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SHORT COMMUNICATION

Adlafia neoniana (Naviculaceae), a new diatom species from forest streams in Puerto Rico

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Background and aims – The samples on which this study is based were collected in two streams in Puerto Rico as part of a long-term monitoring program conducted by the National Ecological Observatory Network (NEON). Detailed study of the diatom assemblages revealed the occurrence of a new diatom species belonging to the genus *Adlafia*.
Methods – The study considered 82 samples of epilithic diatoms from two streams in Puerto Rico (Rio Cupeyes and Rio Guilarte) subjected to different degrees of anthropogenic impacts. The new species description is based on high-quality Nomarski (DIC) LM micrographs which document the full spectrum of shape variability along the size diminution series (including girdle views), and SEM images documenting valve ultrastructure (including detailed internal views). In addition we provide information about ecological preferences with respect to trophic state / nutrients, habitat within the stream channel / microhabitat (riffle, run, pool), and season of maximum occurrence.
Key results – The new species is described as *Adlafia neoniana* Cantonati sp. nov. It resembles *A. drouetiana* but has lanceolate (instead of triundulate) outline, slightly-subcapitate to subrostrate ends (instead of subcapitate to capitate), and higher stria density. It is also similar to *A. muscora* but differs by elliptic-lanceolate (instead of linear-lanceolate) outline, broader valves, and higher stria density. Moreover, the new species was most abundant in an undisturbed, oligotrophic, shaded forest stream whilst *A. drouetiana* is known from Brazil to be tolerant to organic pollution and eutrophication.
Conclusions – *Adlafia neoniana* can be differentiated from other similar species in the light microscope by recognizable characters or character combinations. Its discovery and characterization is a contribution to the knowledge of the still understudied biodiversity of microorganisms in tropical streams.

Key words – Tropical diatoms, *Adlafia*, *Adlafia neoniana*, *Adlafia drouetiana*, *Adlafia muscora*, morphology, new species, ecology, streams, Puerto Rico.

INTRODUCTION

Adlafia Lange-Bert. (in Moser et al. 1998) is a relatively small (26 species listed in DiatomBase, Kociolek et al. 2018) freshwater, isovalvar, isopolar, bilaterally symmetrical ("naviculoid") genus (Jüttner et al. 2018). According to Lange-Bertalot (in Moser et al. 1998) and Lange-Bertalot (2001), the genus typically includes small species (< 25 µm); cells are solitary, with linear to linear-lanceolate outline, and rostrate, subcapitate, or wedge-shaped ends; the raphe is filiform, with at most slightly-expanded central pores, and

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Table 1 – Location, physical and hydrochemical characteristics of the two study streams.
min-max, average (in bold), and N (= total number of available data points). All data available for the study period (2015–2016) were downloaded from https://www.neonscience.org/.

Parameters	Cupeyes	Guilarte
Longitude	66°59′12.30″W	66°47′54.73″W
Latitude	18°06′48.65″N	18°10′26.77″N
Elevation (m a.s.l.)	166	548
Discharge (l/s)	23.1–658.1, **151.3** (29)	72.2, 688.6, **230.1** (25)
Temp. (ºC)	21.3–25.0, **23.2** (45)	12.9–22.8, **20.6** (40)
Dissolved oxygen (%)	87–100, **94** (23)	9–107, **94** (21)
pH	7.80–8.30, **8.08** (12)	7.47–7.92, **7.76** (11)
Conductivity (µS.cm^{-1})	204–400, **271** (12)	125–286, **202** (11)
Alkalinity (meq/l)	1.90–3.75, **2.63** (45)	1.04–2.34, **1.71** (39)
Nitrate + Nitrite N (µg/l)	240–348, **290** (12)	380–613, **493** (11)
Total Dissolved N (µg/l)	126–436, **352** (12)	458–704, **583** (11)
Total Dissolved P (µg/l)	0–9, **2** (12)	1–65, **19** (11)
Silica (mg/l)	0.01–19.99, **14.50** (12)	0.01–15.30, **10.56** (11)
Sulphate (mg/l)	0.53–4.12, **2.80** (12)	1.52–5.61, **4.67** (11)
Chlorine (mg/l)	1.72–10.08, **5.71** (12)	0.45–7.29, **5.56** (11)
Calcium (mg/l)	2.65–6.23, **4.09** (12)	15.12–33.10, **25.55** (11)
Magnesium (mg/l)	21.74–42.08, **31.53** (12)	3.16–6.92, **5.50** (11)

