

***Mesospora elongata* sp. nov. (Ralfsiales, Phaeophyceae), a new crustose brown algal species from the Indo-Pacific region**

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Crustose brown algae have relatively simple morphology and anatomy, and the limited characters were a major obstacle in delineating species of *Mesospora* (Ralfsiales, Phaeophyceae). The type species, *Mesospora schmidtii*, was described using material collected in Indonesia. A subsequent study, using material from Japan, was identified as *M. schmidtii*, but we questioned the identity. Therefore, we analyzed morphological characters as well as the plastid-encoded RuBisCO large subunit (*rbcL*) and mitochondrial-encoded cytochrome *c* oxidase subunit 1 (*cox1*) genes using new material obtained from the type locality (Indonesia) and new material obtained from Japan. The molecular phylogenetic analysis distinguished two clades, one representing material from the type location and the other representing an undescribed species from Japan. Therefore, we described *Mesospora elongata* sp. nov. to encompass the Japanese material. Morphologically, the two species were very similar (i.e. cryptic species); the only obvious difference was the number of cells. We also discussed *Hapalospongidion* but we concluded that *Mesospora* should be a distinct genus based on morphology; gene sequence data will be required for *Hapalospongidion* before the relationship of the two genera is more thoroughly resolved.

KEY WORDS: *cox1*, Cryptic species, *Mesospora elongata*, *Mesospora schmidtii*, Ralfsiales, *rbcL*

INTRODUCTION

The marine crustose brown algal genus *Mesospora* was first described by Weber-van Bosse (1911) on the basis of materials collected from several localities in Indonesia during the Siboga Expedition. Weber-van Bosse (1911, 1913) initially placed *Mesospora* in the family Ralfsiaceae with *M. schmidtii* Weber-van Bosse as the genotype albeit with some uncertainty in view of the thin horizontal basal layer of the thallus, which consisted of generally two and rarely four layers of cells. Other generic characteristics provided by the author include (1) a mucilaginous thallus wholly adherent to the substratum, (2) loosely adherent clavate erect filaments, (3) intercalary plurilocular sporangia near the apex of filaments and (4) ovate, unilocular sporangia laterally borne at the base of filaments. *Mesospora* has been considered as a synonym of *Hapalospongidion* (Womersley 1987; León-Alvarez & González-González 1993; Silva *et al.* 1996); however, based upon morphological details, we consider the genus *Mesospora* to be distinct from *Hapalospongidion* (see Discussion).

Currently, five species of *Mesospora* are recognised (Guiry & Guiry 2012): *M. schmidtii* Weber-van Bosse (1911) from Indonesia is the type species, *M. vanbosseae* Børgesen (1924) from Easter Island, *M. macrocarpa* (Feldmann) den Hartog (1968) from the Mediterranean, *M. pangensis* (Setchell) Chihara & Tanaka from Tutuila Island and its variety *M. pangensis* var. *galapagensis* (Setchell & Gardner) Chihara &

Tanaka (Tanaka & Chihara 1982) from the Galapagos Islands, and *M. negrosensis* West & Calumpang (1996) from the Philippines. The known distribution of *M. schmidtii* is confined to the warmer waters of the Indian Ocean and the (sub)tropical western Pacific Ocean. In addition to the original description, *M. schmidtii* has been reported in Vietnam (Dawson 1954), Hong Kong (Kaeher 1994), Japan (Tanaka & Chihara 1982; Yoshida *et al.* 1990), the Solomon Islands (Womersley & Bailey 1970), Australia (Phillips 2002) and Malaysia (Lim *et al.* 2008). A detailed study by Tanaka & Chihara (Tanaka & Chihara 1982) led to the establishment of the family Mesosporaceae to accommodate *Mesospora*, *Hapalospongidion* and *Basispora*. However, a number of discrepancies in the descriptions of *M. schmidtii* by Weber-van Bosse (1911, 1913) and Tanaka & Chihara (1982) suggest that the two may be distinct entities. The present study addresses the issue using morphological observations and molecular analysis of the plastid RuBisCO large subunit (*rbcL*) and mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) genes. We recollected *M. schmidtii* specimens from Batukijok, near the syntype locality: bay of Labuan Terang [Labuan Tring in Weber-van Bosse (1911, 1913)], Lombok Island, Indonesia. *Mesospora schmidtii* specimens were also collected from Malaysia.

MATERIAL AND METHODS

Brown algal crusts on rocks were collected from Lombok Island, Indonesia; Malaysia; and Ishigaki Island, Japan.

