1	Current status of the freshwater red algal diversity (Rhodophyta) of the
2	African continent including description of new taxa (Batrachospermales)
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17	Running title: Freshwater Rhodophyta diversity in Africa
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ABSTRACT

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Freshwater red algae have been collected on the African continent since the early 1800s. However, the collections have been sparse and geographically restricted. The present study sought to bring together information from the literature, herbarium specimens and newly collected specimens to provide an updated assessment of the freshwater red algal diversity of the African continent with a focus on the species rich Batrachospermales. DNA sequence data and morphological observations were conducted for recently collected specimens. From these analyses, four new taxa are proposed: Kumanoa bouwmanii, Sheathia murphevi, Sirodotia kennedyi and the form taxon 'Chantransia azurea'. DNA sequence data had been previously published for Kumanoa iriomotensis, Sirodotia aff. huillensis and S. suecica. With this study, we have added sequence data for *Torularia atra* as well as a second location for *S. suecica*. In total, there are eight taxa with sequence data, of which five appear to be endemic. From our assessment of literature reports and herbarium specimens, we conclude that Kumanoa, Sirodotia and Torularia have often been collected and are relatively geographically widespread with two or more species present. In addition, Montagnia, Nothocladus, Paralemanea, Sheathia and Visia as well as Batrachospermum section Gonimopropagulum are represented in the flora. We estimate that 14 to 19 batrachospermalean taxa can be recognized for the African flora, and that with more study, that number could easily double or triple based on the diversity known from other well-studied continents.

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KEY WORDS Africa; Batrachospermales; Biogeography; COI-5P; Freshwater red algae; rbcL

gene; Molecular systematics; Morphology; Rhodophyta; Taxonomy

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INTRODUCTION

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Most members of the Rhodophyta are marine seaweeds, but there is a substantial number of taxa that inhabit freshwater and terrestrial environments (Kumano 2002). These freshwater and terrestrial red algae are classified in 14 orders (Kumano 2002; Guiry & Guiry 2019). Some orders have primarily marine members with a few taxa that have transitioned to freshwater habitats. For example, species of *Bostrychia* and *Caloglossa* in Ceramiales occur in estuarine habitats, but have been reported from strictly freshwater water habitats (Sheath et al. 1993, Johnston et al. 2014; West et al. 2015). Audouinella and Ottia are the only freshwater genera in the marine order Acrochaetiales (Entwisle et al. 2017; Saunders et al. 2018). In Hildenbrandiales, Hildenbrandia has both marine and freshwater species (Sherwood & Sheath 2003). There are only four orders in which all members are strictly freshwater: Balbianiales, Batrachospermales, Compsopogonales and Thoreales. Balbianiales, Compsopogonales and Thoreales all contain less than three genera, but the Thoreales has c.18 species with the others having three or fewer (Sheath et al. 1994; Necchi et al. 2013; Johnston et al. 2018). The bulk of species diversity for freshwater red algae is in Batrachospermales with approximately 140 species in 19 genera (Guiry & Guiry 2019). Although freshwater red algae have been reported from a variety of habitats including specialized environments such as hot springs, the majority tend to inhabit streams and rivers, with fewer taxa in lakes and bogs (Sheath & Vis 2015). These environments have been examined and specimens collected from most continents (except Antarctica; Kumano 2002). Some continents, areas and regions have been well-studied. For example, there are recent floristic publications on Europe (Eloranta et al. 2011; Eloranta 2019), North America (Sheath & Vis

2015), the British Isles (Sheath & Sherwood 2011), and India (Ganesan et al. 2018). However,

there are still gaps in our knowledge. Vis (2016) used the Batrachospermales as a biogeographic case study to examine the distribution of *Batrachospermum* sections and genera among the continents. Only Australia had fewer taxa than Africa and it was hypothesized that the low numbers for Africa may have been due to lower sampling effort than the other continents (Vis 2016). Africa shared many of these taxa with other continents, but of note was *Nothocladus* sensu stricto that has only been reported from Africa and Australia and *Batrachospermum* section *Gonimopropagulum* which appears to be endemic (Vis 2016). This case study at the generic level provided some information on the African flora and its relationship with the flora of other continents, but a more in depth understanding of species diversity could yield more biogeographic insights.

The first records of freshwater red algae exclusive of specialized habitats (i.e. aerophytic, hot springs) from Africa come from the early to mid 1800's with species described by Bory (1808). More collections and species descriptions followed in the mid to late 1800s (Table 1). There were a handful of records published between 1900 to the 1960's and subsequently, there have been a few papers documenting freshwater red algae from parts of the continent (Table 1). Most taxa reported are from Batrachospermales (16), but there are records of some taxa in Acrochaetiales (2), Ceramiales (4), Compsopogonales (1), Hildenbrandiales (2) and Thoreales (1). Geographic records are most common from South Africa and the islands of Madagascar, Mauritius and Réunion, but reports from eleven countries are present in the literature (Table 1).

The order Batrachospermales, which comprises most of the taxon records for the African continent, has undergone substantial taxonomic change in recent years. Most of the sections of *Batrachospermum* have been raised to genera and there have been proposals for new genera and species (e.g. Entwisle *et al.* 2009; Salomaki *et al.* 2014; Necchi *et al.* 2019a, b). Prior to

establishment of *Kumanoa*, there were nine genera recognized in the order, whereas currently there are 19 (Entwisle *et al.* 2009; Guiry & Guiry 2019). In addition, many recent studies used DNA sequence data and showed that taxa previously thought to be widespread harbored cryptic species with more restricted distributions (e.g. Salomaki *et al.* 2014; Necchi *et al.* 2019a, b). Nevertheless, studies have also confirmed a few species, such as *Sirodotia suecica* Kylin, as cosmopolitan, occurring on numerous continents (Lam *et al.* 2012). Most of the herbarium collections and literature records from Africa occurred prior to this new taxonomy and would benefit from updating the nomenclature as well as determining the identity of specimens using recent taxonomic literature.

