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Molecular phylogeny and taxonomy of the fern genus *Anisocampium* (Athyriaceae)

Yea-Chen Liu,[1,2] Wen-Liang Chiou[1] & Masahiro Kato[3]

1 *Division of Botanical Garden, Taiwan Forestry Research Institute, 53 Nan-Hai Road, Taipei, Taiwan*
2 Current address: *Department of Biological Resources, National Chiayi University, Chiayi, Taiwan*
3 *Department of Botany, National Museum of Nature and Science, Tsukuba 305-0005, Japan*
Authors for correspondence: *Wen-Liang Chiou, chiou@tfri.gov.tw*; *Masahiro Kato, sorang@kahaku.go.jp*

Abstract We redefine the fern genus *Anisocampium* based on the results of a phylogenetic analysis using two regions of the chloroplast genome. The redefined genus is separate from *Athyrium* s.str. and comprises four species, two of which were previously assigned to *Athyrium* or *Kuniwatsukia*. *Anisocampium paucijugum* is reduced to a synonym of *A. cumingianum*. The four accepted species in *Anisocampium* are morphologically distinct but the putative hybrid *An.* ×*saitoanum* suggests low reproductive isolation in the genus. *Anisocampium* can be distinguished from related genera by a combination of characters only.

Keywords *Anisocampium*; *Athyrium*; *Kuniwatsukia*; *rbcL*; *trnL-F*

■ INTRODUCTION

The classification of the athyrioid ferns remains unsettled, even though molecular phylogenetic data have been increasingly used to determine relationships. One ambiguous genus is *Anisocampium* C. Presl. (1849), described based on the imparipinnate leaves and round sori of *An. cumingianum* C. Presl. Copeland (1947) added a second species, *An. paucijugum* (Aderw.) Copel., and stated that *Anisocampium* shows an athyrioid affinity.

In 1973, Pichi Sermolli proposed the genus *Kuniwatsukia* as a replacement name for *Microchlaena* Ching (1938), a later homonym of *Microchlaena* Wight & Arn. (Sterculiaceae). *Microchlaena* Ching was based on *M. yunnanensis* (Christ) Ching (= *Microchlaena cuspidata* (Bedd.) Ching). Iwatsuki (1970) considered *M. cuspidata* to be related to *An. cumingianum* and *Athyrium sheareri* (Baker) Ching, based on vascular anatomy of rhizome and stipe, leaf architecture, and chromosome number. Hsieh (1985) formally transferred *Athyrium sheareri* to *Anisocampium*.

Recently, Fraser-Jenkins (2006) referred *Anisocampium cumingianum* to *Athyrium* sect. *Niponica* Ching & Y.T. Hsieh, a section established for *Athyrium niponicum* (Mett.) Hance (Hsieh, 1986). *Athyrium niponicum* has been recognized as a member of *Athyrium* for more than a century after its transfer from *Asplenium*, but recent molecular analyses have shown it to have a closer affinity with *Anisocampium* and *Kuniwatsukia*. In his floristic treatment of Chinese *Athyrium*, Wang & al. (1999) assigned ten species to sect. *Niponica*, in four series: ser. *Niponica* with two species (*Ath. niponicum*, *Ath. brevisorum* (Wall. ex Hook.) Moore) and ser. *Biserrulata*, ser. *Fallaciosa* and ser. *Yokoscentia* with the remaining eight species. Series *Niponica* was characterized by the creeping rhizomes, the abruptly narrowed apex of the bipinnate leaf, the shortened basal pinnules of the stalked, basal pinnae. Except for *Athyrium niponicum*, all species differ from the remaining 160–220 species of *Athyrium* in leaf dissection and shape of sori.

A putative hybrid between *Athyrium niponicum* and *Ath. sheareri* (≡ *An. sheareri*) was reported by Serizawa (1986). Hybridization suggests close affinities of the two species. The free venation of *Athyrium sheareri* is similar to that of *Athyrium* and differs from the anastomosing venation of *Anisocampium cumingianum*.

Recent molecular phylogenic studies have shown that *Athyrium* s.l. (including *Anisocampium*) is paraphyletic. Based on *rbcL* sequences, Sano & al. (2000) found *Ath. sheareri* and *Ath. niponicum* to form a clade sister to a clade comprising *Cornopteris* Nakai and *Athyrium* that includes *Pseudocystopteris* Ching. Based on the *trnL-F* regions, Wang & al. (2003) found *Kuniwatsukia cuspidata* to be clustered with *Ath. niponicum*. In a molecular analysis using a larger dataset, Adjie & al. (2008) confirmed that *Ath. niponicum*, *Ath. sheareri* and *Ath. cuspidatum* form a monophyletic clade. *Anisocampium cumingianum*, the type species of *Anisocampium*, was lacking in all previous molecular phylogenetic analyses, however, leaving the relationship between *Anisocampium* and *Athyrium* unclear.

In this paper, we describe the molecular phylogeny of the species considered to be related to *Anisocampium*. Based on the results of the analysis and comparative morphology, we redefine the genus *Anisocampium* as comprising four species, the Sino-Japanese *An. niponicum* and *An. sheareri*, the Sino-Himalayan *An. cuspidatum*, and the tropical and subtropical Asian *An. cumingianum* (Fig. 1).

■ MATERIALS AND METHODS

Taxon sampling and source of sequences. — A total of 20 athyrioid species of ferns, including eight species of *Athyrium*, three of *Cornopteris*, two of *Diplazium*, three of *Deparia*, and all four species of *Anisocampium* were sampled. The species of

Athyrium represent all five sections recognized by Ching and Hsieh (Hsieh, 1986), or six of the 14 sections proposed by Wang (1997). Three species of sect. *Niponica*, i.e., *Ath. niponicum*, *Ath. biserrulatum*, and *Ath. yokoscense*, are the type species of ser. *Niponica*, ser. *Biserrulata*, and ser. *Yokoscentia*, respectively. The species of *Diplazium* and *Deparia* were selected as outgroups, because they are the closest relatives of *Athyrium* and *Anisocampium* (Sano & al., 2000; Wang & al., 2003; Adjie & al., 2008). In addition to the *rbcL* and *trnL-F* sequences available in GenBank (Appendix), we obtained new sequences of *Anisocampium cumingianum* (*rbcL*, HM156338) and *Athyrium biserrulatum* (*rbcL*, HM156336; *trnL-F*, HM156337). Voucher specimens are deposited in the Herbarium of Taiwan Forestry Research Institute (TAIF).

