BLUMEA 46 (2001) 513–525

TAXONOMIC STUDIES OF CHEIROPLEURIA (DIPTERIDACEAE)

MASAHIRO KATO[1], YOKO YATABE[2], NORIO SAHASHI[3] &
NORIAKI MURAKAMI[2]

SUMMARY

Morphological and molecular studies are made on the fern genus *Cheiropleuria*, which was treated in the past as monotypic. We describe *C. parva*, a new species from Borneo, and separate *C. integrifolia*, distributed in Japan and Taiwan and probably in China too, from Southeast Asian *C. bicuspis*. The three species differ from each other in the size, shape and texture of lamina, divergence angles between lobes, frequency of bilobed leaves, and spore size and morphology. *Cheiropleuria parva* is distinct from *C. bicuspis* populations sympatric on Mt Kinabalu in the leaves of juvenile and adult plants, suggesting reproductive isolation. Nucleotide differences in the *rbcL* gene support separation of the three species.

Key words: *Cheiropleuria*, Dipteridaceae, fern, *rbcL*, taxonomy.

INTRODUCTION

Cheiropleuria is an isolated fern genus that is generally referred to the monotypic family Cheiropleuriaceae (e.g., Kramer, 1990; Laferrière, 1998), rarely to Dipteridaceae together with *Dipteris* (Parris et al., 1992), or to Polypodiaceae in traditional classifications (Copeland, 1947; Holttum, 1955). Among these different family treatments it is usually agreed that *Cheiropleuria* is more or less closely related to *Dipteris*. Their close intergeneric relationship is supported by recent molecular and morphological studies (Hasebe et al., 1994, 1995; Pryer et al., 1995).

The morphology of *Cheiropleuria* is simple and provides few diagnostic characters for species delimitation. The sterile leaves are simple or bilobed, or rarely tetralobed, and lobed ones are usually seen in old plants (Tagawa, 1959), while the fertile leaves are always simple and much narrower than the sterile. *Cheiropleuria* appears to be so uninterruptedly variable as to have been treated as comprising a single species, *C. bicuspis* (Blume) C. Presl (Copeland, 1947; Holttum, 1955; Tagawa & Iwatsuki, 1989; Kramer, 1990; Iwatsuki, 1992; Nakaike, 1992; Parris et al., 1992; Iwatsuki et al., 1995; Laferrière, 1998; Ling, 2000). Local populations with simple sterile leaves, which are distributed in Japan (including Ryukyu) and Taiwan, were separated as var.

---

1) Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo 113-0033, Japan; author for correspondence (sorang@biol.s.u-tokyo.ac.jp).
2) Department of Botany, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan.
3) Department of Biology, School of Pharmaceutical Science, Toho University, Funabashi, Chiba 274-0072, Japan.

integrifolia D.C. Eaton ex Matsum. & Hayata (Nakai, 1928; see also Van Alderwerelt van Rosenburgh, 1908), but this treatment has not been accepted by most pteridologists (e.g., Tagawa, 1959; Kramer, 1990; Iwatsuki, 1992; Nakaike, 1992; De Vol & Shieh, 1994; Iwatsuki et al., 1995; Laferrière, 1998). *Cheiropleuria bicuspis* s.l. is distributed widely in SE and E Asia ranging from Malesia (Sumatra to New Guinea), Thailand, Vietnam, S China, Taiwan, and Japan. Copeland (1917) and Christensen & Holttum (1934) noted that *C. bicuspis* of Mt Kinabalu in N Borneo is very variable, and later Holttum (1955) stated that "In Borneo there is a variety with quite small very tough fronds, if bilobed only about 6–8 cm wide." Iwatsuki & Kato (1984) recorded the same form from northwestern E Kalimantan.

We found the two forms differing distinctly in leaf form; not only of adults but also juveniles, as described below. There are also significant differences in quantitative characters of var. *bicuspis* and var. *integrifolia.* Based on these morphologies, along with field observations and molecular data, we propose a new species, *C. parva*, from Borneo, and a new combination for *C. integrifolia.*

MATERIALS AND METHODS

Materials and morphological observations

For a comparison of leaf characters, we examined specimens deposited in the herbaria of the University of Tokyo (TI) and Kyoto University (KYO) and plants collected from Mt Kinabalu, Malaysia. A number of young plants of *C. bicuspis* (*Kato et al. 1622*) and *C. parva* (*Kato et al. 1620, 1621*) were collected from Mt Kinabalu populations 50–100 m apart from each other, and used for a morphological observation of juvenile leaves. We measured the length (mean length if bilobed) and width of lamina, the length from the apex to the point where the lamina is the widest if simple, and the length (mean) of lobes and the divergence angle between lobes if bilobed, and the thickness of lamina between veins (Fig. 1).

Spores were collected from herbarium specimens deposited in KYO and TI. They were coated with a thin layer of carbon and then with gold. Spore morphology was observed in a scanning electron microscope (SEM) JSM-U3 at 15 kV. At least 30 spores per specimen were measured at × 1000 magnification.

For a molecular analysis, two individuals of each of *C. bicuspis* and *C. parva* were collected from Mt Kinabalu, and an individual of *C. integrifolia* was collected from Miyazaki, Japan (Table 1). For comparison *Dipteris conjugata* Reinw. and *D. lobbiana* (Hook.) T. Moore were analyzed. One leaf from each individual was used for DNA

Table 1. Material used in molecular phylogenetic analysis. *C.* = *Cheiropleuria*; *D.* = *Dipteris.*

Species	Locality and voucher	Accession No.
C. bicuspis	Mt Kinabalu, Sabah, Malaysia; *Murakami 98M-12, 98M-13*	AB042570
C. integrifolia	Miyazaki Pref., Kyushu, Japan; *Suzuki 00-01*	AB042569
C. parva	Mt Kinabalu, Sabah, Malaysia; *Murakami 98M-23, 98M-26*	AB042572
D. conjugata	Okinawa, Japan; *Hasebe 27618* (Hasebe et al., 1995)	U05620
D. lobbiana	Tahan National Park, Pahang, Malaysia; *Kato 0926*	AB042561

