An oomycotan disease, tentatively attributed to *Lagenidium callinectes*, is striking barnacles (Crustacea, Cirripedia) in the Morbihan gulf (South Brittany, France)

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Abstract

A disease due to an oomycota whose first symptoms were detected in 2016 in the barnacle *Chthamalus stellatus* strikes four other species, *Chthamalus montagui*, *Austrominius modestus*, *Perforatus perforatus* and *Amphibalanus eburneus* in the Morbihan gulf. The hyphae develop inside the eggs or the embryos which they digest. Outside they form a very dense network especially on the outer layers of the egg masses and entrap the healthy embryos and nauplius larvae, generally leading to their death. The parasite, most probably introduced into the Morbihan gulf, presents characters compatible with those of *Lagenidium callinectes*, a species to which it is tentatively attached.

If this identification is confirmed, it would be the first record of *L. callinectes* in European waters. It would be also a source of concern, not only for cirripedes, notably *Pollicipes pollicipes*, but also for commercially important decapods that the parasite may contaminate. Monitoring of the spawning of these species would be highly desirable.

The author proposes to call this pathology "the barnacle disease of Mor-Bihan" (with a hyphen, to indicate that it concerns the "little sea" and not the French department of Morbihan).

Keywords: *Amphibalanus*; *Austrominius*; barnacles; *Chthamalus*; *Lagenidium*; Morbihan gulf; parasite; *Perforatus*

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Reçu le 15 novembre 2021 ; accepté après révision le 9 mai 2022 ; en ligne le 15 juin 2022.
Received 15th November 2021; accepted in revised form 9th May 2022; online 15th June 2022.

Un oomycète, susceptible d'être *Lagenidium callinectes*, s'attaque aux œufs de cinq espèces de « balanes » (crustacés, cirripèdes) dans le golfe du Morbihan (France)

Résumé

Une maladie due à un oomycète dont les premiers symptômes ont été détectés en 2016 sur la balane *Chthamalus stellatus* frappe quatre autres espèces, *Chthamalus montagui*, *Austrominius modestus*, *Perforatus perforatus* et *Amphibalanus eburneus* dans le golfe du Morbihan. Les hyphes se développent à l'intérieur de l'œuf ou de l'embryon qu'ils digèrent. À l'extérieur ils forment ensuite un réseau très dense particulièrement près de la surface des masses d'œufs et y emprisonnent les embryons sains ou les larves nauplius, entraînant souvent leur mort. Le parasite, très probablement introduit dans le golfe du Morbihan, présente des caractères compatibles avec ceux de *Lagenidium callinectes*, espèce à laquelle il a été, au moins à titre provisoire, rattaché.

Si cette identification est confirmée, ce serait la première mention de *L. callinectes* dans les eaux européennes. Elle serait une source d'inquiétude, non seulement pour les cirripèdes, notamment *Pollicipes pollicipes*, mais aussi pour les décapodes d'importance commerciale que le parasite est susceptible de contaminer. Une surveillance des pontes de ces espèces serait hautement souhaitable.

L'auteur propose d'appeler cette pathologie « la maladie des balanes du Mor-Bihan » (avec un trait d'union, pour indiquer qu'elle concerne la « petite mer » et non le département du Morbihan).

Mots-clés : *Amphibalanus* ; *Austrominius* ; balanes ; *Chthamalus* ; golfe du Morbihan ; *Lagenidium* ; parasite ; *Perforatus*

Introduction

For some four decades limpets (*Patella vulgata* Linnaeus, 1758) have been browsing the seaweeds belts, mainly that of *Ascophyllum nodosum* (Linnaeus) Le Jolis 1863 on the rocky shores of the Morbihan gulf (47°34′N 2°48′W) (Le Roux 2005, 2008, 2012). The rocky surfaces left bare are rapidly colonized by barnacles, mainly *Chthamalus montagui* Southward, 1976, *Semibalanus balanoides* (Linnaeus, 1767) and *Austrominius modestus* (Darwin, 1854). The question arose whether *Chthamalus stellatus* (Poli, 1791) a species preferring exposed shores, settled too in the gulf, was able to breed successfully in it and, theoretically at least, to form a self sustaining population. To this end the species was looked for and the presence of egg masses (= egg lamellae) in the mantle cavity was checked. It appeared that they exhibited some abnormalities which were later found in other species.

Material and techniques

The observations about *C. stellatus*, not strictly planned, were performed around the gulf according to the opportunities. The species was looked for in the rough and generally dense carpet of barnacles covering the rocky heads and some artificial structures with the naked eye and recognized from other species, particularly from *C. montagui*, using the criteria described by Southward (1976). When available, a sample of 20 to 30 specimens was collected for later examination either in the fresh state or preserved in French rubbing alcohol. Fixation in formalin 4 % in seawater was also used.

The egg lamellae were torn apart with fine needles under a dissecting microscope (×40) and mounted in water for microscopic examination (×20, ×80 and ×120). No staining or other histological techniques were used.

Photographs were taken with a camera Nikon coolpix L101 maintained by hand upon the eyepiece of the optical apparatus. All measurements were determined from images projected on a computer screen. This process is not very accurate for small dimensions.

Results

The case of *Chthamalus stellatus*. Discovery of the disease

Distribution and reproduction in the Morbihan gulf

C. stellatus is present in the Morbihan gulf and this is no wonder since Southward (1976) reported its occurrence on sheltered sites at the mouth of the River Yealm near Plymouth and in Scottish lochs. Figure 1 shows the places where it was found and although most of the isles were not prospected, it appears that it is widely distributed at the west of a line joining points 9 to 15 included.

Densities are always low, between 0 to 5 specimens per square decimetre, rarely 7 or 8. This implies that individuals are often isolated or several centimetres apart from each other. The level occupied by the species is difficult to determine with precision owing to the low density and lack of continuity of the settlement. It lies however, as reported by Southward (1976), much lower than the maximum density level of *C. montagui*, below mean tide level (about the lower extent of the *Ascophyllum* belt) where both species coexist.

Reproduction, with healthy egg masses, was observed between April and August as shown in figure 2. Concurrently, various abnormalities were noticed but in September and November only "cushions", described below, were to be found.

