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Morphological and genetic characteristics of erect subtidal species of *Alcyonidium* (Ctenostomata: Bryozoa)

J.S. Porter

School of Biological Sciences, University of Wales Swansea, Singleton Park, Swansea, Wales, SA2 8PP.
E-mail: bdporter@swansea.ac.uk

The ctenostome bryozoan *Alcyonidium diaphanum* is widespread and abundant in the coastal waters of the British Isles. During the Centre for Environment, Fisheries and Aquaculture Science groundfish surveys some atypical colonies were found and subsequent histological and genetic analyses have shown that these represent two previously undescribed species of erect subtidal *Alcyonidium*. They are described here fully and named *Alcyonidium condylocinereum* and *Alcyonidium hydrocoalitum*. Comparisons with further museum specimens of erect *Alcyonidium* species have shown that *A. proliferans*, *A. topsenti* and *A. gelatinosum* form *lacourti* can be synonymized with *A. diaphanum*. The northern and southern limits of *A. diaphanum* are here more clearly defined; however, the identities of Arctic erect *Alcyonidium* species have yet to be fully investigated using histological and genetic methodologies. It is clear from this study that gross colony morphology is not a good indicator of species identity, but that tentacle number and reproductive mode can be useful taxonomic characteristics in these species.

INTRODUCTION

The carnose ctenostome bryozoan genus *Alcyonidium* Lamouroux, 1813 has long been taxonomically problematic. Particular attention has been paid to the thin encrusting species of the genus, found in littoral habitats, leading to the eventual redescription of *A. mytili* Dalyell, 1848 (Cadman & Ryland, 1996a,b), *A. gelatinosum* (Linnaeus, 1761) (=*A. reticulum* Ryland & Porter, 2000) and *A. polyoum* (Hassall, 1841) (Ryland & Porter, 2003). Significantly less attention has been paid to the large subtidal species characterized by lobate colony forms. Allozyme electrophoresis indicated that the erect, lobate *A. diaphanum* (Hudson, 1762) consisted of two genetic types (Thorpe et al., 1978, as *A. gelatinosum*), which appeared to correspond to two morphotypes, Type I and Type II. During the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) groundfish surveys in 1999–2001, the by-catch of beam trawl surveys was analysed and samples of *A. diaphanum* were collected for a population genetic survey. A preliminary study by Porter et al. (2001) distinguished three colony morphotypes, which could be confidently matched to the herbarium specimens selected as lectotype and paralectotypes of the newly redescribed taxon *A. diaphanum* (Hudson, 1762). Reviewing the whole of the CEFAS samples Porter et al. (2002) recognized a further three morphotypes, and plotted the geographical distribution of all six in the southern North Sea, English Channel and Irish Sea. The six morphotypes of *A. diaphanum*, two of which corresponded to the Types I and II of Thorpe et al. (1978) were genetically indistinguishable on the basis of the 12s rRNA and Cytochrome Oxidase I mitochondrial DNA sequence data (J.S.P., unpublished data).

At four locations, in a total of 188 sampled, specimens of an *Alcyonidium* species with an unusual 'grey knobbly' colony form were found: in the Dover Strait, at two locations in the English Channel and at one location off south-eastern Ireland. These are here compared with *A. diaphanum* using morphological and molecular genetic techniques. Two other nominate species of *Alcyonidium* with erect, lobate colony forms, and reported from sublittoral habitats, are also considered, viz *A. proliferans* Lacourt, 1949 and *A. topsenti* Roehrich, 1910. These have been distinguished from *A. diaphanum* on morphological grounds (d'Hondt, 1983) but have never been examined using molecular genetic techniques. A specimen of a translucent, rubbery species of *Alcyonidium* investing the stems of large, subtidal hydroids, collected from Oxwich Bay, South Wales, and attributed to *A. cellarioides* Calvet, 1900 (Hayward, 1985) was also re-examined, and compared with two further specimens from the Dover Strait which appeared to represent the same species. While *A. proliferans* and *A. topsenti* are shown to be genetically indistinguishable from *A. diaphanum*, the grey knobbly *Alcyonidium* and the translucent '*A. cellarioides*' from Oxwich Bay differed significantly from *A. diaphanum* and from each other, and are here accordingly described as new species.

MATERIALS AND METHODS

Data on the relative abundance and distribution of specimens of the grey knobbly *Alcyonidium* originated from the CEFAS groundfish surveys in the southern North Sea and eastern English Channel (August) and the Bristol Channel and Irish Sea (September) from 1997 to 2001. Material was collected with a 4-m beam trawl and tows were of 30 min duration. Further details of the gear and sampling protocol are given in Ellis et al. (2000). The unusual grey knobbly form (Figure 1A) was found at four locations (Table 1) in small quantities (16 colonies per location). Topotype specimens of *Alcyonidium diaphanum* (Hudson, 1778), which have been fully described elsewhere (Porter et al., 2001), were used as a comparison. A specimen designated as *Alcyonidium cellarioides* Calvet, described in the *Linnean Society Synopsis of*

