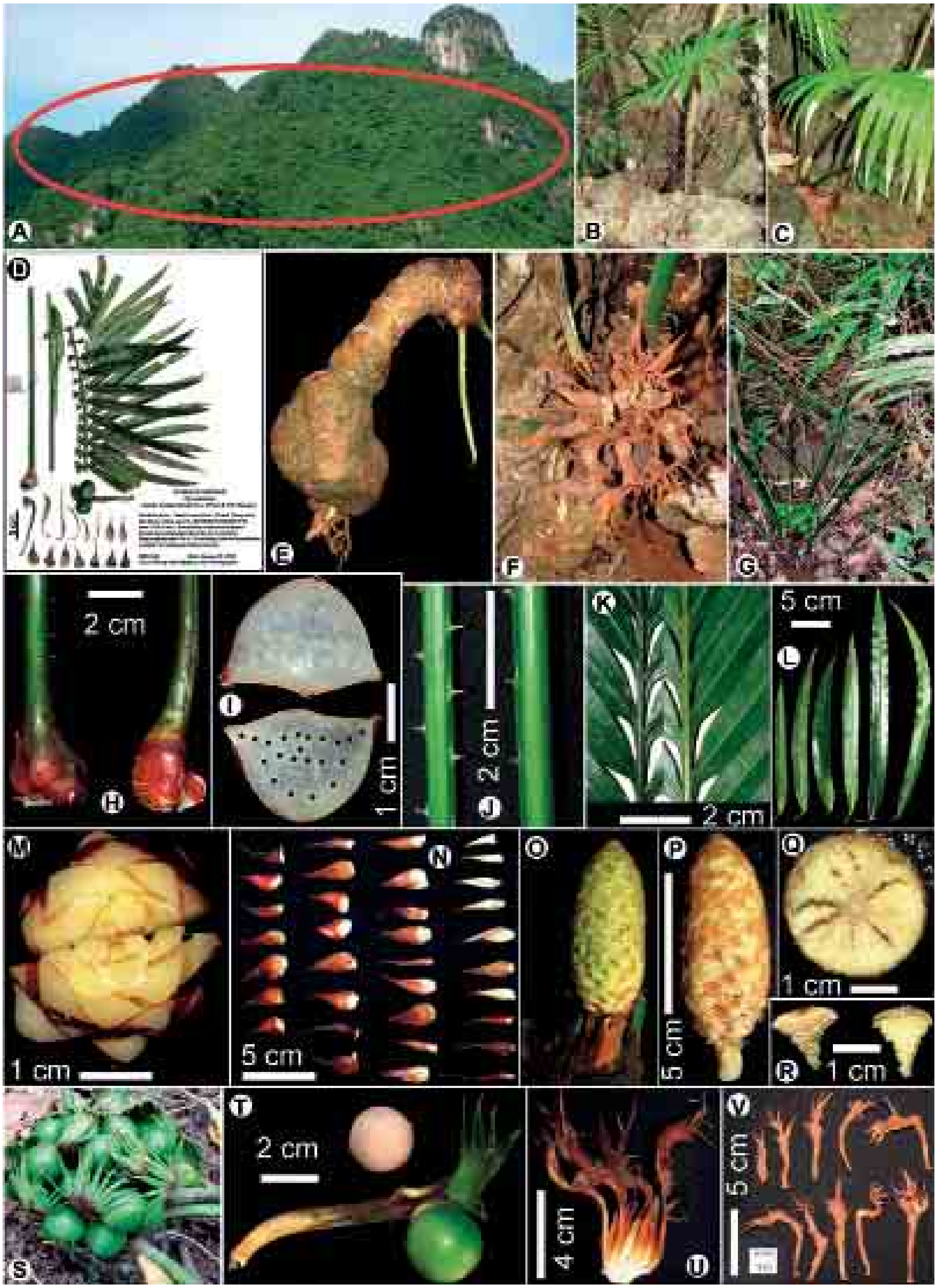

Figure 2. *Cycas hoabinhensis* in shaded habitat. A – habitat; B, C, G – mature plants growing on rock and soil; D – herbarium sheet; E, F – stem; H – petiole base; I – cross section of petiole; J – spines on petiole; K – part of leaf; L – leaflets; M – cross section of cataphylls; N – cataphylls; O, P – microsporangiate cone; Q, R – microsporophylls; S – megasporangiate cone; T – megasporophyll lamina with ripe seeds; U, V – megasporophylls.