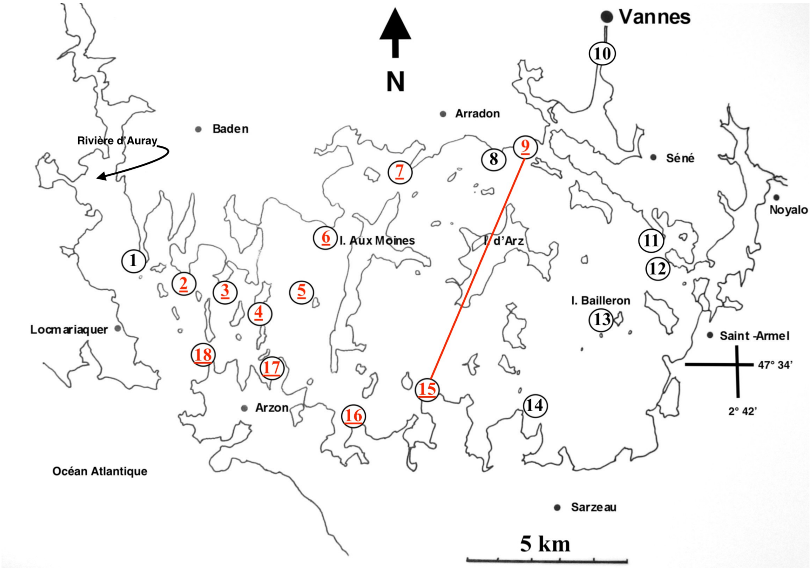

Figure 1: Map of the Morbihan gulf. French names not translated. 1, pointe du Blaire; 2, pointe de Locmiquel; 3, pointe du Berchis; 4, Berder island; 5, Creizic island; 6, pointe de Port Jakez; 7, pointe d'Arradon; 8, cale de Penboc'h; 9, pointe de Roguedas; 10, Kérino; 11, pointe du Bile (aka "Grand Pont"); 12, le Péchit; 13, Bailleron island; 14, pointe du Ruaud; 15, pointe de l'Ours; 16, pointe du Logeo; 17, pointe de Penbert; 18, pointe de Bilgroix. Red underlined numbers: *Chthamalus stellatus* (Poli, 1791) present; black numbers: *C. stellatus* not met with.

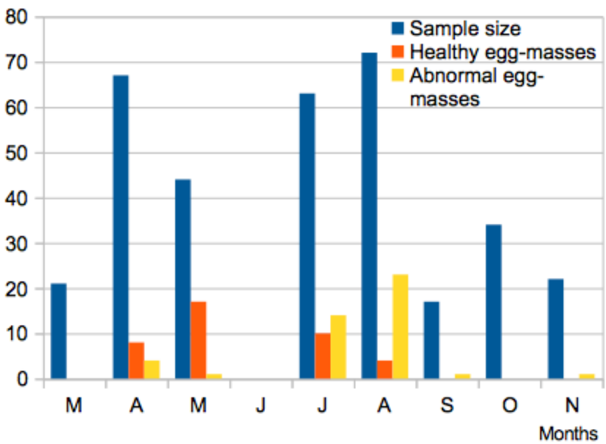

Figure 2: *Chthamalus stellatus* (Poli, 1791). Reproductive activity. Data pooled by month from different places in the Morbihan gulf from 2016 to 2021. Abnormal egg masses include all forms except in September and November, when only "cushions" were found.

Egg masses abnormalities

The eggs are laid in a pouch secreted by the oviductal gland, they stick to each other by their fecundation membrane (Walley, White & Brander 1971; Barnes, Barnes & Klepal 1977).When freshly laid healthy egg masses are bright orange with clear-cut egg outlines. The spaces between eggs seem empty and quite clear (Figure 3A–B). Abnormal egg masses exhibit more or less large areas with a milky colour in which eggs contours are blurred and the inter-eggs spaces more or less occluded. This aspect may extend to the whole surface (Figure 3C–D). The first observations of lamellae abnormalities were made in 2016.

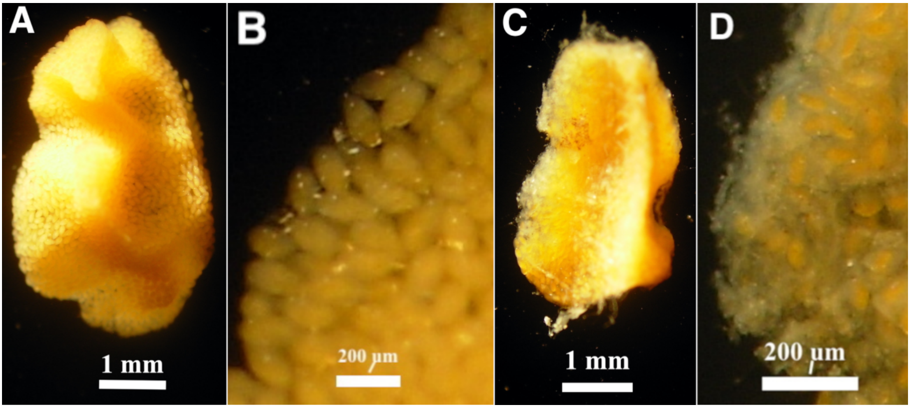

Figure 3: *Chthamalus stellatus* (Poli, 1791). A & B, normal aspect of a healthy lamellae. C & D, abnormal lamellae. A is seen from the outer, B from the innerside. The notch, top left in A and bottom right in B, corresponds to the tergal depressor muscle.

In a more advanced stage the egg lamellae become entirely whitish, often with hairy margins (Figure 3C, top). The embryos, more or less recognizable, are generally dead, and partially disintegrated. It appears that the lamellae are limited by an outer felting of matted threads reminiscent of cotton wool, which encloses embryos either in a medium or late stage of development or even nauplii that are unlikely to escape from such a pouch (Figure 4A–B). This kind of abnormal lamellae may be found alone inside the mantle cavity or next to freshly laid ones (Figure 4C). The presence of supernumerary lamellae pairs in the mantle cavity of barnacles is extremely rare. Crisp & Davies (1955) cite one or two cases out of several hundred specimens in *C. "stellatus"* [note that in their paper *C. stellatus* refers mainly to *C. montagui* from the upper shore levels, since the two species were recognized as distinct but in 1976 (Southward in 1976)] however these were healthy lamellae, one pair freshly laid the other ripe and about to release larvae. O'Riordan, Myers & Cross (1995) also found a case of 3 healthy lamellae, in *C. stellatus*.

The microscopic structure of the abnormal lamellae will be dealt with later. However it may be stated here that, at an advanced stage, the tangle of threads is very similar to mould hyphae (or mycelium).