DNA extraction, PCR amplification and sequencing. — Total genomic DNA of *Anisocampium cumingianum* and *Athyrium biserrulatum* was extracted from silica-gel-dried leaves using the modified cetyl trimethyl ammonium bromide (CTAB) procedure of Doyle & Doyle (1987). Two plastid genome regions (*rbcL*, *trnL-trnF*) were amplified separately using the standard polymerase chain reaction (PCR) and published primer sets (primer ESRBCL1F, ESRBCL1361R from Schuettpelz & Pryer, 2007 for the *rbcL* gene; primer f from Taberlet & al., 1991; Fern-1 from Trewick & al., 2002 for the *trnL-trnF* region including the *trnL* intron and the *trnL-trnF* IGS). The resulting PCR products were purified using GFX PCR DNA and Gel Band Purification kits according to the manufacturer's instructions, then directly sequenced. Sequencing reactions were conducted using the DYEnamic ET Dye Terminator Cycle Sequencing Kit. Sequences were analyzed using MegaBACE 1000 DNA Analysis Systems (Amersham Biosciences, Taipei, Taiwan) following the manufacturer's protocol.

Sequence alignment and phylogenetic analyses. — Alignments of all sequences were generated using CLUSTAL X (Thompson & al., 1997) and subsequently edited manually in BioEdit (Hall, 1999) for both intron and IGS of the *trnL-F* region. Ambiguously aligned regions were excluded from all subsequent analyses. Phylogenetic analyses were conducted using both maximum likelihood and Bayesian inference of phylogeny. Maximum likelihood analyses were conducted using PHYML v.2.4.3 (Guindon & Gascuel, 2003) under the optimal model of sequence evolution. For each dataset, an Akaike information criterion as implemented in jModeltest v.0.1.1 (Posada, 2008) was used to select the model of nucleotide substitution, including gamma shape and proportion of invariant sites. Maximum likelihood bootstrap support (MLBS) values were estimated from 1000 replicates in PHYML. Bayesian inference was performed with MrBayes v.3.0b4 (Ronquist & Huelsenbeck, 2003). Markov chains were run for 10,000,000 generations and sampled every 1000 generations, and the first 1000 trees were the "burn-in" of the chain. An allcompat consensus tree was calculated to obtain topology with average branch lengths as well as posterior probabilities (PP) for all resolved nodes.

■ RESULTS

The phylogenetic relationships of *Anisocampium* were deduced from the combined *rbcL* and *trnL-F* sequences (in total 2003 base pairs) (Fig. 2). *Anisocampium* was sister, with high support (PP = 1.0, MLBS = 98%), to a clade comprising *Athyrium* and *Cornopteris* which showed high support (PP = 1.0, MLBS = 99%). Monophyly of each of the three genera was also robust with high support for each (PP = 1.0, MLBS = 100%). In *Anisocampium*, a clade of *An. sheareri* and *An. cumingianum* was sister to *An. cuspidatum* (≡ *Ath. cuspidatum*), and together sister to *An. niponicum* (≡ *Ath. niponicum*), although support for the first two clades was not very high.

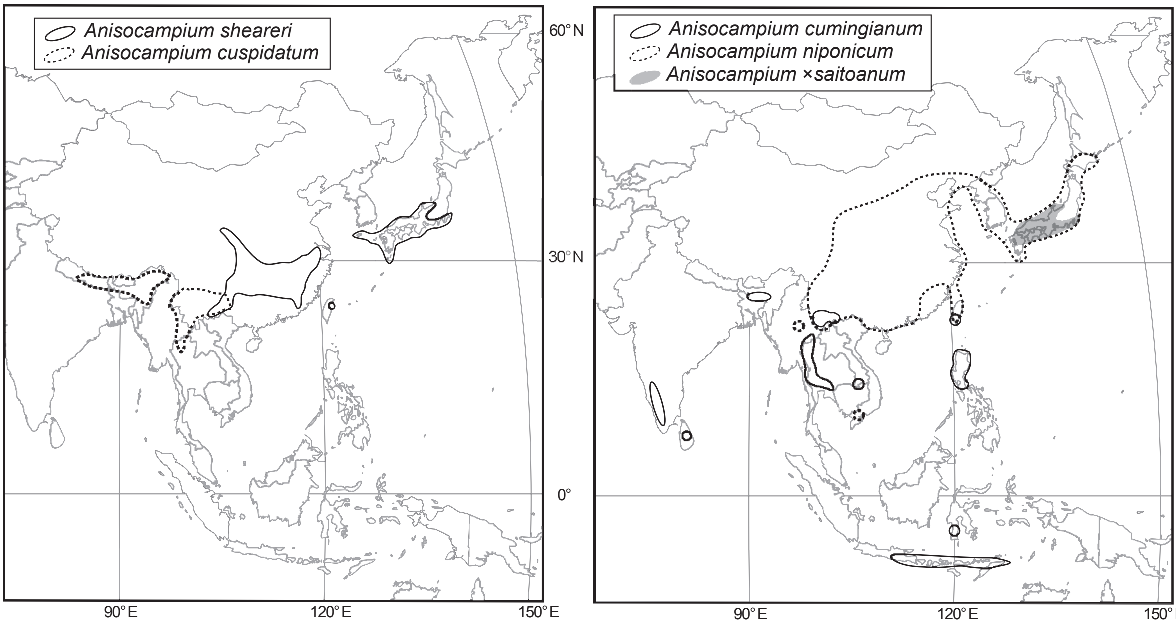

Fig. 1. Distribution of *Anisocampium*.