terminal fissures strongly curved unilaterally (Cantonati et al. 2017); the axial area is very narrow, the central area is variable but never reaching the margins; the dense striae are radiate in the central part, becoming abruptly convergent towards the ends; under the SEM, the uniseriate striae proceed uninterrupted from the valve face to the mantle, the areolae are closed by hymenes outside, and the narrow girdle of each theca is formed by two copulae, each with a biseriate row of poroids; the genus is named after the Association of French Speaking Diatomists (ADLAF - Association des Diatomistes de LAngue Française).

Some species are widely distributed, and apparently cosmopolitan, e.g. *Adlafia bryophila* (J.B.Petersen) Gerd Moser, Lange-Bert. & Metzeltin (e.g. Metzeltin & Lange-Bertalot 1998, Cantonati et al. 2017, Tusset et al. 2017). *Adlafia drouetiana* (R.M.Patrick) Metzeltin & Lange-Bert. is so far known only from Brazilian streams (Tusset et al. 2017). *Adlafia sinensis* Bing Liu et D.M.Williams was described from China (Liu et al. 2017) but was recently found in Brazil (Tusset et al. 2017).

With respect to their ecology, many *Adlafia* species are considered pseudoaerial (e.g. Spaulding & Edlund 2009), and typically colonize alkaline, oligotrophic, headwater streams (e.g. Spaulding & Edlund 2009, Liu et al. 2017, Tusset et al. 2017). However, they are also found over a wide range of electrolyte content (e.g. *Adlafia lange-bertalotii* occurring in fairly low to intermediate mineralization levels; Monnier et al. 2012). A few taxa colonize environments with higher trophic state (e.g. *Adlafia minuscula* var. *muralis* (Grunow) Lange-Bert. (in Lange-Bertalot & Genkal 1999) which is tolerant of organic pollution up to the transition between α-mesosaprobic and polysaprobic conditions and typical of running waters with high concentrations of nutrients; Cantonati et al. 2017).

Other new species of *Adlafia* were described recently, in particular from streams in Brazil (Tusset et al. 2017), China (Liu et al. 2017), central Europe (Grand-Duchy of Luxembourg, Monnier et al. 2012), the United States (Morales & Le 2005), and Lake Baikal (Russia, Kulikovskiy et al. 2012).

Adlafia drouetiana was described, as *Navicula Drouetiana*, by Ruth Patrick in 1944. In 1998 Ditmar Metzeltin and Horst Lange-Bertalot provided the first SEM image. For many years, well-documented reports remained scarce, and only recently have new data on its morphology (Tusset et al. 2017) and ecology (Heinrich et al. 2014) been published.

The aim of the present paper is to describe and characterize a new *Adlafia* species found in two streams in SW Puerto Rico subjected to different degrees of anthropogenic impacts. Sample material and ecological data for this study are from the USA National Ecological Observatory Network (NEON) program, which regularly surveys the streams. The species description is based on high-quality Nomarski (DIC) LM micrographs of the full spectrum of shape variability along the size diminution series (including girdle views), and SEM images documenting valve ultrastructure (including detailed internal views). In addition we provide ecological data on trophic state / nutrients, within-stream habitat character / microhabitat (riffle, run, pool), and season of maximum occurrence.

MATERIAL AND METHODS

Study streams

The two tropical streams studied in southwest Puerto Rico have low-to-medium discharge, warm water, and medium conductivity (table 1). They are part of NEON (National Ecological Observatory Network) Domain D04, Atlantic Neotropical.