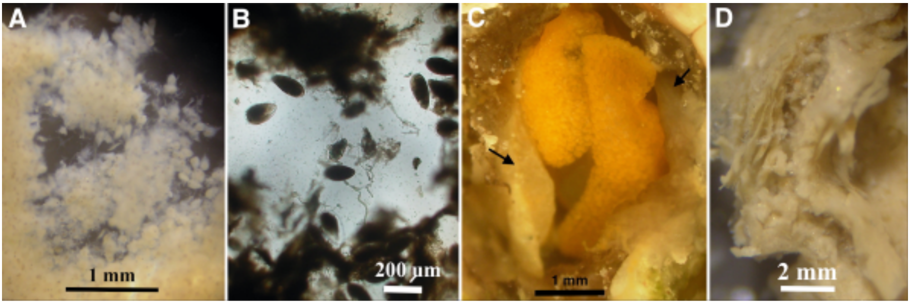

Figure 4: *Chthamalus stellatus* (Poli, 1791). A, dissociated lamella showing the cotton wool like matter embedding dead nauplii. B, eggs, partly destroyed, threads and fluffy matter. Microscopic view. C, abnormal lamellae (arrows) next to freshly laid normal ones (orange colour) in the mantle cavity. Somewhat displaced. D, lamellar structure of a cushion.

It seems that eventually addition of several egg lamellae give rise to a massive structure made of the two symmetrical masses joined, even welded along the dorsal mid-line, filling the mantle cavity and taking a cast of it. Owing to its position, underneath the barnacle soma, this structure is referred to as a "cushion" (Figure 5A–B).

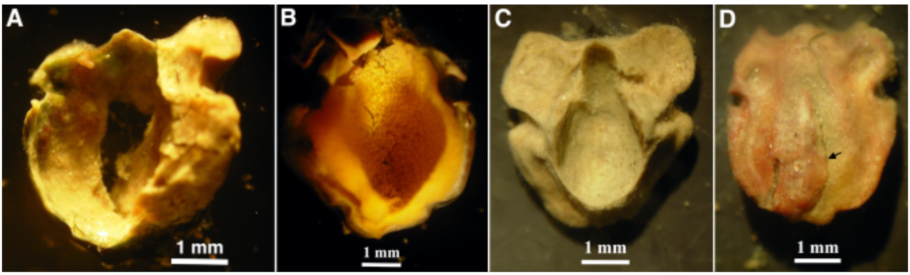

Figure 5: Cushions. A, B, *Chthamalus stellatus* (Poli, 1791), ventral (upper face) view. The two lamellae are still well separated in A. The upper depression is coated with dead eggs in B. C, *Austrominus modestus* (Darwin, 1854), ventral view. D, *Chthamalus montagui* Southward, 1976, dorsal view. The mid-line (arrow) marks the limit of the two lamellae. The form of the soma is quite well moulded in A, B and C. The scutal depressor muscle notch is well marked in all cases.

The cushions may contain mycelium and embryo remains in its upper part or even freshly laid eggs (Figure 5B) however the large ones are of a brittle consistance and mostly made of a fluffy or muddy-like dead matter in which the original elements are difficult to identify. They may also contain some foreign particles such as mica crystals, sponge spicules or diatom frustules which however appear to constitute but a very small proportion of the total volume. In a broken cushion several layers (up to 6–8), assumed to represent successive lamellae, are to be seen (Figure 4D).

Abnormal clutches were found every year, except 2020 for lack of collection, between 2016 and 2021 at all points where *C. stellatus* was collected (except in points 5 and 9 where only one animal was observed) and throughout the breeding season. This indicates that the anomalies are probably not mere accidents possibly induced, for example, by adverse weather events. Other causes had to be sought.

Barnacles are hermaphroditic organisms that normally cross-fertilize, thanks to an extensible penis which drives the sperm from one animal to another within a few centimetres reach. Barnes & Crisp (1956) report 5 cm as a critical maximal distance allowing cross-fertilization in *A. modestus* and *S. balanoides*, two species which approximate the size of *C. stellatus*. In more distant animals self-fertilization is possible in certain species, however "eggs produced without copulation appear to be less viable than normally fertilized eggs". The authors add: "In the early summer most of the eggs in the mantle cavities of isolated individuals were incompletely developed and in many cases the whole egg mass had begun to degenerate in situ; on being touched with a needle the egg mass broke down into an oily suspension of tissue". According to these statements the "fungal" [1] filaments in diseased lamellae were first considered to be a saprophytic mould growth on unfertilized or autofertilized dead eggs. However in many cases spoiled lamellae appeared in contiguous or very close (less than 5 cm) animals as well. Remotely isolated ones on the contrary could accomodate healthy egg masses. On the other hand the structure of the diseased lamellae in the Morbihan gulf was quite different from that described by Barnes & Crisp in not breaking in "an oily suspension tissue". All of this strongly suggested that abnormalities are not a consequence of autofertilization.

Moreover, casual observations on *C. montagui* collected together with *C. stellatus* revealed the presence of similar abnormalities, consequently other species were checked.

Further species

Five other species of barnacles are readily collected on the rocky shores of the gulf: *C. montagui*, *A. modestus*, *Perforatus perforatus* (Bruguière, 1789) and *S. balanoides*. Another species, recently introduced, *Amphibalanus eburneus* (Gould, 1841) is also abundant at Kérino and Vannes harbour (Le Roux 2013). *S. balanoides* is a winter-breeder (Southward 2008) and hence was not concerned by this study mainly carried out during spring and summer. In the remaining four species abnormal lamellae, very similar to those of *C. stellatus*, were observed (Figure 6).

In *A. eburneus* however the web of "fungal" filaments forms a less resistant fabric than in other species. In *C. montagui* lamellae infection may be asymmetric (one side contaminated, the other healthy) or a lamella may be only partially contaminated (for example on its hinder part, posterior to the tergal depressor notch) this suggests

1. Fungal in quotation marks, since oomycota are not fungi.

that contamination could be due, in part, to a non mobile agent and performed by the "fungal" threads themselves running from egg to egg.

