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RESEARCH ARTICLE

New record of the benthic marine mucilage aggregates *Phaeocystis giraudii* from Turkish Eastern Mediterranean Sea

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Abstract

This study presents a new record of *Phaeocystis giraudii* Derbes et Solier (Phaeocystales, Prymnesiophyceae), which forms gelatinous aggregates and is found as clusters in various sea substrates in the coastal zones of Turkey. The species has been observed at various locations in the central Aegean Sea (Sığacık, Gümüldür and Çeşme) and Mediterranean Sea (Kaş and Adrasan) since 1994. Mucilaginous aggregate colonies at these stations cover 5-60% of the substrate at a depth range of 8-25 m. This paper also discusses the taxonomic problems in the nomenclature of the species.

Keywords: *Phaeocystis giraudii*, *Chrysoreinhardia graudii*, new record, mucilage, sea snot, Turkey

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Introduction

In recent years, mucous and gelatinous structured aggregates in the sea formed by some pelagic and benthic organisms have been called "mucilage events" (Deserti *et al.* 2005) or "mucilage phenomena" (Precali *et al.* 2005) and "mucilaginous life". Aggregate events have negatively impacted the development of seagrass meadows (Lorenti *et al.* 2005; Ozalp 2021), gorgonian corals (Rinaldi *et al.* 1995; Giuliani *et al.* 2005; Ozalp 2021), mussels (Cornello *et al.* 2005), sponges, molluscs, and several other benthic organisms (Giani *et al.* 2012) at various locations in the Mediterranean. Aggregate phenomena formed by some pelagic species like phytoplankton, bacteria and dinoflagellates when precipitated damage both pelagic and benthic ecosystem, tourism, fisheries and other maritime sectors (Alldredge and Cracker 1995; Innamorati *et al.* 2001; Aktan *et al.* 2008; Balkıs *et al.* 2010). Both epibenthic microalgae (diatom colonies, cyanobacteria) and macrophytes have contribution in the origin of benthic aggregates (De Phillippis *et al.* 2005; Sartoni *et al.* 2008). Although the sources triggering the aggregate phenomenon are still not clearly understood, long-lasting monitoring and several studies in the Adriatic Sea indicate that this phenomenon is related to the nutrient burden entering the sea from several sources, but changes in the seasonal, meteorological and oceanographic conditions also play an important role, supporting the conclusion that this event is an environmental problem (Degobbis *et al.* 1995; Innamorati *et al.* 2001; Precali *et al.* 2005). Recently in 2003, the heat wave in Europe caused increases in sea water temperatures, and it is reported that this triggered the mucilage event (Schiaparelli *et al.* 2007). Again in Europe, it is known that nutrient entering into the coastal water through rivers played a role in the formation of "foam aggregate" by the *Phaeocystis* colonies in the North Sea (Lancelot 1995). Consequently, there is an increasing consensus that the synergetic combinations of the above-mentioned factors play a role on the formation of the mucilage or gelatinous aggregate phenomenon (Degobbis *et al.* 1999).

The oldest record of the accumulation of gelatinous aggregate and mucilage phenomena in the Mediterranean dates back to 200 years ago (Rinaldi *et al.* 1995). Since then, reports of benthic mucilage aggregates have been more frequent particularly in some areas and become nowadays an important concern in the entire Mediterranean (Cinelli 1992; Rinaldi 1992; Sartoni *et al.* 1995; Hoffmann *et al.* 2000; Giani *et al.* 2005a, b; Schiaparelli *et al.* 2007; Aktan and Topaloğlu 2011). The Adriatic Sea and the coasts and islands of Italy and France are locations which are frequently the subject of studies both for a checklist and for a mucilage phenomenon (Giaccone 1992; Innamorti 1992; Frick *et al.* 1996; Degobbis *et al.* 1999).

Mucilage and gelatinous aggregate events on the Turkish coasts were noticed for the first time in 1994 in Sığacık Bay, and subsequently filamentous structured mucilage aggregates were observed in 1997 on Hekim Island (İzmir Bay) at a depth of 18 m, and on Melina Island (Ayvalık) below a depth of 40 m (1997-1998).