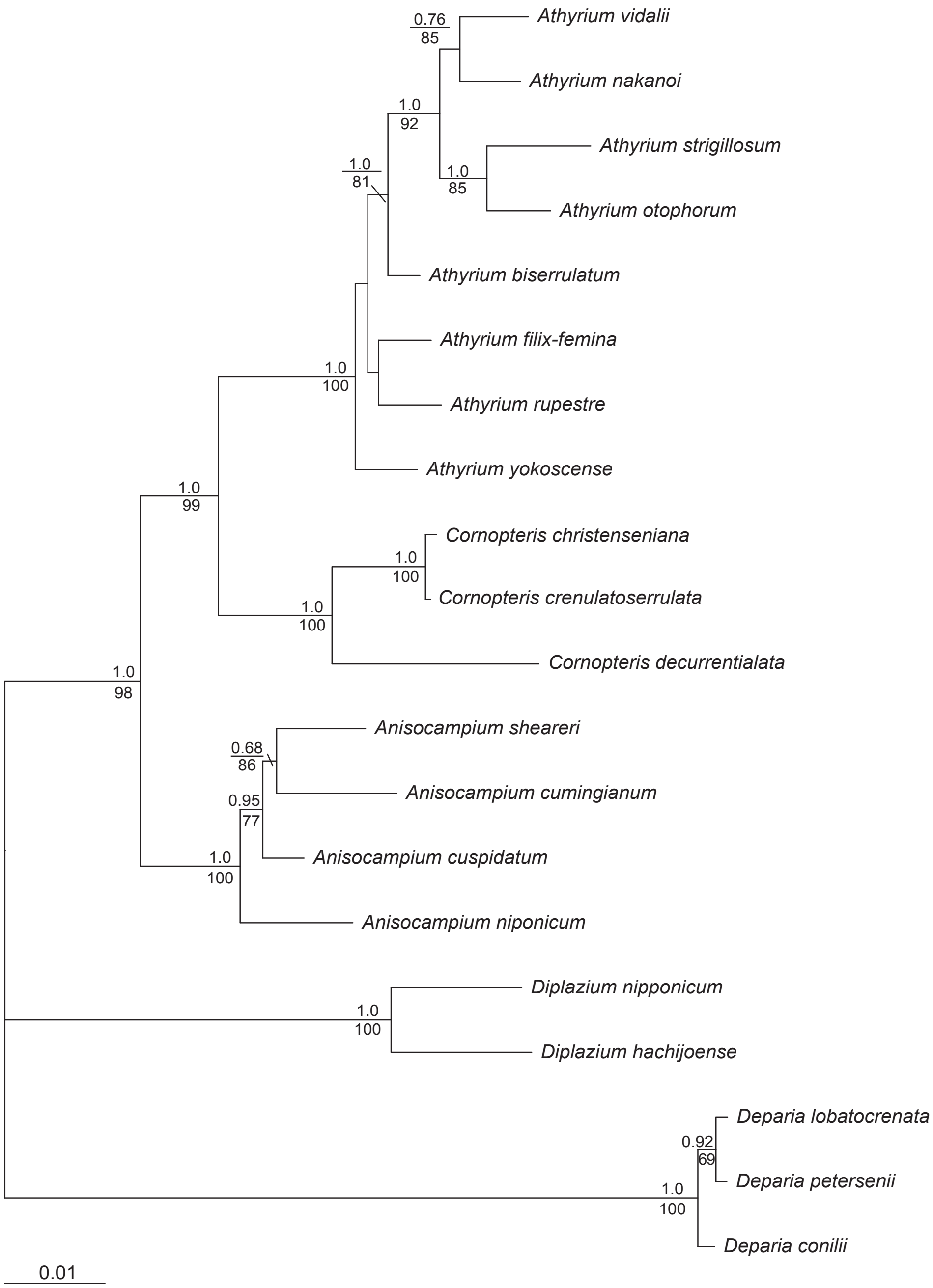

Fig. 2. Phylogenetic tree of *Anisocampium* and allied genera based on *rbcL* and *trnL-F* region sequences. Tree topology is deduced from Bayesian inference analysis; numbers indicate values of posterior probabilities (above branches) and bootstrap (below branches); numbers less than 0.5 or 50 are not shown. Three species of *Deparia* and two of *Diplazium* are outgroups.

■ DISCUSSION

The *Anisocampium* clade is distinct from the *Athyrium* clade, and similar to the topology of previous analyses (Sano & al., 2000; Wang & al., 2003; Schuettpelz & Pryer, 2007; Adjie & al., 2008). In the taxonomic definition below, we redefine the genus *Anisocampium* to include *An. cuspidatum* (≡ *Kuniwatsukia cuspidata*) and *An. niponicum* (≡ *Athyrium niponicum*), and separate the genus from *Athyrium* and *Cornopteris*. The results are not consistent with current taxonomic treatments based on morphological comparisons (Kato, 1977; Ching, 1978; Kato & Kramer, 1990; Wang, 1997; Wang & al., 1999). *Kuniwatsukia* and *Anisocampium* have been treated as synonyms of *Athyrium* (Kato, 1977; Fraser-Jenkins, 2006), while *Cornopteris* has long been presumed to be related to *Diplazium* (Ching, 1945; Wang, 1997) or *Athyrium* (Tagawa, 1959; Kato, 1979). Based on morphological similarity, Fraser-Jenkins (2006) placed *Ath. cumingianum* (≡ *An. cumingianum*), along with *Ath. niponicum*, in *Athyrium* sect. *Niponica*, while recognizing *Cornopteris*. He also noted the similarity of *Ath. cumingianum* with *Ath. cuspidatum* and *Ath. sheareri*.

