European Journal Of Phycology
October 2023, Volume 58 Issue 4 Pages 399-426
https://doi.org/10.1080/09670262.2023.2164907
https://archimer.ifremer.fr/doc/00822/93398/

Diversity of the genus *Avrainvillea* (Dichotomosiphonaceae, Chlorophyta): new insights and eight new species

Lagourgue Laura [1, *], Rousseau Florence [2], Zubia Mayalen [3], Payri Claude E. [1]

[1] Institut de Recherche pour le Développement, UMR 9220 ENTROPIE (Institut de Recherche pour le Développement, Université de la Réunion, IFREMER, Université de la Nouvelle-Calédonie, Centre National de la Recherche Scientifique), New Caledonia
[2] Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France
[3] Université de la Polynésie française, UMR Ecosystèmes Insulaires Océaniens, LabEx CORAIL, BP6570, Faa'a, Tahiti 98702, French Polynesia

* Corresponding author : Laura Lagourgue, email address : lagourgue.l@gmail.com

Abstract :

Avrainvillea is a green macroalgal genus of the family Dichotomosiphonaceae (order Bryopsidales). Many species have been morphologically described, but few studies have addressed the genetic diversity of this genus. Based on a rich collection of specimens from the tropical Western Atlantic, Indian and Pacific Oceans, we aimed to (1) reassess Avrainvillea species diversity through species delimitation analyses, (2) update their distribution ranges, (3) reconstruct the species phylogenetic relationships, based on a concatenated multilocus matrix (tufA, rbcL and 18S rDNA) and (4) revise their taxonomy and describe new species where necessary. Our species delimitation approach highlighted 23 secondary species hypotheses in our collection, including nine known and currently accepted species, four species complexes (A. amadelpha, A. lacerata, A. erecta-obscura and A. mazei-nigricans), and eight new species for which we provide descriptions: A. laciniata (Papua New Guinea), A. minima and A. pyrochroma (Madagascar), A. mollis and A. kanakiensis (New Caledonia), A. pavonina (Fiji), A. spongiosa (Pacific) and A. corticata (Indo-Pacific). We also propose the resurrection of A. gracillima Børgesen, the reinstatement of Avrainvillea lacerata var. robustior A.Gepp & E.S.Gepp, and the synonymy of A. rotumensis A.D.R.N'Yeurt, D.S.Littler & Littler with A. pacifica A.Gepp & E.S.Gepp. We complemented the taxonomic work by providing a contemporary dichotomous key for morphological identification of all extant species. Our multilocus phylogeny included 25 species of Dichotomosiphonaceae and recovered Avrainvillea as a polyphyletic group, divided into three distinct clades, with Cladocephalus luteofuscus positioned within the group. The species determined using the species delimitation approach were all monophyletic and 19 of them were highly supported. For the first time, this study also provided genetic sequences for A. asarifolia, A. clavatiramea, A. digitata, A. elliottii, A. fulva, A. gracillima, A. geppiorum, A. pacifica and A. obscura.

HIGHLIGHTS

► *Avrainvillea* is not monophyletic. ► Reassessment of *Avrainvillea* species diversity delimited 23 secondary species hypotheses. ► Eight new species of *Avrainvillea* were discovered in the Indo-Pacific.

Keywords : Biodiversity, green macroalgae, Indo-Pacific, species delimitation, systematics, Tropical Atlantic

INTRODUCTION

Avrainvillea is a green siphonous marine macroalgal genus belonging to the family Dichotomosiphonaceae (order Bryopsidales) which also includes *Dichotomosiphon* (type genus) and *Cladocephalus*. The genus was proposed by Decaisne in 1842, and currently, 30 species of *Avrainvillea* are taxonomically accepted based on morphology and, for eight of them, on DNA data (Guiry & Guiry, 2022). The genus has a worldwide tropical and subtropical distribution across the Atlantic, Indian and Pacific oceans. Ecologically, species of *Avrainvillea* are found from the intertidal down to 60 m on sand mud, reefs or in seagrass beds (Olsen-Stojkovich, 1985; Guiry & Guiry, 2022). The distribution in the seascape depends on species and environmental conditions and ranges from physically distant individuals to dense mound-building colonies (Littler & Littler, 2004). Some species, such as *Avrainvillea* cf. *erecta* and *A. amadelpha* (Montagne) A.Gepp & E.S.Gepp in Hawaii (Wade *et al.*, 2018; Veazey *et al.*, 2019) or '*A. amadelpha'* in the Mediterranean Sea (Verlaque *et al.*, 2017) can proliferate or become invasive. Cabrera & Suarez (2006) listed 10 species as restricted to the tropical and subtropical Atlantic and 11 species found only in the Indo-Pacific, including six endemic species each known from only one locality. In addition, Cabrera & Suarez (2006) suggested that *A. calathina* Kraft & Olsen-Stojkovich and *A. rotumensis* A.D.R.N'Yeurt, D.S.Littler & Littler are relict species found only in Lord Howe Island (Australia) and Rotuma Island (Fiji), respectively, and that *A. canariensis* A.Gepp & E.S.Gepp may be neo-endemic to the Canary Islands (Eastern Atlantic).

Morphologically, *Avrainvillea* species are characterized by uncalcified, single or multiple blades, with or without a stipe and anchored to the substrate by a holdfast. Siphons are cylindrical to moniliform, dichotomously divided and forming a cohesive blade, although they have no secondary adhesion structures, contrary to closely related families such as the Udoteaceae and the Rhipiliaceae (or tribe Rhipileae). *Avrainvillea* species are holocarpic, i.e. the thallus dies after the gametes are released. Sexual reproduction has been observed in *A. asarifolia* Børgesen (Littler & Littler, 1992), *A. erecta* (Berkeley) A.Gepp & E.S.Gepp (Howe, 1907; Verlaque, 2017), *A. mazei* G.Murray & Boodle (Young, 1977), *A. nigricans* Decaisne (Howe, 1907), *A. obscura* (C.Agardh) J.Agardh (Young, 1977) and *A. rawsonii* (Dickie) M.Howe (Young, 1977). Vegetative multiplication is also common in *Avrainvillea* (Olsen-Stojkovic, 1979) and is characterized by a habit in clusters or colonies of several individuals.

Most previous studies of the genus include systematics and morphology (Murray & Boodle, 1889; Howe, 1905, 1907; Børgesen, 1908; Cabrera & Suarez, 2006); they include one global monograph (Olsen-Stojkovich, 1985) and two others focused on the Indo-Pacific (Gepp & Gepp, 1911) and the Western Atlantic (Littler & Littler, 1992). Only two molecular studies are available (Curtis *et al.*, 2008; Wade *et al.*, 2018). Additional molecular data is available in the more extensive Bryopsidales studies of Sauvage *et al.* (2016) and Verbruggen *et al.* (2017) (*tufA* barcodes and chloroplast genomes, respectively). However, there is still a lack of genetic data for this genus, with only 8 of the 30 supposed species having sequences. Many rearrangements of *Avrainvillea* species have occurred over time, such as proposal of synonymies (e.g. *A. sordida* under *A. longicaulis* (Kützing) G.Murray & Boodle or *A. capituliformis* under *A. obscura*) or transfers to or from other genera (*e.g.*, *Udotea* or *Chlorodesmis*). This highlights the challenges of species identification and taxonomic determination in this genus due to species polymorphism and plasticity.

In this study, based on the extensive collections of the New Caledonia Herbarium (IRD, Noumea, New Caledonia; NOU), the Paris cryptogamic collection (MNHN, Paris, France; PC) and the University of French Polynesia Herbarium (Faa'a, Tahiti, FP; UPF), we (i) reassess *Avrainvillea* species diversity based on molecular species delimitation approaches and morpho-anatomical observations and (ii) reconstruct a multilocus phylogeny (*tuf*A, *rbc*L and 18S rDNA) to investigate relationships between species and confirm the monophyly of the genus and its position within the Dichotomosiphonaceae.

MATERIAL AND METHODS

Sampling

Samples of *Avrainvillea* were collected from various localities worldwide using SCUBA or snorkelling. Sampling localities included the Atlantic (Lesser Antilles, Caribbean), Indian Ocean (Juan de Nova, Madagascar, Mayotte, Reunion Island), and the Pacific Ocean (Indonesia, Papua New Guinea, New Caledonia, Vanuatu, Solomon Islands, Fiji, Tuvalu, French Polynesia) (Fig. 1; Table S1). Subsamples of fresh specimens were preserved in 95% ethanol and/or silica gel for DNA extractions and in a 5% formaldehyde solution in seawater for later morpho-anatomical observations. Vouchers were pressed and dried on herbarium sheets and housed in the collections of NOU, UPF and PC (herbarium abbreviations follow Thiers (2022, continuously updated).

Extraction, PCR Amplification and Sequencing

DNA extractions were done using a Qiagen Plant mini-Kit (Qiagen Inc., Valencia, CA, USA) or a cetyl trimethyl ammonium bromide (CTAB) protocol. Two chloroplast markers, *tuf*A and *rbc*L, and the 18S rDNA nuclear gene were chosen for this study, following previous studies on neighbouring families, such as the Udoteaceae, Rhipiliaceae, or Caulerpaceae (Lagourgue & Payri, 2020, 2021; Sauvage *et al.*, 2013). The primers HtufA-F/HtufA-R were used for *tuf*A, rbcL1/ rbcL223R, 204F/ 400R, 381F/rbcL2, and rbcL-712F/rbcL-1391R for *rbc*L and TW3F, SSH011R, SSH09F, and H1R for 18S rDNA (Table S2). The Polymerase Chain Reaction (PCR) was conducted in a final volume of 25 µl, including 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, California, USA), 1 µl of each relevant F and R primer (10 µM), 0.75 µl of dimethylsulfoxide (DMSO), 1 µl of bovine serum albumin (BSA), 2.5 µl of DNA, and 6.25 µl of ultra-pure water. PCR programmes follow Pierce *et al.* (2006) and Lagourgue *et al.* (2018). PCR products were sent to Genoscreen (Lille, FRANCE) for Sanger sequencing, and the sequences obtained were edited in Geneious version 7.1.9 (http://www.geneious.com, Kearse *et al.*, 2012). All sequences available on GenBank for the genus were also added to our dataset. Datasets were aligned separately for each marker using the MUSCLE algorithm.

Species Delimitation Analyses

For the molecular species delimitation, phylogenetic trees were inferred from *tuf*A and *rbc*L separately, with either the whole set of sequences or with only the unique haplotypes by using the Collapsetypes v4.6 perl script (Chesters *et al.*, 2013). Three species delimitation methods were used to assess the diversity of the genus *Avrainvillea*: the Assemble Species by Automatic Partitioning (ASAP; Puillandre *et al.*, 2021), the General Mixed Yule Coalescent (GMYC) (Pons *et al.*, 2006) and the Poisson tree process model (PTP, Zhang *et al.*, 2013).

Maximum Likelihood (ML) trees were reconstructed using RAxML (Stamatakis, 2014) through the CIPRES web portal (Miller *et al.*, 2010). ML analyses were launched using the “rapid bootstrapping and search for the best scoring ML tree” algorithm, with the GTR + G evolutionary model and 1,000 bootstrap (bs) iterations (Stamatakis *et al.*, 2008). Bayesian ultrametric trees were estimated using BEAST v.2.6.3 (Drummond *et al.*, 2012). Two independent analyses of 20 and 30 million generations were run for *tuf*A and *rbc*L, respectively, and sampled every 1,000 generations. A relaxed lognormal molecular clock (Drummond *et al.*, 2006) with a coalescent constant size tree prior was used. The run outputs were checked on Tracer v.1.5 (Rambaut & Drummond, 2007) for convergence of the Markov-

Chain Monte Carlo (MCMC) and values of the effective sample size (ESS >200). After removing 10% generations as burn-in, Log Combiner was used to combine the trees. We calculated the Maximum Clade Credibility Tree (MCCT) with Tree Annotator (available on the Beast package).

The ASAP program was also launched directly on gene alignments, through the ASAP website (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html#), with the simple distance model. The PTP method was conducted through the Exelixis Lab webserver (http://sco.h-its.org/exelixis/web/software/PTP/index.html) on ML rooted trees and run for 500,000 generations, sampling every 1,000 generations and without considering the outgroups. GMYC was performed using the package "splits" in R (R Development Core Team, 2019), with the "single" method on Bayesian MCCTs.

Each method's partition results were called primary species hypotheses (PSHs). Secondary species hypotheses (SSHs) were defined by comparing and searching for congruence in the PSHs between methods and markers. When results were not congruent, we applied a majority rule, and the most prevalent PSH was selected as SSH (except for particular cases explained below). When this rule was not applicable or relevant, we generally preferred the most conservative partition in order not to multiply the number of assumptions of unverified species. For a more practical and clearer presentation of the exploratory species delimitation results, the term "clade" refers to SSHs defined at the end of the process. Finally, molecular-based SSHs (i.e. molecular species) were confirmed using morpho-anatomical observations when results were congruent and, when possible, assigned species names or described as new species when necessary.

Morphological Observations

Morpho-anatomical observations were carried out on specimens of each SSHs defined above for species identification and/or determination of diagnostic characters. Fragments of the blade, stipe and holdfast preserved in formaldehyde or directly collected on herbarium specimens were observed and measured using an Axio Imager A2 microscope (ZEISS, Oberkochen, Germany) fitted with a Canon EOS 100D camera (Canon, Tokyo, Japan). Morpho-anatomical observations were mainly based on the monographies of Olsen-Stojkovich (1985), Gepp & Gepp (1911) and Littler & Littler (1992), as well as occasional species descriptions available in the literature (e.g. Yamada, 1932; Trono, 1971; Kraft & Olsen-Stojkovich, 1985). Characters of interest included habit types (individual or cluster), the external morphology of the thallus (with or without stipe; blade, stipe and holdfast aspect, etc.), siphon shape (cylindrical, tortuous, torulose, moniliform), the colour of the different parts of the thallus, and the shape of the dichotomies and apices. Measurements included the width and height of the thallus, blade, stipe, and holdfast, the diameter of the siphons (cortex and medulla of the blade and stipe) and the diameter of the rhizoids.

Phylogenetic Reconstruction

We build a concatenated multi-locus matrix using, specimens for which at least two sequences of the three markers were available (*tuf*A, 18S rDNA, and, when possible, the entire *rbc*L sequence) (Table S1). The dataset was analysed with Partition Finder v1.1.0 (Lanfear *et al.*, 2012) using the Akaike information criterion (AIC) to determine the best partition schemes and the most suitable evolutionary models. Sequences of *Bryopsis plumosa* (Hudson) C.Agardh, *Codium taylorii* P.C.Silva, *C. duthieae* P.C.Silva, *C. platylobium* Areschoug, *Caulerpa taxifolia* (M.Vahl) C.Agardh, *C. sertularioides* (S.G.Gmelin) M.Howe, and *C. verticillata* J.Agardh were added as outgroups of the Dichotomosiphonaceae. We also included *Dichotomosiphon* and *Cladocephalus* GenBank sequences to better assess the position of *Avrainvillea* within the family. Bayesian Inference (BI) was performed using MrBayes v.3.2 (Ronquist & Huelsenbeck, 2003) through the CIPRES portal on the partitioned multilocus matrix with the following evolutionary models: GTR+G, GTR+I+G, GTR+G for *tuf*A, HKY+I, GTR+I+G, GTR+I, GTR+G, JC+G, GTR+G, for *rbc*L and GTR+I+G for 18S rDNA (N.B.: PartitionFinder favoured a six-partition scheme for *rbc*L (i.e. by fragments and codon positions) probably due to the heterogeneity of the available sequences between the different samples. The analysis was carried out in two independent runs of four incrementally heated chains of 40 million generations, sampled every 1,000 generations, with a 10% burn in. After checking the run outputs with Tracer v.1.5 (Rambaut & Drummond, 2007), we computed the consensus topology and posterior probabilities. ML trees were reconstructed using RAxML (Stamatakis, 2014) on the partitioned dataset (i.e. 9 partitions for the chloroplast *tuf*A + *rbc*L tree; 10 partitions for the multilocus *tuf*A + *rbc*L +18S tree), with the "Multiparametric bootstrapping" algorithm, the GTR + G evolutionary model, and 1,000 bootstrap (bs) iterations (Stamatakis *et al.*, 2008).

RESULTS

Species Delimitation Results and Species Identification

DNA data were successfully obtained for 183 *Avrainvillea* samples, including 156 *tuf*A sequences, 51 sequences for the *rbc*L5' fragment, 106 sequences for the *rbc*L3' fragment and 21 18S rDNA sequences. A total of 170 sequences were submitted to GenBank with accession numbers OM313015 to OM313096 for *tuf*A, OM313097 to OM313164 for *rbc*L and OM313165 to OM313184 for 18S rDNA. The collapsed datasets with unique haplotypes consisted of 80 sequences for *tuf*A and 79 for *rbc*L. Species delimitation results for each chloroplast marker (Figs S1 to S4) are presented in Fig. 2 next to the ML tree built on the concatenated (*tuf*A + *rbc*L) dataset. For the *tuf*A dataset, GMYC delimited 24 PSHs, while ASAP resulted in 26 PSHs and hPTP in 20 PSHs (Figs 2 and S1). For this marker, a total of 14 PSHs were common to the three methods, and differences in delimited partitions between methods were observed for 6 clades (clades 9, 10, 12, 19, 20 and 23). For the *rbc*L dataset, 25 PSHs were delimited by GMYC, while ASAP and hPTP resulted in 33 PSHs (Figs 2 and S3). For this marker, 11 PSHs were common to the three methods, while differences in partitions between methods were observed for 16 clades (clades 3-5, 7, 9, 10, 13, 14, 17, 19-24 and 26).