In this study, *Phaeocystis giraudii* is reported for the first time from Turkey, in the central Aegean Sea and Turkish part of the Eastern Mediterranean Sea. The confusion caused by the debatable nomenclature of the species is discussed and the final status of the systematic position is stated. Furthermore, substrate type, depth range, morphological variations and microscopic features of the cells of the species are described.

Materials and Methods

Phaeocystis giraudii, has been observed at five locations in the coastal zones of the central Aegean Sea and Mediterranean Sea in Turkey (Figure 1). Year-round dynamics of the aggregates were followed at Sığacık (Akkum) between June 1994 to November 1998 in the Aegean Sea. Additional stations visited once in May-August to check for aggregates were Gümüldür (Kalemlik) in 2005 and 2007, Çeşme (Eşek Island) in 2011 in the Aegean Sea; Kaş (Camel Reef) in 2009 and Adrasan in 2010 in the Mediterranean coast (Table 1). The species was observed and collected by Scuba diving from the surface to 25 m. The Braun-Blanquet and Pavillard (1922/28) scale was used to determine the coverage rate of the species in different substrates for *in situ* observations (Ciecierska *et al.* 2010).

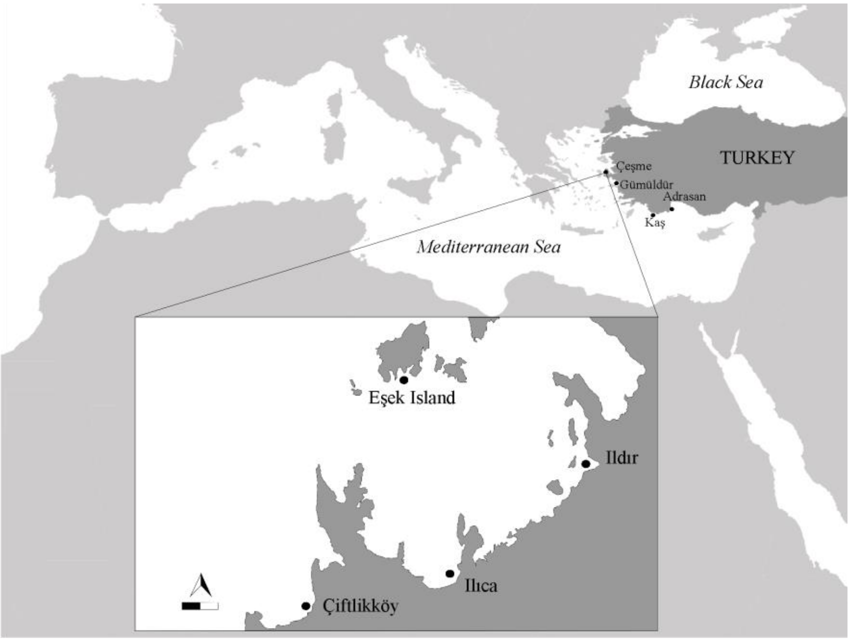

Figure 1. Sampling localities in this study

The samples were transported to the laboratory and quickly analysed since the species is sensitive to oxygen, temperature and pH changes in the seawater, and turns into a thallus gelatinous mass, losing its original structure. Upon completion of the microscopic measurements, vegetative cell formation was photographed. Afterwards, gelatinous thalli were fixed in 4% formaldehyde/sea water mixture and stored in the laboratories of Ege University and Akdeniz University.

Results and Discussion

Classification

Divisio: Haptophyta D.J. Hibberd *ex* Edvardsen *et* Eikrem. (in Edvardsen *et al.* 2000: 26) [=Pyrmnesiophyta (Van den Hoek *et al.* 1995: 219)]

Classis: Prymnesiophyceae D.J. Hibberd, 1976 [=Haptophyceae in Throndsen 1997: 633]

Ordo: Phaeocystales Medlin (in Edvardsen *et al.* 2000: 28)

Familia: Phaeocystaceae Lagerheim, 1896: 288

Genus: *Phaeocystis* Lagerheim, 1893: 93

***Phaeocystis giraudii* (Derbes et Solier) De Toni 1895: 593**

Type locality: France: Marseille: Estaque; Madrague, on various algae (Silva 1996-to date).

