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PAGES: 43-51

ORIGINAL PDF URL: <https://dergipark.org.tr/tr/download/article-file/1532604>

New records of Pennate Diatom Species from the Mediterranean Sea

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ABSTRACT

Nanoplanktic diatom species are generally overlooked in conventional microscopy analyses. Phytoplankton samples were collected from surface water of a shallow coastal region in the northeastern Mediterranean Sea and analyzed with scanning electron microscopy. Two new nanoplanktic diatom species were recorded; *Cocconeis sigillata* Riaux-Gobin et Al-Handal and *Amphicocconeis rodriguensis* Riaux-Gobin et Al-Handa. In addition, *Delphineis australis* (Petit) Watanabe, Tanaka, Reid, Kumada et Nagumo, *Amphicocconeis disculoides* (Hustedt) De Stefano et Marino which was previously observed in the Mediterranean Sea was also presented here. A specimen previously recorded as *Psmammococconeis* cf. *disculoides* from Argentina waters were first time recorded in the Mediterranean Sea from the external sternum valva view in this study. Differences between this *Psmammococconeis* cf. *disculoides* species and *Amphicocconeis disculoides* were reported. *Cocconeis* cf. *peltoides* found in this study shows different characteristics than previously identified specimens.

KEY WORDS: *Cocconeis*, *Amphicocconeis*, Northeastern Mediterranean Sea, SEM analysis.

How to cite this article: Konucu, M., Eker Develi, E. (2021).
New records of Pennate Diatom Species from the
Mediterranean Sea,
MedFAR,4(2):43-51.

1. Introduction

Delphineis Andrews is a genus of araphid pennate diatoms pertaining to the family Rhaphoneidaceae, which lives over sandy shores and mudflat in coastal marine waters as attached to sand grains, seagrasses (Chung and Lee, 2008), on other diatoms or as plankton through water turbulence (Cefarelli et al., 2016; Sar et al., 2007). Cells of *Delphineis* may appear in short or long-chain or sometimes as solitary (Hilaluddin et al., 2011). Whereas, *Cocconeis* is a monoraphid diatom genus within the family Cocconeidaceae Kützing and has both marine and freshwater species. Cells are solitary and adnate to hard substrata with the raphid valve, having the ability of very slow movement (Kingston, 1980; Kingston, 2003; Svensson et al., 2014). *Cocconeis* species can be epilithic, epipsammic, epiphytic or epizoid (Kingston, 1980; Car et al., 2012; Kaleli et al., 2016) and they are among the most diverse diatom genera (Al-Handal et al., 2019). It has a worldwide distribution (Round et al., 1990; Suzuki et al., 2001) and the highest number of marine *Cocconeis* species is suggested to be reported in tropical and sub-tropical regions if this distribution is not biased with uneven samplings regions (Majewska et al., 2014).

Following the construction of the Suez Canal in 1869, the Mediterranean and the Indian Ocean were connected to each other (Gomez, 2006). However, migration of many Erythrean organism commenced few decades later following dilution of natural hypersaline lakes which are parts of the man-made

channel (Galil, 2000). New phytoplankton species can also be transported to the Mediterranean Sea from the Indo-Pacific region via ship fouling or attaching on migrating organisms such as turtles (López-Fuerte et al., 2017; Kaleli et al., 2016). The aim of the present study was to investigate new nanoplanktic diatoms species in Erdemli coastal waters and to clarify the characteristics of identified species.

2. Material and Methods

Phytoplankton samples were collected into 1 L amber glass bottles from the surface water of a shallow pier on the Erdemli coast, Turkey (36°36' N, 34°19' E) in the north-eastern Mediterranean Sea during September 2015-September 2016 (Figure 1). The sampling region is reported to have good environmental conditions based on different Eutrophication Assessment Tools (Tugrul et al., 2018) and located around 40 km far from the Mersin port. 31% buffered formaldehyde was used to fix samples to become 1.5% final concentration. 300 mL of this seawater was filtered through 0.2 µm pore size polycarbonate filters and examined with a Zeiss Supra55 field emission scanning electron microscope (FESEM) after coating with platinum using the Quorum Q150R Sputter Coater instrument (Şahin et al., 2019). Light microscope image was taken under a Nikon/eclipse TS100 inverted microscope.

