

1 **The ecology of one cosmopolitan, one newly introduced and one occasionally advected**
2 **species from the genus *Skeletonema* in a highly structured ecosystem, the northern**
3 **Adriatic**

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15 **Key words:** phytoplankton, northern Adriatic, diatoms, *Skeletonema marinoi*, *Skeletonema*
16 *grevillei*

17 **Abbreviations:** FP, fultoportula; FPP, fultoportula process; IFPP, intercalary fultoportula
18 process; IRP, intercalary rimoportula; IRPP, intercalary rimoportula process; RP, rimoportula;
19 RPP, rimoportula process; TFP, *terminal* fultoportula; TFPP, terminal fultoportula process;
20 TRP, terminal rimoportula; TRPP, terminal rimoportula process

21 **Abstract**

22 The diatom genus *Skeletonema* is globally distributed and often an important constituent of the
23 phytoplankton community. In the marine phytoplankton of the northern Adriatic Sea, we found three
24 species of the genus *Skeletonema*: *S. menzelii*, *S. marinoi*, and *S. grevillei*. Making use of the steep
25 ecological gradients that characterize the northern Adriatic, along which we could observe those
26 species, we report here on the ecological circumstances under which those species thrive and how their
27 respective populations are globally connected. This is the first detailed ecological study for the species
28 *S. grevillei*. This study is also the first report for *S. grevillei* for the Adriatic Sea and Mediterranean
29 together with additional electron microscopic details on fresh *in situ* samples for this species. *S.*
30 *marinoi* appears to clearly prefer strong freshwater influence and high nutrient concentrations

31 delivered by low salinity waters. It can outcompete other diatom species and dominate
32 microphytoplankton blooms. *S. grevillei* on the other hand appears to thrive in high nutrient
33 concentrations triggered by water column mixing. It also appears to prefer higher salinity waters and
34 coastal embayments. Genetic analysis of *S. grevillei* demonstrated a peculiar dissimilarity with isolates
35 from coastal waters off Yemen India, Oman, and China. However, a closely related sequence was
36 isolated from coastal waters off Japan. These results indicate that *S. grevillei* is an introduced species,
37 possibly transported by ballast waters. *S. menzelii* is a sporadic visitor in the northern Adriatic,
38 advected from rather oligotrophic middle Adriatic waters and never dominates the phytoplankton
39 community in the northern Adriatic.

40 [Introduction](#)

41 Diatoms are ecologically one of the most important phytoplankton groups, responsible for
42 nearly one quarter of global primary production, and 40 % of marine primary production [1].
43 The major diatom blooms are typical of coastal oceans and upwelling zones, in which nutrient
44 levels are high [2]. *Skeletonema* is one of the globally most common/abundant coastal diatom
45 genera, together with *Nitzschia*, *Achnanthes*, and *Cocconeis* [3]. Species from the genus
46 *Skeletonema* are reported to often form dense blooms [4-9].
47 More than 150 years have passed since the original description of the genus *Skeletonema* [10],
48 and until the early 2000s it was usually referred to as *S. costatum* due to the difficulty of light
49 microscope identification [11]. In the early 2000s a more detailed morphological
50 investigations together with new molecular insights revealed a more complex taxonomic and
51 genetic diversity within the genus *Skeletonema* [12], and to this day there are more than 20
52 different species described, which formerly were recognized as only one species [13]. Those
53 new findings have raised the question which *Skeletonema* we are/were counting as *costatum*,
54 and what are the methods for proper but effective species identification? Recently Hevia-
55 Orube et al [14] recognized those questions and used molecular and microscopical techniques
56 on three species *S. costatum*, *S. dohrnii*, and *S. menzelii*. These methods are necessary for
57 deciphering the ecology of this cryptic genus, but we are far from understanding the ecology
58 for the whole diversity of the genus. There are currently 1450 scientific reports available
59 containing information about *S. costatum*, 138 report with information about *S. marinoi*, 12
60 reports with information on *S. menzelii* and 7 reports with information on *S. grevillei*.
61 Species from the genus *Skeletonema* are characterized by cylindrical cells, with long tubular
62 processes associated with a peripheral ring of fultoportules. The tubular processes run
63 perpendicular to the valve and link to those of sibling valves to form permanent colonies of
64 variable length [3]. During the revision of the genus, *Skeletonema marinoi* was described and

65 the Adriatic Sea was named as its type locality [12]. *Skeletonema marinoi* is one of the key
66 diatom species in the Adriatic Sea. In the northern Adriatic it regularly occurs during winter
67 months being the major constituent of the winter-early spring bloom. But it has been found in
68 Hong Kong and at the east coast of the United States as well. Thus, *S. marinoi* is considered a
69 cosmopolitan species. Moreover, *Skeletonema marinoi* is generally considered to be a fast
70 bloom forming species in rather eutrophic conditions.

71 The northern Adriatic is a shallow basin, and the most northern part of the Mediterranean. It is
72 characterized by strong and dynamic ecological gradients under the governing influence of
73 the Mediterranean's largest freshwater and nutrient input, the Po River. It is, furthermore,
74 prone to expressed changes in water temperature due to its shallowness and strong, cold wind
75 situations [15-18]. This wide range of conditions makes the Adriatic well suited for the study
76 of ecological preferences of phytoplankton species. The aim of this paper is to take a closer
77 look into the diversity and ecology of the genus *Skeletonema* in the northern Adriatic Sea, and
78 to show that the increased taxonomic resolution helps explaining the ecological range of
79 *Skeletonema* species in the northern Adriatic. For this we inspected the monthly long term
80 phytoplankton records collected in the northern Adriatic Sea. And due to the cryptic nature of
81 the genus we also undertook genetic analysis and electron microscopy on isolates from the
82 selected stations and different bloom and non-bloom events.