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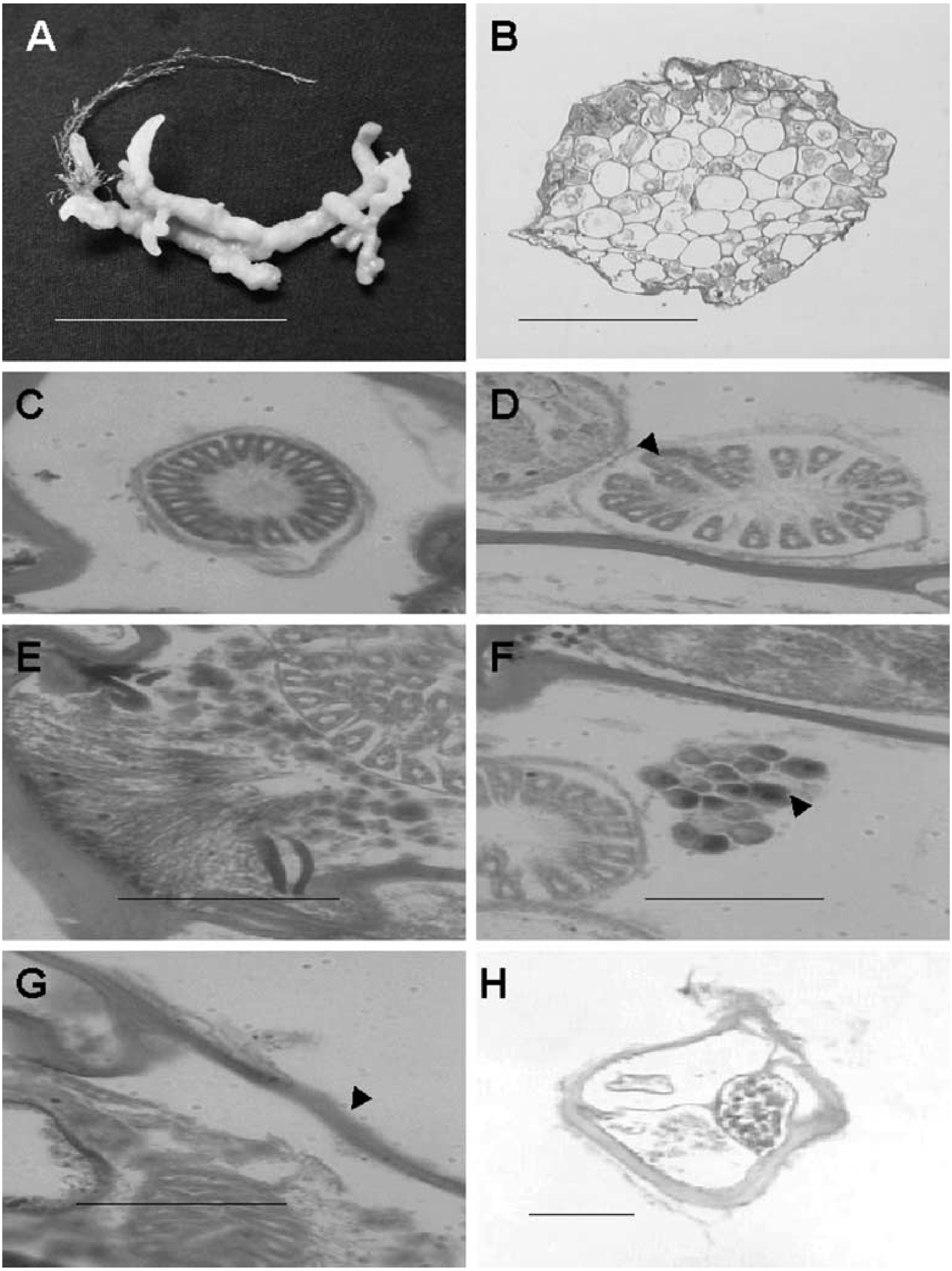

Figure 1. (A) Whole holotype colony of *Alcyonidium condylocinereum*; (B) histological section through the main column of a colony of *A. condylocinereum* showing the arrangement of the zooids; (C) histological section showing a lophophore of *A. condylocinereum*, in this case with 18 tentacles arranged in a circle and enclosed by the tentacle sheath; (D) histological section showing a lophophore of *A. condylocinereum* with an intertentacular organ on the lophophore as indicated by the arrow; (E) histological section indicating spermatogenesis in *A. condylocinereum*; (F) histological section indicating young ovary with 15 oocytes developing, note that the polypides are present at this stage in development; (G) the arrow indicates the external colony wall, it is smooth and without folding or crenulations; (H) this histological section shows the apical budding at the growing tip of a nodule of *A. condylocinereum*. Scale bars: A, 4 cm; B, 0.5 cm; E, 50 μm; F, 70 μm; G, 80 μm; H, 120 μm.

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Table 1. *Locations of* Alcyonidium *specimens used in the study.*

Species	Specimen no.	Date collected	Location	Depth
A. condylocinereum	2002.2.1.3	02 September 1999	50.65°N 01.51°E	26 m
A. condylocinereum	2002.2.1.4	02 September 1999	50.65°N 01.51°E	26 m
A. condylocinereum	2002.2.1.5	02 September 1999	50.65°N 01.51°E	26 m
A. condylocinereum	2002.2.1.6	02 September 1999	50.65°N 01.51°E	26 m
A. condylocinereum	2002.2.1.7	02 September 1999	50.65°N 01.51°E	26 m
A. condylocinereum	2002.2.1.8	02 September 1999	50.65°N 01.51°E	26 m
A. condylocinereum	2002.2.1.9	16 September 2000	51.53°N 03.94°W	27 m
A. condylocinereum	2002.2.1.10	14 September 2001	51.53°N 03.94°W	27 m
A. condylocinereum	2002.2.1.11	15 September 2001	51.47°N 04.08°W	37 m
A. condylocinereum	2002.2.1.12	29 September 2001	52.16°N 06.11°W	77 m
A. hydrocoalitum	1984.2.26.12	06 May 1975	51.47°N 04.23°W	15 m
A. hydrocoalitum	2002.2.1.13	02 September 1999	50.65°N 01.51°E	26 m
A. hydrocoalitum	2002.2.1.14	02 September 1999	50.65°N 01.51°E	26 m