An alternative classification to ours is to recognize *Athyrium* s.l. as including *Anisocampium* and *Cornopteris*. *Cornopteris* is characterized by exindusiate sori and fleshy projections (corni) at the base of costae and costules, by which it is distinguished from *Athyrium*. Kato (1977), Kato & Kramer (1990) and Fraser-Jenkins (2006) treated *Anisocampium* and *Kuniwatsukia* as synonyms of *Athyrium*, but separated *Cornopteris*. Our phylogenetic analysis indicates that such a circumscription results in the paraphyly of *Athyrium*.

Like Adjie & al. (2008), we did not find any unique diagnostic characters to define *Anisocampium*. The genus can only be defined and distinguished from the closely related *Athyrium* and *Cornopteris* by a combination of features of the stipe base, projections, spines and sori (Table 1). The base of the stipe is as broad as the upper part of the stipe in *Anisocampium*, while the stipe is broader near the base with lateral pneumatophores and narrowed to the very base in *Athyrium*, *Cornopteris* and *Deparia* within the athyrioid ferns (Liew, 1972; Kato, 1977, 1979, 1984). The stipe base is persistent after the leaves wither. They are arranged tightly around the short, erect rhizomes in most species of these two genera, or are loosely arranged on the creeping rhizomes in, e.g., *Athyrium atkinsonii* Bedd. and *Cornopteris decurrentialata* Nakai. The persistent stipe base, called a trophopod (Johnson, 1986), is possibly a derived, adaptive trait in plants in the temperate zone. The persistent stipe base is absent in *Anisocampium* and *Diplazium*, even in species with erect rhizomes.

Fleshy projections (*corni*) in the groove on the adaxial surface at the base of the costae and costules are characteristic of *Cornopteris*, while they are absent from *Anisocampium* and *Athyrium*. There are spines in many species of *Athyrium* and *Cornopteris*, but none in *Anisocampium.* The spines are continuations of the interrupted ends of ridges along the groove of the costae and costules on the adaxial surface of the leaf. The sori are rounded-reniform, shortly U- or J-shaped and indusiate in most species of *Anisocampium*, but they are also oblong-linear in *An. niponicum*. In *Cornopteris*, however, they are rounded to linear and exindusiate and in *Athyrium* usually linear or J-shaped and indusiate.

Diagnostic characters separating *Anisocampium* and related genera are shown in Table 1. A key for their identification and a brief taxonomic treatment of the revised genus *Anisocampium* are given below.

■ KEY TO *ANISOCAMPIUM* AND RELATED GENERA

1. Groove of costa interrupted at base and not confluent with groove of rachis; multicellular hairs present on stipe, rachis and costae, rarely few or absent .... ***Deparia***
1. Groove of costa continuous with groove of rachis; multicellular hairs absent or sometimes present **2**
2. Sori exindusiate; fleshy projections present at base of costa .... ***Cornopteris***
2. Sori indusiate; fleshy projections absent................. **3**
3. Sori linear or oblong, not J- or U-shaped; groove of rachis and costa U-shaped with flat base in cross section .... ***Diplazium***
3. Sori various in shape, linear, oblong, J- or U-shaped, or round-reniform; groove of rachis and costa V-shaped in cross section.. **4**
4. Rhizome ascending or shortly creeping; stipe base usually broadened, with pneumatophores on sides, narrowed downward; spines at base of costules and midvein present or absent adaxially; lamina 2- or 3-pinnate, sometimes pinnate or bipinnatifid, usually gradually narrowed to apex .... ***Athyrium***
4. Rhizome creeping or ascending; stipe base not broadened, devoid of pneumatophores; spines absent; lamina pinnate or bipinnatifid, in *A. niponicum* bipinnate to tripinnatifid, abruptly narrowed to apex or terminated by pinna conform to lateral pinnae ....***Anisocampium***

Anisocampium C. Presl, Epimel. Bot.: 52. 1849; Tardieu & Christensen in Lecomte, Fl. Gén. l'Indo-Chine 7(7): 281. 1940; Copeland, Gen. Fil.: 151. 1947; Ching in Acta Phytotax. Sin. 16(3): 13. 1978 – Type: *Anisocampium cumingianum* C. Presl.

= *Kuniwatsukia* Pic. Serm. in Webbia 28: 455. 1973; Ching in Acta Phytotax. Sin. 16(3): 13. 1978 ≡ *Microchlaena* Ching in Bull. Fan Mem. Inst. Biol. 8: 322. 1938, non *Microchlaena* Wall. ex Wight & Arn. 1834; Iwatsuki in Acta Phytotax. Geobot. 24: 188. 1970 – Type: *Kuniwatsukia cuspidata* (Bedd.) Pic. Serm., **syn. nov.**

= *Athyrium* Roth sect. *Niponica* Ching & Y.T. Hsieh in Bull. Bot. Res., Harbin 6(4): 131. 1986, pro parte – Type: *Athyrium niponicum* (Mett.) Hance, **syn. nov.**

Rhizome creeping or ascending to shortly erect (in *A. cuspidatum*), radially symmetrical or dorsiventral (in *A. cumingianum*), apex scaly. Leaves more or less distant from each other, stipe base scaly, not swollen, with 1 (in *A. niponicum*) or 2 leaf traces in rhizome, deltoid-ovate or ovate-oblong, imparipinnate to 3-pinnatifid, abruptly reduced to pinnatifid apex or bearing terminal pinna conforming with lateral pinnae; pinnae lanceolate or narrowly elliptic, base of lower pinnae inequilateral; veins free or anastomosing (in *A. cumingianum*); spines absent on adaxial surface at base of costae, costules and midvein. Sori oblong, J-shaped, horseshoe-shaped, round-reniform, indusiate; indusia conforming with sori, margin ciliate, or erose, often fugacious. Spores bilateral, with perispore. Chromosome number $x = 40$. Four species.

Habitat. – Mesic forests from near sea level to 1900 m alt.

Distribution. – Japan, Korea, China, Taiwan, Vietnam, Laos, Thailand, Myanmar, India, Bhutan, Nepal, Sri Lanka, Philippines, Indonesia (Fig. 1).