When comparing results between markers and methods, seven PSHs (1, 2, 6, 8, 11, 12, 15) were congruent and were defined as SSHS. Differences found for the other clades are detailed in Table S3, along with the resolution process and the species assignment decision.

Interspecific distances between delimitated *Avrainvillea* species were also analysed to aid in conflict resolution. Interspecific similarity values ranged from 91.39 to 99.82% for the *tuf*A marker and from 93.50 to 99.84% for the *rbc*L marker (Table S4). For the *rbc*L marker, the highest similarity value was found with *A. spongiosa* and *A. clavatiramea*. For the *tuf*A marker, the highest similarity value was found between *A. pacifica* and *A. mollis* (also high for *rbc*L, 99.67%). Among the species sharing the highest interspecific similarity values are species complexes. In contrast, *A. spongiosa* and *A. minima* showed the highest interspecific divergence for both markers. Finally, in our systematic integrative approach to the genus *Avrainvillea*, we assigned 15 clades to current species names (Fig. 2) and 7 clades (8, 15, 17, 18, 19, 21, 23) were considered as new species and are described below. For some cases requiring the sequencing of types for a better determination, we have created species complexes, namely: "*A. amadelpha* complex", "A. *erecta-obscura* complex", "*A. lacerata complex*" and "*A. mazei-nigricans* complex". Morpho-anatomical observations for all clades are reported in Table S5, with the descriptions of currently accepted species.

Phylogeny of the Dichotomosiphonaceae

A total of 92 taxa were included in our concatenated multilocus matrix (3,490 base pairs (bp): *tufA*: 838 bp; *rbc*L: 1,342 bp and 18S rDNA: 1,310 bp) and represented one species of *Cladocephalus* (*C. luteofuscus*), one of *Dichotomosiphon* (*D. tuberosus*), 18 *Avrainvillea* species, and four species complexes. In both BI and ML phylogenetic reconstructions, *Dichotomosiphon* was the first diverging genus among the family (Fig. 3). The representatives of *Avrainvillea* were distributed into three fully supported groups (A, B, C; Fig. 3) and appeared as a non-monophyletic group since *Cladecephalus* was either a sister clade of group A in the BI tree or of group B in the ML tree, with low support values (Figs 3 and S6).

All the species or complexes delimited in this study appeared monophyletic, including 13 highly supported in both phylogenetic trees (bs ≥ 95; PP ≥ 0.98), and five strongly supported in the BI tree (bs <95; PP ≥ 0.98). The two complexes *A. erecta-obscura* and *A. mazei-nigricans* were moderately to not supported (bs <95; PP <0.98) and three species were represented by singletons (*A. geppiorum, A. clavatiramea* and *A. spongiosa*; *rbc*L and *tuf*A sequences could not be obtained for *A. geppiorum* and *A. clavatiramea*, respectively, and are not represented in corresponding gene-trees).

Maximum Likelihood and Bayesian analyses resulted in similar species phylogenetic relationships, except for (i) *A. gracillima*, which was sister of *A. kanakiensis* in the ML tree and of a group including *A. elliottii, A. hollenbergii, A. calathina* and *A. digitata* in the BI tree (Fig. S6); (ii) *A. obscura*, which diverged before *A. pyrochroma* sp. nov. and *A. spongiosa* sp. nov. in the BI tree, but not in the ML tree; (iii) *A. pacifica*, which was sister of *A. mollis* sp. nov. and *A corticata* sp. nov. in the BI tree (Fig. S6), while in the ML tree, *A. corticata* sp. nov. was sister of *A. pacifica* and *A. mollis* sp. nov.

Finally, although *Avrainvillea clavatiramea* could not be confirmed by the species delimitation approach, we chose to maintain it as distinct based on the results of the multilocus analysis and morpho-anatomical observations that clearly set it apart from other SSH17 specimens identified as *A. spongiosa*.

Geographical Distribution of* Avrainvillea *Species

Among the 37 currently accepted species names (including those newly described here and excluding *A. rotumensis*), 29 species are restricted to one ocean basin (Table 1): 15 species are found exclusively in the Tropical Atlantic (with 7 included in this study), four in the Indian Ocean (two represented here) and 10 in the Pacific Ocean (9 represented) (Table 1). The distribution ranges of *Avrainvillea amadelpha, A. gracillima, A. lacerata* and *A. obscura* extend across the Indo-Pacific basin. The Indo-Pacific distribution of *A. erecta*, *A. ridley* and *A. riukiuensis* requires confirmation as the species limits are questioned. *Avrainvillea longicaulis* is assumed to be present in both the Atlantic and Indian oceans, but we could not verify this. No *Avrainvillea* species appeared to be cosmopolitan. Distribution maps of *Avrainvillea* species based on DNA data are available in Fig. S8.

Our extensive collection of *Avrainvillea* samples made it possible to update the distribution of some species and record new occurrences: the distribution range of *A. amadelpha* is completed with the Scattered Is. (Juan de Nova), Mayotte, Papua New Guinea, New Caledonia (Isle of Pines, Chesterfield Is., Entrecasteaux-Surprise Is.), and Tuvalu; *A. clavatiramea*, previously restricted to Australia, and *A. hollenbergii*, recorded from several Pacific Islands, are now also reported from New Caledonia (Chesterfield Is.); the Caribbean species *A. digitata* is confirmed in the Lesser Antilles with two new localities, Guadeloupe and Saint Vincent and the Grenadine (Union Is.); *A. lacerata* is newly reported from Mayotte, Madagascar and Vanuatu; *A. obscura* is newly reported from Madagascar and Mayotte, and *A. pacifica* is confirmed from Fiji and newly reported from Vanuatu; *A calathina* is no longer endemic to Lord Howe Is. as its distribution is extended to New Caledonia (Isle of Pines) and French Polynesia (Gambier).

Taxonomic description of new species

We propose eight new species that we describe as follows (specimens examined for each species are detailed in Supplementary Data S1):

Avrainvillea minima Lagourgue, Rousseau & Payri **sp. nov. (Figs 4-13) (SSH 8)**
Holotype: NOU203655. Collected by C.E. Payri, on 25 October 2016. Housed at NOU.
Type locality: Madagascar, Nosy Mitsio.
Description: Small thalli, 1.6–5.8 cm tall, composed of a very slender unbranched stipe (8 to 15 mm long; 3 to 4 mm wide) bearing single green (brown when dried) blade, 1-1.3 cm high, 1.9–3.5 cm wide (Figs 4-5), and a bulbous holdfast (small or more developed) (Fig. 6). Several stipitate blades arising on the same holdfast (Fig. 6). Blade not zonate, but with irregular zonation restricted to the margin, oblong, papery to spongy. Blade medullary siphons are thin, 10–20 (40) µm in diameter and cortical siphons 5–8 (10) µm in diameter; siphons tortuous or torulose with or without supradichotomal constrictions (Fig. 7); when present, constrictions slight elongated or asymmetrical (Figs 8-9); apices rounded, clavate or blunt. Stipe medullary siphons are 15–30 µm in diameter and cortical siphons 8-10 µm or less; stipe siphons torulose, sometimes with isolated constrictions (Figs 10-11); Rhizoids brown-orange,

cylindrical, tortuous or distorted (Figs 12-13); primary rhizoids 20–40 µm in diameter, secondary rhizoids 10–15 µm.

Notes: The species is composed of clusters of small stipitate blades, like *A. carteri* or *A. amadelpha* (2 to 4 uprights per holdfast in the species *vs.* several or numerous in the two others). The thallus of *A. minima* is smaller than *A. carteri*, and its blades are not zonate; its stipe is unbranched, slender and thinner than in the two aforementioned species. The siphons of *A. minima* are cylindrical or torulose (even in the medullary part) while they are somewhat cylindrical in the medulla and torulose in the cortex or near the apices in both other species. Finally, unlike *A. amadelpha*, there is no pseudo-cortex.

Etymology: In reference to the small size of the species.

Distribution: Madagascar (northwestern coast).

Habitat: On coarse detrital sediments or seagrass beds, mixed with other algae (*Caulerpa*, *Halimeda*), 20-15 m depth, isolated or in patches of several individuals.

Representative sequences : NOU203655 (*tuf*A: OM313045; *rbc*L: OM313120; 18S: OM313169), NOU203725 (*tuf*A: OM313091; *rbc*L: OM313160; 18S: OM313184), NOU203702 (*tuf*A: OM313041; *rbc*L: OM313116), NOU203672 (*tuf*A: OM313038; *rbc*L: OM313113), NOU203717 (*tuf*A & *rbc*L: LLAVR105-22), NOU203726 (*tuf*A: LLAVR108-22).

Avrainvillea corticata Lagourgue & Payri, **sp. nov. (Figs 14-26) (SSH 19)**

Holotype: NOU203638. Collected by C.E. Payri, on 23 October 2016. Housed at NOU.

Type locality: Madagascar, Nosy Hara.

Description: Thallus green (dark green to brown when dried), 11–15.5 cm tall, composed of a bulbous holdfast (3 cm long; 2.5 cm wide) with unbranched stipe (4.7–4.9 cm tall; 3–6 mm wide) and flabellate blade (3.8–7.5 cm high; 4.2–8.5 cm wide) (Figs 14-15). Sometimes, several individuals arise from the same holdfast (Fig. 15). Blade suborbicular, ovate, reniform, oblong with truncate base or cuneate, not zonate, papery to spongy, thick, with entire or lobed or lacerate margins. Stipe/blade junction continuous and indistinct. Blade siphons are 25–50 µm in diameter in the medullary part and 20 µm in the cortex; siphons moniliform (or slightly moniliform) (Fig. 16), brown or colourless with visible plastids (Figs 17-19); with isomorphic dichotomies and slight symmetrical supradichotomal constrictions (Figs 17-20); apices rounded or keel-shaped (Fig. 20). Stipe medullary siphons tortuous, with elongated supradichotomal constrictions, 40–60 µm in diameter; cortical siphons moniliform, 20–30 µm in diameter (Figs 21-22). In the cortical parts of both stipe and blade, siphons are orange or fulvous, with numerous close dichotomies (Figs 21-22); apices rounded, blunt or distorted and thin (8 µm); the network of branches forms a pseudo-cortex (Figs 23-24). Rhizoids brown/orange, black, moniliform or tortuous, 50–60 µm in diameter, tapering and becoming cylindrical towards the apices (to 10 µm in diameter) (Figs 25-26).

Notes: The species resembles *A. longicaulis* in its external morphology, colour, blade texture and siphon diameter. It differs from *A. longicaulis* in having siphons that are moniliform in the blade and moniliform or tortuous in the stipe. The rhizoids are also larger (50–60 µm for primary moniliforms rhizoids and 10 µm for secondary rhizoids *vs*. 5–10 µm in *A. longicaulis*). *Avrainvillea corticata* also resembles the solitary *A. mollis* in external habit and siphon aspect but it can have several uprights on same holdfast, the blade texture is papery to spongy *vs*. velvet-like and soft in *A. mollis,* and the siphon diameter is slightly smaller in *A. corticata* in the blade (25–45(55) µm *vs*. 40–60 µm in *A. mollis*), stipe (30–60 µm *vs*. 40–130 µm in *A. mollis*) and rhizoids (50–60 µm vs 40–90 µm in *A. mollis*); blade and stipe siphons form a pseudocortex in *A. corticata*. Finally, the species also looks like *A. pavonina* in external aspect and size of blade siphons but differs in blade texture (papery to spongy *vs*. velvet-like for *A. pavonina*), morphology of blade siphons (moniliform *vs*. torulose, cylindrical or tortuous in *A. pavonina*), the presence of a pseudocortex, and the smaller rhizoid diameter (50–60 µm *vs*. 60–110 µm in *A. pavonina*).

Etymology: In reference to the pseudo-cortex formed by the siphons in the cortical part of the blade and stipe.

Distribution: Madagascar, Mayotte, New Caledonia (Grande Terre).

Habitat: On coarse detrital sediments or muddy bottom, or seagrass beds, mixed with other algae (*Caulerpa*, *Halimeda*) and seagrass (e.g. *Halophila)*, 1–7 m depth; isolated or numerous individuals.

Representative sequences: NOU218895 (*tuf*A: OM313042; *rbc*L: OM313117), NOU203573 (*tuf*A: OM313070; *rbc*L: OM313140), NOU203670 (*tuf*A: OM313028; *rbc*L: OM313104; 18S: OM313166), NOU203635 (*tuf*A: LLAVR093-22), NOU203638 (*tuf*A: OM313035; *rbc*L: OM313111).

Avrainvillea mollis Lagourgue & Payri, **sp. nov. (Figs 27-43) (SSH 23)**

Holotype: NOU203077. Collected by C.E. Payri, on 12 October 2015. Housed at NOU.

Type locality: New Caledonia, Ouen Isle, Pûmbo Islet.

Description: Thallus green (brown when dried), 15.5-21 cm tall, composed of bulbous holdfast (3–10.3 cm tall; 2–2.5cm wide), with large flattened or cylindrical, unbranched stipitate (stipe 3.8–8 cm tall and 4–8 mm wide) flabelliform blade (5.5–8.5 cm tall and 8.5–9 cm wide) (Figs 27-28). Stipe/blade junction continuous. Blades are cuneate, ovate or oblong, sometimes lobed, with lacerate margins, felted to velutinous and smooth, not zonate. Blade siphons are moniliform, yellow or colourless with visible plastids (Fig. 29), 40–60 µm in diameter in medulla and 20–40 µm in the cortex; siphon dichotomies isomorphic with symmetrical supradichotomal constrictions (Fig. 30); apices rounded, clavate, keel-shaped or slightly blunt (Figs 31-33). Stipe siphons are 50–90(130) µm in diameter in the medulla and 25–40 µm in the cortex (some individuals have cortical siphons 40–80 µm in diameter); stipe siphons tortuous and distorted or cylindrical (Figs 34-35), with symmetrical slight supradichotomal constrictions above widened dichotomies (Figs 36-38), and rounded, blunt or bulbous apices. Primary rhizoids are 40–90 µm in diameter, distorted, tortuous or moniliform; secondary rhizoids are 10–20 µm and cylindrical, tortuous or slightly moniliform. Rhizoids are brown, with symmetrical slight constrictions; they taper towards the apices (e.g. from 50 to 10 µm) and become increasingly less moniliform (Figs 39-41); apices are rounded or blunt; some rhizoids terminate in expanded pad apices (Figs 42-43).

Notes: The species resembles *A. longicaulis* in its external morphology but is composed of mostly moniliform siphons in the blade, and stipe siphon diameter are larger than those of *A. longicaulis* (40–130 µm in the present species *vs.* 38–46 µm in *A. longicaulis*), as are the rhizoids (40–90 µm *vs.* 5–10 µm in *A. longicaulis*). *Avrainvillea mollis* is also morphologically close to *A. corticata* (see description of the latter for differences between the two). *Avrainvillea mollis* can also be confused with *A. pavonina* in the field due to similar thallus sizes and blade texture. However, *A. mollis* is differentiated from *A. pavonina* in having solitary thalli with entire blades (vs. the numerous stipitate blades with deep indentations of *A. pavonina*. *Avrainvillea mollis* has moniliform blade siphons whereas they are torulose, cylindrical or tortuous in *A. pavonina*. Siphons of *A. mollis* are of larger diameter in both the blade (40–60 µm *vs*. 20–50 µm in *A. pavonina*), and stipe (40–130 µm *vs*. 30–90 µm in *A. pavonina*).

Etymology: In reference to its soft and silky appearance.

Distribution: New Caledonia (Grande Terre).

Habitat: Growing solitary or in small group of individuals on sandy bottoms in lagoon area and channel down to 15 m; mixed with other algae (e.g. *Halimeda, Caulerpa, Udotea*) and solitary corals (e.g. *Heteropsammia, Heterocyatus*).

Representative sequences: NOU203077 (*tuf*A: OM313040; *rbc*L: OM313115), NOU203078 (*tuf*A & *rbc*L: LLAVR024-22), NOU203091(*tuf*A: LLAVR025-22), NOU203092 (*tuf*A & *rbc*L: LLAVR026-22), NOU203841 (*tuf*A: OM313026; *rbc*L: OM313102; 18S: OM313165), NOU203839 (*tuf*A: OM313037), NOU203840 (*tuf*A: LLAVR127-22), NOU218900 (*tuf*A & *rbc*L: LLAVR001-22), NOU218901 (*tuf*A & *rbc*L: LLAVR129-22), NOU218891 (*tuf*A & *rbc*L: LLAVR112-22).

Avrainvillea pavonina Lagourgue & Payri, **sp. nov. (Figs 44-54) (SSH 15)**

Holotype: NOU213732. Collected by C.E. Payri, on 10 May 2007. Housed at NOU.
Type locality: Fiji, Vanua Levu, Kakaulau reef.
Description: Thallus dark-green (brown when dried), 10 to 29.5 cm tall, composed of a bulbous holdfast from which one or several stipitate blades arise (Fig. 44); some individuals have remnants of former dead individuals (Fig. 45). Stipe (1.6–5 cm tall; 4–9 mm wide) is unbranched, large and flattened. Stipe/ blade junction continuous. Blade flabellate and cuneate, thick and velvet-like or velutinous, becoming thinner and papery at the margin; 6–10.5 cm tall, 3–11 cm wide; lacerate, eroded or smooth growing margins; lobed indentations deep into the blade, making a heart-shaped appearance when bilobed (Figs 44-45). Blade medullary siphons are 30–50 µm in diameter and 20 µm in the cortex. Blade siphons, orange or yellow, cylindrical to torulose and tortuous (Fig. 46); isomorphic and triangle-shaped dichotomies with symmetrical constrictions (Figs 47-48); apices rounded or blunt, some hook-shaped and torulose. Stipe medullary siphons 30–90 µm in diameter and torulose; cortical stipe siphons 10–20 µm in diameter, cylindrical or torulose (Figs 51-52), with blunt or pointed apices. Primary rhizoids (60–110 µm in diameter) torulose with isolated constrictions and taper towards the apices; secondary rhizoids (10-20 µm in diameter) cylindrical with blunt or pointed apices (Figs 53-54).
Notes: The species looks like *A. longicaulis* in its thallus habit, blade shape (although it differs in its indentations) and blade and stipe siphon diameter. But it has a velvet-like and velutinous rather than coarse and granular blade, the blade margins are eroded, blade and stipe siphons are cylindrical to torulose but not moniliform (present in *A. longicaulis)* and the rhizoids differ in size (60–110 µm tapering 10–20 µm *vs.* 5–10 µm), colour (orange *vs*. hyaline) and apices (blunt or tortuous *vs*. expanded pad or bulbous apices). *Avrainvillea pavonina* also resembles *A. corticata* in its external aspect and size of blade siphons as well as

resembling *A. mollis* in thallus size and blade texture. Differences are further detailed in the description of the two species.