A study documenting the diversity of freshwater red algae in Africa would complement research on other continents, provide a better understanding of the taxonomic diversity and potentially offer valuable biogeographic insights. Therefore, this research was undertaken to identify newly collected specimens and place them within the context of the known diversity on the African continent and to use them to understand biogeographic trends in Batrachospermales as well as individual species' distributions. When possible, specimens of Batrachospermales from herbarium collections were accessed to verify taxon records and distributions. Although the focus of the current study was primarily the Batrachospermales due to the diversity of taxa, the literature for other freshwater red algal species (exclusive of extremophiles) was surveyed and presented.

MATERIALS AND METHODS

Recent collections of specimens were made from 12 locations as follows: Nigeria (1), South Africa (6), and Zambia (5) (Table S1; Fig. 1); these include three specimens with sequence data

previously published (Lam *et al.* 2012; Vis *et al.* 2012). Collections were pressed on herbarium sheets or dried in silica desiccant. A portion of each specimen was deposited in the Floyd Bartley Herbarium (BHO); specimens from South Africa and Zambia were also deposited in South African National Diatom Collection (SANDC), and those from Nigeria in the herbarium of the University of Lagos (LUH).

Morphological identifications of the recent collections were made to the lowest taxonomic level possible. Specimens were examined using a BX-40 compound microscope (Olympus America, Center Valley, Pennsylvania, USA) and SZ-40 stereomicroscope (Olympus America). Measurements and photomicrographs of morphological characters were performed using the cellSens program (Olympus America) and the microscope mounted SC-100 camera (Olympus America). Recent taxonomic literature was consulted including Kumano (2002), Lam et al. (2012), Necchi & Vis (2012), Salomaki et al. (2014) and Rosignolo & Necchi (2016). When possible, measurements of quantitative characteristics were made as follows: whorl diameter, fascicle cell number, carpogonium diameter/length/branch cell number, spermatangial diameter/length, carposporophyte diameter/length, carposporangial diameter/length and gonimoblast cell number. Qualitative characteristics of thallus color after drying, placement of the carposporophytes relative to the whorl, shape of whorls, trichogynes and fascicle cells as well as any other distinguishing features were noted.

For these recent collections, DNA sequence data were generated. A portion of the thallus was wetted and removed from herbarium sheets, placed in a 1.5 ml microcentrifuge tube with silica desiccant. Some specimens had been placed in desiccant in the field. The desiccated material was ground in liquid nitrogen using a mortar and pestle and processed using the Nucleospin Plant II DNA kit (Macherey-Nagel, Bethlehem, PA) according to manufacturer's

protocol. PCR was performed to amplify a 1282 base pair (bp) fragment of the chloroplast encoded rubisco large subunit gene (rbcL) and a 664 bp portion of the mitochondrial encoded cox1 gene (COI-5P). The primers used for rbcL amplification were F160 and rbcLR (Vis et al. 1998). When a PCR product was not obtained from this primer pair, the region was amplified as two separate fragments pairing F160 with R897.1 and F650 with rbcLR to achieve the same sequenced region (Salomaki et al. 2014). The primers used for COI-5P were GazF1 and GazR1 (Saunders 2005). The PCR master mix contained 19 µl of autoclaved deionized water, 1.5 µl of each primer and 2 μl of DNA with an IllustraTM PuReTaq Ready-To-Go PCR Bead (GE Healthcare, Pittsburgh, Pennsylvania, USA). The thermocycler settings for rbcL were as follows: 95 °C for 1 min, then 35 cycles of 93 °C for 30 sec, 50 °C for 30 sec, 72 °C for 1 min, and a final hold for 10 min at 72°C; the settings for COI-5P were 95 °C for 1 min, 35 cycles of 93 °C for 30 sec, 50 °C for 30 sec, 68 °C for 1 min, and a final hold for 10 min at 72 °C. PCR products were purified using the PureLinkTM Quick PCR Purification kit and sequenced at the Ohio University Genomics Facility. For the longer rbcL fragment, two internal primers F650 and R897.1 or R897 were used in the sequencing reactions to obtain full length sense and anti-sense strand sequence (Stewart et al. 2007). Sequence data were compiled using SequencherTM v5.2.4 (GeneCodes Corp, Ann Arbor, Michigan, USA) and compared to published DNA sequences using the BLAST feature of GenBank (Benson et al. 2018). All new sequence data generated were submitted to GenBank. For phylogenetic analyses, numerous data sets were created utilizing rbcL and COI-5P sequences data from GenBank. To examine the placement of the African specimens in

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sequences data from GenBank. To examine the placement of the African specimens in Batrachospermales, 54 previously published specimens of Batrachospermales and 4 specimens of Thoreales were utilized with both *rbc*L and COI-5P data. There were two new specimens of

Sirodotia with only rbcL or COI-5P data, and all other specimens had both genes (Table S2). To examine the relationship of the African specimens with all species within Kumanoa, Sheathia and Sirodotia, rbcL data alone were utilized and single gene trees constructed since there are many fewer species with COI-5P data. For Kumanoa, all rbcL GenBank sequences were downloaded and a single sequence was used to represent each of the Kumanoa species with DNA sequence data; this resulted in 32 sequences including those generated in this study, as well as nine outgroup taxa for a total of 41 sequences. For Sheathia, all unique sequences for S. arcuata and at least one representative from the other Sheathia species were used. For Sirodotia, only unique rbcL sequences with more than 1000 bp were used. These data sets were analyzed using maximum likelihood (ML) implemented in RAxML (Stamatakis 2014) and Bayesian Inference (BI) implemented in MrBayes v.3.2 (Ronquist et al. 2012) as plug-ins in Geneious Prime 2019.2.1 (https://www.geneious.com). For ML and BI analyses, a GTR+G model was employed and support values were determined from 1000 ML bootstrap replicates and BI posterior probabilities of 3×10^6 generations sampling every 100 generations with the first 250 generated trees removed as burn-in.