The Rio Cupeyes study site (NEON code: CUPE; Academy of Natural Sciences site code: NFS008, site_id: 207563; nearest city: San German, Puerto Rico, PR, USA) is in the Maricao State Forest and is a NEON aquatic core field site. The Site Host is the Commonwealth of Puerto Rico. The lithology is serpentinite rocks (Cretaceous). The area is made up of old and second-growth tropical, moist and wet forest. It is considered a good example of an increasingly rare intact Puerto Rican stream (https://www.neonscience.org/field-sites/field-sites-map/CUPE). Average total dissolved phosphorus (TDP) is 2 µg/l, and average total dissolved nitrogen (TDN) is 352 µg/l. The average magnesium concentration (31.53 mg/l) is high (table 1).

The Rio Guilarte study site (NEON code: GUIL; Academy of Natural Sciences site code: NFS009, site_id: 207564; nearest city: Adjuntas, Puerto Rico, PR, USA) is a relocatable (= may be re-deployed periodically throughout the 30-year lifetime of the Observatory) NEON aquatic field site. The Site Host is the University of Puerto Rico. The lithology is characterized by the Anon formation: interbedded andesite/volcanic brecia, rhyodacite, and sandstone/siltstone of the Upper Eocene (https://www.neonscience.org/field-sites/field-sites-map/GUIL). Average TDP is 19 µg/l, and average TDN is 583 µg/l (table 1).

Slides studied, microscopy, new species description

The samples on which this study is based were taken at the CUPE and GUIL sites from 20 Jan. 2015 to 20 Dec. 2016 and from 22 Jan. 2015 to 29 Nov. 2016, respectively. All sample information and diatom counts can be downloaded from the NEON Data Portal: http://data.neonscience.org/home. In the counts, the new species described here was called *A. drouetiana*, as counts were carried out before the new species was recognized.

Eighty-two epilithon samples from the Cupeyes (37 samples) and Guilarte (45 samples) streams, collected on 11 sampling dates, were studied. On each sampling date, a primary (5 composite samples) and, usually but not always, a secondary (3 samples) habitat were sampled. Primary habitat was riffle in both streams. Secondary habitat was run in Cupeyes and pool in Guilarte. The sampling stations are long stream reaches (1 km); riffle (and run and pool) samples are taken along this stretch. Three cobbles for each composite sample were collected randomly. A halved 35-mm slide cassette was used as template to delineate sampling area. Periphyton was scraped from the cobbles with a brush (Parker 2018).

Samples were digested following the NAWQA protocols (Charles et al. 2002). The cleaned material was mounted in Naphrax (refractive index of 1.74). At least 600 valves were identified and counted using a Zeiss Ser. N. 800392 (Zeiss,

Table 2 – **Morphometric characteristics of *Adlafia neoniana* Cantonati sp. nov., and comparison with the most similar species, *Adlafia drouetiana* and *A. muscora*.**
When available, min-max, average, and total number of observations are provided (averages are in bold). For the stria density, the mode is provided instead of average.

Species	*Adlafia neoniana*	*Adlafia drouetiana*		*Adlafia muscora*		
Source of information / character	This paper	Tusset et al. (2017)	Patrick (1944)	Kociolek & Reviers (1996)	Lange-Bertalot (2001)	Tusset et al. (2017)
Valve length (µm)	9.4–18.5, **14.5**, n = 51	14.7–20.3	17.4	19.5	13.6–18.7*	10–17.6
Valve width (µm)	3.7–5.1, **4.2**, n = 51	4.0–5.4	4	3.5	3–4*	3.1–4.6
Valve shape	elliptic-lanceolate	linear-lanceolate typically slightly triundulate	linear to lanceolate	elliptic-lanceolate	linear-lanceolate*	elliptic-lanceolate
Apex	rostrate to subcapitate	capitate to subcapitate	rostrate	subrostrate	rostrate to subrostrate*	rostrate to subcapitate
Striae (in 10 µm)	30–32, **32**, n = 77	22–28	29–30	23–26	–	28–32
Distance between central raphe ends (µm)	0.5–1.2, **0.7**, n = 51	0.6–1.2, **0.9**,* n = 10	–	–	0.6–0.7, **0.6**,* n = 5	0.7–1.2, **0.9**,* n = 9
Areolae (in 10 µm)	45–50	30–50	–	–	–	50–62

