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First report of the Hawaiian genus *Newhousia* (Dictyotales, Phaeophyceae) from Madang, Papua New Guinea and description of the new species *N. yhaga* sp. nov.

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Abstract: The calcified encrusting brown-algal genus *Newhousia* was collected in 2012 from two sites in Madang Lagoon, northern Papua New Guinea. This is the first report of that enigmatic genus since its original description in 2004 from Oahu in the Hawaiian Islands. Morphologies of thalli from both Papuan localities agree with the original diagnosis and the published figures of the Hawaiian *Newhousia imbricata*. Ribulose-1,5-biphosphate carboxylase large subunit (*rbc*L) sequences of Papuan specimens were 96% identical to those of *N. imbricata*, and although there were no outstanding morphological differences between the Hawaiian and the Papuan specimens, the genetic divergence between the two strongly indicates they are not conspecific. A formal description of the new species, *Newhousia yhaga*, is provided and the phylogenetic implications of concatenated *cox*3, *psb*A and *rbc*L sequences evaluated. A multimarker phylogeny was generated in the hope to establish the precise phylogenetic position of *Newhousia* within the order Dictyotales. Even though both Bayesian inference (BI) and maximum likelihood (ML) analysis point to *Newhousia* being sister to *Lobophora*, these results are not supported by ML bootstrap values. The present documentation of *Newhousia* in a southwest Pacific locality considerably extends the geographical range of this unusual genus.

Keywords: BI and ML multilocus phylogenies; Dictyotales; molecular-assisted taxonomy; *Newhousia*; Papua New Guinea.

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Introduction

In 2004, Kraft, Saunders, Abbott *et* Haroun described *Newhousia imbricata* as a member of the brown-algal order Dictyotales and the first encrusting representative of its class to exhibit calcification. At that time endemic to Oahu in the Hawaiian Islands, it was only the second genus of calcified species in the Phaeophyceae, although in a form differing from that of any other alga. Unique was the laying down of calcium carbonate as both an extracellular horizontal layer between the overlapping frond and as intracellular deposits laid down within the cell-wall matrix, an additional unique feature being the production of calcium carbonate in the form of both calcite and aragonite (Kraft et al. 2004). Based on small and large subunit rDNA phylogenies, Kraft et al. (2004) determined that *Newhousia* was in a strongly supported monophyletic clade that includes *Distromium*, *Lobophora* and *Zonaria*.

Since its discovery, the monotypic genus *Newhousia* has not been reported outside the island of Oahu. During an expedition led by the last author as part of the research program "La Planète Revisitée" ("Our Planet Reviewed") conducted between 10 and 24 November 2012 in northern Papua New Guinea (Figure 1), a crustose brown alga was collected at two neighbouring sites in Madang Lagoon. The morphology and phylogenetic position of these specimens are addressed in this study.

