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PROOF RESEARCH PAPER

Morphological and Molecular Characterization of Scenedesmus-Like Species from Ergene River Basin (Thrace, Turkey)

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Abstract

The genus *Scenedesmus* and other allied morphospecies can not only been identified morphologically due to their smaller sizes and phenotypic plasticity. In this study, six *Scenedesmus*-like species were isolated and investigated from the inland freshwater of Ergene River Basin (Thrace, Turkey). These strains were studied in-depth using the light microscopy (LM) and the molecular ITS1-ITS4 gene sequencing. The combined morphological and phylogenetic analysis revealed that the strains studied belonged to the genera *Desmodesmus* (2 strains were characterized as *D. communis* and one strain identified as *D. armatus* var. s*ubalternas*), *Acutodesmus* (2 strains related to *A. obliquus*), and only one strain of the genus *Pectinodesmus* that identified as *P. pectinatus.*This study confirms that morphological identification of *Scenedesmus*-like species is considered not only enough for accurate species delineation but also the molecular approaching techniques should be applied. However, more deep molecular studies using other genes sequencing should be integrated for more easily species identification.

Keywords: ITS; microalgae; morphotaxonomy; Scenedesmaceae; phylogeny; Ergene River (Turkey).

Introduction

The classification of the genus *Scenedesmus* Meyen is still unclear. To date, this genus has been investigated from different morphotaxonomic standpoints and placed into different algal groups (e.g., Hegewald, *et al.*, 2010; 2013). Early, all autosporic coccal green algae characterized by flat and/or curved coenobia were described by Meyen (1829) as *Scenedesmus* (as cited in Hegewald and Wolf 2003). Turpin (1828) described some species of the genus *Scenedesmus* and transferred them into diatoms. Then later, Ehrenberg (1834) placed them into family Desmidiaceae. Nevertheless, Nageli (1849) sticked the genus *Scenedesmus*, and its relative species, to order Chlorococcales and family Hydrodictyaceae. In 1926, Chodat split the genus *Scenedesmus* into a number of subgenera and these were reduced by Hegewald and Silva (1988) later to just three subgenera: *Scenedesmus*, *Desmodesmus* and *Acutodesmus*. Recent molecular techniques strongly supported this taxonomical standpoint. *Desmodesmus* was separated as a genus of its own by An *et al.* (1999) based on ITS-2 rDNA sequence analysis. Hegewald (2000) has transferred 32 species and 22 varieties to *Desmodesmus* from the sub-genus *Scenedesmus*. The subgenus *Acutodesmus* was raised to generic rank according to contributions of Tsarenko (2000), and Tsarenko and Petlovany (2001), and this has been supported by 18s rDNA and ITS-S sequence comparisons (Hegewald and Wolf, 2003).

Scenedesmus-like taxa are usually characterized by a high degree of polymorphism in both field and culture conditions. This distinct feature is easily recognized by high variations in number of cells forming coenobia, cell dimensions and cell wall ornamentations (Hegewald 1997). For example, some researchers discussed the aforementioned characteristics in some cultured strains of *D. communis* (e.g., Komarek and Ruzicka, 1969; Brezina *et al.*, 1972; Steenbergen, 1978; Hegewald, 1989; Trainor, 1992; Bica, *et al.*, 2012). Kessler *et al.* (1997) pointed out that the morphological characteristics and metabolic capacities are considered not only enough to clearly distinguish the different *Scenedesmus* and *Desmodesmus* taxa. Recently, the molecular phylogenetic analysis was implemented to solve some this taxonomic puzzle (e.g., An *et al.*, 1999; Van Hannen *et al.*, 2002; Hegewald and Wolf, 2003; Johnson *et al.*, 2007; Vanormelingen *et al.*, 2007; Fawley *et al.*, 2011; Bica *et al.*, 2012). The recent study of Radha *et al.* (2013)

confirmed that the combined molecular and morphological identification of microalgae is a very important approach for species delineation. Moreover, some abiotic (e.g., light intensity, nutrient concentrations, heavy metals and temperature), and biotic factors (e.g., herbivores) significantly trigger the morphological plasticity in *Scenedesmus*, *Desmodesmus* and other related morphospecies (Jonson *et al.*, 2007).

In Turkey, there are a few phylogenetic studies on different microalgal groups but none of them gave more interest to members of family Scenedesmaceae (e.g., Tüney and Sukatar, 2010; Soylu and Gönülol, 2011; Kesici *et al.*, 2013; Kızılkaya-Tüney *et al*., 2016). Thus, the main aim of the present study was to investigate the molecular and morphological relationships of some *Scenedesmus*-like species isolated from the River Basin of Ergene (Turkey) using the light microscopy (LM) and PCR analysis. Another goal is to increase our knowledge about the taxonomy and autecology of the Turkish freshwater microalgae.

Materials and Method

Materials Collection, Identification and Isolation

Water samples were collected from inland waters in River Basin of Ergene (Thrace, Turkey) in May 2012. Coordinates and physical parameters of sampling localities are shown in Table 1. The algal samples were collected via 25 µm-planktonic net and then kept in clean plastic bottles and stored at 18˚C until transferring to the laboratory using a portable cooler. The algal samples collected were examined morphologically using Olympus CX31 light microscopy. The taxonomic identification was performed following the classification systems adopted by Prescott (1973), Komarek and Fott (1983), and Soeder and Hegewald (1989). The cell shapes and arrangement, cell length and width, details of the outer cells, and the shape and length of the spines were mainly used as the main diagnostic features in species delineation. A total of 40 measurements were made for each of this taxonomically important morphometric features. The scenedesmoid specimens were isolated using the agar plate method (Bold, 1942). Axenic algal cultures were grown in Bold Basal Medium (Stein, 1973) under 16: 8 L/D illumination photocycle with time cycled socket and provided by cold white fluorescent lamps with about 150 µmol photons m^{-2} s^{-1}, and at a temperature of 25 ± 2 º C. The algal photomicrographs were taken by Olympus CH 51 digital camera. The scenedesmoid cultures were daily followed and identified morphologically and then harvested for DNA identification when reached to the appropriate cell density at the exponential growth phase.