Basionym: *Tetraspora giraudii* Derbès & Solier in Castegnea, 1851: 94

Original publication: Derbès, A. & Solier, A.J.J. 1851. Algues. In: Supplément au catalogue des plantes qui croissant naturellement aux environs de Marseille. (Castagne, J.L.M. Eds), pp. 93-121. Aix: Nicot & Pardigon.

Homotypic synonyms: *Phaeocystis giraudii* (Derbès *et* Solier) cfr. Leigerheim 1.c., (De Toni 1895: 593), *Phaeocystis giraudii* (Derbès *et* Solier) Leigerheim (Hamel 1930: 51-52), *Phaeocystis giraudii* (Derbès *et* Solier) Hamel (Throndsen 1997: 636), *Pulvinaria giraudii* (Derbe`s *et* Solier) Bourrelly 1957: 373 (invalid combination: incomplete reference) (Hoffmann *et al.* 2000).

Discussion on taxonomic status
The name of the class was given by Casper in 1972 (with a German description, not Latin). *Pulvinaria* was named as a genus by Reinhard, and continued to be used debatably by other authors for a long time (Hoffman *et al.* 2000). To eliminate this confusion, Billard created the genus name *Chrysoreinhardia* as

"nom. nov." (Hofmann *et al.* 2000). To locate other species belonging to the suggested genus, *Pulvinaria algicola* is shown as the type species basionym: Reinhard 1885; p. 248 and named *Chrysoreinhardia algicola* (Reinhard) Billard comb. nov. Basionym: *Tetraspora giraudii* Derbes et Solier, 1856 is used for another species *Chrysoreinhardia giraudii* (Derbes et Solier) Billard comb. nov., whereas the debatable synonyms *Phaeocystis giraudii* (Derbes et Solier) Lagerheim and *Pulvinaria giraudii* (Derbes et Solier) Bourrelly (invalid combination; incomplete reference) are used (in Hoffmann *et al.* 2000). However, in Hoffman *et al.* (2000), the valid nomenclature (*Phaeocystis giraudii*) used by De Toni (1895) is not mentioned. Suspiciousness of taxon is removed through the previous nomenclature by De Toni, and the validity has gained more strength.

In Edvardsen *et al.* (2000), Medlin presented the diagnostic features of the order effectively while using the order Phaeocystales "ord. nov." as a new classification. Lagerheim placed the family Phaeocystaceae and the species *Phaeocystis* in this order (1896). Edvardsen *et al.* (2000) cited the study in 1988 published by Saurnia which discusses the presence of nine validly published (undebatable) species. Medlin and Zingone (2007) analysed the same study and highlighted that two species (*Phaeocystis fuscescens* and *Phaeocystis giraudii*) of the genus had not been evaluated by Saurnia on grounds of indecisive genus names. However, the genus *Phaeocystis* today maintains its validity as it did in the past, with nine known species (Edvarsen *et al.* 2000; Medlin and Zingone 2007; Rousseau *et al.* 2007; Gaebler-Shwarz *et al.* 2010). The basionym *Tetraspora giraudii* Derbes et Solier (in 1856) recommended by Billard for the species *Chrysoreinhardia giraudii* (Derbes et Solier) "comb. nov." belonging to the genus *Chrysoreinhardia* is in fact the species *Phaeocystis giraudii* placed in the genus *Phaeocystis* by Lagerheim with the addition of the note "cfr" (De Toni 1895: 593). In other words, by adding the note "cfr" not after the genus but following the species, Lagerheim expresses the doubt that the sample analysed was a *giraudii*.

