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Short Communication

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First report of any species of the red algal order Nemaliales from mainland Ecuador: *Neoizziella asiatica* (Liagoraceae, Rhodophyta)

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Abstract: The benthic marine algae of the mainland coast of Ecuador are poorly known mainly due to a lack of collections. Currently, DNA barcoding is the preferred method to identify species of benthic marine algae worldwide, as morpho-anatomical characters are inadequate to distinguish many species of macroalgae. We used the red algal barcode *rbc*L-3P to identify specimens collected in January 2020 from Manabí, Ecuador as *Neoizziella asiatica*. This is the first member of the red algal order Nemaliales to be reported from the mainland coast of Ecuador and extends the distribution of this alga by 1,100 km to the south from Panama.

Keywords: DNA barcoding; Ecuador; *Neoizziella asiatica*; range extension; *rbc*L.

The first comprehensive treatment of the marine macroalgal flora of Ecuador was by Müller-Gelinek and Salazar (1996), including taxonomic keys to the marine green, brown and red algae. These algae were identified using morphological characters visible to the naked eye as well as anatomical characters examined by light microscopy. The vast majority of the taxa in this treatment were from the Galápagos Archipelago, with only a handful of historical records from mainland Ecuador. More recently Cárdenas-Calle et al. (2020) produced a checklist based on their comprehensive survey of the marine invertebrates and algae from rocky intertidal and subtidal habitats along the mainland coast of Ecuador. They reported 37 green, 13 brown and 78 red algal species that were identified using the morpho-anatomical characters from the taxonomic keys in Müller-Gelinek and Salazar (1996).

Since the mid-2000s DNA barcoding has become a preferred method worldwide to identify marine macroalgae (Bartolo et al. 2020; McDevit and Saunders 2009; Saunders and Kucera 2010; Torrano-Silva et al. 2018; Vieira et al. 2021). Many species of marine macroalgae lack diagnostic morpho-anatomical characters (Gabrielson et al. 2018; Hind et al. 2015; Vieira et al. 2014) and/or are morphologically plastic (Gao et al. 2016; Hind et al. 2014; Lewis et al. 1987), so that accurate species identifications are impossible. Therefore, DNA barcoding was used to identify the macroalgae collected along mainland coast of Ecuador in January 2020. Herein we report the first record of *Neoizziella asiatica* Showe M.Lin, S.-Y.Yang *et* Huisman from the mainland coast of Ecuador, a range extension of 1,100 km from previous reports of this species from Costa Rica (Costa et al. 2016) and Panama (Freshwater et al. 2017).

Two specimens that were not reproductive (Figure 1) were collected by hand in the low and mid-intertidal zones of Ciriales (1°28'53.2554"S, 80°47'6.3594"W), a rocky beach south of the small town of Machalilla, in the Province of Manabí, Ecuador. Voucher specimens were pressed in the field and a fragment was removed, patted dry and placed into silica gel desiccant. Duplicate voucher specimens from the same individual were deposited in NCU (herbarium acronyms follow Index Herbariorum online, Thiers 2021) and at Universidad San Francisco de Quito. See Table 1 for collection data and GenBank accession numbers.

Total genomic DNA was extracted and amplified from the field-collected specimens at the University of North Carolina Chapel Hill using the method in Hughey et al. (2001). Plastid encoded *rbc*L was amplified in two sections using the primer pairs F57/R1150 and F753/RrbcS start

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Figure 1: Habit of freshly collected specimen #E341 of *N. asiatica* collected from Manabí, Ecuador. Scale numbers in cm.

(Freshwater and Rueness 1994), with the final sequences trimmed at the 3' end to base pair (bp) 1467, the terminus of the *rbc*L gene in red algae. PCR products were cleaned with the Qiagen PCR Purification Kit, cycle sequenced, and sent to the DNA Analysis Core Facility at the Marine Sciences Center, University of North Carolina Wilmington for final sequencing. Sequences were manually aligned and compiled using Sequencher 5.4.6 (Gene Codes Corp., Ann Arbor, Michigan, USA).