Molecular Analysis

DNA Isolation and PCR Analysis

Total genomic DNA from the cultured samples were isolated by the Gene JET Plant Genomic DNA Purification Mini Kit (Thermo Scientific, USA) according to the manufacturer's instructions and then stored at -20 ºC until further analysis. 25 µl PCR mix contained 1U Dream Taq DNA polymerase (Thermo Scientific, USA), 10× reaction buffer (Thermo

Table 1. Physical parameters of the sampling sites

Algal Taxa	Sampling Sites	Coordinates	Temp. (°C)	pH	Conduct. (µS cm^{-1})	Dissolved O_2 (mg l^{-1})
Scenedesmus acutus Meyen (sample 1)	Sazlıdere	41° 13' 22.541" N 26° 41' 12.122" E	13.20	7.80	195.3	8.90
Scenedesmus antennatus Brebisson (sample 2)	Kaşıkçı Village pool	40° 59' 46.499" N 27° 11' 21.466" E	16.30	7.25	2038	5.21
Scenedesmus quadricauda Chodat (sample 3)	Bahçedere	41° 22' 5.830" N 27° 44' 45.193" E	20.70	7.47	760	0.98
Scenedesmus opoliensis P.G. Richter (sample 4)	Kaşıkçı Lake	41° 1' 17.435" N 27° 13' 25.988" E	16.30	7.25	2038	5.21
Scenedesmus acuminatus (Lagerheim) Chodat (sample 5)	Kaşıkçı Lake	41° 1' 17.435" N 27° 13' 25.988" E	16.30	7.25	2038	5.21
Scenedesmus armatus Chodat (sample 6)	Hacıfaklı stream	41° 42' 35.078" N 27° 28' 43.904" E	15.30	7.30	538	8.99

Scientific, USA), 0.2 mM dNTP mix (Thermo Scientific, USA), 0.25 µM forward and reverse primers (Alpha DNA and IONTEK), 50 ng DNA and ultrapure water (HyClone). PCR analysis was performed by T100™ Thermal Cycler (Bio- Rad, USA).

ITS1 (5'-TCC GTA GGT GAA CCT GCG G -3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC -3') primers (White *et al.* 1990) were used to amplify ITS (Internal Transcript Spaces) region of the identified species.

PCR conditions for ITS1-ITS4 primer pair was started with initial denaturation at 95°C for 30 sec. and then it was followed by 35 cycles of denaturation at 95°C for 5 sec., annealing at 55°C for 5 sec., extension at 72°C for 10 sec. and a final extension at 72°C for 1 min.

The PCR products were analyzed by 1% agarose gel electrophoresis in 1× Tris-Boric acid-EDTA (TBE) buffer at 5 Vcm^{-1}, stained with Safe View (ABM) and visualized under UV illumination.

Sequence Analysis and Phylogenetic Analysis

Sequence analyses of PCR amplicons were performed at RefGen Biotechnology Research Laboratories (Ankara, Turkey) using the eukaryotic primers (ITS1-ITS4). The sequences obtained were compared to those deposited in the GenBank using the BLAST algorithm (Altschul *et al.*, 1997). ITS gene region sequences were aligned using the ClustalW algorithm. Aligned data set was used for creating phylogenetic trees in MEGA 7 software (Tamura *et al.*, 2011), Unweighted Pair Group Method with Arithmetic Mean (ML) (Michener and Sokal, 1957) and Neighbor Joining (NJ) (Tamura *et al.*, 2004) algorithms were used to infer phylogenetic relationships.

Results

Morphological Identification

The morphological identifications of samples were done by Komarek&Fott, 1983. Length and width of forty replicates were measured and the results are in Table 2. The *Scenedesmus*-like isolates included in this study (Table 2; Figure 1) were morphologically identified as the following: *Scenedesmus acutus* Meyen (sample 1), *S. antennatus* Brebisson (sample 2), *S. quadricauda* Chodat (sample 3), *S. opoliensis* P.G. Richter (sample 4), *S. acuminatus* (Lagerheim) Chodat (sample 5), *S. armatus* Chodat (sample 6).

DNA Isolation and PCR Analysis

The presence of only one DNA band on the agarose gel indicated that there were no other contaminants in the sample. The quantity of isolated DNAs was measured by Nanodrop (Thermo Scientific, USA). The DNA samples were diluted with ultra-pure water (HyClone) to obtain 50 ng DNA for PCR analysis. We obtained approximately 700 base pair amplicons with ITS1-ITS2 primer pairs.

Sequence Analysis and Phylogenetic Analysis

Sequenced ITS gene regions of our samples compared with others in Genebank using BLAST algorithm. As seen in Table 3 First sample which was morphologically identified as *S. acutus* appeared as *A. obliquus*, while the second sample *S. antennatus* apeeared as *A. obliquus*, the third sample *S. quadricauda* appeared as *D. communis*, forth sample *S. opoliensis* appeared as *D. communis*, fifth sample *S. acuminatus* appeared as *P. pectinatus* and finally sith sample *S. armatus* appeared as *D. armatus* var. *subalternas* in GeneBank data. The sequenced *Scenedesmus*-like strains were deposited in the GenBank (Table 3 and 4). After BLAST analysis we aligned our sequences within themselves and our sequences with the ones from GeneBank using ClustalW algorithm. Afterwards aligments used for phylogenetic tree construction in Mega 7 programme. Substitutions model was Tamura-Nei model and Bootstrap value was 1000.

ML and NJ phylogenetic trees gave identical results for our isolates since only the NJ tree is demonstrated (Figure 2) and for our isolates and GenBank data together (Figure 3 and Figure 4).

The tree that constructed with only our samples has two clades (Figure 2). First clade has two sister clades *A. obliquus* clade consist of two *A. obliquus* samples and *P. pectinatus* clade.

The other clade again has two sister clades; *D. communis* clade formed by two samples and *D. armatus* var. *subalternas*.