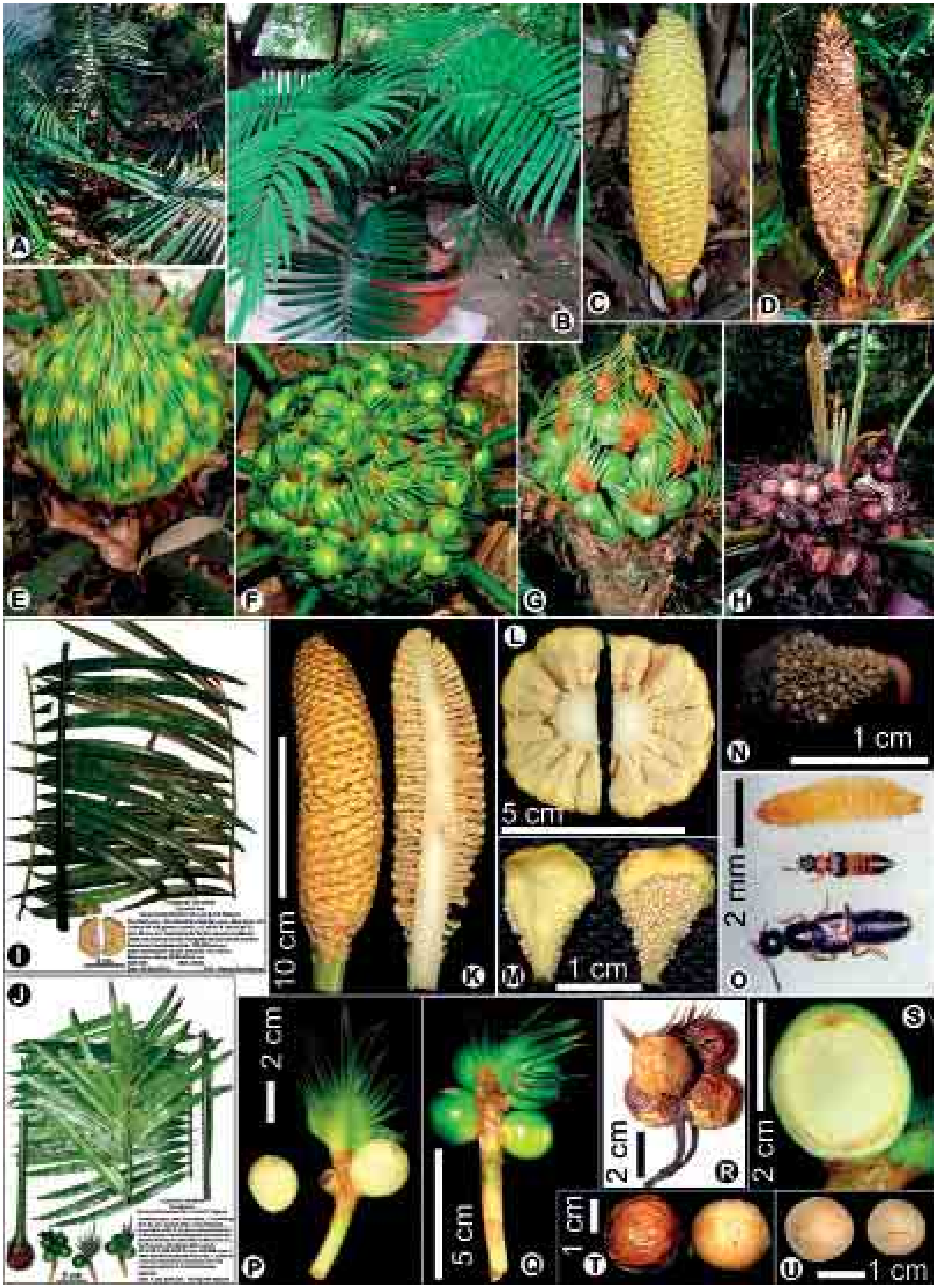

Figure 3. *Cycas hoabinhensis* in cultivation. A – plant cultivated on ground; B – plant cultivated in a pot; C, D – plant with microsporangiate cone; E, F, G, H – plant with megasporangiate cone; I, J – herbarium sheet; K – microsporangiate cone; L, M – microsporophylls; N, O – beetles and larva destroying microsporophylls; P, Q, R – megasporophylls; S, T, U – seeds.

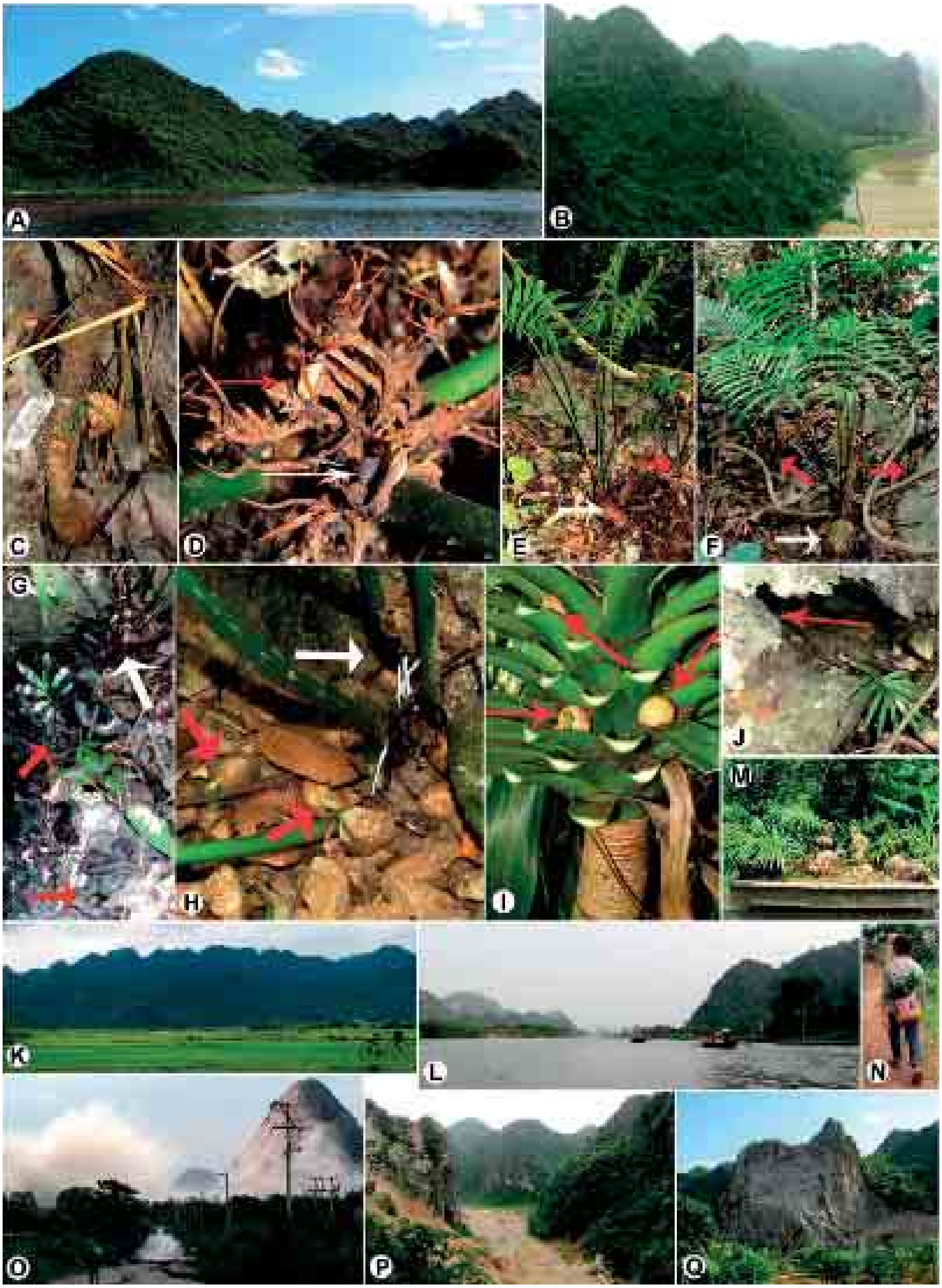

Figure 4. Subpopulations of *Cycas hoabinhensis* in nature. A, B – habitat; C – mature plant; D – red arrow shows relic of a fallen ripe seed, white arrow indicates sterile seeds; E, F, G, H – seedlings around parental plant (red arrows; parental plants = white arrows); I, J – the evidence of seed dispersal by rodents or other small mammals (red arrows = seeds in the leaf base of a trunk of *Dracaena cambodiana*; a seedling in a possible shelter of rodents); K, L – limestones isolated by plain, river; M, N – wild collected plants for sale; O, P – blasting limestones for cement factories; Q – excavation of lime for gravel and construction.