the British Fauna, no. 33 by Hayward (1985:50) was obtained from the Natural History Museum, London (BMNH 1984.2.26.12). Paratype specimens of *Alcyonidium proliferans* Lacourt were obtained from the Institute voor Taxonomie, Amsterdam (nos. v.Br. 239 and v.Br. 240), as were single specimens of *Alcyondium topsenti* Roehrich (no. v.Br. 168) and *A. gelatinosum* f. *lacourti* d'Hondt (1983) (no. v.Br. 201). Histological sections for each specimen were cut according to the methodology of Porter et al. (2000). Data such as tentacle number, embryo diameter and presence of an intertentacular organ (ITO) or other reproductive structures were recorded. External measurements of a haphazard selection of 50 autozooids were made for each specimen. Morphological descriptions are accompanied by DNA sequence data from the mitochondrial 12s rRNA and CO1 genes. Mitochondrial DNA was extracted from each contemporary specimen following the methodology of Porter et al. (2001). In the case of the museum specimens, however, a different method of DNA extraction was followed as it was unknown exactly what preservatives would have been used to curate the samples. The methodology used was adapted from that described by Vachot & Monnerot (1997) to extract DNA from museum specimens of amphibians: in our method, during the initial digestion, a 25% solution of sodium dodecyl sulphate (SDS) was used instead of the usual 2% and 20 mg/ml of proteinase K instead of the usual 0.8 mg/ml. Samples were then shaken overnight during the digestion process, at 37°C in an incubator. This technique is reported to facilitate the breakdown of cross-links formed in the DNA helix structure during preservation by formaldehyde-based solutions, thus allowing the subsequent uncoiling of the DNA strands. The polymerase chain reaction (PCR) was subsequently utilized to obtain copies of the 12s rRNA and CO1 genes and these PCR products were cloned and sequenced as described by Porter et al. (2001). The DNA sequences obtained were aligned using the Clustal W program (Higgins & Sharpe, 1988).

RESULTS

DNA sequence data

Sequence differences are summarized in Table 2 for the 12s gene and Table 3 for the CO1 gene. For the 12s gene there were two taxonomically informative positions, at 122 and 177 base pairs. At position 122 *Alcyonidium diaphanum* and *A. proliferans* had the nucleotide thymine (T) whilst specimens of the 'grey knobbly' morphotype, and also as a comparison *A. mytili* (an encrusting *Alcyonidium* species), had a transversion to cytosine (C). At position 177 *A. diaphanum* and *A. proliferans* again had T whilst the 'grey knobbly' morphotype and *A. mytili* had a transversion to guanine (G). DNA of sufficiently good quality for PCR amplification could not be obtained from *A. topsenti* or '*A. cellarioides*'.

For the CO1 gene, 12 specimens of *A. diaphanum*, six each from Whitstable and from Station 104 from the North Sea (Porter et al., 2001) were compared with the two *A. proliferans* samples, a single specimen of the grey knobbly morphotype, the '*A. cellarioides*' from Oxwich Bay and an undescribed Antarctic *Alcyonidium*. Seven taxonomically informative positions were identified from a 602 base pair fragment of the CO1 gene. These differences are detailed in Table 3 and it is clear that there are two haplotypes within *A. diaphanum*, these haplotypes also encompass both of the *A. proliferans* specimens. The grey knobbly morphotype however shows a single haplotype which differs in two positions from both *A. diaphanum* haplotypes, these being at positions 586 and 587, the change being from a G to a C at 586 and from a C to a G at 587. All of these specimens differed in sequence from an undescribed Antarctic species which showed differences at positions 194, 215 and 327 where all bases were T instead of G. However, at positions 586 and 587 the Antarctic taxon had bases identical with *A. diaphanum* but differed from the grey knobbly morphotype.

Both sets of results indicate that there are no significant genetic differences between *A. diaphanum* and *A. proliferans* but that there are significant differences between *A. diaphanum* and the grey knobbly morphotype. The grey knobbly morphotype should therefore be considered to be non-conspecific with *A. diaphanum*, whilst *A. proliferans* should be synonymized with *A. diaphanum*. *Alcyonidium topsenti* and '*A. cellarioides*' could not be characterized genetically due to the poor quality of DNA extractions.

Morphological and reproductive data for all taxa considered are given in Table 4, the most important being the number of tentacles in the lophophore and the reproductive mode. In *A. diaphanum* the lophophore has 14 to 16 tentacles, and embryos are brooded, being released

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Table 2. *DNA sequence differences in the 12s rRNA gene sequences for different species of erect subtidal* Alcyonidium *collected from British coastal waters.*

Species/sample location/no.	Museum no.	Position 122	Position 177	Haplotype
Alcyonidium diaphanum				
Whitstable Topotype	BM (NH) 2000.12.19.1	T	T	1
Whitstable Topotype	BM (NH) 2000.12.19.2	T	T	1
Whitstable Topotype	BM (NH) 2000.12.19.5	T	T	1
Whitstable Topotype	BM (NH) 2000.12.19.6	T	T	1
Alcyonidium proliferans				
North Sea Paralectotype	v.Br 240	T	T	1
North Sea Paralectotype	v.Br 239	T	T	1
Alcyonidium condylocinereum				
English Channel Holotype	BM (NH) 2002.2.1.3	C	G	2
English Channel Paratype	BM (NH) 2002.2.1.4	C	G	2
English Channel	BM (NH) 2002.2.1.5	C	G	2
Alcyonidium mytili				
Longniddry, Scotland	na	C	G	2

na, not available.

as lecithotrophic larvae with an inferred short free-swimming period. The two paralectotype specimens of *A. proliferans* have 1516 and 1415 tentacles respectively, and were also found to display brooded development. These data, together with the molecular genetic data, provide firm grounds for synonymizing *A. diaphanum* and *A. proliferans*. The taxonomic identity of *A. topsenti* is less clearly established but, on the basis of Roehrich's (1910, in d'Hondt, 1983) description it is most likely to represent *A. diaphanum*. The grey knobbly *Alcyonidium* showed clear genetic differences from *A. diaphanum*. Both the grey knobbly species and '*A. cellarioides*' were found to display a reproductive mode incorporating a planktonic larval phase, but had differing ranges of zooidal size and tentacle number. Accordingly, they are formally described as new species, *A. condylocinereum* sp. nov. and *A. hydrocoalitum* sp. nov.