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Smaller rocks were air-dried prior to desiccation in silica gel; they were maintained at ambient temperature. Rocks too large to be transported back to the laboratory were collected by either breaking off pieces of the rock or scraping crusts from the rock with razor blades. Specimens were deposited in the University of Malaya Seaweeds and Seagrasses Herbarium and the herbarium of the Kobe University Research Center for Inland Seas (KURCIS). Gene sequences were either newly generated or retrieved from the GenBank (Table S1).

Light microscope observations were made using squash preparations mounted in corn syrup. Characters examined included number of cells, cell size, position and dimensions of unilocular and plurilocular sporangia, number of sterile terminal cell(s), number of stalk cell(s) and length of erect filament.

Approximately 3–5 mg of dried thalli was ground in liquid nitrogen and genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The *rbcL* gene (approximately 1340 nucleotides) was amplified as two or three overlapping fragments using the primer sets NDrbcL2-NDrbcL9 and PRBF3-PRBR3 or *rbcF0-RalR952*, PRBF2-PRBR2 and PRBF3-PRBR3. The first half of the *cox1* gene (approximately 650 nucleotides) was also sequenced. PCR amplification was carried out using MultiGene Thermal Cycler TC9600-G (Labnet International Inc., USA). The total volume was 20 μ l: 2 μ l of 10x *i*-Taq plus reaction buffer, 0.75 μ l of dNTP mixture (consisting of 2.5 mM each of dNTP), 1 μ l of each forward and reverse primers (10 pmol/ μ l) (see Table S2), 0.25 μ l of *i*-Taq plus DNA polymerase (iNtRON Biotechnology, Korea), 1 μ l (25–50 ng) genomic DNA and 14 μ l ultrapure water. Using a modified PCR routine for the amplification of the *rbcL* gene with the primer set NDrbcL2/NDrbcL9, an initial denaturation for 3 min at 94°C was followed by five cycles of denaturation at 94°C for 30 s, annealing at 47°C for 30 s, extension at 72°C for 1 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 1 min; and a final extension at 72°C for 10 min. Thermal cycling performed using other primer pair combinations followed Bittner *et al.* (2008) but used an annealing temperature of 50°C for *rbcL* amplifications (see Table S2 for *cox1*). PCR products were checked for length and yield by electrophoresis on 1% agarose gels stained with SYBR Safe DNA gel stain (Invitrogen, USA). The resulting products were purified using LaboPass Gel and PCR Clean-up kit (Cosmo Genetech, South Korea) and sequenced commercially by First BASE (<http://www.base-asia.com>).

The raw sequences were assembled and edited in the program ChromasPro ver. 1.42 (Technelysium Pty Ltd, Australia). Consensus sequences were preliminarily aligned in ClustalX v. 2.0.8 (Larkin *et al.* 2007) and subsequently manually revised in Bioedit v. 7.0.9.0 (Hall 1999). Two data sets were employed for the phylogenetic analyses: 13 taxa for *rbcL* and 10 taxa for *cox1*–5'. *Ralfsia fungiformis* (Gunnerus) Setchell & Gardner and *Analipus japonicus* (Harvey) Wynne sequences for both genes were obtained from GenBank and used as outgroup taxa (Table S1).

Maximum Parsimony (MP) analyses were accomplished in PAUP* 4.0b10 (Swofford 2001) using heuristic search

with 100 random sequence addition replicates and a tree bisection reconnection (TBR) branch-swapping algorithm. Gaps in the alignment were treated as missing data. All characters were treated as unordered and equally weighted, the Multrees option active and branches with a maximum length of zero collapsed to yield polytomies. To assess support for the resulting nodes, bootstrap percentage (BP) was computed with 1000 replications using one random taxon additions under the heuristic search method with TBR swapping.

Prior to Maximum Likelihood (ML) analyses using PAUP* 4.0b10 (Swofford 2001), the program Modeltest v.3.7 (Posada & Crandall 1998) was used to determine the sequence evolution model that best fits our data, using the hierarchical likelihood ratio test (hLRT) ($\alpha = 0.01$). For the *rbcL* data set, the best model selected was a general time reversible (GTR) model with a gamma distribution. The chosen model for *cox1*–5' data set was the HKY + G model. One hundred random sequence addition replicates were analysed under the heuristic search algorithm, and subsequently 100 bootstrap replicates were performed with one random sequence addition replicates.

