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Molecular study of the rare genus *Neoabbottiella* (Rhodophyta) reveals its assignment to the Halymeniaceae is incorrect

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SUMMARY

The rare red algal genus *Neoabbottiella* from the Russian Pacific coast was taxonomically re-examined. Currently, *Neoabbottiella* is assigned to the Halymeniales; however, some features of the reproductive anatomy cast doubt on the correctness of assigning the genus to Halymeniaceae *sensu lato*. We investigated the phylogenetic affinities of *Neoabbottiella* using four DNA markers (nuclear LSU and SSU rDNA, *rbc*L and COI-5P). *Neoabbottiella* failed to group with any families of the Halymeniales, but it was nested in a highly supported clade containing representatives of the genus *Schmitzia* (Calosiphoniaceae *incertae sedis*). Reproductive anatomy and post-fertilization development also separate *Neoabbottiella* from the Halymeniaceae. The main characteristics distinguishing *Neoabbottiella* from the Halymeniales include the intercalary position of the auxiliary cell, the contact of the connecting filament with the auxiliary cell via the lateral process, the origin of the initial gonimoblast cell from the connecting filament at a distance from the auxiliary cell and a lack of a pericarp of branched ampullar filaments. These traits bring *Neoabbottiella* close to its genetic sister genus *Schmitzia*, making the classification of *Neoabbottiella* at the family and order levels difficult. The eventual taxonomic classification of *Neoabbottiella* awaits the input of data of other species of *Schmitzia* as well as *Calosiphonia*, the type genus of the Calosiphoniaceae; thus, *Neoabbottiella* should be provisionally excluded from the Halymeniales and considered as *incertae sedis* in the Rhodymeniophycidae. Further, the results of the present study showed that *Neoabbottiella* is monotypic; *Neoabbottiella decipiens* should be recognized as a synonym of the generitype *Neoabbottiella araneosa*.

Key words: endemic, *Neoabbottiella araneosa*, *Neoabbottiella decipiens*, Russian Pacific, *Schmitzia*.

INTRODUCTION

The genus *Neoabbottiella* Perestenko was proposed in 1975 on the basis of material collected from the Paramushir Islands (Kuril Islands), on the Russian coast of the Pacific Ocean (Perestenko 1975). This genus was initially described as *Abbotia* Perestenko (Perestenko 1975), but Perestenko (1977a) reported that the generic name *Abbotia* was a later homonym already occupied by an angiosperm genus, and she renamed the genus *Abbottia* Perestenko, which was likewise occupied by an angiosperm genus. In a subsequent publication, Perestenko (1977b) stated that the name *Abbottia* Perestenko was incorrect in Latin and orthographically corrected it to *Abbottea* Perestenko (Perestenko 1977b). Later, Perestenko (1982: 30) noted that '…a change in the name of one letter looks like a spelling mistake', and she again renamed the genus *Neoabbottiella* (Perestenko 1982). Moreover, the name *Abbottea* was not validly published as it was not accompanied by a description or diagnosis of the taxon or by a reference to a previously and effectively published description or diagnosis (Turland *et al*. 2018, ICN, Art. 38.1). Lindstrom (1985) validated the generitype name *Neoabbottiella araneosa* as the only species known at that time. Later, two additional species, *Neoabbottiella valentinae* Pisareva & Kloczkova and *Neoabbottiella decipiens* Pisareva & Kloczkova, were described (Pisareva and Klochkova 2013). Further morphological and molecular studies have shown that *N. valentinae* shares features with *Schizymenia* rather than *Neoabbottiella* and it was transferred to *Schizymenia* as *Schizymenia valentinae* (Pisareva & Kloczkova) O.S. Belous, Skriptsova & Shibneva (Belous *et al*. 2022).

The main feature of *N. araneosa* is the arrangement of carposporophytes in the form of stars with multiple rays, which fuse and form a web-like pattern. *Neoabbottiella decipiens* Pisareva & Kloczkova is morphologically similar to the generitype, differing only slightly in color. The main distinguishing features of *N. decipiens* are a rare occurrence of stellate refractive cells in the medulla as compared to *N. araneosa*, and stars with multiple rays formed by carposporophytes do not fuse and are often surrounded by the ring of carposporophytes in *N. decipiens* (Pisareva and Klochkova 2013).

The taxonomic placement of *Neoabbottiella* has changed several times following changes in ordinal-level classification and point of view of researchers on the organization of primary female reproductive structures of the algae. Based upon the morphology of female reproductive structures and the vegetative anatomy of *Neoabbottiella*, Perestenko (1975) tentatively assigned it to the Dilseaceae (order Cryptonemiales). This family was segregated from Dumontiaceae by Bert (1965); however, this proposal did not find full support and Dilseaceae was recognized as a synonym of Dumontiaceae

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(Abbott 1968). Abbott (1982) transferred *Abbotia araneosa* to the genus *Neodilsea* (as *Neodilsea araneosa* (Perestenko) I.A. Abbott) of the family Dumontiaceae (order Cryptonemiales). Perestenko (1982) claimed the generic uniqueness of this taxon proposing the name *Neoabbottiella*. Later, Lindstrom (1985: 264) studied the holotype of *N. araneosa* and found that '…the carpogonia and auxiliary cells occur among compact and highly branched clusters of accessory filaments reminiscent of the ampullae characteristic of Cryptonemiaceae'. On this base, Lindstrom (1985) proposed to transfer *N. araneosa* to the family Cryptonemiaceae (=Halymeniaceae, see Guiry 1978; Schneider and Wynne 2007: 227, note 47). Later, the orders Cryptonemiales and Gigartinales were combined as the latter (Kraft and Robins 1985). Based on nrSSU sequence data, it was shown that the family Halymeniaceae was not allied to Gigartinales, and it was proposed to reinstate the Cryptonemiales as the Halymeniales and to include two families, Halymeniaceae and Sebdeniaceae, in it (Saunders and Kraft 1996). Dumontiaceae was retained within Gigartinales (Saunders and Kraft 1996). This resulted in *Neoabbottiella* being assigned to the Halymeniaceae (order Halymeniales) (Schneider and Wynne 2007; Pisareva and Klochkova 2013; Guiry and Guiry 2023). Nevertheless, some features of the reproductive anatomy of *Neoabbottiella*, namely, the intercalary position of the auxiliary cell fourth from the distal end of the auxiliary branch and its indistinguishability before contact with the connecting filament, as well as the absence of a pericarp or involucre of elongated ampullary filaments around the gonimoblast (Perestenko 1975, 1994), clearly distinguish this genus from Halymeniaceae *sensu lato* (Chiang 1970; Kim *et al*. 2021). This allows us to hypothesize that the assignment of *Neoabbottiella* to Halymeniales may be incorrect. To test this, we re-examined the morphology and reproductive anatomy of *Neoabbottiella* and employed phylogenetic analyses based on the large subunit (28S) of nuclear ribosomal DNA (nrLSU), the small subunit (18S) of nuclear ribosomal DNA (nrSSU), the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (*rbc*L) and the cytochrome oxidase subunit 1 gene (COI-5P).

