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Observations of the diatoms *Sceptronema orientale* Takano and *Tabularia parva* (Kützing) D.M.Williams & Round on the exoskeleton of copepods in the English Channel and coastal Celtic Seas

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Sceptronema orientale (photo: Fernando Gómez).

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Observations of the diatoms *Sceptronema orientale* Takano and *Tabularia parva* (Kützing) D.M.Williams & Round on the exoskeleton of copepods in the English Channel and coastal Celtic Seas

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ABSTRACT
The consortium of the epizoic diatom *Sceptronema orientale* Takano on living individuals of the harpacticoid copepod *Euterpina acutifrons* Dana was found in summer 2018 in the port of Brest, Celtic Seas, and in several locations of the open English Channel during the summer-autumn transition. Diatoms as epibionts on copepods remained unreported up to date in the region despite the high tradition of plankton observations. *Tabularia parva* (Kützing) D.M.Williams & Round was attached to the empty carapace of a copepod in the port of Brest. The detailed morphology is illustrated by scanning electron microscopy.

KEY WORDS
Araphid pennate diatom, epibiosis, epiphytic diatom, epibiont, symbioses, North Atlantic.

RÉSUMÉ
Observations des diatomées Sceptronema orientale *Takano et* Tabularia parva *(Kützing) D.M. Williams & Round sur l'exosquelette de copépodes dans la Manche et la frange côtières des mers Celtiques.*
Le consortium de la diatomée épizoïque *Sceptronema orientale* Takano sur des individus vivants du copépode harpacticoïde *Euterpina acutifrons* Dana a été retrouvé durant l'été 2018 dans le port de Brest, mers Celtiques, et à plusieurs endroits dans les eaux au large de la Manche pendant la transition estivo-automnale. Les diatomées épibiontes de copépodes n'ont pas été signalées à ce jour dans la région malgré une grande tradition d'observations du plancton. *Tabularia parva* (Kützing) D.M.Williams & Round a été retrouvée attachée à la carapace vide d'un copépode dans le port de Brest. La morphologie détaillée est illustrée par microscopie électronique à balayage.

MOTS CLÉS
diatomées pennées araphides, épibiose, diatomée épiphyte, épibionte, symbioses, Atlantique Nord.

INTRODUCTION

Copepods dominate the zooplankton biomass and are considered to be the most abundant animals in the ocean (Mauchline 1998). Some species of araphid diatoms have developed a symbiotic relationship with copepods using the host external surface for the settlement and growth (Hiromi *et al.* 1985; Gómez *et al.* 2018). The diatom *Sceptronema orientale* Takano, characterized by a distinctive shape of an Egyptian sarcophagus, is only known as epibiont of the harpacticoid copepod *Euterpina acutifrons* Dana. The epibiosis was first described from coastal waters of Japan influenced by warm waters of the Kuroshio Current (Takano 1983; Hiromi *et al.* 1985), and further reported in the Mediterranean Sea (Kimor *et al.* 1992; Skovgaard & Saiz 2006), and it was also found in the coastal waters of Argentina (Sar & Sunensen 2014). In the Mediterranean Sea, Skovgaard & Saiz (2006) reported that from late summer to early winter, 20 to 65% of all *Euterpina acutifrons* adults carried the diatom. The distinctive shape of *Sceptronema orientale* and its habitat, only known as epibiont of copepods, facilitate the identification. In contrast, the identification of diatom species of the genus *Tabularia* (Kützing) D.M.Williams & Round, commonly found as epiphytic on benthic macroalgae, is more difficult during the routine light microscopical observations (Williams & Round 1986; Snoeijs & Kuylenstierna 1991; Snoeijs 1992). From samples collected in the English Channel and Celtic Seas, this study describes the first record of *Sceptronema orientale* attached to live copepods in the English Channel, and the first observation of *Tabularia parva* (Kützing) D.M.Williams & Round attached to the empty exoskeleton of a copepod.