Etymology: From the Latin 'pavo', meaning peacock, in reference to its frond resembling a peacock tail.

Distribution: Fiji, Micronesia.

Habitat: Growing on sandy bottom in lagoon area and channel, down to 22 m; mixed with other algae (e.g., *Halimeda*, *Caulerpa*, *Udotea*).

Representative sequences: NOU213732 (*tuf*A: OM313093; this study), ARS08608 (*tuf*A: MF872103; *rbc*L: MF872078; Wade *et al.*, 2018).

Avrainvillea pyrochroma Lagourgue & Payri, **sp. nov. (Figs 55-64) (SSH 5)**

Holotype: NOU203688. Collected by C.E. Payri, on 27 October 2016. Housed at NOU.

Type locality: Madagascar, Nosy Lava.

Description: Thallus small, 6.7–9.5 cm tall, composed of a long and bulbous immersive holdfast (3–5.5 cm tall; 0.7–1.3 cm wide), subterete, unbranched, short and very slender stipe (6–8 mm tall; 1.5–3 mm wide) and a single blade (2.7–2.8 cm tall; 3.5–5.1 cm wide) (Fig. 55). Blade green and of different colours once dried, from yellowish-orange to red (Fig. 56); blade reniform or half-moon-shaped, with lacerate margins and visible siphons, zonate, membranous and friable when dried. Blade siphons yellow and cylindrical (Fig. 57), with clavate, rounded or swollen apices (Fig. 58); isomorphic dichotomies with symmetrical constrictions (Figs 59-60); diameter of blade siphons 30–40 µm in the medulla and 10–20 µm in the cortex. Stipe siphons cylindrical or torulose (Figs 61-62); 30–80 µm in diameter in the medulla and 10–25 µm in the cortex. Rhizoids are red/brown or hyaline, moniliform, torulose or cylindrical (Figs 63-64); primary rhizoids measure 40–60 µm in diameter and secondary ones are 10–20 µm.

Notes: The species resembles *A. erecta* with its reniform blade, yellow to orange siphons, and in their diameter, however it is stipitate with a very slender cylindrical stipe and blades are zonate with brown to red/orange margin when dried; there is no pseudo-cortex; only known from Madagascar.

Etymology: In reference to the fiery colours of the blades as they dry.

Distribution: Madagascar.

Habitat: Growing on sandy bottom in lagoon area and channel or seagrass beds, down to 11 m; mixed with other algae (e.g. *Halimeda*, *Caulerpa*, *Udotea*) and seagrass (e.g. *Halophila, Thalassodendron*).

Representative sequences: NOU203671 (*tuf*A: LLAVR097-22), NOU203685 (*tuf*A: OM313062; *rbc*L: OM313134; 18S: OM313173), NOU203686 (*tuf*A: OM313094; *rbc*L: OM313162), NOU203687 (*tuf*A: OM313095; *rbc*L: OM313163), NOU203688 (*tuf*A: OM313071; *rbc*L: OM313141; 18S: OM313175).

Avrainvillea laciniata Lagourgue & Payri, **sp. nov. (Figs 65-84) (SSH 18)**

Holotype: NOU203381. Collected by C.E. Payri, on 20 August 2014. Housed at NOU.

Type locality: Papua New Guinea, New Ireland, Kavieng.

Description: Small thallus, 3.9–6.8 cm tall, composed of a long and bulbous immersive holdfast (1.3–3.7 cm tall; 0.6–1.3 cm wide), a terete to subterete, unbranched, short and very slender stipe (2–12 mm tall; 0.1.5–3 mm wide) and a single blade (1.4–2.8 cm high; 2.7–4.1 cm wide) (Figs 65-66). Blade green and becoming pale green to yellowish when dried, flabellate, cuneate or reniform (for some individuals) (Fig. 67); margin lacerate or lobed (some samples with very lacerate margins, given a free-siphons aspect) (Fig. 67). Blade siphon diameter is 40–50 µm in the medulla and 15–30 µm in the cortex; siphons cylindrical (some torulose) (Figs 68-69), with isomorphic dichotomies with symmetrical constrictions and supradichotomal swelling (Figs 70-71); blade siphons brown or pale orange or uncoloured except at the apices where tips are brown to orange; apices rounded, swollen, slightly blunt or clavate (Figs 72-75). Stipe siphons torulose (Figs 76-78) and 30–55 µm in diameter in the medulla and 10–20 µm in the cortex. Primary rhizoids measure 45–60 µm in diameter and are brown/red or black, moniliform, tortuous or distorted; secondary rhizoids are 10–20 µm in diameter, hyaline, cylindrical or torulose (Figs 79-84).

Notes: The species resembles *A. erecta* as some specimens have reniform blades, but they are all stipitate with a slender cylindrical stipe. The species also resembles *Rhipilia andersonii* with coloured siphons (yellow/orange) and the presence of two ectomorphs, but Murray (1886) pointed out that *R. andersonii* corresponds to Cumming's samples, from which *A. erecta* was described and the former species was therefore considered synonymous with the latter.

Etymology: In reference to the shape of the blade cut thinly at the margin.

Distribution: Papua New Guinea (Kavieng, Madang).

Habitat: growing on sandy/muddy bottom in lagoon area down to 28 m; mixed with other algae (e.g. *Halimeda, Caulerpa, Udotea*).
Representative sequences: NOU203381 (*tuf*A: OM313079; *rbc*L: OM313150; 18S: OM313178), NOU203382 (*tuf*A: OM313017; *rbc*L: OM313098), NOU203383 (*tuf*A: OM313016; *rbc*L: OM313097), NOU203519 (*tuf*A: OM313020).

Avrainvillea spongiosa Lagourgue, Zubia & Payri, **sp. nov. (Figs 85-94) (SSH 17)**

Holotype: UPF4066. Collected by C.E. Payri, on 10 October 2008. Housed at UPF.
Type locality: French Polynesia, Society Islands, Moorea, Haapiti reef.
Description: Thallus (3.5–7.5 cm tall), dark green (brown when dried), with big bulbous and immersive holdfast (1.7–3 cm tall; 1.3–3.5 cm wide), sessile or with small, stout and flattened stipe (2–4 mm tall; 2 mm to 2 cm wide; when present) and a single blade (1.5–5 cm tall; 0.9–8.5 cm wide) (Figs 85-87). Blade flabellate, subrotundate or cuneate, or lobed; spongy to velutinous. Some individuals have a very reduced and rounded/ball-shaped blade with the holdfast representing 75% of the thallus. Blade siphons measure 50–60 (to 70–80) µm in diameter in the medulla and 20–30 µm in the cortex; blade siphons yellow/orange, cylindrical (Fig. 88) with larger and swollen rounded apices (Figs 89-90); isomorphic dichotomies isomorphic with deep symmetrical constrictions (Figs 91-92); isolated constrictions along the siphons (often near the apex) (Fig. 90); Some parts are full of plastids and still green. Stipe siphons torulose to tortuous (Figs 93-94) with rounded to swollen and bulbous apices, 30–50 µm in diameter in the medulla and 20 µm (up to 50 µm at swollen apices) in the cortex. Primary rhizoids are tortuous and distorted, and measure 30–50 µm in diameter while secondary rhizoids are 20 µm in diameter.
Notes: The species looks like *A. erecta* in its subsessile or shortly stipitate form, but its blades are cuneate, lobed or ball-shaped rather than reniform and they are never zonate. It likewise has yellow/orange or fulvous siphons, with rounded apices but in some specimens, siphon diameter was larger (70–80 µm) than in *A. erecta* (30–60 µm) and tapered towards the cortex. The species also resembles *A. obscura* based on siphon diameter (40–60 to 80 µm in medulla and 30 to 50 µm at the apices in the cortex in *A. obscura*) and some individuals have cuneate blades. Numerous specimens are sessile or subsessile, have yellow/orange siphons and rounded rather than clavate apices.
Etymology: In reference to the spongy texture of the blade.
Distribution: French Polynesia (Moorea), New Caledonia (Grande Terre).

Habitat: Growing in shallow sandy bottom in coarse sediment, isolated or in small groups of individuals on fringing reefs (Moorea), 1 m depth; mixed with other algae (mostly brown algae; e.g. *Padina, Dictyota*), and or in coral sand on the inner slope on the barrier reef (Tahiti, Faa'a; New Caledonia, Koumac), down to 5 m.

Representative sequences: UPF4066 (*tuf*A: OM313050; *rbc*L: OM313125), UPF4154 (*rbc*L: LLAVR163-22), NOU218887 (*tuf*A: OM313021), NOU218888 (*rbc*L: LLAVR014-22), NOU218890 (*rbc*L: OM313143).

Avrainvillea kanakiensis Lagourgue & Payri, **sp. nov. (Figs 95-103) (SSH 21)**

Holotype: NOU087408. Collected by C.E. Payri, on 05 April 2012. Housed at NOU.

Type locality: New Caledonia, Western Lagoon, Poya

Description: Thallus solitary or in clusters of several uprights on same holdfast, 4–7.5 cm tall, green yellowish to greyish green, sometimes yellow at the margin when dried (Figs 95-96). Blades (1–2.8 cm tall and 0.4–3.3 cm wide) oblong, reniform or cuneate, thin, membranaceous and almost diaphanous, with papery texture, inconspicuously zonate or without zonation; margin smooth, eroded or lacerate, thinner with visible siphons, sometimes with outgrowths. Stipe slender and cylindrical (1–2 mm wide; 1.5–2 cm tall). Rhizoidal mats, long or small (1–3 cm tall; 0.6–1 cm wide). Medullary blade siphons 10–18(30) µm, moniliform (Fig. 97) and 5-10 µm in the cortex, cylindrical, tortuous or torulose; siphons hyaline, with visible plastids in medulla; dichotomies with symmetrical supradichotomal constrictions elongated to deep (Fig. 98); apices rounded and swollen or torn away (Fig. 99). Stipe siphons cylindrical, tortuous, torulose to slightly moniliform, orange/brown or hyaline (Figs 100-101), with rounded or blunt apices, 20–40 µm in medulla and 10–20 µm in cortex. Primary rhizoids 20–40 (70) µm, pale orange to brown, deformed, large, flat, tortuous and with outgrowths (Fig. 102); secondary rhizoids cylindrical and thin, pale orange or hyaline, 8–10 µm in diameter (Fig. 103).

Notes: the species looks like *Avrainvillea riukiuensis* in its anatomical character but not in the habit (can be found in clusters whereas *A. riukiuensis* is solitary), in the sizes of the thallus, blade and stipe.

Etymology: The species was named in reference to the locality of its discovery (and for now only known distribution) in New Caledonia, called 'Kanaky' by the local Melanesian population.

Distribution: New Caledonia (Grande Terre; Isle of Pines; Entrecasteaux reefs (Surprise Islands)).

Habitat: Growing on hard substrata and often holdfasts firmly embedded in crevices of massive corals with only the clusters of blades apparent.

Representative sequences: NOU087408 (*tuf*A: OM313043; *rbc*L: OM313118), NOU218896 (*rbc*L: LLAVR118-22), NOU218897 (*tuf*A: OM313029; *rbc*L: OM313105); NOU203987 (*tuf*A: OM313066; *rbc*L: OM313137; 18S: OM313174).

We also propose the resurrection of *A. gracillima*, relegate *A. robustior* to a variety of *A. lacerata*, and synonymize *A. rotumensis* under *A. pacifica*, as follows:

***A. gracillima* Børgesen 1940 (Figs S9-19)** (SSH 13)

Holotype: Mauritius, between Gunner's Quoin and Flat Island, at a depth of 25 fathoms, 15/10 29, Th. M.

Emended description: Thallus small and solitary (2–5.7 cm tall), brownish to olive-green and dull, composed of a small disc of rhizoids (2–9 mm tall; 2–11 mm wide) attached to pieces of calcareous algae, a slender cylindrical terete and unbranched stipe (4–10 mm tall; 0.75–2 mm thick), and a single blade (1–2.9 cm tall; 1.1–3.8 cm wide) (Figs S9-10). Stipe/blade junction continuous. Blade oblong, reniform, or subcordate, with a cuneate base and broadly subentire, lacerate or fibrillous upper margins (Fig. S10); thin and membranaceous, with a rather gritty surface, firm below, of a looser consistency above; indistinct zonation near the upper margin of the frond. Blade siphons subcylindrical, often tortuous, moniliform and torulose (Figs S11-12), more or less firmly interwoven; yellowish-brownish to dark brown; medullary blade siphons 20–25 µm in diameter, rarely up to 30 µm below the dichotomies, and cortical siphons 5–10(15) µm; dichotomously divided, with or without supradichotomal constrictions; when present, constrictions are symmetrical (Fig. S13) or asymmetrical, and some are long-necked or elongated; apices rounded, blunt, bent or tortuous (Figs S14-15). Stipe siphons cylindrical to torulose (Figs S16-17), pale to dark brown or hyaline (cortical siphons), (20)30–40 µm in diameter in the medulla and 10–15(20) µm or less in the cortex; dichotomies unconstricted or with elongated and symmetrical constrictions (Fig. S18). Primary rhizoids 40–60 µm in diameter, orange and distorted; secondary rhizoids

10 µm or less in diameter, hyaline, torulose or cylindrical, forming a network (Fig. S19); rhizoid dichotomies constricted or unconstricted; rhizoids apices blunt or tortuous.

Notes: This species differs from *A. riukiuensis* by the smaller sizes of its thallus (2–5.7 cm tall *vs*. up to 17 cm high and 16 cm broad in *A. riukiuensis*), blade (and 0.75–2 wide *vs*. 16 cm broad) and stipe (4–10 mm long and 0.75–2 mm thick *vs*. up to 5 cm long, 6 mm in diam.) and the larger siphon diameter (20–30 µm *vs*. 10–18(20) µm).

Distribution: Mauritius (Børgesen, 1940), Reunion Island (this study), Madagascar (this study); Taiwan (Shao, 2003-2014; to be verified), New Caledonia (Chesterfield Islands; this study).

Habitat: Growing on hard substratum or dead corals; mixed with other benthic organisms in spurs and grooves area on outer slope, down to 35 m, locally abundant.

Representative sequences: NOU203318 (*tuf*A: OM313096; *rbc*L*:* OM313164), NOU203319 (*tuf*A: OM313032), NOU203335 (*tuf*A: OM313092; *rbc*L: OM313161), NOU204152 (*tuf*A: LLAVR003-22), NOU203472 (*rbc*L: OM313146).

Avrainvillea lacerata **var.** ***robustior*** **of Gepp & Gepp 1911 (Figs S20-27)**

Emended description: Plant green to greenish brown, up to 10 cm. high, forming compact clump of shortly stipitate blades (Figs S20-21). Small rhizoidal system (8–18 mm tall; 1.2–2 cm wide), stipes short and slender, almost indistinct (3–10 mm long; 1.5–3 mm wide), and blades cuneate, oblong to rotund, with truncate or cordate base, sometimes intersecting one another (2.2–3.8 cm tall; 2–4.8 cm wide) (Fig. S21). Blade thin, slightly zonate, with entire, eroded, or lacerate and fringed margins. Blade siphons cylindrical, pale brown or yellowish-brown, 20–30 µm in diameter in medulla; cortical siphons frequently irregularly torulose and tortuous and colourless (Fig. S22), 10 µm in cortex and tapering to about 5–6 µm at apices; dichotomies with distinctly long-necked constrictions and supradichotomal swelling (Figs S23-24); apices rounded, tortuous or torulose. Stipe siphons cylindrical to torulose (Fig. S25), brown, orange, or colourless, 30–40 µm in diameter in medulla, 10–25 µm in cortex; dichotomies and apices same as blade siphons. Primary rhizoids torulose and tortuous, pale orange to dark brown, 30–40 µm (Figs S26-27); secondary rhizoids cylindrical, torulose to distorted hyaline, 10–20 µm; elongated/long-necked constrictions when present above dichotomies; rhizoids apices rounded or with expanded pads.

Notes: The whole plant is stouter and more luxuriant than f. *typica*. Supradichotomal constrictions are more long-necked than in f. *typica*.

Synonym: *Avrainvillea amadelpha* (Montagne) A.Gepp & E.S.Gepp *fide* Oslen-Stojkovich 1985.

Distribution: Singapore (Gepp & Gepp, 1911), Madagascar (This study).

Habitat: Growing on coarse sandy bottom mixed with other algae and benthic organisms. Forming clumps of densely packed fronds attached by short and thick stalks.

Representative sequences: NOU203628 (*tuf*A: OM313080; *rbc*L: OM313151, 18S: OM313179), NOU203629 (*tuf*A & *rbc*L: LLAVR089-22), NOU203632 (*tuf*A: LLAVR090-22), NOU203633 (*tuf*A: LLAVR091-22), NOU203634 (*tuf*A: LLAVR092-22).