Kakusan v.3 (Tanabe 2007) was used to select the optimal model of sequence evolution to fit our data based on Bayesian Information Criterion (BIC) for Bayesian Inference (BI) analyses. Phylogenetic trees were then constructed using MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001). Bayesian analyses for both data sets were performed using the GTR + Gamma model. Two parallel independent runs of four Markov chains were performed for 2,000,000 generations and trees were sampled every 100th generation. Log likelihood values reached a plateau within the first 20,000 generations in both *rbcL* and *cox1*–5' analyses. To ensure stabilization, the first 200 trees were discarded as burn-in and the remaining trees of both runs were used to compute the consensus trees. Node credibility of the BI tree was evaluated by Bayesian posterior probabilities (PP).

For bootstrapping comparison purposes, nodal support was deemed strong for those with $BP \geq 85\%$ and $PP > 0.95$, moderate for $70\% \leq BP < 85\%$ and $0.90 \leq PP \leq 0.95$ and weak for $BP < 70\%$ and $PP < 0.90$. To assess the level of intra- and interspecific variations in the *rbcL* and *cox1* sequences, uncorrected (*p*) pairwise genetic distances were estimated using PAUP* 4.0b10 software (Swofford 2001).

RESULTS

Mesospora schmidtii Weber-van Bosse

Figs 1–3

In the protologue, Weber-van Bosse (1911) did not designate a holotype among specimens from the original six syntype localities. Here we designated as lectotype of *Mesospora schmidtii* Weber-van Bosse the microslide L0790155 (Fig. 1) held in the Herbarium of Weber-van Bosse, included in the Nationaal Herbarium Nederland (L). We chose this material because it was part of the original material of this taxon (ICBN, Art. 9.2, Note 2) and corresponded to the drawings that Weber-van Bosse included in her later publication (Weber-van Bosse 1913: 143–145, fig. 43 and pl. II, figs. 2, 3). Annotations on the microslide: 'Siboga-Expeditie,' '*Mesospora*



Figs 1–3. *Mesospora schmidtii*.

Fig. 1. Image of the lectotype of *M. schmidtii*, microslide L0790155 (L, in Herbarium Weber-van Bosse) with annotations.

Fig. 2. Squash preparation of *M. schmidtii* showing erect filaments bearing plurilocular sporangia with sterile terminal cells. Voucher number = PSM 12207. Slide PSM 12207-96-1. Scale bar = 50 μ m.

Fig. 3. Squash preparation of *M. schmidtii* showing a unilocular sporangium terminally borne on stalk cells adjacent to longer erect vegetative filaments. Voucher number = PSM 12246. Slide PSM 12246-GB7A-2. Scale bar = 50 μ m.

Figs 4–6. *Mesospora elongata* sp. nov.

Fig. 4. Crusts of *Mesospora elongata* sp. nov. on rocks. Scale bar = 2 cm.

Fig. 5. Squash preparation of *Mesospora elongata* sp. nov. showing conspicuously longer erect filaments bearing plurilocular reproductive structures with sterile terminal cells. Voucher number = KU-d112206 (holotype). Slide KU-d112206-FUS3-1. Scale bar = 50 μ m.

Fig. 6. Squash preparation of *Mesospora elongata* sp. nov. showing unilocular sporangia terminally borne on stalk cells, lateral to the erect vegetative filaments. Voucher number = PSM 12221. Slide PSM 12221-38-1. Scale bar = 50 μ m.

Table 1. Summary of statistics from MP analyses of *rbcL* and *cox1* data sets.

| | <i>rbcL</i> | <i>cox1-5'</i> |
|--|-------------|----------------|
| No. of taxa | 13 | 10 |
| Length of alignment | 1340 | 657 |
| No. of parsimony informative characters | 203 (15%) | 200 (30.5%) |
| No. of variable uninformative characters | 105 (8%) | 56 (8.5%) |
| No. of constant characters | 1032 (77%) | 401 (61%) |
| No. of MP trees | 1 | 4 |
| MP tree length | 494 | 462 |
| Consistency index | 0.7206 | 0.7900 |
| Retention index | 0.7625 | 0.7954 |

schmidtii Weber-van B.' and 'antheridia' were in her own writings. An epitype was also designated (ICBN, Art. 9.7) because the exact type locality for the lectotype was unknown although all syntype localities were in Indonesia. After careful consideration, microslide PSM 12246-GB7A-1, housed at the University of Malaya Seaweeds and Seagrasses Herbarium, was designated as epitype of *M. schmidtii* in support of the above designated lectotype (L0790155). The epitype was collected from a distinct geographic point near to one of the syntype localities. Epitype collection details are: Batukijok, Lombok Island, Indonesia (*P.-E. Lim*, 1 May 2006, PSM 12246-GB7A-1).