MATERIAL AND METHODS

Plankton samples were collected from surface waters with a phytoplankton net (20-µm mesh size) during two research cruises: ECOPEL Manche Leg-2 (https://doi.org/10.17600/18000443) on-board R/V "Antea" (IRD) from July 16 to July 31, 2018; and CGFS (https://doi.org/10.17600/18000517) on-board R/V "Thalassa" (IFREMER) from September 11 to October 11, 2018. During the first cruise, aliquots of the living plankton concentrate were examined on-board with an inverted microscope (Eclipse TS-100, Nikon Inc., Tokyo, Japan) and photographed with a digital camera (Nikon D5000). In the second cruise, concentrated samples were preserved with acid Lugol's solution and examined with an inverted microscope (Nikon Eclipse TE2000-S, Tokyo) and photographed with a Nikon Digital Sight DS-2M camera. In order to establish cultures, during the first cruise (16-31 July, 2018), the copepod exoskeletons with epizoic diatoms were isolated, washed several times into a series of drops of 0.2-µm-filtered seawater to remove other organisms, and placed in bottles during the transport between Brest and Wimereux (*c.* 700 km). At the lab, the epizoic diatoms were detached from the host, and placed in a 12-well tissue culture plate with 0.2-µm filtered and sterilized seawater supplemented with f/2 medium with silicate and incubated at 18°C, with 80 µmol photons m^{-2} s^{-1} from cool-white tubes; the photoperiod was 14:10 light:dark. The diatoms were re-isolated and placed into a six-well tissue culture plate. For scanning electron microscopy, the copepods with epizoic diatom *Sceptronema orientale* and the culture material of *Tabularia parva* were placed on 3 µm pore size polycarbonate membrane filters (Millipore Ltd., Middlesex, United Kingdom). In the case of the culture of *Tabularia parva*, one subsample was treated using the standard nitric acid/potassium dichromate method (Carr *et al.* 1986). Filters were mounted on an aluminium stub, sputter-coated with Au/Pd (Polaron SC7620, Quorum Technologies Ltd., Ashford, United Kingdom) and observed at 15 kV with a SEM LEO 438 VP (Carl Zeiss AG, Oberkochen, Germany). Images were presented on a black background using Adobe Photoshop CS3 (Adobe Systems Inc., San Jose, CA, United States).

RESULTS

BACILLARIOPHYTA, *incertae sedis*
Genus *Sceptronema* Takano

Sceptronema orientale Takano

DISTRIBUTION. — The consortium of the epizoic diatom *Sceptronema orientale* was observed in the port of Brest on the copepod *Euterpina acutifrons* in July 29-30, 2018. In September 2018, the epibioses were found in four stations near the coasts of England and France (Fig. 1, Table 1). The percentage of copepods that carried the epizoic diatom never exceeded the 30% of the population. The attempt to culture *S. orientale* was unsuccessful.

DESCRIPTION

The cells were solitary or forming a linear chain of two cells joined with a mucilaginous pad (Fig. 2A-L, N, P-Q). Chains of three or more cells or the connection between the foot (narrow) poles of two cells were not observed. The cells were attached to the whole body of the host. The cells attached to the cephalosome showed short stalks (Fig. 2J, O) whilst the cells attached to the caudal setae showed stalks with more variable length (Fig. 2K, L).

Cells were heteropolar in valve view and slightly wedge-shaped. Valve was narrowly obovate with a wide rostrate head-pole and a narrow capitate foot-pole, showing a distinctive outline that resembles an Egyptian sarcophagus (Fig. 2M, N). The frustules were 28-58 µm long and 7-10 µm wide. Sternum was central, straight and spathulate at the ends (Fig. 2T). The striae were uniseriate, *c.* 40 in 10 µm, extending almost to the valve mantle edge. The striae were perpendicular to the sternum, except at the poles. Areolae were elliptical, originated in the transapical direction and occluded. There was an apical slit field in the valve mantle composed of longitudinal bars at each valve pole (Fig. 2P-U). The head pole contained more longitudinal slits (*c.* 17) than the basal pole (*c.* 13 slits) (Fig. 2P). The mucilage pad