Avrainvillea pacifica A.Gepp & E.S.Gepp 1911 (Figs S28-36) (SSH 20)

Type locality: Fualope, Ellice Island.

Holotype: A.14, David, Funafuti Expedition, 16/09/1898 (BM000685721, Natural History Museum).

Heterotypic synonym: *A. rotumensis* A.D.R.N'Yeurt, D.S.Littler & Littler, 1996

Emended description: Plant brownish or dark olive-green (pale brown when drying), up to 18 cm tall, consisting of solitary fronds or 2 or more stipitate fronds arising from a thickened base (1 to 4 cm tall; 1.5 to 5.5 cm wide) (Figs S28-29); stipes unbranched (or seldom branched), cylindrical, and stout (up to 12 cm long; up to 4 cm thick), bearing a cordato-semirotundate or peltate blade (1.4–5.6 cm long, 4–9 cm wide) (Figs S28-29). Blade with or without zonation, smooth and velvet-like, rather thick (3–4 mm; not membranaceous) and with margin entire. Blade siphons thin, markedly torulose to moniliform or tortuous, becoming cylindrical towards the apices (Figs S30-31); colourless to light-brown or light-orange; 20–70 µm in medulla, tapering to about 6–20 µm at their apices, and 8–30 µm in the cortex; dichotomies wide-angled (Fig. S32), with or without constrictions (more or less deep when present); apices rounded or blunt, often tortuous or hooked, not interwoven into a pseudo-cortex, but easily teased asunder. Stipe medullary siphons moniliform (Figs S33-34) and pale brown/orange, 40–80 µm in diameter; cortical siphons cylindrical, tortuous or moniliform (Fig. S34), and hyaline, 8–20 µm in diameter; stipe siphons dichotomously divided, with or without constrictions and with rounded apices. Rhizoids are 50–60 µm in diameter and tapering to 10–20 µm; rhizoids moniliform, tortuous and rather cylindrical towards apices, with or without supradichotomal constrictions, and blunt apices (Figs S35-36).

Distribution: Pacific Ocean: Federated States of Micronesia (Lobban & Tsuda 2003, Tsuda 2006, Lobban & Tsuda 2003, to be verified), French Polynesia (Gepp & Gepp, 1911; Payri & N'Yeurt 1999), Tuvalu (Gepp & Gepp, 1911; this study), Fiji (N'Yeurt *et al.*, 1996; this study), Vanuatu (this study).

Habitat: Growing on hard substratum in coral structures mixed with other benthic organisms, on shallow reefs or deeper part of barrier reefs, pass edges and outer slopes, 10 m depth.

Representative sequences: NOU203450 (*tuf*A: OM313072; *rbc*L*:* OM313142), NOU203445 (*tuf*A: OM313064; *rbc*L*:* OM313136), NOU213748 (*tuf*A: OM313022), NOU213742 (*tuf*A: OM313015).

We propose the following dichotomous species key based on morpho-anatomical characters only. Varieties and forms are not included. The species that we have studied morpho-anatomically are indicated in bold, while new species proposed above are indicated by an asterisk.

1. Blades digitate (finger-like) or somewhat clavate (club-shaped)........***A. digitata***

1. Blades flattened, peltate (umbrella-shaped) or expanded 2

    2. Blades thickened and peltate or expanded................................*A. rawsonii*

    2. Blades flattened and paddle-shaped..3

3. Blades clustered (5-8) atop stipes..*A. fenicalli*

3. Blades borne singly on stipes..4

    4. Holdfast rhizomatous, submerged 5

    4. Holdfast mat-like or bulbous, emergent 6

5. Stipe long, 6–16 cm, slender..24

5. Stipe short, 0.5–5 cm, often stout or almost lacking..........................31

    6. Thalli forming gregarious carpets, stipes branched several times . 7

    6. Thalli discrete, stipes mostly unbranched, or branched once or twice near the base forming a cluster of up to four blades 13

7. Blade funnel-shaped..*A. cyathiformis*

7. Blade fan-shaped 8

8. Blade thin and papery 9

8. Blade thick and spongy...10

9. Blade margin lacerate ....***A. lacerata***

9. Blade margin lobed or smooth ....***A. kanakiensis****

10. Pseudocortex present 11

10. Pseudocortex absent..*A. mazei*

11. Non-zonate blade, surface siphons tapering to 5–7 µm ....*A. carteri*

11. Distinctly zonate blade 12

12. Blade siphons cylindrical, rounded or swollen apices, tapering to 12–15 µm. ***A. amadelpha***

12.Blade siphons torulose or cylindrical, secondary constrictions, apices clavate, tapering to 5–13 µm ....*A. xishaensis*

13. Blade siphon diameter <20 µm 14

13. Blade siphon diameter > 20 µm 15

14. Blade siphons mostly weakly torulose, secondary lobes arising from margin or face of blade ....***A. hollenbergii***

14. Blade siphons mostly cylindrical, no secondary lobes arising from margin or face of blade ....*A. riukiuensis*

15. Blades thick > 2mm...16

15. Blades thick ≤ 2mm...17

16. Blade subcuneate, 0.5–5 cm wide, plants to 5 cm tall, siphon diameter 28–38 µm, apices rounded or clavate ....*A. ridley*

16. Blade cuneate-obovate, 2–8 cm wide, plants to 12 cm tall, siphon diameter 19–28 µm, apices pointed ....*A. longicaulis*

17. Distal portion of blade with compact cortex 18

17. Distal portion of blade lacking compact cortex................................ 19

18. Blade not zonate, with shallowly cordate base, cortical siphons generally moniliform tapering to 8–12 µm in diameter ....*A. levis*

18. Blade distinctly zonate, subpeltate and strongly concave at the base, siphons torulose, occasionally cylindrical or moniliform, 12–20(25–30) µm in diameter in medulla and 5–8(12–16) µm in diameter in cortex...***A. calathina***

19. Blade > 10 cm tall 20

19. Blade < 10 cm tall 22

20. Margin lobed...21

20. Margin not lobed, subentire to fimbriate or slightly lacerate...*A. canariensis*

21. Margin with lobed indentations extending 1/3 into the blade, siphons with expanded, subclavate or obtuse apices ....***A. geppiorum***

21. Margin often smoothly lobed, but with indentations < 1/3 deep into the blade, siphons with rounded apices...*A. hayi*

22. Individual thallus 23

22. Thallus consisting of a cluster of two to several uprights from same holdfast…*A. minima*

23. Blade oblong, reniform or subcordate, surface a little gritty, blade siphons 20–30 µm in diameter in medulla and 5–15 µm in cortex...***A. gracillima***

23. Blade orbicular to slightly oblong, finely fibrous, blade siphon diameter of 32–48 µm in diameter medulla and cortex and 44–60 µm in growing margins..........*A. sylvearleae*

24. Blade cuneate or rhomboid, not zonate, growing margins eroded to slightly lacerate… 25

24. Blade markedly reniform, zonate, growing margins smooth...***A. asarifolia***

25. Blade olive, with siphons predominantly cylindrical in cortex, apices clavate ***A. clavatiramea***

25. Blade black, with siphons predominantly torulose or moniliform, apices rounded, swollen or blunt 26

26. Blade siphons moniliform..27

26 Blade siphons torulose or tortuous...***A. pavonina****

27. Blade siphons not tapered towards the surface of the frond, or few .***A. nigricans***

27. Blade siphons extremely tapered towards the surface of the fronds .28

28. Blade thin (1–2mm) ....*A. silvana*

28. Blade thick > 2mm...29

29. Siphon diameter 30–60 µm, siphons abruptly tapering to form a tight cortex ***A. corticata****

29. Siphon diameter 40–130 µm, tapering to form a loose cortex 30

30. Stipe siphons mainly moniliform, with symmetrical and deep constrictions (some lighter) .... ***A. fulva***

30. Stipe siphons mainly tortuous, with widened dichotomies and light symmetrical constrictions ....***A. mollis****

31. Blade split to stipe forming distinctive heart-like lobes...................***A. elliottii***

31. Blade reniform, cuneate, orbiculate, rhomboid or capitular.............32

32. Siphon diameter > 28 µm, dichotomies deeply constricted.....33

32. Siphon diameter < 28 µm, dichotomies shallowly constricted 37

33. Blade reniform, faintly zonate, siphons bright yellow, blade siphons 30–60 µm in diameter in medulla… 34

33. Blade cuneate or cuneate-rotundate, seldom to not zonate, siphons coloured, blade medullary siphons > 40 µm in diameter...35

34. Subsessile or shortly stipitate, siphons not tapering, rounded apices *A. erecta*

34. Very slender cylindrical stipe (6–8 mm tall), blade brown to red/orange when dried, siphons tapering towards the apices, clavate or rounded apices ....***A. pyrochroma****

35. Subsessile or shortly stipitate, lobed, not zonate, blade siphons yellow/orange or fulvous, 50–60 (to 70–80) µm in diameter, rounded apices ....***A. spongiosa****

35. Distinctly stipitate 36

36. Short and stout stipes, thick blades with lightly lacerate margins, seldom zonate, blade medullary siphons up to 65 µm in diameter, olive to light brown, clavate apices ***A. obscura***

36. Very slender cylindrical stipe, thin blade with markedly lacerate margins, not zonate, becoming grey when dried, blade medullary siphons up to 50 µm in diameter, siphons brown to pale orange, apices rounded, swollen, slightly blunt or clavate... ***A. laciniata****

37. Blade rhomboid or orbicular, thin and membranaceous, siphons predominantly cylindrical to weakly torulose, apices slightly tortuous, rounded… ....*A. gardineri*

37. Blade cordate-semirotundate or peltate, thick, siphons markedly torulose to moniliform, apices distinctly hooked ....***A pacifica***

DISCUSSION

Phylogeny of the family Dichotomosiphonaceae and diversity of the genus Avrainvillea

A total of 25 species of Dichotomosiphonaceae are represented in this study (i.e. 23 *Avrainvillea* species, one *Cladocephalus* species and one *Dichotomosiphon* species), with well-supported nodes (bs ≥ 95; PP ≥ 0.98) for most of them (20/25 for at least one reconstruction method). When comparing trees (single gene-trees, vs. chloroplast *tuf*A + *rbc*L tree vs. multilocus *tuf*A + *rbc*L + 18S tree), the combination of markers, especially from different genetic compartments, allows a better resolution of the topology with higher node support. As chloroplast markers are more variable, they allow for better resolution of phylogenetic relationships at the species level, while the more conserved 18S allows for deeper node resolution.

For the first time, our study includes DNA data and analyses of the phylogenetic relationships of the following nine *Avrainvillea* species: *A. asarifolia, A. clavatiramea, A. digitata, A. elliottii, A. fulva, A. gracillima, A. geppiorum, A. pacifica* and *A. obscura*. The significant sampling effort and the integrative systematics approach of our study revealed 8 new species, adding to the 29 current *Avrainvillea* species (excluding *A. rotumensis*): *A. laciniata* (Papua New Guinea), *A. minima* and *A. pyrochroma* (Madagascar), *A. mollis* and *A.*

kanakiensis (New Caledonia), *A. pavonina* (Fiji), *A. spongiosa* (Pacific) and *A. corticata* (Indo-Pacific). Despite our sampling efforts, 13 species remain unsequenced (*A. carteri, A. cyathiformis, A. canariensis, A. fenicalii, A. gardineri, A. hayi, A. levis, A. longicaulis, A. rawsonii, A. ridleii, A. silvana, A. sylvearleae* and *A. xishaensis*). Like other groups with complex systematics and a limited number of morpho-anatomical characters the variation of which is difficult to interpret as intra- or interspecific level [e.g. *Lobophora* (Vieira *et al.*, 2014), or Udoteaceae (Lagourgue & Payri, 2020)], it is impossible to avoid a profound revision of the definition of *Avrainvillea* species using molecular characters. However, there is very strong support for the interpretation of distances between *Avrainvillea* species as interspecific, with very high scores (> 99%) for some markers. This is similar to what was highlighted in other close genera, such as *Caulerpa* (82.7-99.7 % for *tuf*A and up to 99.6 % for *rbc*L; Kazi *et al.*, 2013), *Rhipilia* (up to 99.4% for *rbc*L and 98.9% for *tuf*A; Verbruggen & Schils, 2012) or *Codium* (up to 99.3% for *rbc*L and *tuf*A; Verbruggen *et al.*, 2007) and can reflect the variability in limits of resolution of molecular markers for closely related or cryptic species. Although *tuf*A has slightly higher divergence values and could be considered slightly more informative for species delineation, combining different molecular markers in species delineation approaches is still needed.

In terms of distribution ranges, 24 species of the 30 taxonomically accepted species appear restricted to a single ocean basin and no *Avrainvillea* species was cosmopolitan. This is similar to what has been shown for related families, such as the Udoteaceae and Rhipiliaceae (or Rhipileae tribe; Lagourgue & Payri, 2020, 2021), but contrasts with genera from other bryopsidalean families (e.g. *Caulerpa,* Belton *et al.*, 2019), or *Codium* (Verbruggen *et al.*, 2007). This may be partly due to the fact that *Avrainvillea,* despite its capacity for vegetative propagation, has less reproductive and dispersal capacity than these other genera (*e.g.*, no multiplication by fragmentation). From our data set, the Atlantic and Indo-Pacific species of *Avrainvillea* appear to be interspersed and occur within the three major clades (A, B, and C). A time-calibrated phylogeny and biogeographic analysis of *Avrainvillea* species will be needed to elucidate the spatial origin of the most recent common ancestor (MRCA) and to explore the spatial and temporal diversification dynamics and highlight the drivers that have played a role in the current distribution of the species.

Incidence of the placement of* Cladocephalus *within* Avrainvillea *species?

In the molecular phylogenetic trees inferred in this study, the genus *Avrainvillea* does not appear monophyletic, with *Cladocephalus* nesting among its species, which is congruent with the results of Wade *et al.* (2018). However, only one *Cladocephalus* species is represented here (*C. luteofuscus*; cf. Fig. S7), and we do not know the phylogenetic position of the type species (*C. scoparius*), nor of the third species, *C. excentricus*. Therefore, we cannot be sure that the position of *C. luteofuscus* represents the position of the genus, which does not allow us to draw a taxonomic conclusion.

In future studies, if the type species of *Cladocephalus* clusters with *C. luteofuscus*, it may be necessary to either split *Avrainvillea* into three genera or expand the definition of *Avrainvillea* to accommodate *Cladocephalus* species.

Species assignments that remain to be resolved

Throughout this study, we have been confronted with species identifications for which published data diverge from our own results and observations, or species boundaries that are still unclear to us. We summarize below the cases that require more data and specimens.

Based on morphological observations, SSH4 specimens were assigned to *A. obscura,* as they have cuneate (and not reniform) blades, short and stout stipes, rhizomatous holdfast, blade medullary siphon diameter between 40 and 65 µm and clavate apices. Some specimens have a capitulum of free siphons i.e. the second ectomorph of *A. obscura* (Table S5). These characters correspond to the diagnosis of *A. obscura* (Agardh, 1823; Agardh, 1887) and differentiates them from *A. erecta*. However, the distinction from *A. erecta* remains narrow and confusing (Table S5) which was pointed out by Gepp & Gepp (1911) when they transferred *Dichonema erectum* to *Avrainvillea erecta* and suggested its possible synonymy with *A. obscura*. In our multilocus concatenated phylogeny, sequences of *A. obscura* appeared closely related to the sequences of *A.* cf. *erecta* and *Avrainvillea* sp. of Wade *et al.* (2018). However, delimitation analyses resulted in two different hypotheses depending on the genetic marker. Interestingly, the type sequence of "*Chloroplegma papuanum"* (BM000561613), currently considered as a heterotypic synonym of *A. erecta*, was recovered either as the same or as a distinct species of *A. erecta* (Figs 2 and S3). These inconsistent results may be explained by the insufficient phylogenetic signal provided by the short *rbc*L sequence of *C. papuanum.* Species delimitation between *A. erecta* and *C. papuanum* should be considered as unresolved. Therefore, although our molecular analyses confirm the close relationship between *A. obscura* and *A. erecta,* they cannot confirm their status and additional molecular markers are needed to decide whether they should be merged into a single species or if *A. erecta* should be split into two distinct species. For the time being, this case is considered as the "*A. erecta-obscura* complex".

The delimitation of *A. mazei* (sequences from Curtis *et al.*, 2008 and Verbruggen *et al.*, 2017) and *A. nigricans* differed according to markers and methods (either a single species or up to three different species hypotheses). Although we are probably close to the limit of resolution of both molecular markers, the close relationships between the sequences of *A. mazei* and *A. nigricans* questioned the morphological identification of these specimens. All our morpho-anatomical observations on our vouchers belonging to the clade matched with the description of *A. nigricans*. Ideally, meticulous morpho-anatomical observations of the specimens of Curtis *et al.* (2008) and Verbruggen *et al.* (2017) (especially for *A. mazei*) are needed to verify whether they represent two distinct species (genetically very close) or a single species. If they correspond to *A. mazei*, the validity of the species will be questioned, as it is morphologically close to *A. nigricans* but differs in the siphons (strikingly torulose and moniliform in *A. nigricans vs*. predominantly cylindrical in *A. mazei*) (Olsen-Stojkovich, 1985). More representatives of *A. mazei* (samples and sequences) are needed to conclude on this point, and for now, we consider this the "*A. mazei-nigricans* complex".