In NJ and ML trees constructed with the sequences from GeneBank data gave similar results. In these trees our *D. armatus* var. *subalternas* sample created a clade with other *D. armatus* var. *subalternas* species from GeneBank. Our *D. communis* samples also appeared in the same clade with other *D. communis* sequences. Similarly, our *A. obliquus* sequences appeared in the same clade with other *A. obliquus* sequences obtained from GeneBank. Our *P. pectinatus* isolate formed a clade with other *P. pectinatus* sequences.

Discussion

The genus *Scenedesmus* is characterized by the following morphological features: flat or slightly curved coenobia which are composed of 2-32 cells that are linearly or laterally-arranged in 1 or 2 rows and usually surrounded by mucilage; cells elongate or cylindrical, ovoid, ellipsoid to ovoid, with apices usually rounded;walls smooth, granular or toothed; cell apices usually rounded. The chloroplast is parietal and usually with a single pyrenoid. Asexual

Table 2. Synonyms, current names and morphometric diagnostic features of specimens studied based on the morphological identification.

Morphological identification (Komarek & Fott 1983)	Synonyms (Guiry & Guiry 2015)	Taxonomically-accepted current names (Guiry & Guiry 2015)	Length×Width (µm) (Komarek & Fott 1983)	Length×Width (µm) (in this study)	Average ±Standard Deviation (length-width)
Scenedesmus acutus Meyen (sample 1)	*Scenedesmus dimorphus* f. *granulatus* Isabella & R.J.Patel 1989 *Arthrodesmus acutus* Ehrenberg ex Ralfs *Scenedesmus crassus* Chodat 1926 *Scenedesmus scenedesmoides* Chodat 1926	*Scenedesmus obliquus* (Turpin) Kützing	(5-) 7-25 (-27)×2-7.5 (-14)	10×4	10.4±2.1 3.8 ±1.4
Scenedesmus antennatus Brebisson (sample 2)	-	*Scenedesmus dimorphus* (Turpin) Kützing	9-28×1.5-5	15×4	14.6±1.9 4.1± /1.5
Scenedesmus quadricauda Chodat (sample 3)	*Desmodesmus quadricaudatus* (Turpin) *Achnanthes quadricauda* Turpin 1828	*Scenedesmus quadricauda* Chodat	(5.5)10-36×(2.1) 3-8 (-12)	11×5	10.6±1.8 4.6±1.6
Scenedesmus opoliensis P.G. Richter (sample 4)	*Scenedesmus carinatus* f. *brevicaudatus* Uherkovich *Scenedesmus opoliensis* var. *setosus* Dedusenko *Selenastrum acuminatum* Lagerheim	*Desmodesmus opoliensis* (P.G.Richter) E.Hegewald	8-36.5×2-9	20×7	20.4±4.2 7.1±2.1
Scenedesmus acuminatus (Lagerheim) Chodat (sample 5)	*Scenedesmus obliquus* var. *acuminatus* (Lagerheim) Chodat *Scenedesmus falcatus* Chodat 1894 *Scenedesmus acuminatus* var. *minor* G.M.Smith 1916 *Scenedesmus acuminatus* var. *elongatus* G.M.Smith 1926	*Acutodesmus acuminatus* (Lagerheim) P.M.Tsarenko	9.6-48 (-50)×1.5-9	22×5	22.4±4.6 5.3±1.6
Scenedesmus armatus Chodat (sample 6)	-	*Scenedesmus armatus* (Chodat) Chodat	5-13×2.3-6	11×6	10.8±1.7 6.2±1.9

reproduction usually occurs with autospores which are released by fracture of lateral cell walls (Komarek and Fott, 1983; Skaloud, 2008; Sakthivel, 2016).

The genus *Acutodesmus* is, to a lesser extent, sharing some morphological characteristics with the genus *Scenedesmus* but there are still some remarkably distinct differentiations such as acute cell poles and polar thickenings of the cell walls (Komarek and Fott, 1983; Hegewald and Hanagata, 2000). Neither *Scenedesmus* nor *Acutodesmus* (sensu

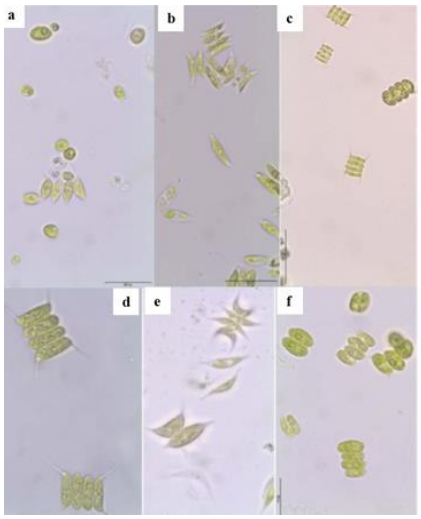

Figure 1. LM photomicrographs of *Scenedesmus*-like species investigated in this study. a: *Acutodesmus obliquus* (= sample 1), b: *A. obliquus* (= sample 2), c: *Desmodesmus communis* (= sample 3), d: *D. communis* (= sample 4), e: *Pectinodesmus pectinatus* (= sample 5), f: *Desmodesmus armatus* var. *subalternas* (= sample 6)**.** Scale bars = 20 µm.

Table 3. Morphological and molecular identifications of the *Scenedesmus*-like species studied and their accession numbers in the GenBank NCBI

Samples	Morphologic Identification	Molecular Identification	GenBank Accession Numbers
1	*Scenedesmus acutus* Meyen	*Acutodesmus obliquus*	KF470790
2	*Scenedesmus antennatus* Brebisson	*Acutodesmus obliquus*	KF470791
3	*Scenedesmus quadricauda* Chodat	*Desmodesmus communis*	KF470792
4	*Scenedesmus opoliensis* P. G. Richter	*Desmodesmus communis*	KF470793
5	*Scenedesmus acuminatus* (Lagerheim) Chodat	*Pectinodesmus pectinatus*	KF470794
6	*Scenedesmus armatus* Chodat	*Desmodesmus armatus* var. *subalternas*	KF470795