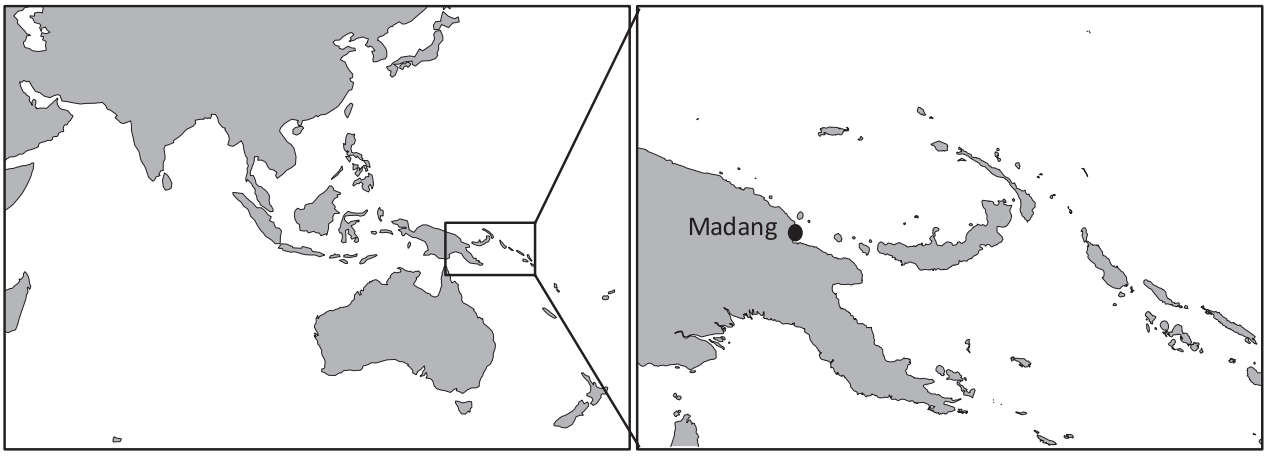

Figure 1: Map showing the location of Madang, Papua New Guinea, where *Newhousia yhaga* C.W.Vieira, De Clerck *et* Payri, sp. nov. was collected.

Materials and methods

Taxon sampling

Sampling was carried out by scuba diving to 10-m depths. Two specimens of *Newhousia* (IRD11128 and IRD11129, *leg*. Claude Payri) were secured from two sites, located ca. 6 km apart: Paeowa Island (PCT29. 5.1745° S; 145.833° E) and Malamal Anchorage (PCT12. -5.11995° S; 145.823° E) on 13 and 18 November 2012, respectively. Since the algae were tightly adherent crusts on dead corals (Figure 2A, B, D), specimens had to be removed with a chisel and hammer. Voucher specimens were preserved in silica gel and are presently housed at the herbarium of the Institut de Recherche pour le Développement in Noumea, New Caledonia (NOU).

Morphological analyses

Photographs of habit and anatomy were taken using a Leica EC3 camera (Leica Microsystems, Wetzlar, Germany) mounted on a Leica stereomicroscope (Leica MDG 33, Leica Microsystems, Wetzlar, Germany). Specimens were decalcified over a five-day period in a solution of potassium dichromate and acetic acid (3 g potassium dichromate dissolved in 5 ml acetic acid and diluted to 100 ml with distilled water). Tissue layers were carefully peeled after decalcification. Longitudinal and transverse sections of decalcified thalli were made using a medical portable freezing microtome (Labonord®). Photographs of the sections were taken with a digital camera (Olympus Camedia C-5050 5.0 Megapixel, Tokyo, Japan) attached to a compound microscope (Olympus BH-2, Tokyo, Japan). Cortical- and hypodermal-cell thicknesses, widths and lengths were measured.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from tissue samples dried in silica gel using a cetyl-trimethyl ammonium bromide-extraction method following De Clerck et al. (2006). Sequences were generated from the mitochondrial encoded cytochrome c oxidase subunit III gene (*cox*3), and the chloroplast encoded ribulose-1,5-biphosphate carboxylase large subunit (*rbc*L) and the photosystem II protein D1 (*psbA*) genes. Sequences were blasted to the NCBI database. Sequences were aligned with those of other phaeophycean algae (Supplementary Table S1) using MUSCLE (Edgar 2004) implemented in eBioX 1.6 beta (Barrio et al. 2009).

Phylogenetic analyses

Maximum likelihood (ML) and Bayesian inference (BI) phylogenetic species trees were generated from individual genes (*cox*3, *psb*A and *rbc*L) and from a concatenated alignment including *cox*3 (610 bp), *psb*A (919 bp) and *rbc*L (1360 bp) genes, partitioned by gene and codon position. Bayesian phylogenetic inference was analyzed using MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003), initiated with a random starting tree and four chains of MCMC iterations were run simultaneously for 100 million generations. The first 100,000 (25%) trees sampled were discarded as