83 2. Materials and methods

84

85 Study area

86

87 All sampling stations are within the northern Adriatic (NA) (Fig. 1 A). The NA is the northern
88 most, semi-enclosed part of the Mediterranean (Fig. 1 B). It is characterized by strong
89 gradients of nutrient concentrations and its Plankton can be generally considered to be
90 phosphate limited. However, the Mediterranean's largest freshwater input, the river Po, is a
91 strong nutrient source for the area [17, 19, 20]. The study area is generally shallow with
92 maximum depths of 45-60 m.

93

94

95 Sampling

96

97 As part of a Croatian long-term monitoring program of phytoplankton assemblages in the
98 northern Adriatic Sea [21], water and net samples were collected monthly at seventeen
99 stations across the northern Adriatic through the period 1998 – 2009. Additional fifteen
100 stations were sampled during 2014 and 2015 in Lim Chanel, Pula harbour, Rijeka harbour and
101 Kvarner Bay (Fig. 1). Water and phytoplankton samples were taken at the water surface, in
102 5m, 10m, 15m, 20m depth as well as 1m above the seafloor. Overall 9599 samples were
103 analysed. In 1718 samples we found *Skeletonema* species.

104 Conductivity–Temperature– Depth (CTD) profiles were recorded with an SBE 25 Sealogger
105 CTD probe (Sea-Bird Electronics, Inc., Bellevue, Washington, USA) including oxygen
106 saturation.

107

108 Sample analysis

109

110 Nutrients: nitrate (NO_3), nitrite (NO_2), orthophosphate (PO_4) and orthosilicate (SiO_4) were
111 measured by spectrophotometric methods [22]. Ammonium (NH_4) was analysed by a
112 modified technique of the indophenol method [23]. Measurements were performed on a
113 Shimadzu UV-Mini 1240 spectrophotometer with 10 cm cells. In statistical analyses total
114 inorganic nitrogen (TIN, sum of NO_3 , NO_2 , and NH_4) was used. A 500 mL subsample for the
115 determination of chlorophyll *a* was filtered onto Whatman GF/C filters and immediately
116 frozen at -20°C until analysis (within a week). Total chlorophyll *a* concentrations were
117 determined on a Turner TD-700 fluorimeter [22] after three hours of extraction in 90%
118 acetone (in the dark, with grinding). Further details were described earlier [24].

119

120 Phytoplankton samples, 200 mL, were fixed with neutralised formaldehyde (2% final
121 concentration). Phytoplankton cells were counted in 50 mL subsamples after 40 h of
122 sedimentation time [25], using an Axiovert 200 microscope (Zeiss GmbH, Oberkochen,
123 Germany) and following the Utermöhl [26] method. Prior to the description of *S. marinoi* in
124 2005 *S. marinoi* was identified as *S. costatum* in our dataset. However genetic and electron
125 microscopical analysis on samples from 2006 on demonstrated that the taxon *S. costatum*
126 from our analysis prior to the description of *S. marinoi* in fact had to be attributed to the taxon
127 *S. marinoi*. *S. costatum* as delineated in the was never observed during ultrastructural

128 analyses of our samples from the NA. For the here reported analyses we attributed all
129 abundances recorded for the taxon *S. costatum* prior to 2005 to the taxon *S. marinoi*. Overall
130 9599 samples were analysed. In 1718 samples we found *Skeletonema* species.

131

132 Colonies of the *Skeletonema* species were manually isolated with a micropipette from live net
133 samples collected at various stations in the northern Adriatic Sea. Colonial cells were grown
134 into monoclonal batch cultures in 100 ml f/2 medium [27] and incubated at 18 ° C and 75 μ
135 mol photons m⁻² s⁻¹ on 12:12 h light/dark photoperiod.

136

137 Net sample material and cultures were acid cleaned of organic matter for electron microscopy.
138 For EM preparation, samples were treated with acids (1:1:4, sample: HNO₃: H₂SO₄), boiled
139 for a few minutes, and then washed with distilled water three times. Frustules were allowed to
140 sink for a few minutes between washing steps. For transmission electron microscopical
141 (TEM) examination, a drop of cleaned material was mounted on a 100-mesh copper grid
142 covered with pioloform (Agar Scientific Ltd., Stansted, UK), air-dried, and observed with an
143 FEI Tecnai TEM (FEI Co., Eindhoven, The Netherlands). For scanning electron
144 microscopical (SEM) examination, the cleaned diatom material was dropped on silica waver
145 or directly on aluminium object carriers. The object carriers were air-dried and examined
146 without sputter coating. When needed samples were gold coated with a sputter coater (S150A
147 Sputter coater; Edwards Ltd., Crawley, UK), and observed with a Philips 515 SEM (FEI Co.).
148 Morphological features were observed in LM, TEM, and SEM. Ultrastructural morphometric
149 data were obtained in TEM and SEM. All LM observations were carried out on field samples
150 and monoclonal cultures (in exponential phase) using a Zeiss Axiovert 200 microscope (Carl
151 Zeiss, Oberkochen, Germany) equipped with Nomarski differential interference contrast
152 (DIC), phase contrast, and bright-field optics. Light micrographs were taken using a Zeiss
153 Axiocam digital camera. The terminology used to describe ultrastructural features of
154 *Skeletonema* species follows Anonymous [28] , Ross et al. [29] and the original descriptions
155 in Sarno [12] and Zingone et al. [30].