SYSTEMATICS

Alcyonidium condylocinereum sp. nov.
(Figure 1A–H)

Material

Holotype: BMNH 2002.2.1.3 Dover Strait, English Channel, 50.65°N 01.51°E collected by J.S. Porter.

Paratype: BMNH 2002.2.1.4 Dover Strait, English Channel.

Other material: BMNH 2002.2.1.5-8 Dover Strait, English Channel; 2002.2.1.910 Mumbles, Bristol Channel; 2002.2.1.11 central Bristol Channel; 2002.2.1.12 Rosslare, south-east Ireland.

Description

The holotype (BMNH 2002.2.1.3) measures 8×4.5×1 cm, the paratype (BMNH 2002.2.1.4) 6.5×4.5×1 cm (see Figure 1A). As the specimens were collected by trawling and both are slightly damaged at the base, basal diameter is not measured. The holotype has a central column with branches differentiating from the column at various intervals along its length. Approximately halfway along its length, the central column splits into two and further branches emanate from both columns. The columns themselves have a knobbly surface, and these knobs measure approximately 0.5 cm in diameter and protrude 2–3 mm from the column surface. These knobbly structures also occur on the branches. External zooidal measurements are mean width 200.6±62.8 μm (SD) and mean length 309.5±59.9 μm (SD) (N=50) (Figure 1B). Tentacle counts are 18–19, with a mode of 18 (N=30) (Figure 1C). The reproductive mode is clearly planktotrophic, with the development of an ITO (Figure 1D, see arrow) on the polypides and the presence of ovarian tissue emanating from the gut wall and containing many microlecithal oocytes (Figure 1F). Whilst larvae have not been observed in this study, it is generally the pattern in the genus *Alcyonidium* that this developmental mode gives rise to precociously inseminated late stage oocytes (Temkin, 1996) which are spawned via the ITO and develop into modified cyphonautes larvae in the water column, as in *A. mytili* (Cadman & Ryland, 1996a,b).

Etymology

Latin: *condylus*, knob; *cinereus*, grey.

Remarks

Alcyonidium condylocinereum is clearly distinguishable from *A. diaphanum* in several aspects. There are significant differences in gene sequence in both the 12s rRNA and CO1 genes (Tables 2 & 3). These genetic differences are supported by differences in tentacle number, zooid size, and perhaps most significantly, reproductive mode, i.e. *A. condylocinereum* is here inferred to shed planktonic larvae, whilst it is known that *A. diaphanum* is a brooder (Porter et al., 2001) (summarized in Table 4). Gross colony morphology, however, is clearly not a good indication of species status for these erect subtidal *Alcyonidium* species.

Alcyonidium diaphanum (Hudson, 1778)
(Figure 2A)

Fucus gelatinosus Hudson, 1762: 471
not *Alcyonium gelatinosum* Linnaeus, 1761: 538
Alcyonium gelatinosum Linnaeus, 1767: 1295 part

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Table 3. *DNA sequence differences in the CO1 gene sequences for different species of erect subtidal* Alcyonidium *collected from British coastal waters.*

Species/sample location/no.	Museum no.	Position 194	Position 215	Position 327	Position 359	Position 419	Position 586	Position 587	Haplotype
Alcyonidium diaphanum									
Whitstable 1	BM(NH) 2000.12.19.1	G	G	G	T	T	G	C	1
Whitstable 2	BM(NH) 2000.12.19.2	G	G	G	T	T	G	C	1
Whitstable 3	BM(NH) 2000.12.19.3	G	G	G	C	C	G	C	2
Whitstable 4	BM(NH) 2000.12.19.4	G	G	G	C	C	G	C	2
Whitstable 5	BM(NH) 2000.12.19.5	G	G	G	T	T	G	C	1
Whitstable 6	BM(NH) 2000.12.19.6	G	G	G	T	T	G	C	1
*104 1	na	G	G	G	T	T	G	C	1
*104 2	na	G	G	G	T	T	G	C	1
*104 3	na	G	G	G	T	T	G	C	1
*104 4	na	G	G	G	T	T	G	C	1
*104 5	na	G	G	G	T	T	G	C	1
*104 6	na	G	G	G	T	T	G	C	1
Alcyonidium proliferans									
North Sea 1	v.Br. 240	G	G	G	T	T	G	C	1
North Sea 2	v.Br. 239	G	G	G	T	T	G	C	1
Alcyonidium condylocinereum Holotype	BM (NH) 2002.2.1.3	G	G	G	T	T	C	G	3
Antarctic sp.	na	T	A	A	T	T	G	C	4

*, These samples were collected from the English Channel on CEFAS Groundfish survey cruise no. 8/99 on RV 'Corystes'. na, not available.