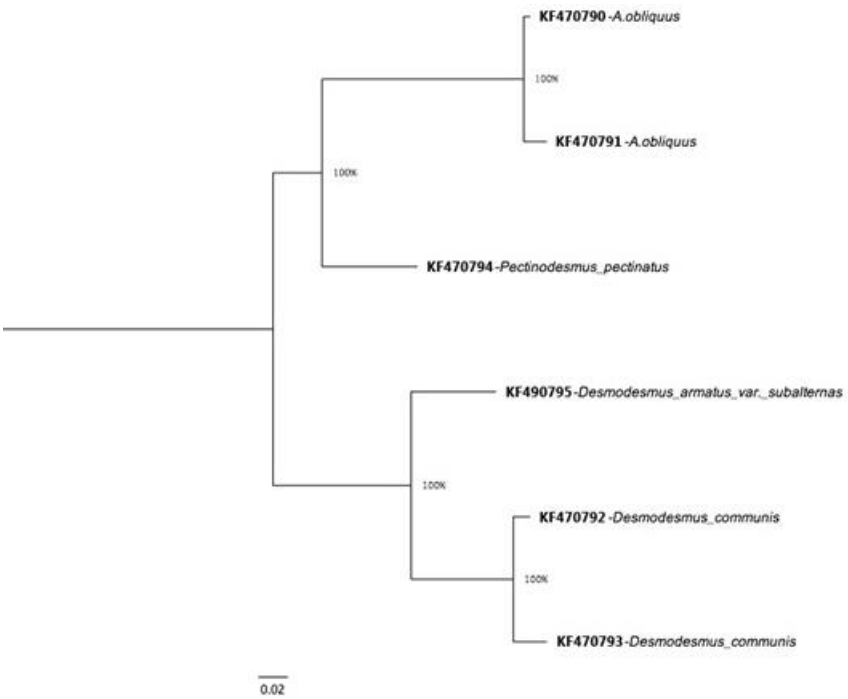

Figure 2. Neighbour Joining (NJ) tree of *Scenedesmus*-like isolates included in this study based on the partial sequences of the ITS regions constructed by Tamura-Nei method with 1000 bootstrap value.

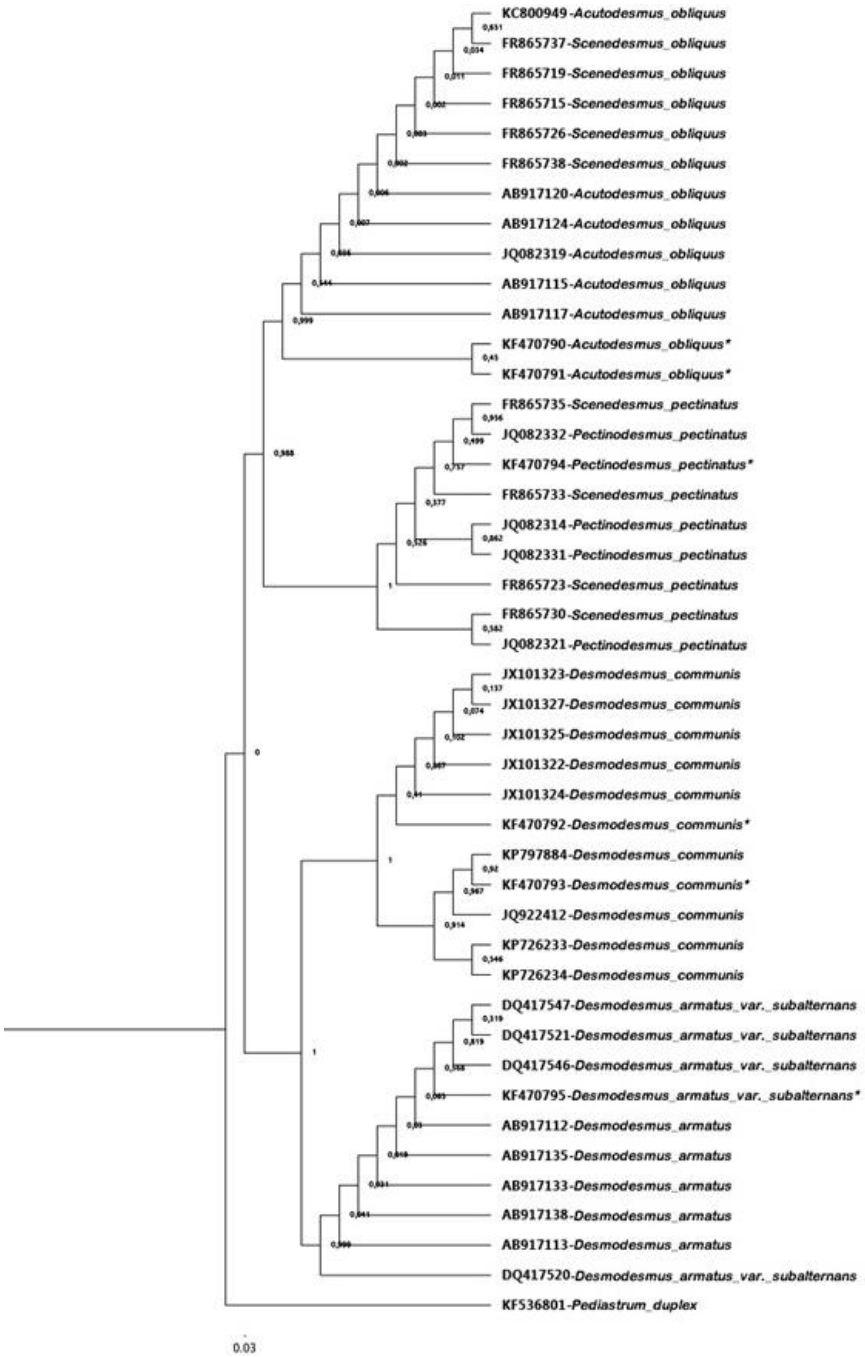

Figure 3. Neighbour Joining (NJ) phylogenetic tree between *Scenedesmus*-like species included in this study and the other related taxa in GenBank database with 1000 bootstrap values (KF536801 is outgroup). Our isolates were indicated with asterix (*).

Tsarenko) is monophyletic in the light of molecular data (Hegewald and Wolf, 2003). Therefore, interpretation of the morphological evolution of family Scenedesmaceae is still need a lot of work due to the high degree of plasticity or convergence among its algal taxa (Elias *etal.*, 2010).