cultivated plants is usually 50–60 cm tall, (5)7–12(15) cm in diameter at the narrowest point, 2–4 mature seeds on a laminar megasporophyll. Specimens in the wild have small, twisted and narrow stems, (10)15–30(40) cm in height, (4)5–7(10) cm in diameter at the narrowest point, usually 0–1(rarely 2) mature seeds. Moreover, individuals growing in the wild on exposed rocks have leaves that are smaller, thicker and harder than those on rocks or soil in shaded places. The leaflet margins are flat, not undulate.

Specimens examined. Vietnam. Ha Noi, My Duc distr., Chua Huong, Yang & Hiêp SLY 757; 22°36'37"N 105°44'14"E, elev., 160 m a.s.l., CPC 4848 & 4849. Ha Nam prov., Thanh Liem distr., Thanh Thuy com., 20°29'22"N 105°52'11"E, elev. 162 m a.s.l., NSK 540. Kim Bang distr., Kha Phong com., collected from Ba Sao com., NSK 544; Ba Sao com., 20°32'37"N 105°48'06"E, elev. 125 m a.s.l., NSK 545. Hoa Binh prov., Luong Son distr., Yang & Hiêp SLY 519. Lac Thuy distr., Chi Ne, Yang & Hiêp SLY 425; Phu Lao com., cultivated in Thap's garden, collected in Phu Lao com., 20°33'19"N 105°45'42"E, elev. 100 m a.s.l., NSK 560, 562 & 698; Phu Thanh com., 20°33'17"N 105°43'20"E, elev. 80 m a.s.l., NSK 563 & 564; 20°34'55"N 105°40'56"E, elev. 120 m a.s.l., NSK 694, 695, 696 & 697; 20°32'37"N 105°48'07"E, elev. 130 m a.s.l., NSK 705b; 20°35'47"N 105°33'20"E, elev. 152 m a.s.l., NSK 773; Lien Hoa com., 20°28'22"N 105°44'23"E, elev. 150–180 m a.s.l., NSK 589 & 590. Lac Son distr., Binh Chan com., 20°27'09"N 105°31'55"E, elev. 78 m a.s.l., cultivated in Mr. Tan's garden, collected in Lac Hung com., NSK 574. Kim Boi distr., Cao Duong com., 20°27'09"N 105°31'55"E, elev. 85 m a.s.l., NSK 575 & 576; Sao Bay com., 20°36'01"N 105°37'07"E, elev. 120 m a.s.l., NSK 648 & 699; 20°36'05"N 105°37'08"E, elev. 150–180 m a.s.l., NSK 750, 751, 752 & 752B. Yen Thuy distr., Lac Luong com., 20°28'02"N 105°38'01"E, elev. 205 m a.s.l., NSK 626, 627, 742, 743, 744, 745 & 746; Da Phuc com., 20°27'10"N 105°35'17"E, elev. 150 m a.s.l., NSK 641 & 642. Ninh Binh prov., Nho Quan distr., Cuc Phuong N.P., Hiêp 2056; ridge crest near gate, 20°15'54"N 105°41'48"E, Hill 5050 & Loc; Yen Quang com., Cuc Phuong N.P., 20°28'05"N 105°37'59"E, elev. 200 m a.s.l., NSK 568; Ex-situ conservation area of Cycads, 20°15'54"N 105°41'48"E, elev. 40 m a.s.l., collected from Ky Phu com., NSK 753, 753B, 753C & 755; Ky Phu com., 20°12'52"N 105°44'30"E, elev. 150 m a.s.l.; NSK 756; Cuc Phuong com., 20°15'10"N 105°41'54"E, elev. 165 m a.s.l., NSK 770 & 772; Phu Long com., cultivated in Hoa's garden, NSK 548; village 7, 20°10'46"N 105°48'20"E, elev. 150 m a.s.l., NSK 567. Hoa Lu distr., Ninh Hai com., 20°12'58"N 105°54'01"E, elev. 70 m a.s.l., NSK 570, 571 & 572. Tam Diep distr., Quang Son com., 20°27'09"N 105°31'55"E, elev. 85 m a.s.l., NSK 650; Dong Son com., 20°7'40"N 105°55'47"E, elev. 160 m a.s.l., NSK 664, 665, 666, 667 & 668; Nam Son com., 20°8'04"N 105°53'43"E, elev. 135 m a.s.l., NSK 659, 660, 661 & 662.

Phenology. Mature pollen cones are usually observed in April–May and they are decaying in May–June. Our study of cultivated samples indicates that the period from the emergence of the male cone to the pollination phase was about 45 days. After the separation of the microsporophylls from each other in a spiral fissure around the cone, the ripe pollen is released for about 10 days. Later, adult beetles (*Staphylinidae*) and unidentifiable larvae (Fig. 3 N, O) completely destroy the pollen cones. The megasporophylls appear in May and possibly receive pollen in late May or early June. Seeds mature in December–January of the following year. Dry megasporophylls bearing seeds remain until February–April of the second year. Plants with mature megasporophylls on their trunks usually sprout young leaves in August of the first year or in April of the second

Table 1. The age structure and conservation status of selected subpopulations.