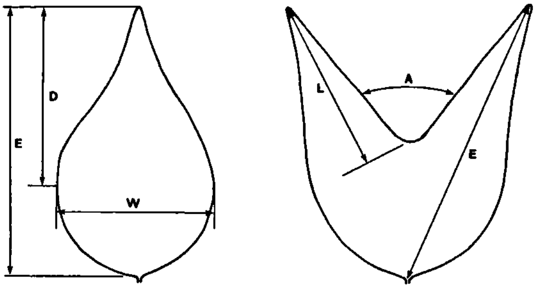

Fig. 1. Leaf-lamina characters measured. A = divergence angle between lobes; D = length from the widest point to the apex; E = entire length; L = length from the base of lobe to the apex; W = width.

extraction, and the remaining part was used for voucher specimen. Vouchers are deposited in KYO, and sequences are deposited in GenBank (see Table 1 for accession numbers).

Molecular phylogenetic analysis

Total DNA was extracted using 2X CTAB solution according to Doyle & Doyle (1987). The DNAs were purified using Qiagen Column Tip-20 (Qiagen GmbH) according to the manufacturer's instruction. We conducted Hot Start PCR (Mullis, 1991) using anti-Taq high (Toyobo). The *rbcL* gene was amplified and sequenced with the following eight primers:

aF (5'-ATGTCACCACAAACAGAGACTAAGC-3')
bF (5'-TATCCCCTGGATTTATTTGAGGAAGGTTC-3')
cF (5'-TGAAAACGTGAATTCCCAACCGTTTATGCG-3')
sF (5'-ACTGTAGTGGGCAAATTGGAAGGCGAACG-3')
sR (5'-GAACCTTCCTCAAATAAATCCAGGGGATA-3')
aR (5'-CTTCTGCTACAAATAAGAATCGATCTCTCCA-3')
bR (5'-CGTTCGCCTTCCAATTTGCCCACTACAGT-3')
cR (5'-GCAGCAGCTAGTTCCGGGCTCCA-3')
[see Hasebe et al. (1994) for details about primer location].

The thermal cycling protocol comprised 35 cycles, each with 1-min denaturation at 94° C, 2-min annealing at 50° C, an extension of 3 min at 72° C, concluding with an extension of 7 min at 72° C. The PCR products were purified using QIA quick gel extraction kit (QIAGEN) after electrophoresis in 1% agarose gel, and then used as templates for direct sequencing. Sequencing reaction was conducted using a Big Dye terminator cycle sequencing kit (Perkin Elmer). The reaction mixtures were analyzed on an Applied Biosystems Model 377 automated sequencer (Perkin Elmer). Sequence data were aligned using the Sequence Navigator program (Perkin Elmer).

Phylogenetic analysis was performed by the maximum parsimony method using PAUP version 3.1.1 (Swofford, 1993). Branch and bound searches were performed with character states specified as unordered and equally weighted, further sequence addition and MULPARS on. To evaluate the relative robustness of the clades found in the most parsimonious tree, bootstrap analyses (Felsenstein, 1985) were conducted on 10,000 replicates using a general heuristic search with equally weighted and simple sequence addition. All minimal trees were held for each replicate. We used *Dipteris conjugata* and *D. lobbiana* as outgroups to root the tree. Its relevance as an outgroup was shown by a comprehensive molecular work on most fern families by Hasebe et al. (1995).

COMPARATIVE LEAF MORPHOLOGY

Cheiropleuria parva vs. C. bicuspis and C. integrifolia

In mature or semimature plants, the simple lamina of *C. parva* is much shorter and moderately wider than that of *C. bicuspis* and *C. integrifolia*, and consequently the ratio of lamina length to width in *C. parva* is smaller (Table 2). The bilobed lamina of *C. parva* is also much shorter. The ratio of the lobe length to the entire lamina length is a little smaller than in *C. bicuspis* and a little larger than in *C. integrifolia*, but without a significant difference (Table 3). The divergence angles of the lobes of *C. parva* are larger than those of the other two species.

The thickness of lamina and the distinctness of veins also differ. In *C. parva* the laminae are 0.5–0.7 mm thick in a dried condition and thick-coriaceous, while they are 0.2–0.4 mm and firm-papyraceous in *C. bicuspis* distributed in Mt Kinabalu together with *C. parva*. In *C. parva* the veinlets are hardly or not visible on the abaxial side of laminae, while in *C. bicuspis* and *C. integrifolia* they are visible.

There is another difference in leaf morphology between juvenile plants of *C. bicuspis* and *C. parva*, which are sympatric in Mt Kinabalu (Fig. 2). There is a heteroblastic change of leaves in both species, but the heteroblastic patterns differ. In *C. parva* the youngest or occasionally the second youngest leaves of the juvenile plants examined

Table 2. Measurements (means ± SDs) of leaf-lamina characters* in simple-leaved *Cheiropleuria*.

Species (populations)	Length (E) (mm)	Width (W) (mm)	Length (D) (mm)	Ratio: E/W	Ratio: D/E
C. integrifolia (N = 165)	155 ± 31	52 ± 16	101 ± 21	3.2 ± 0.8	0.65 ± 0.06
C. integrifolia (Japan; N = 150)	155 ± 31	50 ± 15	101 ± 21	3.3 ± 0.7	0.65 ± 0.06
C. integrifolia (Japan excl. Ryukyu; N = 80)	147 ± 26	44 ± 13	95 ± 17	3.5 ± 0.7	0.65 ± 0.06
C. integrifolia (Ryukyu; N = 70)	164 ± 35	57 ± 15	108 ± 24	3.0 ± 0.7	0.66 ± 0.06
C. integrifolia (Taiwan; N = 15)	152 ± 28	73 ± 17	103 ± 20	2.2 ± 0.5	0.68 ± 0.05
C. bicuspis (N = 21)	118 ± 22	58 ± 16	86 ± 16	2.1 ± 0.5	0.73 ± 0.06
C. bicuspis (SE Asia excl. Mt Kinabalu; N = 8)	124 ± 29	66 ± 18	85 ± 21	2.0 ± 0.4	0.68 ± 0.07
C. bicuspis (Mt Kinabalu; N = 13)	113 ± 16	53 ± 13	87 ± 13	2.3 ± 0.6	0.76 ± 0.03
C. parva (Borneo; N = 16)	77 ± 15	60 ± 12	57 ± 10	1.3 ± 0.2	0.74 ± 0.05

*) Length (E) = entire length; length (D) = length from the widest point to the apex (see Fig. 1).

