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A new *Euastrum* species (Conjugatophyceae, Streptophyta) from the Western Desert of Egypt

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Abstract – An *Euastrum* species (Conjugatophyceae, Streptophyta) was found in an agricultural ditch fed by a rheocrenic, slightly-hot spring 'Ain El-Balad' in the El-Farafra Oasis, Western Desert of Egypt. Morphotaxonomic diagnosis and autecology of this desmid species are hereby documented in detail and compared to other morphologically similar species and infraspecific taxa, particularly those of the species complex *E. spinulosum*. As compared to most other species of that complex, the newly described *Euastrum* species is mainly characterized by its distinctive small-sized cells, rather shallow and widely open invaginations between the semicell lobes and less pronounced apical lobes. Moreover, the basal lobes are broadly rounded and larger than the lateral lobes. We have named this interesting species *E. elfarafraense* sp. nov. after the type locality, the El-Farafra Oasis.

Desmids / Egypt / *Euastrum elfarafraense* / new species

INTRODUCTION

The genus *Euastrum* Ehrenberg ex Ralfs is generally considered one of the most representative taxa in the family Desmidiaceae (Gontcharov, 2008; Guiry, 2013). To date, there are about 244 species and infraspecies which are accepted taxonomically (Guiry & Guiry, 2017). *Euastrum* is highly heterogeneous from the morphological point of view. Accordingly, the recent molecular concatenated study of Gontcharov & Melkonian (2008) showed using multi-locus data that this genus is polyphyletic in origin, forming three well-delimited phylogenetic lineages.

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From ecological and biogeographical standpoints, a number of *Euastrum* species, like those of the *E. mononcylum* group, seem to be largely confined to particular regions in their distribution and generally prefer subtropical-to-tropical habitats (Růžička, 1981; Coesel, 2000; Shukla *et al.*, 2008). This might be related to dispersal barriers and failures in interbreeding sexual reproduction between co-existing related taxa. They therefore are highly susceptible to loss and disappearance in terms of biodiversity (Coesel, 2000). Nevertheless, Kouwets (1998), in his floristic survey on desmids in some temperate regions of France, recorded *E. spinulosum* in meso-eutrophic habitats, and stressed that this temperate form represents a comparatively recent adaptation of the original (sub)tropical species transported by migrating birds, and its wider distribution or disappearance in temperate areas might be a matter of time. Furthermore, he indicated the urgent re-evaluation of varieties and forms belonging to the species complex *E. spinulosum* which may belong to other different *Euastrum* species. Kouwets (2008) also threw light on these taxonomic problems in desmid systematics due to the existence of a very large number of infraspecific taxa obscuring a clear species definition and suggested the strict use of a monothetic species definition to delimit characteristics of ill-defined infraspecific taxa.