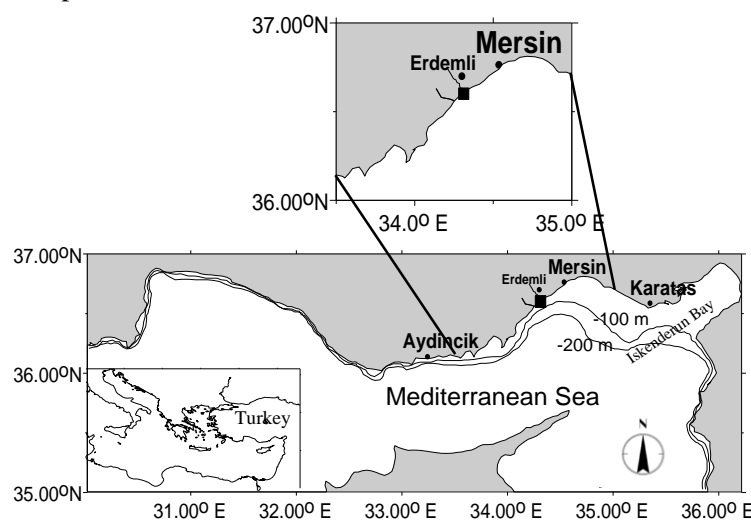


Figure 1. The sampling region (black square).

3. Results

Two new tychoplankton species, *Cocconeis sigillata* Riaux-Gobin et Al-Handal and *Amphicocconeis rodriguensis* Riaux-Gobin et Al-Handa, were identified. In addition, *Delphineis australis* (Petit) Watanabe, Tanaka, Reid, Kumada et Nagumo, *Amphicocconeis disculoides* (Hustedt) De Stefano et Marino from internal sternum valva view and *Psmammococconeis* cf. *disculoides* from external sternum valva view were recorded. *Cocconeis* cf. *peltoides* Hustedt found in the present study has different characteristics than previously reported specimens of *Cocconeis peltoides*.

Cocconeis sigillata Riaux-Gobin et Al-Handal 2011

Description: Valva is elliptic having round apices, 11-12 µm long and 7-8 µm wide. There are 15-16 striae in 10 µm and 60 areolae in 10 µm in quincunx on sternum valve. Sternum valva is inclined from the apical axis, described by low longitudinal costae ending near the apices. Striae are biseriate in quincunx. The axial region is rather enlarged (Figure 2a).

Amphicocconeis rodriguensis Riaux-Gobin et Al-Handal

Description: Valva is elliptical and slightly protruded in the apices (Figure 2b). 9.5-10 µm long, 4.5-5 µm wide, 17 striae in 10 µm, ~16 areolae in 10 µm. Sternum of araphid valva is constricted and linear, slightly compressed along the axis. Striae are uniseriate and faintly radiate near apices. Areolae are rectangular to oblong, having higher density along the margin. Interstriae have outgrowths..

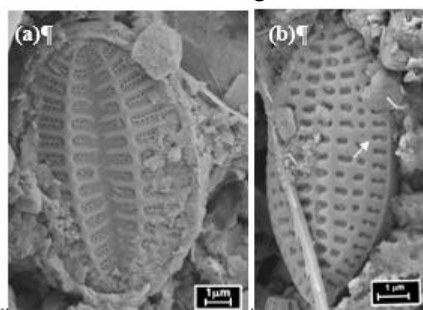


Figure 2. (a) *Cocconeis sigillata* Riaux-Gobin et Al-Handal, external sternum valve (Sv), (b) *Amphicocconeis rodriguensis* Riaux-Gobin et Al-Handal external view of sternum valve, virgae have outgrowths (white arrow).

Amphicocconeis disculoides (Hustedt) De Stefano et Marino 2003

Basionym: *Cocconeis disculoides* Hustedt

Non *Psmammococconeis disculoides* (Hustedt) Garcia (2001).

Description: Areolae externally depressed and occluded by hymenes. One type of areolae is present around the margin as shown by Sar et al. (2003) and De Stefano and Marino (2003). Pars interior of SV valvocopulae covers about 2/3 of the valva surface. Striae are uniseriate (12-13 in 10 µm) and slightly radiate having elongated areolae (~9 in 10 µm) occluded by hymens (Figure 3a).