Ulva diaphana Hudson, 1778: 570
Alcyonidium diaphanum: Lamouroux, 1813: 71; Thorpe & Winston, 1986: 846.
Hayward, 1985 (*cum syn.*): 44; Porter, Hayward & Spencer-Jones, 2001.
Alcyonidium proliferans Lacourt, 1949: 289–321
Alcyonidium topsenti Roehrich, 1910: 164–169

Material

Alcyonidium diaphanum Lectotype: BMNH 1963.2.16.1a. Sheerness, 1729.
Alcyonidium diaphanum Paralectotypes: BMNH 1963.2.16.1bc. Sheerness, 1729.
Alcyonidium diaphanum Topotypes: BMNH 2000.11.17.13, Whitstable, 3 November 1998, beached on strandline, Porter & Ryland.
Alcyonidium proliferans Paralectotypes: v.Br 239, Hornzif, Noordsee 1913; v.Br 240, Scheveningen, 1879, det. Lacourt.
Alcyonidium topsenti Roehrich: v.Br. 168
Alcyonidium gelatinosum f. *lacourti* d'Hondt 1983 v.Br. 201

Remarks

The genetic identity of *Alcyonidium diaphanum* was established by Porter et al. (2001) and paratype specimens of *Alcyonidium proliferans* have been researched by mitochondrial DNA sequencing methods which reveal that they do not differ from *A. diaphanum.*

The specimen v.Br. 239 (Figure 2B) is brownish in coloration and measures approximately 7 cm in length and 2 cm at its maximum width, the thickness is approximately 0.5 cm, it is colonized by several sertularian hydroid colonies. The specimen is, however, not completely intact, and there is no point of attachment present: the total length of the colony may therefore be longer than that measured here. The gross colony morphology consists of a flattened subcylindrical column, reminiscent of the knobbly column morphotype described by Porter et al. (2001). Zooid length ranged from 0.132 to 0.383 mm with a mean of 0.233 ±0.045 (SD) mm. Zooid width ranged from 0.111 to 0.226 mm with a mean of 0.148 ±0.024 (SD) mm. Tentacle counts for 14 lophophores gave a range of 15–16 (mode=15) (Figure 1F). Reproductive mode is clearly lecithotrophic with the presence of large oocytes surrounded by follicle cells (Figure 2G).

Specimen number v.Br. 240 is honey-coloured (Figure 2C), much paler than specimen v.Br. 239, and measures approximately 10 cm long and 3 cm wide, its thickness does, however, vary between 0.4–0.8 cm along its length. The gross colony morphology consists of a flattened cylindrical column with numerous small protuberances emerging from the main column along its entire length. These protuberances are 2–4 mm in height and perpendicular to the column axis; there are no lateral

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Table 4. *Summary of morphological and reproductive data for erect subtidal* Alcyonidium *species found in British coastal waters.*

Species/specimen no.	Tentacle count	Zooid length (μm)	Zooid width (μm)	Reproductive mode	Reference
A. diaphanum					
Hudson BMNH 2000.11.17.1	14–16 mode 14	214–289	150–203	Lecithotrophic	Porter et al., 2001
A. proliferans					
Lacourt	14–16	100–150	na	Unknown	d'Hondt, 1983
A. proliferans					
Lacourt v.Br. 239	15–16 mode 15 (N=14)	132–384 mean 223 (N=50)	110–226 mean 149 (N=50)	Lecithotrophic	This study
A. proliferans					
Lacourt v.Br. 240	14–15 mode 14 (N=8)	188–472 mean 310 (N=50)	109–309 mean 193 (N=50)	Lecithotrophic	This study
A. topsenti					
Roehrich 1910 v.Br.168	16	350–600	na	na	d'Hondt, 1983
A. gelatinosum					
form *lacourti* d'Hondt v.Br. 201	na	420–450	na	na	d'Hondt, 1983
A. condylocinereum					
Holotype BMNH 2002.2.1.3	18–19 mode 18 (N=30)	309.5 ±59.9 (SD) (N=50)	200.6 ±62.8 (SD) (N=50)	Planktotrophic	This study
A. hydrocoalitum					
Holotype BMNH 1984.2.26.12	14–16 mode 16	530	340	Planktotrophic	Hayward 1985 as *A. cellarioides* Calvet and this study
A. cellarioides Calvet	20	700	na	Planktotrophic	Calvet, 1900

na, not available.

branches. Zooid length ranged from 0.188 to 0.472 mm with a mean of 0.310 ±0.004 (SD) mm. Zooid width ranged from 0.109 to 0.309 mm with a mean of 0.193 ±0.050 (SD) mm. Tentacle counts for 8 lophophores gave a range of 14–15 (mode=14).

The specimen v.Br. 168, *A. topsenti*, Roehrich, is shown in Figure 2D. DNA sequences could not be obtained from this specimen. Morphological characters had already been obtained by Roehrich, 1910 and are summarized in Table 4. Tentacle counts and zooid sizes overlap with those for *A. diaphanum*, suggesting that *A. topsenti* is likely to be conspecific with *A. diaphanum*.

d'Hondt (1983) introduced the taxon *Alcyonidium gelatinosum* form *lacourti*, describing the holotype colony as 120 mm long with a breadth of 56 mm. His plate VII, figure 3 illustrates the holotype and shows a slender cylindrical colony with lateral processes and knobs resembling the 'knobbly column' morphotype of *A. diaphanum*. However, the registration number given, v.Br. 201, proves to be a specimen labelled *A. hirsutum*, Zuiderzee, and is an unidentifiable specimen of what might have been an *Alcyonidium* (Figure 2E). The specimen appears at some stage in its history to have dried out; it is completely rigid and brittle, and no details of autozooids can be made out. It is not possible to resolve the identity of 'form *lacourti*' although it should be noted that it resembles the 'knobbly column' morphotype of *A. diaphanum*. However, this is an unimportant detail as, according to the International Code of Zoological Nomenclature Article 15.2 (1999), 'forms' are deemed to be infrasubspecific and excluded from the provisions of the code.