The *rbc*L tree included the two sequences from the mainland of Ecuador along with 14 others from the family Liagoraceae downloaded from GenBank (Table 1), including species of *Akalaphycus* Huisman, I.A.Abbott *et* A.R.Sherwood, *Titanophycus* Huisman, G.W.Saunders *et* A.R.Sherwood and *Macrocarpus* Showe M.Lin, Y.Yang *et* Huisman that were shown to be most closely related to *Neoizziella* Showe M.Lin, Y.Yang *et* Huisman by Lin et al. (2011). Two more distantly related species in Liagoraceae, *Dermonema virens* (J. Agardh) Pedroche *et* Avila Ortíz and *Liagora albicans* J.V.Lamouroux were used as outgroups. Sequences ranged in length from 694–1,387 bp. The dataset was compiled and aligned using MUSCLE (Edgar 2004) as implemented in Geneious Prime (2020.2.4, Biomatters, Auckland, New Zealand).

A BLAST analysis of GenBank sequences indicated that the *rbc*L sequences of two specimens from mainland Ecuador were 99.63–100% matches to *N. asiatica*. Both phylogenetic analyses gave the same phylogram, and only the Bayesian is presented (Figure 2) with both the posterior probabilities (PP) and bootstrap percentages (BP) at the nodes. The analyses of the *rbc*L sequences placed the field-collected specimens of *N. asiatica* (Figure 1) in a clade with other specimens of this species, including the holotype specimen from Taiwan (Figure 2). The Ecuadorean sequences were identical to each other and to the specimens from Panama, Costa Rica and Indonesia and differed from the specimen from Western Australia by 1 bp, from New Caledonia by 4 bp, from the Philippine Islands by 2 bp, and from the holotype from Taiwan by 3 bp.

This is the first report from mainland Ecuador of any taxon in the red algal order Nemaliales, an order commonly found in subtropical and tropical waters. Scant attention has been paid to the benthic marine algae of mainland Ecuador, with only Taylor (1945) collecting at three sites before proceeding to the Galápagos Archipelago followed by the recent checklist of Cárdenas-Calle et al. (2020). This absence of representatives of Nemaliales is due primarily to lack of collecting. Many taxa of Nemaliales can readily be identified as belonging to this order, even if they are incorrectly identified to genus or species. These are not cryptic taxa at the ordinal rank.

Neoizziella was one of four genera segregated in the last 20 years from *Liagora sensu lato*. These genera (*Macrocarpus*, *Neoizziella*, *Titanophycus* and *Yoshizakia* Showe M.Lin, Huisman *et* Payri) were distinguished based on a combination of DNA sequences and morpho-anatomy of female post-fertilization development (Lin et al. 2011, 2013). *Neoizziella* contained two species, the generitype, *N. asiatica* and *N. divaricata* (C.K.Tseng) Showe M.Lin, S.-Y.Yang *et* Huisman. The former was reported from the tropical eastern Indian Ocean (Bali, Indonesia) and subtropical and tropical western Pacific Ocean (Taiwan and Cebu Island, Philippines); the latter from subtropical (Taiwan) and tropical (Hainan) islands in the western Pacific Ocean. The range of *N. asiatica* was expanded eastward to New Caledonia in the tropical western Pacific Ocean (Lin et al. 2013) and westward to Madagascar in the western Indian Ocean (Vieira et al. 2021).

Costa et al. (2016) were the first to report *N. asiatica* from the Eastern Tropical Pacific (Costa Rica), followed by Freshwater et al. (2017) from Punta Burica, Panama, just across the border from Costa Rica. Finding *N. asiatica* at Manabí, Ecuador extends the range of this species 1,100 km further south in this region. The west Pacific specimens of *N. asiatica* were found in the very shallow subtidal, 1–2 m

Table 1: List of taxa with herbarium accession number or collection number (indicated by a # before the number), collection data, and GenBank accession numbers for *rbc*L sequences used in this paper.