■ KEY TO THE SPECIES OF *ANISOCAMPIUM*

1. Lamina pinnate, apex of lamina pinnatifid, not conforming with lateral pinnae **2**
1. Lamina terminated by a pinna conforming or subconforming to lateral pinnae....................................... **4**
2. Lamina pinnate throughout; pinnae usually shallowly lobed; sori round .... 1. ***A. sheareri***
2. Lamina bipinnatifid or bipinnate; pinnae lobed more than 2/3 to costa; sori oblong, J-shaped, or round **3**
3. Lamina bipinnate; pinnules free, lobed... 2. ***A. niponicum***

Table 1. Comparison of *Anisocampium* and related genera.

Character	*Anisocampium*	*Cornopteris*	*Athyrium*[a]	*Diplazium*[b]	*Deparia*[c]
Rhizome	Creeping or erect	Creeping or erect	Erect or ascending, few creeping	Creeping, ascending or erect	Creeping, ascending or erect
Stipe base	Not persistent	Persistent	Persistent, rarely not	Not persistent	Persistent or not
Scale margin	Entire	Entire	Entire	Toothed, rarely entire	Entire
Multicellular hairs	Absent	Present or absent	Absent, rarely present	Absent, rarely present	Present, rarely absent
Unicellular hairs	Present	Present or absent	Present or absent	Absent	Absent
Pinna- or pinnule base	Inequilateral	Equilateral	Inequilateral	Equilateral	Equilateral
Spine[d]	Absent	Present	Present or absent	Absent	Absent
Continuation of groove[e]	Confluent	Confluent	Confluent	Confluent	Not confluent
Shape of groove[f]	V-shaped	V-shaped	V-shaped	Widely U-shaped, rarely V-shaped	Shallowly V-shaped
Projections[g]	Absent	Present	Absent	Absent	Absent
Veins	Free or anastomosing	Free	Free	Free or anastomosing	Free or anastomosing
Sori	Round-reniform, U- or J-shaped, or oblong	Round or oblong	Round-reniform, U- or J-shaped, linear, or oblong	Linear or oblong	Round-reniform, U- or J-shaped, linear, or oblong
Indusium	Present	Absent	Present, rarely absent	Present	Present
Indusium margin	Ciliate, lacerate, erose or subentire	—	Entire, erose, rarely ciliate	Entire or erose	Entire, erose, lacerate or ciliate
Chromosome number	40	40 or 41	40	41	40 or 41

[a] Including *Pseudocystopteris*.
[b] Including *Allantodia*.
[c] Including *Athyriopsis*, *Dictyodroma*, *Dryoathyrium*, *Lunathyrium*, and *Triblemma*.
[d] Spines are continuations from ends of interrupted ridges embanking grooves at insertions of costae, costules and midveins on adaxial surface of lamina.
[e] Grooves on adaxial surface of costae and costules are confluent with grooves on costules and midveins or not confluent because of a separating ridge.
[f] Grooves are V-shaped or U-shaped with flat bottom in cross section.
[g] Fleshy projections (*corni*) in grooves at base of pinnae and/or pinnules differ from spines, which are continuous with ridges.

3. Lamina bipinnatifid; pinnules not free, at least adnate to costa by broad base .... 2a. ***A. ×saitoanum***
4. Pinnae to 7 pairs, lanceolate, base cuneate or round; veins anastomosing .... 3. ***A. cumingianum***
4. Pinnae 10 pairs or more, linear, base truncate; veins free .... 4. ***A. cuspidatum***

1. ***Anisocampium sheareri*** (Baker) Ching in Acta Bot. Yunnan. 7(3): 314. 1985; Wang & al., Fl. Reipubl. Pop. Sin. 3(2): 75, pl. 15, f. 1–6. 1999 ≡ *Nephrodium sheareri* Baker in J. Bot. 13: 200. 1875 ≡ *Dryopteris sheareri* (Baker) C. Chr., Index Filic.: 292. 1905 ≡ *Athyrium sheareri* (Baker) Ching in Christensen, Index Filic., Suppl. 3: 44. 1934; Iwatsuki, Ferns Fern Allies Jap.: 232, photo 151(1, 2). 1992; Kato in Iwatsuki & al., Fl. Jap. 1: 203. 1995 – Type: China, *Shearer s.n.* (K, photo!).

= *Nephrodium polypodiforme* Makino in Bot. Mag. (Tokyo) 13: 58. 1899 ≡ *Dryopteris polypodiformis* (Makino) C. Chr., Index Filic.: 285. 1905 – Syntypes: Japan, *Z. Matsumura s.n.*, Jul. 17, 1882 (TI), *Y. Yoshinaga s.n.*, 1887, *M. Makino s.n.*, Aug. 1887, 1889, Nov. 1892 (K, photo!).

= *Aspidium otarioides* Christ in Bull. Acad. Int. Géogr. Bot. 11: 247. 1902 ≡ *Dryopteris otarioides* (Christ) C. Chr., Index Filic.: 282. 1905 – Type: China, *E. Bodinier 2050* (P, photo!).

= *Nephrodium isolatum* Baker in Gard. Chron., n.s., 14: 494. 1880 – Type: China, *Maries s.n.* (K, photo!).

Distribution. – Japan, South Korea (Cheju Is.), China (North Central, Southeast, South Central), Taiwan (new record, Hualien Co., *Y.Y. Huang 620*; deposited in HAST).