Table 3. Measurements (means ± SDs) of leaf-lamina characters* in bilobed-leaved *Cheiropleuria.*

Species (populations)	Length (E) (mm)	Length (L) (mm)	Angle (A) (°)	Ratio: L/E
C. integrifolia (N = 19)	156 ± 20	57 ± 12	33 ± 9.1	0.37 ± 0.07
C. integrifolia (Japan excl. Ryukyu; N = 7)	144 ± 16	53 ± 15	34 ± 3.5	0.37 ± 0.09
C. integrifolia (Ryukyu) (N = 12)	162 ± 20	60 ± 10	33 ± 11	0.37 ± 0.05
C. bicuspis (N = 63)	146 ± 29	88 ± 26	81 ± 21	0.60 ± 0.09
C. bicuspis (SE Asia excl. Mt Kinabalu; N = 44)	147 ± 30	90 ± 27	85 ± 18	0.60 ± 0.08
C. bicuspis (Mt Kinabalu; N = 19)	143 ± 28	85 ± 24	68 ± 23	0.58 ± 0.10
C. parva (Borneo; N = 41)	80 ± 17	37 ± 11	106 ± 23	0.47 ± 0.10

*) Length (E) = entire length; length (L) = length from the base of lobe to the apex; angle (A) = divergence angle between lobes (see Fig. 1).

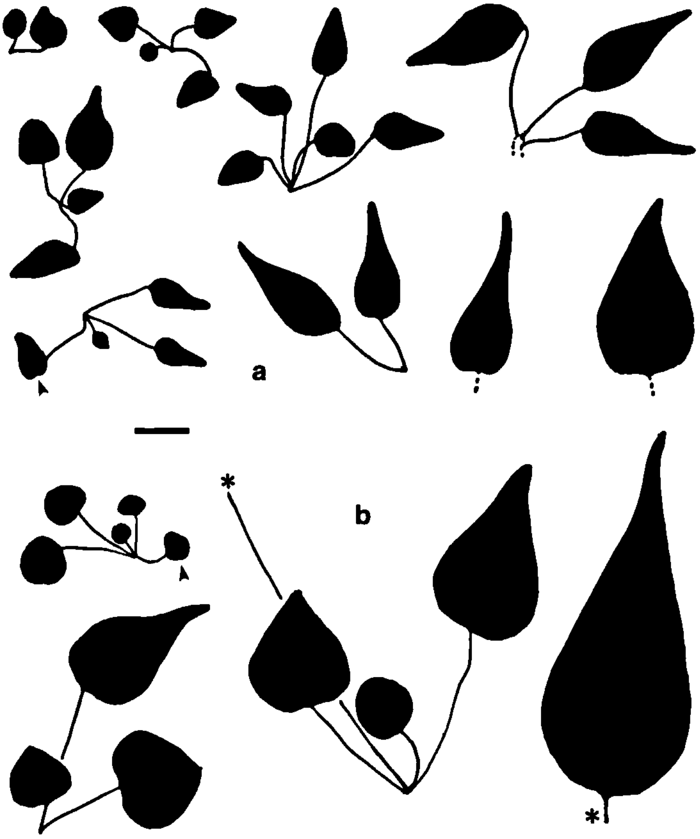

Fig. 2. Silhouettes of selected leaves of Mt Kinabalu sympatric juvenile plants of *Cheiropleuria.* a. *C. parva* M. Kato, Y. Yatabe, Sahashi & N. Murak.; b. *C. bicuspis* (Blume) C. Presl. Arrowheads indicate attachment of lamina to petiole. — Scale bar = 1 cm.

are usually round. Their laminae are not more than 7 mm long and wide, and are round at the apex. The older leaves are longer with the apices subacute, then acute to acuminate. In Mt Kinabalu populations of *C. bicuspis*, the young leaves with laminae up to 11 mm (rarely 13 mm) long and wide still have round apices (see also Wagner, 1952) and are much larger than those of *C. parva* at comparable heteroblastic stages. Then the leaves change to those with acute to acuminate apices.

Cheiropleuria integrifolia vs. C. bicuspis

A quantitative comparison shows that the two species are separable in the relative length of simple sterile leaves and the frequency of bilobed-leaved plants. The leaves of *C. integrifolia* are longer and relatively narrower than those of *C. bicuspis* (Table 2) and simple-leaved plants are more frequent in *C. integrifolia* than in *C. bicuspis* (Fig. 3). All three species take a heteroblastic change from simple young leaves toward bilobed old leaves. Northern or temperate plants of *C. integrifolia* show a tendency toward having generally simple-leaves, as pointed out by Tagawa (1959), indicating that plants become reproductively mature at heteroblastically young stages. It is noted that no Taiwanese materials examined are bilobed-leaved, as described in a local flora (De Vol & Shieh, 1994), but Taiwanese *C. integrifolia* is similar to *C. bicuspis* in the relative length of leaves.