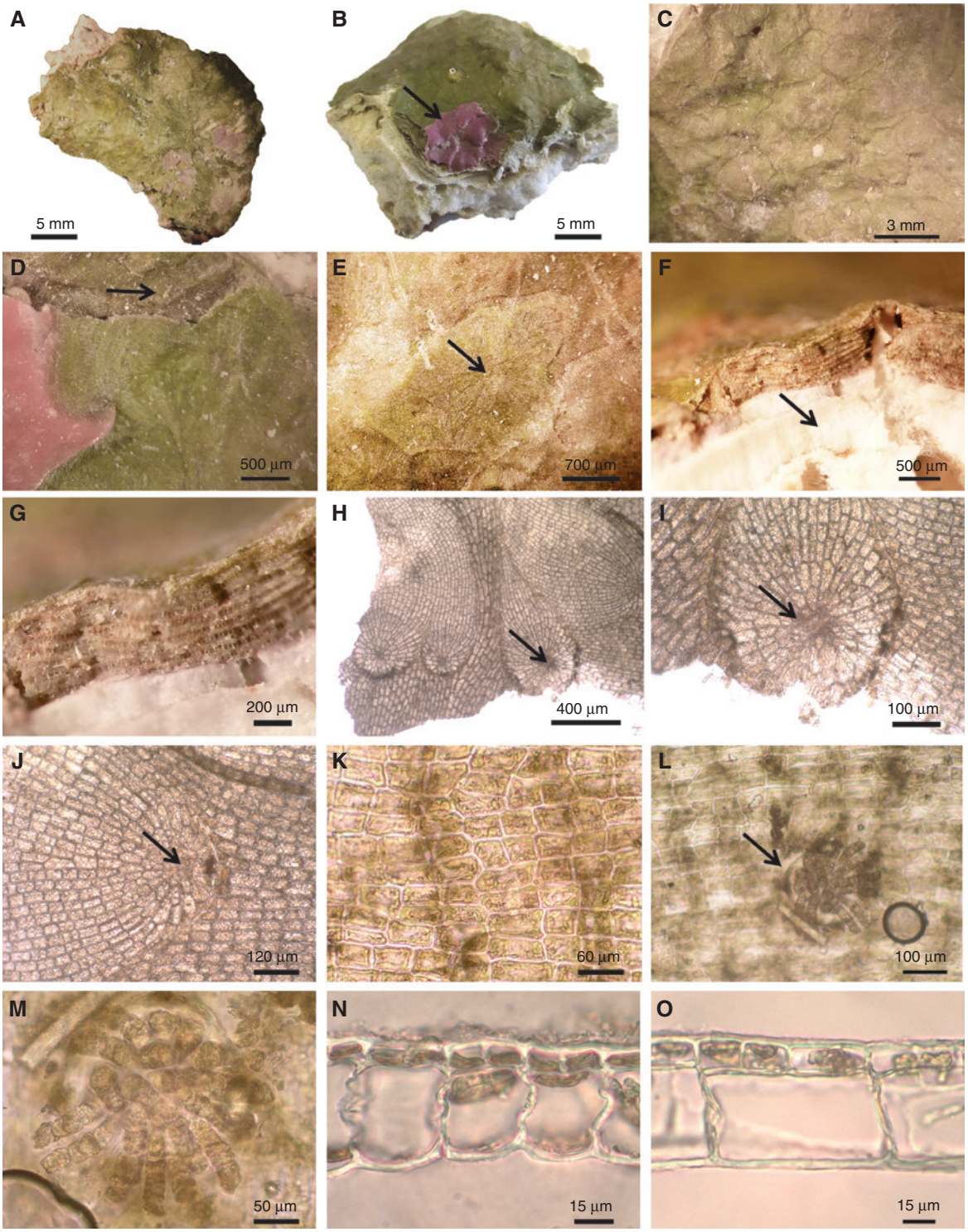

Figure 2: *Newhousia yhaga* sp. nov. habit and anatomical features. (A, B) Two aspects of the holotype specimen, showing its predominantly olive-green hue. A coralline red alga (Figure 2B, arrow) is epiphytic on the *Newhousia* crust. (C) Detail of radially growing adjacent and overlapping blades on the surface of the holotype. (D) Fracture of the *Newhousia* crust exposing underlying blades (arrow). (E) Detail of one of the single surface blades irregularly spreading from the central point (arrow) that initiated its growth. (F, G) Medium and high-power magnifications of banding shown by overlapping layers of blades (dark lines) and calcium carbonate (white lines) that comprise the consolidated crusts of a *Newhousia* thallus. *Newhousia* is cemented to coral substratum (Figure 2F, arrow). (H–J) Stages in radial development of surface blades from points of origin (arrows). (K) Surface view of aligned, rectilinear cells of the epithallus. (L, M) Low- and high-power details of cluster of erumpent filaments that may represent juvenile hairs. (N) Cross section of single blade showing double row of rectilinear epithallial cells overlying each cuboidal hypothallial cell. (O) Longitudinal section of single blade showing aligned epithallial row overlying a rectilinear hypothallial cell.