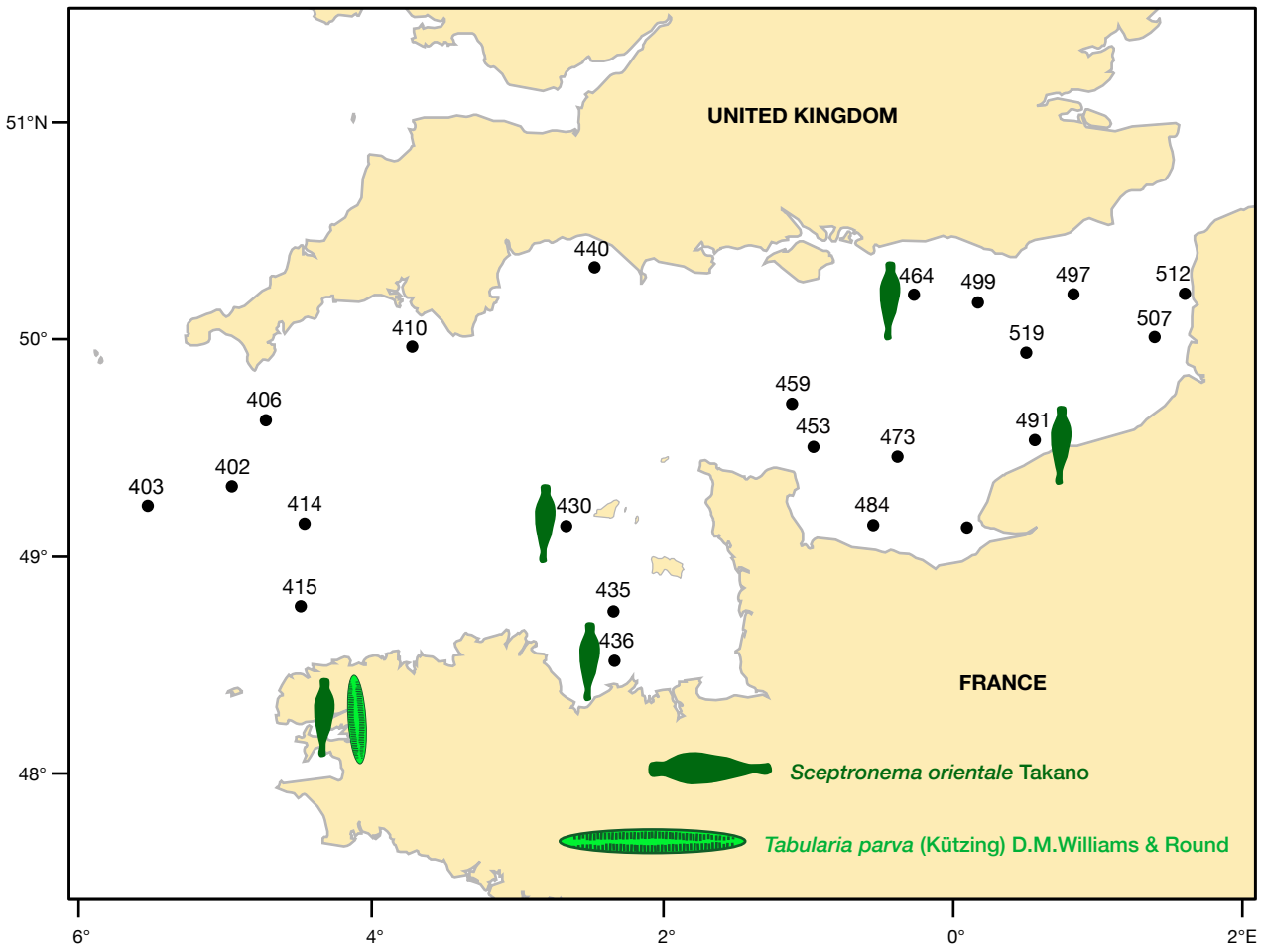

FIG. 1. — Map of the sampling stations in the English Channel and Celtic Seas during the CGFS cruise (on the R/V "*Thalassa*", IFREMER) in September-October 2018 and records in the port of Brest in July 2018.

emerged from the slit fields (Fig. 2P, Q). There was numerous rimoportulae with an elliptical shape on the external valve surface near the poles (Fig. 2R, S).