156

157

158

159 [DNA extraction, PCR amplification, sequencing](#)

160

161 For molecular analysis, monoclonal cultures of *Skeletonema* species were harvested by

162 centrifugation at 5000 rpm for 5 min (5417R, rotor F453011; Eppendorf AG, Hamburg,
163 Germany). DNA was isolated with the DNA plant mini kit (Qiagen GmbH, Hilden, Germany)
164 according to the producers' s recommendations. The hypervariable V4 region of the 18S
165 rRNA gene was amplified using the primers 5-ATTCCAGCTCCAATAGCG-3 and 5-
166 GACTACGATGGTATCTAATC- 3 according to Zimmermann et al. [31] and sequenced
167 (using the same primers) on an ABI PRISM 3100 Avant Genetic Analyzer (Applied
168 Biosystems, Foster City, CA, USA) according to the company's recommendations. The D1-
169 D3 region of the 28S rRNA gene was amplified using the primers 5-
170 ACCCGCTGAATTTAAGCATA-3 and 5-ACGAACGATTTGCACGTCAG-3 and
171 sequenced like described above [32]. The resulting sequences from two runs for each
172 direction were compared to exclude sequencing mistakes by majority rule (3:1).

173

174

175 Genetic marker analysis

176

177 The resulting sequences were aligned into an alignment of near full-length 18S and 28S
178 rDNA genes. The alignments were based on the alignment of all publicly available full-length
179 18S and 28S rRNA gene sequences, including more than 1200 diatom sequences
180 (SSURef_98_Silva_20_03_09_opt database and LSURef_98_Silva_20_03_09_opt; [33].
181 Sequences were manually aligned and compared using the ARB 5.1 software package [34]
182 following the protocol suggested by Peplies et. al., specifically using the neighbour joining
183 algorithm included in the Arb software package [35]. The alignment for the 18S rDNA
184 includes 437 positions, while the alignment for the 28S rDNA fragment includes 680
185 positions. Genetic distances were calculated as percentages.

186

187 [Statistical analysis](#)

188

189 Statistical analysis as well as graphical presentation of the results was performed using the
190 software package R and included core packages as well as programs from the packages
191 Hmisc, base and ggplot2 [36-39]. In box and whisker plots, the top and the bottom of the
192 boxes represent the 25th and 75th percentile respectively. The centre line delineates the 50th
193 percentile. Outliers are shown as dots and where defined as either greater than the 3rd quantile
194 + 1.5x(quantile 3 – quantile 1) or smaller than quantile 1 – 1.5x(quantile 3-quantile1).

195 Whiskers (notches) extend to the most extreme data point that is less than 1.5 times the box
196 size away from the box. Correlation graphs were produced using the package
197 PerformanceAnalytics [40]. In the correlations graphs result were grouped according to their
198 p-value in three groups: $P < 0.001$, $P < 0.01$, $P < 0.05$, following the discussion by R. A. Fisher
199 [41]. In the reported cases, correlations with a p-value smaller than 0.05 were considered
200 significant.

201

202 Results

203 Taxonomy and morphometrics of *Skeletonema* spp. in the northern Adriatic.

204 All three detected species, *S. marinoi*, *S. grevillei*, and *S. menzelii*, were inspected
205 microscopically. The morphology of the observed *S. marinoi* fits and falls within the details
206 and ranges described earlier [12, 30]. Cells formed long curved or coiled chains. Valve
207 diameter varied from 2 to 12 μm . Ultrastructural details observed in *S. marinoi* during this
208 survey are presented in the supplementary figure 1. External processes of the fuloportulae
209 were open, with flat and flared tips and jagged distal margins. Each process connected with
210 one or two processes of the sibling valve. The rimoportula was close to the valve face margin
211 in intercalary valves and subcentral in terminal valves. External process of the rimoportula
212 was short in intercalary valves, long in terminal valves of the colony. Copulae with transverse
213 ribs were interspaced by rows of pores. The valve face was slightly convex; the mantle was
214 vertical. The FPPs were open along their entire length. Their distal end was flattened and
215 flared, with a dentate margin. The IFPPs of sibling valves were either aligned, with a 1:1
216 linkage, or displaced, with a 1:2 linkages and a zigzag connection line. The interlocking
217 between IFPPs was in all cases a plain joint, with no intricate knots or knuckles. The flared
218 tips of the IFPPs overlapped with edges that interdigitated with one another. The TRP was
219 located close to the central annulus or midway between the center and the margin of the valve
220 and had a long tubular process with a slightly flared or trumpet-or cup-shaped apex. The IRP
221 was short and at the edge of the valve face. The copulae showed the typical central ridge,
222 which was flanked on both sides by transverse ribs interspaced by rows of pores.

223 For *S. grevillei* detailed electron microscopic analysis revealed new characteristics of the
224 species. In the original description of *S. grevillei* based on the type material the authors state
225 that in light micrographs cells have a delicate aspect, with the cingulum often collapsed [30].
226 In our samples the cingulum in LM was intact (see Fig. 2 A for an SEM aspect of the
227 cingulum). We observed colonies with 3-28 cells in both monoclonal cultures and *in situ*

228 samples. In our results the valve face was slightly convex, and the perivalvar axis was
229 generally longer than or as long as the cell diameter like in the original description as well
230 [12, 30, 42]. In our results the cell diameter was from 5-19 μm , which extends the
231 measurements from original descriptions 6-12 (Table 1). The IFPPs were rather long ($8.5 \pm$
232 $1.6 \mu\text{m}$ $n=33$ against $6.7 \pm 1.6 \mu\text{m}$, $n=35$), each joining one IFPP of the adjacent cells (1:1
233 junction), with a thickening at the joint (Fig. 2, A- F). Only rarely one IFPP joined two IFPPs
234 of the next valve (1:2 junction) (not shown). A zigzag line at the level of the connection was
235 never observed which is in accordance with the original description [30].