SPORE MORPHOLOGY

The spore morphology is similar among the species, except for the length of the laesurae. Those of *C. parva* are longer relative to spore size than those of the other species, which are 2/3 of the equatorial radius. There is a difference in spore size between *C. integrifolia* and the other two species. Erdtman & Sorsa (1971) noted that *C. bicuspis* from Sumatra has smaller spores (23 by 38 µm for acetolyzed spores) than *C. integrifolia* from Taiwan (as var. *integrifolia*; 39 by 49 µm). Other reports verify the difference [Kawasaki, 1963 for Taiwanese *C. integrifolia*; Nayar & Devi,

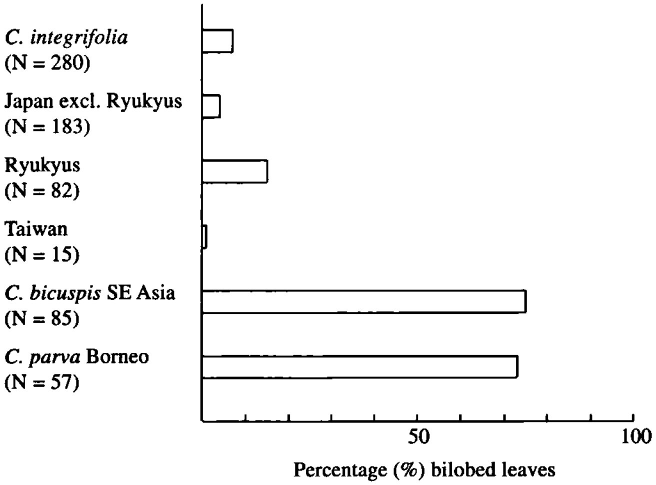

Fig. 3. Frequencies of bilobed specimens of three *Cheiropleuria* species deposited in KYO and TI.

1964 for Bornean *C. bicuspis*; Kremp & Kawasaki, 1972 for Japanese (Ryukyu, Honshu) *C. integrifolia*; Botanical Institute of Beijing, 1976 for Chinese (Hainan) *C. integrifolia*; Huang, 1981 for Taiwanese *C. integrifolia*]. Our observations also show such a spore size difference. Spores of *C. integrifolia* are 26 (23–29) by 35 (31–40) µm, while spores of *C. bicuspis* are 22.4 (20.3–24.8) by 29.6 (26.5–32.7) µm (for *C. parva*, see description).

The difference in spore size may be correlated to ploidy level, as in many other plants. Nakato (1996) reported that *C. integrifolia* (as *C. bicuspis* s.l.) from the Ryukyu, Japan, has 232 chromosomes in mitosis (2n = 232, 4×), while Walker (1984, pers. comm.) reported n = c. 57 (57–60) for *C. bicuspis* (2×) collected from Mulu National Park, Sarawak. Taken together, it might be possible that *C. bicuspis* and *C. parva* are diploid and different from the tetraploid *C. integrifolia*, pending further chromosomal study.

SEQUENCE VARIATION AND MOLECULAR PHYLOGENETIC ANALYSIS

Boundaries of the *rbcL* gene were estimated according to Hasebe et al. (1994). The final alignment has a total length of 1250 sites (see Fig. 5), of which 13 are variable. The two individuals of *C. parva* and those of *C. bicuspis* from Mt Kinabalu each showed the same sequences. Seven sites of the *rbcL* sequences were variable between *C. parva* and *C. bicuspis*. The *C. integrifolia* plant showed 10 and 9 nucleotide differences from *C. bicuspis* and *C. parva*, respectively.

The single most parsimonious tree (length = 63 steps; CI = 0.968; RI = 0.951) was obtained by the Wagner parsimony method (Fig. 4). *Cheiropleuria parva* and *C. bicuspis* from Mt Kinabalu did not make a clade. The *rbcL* sequence variation at 7 nu-

