Nordic Journal of Botany 25: 145–151, 2007
doi: 10.1111/j.2008.0107-055X.00109.x,

Subject Editor: Bertil Ståhl. Accepted 30 October 2007

Ecological and genetic notes on *Lindsaea digitata* (Lindsaeaceae), a new fern species from western Amazonia

Samuli Lehtonen and Hanna Tuomisto

S. Lehtonen (samuli.lehtonen@utu.fi) and H. Tuomisto, Dept of Biology, Univ. of Turku, FI–20014 Turku, Finland.

Morphological, ecological and genetic evidence have revealed the existence of two sympatric species in what has previously been recognised as *Lindsaea divaricata* Klotzsch. The new species, *Lindsaea digitata* Lehtonen & Tuomisto, is described here, and its ecological and genetic differences from the apparently closely related *L. divaricata* are documented.

Lindsaea Dryand. is a pantropical fern genus of ca 150 species, some of which extend to the subtropics in Japan and Australia. With the exception of a few species of open habitats, most *Lindsaea* species grow in moist forests at elevations below 3000 m (Kramer 1971). Most of the species are terrestrial, but especially at higher elevations some species may grow as epiphytes (Kramer 1971), and some lowland species grow on decaying wood (Tuomisto 1998).

The latest revision of South American *Lindsaea* accepted 45 species, distributed from southern Mexico to southern Brazil with the main centre of diversity in the Guiana shield (Kramer 1957). However, botanical collections in tropical America are highly concentrated in the vicinity of major cities, even at present (Nelson et al. 1990, Schulman et al. 2007), and the high-diversity regions recognised 50 years ago may be even more heavily biased towards areas that were not too difficult to access in earlier times.

Indeed, several new *Lindsaea* species have been described from South America after Kramer's (1957) revision (Vareschi 1966, Kramer 1989, 1991, Marcano 1989). In addition, extensive inventory in the western Amazonian lowlands has increased the number of species known from this area by documenting a broader range for known species and revealing several possibly new species (Tuomisto and Poulsen 1996, Tuomisto 1998, Tuomisto et al. 2002, Smith et al. 2005, Cárdenas et al. 2007).

On the basis of morphological and ecological data, it has been suspected for some time that the traditionally circumscribed *L. divaricata* comprises two species (Tuomisto 1998). In this paper, we confirm their distinctness using molecular evidence, and describe a new species, *Lindsaea digitata*.

Lindsaea digitata Lehtonen & Tuomisto, sp. nov.

A Lindsaea divaricata Klotzsch pinnulis in statu juvenili profunde dissectis (non crenatis), ad maturitatem apice acutiusculis digitiformibus (non rotundatis) differt.

Type: Peru, Madre de Dios, Manu, Río Madre de Dios, 1–2 km north of the river and 10 km east from the mouth of río Manu, 12°15′S, 70°48′W, 250–350 m, 26 Oct 1998, Tuomisto et al. 13510. (holotypus USM!, isotypi AAU!, CUZ!, TUR!, UC!).

Rhizomes short-creeping, ∅ 2–3 mm; scales lanceolate, to 1.5 mm × 0.2 mm. Leaves 1–2 mm apart; petiole ca 10–40 cm long, somewhat longer than the laminae, ∅ 1–2 mm, castaneous to black, lustrous, proximal part terete, distal part with the abaxial side grooved and with two membraneous pale wings. Laminae dark green, triangular, ca 15–30 cm long and equally wide at base, bipinnate with a conform terminal pinna; rachises castaneous to dark brown, with two pale lateral spreading wings abaxially. Pinnae 1–4 per side, alternate, oblong, 10–19 cm × 3–4 cm. Pinnules spreading to ascending, subcontiguous to slightly overlapping, ca 17–25 per side, 13–19 mm × 5–7 mm, inner margin straight, quite parallel to the rachis, lower margin straight or somewhat concave, upper margin ± straight, apex subacute. Proximal and distal pinnules on each pinna somewhat reduced, the distalmost about 1/2 the size of the largest, one or two sometimes connected by a narrow wing with the terminal segment. Terminal pinnule on each pinna triangular with asymmetrically hastate base, sterile or soriferous, 10–23 mm × 7–21 mm. Veins ± immersed, mostly 2-forked, free. Sori continuous along the upper margin; indusia ca 0.2–0.3 mm wide, entire, ending