236 The length of the observed TFPPs was $5.6 \pm 1.8 \mu\text{m}$ ($n=10$), and that is very close to the
237 original description and they are visibly thickened at their tips (Fig. 2 A-B). A transverse
238 ridge forms a straight line across the bases of the processes, and other ridges are visible on the
239 valve mantle (Fig. 2, A and D). With EM, the mantle ridges are seen as a scalloped edging of
240 ridges at the base of the FPPs (Fig. 2, A–D and F–H). The straight line visible in LM
241 corresponds to a series of silica ridges with concave rims that connect the internal faces of the
242 bases of the FPPs [30]. A second and at times a third series of ridges, more or less parallel to
243 the first one, may join the lateral bases of the FPPs (Fig. 2, F–H). Finally, two opposite
244 concave ridges at the external base of each FPP delimit a circular or oval hole (Fig. 2, A, D,
245 E). These structures were more or less developed in different individuals. Those findings are
246 in accordance to Naik [43] who also observed larger silica ridges compared to original
247 description.

248 The valve face showed a central annulus. Its solid area was interspaced with an irregular
249 agglomeration of small, round pores. Radial, bifurcating and delicate ribs covered the valve
250 face and were separated by small, round pores. On the valve margin delicate ribs connected
251 the radial, delicate ribs perpendicular to those. Thus rectangular areas were formed between
252 the delicate ribs, which were entirely filled with small, round pores (Fig. 2, C, H).

253 The FPPs open along their entire length (Fig. 2, A–H) and the distance between them was 1.5-
254 2.5 μm . The fuloportulae processes in the terminal valves extremities were irregularly
255 truncated and pointed at their lateral ends (Fig. 2, A) we observed generally one small spine.
256 The interlocking between IFPPs was particularly intricate and tight, resembling a bone
257 knuckle (Fig. 2, D and F), same as documented in original description of the species. The
258 TRP was located just inside the marginal ring of TFPs (Fig. 2, B) and beared a long tubular
259 process ($6.1 \pm 1.8 \mu\text{m}$, $n=5$) even longer than in original description, which was wider and
260 obliquely truncated at its top, with a tubular end (Fig. 2B). The intercalary RP was located
261 marginally and had a short ($0.6 \mu\text{m}$) and tubular external process (Fig. 2, E) similar as

262 described before. We also found incidents where the IRP was entirely incorporated in the
263 rather massive silica ridge between IFPP. Such IRP was not described before. Sometimes, it
264 was incorporated in silica ridges and was very difficult to spot and measure. A ridge went
265 medially along the whole length of the copula (Fig. 2 K). Thin transversal ribs, generally
266 bifurcate at their ends and interspaced with a hyaline area were observed. A comparison
267 between the here reported values and the original description of *S. grevillei* is presented in
268 Table 1.

269 For *S. menzelli* no cultures were isolated and no electron microscopical analysis was
270 performed for the species. The observed cells from in situ samples however showed no
271 apparent divergence from the original morphological descriptions and recent reports [44, 45].
272 We observed single cells and chains of two cells, longer chains were never observed. The FPs
273 were located marginally near the transition from the valve face to the mantle. Ultrastructural
274 traits like the missing costae and areolae as well as the two satellite pores (as opposed to three
275 in other *Skeletonema* species) were not analysed [12].

276

277

278 Ecology

279 Figure 3. shows a box and whiskers plot of all abundances of *S. marinoi* observed between
280 1999 and 2016 on a transect across the northern Adriatic (black). It demonstrates that *S.*
281 *marinoi* was a regular component of the northern Adriatic phytoplankton community with an
282 expressed winter-early spring bloom.

283

284 There was an irregularly occurring late summer bloom of *Skeletonema* sp. (July-September)
285 (Fig. 3), assigned to *S. marinoi*, however it was never extensively characterized via electron
286 microscopy or using molecular markers and hence it cannot be excluded that also *S. grevillei*
287 is found during such blooms.

288

289 *Skeletonema grevillei* Sarno and Zingone was observed for the first time in the Adriatic Sea
290 during the autumn bloom 2014 when it reached high abundances. This is simultaneously the
291 first record of *S. grevillei* in the Mediterranean. Figure 3 shows a box and whiskers plot for
292 abundances of *S. grevillei* when observed between the years 2014 and 2016 (grey).

293 The highest abundance was $2,5 \times 10^5$ cells L⁻¹. *S. grevillei* appeared in September with peak
294 abundances in November/December and lasted until January/February.

295 *S. menzelii* was found only sporadically and in very low abundances across the entire study
296 area.

297 Figures 4 and 5 show the geographical distribution of abundances for *S. marinoi* (fig. 4) and *S.*
298 *grevillei* (Fig. 5) in the study area. We found highest abundances for *S. marinoi* near the
299 western Adriatic coast in waters close to the mouth of the Po River, while highest abundances
300 for *S. grevillei* were observed in the harbours on the eastern Adriatic coast. The highest
301 abundances were found in the surface layer from 0-10 m depth in Rijeka harbor (Fig. 5).

302 Figure 6 shows box and whiskers plots for environmental parameter recorded when *S.*
303 *marinoi* or *S. grevillei* respectively were found in the samples. A significant difference was
304 observed in the oxygen saturation values accompanying *S. marinoi* and *S. grevillei*. A two
305 sample t-test showed the two sets of oxygen saturation values to be significantly different (p-
306 value < 2.2e-16). Figure 7 shows a scatter plot of oxygen saturation values and abundances
307 for *S. marinoi* and *S. grevillei*. For all other, oxygen unrelated parameters we found rather
308 overlapping ranges for both species. Table 2 reports descriptive statistics for both species and
309 for all analysed parameters.

310 Table 3 shows the correlations between group abundances of total microphytoplankton,
311 diatoms, dinoflagellates, and coccolithophorids during appearances of *S. marinoi* and *S.*
312 *grevillei* respectively. High correlation coefficients of 0.9 between *S. marinoi* and
313 microphytoplankton as well as diatom abundances demonstrate *S. marinoi* dominating diatom
314 blooms. *S. grevillei* however never dominates the microphytoplankton community.
315 Supplementary figure 2 gives a graphical representation of the correlations for *S. marinoi* (a)
316 and *S. grevillei* (b) summarized in table 3.