In the same study, when the species *Phaeocystis fulvescens* (A.Br.) cfr. Born. is cited (De Toni 1895; 593), the doubt about the genus *Phaeocystis* is mentioned. However, in his study dated 1930, Hamel overlooked an important particularity quoting the "cfr" note after the species, which is a debatable situation for the species *Phaeocystis giraudii* in De Toni's study. Another conflict is that the same researcher emphasizes the debatable situation of the species based on the figures given by Derbes and Solier (Figures 51, 53; D, E, F). Thereafter, the taxon is supported by Papenfuss (1968: 28 p.), who included *Phaeocystis giraudii* (Derb. et Solier) Lagerheim in the floristic list without any cautionary note. When the species was included in the list, however, both the "cfr" note for debatable place by Lagerheim and the correction by De Toni were disregarded. In response to such a complicated situation, Bourrelly (1957: 373 nom. invalid) proposed species *Pulvinaria giraudii* based on the genus *Pulvinaria* designated by Reinhard (1885). Coppejans and Boudouresque (1983) also used this nomenclature. Meanwhile, Guiry and Guiry (2019) accepted *Pulvinaria giraudii* (Derbes et

Solier) Bourelly (1957; 373 nom. invalid), as “current name” giving Hoffman *et al.* (2000) as key reference.

This situation shows that the same species is cited as two different species in the “algae base”, given the current basionym *Tetraspora gelatinosa* for both. Palmelloid phasen[1] being dominant in the family Phaeocystaceae and being able to adjust to benthic life from planktonic life supports the placement of the species in the marine plankton for the first time (Throndsen 1997). The researcher listed six species belonging to the genus, but included the species *Phaeocystis giraudii* (Derbes et Solier) Hamel under Hamel nomenclature, not mentioning any information about De Toni.

On the basis of the above-mentioned sources, some of the planktonic and benthic species among the mucilage aggregates are cited under the genus name *Phaeocystis*. This situation is an important piece of evidence for the validity of the genus name (Edvardsen *et al.* 2000). Five taxa belonging to the genus *Phaeocystis* are given by Medlin based on 18 S ribosomal DNA sequences and available morphological data (Edvardsen *et al.* 2000: 23,28,31), and Billard mentions the species *Phaeocystis giraudii* (Derbes et Solier) Lagerheim, as a synonym under *Chrysoreinhardia giraudii* (Derbes et Solier) Billard (Hoffman *et al.* 2000: 224-228). The researcher justifies this based on the invalid combination (incomplete reference) available in the sources.

In this paper, we conclude based on evaluation of all the references mentioned so far that there is not a real debatable situation for the nomenclature of the species. Thus, the genus *Phaeocystis* has been maintained in most of the studies, whereas it is understood from their own notes that some of the authors accepted inaccurate and incomplete nomenclature usage in successive years, as mentioned above. Although it seems to be a complex situation, when the studies are reviewed, placing the species under *Phaeocystis* is not a debatable case in reality. Therefore, we conclude that instead of *Chrysoreinhardia*, it is valid to use the historical taxon for the species under the genus *Phaeocystis*, as *Phaeocystis giraudii* (Derbes et Solier) De Toni.

Seasonal Dynamics and Distribution

Phaeocystis giraudii was identified and photographed for the first time on the Turkish coasts on June 1994 at Akkum (Sığacık), and it was monitored at this location until 1998. The species was found at all locations with a percent cover varying from Adrasan (5%) in August 2010 to Kaş (60%) at Camel Reef location in August 2009, when the abundance was highest.

Year-round monitoring at a depth of 8-25 m showed that the population develops seasonally following the same pattern in the Aegean and Mediterranean coasts (Table 1). Macroscopically visible gelatinous aggregates started to be formed in May, showed maximum growth in August, and completed their development throughout the month. Starting in September, they start to break off the substrates and form clusters in the intermatte, finally disappearing gradually in October. The densest colonization is observed between 10-15 m and becomes less common below 15-20 m.