Subp. No	Coordinates	Age structure of individuals in a subpopulation			Exploitation for sale and bonsai/Present in protected area
		Adults (**N**/%)	Juveniles (**N**/%)	Seedlings (**N**/%)	
Subp. 1	20°27'09"N 105°31'55" E	4 (22.22%)	13 (72.22%)	1 (5.56%)	Yes/No
Subp. 3	20°36'01"N 105°37'07" E	24 (85.71%)	3 (10.71%)	1 (3.58%)	Almost None/Yes
Subp. 4	22°36'37"N 105°44'14" E	6 (33.33%)	12 (66.67%)	0 (0.0%)	Yes/Yes
Subp. 6	20°34'55"N 105°40'56" E	7 (26.92%)	17 (65.38%)	2 (7.70%)	Yes/No
Subp. 7	20°32'37"N 105°48'06" E	10 (37.04%)	16 (59.26%)	1 (3.70%)	Yes/No
Subp. 10	20°28'22"N 105°44'23" E	13 (38.24%)	18 (52.94%)	3 (8.82%)	Yes/No
Subp. 11	20°28'02"N 105°38'01" E	11 (31.43%)	21 (60.00%)	3 (8.57%)	Yes/No
Subp. 12	20°27'10"N 105°35'17" E	7 (29.17%)	17 (70.83%)	0 (0.0%)	Yes/No
Subp. 14	20°15'03"N 105°41'58" E	9 (32.14%)	14 (50.00%)	5 (17.86%)	Yes/Yes
Subp. 15	20°12'58"N 105°54'01" E	10 (30.30%)	19 (57.58%)	4 (12.12%)	Yes/Yes
Subp. 17	20°10'50"N 105°48'24" E	12 (32.43%)	23 (62.16%)	2 (5.41%)	Yes/No
Subp. 19	20°07'40"N 105°55'47" E	10 (37.04%)	13 (48.15%)	4 (14.81%)	Yes/No

year and do not form new megasporophylls in the second year. A megasporophyll cycle of *C. hoabinhensis* probably takes 2–3 years.

Population structure and seed delivery. Field studies show that the age structure of *C. hoabinhensis* subpopulations essentially differs from each other, especially in the number of adults and juveniles (Table 1). The number of mature individuals per subpopulation was remarkably high in subpopulation 3 (24 = 85.71%) and low in the other subpopulations (4 = 22.22% to 13 = 38.24%). Conversely, the number of juveniles in subpopulation 3 (10.71% juveniles) was much less than in other subpopulations (48.15%–72.22% juveniles). In general, the number of juveniles in a subpopulation was much higher than the number of seedlings, and the percentage of seedlings per a subpopulation was very low, from 0% in the subpopulation 4 to 17.86% in subpopulation 14. Based on interviews of local people who live around the subpopulations, on our investigation of 16 subpopulations in the field and on the data presented in Table 1, it is conceivable that subpopulation 3 consisting of 24 (85.71%) adults, is a relatively primary population with minimal disturbance and that all other subpopulations have been excessively exploited over time by plant collectors.

It is very surprising that nearly all adult individuals of all subpopulations observed in 2013 and 2014 did not produce cones. Only 20 of 150 mature plants studied in 16 subpopulations produced reproductive structures, 7 individuals with pollen cones and 13 individuals with megasporophylls. Moreover, there were only 30 fertile seeds with a developed embryo on 10 megasporophylls, all others were sterile.

In the field surveys, we found that seedlings and saplings usually grow around adult plants (parents), commonly within radius of (0.2)1–2.5(<3) m. In one observation, 3 germinating seeds were found, with sclerotesta split and primary root emergent, within 3–10 cm apart from the parent. Three other seeds were found at 50 cm height in leaf bases of a trunk of *Dracaena cambodiana*, which was 60 cm away from a cycad with dry megasporophylls. It should be expected that gravity is the main factor in *C. hoabinhensis* seed dispersal. Seeds simply roll down on steep slopes (barochory). However, some observations like in *Dracaena cambodiana* indicate that rodents or other small mammals may also disperse seeds but this is not the regular way of seed dispersal.

Ecological characteristics (Tables 2 & 3; Figs 4, 5 & 6)

Distribution. Field studies of 40 different localities show that *C. hoabinhensis* subpopulations are scattered at elevations from (70)100–200(<250) m a.s.l. in the limestone areas of the four provinces Ninh Binh (Tam Diep, Nho Quan, Hoa Lu districts), Hoa Binh (Yen Thuy, Lac Thuy, Kim Boi, Luong Son districts), Ha Noi (My Duc district) and Ha Nam (Kim Bang, Thanh Liem districts). The distribution range of this species is shown in Figs 5 & 6.

Rock and soil composition. Some characteristics of calcareous rock and soil of selected localities (see Table 3) in the areas with and without *C. hoabinhensis* are presented in Table 2 and 3. In the case of chemical composition of rock and soil samples (R & S), localities 3–7 with occurrence of *C. hoabinhensis* don't differ much from localities 1 and 2 without *C. hoabinhensis*: all value variations in R1 & R2 are within the values of R3 to R7 (CaO = 54.3–60.1%; MgO = 214–1012 mg/kg; loss on ignition at 1000°C = 30.5–43.8%), and similarly the soil values of S1 & S2 are within the range of S3 to S7 (pH (KCl) = 6.28–7.25; OM = 2.29–7.34 mg/kg; TN = 0.2–0.41%; AN = 59.4–218 mg/kg; TP = 0.08–0.19%; AP = 18.1–34.5 mg/kg; K^+ = 33.2–165 mg/kg; Ca^{2+} = 2136–10 090 mg/kg; Mg^{2+} = 155–1183 mg/kg, and CEC = 7.65–20.0 cmol/kg). The difference can be only seen in the soil texture of the samples: the percentage of Limon in S1 = 16%, S2 = 13.3% is lower than in S3–S7 = 18.8–49.3%. Fine sand and coarse sand maximum values in S2 = 69.6% and S1 = 24%, respectively. The soil property of samples (S3–S7) in the wild resembles to a sample in the local garden (S8); all values of S8 are within the limits of those in S3–S7, with the exception of one value in soil texture (coarse sand of S8 = 20 > S3 = 3.6, S4 = 17, S5 = 7.4, S6 = 11.5, S7 = 5.6).

Table 2. Chemical composition of rock samples.

No	Rock variable	Unit	Rock sample							
			R1	R2	R3	R4	R5	R6	R7	R8
1	CaO	%	56.7	59.7	60.1	59.2	54.6	57.2	54.3	54.7
2	MgO	mg/kg	334	413	214	237	186	266	1012	516
3	LOI_{1000}	%	35.6	41.2	43.8	42.3	34.1	36.1	30.5	31.5

Loss on ignition at 1000°C (LOI_{1000})

Table 3. Physical and chemical composition of soil samples.