2. ***Anisocampium niponicum*** (Mett.) Y.C. Liu, W.L. Chiou & M. Kato, **comb. nov.** ≡ *Asplenium niponicum* Mett. in Ann. Mus. Bot. Lugduno-Batavi 2: 240. 1866 ≡ *Athyrium niponicum* (Mett.) Hance in J. Linn. Soc., Bot. 13: 92. 1872; Tardieu & Christensen in Lecomte, Fl. Gén. l'Indo-Chine 7(7): 273. 1940; Dickason in Ohio J. Sci. 46: 123. 1946; Iwatsuki, Ferns Fern Allies Jap.: 239, photo 160(1, 2). 1992; Shieh & al. in Editorial Committee of the Flora of Taiwan, Fl. Taiwan, ed. 2, 1: 424. 1994; Kato in Iwatsuki & al., Fl. Jap. 1: 210. 1995; Wang & al., Fl. Reipubl. Pop. Sin. 3(2): 132, pl. 25, f. 1–8. 1999; Liu & al., Fern Fl. Taiwan, Athyrium: 34, fig. 14, map 14, photo 14. 2009 – Type: Japan, *Keiske (K. Ito)*, *Siebold, Buerger s.n.* (L, photo!).

= *Asplenium uropteron* Miq. in Ann. Mus. Bot. Lugduno-Batavi 3: 174. 1867 ≡ *Athyrium uropteron* (Miq.) C. Chr., Index Filic.: 147. 1905 – Type: Japan, *Keiske (K. Ito) s.n.* (L, photo!).

= *Athyrium biondii* Christ in Nuovo Giorn. Bot. Ital., n.s., 4: 91. 1897 – Type: China, *G. Giraldi s.n.* (B, photo!).

= *Athyrium yunnanense* Christ in Bull. Geogr. Bot. Mans. 17: 134. 1907 – Type: China, *Cavalerie 3864* (K, photo!).

= *Athyrium fissum* Christ in Notul. Syst. (Paris) 1: 47. 1909 – Type: China, *Henry 13107* (P, photo!).

Distribution. – Japan, Korea, China (Northeast, North Central, Southeast, South Central), Taiwan, southern Vietnam, Myanmar (central), northeast India (Arunachal Pradesh). *Anisocampium niponicum* is also in northeast India (Manipur and Tripura) (C.R. Fraser-Jenkins, pers. comm.).

Note. – Ogura (1921) and Kato (1977) found single-leaf trace vasculature in *An. niponicum*, *Ath. yokoscense* (Franch. & Sav.) Christ, and *Ath. vidalii* (Franch. & Sav.) Nakai, which is contrasted with the binary leaf trace in most athyrioid ferns. The leaf trace is divided into two and united to a single U-shaped bundle in cross section in the upper part of the stipe.

2a. ***Anisocampium* ×*saitoanum*** (Sugim.) M. Kato, **comb. nov.** ≡ *Athyrium sheareri* f. *saitoanum* Sugim., Keys Herb. Pl. Jap. 3: 404. 1966 ≡ *Athyrium* ×*saitoanum* (Sugim.) Seriz. in J. Phytogeogr. Taxon. 34: 68, f. 1–3. 1986 – Type: Japan, *Y. Saito s.n.* (Herb. Sugimoto, photo!).

Distribution. – Western Japan; the distribution of *Anisocampium* ×*saitoanum* extends over most of the range of overlap of *A. niponicum* and *A. sheareri*.

Note. – *Anisocampium* ×*saitoanum* is a putative hybrid derived from crosses between *An. niponicum* and *An. sheareri*. It has been recorded from 18 prefectures in western Japan (Fig. 1; Serizawa 1986; Kurata & Nakaike, 1997). The parental species are phylogenetically the farthest from each other in the genus in Japan. *Anisocampium* ×*saitoanum* can reproduce to a certain degree in culture, indicating incomplete reproductive isolation between the species (T. Oka, pers. comm.). The hybrid may therefore be reproducing in nature or may be the result of multiple origins. Either case suggests a close affinity of the two parental species.

3. ***Anisocampium cumingianum*** C. Presl, Epimel. Bot.: 59. 1849; Tardieu & Christensen in Lecomte, Fl. Gén. l'Indo-Chine 7(7): 281. 1940; Copeland, Fern Fl. Philipp. 3: 418. 1960; Sledge in Bull. Brit. Mus. (Nat. Hist.) Bot. 2(11): 281. 1962; Tagawa & Iwatsuki, Fl. Thailand 3(3): 444, f. 47(5–7). 1988; Manickam & Irudayaraj, Pterid. Fl. W. Ghats: 231, pl. 178. 1991; Wang & al., Fl. Reipubl. Pop. Sin. 3(2): 77, pl. 15, f. 7–8. 1999 ≡ *Athyrium cumingianum* (C. Presl) Ching in Christensen, Index Filic., Suppl. 3: 40. 1934; Fraser-Jenkins in Shaffer-Fehre, Rev. Handb. Fl. Ceylon 15(B): 548. 2006 ≡ *Aspidium otaria* Kunze ex Mett., Abh. Senckenberg. Naturf. Ges. 2: 318. 1858 ≡ *Nephrodium otaria* (Kunze ex Mett.) Baker in Hooker & Baker, Syn. Fil.: 288. 1867 – Type: Philippines, *Cuming 239* (K!).

= *Phegopteris paucijuga* Alderw. in Bull. Jard. Bot. Buitenzorg, ser. 2, 16: 26. 1914 ≡ *Anisocampium paucijugum* (Alderw.) Copel., Gen. Fil.: 151. 1947 – Type: Java, *C.A. Backer 8290* (BO, photo!), **syn. nov.**

Distribution. – China (southern Yunnan), Taiwan (Liu & al., 2000), Laos, Thailand, southern Myanmar, southern and northeast India, Philippines (Luzon), Indonesia (Bali, East Java, newly recorded to South Sulawesi [Bantimurung], *T. Oka 100608011*, TNS), Sri Lanka. *Anisocampium cumingianum* also occurs in the Lesser Sunda Islands (Sumbawa, Timor).

Note. – The lamina of *An. paucijugum*, described from eastern Java, is relatively small, i.e., 12 cm long and has two or three pinnae on each side of the rachis. The size of the leaf and the number of pinnae are within the range of variation of *An. cumingianum*. The other characteristics, e.g., the imparipinnate leaf, anastomosing veins, and round sori, are shared with *An. paucijugum* and *An. cumingianum*.