Figure 2. *In situ* underwater pictures; *Phaeocytis giraudii* colonies on the *Posidonia oceanica* leaves and the bottom, (A) Gümüldür (B) Çeşme (C, D) Sığacık

Gelatinous aggregates settled in the Mediterranean and central Aegean Sea are seen especially on *Posidonia* leaves, starting mid-May. They reach 1-2 mm by the end of May, and in June they grow enormously in a couple of weeks. In August, they attain a diameter of 14-15 mm. The colonies observed in Sığacık are greenish yellow in colour. They start to disappear in September, forming shapeless yellowish-brown layers on the bottom. The appearance of the aggregates in Gümüldür and Çeşme samples is yellowish-brown from May to September (Figure 2). The aggregates developing in the basins of Çeşme, Kaş and Adrasan form gelatinous colonies resembling cloud or foam (Figure 3). Cells are spherical to hemispherical, and 7-11 μm in diameter. A mucilage layer forms concentric circles around the cells, and this protects the colony from environmental factors (Figure 4). The light brownish coloured mucilaginous colonies reach a size of up to 14 mm in diameter. The colonies are pulvinate and occur on sublittoral solid substrates and rocks, or epiphytic on seagrass leaves and marine macroalgae.[1]

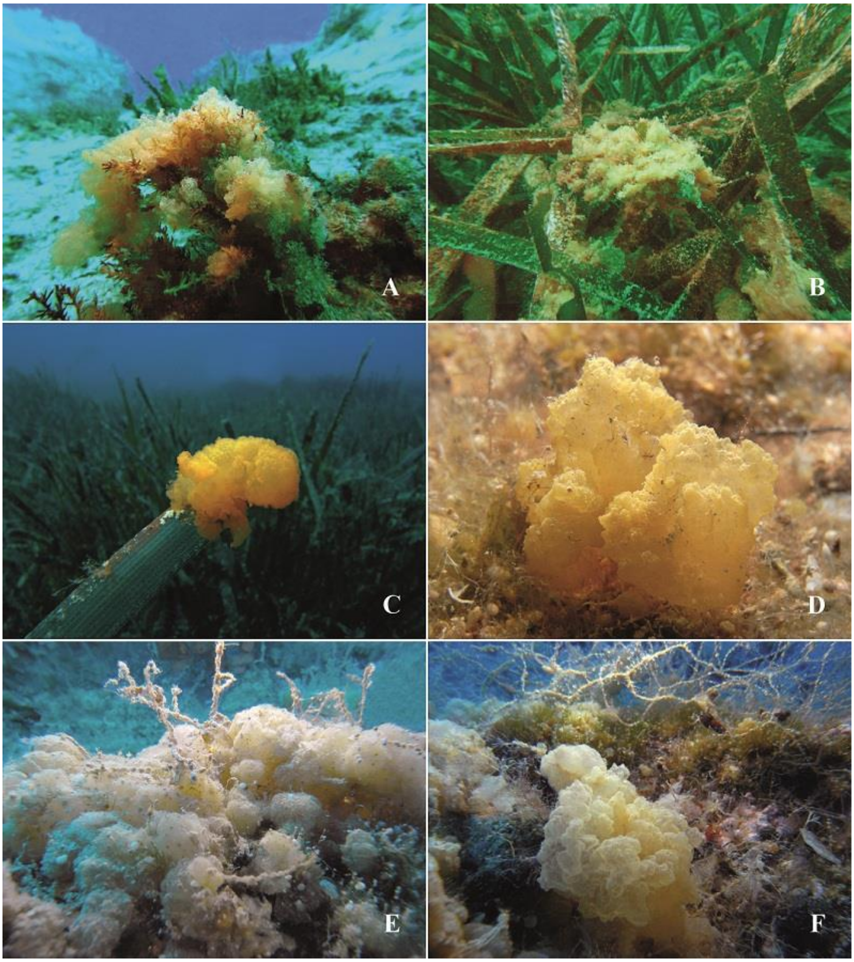

Figure 3. *In situ* underwater pictures; *Phaeocytis giraudii* colonies on the marine algae, the *Posidonia oceanica* leaves and the bottom (A, B, C) Kaş, and (D, E, F) Adrasan

Average water temperatures in 1998 at a depth of 5-20 m were recorded as 18.5 - 21.5°C. The maximum depth limit for the population on the Akkum coast is 15

[1] ["The palmelloid stage refers to the massing of cells. One of the basic characteristics of this stage is the production of mucilaginous material that causes the cells in the aggregate to become separated from one another. The cells are embedded in a homogeneous matrix" (Bausor and Agona 1973)]

m. The densest colonization pycnocline is observed at a depth of 9-12 m with a percentage coverage of 30-40% on the bottom (Table 1).