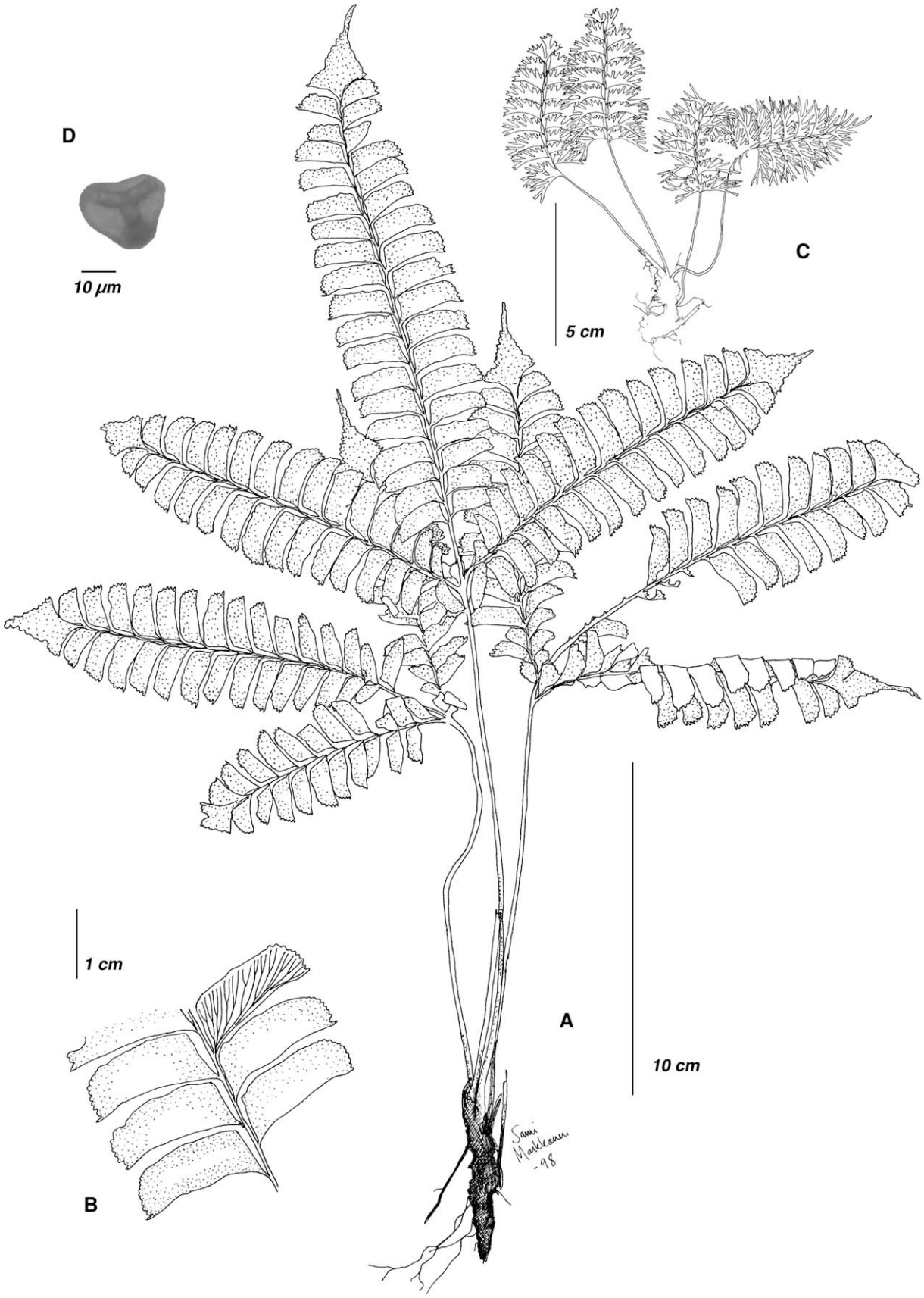

Fig. 1. *Lindsaea digitata*. (A) habit of an adult plant (Lusa 43, TUR), (B) pinnules from the middle of terminal pinna (Lusa 43, TUR), (C) habit of a juvenile plant (Lusa 21, TUR), (D) spore (Tuomisto et al. 8144, TUR). Drawn by S. Markkanen.