HOMOTYPIC SYNONYM: *Hapalospongidion schmidtii* (Weber-van Bosse) P.C. Silva.

TYPE LOCALITY: Indonesia (Siboga Expedition).

SPECIMENS EXAMINED: Batukijok, Lombok Island, Indonesia, 1 May 2006 (PSM 12246), 10 June 2010 (PSM 12235); Pulau Che Kamat, Johor, Malaysia, 29 May 2009 (PSM 12173); Port Dickson, Negeri Sembilan, Malaysia, 16 December 2009 (PSM 12201, PSM 12203, PSM 12207); Pulau Merambong, Johor, Malaysia, 24 August 2009 (PSM 12179, PSM 12187, PSM 12197); Tanjung Langsat, Johor, Malaysia, 27 May 2010 (PSM 12211); Kampong Dandulit, Sabah, Malaysia, 9 November 2010 (PSM 12244, PSM 12245).

The plants were epilithic and formed smooth (at times, flaky), dark brown-black crusts; crusts were sticky upon contact with water. Thalli were firmly adherent to the rock substrate over the entire under surface, i.e. there were no rhizoids. The crust outline was discrete and orbicular in juvenile plants; the crust often became irregular in older thalli as a result of confluence with surrounding thalli. In squash preparations, the thallus comprised a horizontal basal layer of one to four (generally two) layers of adjoined rectangular cells. The basal region gave rise to erect, free, simple and unbranched filaments that were composed of 10–19 cells. These loosely associated filaments, held within a gelatinous matrix, were readily separated under light pressure. The erect filaments were slightly clavate in appearance. Cells at the basal end of the filaments were cylindrical; cells measured 7.2–14.8 (–17.7) μm in length and 2.8–6.8 (–10.0) μm in width; the length to diameter ratio was 1.5–3.5. The upper region cells were subglobose, and they

were 5.7–10.7 (–13.2) μm in length and 4.3–8.8 (–11.2) μm in width; the length to diameter ratio was between 0.8:1 to 2.5:1. Intercalary plurilocular sporangia occurred in the upper portion of erect filaments, and the sporangia terminated with two to three sterile terminal cells. Plurilocular sporangia were 23.3–33.5 (–42.9) μm in length, 6.6–10.0 (–12.3) μm in width; the locules were somewhat regularly arranged. Unilocular sporangia were terminally inserted on stalks of up to four cells; they were lateral and basal to the surrounding filaments. The unilocular sporangia were 63.3–143.2 μm in length and 20.2–48.8 μm in diameter. The stalk cells were shorter than other cells of the vegetative filaments. The two types of sporangia were not conclusively found on the same plant; it was difficult to separate confluent individuals because of the continuous crust. Infrequently, colourless hairs emerged as tufts from depressions in the basal layer. Sheath-like cell wall remnants were attached prominently above and/or below the plurilocular sporangia; occasionally sheath-like remnants were attached to sterile terminal cells and other vegetative cells.

In addition, we examined specimens that were distinguished from *Mesospora schmidtii* (Figs 4–6). These plants had erect filaments that were comprised of 11–29 cells. Cells near the basal end of the erect filaments were 6.3–15.9 (–23.7) μm in length and 2.5–6.0 (–9.2) μm in width; cells at the distal end were 5.8–10.7 (–17.1) μm in length and 3.4–7.9 (–13.2) μm in width. Plurilocular sporangia were (13.8) 22.1–49.9 μm in length, (4.8) 8.1–10.9 μm in width, and some of the locules were uniseriate. Unilocular sporangia were 25.9–76.7 μm in length and 11.5–26.9 μm in diameter; they were terminally inserted on stalks of up to eight cells, lateral to the surrounding filaments.