This study revealed that *Scenedesmus*-like species identification is considered to be very difficult depending only upon the light microscopy observations. It is well-known that scenedesmoid species can be transformed into unicellular and small 2- or 3- celled coenobian forms under different culture conditions (Soeder and Hegewald, 1989). Therefore, the recent molecular techniques should be applied to preciously identify the *Scenedesmus*-like taxa.

In this work, each strain studied has its main characteristic features. *Scenedesmus acutus* specimens (sample 1; Fig. 1a) has elongated fusiform cells, concave middle and convex ends of outer cells, smooth cell wall. *S. antennatus* (sample 2; Fig. 1b) can be easily confused with *S. acutus* (Fig. 1). To differentiate these species, colorless mucilage knobs at poles of cells have to be examined (Komarek and

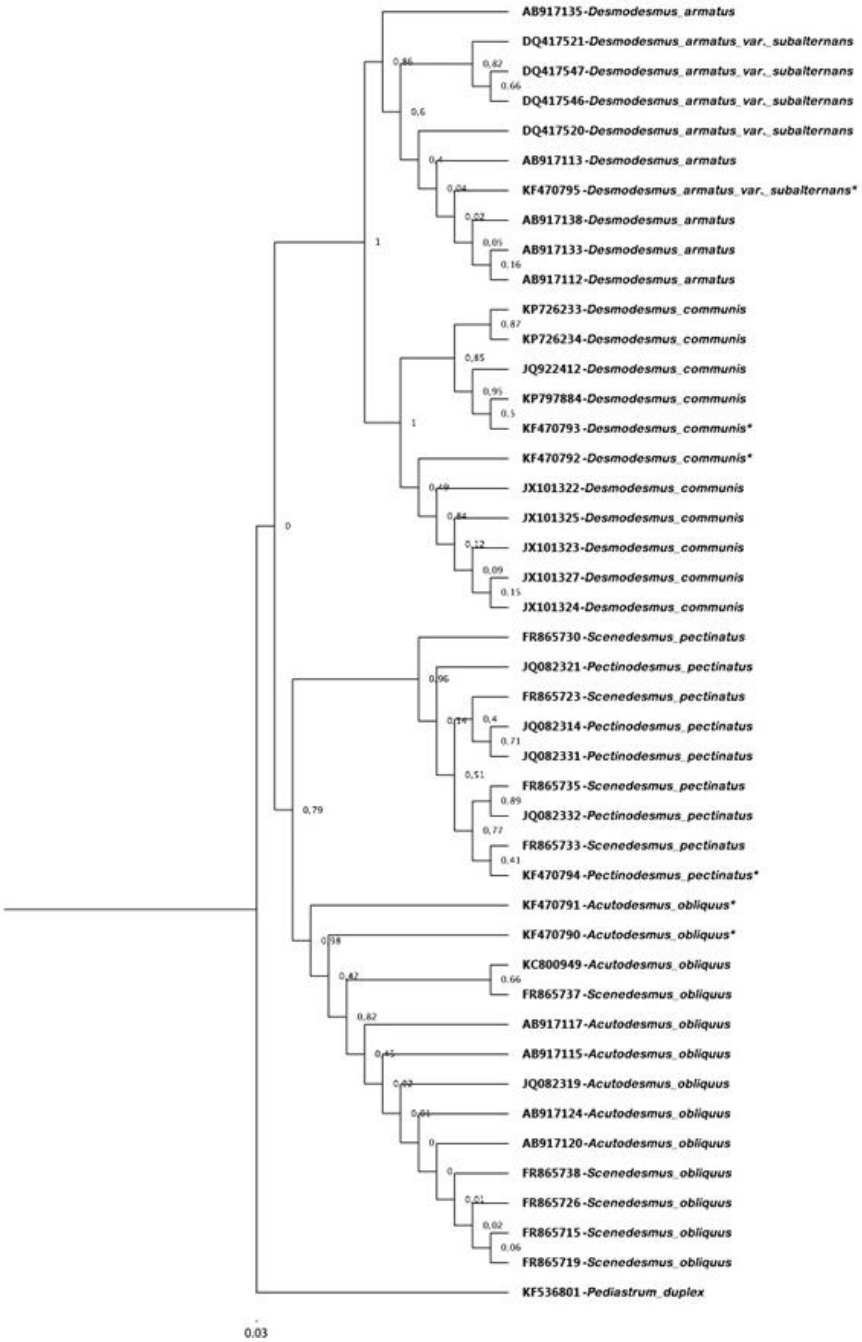

Figure 4. Maximum Likelihood (ML) phylogenetic tree between *Scenedesmus*-like species included in this study and the other related taxa in GenBank database with 1000 bootstrap values (KF536801 is outgroup). Our isolates were indicated with asterix (*).

Fott, 1983).

According to Prescott (1973), *S. quadricauda* (sample 3; Fig. 1c) is characterized by its oblong-cylindrical cells usually in one series; outer cells with long curved spines at each pole and the inner cells without spines. These distinctive features are in good agreement with our specimens. The morphological similarities between *S. opoliensis* (sample 4; Fig. 1d) and *S. quadricauda* (sample 3; Fig. 1c) might lead to a misidentification. However, they could be separated based on the distinctive poles of outer cells. For more details, outer cells of *S. opoliensis* are fusiform-naviculoid (Prescott, 1973) and the coenobium is curved at the top view (Komarek and Fott, 1983). All of these features are compatible with our strain identified.

S. acuminatus (sample 5; Fig. 1e) can be easily distinguished by the presence of lunate cells with sharp apices in a single row (Prescott, 1973).

According to Komarek and Fott (1983), *S. armatus* (sample 6; Fig. 1f) is defined by cylindric-oval cells and spines are up to 2 µm. These characteristics helped us to discriminate *S. armatus* from the other species, *S. quadricauda* (sample 3).