Psmammococconeis cf. *disculoides* (Hustedt) Sar, Romero et Sunesen 2003

Description: Valva is broadly elliptical to lanceolate as described by Sar et al. (2003), which was observed in Patagonian waters and in Hustedt's type material (Ztl/79 and Am792), 5-10 µm long, 3.5-6 µm wide (Figure 3b-c). There are 18-20 striae in 10 µm. Sternum valva is somewhat convex with a thin, linear sternum without a central area. Striae are rough and uniseriate, straight to slightly radiate in the middle of the valva and arched radiate towards apices. Areolae extended, subrectangular, oval or circular, 14 in 10 µm (Figure 3b,c) similar to specimens shown by Sar et al. (2003). Areolae externally depressed and occluded by hymenes. One type of areolae is present around the margin as shown by Sar et al. (2003) and De Stefano and Marino (2003).

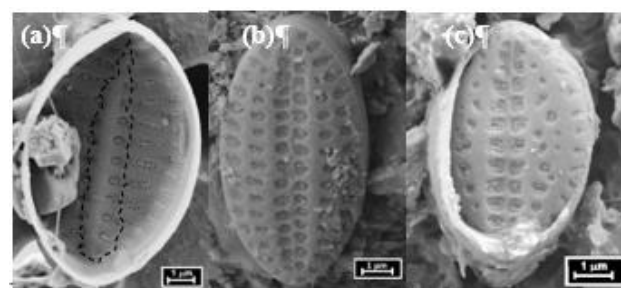


Figure 3. (a) *Amphicocconeis disculoides* with pars interior of valvocopulae (dashed line), internal sternum valva view (b) *Psmammococconeis* cf. *disculoides* sensu Sar et al. (2003), external Sv (c) a small individual of *P. cf. disculoides*.

Delphineis australis (Petit) Watanabe, Tanaka, Reid, Kumada and Nagumo 2013

Basionym: *Rhaphoneis fasciolata* var. *australis* Petit 1877

Synonyms. *Rhaphoneis surirella* Ehrenberg var. *australis* (Petit) Van Heurck 1880-1885,

Delphineis surirella var. *australis* (Petit) Tsarenko 2009

Description: Cells are rectangular in girdle view and form ribbon-shaped colonies containing a couple of plastids (Figur. 4a). Valves elliptical-lanceolate with obtusely rounded apices (Figure 4b). Valva length is 18-24 μm and width 11-12 μm . Sternum width is 2.5-3 μm and upright to elliptical, faintly thinner in the middle of the valva (Figure 4b-d). The ratio of length to width 1.8-1.9. Areolae are elliptical to rectangular, occluded by rotae with two spokes. Two apical pores and a rimoportulae are visible in apices. There are minute papillae on the valva edges (Figure 4b). 11- 13 uniseriate striae and 10 areolae in 10 μm are present. Striae are either straight or slightly arched. There are four girdle bands, first, second and third ones encircle the cell while the forth one is short on one apex. Areola is not present on any of the girdle bands (Figure 4e).

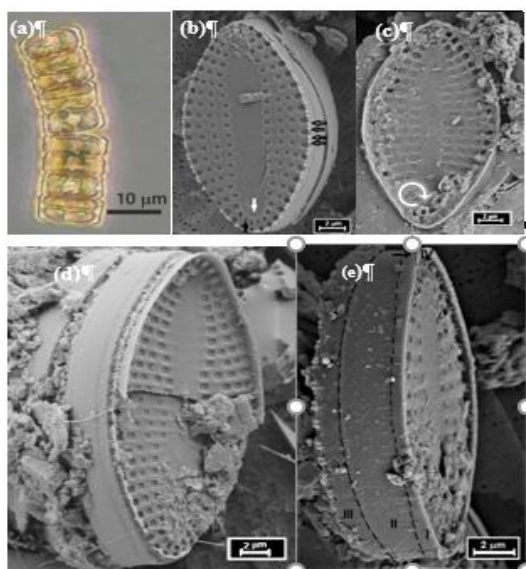


Figure 4. *Delphineis australis* (a) light microscope image, (b) external valva with two apical pores (white arrow) and a rimoportulae (black filled arrow), minute papillae (empty arrows), (c) internal valva view, (d) internal valva seen over the external valva, (e) the first (I), second (II), third (III) and fourth (IV) girdle bands (arrow).