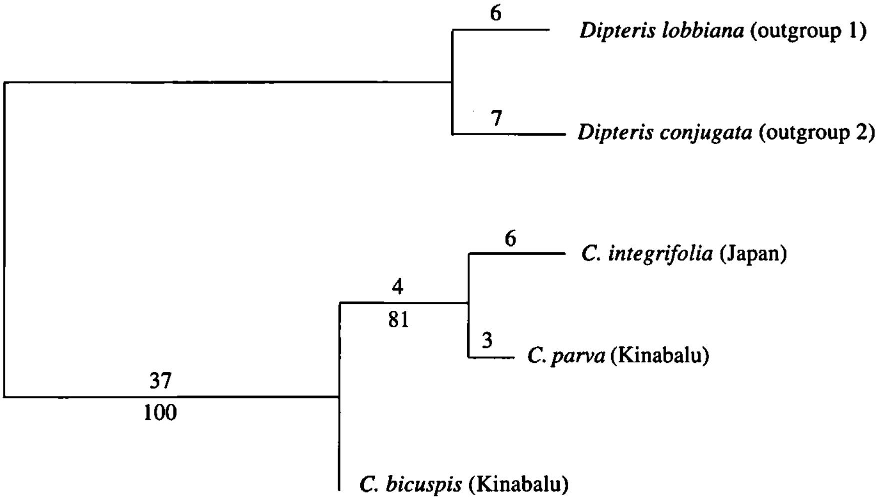

Fig. 4. The single most parsimonious tree (length = 63 steps; CI = 0.968; RI = 0.951) from analysis of *rbcL* sequence data of *Cheiropleuria* species. Numbers above and below branches indicate numbers of nucleotide substitutions (ACCTRAN optimization) and bootstrap percentages, respectively. *Dipteris conjugata* Reinw. and *D. lobbiana* (Hook.) T. Moore were treated as outgroup.

```
                                                                                                                   100
C. bicuspis (Kinabalu)     CTCACTACAC TCCCGATTAT GAGACCAAAG ATACTGACAT CTTGGCAGCC TTCCGGATGA CTCCGCAGCC CGGGGTACCG CCTGAGGAAG CTGGAGCTGC
C. parva (Kinabalu)        A......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
C. integrifolia (Japan)    A......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
                                                                                                                   200
C. bicuspis (Kinabalu)     AGTAGCTGCG GAATCTTCCA CAGGTACGTG GACCACTGTG TGGACGGATG GACTCACTAG CCTCGATCGT TACAAGGGTC GATGCTACGA TATCGAACCC
C. parva (Kinabalu)        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......... .......... .......... .......... .......... .......... .......... .......... .C........
                                                                                                                   300
C. bicuspis (Kinabalu)     GTCGCTGGGG AAGAGAATCA ATATATCGCA TATGTAGCTT ATCCTTCGGA TCTATTTGAA GAAGGTTCCG TCACCAATAT GTTCACTTCC ATTGTAGGTA
C. parva (Kinabalu)        .......... .......... .......... .......... .......... ..C....... .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
                                                                                                                   400
C. bicuspis (Kinabalu)     ACGTATTTGG ATTTAAAGCC CTACGAGCTC TACGTTTGGA GGACCTGAGA ATTCCTCCTG CTTATTCCAA AACTTTCATA GGGCCGCCCC ACGGTATCCA
C. parva (Kinabalu)        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......... .......... .......... .......... ........C. .......... .......... .......... ..........
                                                                                                                   500
C. bicuspis (Kinabalu)     AGTCGAGAGG GATAAACTGA ACAAATATGG TCGTCCCTTG CTGGGATGTA CAATCAAGCC AAAATTGGGC TTATCTGCCA AAAACTATGG CAGGGCTGTT
C. parva (Kinabalu)        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
                                                                                                                   600
C. bicuspis (Kinabalu)     TACGAATGTC TCCGTGGCGG ACTTGATTCT ACGAAGGATG ATGAGAACGT AAATTCTCAA CCATTCATGC GTTGGAGGGA CCGGTTCCTG TCTGTGGCGG
C. parva (Kinabalu)        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......... .......... .......... .......... .......... .......... .......... .......G.. ..........
                                                                                                                   700
C. bicuspis (Kinabalu)     AAGCTCTTTT CAAGGCTCAG GCCGAAACGG GCGAAATAAA AGGACATTAT CTAAATGCCA CTGCGGGTAC GTGTGAGGAA ATGATGAAAA GAGCAGTCTT
C. parva (Kinabalu)        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......... .......... .......... .......... .......... .......... .......... .......... ......C...
                                                                                                                   800
C. bicuspis (Kinabalu)     TGCTAGAGAA TCGGGAGCAC CCATCGTCAT GCATGATTAC TTGACAGGAG GCTTCACTGC AAATACTAGC TTGGCCTTTT ATTGTCGAGA TAATGGGCTA
C. parva (Kinabalu)        .......... .......T.. .....AC..C .......... .......... .......... .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......T.. .....AC... .......... .......... .......... .......... .......... .......... ..........
                                                                                                                   900
C. bicuspis (Kinabalu)     CTGCTTCATA TCCACCGCGC GATGCATGCT GTTATCGATA GACAGAGAAA TCACGGTATC CATTTCCGTG TCCTGGCCAA AGCGTTGCGT ATGTCCGGTG
C. parva (Kinabalu)        .......... .......... .......... .......... .......... .........G .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......... .......... .......... .......... .......... .......... .......... ...A...... ..........
                                                                                                                   1000
C. bicuspis (Kinabalu)     GGGATCATGT CCACGCCGGG ACGGTAGTGG GTAAACTGGA GGGAGAACGA GACGTCACAT TGGGTTTCGT CGATTTGCTA CGCGACGATT ATATCGAGAA
C. parva (Kinabalu)        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
                                                                                                                   1100
C. bicuspis (Kinabalu)     AGACCGAAGC CGGGGTATAT ATTTCACTCA GGATTGGGTA TCCATGCCAG GTGTATTTCC CGTAGCCTCG GGAGGTATCC ACGTCTGGCA TATGCCCGCT
C. parva (Kinabalu)        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
                                                                                                                   1200
C. bicuspis (Kinabalu)     CTAACTGAAA TCTTCGGAGA TGATTCTGTC TCACAGTTTG GCGGAGGAAC CTTGGGACAC CCCTGGGGAA ACGCACCAGG CGCTGTAGCT AATCGAGTTG
C. parva (Kinabalu)        .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......... .......... ........C. .......... .......... .......... .......... .......... ..........

C. bicuspis (Kinabalu)     CATTGGAGGC TTGTGTACAA GCCCGTAATG AGGGACGCGA CCTTGCTCGT
C. parva (Kinabalu)        .......... .......... .......... .......... ..........
C. integrifolia (Japan)    .......... .......... .......... .......... ..........
```

Fig. 5. Alignment of three *rbcL* sequences of *Cheiropleuria parva* M. Kato, Y. Yatabe, Sahashi & N. Murak., *C. bicuspis* (Blume) C. Presl, and *C. integrifolia* (D.C. Eaton ex Hook.) M. Kato, Y. Yatabe, Sahashi & N. Murak., showing different nucleotides. Dots indicate the same nucleotides.

cleotide positions and a remote relationship between *C. parva* and *C. bicuspis* suggest a strong isolation between the two populations even though they are sympatric, and support a taxonomic segregation of *C. parva* from *C. bicuspis*.

Cheiropleuria integrifolia has 6 unique nucleotide changes and 9 and 10 nucleotide differences from *C. parva* and *C. bicuspis*. *rbcL* is estimated to be a slowly evolving gene. Yatabe et al. (1999) reported that the *rbcL* gene of *Osmunda* changed at the rate of one nucleotide substitution per five million years. Thus, the pattern of the nucleotide changes supports a proposed taxonomic segregation of *C. integrifolia* from *C. bicuspis*. If *C. integrifolia*, like *C. parva*, is a derivative from local (northern) populations of *C. bicuspis*, the last species is a paraphyletic group. Samples of *C. bicuspis* from two other localities show similarly large nucleotide differences not only from *C. bicuspis* of Mt Kinabalu but also from *C. integrifolia* and *C. parva* (data not shown), suggesting more genetic variations of *C. bicuspis* than we are aware. Further morphological and molecular studies may find variations that merit separation of local species in *Cheiropleuria*, even if morphological differences are small.