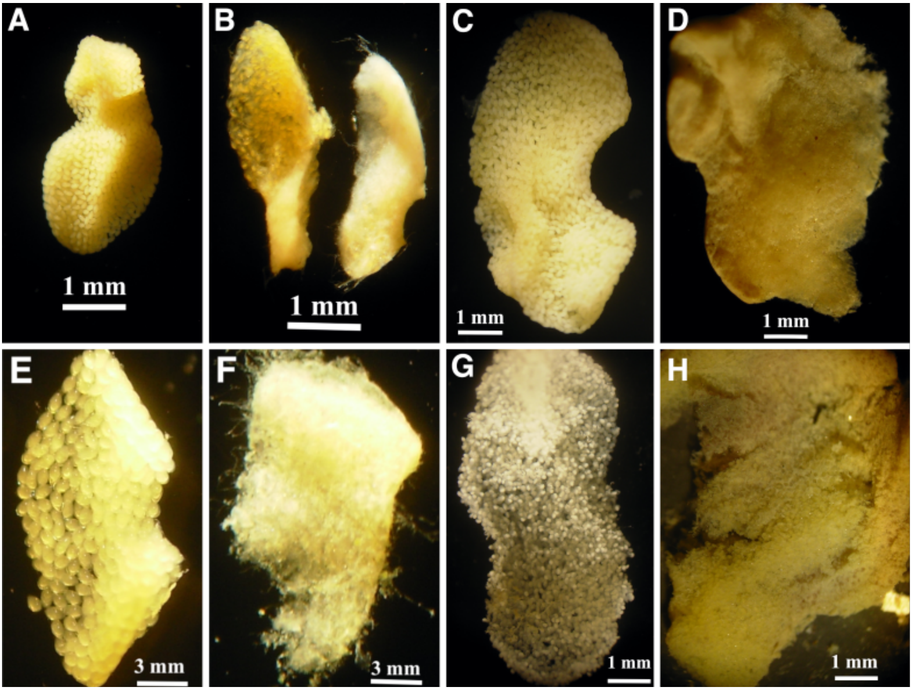

Figure 6: Healthy and diseased lamellae. A & B, *Chthamalus montagui* Southward, 1976: A, healthy; B, diseased. Two stages of development of the disease are figured; right lamella: terminal stage. C & D, *Perforatus perforatus* (Bruguière, 1789): C, healthy; D, diseased. E & F, *Austrominius modestus* (Darwin, 1854): E, healthy; F, diseased. G & H, *Amphibalanus eburneus* (Gould, 1841): G, healthy; H, diseased.

Prevalence

Table 1 gives some examples of contamination levels recorded in July and August 2021. Although based on small numbers of broods it shows clearly that the parasite is quite common in most of the prospected places. However it does not reflect accurately the toll paid by the barnacles to their parasite, first because some lamellae are only partially infected and second because freshly laid lamellae are reported as healthy for not exhibiting signs of infection but they may get striken later by the disease. Nevertheless the loss of eggs may represent a significative part of the brood reaching perhaps as far as 25 to 50 per cent. In spite of such a cull on recruitment, barnacle communities are thriving in the gulf and it may be said that the disease is a silent one. However, according to the susceptibility of the different species to the parasite, the balance between their populations may have been affected but remained unnoticed due to a lack of monitoring program.

Table 1: Examples of contamination levels recorded in July and August 2021 for five species of barnacles : *Chthamalus stellatus* (Poli, 1791), *Chthamalus montagui* Southward, 1976, *Austrominius modestus* (Darwin, 1854), *Perforatus perforatus* (Bruguière, 1789), *Amphibalanus eburneus* (Gould, 1841). In column "place", low and high refer to the relative level in the species belt.

Species	Place	Date	Checked	Healthy	Contaminated	Cushion
C. stellatus	3	07-14	25	16	14	0
C. stellatus	2	07-17	24	3	9	4
C. montagui	3, low	07-23	51	8	12	0
C. montagui	3, low	08-17	61	14	15	0
C. montagui	3, high (*Pelvetia* belt level)	08-17	12	1	5	0
C. montagui	11, medium	08-04	38	20	0	0
A. modestus	11	08-04	37	12	2	2
A. modestus	10	08-02	24	5	3	7
P. perforatus	2	07-28	23	4	6	0
A. eburneus	10	07-30	36	9	7	0

The lack of contamination of *C. montagui* at point 11 as well as the low level for *A. modestus* probably point to a lower aggressiveness of the parasite in the eastern part of the gulf. This possibility is further upheld by the fact that in *C. montagui* 16 lamellae out of 20 were in an advanced developmental stage with nauplii visible in the eggs which therefore had been for a long time at risk of being infected.

The number of cushions in *C. stellatus* at point 2 and still more in *A. modestus* at point 10 is puzzling. Local environmental conditions (mud incorporated into them at point 10?) may be involved but clarifying this point would need further investigations.

It is worth noting that, owing to their position and large volume, the cushions mechanically prohibit any moulting to the barnacle and that anyway moulting absence is a condition to their building up. In *C. montagui* at least the infected lamellae can be expelled at moult with the exuviae (Figure 7A). They can be easily retrieved by putting a recently collected stone carrying a group of barnacles in a container with sea water during one hour or so. One finds however cushions in this species (Figure 5D) and it is unclear how the moult inhibition necessary to their formation is ordered. If the cushion layers correspond to one brood this means that the period without moulting may last probably one year or more: *C. stellatus* and *C. montagui* release 1 to 4.4 broods per year at Plymouth (Burrows, Hawkins & Southward 1992) and *A. modestus*, in favourable conditions, as much as 26 (Barnes & Barnes 1968). Such a long while of anecdysis is likely to be a terminal one in a barnacle life.

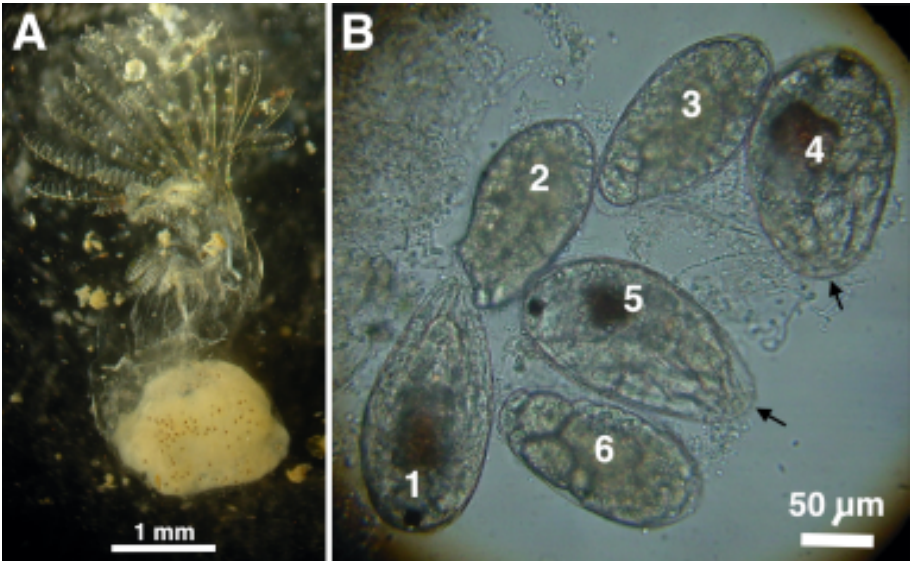

Figure 7: *Chthamalus montagui* Southward, 1976. A, contaminated lamellae inside an exuvia. Remains of vitellus in the larval digestive gland appear as red dots. B, diseased and healthy embryos, late stage of development. 1, healthy; 4 & 5 probably infected with signs of a delayed development (embryos shorter than 1, eggs with rounded posterior ends, pointed at by arrows); 2, 3 & 6, certainly infected (stopped embryogenesis) but hyphae limits still indistinct and no extramatrical extensions emitted.