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Historical reports of freshwater red algae from Africa were investigated using a variety of data sources. Published literature was researched by keywords (algae, freshwater and Africa) in ISI web of science and the references in Kumano (2002) and Levanets & van Rensburg (2010). As well, records in an unpublished manuscript of M. Pocock were utilized (Table 1). Specimen names were updated to currently accepted taxonomy based on AlgaeBase (Guiry & Guiry 2019). Herbarium records for taxa in Batrachospermales and Thoreales from the Botanic Garden Meise (BR), Selmar Schonland Herbarium in South Africa (GRA), the Muséum National d'Histoire Naturelle in France (PC) and the herbaria consortium Macroalgal Herbarium Portal

(macroalgae.org accessed March 2019) were reviewed (Table 2). Specimens were requested from University of Michigan (MICH) and Harvard University (FH). When possible, herbarium specimens were examined microscopically and identified to the lowest taxonomic level possible similar to recent collections. If the specimens could not be microscopically examined only the nomenclature was updated.

RESULTS

DNA sequence analyses

Sequence data for the *rbc*L gene of specimens identified as *Kumanoa iriomotensis* (Kumano) M.L.Vis, Necchi, W.B.Chiasson & Entwisle, *Sirodotia* aff. *huillensis* (Welwitsch ex West & G.S.West) Skuja and *S. suecica* from Africa were previously published and new COI-5P data were generated for *Sirodotia* (supplemental Table S1). New sequence data for *rbc*L gene and COI-5P were generated for four more specimens. For two specimens, DNA sequence data from only one of the two regions were obtained. Unfortunately, no DNA sequence data were obtained for eight specimens after multiple attempts at DNA extraction and PCR. In total, DNA sequence data of *rbc*L are available for eight specimens and data of COI-5P for seven specimens (supplemental Table S1).

Phylogenetic analyses of the *rbc*L and COI-5P concatenated sequences using both ML and BI for specimens from Africa with sequence data and other batrachospermalean taxa showed similar tree topologies, and only the ML tree with support values is shown (Fig. 2). Specimens were placed in clades for *Kumanoa*, *Sheathia*, *Sirodotia*, and *Torularia* with high support values. However, one specimen of '*Chantransia*', was on a long branch and sister to *Virescentia*, but this placement was only supported by posterior probabilities (Fig. 2). *Torularia atra* (Hudson)

M.J.Wynne from South Africa was sister to another specimen from Australia identified as *T. atra*.

Further ML and BI phylogenetic analyses were conducted for Kumanoa, Sheathia and Sirodotia using only rbcL sequences since there are data for more species than in the concatenated alignment (Figs 3-5). The ML and BI analyses of Kumanoa species and specimens from Africa showed similar tree topologies and only the ML with support values is shown (Fig. 3). The new species of *Kumanoa* was within a clade with *K. montagnei* Entwisle, M.L.Vis, W.B.Chiasson, Necchi & A.R.Sherwood and K. nodiflora (Montagne) Entwisle, M.L.Vis, W.B.Chiasson, Necchi & A.R.Sherwood with high support values. The other specimen, K. iriomotensis was sister to K. curvata (Z.-X.Shi) M.L.Vis, Necchi, W.B.Chiasson & Entwisle, but with low support in both analyses (Fig. 3). The ML and BI analyses for *Sheathia* species and the specimen from Africa showed similar tree topologies and only the ML with support values is shown (Fig. 4). The new species of *Sheathia* was within a large clade of specimens of *S. arcuata* (Kylin) Salomaki & M.L.Vis. This S. arcuata complex has specimens from worldwide locations and the sequence diversity is greater than other species of Sheathia (Fig. 4). The ML and BI analyses for Sirodotia species and specimens from Africa showed similar tree topologies and only the ML tree with support values is shown (Fig. 5). One specimen, S. suecica, was within a large clade of highly similar sequences of other specimens identified as S. suecica. S. aff. huillensis did not seem to be closely allied to any particular species (Fig. 5). The new species of Sirodotia was sister to a clade from South America identified as S. delicatula Skuja, but that relationship was only supported by posterior probability.

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Morphological analyses

Both the specimens that had DNA sequence data and the specimens that did not yield those data were examined for microscopic characters that could be used for identification. The morphological data for those specimens identified as new to science using the DNA sequence data is provided in the taxonomic proposal section. The specimen recognized as *Torularia atra* via DNA sequence data had morphological characters consistent with that species; likewise, the specimen of Sirodotia suecica from Zambia was confirmed with morphology. The specimens of Kumanoa iriomotensis, Sirodotia suecica (South Africa) and S. aff. huillensis had been examined and confirmed in previous studies. The other eight specimens without sequence data were identified to the lowest taxonomic level possible with the morphological characters present (Table S1). Three of the specimens were identified as *Montagnia* cf. *macrospora* (Montagne) Necchi, M.L.Vis & A.S.Garcia based on overall gametophyte morphology and large carposporangia. Two specimens had the overall gametophyte morphology of Visia, but identification of species requires sequence data so they were labelled Visia sp. (Necchi et al. 2019a). One specimen was identified as *Sirodotia* sp. based on an asymmetrical carpogonium and an indeterminant carposporophyte; however, no spermatangia were observed in order to determine the species. A specimen of *Thorea* sp. was identified, but no reproductive structures were observed. A specimen with well-developed barrel-shaped whorls was identified as Batrachospermum sp. as it had spermatangia and a few carpogonia on undifferentiated carpogonial branches and regular cortication. However, this specimen could be *Sheathia* as some species do not have heterocortication. When possible, the historical specimens were morphologically examined to confirm

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species identification and determine morphological characters that might warrant new taxon status. Specimens of *Batrachospermum africanum* Rabenhorst were examined from PC and FH.

These were determined not to be *Batrachospermum* and most likely not a red alga. Specimens were sought for *Batrachospermum bohneri* Schmidle from the most likely herbarium of deposit, Eidgenössische Technische Hochschule Zürich (ZT), but none were found. The description of *B. bohneri* and the subsequent paper devoted to carpogonial development note large carpospore size and enlarged pit connections between cells of the carpogonial branch (Schmidle 1899a, b). These characters would place it in the genus *Montagnia*, and most likely synonymous with *M. macrospora* as that species is known from South America and Asia (Necchi *et al.* 2019b). Specimens of *Batrachospermum patens* Suhr showed the overall gametophyte morphology of *Torularia atra* with reduced whorls that are not adherent. One carpogonium was observed and a few carposporophytes, which were within the species circumscription of this taxon.