In the observations from 2005 to 2007 on the Gümüldür (Kalemlik) coast in the central Aegean Sea, the average water temperature of the coast from a depth of 3 m to the thermocline was 23.6°C, without much variation. Gelatinous formation covered 5-10% of the area with dead matte and photophilic algal substrates at a depth of 8-10 m, and 20-50% at a depth of 15 m (Table 1). This species forms 80% of the epiphyte on *Posidonia* leaves, and it is not observed on rhizomes with low light intensity and other substrates in the shade in August.

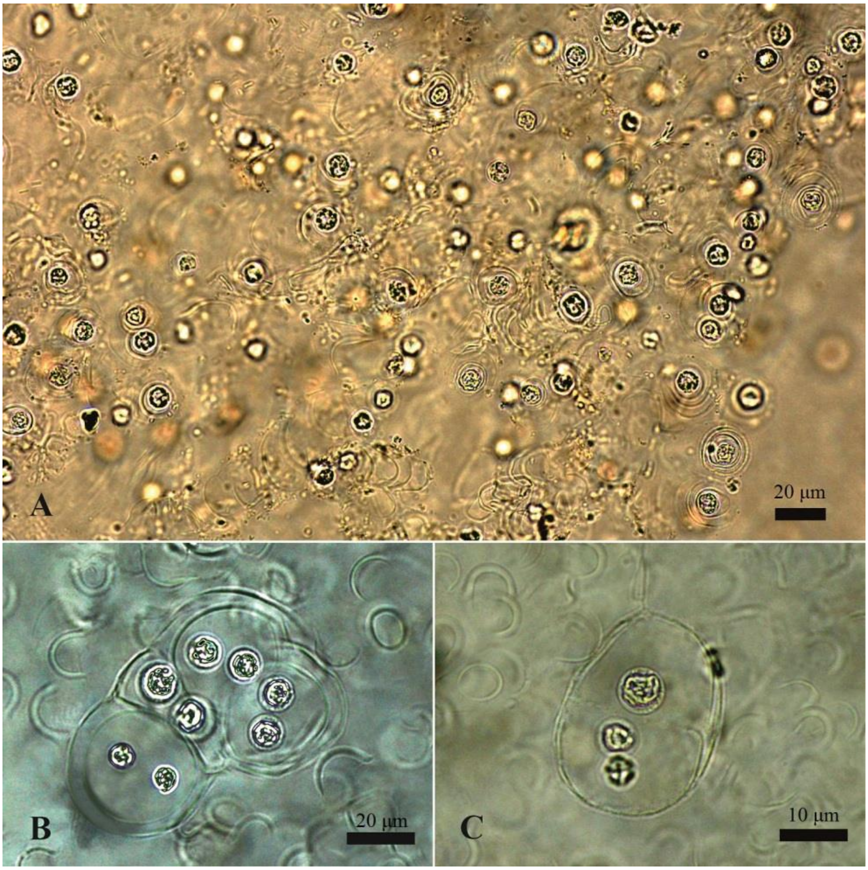

Figure 4. Microscopic cell formation of *Phaeocytis giraudii* (A) Cell formation in dense gelatinous aggregate, (B, C); Mucilage layer forming concentric circles around the cells and diatomic cell colonies

The phytobenthos in Çeşme, Eşek Island (Jandarma Bay), which is in the middle of the central Aegean Sea coast, was searched down to a depth of 25 m in July

2011. Water temperature to the thermocline was 24°C, and the upper limit of *Posidonia* meadows started at a depth of 5 m. Aggregates started from a depth of 9 m and continued to 20 m with decreasing density. They covered 5-20% of the area on *Posidonia* leaves or intermatte, dead rhizomes, and hard or photophilic algal substrates (Table 1).