Avrainvillea amadelpha and *A. lacerata* are two genetically distinct but morphologically close species, although anatomical characters distinguish them (e.g. pseudo-cortex in *A. amadelpha*, larger siphon apices (10-20 µm) than in *A. lacerata (*10 µm maximum). They also both seem to represent species complexes. In this study, *A. amadelpha* was designated for SSH10. We agree with the *A. amadelpha* identification of Verlaque *et al.* (2017) and therefore disagree with the previous identification of Brostoff (1989), whose specimens are attributed to *A. lacerata* in our analyses. However, we have noticed an incongruence in the position of the Hawaiian specimens depending to the marker analysed: ARS08383 and ARS08510 cluster in SSH10 with *rbc*L while ARS08418 clusters in SSH9 with *tuf*A and in the concatenated phylogeny. In addition, depending on species delimitation methods and markers, some GenBank sequences assigned to *A. lacerata* group within SSH10 (e.g. HV0599, TS1543; Fig. S1). We therefore cannot conclude on their identity without morpho-anatomical analyses, and we believe it may not be *A. lacerata* (SSH 9 in this study).

Avrainvillea lacerata was represented by SSH9 and included *A. lacerata* f. *typica* and *A. lacerata* var. *robustior* (e.g. NOU203628 and NOU203629). The latter was designated as a synonym of *A. amadelpha* by Oslen-Stojkovich (1985). Here, the sequence available for the variety clustered within *A. lacerata* and we therefore propose to reinstate *Avrainvillea lacerata* var. *robustior* A.Gepp & E.S.Gepp. The two morphologies "f. *typica*" and "var. *robustior*" were recovered as two distinct entities with the majority of species delimitation methods (Figs 2, S1 and S3). While samples of "var. *robustior*" form a monophyletic group, this is not the case for *A. lacerata* f. *typica* (Figs 2, S5 and S6). Additional samples, especially of var. *robustior,* are needed to decide whether this variety should remain included in *A. lacerata* or elevated to the rank of species (i.e. "*A. robustior"*), as previously suggested by Gepp & Gepp (1911).

Considering the above and the taxonomic ambiguities raised by these two species, we proposed to consider the whole clade 10 as a single SSH attributed to the "*A. amadelpha* complex" and the whole clade 9 as a single SSH, assigned to the "*A. lacerata* complex". More data (samples and sequences, especially of the type specimens) is needed for complete confidence.

Finally, and because species identification in the genus *Avrainvillea* is particularly hampered by polymorphism or plasticity, we propose, for each species we have directly studied, a combination of morpho-anatomical character types that may be useful to facilitate their identification (Table 2).

Morphological clusters and future questions

The morphological clusters proposed by Olsen-Stojkovic (1985) to classify *Avrainvillea* species were only partially confirmed by our results (Table S6).

1/The "obscura" group (*A. obscura, A. erecta, A. clavatiramea, A. gardineri and A. elliottii*) was defined morphologically by Olsen-Stojkovich (1985) as "solitary thalli, well-developed stipes and extensive submerged rhizomatous holdfast, siphons structures predominantly cylindrical, but occasionally torulose, and apices rounded to strikingly clavate". We found that *A. obscura* and *A. erecta* are genetically closely related. *Avrainvillea clavatiramea* is phylogenetically close, as well as *A. pyrochroma* sp. nov., *A. laciniata* sp. nov. and *A. spongiosa* sp. nov., which also have morpho-anatomical features matching this cluster. However, these species did not form a monophyletic group. *Avrainvillea elliottii* clustered closer to *A. hollenbergii*. Sequences for *A. gardineri* were not available for this study.

2/ The "longicaulis" of Olsen-Stojkovich (1985) is composed of *A. longicaulis, A. pacifica, A. asarifolia, A. lacerata* and *A. amadelpha* and characterized by "well-develop branched stipes, emergent holdfasts, cylindrical, torulose and tortuous siphons". We had no representative of *A. longicaulis.* We found *A. lacerata* closely related to *A. amadelpha*. *Avrainvillea asarifolia* did not fall within this group but between the "obscura" and "nigricans" groups. *Avrainvillea pacifica* appeared closer to species with moniliform siphons (*A. fulva, A. corticata* sp. nov. *and A. mollis* sp. nov.) than of *A. lacerata/A. amadelpha.*

3/ The “nigricans” group, composed of *A. nigricans, A. mazei* and *A. rawsonii*, was characterized by dark spongiose thalli occurring in patches, holdfasts consisting of extensive emergent mats, siphons predominantly moniliform (Olsen-Stojkovich, 1985). Here, we found *A. nigricans* closely related to *A. mazei* (GenBank accessions which we could not check morphologically) and *A. rawsonii* could not be included. Morphologically, the species composing our group B also match the descriptive characters of the “nigricans” group, but they did not appear closely related phylogenetically.

4/ Finally, *A. hollenbergii* was not phylogenetically close to *A. riukiuensis*, although this was suggested by Olsen-Stojkovich (1985). However, we suspect the GenBank sequence for the latter could have been misidentified. Morphologically, both species are characterized by very small siphon diameter. *Avrainvillea calathina, A. gracillima* and *A. minima* sp. nov. also shared this particular character but these species did not form a monophyletic group in our phylogenetic inferences.

Olsen-Stojkovic’s classification then becomes obsolete in view of the DNA-based classification (see Table S6 summarizing Olsen-Stojkovic’s groups and our three phylogenetic clusters (A, B, C) with corresponding species and shared morpho-anatomical characters). These results confirm that classifications based on morpho-anatomical characters alone are misleading and not supported by genetic and phylogenetic analyses. Nevertheless, it is worth noticing that similar morpho-anatomical characters were found several times independently in phylogenetically distant species (e.g. moniliform siphons in *A. fulva, A. corticata* and *A. nigricans* or deep supradichotomal constrictions in *A. digitata, A. geppiorum, A. fulva, A. laciniata*). It would be interesting to further investigate the distribution and evolution of the morpho-anatomical characters along the lineages and be able to determine if they are ancestral (plesiomorphic) or derived states (in this case, are they homoplasious or synapomorphic?).

Littler & Littler (2004) highlighted the influence of the environment on the general habit (solitary or colonial/mound-building) of some *Avrainvillea* species (*A. longicaulis* and *A. asarifolia*). It would be worth investigating further whether the environmental conditions can influence other morpho-anatomical traits (e.g. colour, siphon size or siphon morphology). Another interesting question would be to assess the evolutionary role in the distribution of morpho-anatomical traits in *Avrainvillea.*

Acknowledgments

Samples were collected by several collectors and the authors are particularly grateful to Line Le Gall, Hélène Magalon, Lydiane Mattio, Serge Andréfouët, Elvan Ampou, Gustave Paulay and Heroen Verbruggen for providing additional samples or sequences.
For their help with the Latin translations to name the new species, we thank Francesca Benzoni and Lydiane Mattio. We are also grateful to Lydiane Mattio (blue[c]weed) for revising the English and providing useful comments to improve the manuscript.
Most of the molecular analyses were performed at the Plateforme du Vivant (CRESICA-IRD Noumea). Although most of the sequences were obtained by LL, we also would like to thank Claire Bonneville, and Gregoire Davignon for the additional sequences they produced. Molecular data of a few other samples (PC) were produced by the Service de Systématique Moléculaire of the Muséum National d'Histoire Naturelle (CNRS - UMS 2700).
The following campaigns made it possible to build this rich dataset: Bunaken, 2014 : INDESO project (research permits 133/SIP/FRP/SM/V/2015 and 918/BLITBANKKP/II/2016 issued by the Indonesian government and under a material transfer agreement between BALITBANG KP (now BRSDM KP, Ministry of Maritime Affairs and Fisheries) and IRD); Caribbean Islands, 2015: R/V Antea , PACOTILLES, doi: 10.17600/15005200); Fiji, 2007: R/V Alis, BSM-Fidji, doi: 10.17600/7100030; French Polynesia, 2008: Moorea-biocode; Guadeloupe, 2014: R/V Antea , KARUBENTHOS organized by Onema and MNHN, doi : 10.17600/15005400; Kavieng, 2014: doi:10.17600/14004400; Madagascar, 2010 : Atimo Vatae, doi:10.17600/10110040; 2016: R/V Antea, MAD, doi: 10.17600/16004700; Madang, 2012: R/V Alis, NUIGUINI campaign, doi:10.17600/12100070; Mayotte, 2010 : TARA; 2016: SIREME; New Caledonia, 2005 : R/V Alis, BSM-LOYAUTE, doi:10.17600/5100030; 2012: CORALCAL4, doi:10.17600/12100060; 2013: LOF; 2015: R/V Alis, CHEST, doi:10.17600/15004500; 2017: R/V Alis PostBlanco1 & TARA-NC; Scattered Islands, Juan de Nova Is., 2013: BIORECIE; Tuvalu: CLS, ESA grant number no. 4000110904/14/I-AM; Vanuatu, 2006: SANTO, doi:10.17600/6100100.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding Authors

This work was supported by the DUNE Labex-CORAIL project and UMR ENTROPIE (IRD) funds.

Supplementary Material

Table S1: List of vouchers included in the study with sample ID, Herbarium ID, species identification, collection localities, GenBank accession numbers (or BOLD sequence ID for those not submitted), and the corresponding SSH number.

Table S2: Name, nucleotide sequence and reference of the primers used for the amplification of the *tuf*A, *rbc*L, and 18S rDNA markers.

Table S3: Details of incongruence resolution process and species assignment decision.

Table S4: Table of interspecific genetic distances between species for *tuf*A and *rbc*L markers (presented as % similarity).

Table S5: Morpho-anatomical observations for all clades, along with currently accepted species descriptions.

Table S6: Summary of Olsen-Stojkovic's groups and the three phylogenetic clusters (A, B & C) found in this study, with the list of the corresponding species and the shared morpho-anatomical characters.

Fig. S1: Species delimitation results obtained with the three methods (GMYC, ASAP and PTP) for the *tuf*A dataset. The tree is the MCCT from the BEAST analyses. The numbers in brackets indicate the supplementary identical haplotypes. Black bars indicate PSHs retained as SSHs, following the majority rule, while blue bars represent the PSHs retained as SSHs although not favoured by the majority rule. Grey bars are alternative PSHs not retained. The defined SSHs (clades) are indicated in the right column, with morpho-anatomical congruence (M column, v: specimens observations matched with existing species diagnoses; x: no match with existing diagnoses; R: proposition of species resurrection; S: proposition of synonyms; *: incongruences), as well as species identification.

Fig. S2: ML gene-tree obtained with RaxML for *tuf*A dataset, using the GTR + G evolutionary model and 1,000 bootstrap (bs) iterations. Bootstraps values (bs) are reported at the nodes if > 50.

Fig. S3: Species delimitation results obtained with the three methods (GMYC, ASAP and PTP) for the *rbc*L dataset. The tree is the MCCT from the BEAST analyses. Numbers in brackets indicate additional identical haplotypes. Black bars indicate partitions retained as SSHs, following the majority rule; blue bars represent the partition retained as SSHs but not favoured by the majority rule. Grey bars are alternative partition schemes not retained. The defined SSHs (clades) are indicated in the column to the right, with morpho-anatomical congruence (v: specimens observations matched with existing species diagnoses; x: no match with existing diagnoses; R: proposition of species resurrection; S: proposition of new synonyms; *: incongruences), and species identification.

Fig. S4: ML gene-tree obtained with RaxML for *rbc*L dataset using the GTR + G evolutionary model and 1,000 bootstrap (bs) iterations. Bootstraps values (bs) are reported at the nodes if > 50.

Fig. S5: ML phylogeny of the Dichotomosiphonaceae obtained from the multilocus matrix (*tuf*A, *rbc*L, and 18S rDNA) with bootstrap values (bs) indicated at the nodes. The position of *Cladocephalus luteofuscus* among the *Avrainvillea* species is framed in blue. GenBank sequence assignments that need verification are indicated in grey. Nodes A, B, C indicate *Avrainvillea* species groups. The outgroup species were *Bryopsis plumosa*, *Codium taylorii, C. duthieae, C. platylobium, Caulerpa taxifolia, C. sertularioides*, and *C. verticillata*. Abbreviations: FP: French Polynesia, NC: New Caledonia, PNG: Papua New Guinea

Fig. S6: BI phylogeny of the Dichotomosiphonaceae obtained from the multilocus matrix (*tuf*A, *rbc*L, and 18S rDNA) with Posterior probabilities (PP) indicated at the nodes. The position of *Cladocephalus luteofuscus* among *Avrainvillea* species is framed in blue. GenBank sequence assignments that need verification are indicated in grey. Nodes A, B, C indicate the different *Avrainvillea* species groups. The outgroup species were *Bryopsis plumosa, Codium taylorii, C. duthieae, C. platylobium, Caulerpa taxifolia, C. sertularioides*, and *C. verticillata*. Abbreviations: FP: French Polynesia, NC: New Caledonia, PNG: Papua New Guinea

Fig. S7: *Cladocephalus luteofuscus* (PC0143565). **A:** Overall view of the pseudo-cortex formed by interwoven cortical siphons and appendages in the blade; **B-D:** Detail of the appendages of blade siphons. Scale bars: A: 167 µm; B: 66 µm; C: 88 µm; D: 45 µm

Fig. S8: Distribution map of *Avrainvillea* species included in this study, based on DNA data.

Figs S9-S19. *Avrainvillea gracillima.* **Fig. S9.** Habit *in situ* (NOU203318). **Fig. S10.** Habit *ex situ* (NOU203318). **Figs S11-S12.** Blade siphons subcylindrical, torulose or tortuous (S11: NOU203318; S12: NOU203335). **Fig. S13.** Dichotomies with supradichotomal constrictions (NOU203335). **Figs S14-S15.** Tortuous or bent apices (NOU203318). **Figs S16-S17.** Stipe siphons cylindrical to torulose (S16: NOU203318; S17: NOU203335). **Fig. S18.** Dichotomies with symmetrical constrictions in stipe siphons (NOU203318). **Fig. S19.** Primary rhizoids distorted, secondary rhizoids torulose to cylindrical (NOU203335). Scale bars: 9: 0.6 cm; 10: 0.45 cm; 11-12: 125 µm; 13: 80 µm; 14-15: 66 µm; 16-18: 100 µm; 19: 150 µm.

Figs S20-27. *Avrainvillea lacerata* var. *robustior.* **Fig. S20**. *In situ* habit (NOU203629). **Fig. S21.** Herbarium specimen (NOU203629). **Fig. S22.** Torulose cortical blade siphons (NOU203629). **Figs S23-S24**. Long-necked constrictions at dichotomies and supradichotomal swelling (S23 : NOU203628, S24 : NOU203629). **Fig. S25.** Cylindrical to torulose stipe siphons (NOU203628). **Figs S26-S27.** Rhizoids tortuous to distorted (NOU203629). Scale bars: 20: 0.93 cm; 21: 1.28 cm; 22: 250 µm; 23: 40 µm; 24: 37.5 µm; 25: 300 µm; 26: 133 µm; 27: 266 µm.

Figs S28-36. *Avrainvillea pacifica*. **Fig. S28.** *In situ* habit of a peltate individual (NOU213749). **Fig. S29.:** Herbarium specimen with cordato-semirotundate habit (NOU203445). **Figs S30-31.** Blade siphons torulose to moniliform or tortuous (NOU203450). **Fig. S32.** Dichotomies wide-angled (NOU203450). **Figs S33-S34.** Moniliform medullary stipe siphons and cortical cylindrical, tortuous or moniliform cortical siphons (NOU203450). **Fig. S35.** Rhizoids moniliform to tortuous (NOU203450). **Fig. S36.** Rhizoids with or without supradichotomal constrictions and blunt apices (NOU203450). Scale bars: 28: 1,375 cm; 29: 2,59 cm; 30: 150 µm; 31: 166 µm; 32: 60 µm; 33: 171 µm; 34-35: 166 µm; 36: 250 µm.

Data S1 : List of specimens examined for each new or revised species.

Author contributions

L. Lagourgue: treatment and analyses of molecular data (species delimitation, phylogeny), morphological analyses, taxonomic diagnosis, original concept, drafting and editing manuscript; F. Rousseau: sample collection, acquisition of genetic sequences, morphological observations, review and editing of manuscript; M. Zubia: sample collection, review and editing of manuscript; C.E. Payri: sample collection, morphological observations, funding acquisition, original concept, review and editing of manuscript.

References

Agardh, C.A. (1823). Species algarum. Rite cognitae, cum synonymis, differentiis specificis et descriptionibus succinti. Volumen Primum. Lund. pp. 399-531

Agardh, J.G. (1887). Till Algernes Systematik. Nya bidrag. *Acta Universitatis Lundensis* **23**: 1–174. 5 plates.

Belton, G.S., Draisma, S.G.A., Prud'homme van Reine, W.F., Huisman, J.M. & Gurgel, C.F.D. (2019). A taxonomic reassessment of *Caulerpa* (Chlorophyta, Caulerpaceae) in southern Australia, based on *tuf*A and *rbc*L sequence data. *Phycologia* **58**, 234-253. https://doi.org/10.1080/00318884.2018.1542851

Børgesen, F. (1908). The species of *Avrainvillea* hitherto found on the shores of the Danish West Indies. *Videnskabelige meddelelser fra den Naturhistoriske forening i Kjöbenhavn* 27–44, 8 Figs, Plate III.

Brostoff, W.N. (1989). *Avrainvillea amadelpha* (Codiales, Chlorophyta) from Oahu, Hawaii. *Pacific Science* **43**, 166–169.

Cabrera, R. & Suárez, M. (2006). Lista sistemática y distribución mundial del género *Avrainvillea* (Chlorophyta). *Revista de Investigaciones Marinas* **27**, 103–114.

Chesters, D. (2013). Collapsetypes.pl. Available at: https://sourceforge.net/projects/collapsetypes (last accessed 23 March 2021)

Curtis, N.E., Dawes, C.J. & Pierce, S.K. (2008). Phylogenetic analysis of the large subunit rubisco gene supports the exclusion of *Avrainvillea* and *Cladocephalus* from the Udoteaceae (Bryopsidales, Chlorophyta). *Journal of Phycology* **44**, 761–767. https://doi.org/10.1111/j.1529-8817.2008.00519.x

Decaisne, J. (1842). Mémoire sur les corallines ou polypiers calcifères. *Annales des Sciences Naturelles, Botanique, Seconde Série* **18**, 96–128.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J.& Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4**, 699–710. https://doi.org/10.1371/journal.pbio.0040088

Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**:1969–73.