317 Genetic analysis

318 We analysed the hypervariable V4 Region of the SSU rDNA as well as the D1-D3 region of
319 the LSU rDNA.

320 The V4 region of the SSU rDNA of *S. marinoi* from our samples (Genbank accession number
321 MF772522) was found to be identical to the available sequences published earlier from the
322 western coast of the Adriatic sea (NCBI accession numbers: AJ632213, EF433521,

323 AF462060, AJ632212, AJ632216, AJ632214, EF138932, EF138940, EF138939, EF433519,
324 EF138934, HM236346, HM236347, JF489952, JF489958, HM236345, HM236349,
325 HM236348, JF489953, KJ671706, KJ671705, KJ671707, KT860966, KJ671708) as well as
326 to sequences published from isolates from the western Mediterranean (KR091067) and the
327 Baltic Sea (HH805045).

328 The D1-D3 region of the LSU rDNA of *S. marinoi* from our samples (Genbank accession
329 number MF772714) was found to be identical to the available sequences published earlier
330 from Hong Kong bay (AJ633529) and from the western coast of the Adriatic Sea (NCBI
331 accession numbers: AJ633533, AJ633536, AJ633532, AJ633535, AJ633530, AJ633531,
332 AJ633534, Q396506, EF433522, EF655656, FR823443, FR823447, EF433524, FR823444)

333 Supplementary figure 3 shows a tree-representation of neighbour joining analysis of all
334 available sequences for the V4 region of the SSU rDNA (a) and the D1-D3 region of the LSU
335 rDNA (b) of *S. grevillei*. The results clearly demonstrate that for both regions the strains
336 isolated from the northern Adriatic represent a genotype different from those found elsewhere
337 (SSU Genbank accession number MF772521, LSU Genbank accession number MF772715).
338 Figure 10 shows the genetic distance of *S. grevillei* isolates from various areas if compared to
339 the northern Adriatic isolate. Close relatives are reported from Yemen and Japan.

340

341

342 Discussion

343

344 Taxonomy and morphometrics of *Skeletonema* spp. in the northern Adriatic.

345 Morphological characteristics of *S. marinoi* were found to be within the ranges so far
346 described for the species. This allows to assume, that the ecological conditions found in the
347 northern Adriatic do not invoke dramatic morphological responses, altering its silica
348 structures from the details laid out in its species descriptions [12, 46]. For *S. grevillei*
349 however, we found morphological characteristic that are either newly observed or are slight
350 aberrations from the original description. We uncovered that *S. grevillei* is capable of forming
351 rather long chains (*in situ* and in cultures), which was not found in the type material nor was
352 that earlier described. We extended the range for cell diameters to 5-19µm. We can also report

353 larger values for IFPP length ($8.5 \pm 1.6 \mu\text{m}$). Contradicting the original description, we found
354 that the valve face shows a central annulus. Its solid area is interspaced with an irregular
355 agglomeration of small, round pores. Radial, bifurcating and delicate ribs cover the valve face
356 and are separated by small, round pores. On the valve margin, delicate ribs connect the radial,
357 delicate ribs perpendicular to those. Thus, rectangular areas are formed between the delicate
358 ribs, which are entirely filled with small, round pores (Fig. 2, C, H). These incidentally also
359 represent a combination of features described for other *Skeletonema* species, which either
360 show bifurcating radial ribs or rectangular areas only. The original description mentions that
361 on the valve face, radial rows of rectangular areolae branch off from the central annulus.
362 However, no EM micrograph was shown to support this statement. Our findings differ from
363 the original description in irregular agglomeration of pores in the central annulus and more
364 importantly in the observation that the valve face is not ornamented with rectangular areolae.
365 For the FPP length we report again larger values ($1.5 - 2.5 \mu\text{m}$ as opposed to $0.7-1.5 \mu\text{m}$) than
366 the original description. Incidentally, we found cells, where the IRP is entirely incorporated in
367 the rather massive silica ridge between IFPP. Such IRP was not described before.

368 Owing to the overall similarity of the characteristics to those described to *S. grevillei* and the
369 age of the type material, we nevertheless still assume that we here describe *S. grevillei* (Fig.
370 2). That would indicate that in some instances we might have observed morphological
371 reactions to the ecological conditions found in the northern Adriatic. However, we think that
372 we added substantial information to the morphological characterization of *S. grevillei*.

373

374

375 Ecology

376 The distribution of *Skeletonema* species identified in Sarno et al. [12] provides, in some cases,
377 evidence of distinct ecological characteristics. The four *Skeletonema* species found in the Gulf
378 of Naples tend to occupy different seasonal niches: *S. dohrnii* has only been found in winter,
379 *S. pseudocostatum* blooms in late spring, early summer, *S. tropicum* is recorded in late
380 summer, early autumn, and *S. menzelii* is typical of autumn. These periods are characterized
381 by markedly different conditions in terms of temperature ($13-30^\circ\text{C}$), salinity (25–38 psu),
382 water column stability, photoperiod, and nutrient concentrations [47]. In the northern Adriatic
383 we recorded three different *Skeletonema* species: *S. marinoi*, *S. grevillei*, and *S. menzelii*.
384 In the northern Adriatic *S. marinoi* can be found frequently from February to April and less