MATERIAL AND METHODS

Morphological analysis

We examined 19 specimens of *Neoabbottiella* (see Table S1) as well as the holotype of *N. decipiens* (LE A0000657) at the V.L. Komarov Botanical Institute (LE, St. Petersburg, Russia). Fertile specimens were identified based on the descriptions in Perestenko (1975, 1994) and Pisareva and Klochkova (2013). The specimens of wine-red or reddish-chestnut color in which stars of gonimoblasts formed web-like patterns were assigned to *N. araneosa*, and specimens of reddish-chestnut or brown-chestnut color with separate stars of gonimoblasts surrounded by a ring of gonimoblasts were identified as *N. decipiens* (Pisareva and Klochkova 2013). Species identification of vegetative specimens was impossible; therefore, they were listed in the phylogenetic trees as *Neoabbottiella* sp.

Cross-sections of blades were made by hand with a razor blade. Squashed preparations were prepared after soaking fragments of dry tissue in distilled water for 20 min, after which the tissue was squashed under a cover slip and then stained with Hematoxylin Carazzi (Bancroft and Layton 2019) for 1 min and flushed with distilled water. For aniline staining, fragments of dry algae were soaked in distilled water for 20 min and then stained with 1% aniline blue for 5 min and acidified with 1% HCl for 10 s prior to flushing with distilled water. Alternatively, we rehydrated fragments of specimens in seawater, sectioned them with a razor blade and stained them with hematoxylin or aniline as above. Reproductive anatomy and post-fertilization processes were studied only in *N. araneosa*.

Molecular analyses

Genomic DNA was extracted from thallus fragments of herbarium specimens using the DNA Easy Plant mini-kit (Qiagen, Germantown, USA) or by the CTAB method (Wang *et al*. 2006). A partial *rbc*L gene fragment was amplified with specific primers F57 (forward) and rbcLrevNEW (reverse) (Saunders and Moore 2013). A fragment of COI-5P was amplified with the primer pair GazF1 and GazR1 (Saunders 2005). Primers G01 and G07 were used to amplify nrSSU (Saunders and Moore 2013), while T01N-T20 and T04-T15 were used to amplify a fragment of nrLSU following a previously published protocol (Saunders and Moore 2013).

The preparation of samples for sequencing was carried out as described by Shibneva *et al*. (2020). The PCR products were bidirectionally sequenced using ABI 3110 and ABI 3500 sequencers (Applied Biosystems). Sequences of species belonging to most orders of Rhodymeniophycidae and most families of Gigartinales and Halymeniales, the taxa with which *Neoabbottiella* has historically been associated, were downloaded from GenBank (see Table S2). The sequences of *rbc*L and COI-5P were aligned in MEGA-X (Kumar *et al*. 2018) using the ClustalW algorithm; nrLSU and nrSSU were aligned using MAFFT (Katoh and Standley 2013) at https://www.ebi.ac.uk/Tools/msa/mafft/. nrLSU and nrSSU sequence alignments were further refined by eye using MEGA-X (Kumar *et al*. 2018), and ambiguous regions of the alignments were removed. MEGA-X (Kumar *et al*. 2018) was used for calculating genetic distances (p-distance). *Ahnfeltia* spp. (see Table S2) were included as an outgroup for the phylogenetic analysis following Withall and Saunders (2006) and Yang *et al*. (2016).

Six initial alignments were constructed: (1) nrSSU (1823 bp), (2) nrLSU (1350 bp), (3) *rbc*L (1318 bp), (4) COI-5P (588 bp), (5) COI-5P + *rbc*L + nrLSU combined (3254 bp) and (6) nrLSU + nrSSU combined (3174 bp), including species for which complementary data were available.

Final COI-5P + *rbc*L + nrLSU and nrLSU + nrSSU concatenated alignments were constructed and analyzed with partitioning by gene and then codon for *rbc*L and COI-5P.

For each alignment, phylogenetic trees were reconstructed by the maximum likelihood (ML) method using IQ-TREE 2.2.0 (Minh *et al*. 2020) according to automatically selecting substitution models. The bootstrap values were estimated using ultrafast bootstrap, non-parametric and aLRT approximations (Minh *et al*. 2013; Hoang *et al*. 2018) with 1 million replications in IQ-TREE. The Bayesian posterior probabilities (BPPs) were calculated by the Bayesian inference method in MrBayes v3.2.7 (Ronquist *et al*. 2012) using the best-fit models for each codon of COI-5P and *rbc*L or nrLSU and nrSSU. MrModeltest 2.4 (Nylander 2004) was used to select the best-fit partitioning scheme and models for Bayesian analyses. The best-fit model for nrLSU, nrSSU, for each codon position of COI-5P and for the first and third codon positions of *rbc*L was GTR + I + G (Tavaré 1986), while the best-fit model for the second codon positions of *rbc*L was F81 + I + G (Felsenstein 1981). Bayesian analysis was performed with two independent runs of Metropolis-coupled Markov chain Monte Carlo analyses. The chains were run for 5 million generations and sampled every 500 generations. A burn-in of 1 250 000 generations (or 25% of the sampled trees) was used. Moreover, trace files were visually inspected in Tracer 1.7 (Rambaut *et al.* 2018). FigTree v. 1.4.4 (Rambaut 2016) was used to visualize phylogenetic trees after analysis. BPPs of the nodes were added to ML trees. The obtained sequences have been deposited in GenBank with accession numbers OQ557647–OQ557651 (COI-5P), OQ557652–OQ557656 (*rbc*L), OQ557399–OQ557405 (nrLSU) and OQ557398 (nrSSU) (see Table S2).