The ITS molecular analysis revealed that *S. acutus* (sample 1 that is morphologically identified) and *S. antennatus* (sample 2) were clustered well with other *Acutodesmus obliquus* species in the GenBank with a 99% similarity value (Table 4); *Scenedesmus*-like specimens ofsample 3 (*S. quadricauda*) and sample 4 (*S. opoliensis*) were related to other *Desmodesmus communis* taxa.*S. acuminatus* (sample 5) was phylogenetically clustered with *Pectinodesmus pectinatus* with a high similarity value ranged between 98-99%.Sample 6 (*S. armatus*) was also characterized as *Desmodesmus armatus* var. *subalternas* (for more details check Tables 3 and 4).

The NJ clustering analysis based on the sequenced ITS regions (Figure 2) pointed out that samples 1 and 2 (with GenBank accession numbers KF470790 and KF470791, respectively) were phylogenetically identical and are belonging to the same species *Acutodesmus obliquus*. The same point for samples 3 and 4 (accession numbers: KF470792 and KF470793, respectively) where they separated in a distinctive clade as *Desmodesmus communis*. Sample 6 (*D. armatus* var. *subalternas*) was placed in the same clade containing other identified *Desmodesmus* species but with a relatively low phylogenetically support (with bootstrap value 56). Sample 5 (KF470794) was specific in its position as a distinctive species identified as *Pectinodesmus pectinatus*.

Comparison of scenedesmoid algal taxa in this study with other allied sequenced taxa deposited in the GenBank showed that samples 1 and 2 (with accession numbers KF470790 and KF470791, respectively) could be identified as *Acutodesmus obliquus* from the morphological and phylogenetic standpoints, where they are clustered well with other taxa from the GenBank (Figure 3 and 4). As expected, samples 3 and 4 (KF470792 and KF470793) also located in the same clade with other *D. communis* species from GenBank. Sample 5 (KF470794)

Table 4. Similarity and e-values as blast results of ITS regions of our isolates and GenBank data

Accession No	Taxa Identified	GenBank Data		
		Accession no	e-value	Similarity
KF470790	*Acutodesmus obliquus*	AB917115	0.0	99%
KF470791	(sample 1 and 2)	AB917115	0.0	99%
		AB917117	0.0	99%
		AB917120	0.0	99%
		AB917124	0.0	99%
		KC800949	0.0	99%
		JQ082319	0.0	99%
		FR865715	0.0	99%
		FR865719	0.0	99%
		FR865726	0.0	99%
		FR865737	0.0	99%
		FR865738	0.0	99%
KF470792 and	*Desmodesmus communis*	JX101322	0.0	99%
KF470793	(samples 3 and 4)	JX101323	0.0	98%
		JX101324	0.0	98%
		JX101325	0.0	98%
		JX101327	0.0	98%
		KP726233	0.0	98%
		KP726234	0.0	98%
		KP797884	0.0	98%
		JQ922412	0.0	98%
KF470794	*Pectinodesmus pectinatus* (sample 5)	FR865723	0.0	98%
		FR865730	0.0	98%
		FR865733	0.0	99%
		FR865735	0.0	99%
		JQ082314	0.0	98%
		JQ082321	0.0	98%
		JQ082331	0.0	98%
		JQ082332	0.0	99%
KF470795	*Desmodesmus armatus* var. *subalternas* (sample 6)	AB917112	0.0	99%
		AB917113	0.0	99%
		AB917133	0.0	99%
		AB917138	0.0	99%
		FR865727	0.0	99%
		DQ417520	0.0	99%
		DQ417521	0.0	99%
		DQ417546	0.0	99%
		DQ417547	0.0	99%

identified as *D. armatus* var. *subalternas* is in the same clade with the other genotypes. Sample 6 (KF470795) identified as *P. pectinatus* by ITS region analysis,and it was grouped in the same clade with the previously-identified taxa as *S. pectinatus* and *D. pectinatus*. In a good agreement with our results, Hegewald and Wolf (2003) studied different *Scenedesmus* and *Acutodesmus* species and classified them according to their 18S rDNA and ITS-2 regions. They demonstrated that *S. arcuatus*, which is morphologically might be similar to *S. hindakii*, was distinctive and embedded in a separate clade and has a high bootstrap value with *A. regularis* and *A. pectinatus.* The recent contribution of Hegewald *et al*. (2013) on some *Scenedesmus*-like species also revealed that the *Acutodesmus* species studied (*A. distendus, A. acuminatus, A. reginae* and *A. nyygaardii*) were branched with the other investigated *Scenedesmus* species (*S. bernardii* and *S. bajacalifornicus*). All of these specimens were previously identified as *Scenedesmus* in Lewis and Flechtner (2004)'s study.

As we have been experienced, it is inaccurate to identify the genus *Scenedesmus* and other allied morphospecies using only the light microscopy. Recent molecular techniques should be applied to solve this taxonomic problem. This study unveiled 6 different scenedesmoid isolates using the detailed combined LM and phylogenetically ITS1-ITS4 molecular regions. For more details, all isolates were firstly identified morphologically as *Scenedesmus* spp. using the light microscopy but later their identifications were confirmed on the basis of molecular techniques: samples (1 and 2) were belonged to the genus *Acutodesmus*; samples (3, 4, and 6) were related to the genus *Desmodesmus*, and sample (5) was sticked to the genus *Pectinodesmus*.

This kind of investigation on some Turkish *Scenedesmus*-like species bring out a question as asked before in Hegewald *et al.* (2013): To which extent is the genetic variation possible in *Scenedesmus*-complex species? This algal group usually reproduce vegetatively and not allow a genetic diversity at high levels. Zoospores, tools which allow genetic diversity, were rarely observed within this algal group (Trainor, 1963; Trainor 1996). However, Corradi *et al.* (1995) indicated to the zoospores formation under stress conditions and all of these observations answered well this question even if just a little bit. In other words, this means the formation of zoospores and genetic crossing-over might be not rare in the *Scenedesmus*-complex species. To provide this insight, more deep studies should be carried out on this algal group.