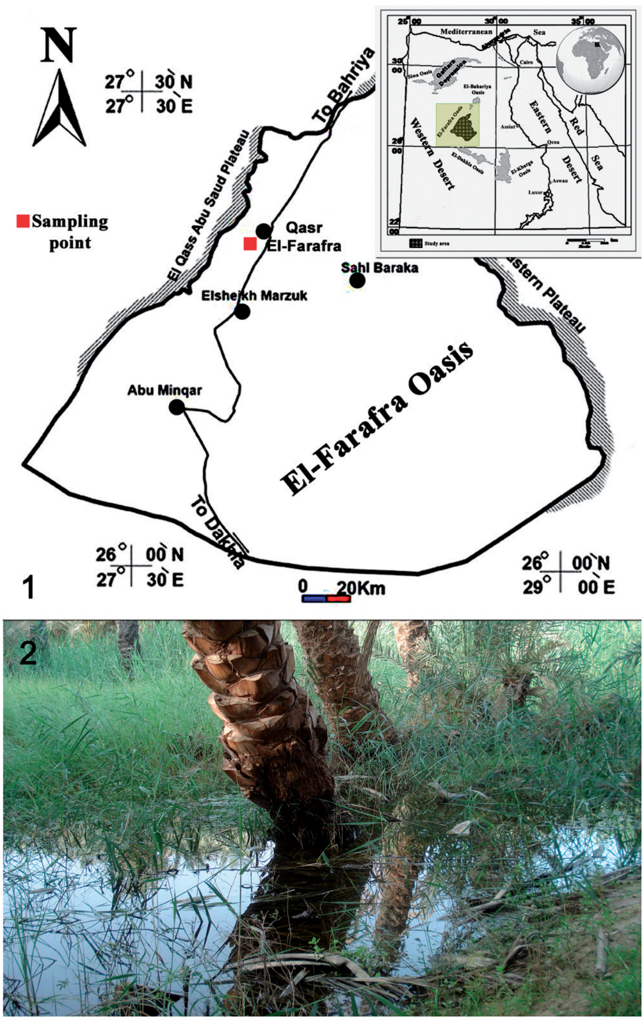
Apart from the restricted biogeographical distribution of some specific desmids, the phenomenon of environmentally-induced phenotypic plasticity has been well known and discussed in this genus and other desmids (e.g., Kouwets, 1998 & 2008; Černá & Neustupa, 2010; Štěpánková *et al.*, 2012; Barbosa *et al.*, 2014). Vidyavati (1985) presumed that the desmid cells are narrowly capable of maintaining their morphological specificity, which is genetically controlled, only under favourable culture conditions, but whenever there is a dramatic alteration in some environmental gradients such as temperature and illumination intensity, it will upset the metabolic pathways and lead to abnormal forms. Consequently, more stringent diagnosis of the morphotaxonomic characteristics of some cryptic desmid species should undoubtedly be made to resolve the confused taxonomy, especially when using only traditional taxonomic criteria and in case of discovering new species (Šťastný, 2010; Šťastný & Kouwets, 2012; van Geest & Coesel, 2012; Coesel & Meesters, 2015; Coesel *et al.*, 2017). However, the use of different gene biomarkers is nowadays becoming increasingly common in algal species delimitation and pointing phycologists towards more effective and realistic species boundaries (Leliaert *et al.*, 2014; Leliaert & De Clerck, 2017) but its full potential on desmids obviously hasn't been realized yet.

Considerable work has already been conducted on the biodiversity and species composition of the genus *Euastrum* in most African countries, resulting in the identification of some new and interesting species (e.g., Grönblad & Croasdale, 1971; Compère, 1977; Coesel, 2000; Coesel & van Geest, 2008; van Geest & Coesel, 2012). Information about the distribution of desmids, particularly *Euastrum* species, in Egyptian habitats is still very scarce in spite of the relatively species-rich inventory of well identified freshwater algal taxa (e.g., El-Otify *et al.*, 2003; Mansour *et al.*, 2015; Shaaban *et al.*, 2012 & 2015; Ali & El Shehawy, 2017). This may be due to the extremely small number of high-quality, specialized publications focused on desmid studies that appeared over the last century and start of this millennium. In addition, most of the Egyptian habitats investigated were characterized by neutral to alkaline pH regimes and suffered from different aspects of eutrophication (Saber, unpublished data) which mainly caused severe impacts on non-tolerant desmid species (Šťastný, 2010).

Within our integrated and recent investigations of the phycological diversity of Egyptian oases, the present study aims to describe in detail for the first time the rare *Euastrum* species found in an agricultural ditch fed by a slightly hot spring 'Ain El-Balad' located in the hyper-arid desert of the El-Farafra Oasis, and critically compare its diagnostic features with other similar or related taxa.