Molecular phylogenetic analyses

Mitochondrial *cox1* sequences were more variable and more parsimony informative than plastid *rbcL* sequences (see Table 1). For the *rbcL* sequences, all three methods of phylogenetic inference (ML, MP, BI) yielded congruent, though not identical trees; only the ML tree is shown (Fig. 7). Two strongly supported clades were recovered (clade A and clade B, Fig. 7), and we interpreted this as evidence for two independent species. The basic topologies of the ML and MP trees were similar, and they also resolved a strongly monophyletic clade of *Mesospora* species (100% BP for ML and MP). The only difference between the ML and MP trees was the reverse branching order of clade B and the *Mesospora* sp. C/ *Mesospora* sp. D clade. *Mesospora* sp. G was separated from the rest of the *Mesospora* cluster in the BI tree. Despite that, support for the *Mesospora* cluster excluding *Mesospora* sp. G was strong with PP = 1 (100% BP for ML and MP). Sequences within clade B were more divergent with 6–19 bp (0.45%–1.42%) differences whereas sequences within clade A were identical. Meanwhile the variation between the two clades was 102–116 bp (7.74%–8.66%).

The results of the ML, MP and BI analyses of the *cox1-5'* alignments are summarized in Fig. 8. Consistent with *rbcL* analyses, the *Mesospora* taxa were monophyletic with strong support (BP = 100%) in the ML and MP trees but this relationship was not resolved in the BI tree. The *Mesospora* taxa were likewise split into two major clades, A and B (Fig.

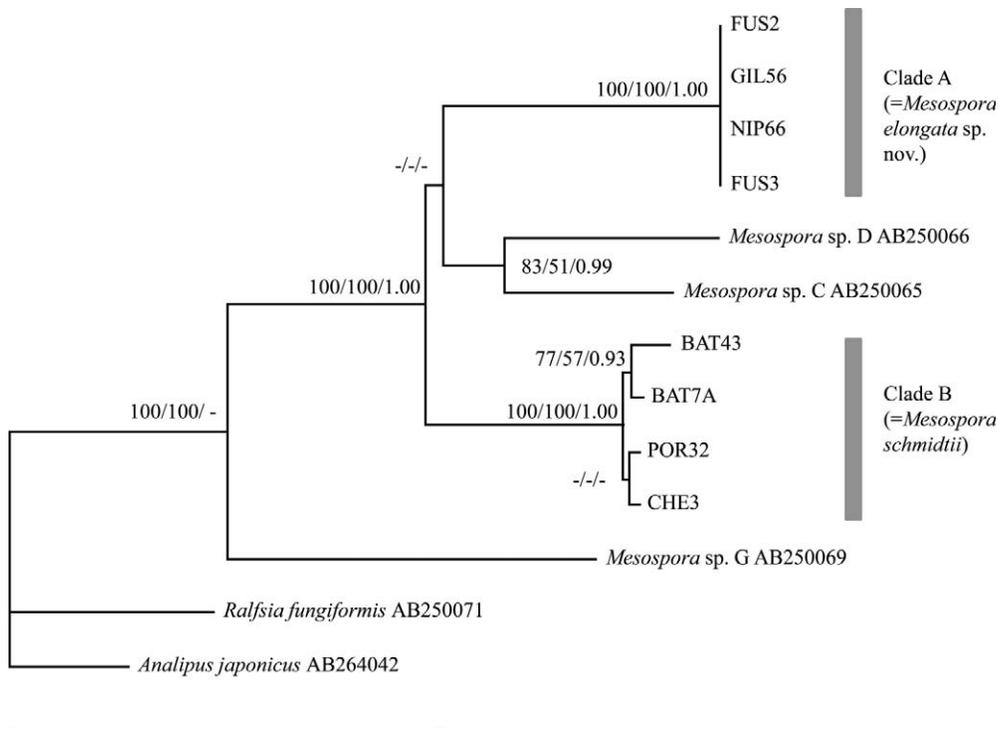


Fig. 7. ML phylogeny inferred based on *rbcL* gene sequences. Numbers associated with each branch indicate bootstrap values (> 50%) for ML (left), MP (middle) and BI (right). Dashes (-) denote percentages of <50% (or that the internode did not occur in the MP or BI tree). -Ln likelihood was 4124.3086; gamma distribution shape parameter (alpha) = 0.1704; nucleotide frequencies: A = 0.2833, C = 0.1667, G = 0.2325, T = 0.3175 and substitution model rate matrix: (R(a) [A-C]: 0.7755, R(b) [A-G] = 9.3795, R(c) [A-T] = 2.8709, R(d) [C-G] = 1.1704, R(e) [C-T] = 23.0905, R(f) [G-T] = 1.0000). Scale bar = 0.1 substitutions per site.