burn-in, based on the stationarity of likelihood values as assessed using Tracer version 1.5 (Rambaut and Drummond 2009). A consensus topology and posterior probability values were calculated from the remaining trees. ML analyses were conducted using RAxML under a GTR+CAT model (Stamatakis 2006). The robustness of the resulting phylogenies was tested using 1000 replicates of a rapid bootstrap heuristic (Stamatakis et al. 2008). All tree searches were conducted using the Cipres web portal (Miller et al. 2010).

Results

Molecular results

The *rbc*L sequence blasted to the NCBI database resulted in the highest sequence similarity with the species *Newhousia imbricata* (GenBank Number: EF990240), with a query cover of 99%, and a maximum identity of 96% (1146/1199, 53 pb differences). The *rbc*L sequence clustered together with the *N. imbricata* sequence in the *rbc*L based ML and BI phylogenetic trees.

The Bayesian phylogeny inferred from the small-subunit r*DNA* in Kraft et al. (2004) positioned *N. imbricata* as a sister taxon to the genus *Zonaria* and the authors recommended that the phylogenetic position of *Newhousia* be refined by further investigation with more variable genes. The Bayesian and ML phylogenies (Figure 3) based on the concatenation of the *rbc*L+*psb*A+*cox*3 genes positioned *Newhousia* as sister species to *Lobophora*, with support values of 100% for the BI phylogeny but only 56% for the ML phylogeny (Figure 3). Although our results point to *Newhousia* being sister to *Lobophora*, the low ML bootstrap results do not allow us to conclude with certainty about the phylogenetic position of *Newhousia*.

Taxonomic results

The molecular data imply that the two Papua populations represent a separate, second species of *Newhousia*, which is described here.

Newhousia yhaga C.W.Vieira, De Clerck *et* Payri sp. nov. (Figure 2A–O).

Description

Thalli tightly adherent to and following the contours of dead-coral substrata (Figure 2A, B), fronds ovoid to irregularly lobed in surface outline (Figure 2C–E), to 3–4 mm in diameter, green khaki-gray (Figure 2A, B, D) or reddish-brown (Figure 2C) in color, consisting of multiple layers of imbricated, marginally meristematic encrusting blades (Figure 2F, G) cemented to the substratum and to each other (Figure 2C) across the whole of the undersurfaces and without anchoring rhizoids; stack of blades to 1 mm thick. Blades extend peripherally from a continuous marginal meristem (Figure 3E, H–J), 45.7±5.1 µm thick, bilayered, cells of the epidermal and hypodermal layers 11.3±2.4 µm and 34.3±3.9 µm thick respectively. Epidermal cells rectilinear in surface (Figure 2H–K), cross-sectional (Figure 2N). Hypodermal cells cuboidal in cross-section (Figure 2N), rectilinear or in long section (Figure 2O), 32.3±3.2 µm in width by 59.7±3.9 µm in length. Juvenile hairs (Figure 2L, M) clustered. Oogonia, antheridia and sporangia unknown. Differing from *N. imbricata* by (1) the distinctive radial development of surface blades from points of origin and (2) the 4% difference in *rbc*L nucleotide sequences [PC0063019 (IRD11128) and IRD11129].

Ethymology

The epithet “yhaga” comes from the word “Yhag”, which is the general name for brown algae in Bel language that is spoken by the villages surrounding Madang Lagoon and in other areas in Madang province.

Holotype

PC0063019 (IRD11128), *leg*. C. Payri (13.xi.2012). Figure 2A, B.

Type locality

Paeowa Island, Madang Lagoon, Papua New Guinea (05.1745° S; 145.833° E), thalli on dead coral at -10 m.

Distribution

Endemic so far to the northeastern shore of Papua New Guinea (Figure 1).