REMARKS

The valve outline of *Sceptronema orientale* is high distinctive when compared to the other epizoic araphid diatoms. In the original description, Takano (1983: fig. 20) illustrated chains of more than two cells, including cells attached by the foot-pole of one cell to a foot-pole of other cells. We have not observed chains of more than two cells or two cells joined by their foot-poles. The length of the stalk of *S. orientale* differed according to its location on the host. The stalks are short for the cells attached to the frontal part of the copepod (Fig. 2H-J), while the stalks are usually longer in the caudal setae (Fig. 2K, L). There, the stalk may be branched because the stalk is used as attachment point for the stalk of other individuals (Fig. 2K, L). This suggests an adaption of the diatom to the epizoic life. *Sceptronema orientale* is only known as epibiont of *Euterpina acutifrons*. In the Gulf of Biscay, Paulmier (1997: pl. 181, fig. 13) reported the diatom *Pseudohimantidium pacificum* Hustedt & Krasske in Krasske, on *Corycaeus* sp. and a line drawing of a diatom identified as *Licmosphenia* sp. that may correspond to *S. orientale*.

Family ULNARIACEAE E.J.Cox
Genus *Tabularia* (Kützing) D.M.Williams & Round

Tabularia parva (Kützing) D.M.Williams & Round

DISTRIBUTION. — Dense clusters of *Tabularia parva* cells were often found associated with the empty exoskeleton of a copepod from a plankton sample collected in the port of Brest (Fig. 3A, Table 1). The epibiosis of this diatom on live copepods was not observed.

DESCRIPTION

Cells were found on the empty carapace of a copepod (Fig. 3A). The cells were isolated and successfully cultured (Fig. 3B-R). The valve outline was linear-elliptical tapering towards rounded poles in valve view, and rectangular in girdle view (Fig. 3F). The cells were 16-23 µm long and 3.2-4.3 µm wide. The valve surface was flat and the sternum was wide lanceolate. The striae (18-19 striae in 10 µm) consisted of biseriate areolae covered by cribra, but with one areola near the sternum (Fig. 3H-N). There was a single rimoportula per valve situated close to one small apical pore field or ocellulimbus, or slightly displaced to one side and often obliquely oriented (Fig. 3H-L). Each valve pole showed a small ocellulimbus that contained four to seven rows of closely packed porelli (Fig. 3O-R). There is one or two uniseriate striae near the ocellulimbus (Fig. 3O-R).