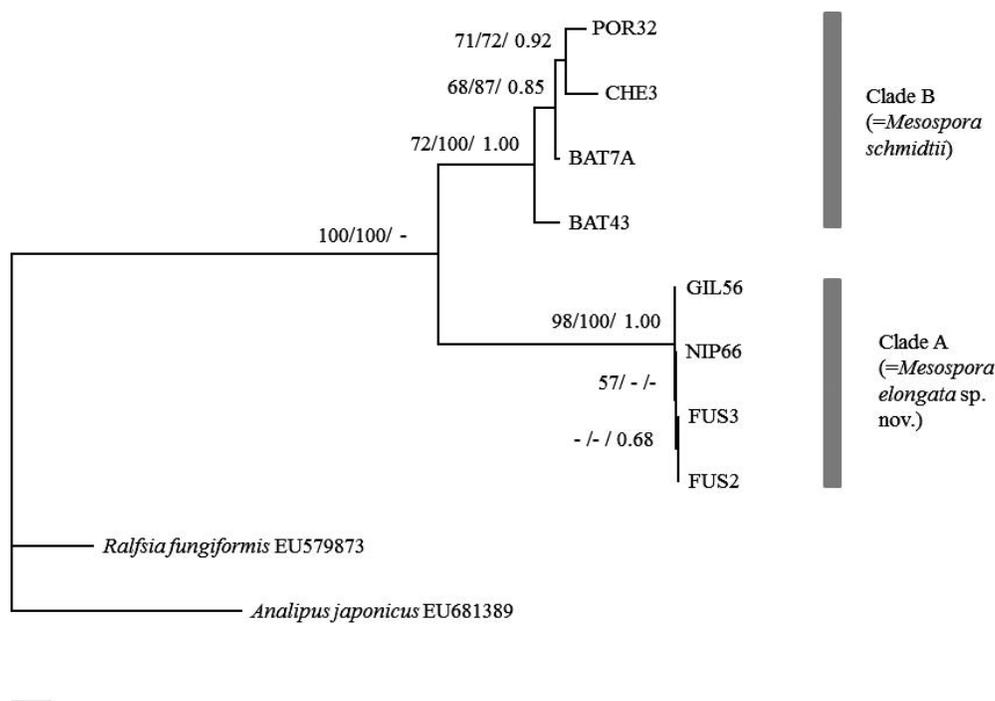


Fig. 8. ML phylogeny inferred based on *cox1-5'* gene sequences. Numbers associated with each branch indicate bootstrap values (> 50%) for ML (left), MP (middle) and BI (right). Dashes (-) denote percentages of <50% (or that the internode did not occur in the MP or BI tree). -Ln likelihood was 2680.3987; gamma distribution shape parameter (alpha) = 0.1528; nucleotide frequencies: A = 0.2095, C = 0.1897, G = 0.1941, T = 0.4068; Ti/tv ratio = 5.9171. Scale bar = 0.1 substitutions per site.

8). Clade A received strong support from all three analyses (98/100% BP for ML/MP, and PP = 1 for BI). Clade B was moderately supported in ML (BP = 72%) but it received strong support from MP (BP = 100%) and BI (PP = 1). ML, MP and BI trees were identical in topology except for the branching order. The uncorrected sequence divergences (*p*-distance) within clades A and B were 0–2 bp (0%–0.30%) and 43–81 bp (6.54%–12.33%) respectively; those between the two clades were 131–145 bp (19.94%–22.07%).

DISCUSSION

The status of the genus *Mesospora* has received considerable attention. Womersley (1987) regarded *Mesospora* and *Basispora* to be closely related to *Hapalospongidion* or even placed in synonymy with the latter genus. He based this conclusion on chloroplast number and position of unilocular sporangia; specifically, *Mesospora* and *Hapalospongidion* have one to three chloroplasts per cell and unilocular sporangia occur terminally on vegetative filaments. *Basispora* is similar; it has several chloroplasts per cell and terminal unilocular sporangia arise on long stalks from near the base of the laterally free erect filaments. Others have pointed out the close relationship among the genera: all include crustose plants with a base of prostrate filaments from which simple, loosely adjoined filaments arise; all have intercalary plurilocular sporangia and terminal unilocular sporangia arising on short erect filaments (John & Lawson 1974; Tanaka & Chihara 1982). Womersley (1987) also placed *Hapalospongidion* in the Ralfsiaceae instead of Mesosporaceae, but a molecular study showed that the two families were separate (Lim *et al.* 2007).