No	Soil variable	Unit	Soil sample							
			S1	S2	S3	S4	S5	S6	S7	S8
1	Soil texture									
	Clay	%	2.5	3.4	5.9	4.8	1.1	2.8	1.0	3.1
	Limon	%	16.0	13.3	43.1	20.9	43.3	18.8	49.3	22.6
	Fine sand	%	57.5	69.6	47.4	57.3	48.2	66.9	44.1	54.3
	Coarse sand	%	24	13.7	3.6	17	7.4	11.5	5.6	20
2	pH (KCl)		6.81	6.68	6.62	6.85	7.11	7.25	6.28	6.57
3	OM	mg/kg	2.89	6.96	7.34	3.78	3.28	3.66	2.29	3.06
4	TN	%	0.24	0.4	0.29	0.28	0.2	0.41	0.2	0.32
5	AN	mg/kg	116	193	59.4	79.2	81.6	218	87.5	203
6	TP	%	0.15	0.06	0.08	0.11	0.16	0.19	0.1	0.17
7	AP	mg/kg	21.1	11.9	26.2	18.1	18.5	34.5	19.4	23.4
8	K^+	mg/kg	135	97	165	124	37.6	154	33.2	41.6
9	Ca^{2+}	mg/kg	4688	7710	10090	5182	4135	5557	2136	3453
10	Mg^{2+}	mg/kg	301	314	384	571	1183	630	155	389
11	CEC	cmol/kg	15.2	11.3	20.0	9.6	16.4	16.7	7.65	7.05

Soil variables: Organic matter (OM), total nitrogen (TN), available nitrogen (AN), total phosphorus (TP), available phosphorus (AP), cation-exchange capacity (CEC).

Localities: R1 & S1 = 20°10’26”N 105°28’3”E; R2 & S2 = 20°17’2”N 105°30’18”E; R3 & S3 = 20°28’2”N 105°37’59”E; R4 & S4 = 20°32’35”N 105°48’12”E; R5 & S5 = 20°07’40”N 105°55’46”E; R6 & S6 = 20°36’01”N 105°37’08”E; R7 & S7 = 20°15’10”N 105°41’54”E; R8 & S8 = 20°33’10”N 105°45’52”E. Localities without *C. hoabinhensis*: R1 & S1, R2 & S2; locality R8 & S8 in a local garden with cultivated trees; localities R3–R7 & S3–S7 from wild subpopulations.

Climatic characteristics. *Cycas hoabinhensis* occurs in a monsoon tropical climate with cold winter and summer rains and with a dry period from December to January or February. According to Van et al. (2000), the annual average temperature is 23.0–23.4°C with the lowest temperature in January and the highest in July. The annual average of rainfall is 1820.8–1986.8 mm (the peak is in September) and the annual average of relative humidity is 84–85% (the minimum is in November or December and the maximum in March).

Vegetation structure. Roughly 130 vascular plant species from 110 genera and 47 families were recorded as plants closely associated with *C. hoabinhensis*. They form a typical assemblage of zonal evergreen broad-leaved lowland limestone mountain forests (Averyanov et al. 2003). In the studied areas, the upper stratum of this vegetation type was over-exploited for timber in the past, so currently it mainly consists of three forest strata.

Highest stratum includes small trees and treelets, for example, *Pistacia weinmanniifolia* J. Poiss & Franch., *Mitrephora calcarea* Ast, *Alstonia mairei* H. Lév., *Dracaena cambodiana* Gagnep., *Alangium chinense* (Lour.) Harms, *Calophyllum balansae* Pit., *Diospyros siderophylla* H.L. Li, *Ficus* sp., *Streblus asper* Lour., *S. macrophyllus* Blume, *Boniodendron parviflorum* (Lecomte) Gagnep., *Sinosideroxylon bonii* Aubrév., *Sterculia lanceolata* Cav., *Heynea trijuga* Sims.