Gepp, A. & Gepp, E.S. (1911). The Codiaceae of the Siboga Expedition. Including a Monograph of Flabellarieae and Udoteae. *Siboga-Expeditie* **62**:1–150

Guiry, M. D. & Guiry, G. M. (2022). AlgaeBase. World-wide Electronic Publication. National University of Ireland, Galway, Ireland. http://www.algaebase.org (last accessed 17 december 2021)

Hillis-Colinvaux, L. (1986). Distribution patterns of some Bryopsidales in the geologie past: their hearing on present distributions. *Botanica Marina* **XXIX**, 271–277.

Howe, M.A. (1907). Phycological Studies-III. Further Notes on *Halimeda* and *Avrainvillea. Bulletin of the Torrey Botanical Club* **34**, 491. https://doi.org/10.2307/2479207

Howe, M.A. (1905). Phycological studies- I. New Chlorophyceae from Florida and the Bahamas. *Bulletin of the Torrey Botanical Club* **32**, 563–586.

Kazi, M.A., Reddy, C.R.K. & Jha, B. (2013). Molecular phylogeny and barcoding of *Caulerpa*(Bryopsidales) based on the *tuf*A, *rbc*L, 18S rDNA and ITS rDNA Genes. *PLoS One* **8**,

e82438. http://dx.doi.org/10.1371/journal.pone.0082438.

Kraft, G.T. & Olsen-Stojkovich, J. (1985). *Avrainvillea calithina* (Udoteaceae, Bryopsidales), a new green alga from Lord Howe Island, NSW, Australia. *Phycologia* **24**, 339–345.

Lagourgue, L. & Payri, C.E. (2021). Diversity and taxonomic revision of tribes Rhipileae and Rhipiliopsideae (Halimedaceae, Chlorophyta) based on molecular and morphological data. *Journal of Phycology* **57**, 1450–1471. https://doi.org/10.1111/jpy.13186

Lagourgue, L. & Payri, C.E. (2020). Large scale diversity reassessment, evolutionary history, and taxonomic revision of the green macroalgae family Udoteaceae (Bryopsidales, Chlorophyta). *Journal of Systematics and Evolution* **60**, 101-127 https://doi.org/https://doi.org/10.1111/jse.12716

Lagourgue, L., Puillandre, N. & Payri, C.E. (2018). Exploring the Udoteaceae diversity (Bryopsidales, Chlorophyta) in the Caribbean region based on molecular and morphological data. *Molecular Phylogenetics and Evolution* **127**, 758–769. https://doi.org/10.1016/j.ympev.2018.06.023

Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. (2012). PartitionFinder: Combined

Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. *Molecular Biology and Evolution* **29**, 1695–1701. https://doi.org/10.1093/molbev/mss020

Littler, D.S. & Littler, M.M. (1992). Systematics of *Avrainvillea* (Bryopsidales, Chlorophyta) in the tropical western Atlantic, *Phycologia* **31**, 375-418

Littler, M.M., Littler, D.S. & Brooks, B.L. (2004). Extraordinary mound-building forms of *Avrainvillea* (Bryopsidales, Chlorophyta): their experimental taxonomy, comparative functional morphology ecological strategies. *Atoll Research Bulletin* **515**, 1–25.

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees, in: 2010 Gateway Computing Environments Workshop (GCE). IEEE, pp. 1–8. https://doi.org/10.1109/GCE.2010.5676129

Murray, G. (1886). On a new species of *Rhipilia* (*R. Andersonii*) from Mergui Archipelago. *Transactions of the Linnean Society of London, Second Serie, Botany.* **2**, 225–227. https://doi.org/10.1111/j.1095-8339.1886.tb01008c.x

Murray, S.N. & Littler, M.M. (1989). Seaweeds and seagrasses of Southern California: distributional lists for twenty-one rocky intertidal sites. *Bulletin of the Southern California Academy of Sciences* **88**, 61–79.

N’Yeurt, A.D.R., Littler, D.S. & Littler, M.M. (1996). *Avrainvillea rotumensis* sp. nov. (Bryopsidales, Chlorophyta), a peltate species from the South Pacific. *Phycological Research* **44**, 81–84.

Olsen-Stojkovich, J. (1985). A systematic study of the genus *Avrainvillea* Decaisne (Chlorophyta, Udoteaceae). *Nova Hedwigia* **41**, 1–68.

Olsen-Stojkovich, J. (1979). Revision of the pantrical algal genus *Avrainvillea* Decaisne (Codiales, Codiaceae). M.S. thesis, University of Guam. 148 p.

Pierce, S.K., Curtis, N.E., Massey, S.E., Bass, A.L., Karl, S.A. & Finney, C.M. (2006). A morphological and molecular comparison between *Elysia crispata* and a new species of kleptoplastic sacoglossan sea slug (Gastropoda: Opisthobranchia) from the Florida Keys, USA. *Molluscan Research* **26**, 23–38.

Pons, J., Barraclough, T.G., Gomez-zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D. & Vogler, A.P. (2006). Sequence-based species delimitation for the

DNA taxonomy of undescribed insects. *Systematic Biology* **55**, 595–609. https://doi.org/10.1080/10635150600852011

Puillandre, N., Brouillet, S. & Achaz, G. (2021). ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources* **21**, 609–620. https://doi.org/10.1111/1755-0998.13281

Rambaut, A. & Drummond, A. (2007). Tracer version 1.5. Available at: http://beast.bio.ed.ac.uk/Tracer (last accessed 18 February 2021)

Ronquist, F. & Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–4.

Sauvage, T., Payri, C., Draisma, S.G., Prud'homme Van Reine W.F., Verbruggen, H., Belton, G.S., Gurgel, F.D., Gabriel, D., Sherwood, A.R. & Fredericq, S. (2013). Molecular diversity of the *Caulerpa racemosa-Caulerpa peltata* complex (Caulerpaceae, Bryopsidales) in New Caledonia, with new Australasian records for *C. racemosa* var. *cylindracea. Phycologia* **52**, 6–13. https://doi.org/10.2216/11-116.1

Sauvage, T., Schmidt, W.E., Suda, S. & Fredericq, S. (2016). A metabarcoding framework for facilitated survey of endolithic phototrophs with *tuf*A. *BMC Ecology* **16**, 1–21. https://doi.org/10.1186/s12898-016-0068-x

Silva, P.C., Basson, P.W. & Moe, R.L. (1996). *Catalogue of the Benthic Marine Algae of the Indian Ocean. Smithsonian Institution Press,* Washington, DC

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stamatakis, A., Hoover, P. & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57**, 758–771. https://doi.org/10.1080/10635150802429642

Thiers, B. (2022). Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available from http://www.sweetgum.nybg.org/ih/ [last accessed 16 December 2021].

Trono, G.C. (1971). Some new species of marine benthic algae from the Caroline Islands, western-central Pacific. *Micronesica* **7**, 45–77.

Veazey, L., Williams, O., Wade, R., Toonen, R. & Spalding, H.L. (2019). Present-day distribution and potential spread of the invasive green alga *Avrainvillea amadelpha* around the main Hawaiian Islands. *Frontiers in Marine Science* **6**, 402. https://doi.org/10.3389

Verbruggen, H., Ashworth, M., LoDuca, S.T., Vlaeminck, C., Cocquyt, E., Sauvage, T., Zechman, F.W., Littler, D.S., Littler, M.M., Leliaert, F. & De Clerck, O. (2009). A multi-locus time-calibrated phylogeny of the siphonous green algae. *Molecular Phylogenetics and Evolution* **50**, 642–653. https://doi.org/10.1016/j.ympev.2008.12.018

Verbruggen H. & Schils T. (2012). *Rhipilia coppejansii*, a new coral reef-associated species from Guam (Bryopsidales, Chlorophyta). *Journal of Phycology* **48**, 1090–1098.

Verbruggen, H., Leliaert, F., Maggs, C.A., Shimada, S., Schils, T., Provan, J., Booth, D., Murphy, S., De Clerck, O., Littler, D.S., Littler, M.M. & Coppejans, E. (2007). Species boundaries and phylogenetic relationships within the green algal genus *Codium* (Bryopsidales) based on plastid DNA sequences. *Molecular Phylogenetics and Evolution* **44**, 240–254. https://doi.org/10.1016/j.ympev.2007.01.009

Verbruggen, H., Marcelino, V.R., Guiry, M.D., Cremen, M.C.M. & Jackson, C.J. (2017). Phylogenetic position of the coral symbiont *Ostreobium* (Ulvophyceae) inferred from chloroplast genome data. *Journal of Phycology* **53**, 790–803. https://doi.org/10.1111/jpy.12540

Verlaque, M., Langar, H., Hmida, A. Ben, Pergent, C. & Pergent, G. (2017). Introduction of a new potential invader into the Mediterranean Sea: the Indo-Pacific *Avrainvillea amadelpha* (Montagne) A. Gepp & E.S. Gepp (Dichotomosiphonaceae, Ulvophyceae). *Cryptogamie, Algologie* **38**, 267–281. https://doi.org/10.7872/crya/v38.iss3.2017.267

Vieira, C., D'hondt, S., De Clerck, O. & Payri, C.E. (2014). Toward an inordinate fondness for stars, beetles and *Lobophora*? Species diversity of the genus *Lobophora* (Dictyotales, Phaeophyceae) in New Caledonia. *Journal of Phycology* **50**, 1101–1119. https://doi.org/10.1111/jpy.12243

Wade, R., Spalding, H., Peyton, K., Foster, K., Sauvage, T., Ross, M. & Sherwood, A. (2018). A new record of *Avrainvillea* cf. *erecta* (Berkeley) A. Gepp & E. S. Gepp (Bryopsidales, Chlorophyta) from urbanized estuaries in the Hawaiian Islands. *Biodiversity Data Journal* **6**, e21617. https://doi.org/10.3897/BDJ.6.e21617

Yamada, Y. (1932). Notes on Some Japanese Algae IV. *Journal of Faculty Science, Hokkaido Imperial University, Serie. V* **II**, 267–284. https://doi.org/10.15281/jplantres1887.46.160

Young, J.R. (1977). Ecological observations on the reproduction of the tropical marine green alga *Avrainvillea* from Panamá (Siphonales/ Codiaceae). *Journal of Phycology* **13**, 76.

Zhang, J., Kapli, P., Pavlidis, P. & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* **29**, 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

Tables

Table 1: Global distribution of _Avrainvillea_ species based on DNA data or the literature when unavailable. Species with DNA data represented in this study are in black, and misrepresented species are in grey. Species for which the distribution area is updated with this study are indicated in bold. Species for which the distribution needs to be verified are marked with *, particularly for the Indo-Pacific or strict Pacific distribution of _A. riukiuensis,_ since we propose to resurrect _A. gracillima_ (several reports of _A. riukiuensis_ in the Indian Ocean were under _A. gracillima_).

Geographical regions	**Tropical Atlantic (exclusively)**	**Indian Ocean (exclusively)**	**Pacific (exclusively)**	**Indo-Pacific**	**Atlantic + Indian Ocean**
Species	_A. asarifolia_, _A. canariensis_, _A. cyathiformis,_ _A. digitata_, _A. elliottii_, _A. fenicalli,_ _A. fulva_, _A. geppiorum_, _A. hayi_, _A. levis,_ **_A. mazei-nigricans_ complex**, _A.rawsonii_, _A. silvana_, _A. sylvearleae_	_A. carteri_, _A. gardineri,_ _A. pyrochoma_ sp. nov., _A. minima_ sp. nov.	_A. calathina_, _A. clavatiramea_, _A. hollenbergii_, _A. laciniata_ sp. nov, _A. pacifica_, _A. kanakiensis_ sp. nov, _A. mollis_ sp. nov, _A. pavonina_ sp. nov, _A. spongiosa_ sp. nov, _A. xishaensis_	_A. amadelpha_ complex, **_A. lacerata_ complex, _A. erecta_*-_obscura_ complex**, **_A. gracillima_**, _A. ridleyi,_ _A. riukiuensis_*,	_A. longicaulis_*
Total	15	4	10	7	1

Table 2: Selection of the most informative characters combined for the species examined during this study. Characters that are sufficient on their own to identify a species are indicated in bold.

SPECIES	COMBINATION OF CHARACTERS FOR IDENTIFICATION
A. amadelpha	Siphon apices and pseudo-cortex
A. asarifolia	**Habit**, blade zonation, colour (when dry), dichotomies/constrictions, pseudo-cortex
A. calathina	Habit, blade texture & colour of the blade (when dry), siphon size, pseudo-cortex
A. clavatiramea	Habit, siphon apices and constrictions
A. corticata	Siphons aspect, colour, apices and pseudo-cortex
A. digitata	**Habit,** siphons constrictions and apices
A. elliottii	Blade margins, zonation, constrictions
A. fulva	**Siphons aspect** and size
A. geppiorum	Habit, siphons apices and constrictions
A. gracillima	Habit, size, blade texture & thickness, blade siphons size
A. hollenbergii	Habit, blade margin & colour (when dry), siphons size, dichotomies aspect & constrictions, pseudo-cortex
A. kanakiensis	Habit, Color & texture (when dry), blade siphons size
A. lacerata	Habit, siphons apices size, constrictions
A. laciniata	Habit, margin, colour (when dry), siphons aspect and apices
A. minima	Habit, size, siphon size
A. mollis	Blade texture, siphons aspect and apices
A. nigricans	Colour (when dry), blade thickness & texture, siphons aspect, constrictions
A. obscura	Habit, blade thickness, siphons aspect, apices and constrictions
A. pacifica	**Habit**, texture, color (when dry), siphons aspect and apices
A. pavonina	Habit, blade thickness & texture, dichotomies aspect, rhizoids size
A. pyrochroma	Habit, **blade color (when dry)**
A. spongiosa	Habit, blade siphons aspect, color and apices, constrictions

Figures

Fig. 1: Geographical origin of samples used in this study for which DNA data is available. Red dots: specimens from our collections; Green triangles: specimens from the GenBank.

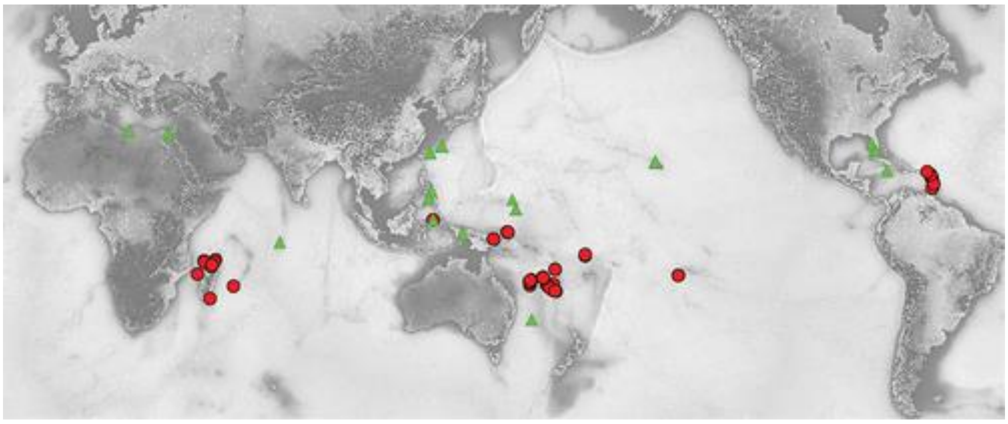


Fig. 2: Species delimitation results obtained with the three methods (GMYC, ASAP and PTP) for the *tuf*A and *rbc*L dataset. The tree is the chloroplast (*tuf*A + *rbc*L) ML tree from the RaxML analyses. Black bars indicate PSHs retained as SSHs, following the majority rule, while bars are alternative PSHs not retained. The defined SSHs (clades) are indicated in the right column, with morpho-anatomical congruence (M column, v: specimens observations matched with existing species diagnoses; x: no match with existing diagnoses; R: proposition of species resurrection; S: proposition of synonyms; *: incongruences), as well as species identification.