Jena, Germany) and x1000 magnification (ANSP Drexel PCER Procedure No. P-13-39 Revision 2 02/17). High-quality Nomarski (DIC) LM micrographs were taken using a ANSP microscope Zeiss Akioskop 50 with objective lens Zeiss Plan-Apochromat 100x/1.40 Oil and digital camera Nikon Digital Sight DS Fi1.

Materials (slides, prepared material, and aliquots of the original samples), including the holotype slide, are held at the ANSP Diatom Herbarium (ANSP). Isotype slides and aliquots of prepared material from the same locality and substratum are being deposited in the NEON Biorepository in Arizona, the Diatom Collection of the Natural History Museum (BM) of London (UK), and the Diatom collection of the MUSE - Museo delle Scienze (TR), Trento, Italy. Numbers of specimens measured along the size diminution gradient to obtain ranges and averages (mode) of morphological and ultrastructural features are indicated in table 2. Stria density was assessed on both sides of the same valve in 26 cases out of 51, for a total of 77 measurements.

SEM observations were made primarily at the University of Frankfurt using a Hitachi S-4500 (Hitachi Ltd., Tokyo, Japan) at high vacuum on gold coated stubs. Terminology for valve morphology is based on Round et al. (1990) and Lange-Bertalot (2001).

Statistical analyses

The ecological preferences of *Adlafia neoniana* were studied assessing three factors: river (Cupeyes, Guilarte; df = 1), habitat (riffle, run, pool; df = 2), and season (autumn, winter, and summer; df = 2) . Since the variance was heterogeneous (Bartlett's K-squared = 6.67, df = 1, p-value < 0.01), the three factors were tested separately using a non-parametric statistic. The Kruskal–Wallis test was used instead of the one-way analysis of variance (ANOVA). Since it is a non-parametric method, the Kruskal–Wallis test does not assume a normal distribution of the residuals, and is often used in the case of different sample sizes (Sokal & Rohlf 1995). All the analyses were carried out using R (R Core Team 2018).

RESULTS

Adlafia neoniana Cantonati, **sp. nov.**

Figs 1–2

Type material – Unites States of America, Puerto Rico, Cupeyes Stream, National Forest, south-eastern part of the Island of Puerto Rico, lithology: serpentinites (metamorphic rocks), (18°06′48.65″N, 66°59′12.30″W, 166 m a.s.l.), 19 Feb. 2015 (holo-: ANSP, slide NEON00323 b, partly shown