Table 1. Sampling stations, dates, substrate types and depth range where *P. graudii* was observed and % cover

(Scale-Covering (%); 5:75-100, 4:50-75, 3:25-50, 2:5-25, 1:1-5, +:0,1-1, r:<0,1)

Station	Coordinate	Date	Substrate	Depth (m)	Cover (%) (Scale)
Çeşme (Eşek Island)	38°25'27.01"N 26°20'07.38"E	2011	*Posidonia oceanica* leaves, marine algae, dead matte	9-20	5-20 (1-2)
Adrasan	36°18'47.75"N 30°28'36.17" E	2010	Sea bottom	10-25	5 (1)
Kaş (Camel Reef)	36°09'28.23"N 29°37'53.39"E	2009	*Posidonia oceanica* leaves, on the marine algae sea bottom	10-25	5-60 (1-4)
Gümüldür (Kalemlik)	38°01'12,74"N 27°04'21.14"E	2005 - 2007	*Posidonia oceanica* leaves, dead matte	8-15	5-50 (1-3)
Sığacık (Akkum)	38°11'48.60"N 26°46'19.94" E	1994 - 1998	Sea bottom	9-15	30-40 (3)

The presence of the species on the Kaş (Camel Reef) coasts of the Mediterranean coast of Turkey has been recorded between 10 to 25 m in May 2009. Average water temperature is measured as 22°C. It develops as small masses, frequently scattered in the basin. Colonies which have not yet formed aggregates with a diameter of maximum 4-5 mm have a coverage percentage of 5%. It is observed that the volume of the colonies becomes larger in June and July and they form aggregates with a coverage reaching 30-40%. In this month, average water temperature is measured as 25-27°C. Maximum development is observed in August, when the coverage may reach 50-60%. Average water temperature is measured as 28.7°C. Colonies are scattered on *P. oceanica* leaves and on some macroalgae, especially *Cystoseria* sp.

Other instances of this species were recorded in the Adrasan coasts in June 2010 at a depth of 10-25 m. The colonies on the Adrasan coast were found in small masses, unlike the expected big aggregates, even in July and August when the maximal development was observed. They were observed rarely and the coverage did not exceed 5%. Average water temperature was measured as 27°C.

Phaeocystis giraudii may form massive aggregates. Its records have been confirmed in macroscopically visible aggregate colonies from May to September. In our data, *P. giraudii* extends to a depth of at least 25 m, but is more rarely below 15-20 m.

Figure 5. Recorded locations of *Phaeocysti giraudii* in the world seas

Hyeres coast in France are among the first records in the Mediterranean Sea (Hamel 1930). The species is described as “parasites on various algae” by Coppejans and Boudouresque (1983), and its occurrence in April at Ghardaqa in the Red Sea is mentioned. It was reported in this study that the colonies were collected from the rhizomes of *P. oceanica* at a depth of 3 m in Revellata Bay. In the Adriatic Sea, Hoffmann *et al.* (2000) stated that mucilage colonies generally appear at a depth of 0-20 m from March to September; however, in 1997-1998, the colonies remained until November, extending to a depth of 38 m along the Corsican coast (Figure 5). Another record outside the Mediterranean Sea is from the Australian coast (Port Phillip Bay, Melbourne), and the species is recorded as *Phaeocystis giraudii* (Derbes et Solier) Lagerheim (King *et al.* 1971; Light and Woelkerling 1992).

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Denizel bentik müsilaj agregat *Phaeocystis giraudii* türünün Doğu Akdeniz Türkiye kıyılarından yeni kaydı

Öz

Denizlerde çeşitli substratlarda kümeler halinde jelatin agregatlar oluşturan *Phaeocystis giraudii* Derbes et Solier (Phaeocystales, Prymnesiophyceae), Türkiye kıyılarında ilk kez kayıt edilmiştir. Tür, orta Ege (Sığacık, Gümüldür and Çeşme) ve Türkiye'nin Akdeniz (Kaş ve Adrasan) kıyılarında 1994 yılından itibaren izlenmiştir. Koloniler, bu istasyonlarda 8-25 metre derinliklerde dağılım göstermekte ve substratın %5-60'ını örtmektedir. Ayrıca bu çalışmada, türün isimlendirmesindeki taksonomik problemler tartışılmıştır.

Anahtar kelimeler: *Phaeocystis giraudii*, *Chrysoreinhardia graudii,* yeni kayıt, müsilaj, deniz salyası, Türkiye

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