Anisocampium cumingianum is the most derived species in the genus in its dorsiventral rhizome (vs. radially symmetrical), the imparipinnate lamina (vs. paripinnate lamina with gradually less dissected apical part), and the anastomosing veins (vs. free veins).

4. ***Anisocampium cuspidatum*** (Bedd.) Y.C. Liu, W.L. Chiou & M. Kato, **comb. nov.** ≡ *Lastrea cuspidata* Bedd., Ferns Brit. India: t. 118. 1870 ≡ *Aspidium cuspidatum* Mett. in Abh. Senckenberg. Naturf. Ges. 2: 376. 1858, non Desv. 1827 ≡ *Nephrodium cuspidatum* Baker in Hooker & Baker, Syn. Fil.: 260. 1867, non C. Presl 1825 ≡ *Dryopteris cuspidata* (Bedd.) Christ in Philipp. J. Sci., C 2: 205. 1907 ≡ *Microchlaena cuspidata* (Bedd.) Ching in Acta Phytotax. Sin. 9: 99. 1964; Iwatsuki in Acta Phytotax. Geobot. 24: 188. 1970 ≡ *Kuniwatsukia cuspidata* (Bedd.) Pic. Serm. in Webbia 28: 455. 1973; Tagawa & Iwatsuki, Fl. Thailand 3(3): 442, f. 47. 1988; Wang & al., Fl. Reipubl. Pop. Sin. 3(2): 78, pl. 16. 1999 ≡ *Athyrium cuspidatum* (Bedd.) M. Kato in Bot. Mag. (Tokyo) 90: 27. 1977 ≡ *Dryopteris khasiana* C. Chr., Index Filic.: 272. 1905 ≡ *Polypodium elongatum* Wall. ex Hook., Sp. Fil. 4: 234. 1862, non Aiton 1789 ≡ *Phegopteris elongata* J. Sm., Hist. Fil.: 233. 1875, non E. Fourn. 1872 ≡ *Lastrea elongata* Bedd. ex Clarke, Trans. Linn. Soc. London, Bot. 1: 513. 1880, non C. Presl 1836 ≡ *Dryopteris elongata* (Bedd. ex Clarke) Kuntze, Revis. Gen. Pl. 2: 811. 1891 – Type: Nepal, *Wallich Cat. No. 309* (NY, photo!).

= *Aspidium yunnanense* Christ in Bull. Herb. Boissier 6: 965. 1898 ≡ *Microchlaena yunnanensis* (Christ) Ching in Bull. Fan Mem. Inst. Biol. Bot. 8: 325, t. 6. f. 1. 1938; Dickason in Ohio J. Sci. 46: 120. 1946 ≡ *Dryopteris yunnanensis* (Christ) Copel., Gen. Fil.: 122. 1947 ≡ *Aspidium stenolepis* Christ, Bull. Herb. Boissier 7: 22. 1899 – Type: China, *A. Henry 9038A* (US, NY, photo!).

Distribution. – China (South Central, Southwest Central), northeast Myanmar, Northern Thailand, Bhutan, northeast India, central and east Nepal. According to C.R. Fraser-Jenkins (pers. comm.), *Anisocampium cuspidatum* also occurs in the western Himalayas (Pithoragash, India). Hooker (1862: 234) thought that the type specimen of *Polypodium elongatum*, collected in Kew Gardens, originated in Sri Lanka, but Clarke (1880: 512) doubted it (Fraser-Jenkins, 2006).

Note. – *Anisocampium cuspidatum* is distinct in having shortly creeping rhizomes with an ascending apex and radially arranged leaves with linear pinnae.

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■ LITERATURE CITED

Adjie, B., Takamiya, M., Ohta, M., Ohsawa, T.A. & Watano, Y. 2008. Molecular phylogeny of the lady fern genus *Athyrium* in Japan based on chloroplast *rbcL* and *trnL-trnF* sequences. *Acta Phytotax. Geobot.* 59: 79–95.

Ching, R.C. 1938. A revision of the Chinese and Sikkim-Himalayan *Dryopteris* with reference to some species from neighbouring regions. *Bull. Fan Mem. Inst. Biol.* 8: 157–334.

Ching, R.C. 1945. The studies of Chinese ferns XXXII. *Lingnan Sci. J.* 21: 31–37.

Ching, R.C. 1978. The Chinese fern families and genera: Systematic arrangement and historical origin. *Acta Phytotax. Sin.* 16(3): 1–19, 16(4): 16–37.

Clarke, C.B. 1880. A review of the ferns of northern India. *Trans. Linn. Soc. London, Bot.* 1: 425–619, pl. 49–84.

Copeland, E.B. 1947. *Genera filicum*. Waltham: Chronica Botanica.

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

Fraser-Jenkins, C.R. 2006. Woodsiaceae (excluding *Diplazium*). Pp. 532–561 in: Shaffer-Fehre, M. (ed.), *A revised handbook to the flora of Ceylon*, vol. 15(B), *Ferns and fern-allies.* Enfield: Science Pub.

Guindon, S. & Gascuel, O. 2003. PhyML: A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52: 696–704.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids. Symp. Ser.* 41: 95–98.

Hooker, W.J. 1862. *Species filicum*, vol. 4. London: Pamplin.

Hsieh, Y.T. 1985. Classification and distribution of *Anisocampium* Presl. *Acta Bot. Yunnan.* 7: 313–316.

Hsieh, Y.T. 1986. The classification of *Athyrium* Roth. *Bull. Bot. Res., Harbin.* 6(4): 129–135.

Iwatsuki, K. 1970. Taxonomic studies of Pteridophyta IX. *Acta Phytotax. Geobot.* 24: 182–188.

Johnson, D.M. 1986. Trophopods in North American species of *Athyrium* (Aspleniaceae). *Syst. Bot.* 11: 26–31.