TAXONOMIC TREATMENT

CHEIROPLEURIA

Cheiropleuria C. Presl, Epimel. Bot. (1851) 189; Copel., Gen. Fil. (1947) 178; Holttum, Rev. Fl. Malaya 2 (1955) 136; Tagawa, Col. Ill. Japan. Pterid. (1959) 154; Copel., Fern Fl. Philip. 3 (1960) 456; Ohwi, Fl. Japan (1965) 97; Walker, Fl. Okinawa (1976) 111; Tagawa & K. Iwats., Fl. Thailand 3 (1979) 484; Kramer in Kubitzki (ed.), Fam. & Gen. Vasc. Pl. 1 (1990) 69; Parris et al., Pl. Mt Kinabalu 1 (1992) 48; K. Iwats., Ferns & Fern Allies Japan (1992) 260; De Vol in Fl. Taiwan 2nd ed. 1 (1994) 467; K. Iwats. in K. Iwats. et al. (eds.), Fl. Japan 1 (1995) 233; Laferr., Fl. Males. II, 3 (1998) 285; Y.X. Ling, Fl. Reip. Pop. Sin. 6, 2 (2000) 5. — Type species: *Cheiropleuria bicuspis* (Blume) C. Presl.

Terrestrial. *Rhizomes* creeping with leaves narrowly spaced, branched, covered by pale-brown multicellular hairs. *Leaves* dimorphic; petioles glabrous except at the hairy base; sterile laminae simple or bilobed or rarely tetralobed, acuminate or caudate at apex, cuneate, round to cordate at base, firm-papyraceous to thick-coriaceous, glabrous on both surfaces; veins reticulate, main veins digitate and parallel, sometimes forked, visible, fine veinlets between them visible or hardly visible; fertile laminae simple, linear, gradually narrowed to both ends. *Sori* acrostichoid, sporangia entirely covering the abaxial surface of lamina except the margin, mixed with club-shaped unicellular paraphyses, exindusiate. *Spores* tetrahedral, trilete, surface psilate by light microscopy (LM).

KEY TO THE SPECIES

1a. Simple laminae 6–11 by 4–8.5 cm (L/B = 1–1.5), bilobed laminae 6–11 by 6–12 cm, thick-coriaceous, divergence angles (70–)90–130(–140)°, veinlets invisible .... **3. C. parva**

b. Simple laminae 10–20 by 3–8 cm (L/B = 2 or more), firm-papyraceous, in lobed lamina divergence angles 30–110°, veinlets visible 2

2a. Laminae often simple, sometimes bilobed, divergence angles 30–40° (Japan, Taiwan, China) .... **2. C. integrifolia**
b. Laminae usually bilobed, divergence angles more than 70°, rarely (in young plants) simple (SE Asia) .... **1. C. bicuspis**

1. Cheiropleuria bicuspis (Blume) C. Presl

Cheiropleuria bicuspis (Blume) C. Presl, Epimel. Bot. (1851) 189; Tardieu & C. Chr. in Lecomte, Fl. Gén. Indo-Chine 7 (1941) 443; Holttum, Rev. Fl. Malaya 2 (1955) 136; Copel., Fern Fl. Philip. 3 (1960) 456; Tagawa & K. Iwats., Fl. Thailand 3 (1979) 484; Parris et al., Pl. Mt Kinabalu 1 (1992) 48, p.p.?; Laferr., Fl. Males. II, 3 (1998) 285. — *Polypodium bicuspe* Blume, Enum. Pl. Javae (1828) 125. — Type: *Blume s.n.* (L, photo), Java.

Petioles 20–50 cm long in sterile leaves, equally long or longer in fertile leaves; sterile leaves bilobed, rarely tetralobed or sometimes simple; simple sterile laminae ovate-lanceolate or ovate, 8–15 by 3–8 cm, L/B = 1.5–2.5, bilobed ones 10–20 cm long, as wide as long, lobed more than 1/2 from the apex, widely cuneate to widely round or sometimes subcordate at base, 0.2–0.4 mm thick, firm-papyraceous, divergence angles 70–110°; fine veinlets visible; fertile laminae 10–25 by 1–1.5 cm. *Spores* 22 by 30 µm, laesurae 2/3 of the equatorial radius.

Distribution — Vietnam, Thailand, throughout Malesia.

2. Cheiropleuria integrifolia (D.C. Eaton ex Hook.) M. Kato, Y. Yatabe, Sahashi & N. Murak., *stat. nov.*

Acrostichum bicuspe (Blume) Hook. var. *integrifolia* D.C. Eaton ex Hook., Sp. Fil. 5 (1864) 272. — *Cheiropleuria bicuspis* (Blume) C. Presl forma β *integrifolia* D.C. Eaton ex Luerss., Bot Jahrb. Syst. 4 (1883) 354. — *Cheiropleuria bicuspis* (Blume) C. Presl var. *integrifolia* D.C Eaton ex Matsum. & Hayata, J. Coll. Sci. Imp. Univ. Tokyo 22 (1906) 641; Nakai, Bot. Mag Tokyo 42 (1928) 214. — Type: *C. Wright* (K?, n.v.), Ryukyu, Japan.

Cheiropleuria bicuspis auct. non C. Presl: sensu Tagawa, Col. Ill. Japan. Pterid. (1959) 154, 189; Ohwi, Fl. Japan (1965) 97; Nakaike, Enum. Pterid. Japon. (1975) 361; Walker, Fl. Okinawa (1976) 111; K. Iwats., Ferns & Fern Allies Japan (1992) 260; Nakaike, New Fl. Japan Pterid. (1992) 634; De Vol, Fl. Taiwan 2nd ed. 1 (1994) 467; K. Iwats. in K. Iwats. et al. (eds.), Fl. Japan 1 (1995) 233; Y.X. Ling, Fl. Reip. Pop. Sin. 6, 2 (2000) 5, p.p.

Petioles 20–50 cm long in sterile leaves, longer in fertile leaves; sterile leaves simple or sometimes bilobed; simple sterile laminae oblong- or ovate-lanceolate, 10–22 by 3–8 cm, L/B = 2–4.5, bilobed ones 13–19 by up to 9(–12) cm, lobed less than 2/5 from the apex, cuneate to round-cuneate at base, 0.2–0.4 mm thick, firm-papyraceous, divergence angles 30–40°; fine veinlets visible; fertile laminae 10–25 by 1–1.5 cm. *Spores* 26 by 35 µm, laesurae 2/3 of the equatorial radius.