Other aspects to take into account are the detrimental effects (hindrance to respiration and food capture) that the presence of cushions has on the barnacle.

The infecting agent

General aspect

Given that the parasite hosts are mostly sympatric, closely related taxonomically, and that their disease symptoms are quite similar it will be assumed that the infecting agent is the same for the five species. Microscopic examination brought no evidence against this opinion however questionable.

C. montagui is the most abundant and easily collected species, morever it yields clearer figures than *C. stellatus*, so unless otherwise stated, the following description and figures refer mainly to it.

Microscopic examination of dissociated lamellae with a milky abnormal aspect does not reveal the presence of the disease agent. It can however be suspected, or detected owing to a delayed development and abnormal aspect of certain eggs or embryos (Figure 7B). Intramatrical hyphae (inside the egg) seem to appear or organize themselves quickly out of a confused mass of cells.

Later the infected eggs get stuffed with knobby contorted and variously ramified intramatrical hyphae (Figure 8).

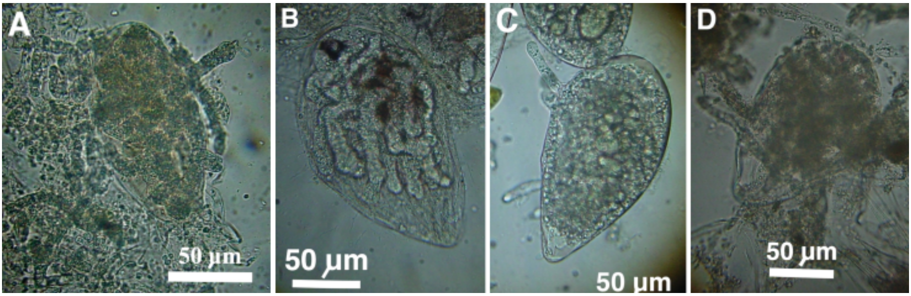

Figure 8: Embryos stuffed with hyphae. A, *Chthamalus stellatus* (Poli, 1791); B, *Chthamalus montagui* Southward, 1976; C, *Austrominius modestus* (Darwin, 1854); D, *Perforatus perforatus* (Bruguière, 1789). A, C and D start emitting extramatrical hyphae. B and C, hyphae contain refractive oily droplets. *Amphibalanus eburneus* (Gould, 1841), not figured, provides similar pictures.

In situ, the morphology of the intramatrical hyphae is quite unclear but it appears more clearly when, as an effect of the dilaceration of the lamellae, the hyphae are extirpated from the egg membrane and more or less extended (Figure 9A–B) however it is then difficult to tell the difference between intra and extramatrical filaments.

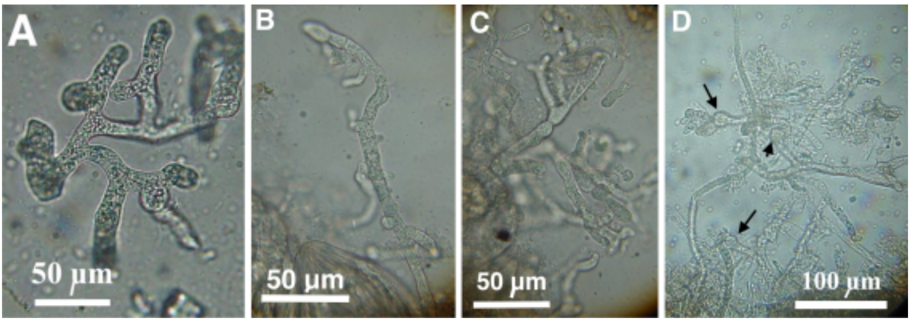

Figure 9: A & B: supposed intramatrical hyphae, extracted from eggs, showing granular cytoplasm with some refringent bodies. A, knobs and ramifications with rounded tips. C & D, abundantly branched extramatrical hyphae with terminal vesicles (arrows) in D. (A & D: *Chthamalus stellatus* (Poli, 1791); B & C: *Chthamalus montagui* Southward, 1976).

The intramatrical hyphae measure from 7.5 µm to 40 µm (mean = 13 µm; σ = 5;

n = 28) in diameter and have a dense, greyish, more or less coarse granular cytoplasm, possibly lined with lipid globules (Figures 8–9). They cross the egg membrane and become extramatrical hyphae (Figure 9C–D) whose diameter vary from about 20 µm to some 5 µm (mean = 10 µm; σ = 3; n = 57). The finest hyphae are often vacuolated and completely empty at the end of the parasite's life cycle. In vivo the extramatrical hyphae are elastic and extensible to lengths up to 600 µm.

Extramatrical hyphae form a tangle between the eggs intramatrical hyphae systems (Figure 10A). They have a rounded or distinctly swollen vesicle end (Figure 10B) measuring from 12 µm to 30 µm in diameter (mean = 20 µm; σ = 4.5; n = 30) and some exhibit intermediate swellings. The terminal vesicles may rest against the hyphae or against a vesicle belonging to the hyphal system of a neighbouring egg, but it is not clear whether these contacts are incidental or have functional significance.

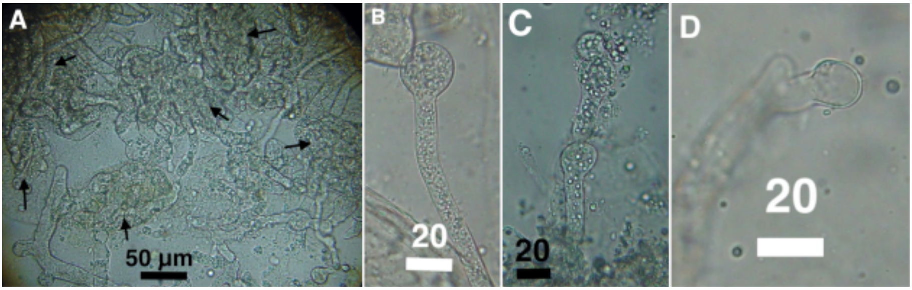

Figure 10: *Chthamalus montagui* (Southward, 1976). A, hyphal tangle between 6 contaminated eggs (arrows). Somewhat stretched by dilaceration; B, vesicle; C, series of vesicles containing lipidic globules; D, empty vesicle. Scales in µm.

The vesicles are frequently extended by a narrower finger-like tube and can also be aligned in series (Figure 10C). Their content is variable, it is either a granular cytoplasm similar to that of hyphae or containing elongated refractive bodies or even a swarm of wriggling elements similar to spermatozoids suggesting that they may constitute antheridia. However such male gametes are not known in oomycota and they could be clusters of large bacteria. According to Crisp, Bland & Bahnweg (1989) resting bodies (i.e. eggs) would be "apparently asexual". At the end of the parasite cycle the vesicles appear empty with a continuous wall and no visible aperture (Figure 10D).