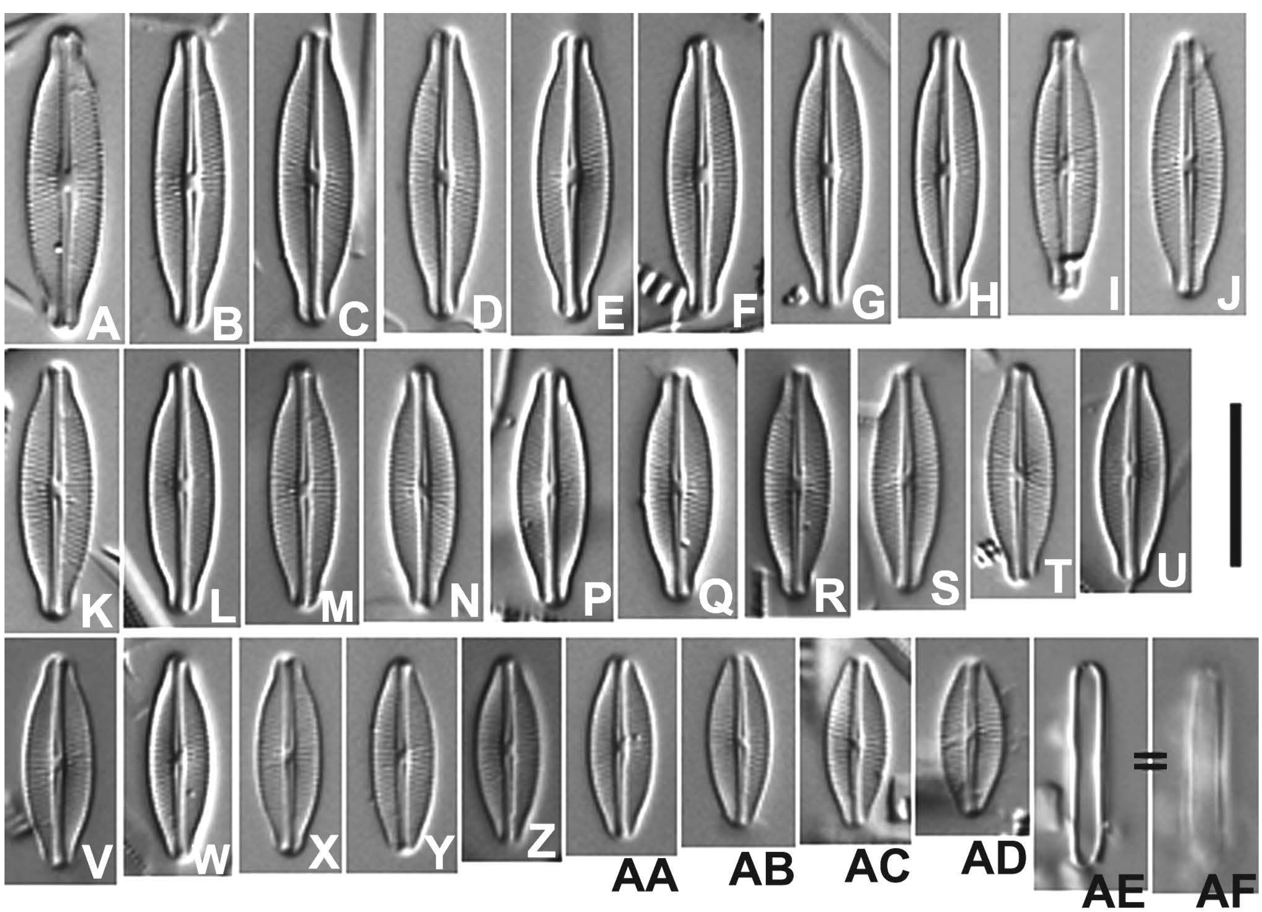

Figure 1 – *Adlafia neoniana*, LM size diminution series from the type locality. Note the rostrate to subcapitate ends and a considerable reduction in cell size: A–AD, valve views; AE–AF, girdle views (the same specimen is shown in two different focal planes). Scale bar = 10 µm.

here in fig. 1; iso-: BM, slide BM-101962; iso-: TR, slide cLIM004 DIAT 3514; iso-: NEON Biorepository, Arizona State University's Natural History Collection in Tempe, AZ, slide NEON00323 a).

Registration – http://phycobank.org/101113

Description: LM – Frustules narrowly rectangular in girdle view; frustule width slightly less than 2 µm (fig. 1AE & AF). Valves elliptic-lanceolate with rostrate to subcapitate apices (fig. 1A–AD). Valve length 9.4–18.5 µm, valve width 3.7–5.1 µm. Axial area narrow linear along the entire length of the valve, central area indistinct. Proximal raphe ends clearly discernible with LM, straight and slight widened. Striae dense, discernible only with good quality optics, 30–32 in 10 µm, radiate, becoming convergent towards the apices.

Description: SEM – Axial area slightly widening towards central area (fig. 2A & B). Raphe with external terminal fissures hooked towards the secondary side; central raphe ends very slightly bent towards the primary side (fig. 2A & B). Striae uniseriate, radiate, becoming abruptly convergent near the valve ends, Striae continuing onto the mantle without interruption (fig. 2A). A few shortened striae present on each side of the central part of the valve (fig. 2A & B). Areolae rounded to rectangular, occluded by hymenes outside (fig. 2D & E). Internally, both proximal raphe ends deflected towards the primary side (fig. 2C & D), distal raphe ends terminating in small helictoglossae (fig. 2E & F).