385 frequently during summer. It is a regular component of the northern Adriatic winter-early
386 spring bloom (Fig. 3). Favourable conditions for *S. marinoi* are a strong influence of nutrients
387 mostly from the Po River. It is fast growing and outcompeting other diatom species during the
388 bloom. Often highest abundances are found in the western part of the NA, where nutrient
389 concentrations are highest (Fig.4). It appears to thrive best in open waters under the direct
390 influence of strong freshwater and nutrient inputs (Fig. 6). This explains the geographical
391 distribution with markedly higher abundances near the Po river outflow, where salinity is
392 lowered and nutrients are delivered in high concentrations [17]. This notion is also
393 corroborated by the rather high concentration of total nitrogen concentrations in water
394 samples where *S. marinoi* was present in high abundances. The oxygen oversaturation in the
395 upper layer of the water column indicates the riverine freshwater influence, as well as highly
396 productive conditions (Fig.7). The high and significant correlation between *S. marinoi*
397 abundances and total microphytoplankton as well as diatom abundances in samples containing
398 *S. marinoi* allows the conclusion, that *S. marinoi* if conditions are favourable, will outcompete
399 other diatoms and dominate a diatom bloom (Fig. 8). There is an irregularly occurring late
400 summer bloom of *Skeletonema* sp. (July-September) (Fig. 3) which is currently assigned to *S.*
401 *marinoi*, however it was never extensively characterized via electron microscopy or using
402 molecular markers and hence it cannot be excluded that also *S. grevillei* is found during such
403 blooms

404

405 Our dataset contains abundance data for *S. marinoi* from before the description of *S. grevillei*
406 [30] when most certainly observations of *S. grevillei* were reported under the species name *S.*
407 *marinoi* (Fig. 3). Since the description of *S. grevillei* as new species, it was counted as
408 separate species. Since then, *S. grevillei* was observed from September to January, while *S.*
409 *marinoi* appears from February to August. Only rarely both species are observed
410 simultaneously, and if so with opposite trends in abundances. Oxygen saturation during *S.*
411 *grevillei* blooms was observed to be significantly lower than during *S. marinoi* blooms, which
412 indicates lower primary production rates as well as possibly higher respiration rates.
413 *S. grevillei* was observed for the first time (reported here) in 2014 during an autumn bloom
414 (September-December) along the eastern Adriatic coast reaching relatively high abundances
415 ($2,6 \times 10^5$ cell l⁻¹). Before that *S. grevillei* was only found in Hong Kong Bay (type locality)
416 [30], Xiamen Harbour [48], Bay of Bengal [43], Muscat Oman [9]. Gu and co-authors found
417 [48] *S. grevillei* in Xiamen harbor from august to September but they do not report on
418 abundances or any other ecological factors accompanying the bloom. As *S. grevillei* appeared

419 in the warm season of Xiamen harbor, and in Arabian Sea as well [9], the authors suggested
420 that this is a tropical species that occurs also in the warm season of warm temperate regions
421 [48], and it was characterized as summer/autumn species. But there is only a limited number
422 (7 in total) of publications mentioning this species, and so far none of them reporting on
423 ecology. We observed larger abundances during the autumn bloom shortly after the onset of
424 water column mixing in September and in December, when water temperatures started
425 dropping more rapidly. Water temperature in samples containing *S. grevillei* ranged from
426 10.06°C to 21.79°C with a median of 16.08°C. Highest abundances for *S. grevillei* are
427 observed along the eastern Adriatic coast and specifically in harbor bays (Fig. 5) which is in
428 strong contrast to the preference of *S. marinoi*, which appears to prefer nutrient loaded, open
429 waters closer to the Po River mouth (Fig. 4). *S. grevillei* thrives in elevated nutrient
430 concentrations, but appears to prefer higher salinities (see Fig. 6). Figure 6 demonstrates a
431 nonsignificant trend of elevated nutrient salt concentrations during *S. marinoi* observations.
432 This might be explained by *S. marinoi*'s capability to outcompete other diatom species during
433 bloom conditions, while *S. grevillei* under such competitive conditions rather vanishes. This
434 probably indicates that its tolerance towards reduced salinity is not as pronounced as it is for
435 *S. marinoi* [49].

436
437 *S. menzelii* appears regularly in small abundances during winter months (December-January).
438 It is more prominent along the eastern Adriatic coast where the phytoplankton abundances are
439 lower and biodiversity is higher. However, *S. menzelii* never dominates the
440 microphytoplankton community and is observed rather sporadically. It reaches abundances of
441 up to 5680 cells/L. Given the low frequency and only sporadic observations at this time we
442 cannot extract significant correlations between ecological parameters and the abundances of
443 *S. menzelii*. It is well possible, that *S. menzelii* is not establishing a permanent population in
444 the NA but its presence is rather due to advection with water masses from the middle and
445 southern Adriatic.

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448 Genetic analysis

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450 Both analysed genetic markers for *S. marinoi* were identical to previously published
451 sequences from isolates from other regions. This supported the unequivocal taxonomic
452 identification of *S. marinoi* as such. This result indicates furthermore that the northern

453 Adriatic *S. marinoi* probably is part of a globally distributed and possibly connected
454 population. This observation is in accordance to the finding of Kooistra and colleagues [50].
455 *S. grevillei* on the other hand for both marker regions showed marked sequence differences
456 from earlier published sequences for isolates from different locations (see supplementary
457 figure 2). Supplementary figure 4 shows the genetic distances within the D1-D3 region of the
458 LSU rDNA for all analysed sequences. Most similar sequences are reported for isolates from
459 coastal water off Yemen and off Japan. Sequences that are more dissimilar are reported for
460 isolates from coastal waters off India and China. This observation cannot be explained by
461 ocean currents. Natural genetic drift over or along communicating populations and geographic
462 distances would result in a unidirectional gradient of genetic distance. In this case however, it
463 rather appears that there is a shortcut from Japan waters to the Adriatic that might include
464 coastal water off Yemen. Ballast water transport from Japan through the red sea and the Suez
465 channel into the Adriatic would explain the genetic similarity of Adriatic isolates with isolates
466 from the coast off Yemen at the southern entrance into the red sea and with isolates from
467 Japan.