MATERIALS AND METHODS

Euastrum specimens were collected from an agricultural ditch fed by a typical freshwater (T.D.S. = 210 mg.L^{-1}), rheocrenic, slightly-hot (water temperature = 36.3°C) spring "Ain El-Balad" located in the El-Farafra Oasis, Western Desert of Egypt. This ditch (27°03' 26.1" N, 27°57' 47.4" E, 104 m a.s.l) is mainly used for irrigation of some farm lands in vicinity of Ain El-Bald (Saber *et al.*, 2017). Ain El-Balad can be classified as a slightly-hot spring based on the classification system



Figs 1-2. Location of the sampling point 'agricultural ditch fed by water of the slightly-hot spring Ain El-Balad' in Qasr El-Farafra, Western Desert of Egypt (Fig. 1), and landscape view for the habitat studied (Fig. 2).

reported by Glazier (2009) that distinguished "hot" springs as those with water temperature exceeding that of the human body. El-Farafra Oasis (26°00"-27°30" N, 26°30"-29°00" E) is one of the smallest oases (~ 10,000 km^2) excavated in the limestone plateau occupying the central part of Western Egypt, and located about 650 km southwest of Cairo (El Bastawesy & Ali, 2013; Wanas & Soliman, 2014). This natural depression is in a hyper-arid zone and characterized by a hot desert climate with a mean annual air temperature "MAAT" of ca 22°C and receiving 10 mm or less average annual precipitation (Elsheikh, 2015; Saber *et al.*, 2017). Groundwater in the El-Farafra Oasis is mainly derived from bores discharging from the world's largest non-renewable groundwater resource, the Nubian Sandstone Aquifer (Voss & Soliman, 2014; Powell & Fensham, 2016).

Specimens of *Euastrum* were collected on October 16th 2016 by squeezing the stonewort *Chara vulgaris* and mass growths of *Spirogyra* sp. and *Oedogonium* sp. filaments, sampling algal growths present on the bottom of the agricultural ditch, and finally placing them in sterile clean polyethylene bottles using a brushing syringe as described in Spitale *et al.* (2011). The specimens were transported to the laboratory in a chilled ice-box for investigation of chloroplast details. For observation of cell wall ornamentation, some of the specimens were treated with cold hydrogen peroxide (30% final conc.) for 30 mins. The materials were studied and identified using a BEL® photonics biological light microscope (BEL® Engineering, Monza, Italy). Morphometrical diagnostic features were measured and photographed using Canon Powershot G12 digital camera (Japan) and 30 measurements were made of each morphometric feature. Voucher specimens including the permanent slides and fixed materials (2% v/v formaldehyde final conc.) were deposited in the Phycology Unit (No. 341), the Botany Department, Faculty of Science at Ain Shams University (Cairo, Egypt), and in the Limnology and Phycology Section of the Museo delle Scienze – MUSE in Trento, Italy. The terminology used in the species description follows Růžička (1981) and Coesel & Meesters (2007).