Zoosporogenesis

Despite the large number of specimens examined, belonging to the 5 contaminated species (Tableau 1 counts 73, which is only about half of the total number) the process of spore formation was observed only three times and this in its sub-terminal phase, i.e. as the presence of spore-bearing vesicles. Two of these formations are shown in figure 11A and 11B. It appears in figure 11B as a slightly elongated ball 25 µm × 30 µm containing a number of spores estimated at a few tens and whose size seems to lie between 5 and 10 µm. The spores fill the entire capsule. The release of spores was not observed.

Ovocytes

The so-called oocytes, ovocytes, resting cells or oospores which represent the sexual phase of the life cycle of the oomycota are quite common and even abundant (Figure 11C). When mature they are thick walled and measure from 18 µm to 30 µm in diameter (mean = 25; σ = 4.5; n = 21). They are often wrapped in a flaky material (Figure 11D) that hides radiating blunt spines (Figure 11E) which could be of taxonomic interest.

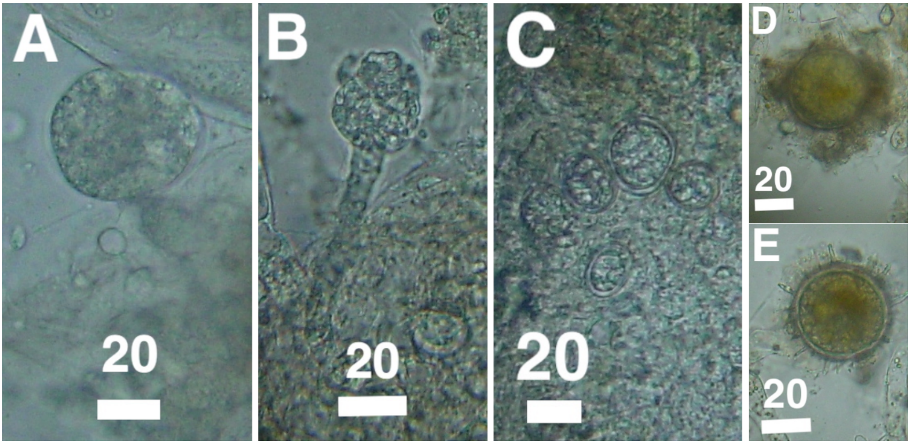

Figure 11: *Chthamalus montagui* Southward, 1976. A & B, spore bearing vesicles; C, thick walled resting cells; D, resting cell wrapped up in a fluffy material; E, resting cell bristling with blunt spines. Scales in µm.

Identification

No pathology of this type affecting barnacles has, to our knowledge, been reported in Europe.

Had it existed in the United Kingdom in the last century, it is impossible that it would not have been noticed and described by researchers such as H. Barnes, M. Barnes, D. Crisp or H. Southward who have studied intensively and extensively the barnacles of its sea shores. H. Barnes and M. Barnes have also carried out studies in France, especially in Arcachon (cf. Barnes 1992) and they have notably (1968) counted the eggs of *Chthamalus stellatus* (i.e. *C. stellatus* and *C. montagui*), *P. perforatus* and *A. modestus* at numerous points along the European Atlantic coast, including, in the case of *C. stellatus*, 9 points in France between Grandville and Hendaye, apparently without encountering anomalies in the appearance of the eggs.

At the end of the century, Burrows, Hawkins & Southward (1992) in their comparative description of the reproduction of *C. stellatus* and *C. montagui* in the Plymouth area, make no mention of it. Similarly, in Ireland, O'Riordan, Myers & Cross (1992, 1995), did a thorough study of the reproduction of *C. stellatus* and *C. montagui* and don't mention any disease. In France studies on parasitism of *C. "stellatus"* carried out by Bocquet-Védrine (1961) and Goudeau (1977) although not involved in reproduction would probably have detected it too.

From the data available in France, it can therefore be deduced that the parasite was not present there before the 1970s and 1980s, unless, of course, it was endemic to southern Brittany or the Gulf of Morbihan, which seems highly unlikely given the natural and artificial exchanges that take place between regions in the marine environment. So it is hightly likely that the parasite is an introduced species. It has to be said that the Morbihan gulf is known as a hot-spot of species introduction in France (Le Roux 2018).

Two species of oomycota parasitic on barnacle eggs have been described:

— *Lagenidium chthamalophilum* (Johnson, 1958) on *Chthamalus fragilis* Darwin, 1854 from the eastern coast of the United States (Johnson 1958, as *C. fragilis denticulata*).
— *Lagenidium callinectes* (Couch, 1942), initially described in eggs of the blue crab *Callinectes sapidus* Rathbun, 1896 (Couch 1942) also from the eastern coast of the United States. Johnson & Bonner (1960) has shown that this is the same species that attacks the eggs of the barnacle *Chelonibia patula* Ranzani, 1818 [now *C. testudinaria* (Linnaeus, 1758)] which attaches itself to the crab carapace.

There are many differences between *L. chthamalophilum* as described by Johnson (1958) and the present species: in the first one, hyphae appear from the beginning on the surface of the embryo, their cytoplasm is foamy and golden hyaline, the sporangia, intramatrical, are clearly differentiated, the spores do not fill the vesicle, oocytes are rare. Moreover, *C. chthamalophilum* seems to be strictly host specific, attempts to contaminate *Balanus amphitrite* [now *Amphibalanus amphitrite* (Darwin, 1854)] failed first (Johnson 1958) but was however successful, in special conditions, later (Johnson 1960). Consequently, it is unlikely that the Morbihan parasite belongs to this species.

L. callinectes has a wide host spectrum, is able to contaminate several decapod eggs (Rogers-Talbert 1948; Nakamura & Hatai 1995; Shields, Williams & Boyoko 2015), barnacle eggs (Johnson & Bonner 1960), a rotifer (Des & Fris 2007) and even algae (Fuller, Fowles & McLaughlin 1964). In the latter case the specific attribution of the parasite seems however uncertain as stated by the authors: "we believe this fungus is *Lagenidium callinectes* Couch".

Johnson & Bonner (1960) do not describe the aspect of the lamellae in *Chelonibia* but emphasize the abundance of extramatrical hyphae and their termination by a bulbous formation (vesicle) which does not seem destined to form a sporangium. These characters are shared with the present species. On the other hand, the diameter of the extramatrical hyphae (8–14 µm) as well as that of the resting cells (18–36 µm) are compatible with it too. The same for the data of Crisp, Bland & Bahnweg (1989) in their emended description of the species about hyphae (5.6–13.7 µm) and resting cells (18–32 µm). Their vesicle however are significantly larger (42.6–50.7 µm) this could be due to a lack of maturity in the present case, or to culture conditions.