Hapalospongidion was described as a gelatinous thallus comprising erect, unbranched filaments arising from a distromatic basal plate (Saunders 1899). There were two types of erect filaments; intercalary plurilocular sporangia were formed on the longer filaments and unilocular sporangia were terminally formed on the shorter filaments. The illustration by Saunders (1899: pl. I, fig. 2b and 2b') illustrated two peculiar structures on the shorter filaments: one was a series of enlarged cells described as rare and immature or abortive unilocular sporangia, the other was a few lateral outgrowths at the apex of the filament. However, Hollenberg (1942) commented that his specimens, collected from the type locality, did not show any seriate intercalary unilocular sporangia as reported by Saunders (1899) and Hollenberg dismissed the structure as abortive plurilocular sporangia. Furthermore, Hollenberg interpreted the lateral outgrowths as aborted accessory unilocular sporangia or the true tip of the fruiting filament. Significantly, neither of these two structures has been reported for *Mesospora* or *Basispora*. There is another important distinction between *Mesospora* and *Hapalospongidion*, often overlooked: terminal unilocular sporangia arise directly from the basal layer on shorter erect filaments in *Hapalospongidion* but terminal unilocular sporangia arise on a morphologically differentiated stalk cell or cells that are issued laterally from erect filaments in *Mesospora* (and not necessarily basal in position, e.g. *M. mesocarpa*). We did observe unilocular sporangia

occurring on thallus with fewer cell numbers per filament but because of the scarcity of unilocular sporangia in our collections, we remain uncertain about this observation. *Mesospora* is also distinguished from *Hapalospongidion* by its vegetative filaments, which are considerably shorter (less than 35 cells) (Womersley 1987). Furthermore, *Hapalospongidion* has a soft and cushion-like thallus (see Saunders 1899: pl. I, fig. 1 and Hollenberg 1942) and reflected in its name derived from the Greek words 'apalos' and 'spongos' meaning soft and sponge, respectively. Conversely, the thallus of *Mesospora* is not sponge-like. *Basispora* was characterised as having terminal unilocular sporangia on distinct stalks that emerge laterally from near the base of erect filaments, several discoid chloroplasts per cell, and no known plurilocular sporangia (John & Lawson 1974); therefore, *Basispora* is distinguished from *Hapalospongidion* by the position of the unilocular sporangia, which are laterally placed on erect filament at the end of stalk cells (in *Hapalospongidion*, they are terminal on the erect filament itself). Based upon these features, we retain *Mesospora* and *Basispora* as genera distinct from *Hapalospongidion*. It is evident that further investigation is necessary to clarify the taxonomic status of the three genera.

The high degree of morphological similarity between our *Mesospora schmidtii* specimens and our specimens that match the description of *M. schmidtii sensu* Tanaka & Chihara (1982) (clade A, Figs 7, 8) suggests that these represent two cryptic species that are clearly separated using molecular phylogenetic analyses. Nevertheless, both groups of specimens were distinguished using cell numbers in filaments. For example, both clades were present on Lombok Island; using squash preparations of *M. schmidtii* from Batukijok, we distinguished *M. schmidtii* from specimens of clade A. Clade A possessed longer erect filaments composed of 11–29 cells, which fitted the description of *M. schmidtii* given by Tanaka & Chihara (1982). We agree with Tanaka & Chihara (1982) that the plurilocular sporangia observed by Weber-van Bosse (1911, 1913) had not reached full maturity, i.e. we found sporangia with more than eight locules from Batukijok. The size of plurilocular sporangia was not given by Weber-van Bosse (1911, 1913) and no size comparison could be made. The size of plurilocular sporangia in our specimens of *M. schmidtii* (clade B, Figs 7, 8) were smaller [23.3–33.5 (–42.9) μm in length, 6.6–10.0 (–12.3) μm in width] than those observed for our specimens in clade A (Figs 7, 8) [(13.8) 22.1–49.9 μm in length, (4.8) 8.1–10.9 μm broad]. Furthermore, plurilocular sporangia from clade A were smaller than those reported by Tanaka & Chihara (1982). Unilocular sporangia of our *M. schmidtii* specimens agreed well with the description by Weber-van Bosse (1911, 1913); however, they were broader and less elongate (ratio-wise) than those of the clade A specimens. Similar to plurilocular sporangia, clade A unilocular sporangia were smaller than those reported by Tanaka & Chihara (1982); this suggests that clade A unilocular sporangia had not reached full maturity. The discrepancies between our clade A specimens and those described by Tanaka & Chihara could also be attributed to small sample size caused by the scarcity of the specimens during the time of our collections.