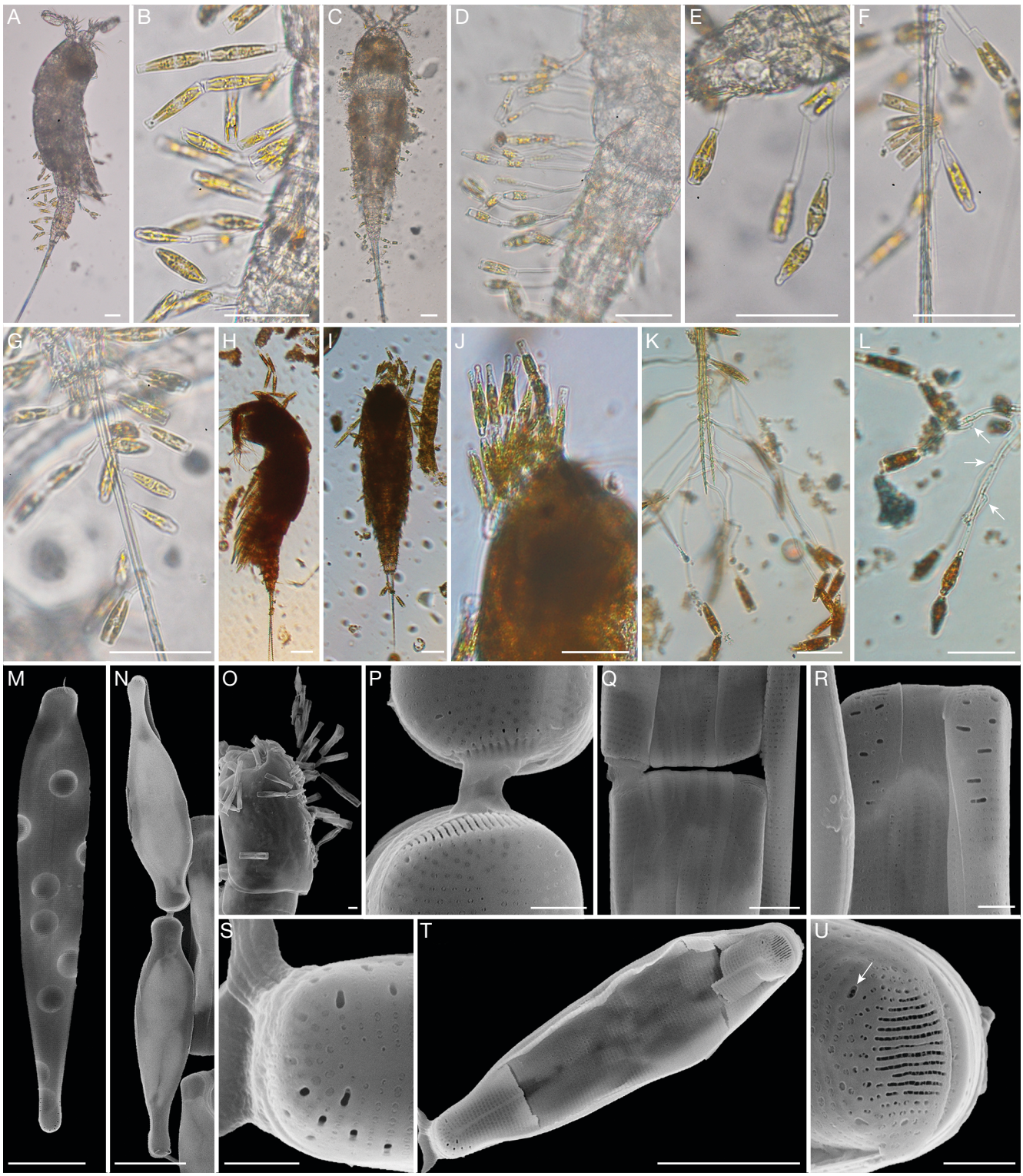

Fig. 2. — Light (A-L) and scanning electron (M-U) micrographs of the diatom *Sceptronema orientale* Takano as epibiont on the copepod *Euterpina acutifrons* Dana: **A**-**G**, live cells from the port of Brest, Brittany, in July 2018; **H**-**L**, Lugol-preserved cells from the open English Channel in September 2018; **H**-**J**, frustules on the cephalosome; **K**, note the long stalks in the copepod caudal setae; **L**, the **arrows** indicate the stalks attached to the stalk of other individuals; **M**, a very elongated cell. The head (wide) and foot (narrow) poles are the top and bottom, respectively. Circles are due to the filter pores; **M**-**O**, **T**, note the Egyptian sarcophagus outline in valve view; **P**-**Q**, head poles of two cells joined by a mucilaginous pad; **R**, note the rimoportulae; **S**-**U**, valve view; **S**, note the mucilaginous pad in the foot pole and rimoportulae; **U**, head pole showing the vertical slits. The arrowhead indicates the rimoportula. Scale bars: A-L, 50 µm; M-O, T, 10 µm; P-S, U, 1 µm.

The mucilage pad was secreted from the ocellulimbus allowing the attachment to the host or surface (Fig. 3G). The wild cells formed bunches of solitary cells joined by the valve poles to the host surface. The cultured cells lie on the bottom of the culture chambers or were arranged perpendicularly (Fig. 3B). When a particle was present, the diatoms formed bunches on