Taxon name	Herbarium accession or collection no.	Collection data	*rbc*L GenBank accession no.
Akalaphycus 'liagoroides'	UNB GWS002064	Leeward side of Ball's Pyramid, Lord Howe Island, Australia, 1.ii.2004, leg. G. T. Kraft	KC134343
Akalaphycus 'setchelliae'	#NTOU16042005As	Xiao Gang Kuo, Kenting National Park, Pingtung Co., Taiwan, 16 April 2005, no habitat data, 9.iii.2008, no habitat data, leg. S.-M. Lin	KP238491
Dermonema 'virens'	#NTOU16iii08LKC	Lung Keng, Pingtung Co., Taiwan, 9.iii.2008, no habitat data, leg. S.-M. Lin	HQ901787
Liagora 'albicans'	#NTOU28iv06MBS	Feng Chui Sha, Pingtung Co., Taiwan, 27.iv.2006, no habitat data, leg. S.-M. Lin	HQ901786
Macrocarpus 'perenni's	#NTOU23xii07WLD125	Wan Li Tong, Pingtung Co., Taiwan, 23.xii.2007, no habitat data, leg. S.-M. Lin	HQ901783
Neoizziella asiatica	NCU 674485 (MB 341b) #USFQ MB 341a	Ciriales, Manabí, Ecuador, 16.i.2020, mid-intertidal, epilithic, leg. A. Eguiguren *et* M. Brandt	OK430878
Neoizziella asiatica	NCU 674486 (MB 362a) #USFQ MB 362b	Ciriales, Manabí, Ecuador, 16.i.2020, low intertidal on mollusk, leg. A. Eguiguren *et* M. Brandt	OK430879
Neoizziella asiatica	WNC 20120-s060	Near Mono Feliz, Punta Burica, Chiriquí, Panama (Pacific), 9.i.2011, epilithic in intertidal pool, leg. C. Fernandez-Garcia *et* Neomi Leon	KY573990
Neoizziella asiatica	MELU SAMEA4358434	Costa Rica, no date, no habitat data, no collector	LT622872
Neoizziella asiatica	#CP08-309	Teti, New Caledonia, 9.xi.2008, no habitat data, leg. C. Payri *et* S.-M. Lin	JX878371
Neoizziella asiatica	#NTOU7xi01Cebu	Cebu, Philippines, 7.xi.2002, no habitat data, leg. S.-M. Lin	HQ901777
Neoizziella asiatica	#NTOU23xii07abcWLD	Won Li Tong, Pingtung Co., Taiwan, 23.xii.2007, no habitat data, leg. S.-M. Lin	HQ901778
Neoizziella asiatica	UNB S9/K10-G	Cassini Island, West Australia, Australia, 25.x.2010, leg. J. Huisman	JX878370
Neoizziella asiatica	#NTOU24vii08Bali_Na	Sarangan Island, Bali, Indonesia, 24.vii.2008, no habitat data, leg. S.-M. Lin	HQ901789
Neoizziella 'divaricata'	#NTOU12ii09HK128	Hai Kou, Pingtung Co., Taiwan, 12.ii.2009, no habitat data leg. S.-M. Lin	HQ901782
Titanophycus 'setchellii'	#CP08-308	Teti, New Caledonia, 9.ix.2008, no habitat data, leg. C. Payri *et* S.-M. Lin	JX878372
Titanophycus 'validus'	#23	French Bay, San Salvador, Bahamas Islands, 26.iii.2006, no habitat data, leg. M. H. Hommersand	GU357692

Herbarium acronyms follow Thiers (2021). Specific epithets in single quotes ' ' have not had their type specimens sequenced. *Neoizziella asiatica* specimens are arranged from east to west.

deep; east Pacific specimens were all collected from mid-to low intertidal habitats, including a tidal pool, on bedrock or on a mollusk shell.

Lin et al. (2011) distinguished the two recognized *Neoizziella* species, *N. asiatica* and *N. divaricata* by DNA sequences and characters of gross morphology, i.e., thallus height (4–6 cm vs. 3–4 cm), number of orders of branches (7–9 vs. 3–5) and the shape of distal ends of branches (blunt versus tapered). Morphologically, the tropical east Pacific specimens of *N. asiatica*, including those from Ecuador, are intermediate in size between the two species (3–5 cm), have the number of orders of branches of *N. divaricata* (3–5), but the ends of distal branches are blunt as in *N. asiatica* (Figure 1). Thus, to correctly assign tropical east Pacific specimens of *Neoizziella* to a species requires DNA sequences.