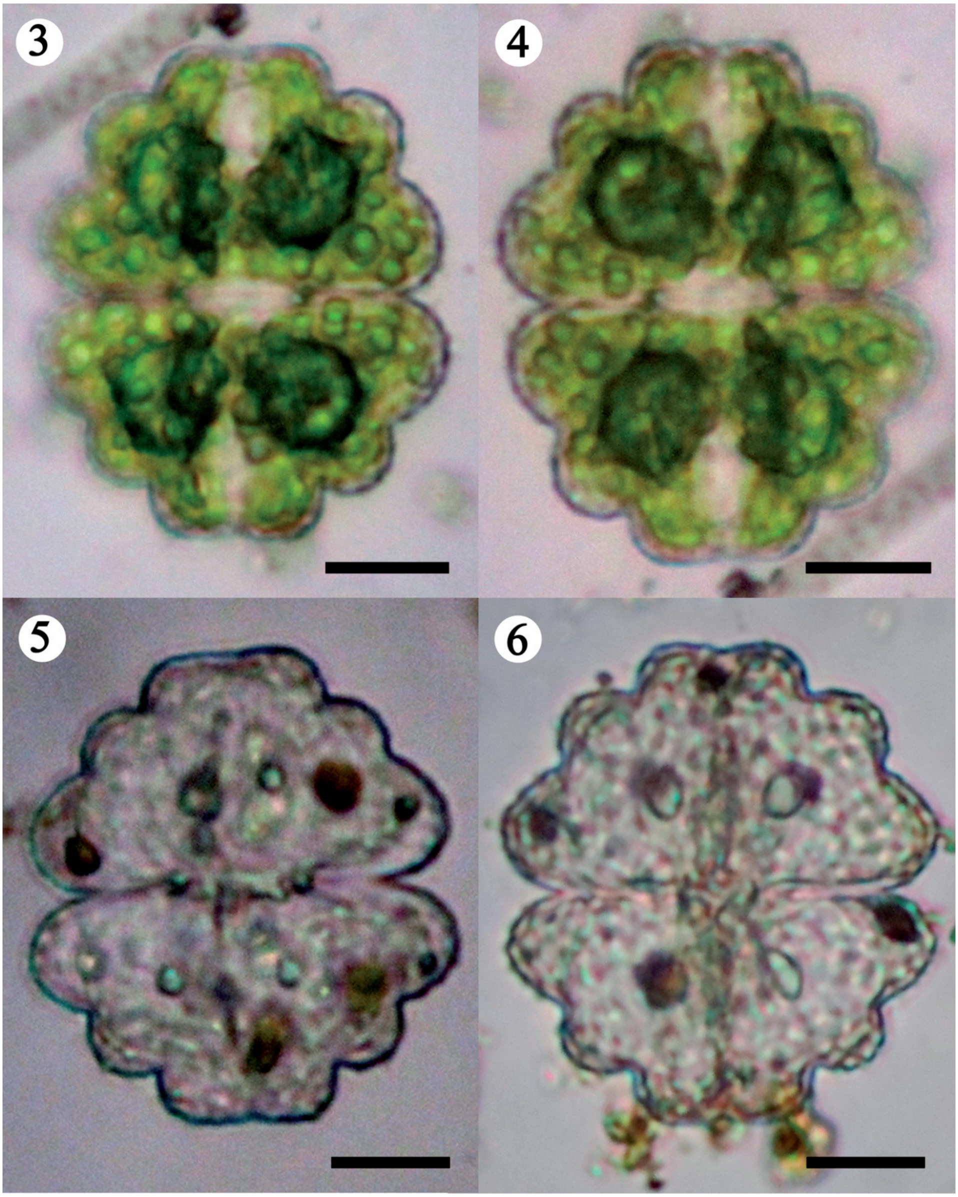
Water sampling was also conducted using clean polyethylene bottles. Water temperature (°C), pH, specific conductivity ($\mu S.cm^{-1}$), and total dissolved solids ($mg.L^{-1}$) were measured *in situ* using the calibrated portable Temp/pH/EC/T.D.S. meter HANNA HI 991301. Dissolved Oxygen (D.O.) was also determined using the in-field calibrated dissolved oxygen meter Lutron® YK-22DO. Detailed hydrochemical characteristics including major ions, nutrients, trace elements and metals were investigated following standard procedures and methods (Chapman & Pratt, 1978; Clesceri *et al.*, 2000).

OBSERVATIONS

Division: Streptophyta
Class: Conjugatophyceae (Zygnematophyceae)
Order: Desmidiales
Family: Desmidiaceae
Genus: *Euastrum*