Etymology – The new species is named after the NEON (National Ecological Observatory Network) programme. This is a 30+ year project dedicated to understanding how environmental change (including climate change) impacts ecosystems at the study sites. It includes > 80 sites from 20 eco-climatic domains representing US ecosystems: Hawaii, Alaska, the continental Unites States, and Puerto Rico. All the materials studied for the present paper were provided by NEON.

Similar taxa – The most similar species is *Adlafia drouetiana*, which has triundulate margins and a lower stria density (22–28 in 10 µm; table 2). *Adlafia sinensis* is also similar, but is typically smaller, and has striae which cannot be counted in LM (32–36 in 10 µm).

Distribution – The wider distribution is not yet known because the species has not been distinguished previously from other *Adlafia* taxa. So far it is known only from the type locality (Rio Cupeyes) and from another stream in Puerto Rico (Rio Guilarte), located 21 km away. It is, however, possible that the the populations from Brazil identified by Tusset et al. (2017) as *A. muscora* also belong to *Adlafia neoniana*, hence it might also occur in several streams and rivers in Brazil.

Adlafia neoniana occurred in 25 out of the 37 samples analyzed from Rio Cupeyes, and in 13 out of the 45 samples from Rio Guilarte. Maximum relative abundance was higher in Rio Cupeyes (6.4%) compared to Rio Guilarte (0.65%).

Only epilithon samples were available for this study but they were collected from different habitats and stream reaches (within the same station). Figure 37 shows the distribution of the species with respect to stream, microhabitat, and season, showing that it was clearly more abundant and frequent in Rio Cupeyes (Kruskal-Wallis chi-squared "streams" = 15.35, d.f. = 1, p < 0.0001). A hypothesized preference for riffles and runs was not statistically significant (Kruskal-Wal-