RESULTS

Molecular analyses

For the phylogenetic analysis we used the multilocus datasets (COI-5P + *rbc*L + nrLSU), as well as gene-specific trees (*rbc*L, nrLSU, nrSSU and concatenated nrLSU + nrSSU), presented in the Supporting information (see Figs S1–S4).

Phylogenetic ML trees constructed for single loci as well as concatenated nrLSU + nrSSU (see Figs S1–S4) showed a correspondence with the tree obtained from the combined COI-5P + *rbc*L + nrLSU data (Fig. 1). All the included orders were strongly monophyletic with high support on the trees except Gigartinales and Peyssonneliales, which form a single monophyletic clade with high statistic support in the COI-5P + *rbc*L + nrLSU-based tree (Fig. 1) and an unsupported clade in the *rbc*L-based tree (Fig. S3). Gigartinales was non-monophyletic.

Unexpectedly, sequences of the holotype of *N. decipiens* were almost identical to those of *N. araneosa*. The divergence ranged from 0 to 0.5% for *rbc*L (while within the genus it did not exceed 0.6%) and it was 0% for COI-5P and nrLSU. The phylogenetic trees showed that *Neoabbottiella* did not group with members of the Halymeniales and was placed in a highly supported clade including *Schmitzia* spp. of the family Calosiphoniaceae (Fig. 1). This clade was sister to the clade containing species of the orders Acrosymphytales and Inkyuleeales (Fig. 1).

Morphological examination

Vegetative morphology of Neoabbottiella araneosa

Thalli were bladed, mucous, smooth, entire, rounded or irregular in shape, with a cordate or narrow-wedge base, perforated

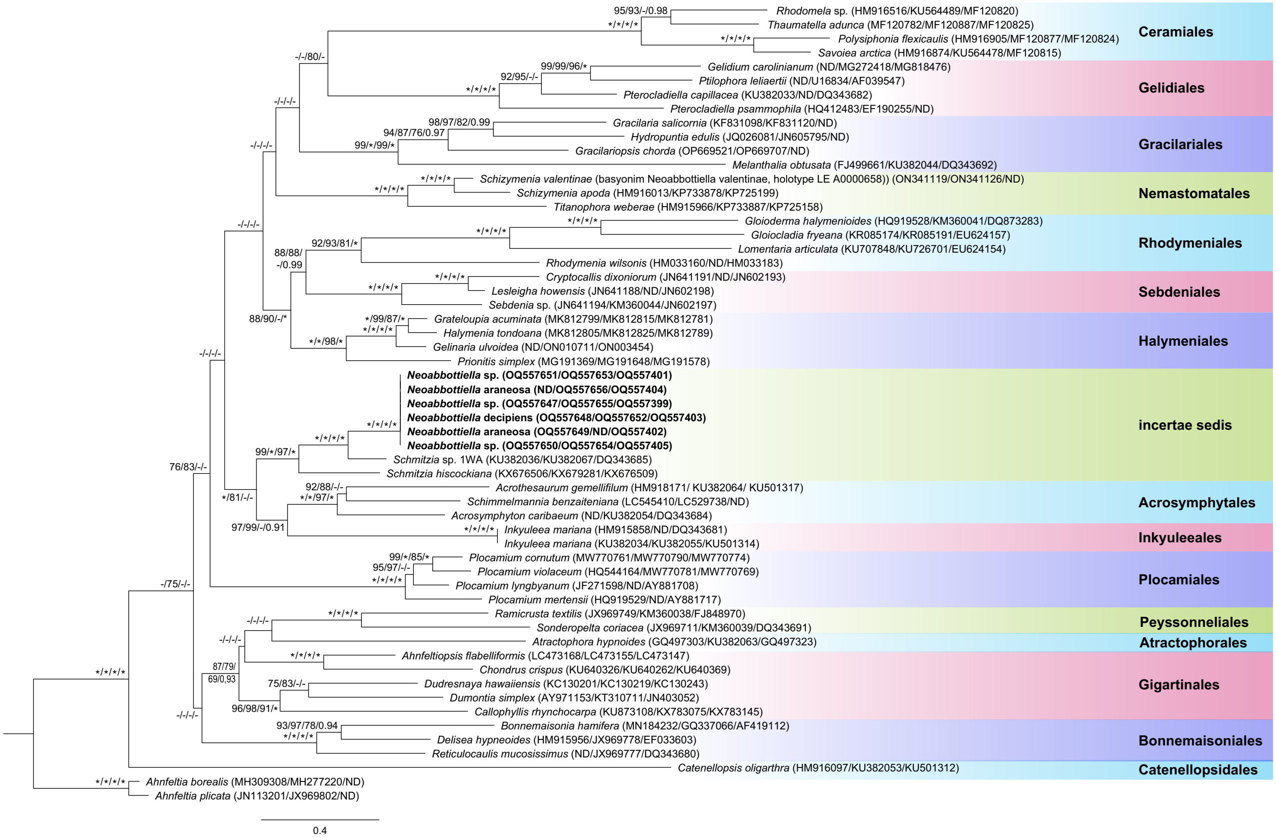

Fig. 1. Maximum likelihood (ML) phylogeny as determined using a combined COI-5P + *rbc*L + nrLSU dataset. Support values are ultrafast bootstrap/aLRT/non-parametric bootstrap/Bayesian posterior probabilities. Only Bayesian posterior probability values of >0.9 and bootstrap support values of >75% are shown. Scale bar: substitutions per site. Numbers in brackets after the species names are GenBank accession numbers (COI-5P/*rbc*L/nrLSU). *Bayesian posterior probabilities = 1, bootstrap = 100%.