Distribution — Japan, Taiwan, China.

3. Cheiropleuria parva M. Kato, Y. Yatabe, Sahashi & N. Murak., *spec. nov.* — Fig. 6, 7

Ab *C. bicuspide* laminis parvis, solidissimis, lobis brevibus, basi rotundatis vel subcordatis, venis aegre visibilibus differt. — Typus: *Kato et al. 1488* (holotype UKMS; isotypes HYO, KYO, TI), Mt Kinabalu, summit trail, open slope, 2100 m, 22 Dec. 1997.

Rhizomes 2.8–4.6 mm thick. *Leaves* 3–6 mm apart; petioles 10–23 cm long in sterile leaves, 15–23 cm long in fertile leaves; simple sterile laminae deltoid-ovate, 6–11 (mean: 7.7) cm long, 4–8.5 (mean: 6) cm wide, L/B = 1–1.5, bilobed ones 6–11 (mean: 8) cm long, 6–12 cm wide, lobed less than 1/2 from the apex, acuminate or caudate at apex, round to cordate at base, thick-coriaceous, 0.5–0.7 mm thick in dried condition, thick-coriaceous, divergence angles (70–)90–130(–140)° (mean: 105°); veins reticulate, fine veinlets between main veins hardly visible on the abaxial surface of laminae; fertile laminae 10–12 by 0.8–1.4 cm. *Spores* 20–25.5 (mean: 22.5) µm in polar diameter, 27–33 (mean: 30) µm in equatorial diameter, laesurae 3/4 of the equatorial radius and bordered by distinct margo, surface psilate by LM, perine more or less smooth and fragile by SEM (Fig. 7).

Habitat — Open or semi-open slopes or ground in montane or mossy forests. In Mt Kinabalu, Sabah (N Borneo), Malaysia, populations occur in patches on open or semi-open, gentle or steep slopes (often along trails), sympatric with *C. bicuspis*. The two species seem to be reproductively isolated.

Distribution — Borneo (Sabah, northwestern E Kalimantan).

Note — *Lau 27379* (KYO) from Hainan is similar to *C. parva* rather than to *C. bicuspis*, and further collecting and observation are needed for Hainan populations.

Other specimens examined:

Kato et al. 1620 (TI), Mt Kinabalu, summit trail, open steep slope, 2100 m; *Kato et al. 1621* (TI), Mt Kinabalu, summit trail, open steep slope, 1900 m; *Suehiro F-814* (KYO), *Yamada 1675* (KYO), *Kokawa & Hotta 5543* (KYO), Mt Kinabalu; *Kato et al. 9510* (KYO), E Kalimantan, Gunung (= Mt) Batu Harun, mossy forests; *Kato et al. 11115* (KYO), E Kalimantan, Gunung (= Mt) Buduk Rakik, mossy forests.

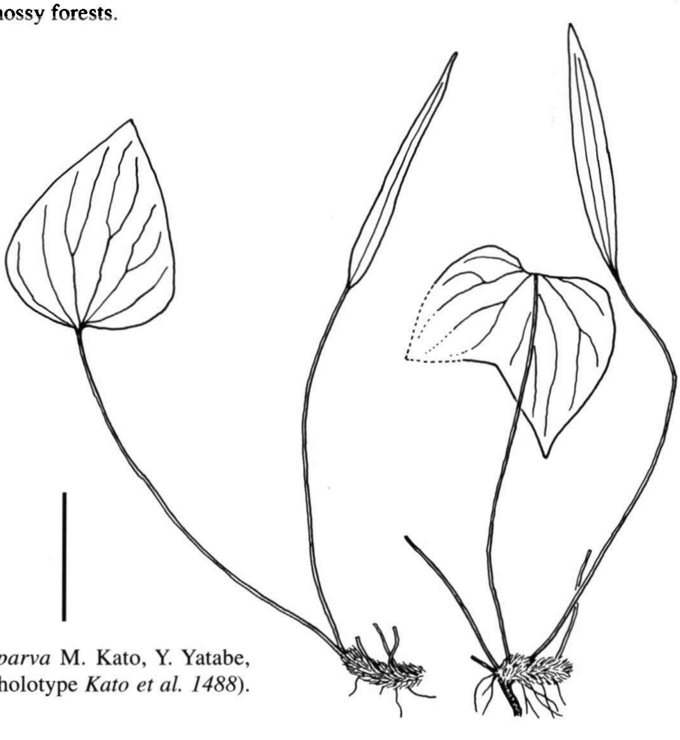

Fig. 6. *Cheiropleuria parva* M. Kato, Y. Yatabe, Sahashi & N. Murak. (holotype *Kato et al. 1488*). — Scale bar = 5 cm.

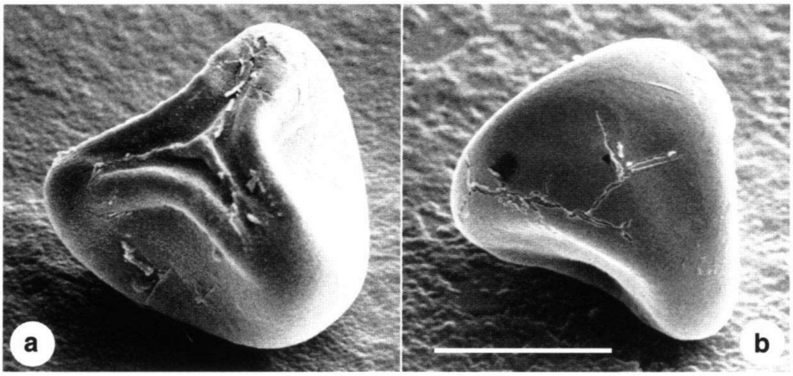

Fig. 7. Spore SEM micrographs of *Cheiropleuria parva* M. Kato, Y. Yatabe, Sahashi & N. Murak. (*Kato et al. 11115*). a. Distal face; b. proximal face. — Scale bar = 20 µm.