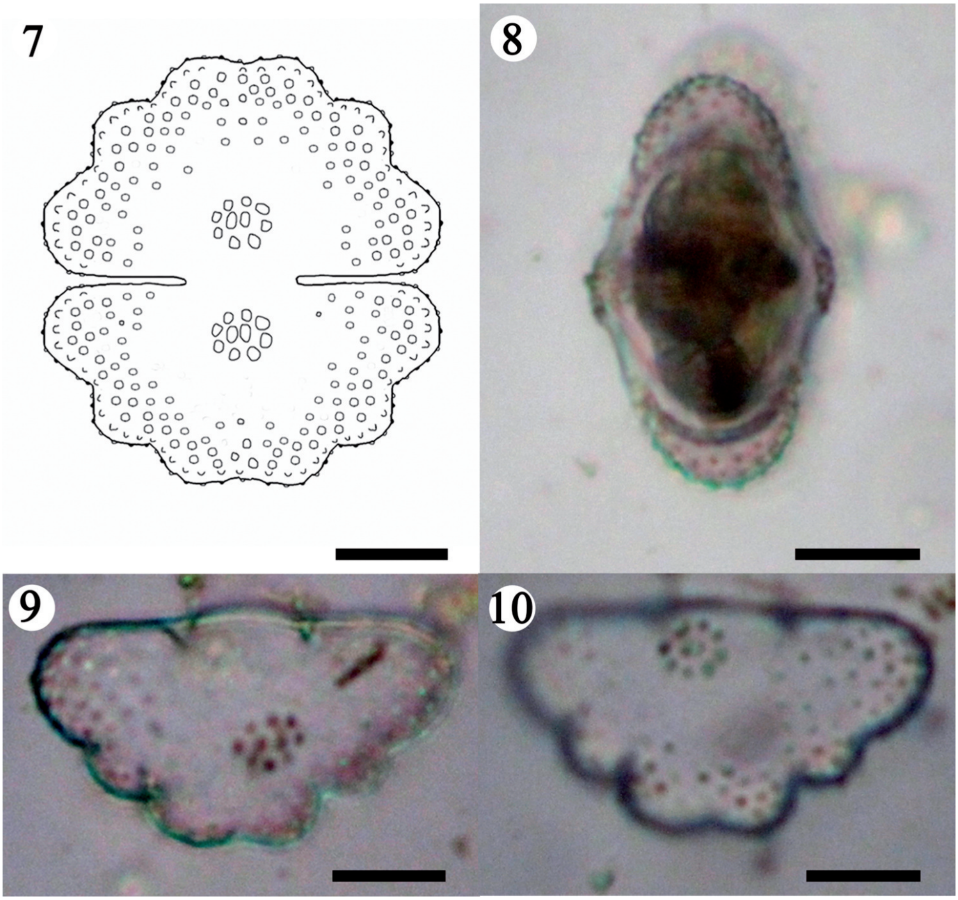
Euastrum elfarafraense Saber, Kouwets, Haworth & Cantonati **sp. nov. Figs 3-10**

Diagnosis: Cells nearly circular to oval in outline with a moderately deep and narrow sinus, closed inwardly but opening acutely to the outside. Semicells more or



Figs 3-6. *Euastrum elfarafraense* sp. nov. **3-4.** Living cells: Note chloroplasts and pyrenoids. **5-6.** Hydrogen peroxide-treated cells showing rather shallow and wide invaginations between the lateral lobes, the basal lobe significantly larger than the upper part and the polar lobe with weakly retuse margin in the midregion. Scale bar = 10 µm.

less trapeziform, 5-lobed and having rather shallow and widely open invaginations between the lateral lobes. Polar lobe somewhat rectangular with rounded margins and slightly retuse in the centre. Basal and lateral lobes in general simple and convex with the basal lobe being broadly rounded and larger than the lateral ones (Figs 3-6).



Figs 7-10. *Euastrum elfarafraense* sp. nov. **7.** Line-drawing in front view. **8.** Cell in apical view. **9-10.** Semicells in different focal planes showing the cell wall sculpture and the granulate tumor. Scale bar = 10 µm.

Cells markedly elliptic in apical view (Fig. 8). Cell walls sparsely covered with small granules distinctly visible on cell margins. Each semicell has a slightly pronounced central protuberance (tumour) with a more or less circular arrangement of about 8 granules surrounding two in the centre (Figs 7, 9-10). Chloroplast with two pyrenoids in each semicell. Dimensions: length 37.5-43.7 µm, breadth 33.8-37.5 µm, isthmus ca 10-11µm, thickness 18-20 µm.
Holotype: collected by Abdullah A. Saber; fixed specimens (2% v/v formaldehyde final conc.) and permanent slides were deposited in the Phycology Unit No. 341 at the Botany Department, Faculty of Science, Ain Shams University, Cairo (Egypt), October 16th 2016. Accession number: PBA-1605.
Isotype: subsample of the holotype material preserved in formaldehyde (2%) final conc. and deposited in the phytobenthic algae collection of the Museo delle Scienze – MUSE Limnology and Phycology Section (Trento, Italy). Accession number: cLIM009 FITB 881.