468 *S. menzeli* was only observed sporadically and no cell culture was established for subsequent
469 genetic analysis.

470

471 Summary

472 We observed three species from the genus *Skeletonema* in the northern Adriatic: *S. marinoi*, *S.*
473 *grevillei* and *S. menzeli*.

474 *S. marinoi* appears throughout large parts of the year with expressed blooms in late winter and
475 early spring when water temperatures are low and nutrient concentrations are high. *S. marinoi*
476 dominates highly productive microphytoplankton blooms in coastal and open waters and
477 appears generally well adapted to steep spatio-temporal ecological gradients as present in the
478 northern Adriatic [51, 52]. Genetic similarity to most available sequences from isolates from
479 other marine areas suggests a large and interconnected population's structure with
480 mechanisms for conservation of genetic markers.

481 *S. grevillei* is observed in autumn and early winter, when temperatures fall and nutrients
482 become available through water column mixing. Like *S. marinoi* it appears to be a stable
483 constituent of the northern Adriatic phytoplankton. However, the species never dominates the
484 phytoplankton community. Highest abundances are observed in harbour bays and along the
485 eastern Adriatic coast. Clearly *S. grevillei* prefers higher nutrient concentrations and harbour
486 areas. Its preference for coastal proximity and inability to dominate massive bloom events

487 might explain a slower distribution rate across large distances and a generally higher genetic
 488 variability between isolated from geographically distant locations. It also might make *S.*
 489 *grevillei* a successful traveller in ballast waters. It certainly appears that the *S. grevillei*
 490 population we observed in the northern Adriatic might be a permanently introduced species to
 491 the area.

492 *S. menzelii* was observed only sporadically and with low abundances in the northern Adriatic.
 493 It probably is not a permanent constituent of the northern Adriatic, but rather appears when
 494 advected from more southern parts of the Adriatic, where temperature is more stable and
 495 generally higher, and where nutrient concentrations are lower and less fluctuating.

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500

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512 Table 1. Main morphometric data of *S. marinoi* and *S. grevillei* from this study compared with the
 513 original description from Zingone et al 2005. and Sarno et al. 2005. Bold numbers exceed ranges from
 514 the original descriptions.

515

		<i>S. marinoi</i> this study	Sarno et al.2005	<i>S. grevillei</i> this study	Zingone et al. 2005
Cell diameter (µm)	Min-max	5 - 12	2 - 12	5 - 19	6 - 12
	Avg±SD	8.95±1.76	4.3±1.9	11.1 ± 2.7	7.4 ± 2.2
	<i>n</i>	39	300	24	20
Distance between cells (µm)	Min-max	6.33 -10.4	0.5-1.5	9.3 - 23	8 – 20
	Avg±SD	8±1.4	0.9±0.2	16.9 ± 4.9	13.4 ± 3.2

	<i>n</i>	12	26	9	17
Cells per colony	Min-max	3-20	2-45	3-28	3-8
	Avg±SD	8.5±7.7	16.2±10.9	13.9 ± 7.4	3.8 ± 1.9
	<i>n</i>	40	125	30	13
FPPs in 10 µm	Min-max	8-12	9-11	6-9	7-12
	Avg±SD		10±1	7.2 ± 0.9	8.2 ± 1.4
	<i>n</i>		5	11	13
Distance between FPPs (µm)	Min-max	0.9-1.8	0.5-1.5	1.4 - 2.5	0.7-1.5
	Avg±SD	1.4±0.3	0.9±0.2	2.0 ± 0.24	1 ± 0.2
	<i>n</i>	15	26	22	9

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Table 2. Environmental parameters found in samples, when *S. marinoi* or *S. grevillei* were present.

	Temp [°C]	PSU	Density anomaly [kg/m ³]	dissolved oxygen [mg/L]	oxygen saturation	P [µM]	total P [µM]	nitrate [µM]	nitrite [µM]	ammonia [µM]	total N [µM]	silica [µM]
mean	15.14	34.91	25.72	6.52	1.14	0.11	0.26	5.42	0.43	0.52	12.54	5.18
sd	6.13	4.27	3.85	1.16	0.17	0.27	0.25	10.72	0.43	0.99	17.10	9.69
median	13.00	36.34	26.74	6.39	1.09	0.05	0.18	1.57	0.28	0.26	7.22	2.30
min	5.15	5.21	3.58	4.17	0.87	0.00	0.03	0.00	0.00	0.00	1.39	0.00
max	29.70	38.54	29.88	11.98	2.09	4.90	1.64	80.67	2.27	9.04	163.50	75.70
mean	16.29	36.34	26.68	5.35	0.97	0.10	0.25	3.20	0.30	0.90	4.40	3.64
sd	2.63	3.34	2.60	0.35	0.60	0.12	0.17	5.70	0.19	1.14	6.29	6.67
median	16.08	37.35	27.54	5.27	0.97	0.05	0.20	1.90	0.27	0.55	2.59	2.47
min	10.06	18.51	12.05	4.77	0.84	0.03	0.09	0.30	0.03	0.10	0.62	0.54
max	21.79	38.21	29.00	6.35	1.11	0.54	0.88	39.91	0.88	6.83	41.58	50.87

S. marinoi

S. grevillei

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Table 3. Correlations between microphytoplankton groups and *S. marinoi* and *S. grevillei* respectively