Alcyonidium gelatinosum (Linnaeus, 1761) var. *diaphanum* (Farre, 1837) was listed by d'Hondt (1983) from Anchorage (Alaska), Arctic Russia, Novaya Zemlaya, Svalbard and Bear Island. However, *Halodactylus diaphanus* Farre (1837) was described from material collected on the coast of Sheppey, Kent and, from Farre's description and illustrations, it is clear that his account was founded on specimens of *A. diaphanum* and another erect species bearing an ITO which might have been *A. condylocinereum*. Farre's material was clearly mixed and cannot be included in any synonymy presented here.

Alcyonidium hydrocoalitum sp. nov.
(Figure 3A–F)

Alcyonidium cellarioides: Hayward, 1985: 51, figure 10.
Not *Alcyonidium cellarioides* Calvet, 1900: 151; 1902:88, plate 3, figures 7–8.

Material

Holotype: BMNH 1984.2.26.12, Oxwich Bay, Gower Peninsula, South Wales 51.47°N 04.23°W collected by P.J. Hayward.

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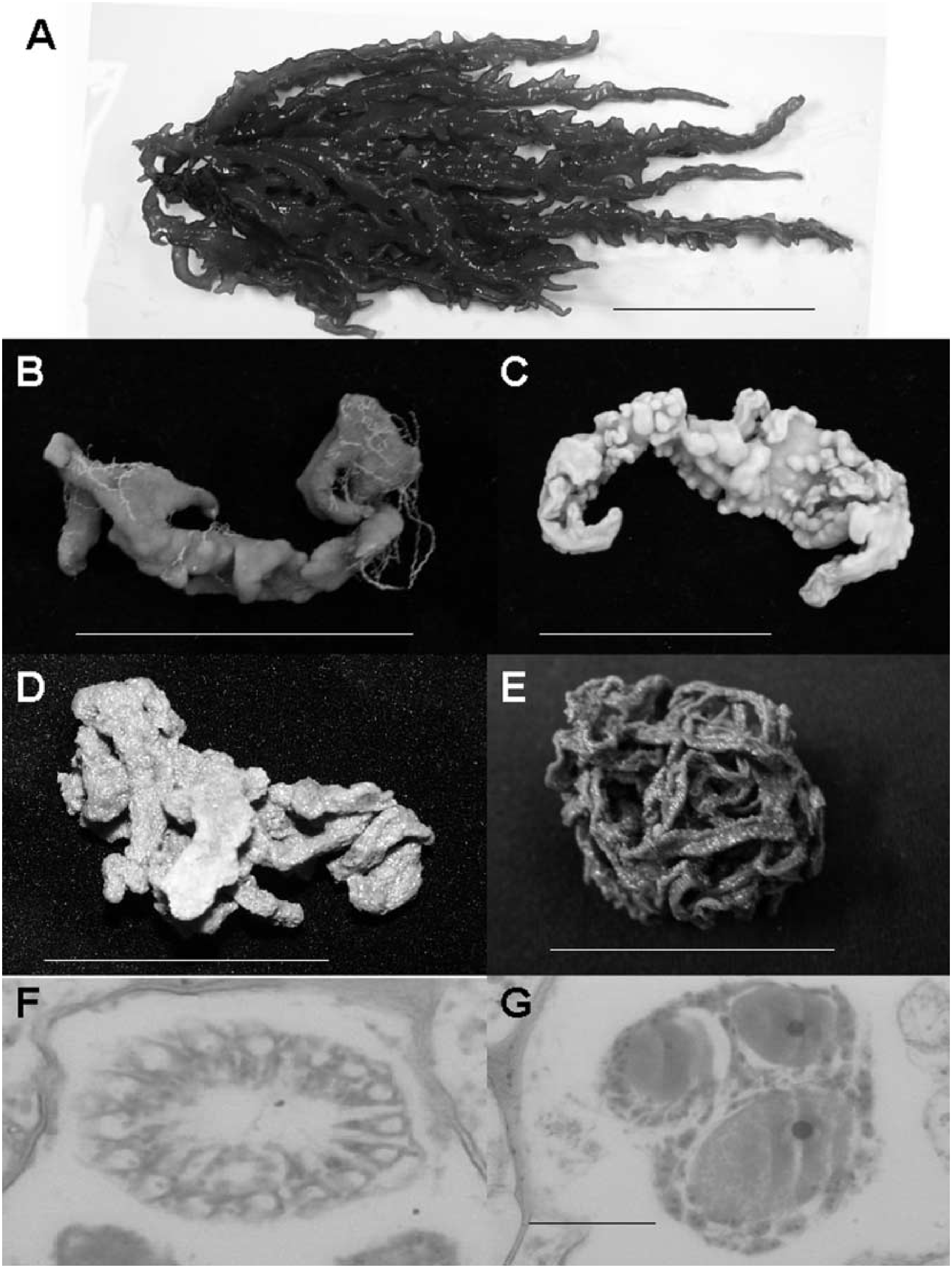

Figure 2. (A) A whole colony of *Alcyonidium diaphanum*; (B) whole colony of *A. proliferans* v.Br. 239; (C) whole colony of *A. proliferans* v.Br. 240; (D) whole colony of *A. topsenti* v.Br. 168; (E) whole colony of *A. gelatinosum* form *lacourti* v.Br. 201; (F) histological section through specimen of *A. proliferans* to show the lophophores with 16 tentacles; (G) histological section through ovarian tissue of *A. proliferans* showing three large embryos surrounded by follicle cells. Scale bars: A, 30 cm; B, 7 cm; C, 4 cm; D,E, 5 cm; G, 50 μm.