Table 1. Main hydrochemical characteristics of the slightly-hot spring 'Ain El-Balad' and of the agricultural ditch fed by its water in El-Farafra Oasis (The Western Desert of Egypt)

Parameters	*Ain El-Balad spring-water-fed agricultural ditch*	*Ain El-Balad spring*
Temp. (°C)	23.8	36.3
pH	6.16	6.74
Conductivity ($\mu S.cm^{-1}$)	960	430
T.D.S. ($mg.L^{-1}$)	460	210
Dissolved oxygen ($mg.L^{-1}$)	1.9	5.7
Na^{+} ($mg.L^{-1}$)	25.5	15.4
K^{+} ($mg.L^{-1}$)	24.8	11.4
Ca^{2+} ($mg.L^{-1}$)	29.9	13.0
Mg^{2+} ($mg.L^{-1}$)	16.6	8.9
Cl^{-} ($mg.L^{-1}$)	131	57
SO_4^{2-} ($mg.L^{-1}$)	36.03	20.11
HCO_3^{-} ($mg.L^{-1}$)	314.41	138.79
CO_3^{2-} ($mg.L^{-1}$)	0.0	0.0
NO_2^{-} ($\mu g.L^{-1}$)	8	12
NO_3^{-} ($\mu g.L^{-1}$)	400	410
NH_4^{+} ($\mu g.L^{-1}$)	150	0.0
TP ($\mu g.L^{-1}$)	100	70
SRP ($\mu g.L^{-1}$)	48	0.0
SiO_2 ($mg.L^{-1}$)	7.1	1.7
Sr ($\mu g.L^{-1}$)	226	74.8
Ba ($\mu g.L^{-1}$)	70.4	73.7
Fe ($\mu g.L^{-1}$)	58.8	242
Rb ($\mu g.L^{-1}$)	19.6	24.7
Li ($\mu g.L^{-1}$)	16.8	12.2
Zn ($\mu g.L^{-1}$)	7.7	9.5
Mn ($\mu g.L^{-1}$)	3.8	37.1
V ($\mu g.L^{-1}$)	0.94	0.18
Cu ($\mu g.L^{-1}$)	0.36	0.08
T ($\mu g.L^{-1}$)	0.16	0.70
Ni ($\mu g.L^{-1}$)	0.05	0.93

Type locality: agricultural ditch fed by freshwater from the slightly hot rheocrenic spring 'Ain El-Bald', Qasr El-Farafra, El-Farafra Oasis, Western Desert, Egypt (27°03' 26.1" N, 27°57' 47.4" E, 104 m a.s.l).
Etymology: the new species is named after the type locality El-Farafra Oasis.

Ecology: a benthic freshwater (E.C. = 960 μS.cm^{-1}) desmid, moderately acidophilous (pH = 6.16), and rather rare in occurrence. The most abundant co-occurring taxa include the desmid *Micrasterias truncata*, the raphid small-sized diatom *Sellaphora nigri*, the heterocytous cyanobacterium *Anabaena oscillarioides*, the pollution-tolerant blue-green alga *Oscillatoria limosa*, unbranched green filaments of *Spirogyra* sp., *Oedogonium* sp. and the stonewort *Chara vulgaris*. Chemically, Ca^{2+}, Na^{+}, K^{+} & HCO_3^{-} were the major ions present with values of 29.9, 25.5, 24.8 and 314.41 mg.L^{-1}, respectively. Nutrients, especially NO_3^{-}, NH_4^{+} & SRP, were also present in relatively high concentrations (400, 150, and 48 μg.L^{-1} respectively), reflecting meso-eutrophic conditions. The heavy elements having notable concentrations were Sr, Ba, and Fe (see Table 1 for more details). Values of major ions and trace elements reflect the hydrochemical features of the Nubian Sandstone Aquifer feeding this area and the bedrock characteristics of the El-Farafra Oasis.