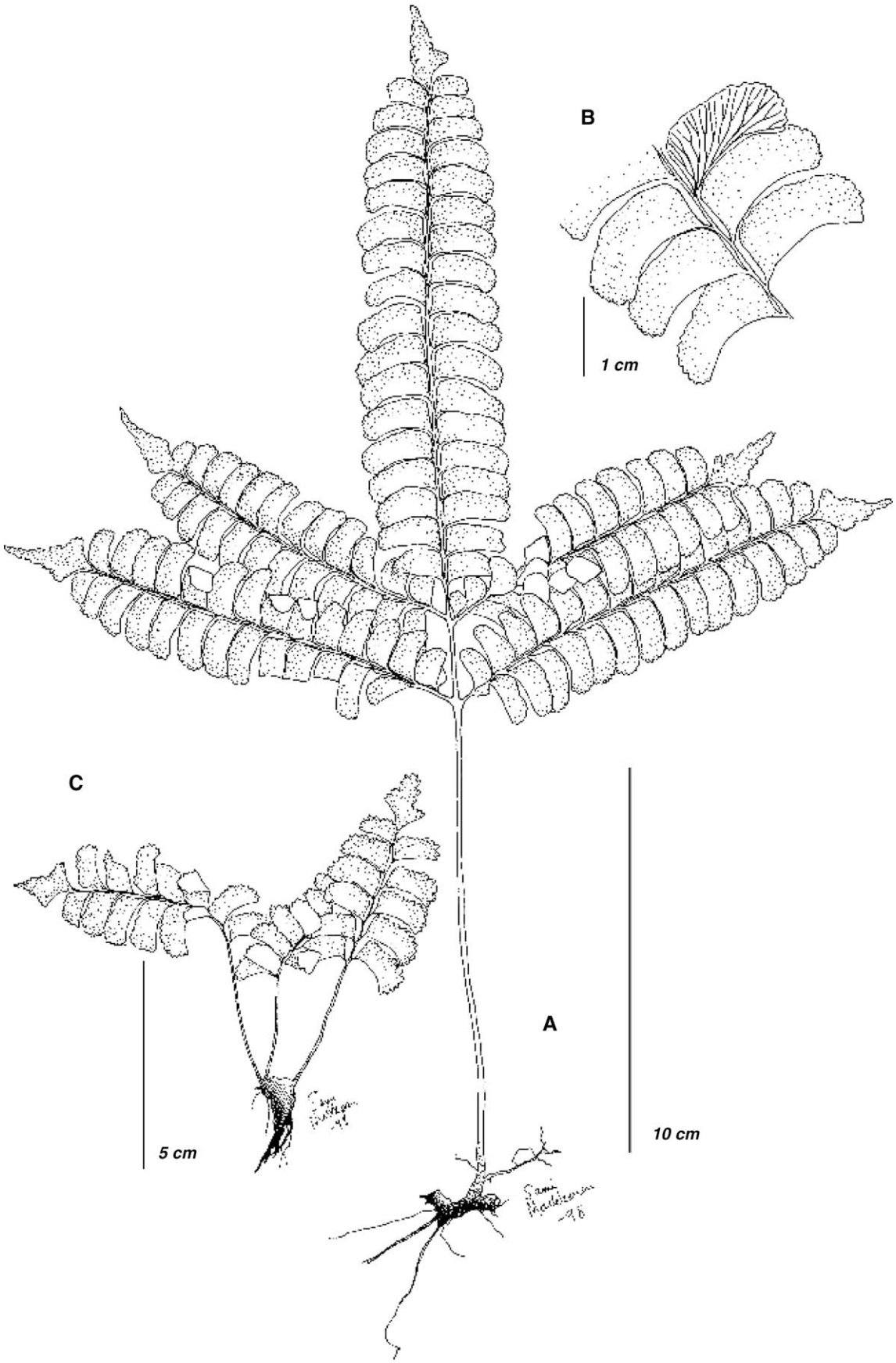

Fig. 2. *Lindsaea divaricata*. (A) habit of an adult plant (Lusa 2, TUR), (B) pinnules from the middle of terminal pinna (Lusa 2, TUR), (C) habit of a juvenile plant (Lusa 130, TUR). Drawn by S. Markkanen.