Numerous specimens from Africa in herbaria had manuscript names from H. Skuja that were never published; as such, they have no priority in the ICN (Table 1) (Entwisle 1992). Since the naming suggests that these may be unique, these specimens were examined to determine to which segregate genus from *Batrachospermum* they belonged, and whether any morphological characters could be identified that warranted a new species. Specimens labelled 'Batrachospermum proximum' and 'B. velatocarpum' showed morphological characters similar to Kumanoa nodiflorum and the new species of Kumanoa sp. nov. such that we have assigned those specimens to the new species of Kumanoa from Africa based on morphology and geography (Table 2). The specimens labelled 'Batrachospermum jadinii' and 'B. mucronatum' had curled carpogonial branches and were designated Kumanoa sp. The specimens of 'Batrachospermum stephensii' and 'B. libericum' fit the description of Visia having carpogonia on long, straight branches, carposporangia not particularly large, and the overall gametophyte morphology. The specimens of 'Sirodotia plumula' and 'Batrachospermum vanderystii' were

276 not examined and have been labelled as *Sirodotia* sp. and *Batrachospermum* sp., respectively. Other specimens examined from herbaria were identified to genus or species when possible, employing morphological characters from recent taxonomic treatments (Table 2). TAXONOMIC AND NOMENCLATURAL PROPOSALS Kumanoa bouwmanii A.L.Szinte, J.C.Taylor & M.L.Vis sp. nov. Figs 6-9 DESCRIPTION: Thalli moderately mucilaginous, delicate; approximately 7 cm high; upon drying a light purple colour; branching irregular and abundant. Whorls reduced, distinct and separate, elongate-obconic, 264-320 µm in diameter. Internode 474-557 (655) µm long. Primary fascicles straight, 5-8 cell-storeys. Secondary fascicles covering the entire internode and as long 286 287 as the primary fascicles. Monoecious. Spermatangia sub-spherical or spherical, terminal or subterminal on primary or secondary fascicles, 5-8 µm in diameter. Carpogonial branches compact, twisted arising from the pericentral cells. Involucral filaments short, if present. Carpogonia 24-40(-50) μm long; trichogynes club-shaped, unstalked, 9-12(-15) μm in diameter. Carposporophytes 1-2 per whorl, dense, hemispherical as high as the whorl radius, 255-302 µm in diameter. Gonimoblast filaments 3-4 cell-storeys, cells barrel-shaped; carposporangia subspherical 16-28 µm long, 12-24 µm in diameter. 294 Representative unique molecular sequences deposited in GenBank: rbcL MN974516; COI-5P MN974521. HOLOTYPE: Zambia, Lufubu River at Chipili Village, M13 road-bridge, North-Western

Zambia, 10°43'44" S, 29°05'40.38" E, Coll. J.C. Taylor. 01.vi.2012, SANDC 19-564.

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ISOTYPE: BHO A-0950.

ETYMOLOGY: The specific epithet is named in honor of Professor H. Bouwman, Professor of Zoology at North-West University who has provided support and encouragement for a variety of projects in the biological sciences, including algal studies.

DISTRIBUTION: Known only from type locality.

REMARKS: This species has molecular data that ally it with *K. montagnei* and *K. nodiflora*. The morphology is distinct from *K. montagnei*, which has well-developed barrel-shaped whorls. The morphology of *K. bouwmanii* and *K. nodiflora* are much more similar with both species having reduced, distinct obconic whorls. It can be distinguished from *K. nodiflora* based on the following characteristics: shorter gonimoblast filaments and somewhat larger carposporangia in

Sheathia murpheyi A.L.Szinte, J.C.Taylor & M.L.Vis sp. nov.

both diameter and length. Although there are slight morphological differences, DNA sequence

312 Figs 10-12

data are recommended for a positive identification.

DESCRIPTION: Thalli delicate; less than 2 cm high; upon drying a light pink colour; branching irregular and abundant. Whorls well-developed, distinct and separate, barrel-shaped, 260-339 μ m in diameter. Thick cortication on the main axis composed only of cylindrical cells. Primary fascicles straight, 4-8 cell-storeys. Secondary fascicles few to none. Potentially dioecious, no spermatangia observed. Carpogonial branch straight composed of 4-5 undifferentiated cells that do not differ from the fascicles. Carpogonium 23-32 μ m long; trichogyne symmetrical or slightly asymmetrical, fusiform to clavate, 6-9 μ m in diameter. Carpogonia present on involucral filaments of carpogonial branch. No carposporophytes observed.

321	Representative unique molecular sequences deposited in GenBank: rbcL MN974517;
322	COI-5P MN974522.
323	HOLOTOYPE: Zambia, Luwumbu River at M14 road-bridge, Nyika Region, Northern Zambia,
324	10°44'45.77" S, 33°26'31.63" E , Coll. J.C. Taylor, 02.vii.2011, SANDC 19-565.
325	ISOTYPE: BHO A-0947
326	ETYMOLOGY: The specific epithet is named in honor of Dr. K. Murphey retired from Glasgow
327	University who is an aquatic macrophyte specialist and project leader for the Southern African
328	River Assessment Scheme (SAFRASS) project during which the samples from Zambia were
329	collected.
330	DISTRIBUTION: Known from type locality.
331	REMARKS: Both S. murpheyi and S. arcuata are distinguished from other Sheathia species
332	based on regular cortication. The most recent species circumscription of S. arcuata is quite broad
333	(Vis et al. 2010) and S. murpheyi is not distinguishable from this taxon without DNA sequence
334	data. In the phylogenetic trees, S. murpheyi was sister to a clade of S. arcuata specimens that
335	were primarily sporophytes and one gametophyte from Taiwan; this specimen and S. murpheyi
336	did differ in carpogonial length (15-20 μm versus 23-32 $\mu m,$ respectively; Chou & Wang 2006;
337	Vis et al. 2010).
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339	Sirodotia kennedyi A.L.Szinte, J.C.Taylor & M.L.Vis sp. nov.
340	Figs 13-16
341	DESCRIPTION: Thalli delicate; less than 2 cm high; becoming brown on drying; branching
342	irregular. Whorls reduced, obconic to pear-shaped, separate to confluent, 198-315 μm in
343	diameter. Internode 82-90 µm long. Primary fascicles straight, 3-5 cell-storeys. Secondary