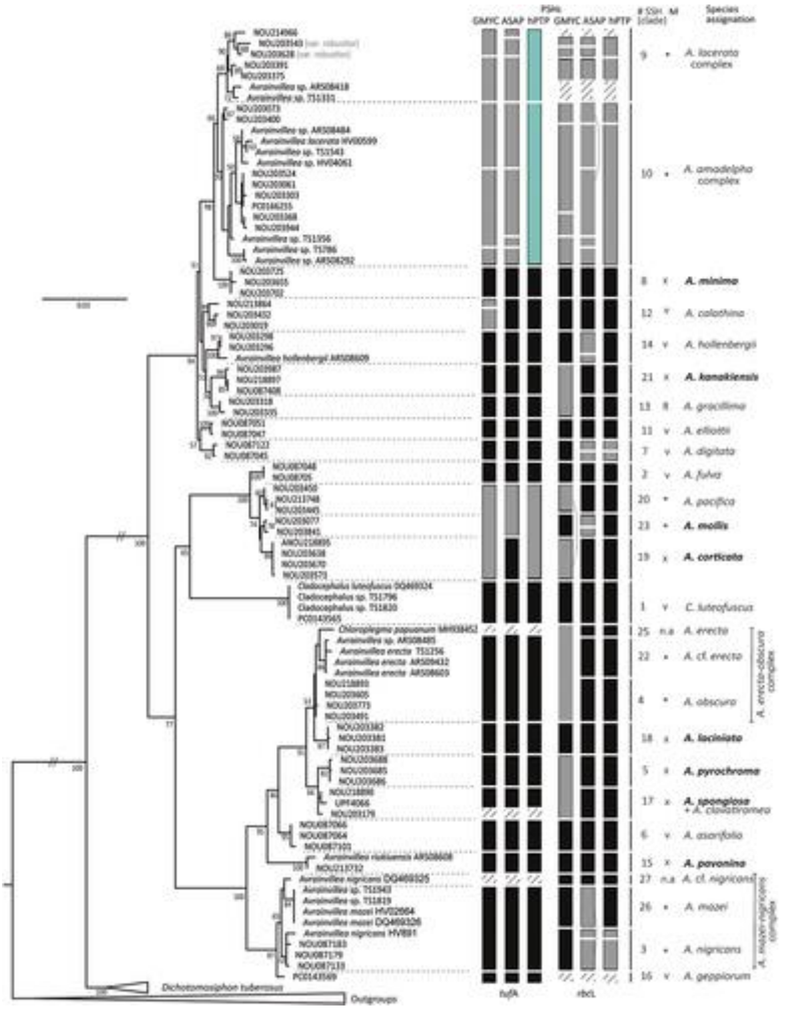
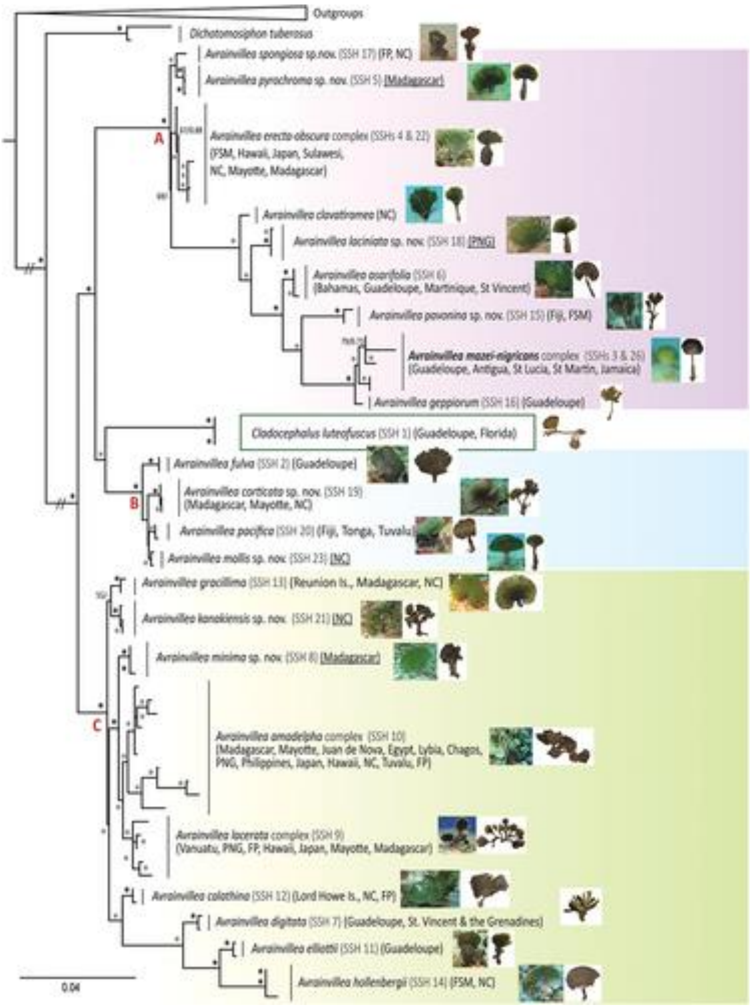
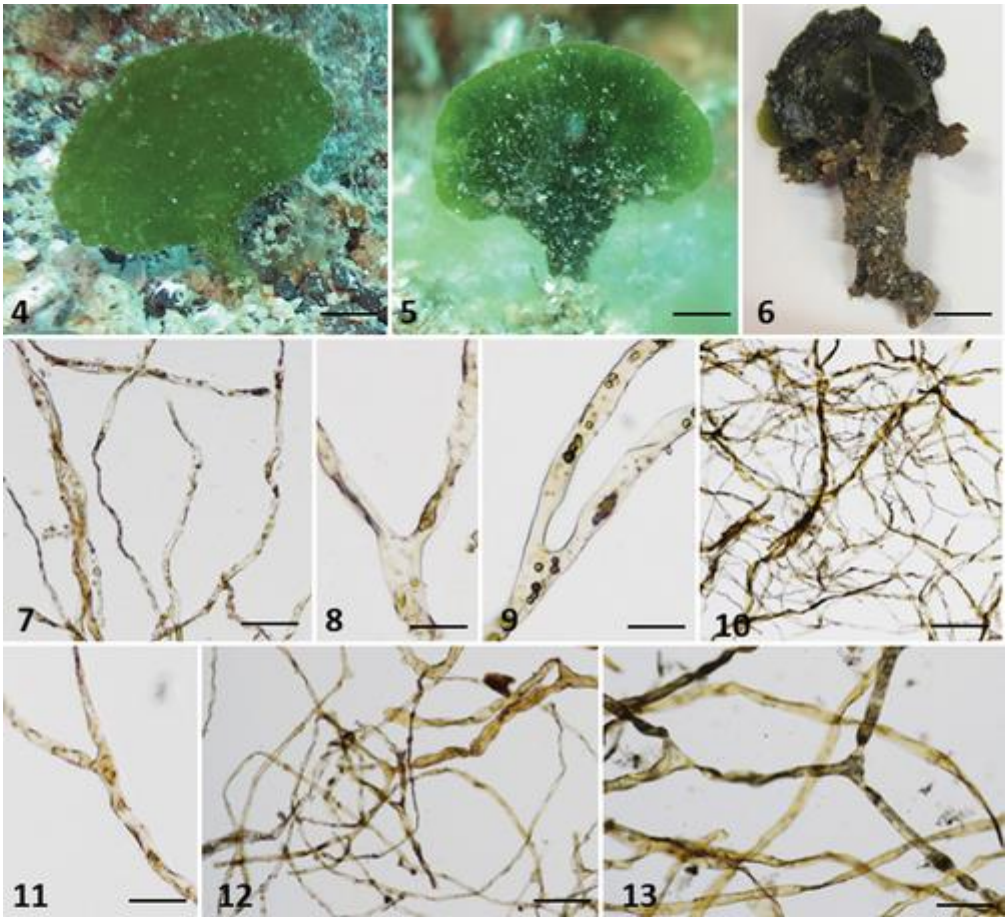


Fig. 3: ML phylogeny of the Dichotomosiphonaceae family obtained from the multilocus matrix (*tuf*A, *rbc*L, and 18S rDNA). Nodes with black stars are highly supported for both ML and BI methods, while grey stars indicate highly supported nodes with BI only. bs/PP are given for not supported nodes. The position of *Cladocephalus luteofuscus* among the *Avrainvillea* species is framed in green Nodes A, B, and C indicate the different *Avrainvillea* species groups. The type species is shown in bold. Species geographical distributions are reported, and endemic localities are underlined. The coloured rectangle indicates geographical regions: Caribbean (*sensu largo*) in yellow, Indian Ocean in red, Pacific in blue and Indo-Pacific in purple. The outgroup species are *Bryopsis plumosa*, *Codium taylorii*, *C. duthieae*, *C. platylobium*, *Caulerpa taxifolia*, *C. sertularioides*, and *C. verticillata*. Abbreviations: FSM: Federated States of Micronesia; FP: French Polynesia, MD: Madagascar, NC: New Caledonia, PNG: Papua New Guinea. Image rights: Menou, J.L, Lagourgue, L., Lasne G., Payri C.E, Rousseau, F., and Zubia, M. (Yusimí Alfonso for the illustration of *A. mazei*).



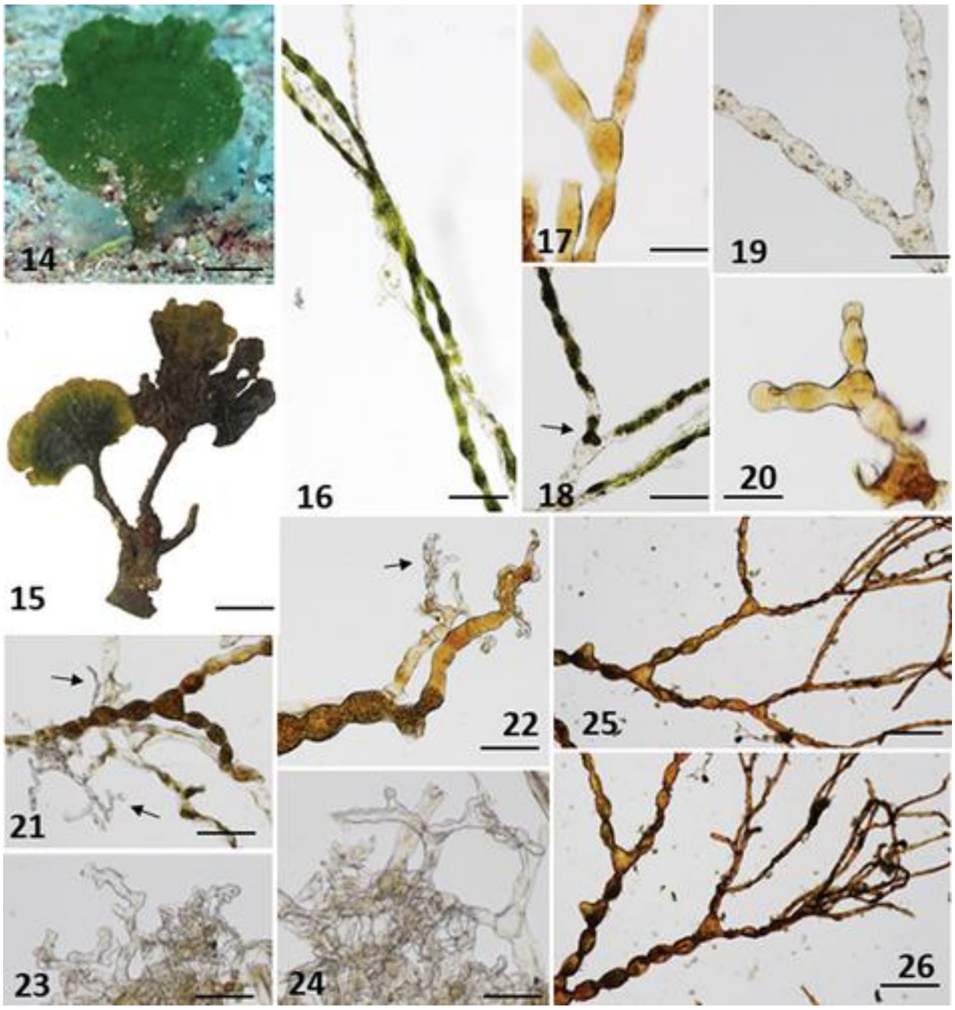
Figs 4-13: *Avrainvillea minima* sp. nov. **Figs 4-5.** *In situ* habit of the species (4: NOU203655, 5: NOU203717). **Fig. 6.** *Ex situ* specimen (NOU203726). **Fig. 7.** Torulose blade siphons (NOU203655). **Figs 8-9.** Slight asymmetrical or elongated constrictions at dichotomies of blade siphons (E: NOU203655; F: NOU203726). **Fig. 10.** Stipe medullary and cortical siphons (NOU203655). **Fig. 11.** Supradichotomal constriction on stipe siphons (NOU203655). **Fig. 12.** Rhizoids (NOU203702). **Fig. 13.** Dichotomies on rhizoids with supradichotomal constrictions (NOU203726). Scale bars: Fig. 4: 0.4 cm; Fig. 5: 0.5 cm; Fig. 6: 1.15 cm; Fig. 7: 130 µm; Fig. 8: 30 µm; Fig. 9: 40 µm; Fig. 10: 150 µm; Fig. 11: 100 µm; Fig. 12: 115 µm; Fig. 13: 160 µm.



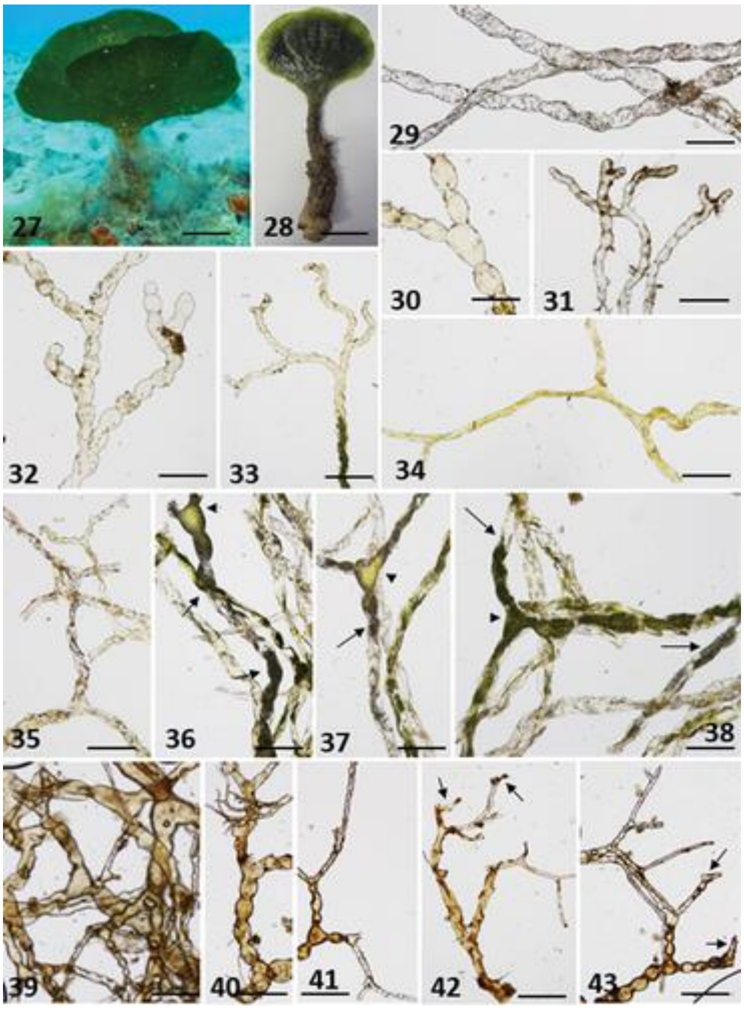
Figs 14-26: *Avrainvillea corticata* sp. nov. **Fig. 14:** *In situ* habit of the species (NOU203670).

Fig. 15. *Ex-situ* specimen (NOU203635). **Fig. 16.** Moniliform blade siphons (NOU203635);

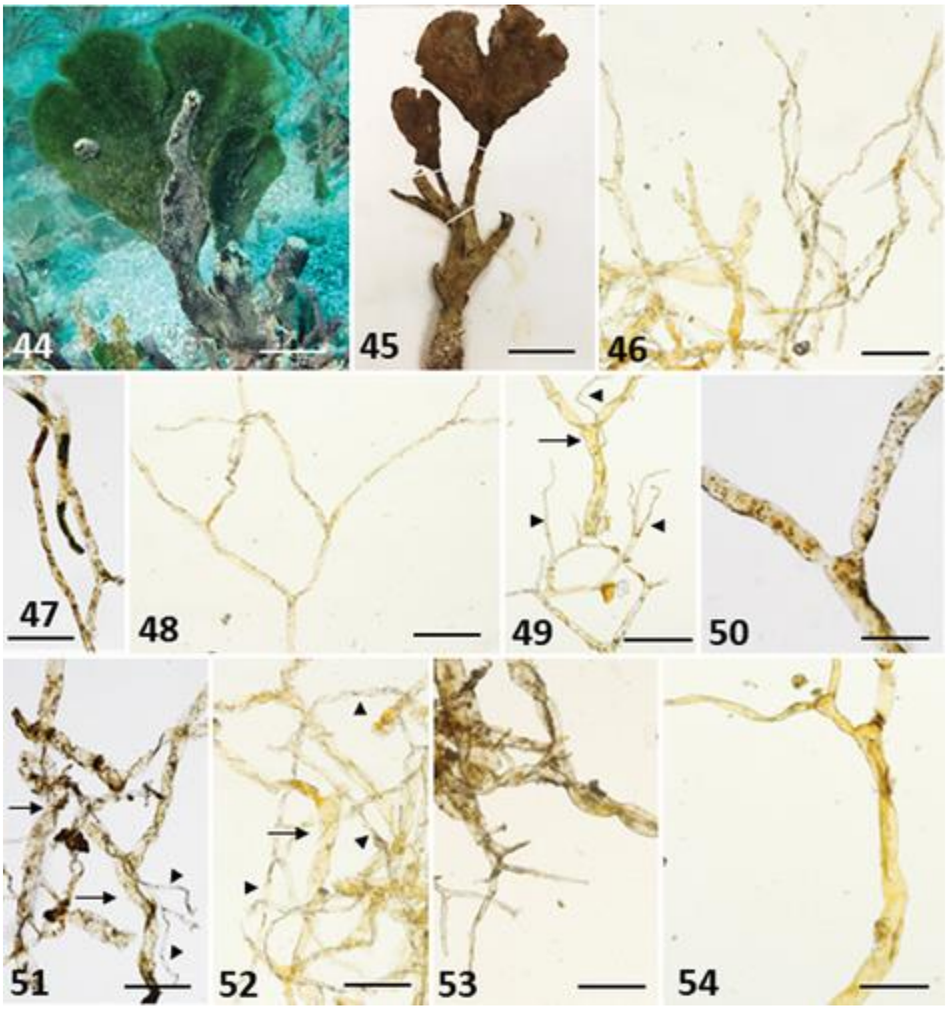
Figs 17-20: Dichotomies with symmetrical constrictions on medullary (18, 19: NOU203635) and cortical blade siphons with rounded or keel-shaped apices (17, 20: NOU203638) and some visible plastids (arrow on Fig. 18). **Figs 21-22.** Moniliform stipe siphons, ending with successive and close dichotomies (arrows; NOU203638). **Figs 23-24**. Pseudocortex (NOU203638). **Figs 25-26.** Rhizoids brown, orange, moniliform and becoming cylindrical near apices (NOU203638). Scale bars: Fig. 14: 1.5 cm; Fig. 15: 2.5 cm; Fig. 16: 150 µm; Fig. 17: 50 µm; Fig. 18: 150 µm; Fig. 19: 75 µm; Fig. 20: 50 µm; Fig. 21: 125 µm; Fig. 22: 120 µm; Fig. 23-24: 100 µm; Fig. 25-26: 125 µm.