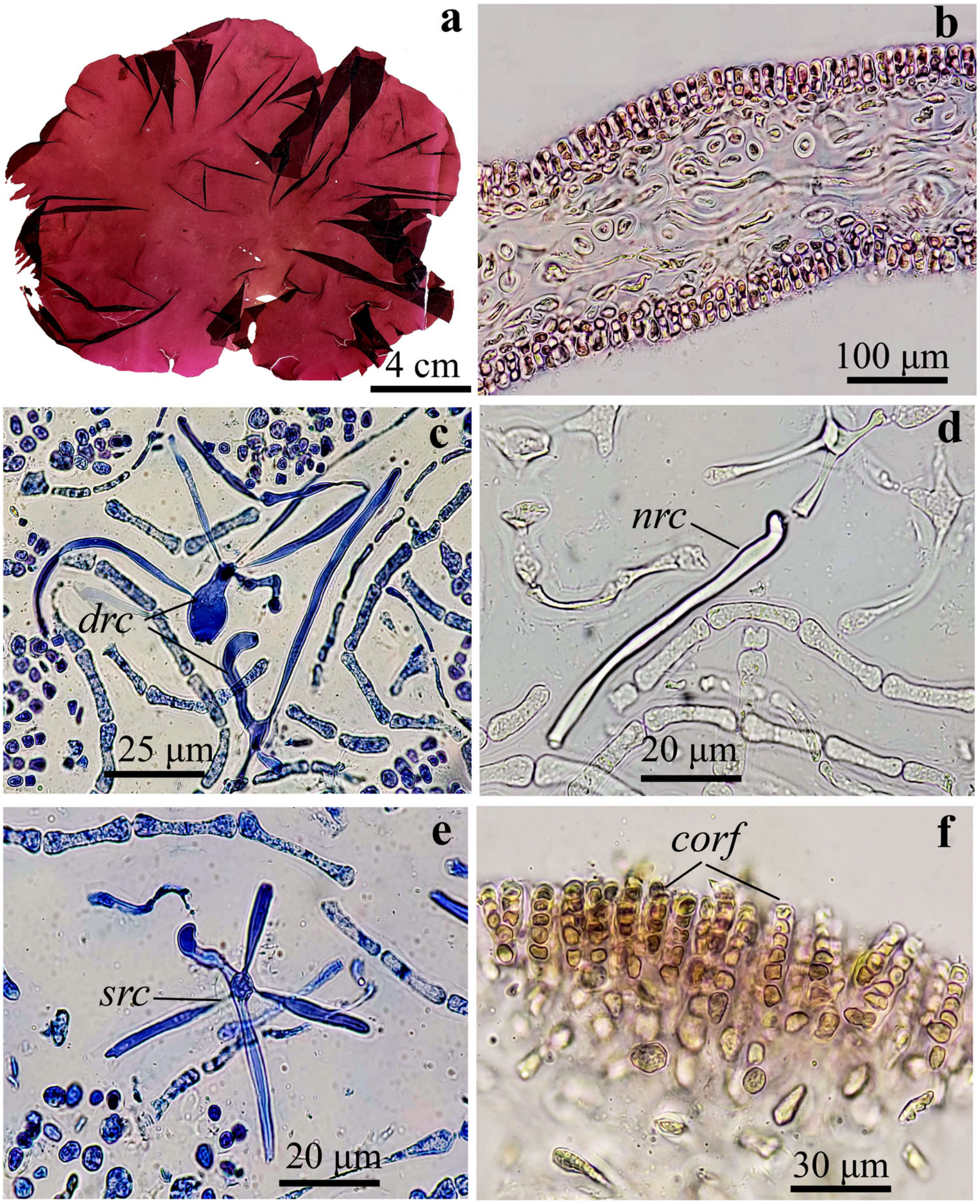

Fig. 2. Vegetative morphology of *Neoabbottiella araneosa*. (a) Habit of a vegetative plant (voucher ASKR-755, collected from aquaria of the Pacific Institute of Geography, Kamchatka Branch, Petropavlovsk-Kamchatsky). (b) Cross-section of a vegetative blade. (c) Squashed preparation showing a dendroidal refractive cell (*drc*) stained with aniline blue. (d) Squashed preparation showing a needle-shaped refractive cell (*nrc*). (e) Squashed preparation showing a stellate-shaped refractive cell (*src*) stained with aniline blue. (f) Outer cortical filaments (*corf*) consisting of three to six rounded cells enclosed in a common mucous membrane.

and dissected into lobes with wavy or pitted edges at maturity. Blades were up to 30 cm high, up to 50 cm wide and 120–600 µm thick (to 900 µm at the base) (Fig. 2a). Blades were attached with small disks on short compressed stipes, but these were sometimes absent. The medulla was composed of anticlinal and periclinal filaments of long and short rectangular cells measuring 8–13 × 15–75 µm (Fig. 2b). Refractive cells were dendroid-, needle- and stellate-shaped and abundant in the medulla (Fig. 2c–e). The number of refractive stellate cells varied within the thallus and it was 8–20 per 9 mm^2 (microscope view ×100) in both *N. araneosa* and *N. decipiens*. The subcortex consisted of one to three (four) rows of oval cells measuring 10–20 × 12–25 µm. The cortex was formed by filaments consisting of three to six cells measuring 3.5–8.0 × 5–10 µm, enclosed in a common mucous membrane (Fig. 2f). Pit connections between the outer cortical cells were not observed.