Table 1. List of sequenced *Lindsaea* specimens and their GenBank accession numbers.

Taxon	Locality	Voucher	GenBank acc. *trnL*	*psbA*	*rpoC1*
L. divaricata	Ecuador, Napo	Tuomisto and Ruokolainen 11735 (TUR)	EU146056	EU146034	EU146045
L. divaricata	Peru, Loreto	Tuomisto and Ruokolainen 12730 (TUR)	–	EU146036	EU146046
L. divaricata	Peru, Loreto	Tuomisto et al. 12771 (TUR)	EU146053	EU146037	EU146047
L. divaricata	Peru, Loreto	Tuomisto et al. 13035 (TUR)	EU146055	EU146033	EU146044
L. divaricata	Peru, Loreto	Tuomisto et al. 14553 (TUR)	EU146052	EU146040	EU146042
L. divaricata	Peru, Loreto	Tuomisto et al. 14976 (TUR)	EU146054	EU146032	EU146043
L. digitata	Ecuador, Sucumbios	Poulsen 78532 (TUR)	EU146059	EU146039	EU146050
L. digitata	Ecuador, Napo	Tuomisto and Ruokolainen 11755 (TUR)	EU146058	EU146035	EU146048
L. digitata	Peru, Loreto	Tuomisto et al. 14121 (TUR)	EU146060	EU146038	EU146049
L. digitata	Peru, Loreto	Tuomisto et al. 14470 (TUR)	EU146057	EU146041	EU146051

0.5–0.7 mm from the margin, reflexed at maturity. Spores trilete, pale, ∅ ca 25 µm. Juveniles initially with 1-pinnate leaves with deeply dissected pinnules, the segments to 8 × ca 1 mm, degree of pinnule dissection gradually diminishing in subsequently produced leaves (Fig. 1).

Distribution

Amazonian lowlands in western Brazil, Colombia, Ecuador and Peru.

Specimens examined

Brazil. Amazonas: Río Curuquetê, São Paulo, 30 km above mouth of Río Coti. Forest on high river banks, 19 Jul 1971, Prance et al. 14420 (US!).

Colombia. Amazonas: 0.5–6 km southeast of Estrecho (9 km east of confluence of Metá river with Caquetá), 0°59′S, 71°31′W, 150–250 m, 28 Apr 1998, Tuomisto et al. 12239 (COAH!, TUR!). 13–18 km southeast of Estrecho (which is 9 km east of confluence of Metá river with Caquetá), 1°5′S, 71°28′W, 150–250 m, 5 May 1998, Tuomisto et al. 12313 (COAH!, TUR!). 4 km westnorthwest of the confluence of the Culebra river with Caquetá, 0°57′S, 71°47′W, 150–250 m, 19 May 1998, Tuomisto et al. 12361 (COAH!, TUR!).

Ecuador. Morona-Santiago: Mutintz, southeast of Makuma, ca 1 km along trail to Tunantza, 2°11′S, 77°44′W, 675 m, 1 Nov 1996, Øllgaard & Navarrete 1927 (AAU!).

Table 2. Primers used for the three loci sequenced in *Lindsaea digitata* and *L. divaricata* specimens.

Locus	5′–3′	Reference
trnL-trnF	e: GGTTCAAGTCCCTC TATCCC	Taberlet et al. 1991
	f: ATTTGAACTGGTG ACACGAG	Taberlet et al. 1991
trnH-psbA	trnH: CGCGCATGGTGG ATTCACAATCC	Tate and Simpson 2003
	psbA3′f: GTTATGCATGA ACGTAATGCTC	Sang et al. 1997
rpoC1	LP1: TATGAAACCAGAA TGGATGG	Chase et al. 2007
	LP5: CAAGAAGCATATCTT GASTYGG	Chase et al. 2007