Habitat

Thalli consisting of multiple layers of tightly adhering crusts several cm^2 in surface area and cemented to hard

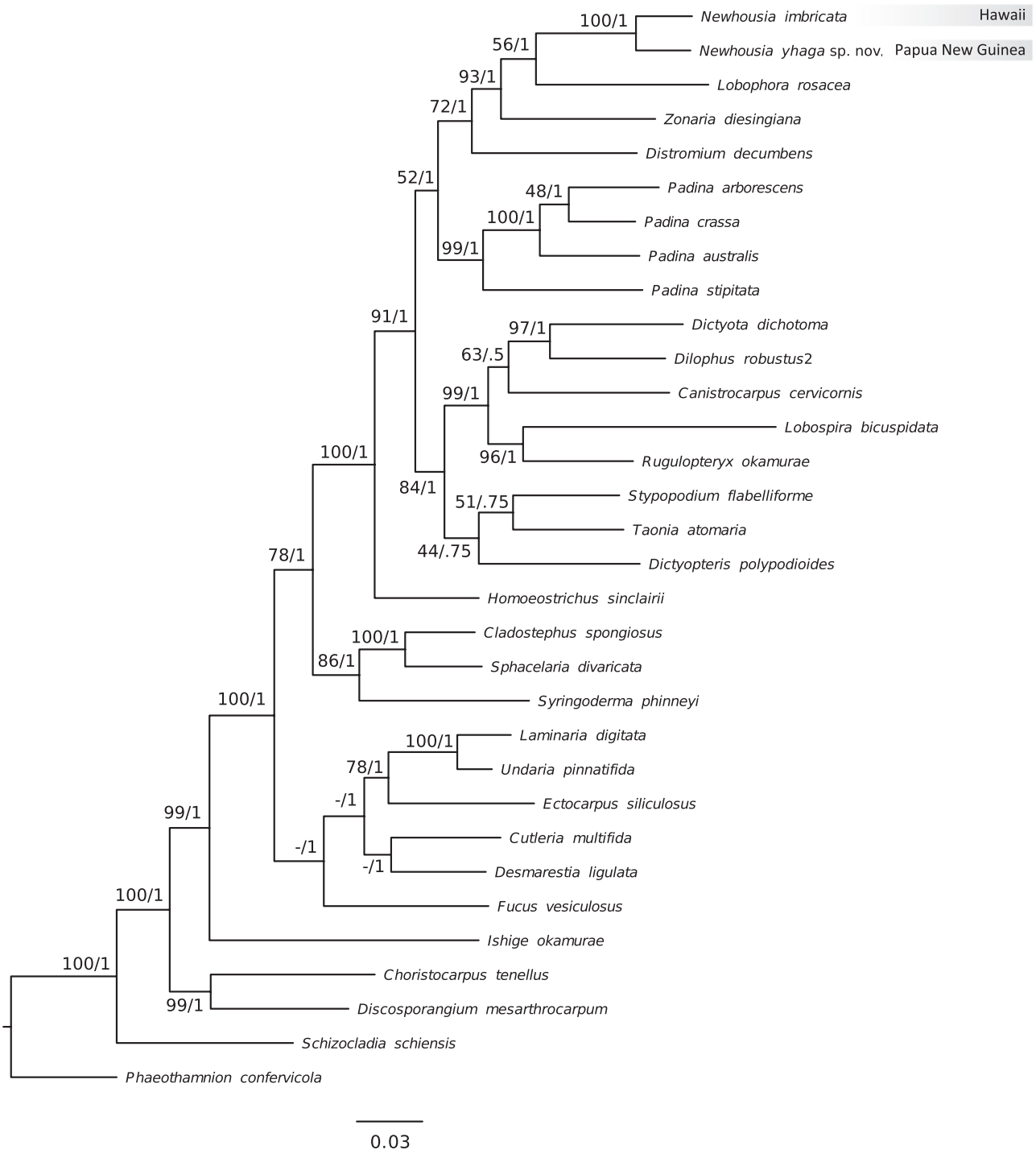

Figure 3: Bayesian tree, generated with MrBayes, based on the concatenation of *cox*3, *psb*A and *rbc*L gene sequences of Phaeophycean species including *Newhousia imbricata* and *Newhousia yhaga* sp. nov. The values shown at each node represent the maximum likelihood (left) and posterior probability (right) values. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

substrata, intermixed with encrusting coralline algae (Figure 2B, D) at upper levels of inner and outer reef slopes from low intertidal to depths of 10 m in areas of strong to moderate water movement.