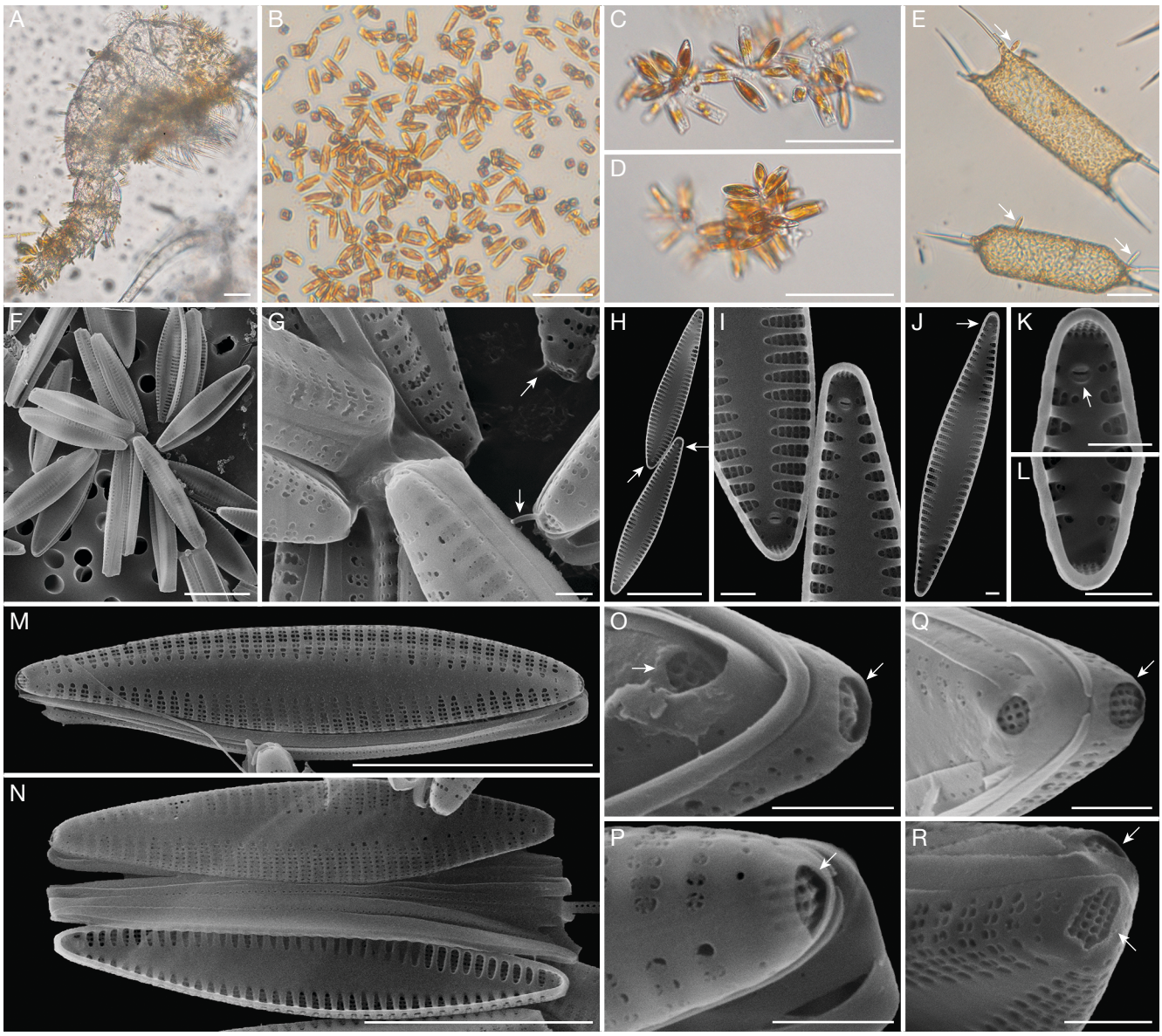

Fig. 3. — Light (**A**-**E**) and scanning electron (**F**-**R**) micrographs of *Tabularia parva* (Kützing) D.M.Williams & Round: **A**, wild cells on the empty carapace of a copepod from the port of Brest, Brittany.; **B**-**R**, cultured cells; **B**, note a part of the cells arranged perpendicularly to bottom; **C**, **D**, cells forming bunches attached to an exopolymeric transparent particle; **E**, the **arrows** indicate cells attached to the diatom *Trieres chinensis* (Greville) Ashworth & E.C.Theriot; **F**, **G**, cells attached by mucilage pads; **G**, the **arrows** indicate the thin mucilage; **H**-**R**, acid-cleaned valves; **H**-**L**, internal view of the valve. The **arrows** indicate the single rimoportula per valve; **M**, **N**, valve view; **O**-**R**, the **arrowheads** indicate the ocellulimbus. Scale bars: A-E, 50 µm; F, H, M, N, 10 µm; G, I-L, O-R, 1 µm.

its surface (Fig. 3C-D). Cells were added to a culture of the large diatom planktonic diatom *Trieres chinensis* (Greville) Ashworth & E.C.Theriot (also spelled as "sinensis"). After a few days, the cells were attached to the large diatom (Fig. 3E).