Reproductive anatomy of Neoabbottiella araneosa

Gametophytic plants were monoecious. Spermatangia were elongate, with pointed claw-like ends, measuring 3–5 × 10–12 µm, developed singly from spermatangial

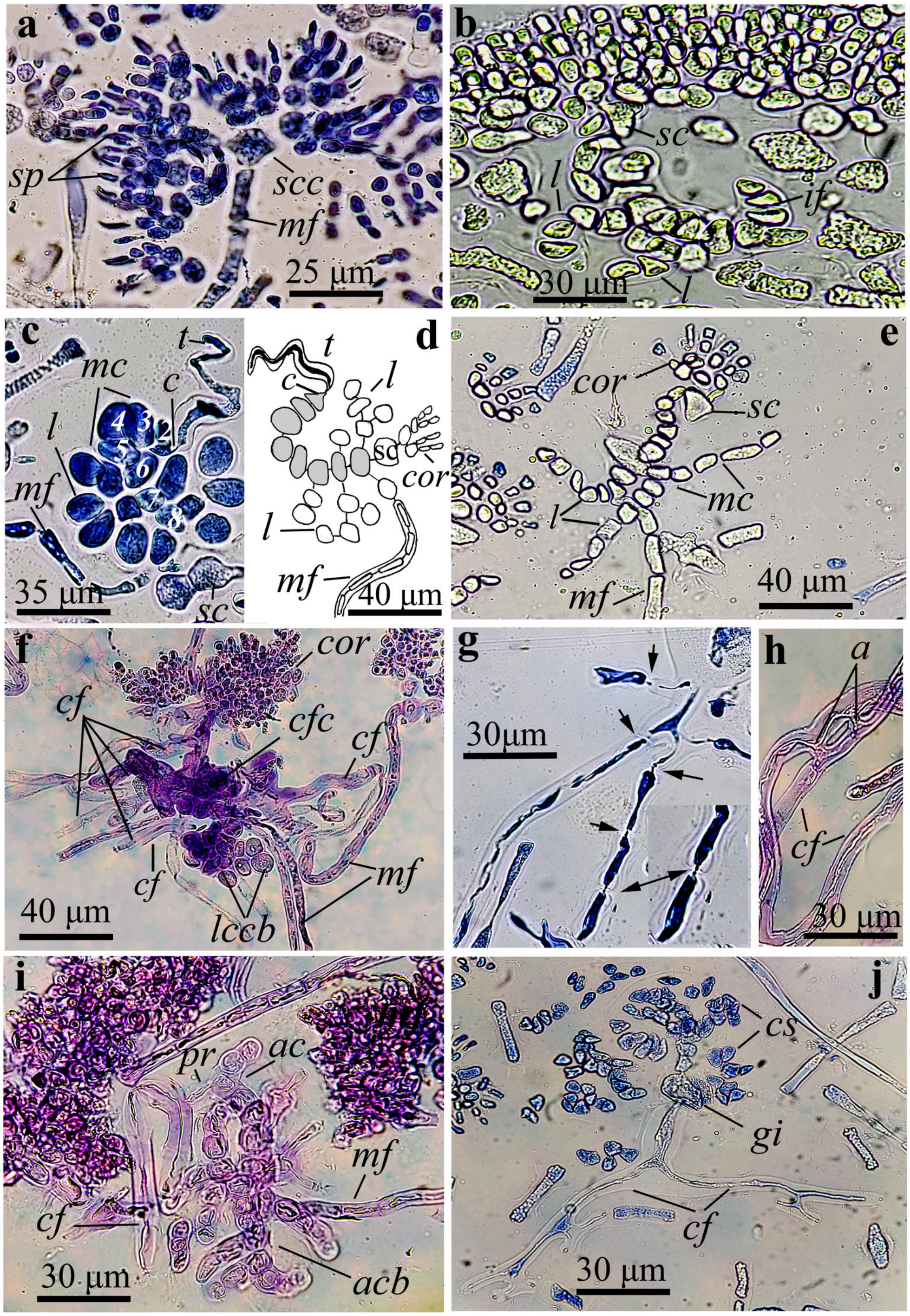

Fig. 3. Reproductive morphology and post-fertilization development of *Neoabbottiella araneosa*. (a, c, e, g, j) Stained with aniline blue. (f, h, i) Stained with hematoxylin. (a) Squashed cortical filaments with claw-like spermatangia (*sp*). *scc*, subcortical cell; *mf*, medullary filament. (b) Cross-sections of the fertile blade showing auxiliary cell branch. *if*, initial filament; *l*, lateral branches; *sc*, supporting cell. (c) Eight-celled carpogonial cell branch. *c*, carpogonium; *l*, lateral branches; *mc*, mucilage coat; *mf*, medullar filament; *sc*, supporting cell; *t*, sinuous wide thrichogyne. (d) Schematic drawing of carpogonial cell branch interpretative of (c). *c*, carpogonium; *cor*, cortical filaments; *l*, lateral branches; *mf*, medullar filament; *sc*, supporting cell; *t*, trichogyne. (e) Auxiliary cell branche arising from the cells of the inner cortex (*cor*) and enclosed in a common mucilage coat (*mc*). *sc*, supporting cell; *mf*, medullar filament; *l*, lateral branches. (f) Carpogonial fusion cell (*cfc*) and connecting filaments (*cf*) arising from it and diverging in rays. *cor*, cortex; *lccb*, laterals of the carpogonial cell branch; *mf*, medullar filaments. (g) Fragment of a branched connecting filament showing a septa (arrows). (h) Fragment of a branched connecting filament (*cf*) showing anastomoses (*a*). (i) Connecting filament (*cf*) contacted by a short lateral process (*pr*) with an auxiliary cell (*ac*). *acb*, auxiliary cell branch; *mf*, medullar filaments. (j) Initial gonimoblast cell (*gi*) on a swelling of the connecting filament (*cf*). *cs*, carposporangia.

mother cells formed terminally on the cortical filaments (Fig. 3a).

Female reproduction was non-procarpic. Carpogonial cell and auxiliary cell branches developed from the inner cortical cells. The initial filaments of the branches grew downwardly from large supporting cells (20–25 μm in diameter) and then arced upwards; the proximal and medial cells of this filament formed short, two- to five-celled ascending branches, not converging distally (Fig. 3b). These branched structures were reminiscent of *Thamnoclonium*-type ampullae (Chiang 1970). Carpogonial cell branches were rare, monocarpogonial and compact, consisting of 12–35 round or oval-elongated cells of 12–16 × 10–14 μm enclosed in a common mucilage coat (Fig. 3c). The carpogonial branch was distally recurved and consisted of five to nine rounded or longitudinally elongated cells, four or five distal cells were free of laterals, and the lower cell carried two- to three-celled lateral branches, which also sometimes branched (Fig. 3c,d). The carpogonium was triangle-shaped, measuring 8–9.5 × 8.5–9 μm (Fig. 3d). The third cell of the carpogonial branch was larger than the other cells and 15–16 μm in diameter (Fig. 3c,d). The trichogyne was short, wide (8–10 × 20–25 μm), sinuous and tapering towards both ends or pinched only near the carpogonium (Fig. 3c,d).