In conclusion, there is still an urgent need for further deep morphotaxonomically and phylogenetically combined and detailed studies on members of family Scenedesmaceae to increase the number of available sequences of different genetic markers for different species. By this way, large-scaled databases will give a reasonable support to identify easily and preciously different scenedesmoid taxa inhabiting different ecosystems

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References

An S. S., Friedl, T., & Hegewald E. (1999). Phylogenetic relationships of *Scenedesmus* and *Scenedesmus*-like coccoid green algae as inferred from ITS-2 rDNA sequence comparisons. *Plant Biology*, 1(4), 418–428. doi: 10.1111/j.1438-8677.1999.tb00724.x

Altschul, S. F., Madden T. L., & Schaffer A. A. (1997). Gapped blast and psi-blast: a new generation of protein database search programs. *Nucleic Acids Research*, 25, 3389–3402. doi:10.1093/nar/25.17.3389

Bica, A., Barbu-Tudoran, L., Druga, B., Coman, C., Nicoara A., Szöke Nagy T., & Dragoş N. (2012). *Desmodesmus communis* (Chlorophyta) from Romanian Freshwaters: coenobial morphology and molecular taxonomy based on the ITS2 of new isolates. *Annals of The Romanian Society For Cell Biology*, 1, 16–28.

Bold, H.C. (1942). The Cultivation of Algae. *The Botanical Review III*, 2, 69-138. https://doi.org/10.1007/bf02879474

Brezina, V., Hrib, J., & Komarek, J. (1972). Morphological changes in the cells of *Scenedesmus quadricauda* during ontogeny. *Archiv für Hydrobiologie*, 41, 57–71.

Chodat, R. (1926). *Scenedesmus*. Étude de génétique de systématique expérimentaleetd hydrobiologie. Schweizerische Zeitschriftfür. *Hydrologie*, 3, 71–258.

Corradi, M. G., Gorbi, G., Ricci, A., Torelli, A., & Bassi, M. (1995). Chromium–induced sexual reproduction gives rise to a Cr–tolerant progeny in *Scenedesmus acutus*. *Ecotoxicology and Environmental Safety* 32, 12–18. https://doi.org/10.1006/eesa.1995.1079

Ehrenberg, C. G. (1834). Dritter Beitragzur Erkenntniss grosser Organisation in der Richtung des kleinsten Raumes. Abhandlungen der Königlichen Akademie der Wissenschaftenzu Berlin, 145–336 pp.

Elias, M., Nemcova, Y., Skaloud, P., Neustupa, J., Kaufnerova1, V., & Sejnohova, L. (2010). *Hylodesmus singaporensis* gen. et sp. nov., a new autosporic subaerial green alga (Scenedesmaceae, Chlorophyta) from Singapore. *International Journal of Systematic and Evolutionary Microbiology*, 60, 1224-1235. doi 10.1099/ijs.0.012963-0.

Fawley, M. W., Fawley, K. P., & Hegewald, E. (2011). Taxonomy of *Desmodesmus serratus* (Chlorophyceae, Chlorophyta) and related taxa on the basis of morphological and DNA sequence data. *Phycologia*, 50, 23–56. doi: http://dx.doi.org/10.2216/10-16.1.

Guiry, M. D., & Guiry, G. M. (2015). AlgaeBase. Retrived from http://www.algaebase.org.

Hegewald, E. (1978). Eineneue unterteilung der gattung *Scenedesmus* Meyen. Nova Hedwigia, (30): 343–376. doi: 10.1127/nova.hedwigia/30/1979/343.

Hegewald, E., & Silva, P. (1988). Annotated catalogue of *Scenedesmus* and nomenclaturally related genera

including original descriptions and figures. *Bibliography Phycology*, 80, 1-587.

Hegewald, E. (1989). The *Scenedesmus* strains of the culture collection of the university of Texas at Austin (UTEX). *Archiv für Hydrobiologie,* 55, 153–189.

Hegewald, E. H. (1997). Taxonomy and phylogeny of *Scenedesmus*. *The Korean Journal of Phycology*, 12, 235–246.

Hegewald, E. (2000). New combinations in the genus *Desmodesmus* (Chlorophyceae, Scenedesmaceae). *Algological Studies*, 96, 1–18.

Hegewald, E., & Hanagata, N. (2000). Phylogenetic studies on Scenedesmaceae (Chlorophyta). *Archiv für Hydrobiologie*, 136, 29–49.

Hegewald, E., & Wolf, M. (2003). Phylogenetic relationships of *Scenedesmus* and *Acutodesmus* (Chlorophyta, Chlorophyceae) as inferred from 18S rDNA and ITS-2 sequence comparisons. *Plant Systematics and Evolution*, 241, 185–191. doi: 10.1007/s00606-003-0061-7.

Hegewald, E., Wolf M., Keller A., Friedl T., & Krienitz L. (2010). ITS2 sequence–structure phylogeny in the Scenedesmaceae with special reference to *Coelastrum* (Chlorophyta, Chlorophyceae), including the new genera *Comasiella* and *Pectinodesmus*. *Phycologia*, 49: 325–335.

Hegewald, E., Bock, C., & Krienitz, L. (2013). A phylogenetic study on Scenedesmaceae with the description of a new species of *Pectinodesmus* and the new genera *Verrucodesmus* and *Chodatodesmus* (Chlorophyta, Chlorophyceae). *Fottea*, 13(2), 149-164. https://doi.org/10.5507/fot.2013.013

Johnson, J. L., Fawley, M., & Fawley, K. P. (2007). The diversity of *Scenedesmus* and *Desmodesmus* (Chlorophyceae) in Itasca State Park, Minnesota, USA. *Phycologia*, 46, 214-229. doi: http://dx.doi.org/10.2216/05-69.1.

Kesici, K., Tuney, I., Zeren, D., Guden, M., & Sukatar, A. (2013). Morphological and molecular identification of pennate diatoms isolated from Urla-Izmir, Coast of Aegean Sea. *Turkish Journal of Biology*, 37, 530–537. doi:10.3906/biy-1205-40.

Kessler, E., Schafer, M., Hümmer, C., Kloboucek, A., & Huss, V. A. R. (1997). Physiological, biochemical, and molecular characters for the taxonomy of the subgenera of *Scenedesmus* (Chlorococcales, Chlorophyta). *Botanica Acta*, 110, 244–250. doı:10.1111/j.1438-8677.1997.tb00636.x.