It should be noted that the description and especially the drawings or photographs (Fig. 3, except the spores) provided by Nakamura & Hatai (1995), Hatai, Roza & Nakayama (2000, Fig. 4) and Bland & Amerson (1973, Fig. 11, 13 and 17) for *L. callinectes* correspond well with the images of the present parasite.

Gotelli (1974a,b) does not indicate precisely the origin of the material he cultivated and does not specify the criteria that were used to determine it. This makes its identification somewhat doubtful. However our figures 11A and 11B are in good agreement with his figures 5 and 6 (Gotelli 1974b).

L. callinectes has a wide geographic distribution (perhaps artificially extended) since it was described in the eastern USA and is also found on the western coast of USA, the Philippines, Tahiti (Crisp, Bland & Bahnweg 1989), in Japan (Nakamura & Hatai 1995) and Indonesia (Hatai, Roza & Nakayama 2000).

Taking into account the above elements, it seems reasonable to consider that the Morbihan parasite belongs to the species *L. callinectes*. However, this is a provisional proposal (made by a zoologist with little experience in the field of oomycota) because obviously much knowledge is still missing, especially concerning sporulation, to put forward a firm denomination. It will be the task of specialists to adopt, clarify or reject this proposal based on further morphological observations, cultures if possible, and, ideally, on DNA sequencing.

Conclusion

The first symptoms of the disease that affects barnacles in Morbihan gulf were detected in 2016 on *C. stellatus* in the form of an anomaly in the egg masses of breeding individuals. The eggs masses are invaded by a network of filaments that penetrate the eggs and destroy them, this leads to attribute the pathology to an oomycota.

The study of the disease in this species shows that it is present during the whole breeding period (spring-summer) and that it concerns the western part of the gulf. The demonstration of the same symptoms in four other species (*C. montagui*, *A. modestus*, *A. eburneus* and *P. perforatus*) shows that the pathology is polyvalent and rages practically over the whole gulf with, however, less aggressiveness in the eastern basin. It will be interesting to monitor the reproduction of *S. balanoides*, which reproduces in winter, to know if the parasite is still active in this season.

The symptoms of the disease as well as certain characteristics of the hyphae, sporulation and resting cells lead, as a hypothesis which needs to be confirmed by future studies, to link the species to *L. callinectes* a species however "quite heterogenous" according to Crisp, Bland & Bahnweg (1989). If it is indeed this species, the Morbihan form is characterized by an important investment in the "sexual" phase of reproduction (abundance of resting cells). Its mention would be the first one in European waters.

L. callinectes has a wide host range and its presence would raise concerns for other cirripedes, notably for the fragile populations of *Pollicipes pollicipes* (Gmelin, 1791 [in Gmelin, 1788–1792]) in southern Brittany and also for commercially important decapods such as crabs, lobsters and shrimps. It is reassuring to note that, although the disease has been present for 5 years, and perhaps much more, to our knowledge, no symptoms have been reported concerning these species. However given the discretion with which this disease is manifested, it is not forbidden to think that its presence in the gulf is ancient and that it dates back to the period of massive importation of the Pacific oyster *Magallana gigas* (Thunberg, 1793) in the 1970s. One may then wonder if there is not a link between the unexplained and dramatic fall in the numbers of two decapod crustaceans, the hermit crab *Pagurus bernhardus* (Linnaeus, 1758) and the shrimp *Palaemon elegans* Rathke, 1836 (Le Roux 2006) in the gulf in the 80's. It will be useful to monitor the susceptibility of the eggs of these two species to the presence of the parasite. In this respect however, it may be noted that Le Roux (2013) did not notice the parasite on *A. eburneus* at Kérino (point 10) that is in favor of a recent introduction.

Barnacles are likely to be transported attached to the hull of ships and also by transfers of mollusks from one shellfish basin to another, so this disease has probably been spread outside the Morbihan Gulf (since the manuscript of this paper was first submitted, the parasite has been spotted at Penvins (47°49′N 2°41′W) and on the western coast of the presqu'île de Quiberon at Penthièvre (47°32′N 3°8′W)) and it will be interesting to try to detect it along the Breton and even European coasts in the coming years. Such an operation would be relatively easy and inexpensive, since barnacles are readily collected and symptoms are easily detected. It would make it possible to establish a distribution map of the parasite and to program studies in parasite free regions in order to be able to measure the effects of the installation of the disease on the barnacle communities.

Samples will be deposited in the collection of the Observatory of the European Institute for Marine Studies - University of Brest, France.

Acknowledgement

The author would like to thank the University of Bretagne Sud for its documentation facilities, Evelyne Koc (university library) for the rapidity of her answers to his requests, Guillaume Gelinaud (Bretagne Vivante, Réserve Naturelle des Marais de Séné) for his friendly help, Lionel Allano (Station de Biologie Marine de Bailleron) for collecting barnacles in Bailleron, and Jean-Yves Monnat. The latter asked him, some years ago, if *Chthamalus stellatus* was to be found in the Morbihan gulf. Had he not, this paper would not be.

References

Barnes, H. & Barnes, M., 1968. Eggs numbers and metabolic efficiency of egg production and fecundity; local and regional variations in a number of common cirripeds. *Journal of Experimental Marine Biology and Ecology,* **2 (2),** 135–153. doi:10.1016/0022-0981(68)90004-X.

Barnes, H., Barnes, M. & Klepal, W., 1977. Studies on the reproduction of cirripeds. I. Introduction: copulation, release of oocytes and formation of the egg lamellae. *Journal of Experimental Marine Biology and Ecology,* **27 (3),** 195–218. doi:10.1016/0022-0981(77)90124-1.

Barnes, H. & Crisp, D. J., 1956. Evidence of self-fertilization in certain species of barnacles. *Journal of the Marine Biological Association of the United Kingdom,* **35 (3),** 631–639. doi:10.1017/s002531540001047x.

Barnes, M., 1992. The reproductive periods and condition of the penis in several species of common cirripedes. *Oceanography and Marine Biology: an Annual Review,* **30,** 483–525.

Bland, C. E. & Amerson, H. V., 1973. Observations on *Lagenidium callinectes*: isolation and sporangial development. *Mycologia,* **65 (2),** 310–320. doi:10.2307/3758103.

Bocquet-Védrine, J., 1961. Monographie de *Chthamalophilus delagei*, Rhizocéphale parasite de *Chthamalus stellatus* (Poli). *Cahiers de Biologie Marine,* **2 (5),** 455–593.