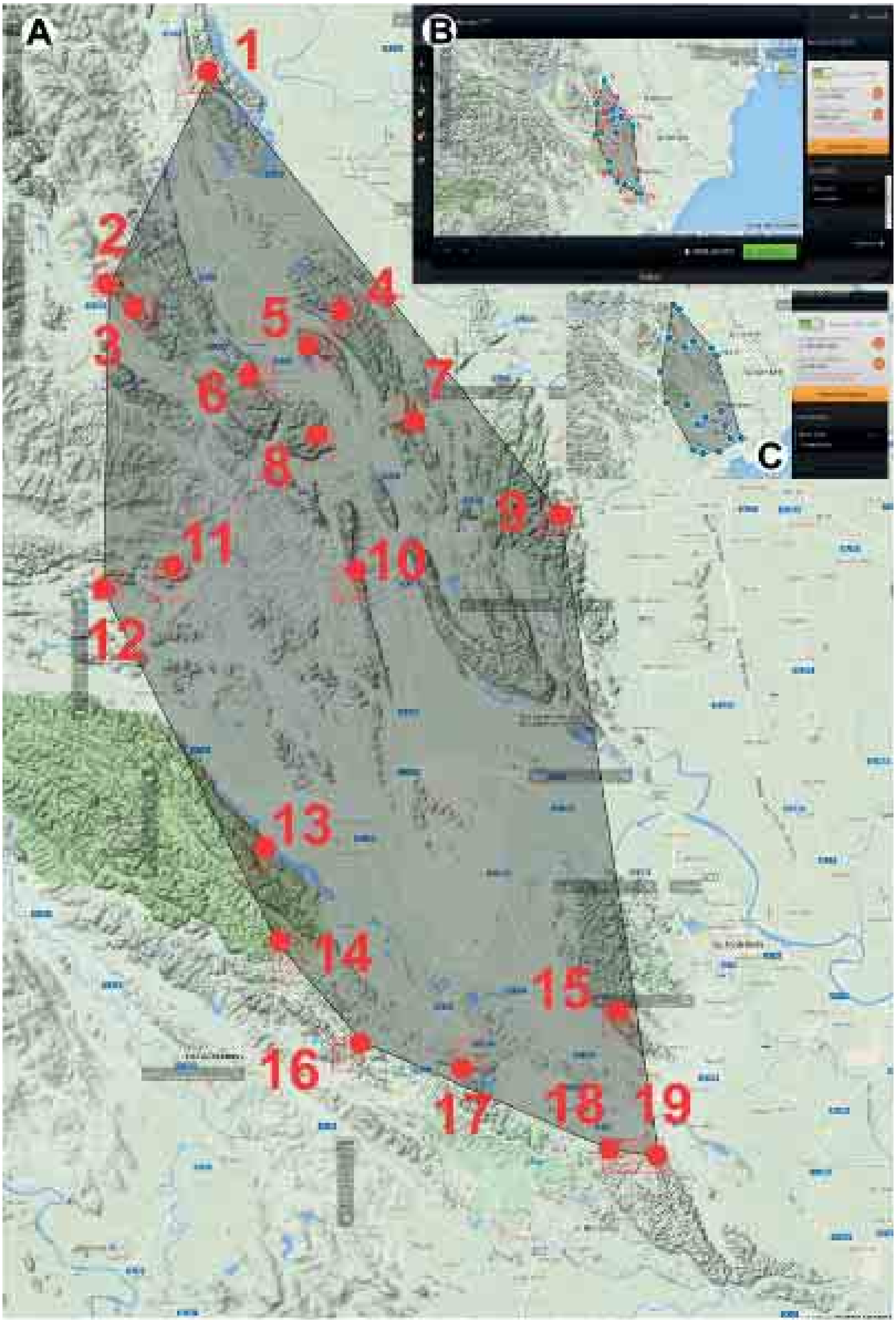

Figure 5. Distribution and estimated extent of occurrence (EOO) of *Cycas hoabinhensis*. A – distribution map of 19 subpopulations; B – EOO of subpopulations; C – maximum of EOO.

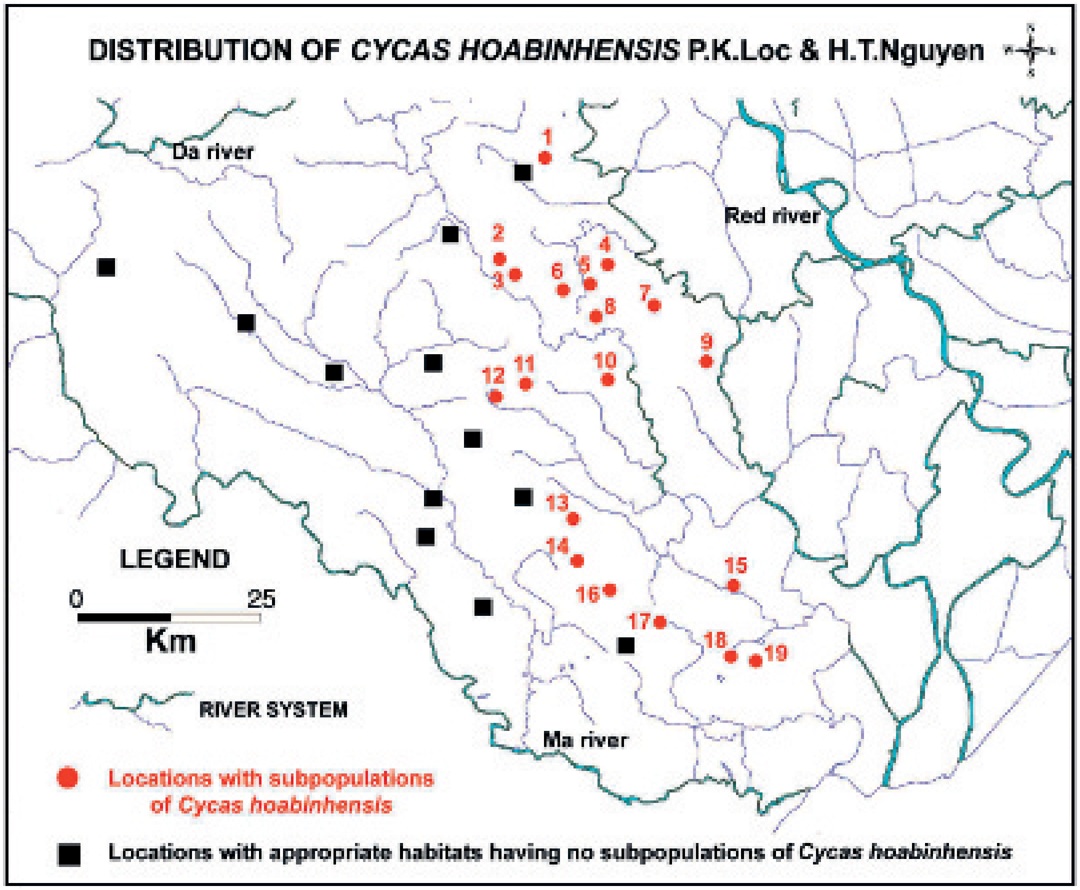

Figure 6. Subpopulations of *Cycas hoabinhensis* isolated by rivers.

The middle stratum is composed of diverse species of shrubs, palms and lianas such as *Cystacanthus pyramidalis* Benoist, *Gymnostachyum listeri* Prain, *Justicia austroguangxiensis* H.S. Lo & D. Fang, *J. grossa* C.B. Clarke, *Ptyssiglottis kunthiana* (Nees) B. Hansen, *Ruellia poilanei* Benoist, *Phyllanthus lingulatus* Beille, *P. songboiensis* Thin, *Campylotropis bonii* Schindl., *Broussonetia kazinoki* Siebold, *Hiptage boniana* Dop, *Ardisia pseudocrispa* Pit., *Mussaenda glabra* Vahl, *Vidalasia tonkinensis* (Pit.) Tirveng., *Atalantia buxifolia* (Poir.) Benth., *Micromelum hirsutum* Oliv., *Maesa* sp., *Clausena* sp., *Schefflera* sp., *Cordia* sp., *Euonymus* sp., *Munronia pinnata* (Wall.) W. Theob., *Strychnos* sp., *Licuala* sp., *Rhapis* sp., *Caryota* sp., *Sinarundinaria* sp., *Dischidia tonkinensis* Costantin, *Melodinus fusiformis* Benth., *Marsdenia tinctoria* R. Br., *Cryptolepis dubia* (Burm. f.) M.R. Almeida, *Urceola napeensis* (Quint.) D.J. Middleton, *Pothos grandis* P.C. Boyce & V.D. Nguyen, *P. repens* (Lour.) Druce, *Artabotrys* sp., *Rhaphidophora hongkongensis* Schott, *Tournefortia montana* Lour., *Gnetum latifolium* Blume, *Bauhinia pyrrhoclada* Drake, *B. scandens* L., *Mucuna hainanensis* Hayata, *Jasminum annamense* Wernham, *Renanthera coccinea* Lour., *Vanilla annamica* Gagnep., *Stemona tuberosa* Lour., *Cayratia japonica* (Thunb.) Gagnep., *Tetrastigma beauvaisii* Gagnep.