344	fascicles present, but not covering the whole internode. Potentially dioecious, no spermatangia
345	observed. Carpogonial branch composed of 3-4 undifferentiated cells. Carpogonia 25-40 μm
346	long with an asymmetrical base. Trichogynes elongate pyriform or irregularly-shaped, unstalked,
347	7-11 µm in diameter. Carposporophytes with carposporangia on one-celled branches from an
348	indeterminate prostrate gonimoblast filament. Carposporangia obovoidal, 9-10 μ m long, 6-7 μ m
349	wide.
350	Representative unique molecular sequence deposited in GenBank: rbcL MN974518.
351	HOLOTOYPE: Zambia, Mutinondo River, Mutinondo Wilderness, 12°16'06.08" S, 31°22'52.15"
352	E, Coll. M.P. Kennedy, 07.vii.2011, SANDC 19-566.
353	ISOTYPE: BHO A-0946.
354	ETYMOLOGY: The specific epithet is named in honor of Dr. M. Kennedy of Coventry
355	University, an aquatic macrophyte specialist/environmental scientist and participant in the
356	Southern African River Assessment Scheme (SAFRASS) project in Zambia and who drove
357	many thousands of kilometers to obtain samples.
358	DISTRIBUTION: Known only from type locality.
359	REMARKS: Sirodotia kennedyi has similar gametophyte morphology and sizes of carpogonia to
360	S. delicatula and S. suecica, and shorter carpogonia than S. huillensis and S. aff. huillensis. The
361	other characters used to distinguish Sirodotia species, the origin of the gonimoblast filaments and
362	spermatangial placement were not observed in S. kennedyi (Lam et al. 2012). Therefore, DNA
363	sequence data is needed to distinguish this species from congeners.
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365	Chantransia azurea A.L.Szinte, J.C.Taylor & M.L.Vis [form] sp. nov.
366	Fig. 17

367	DESCRIPTION: Thalli consisted of erect filaments twining around other algae and moss. Cells
368	were bright blue. Filaments were highly and irregularly branched, with mostly cylindrical cells,
369	19-29 μm long, 5-11 μm wide. Apical cells were 6-13 μm long, 3-8 μm wide rarely terminating
370	in a hyaline hair. No monosporangia observed.
371	Representative unique molecular sequences deposited in GenBank: rbcL MN974515;
372	COI-5P MN974520.
373	HOLOTYPE: South Africa: R321 before Newberg Dam, approximately 10 km from Grabouw
374	near Cape Town, 34°04'46" S, 19°03'29" E, Coll. M.L. Vis, W.B. Chiasson & G. Ellis,
375	22.viii.2005, BHO A-1434.
376	ETYMOLOGY: The specific epithet refers to the brilliant blue color of the specimens in the
377	field and after drying.
378	DISTRIBUTION: Known from type locality.
379	REMARKS: The thalli completely covered the main axis of a Batrachospermum-like
380	gametophyte, but the gametophyte was too immature to identify. This taxon, 'Chantransia
381	azurea' is quite unique in its DNA sequence and if there had been a gametophyte specimen, it
382	would have been described as a new genus within the order; however, given the lack of
383	morphological data, we have chosen to recognize it as a distinct entity as a form taxon.
384	
385	Nomenclatural Changes
386	Torularia atra (Hudson) M.J.Wynne
387	HETEROTYPIC SYNONYM: Batrachospermum patens Suhr 1840: 296, no fig.

REMARKS: *B. patens* is placed in taxonomic synonymy with *Torularia atra* based on an examination of a syntype collection in the Muséum National d'Histoire Naturelle - PC0473547. We hereby designate that collection the lectotype of *Batrachospermum patens* Suhr.

Montagnia macrospora (Montagne) Necchi, M.L.Vis & A.S.Garcia

HETEROTYPIC SYNONYM: Batrachospermum bohneri Schmidle 1899: 2

REMARKS: No physical specimens of *Batrachospermum bohneri* have been located in the pertinent herbarium (ZT). However, the description in the protologue by Schmidle (1899a) and the drawings in Schmidle (1899b) of the same material reveal the morphological characteristics, especially the carposporangium size, to be the same as those of *M. macrospora*, demonstrating that these two taxa are conspecific.

Problematic Taxa

Batrachospermum africanum Rabenhorst 1855: 281 (as 'afrikanum')

Specimens examined in this study from PC and FH were not red algae and it could not be determined whether these were type material. Nevertheless, Entwisle (1992) examined isotype material in MEL noting that it was a mixture of two distinct Batrachospermum taxa and that it was unclear which was B. africanum according to Rabenhorst's description such that this taxon remains uncertain.

Sirodotia angolensis (West & G.S.West) Skuja in Reis 1960: 53

The basionym of this taxon is *Batrachospermum angolensis*. Sheath *et al.* (1993) examined type material of *B. angolensis* and determined it to be a taxonomic synonym of *Torularia*

puiggariana. These authors may not have been aware of the combination of S. angolensis.

Therefore, we denote *S. angolensis* as a heterotypic synonym of *Torularia puiggariana*

(Grunow) M.J. Wynne 2019: 2.

DISCUSSION

Systematics of taxa from Africa

Sheathia murphyi was within a large clade of specimens from locations worldwide that have previously been attributed to *S. arcuata* (Vis *et al.* 2010). However, the sequence variation among *S. arcuata* is much greater than other species of the genus (Salomaki *et al.* 2014). In that study, it was suggested that *S. arcuata* represents a species complex and should be reexamined given the geographic patterns among the subclades. Vis *et al.* (2010) did not observe clear morphological differences among specimens representing the subclades and were reticent to describe new species based on DNA sequence data alone. More recently, species that can only be distinguished via DNA sequence data have become more common in the literature for marine and freshwater red algae (e.g. Diaz-Tapia 2019; Necchi *et al.* 2019a, b). We decided not to add to this species complex but decided to propose a new species based on DNA sequence data and geographic distinctiveness. This species complex will need further investigation as delineating species from this complex is beyond the scope of the current study.