ACKNOWLEDGEMENTS

We thank Prof. K. Iwatsuki, Dr. H. Akiyama, Mr. T. Suzuki, Prof. Maryati Mohamed, and Mr. Razali Jaman for their assistance in the field works and collection of plant materials. We also thank Ms. K. Matsuda for her assistance in the sequencing experiments, and Prof. T. G. Walker for providing cytological data. This study was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science to MK and NM, and for JSPS Fellows to YY.

REFERENCES

Botanical Institute of Beijing. 1976. Sporae Pteridophytorum Sinicorum. Science Press, Beijing.

Christensen, C. & R.E. Holttum. 1934. The ferns of Mt Kinabalu. Gard. Bull. Str. Settl. 7: 191–324, with 12 plates.

Copeland, E.B. 1917. Keys to the ferns of Borneo. Sarawak Mus. J. 2: 287–424.

Copeland, E.B. 1947. Genera filicum. Chronica Botanica, Waltham, Mass.

De Vol, C.E. & W.-C. Shieh. 1994. Cheiropleuriaceae. In: Flora of Taiwan. 2nd ed. Vol. 1: 467–468. Editorial Committee of the Flora of Taiwan, Taipei.

Doyle, J.J. & J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. Phytochem. Bull. 19: 11–15.

Erdtman, G. & P. Sorsa. 1971. Pollen and spore morphology/plant taxonomy. Pteridophyta. Almqvist & Wiksell, Stockholm.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Hasebe, M., T. Omori, M. Nakazawa, T. Sano, M. Kato & K. Iwatsuki. 1994. rbcL gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. Proc. Natl. Acad. Sci. USA 91: 5730–5734.

Hasebe, M., P. Wolf, K. Pryer, K. Ueda, M. Ito, R. Sano, G. Gastony, J. Yokoyama, J. Manhart, N. Murakami, E. Crane, C. Haufler & W. Hauk. 1995. Fern phylogeny based on rbcL nucleotide sequences. Amer. Fern J. 85: 134–181.

Holttum, R.E. 1955. A revised flora of Malaya. Vol. II. Ferns of Malaya. Government Printing Office, Singapore.

Huang, T.-C. 1981. Spore flora of Taiwan (Pteridophyta). Tseng-Chieng Huang, Taipei.

Iwatsuki, K. 1992. Ferns and fern allies of Japan. Heibonsha, Tokyo.

Iwatsuki, K. & M. Kato. 1984. Additions to the enumeration of East Kalimantan pteridophytes 3. Acta Phytotax. Geobot. 35: 59–67.

Iwatsuki, K., T. Yamazaki, D.E. Boufford & H. Ohba (eds.). 1995. Flora of Japan Vol. 1. Pteridophyta and Gymnospermae. Kodansha, Tokyo.

Kawasaki, T. 1963. On the external figures of spores in the Polypodiaceae and their phylogenetical significance (1). Bull. Tokyo Gakugei Univ. Ser. 4, 14: 69–81.

Kramer, K.U. 1990. Cheiropleuriaceae. In: K. Kubitzki (ed.), The families and genera of vascular plants. Vol. I. K.U. Kramer & P.S. Green (eds.), Pteridophytes and gymnosperms: 68–69. Springer-Verlag, Berlin.

Kremp, G.O. & T. Kawasaki. 1972. The spores of the pteridophytes. Hirokawa Publishing Co., Tokyo.

Laferrière, J.E. 1998. Cheiropleuriaceae. Fl. Males. II, 3: 285–286.

Ling, Y.-X. 2000. Cheiropleuriaceae. Fl. Reip. Pop. Sin. 6, 2: 5–6.

Mullis, K.B. 1991. The polymerase chain reaction in an anemic mode, how to avoid cold oligodeoxyribonuclear fusion. PCR Meth. Appl. 1: 1–4.

Nakai, T. 1928. Notes on Japanese ferns VII (Plagiogyriaceae, Cheiropleuriaceae, Dipteridaceae & Polypodiaceae I). Bot. Mag. Tokyo 42: 203–218.

Nakaike, T. 1992. New flora of Japan. Pteridophyta. Shibundo, Tokyo.

Nakato, N. 1996. Notes on chromosomes of Japanese pteridophytes (4). J. Jap. Bot. 163–167.

Nayar, B.K. & S. Devi. 1964. Spore morphology of Indian ferns. III. Polypodiaceae. Grana Palynol. 5: 342–395.

Parris, B.S., R.S. Beaman & J.H. Beaman. 1992. The plants of Mount Kinabalu. I. Ferns and fern allies. Roy. Bot. Gard., Kew.

Pryer, K.M., A.R. Smith & J.E. Skog. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and rbcL sequences. Amer. Fern J. 85: 205–282.

Swofford, D. 1993. Phylogenetic Analysis Using Parsimony, Version 3.1. Illinois Natural History Survey, Champaign.

Tagawa, M. 1959. Coloured illustrations of the Japanese Pteridophyta. Hoikusha, Osaka.

Tagawa, M. & K. Iwatsuki. 1989. Cheiropleuriaceae. Fl. Thailand 3, 4: 481–483.

Van Alderwerelt van Rosenburgh, C.R.W.K. 1908. Malayan ferns: Handbook to the determination of the ferns of the Malayan Islands. Landsdrukkerij, Batavia.

Wagner Jr., W.H. 1952. Types of foliar dichotomy in living ferns. Amer. J. Bot. 39: 578–592.

Walker, T.G. 1984. Chromosomes and evolution in pteridophytes. In: A.K. Sharma & A. Sharma (eds.), Chromosome evolution of eukaryotic groups 2: 103–141. CRC Press, Boca Raton.

Yatabe, Y., H. Nishida & N. Murakami. 1999. Phylogeny of Osmundaceae inferred from rbcL nucleotide sequences and comparison to the fossil evidences. J. Plant Res. 112: 397–404.