Napo: Río Lagarto Cocha. Near Redondo Cocha, 0°33′S, 75°13′W, 190 m, 11–17 Jun 1983, Lawesson et al. 44478 (AAU!); Yasuní National Park, km 92.5 along the oil road, 0°53′S, 76°13′W, 200–300 m, 10 Mar 1998, Tuomisto & Ruokolainen 11755 (TUR!, QCA!, QCNE!); Yasuní National Park, 0–2 km towards northnortheast from km 38 of the oil road traversing the park, 0°38′S, 76°27′W, 200–300 m, 24 Apr 1997, Tuomisto et al. 10863 (TUR!, QCA!, QCNE!); Yasuní National Park, km 101.8 along the oil road, 0°57′S, 76°14′W, 200–300 m, 1 Mar 1998, Tuomisto & Ruokolainen 11651 (TUR!, QCA!, QCNE!).
Pastaza: Río Bobonaza, near outlet into Río Pastaza, between Destacamento Cabo Pozo and La Boca, ca 2°30′–35′S, 76°38′W, 275 m, 21 Jul 1981, Øllgaard et al. 34943 (AAU!). Sucumbíos: Reserva Faunistica Cuyabeno. One-hectare plot ca 1 km north of Laguna Grande and surroundings, 0°0′S, 76°12′W, 265 m, 11 Apr–10 Jun 1988, Poulsen 78532 (TUR!).

Peru. Huánuco: Prov. Leoncio Prado: Rupa Rupa, al este de Tingo María, 700 m, 17 Nov 1971, Schunke 5166 (US!).
Loreto: Prov. Loreto: Mishuyacu, near Iquitos, 100 m, Oct–Nov 1929, Klug 69 (US!); Mishuyacu, near Iquitos, 100 m, May 1930, Klug 1337 (US!); Near Iquitos, Jan–Feb 1996, Lusa 21 (TUR!); Near Iquitos, Jan–Feb 1996, Lusa 43 (TUR); Río Pucacuro, just northeast of the river, 3°17′S, 74°59′W, 100–200 m, 14 Jan 2005, Tuomisto et al. 14121 (AMAZ!, TUR!, USM!); Río Pucacuro, tierra firme forest in hilly terrain within 1 km east of the river, 2°45′S, 75°4′W, 100–200, 19 Jan 2005, Tuomisto et al. 14461 (AMAZ!, BM!, TUR! USM!); Río Pucacuro, tierra firme forest within 1 km west of the river, 2°45′S, 75°5′W, 100–200 m, 19 Jan 2005, Tuomisto et al. 14470 (AMAZ!, TUR!, USM!); about 2 km north of the village San Antonio at lower Río Marañon, 4°32′S, 73°38′W, 100–200 m, 16 Jan 1996, Tuomisto et al. 8132 (AAU!, AMAZ!, TUR!, USM!); about 2 km north of the village San Antonio at lower Río Marañon, 4°32′S, 73°38′W, 100–200 m, 17 Jan 1996, Tuomisto et al. 8144 (AMAZ!, TUR!).
Prov. Mcal. Ramon Castilla: Río Yaguasyacu, 3–4 km northnorthwest from the village of Puerto Izango, 3°16′S, 72°0′W, 100–150 m, 15 May 1997, Tuomisto et al. 10966 (AMAZ!, TUR!, UC!, USM!); Río Yaguasyacu, 3 km northnorthwest from the village of Puerto Izango, 3°16′S, 72°0′W, 100–150 m, 19 May 1997, Tuomisto