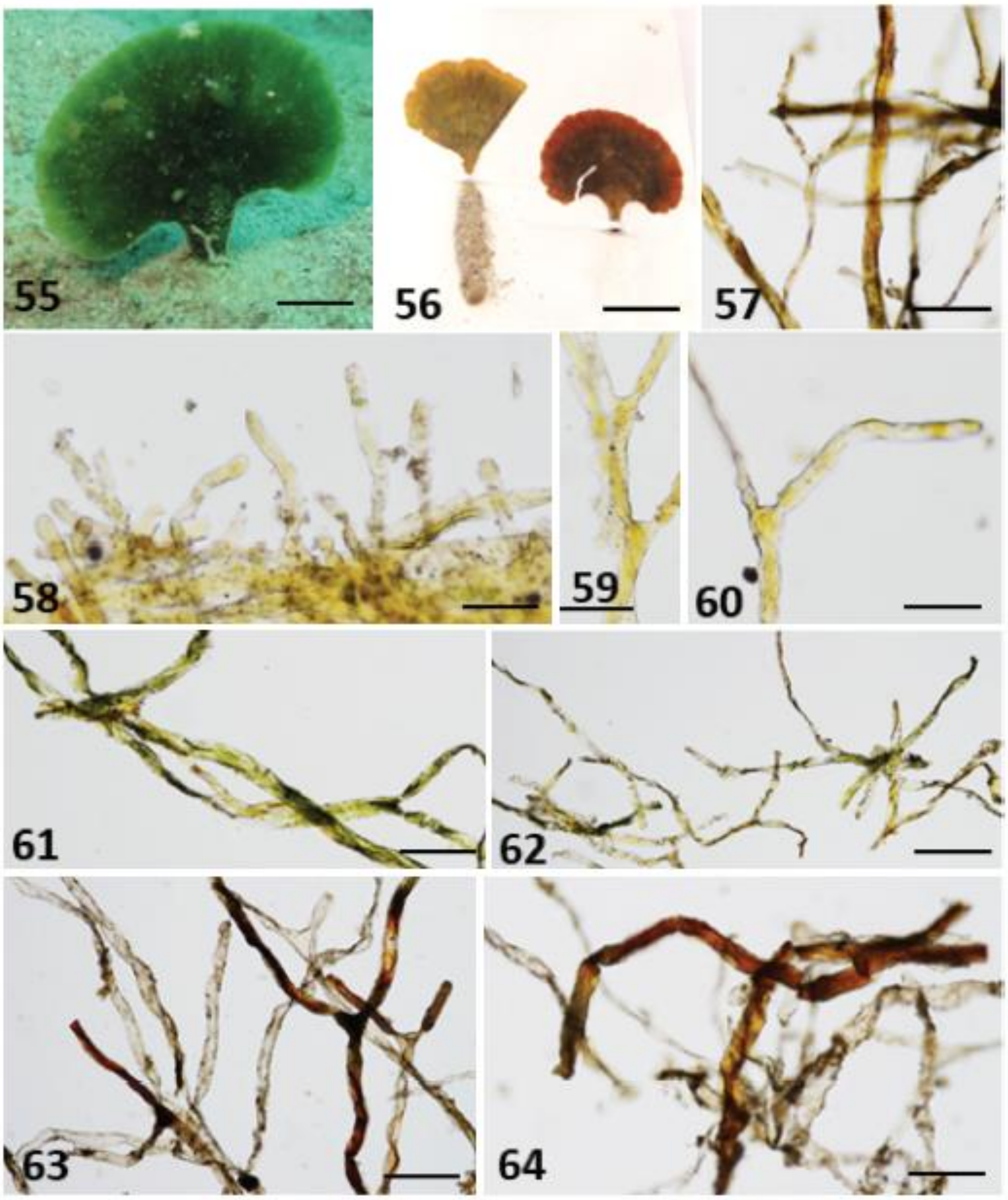
Figs 27-43: *Avrainvillea mollis* sp. nov. **Fig. 27.** In *situ* habit of the species (NOU203077). **Fig. 28.** *Ex-situ* specimen (NOU203077); **Fig. 29:** Blade siphons slightly moniliform and moniliform (NOU203092). **Fig. 30.** Dichotomies with symmetrical constrictions (NOU203839). **Figs 31-33.** Dichotomously divided siphons towards the apices with swollen rounded or keel-shaped apices (31: NOU203092, 32-33: NOU203839). **Figs 34-35.** Tortuous **s**tipe siphons (34: NOU203077, 35: NOU203839). **Figs 36-38:** Moniliform medullary stipe siphons with plastid concentrations (arrows), tortuous uncoloured siphons, and widened dichotomies (arrow heads; NOU203092). **Figs 39-40.** Primary moniliform rhizoids and thinner secondary rhizoids (NOU203092). **Figs 41-42.** Rhizoids moniliform and brown or orange-coloured, tapering towards the apices becoming more translucid and less moniliform (41: NOU203092, 42: NOU203077). **Fig. 43.** Rhizoids terminating in expanded pads (arrows; NOU203092). Scale bars: Fig. 27: 2 cm; Fig. 28: 3.7 cm; Fig. 29: 125 µm; Fig. 30: 80 µm; Fig. 31: 130 µm; Fig. 32: 100 µm; Fig. 33: 150 µm; Fig. 34: 250 µm; Fig. 35: 300 µm; Fig. 36-37: 330 µm; Fig. 38: 250 µm; Fig. 39: 150 µm; Fig. 40: 200 µm; Fig. 41: 100 µm; Fig. 42: 265 µm; Fig. 43: 100 µm.



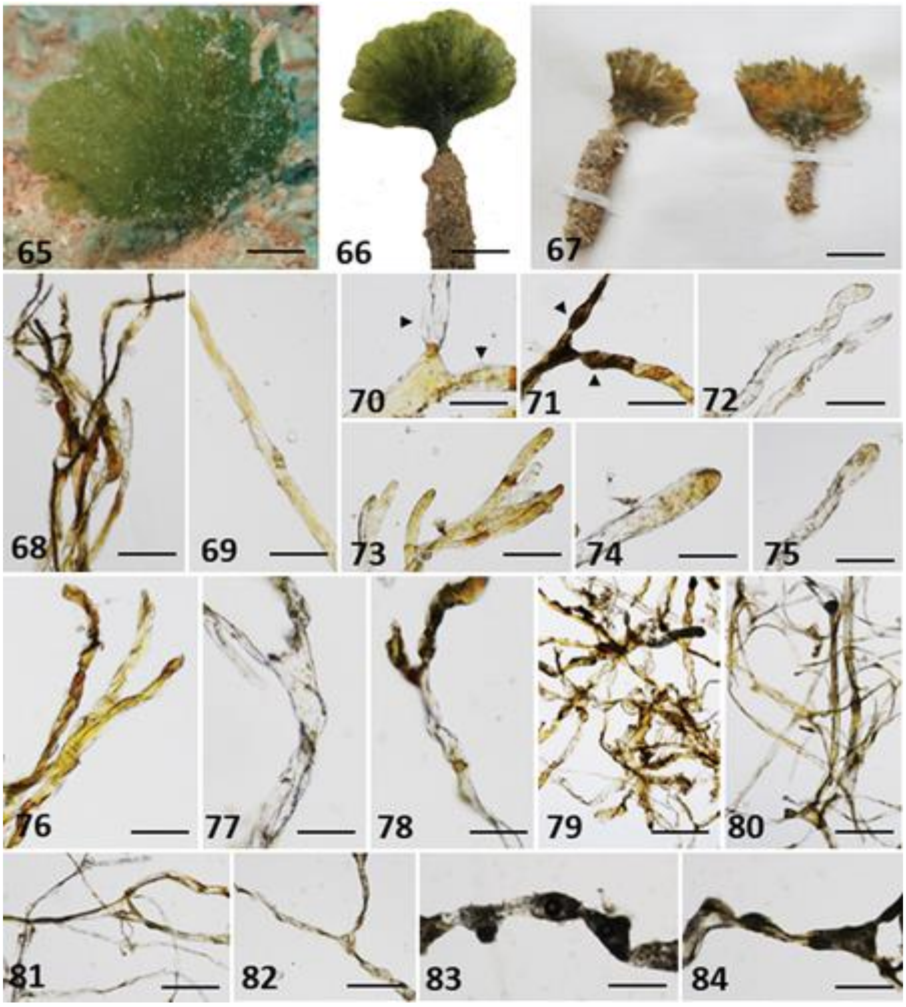
Figs 44-54: *Avrainvillea pavonina* sp. nov. **Fig. 44.** *In situ* habit of the species (NOU213732). **Fig. 45.** Herbarium specimen (NOU213732). **Fig. 46.** Blade siphons cylindrical to torulose and tortuous (NOU213732). **Figs 47-48.** Dichotomously divided blade siphons (47: NOU213733, 48: NOU213732). **Fig. 49.** Large medullary siphons (arrow) with thinner cortical siphons (arrow heads) (NOU213732**). Fig. 50.** Isomorphic dichotomies with symmetrical constrictions (NOU213733). **Figs 51-52.** Large medullary (arrows) and thinner cortical siphons (arrow heads) from stipe, cylindrical to torulose (H: NOU213733, NOU213732). **Figs 53-54.** Rhizoids dichotomously divided and tapering towards the apices (NOU213732). Scale bars: Fig. 44: 2.9 cm; Fig. 45: 4.8 cm; Fig. 46: 160 µm; Fig. 47: 200 µm; Fig. 48: 200 µm; Fig. 49: 200 µm; Fig. 50: 80 µm; Fig. 51: 265 µm; Fig. 52: 165 µm; Fig. 53: 220 µm; Fig. 54: 200 µm. Image rights: Lasne G.



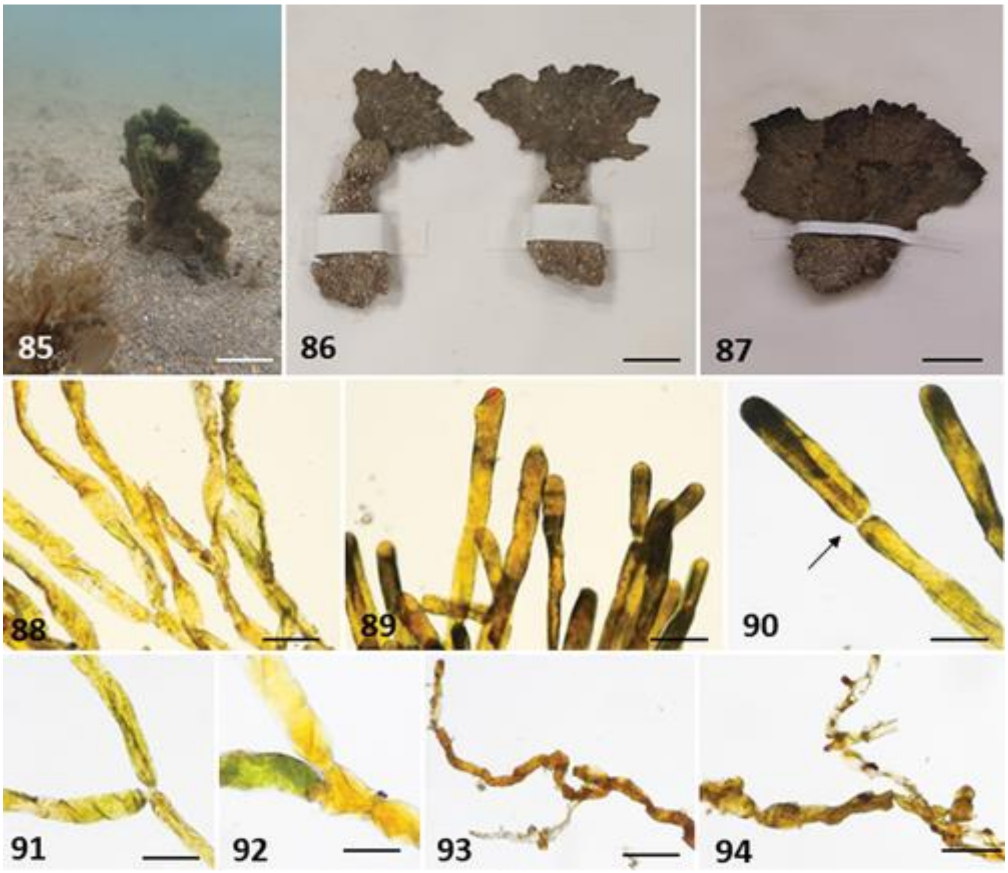
Figs 55-64: *Avrainvillea pyrochroma* sp. nov**. Fig. 55.** *In situ* habit of the species (NOU203686). **Fig. 56.** Herbarium specimen (NOU203688). **Fig. 57.** Cylindrical blade siphons (NOU203686). **Fig. 58.** Clavate, rounded or swollen apices (NOU203688). **Figs 59-60.** Dichotomies with symmetrical constriction on blade siphons (NOU203688). **Figs 61-62.** Stipe siphons cylindrical and torulose (NOU203688). **Figs 63-64.** Rhizoids red or hyaline, cylindrical to torulose (63: NOU203688, 64: NOU203686). Scale bars: Fig. 55: 0.9 cm; Fig. 56: 1.95 cm; Fig. 57: 200 µm; Fig. 58-60: 100 µm; Fig. 61: 160 µm; Fig. 62-63: 200 µm; Fig. 64: 250 µm.



Figs 65-84: *Avrainvillea laciniata* sp. nov. **Fig. 65.** *In situ* habit of the species (NOU203381). **Fig. 66.** *Ex situ* specimen (NOU203381). **Fig. 67.** Herbarium specimens (NOU203382**). Figs 68-69.** Cylindrical brown or pale orange siphons (68: NOU203382, 69: NOU203383). **Figs 70-71.** Isomorphic dichotomies with symmetrical constrictions and supradichotomal branch swelling (arrow heads) (70: NOU203381, 71: NOU203383). **Figs 72-75.** Coloured and rounded, swollen or slightly blunt apices (72, 74, 75: NOU203383, 73: NOU203381). **Fig. 76.** Stipe siphons (NOU203383). **Figs 77-68.** Dichotomies on stipe siphons with supradichotomal constrictions (NOU203381). **Figs 79-80.** Primary and secondary rhizoids (79: NOU203382, 80: NOU203383). **Figs 81-82.** Dichotomies with constrictions on rhizoids (81: NOU203382, 82: NOU203383). **Figs 83-84.** Black moniliform and distorted primary rhizoids (NOU203381). Scale bars: Fig. 65: 1.5 cm; Fig. 66: 2.2 cm; Fig. 67: 1.5 cm; Fig. 68-69: 200 µm; Fig. 70: 70 µm; Fig. 71: 200 µm; Fig. 72: 120 µm; Fig. 73: 125 µm; Fig. 74: 80 µm; Fig. 75-76: 130 µm; Fig. 77: 100 µm; Fig. 78: 130 µm; Fig. 79: 250 µm; Fig. 80: 260 µm; Fig. 81-82: 100 µm; Fig. 83-84: 150 µm.



Figs 85-94: *Avrainvillea spongiosa* sp. nov. **Fig. 85.** *In situ* habit of the species (UPF5775). **Figs 86-87.** Herbarium specimens (86: UPF4066, 87: NOU218888). **Fig. 88.** Cylindrical and dichotomously divided blade siphons (NOU218888). **Fig. 89.** Rounded and swollen apices on blade siphons. **Fig. 90.** Isolated constriction on blade siphons (arrow; NOU218888). **Figs 91-92.** Dichotomies with symmetrical and deep constrictions (91: NOU218888, 92: NOU218650). **Figs 93-94.** Tortuous and distorted rhizoids (NOU218888). Scale bars: Fig. 85: 3.1 cm; Fig. 86: 1.65 cm; Fig. 87: 2.15 cm; Fig. 88: 150 µm; Fig. 89: 65 µm; Fig. 90: 50 µm; Fig. 91-92: 100 µm; Fig. 93: 165 µm; Fig. 94: 125 µm.



Figs 95-103: *Avrainvillea kanakiensis* sp. nov. **Fig. 95.** *In situ* habit of the species (NOU203987). **Fig. 96.** Herbarium specimen, cluster of several uprights (NOU087408). **Fig. 97.** Moniliform blade siphons (NOU203987). **Fig. 98.** Blade siphon dichotomy (NOU087408). **Fig. 99.** Rounded and swollen apices (NOU087408). **Fig. 100.** Moniliform, torulose to tortuous stipe siphons (NOU218897). **Fig. 101.** Stipe siphon dichotomy (NOU218897). **Figs 102-103.** Rhizoids, primary (arrows) and secondary (arrow heads) (102: NOU218896, 103: NOU087408). Scale bars: Fig. 95: 0.7 cm; Fig. 96: 1.8 cm; Fig. 97: 200 µm; Fig. 98: 100 µm; Fig. 99: 16 µm; Fig. 100: 100 µm; Fig. 101: 130 µm; Fig. 102: 175 µm; Fig. 103: 80 µm.