Cocconeis cf. *peltoides* Riaux–Gobin et Compè

Description: 11.5 μm long, 8.5 μm wide, valva is elliptical to slightly lanceolate with rounded apices and axially concave (Figure 5).

External side of sternum valva has 13 striae in 10 μm , 23 areolae in 10 μm . Median area is lanceolate and bordered by two inconspicuous longitudinal costae not extending to apices. Striae are uniseriate and radiate towards the apices. Areola are circular in general and appear to be on the costae as different from the specimens described by Riaux-Gobin et al. (2011a), Majewska et al. (2014) and Sar et al. (2003). Projections of costae extend towards the sternum and some of them are fused in the median area. No areolae is present in the axial region. Interstriae are depressed

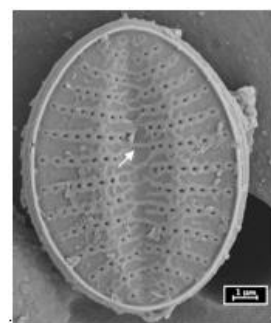


Figure 5. *Cocconeis* cf. *peltoides* Hustedt sternum valve (Sv)

4. Discussion

In this study, two new species of diatoms, *Cocconeis sigillata* and *Amphicocconeis rodriguensis*, are described from the Mediterranean Sea (Table 1). These two species were first found in the western Indian Ocean around Mascarene archipelago (Riaux-Gobin et al., 2011a, b). *Delphineis australis* (Petit) T. Watanabe, J. Tanaka, G. Reid, Kumada and Nagumo was reported in a coastal Lake in the Mediterranean Sea previously (Kaleli, 2019). Here, it was identified in a coastal region in the northeastern Mediterranean Sea by SEM and light microscopy. Larger sternum width, narrower sternum towards the central axis, different minute papillae pattern with two regular minute papillae per interstriae in general, could be among the features differentiating *D. australis* (Figure 2) from

Delphineis surirella (Ehrenberg) Andrews (Watanabe et al., 2013, Figure 21-32). In addition, plain structure of girdle bands in *D. australis* also seems different than undulate pars interior of first and second bands in *D. surirella* (Watanabe et al., 2013, Figure 28).

Genera *Cocconeis*, *Psammococconeis* and *Amphicocconeis* can be differentiated from raphe sternum valva views distinctively. While *Psammococconeis* has simple terminal raphe fissures and coaxial central endings in the internal view, *Amphicocconeis* has curved and elongated terminal fissures in the external view and coaxial central endings. In *Cocconeis* there is no terminal fissures and central nodule endings are deflected in the internal valva view. In the present study, *Amphicocconeis disculoides* (Hustedt) De Stefano et Marino seen in Figure 3a from the internal sternum valva view seems very similar to the individual ascribed to the same species by De Stefano and Marino (2003, Figure 10, 11). However, our Figure 3b showing an individual from external sternum valva view, which seems same with the specimen

identified as *Psammococconeis* cf. *disculoides* by Sar et al. (2003), does not resemble to external sternum valva view of *Amphicocconeis disculoides* (Hustedt) De-Stefano et Marino (Figure 2, 6 in De-Stefano and Marino, 2003). While our individual has circular areolae along the axis in the external sternum valva, *A. disculoides* shown by De-Stefano et Marino (2003) lacks these types of areolae. These two species were suggested to be same by Riaux-Gobin et al. (2011). The reason for referring the species identified as *Psammococconeis* cf. *disculoides* by Sar et al. (2003) to *Amphicocconeis disculoides* was the presence of curved terminal fissures in the external raphe sternum valve observed in the type material of Hustedt (type material Zt1/79 and Am 792, Figure 70 in Sar et al., 2003). It is possible that specimens observed from external sternum views by Sar et al. (2003) are different than *Amphicocconeis disculoides*.

Amphicocconeis disculoides was previously observed in the Mediterranean Sea (De Stephano and Marino, 2003), North America (Hustedt, 1955) and Northern Europe (Hendey, 1964) previously.

Table 1. Geographic distribution of *Delphineis* and *Cocconeis* species found in the present investigation.