Burrows, M. T., Hawkins, S. J. & Southward, A. J., 1992. A comparison of reproduction of co-occuring chthamalid barnacles *Chthamalus stellatus* (Poli) and *Chthamalus montagui* Southward. *Journal of Experimental Marine Biology and Ecology,* **160 (2),** 229–249. doi:10.1016/0022-0981(92)90240-B.

Couch, J. N., 1942. A new fungus on crab eggs. *Journal of the Elisha Mitchell Scientific Society,* **58,** 158–162.

Crisp, D. J. & Davies, P. A., 1955. Observations *in vivo* on the breeding of *Elminius modestus* grown on glass sides. *Journal of the Marine Biological Association of the United Kingdom,* **34 (2),** 357–380. doi:10.1017/S0025315400027715.

Crisp Jr., L. M., Bland, C. E. & Bahnweg, G., 1989. Biosystematics and distribution of *Lagenidium callinectes*, a fungal pathogen of marine crustacea. *Mycologia,* **81 (5),** 709–716. doi:10.2307/3759875.

Des, R. & Fris, J., 2007. *Lagenidium callinectes* infection on rotifers *Brachionus* sp. *Indonesian Aquaculture Journal,* **2,** 15–22.

Fuller, M. S., Fowles, B. E. & McLaughlin, D. J., 1964. Isolation and pure culture study of marine phycomycetes. *Mycologia,* **56 (5),** 745–756. doi:10.1080/00275514.1964.12018163.

Gotelli, D., 1974a. The morphology of *Lagenidium callinectes*. I. Vegetative development. *Mycologia,* **66 (4),** 639–647. doi:10.1080/00275514.1974.12019655.

Gotelli, D., 1974b. The morphology of *Lagenidium callinectes*. II. Zoosporogenesis. *Mycologia,* **66 (5),** 846–858. doi:10.1080/00275514.1974.12019682.

Goudeau, M., 1977. Contribution à la biologie d'un crustacé parasite : *Hemioniscus balani* Buchholz, isopode épicaride. Nutrition, mues et croissance de la femelle et des embryons. *Cahiers de Biologie Marine,* **18 (2),** 201–242. doi :10.21411/CBM.A.BA5B92D.

Hatai, K., Roza, D. & Nakayama, T., 2000. Identification of lower fungi isolated from larvae of mangrove crab, *Scylla serrata*, in Indonesia. *Mycoscience,* **41 (6),** 565–572. doi:10.1007/BF02460922.

Johnson Jr., T. W., 1958. A fungus parasite in ova of the barnacle *Chthamalus fragilis denticulata*. *The Biological Bulletin,* **114,** 205–214. doi:10.2307/1538849.

Johnson Jr., T. W., 1960. Infection potential and growth of *Lagenidium chthamalophilum*. *Botany,* **47 (5),** 383–385. doi:10.2307/2439226.

Johnson Jr., T. W. & Bonner Jr., R. R., 1960. *Lagenidium callinectes* Couch in barnacle ova. *Journal of the Elisha Mitchell Scientific Society,* **76,** 147–149.

Le Roux, A., 2005. Les patelles et la régression des algues brunes dans le Morbihan. *Penn Ar Bed,* **192,** 1–22.

Le Roux, A., 2006. Aperçu de la flore et de la faune marines du Mor-Bihan ou Golfe du Morbihan. *Bulletin de la Société Polymathique du Morbihan,* **132,** 359–405.

Le Roux, A., 2008. Les patelles (*Patella vulgata* L.) agents de la destruction de la couverture algale des estrans rocheux du Golfe du Morbihan. *Bulletin de la Société des Sciences Naturelles de l'Ouest de la France,* **30,** 162–180.

Le Roux, A., 2012. Quoi de neuf chez les patelles *Patella vulgata* L. ? *Penn Ar Bed,* **212,** 29–40.

Le Roux, A., 2013. Observations sur la balane *Balanus (Amphibalanus) eburneus* Gould, 1841 (Crustacé, Cirripède) et les espèces associées dans le port de Vannes (Morbihan, France). *Bulletin de la Société des Sciences Naturelles de l'Ouest de la France,* **35,** 1–10.

Le Roux, A., 2018. Additions à la liste des espèces marines introduites dans le golfe du Morbihan. *Penn ar Bed,* **230,** 37–60.

Nakamura, K. & Hatai, K., 1995. Three species of Lagenidiales isolated from the eggs and zoae of the marine crab *Portunus pelagicus*. *Mycoscience,* **36 (1),** 87–95. doi:10.1007/BF02268577.

O'Riordan, R. M., Myers, A. A. & Cross, T. F., 1992. Brooding in the intertidal barnacles *Chthamalus stellatus* (Poli) and *Chthamalus montagui* Southward in south-western Ireland. *Journal of Experimental Marine Biology and Ecology,* **164 (1),** 135–145.
doi:10.1016/0022-0981(92)90141-V.

O'Riordan, R. M., Myers, A. A. & Cross, T. F., 1995. The reproductive cycle of *Chthamalus stellatus* (Poli) and *C. montagui* Southward in south-western Ireland. *Journal of Experimental Marine Biology and Ecology,* **190 (1),** 17–38.
doi:10.1016/0022-0981(95)00029-Q.

Rogers-Talbert, R., 1948. The fungus *Lagenidium callinectes* Couch (1942) on eggs of the blue crab in Chesapeake Bay. *The Biological Bulletin,* **95,** 214–228.
doi:10.2307/1538026.

Shields, J. D., Williams, J. D. & Boyoko, C. D., 2015. Parasites and diseases of Brachyura. In *Treatise on Zoology - Anatomy, Taxonomy, Biology. The Crustacea, Volume 9 Part C (2 vols).* (Eds. Castro, P., Davie, P., Guinot, D., Schram, F. & von Vaupel Klein, C.) Brill, Leiden. Chap. 71-12, 639–774. 1221 p.
doi:10.1163/9789004190832_015.

Southward, A. J., 1976. On the taxonomic status and distribution of *Chthamalus stellatus* (Cirripedia) in the north-east Atlantic region: with a key to the common intertidal barnacles of Britain. *Journal of the Marine Biological Association of the United Kingdom,* **56 (4),** 1007–1028.
doi:10.1017/S0025315400021044.

Southward, A. J., 2008. Barnacles. (Eds. Crothers, J. H. & Hayward, P. J.) *Synopses of the British Fauna (New Series),* **57**. Field Studies Council, Shrewsbury. 140 p.

Walley, L. J., White, F. & Brander, K. M., 1971. Sperm activation and fertilization in *Balanus balanoides*. *Journal of the Marine Biological Association of the United Kingdom,* **51 (2),** 489–494.
doi:10.1017/S0025315400031933.