DISCUSSION

The profound taxonomic problems around the small-sized *Euastrum* species and their relation to other morphologically similar taxa were highlighted, though mainly for other phylogenetical clades of the genus *Euastrum* as traditionally circumscribed, already several years ago (Kouwets, 1984 & 1987). In addition, Kouwets (1998) stated that there is an urgent need to re-evaluate the taxonomic ranks of the species complex *E. spinulosum* with its varieties and forms where they should be renamed and identified as different species. In this study, the interest in *Euastrum* specimens aims to partly resolve this puzzle by an in-depth comparison of the morphotaxonomy and ecological preferences of the El-Farafra *Euastrum* with other similar taxa. In general, differences in the form and disposition of the cell wall sculpture are considered an important taxonomic value for accurate species delimitation in the genus *Euastrum* (e.g., Brook *et al.*, 2011; van Geest & Coesel, 2012).

Euastrum elfarafraense sp. nov. and related species and infraspecific taxa form a very complex taxonomic problem. The greatest diversity of these forms is found in the tropics and especially in tropical Africa. Coesel (2000) presents a series of seven possible evolutionary lines, all starting from *E. mononcylum* (Nordstedt) Raciborski 1885. In this concept, semicells of *E. mononcylum* have a “basic” morphology, with two lateral lobes and an apical lobe, all emarginate and separated by an almost rectangular incision and one central protuberance. The margins of the lobes are ornamented with series of small conical spinules and the protuberance is ornamented with small warts in concentric circles. The evolutionary lines differ in the extent to which the lateral lobes become prominent or rather reduced.

Evolutionary line 1 *sensu* Coesel (2000) includes an initial deepening of the invagination between the lateral lobes, and a gradual narrowing of the incision between lateral and apical lobes, leading to *Euastrum spinulosum* Delponte 1877. Next, a gradual reduction of the basal semicell lobe is suggested, leading to forms of *E. divergens* Joshua 1886.

In this study, *Euastrum elfarafraense* sp. nov. is obviously closely related to the *E. spinulosum* species complex. However, in its original concept *E. spinulosum* is a much larger form: Delponte (1877) described it from Italy and mentioned dimensions of 79.2 × 68 μm which is about twice as large as *E. elfarafraense*. From South Africa, Nordstedt (1880) described a subspecies *africanum*, measuring 85 ×

73 µm and this is generally considered a synonym of the nominate subspecies (see, however, Croasdale in Grönblad & Croasdale 1971, who reported material from Namibia measuring 86 × 82 µm and suggested that both taxa be kept apart).

In *Euastrum elfarafraense* the invaginations between the lateral lobes are rather shallow and wide. Furthermore, the lower "basal lobe" is significantly larger than the upper "lateral lobe" which is in contradiction with the suggested gradual reduction of this lobe in evolutionary line 1 suggested by Coesel (2000).

A thorough search through the available literature only revealed two more reports of smaller forms of *Euastrum spinulosum* with a more prominent basal lobe. From Chad, Gauthier-Lièvre (1958) reported *E. spinulosum* var. *africanum* f. *minus* (described by Nordstedt 1880 as subsp. *africanum* var. *minus*!) measuring 46-48 × 40-42 µm. The figure presented by Gauthier-Lièvre (l.c.) is very similar to the present material of *E. elfarafraense*. Nordstedt's original description of var. *minus* was of material from Senegal and measured 54-64 × 44-52 µm but unfortunately no figure was given. A similar form was reported from Lake Chad by Compère (1977) as an unnamed form of *E. spinulosum*. As dimensions of the species he gave 42-80 × 38-73 µm (obviously including the "typical" form), but stated that the forma observed was small and its ornamentation reduced. Unfortunately no exact measures of this smaller form were given but the figure is rather similar to the present material and measures approximately 47 × 39 µm.

In addition to the nominate variety, Grönblad & Croasdale (1971) presented figures of var. *minus* from Namibia, measuring 49-60 × 43-52 µm, but these forms lack the significantly larger basal lobe characteristic of *Euastrum elfarafraense*. Additionally, the var. *minus* appears slightly larger than the present form.