et al. 11062 (AMAZ!, TUR!, UC!, USM!, Z!); Río Yaguasyacu, 2 km west from the village of Nueva Esperanza, 3°20′S, 72°0′W, 100–150 m, 23 Aug 1998, Tuomisto et al. 12707 (AMAZ!, TUR!, USM!).
Prov. Maynas: 24 km southwest of Iquitos at Perto Almendrez, 23 Jun 1984, Moran & Vasquez 3635 (AAU!); Between the roads Ex Petroleros and Bello Horizonte, km 38–40 of the road Iquitos-Nauta, 4°4′S, 73°28′W, 100–200 m, 9 Jan 1995, Tuomisto et al. 7206 (AMAZ!, NY!, TUR!); Explorama/ACEER lodge near the mouth of Quebrada Sucusari at lower Napo, 3°16′S, 72°55′W, 100–200 m, 20 Jan 1995, Tuomisto et al. 7278, (AMAZ!, KSP!, NY!, TUR!, UC!).
Prov. Requena: Río Ucayali, 2 km north from the biological station of Jenaro Herrera, 4°52′S, 73°39′W, 100–200 m, 18 Sep 1998, Tuomisto et al. 13001 (AMAZ!, TUR!, USM!).
Madre de Dios: Prov. Manu: Río Madre de Dios, 1–2 km north of the river and 10 km east from the mouth of río Manu, 12°15′S, 70°48′W, 250–350 m, 26 Oct 1998, Tuomisto et al. 13510 (holotype USM!; isotypes AAU!, CUZ!, TUR!, UC!); Río Madre de Dios, less than 1 km north of the river and 5 km west from the mouth of río de los Amigos, 12°33′S, 70°10′W, 200–300 m, 5 Nov 1998, Tuomisto et al. 13634 (CUZ!, TUR!, UC!, USM!).

Habitat

Non-inundated lowland rain forests on clayey to loamy soils, at elevations to 700 m.

Notes

Lindsaea digitata is most similar to *Lindsaea divaricata*, the name applied to nearly all specimens in the past. The two are easily distinguished in the juvenile stage because *L. digitata* has deeply dissected pinnules (Fig. 1C), a character we have not seen in any other South American *Lindsaea*. Pinnules of juvenile *L. divaricata* are crenate, but not dissected (Fig. 2C). The degree of pinnule dissection decreases gradually with plant age in *L. digitata* such that the longest segments remain close to the main vein, which eventually ends in a single finger-like protrusion. In fertile pinnules this protrusion may be hardly noticeable, but even then the pinnule apex is relatively narrow and subacute. In contrast, *L. divaricata* has either entire or crenate pinnule apices, and the main vein ends slightly short of the distinctly rounded and rather broad pinnule apex. *Lindsaea* rhizomes often retain juvenile leaves even in mature plants, and the identification of these two species becomes much easier and more certain if such juvenile leaves are preserved on herbarium specimens. The dried leaves of *L. digitata* often appear darker, greener and more opaque than those of *L. divaricata*, which tend to be yellowish and slightly translucent (at least when preserved with alcohol before drying).

Molecular differentiation

We sequenced three DNA regions from six *L. divaricata* and four *L. digitata* specimens (Table 1) in order to study possible genetic differences of the two species. The studied sequences were *trnL-trnF* intron, non-coding *trnH-psbA* intergenic spacer, and a partial plastid gene *rpoC1* (Table 2). The sequence variation within each species was very limited, but between the two species there was always a consistent difference, such that all sequenced individuals could be reliably identified to species on the basis of any of the three loci. The most noticeable difference between *L. divaricata* and *L. digitata* was a 7 bp inverted sequence in *trnL-trnF* intron (Fig. 3). All studied *L. divaricata* specimens had a TC-rich inversion, and all studied *L. digitata* specimens had an AG-rich inversion.

Ecological differentiation

Both *Lindsaea divaricata* and *L. digitata* occur in the rain forests of the western Amazonian lowlands. We studied possible niche differentiation between the two species by analysing data from 76 transects in which at least one of the two species was present, and both abundance data of the ferns and quantitative data on soil properties were available. Only fern individuals with at least one leaf longer than 10 cm were counted. The data come from 500 m long and 5 m wide transects (surface area 0.25 ha; five transects were originally longer but only 500 m were used here for the sake of consistency) located in Colombia (9), Ecuador (8) and Peru (59). Three surface soil samples (top 5 cm of the mineral soil) were taken from each transect and analysed for pH, loss on ignition at 420°C, sand content and the concentrations of aluminium and exchangeable cations (Ca + K + Mg + Na) using standard methods (van Reeuwijk 1993).