Remarks

Species of the genus *Tabularia* occur as epibionts on benthic animals (Wuchter *et al.* 2003). We found *Tabularia parva* attached to the copepod substrate, but it is uncertain whether the cells were already attached to the living copepod. *Tabularia parva* is placed in the *barbatula* group, with *T. barbatula* (Kützing) Williams & Round, *T. affinis* (Kützing) Snoeijs and *T. ktenoeides* Kuylenstierna, and characterized by biseriate striae, no marginal rib, and always only one rimoportula per valve (Snoeijs & Kuylenstierna 1991; Snoeijs 1992). The striae of *T. barbatula* begin with two areolae, while *T. parva* has one areola near the sternum. The sternum is linear and narrow in *T. barbatula*, while wide and lanceolate in *T. parva*. *Tabularia affinis* showed the lower number of striae in the *barbatula* group (13-14 striae) (Snoeijs 1992). *Tabularia parva* with 18-20 striae in 10 µm is near the upper range of *T. barbatula* (15-18 striae), and in the lower range of *T. ktenoeides* (20-24 striae). The latter taxon with alternate organization of the areolae (Snoeijs & Kuylenstierna 1991). The dimensions, especially the apical axis of *T. parva*, were smaller than the other species of the *barbatula* group, except for *T. ktenoeides* (Williams & Round 1986; Kuylenstierna 1990; Snoeijs & Kuylenstierna 1991; Snoeijs 1992). Our cultured individuals

TABLE 1. — Records of diatoms attached to the exoskeleton of copepods in the English Channel and coastal Celtic Sea.

Taxon	Date of collection	Geographical coordinates
Sceptronema orientale	29.VII.2018	48°22'53"N, 4°29'06"W
Sceptronema orientale	30.VII.2018	48°22'53"N, 4°29'06"W
Sceptronema orientale	20.IX.2018	49°22'01"N, 2°52'16"W
Sceptronema orientale	22.IX.2018	48°46'08"N, 2°27'04"W
Sceptronema orientale	30.IX.2018	50°33'40"N, 0°27'29"W
Sceptronema orientale	05.X.2018	49°53'30"N, 0°27'40"W
Tabularia parva	30.VII.2018	48°22'53"N, 4°29'06"W

of *T. parva* were in the range of size for this species reported by Williams & Round (1986) and Kuylenstierna (1990) from measurements of wild individuals from the Atlantic Ocean, but smaller than the cultured material from the Pacific Ocean (Sato *et al.* 2008). The number of striae is quite constant ranging from 18-20 in 10 µm (Fig. 3M, N; Kuylenstierna 1991; Sato *et al.* 2008).

CONCLUSIONS

Despite the high tradition of plankton studies in the region, this study reports for the first time the presence of the epizoic diatom *Sceptronema orientale* in the English Channel and coastal waters of the Celtic Seas, with numerous records at both sides of the English Channel (Fig. 1). To the best of our knowledge, the epibioses between diatoms and copepods were unreported in the English Channel up to date. This could constitute a biological indicator of climate change as *Sceptronema orientale* is more commonly reported in warm waters such as the Mediterranean Sea (Kimor *et al.* 1992; Skovgaard & Saiz 2006). In the case of *Tabularia parva*, this is the first observation of a copepod carapace as host, but we were unable to observe its presence on the living animal host. Copepods covered by diatoms should be easy to detect in coastal plankton monitoring surveys. In recent years plankton observations using the microscope have been replaced by automated plankton identification systems (i.e. ZooScan) that might not necessarily be calibrated to recognize copepods with diatoms.

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