Sheath-like remnants of old cell walls attached to the cells surrounding the plurilocular sporangia, occasionally on

vegetative filaments, were observed in both *M. schmidtii* and clade A specimens. The sheath-like feature was first clearly attributed to *Mesospora* by Tanaka & Chihara (1982); it was illustrated on the cells of vegetative filaments by Weber-van Bosse (1913). Weber-van Bosse's description was vague but close inspection of her illustrations showed sheath remnants near or surrounding the plurilocular sporangia. Our observations of sheath-like remnants on sterile terminal cells concurred with her description of apical cells, i.e. not smooth but with traces of membranes. We did not observe the sheath-like remnants of cell walls on the stalk cells of unilocular sporangia as reported by Tanaka & Chihara (1982).

Molecular phylogenetic analyses separated clades A and B (Figs 7, 8), and we consider the two clades independent taxa at the species level. Our study included only two of five currently recognised species of *Mesospora* because we did not obtain specimens from the type localities of the other three species. We included all available sequences from GenBank. Furthermore, based upon an unpublished *rbcL* sequence provided by John A. West and Joe Buchanan, we confirmed that none of our specimens were *M. negrosensis*. A comparison of species of *Mesospora* based on morphological data from the literature is also presented here (see Table S3).

The barcoding candidate and faster evolving *cox1* gene (Hebert *et al.* 2003; McDevit & Saunders 2009) showed that clade B (*M. schmidtii*) has a surprisingly high within-species variation (Fig. 8). Nonetheless, there was no overlap between inter- and intraspecific divergence for *cox1*-5' in our samples, further attesting to its efficiency as a barcoding marker; this was the first use of *cox1* as a barcoding marker for the Ralfsiales.

Mesospora schmidtii and clade A are distinguished from *M. vanbosseae* because the latter has a thick basal layer consisting of ten or more layers, lacks unilocular sporangia, has cylindrical cells with thin walls, and an irregular arrangement of locules in the plurilocular sporangia (see Børgesen 1924, p. 259, fig. 9d). *Mesospora negrosensis* can be distinguished from clade A specimens (in addition to previously mentioned unpublished molecular data) because it has fewer cell numbers per filament, no unilocular sporangia and mostly uniseriate plurilocular sporangia (perhaps not fully matured). The position of unilocular sporangia of *M. mesocarpa*, which often arise laterally in the middle of an erect filament, is an important difference between this species and clade A specimens. *Mesospora pangoensis* differs from clade A specimens because it has fewer cells in erect filaments, unknown plurilocular sporangia, a smaller number of stalk cells (three to five cells), and much shorter and broader unilocular sporangia.

Based on molecular and morphological data, we describe clade A specimens as a new species.

***Mesospora elongata* S.W. Poong, P.E. Lim & S.M. Phang sp. nov.**

DIAGNOSIS: Thallus crustose, thin, tightly adherent to the substratum without rhizoids, composed of thin (generally two) layers of horizontal basal cells wider than high and loosely adjoined

erect filaments up to 30 cells long; gelatinous upon contact with water; unilocular sporangia lateral to surrounding filaments, with up to eight stalk cells, without paraphyses; intercalary biseriate plurilocular sporangia with two to four sterile terminal cells; *rbcL* and *cox1* gene sequences distinct from other studied *Mesospora* species.

HOLOTYPE DESIGNATED HERE: KU-d11206, collected by P.E. Lim on 6 February 2005, Herbarium of the Kobe University Research Center for Inland Seas, Rokkodai, Kobe 657-8501, Japan.

DNA SEQUENCES FROM THE HOLOTYPE: JQ620003 (*rbcL*), JQ620011 (*cox1*).

TYPE LOCALITY: rocks, littoral zone, Fusaki, Ishigaki Island, Okinawa Prefecture, Japan.

ETYMOLOGY: *elongata* (Latin) refers to the long filaments arising from the thallus.

ISOTYPE: Nipah, Lombok, Indonesia, 8 June 2010 (PSM 12214).

PARATYPES: Fusaki, Ishigaki Island, Okinawa Prefecture, Japan, 6 February 2005 (KU-d11205); Gili Genting, Lombok, Indonesia, 10 June 2010 (PSM 12221, PSM 12225).

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at <http://dx.doi.org/10.2216/12-42.1.s1>.

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