		Micro-phytoplankton	Bacillario-phyceae	Dinophyceae	Coccolithophoridae
<i>S. marinoi</i>	correlation value	0.90	0.90	0.05	0.01
<i>n=1650</i>	P-value	< 10 ⁻⁸	< 10 ⁻⁸	0.02	0.58
<i>S. grevillei</i>	correlation value	0.03	0.03	-0.12	-0.05
<i>n=68</i>	P-value	0.80	0.80	0.35	0.66
<i>Microphytoplankton</i>	correlation value		1.00	0.30	0.13
<i>n=1650</i>	P-value		< 10 ⁻⁸	< 10 ⁻⁸	1.49E-07
<i>Bacillariophyceae</i>	correlation value	1.00		0.29	0.12
<i>n=1650</i>	P-value	< 10 ⁻⁸		< 10 ⁻⁸	3.01E-07
<i>Dinophyceae</i>	correlation value	0.30	0.29		0.12

<i>n=1650</i>	P-value	< 10 ⁻⁸	< 10 ⁻⁸		8.40E-07
<i>Coccolithophoridae</i>	correlation value	0.13	0.12	0.12	
<i>n=1650</i>	P-value	1.49E-07	3.01E-07	8.40E-07	

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531 Figure captions

532 Figure 1.

533 A: Location of the northern Adriatic in the Mediterranean (arrow).

534 B: Map of the northern Adriatic with the sampling stations.

535 Fig 2

536 SEM micrographs of *S. grevillei* from field samples. (A) Terminal valve of a colony. Distal
537 ends of the TFPPs with one small spine. Scale bar 5 µm. (B) Terminal valve of the colony
538 with the long marginal TRPP (arrow) with its obliquely truncated margin. Scale bar 5 µm. (C)
539 Terminal valve in valve view with the TRP, the annulus (arrow), the TFPPs, and the TFPPs.
540 Scale bar 5 µm. (D) Intercalary valve with the knuckle junctions 1:1. Scale bar 10 µm. (E)
541 Intercalary valve with ridges between IFPP bases and a small IRPP (arrow). Scale bar 1 µm.
542 (F) Detail of knuckle like junction. Scale bar 1 µm. (G) Detail of an intercalary valve with
543 several series of silica ridges (arrows) joining the IFPP bases. Scale bar 4 µm. (H) Detail of an
544 intercalary valve from inner view showing joining the FP bases. Scale bar 4 µm. (I) Internal
545 view of intercalary valve detail showing a FP with three satellite pores (arrows) Scale bar 1
546 µm. (J) Cingular bands with the trasversal ribs interspaced with hyaline areas placed on valve.
547 Scale bar 5 µm. (K) Single cingular band with the trasversal ribs. Scale bar 5 µm.

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550 Fig. 3

551 Box and whiskers plot of recorded abundances for *S. marinoi* (open boxes) between 1999 and
552 2016 as well as for *S. grevillei* (grey) between 2014 and 2016. Observations are grouped by
553 month of observations.

554

555 Fig. 4

556 Spatial distribution of observed abundances of *S. marinoi* between 1999 and 2016.

557

558 Fig. 5

559 Spatial distribution of observed abundances of *S. grevillei* between 2014 and 2016.

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562 Fig. 6

563 Temperature, salinity, oxygen saturation and nutrient salt concentrations recorded in samples
564 when *S. marinoi* (black) and *S. grevillei* (blue) was observed respectively. Temp =

565 Temperature [°C], Psal = practical salinity, Dene = density anomaly [kg/m³], Doxy =

566 dissolved oxygen [mg/L], Osat = oxygen saturation [0-1], Phos = dissolved phosphate [μM],

567 Thps = total dissolved phosphor [μM], Ntra = nitrate [μM], Ntri = nitrite [μM], Amon =

568 ammonia [μM], Ntot = total dissolved N [μM], Slca = silica [μM].

569

570 Fig. 7

571 Oxygen saturations recorded in samples *S. marinoi* (blue) and *S. grevillei* (red) was observed
572 respectively.

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575 Fig. 10

576 Representation of genetic distances (and origin) between different isolates of *S. grevillei*, and

577 the conspecific isolates from the northern Adriatic (location marked by an arrow). Size and

578 colour of the dots correlate to percent sequence difference in the D1-D3 region of the LSU

579 rDNA. The arrowhead indicates the presentation of the sample from Yemen.

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583 Supplementary figure 1

584 Micrographs of *S. marinoi* from culture material at SEM. (A) light micrograph of the colony.

585 Scale bar 20 μm. (B) Colony in girdle view at SEM. Scale bar 10 μm. (C) Terminal valve of

586 the colony with the long marginal TRPP showing flared ends of TFPPs with dentate margins.

587 Scale bar 10 μm. (D) Terminal valve of the colony with subcentral TRPP (arrow). Scale bar 3

588 μm. (E) Intercalary valves with the IFPP connected with 1:1 plain joins or zig-zag (arrow).

589 Scale bar 2 μm. (F) Intercalary valve with ridges between IFPP bases and a small IRPP

590 (arrow). Scale bar 3 μm. (G) Detail of an intercalary valves with several joining the IFPP.

591 Scale bar 2 μm. (H) Cingular bands with the transversal ribs. Scale bar 1 μm.

592

593 Supplementary figure 2

594 Correlation plot between concomitantly observed abundances of *S. marinoi* (A), *S. grevillei*
595 (B) and total microphytoplankton, total diatoms (BACI), total dinoflagellates (DINO), and
596 total coccolithophorids (COCCO). Numbers given are correlation coefficients. *** $P < 0.001$,
597 ** $P < 0.01$, * $P < 0.05$

598

599

600 Supplementary figure 3

601 A: Neighbour joining representation of sequences of *S. grevillei* covering the D1-D3 region of
602 the LSU rDNA. B: Neighbour joining tree representation of sequences of *S. grevillei* covering
603 the V4 region of the SSU rDNA. Isolates from the northern Adriatic are marked with an
604 arrow.

605

606 Supplementary figure 4

607 Genetic distances between all analysed isolates of *S. grevillei*. Marker gene: D1-D3 region of
608 the LSU rDNA.

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