All of the *N. asiatica rbc*L sequences from the east Pacific are identical to the sequence from Bali, Indonesia. They differ from the other west Pacific specimens by 1–4 bp (0.07–0.36% sequence divergence). It is unknown whether *N. asiatica* is a recent introduction to the east Pacific or if it has had a long historical presence but was only recently recognized based on DNA barcoding. *Hommersandiophycus borowitzkae*, another taxon in Liagoraceae, likewise was known only from the Indo-west Pacific (Taiwan, The Philippines, Western Australia) (Lin et al. 2014) until reported from Panama by Freshwater et al. (2017). It is likely that additional Liagoraceae will be found along the mainland coast of Ecuador.

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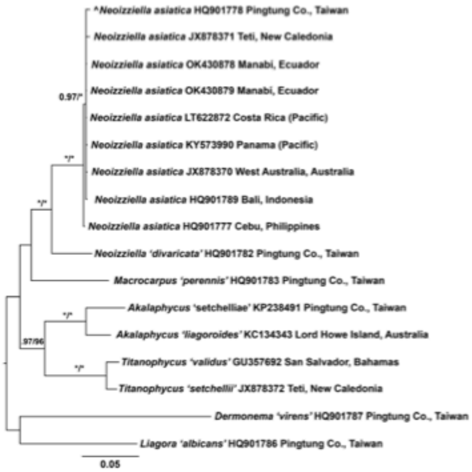

Figure 2: Bayesian phylogram of the *rbc*L sequences showing placement of the two Ecuadorean specimens in *Neoizziella asiatica* with full support. The *rbc*L sequence of the holotype of *N. asiatica* is indicated by a ^. Single quotes indicate species whose type specimens have not been sequenced. Support values at phylogram nodes are posterior probabilities (PP) followed by bootstrap percentages (BP). * at nodes indicate full support; support values below 0.8 PP and 80% BP are not shown. Phylogenetic reconstructions with maximum likelihood (ML) and Bayesian inference (BI) for both data sets were carried out using the RAxML (Stamatakis 2014) and Mr. Bayes (Huelsenbeck and Ronquist 2001) Geneious prime plugins, respectively. The RAxML analyses were performed using the GTR CAT I model and rapid hill-climbing algorithm for 20 random trees to determine the best starting tree for determining node confidence. Node confidence was assessed by 1,000 bootstrap replications and search for best-scoring ML tree. Bayesian analyses were performed using the GTR model, gamma rate variation, a proportion of invariant sites model with 4 heated Monte-Carlo Markov chains for 1,000,000 generations, sampling every 750 generations and with a burn-in length value of 250,000 generations.

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References

Bartolo, A.G., Zammit, G., Peters, A.F. and Küpper, F.C. (2020). The current state of DNA barcoding of macroalgae in the Mediterranean Sea: presently lacking but urgently required. Bot. Mar. 63: 253–272.

Cárdenas-Calle, M., Mora, E., Torres, G., Pérez-Correa, J., Bigatti, G., Signorelli, J. and Coronel, J. (2020). Marine invertebrate and seaweed biodiversity of continental coastal Ecuador. Biodivers. Data J. 8: e53818.

Costa, J., Lin, S.-M., Macaya, E., Fernández-García, C. and Verbruggen, H. (2016). Chloroplast genomes as a tool to resolve red algal phylogenies: a case study in the Nemaliales. BCM Evol. Bio. 16: 205–218.

Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32: 1792–1797.

Freshwater, D.W.and Rueness, J. (1994). Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species based on *rbc*L nucleotide sequence analysis. Phycologia 33: 187–194.

Freshwater, D.W., Idol, J.N., Parham, S.L., Fernández-García, C., León, N., Gabrielson, P.W. and Wysor, B.L. (2017). Molecular assisted indentification reveals hidden red algae diversity from the Burica Peninsula, Pacific Panama. Diversity 9: 19.

Gabrielson, P.W., Hughey, J.R. and Diaz-Pulido, G. (2018). Genomics reveals abundant speciation in the coral reef building alga *Porolithon onkodes* (Corallinales, Rhodophyta). J. Phycol. 54: 429–434.