Numerous herbaceous species of the forest floor are also observed in habitats of *C. hoabinhensis.* Some of them are lithophytes like *Begonia boisiana* Gagnep., *B. morsei* Irmsch., *Chirita linearifolia* W.T. Wang, *Microchirita prostrata* J.M. Li & Z. Xia, *Paraboea swinhoei* (Hance) B.L. Burtt, *Cheirostylis yunnanensis* Rolfe, *Ludisia discolor* (Ker Gawl.) A. Rich, *Paphiopedilum concolor*

(Bateman) Pfitzer, *Drynaria bonii* Christ, *Adiantum caudatum* L., *Stemona kerrii* Craib and some are terrestrial herbs like *Aglaonema modestum* Engl., *A. tenuipes* Engl., *Aspidistra subrotata* Y. Wan & C.C. Huang, *Peliosanthes lucida* Aver., N. Tanaka & K.S. Nguyen, *P. hexagona* Aver., N. Tanaka & K.S. Nguyen, *P. kenhillii* Aver., N. Tanaka & K.S. Nguyen, *Ophiopogon* sp., *Tupistra* sp., *Asplenium polyodon* G. Forst., *A. prolongatum* Hook., *Tectaria* sp., *Neottopteris humbertii* (Tardieu) Tagawa, *Impatiens pingxiangensis* H.Y. Bi & S.X. Yu, *Blumea megacephala* (Randeria) Y. Ling, *Paraboea rufescens* (Franch.) B.L. Burtt, *Nervilia aragoana* Gaudich., *Amomum gagnepainii* T.L. Wu, K. Larsen & Turland, *Costus* sp., *Spiradiclis purpureocaerulea* H.S. Lo. Most common epiphytes are *Cymbidium aloifolium* (L.) Sw., *Liparis averyanoviana* Szlach., *Microsorum steerei* (Harr.) Ching and *Pyrrosia angustissima* (Diels) Tagawa & K. Iwats.

Three new species were found: *Peliosanthes lucida*, *P. hexagona* and *P. kenhillii* (Asparagaceae; Convallariaceae s.str.). Four other species, *Microchirita prostrata, Impatiens pingxiangensis*, *Justicia austroguangxiensis* and *Spiradiclis purpureocaerulea* have been recorded new for the flora of Vietnam.

Assessment of conservation status (Figs 4, 5 & 6)

The current study of all discovered subpopulations of *C. hoabinhensis* provides evidence that the average percentage of adult plants has been reduced during the last three vegetation periods to at least 53.87%. Data obtained from field explorations based on coordinates of studied localities and the GeoCAT website show the extent of occurrence (EOO) of *C. hoabinhensis* (1343–2730 km^2). It is noteworthy that all subpopulations are severely fragmented by vast plains, non-limestone mountain ridges and well-developed river systems. Within its distribution range the species also meets a serious decline of the quality of its habitat and the number of mature individuals caused by excavations of lime, forest overexploitation and commercial collections of plants. Corresponding to the IUCN Red List (IUCN 2012), the global conservation status of *C. hoabinhensis* is assessed as Endangered A2cd; B1ab (ii, iv, v).

Discussion

Morphological variation. It is evident that microclimate causes essential variation of leaf morphology by alteration of leaflet structure. In dry exposed habitats leaflets become smaller, thicker and straight, whereas in shady conditions they are larger, thinner and usually falcate. Cultivated plants are commonly larger in both stem and leaves. The reasons causing cultivars to grow better than plants in the wild are richness of available resources in cultivation (moderate sunlight, optimal shading, richer and not too dry soils), when a well-developed root system can more effectively absorb available nutrients. These advantageous features enable cultivated plants to form more leaves and larger vegetative and reproductive organs than those in the wild. The correlation of leaf shape and size with water and light availability has been well proven by numerous investigations. For example, *Quercus acutissima* Carruthers plants growing in water-limited environments with high light, generally have thick leaves caused by extra layers of palisade mesophyll or longer palisade cells to protect them from high light damage and to reduce water loss (Xu et al. 2009). Leaf size and shape potentially have large effects on leaf temperature because the two-dimensional proportions of a flattened leaf determine the rate of heat transfer across the leaf–air interface influenced by the thickness of its boundary layer (Nicotra et al. 2011). In contrast, in shaded places without water limitation, but with limited light conditions, *Hyptis* *emoryi* Torrey has large, thin leaves to capture photo-synthetically active radiation effectively to maximize the photosynthetic process (Nobel 1976). Briefly, *C. hoabinhensis* adapts to the shade environment as shade-tolerant species, conversely it works as a drought-tolerant species in water-limited places with sunlight exposure.

Pollination and seed delivery. It is clear that wind and insects are the most important factors of a successfull cycad pollination. Wind is a major pollination agent in *Cycas seemanni* A. Braun (Keppel 2002). *Cycas panzhihuaensis* L. Zhou & S.Y. Yang is pollinated by wind and insects (Wang et al. 1997). Insects as pollinators, for example, *Tranes* weevil for *Lepidozamia peroffskyana* Regel (Hall et al. 2004), *Pharaxonotha zamiae* and *Rhopalotria slossoni* for *Zamia pumila* L. (Tang 1987), *Porthetes* weevil for *Encephalartos villosus* Lem. (Donaldson 1997), *Carpophilus chalybeus* for *Cycas revoluta* Thunb. (Kono & Tobe 2007) and *Alphitobius* beetles in *Cycas beddomei* Dyer (Raju & Jonathan 2010). In Vietnam, *Tychiodes* and *Xenocryptus* were recorded as insect pollinators for cycads (Osborne et al. 2007). Many individuals of a beetle species (family Staphylinidae) and unidentifiable larvae were only seen in mature pollen cones of *C. hoabinhensis*. These beetles destroyed microsporophylls during the pollination time. The existence of these beetles and larvae on male cones has not been carefully studied. Therefore, there is an urgent need to study this aspect in future to fulfill our gap in understanding of the biology of *C. hoabinhensis*.