A still smaller form was described from Madagascar by West & West (1895) as *Euastrum spinulosum* subsp. *africanum* var. *duplo-minus* (misspelled "*duplo-minor*"), measuring 42 × 38 µm but this form also lacks the larger basal lobe. *E. elfarafraense* is also distinguished from the morphologically much more similar species *E. panamense* (Coesel 1978) by its distinctive small-sized cells (37.5-43.7 × 33.8-37.5 µm vs 78-85 × 72-82 µm), shallower invaginations between the semicell lobes and the absence of the supra-isthmial tubercle that is considered a key taxonomic feature for *E. panamense* and the absence of granules in the region between its central protuberances and lateral lobes (Coesel, 1978).

Finally, specimens of *Euastrum elfarafraense* should also be compared with forms of *Cosmarium alatum* Kirchner 1878, described from Germany, and especially its var. *aequatoriense* Nordstedt *in* Wittrock & Nordstedt 1893, which was described from Ecuador and var. *gostyniense* Raciborski 1885, described from Poland. However, *C. alatum* and its var. *aequatoriense* were originally described without figures and are hence considered doubtful (although West & West 1908 published a figure of the nominate variety after a drawing by Kirchner). Remarkably, West & West (1908) in addition presented a figure of *C. alatum* var. *aequatoriense* that they had described previously from Central Africa as *Euastrum hexagonum* (West & West, 1896) with dimensions of 44-53 × 38-46 µm. In this form, the basal lobe is significantly larger than the lateral lobe but the incisions between the lobes are less deep compared to those of *E. elfarafraense,* and these taxa are considered dissimilar; they may, however, be part of a separate evolutionary line, supplementary to the lines presented by Coesel (2000). *C. alatum* var. *gostyniense* is also distinguished by its wider cells and more numerous and densely crowded granules. However, its dimensions are much smaller than those of *E. elfarafraense*. The forms presented by Gauthier-Lièvre (1958) and Compère (1977) may be conspecific with *E. elfarafraense*: this needs further study but the name of the latter would still have priority at species level.

Information on the *Euastrum* species occurring in Egypt and their distribution, particularly in desert habitats, is still obscure (e.g., El-Nayal, 1932 & 1936; Shaaban, 1994; Gharib, 2004; Shaaban *et al.*, 2015; Saber, 2016). Only three different *Euastrum* species have so far been identified: *E. denticulatum* var. *stictum* Børgesen 1890 (now regarded as *E. turneri* var. *stictum* (Børgesen) Scott & Prescott 1952), *E. insulare* (Wittrock) Roy 1877 and *E. spinulosum* var. *inermius* (Nordstedt) Bernard 1908 but no detailed morphotaxonomic descriptions are available for these taxa.

Discovery of the interesting species *Euastrum elfarafraense* from the hyper-arid desert habitat of the El-Farafra Oasis in the present study is highly compatible with the hypothesis suggested by Coesel (2000) and Coesel & van Geest (2008) on the restricted 'endemic' biogeographical distribution of some *Euastrum* morphospecies in subtropical and tropical African countries. They clearly confirmed that *E. spinulosum* and *E. mononcylum* species complexes are polymorphic, widely-distributed in subtropical-to-tropical African countries, and need major taxonomic revision with some species separation.

From the ecological standpoint, the finding of this new *Euastrum* species in a meso-eutrophic habitat, in terms of the N and P concentrations, and its rare co-occurrence with other pollution-tolerant taxa is compatible with the findings of Kouwets (1987 & 1998). Coesel (1982) also pointed out that desmids in meso- and eutrophic habitats are usually present in lower numbers and diversity. Several desmids are well known to prefer rather mesotrophic and moderately acid environments (Růžička, 1981; Coesel & Meesters, 2007; Coesel *et al.*, 2017). In conclusion, the desmid flora of the Egyptian desert habitats, and of North Africa in general, should be subjected to more detailed investigations in the future, especially in environments which are still less-polluted (compared to urban communities) and where the probability of finding further interesting and novel desmid species might be relatively high, at least, from the biogeographical standpoint.

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