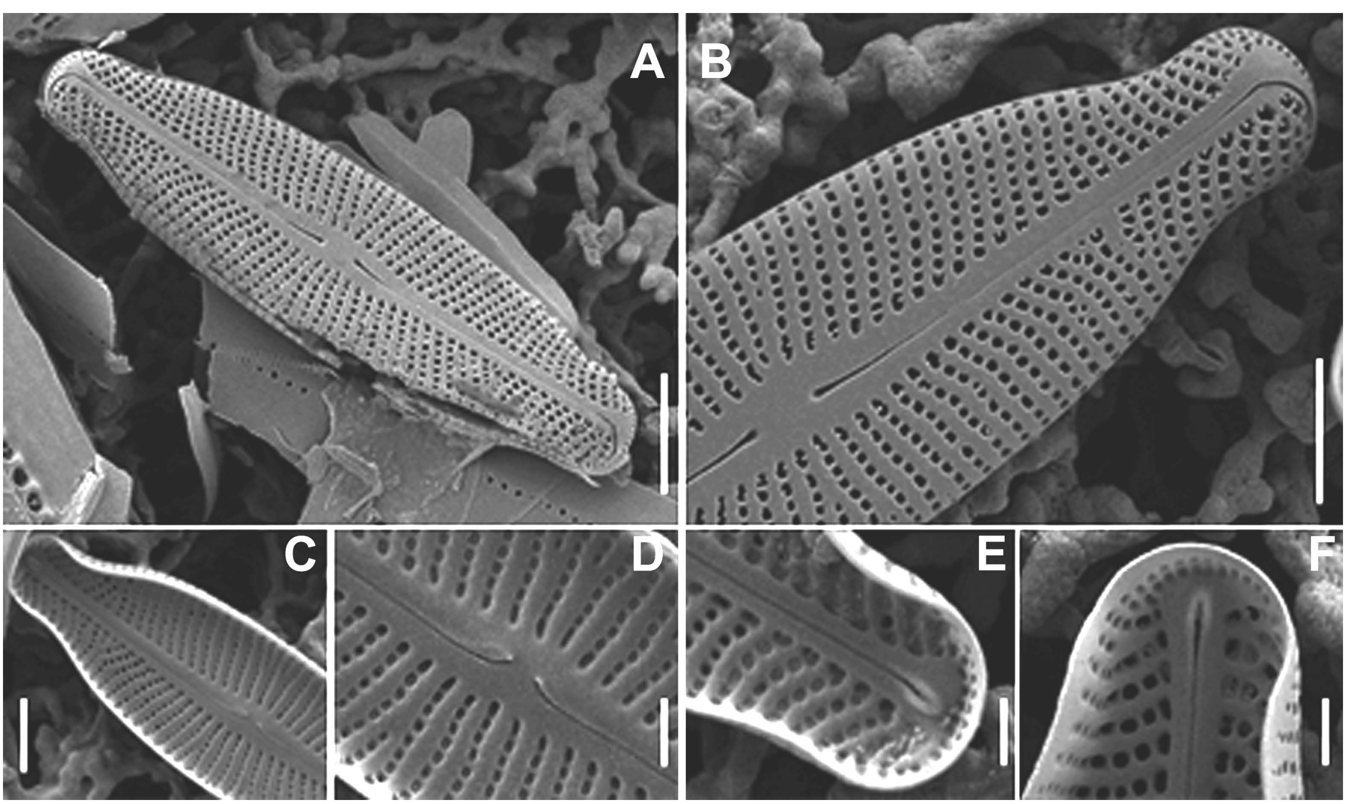

Figure 2 – *Adlafia neoniana*, SEM: A & B, external views; C–F, internal views. Scale bars: A = 3 µm; B & C = 2 µm; D–F = 1 µm.

lis chi-squared = 5.43, d.f. = 2, p = 0.07), or a higher abundance in winter (February) or summer (July) (Kruskal-Wallis chi-squared = 0.42, d.f. = 2, p = 0.81).

Ecology and co-occurring diatom species – The Rio Cupeyes is a shaded, Puerto Rican forest stream of high ecological quality with low to medium discharge and conductivity. This stream differs from the impacted (agriculture in the surroundings) Rio Guilarte, in which the new species was much less abundant, mainly by having lower TDN and TDP values, consistently high oxygen concentrations (% saturation can, on the contrary, drop down to 9% in the Rio Guilarte), and six times higher magnesium concentrations due to the special lithology – serpentinites – of the Rio Cupeyes) (table 1).

The dominant diatom species of the most common genera at the type locality were (species in the Guilarte stream are shown in brackets): *Achnanthidium jackii* Rabenh. [*A. eutrophilum* (Lange-Bert. in Lange-Bertalot & Metzeltin) Lange-Bert.], *Gomphonema* sp. 1 NEON Puerto Rico MC unpublished [*G. kobayasii* Kociolek & Kingston], *Navicula parablis* M.H.Hohn & Hellerman [*N. simulata* Manguin], *Nitzschia paleacea* Grunow in Van Heurck [*Nitzschia* cf. *palea*], *Ulnaria lanceolata* (Kütz.) Compère [*Ulnaria monodii* (Guermeur) Cantonati & Lange-Bert. + *Ulnaria ramesii* (Hérib.) T.Ohtsuka].

DISCUSSION

The established species most similar to our proposed new species *Adlafia neoniana* is *Adlafia drouetiana*, which, however, has: – lower stria density (22–28 in 10 µm vs. 30–32 in 10 µm, with 32 in 10 µm being the mode for *Adlafia neoniana*; table 2); – typically a triundulate outline, as compared to a lanceolate outline in *A. neoniana*; – subcapitate to capitate ends vs. slightly-subcapitate to subrostrate ends in smaller specimens of *A. neoniana*; – larger dimensions, in particular length: 15–20 µm vs. 9–18 µm for *A. neoniana* (table 2); – typically lower areola density: 30–50 in 10 µm vs. 45–50 in 10 µm for *A. neoniana*; – a high tolerance to organic pollution and eutrophication, as shown in subtropical temperate streams (Lobo et al. 2004, as *Adlafia bryophila*, 2010) and headwaters (Heinrich et al. 2014) in Brazil.