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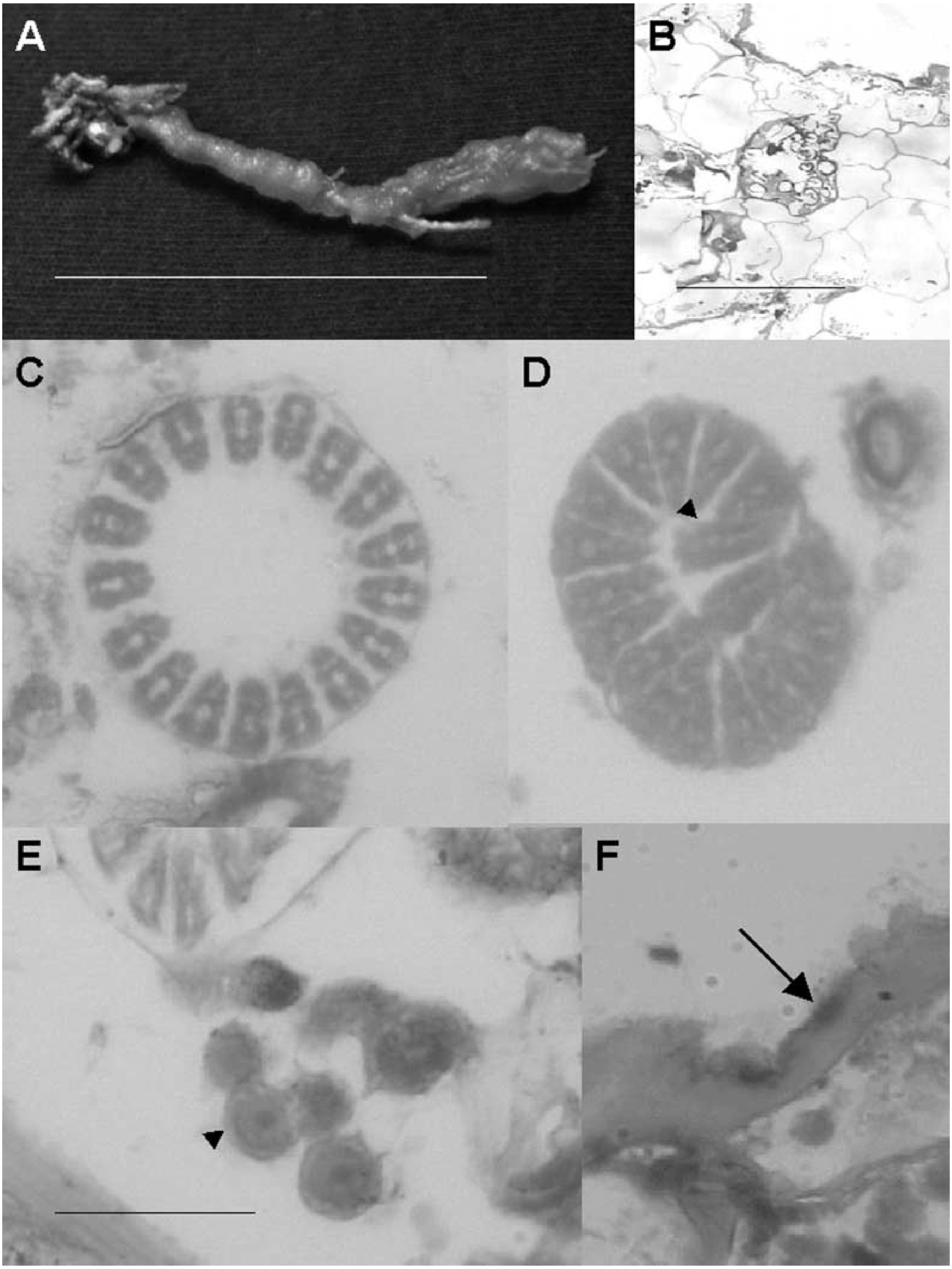

Figure 3. (A) Whole colony of *Alcyonidium hydrocoalitum*; (B) section through colony of *A. hydrocoalitum* indicating zooids growing around the hydroid stem; (C) histological section through a lophophore of *A. hydrocoalitum* indicating 17 tentacles present; (D) histological section through a lophophore of *A. hydrocoalitum* indicating the presence of an intertentacular organ shown by the arrow; (E) histological section through ovarian tissue of *A. hydrocoalitum* showing small oocytes developing and the presence of a lophophore; (F) arrow indicates the external wall of a colony of *A. hydrocoalitum*, it is crenulated with debris loosely attached. Scale bars: A, 6 cm; B, 140 μm; E, 60 μm.

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Paratype: BMNH 2002.2.1.13, Dover Strait, English Channel.

Other material: BMNH 2002.2.1.14, Dover Strait, English Channel.

Description

The holotype colony was described and figured by Hayward (1985) (Figure 3A). It forms a firm gelatinous incrustation around the stems of the hydroid *Tubularia indivisa* (Figure 3B), up to 6 mm thick, smooth-surfaced with an irregular outline; light brownish-grey, with the encrusted stem of the hydroid clearly visible. Zooids measured 0.53×0.34 mm, with frontal buds 0.45×0.34 mm. The orifice is subterminal and papillate with fine plications around its base when closed. The gut is slender, and there are 14–16 tentacles (mode 16) (Figure 3C). The paratype colony was collected in the CEFAS groundfish survey in August 1999 (see Table 1 for details of exact location). The specimen measures 4.5 cm long and 0.3 cm wide, and is investing an unidentified hydroid colony. The colony is almost transparent, consisting of a slender tube-like arrangement investing the main stem of the hydroid, and extending on to its branches. Some of the hydroid branches are completely covered by the bryozoan, and the hydroid itself can only be seen by cutting through the colony in section; the covered branches look like small knobs protruding 2–3 mm from the surface of the bryozoan, and measuring 2–3 mm in diameter. One knob is located at the base of the colony; there are two more approximately 1 cm from the base and a further three knobs approximately 4 cm from the base. The hydroid colony divides into three slender branches; two of the hydroid branches are barely covered by the bryozoan whilst the main stem continues to be covered for a further 2 cm. Zooidal measurements are 241.39 ±86.45 (SD) μm mean width and 413.34 ±126.75 (SD) μm mean length. Only three lophophores could be counted, two had 18 tentacles and the other had 16. Histological sections indicate that this species reproduces following a planktotrophic mode of development and an ITO can be seen in histological sections (Figure 3D). Figure 3E shows microlecithal oocytes in the autozooids, whilst at the same time the polypide is still present, in contrast with brooded mode of development where the polypide is resorbed and a polypide rudiment develops with a coelomopore which acts as a conduit for the larvae to be released, e.g. *A. hirsutum*. The outer colony wall is somewhat crenulated and adherent debris can be seen (Figure 3F).