Gao, G., Zhong, Z., Zhou, X., and Xu, J. (2016). Changes in morphological plasticity of *Ulva prolifera* under different environmental conditions: a laboratory experiment. Harmful Algae 59: 51–58.

Hind, K.R., Gabrielson, P.W., Lindstrom, S.C., and Martone, P.T. (2014). Misleading morphologies and the importance of sequencing type specimens for resolving coralline taxonomy (Corallinales, Rhodophyta): *Pachyarthron cretaceum* is *Corallina officinalis*. J. Phycol. 50: 760–764.

Hind, K.R., Miller, K.A., Young, M., Jensen, C., Gabrielson, P.W., and Martone, P.T. (2015). Resolving cryptic species of *Bossiella* (Corallinales, Rhodophyta) using contemporary and historical DNA. Am. J. Bot. 102: 1–19.

Huelsenbeck, J.P. and Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Hughey, J.R., Silva, P.C., and Hommersand, M.H. (2001). Solving taxonomic and nomenclatural problems in Pacific Gigartinaceae (Rhodophyta) using DNA from type material. J. Phycol. 37: 1091–1109.

Lewis, S.M., Jorris, J.N., and Seales, R.B. (1987). The regulation of morphological plasticity in tropical reef algae by herbivory. Ecology 68: 636–641.

Lin, S.-M., Yang, S.-Y., and Huisman, J.M. (2011). Systematics of *Liagora* with diffuse gonimoblasts based on rbcL sequences and carposporophyte development, including the description of the new genera *Neoizziella* and *Macrocarpus* (Liagoraceae, Rhodophyta). Eur. J. Phycol. 46: 249–262.

Lin, S.-M., Huisman, J.M., and Payri, C. (2013). Characterization of *Liagora ceranoides* (Liagoraceae, Rhodophyta) on the basis of *rbc*L sequence analyses and carposporophyte development, including *Yoshizakia indopacifica* gen. et sp. nov. from the Indo-Pacific region. Phycologia 52: 161–170.

Lin, S.-M., Huisman, J.M., and Ballantine, D.L. (2014). Revisiting the systematics of *Ganonema* (Liagoraceae, Rhodophyta) with emphasis on species from the northwest Pacific Ocean. Phycologia 53: 37–51.

McDevit, D.C. and Saunders, G.W. (2009). On the utility of DNA barcoding for species differentiation among brown macroalgae (Phaeophyceae) including a novel extraction protocol. Phycol. Res. 57: 131–141.

Müller-Gelenik, H. and Salazar, M. (1996). Algas marinas del Ecuador. Instituto Nacional de Pesca, Guayaquil, Ecuador.

Saunders, G.W. and Kucera, H. (2010). An evaluation of *rbc*L, *tuf*A, UPA, LSU and ITS as DNA barcode markers for the marine green macroalgae. Cryptogam. Algol. 31: 487–528.

Stamatakis A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313.

Taylor, W.R. (1945). Pacific marine algae of the Allan Hancock expeditions to the Galapagos islands. Allan Hancock Pac. Exped. 12: 1–528.

Thiers, B. (2021). Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium, Available at: <http://sweetgum.nybg.org/ih/> (Accessed 1 November 2021).

Torrano-Silva, B.N., Vieira, B.R., Riosmena-Rodríguez, R., and Oliveira, M.C. (2018) Guidelines for DNA barcoding of coralline algae, focusing on Lithophylloideae (Corallinales) from Brazil. Bot. Mar. 61: 127–140.

Vieira, C., D'Hondt, S., De Clerck, O. and Payri, C.E. (2014). Toward an inordinate fondness for stars, beetles and *Lobophora*? Species diversity of the genus *Lobophora* (Dictyotales, Phaeophyceae) in New Caledonia. J. Phycol. 50: 1101–1119.

Vieira, C., N'Yeurt, A.De R., Rasoamanendrika, F.A., D'Hondt, A., Thi Thram, L.-A., Van de Spiegel, S., Kawai, H., and De Clerck, O. (2021). Marine macroalgal biodiversity of northern Madagascar: morpho-genetic systematics and implications of anthropic impacts for conservation. Biodivers. Conserv. 30: 1501–1546.

Bionotes

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