In total, 1234 individuals of *L. divaricata* and 344 individuals of *L. digitata* were observed in the 76 transects. Co-occurrence of the two species was measured with Sørensen and Steinhaus indices (Legendre and Legendre 1998). The Sørensen index only takes into account the presence or absence of the species, whereas the Steinhaus index also considers the species abundance. The Sørensen index was 0.26, which means that the species shared only a quarter of the transects in which they occurred. The Steinhaus index was 0.03, which indicates that even when the two species were present in the same transect, their abundance patterns were different, such that one species was abundant when the other was rare. Plotting the species abundance against soil cation content (Fig. 4) revealed that *L. divaricata* was most abundant in soils with the lowest

5′- TGTTAAAACTATCTAACCAGTCGGTTTATTCTCCAAACCGACTGGTTAGATAGTTTGACAC-3′

5′- TGTTAAAACTATCTAACCAGTCGGTTTGGAGAATAAACCGACTGGTTAGATAGTTTGACAC-3′

Fig. 3. Part of the *trnL-trnF* intron. The 7 bp inversion marked with grey, note that the inversion may actually extend up to 50 bp (light grey). Upper sequence (TC rich) belongs to *Lindsaea divaricata*, lower sequence (AG rich) to *L. digitata*.

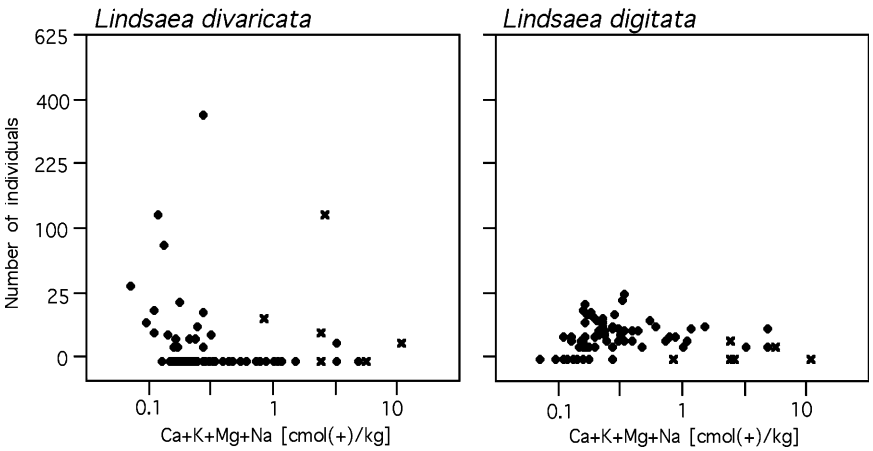

Fig. 4. of *Lindsaea divaricata* and *L. digitata* in 76 western Amazonian transects (each 0.25 ha) plotted on a soil cation content gradient. Transects in non-inundated (terra firma) forests are shown with diamonds, transects in floodplain or swamp forests with crosses. Note the square root scale of the y-axis, and the logarithmic scale of the x axis.

cation content. In contrast, *L. digitata* was found in a wide range of soil cation content, but was absent from those with the lowest cation content. *Lindsaea digitata* was found almost exclusively in well-drained sites, but *L. divaricata* was often found in waterlogged sites. Notably, all occurrences of *L. divaricata* in sites with soil cation content exceeding 0.5 cmol(+)kg^{-1} were in swamp or river floodplain transects, or (in one case) in a locally waterlogged site within an otherwise well-drained transect.

The present paper contributes to the growing number of studies that have documented ecological segregation among South American *Lindsaea* species (Tuomisto and Poulsen 1996, Tuomisto 1998, Salovaara et al. 2004, Cárdenas et al. 2007). Several studies have suggested that plant speciation in Amazonia may be at least partly driven by ecological specialization to different parts of a heterogeneous edaphic mosaic (Gentry 1981, Schulman et al. 2004, Fine et al. 2005, Tuomisto 2006). *Lindsaea divaricata* and *L. digitata* are clearly differentiated ecologically, and based on both morphological and molecular evidence they seem closely related. A phylogenetic study of the entire genus *Lindsaea*, which is currently underway, will shed light on whether they are actually sister species and thereby can be considered as a potential example of ecological speciation.

Etymology

The name refers to the finger-like dissections of the pinnule apices; in ecological inventories, this species has been identified with the field name 'fingers'.

Acknowledgements – We thank numerous persons for collaboration in the field in Peru, Ecuador and Colombia, especially Kalle Ruokolainen, Glenda Cárdenas and Mark Higgins. Maarten Christenhusz helped with the Latin diagnosis and Alan R. Smith gave constructive comments on the manuscript. The study was funded by the Academy of Finland.