Batrachospermum breutelii Rabenhorst appears to be endemic to South Africa and has only been reported from two localities worldwide (Sheath & Whittick 1995). This species is distinct with very large zonate carposporangia that resemble fungal spores. There is no doubt that it would have been easily identified if collected at other locations. Due to its unique morphology, Batrachospermum section Gonimopropagulum was erected. Like many of the sections, it

probably deserves generic status. However, no DNA sequence data exist in order to verify its placement among the genera of Batrachospermales. So, at present it remains a species of *Batrachospermum*, but it does not share the characters of the species of *Batrachospermum* section *Batrachospermum*.

African Flora and Biogeography

With this study, the number of Batrachospermales taxa from Africa with sequence data totals eight: 'Chantransia azurea', *Kumanoa bouwmanii*, *K. iriomotensis*, *Sheathia murpheyi*, *Sirodotia* aff. *huillensis*, *S. kennedyi*, *S. suecica* and *Torularia atra*. Although this is a small number of species in the order and probably on the continent, it would appear that the flora has numerous endemic species as five of the taxa had unique DNA sequences. *Sirodotia suecica* and *Torularia atra* are cosmopolitan species with identical or similar sequences from other continents i.e. Australia, Europe and North America and Asia, Australia, Europe (Lam *et al.* 2012; Rossignolo & Necchi 2016). Although *Kumanoa iriomotensis* is known from Asia based on morphology, there is only sequence data from a South Africa specimen, so no conclusion can be drawn based on molecular data as to whether it is more widespread (Necchi & Vis 2012). Even without *K. iriomotensis*, five of eight (*c.* 62%) specimens sequenced were distinctive, suggesting a potentially unique flora. The diatoms also show similar patterns in terms of endemism with certain taxa being regionally endemic and others cosmopolitan (e.g. Taylor *et al.* 2016).

There have been a modest number of literature reports and herbarium specimens collected in Africa. From our assessment of these records, we conclude that *Kumanoa*, *Sirodotia* and *Torularia* have often been collected and are relatively geographically widespread with two or more species present. *Kumanoa* is represented by *K. bouwmanii*, *K. gracillima* (West &

457 G.S.West) Entwisle, M.L.Vis, W.B.Chiasson, Necchi & A.R.Sherwood and K. iriomotensis, but 458 there were numerous other specimens that could not be confidently identified to species such that 459 there is a high probability of more species. Likewise, there are three species of *Sirodotia*: S. 460 kennedyi, S. aff. huillensis and S. suecica, and numerous other specimens that had to be labelled Sirodotia sp. Two of the three species in Torularia [T. atra and T. puiggariana (Grunow) 461 462 M.J. Wynne] are part of the African flora. The genera *Montagnia*, *Nothocladus*, *Paralemanea*, 463 Sheathia and Visia each are represented by at least one species in the flora. Batrachospermum 464 breutelii has a distinctive carposporangium such that it can be recognized by morphology alone. 465 Although many reports could be verified using DNA sequencing or morphology, some reports could not be verified, in particular, Batrachospermum turfosum Bory from Batrachospermum 466 section *Turfosa*. It is probable that a member of this section would be part of the flora given that 467 this section is known to inhabit streams and bogs with acidic brown waters (Sheath et al. 1994; 468 469 Eloranta et al. 2011). Two reports of Tuomeya and Batrachospermum gelatinosum (L.)DC., are 470 dubious. Tuomeya is a monospecific genus first described from and widespread in eastern North America (Kaczmarczyk et al. 1992). It is unlikely that this species has a disjunct distribution in 471 472 South Africa. Batrachospermum gelatinosum is a northern hemisphere taxon occurring in North 473 America and Europe; other reports from the southern hemisphere were shown to be a cryptic 474 taxon, Nothocladus pseudogelatinosus (Entwisle & M.L.Vis) Entwisle & M.L.Vis (Entwisle et 475 al. 2004, 2016). It is unclear if Lemanea may be part of the flora or not based on the report of 476 Montagne (1846). In the present study, we examined a specimen attributed to *Lemanea* and 477 determined it was *Paralemanea*, a sister genus similar in gross morphology. Since, both genera 478 are widespread on other continents, it is equivocal in our opinion as to whether the report is

Lemanea or *Paralemanea* without a specimen to examine. Excluding the dubious records of taxa, we estimate between 14-19 batrachospermalean taxa in the African flora.

The current number of taxa attributable to the African flora is considerably lower than other continents and large countries which have been the focus of freshwater red algal studies. The floras of North America and Europe each have over 40 taxa currently recognized (Eloranta et al. 2011, Sheath & Vis 2015, Chapuis et al. 2017, Evans et al. 2018). Australia, which is more isolated, has over 25 taxa (Vis & Entwisle 2000; Necchi & Vis 2012; Entwisle et al. 2018). Over 20 species have been recorded from Brazil and Finland (Necchi & Vis 2012, Necchi et al. 2019a, b; Rossignolo & Necchi 2016; Necchi et al. 2018; Eloranta 2019). Therefore, it seems likely that with more study, the number of recognized species from Africa will grow and could easily double or triple based on the numbers from these other studies.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at http://

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FIGURE LEGENDS

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Fig. 1. Map of freshwater red algae reported from Africa with the taxonomy updated as per the results of this study. Symbols with numbers are multiple reports or specimens and symbols with "*" are reports from a country without locality information. More detailed information in Tables 1, 2 and Supplementary Table S1. Fig. 2. Maximum likelihood (ML) phylogeny showing relationships of specimens from Africa with all other genera and select species of Batrachospermales based on rbcL and COI-5P concatenated sequence data. Support values shown as ML bootstrap / posterior probability from Bayesian inference. Dashes (-) indicate support <80% bootstrap / 0.8 posterior probability. Outgroup taxa of Thoreales (Thorea hispida, Thorea okadae, Nemalionopsis shawii) not shown in tree. GenBank accession numbers for included taxa given in Supplementary Tables S1, S2. Scale represents substitutions per site. Fig. 3. Maximum likelihood (ML) phylogeny showing relationships of specimens from Africa with all other species of Kumanoa based on rbcL sequence data. Support values shown as ML bootstrap / posterior probability from Bayesian inference. Dashes (-) indicate support <80% bootstrap / 0.8 posterior probability. Outgroup taxa and GenBank accession numbers not shown in tree but are included in Supplementary Table S3. Scale represents substitutions per site. Fig. 4. Maximum likelihood (ML) phylogeny showing relationships of specimens from Africa with all other species of *Sheathia* based on rbcL sequence data. Support values shown as bootstrap / posterior probability from Bayesian inference. Dashes (-) indicate support <80% bootstrap / 0.8 posterior probability. Outgroup taxa and GenBank accession numbers not shown in final tree but are included in Supplementary Table S4. Scale represents substitutions per site.