Kızılkaya-Tüney, I., Demirel, Z., Kesici, K., Kesici, E., & Sukatar, A. (2016). Morphological, molecular and toxicological characterization of *Nodularia spumigena* Mertens in Jungens (1822) from Brackishwater Lake Bafa (Turkey). *Sinop University Journal of Natural Science*, 1(1), 39-52.

Komarek, J., & Ruzicka, J. (1969). Effect of temperature on the growth and variability of *Scenedesmus quadricauda* (Turp.) Breb. In Fott, B. (Ed) Studies in Phycology, Stuttgart, 262–292.

Komarek, J., & Fott, B. (1983). Chlorophyceae (Grunalgen) ordnung: Chlorococcales. In G. Huber-Pestalozzi, (Ed) *Das Phytoplankton des Suswassers*. Stuttgart, Germany, 1044 pp.

Lewis, L.A., & Flechtner, V.R. (2004). Cryptic species of *Scenedesmus* (Chlorophyta) from desert soil communities of Western North America. *Journal of Phycology*, 40, 1127–1137. https://doi.org/10.1111/j.1529-8817.2004.03235.x

Michener, C. D., & Sokal, R. R. (1957). A quantitative approach to a problem of classification. *Evolution* (11): 490–499. doi: 10.2307/2406046. https://doi.org/10.2307/2406046

Nageli, C. (1849). Gattungeneinzelliger algen, physiologisch und systematischbearbeitet. Neue Denkschriften der Allg. *Schweizerischen Gesellschaftfür die Gesammten Naturwissenschaften* (10): 1–139. https://doi.org/10.5962/bhl.title.6805

Prescott, G. W. (1973). Algae of the Western Great Lake area. W:M.C. Brown Company Publishers, Dubuque, Iowa.

Radha, S., Fathima, A. A., Iyappan, S., & Ramya, M. (2013). Direct colony PCR for rapid identification of varied microalgae from freshwater environment. *Journal of Applied Phycology*, 25, 609–613. doi: 10.1007/s10811-012-9895-0.

Sakthivel, R. (2016). Biodiversity of Chrococcales (Chlorophyceae) from Cement Factories in and Around Areas of Ariyalur District, Tamil Nadu. *European Journal of Biomedical and Pharmaceutical Science*, 3, 267-284.

Skaloud, P., Neustupa, J., & Skaloudova, M. (2008). Species composition and diversity of algae on anthropogenic substrata. *Novitates Botanicae Universitatis Carolinae*, 19, 33-37.

Soeder, C. J., & Hegewald, E. (1989). Scenedesmus. In: Borowitzka, M.A. and L.J. Borowitzka (Eds), Microalgal Biotechnology (pp. 59–84). Cambridge, UK, Cambridge University Press.

Soylu, E. N., & Gönülol, A. (2011). Morphological and 18S rRNA analysis of coccoid green algae isolated from lakes of Kızılırmak Delta. *Turkish Journal of Biology*, 35, 1–7. doi:10.3906/biy-1001-19.

Steenbergen, C. L. M. (1978). Pleomorphism of *Scenedesmus quadricauda* (Turp.) Breb. (Chlorophyceae) in synchronized cultures. *Verhandlungen des Internationalen Verein Limnologie*, 21, 216–223. doi: 10.1007/BF02308492.

Stein, J. R. (1973). Handbook of Phycological Methods, Culture Methods and Growth Measurements. London, UK, Cambridge Univ. Press.

Tamura, K., Nei M., & Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceeding of National Academy of Science of USA*, 101, 11030–11035. doi: 10.1073/pnas.0404206101.

Tamura, K., Peterson D., & Peterson N. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evoluation*, 28, 2731–2739. doi: 10.1093/molbev/msr121.

Trainor, F. R. (1963). Zoospores in *Scenedesmus obliquus*. *Science*, 142: 1673–4. https://doi.org/10.1126/science.142.3600.1673

Trainor, F. R. (1996). Reproduction in *Scenedesmus obliquus*. *Algae*, 11: 183–201.

Trainor, F. R. (1992). Cyclomorphosis in *Scenedesmus communis* Hegew. Ecomorph expression at low temperature. *British Phycological Journal*, 27, 75–81. doi:10.1080/00071619200650091.

Tsarenko, P.M. (2000). Chlorococcales. In: S.P. Wasser and P.M. Tsarenko (Eds), Diversity of algae of Ukraine. *Algologia*, 10(4), 1–309.

Tsarenko, P.M., & Petlevany, O.A. (2001). Addition to the 'Diversity of algae of Ukraine'. *Algologia*. Institute of Botany Press, Kiev, Russia, 130 pp.

Turpin, P. (1828). Aperçuorganographiquesur le nombredeux, considere commemultiplicateur de quatre, huit, douze, seize, trente-deuxetsoixante-quatredans la structure des vegetaux d'un ordreinférieur. *Memoires du Museum National d'Histoire Naturelle Paris,* 16, 295–344.

Tuney, I., & Sukatar, A. (2010). DNA Extraction Protocol from Brown Algae. *Biological Diversity and Conservation*, 3, 51–55.

Van Hannen, E. J., Fink, P., & Lurling, M. A. (2002). A revised secondary structure model for the internal transcribed spacer 2 of the green algae *Scenedesmus* and *Desmodesmus* and its implication for the phylogeny of these algae. *European Journal of Phycology*, 37, 203–208. doi:10.1017/S096702620200361X.

Vanormelingen, P., Hegewald, E., Braband, A., Kitschke, M., Friedl, T., Sabbe, K., & Vyverman, W. (2007). The systematics of a small spineless *Desmodesmus* species, *D. costatogranulatus* (Sphaeropleales, Chlorophyceae), based on ITS2 rDNA sequence analyses and cell wall morphology. *Journal of Phycology* 43, 378–396. doi: 10.1111/j.1529-8817.2007.00325.x.

White, T. J., Bruns T., & Lee, S. (1990). PCR Protocols: A guide to methods and applications. New York, USA, Academic Press Inc.