References

Cárdenas, G. G. et al. 2007. Riqueza y distribución ecológica de especies de pteridofitas en la zona del Río Yavarí-Mirín, Amazonía peruana. – Biotropica 39: 637–646.

Chase, M. W. et al. 2007. A proposal for a standardised protocol to barcode all land plants. – Taxon 56: 295–299.

Fine, P. V. A. et al. 2005. The contribution of edaphic heterogeneity to the evolution and diversity of Burseraceae trees in the western Amazon. – Evolution 59: 1464–1478.

Gentry, A. H. 1981. Distributional patterns and an additional species of the *Passiflora vitifolia* complex: Amazonian species diversity due to edaphically differentiated communities. – Plant Syst. Evol. 137: 95–105.

Kramer, K. U. 1957. A revision of the genus *Lindsaea* in the New World with notes on allied genera. – Acta Bot. Neerl. 6: 97–290.

Kramer, K. U. 1989. *Lindsaea mesarum*, a new fern species from the Roraima sandstone. – Ann. Miss. Bot. Gard. 76: 605–607.

Kramer, K. U. 1991. Two remarkable new lindsaeas from South America. – Bot. Helv. 101: 227–233.

Kramer, K. U. 1971. Flora Malesiana series II – Pteridophyta. Vol. 1, part 3: *Lindsaea*-group. – Wolters-Noordhoff Publishing, Groningen, pp. 177–254.

Legendre, P. and Legendre, L. 1998. Numerical ecology (2nd English ed.). – Devel. Environ. Modell. 20. Elsevier.

Marcano, V. 1989. Novedades pteridofiticas neotropicales II. Lindsaeaceae de Venezuela. – Bol. Soc. Venez. Ci. Nat. 146: 241–270.

Nelson, B. W. et al. 1990. Refugia, endemism centers and collecting density in Brazilian Amazonia. – Nature 345: 714–716.

Salovaara, K. J. et al. 2004. Forest classification in an Amazonian rainforest landscape using pteridophytes as indicator species. – Ecography 27: 689–700.

Sang, T. et al. 1997. Chloroplast DNA phylogeny, reticulate evolution and biogeography of *Paeonia* (Paeoniaceae). – Am. J. Bot. 84: 1120–1136.

Schulman, L. et al. 2004. Spatio-ecological niche segregation of two sympatric species of *Clidemia* (Melastomataceae) in western Amazonian non-flooded rainforests. – Folia Geobot. 39: 143–160.
Schulman, L. et al. 2007. Analysing botanical collecting effort in Amazonia and correcting for it in species range estimation. – J. Biogeogr. 34: 1388–1399.
Smith, A. R. et al. 2005. New records of pteridophytes for the flora of Peru. – Sida, Contr. Bot. 21: 2321–2342.
Tate, J. A. and Simpson, B. B. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. – Syst. Bot. 28: 723–737.
Taberlet, P. et al. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. – Plant Mol. Biol. 17: 1105–1109.
Tuomisto, H. and Poulsen, D. 1996. Influence of edaphic specialization on pteridophyte distribution in neotropical rain forests. – J. Biogeogr. 23: 283–293.
Tuomisto, H. 1998. What satellite imagery and large-scale field studies can tell about biodiversity patterns in Amazonian forests. – Ann. Miss. Bot. Gard. 85: 48–62.
Tuomisto, H. et al. 2002. Distribution and diversity of pteridophytes and Melastomataceae along edaphic gradients in Yasuni National Park, Ecuadorian Amazonia. – Biotropica 34: 516–533.
Tuomisto, H. 2006. Edaphic niche differentiation among *Polybotrya* ferns in western Amazonia: implications for coexistence and speciation. – Ecography 29: 273–284.
Van Reeuwijk, H. 1993. Procedures for soil analysis. – Int. Soil Ref. Info. Centre. Technical paper 9 (4th ed.). Wageningen, the Netherlands.
Vareschi, V. 1966. Pteridofitas nuevas de la flora de Venezuela. – Acta Bot. Venez. 1: 83–120.