Kato, M. 1977. Classification of *Athyrium* and allied genera of Japan. *Bot. Mag. (Tokyo)* 90: 23–40.

Kato, M. 1979. Taxonomy study of the genus *Cornopteris* (Athyriaceae). *Acta Phytotax. Geobot.* 30: 101–118.

Kato, M. 1984. A taxonomic study of the athyrioid fern genus *Deparia* with main reference to the Pacific species. *J. Fac. Sci. Univ. Tokyo Sect. 3, Bot.* 13: 371–430.

Kato, M. & Kramer, U.K. 1990. Subfamily Athyrioideae. Pp. 225–234 in: Kramer, K.U. & Green, P.S. (eds.), *The families and genera of vascular plants*, vol. 1. *Pteridophytes and gymnosperms*. Berlin: Springer.

Kurata, S. & Nakaike, T. (eds.). 1997. *Illustrations of pteridophytes of Japan*, vol. 8. Tokyo: University of Tokyo Press.

Liew, F.S. 1972. Numerical taxonomic studies on North American lady ferns and their allies. *Taiwania* 17: 190–221.

Liu, Y.C., Kuo, C.M. & Liu, H.Y. 2000. SEM studies on spore in Taiwanese fern genera I. Athyrioids. *Taiwania* 45: 181–200.

Ogura, Y. 1921. On the gaps in the stele of some Polypodiaceae. *Bot. Mag. (Tokyo)* 35: 113–125.

Pichi Sermolli, R.E.G. 1973. Fragmenta pteridologiae–IV. *Webbia* 28: 445–477.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molec. Biol. Evol.* 25: 1253–1256.

Ronquist, F. & Huelsenbeck, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Sano, R., Takamiya, M., Ito, M., Kurita, S. & Hasebe, M. 2000. Phylogeny of the lady fern group, tribe Physematieae (Dryopteridaceae), based on chloroplast *rbcL* gene sequences. *Molec. Phylog. Evol.* 15: 403–413.

Schuettpelz, E. & Pryer, K.M. 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. *Taxon* 56: 1037–1050.

Serizawa, S. 1986. On *Athyrium sheareri* form. *saitoanum*. *J. Phytogeogr. Taxon.* 34: 65–69.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primer for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

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

Thompson, J.D, Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. 1997. The CLUSTALX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25: 4876–4882.

Trewick, S.A., Morgan-Richards, M.S., Russell, J., Henderson, S., Rumsey, F.J., Pintér, I., Barrett, J.A., Gibby, M. & Vogel, J.C. 2002. Polyploidy, phylogeography and Pleistocene refugia of the rockfern *Asplenium ceterach*: Evidence from chloroplast DNA. *Molec. Ecol.* 11: 2003–2012.

Wang, M.L., Chen, Z.D., Zhang, X.C., Lu, S.G. & Zhao, G.F. 2003. Phylogeny of the Athyriaceae: Evidence from chloroplast *trnL-F* region sequences. *Acta Phytotax. Sin.* 41: 416–426.

Wang, Z.R. 1997. A subdivision of the family Athyriaceae Alston. *Acta Phytotax. Sin.* 35: 32–36.

Wang, Z.R., Chu, W.M., He, Z.R. & Hsieh, Y.T. 1999. Athyriaceae. Pp. 32–555 in: Chu, W.M. (ed.), *Flora Reipublicae Popularis Sinicae*, vol. 3(2). Beijing: Science Press.

Appendix. Species, sources and GenBank accessions (*rbcL*, *trnL-F*) used in this study.

Anisocampium cumingianum C. Presl, *J.M. Chen s.n.*, Taiwan (TAIF), HM156338, FJ807659. ***Anisocampium cuspidatum*** (Bedd.) Y.C. Liu, W.L. Chiou & M. Kato, EU329029, EU329073 (Adjie & al., 2008). ***Anisocampium niponicum*** (Mett.) Y.C. Liu, W.L. Chiou & M. Kato, EU329042, EU329085 (Adjie & al., 2008). ***Anisocampium sheareri*** (Baker) Ching in Y.T. Hsieh, EU329047, EU329090 (Adjie & al., 2008). ***Athyrium biserrulatum*** H. Christ, *YCLiu 9370*, China (TAIF), HM156337, HM156336. ***Athyrium filix-femina*** (L.) Roth, EU329032, EU329076 (Adjie & al., 2008). ***Athyrium nakanoi*** Makino, EU329039, EU329082 (Adjie & al., 2008). ***Athyrium otophorum*** (Miq.) Koidz., EU329044, EU329087 (Adjie & al., 2008). ***Athyrium rupestre*** Kodama, EU329045, EU329088 (Adjie & al., 2008). ***Athyrium strigillosum*** (Moore ex Lowe) Moore ex Salom, EU329049, EU329092 (Adjie & al., 2008). ***Athyrium vidalii*** (Franch. & Sav.) Nakai, EU329052, EU329095 (Adjie & al., 2008). ***Athyrium yokoscense*** (Franch. & Sav.) H. Christ, EU329055, EU329098 (Adjie & al., 2008). ***Cornopteris christensenianа*** (Koidz.) Tagawa, EU329061, EU329104 (Adjie & al., 2008). ***Cornopteris crenulatoserrulata*** Nakai, EU329062, EU329105 (Adjie & al., 2008). ***Cornopteris decurrentialata*** (Hook.) Nakai, EU329063, EU329106 (Adjie & al., 2008). ***Deparia conilii*** (Franch. & Sav.) M. Kato, EU329065, EU329108 (Adjie & al., 2008). ***Deparia lobatocrenata*** (Tagawa) M. Kato, EU329066, EU329109 (Adjie & al., 2008). ***Deparia petersenii*** (Kunze) M. Kato, EU329064, EU329107 (Adjie & al., 2008). ***Diplazium hachijoense*** Nakai, EU329068, EU329111 (Adjie & al., 2008). ***Diplazium nipponicum*** Tagawa, EU329067, EU329110 (Adjie & al., 2008).