Etymology

Latin: *hydro*-, hydroid; *coalitus*, united.

Remarks

This species has been clearly distinguished from *A. condylocinereum* using zooidal size and tentacle number; its growth form is also different from those of *A. condylocinereum* and *A. diaphanum*. Its attribution to the Mediterranean species *A. cellarioides* Calvet by Hayward (1985) is now considered inappropriate. Although no material of the latter has been available for molecular genetic investigations, its larger polypides, with modal tentacle number 20, is firm evidence for a separate taxonomic identity of *A. hydrocoalitum*, with modal tentacle number 16.

DISCUSSION

Alcyonidium diaphanum is distributed abundantly around the British Isles and off the coasts of north-western Europe, and is especially abundant close to the mouths of estuaries such as the Thames, Severn and Seine (Porter et al., 2002). *Alcyonidium diaphanum* appears to have a defined southern limit on the coasts of Europe at Glénan, Brittany (Porter et al., 2002) and records from further south, including the Mediterranean, should be viewed with caution. Its northern geographical limit remains to be established. What may be the same species is common in the coastal waters of Svalbard (P. Kuklinski, personal communication), but the morphology, reproductive mode and genetic identity of subarctic and Arctic erect, subtidal *Alcyonidium* need to be investigated before the northern range of *A. diaphanum* can be established.

Kluge (1962) recorded erect *Alcyonidium* from throughout the Arctic seas under the taxon *A. gelatinosum* Linnaeus 1767, which he synonymized with the '*A. gelatinosum*' of Hincks (1880). He also recognized three varieties of *A. gelatinosum* distinguished principally by colony morphology, var. *diaphanum*, var. *anderssoni* and var. *pachydermatum*. A further three species of erect *Alcyonidium* were documented by Kluge (1962): *A. radicellatum* Kluge, 1946, *A. vermiculare* Okada, 1925 and *A. excavatum* Hincks, 1880. It is highly unlikely that any of the taxa represent any of the species here described from British coastal waters and it is clear that the Arctic species of erect *Alcyonidium* require re-investigation using morphological, reproductive and genetic characteristics before their taxonomic identities can be confirmed.

Alcyonidium diaphanum is a plastic species, with colony morphologies which may be loosely categorized into morphotypes, perhaps representing phenotypic responses to local environmental characteristics. Porter et al. (2002) showed that six morphotypes of *A. diaphanum* could be recognized; the laminar, cylindrical and knobbly column morphotypes were present throughout the geographical region sampled by CEFAS groundfish surveys but the bushy morphotype was restricted to the area around Lundy, while the bootlace form was found only around the Irish coast and the sausage morphotype was found only off the Isle of Wight and in the bay of the Seine. Colony size also appears to vary with location; for example, in the mouths of estuaries colonies can exceed 50 cm in height, though these observations have not been formally quantified. However, the current study demonstrates that colony morphology as a character is not sufficient for the definition of erect, sublittoral *Alcyonidium* species.

Two additional subtidal species are described here. Each has a recognizable colony form, which without morphological, reproductive and in the case of *A. condylocinereum*, genetic data, might have been defined as simply *A. diaphanum* morphotypes. *Alcyonidium hydrocoalitum* achieves erect growth by investing erect hydroids, to the extent that the hydroid substratum may be completely obscured. In *A. diaphanum* colony growth proceeds partly through the expansion of the outer body wall, with

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frequent fusion of adjacent lobes to enclose external space, but this does not occur in *A. condylocinereum*. Taxonomic identity is readily established through use of molecular genetic techniques, but, in the subtidal species of *Alcyonidium,* as with the intertidal species, tentacle number and reproductive mode prove to be good indicators of taxonomic identity. For example, *A. mytili* Dalyell encrusting the valves of *Mytilus edulis* has a modal tentacle number of 16 and sheds late stage oocytes through an ITO which then develop in the water column into planktonic larvae which resemble cyphonautes larvae. In contrast, the fucoid-encrusting *A. polyoum* Hassall (=*A. gelatinosum,* Hayward, 1985) has a modal tentacle number of 19 and broods coronate larvae. Another brooding species, *A. gelatinosum* (Linnaeus) (=*A. reticulum* Ryland & Porter, 2000) is distinguished by the fewer eggs in its brood chamber, and a modal tentacle number of 17.

Tentacle counts, zooidal measurements, reproductive data and genetic characteristics have thus been utilized to characterize the erect subtidal *Alcyonidium* species in British coastal waters. However, whilst their geographical distributions might now be more clearly documented, their ecology and biology are still poorly understood. In particular, patterns of budding and colony growth remain entirely unknown, and may provide further useful taxonomic characters. While reproductive mode is now known, for no erect species is there any information on reproductive seasonality and periodicity, and rates of growth and longevity are also completely unstudied.

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