- Fig. 5. Maximum likelihood (ML) phylogeny showing relationships of specimens from Africa
- with all other species of *Sirodotia* based on *rbc*L sequence data. Labelling of specimens of
- 688 Sirodotia sp. follows Paiano & Necchi 2013. Support values shown as bootstrap / posterior
- probability from Bayesian inference. Dashes (-) indicate support <80% bootstrap / 0.8 posterior
- 690 probability. Outgroup taxa and GenBank accession numbers not shown in final tree but are
- included in Supplementary Table S5. Scale represents substitutions per site.
- Figs 6-17. Morphology of new species in *Kumanoa*, *Sheathia* and *Sirodotia*.
- 693 Figs 6-9. Morphology of Kumanoa bouwmanii sp. nov.,
- Fig. 6. Elongate-obconic whorls with large dense carposporophytes (arrows) that are as high as
- the whorl. Scale = $150 \mu m$.
- 696 Fig. 7. Fascicles with spermatangia at tips (arrows). Scale = 20 μm.
- Fig. 8. Carpogonia with club-shaped trichogynes (arrows) on compact, twisted carpogonial
- branch (arrowhead). Scale = $20 \mu m$.
- 699 Fig. 9. Carposporophyte with sub-spherical carposporangia (arrows) at the tips of multi-celled
- 700 gonimoblast filaments. Scale = $20 \mu m$.
- 701 Figs 10-12. Morphology of *Sheathia murpheyi sp. nov.*
- 702 Fig. 10. Barrel-shaped whorls (arrow) of main axis with no secondary fascicle evident such that
- whorls appear well separated. Scale = $150 \mu m$.
- Fig. 11. Carpogonium with slightly asymmetrical clavate trichogyne (arrow). Scale = $20 \mu m$.
- Fig. 12. Carpogonium with clavate trichogyne (arrow) on carpogonial branch with
- undifferentiated cells (arrowhead). Involucral filament of primary carpogonium with secondary
- carpogonium with fusiform trichogyne (double arrowhead). Scale = $20 \mu m$.

- 709 Figs 13-16. Morphology of Sirodotia kennedyi sp. nov.
- 710 Fig. 13. Reduced obconic whorls (arrow). Scale = $100 \mu m$.
- Fig. 14. Carpogonium with elongate pyriform trichogyne (arrow) with asymmetrical base
- 712 (arrowhead) on few-celled carpogonial branch (double arrowhead). Scale = $20 \mu m$.
- 713 Fig. 15. Carpogonium with irregular trichogyne (arrow) with asymmetrical base (arrowhead) on
- few-celled carpogonial branch (double arrowhead). Scale = $20 \mu m$.
- Fig. 16. Carposporophyte with carposporangia (arrows) on one-celled branch from indeterminate
- 716 prostrate gonimoblast filament (arrowheads). Scale = $20 \mu m$.
- 717 Fig 17. Habit of *Chantransia azurea* [form] *sp. nov.* with highly branched audouinelloid
- 718 filaments twining around gametophyte of *Batrachospermum* sp. Scale = $50 \mu m$.
- 720 Supplemental Figures and Tables.

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- Fig. S1. Maximum likelihood (ML) phylogeny showing relationships of specimens from Africa
- with all other genera and select species of Batrachospermales based on *rbc*L sequence data.
- 723 Support values shown as ML bootstrap / posterior probability from Bayesian inference. Dashes (-
- 724) indicate support <80% bootstrap / 0.8 posterior probability. Outgroup taxa of Thoreales
- 725 (Thorea hispida, Thorea okadae, Nemalionopsis shawii) not shown in tree. GenBank accession
- numbers for included taxa given in Supplementary Tables S1, S2.
- 727 Fig. S2. Maximum likelihood (ML) phylogeny showing relationships of specimens from Africa
- with all other genera and select species of Batrachospermales based on COI-5P concatenated
- sequence data. Support values shown as ML bootstrap / posterior probability from Bayesian
- inference. Dashes (-) indicate support <80% bootstrap / 0.8 posterior probability. Outgroup taxa

- of Thoreales (*Thorea hispida, Thorea okadae, Nemalionopsis shawii*) not shown in tree.
- 732 GenBank accession numbers for included taxa given in Supplementary Tables S1, S2.

Table 1. Freshwater red algae previously reported in the literature and an unpublished manuscript by Pocock dated 1966^a. Nomenclature updated per AlgaeBase (Guiry & Guiry 2019).

Taxon	Country	Reference	
Acrochaetiales Audouinella chalybea (as Chantransia chalybea)	South Africa	Pocock (1966)	
Audouinella hermannii (as Audouinella violacea)	Chad	Compère (1975)	
Batrachospermales Batrachospermum africanum (as B. afrikanum)	South Africa	Rabenhorst (1855)	
Batrachospermum bohneri	Cameroon	Schmidle (1899)	
Batrachospermum breutelii (as B. dillenii)	South Africa	Rabenhorst (1855); Pocock (1966); Sheath & Whittick (1995)	
Batrachospermum gelatinosum (as B. moniliforme)	Madagascar	Fritsch (1914)	
Batrachospermum patens	South Africa	Suhr (1840)	
Batrachospermum turfosum (as B. vagum)	Ivory Coast, Nigeria Alika & Akoma (2012); D (1999); Ewebiyi <i>et al.</i> (201		
Batrachospermum sp.	Madagascar	Bourrelly (1964); Bixby <i>et al</i> . (2009)	
'Chantransia' sp.	South Africa	Pocock (1966)	
Kumanoa gracillima (as Batrachospermum gracillimum)	Angola, South Africa	West & West (1897); Pocock (1966)	
Kumanoa iriomotensis	South Africa	Vis et al. (2012)	
Lemanea fluviatilis	Algeria	Montagne (1846)	
Nothocladus afroaustralis	Madagascar	Skuja (1964)	