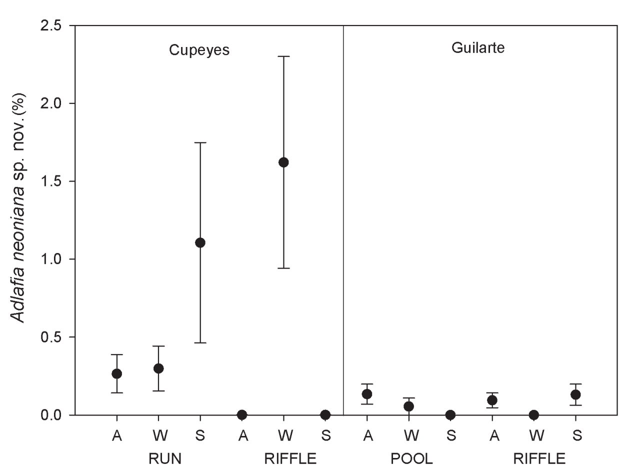

Figure 3 – Relative abundance (%) of *Adlafia neoniana* in the two streams, in the different habitats, and in the different seasons (A = Autumn, W = Winter, and S = Summer).

Since *Adlafia neoniana* was most abundant in the stream on serpentinite rocks, which in Puerto Rico occurs only in the area where the Rio Cupeyes (= type locality of *A. neoniana*) is located (Torres & Quiñones 2003), it might have a preference for increased magnesium concentrations, which is not known for *A. drouetiana*. Preference for higher magnesium contents has been reported in the literature for other diatom species (e.g. *Achnanthidium dolomiticum* Cantonati & Lange-Bert.; Cantonati & Lange-Bertalot 2006, Cantonati et al. 2017).

Other similar species are *Adlafia sinensis* and *Adlafia multnomahii* E.Morales & Le that are typically smaller, and have striae which are uncountable with LM, and *Adlafia bryophila* which is narrower (2.5–4 µm) (e.g. Cantonati et al. 2017).

As concerns *Adlafia muscora* (Kociolek & Reviers) Lange-Bert. in Moser et al. (1998), we think that in the recent publication by Tusset et al. (2017) there might have been some taxonomic drift from the original concept. This is likely to have been caused by the fact that figures in Moser et al. (1998: pl. 6, figs 3–8) display ample morphological variability for *Adlafia muscora*, and these figures are likely to include more than one species. For this reason, we used Lange-Bertalot (2001), instead of Moser et al. (1998), as one of the references for *A. muscora* in table 2.

If only LM micrographs taken from the type slide (i.e. Moser et al. 1998: pl. 6, figs 7 & 8) are considered, it appears clear that the original taxonomic concept for this species refers to linear-lanceolate forms with comparatively low width and stria density (23–26 in 10 µm; Kociolek & Reviers 1996: table 2). If this original concept is kept in mind, Lange-Bertalot's (2001) concern that *Adlafia muscora* is difficult to distinguish from *A. bryophila* appears to be well justified.

Considering the original taxonomic concept for *A. muscora* discussed above, comparing the morphometric data in table 2, and carefully examining the micrographs and data provided in Tusset et al. (2017) for *A. muscora*, we conclude: (1) our proposed new species differs from *A. muscora* mainly because it has an elliptic-lanceolate (instead of linear-lanceolate) outline, broader valves, and higher stria density; (2) the population from Brazil identified by Tusset et al. (2017) as *A. muscora* does not belong to this species but likely represents a Brazilian population of *Adlafia neoniana*.

Adlafia neoniana can thus be differentiated from other similar species by recognizable characters or character combinations. Its discovery and characterization is a contribution to the knowledge of the still understudied biodiversity of tropical stream microorganisms (compare Jyrkänkallio-Mikkola et al. 2018).

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