Auxiliary cell branches were numerous, consisting of 20–40 (70) rounded cells, 10–17 μm in diameter, enclosed in a

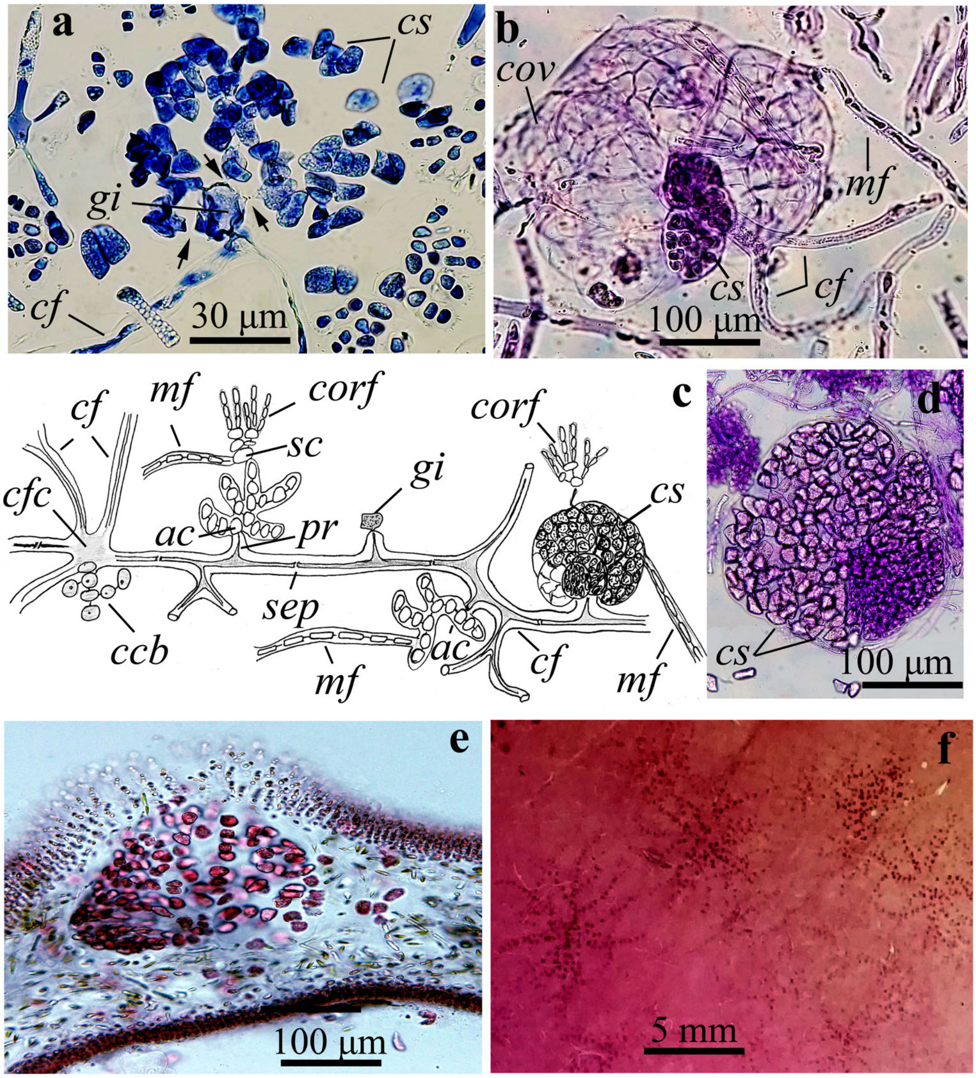

Fig. 4. Post-fertilization development of *Neoabbottiella araneosa*. (a) Initial gonimoblast cell (*gi*) giving rise to several gonimolobes (arrows) stained with aniline blue. *cf*, connecting filament; cs, carposporangia. (b) Gonimoblast surrounded by a transparent gelatinous covering (*cov*) and contacting with medullar filaments (*mf*) stained with hematoxylin. *cf*, connecting filaments; cs, carposporangia. (c) Schematic of post-fertilization development. *ac*, auxiliary cell; *ccb*, carpogonial cell branch; *cf*, connecting filaments; *cfc*, carpogonial fusing cells; *corf*, cortical filaments; *gi*, initial cell of a gonimoblast; *mf*, medullar filaments; *pr*, process to contact connecting filament with auxiliary cell; *cs*, carposporangia; *sc*, supporting cell; *sep*, septum. (d) Carposporophyte with carposporangia (*cs*) stained with hematoxylin. (e) Cross-sections of the fertile blade showing mature gonimoblasts. (f) An enlarged fragment of the surface of the blade with gonimoblasts of *N. araneosa* (ASKR-M155) forming a pattern in the form of multiray stars.

common mucilage (Fig. 3e). The laterals of the auxiliary cell branches were two- to four-celled, occasionally branched. The auxiliary cell was intercalary, and the third or fourth cell from the distal end of the branch, it did not differ in shape and size from other cells of the auxiliary cell branch.