Species	Indian Ocean	Mediterranean	North Sea	Atlantic Ocean	Mexico	Pacific Ocean
<i>Cocconeis sigillata</i>	X ³	X ²¹				
<i>Amphicocconeis rodriguezianis</i>	X ⁴	X ²¹				
<i>Delphineis australis</i>		X ^{19,20,21}				X ^{1,2}
<i>Amphicocconeis disculoides</i>		X ^{8, 21}	X ⁵	X ^{6, 7, 9}	X ⁸	X ¹⁰
<i>Psammococconeis</i> cf. <i>disculoides</i> sensu Sar et al. (2003)		X ²¹	X ⁵	X ^{11, 18}		
<i>Cocconeis</i> cf. <i>peltoides</i>	X ³	X ^{17, 21}	X ¹⁶	X ¹²		X ^{13, 14, 15}

1-Watanabe et al., 2013, 2-Tanaka and Nagumo, 2015, 3- Riaux-Gobin et al., 2011a, 4-Riaux-Gobin et al., 2011b, 5- Hendey, 1964, 6- Moita et al., 1999, 7- Sullivan, 1981, 8- De Stefano and Marino, 2003, 9-Sar, 1996a, 10- Lee et al., 1995, 11- Sar et al., 2003, 12- Gil-Rodríguez et al., 2003, 13-Joh, 2012, 14-McCarthy, 2013, 15- Riaux-Gobin et al., 2015, 16-Scholz and Liebezeit, 2012, 17-Majewska et al., 2014, 18-Hustedt, 1955, 19-Kaleli 2019, 20-Kaleli and Akçaalan, 2021, 21-This study

Cocconeis peltoides has previously been recorded in several different locations including the North Sea (Hustedt 1939), Indian Ocean (Riaux-Gobin et al., 2011a), North Atlantic (Witon and Witkowski,

2006), Argentina coasts (Sar et al., 2003) and the Mediterranean Sea (Majewska et al., 2014) (Table 1). This species was reported to display similarities to *Cocconeis sigillata* except pertaining uniseriate striae

in sternum valve (Riaux-Gobin et al., 2011a). Our specimen displays some different properties than *C. peltoides* and *C. peltoides* var. *archaeana* Riaux-Gobin et Compère reported in previous investigations (Riaux-Gobin et al. 2011a, Figures. 1-6, 23-25; Sar et al., 2003, Fig. 34-38). Major difference seems to be the location of areolae within costae and extension of costae towards the sternum and inter-connection of striae projections in sternum. Similar to *Cocconeis peltoides*, two longitudinal costae separate areolae in the median part and there are two areolae near the sternum in our species as well (Figure 5). Our species either could be autochthonous or allochthonous not reported previously.

Identification of some small phytoplankton taxa such as *Cocconeis* species with a light microscope is not possible since fine structures (e.g. areolae, hymen, fimbriae) are not clearly visible (Romero and Van de Vijver, 2011; De Stefano et al., 2006). Thus, there is limited number of studies in the literature about small species in certain geographical locations (Romero and Van de Vijver, 2011; Majewska et al., 2014). With this investigation, we add two new nanoplanktic diatom species to the benthic flora of the Mediterranean Sea.

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5. Conclusions

Two new monoraphid diatom species, *Cocconeis sigillata* Riaux-Gobin et Al-Handal *Amphicocconeis rodriguensis* Riaux-Gobin et Al-Handal are reported in the Mediterranean Sea. *Cocconeis* cf. *peltoides*, identified in this study, demonstrates a more distinct property, which have areolae within costae and extended costae on sternum, than in *Cocconeis peltoides* Hustedt. External sternum valva of *Psammococconeis* cf. *disculoides* observed in this study seems similar to the individuals recorded by Sar et al. (2003) but different than *Amphicocconeis disculoides* described by De Stephano and Marino (2003). Although *Delphineis australis* was recorded in a coastal lake (23.4 psu) in the Mediterranean region previously, it has not been recorded in coastal waters of the Mediterranean Sea (~38 psu) before this study.

Acknowledgments

This study was supported by TÜBİTAK 115Y767, Mersin University BAP 2016-2-TP-2-1912 and Mersin University BAP 2019-2-AP2-3595 projects.

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