Sirodotia huillensis (as Batrachospermum huillense)	Angola, Madagascar, Réunion (France), South Africa	West & West (1897); Fritsch (1914); Pocock (1966); Bourrelly & Couté (1986b)	
Sirodotia aff. huillensis	South Africa	Lam et al. (2012)	
Sirodotia suecica	South Africa	Lam et al. (2012)	
Sirodotia sp.	Madagascar	Bourrelly (1964)	
Torularia atra (as Batrachospermum dillenii)	South Africa	Pocock (1966)	
Torularia puiggariana (as Batrachospermum angolense, B. nigrescens)	Angola	West & West (1897)	
Tuomeya americana	South Africa	Borge (1928)	
Tuomeya sp.	Madagascar	Bourrelly (1964)	
Ceramiales			
Bostrychia moritziana	South Africa	Pocock (1966)	
Bostrychia scorpioides	South Africa	Pocock (1966)	
Caloglossa leprieurii	South Africa, Madagascar, Mauritius	Bourrelly (1964); Bourrelly & Couté (1986b); Pocock (1966)	
Caloglossa ogasawaraensis	Madagascar	Bourrelly (1964)	
Compsopogonales			
Compsopogon caeruleus	Algeria, Mauritius	Montagne (1846); Bourrelly & Couté (1986b)	
Hildenbrandiales		,	
Hildenbrandia angolensis	Angola	West & West (1897)	
<i>Hildenbrandia rivularis</i> Thoreales	DR Congo	Kumano (2002) ^b	
Thorea violacea **Blanch light of manuscript December 1. Manuscript	Réunion (France)	Bory (1808); Bourrelly & Couté (1986a)	

^aUnpublished manuscript – Pocock, M. 1966 "Freshwater Algae in Southern Africa."

^bReported in Kumano (2002), but no original reference was found.

Table 2. Specimens of freshwater red algae of the order Batrachospermales from herbaria. Specimens examined microscopically in italics. If the specimen was not examined, the nomenclature updated per AlgaeBase (Guiry & Guiry 2019).

Name on Specimen label ^a	Identification or nomenclature update	Country	Herbarium specimen(s) ^b
Batrachospermum africanum	chlorophyte	South Africa	BR (1 specimen), <i>PC0473751-PC0473753</i> , <i>FH00776984</i>
Batrachospermum breutelii	Batrachospermum breutelii	South Africa	PC0658124-PC0658126
Batrachospermum cayennense	Visia sp.	Ivory Coast	PC0473719
Batrachospermum dillenii	Torularia atra	South Africa	GRA9933
Batrachospermum gracillimum	Kumanoa gracillima	DR Congo, South Africa	BR (1 specimen), GRA9934
Batrachospermum guyanense	Kumanoa cf. montagnei	Liberia	MICH642679, MICH642680
Batrachospermum jadini ^c	Kumanoa sp.	Mauritius, Reunion (France)	PC0473640-PC0473652
Batrachospermum libericum ^c	Visia sp.	Liberia	MICH642707, MICH642708
Batrachospermum moniliforme	B. gelatinosum	Liberia	FH00778104 ^d
Batrachospermum moniliforme	Kumanoa spp.e	DR Congo, Liberia	FH00778102, FH00778103, FH00778105
Batrachospermum mucronatum ^c	Kumanoa sp.	Liberia	MICH642719, MICH642720
Batrachospermum patens	Torularia atra	South Africa	PC0473547
Batrachospermum proximum ^c	Kumanoa cf. bouwmanii	Liberia	MICH642925

Batrachospermum pulchrum	Batrachospermum pulchrum	R Congo	PC0473541
Batrachospermum velatocarpum ^c	Kumanoa cf. bouwmanii	R Congo	PC0473407
Batrachospermum radians	Batrachospermum gelatinosum	DR Congo	BR (4 specimens)
Batrachospermum stephensii ^c	Visia sp.	Mauritius, Reunion (France)	PC0658087, PC0658088
Batrachospermum torridum	Kumanoa sp.	R Congo, DR Congo, Ivory Coast, Mauritius, Reunion (France)	BR (10 specimens), PC0473470-0473474
Batrachospermum vagum	Batrachospermum turfosum	South Africa	GRA9936
Batrachospermum vanderystii ^c	Batrachospermum sp.	DR Congo	BR (1 specimen)
Batrachospermum sp.	Batrachospermum sp.	DR Congo, Madagascar, South Africa	BR (3 specimens), GRA9935, GRA9937
Lemanea sp.	Paralemanea sp.	Ethiopia	BR (1 specimen), MICH661437
Sirodotia huillensis	Sirodotia huillensis	DR Congo, South Africa	BR (3 specimens)
Sirodotia huillensis/ Batrachospermum dillenii	Sirodotia huillensis	R Congo ^f , Ivory Coast, Madagascar	PC0473382-PC0473390
Sirodotia plumula ^c	Sirodotia sp.	DR Congo, Ivory Coast	BR (4 specimens), PC0473381
Sirodotia angolensis	Torularia puiggariana	DR Congo, Liberia	BR (2 specimens) MICH672223
Sirodotia huillensis	Torularia puiggariana	Liberia	MICH672224

Torularia or Sirodotia sp.

^aPlease note the changes of the names applied to a specific specimen does not denote synonym of names; all nomenclatural changes are in the nomenclatural changes section.

^bHerbaria abbreviations as follows: BR = Meise Botanic Garden, GRA = Selmar Schonland Herbarium, PC = Muséum National d'Histoire Naturelle, FH = Harvard University, MICH = University of Michigan Herbarium.

^cManuscript name or new combination by Skuja, not validly published.

^dFH00778104 was examined but the material was too degraded to identify whether it is a freshwater red alga; the nomenclature is only updated.

eThese three specimens represented different collections and although they could not be identified to species, it was clear that FH00778102 was a different species than the other two based on carpogonium length being much longer and the carposporophyte being loose instead of dense.

fIt is unclear from the herbarium sheet if the record is from R Congo or DR Congo but the sheet stated "Congo."