The post-fertilization process was not observed in detail. Similarly, no detailed data on this process were obtained previously (Perestenko 1975; Lindstrom 1985). Fertilized carpogonia were very rare; we examined 100 of slides and saw only three carpogonial fusion cells in advanced stages. However, some new details have been revealed. We found that the connecting filaments arose from a large carpogonial fusion cell, and a gonimoblast initial cell developed from the connecting filament and not from an auxiliary cell, as shown by Perestenko (1975). Here we give an emended description of the post-fertilization process, based on our own observation and those of Perestenko (1975). After presumed fertilization, the large carpogonial fusion cell was formed, with lateral cells not incorporated. The early stages of the development of the carpogonial fusion cell were not observed. Subsequently, six to eight connecting filaments arose from the carpogonial fusion cell and diverged in rays (Fig. 3f). Connecting filaments were 10–25 μm thick, transparent, septate, branched and sometimes anastomosing with medullary filaments (Fig. 3g,h). The connecting filament attached to the auxiliary cell by a short lateral process (Fig. 3i). No new connecting filaments developed as a result of these connectins (Fig. 3i). The gonimoblast initial arose from the top of swelling or protrusion, which formed directly on the connecting filament (Fig. 3j), as we suggested, at some distance from the auxiliary cell. We did not find any auxiliary cell branches near the gonimoblast initial. Several gonimolobes arose from the gonimoblast initial (Fig. 4a). The pattern of post-fertilization development is illustrated in Fig. 4c. Gonimoblats were enclosed in a mucilage sheath, clearly visible when stained with hematoxylin (Fig. 4b). Carpospores matured asynchronously and small clusters of unmatured carposporangia were evident in the carposporophyte (Fig. 4d). Mature carposporangia were 17.5–25 μm in diameter. Carposporophytes were round (190–280 μm) or slightly flattened (130–170 × 160–280) and lacked a pericarp and ostiole (Fig. 4e). The carposporophytes formed in chains along the radius of a circle with fertilized carpogonium in the center, creating a pattern in the form of multiray stars on the surface of the blade (Fig. 4f); the stars were separate in younger plants of *N. araneosa*, whereas in old blades the stars fused into a uniform pattern. Tetrasporangia were not seen.

Table 1. Comparison of *Neoabbottiella*, *Schmitzia* spp. and Halymeniales

Characters	*Neoabbottiella*[a,b]	*Schmitzia*[c,d,e]	Halymeniales[f,g,h,i,j]
Structure of the thalli	Multiaxial	Uniaxial	Multiaxial
Carpogonial branch	Five- to nine-celled	Three- or five- to nine-celled	Three- to four-celled, in ampulla
Carpogonial fusion cell	+, of all cells of carpogonial branch except laterals	±, of carpogonium and supporting cells[c]	±, of carpogonia and hypogenous cells[h,i], of cells of carpogonial branch[g]
Connecting filaments (cf)	Septate, branched	Septate, branched	Septate, branched
Auxiliary cell	Indistinguishable before fertilization, fourth or third distal cell of auxiliary cell branch in *Thamnoclonium*-type ampulla	Indistinguishable before fertilization, intercalary vegetative cell	Basal cell of second- or third-order ampullary filament or intercalar cell of first-order ampullary filament (vegetative cell in Tsengiaceae[j])
Fusion of cf with auxiliary cell (AC)	Via short lateral tubular process	Via short lateral tubular process	Via the terminal cell of the connecting filament (lateral fusion in Tsengiaceae[j])
Auxiliary fusion cell	–	–	+, Develops via fusion of AC with neighboring cells of ampullar filaments following initiation of the gonimoblast
Origin of gonimoblast	From a raised protuberance of the connecting filaments at a distance from the auxiliary cell	From a raised protuberance of the connecting filaments at a distance (within 40 μm) from the auxiliary cell	From auxiliary cell
Pericarp	Absent, gonimoblast surrounded by a gelatinous covering	Absent, gonimoblast surrounded by a weakly gelatinous, laminated covering	Of lateral ampullary branches and secondary medullary filaments
Chains of carposporophytes	+, As multiray stars	+	–

[a] Perestenko 1975.
[b] This study.
[c] Maggs and Guiry 1985.
[d] Wilce and Sears 1991.
[e] Hawkes 1982.
[f] Saunders and Kraft 1996.
[g] Rodríguez-Prieto *et al.* 2018.
[h] Rodríguez-Prieto *et al.* 2022.
[i] Chiang 1970.
[j] Saunders and Kraft 2002.

DISCUSSION

The present study clearly shows that *Neoabbottiella* cannot be assigned to Halymeniales, the order in which the genus is currently placed (Lindstrom 1985; Schneider and Wynne 2007; Guiry and Guiry 2023). Our molecular studies clearly separate *Neoabbottiella* from the Halymeniales. Certain features of post-fertilization development in *Neoabbottiella*, the process which is an important diagnostic characteristic in the taxonomy of red algae, also separate *Neoabbottiella* from the Halymeniales. These include the intercalary position of the auxiliary cell, the contact of the connecting filament with the auxiliary cell via a lateral process, the production of the gonimoblast initial cell from the connecting filament at some distance from the auxiliary cell and the lack of pericarp of branched ampullar filaments around carposporophytes in *Neoabbottiella* (Table 1). In this alga the gonimoblast is surrounded by thick genalatinous covering. In contrast, in the Halymeniales, the auxiliary cell is basal to a second- or third-order or intercalary in a first-order filament of auxiliary cell ampullae and it is visible in intact auxiliary cell branches; connecting filaments terminally fuse with an auxiliary cell; the gonimoblast initial cell develops from the auxiliary cell or auxiliary fusion cell; and the carposporophyte has a pericarp, which is formed by secondary medullary filaments and lateral ampullary filaments borne from an auxiliary fusion cell complex (Kawaguchi *et al.* 2004; Gargiulo *et al.* 2013; Manghisi *et al.* 2014; Rodríguez-Prieto *et al.* 2018, 2022; Kim *et al.* 2021). This comparison shows that *Neoabbottiella* is improperly assigned to Halymeniales on the basis of reproductive characteristics.