Specimens examined

1) Paeowa Island (PCT29; 5.1745° S; 145.833° E), (*C. Payri*, 13.xi.2012; IRD11128). Malamal Anchorage, Madang,

Papua New Guinea (PCT12; -5.11995° S; 145.823° E), Madang, Papua New Guinea, 18 November 2012, *leg*. C. Payri (*C. Payri*, 18.xi.2012; IRD11129).

Discussion

The calcified encrusting brown algal genus *Newhousia* was discovered in Madang Lagoon, northern Papua New Guinea. This is the first report of that enigmatic genus since its original description in 2004 from Oahu in the Hawaiian Islands.

While the molecular results confirm the affiliation of the Papuan specimens with *Newhousia*, the rather low identity value between the Papuan and Hawaiian *rbc*L sequences (4% difference) suggests that these two lineages are not conspecific. The genetic divergence between these two molecular operational taxonomic units strongly supports the establishment of the new *Newhousia* species.

The multi-marker phylogenies confirmed the position of *Newhousia* in the monophyletic clade that includes *Distromium*, *Lobophora* and *Zonaria*, and points to *Newhousia* being sister to *Lobophora*. However, the ML analyses are equivocal on the relative positions of *Newhousia*, *Lobophora* and *Zonaria*, and we are still no closer to resolving *Newhousia*'s nearest neighbor.

Newhousia has more morphological features in common with *Lobophora* than with *Zonaria*, which is reflected in our phylogenetic results. Indeed, Kraft et al. (2004) already commented that "the frond structure of *Newhousia* is closest to tightly adherent forms of *Lobophora*". Both *Lobophora* and *Zonaria* have cortical layer on all surfaces and tiers of regularly aligned rectilinear medullary cells, those in *Zonaria* consisting of 5–8 layers of cells that are of uniform size (Womersley 1987), whereas *Lobophora* has a 3–25 layered medulla of stacked and aligned cells, in which those of the central layer are twice or more the height of medullary cells on both sides (Vieira et al. 2014). Both genera are unlike *Newhousia* in that the latter's epidermal layer is restricted to the dorsal surface and its single hypodermal layer is applied and cemented directly to the substratum. In some forms and reputed species of *Lobophora*, however, thalli are crustose and lack a ventral cortical layer, although they attach to solid substrata by ventral, often moniliform rhizoids (Kraft 2009, p. 210, fig. 67C–F, Vieira et al. 2014) rather than cementing directly as in the case of *Newhousia*.

As stated in the introduction, *Newhousia* and *Padina* are so far the only two brown algal genera to display calcification. They are not, however, sister taxa, nor is the mode of calcification in *Padina* (which is by means of usually light coatings of aragonite crystals laid down primarily on ventral surfaces within the coiled apical meristem) at all similar to the extra- and inter-cellular depositions of calcium carbonate in *Newhousia*. Calcification has thus evolved more than once and very differently in the brown algal order Dictyotales and is lacking completely in all other orders of the Phaeophyceae.

Our report of *Newhousia* from Papua New Guinea reveals that the genus is not restricted to Hawaii but may prove to be widespread in the warm waters of the southwest Pacific. One possible reason for its having been overlooked is the fact that the genus can be easily mistaken for an encrusting *Lobophora*, as Kraft (pers. com.) did on first collecting specimens in 1991, filing them as *Lobophora*, and not recognizing the error until new collections were made in 2003. Other genera of superficially similar habit that *Newhousia* might be confused with include *Ralfsia* (although unlikely because the genus is infrequently found in deep waters and has a very different surface structure) or even the red alga *Peysonnellia*, which frequently encrusts and can take on the same greenish-gray hues.

When its authors first elected to name *Newhousia* in honor of Dr. Jan Newhouse, a revered University of Hawaii lecturer in General Science and student of tropical brown algae, they wondered if the name might someday be pre-empted by a pre-existing fossil genus, so amenable to preservation would its growth form and heavy calcification seem to be. This has not yet happened, and the present reports calls yet more attention to a unique and one of the most unusual of all the brown algae.

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