Available observations show that main agents for seed dispersal in cycads are rodents, gravity and floodwater (Raju & Jonathan 2010; Pérez-Farrera et al. 2000; Aguilar et al. 2008). For example, over 90% of *Macrozamia miquelii* (F. Muell.) A. DC. seeds remained within 1 m off the parent. Only brushtail opossums were recorded as extant dispersal fauna and no seeds at all were found 5–10 m away from parent plants (Hall & Walter 2013). The seed coat of *C. circinalis* L. is four-layered and the layer of the spongy tissue seems to be important to cause floatation in water for dispersal. Seeds fall to the ground during late winter and may be washed down to other places by rainwater during the rainy season (Raju & Rao 2011). For *C. hoabinhensis*, observations on 15 populations have shown that seedlings and saplings grow very closely to adult individuals (within the range of (0.5)1–2(2.5) m; no more than 3 m). These data support the assumption that seed dispersal agents of *C. hoabinhensis* are most likely gravity and rodents or other small mammals. A megasporophyll cycle of *C. hoabinhensis* probably takes 2–3 years. This also occurs in other cycads. For example, Newell (1983) found out that none of the *Zamia pumila* females produced cones in two consecutive years and it seems that there is a variation in cone production from year to year. In addition, the production of a cone requires both energy and nutrients, and a female cone is probably more costly than a male cone since a female cone is maintained on the plant for a much longer time and the seeds contain copious storage tissue. When studying demography and cone production of *C. beddomei* for two consecutive years at two sites, Raju & Jonathan (2010) showed that not all of the plants participated in the annual coning event and the percentage of participating plants varied from year to year.

Distribution. The geographic distribution of *C. hoabinhensis* is limited to limestone areas of the southeastern part in the Song Da Rift Zone (Metcalfe 2012). These karst areas, composed of mainly Triassic carbonate rocks of the Dong Giao (T2a dg) formation (Tuyet et al. 2004a; Lam 2010), have two landscape types. These are a tower karst landscape variously shaped vertically-sited and rugged hills with elevation usually less than 150 m (Khang 1985) in My Duc (Huong pagoda near Hanoi), Ha Nam (Kim Bang district; Kha Phong and Ba Sao municipalities), Ninh Binh

(Hoa Lu district, Ninh Hai municipality), Hoa Binh (Lac Thuy district, Chi Ne municipality) provinces, and a residual karst landscape on the corrosional plains that is diverse in morphology and size, from 200–300 m a.s.l., with steep slopes (mostly 20–45°), exposed bedrocks, tills and blocks of limestone on the foot slopes (Tuyet et al. 2004b), found in Ninh Binh province (Tam Diep district, Dong Son municipality), Ha Nam province (Thanh Liem district, Thanh Thuy municipality) and Yen Thuy (Bao Hieu, Da Phuc and Lac Thinh municipalities), Lac Thuy (Phu Thanh municipality), Kim Boi (Sao Bay and Thanh Nong municipality) districts of Hoa Binh province. A comparison of the described data of rock and soil samples, climatic elements in localities with and without the occurrence of *C. hoabinhensis* shows that there are very little differences in rock, soil and climate features. This means that the distribution range of *C. hoabinhensis* possibly does not dependent on those factors. Numerous studies on the correlation between the spatial distribution of plant and environmental factors showed that soil properties clearly affect the density, growth and development of plants but do not effect the distribution ranges (Nazre et al. 2009; Alvarez-Yepiz et al. 2011).

So, what has caused the present limitation of the distribution range of *C. hoabinhensis* and why this cycad does not extend its distribution range to the W, NW or S, where ecological conditions are almost identical (same vegetation, rocks, soil and climate)? The reason could be that the historic formation and evolution of geotectonics of the Song Ma suture and the upper parts of the Song Da Rift Zone (where *C. hoabinhensis* does not occur) took place earlier and more complicated than those of the lower parts of the Song Da Rift Zone with the occurrence of *C. hoabinhensis* (Findlay & Trinh 1997; Hall 2002; Carter & Clift 2008; Osanai et al. 2008; Metcalfe 2012). Also, the well-developed river system (Red river and Day river in the North, Buoi river in the West and Ma river in the south) and many large plains are significant barriers that constrain *C. hoabinhensis* to these areas because of limited seed dispersal (barochory and a seed structure without spongy layer that allows the seeds to float).

Conclusion

Cycas hoabinhensis is a small, usually unbranched, palm-like, lithophytic or terrestrial woody plant. It is a typical local paleoendemic with strictly limited distribution spreading to the southwest and south of Hanoi in northern Vietnam. It grows in evergreen broad-leaved lowland limestone forests at elevations of (70)100–200(<250) m a.s.l. About 130 native species of vascular plants (110 genera and 47 families) are usual associated with *C. hoabinhensis* in its typical habitats. Among them 3 species (*Peliosanthes lucida*, *P. hexagona*, and *P. kenhillii*) were recognized as new for science and 4 species were reported as new records for the flora of Vietnam (*Microchirita prostrata, Impatiens pingxiangensis*, *Justicia austroguangxiensis* and *Spiradiclis purpureocaerulea*). The pollen cone of *C. hoabinhensis* remains about 2 months, a megasporophyll cycle probably takes 2–3 years, seeds become mature from December to January of the following year, possibly dispersed by gravity and/or small rodents. The variation of morphology of *C. hoabinhensis* depends on the adaptation to a habitat with different microconditions. The low regeneration and progressive decrease of the number of mature individuals in all natural populations is caused in part by commercial collecting supported by market demands and in part by habitat loss. These are the main threats for the extinction of this species. The updated global conservation status for this narrowly endemic cycad is assessed as Endangered A2cd; B1ab(ii, iv, v).

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