The molecular-phylogenetic analyses grouped *Neoabbottiella* with representatives of the genus *Schmitzia* (family Calosiphoniaceae *incertae sedis*, Withall and Saunders 2006). Unlike multiaxial *Neoabbottiella*, *Schmitzia* exhibits uniaxial construction of the thallus and an auxiliary cell is an intercalary vegetative cell. Nevertheless, the genera share several common features of female reproductive structures and post-fertilization development (Table 1). Carpogonial cell branches have larger third or fourth cells distally and one- to four-celled laterals; connecting filaments are septate and branched. They also have connecting filaments attached to the auxiliary cells via lateral tubular processes, the gonimoblast initial cell develops from a raised protuberance of the connecting filaments at a distance from the auxiliary cell, a gelatinous covering is present around the carposporophyte and carposporophytes aggregate in long chains because they develop from one connecting filament. All these features are common among *Neoabbottiella* (Perestenko 1975; this study) and *Schmitzia* (Feldmann 1954; Hawkes 1982; Maggs and Guiry 1985; Wilce and Sears 1991).

Since there are still no detailed data on post-fertilization events in *Neoabbottiella*, any taxonomic conclusions are premature. Despite the fact that *Neoabbottiella* was genetically grouped with *Schmitzia* sp. from Australia forming a sister group to *Schmitzia hiscockiana*, we do not propose to synonymize these two genera in the absence of sequences of the generitype, *Schmitzia neapolitana*. Moreover, it has been noted that the '… retention of the Pacific/Caribbean/western Atlantic and the eastern Atlantic groups of *Schmitzia* species in one genus is problematic' (Wilce and Sears 1991: 167), because they are readily divided into two groups on the basis of reproductive morphology (Maggs and Guiry 1985; Wilce and Sears 1991). Members of an eastern Atlantic group including *S. neapolitana* and *S. hiscockiana* have three-celled carpogonial branches lacking secondary laterals. Members of a Pacific group composed of *Schmitzia evanescens* and *Schmitzia japonica* are characterized by four- to nine-celled carpogonial branches with secondary laterals (Maggs and Guiry 1985; Wilce and Sears 1991). In our phylogenetic tree, *Schmitzia* was not monophyletic. Sequences from Australian species were more closely related to those of *Neoabbottiella* than to those of *S. hiscockiana* from France. Further study incorporating topotype sequence data for other species of *Schmitzia* is needed to resolve relationships within *Schmitzia* and its association with *Neoabbottiella*. In the absence of such data the placement of *Neoabbottiella* within *Schmitzia* would be premature due to insufficient sampling.

The classification of *Neoabbottiella* at the family and order levels is difficult. Although this genus was clustered with *Schmitzia*, which is a member of the family Calosiphoniaceae, the trees do not include sequence data of any species of *Calosiphonia*, the type genus of the family. Further studies are needed incorporating sequence data for *Calosiphonia* and additional species of *Schmitzia* to resolve the position within Rhodymeniophycidae.

One finding of the present study is that *N. decipiens* needs to be synonymized with *N. araneosa*. We molecularly analyzed specimens having typical for *N. araneosa* morphology and type material of *N. decipiens* and found the COI-5P sequences of both species were identical. *Neoabbottiella araneosa* and *N. decipiens* are sympatric and exhibit similar morphological features except for the abundance of stellate refractive cells in the medulla and the position of the stars of carposporophytes (Pisareva and Klochkova 2013; Lopatina *et al.* 2016). Also, it was noted slight differences in the shape and color of the blades of these species, which are entire, round or irregular in shape and red to reddish-chestnut in *N. araneosa* and round to dissected in shape and reddish-chestnut to brownish-chestnut in *N. decipiens* (Pisareva and Klochkova 2013). We found that the abundance of stellate refractive cells varied in the thalli of both *N. decipiens* and *N. araneosa* from eight to 20 per view area (×100 magnification = 9 mm^2), and the density of the stars with multiply rays formed by carposporophytes in *N. araneosa* may range from solitary to network forming, depending on maturity. These results imply that *N. araneosa* and *N. decipiens* constitute a single species.

In conclusion, we would like to summarize the main findings of the study. (1) The genus *Neoabbottiella* is monotypic, the name *N. decipiens* being an later heterotypic synonym of *N. araneosa*; and (2) the genus *Neoabbottiella* should be removed from the order Halymeniales. The classification of the genus at the family and order levels is difficult, and further studies are required to resolve this issue. Until this, *Neoabbottiella* should be recognized as *incertae sedis* in Rhodymeniophycidae.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. List of analyzed specimens of *Neoabbottiella* with collection details.

Table S2. List of examined specimens and accession numbers in GenBank. Newly generated sequences are shown in bold.

Fig. S1. ML phylogeny as determined using the nrLSU dataset. Support values are ultrafast bootstrap/aLRT/non-parametric bootstrap/Bayesian posterior probabilities. Only Bayesian posterior probability values of >0.9 and bootstrap support values of >75% are shown. Scale bar: substitutions per site. Newly generated sequences are shown in bold. Numbers in brackets after the species names are GenBank accession numbers. *Bayesian posterior probability = 1, bootstrap = 100%.

Fig. S2. ML phylogeny as determined using the nrSSU dataset. Support values are ultrafast bootstrap/aLRT/non-parametric bootstrap/Bayesian posterior probabilities. Only Bayesian posterior probability values of >0.9 and bootstrap support values of >75% are shown. Scale bar: substitutions per site. Newly generated sequences are shown in bold. Numbers in brackets after the species names are GenBank accession numbers. *Bayesian posterior probabilities = 1, bootstrap = 100%.

Fig. S3. ML phylogeny as determined using the *rbc*L dataset. Support values are ultrafast bootstrap/aLRT/non-parametric bootstrap/Bayesian posterior probabilities. Only Bayesian posterior probability values of >0.9 and bootstrap support values of >75% are shown. Scale bar: substitutions per site. Newly generated sequences are shown in bold. Numbers in brackets after the species names are GenBank accession numbers. *Bayesian posterior probabilities = 1, bootstrap = 100%.

Fig. S4. ML phylogeny as determined using the concatenated nrLSU + nrSSU dataset. Support values are ultrafast bootstrap/aLRT/non-parametric bootstrap/Bayesian posterior probabilities. Only Bayesian posterior probability values of >0.9 and bootstrap support values of >75% are shown. Scale bar: substitutions per site. Newly generated sequences are shown in bold. Numbers in brackets after the species names are GenBank accession numbers (nrLSU/nrSSU). *Bayesian posterior probabilities = 1, bootstrap = 100%.